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Overcoming lignin heterogeneity: reliably characterizing the cleavage of technical lignin†

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The cleaving of technical lignin by different technologies is a promising but complex approach for the production of value-added compounds from biomass. A rigorous description of the underlying mechanisms and influencing parameters is complicated by lignin's heterogeneity, hampering comparability and reproducibility. This frequently leads to contradicting data and theories in the scientific community. By applying a statistical design of experiment approach to the electro-oxidation of Kraft lignin, the statistical variation of the experimental data can be separated from the systematic effects of reaction parameters on lignin properties. The data allow a clear quantification of the effect of temperature, alkalinity, catalyst, lignin concentration and current density on the molecular weight, monomer production, UV absorbance as well as acid-solubility of the treated lignin. The method described can be applied to reliably investigate the whole range of lignin cleavage technologies in spite of the pronounced heterogeneity of lignin.

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Introduction

In light of diminishing fossil resources, global warming and an ever increasing demand of society for sustainability, the chemical industry is undergoing a fundamental paradigm change from fossil-based fuels to biorenewables. The global advancement of biorefinery concepts paves the way for industrial-scale production of lignocellulose-based fuels, chemicals and polymers. Most importantly, the new fractionation processes lead to clean and well-defined process streams of cellulose, hemicellulose and lignin, simplifying the further use of these fractions.¹ These new process designs become technically and economically viable, when lignin is utilized in a more efficient way than simply as a fuel.²

The lignin fraction is already used industrially in a multitude of applications, such as for the controlled release of fertilizers, as a basis for bioplastic materials, nanoparticles and dispersants.³ If lignin itself is not to be utilised as a product in its polymeric state, one of the most heavily-pursued research topics concerning lignin is the production of monomeric aromatic compounds by the cleavage of the intramolecular bonds between the molecule's aromatic rings.⁴

Different approaches for the selective cleavage of these bonds are currently investigated: cleavage by wet aerobic oxidation,⁵ homogeneous⁶ and heterogeneous catalysis,⁷ biological approaches⁸ or, as used in this work, electrochemical cleavage, just to name a few. For an overview of the current methods of lignin depolymerization see Zakzeski *et al.*⁴

Due to the difficulties related to the complex structure of lignin, many of these depolymerization techniques are applied to model substrates that represent specific characteristics of lignin's spectrum of chemical bonds. However, to our best knowledge it has not yet been shown that the results from these model compound studies can directly be transferred to a technical lignin substrate. As such, the investigation of the cleavage of technical substrates is more complex, but equally, if not more, relevant.

For the quantification of the lignin cleavage, many different analytical methods have been applied by the scientific community, such as UV spectroscopy,⁹ the determination of acid-solubility or solubility in organic solvents (*e.g.* ethyl acetate⁶), aqueous^{10–12} and organic size exclusion chromatography^{13,14} (SEC), gas chromatography¹⁵ (GC), high pressure liquid-chromatography¹⁶ (HPLC), 2D-nuclear magnetic resonance spectroscopy^{17,18} (2D-NMR) as well as electrospray ionization^{19,20} (ESI) and matrix-assisted laser desorption/ionization^{21–23} (MALDI). Some of these techniques, for instance 2D-NMR and SEC clearly indicate functional groups and molecular weight, respectively, whereas UV spectroscopy and acid-solubility determination are more easily performed but return rather unspecific information that might or might not relate to specific molecule characteristics.

Yet, even if the most sophisticated analysis techniques are applied to technical lignin, its impurities, complex structure and

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heterogeneity render the identification of the cleavage mechanisms difficult. In addition, the reproducibility of the lignin cleavage can be extremely low: in one of the few studies showing repeated measurements, Smith and co-workers achieved lignin depolymerization yields by electrochemical treatment varying from 0.36% to 30% under exactly similar conditions.²⁴

These difficulties and reproducibility issues can be well seen when studying the history of the electrochemical degradation of lignin: conflicting information is documented by the scientific community. This might stem from differing wood types and extraction methods, which are known to have a strong effect on lignin structure,²⁵ differing measurement methods and interpretation of their complex results, but also from the pronounced heterogeneity that lignin exhibits. The following paragraphs give a short review of the state of the art of electrochemical lignin oxidation, with the most important aspects summed up in Table 1.

In 1989 Smith and coworkers²⁴ depolymerized organosolv lignin by electrochemical oxidation in alkaline solutions and found that without additives, high temperatures (170 °C) and high alkalinity (3 M NaOH) were needed to effectively reduce lignin to monomeric compounds. However, the authors also reported that the results differed strongly from experiment to experiment, even if all parameters were kept constant. In a follow-up study of the same group, Pardini and coworkers²⁶ investigated the mechanism of the previously observed oxidative depolymerization on nickel electrodes and found that the lignin polymerizes to higher chain lengths while simultaneously aldehydes and carboxylic acids are being formed. The study also showed that heating up the lignin solution to 150 to 160 °C prior to the electrochemical treatment induces chemical transformations that facilitate the electrochemical cleavage, explaining the results from their previous work. Pardini *et al.* have also proposed two different pathways for the cleaving mechanism of lignin at Ni electrodes. The first is based on an electron transfer from the deprotonated hydroxyl group to the electrode, which eventually leads to aromatic aldehydes. The second pathway is based on the formation of NiOOH at the electrode surface in the alkaline solution, which then reacts with lignin to eventually form carboxylic acids.

The influence of current density, electrode material and lignin concentration on the electrochemical oxidative depolymerization of Kraft lignin was investigated by Parpot and co-workers.²⁷ They found that high current densities were needed, leading to the formation of oxygen, in order to effectively cleave the lignin. The study also showed that an increase in current density lead to an increase in generated monomers and the authors reasoned that this is a consequence of the higher amount of oxygen that is generated at the anode at higher current densities. However, they also reported that there was a maximum of monomer yield that could be achieved, followed by a degradation of the intended products. Interestingly the study could not identify a correlation between the lignin concentration and the monomer yield.

In contrast to the findings of Parpot *et al.*²⁷ two other studies^{28,29} came to different conclusions: with the electro-oxi-

Table 1 Overview over the existing works on the electrochemical lignin cleavage of selected authors. Arrows pointing up and down indicate that the increase of the specific parameter enhances and reduces the effect of the oxidative treatment, respectively. Where no entry is given for specific parameters, the influence on the oxidation was not given in the study

No.	Temp.	Alkalinity	Lignin conc.	Current	Lignin type	Analysis	Author
1	~170 °C	3 M NaOH			Organosolv	HPLC-UV	Smith <i>et al.</i> ²⁴
2	~150 °C	2 M NaOH			Model Comp.	HPLC-UV	Pardini <i>et al.</i> ²⁶
3	RT	1 M NaOH	No effect	↑	Kraft	HPLC-UV, GC-MS	Parpot <i>et al.</i> ²⁷
4	>145 °C, ↑	3 M NaOH (optimum)	↓	↓	Lignosulfonate	HPLC-UV, SEC-UV	Smith, Utley & Hammond ²⁸
5	RT	pH 5–10		↓	Lignosulfonate	SEC-RI, UV, IR	Chen, Lu & Tu ²⁹
6	2–60 °C, ↑	0.5 M NaOH		↑	Kraft	HPLC-UV, UV	Tolba <i>et al.</i> ⁹
7	na	0.5 M NaOH			Kraft	HPLC-UV, UV, acid insolubility	Tian <i>et al.</i> ³⁰
8	RT	pH 12			Enzymatically derived	SEC-?, GC-MS	Zhu <i>et al.</i> ³¹
9	20–80 °C, ↑	1 M NaOH		↑	Enzymatically derived	SEC-?, GC-MS, ESI-MS/MS, acid-/ether insolubility, UV	Zhu <i>et al.</i> ³²

dation of lignosulfonate, they achieved higher vanillin yields at lower current densities and lower lignin concentrations, facilitated by increased temperatures. In addition, Smith, Utley & Hammond²⁸ also reported an influence of the sodium hydroxide concentration and stated that poor conversion was achieved at 1 M and 6 M sodium hydroxide solutions in comparison to using 3 M solutions.

In yet another study, the cleavage of Kraft lignin by a combination of photochemical and electrochemical oxidation was quantified by UV absorbance as well as by acidifying the reaction solution and subsequent determination of acid-insoluble lignin.³⁰

UV-measurements were also used by Tolba *et al.*⁹ in order to quantify the electrochemical oxidation of Kraft lignin at different iridium-oxide-based catalysts. In their study they report, based on the UV measurements, that increasing temperatures lead to increased reaction rates. They also state that the electrode with the highest activity for oxygen evolution shows the lowest activity for lignin oxidation, a finding contrary to the one presented by Parpot *et al.*²⁷ Tolba and co-workers also state that a higher current density leads to higher monomer yields in accordance with Parpot and coworkers²⁷ but in contrast to Smith, Utley and Hammond.²⁸

Another study investigated not only the oxidation, but the combined oxidation and reduction of lignin directly at the electrodes and by *in situ* generated hydrogen peroxide.^{31,32} Here, an increase in temperature and current density also lead to a more pronounced cleavage.

The conflicting information gathered from the scientific community combined with either unreported or low reproducibility, currently makes the task of establishing reliable facts about the electrochemical cleavage of lignin difficult.

The aim of this work is to present a method that reliably quantifies how certain reaction parameters influence the different aspects of the valorization of technical lignin despite its natural heterogeneity. While the methodology is presented for an electrochemical process, the methodical framework can also be extended to other methods of lignin valorization. To this aim, statistical design of experiment (DoE) was utilized to investigate the influence of five reaction parameters, on which conflicting reports exist in literature: current density (CD), temperature (*T*), electrode material (EM), lignin concentration (*c_L*) and sodium hydroxide concentration (*c_{NaOH}*).

In a further step, the informational value of some of the measurement methods used in the scientific community to quantify this cleavage will be investigated: UV absorbance, determination of acid-solubility, SEC and LC-MS. In addition, the correlation between the different analysis techniques shall be elucidated to see if the informational value of the less complex techniques might be enough to quantify the lignin cleavage process.

Materials and methods

Design of experiment

In order to reliably characterize the influencing parameters on the different aspects of lignin cleavage, the statistical toolbox

of design of experiments has been applied. This toolbox allows the separation of statistical deviations – in other words, noise – in a set of experimental data from the underlying statistically significant interactions between the chosen parameters and the experimental results.³³ The idea behind statistical DoE is to derive the maximum amount of information from a given set of experimental runs or to minimize the number of experiments to achieve a desired information density. In DoEs, the experimental parameters that can be actively controlled are termed factors (*e.g.* temperature) and the specific values that these factors can take are termed levels (*e.g.* 30 and 80 °C). So-called factorial designs, where several factors are set on two distinct levels (defined as low and high), are especially useful when

- investigating a large set of parameters in confined boundaries
- investigating the interaction of several factors
- conducting screening experiments to identify which parameters are most worthwhile to be further investigated.³⁴

A full factorial design of, as in this case, five factors (CD, *T*, EM, *c_L*, *c_{NaOH}*) and two levels consists of all possible factor combinations, leading to 32 experiments (2⁵: 2 levels, 5 factors). This rather high number can be reduced by a factor of two if one is willing to accept a slightly lower information density, leading to 16 experiments in a fractional factorial design. This design has been used for the presented work.

The core of such a DoE is a null hypothesis, which will either be proved or disproved by the gathered data. The results of the DoE are so-called *p*-values that signify the chance that the examined data can be gathered if the null hypothesis is valid. High *p*-values (0.05 and above) indicate that the investigated data correspond to the validity of the null hypothesis. A low *p*-value (below 0.05) means that the resulting data can only be gathered if the null hypothesis is false.

Here, the null hypothesis states that the investigated parameters have no influence on the different measurement signals that are utilized to follow the cleavage of lignin (MW, UV absorbance, acid solubility, monomer yield). If the *p*-value of the resulting data is below 0.05 for the specific parameters, the null hypothesis is disproven and these parameters have a statistically significant influence on the signal. In general, the data investigated in the DoE is always the difference in a measurement signal from the beginning to the end of the electrochemical reaction. Data relevant to the DoE and its analysis can be found in the ESI.†

Electrochemical cleavage of lignin

Based on the state of the art of electrochemical lignin cleavage, five process parameters have been identified for further investigation: temperature, current density, lignin concentration, sodium hydroxide concentration and the catalyst material. Table 2 gives an overview over the chosen factors and the distinct levels. In order to take advantage of the fact that electrochemical reactions can be run under mild conditions, the temperature range has been chosen so that the reaction can be conducted at ambient pressure, which limits the upper

Table 2 Investigated reaction parameters and their respective levels (low/high)

Level	Temperature [°C]	Current [mA cm ⁻²]	Lignin concentration [g L ⁻¹]	Sodium hydroxide concentration [mol l ⁻¹]	Catalyst material
Low	30	2.1	5	1	Nickel
High	80	38.3	20	4	Platinum

temperature to the boiling point of aqueous sodium hydroxide. This leads to a safer and cheaper process, with a considerably lower demand for the process equipment. As electrodes Ni and Pt were chosen. Ni is relatively cheap, long-term stable and one of the most thoroughly investigated electrodes for lignin cleavage. Pt is well established in the electrochemical waste water treatment, where the oxidation of organic compounds plays a major role.

The electro-oxidative cleavage of lignin was conducted in a two-compartment double-walled glass reactor, with an ion exchange membrane (fumasep FAB) separating anolyte and catholyte. For each experiment, 2.5 or 10 g of Kraft lignin (Beech, Sigma Aldrich 370959-500G, Lot# MKBJ0452 V) was weighed (Sartorius CPA2259, accuracy 0.01 mg) and dissolved in 500 ml of a 1 or 4 M sodium hydroxide solution (VWR 28245.320 assay 99) and was then poured into the reactor compartment of the reactor. The counter electrode compartment was filled with sodium hydroxide solution of the same molarity to prevent concentration gradients. The electrodes used for the electro-oxidations were an expanded platinum mesh and a nickel plate with a surface area of 25 cm². As counter-electrode a nickel plate with an area of 30 cm² was inserted. A thermostat (Julabo F25-ME) was used to control the reaction temperature. The current density was set at either 2.1 mA cm⁻² or 38.3 mA cm⁻² by a potentiostat (Autolab PGSTAT302N). Reference measurements were done in order to exclude the reaction of lignin in alkaline solution at the electrodes without an applied current. A scheme of the utilized reactor with the most important characteristics can be found in the ESI (Fig. SI 1†).

Analysis

Four different measurement methods were used to characterize the lignin cleavage:

- Aqueous Size Exclusion Chromatography to quantify the change in the molecular weight of the lignin molecules
- Liquid Chromatography – Mass Spectrometry to quantify target molecules that are generated during the cleavage process
- Total amount of acid-insoluble lignin to determine acid-solubility
- UV/vis spectra and absorbance at a wavelength of 280 nm of the reaction samples to investigate the change to chromophoric elements.

Whereas SEC and LC-MS are specifically targeted on certain aspects (molecular weight and monomeric compounds, respectively), acid-insoluble lignin and UV/vis are rather un-

specific measurement techniques. However, these unspecific techniques are much faster, cheaper and easier to perform than their specified counterparts. Next to the quantification of the cleavage process, the aim of this study was to investigate if acid-insoluble lignin amount and UV/vis absorbance at a wavelength of 280 nm can be used as easy methods to quantify the lignin cleavage.

Due to the difficulty of quantifying all monomeric compounds that might occur in the product stream of the electrochemical depolymerization, a representative subset of seven monomers has been chosen as markers. This subset represents the three monomeric building blocks guaiacyl-, syringyl- and coniferyl-alcohols with three different functionalities, that can be derived from lignin oxidation: 4-hydroxybenzaldehyde (Hy), vanillic acid (4-hydroxy-3-methoxybenzoic acid, VAc), syringic acid (4-hydroxy-3,5-dimethoxybenzoic acid, SAc), vanillin (4-hydroxy-3-methoxybenzaldehyde, V), syringaldehyde (4-hydroxy-3,5-dimethoxybenzaldehyde, S), acetovanillone (4-hydroxy-3-methoxyacetophenone, VO) and acetosyringone (3,5-dimethoxy-4-hydroxyacetophenone, SO).³⁵

Average Standard Errors of Measurement (SEM) have been determined for all the analytical techniques to quantify the reproducibility of the measurements and can be found in the ESI (Table SI 4†). During the electrochemical lignin treatment, samples have been removed at appropriate times and were adequately pre-treated for the subsequent analyses. A scheme of the sampling and subsequent analysis technique can be found in the ESI (Fig. SI 3†).

Size exclusion chromatography. Molecular weights (M_n and M_w) and polydispersity (M_w/M_n) were determined by SEC. Analyses were performed using an Agilent 1200 system equipped with a refractive index detector and a UV detector (VWD-UV, Agilent) at a wavelength of $\lambda = 280$ nm. SEC analyses were carried out with water (HPLC grade, Carl Roth) containing 0.1 mol L⁻¹ sodium hydroxide (NaOH, 99.5%, VWR chemicals) and 0.01 wt% sodium azide (NaN₃, extra pure, Merck KGaA) as eluent. As internal standard 12.5 mg mL⁻¹ glucose monohydrate (biochemistry, Merck KGaA) was used. One pre-column (8 × 50 mm) and three MCX gel columns (8 × 300 mm) were used at a flow rate of 1.0 mL min⁻¹ at 40 °C. The diameter of the gel particles measured 5 µm, the nominal pore widths were 1000, 1000 and 1000 Å. Calibration was achieved using narrowly distributed poly(styrene sulfonate) standards (Polymer Standards Service). Results were evaluated using the PSS WinGPC UniChrom software (version 8.1.1).

UV absorbance. For UV absorbance measurements, samples of 0.5 mL volume have been taken and have been appropriately

diluted (5 g L⁻¹ samples: 1:40; 20 g L⁻¹ samples: 1:160), transferred to 96-well-plates (greiner bio-one UV-star micro-plate, 655801) in triplicates and were measured *via* a wellplate reader (Synergy MX Biotek, spectrum wavelength 230 to 700 nm).

Acid solubility. For acid solubility and LC-MS measurements, 5 mL samples have been removed from the reactor and were acidified to pH 2 utilizing a titrating device (Titrand, Metrohm Autolab) and 1–4 M H₂SO₄ to precipitate the remaining lignin which allows a separation of insoluble lignin and the soluble low-molecular reaction products. The samples were subsequently centrifuged three times at 17 000g (Sigma Laboratory Centrifuge 3K30) with the removal of supernatant and addition of 5 mL H₂SO₄ between the centrifugation steps. The precipitated lignin has been weighed whereas the supernatant was prepared for LC-MS measurements by solid phase extraction. The precipitated lignin has been dried at least 24 hours at 50 °C (memmert drying closet) before weighing (Acculab Atilon, accuracy 0.1 mg). All measurements were done in triplicate.

Solid phase extraction. The supernatant of the triplet centrifugation was pre-treated *via* a modified solid phase extraction based on Pinto, da Silva & Rodrigues.³⁵ The acidic supernatant was transferred onto solid phase extraction columns (LiChrolut En, 40–120 µm, 500 mg, 6 ml, Merck). Chemicals used for SPE were water (EMSURE®, 1.16754.9010, VWR), methanol (Optigrade®, SO-9356-B025 LGC-Standards) and formic acid (EMSURE®, 1.00264.0100). Eluent A contained 90/10 (v/v) water/methanol with 0.1% (v/v) formic acid and eluent B the opposite, 90/10 (v/v) methanol/water with 0.1% (v/v) formic acid. Cartridges were conditioned with 7 ml methanol and afterwards rinsed with 10 ml of eluent A for column equilibration. The samples were filled in the cartridge and 10 ml of eluent A were added for interference elution. For elution of analytes 9 ml of eluent B were applied. The volume was collected and filled up to 10 ml with eluent B for determination of concentrations. Recovery rates after SPE were determined and taken into account when calculating monomer yields.

Liquid chromatography-mass spectrometry. The used equipment was a surveyor liquid chromatography system from Finnigan consisting of a MS binary pump, autosampler plus and a temperature controlled column compartment. A Finnigan mass spectrometer LXQ ion trap with APCI, positive polarity mode, at a temperature of 290 °C, was used as detector. Helium 5.0 was used as a collision gas. The used software was Xcalibur 2.0.

HPLC-parameters: Column Hypersil Gold from Thermo Scientific, C18150 mm × 2.1 mm, 5 µm. Mobile phases used were (A) water/methanol (95/5) with 0.1% formic acid and (B) water/methanol (5/95) with 0.1% formic acid. The gradient started at 5% B then ramped to 50% eluent B. Flow rate was 0.2 ml min⁻¹. Injection volumes were 20 to 50 µl. Column temperature was set to 40 °C and run time was 37 minutes. All calibration samples were dissolved in water/methanol (90/10) + 0.1% formic acid. Calibration range was 0.005–10 mg l⁻¹ for

each component. Quantification was executed with the daughter ions.

Results and discussion

Factors governing the electrochemical lignin cleavage

Table 3 shows the *p*-values of the investigated reaction parameters on the chosen target signals. The columns depict the signal that is investigated and the rows the reaction parameter. The *p*-values are an indication how strongly a reaction parameter influences the measured signal, but not if it influences it in a negative or positive direction. So an effect can be either negatively correlated (the higher the current density, the lower the UV signal) or positively correlated (the higher the current density, the higher the monomer yield). When considering the one factor interactions, it is striking that the sodium hydroxide concentration does not seem to influence any of the investigated lignin characteristics in the investigated parameter space. This result does not correspond with the research of Smith, Utley and Hammond.²⁸ Next to system-inherent heterogeneity, reasons for this might lie in the different nature of the utilized lignin or a parameter window that does not fully overlap with the one in the above mentioned work.

The temperature in the range investigated (30 °C to 80 °C) only shows a significant influence on the acid solubility. This is especially interesting since an influence of the temperature on the monomer yield and the molecular weight was expected. Yet, these parameters do not seem to change significantly in this temperature regime. It is important to note that this trend will substantially alter as soon as a change in reaction mechanism occurs. Temperatures in the range of 150 °C are accompanied by chemical reactions occurring before the electrochemical reaction,²⁴ so these results cannot be extended to these temperature ranges.

One of the main influences that were identified is the current density. An increased current density leads to an increased monomer generation, a stronger UV absorbance decrease as well as a higher grade of depolymerization. The amount of lignin that is soluble in the acid regime is increased as well. This seems to underline the fact that in mild electrochemical reactions the influence of the electrochemical potential is significantly higher than that of the temperature,

Table 3 *p*-Values of the factors concerning the different measurement methods (<0.05: significant, <0.001: highly significant, ns: not significant)

Factor	UV absorbance	Monomer yield	Acid insoluble lignin	Relative molecular weight
Temperature	ns	ns	0.0243	ns
Current density	0.0085	0.0293	<0.0001	0.0039
Lignin concentration	ns	0.0025	ns	ns
Sodium hydroxide concentration	ns	ns	ns	ns
Electrode material	0.005	0.0017	ns	ns

spotlighting promising process improvements. One challenge that remains is the identification of the underlying cause of these observations: higher current densities lead to a higher rate of oxygen generation at the electrodes as well as a higher grade of lignin cleavage. However, it is yet unclear if the generated oxygen is responsible for the lignin cleavage, or if the oxygen evolution is purely a secondary effect without influence on the cleavage result.

The influence of the lignin concentration can mainly be seen in the amount of target monomers that are produced. A decrease in dissolved lignin leads to a higher vanillin yield and monomer yield in general, rendering the process more economical in terms of substrate utilization. However, the lower product concentration can lead to a significant increase in the cost of downstream processing and thus opposes benefits gained from a more efficient material usage.

The catalyst material is influencing the UV absorbance as well as the monomer yield. Here, only two catalyst materials have been investigated: Platinum (Pt) and nickel (Ni). The use of Pt leads to lower monomer yields than Ni, rendering the Pt catalyst for the prevalent target less efficient.

Two-factor interactions could not be identified, which means that there is no specific effect when two of the investigated parameters are set to a certain level compared to the sum of their effect (see Fig. SI 7–10†).

Intermediate conclusions that can be drawn from these results are the following: for a maximum efficient process in terms of monomer yield, a Ni catalyst should be employed at a temperature of 30 °C, a current density of 1.8 A and a lignin concentration of 5 g l⁻¹. The sodium hydroxide concentration can be kept at a necessary minimum for solubility and conductivity to simplify the process conditions. If energy efficiency is of interest, a reduction of the current density to the lower level will be favored. Even though the study focuses exclusively on Kraft lignin and the influence of the pretreatment method on the results have not been investigated, the authors expect that the general trends hold true for other lignin types as well.

Single parameter evaluation

After having identified the magnitude of the influence that the chosen parameters have on the lignin cleavage, the statistically insignificant factors can be clustered, so that the cleavage process is only dependent on the remaining significant reaction parameters. As mentioned in the last section, the significance of a reaction parameter depends on the reaction characteristic that is measured. In the following paragraphs, the characteristic of the cleaving process is investigated as a function of reaction time and the significant effects.

Relative molecular weight. Fig. 1 shows the relative molecular weight of the measured samples over the course of the reaction for the two applied current densities. The error bars in this and all subsequent graphs depict the standard deviation of the investigated samples (sample size: 8 experiments). According to the statistical analysis in the last section the temperature, electrode material, lignin and sodium hydroxide concentration have no significant influence on the change in

Fig. 1 Relative molecular weight of Kraft lignin over the course of 4 hours reaction in an electrochemical reactor compared to the starting weight as measured by SEC. Error bars depict the standard deviation (sample size: 8). Temperature, electrode material as well as lignin and sodium hydroxide concentrations have no influence on the molecular weight. Lines are only visual help and not calculated.

molecular weight. At a current density of 2.1 mA cm⁻² an initial polymerization of the lignin can be observed, leading to increased molecular weights by up to 33.5% (run 9), stabilizing after approximately two hours. At higher current densities, the molecular weight decreases linearly over the reaction by up to 75.7% (run 8) of the original MW.

The fact that the electrocatalytic material does not have a major effect on the result of the electrocatalytic cleavage seems to support the hypothesis that the oxygen activity has a higher impact on the cleavage than the interaction of the lignin molecule with the electrocatalytic material itself. However, due to the large size and low diffusion coefficient of lignin, the influence of the electrode material could also be reduced by mass transport limitations.³⁶

As lignin is originally formed by oxidative polymerization from its base units, it is assumed that too mild oxidative environments lead to a further polymerization of the molecule. These results suggest that the oxidative conditions need to be severe enough to counter the oxidative polymerization mechanism and allow the cleavage of the intramolecular bonds. If the polymerization is a direct consequence of a reactive site that occurs during the removal of the monomeric compound from the lignin or the polymerization occurs independently of the cleavage has yet to be determined.

Important to note is the quite large standard deviation that the investigated sample depicts, which is in accordance to the low reproducibility reported in literature. To draw conclusions from a single experiment is almost impossible under the presented circumstances, rendering the clustering of a number of experiments, as done here, a necessity.

Acid soluble lignin fraction. Fig. 2 shows the amount of lignin that is precipitated after given reaction times by acidify-

Fig. 2 Precipitated lignin fraction after sample acidification over the course of 4 hours reaction in an electrochemical reactor (100% equals the amount precipitated from untreated samples). Error bars depict the standard deviation (sample size: 4). (a) Reaction at $T = 30\text{ }^{\circ}\text{C}$ (b) reaction at $T = 80\text{ }^{\circ}\text{C}$. Electrode material and lignin and sodium hydroxide concentrations have no influence on the amount of precipitated lignin. Lines are only visual help and not calculated.

ing the solution to pH 2 with sulphuric acid. According to the results from the screening experiment, the current density and the reaction temperature are the two dominating reaction parameters. The left graph shows the results from reactions at $30\text{ }^{\circ}\text{C}$, the right graph at $80\text{ }^{\circ}\text{C}$ each at two different current densities.

The acid soluble fraction of the Kraft lignin clearly increases over the course of the electrochemical treatment for the higher current density, with a stronger effect at the higher temperature. In run 8 a reduction of the insoluble lignin fraction to 20.64% of the untreated sample was achieved, which in some publications is labelled as a 79.36% yield. In contrast, the electrochemical treatment at the lower current density of 2.1 mA cm^{-2} and $30\text{ }^{\circ}\text{C}$ even leads to a slight increase in the insoluble fraction. At 2.1 mA cm^{-2} and $80\text{ }^{\circ}\text{C}$ the acid solubility is roughly constant over the course of the reaction.

Interestingly, the increase in solubility shows the same trends in respect to the current density as the reduction in molecular weight, which will be further investigated. Moreover, this solubility increase corresponds to a decrease of the UV signal, further hinting to a change in the molecular structure and functional groups.

UV absorbance. Fig. 3 shows the relative UV signal of the Kraft lignin samples removed from the reactor over the course of the reaction. The samples are normalized with the UV absorbance of the untreated samples. The screening experiments showed the electrocatalytic material and the current density as the governing reaction parameters. The left graph (a) of Fig. 3 shows the relative UV absorbance for the electrochemical treatment with nickel, the right graph (b) with platinum. Interesting to note is the increase in UV absorbance of the lignin sample when treated with nickel as electrocatalyst at low current density, indicating an increase in electron delocalization. When increasing the current density, the UV absorbance shows no change over the reaction from the untreated sample. Almost no change in absorbance also occurs with platinum at low current density. In contrast, at high current

Fig. 3 UV absorbance at a wavelength of 280 nm of Kraft lignin dissolved in 1 M NaOH over the course of 4 hours reaction in an electrochemical reactor compared to the absorbance of the untreated sample. Error bars depict the standard deviation (sample size: 4). Temperature and lignin and sodium hydroxide concentrations have no influence on the UV absorbance. (a) Nickel as electrocatalyst (b) platinum as electrocatalyst. Lines are only visual help and not calculated.

density platinum exhibits a strong bleaching effect, reducing the UV absorbance over the course of the reaction by up to 50% (run 8). This indicates a strong change in the amount of chromophoric groups present in the molecule, which can for example be attributed to opening of aromatic rings and the accompanying disruption of the π -electron structure.

Although UV absorbance measurements are classically utilised as a state of the art technique to determine lignin concentration in the pulp & paper industry,³⁷ this method is obviously no longer valid when a chemical transformation in the lignin occurs. A comparison to the change in molecular weight shows clearly that the chain lengths of the investigated samples are reduced at the nickel electrode even though no change in UV absorbance can be detected. To conclude, there seems to be no causal relation between the UV absorbance and the chain length of the investigated Kraft lignin.

Target monomer yield. The quantified product mix gained by the electrochemical treatment consists mainly of vanillin (61–67%) and vanillic acid (23–30%), corresponding well to the typical S:G:H distribution in softwood lignin³⁸ (the detailed composition of the products can be found in the ESI, Table SI 7†). It is important to note that it is probable that some major component of the lignin cleavage was not identified in the utilised LC-MS measurement. For example, Rahimi and coworkers⁶ showed that the formic-acid-induced depolymerization of lignin leads to a product stream that contains up to 19.8% of the initial mass of lignin in the form of two syringyl- and guaiacyl-derived diketones, which are not measured here.⁶ As such, the calculated yield of the investigated compounds does not represent the whole product mix of the electrochemical treatment.

Fig. 4 shows the yield of monomers quantified by LC-MS as a function of the reaction time and catalyst material. Each point presents the mean of two measurements, so error bars cannot be given. In general, the nickel catalyst shows a higher yield under almost all circumstances than the platinum electrode. Also, a lower lignin concentration seems to favor higher

Fig. 4 Yield of investigated monomer compounds (in relation to mass of treated lignin, corrected with the amount of monomers present in the untreated sample) over the course of 4 hours reaction in an electrochemical reactor. Temperature and sodium hydroxide concentration have no influence on the yield. Figures (a) and (b) are results for the nickel catalyst, figures (c) and (d) for platinum. (a) and (c) are for high current densities (38.3 mA cm⁻²), (b) and (d) for low current densities. The sample size for each point is 2. Lines are only visual help and not calculated.

yields independently from the electrode material or the current density. However, if the absolute mass of produced monomers is calculated, the runs with a nickel electrode, a current density of 38.3 mA cm⁻² and a lignin concentration of 20 g l⁻¹ seem to perform best, only exhibiting a lower yield due to the higher amount of lignin present in the solution. Lower current densities also lead to lower monomer yields by around 29% in the case of nickel as electrocatalyst and a lignin concentration of 5 g l⁻¹. Important to note is that the nickel electrode at a CD of 38.3 mA cm⁻² as well as the platinum electrode in general exhibit a maximum in the measured monomer yield. After a certain reaction time (between 30 and 120 minutes), the monomer yield starts to decline. This behavior also occurs on nickel electrodes, starting at longer reaction times of around 5 hours (data not shown).

Apparently, the phenolic cleaving products are not stable under the present reaction conditions and undergo further reaction as reported elsewhere.²⁷ This phenomenon is not confined to electrochemical processes: similar observations have been shown, for instance, by Deng and co-workers^{39–41} for the heterogeneous catalyzed oxidation and by Fargues *et al.*⁴² for the wet aerobic oxidation.

For electrochemical applications, it is generally believed that the phenolic compounds again undergo oxidation by a direct electron transfer and indirect oxygen atom transfer.^{43–47}

Li and co-workers⁴⁸ have proposed two competing reaction pathways, leading to (a) polymeric products and (b) to over-

oxidation and thus mineralization of phenols. The pathway to polymerization occurs *via* radical polymerization of the phenoxy radicals and hydro- and benzoquinone intermediates.

The reaction pathway to a mineralization of phenols goes *via* the formation of different acids (e.g. maleic, succinic, malonic, oxalic and acetic acid), which in turn can be mineralized to carbon dioxide and water. Other phenolic compounds are shown to form tartaric and fumaric acid, also *via* hydro- and benzoquinone intermediates.⁴⁹

According to Rodgers *et al.*⁴⁵ and Cui, Li & Chen,⁴⁹ electrodes such as graphite and Ni are less effective than Pt at mineralizing phenolic compounds, which is a possible explanation for the increased yield of phenolic substances at the Ni electrode even though the actual cleavage seems to be more defined by the oxygen activity than the electrocatalytic material itself.

Viability of shortcut methods to evaluate the lignin cleavage process

In the previous sections, the influence of several reaction parameters on the UV absorbance, the acid insolubility, the yield of a given set of monomeric compounds and the molecular weight of the lignin itself has been quantified. Two of these methods, namely UV absorbance and acid insolubility are doable without sophisticated analytical equipment and are rather easier to perform than LC-MS and SEC. The question is if these methods allow an interpretation of the cleavage process *via* a non-causal correlation of these signals to the monomer yield and the molecular weight of lignin.

To answer this question, the change of the acid solubility and UV absorbance that was induced by the electrochemical treatment after four hours of electrochemical treatment is plotted against the change in molecular weight for all the experimental runs in parity plots.

Acid solubility compared to molecular weight. Fig. 5 shows the correlation between the relative molecular weight and the relative acid insolubility after four hours of electrochemical treatment for all the experiments conducted in this study. The linear regression line for these data points shows a loose linear correlation according to equation

$$y_{\text{acid}} = 10.70 + 78.8x_{\text{MW}}$$

with a coefficient of determination of

$$r_{\text{acid.MW}}^2 = 0.739.$$

The data show a rough correlation between acid solubility and the molecular weight. However, runs 9 and 12 differ in molecular weight by roughly 35% (run 9: 133% of initial MW; run 12: 98.5% of initial MW) whereas they show almost the same value concerning the change of acid solubility (98.6 (run 9) vs. 99.8% (run 12)). It is clear that there have to be other factors involved that influence the acid solubility than the molecular weight alone, such as the change of functional groups. An investigation of the parity plots for additional reaction times (30, 60 and 90 minutes, see Fig. SI 7†) also shows

Fig. 5 Parity plot of the relative molecular weight in comparison to the relative acid insolubility of the experimental runs 1 to 16 after 4 hours of electrochemical reactions at different reaction parameters. The numbers next to the points depict the run number. The equation for the linear correlation as well as the r^2 of the correlation can be found in the top left corner.

that this correlation is weak in the beginning and grows stronger over time. However, the hypothesis that a lower molecular weight leads to a better acid solubility seems to be valid to a certain degree. The correlation between the solubility of lignin in organic solvents, *e.g.* ethyl acetate, has not been investigated and might give further clues concerning the interaction between molecular weight and solubility. At the same time, 2D-NMR of the soluble and the insoluble fractions might give valuable insight into the governing cleavage mechanisms and primarily involved bonds and functional groups.

UV absorbance compared to molecular weight. A similar plot for the comparison of UV absorbance at a wavelength of 280 nm and molecular weight (see Fig. SI 5†) leads to a coefficient of determination of

$$r^2 = 0.426.$$

In this case, the linear regression is too low to adequately replace the molecular weight determination by a UV measurement. Clearly, under the investigated circumstances the UV signal is an unsuitable method of characterization to determine the progress of the lignin depolymerization. An important conclusion from this investigation is the fact that SEC-measurements only coupled with UV detectors might give an incomplete picture of the molecular weight of lignin, because different fractions might undergo different reactions that lead to changes in the UV spectra and absorbance.

Monomer yield compared to molecular weight. A comparison between the measured monomer yield and the molecular weight shows no correlation (see Fig. SI 6†).

One would expect that a higher degree of cleavage that results in smaller lignin fragments and a lower molecular weight would correlate with an increased amount of low-

molecular compounds. This missing correlation between the effectiveness of lignin cleavage and the production of monomeric compounds again stresses the fact, that it is not enough to cleave the intramolecular bonds but that an effective process needs to be tuned so that products are not overoxidized.

However, the difficulty in the quantitative determination of all the compounds that might occur during the depolymerization of lignin prevents a full characterization of the product stream on the level of mono-, di- and trimeric compounds, which must be taken into account when evaluating the depolymerization from the perspective of the products.

Conclusions

The use of statistical design of experiment allows the clear identification of reaction parameters that influence the electrochemical oxidative cleavage of lignin despite large experimental fluctuations due to lignin heterogeneity. We showed that an increase in current density leads to a stronger reduction of molecular weight, down to 20% of the initial weight. In addition, higher current densities also lead to a higher monomer yield additionally influenced by the electrocatalyst and lignin concentration. The methodical framework presented in this work can be applied to all lignin valorization methods and thus allows the reliable characterization of lignin cleavage, independently of the depolymerization method.

The comparison of the four aforementioned measurement methods showed that a rough correlation exists between the acid-solubility and the molecular weight of the lignin, but that additional aspects must also play a role. No correlation could be shown between the molecular weight and the UV absorbance as well as between the molecular weight and the monomer yield. In addition, the use of the UV absorbance to characterize the lignin cleavage – directly or in SEC-measurements – demands special attention in case the reaction induces changes in the UV properties of the molecules that distort the measurement signal. The methodical framework presented in this work can be applied to all lignin valorization methods and thus allows the reliable characterization of lignin cleavage, independently of the depolymerization method.

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