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Fluorescence analysis of wood chips and their constituents

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Abstract: Micro-spectral analysis and fluorescence decay experiments of beech and oak chips as well as their main constituents lignin and cellulose are reported. While the lignin spectrum shows two fluorescence bands of almost identical intensity around 500 nm and 560 nm, the cellulose spectrum is dominated by a band around 500 with a less pronounced shoulder at 560 nm, whose intensity increases with the amount of residual lignin. Fluorescence decay kinetics are bi-exponential with lifetimes around 0.5 ns and 2.5 ns and an increasing contribution of the short-lived component with an increasing amount of lignin. Both data sets indicate that a quantification of residual lignin in cellulose samples obtained after decomposition of beech wood appears possible in a concentration range of 2–10 %. This may provide a non-destructive and label-free test of the quality of decomposition.

Keywords: beech wood; cellulose; lignin; fluorescence spectroscopy; fluorescence lifetimes

1 Introduction

Cellulose, hemicellulose and lignin are the polymeric structural substances of plants. Cellulose is a homopolymeric compound of glucose units which are connected by glycosidic bonds in 1,4-position (see Figure 1). Hemicelluloses (polyoses) are complex heteropolymeric compounds, which contain linear and branched structures. Building blocks are hexoses, e.g. glucose, galactose and manose, and pentoses, e.g. xylose and arabinose; additionally the partially methylated and acetylated corresponding uronic acids are present. Lignins are complex highly polymeric compounds (see Figure 2), which are set up from three building blocks: cumarylalcohol, coniferylalcohol and sinapinalcohol. Hardwoods and softwoods differ from each other in their contents of the three components, two of which – lignin and hemicellulose – are typical of wood in terms of their chemical composition. Processes for extracting cellulose from wood were developed very early and are still used industrially (Kraft digestion with sodium sulfide and sulfite digestion).[1] The denatured by-products – lignin and hemicellulose – are commonly burnt, so that essentially cellulose (approx. 40 % wood mass) is further used. In both procedures foul-smelling waste caustics are produced, which are evaporated. Further pulping methods using e.g. calcium or magnesium sulfite[2] or soda (Na_2CO_3)[3,4] as digesting compounds have been developed. Experiments aiming to an extraction of sulfur-free lignin from wood by means of organic compounds, such as acetic acid,[5] mixtures of formic acid and performic acid,[6,7] or mixtures of formic and acetic acid (formacell process),[8] have only recently been described. Sulfur-free lignin could also be extracted from hardwood with ethanol-water mixtures in the presence of oxalic acid at 150–200 °C.[9] However, the cellulose resulting in this process is unsuitable for pulp production. The decomposition of biomass using ionic liquids (IL) is a current branch of research and has already been subject of several review articles.[10–14] Lignin can be extracted from wood with “alkaline glycerine”.[15] Catalytic pulping is the subject of a review article.[16] For example, lignin-producing wood digestion is described, running in a two-phase system (water-2-methyltetrahydrofuran) in the presence of oxalic acid at a temperature of 80–140 °C. In another new wood digestion (LX process), 80 % phosphoric acid is used as a reagent. Cellulose and lignin are separated using organic solvents.[17,18] Lignin, which yields a relatively large number

Dedicated to Professor Hubert Schmidbaur on the occasion of his 90th birthday.

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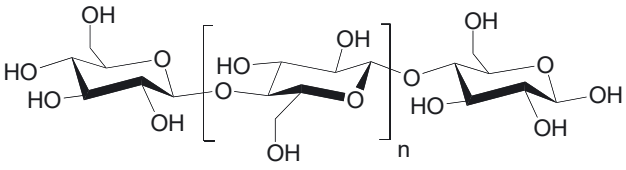


Figure 1: Structure of cellulose.

of monomeric products during hydrogenolysis, is formed when wood is treated with aqueous hydrochloric acid in dioxane upon addition of formaldehyde (5 h, 80 °C).[19,20]

By means of the so-called LICIL process, all wood components can be obtained almost unchanged. Pulping systems are used that consist of organic solvents, such as ethylglycol or tetrahydrofurfurylalcohol and catalytic amounts of reactive, iminium salt-based ionic liquids (RIBIL's),[22] such as the DMF-H_2SO_4 (1:1) adduct. As this compilation shows, new wood pulping processes are constantly being developed. The quality of a digestion process can be estimated by assessing the purity of the wood components obtained. For this purpose, the lignin content in the fiber fraction or the cellulose content in the lignin fraction is determined using classical wet-chemical methods. So far, spectroscopic methods that can be carried out quickly, have only rarely been used for these investigations.

However, in contrast to established wet-chemical methods, rapid optical methods would offer numerous advantages: they are non-destructive (in a sense that they need no further chemical treatment) and often do not require any special labelling, e.g., for infrared spectroscopy, Raman spectroscopy or autofluorescence measurements. Intrinsic fluorescence of lignin and cellulose has been well documented in the literature (for a review see, e.g., refs 23–25). In the present paper their spectral characteristics as well as their fluorescence lifetimes will be used to distinguish the individual components prior to and subsequent to decomposition by various procedures. This paper focuses on wood chips of beech and oak as well as on cellulose samples with various amounts of residual lignin obtained after decomposition of beech chips by different methods. Its main purpose is to assess the quality of decomposition and to estimate the concentration of lignin in an online procedure.

2 Materials and methods

2.1 Sample preparation

A total of 24 chips of beech and oak (12 in each case) with a size of 3–6 mm were obtained from the companies

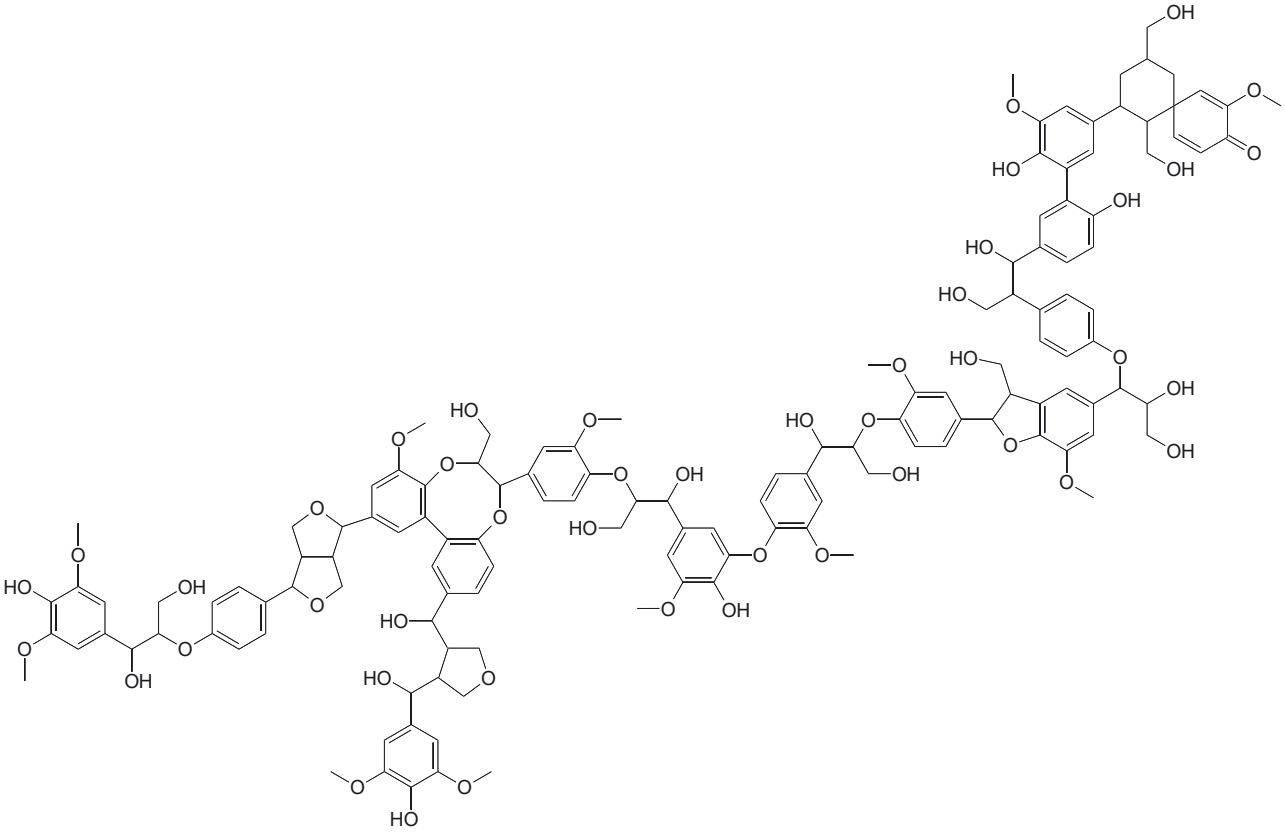


Figure 2: Structural elements present in lignins.[21]

J. Rettenmaier & Söhne JRS, Rosenberg and JELU, J. Ehrler GmbH & Co.KG, Rosenberg, Germany. Various methods for preparation of fiber samples from beech wood chips and beech wood flour are described in Table 1. These samples contained between 72 % and 96 % cellulose and between 1.4 % and 9.0 % lignin. Samples containing between 66 % and 82 % lignin ("lignin fraction" prepared according to ref. 6) served as a reference. Further, we used samples of cellulose (Arbocel B600, Rettenmaier, Rosenberg, Germany) and lignin powder (prepared at Hochschule Aalen according to the LICIL process) as references.

In a 500 mL two-necked round bottomed flask 50 g of wood preparation (chips, flour) was overlayed with 250 mL of the concerning solvent (see Table 1) upon addition of 3–12 g DMF-H_2SO_4 adduct[26,27] (ionic liquid) as catalyst. The flask was provided with a mechanical stirrer (PTFE-crescent stirrer) and an intensive condenser. The mixture was heated upon stirring in an oil bath. When the oil bath temperature had reached 140 °C, counting of the digestion (pulping) time was started. At the end of the given digestion time the mixture was allowed to cool down. The solid part was removed by vacuum filtration (glass frit G3) and washed with acetone. The raw fiber fraction thus obtained was freed from the so-called splinters by subsequent sieving procedures (mesh size 800 > 100 > 20 µm). The acetone was removed from the filtrate by means of a rotatory evaporator. The residue was poured in water to precipitate the lignin. After standing for 24 h the lignin was filtered off (glass frit G4) and washed with water. The fiber and lignin fractions were pre-dried at 50 °C in a drying cabinet for at least two days and dried finally for a minimum of 3 days in a desiccator to constant mass. The cellulose samples – containing flakes of up to 3–5 mm diameter – were dispersed in water. This dispersion was then homogenizeds with a laboratory mixer for 15 min at 10,000 rpm. Subsequently, the dispersion was placed in a watch glass, and the water was evaporated at 45 °C in a drying cabinet.

2.2 Imaging and spectral analysis

Specimens of beech and oak were visualized by a confocal laser scanning microscope (Axiovert 200 M with scanning unit Pascal 5, Carl Zeiss Microimaging GmbH, Jena, Germany) using an excitation wavelength λ_{ex} = 488 nm and fluorescence detection at λ = 505–530 nm as well as at $\lambda \geq 560$ nm. Use of a 10 × /0.30 objective lens allowed sample areas of 920 × 920 µm^2 and layers on their surface of 5–10 µm thickness to be recorded. For micro-spectral analysis a mercury high-pressure lamp operated with a bandpass filter for 395–440 nm was used for excitation and a long pass filter for $\lambda \geq 470$ nm for detection. A custom-made polychromator (resolution: $\Delta\lambda \leq 10$ nm) was placed on top of the microscope (Axioplan 1, Carl Zeiss Microimaging GmbH, Jena, Germany) and combined with an image intensifying system (IMD D4562, Hamamatsu Photonics, Ichino-Cho, Japan). This system allowed for detection of fluorescence spectra from 50 µm × 500 µm object fields. For measuring fluorescence

Table 1: Conditions for preparation of samples from beech wood chips and beech wood flour (sample no. 4) and their composition. In all cases DMF-H_2SO_4 (ionic liquid) was used as a catalyst. Samples 1 to 9 with increasing lignin content in the cellulose fiber fraction.

			Digestion condition					Composition fiber fraction			Lignin fraction
Sample no.	Solvent	Amount of catalyst / g	Temp./ °C	Reaction time / h	Splinter / %	Fiber fraction / %	Lignin fraction / %	Cellulose / %	Hemi-cellulose / %	Lignin / %	Lignin content / %
1	THFA	6	150	3	14.2	35.3	20.5	96.9	5.8	1.4	78.4
2	THFA	12	140	3	6.6	39.8	19.7	90.4	2.0	2.4	71.8
3	THFA	6	140[b]	3	11.6	36.2	17.3	87.8	8.3	2.5	80.3
4	THFA	6	140[c]	3	–	5.0	20	78.1	7.6	3.0	81.7
5	THFA	12	150	3	6.3	38.9	24.4	94.7	6.9	3.1	77.3
6	THFA	6	140[a]	3	17.7	31.7	17.1	83.9	6.1	5.0	70.7
7	Ethylene-glycol	6.4	140	3	54	15	8	78.8	9.6	6.0	80.1
8	Butyl-glycol	6	140	3	56	12	14	72.4	13.2	9.0	66.5
9	Glycerol	6	140	3	85	11	2	78.1	7.0	9.0	80.1

[a]After the wood chips were kept in the digestion flask together with tetrahydrofurfurylalcohol (THFA) for 24 h at 40 °C, the catalyst was added. [b]In the digestion flask the wood chips were evacuated for 0.5 h at room temperature. The connection with the vacuum pump was interrupted, and 250 mL THFA was added to the flask, the DMF-H_2SO_4 adduct was added whereupon the digestion started. [c]Beech wood flour (≤0.5 mm) was used instead of beech wood chips.

decay kinetics, we used a super-continuum fiber laser operated at 450–490 nm with 5 ps pulse duration and 78 MHz repetition rate (NKT Photonics, Birkeröd, Denmark) in combination with an image intensifying camera system (Picostar HR12 image intensifier coupled to a cooled ICCD camera; LaVision, Göttingen, Germany). This system permitted to record fluorescence lifetime images and decay kinetics with a time resolution of 200 ps.

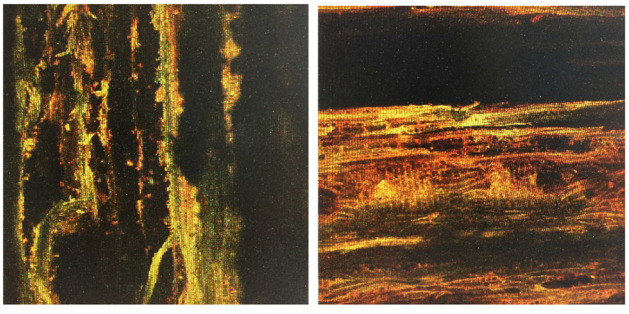
3 Results

3.1 Laser scanning microscopy and micro-spectral analysis

Figure 3 shows the fluorescence of a beech (a) and an oak (b) chip upon recording of thin surface layers by confocal laser scanning microscopy (CLSM) with an excitation wavelength λ_{ex} = 488 nm. An overlay of red and green fluorescence arising from fibrillar structures is shown in the Figure 3(a) and (b).

The fluorescence spectrum from a central part of a beech wood chip (50 × 500 µm^2) is depicted in Figure 4 upon excitation at 395–440 nm. It shows a broad maximum at 490–530 nm and a shoulder around 560 nm. Excitation around 488 nm and use of a long pass filter for $\lambda \geq 510$ nm would result in the same spectrum apart from the short-wavelength part. The fluorescence spectra of all 12 beech and all 12 oak chips were similar and varied only in their absolute intensity up to a factor 2.

Figure 4 also shows the fluorescence spectra of the reference samples lignin and cellulose (Arbocel B600), proving that their addition would result in the spectrum of the beech chip. While the lignin spectrum shows two



a b

Figure 3: Slices of wood chips; overlay of green (λ = 505–530 nm) and red ($\lambda \geq$ 560 nm) fluorescence of beech (a) and oak (b) recorded from a layer Δz = 10 µm by CLSM. Image size: 920 × 920 µm^2; excitation wavelength: λ_{ex} = 488 nm.

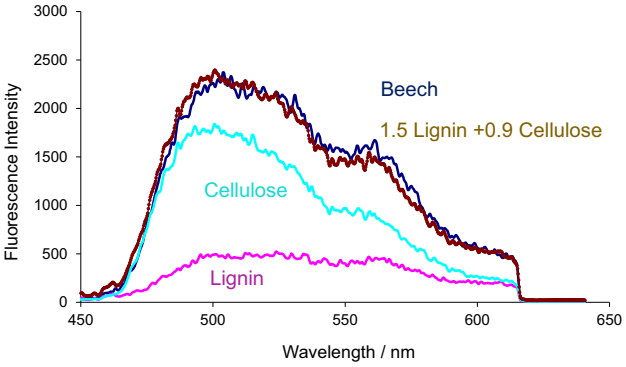


Figure 4: Fluorescence spectra of a beech slice, lignin, cellulose (arbocel B600) and a sum of 1.5 lignin + 0.9 cellulose (spectral contributions); excitation range: λ_{ex} = 395–440 nm.

fluorescence bands of almost identical intensity around 500 nm and 560 nm, the cellulose spectrum is dominated by the band around 500 nm and possesses only a less pronounced shoulder at 560 nm. This suggests that the fluorescence intensity around 560 nm may contain some information about the content of lignin.

In a subsequent experiment we measured the fluorescence spectra of cellulose samples obtained by decomposition of beech wood ("fiber fraction", see Table 1) and containing various concentrations of residual lignin of 1.4 %, 2.4 %, 2.5 %, 3.0 %, 3.1 %, 5.0 %, 6.0 % and in two cases 9.0 %. Spectra were normalized at 520 ± 5 nm and plotted for 2.4 %, 3.0 %, 5.0 %, 6.0 % and 9.0 % lignin in Figure 5. For each specimen we performed 10 independent experiments and evaluated the fluorescence intensity as an average of counts per channel in the spectral range 540–580 nm (Figure 6) as well as at 540–630 nm. An average spectrum resulting from 10 measurements of the sample no. 2 (2.4 % lignin) served as a reference and was subtracted from all other measured spectra. Figure 6 shows an evaluation with median values and median absolute deviations (MADs) determined from 10 spectra in each case in the range of 540–580 nm. The figure proves that fluorescence intensity increases continuously with lignin concentration, but reveals a few deviations: (i) the spectrum of sample no. 1 with 1.4 % lignin shows a higher median value than the spectrum of sample 2 (2.4 % lignin), but a large MAD prevents further evaluation for this low lignin concentration. (ii) the wood flour sample no. 4 with 3.0 % lignin shows comparably high fluorescence intensity. Visually, the color of the cellulose samples changes from dark brown to beige with decreasing lignin concentration. However, the wood flour sample no. 4 differs from this general behavior and appears rather dark, which is concomitant with the high fluorescence intensity. Evaluation in the spectral range 540–630 nm gave similar results.

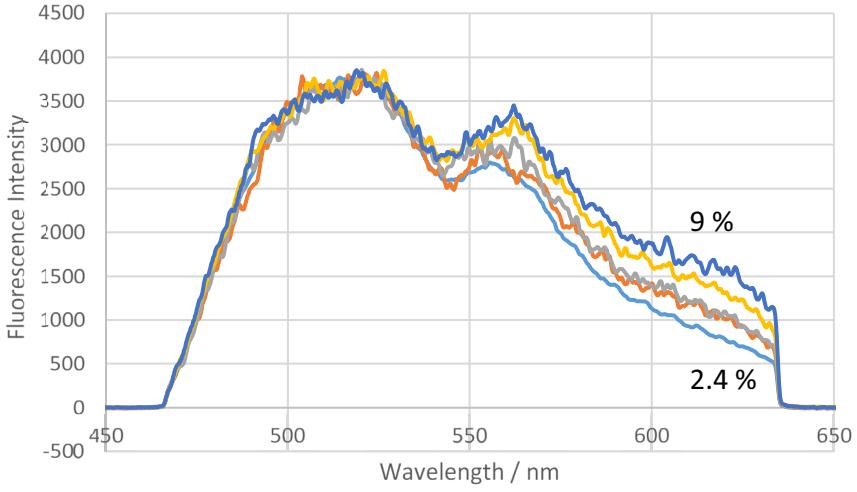


Figure 5: Fluorescence spectra (normalized at 520 ± 5 nm) of cellulose samples containing increasing concentrations of lignin (2.4 %, 3.0 %, 5.0 %, 6.0 % and 9.0 % from bottom to top); excitation range λ_{ex} = 395–440 nm.

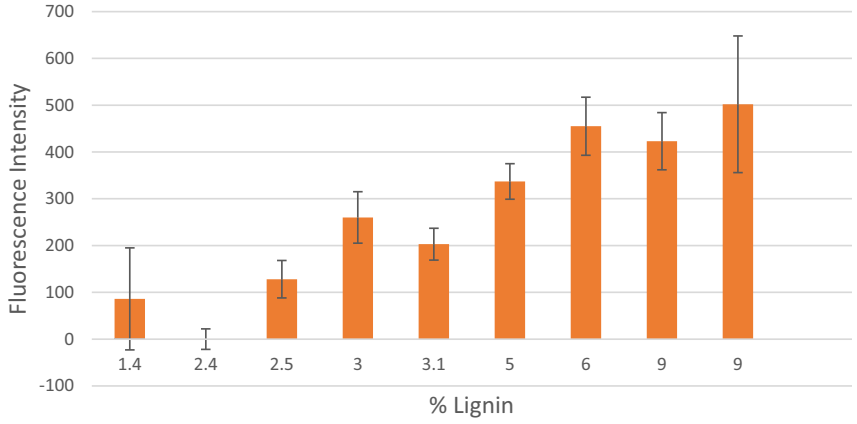


Figure 6: Fluorescence intensity (average counts per channel) of individual cellulose samples with the indicated concentrations of lignin in the spectral range 540–580 nm with reference to sample no. 2 (2.4 % lignin). Median values and median absolute deviations (MADs) of 10 fluorescence spectra each.

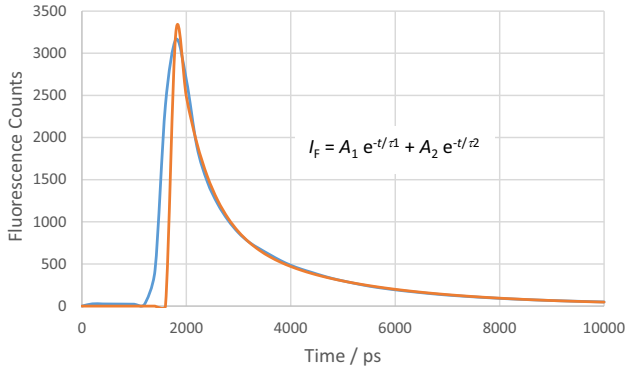


Figure 7: Fluorescence decay kinetics of a beech chip (blue) and bi-exponential curve fitting (red) after excitation by picosecond laser pulses; excitation range: 450–490 nm; detection: $\lambda \geq 510$ nm.

Fluorescence spectra of samples obtained by decomposition of beech and containing between 66 % and 82 % lignin ("lignin fraction") showed broad emission bands with maxima around 520 nm and 560 nm and were similar to the reference spectrum of lignin (Figure 4).

3.2 Fluorescence decay kinetics

Following excitation by short laser pulses (λ_{ex} = 450–490 nm, Δt = 5 ps) the fluorescence decay kinetics recorded at $\lambda \geq 510$ nm from 920 × 920 µm^2 object fields (Figure 7) showed a bi-exponential behavior according to $I_F = A_1\, e^{-t/\tau_1} + A_2\, e^{-t/\tau_2}$ with the fluorescence lifetimes τ_1 and τ_2 and the corresponding amplitudes A_1 and A_2. For all beech and oak chips τ_1 was in the range of 0.45–0.65 ns, and τ_2 varied between 2.30 ns and 2.75 ns. As is shown in Table 2, fluorescence lifetimes of the cellulose samples (with residual amounts of lignin) were similar or only slightly shorter with τ_1 ranging between 0.36 ns and 0.50 ns and τ_2 between 2.10 ns and 2.76 ns, whereas the ratio of amplitudes A_1/A_2 decreased with decreasing lignin concentration from 200–300 (9.0 %) to about 30 (2.4 %). Only sample no. 5 with 3.1 % lignin showed a slight deviation from this general behavior, and sample no. 1 is again regarded to be at the limit of detection. The reference sample of lignin as well as specimens obtained after decomposition of beech chips with lignin concentrations

Table 2: Cellulose obtained by decomposition of beech wood with various concentrations of residual lignin. Fluorescence lifetimes τ_i and corresponding amplitudes A_i, as obtained from bi-exponential curve fitting. Samples are listed from no. 9 to no. 1 with decreasing lignin content.

Sample no.	Lignin / %	A_1	τ_1 / ns	A_2	τ_2 / ns	A_1/A_2
9	9.0	342,782	0.36	1,054	2.10	325
8	9.0	221,630	0.36	1,111	2.10	199
7	6.0	137,186	0.44	1,136	2.36	121
6	5.0	123,132	0.41	1,177	2.14	112
5	3.1	54,642	0.38	477	2.22	114
4	3.0	110,237	0.44	1,483	2.26	74.3
3	2.5	44,459	0.48	922	2.68	48.2
2	2.4	28,606	0.53	901	2.76	31.7
1	1.4	33,212	0.50	1,020	2.62	32.5

between 66 % and 82 % showed a mono-exponential fluorescence decay with a short lifetime of 0.32–0.49 ns, which corresponds to the mean lifetime of lignin reported in the literature.[28] Therefore, the ratio A_1/A_2 of the cellulose specimens seems to reflect the different amounts of residual lignin.

4 Discussion

The main aim of this paper was to characterize beech and oak wood chips as well as the constituents lignin and cellulose of beech wood chips on the basis of their fluorescence spectra and lifetimes and to estimate the concentration of lignin upon decomposition. This may provide a non-destructive label-free test without further chemical treatment of the quality of decomposition in view of numerous applications. Presently, an estimate of residual lignin within cellulose samples appears possible within a range of concentrations between about 2 % and 10 %. In view of a more reliable quantification the reasons for some divergences remain to be investigated: does the preparation method of some samples or the special nature of the beech wood flour (sample no. 4) have any influence on the structure and composition of the lignin molecules? Different treatment of the samples may have an impact on individual chromophores within the lignin molecule, e.g., stilbene and phenylcoumarone.[24] These chromophores (and not only the overall concentration of lignin) are responsible for reflection as well as for fluorescence properties including spectra, lifetimes and quantum yields. Their detailed study may improve the quantification of lignin. In this context it is worth mentioning that two additional samples containing 4.1 % or 5.1 % lignin did not fit the general behavior with a color changing from beige to brown upon increase of the lignin content, and subsequently showed a lower fluorescence intensity at 540–580 nm and a lower amplitude ratio A_1/A_2 (in fluorescence decay kinetics) than expected for this concentration. Apart from these deviations fluorescence lifetime parameters seem to be rather promising for estimation of the lignin content. Spectral data may have an even larger potential, because in principle they are easier to obtain.

In general, fluorescence measurements are rather easy to perform, and the present complex setup may be replaced in the future by a miniaturized sensor system with integrated spectrometer for online experiments. Even cameras with appropriate spectral filters and image processing software appear well applicable to estimate the content of residual lignin in cellulose samples. Further techniques, e.g., infrared or Raman spectroscopy, may represent a valuable addition. Raman techniques have been improved considerably during the last decade,[29] and in combination with multivariate analysis they were used to analyze and image the main components of plant cell walls including lignin, cellulose and hemicellulose.[30] Only in a few cases, Raman measurements of wood meal samples or wood chips have been reported in the literature.[31] In general, Raman techniques, in particular in combination with multivariate methods, are often quite complex and may suffer from a strong fluorescence background, thus making online experiments more difficult. A further option would be near-infrared (NIR) spectroscopy, as reported e.g., in refs 32,33. In comparison with fluorescence measurements (in the visible part of the spectrum), NIR detectors are usually less sensitive, so that many authors use Fourier Transform (FT-IR) Spectroscopy[34] as an alternative technique. FT-IR Spectroscopy needs additional mathematical algorithms, and in combination with statistical methods used for evaluation, IR spectroscopy may also become quite complex, so that fluorescence measurements possibly present an easy and low-cost alternative in the near future.

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