Polysaccharide degradation and lignin modification during brown rot of spruce wood: a polarised Fourier transform near infrared study

Karin Fackler a, * and Manfred Schwanninger b

a Institute of Chemical Engineering, Vienna University of Technology, Vienna, Austria. E-mail: k.fackler@tuwien.ac.at
b Department of Chemistry, BOKU-University of Natural Resources and Life Sciences, Vienna, Austria

Radial thin sections of spruce wood that had been degraded by brown-rot fungi (Gloeophyllum trabeum or Poria placenta) exhibiting mass losses up to 16% were investigated by polarised Fourier transform near infrared (FT-NIR) transmission spectroscopy. This method allowed for the analysis of the changes within wood caused by the decay fungi. The main features of brown rot visible in the FT-NIR spectra were the reduction of amorphous wood polysaccharides in preference to crystalline, an overall loss of molecular orientation of the structural polymers, and the formation of phenolic groups in lignin through cleavage of aryl-ether bonds or demethoxylolation. Furthermore, polarised FT-NIR spectroscopy allowed us to examine the assignments of the first overtones of O–H stretching vibrations in more detail and to suggest a number of new band assignments in this spectral region. Among them were the bands of the O(3)–H(3)···O(5) intramolecular hydrogen bond of glucomannan and the strong parallel intramolecular hydrogen bond O(2)–H(2)···O(6), which is only accessible with polarised NIR spectroscopy because it overlaps with a number of perpendicular bands, among them the perpendicular O(6)–H(6)···O(3) intermolecular hydrogen bond of crystalline cellulose. First overtones of strongly H-bonded O–H groups of cellulose I α (6340 cm⁻¹) and cellulose I β (6270 cm⁻¹) were tentatively assigned.

Keywords: brown-rot fungi, polarised FT-NIR spectroscopy, cellulose I alpha, cellulose I beta, glucomannan, lignin, softwood

Introduction

Brown-rot fungi are aggressive colonisers of wood. These decay fungi have developed molecular mechanisms that enable them to degrade and mineralise the wood polysaccharides very efficiently. Lignin is not degraded but is extensively modified. Brown-rot degraded lignin is highly depleted in methoxyls and arylglycerol-3-ether structures and has a higher content of free phenolic groups. Early in the decay, mechanical wood properties are highly affected. This is mainly due to the depolymerisation of wood polysaccharides. Recently, we were able to show that early decay processes can be traced with mid infrared spectroscopy by their differences in hydrogen bonding within and between the wood polymers. These differences were reflected in the O–H stretching vibration region of the mid infrared spectra. The first overtones (0Ts) of these vibrations are better separated in the near infrared (NIR) region than in the mid infrared (mid-IR) region. Therefore, wood decay processes affecting these cellulose bands and bands of other polysaccharides are expected to become more significant when investigated by means of Fourier transform (FT)-NIR spectroscopy. Furthermore, FT-NIR allows for the measurements of relatively thick wood sections with intact morphology.
these advantages, NIR studies on the H-bonding of wood and polysaccharides are rare.6,7,10–12 These publications, together with that of Mitsui et al.,13 are particularly valuable because a number of O–H stretching vibration first overtone bands occurring in wood and cellulose were assigned and, therefore, were a sound basis for the study of wood degradation processes in this work.

In the present work, FT-NIR transmission spectroscopy was used and the overtones of C–H and O–H stretching vibrations were investigated; in particular, to better understand degradation processes of lignin, cellulose and hemicelluloses during brown rot. For this purpose, a considerable number of wood samples were subjected to brown-rot degradation to different extents. Spectra of radial sections were recorded in polarised modes parallel (0°) and perpendicular (90°) to the direction of tracheid cells. This allowed not only the analysis of anisotropic effects of the degradation of the orthotropic material softwood but also an analysis of oriented O–H and C–H bonds of spruce wood in more detail. The interpretation and analysis of the spectral data was supported by the use of principal component analysis (PCA).

Materials and methods
Fungal cultures
Porina placenta (syn. Oligoporus placenta) MAD 698 and Gloeophyllum trabeum CBS 900.73 that had been maintained on agar slants at 4°C were pre-incubated on malt extract agar plates (Fluka 70145, www.sigma-aldrich.com) prior to their use.

Preparation of degraded spruce wood samples
Spruce (Picea abies L. Karst.) wood blocks from the outermost sapwood (3.5 radial × 3 tangential × 4 longitudinal cm³) from a 120-year-old tree grown near St Pölten, Lower Austria, Austria, were dried for three days at 50°C to a constant weight. After weighing, the blocks were imregnated in a vacuum with water and gamma-sterilised (25.5 kGy min). Sterilised blocks were transferred into glass jars (900 mL, approx. 12 cm diameter), in which fungal cultures had been pre-cultivated on malt extract agar for 14 days. A sterile plastic grid served as a spacer between the agar surface and the wood block. The samples were incubated for up to several weeks at 28°C (Table 1) in a moisture-saturated atmosphere. After incubation, mycelia were carefully wiped off the wood surfaces and wood samples were dried again for one day at 50°C to determine the mass loss (ML) due to degradation. Samples were impregnated with water and thin sections of 200 µm thickness were carefully cut in the radial direction by means of a sliding microtome from a region at least 1 mm from the surface of the wood block. Samples were dried for 24 h at 40°C and 10 mbar vacuum before transmission NIR spectra were recorded. As references, sections of non-degraded samples were prepared after storage.

Cellulose paper samples and powdered model compounds
Cellulose paper samples enriched in cellulose Iα and cellulose Iβ were kindly provided by Lennart Salmén, Innventia, Stockholm, Sweden. The preparation and the characteristics of these samples are described by Åkerholm et al.14 The cellulose Iα enriched sample from Cladophora sp. exhibits a crystallinity of 74%, of which 53% was attributed to the Iα allomorph; the second sample was Iβ-enriched cotton linters, 34% crystallinity, of which 82% was attributed to the Iβ allomorph. Powdered microcrystalline cellulose (MCC), Hewetten type 101, was purchased from JRS Pharma (www.jrs.de) and birch xylan was obtained from Lactan (www.lactan.at). Powdered spruce glucomannan was a gift from Claudia Strobel, TU Munich, Germany. Spruce milled wood lignin was kindly provided by

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time (days)</th>
<th>Number of wood sections investigated</th>
<th>Mass loss (%)</th>
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<tr>
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<td>—</td>
<td>3</td>
<td>—</td>
</tr>
<tr>
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<td>21</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>G. trabeum</td>
<td>21</td>
<td>3</td>
<td>4</td>
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<tr>
<td>G. trabeum</td>
<td>21</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>G. trabeum</td>
<td>28</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>G. trabeum</td>
<td>28</td>
<td>3</td>
<td>16</td>
</tr>
<tr>
<td>P. placenta</td>
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<td>1</td>
<td>&lt; 1</td>
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<tr>
<td>P. placenta</td>
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<td>2</td>
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</tr>
<tr>
<td>P. placenta</td>
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<td>1</td>
<td>8</td>
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<td>14</td>
</tr>
<tr>
<td>P. placenta</td>
<td>56</td>
<td>4</td>
<td>16</td>
</tr>
</tbody>
</table>

Table 1. Spruce wood samples. Decay fungus, incubation time, number of sections investigated, and mass loss due to decay.
Fourier transform near infrared spectroscopy

FT-NIR transmission spectra of radial wood thin sections and cellulose papers were recorded in 16 cm\(^{-1}\) spectral resolution, zero-filling factor (ZF) 1, with a Bruker Vertex 70 FT-IR spectrometer (www.brukeroptics.com), equipped with a CaF\(_2\) beam splitter and a liquid N\(_2\) cooled MCT detector. For each spectrum 400 scans were averaged. For some samples two to four sections were analysed. Four spectra were recorded from each section with IR radiation in the range from 10,000 cm\(^{-1}\) to 3800 cm\(^{-1}\) polarised with a polariser, KRS-5 (www.brukeroptics.com) parallel and perpendicular to the direction of tracheid cells. The wood sections were mounted on a magnetic sample holder to which the polariser was attached. Ten minutes before and during the measurement of the samples and backgrounds the sample compartment of the spectrometer was flushed with dry and oil-free air (200 l h\(^{-1}\)) to avoid any influence of moisture on the spectra. Bands of vibrations that are more pronounced in 0° mode than in 90° mode are due to vibrations with an angle between 0° and 45° relative to the tracheid direction and are referred to as parallel bands, those being more pronounced in 90° mode (angle between 45° and 90°) as perpendicular bands.

For the measurements of Cladophora cellulose and cotton linters, the papers were folded into four layers. Spectra of powdered substances (vanillin, glucomannan, xylan and MCC) were recorded in diffuse reflectance mode (DRIFT-NIR). A total of 100 scans per measuring area were collected in 8 cm\(^{-1}\) spectral resolution, ZF=1 with a Bruker FT-IR spectrometer (Equinox 55, Peltier cooled InGaAs diode detector) by means of a fibre-optic probe. Spectralon (www.labsphere.com/productdetail.aspx?id=226) served as reference.

Fourier transform mid-infrared spectroscopy

ATR-FT-mid-IR spectra from celluloses (64 scans per sample; spectral resolution, 4 cm\(^{-1}\); wavenumber range, 4000–600 cm\(^{-1}\); ZF = 1 using a single reflection attenuated total reflectance (ATR) device (Miracle, Pike Technologies, www.piketech.com) and FT–mid-IR absorbance spectra in polarisation mode (200 scans per sample; spectral resolution, 4 cm\(^{-1}\); wavenumber range, 4000–700 cm\(^{-1}\); ZF = 1; KRS-5 polariser, liquid N\(_2\) cooled MCT detector) were collected but are not shown here.

Pre-treatment and analysis of spectral data

Raw transmission spectra were baseline corrected with a three-point linear baseline (7200 cm\(^{-1}\), 6080 cm\(^{-1}\) and 5380 cm\(^{-1}\); Figure 1). For PCA, second derivatives of raw spectra were calculated using the method of Savitzky and Golay\(^{13}\) (13 smoothing points, second-order polynomial fit) before principal component analyses were carried out with The Unscrambler (Vsn. 9.8, www.camo.com). For the calculation of difference spectra between the polarisation modes, the second-derivative spectra were vector normalised in the range of the amplitude of the aromatic C–H vibration assigned to lignin (6056–5925 cm\(^{-1}\)). This procedure is feasible, because lignin is the least oriented polymer within wood\(^{14,17}\) and aromatic moieties are least affected by brown-rot degradation.

Results and discussion

Fourier transform near infrared absorbance spectra

Wood is a highly anisotropic material. Therefore, clear differences between spectra recorded in the direction of the
longitudinally oriented tracheid cells (0°) and those recorded in perpendicular direction to them (90°) are obvious. Figure 1(a) and (b) shows examples for baseline corrected absorbance spectra of spruce wood sections. Most of the first OTs of O–H stretching vibration modes (7190–6080 cm$^{-1}$) exhibit a higher overall absorbance in parallel polarisation. This is due to the intramolecular H-bonds of cellulose and glucomannan O(3)–H(3)···O(5) that are oriented parallel to the polysaccharide main chains. Between 7000 cm$^{-1}$ and 6900 cm$^{-1}$, however, the perpendicular orientation of non-H-bonded or weakly H-bonded O–H groups of amorphous polysaccharides leads to higher absorbance of the 90° spectrum. c–H stretching vibration first OTs and combination bands between 5700 cm$^{-1}$ and 5350 cm$^{-1}$ are dominated by c–H bands of cellulose whose vibrations are 90° oriented in relation to the main chains. Therefore, the 90° spectrum is more intense in this region.

Only small differences between the polarisation modes are found between 6080 cm$^{-1}$ and 5740 cm$^{-1}$, the region of the aromatic C–H and C–H stretching vibration overtones from methyl groups. In spruce wood, these vibrations are mainly assigned to lignin. Although it was reported that the preferred molecular orientation of the phenylpropane units of lignin in wood is along the fibre direction, it is also known that among all wood polymers of S2 cell walls, lignin shows the least orientation.

The main effect of brown rot that becomes visible in the absorbance spectra is an additional band near 6890 cm$^{-1}$ which is due to an increase of phenolic groups in lignin.

To see the effects of brown-rot degradation and differences between polarisation modes, however, it is beneficial to take a closer look at the second-derivative spectra in which the highly overlapping bands of the absorbance spectra are better resolved as amplitude minima.

Second-derivative spectra

To allow for the analysis of the changes in the polysaccharide content relative to the lignin content in a better way pre-treatment of the spectral data was necessary. Vector normalisation over the whole range of the first OTs was not appropriate due to large differences in total absorbance between spectra recorded in 0° and 90° polarisation (Figure 1). Therefore it was straightforward to normalise the spectra in the range of the amplitude minimum of aromatic C–H stretching vibration overtones, because aromatic compounds are least affected by brown-rot fungi and, although some molecular orientation was also reported for lignin, it also shows the least orientation of all structural polymers.

Figure 2. Second-derivative spectra (vector normalised (VN) between 6056 – 5925 cm$^{-1}$) in 0° (A) and 90° (B) mode of non-degraded radial spruce wood sections (black lines) and average spectra of spruce wood degraded by the brown-rot fungi P. placenta and G. trabeum. Red, early-stage degradation (approx. 5% ML); blue, pronounced degradation (approx. 15% ML). (Figures are printed in colour on the web.)
Table 2. Summary of FT-NIR bands of this study and band assignments from the literature.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Second derivative</th>
<th>Difference spectrum</th>
<th>PCA loading</th>
<th>Orientation</th>
<th>Wavelength</th>
<th>Assignment</th>
<th>Fundamental vibration</th>
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<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>First OT of O–H stretching vibrations</td>
<td>First OT of O–H stretching vibrations</td>
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<td>Wavenumbers (cm⁻¹)</td>
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<tr>
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<td>6990–6980</td>
<td>6990</td>
<td>Mainly ⊥</td>
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<td>Amorphous polysaccharides of wood⁴</td>
<td>Free and weakly H-bonded OH of cellulose¹¹</td>
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<td>1534, 1550</td>
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<td>3234 [this study] 3266, 3234 [this study]</td>
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<td>1610</td>
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<td>1632</td>
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Table 2 (continued). Summary of FT-NIR bands of this study and band assignments from the literature.

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<th>Reference</th>
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<th>Difference spectrum</th>
<th>PCA loading</th>
<th>Orientation</th>
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<td><strong>Wavenumbers (cm⁻¹)</strong></td>
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<td>5560</td>
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</tbody>
</table>

**Combination band**

| 5495 | 5470–5460 | 5480 | 5493 | || | 1825 | O–H + 2 × ν(C–O)²³ |

*The wavelength of the band maximum refers to an approximate mean value of the band.
†In brown-rotted spruce, no polarisation was found for this band.
Orientation in relation to the tracheid direction: ||, 0°; ‖, 90°; s, shoulder.
Compared to the lignin band polysaccharide bands are reduced [Figure 2(a) and (b)], because brown-rot fungi degrade wood polysaccharides. Amplitude minima of various O–H stretching vibrations first OT bands [Table 2] assigned to amorphous (6990 cm\(^{-1}\)), semi-crystalline (near 6720 cm\(^{-1}\)) and crystalline cellulose (\(C_\text{I}\) = 6450 cm\(^{-1}\) and \(C_\text{II}\) = 6288 cm\(^{-1}\)) decreased in relation to the lignin band (5980 cm\(^{-1}\)). Furthermore the polysaccharide bands in the region of C–H stretching vibrations first overtones (5900–5500 cm\(^{-1}\)) were also reduced. The results of degradation are strongly reflected in the spectra of the samples with 15% ML that were recorded in 0° and 90° polarisation mode. As an additional effect of pronounced brown-rot degradation, a shoulder due to phenolic structures of lignin at \(\approx 6900\) cm\(^{-1}\) became visible.

Brown-rot spectra from an earlier stage (\(\approx 5\%\) ML) show larger differences between the polarisation modes. The changes due to degradation were more pronounced in the 90° spectra [Figure 2(b)]. Early degradation of free or weakly H-bonded O–H groups of amorphous polysaccharides (6990 cm\(^{-1}\)) even becomes visible only in the 90° spectra. These O–H groups, mainly O(2)–H(2) and O(6)–H(6), show a perpendicular orientation in relation to the main chains. Significant reduction of O–H groups in 90° orientation to the longitudinal direction suggests that, in these samples, mainly polysaccharides oriented parallel to the tracheid direction were degraded. Polysaccharide degradation was also reflected in other overtone bands from O–H and C–H stretching vibrations. This will be discussed in more detail in the next section.

**Differences of polarised second-derivative spectra of native spruce wood**

Although the bulk cellulose of S2 cell wall layers is oriented along the direction of tracheid cells, and these cell wall layers make up most (80–90%) of the wood substance,\(^{13}\) some features of wood that interfere with this high degree of orientation have to be kept in mind when interpreting the results of polarised spectroscopy of spruce wood. First, it is the perpendicular orientation of the ray cells that are also high in cellulose content and make up approximately 5% of the spruce cells.\(^{13}\) Second, cellulose orientation in primary (P1) and outer secondary cell walls (S1) differs markedly from the parallel orientation. Whereas in primary cell walls cellulose and other wood polysaccharides are highly unordered, cellulose microfibrils of S1 layers are oriented perpendicular to the tracheid direction. Finally, the parallel orientation of cellulose microfibrils in S2 cell wall layers follows an angle [microfibrillar angle] between 4° and 20° in relation to the fibre axis. Nevertheless, clear differences between spectra recorded parallel and perpendicular to the fibre direction were observed; these differences can be clearly assigned to the different pattern of O–H and C–H groups of wood.

Figure 3(a)–(c) shows second-derivative spectra of spruce radial sections in various decay stages recorded in parallel (0°) and perpendicular (90°) mode in the region of the first OT of O–H stretching vibrations and the first OT of the C–H stretching vibrations and a combination vibration assigned to lignin and polysaccharides.

In the difference spectrum [0–90° Figure 3(a)], minima of the O–H stretching vibration first OT region corresponding to parallel oriented O–H vibrations were found at 6700 cm\(^{-1}\), 6680 cm\(^{-1}\) and 6202 cm\(^{-1}\). Tsuchikawa and Siesler\(^{6}\) assigned the region 6720 ± 20 cm\(^{-1}\) to semi-crystalline regions of wood/cellulose. The parallel orientation of this allowed us to conclude that this band can be attributed to one of the intramolecular hydrogen bonds in polysaccharides. The three cellulose preparations [Figure 4(b)] do not exhibit a distinct amplitude minimum near 6700 cm\(^{-1}\). The DRIFT-NIR second-derivative spectrum of powdered glucomannan [Figure 4(a)], however, has a minimum near 6710 cm\(^{-1}\). Mannan I was shown to exhibit intramolecular H bonding O(3)–H(3)–O(5) with a parallel polarised fundamental vibration at 3462 cm\(^{-1}\).\(^{20}\) Therefore, the parallel O–H stretching vibrations first OT band can be assigned to O(3)–H(3)–O(5) intramolecular H-bonding of glucomannan. This assignment is also supported by the ratio between the first OT band and the reference value from the literature for the fundamental vibration (6710 cm\(^{-1}\): 3462 cm\(^{-1}\) = 1.94), which would be similar to the corresponding cellulose band (6612 cm\(^{-1}\): 3342 cm\(^{-1}\)).\(^{11,21}\) A minimum due to this intramolecular hydrogen bond O(3)– H(3)–O(5) band of cellulose was found in second-derivative spectra here near 6480 cm\(^{-1}\) [Figure 3(a)]. An additional strongly H-bonded O–H stretching vibration OT with 0° orientation was found in the difference spectrum at 6202 cm\(^{-1}\), which we were not able to assign.

The difference spectrum [Figure 3(a)] shows maxima at 6990 cm\(^{-1}\), 6800 cm\(^{-1}\), 6612 cm\(^{-1}\) and 6303 cm\(^{-1}\) and a shoulder at 6900 cm\(^{-1}\) indicating that a higher share of the corresponding O–H groups is oriented perpendicular to the tracheid direction. Watanabe et al.\(^{11}\) assigned the band near 7000 cm\(^{-1}\) to free or weakly H-bonded OH groups of cellulose, while Tsuchikawa and Siesler\(^{6}\) assigned it to amorphous wood polysaccharides in general (Table 2). Weakly H-bonded OH groups are prevalent in amorphous polysaccharides [amorphous cellulose, glucomannan, xylan and pectic substances] that are less ordered than crystalline cellulose. According to the studies carried out by Salmén’s group,\(^{17,22}\) in S2 cell wall layers the bulk of the hemicellulose backbone [xylan and glucomannan] is like cellulose overall oriented along the direction of the tracheids; glucomannan shows a higher extent of parallel orientation than xylan.\(^{17,22}\) Therefore O–H groups absorbing NIR radiation near 7000 cm\(^{-1}\) can be mainly assigned to free or weakly H-bonded O(2)–H(2) of glucan and xylan and to O(6)–H(6) of glucan and mannann, that are all oriented in 90° related to the respective backbone. Perpendicular bands in the difference spectrum near 6800 cm\(^{-1}\) [6800–6770 cm\(^{-1}\)] are related to intermolecularly H-bonded O–H groups of intermediate strength. MCC, Cladophora cellulose, cotton linters and glucomannan showed second-derivative minima between 6800 cm\(^{-1}\) and 6740 cm\(^{-1}\) [Figure 4]. In the mid-IR a band at 3497 cm\(^{-1}\) had been assigned to the fundamental vibration of an intermolecular H-bond of mannann.\(^{15}\) Corresponding first OT bands would be expected.
to be located near 6780 cm\(^{-1}\) if a factor of OT : fundamental vibration = 1.94 was applied. This factor was found before for the ratios of the intramolecular H-bonds \(O(3)\)–H(3)···O(5) of mannan and cellulose. We therefore suggest that the perpendicular band near 6780 cm\(^{-1}\) is due to weakly H-bonded \(O(6)\)–H(6) of cellulose and glucomannan. Another perpendicular band at 6612 cm\(^{-1}\) becomes visible in the difference spectrum. This band had been assigned to intermolecularly H-bonded \(O(6)\)–H(6)···O(3)' of cellulose by Watanabe et al.\(^{11}\) who found this band in MCC located at 6622 cm\(^{-1}\). This assignment is in perfect agreement with the perpendicular orientation of the band found here and with the corresponding fundamental vibration at 3410 cm\(^{-1}\) due to the main conformation \(I^{12}\) of the \(O(6)\)–H(6) group of cellulose that forms weak intermolecular H-bonds, but does not accept intramolecular H-bonding from the \(O(2)\)–H(2) group (Table 2).
of the conformations of intermolecular H-bonded O–H groups (O(6)–H(6)···O(3)).

As in the transmission spectra (Figure 1a) and (b) first OTs of C–H stretching vibrations of polysaccharides showed perpendicular orientation. This applied also to the band at 5800 cm⁻¹, which, according to the literature, is due to pyranoses and furanoses of hemicelluloses and lignin. Birch xylan (5805 cm⁻¹) and spruce glucomannan (5810 cm⁻¹) [Figure 4(a)] and spruce milled wood lignin (5793 cm⁻¹) [Figure 4(c)] show amplitude minima here, whereas celluloses do not [Figure 4(b)].

The perpendicular band [Figure 3(a)] between 5700 cm⁻¹ and 5660 cm⁻¹ is due to the C(6)-methylene group of the hexoses, the one near 5590 cm⁻¹ due to the C–H groups of crystalline cellulose. Finally, near 5480 cm⁻¹, a perpendicularly oriented combination vibration O–H + 2 × ν(C–O) was found.

Spruce wood degraded by brown-rot fungi: second-derivative difference spectra

For Figure 3(b), polarised spectra from P. placenta (14% and 16% ML) and spectra from G. trabeum (16% ML) were averaged. This is feasible because we were not able to distinguish differences between wood degradation caused by the two different brown-rot species by means of FT-NIR spectroscopy and PCA [Figure 5(a)]. The main features of brown-rot samples after pronounced decay were weaker amplitude minima of the O–H stretching vibrations first OT and polysaccharide-assigned C–H stretching vibrations first OT due to degradation. Assuming that in these samples all ML had led to polysaccharide degradation, 15% of the wood polysaccharides had been lost. As a result, lignin that is not degraded by brown-rot fungi was accumulated. Of all O–H first OTs, the vibrations near 6295 cm⁻¹ [Figure 3(b)] from cellulose crystalline structures [6307–6267 cm⁻¹] (Table 2) were least affected by degradation. It is known that cellulose crystalline structures are less susceptible to brown-rot degradation than amorphous polysaccharides.

The difference spectrum [Figure 3(b)] between the polarisation modes (0°–90°) was much less intense than in non-degraded spruce wood and also less intense in relation to the second-derivative spectra, indicating that the wood structure, as a whole, was strongly modified during degradation and also in terms of molecular orientation of the structural polymers. No residual orientation of the bands assigned to amorphous polysaccharides was found. It was shown previously that, in these samples, the glucomannan content was reduced by more than one-third, whereas the xylan content was not significantly changed.

Intramolecular O(3)–H(3)···O(5) H-bonds of glucomannan and cellulose showed some residual parallel orientation. Surprisingly, also the 90° orientation between 6325 cm⁻¹ and 6250 cm⁻¹, thought to be assigned to recalcitrant intramolecular H-bonds of cellulose crystals, was lost, although the crystallites were expected to be the least susceptible to degradation. Instead, a parallel band at 6295 cm⁻¹ became significant. By
means of PCA it will be concluded later that several band maxima overlap here, among them the 90° oriented intermolecular H-bond of crystalline cellulose and a strong 0° oriented H-bonded O–H of cellulose, a molecular structure that is accumulated during degradation.

The difference spectrum between the 0° and 90° polarisation modes recorded from samples in an earlier stage of brown rot (average spectra of G. trabeum with 4% ML and 6% ML) is plotted in Figure 3(c). Of the O–H stretching vibrations first overtone (6288 cm\(^{-1}\)) was least affected, whereas amplitude minima 6670 cm\(^{-1}\) and 6480 cm\(^{-1}\) were reduced to a greater extent. The band of amorphous polysaccharides with O–H groups oriented perpendicularly was also reduced.

The degree of orientation of O–H and C–H stretching vibration overtones (wood polysaccharides) as reflected in the difference spectrum was lower and more diffuse than in non-degraded spruce but was still present. The orientation of O–H groups of amorphous polysaccharides (6990 cm\(^{-1}\)) and of cellulose intramolecular H-bonds suffered most from degradation. Interestingly, near 6300 cm\(^{-1}\), the difference spectrum showed two maxima (90°) at 6334 cm\(^{-1}\) and 6257 cm\(^{-1}\) and a local minimum (0°) at 6303 cm\(^{-1}\), indicating again that several vibration modes of strongly H-bonded O–H groups overlap here.

**Principal component analysis of the C–H and O–H stretching vibrations first overtone region**

Polarised FT-NIR spectra (second derivatives) were subjected to PCA (7044–5447 cm\(^{-1}\)). The bulk of the variance (77%) was found on the first PC. As spectra were recorded from samples of slightly different thickness also exhibiting a different distribution of annual rings, differences in total absorbance were observed. As any normalisation of the spectra was omitted, the variance due to these effects was reflected in PC1. The first PC loading [not shown here] therefore had a shape similar to the average second-derivative spectrum of all spectra.

Along PC2 [Figure 5(a)], second-derivative spectra were separated according to the extent of brown-rot degradation. PC2 explained 9% of the variance. Different degradation effects caused by the two fungal species were not detected. Second derivatives of spectra recorded in the different polarisation modes were separated along PC3 (5% of the variance). Second-derivative spectra of samples in early degradation stages and spruce scored positively on PC2, spectra of more severely degraded samples scored negatively. In addition, a few samples of early degradation stages scored more positively than non-degraded spruce.

On PC3, second derivatives of spectra recorded in 90° polarisation scored positively [Figure 5(a)], spectra recorded in 0° polarisation scored negatively.

PC2 loadings [Figure 5(b)] showed positive bands at lignin band positions, indicating the increase of these bands during brown rot: 6900 cm\(^{-1}\) is assigned to lignin derived phenolic O–H.\(^{13}\) In relation to the intensity of the second-derivative amplitude minimum that becomes visible only as a shoulder, the intensity of the corresponding band in the PC2 loading is very high, almost as high as that of lignin that has a much more pronounced second-derivative amplitude minimum. This indicates that the increase in phenolic groups of lignin

![Figure 5. PCA scores (a) and loadings plot (b) calculated from second derivatives of polarised FT-NIR spectra (7044–5447 cm\(^{-1}\)]. Triangles, *P. placenta* (P); diamonds, *G. trabeum* (G); circles, non-degraded spruce (S). The labelling in the scores plot indicates the fungal strain (P or G) or spruce wood (S), the mass loss of the sample in per cent, and the number of the section from which the spectrum was recorded. In case of spruce wood the number only indicates the section. The PC2 loading spectrum is plotted in grey; the PC3 loading spectrum is in black.
is a result of lignin demethoxylation and modification during brown rot.3

The vibration energy of this band at ~6900 cm\(^{-1}\) suggests a weakly H-bonded O–H group rather than a free one. Kubo and Kadla27 introduced a fundamental vibration for phenolic O–H groups with intermolecular H-bonds to an alkoxyl group in ortho position near 3555 cm\(^{-1}\). In a DRIFT-NIR reference spectrum (not shown here), the guaiacyl lignin structure vanillin shows, like brown-rotted wood, a second-derivative amplitude minimum near 6885 cm\(^{-1}\). It is straightforward to propose that O–H stretching vibrations first OT of degraded spruce wood near 6900 cm\(^{-1}\) (6890–6913 cm\(^{-1}\)) are due to intramolecularly H-bonded phenol of guaiacyl(G) residues of lignin or due to phenolic groups formed during ether cleavage processes during brown rot demethoxylation or aryl-ether cleavage.3 These phenolic groups are expected to form H-bonds with the oxygen of methoxyl groups or lignin ether linkages in the ortho position. Other positive loadings follow the C–H stretching vibration first OT reported for lignin23 with maxima at 5987, 5902 cm\(^{-1}\), 5778 cm\(^{-1}\) and 5580 cm\(^{-1}\), bands that are also found in spruce milled wood lignin at 5971 cm\(^{-1}\), 5890 cm\(^{-1}\), 5793 cm\(^{-1}\) and 5578 cm\(^{-1}\) (Figure 4(c)).

Polysaccharide degradation during brown rot is reflected in corresponding negative loading bands (Figure 5). Compared to strong H-bonded O–H bands (~6300 cm\(^{-1}\)) the loading bands at positions of H-bonds of weak and intermediate strength (6750–6665 cm\(^{-1}\) and 6480 cm\(^{-1}\)) were more pronounced than corresponding bands in second-derivative spectra, indicating that these bands were preferentially reduced during degradation. The loading band at 6665 cm\(^{-1}\) is related to O–H bonds of intermediate strength. Cladophora cellulose, cotton and MCC had minima or shoulders at 6660 cm\(^{-1}\) (Figure 4), whereas glucomannan and xylan do not exhibit such a band. One of the O(2)–H(2) conformations of cellulose \(\Gamma_2\) has a mid-IR absorbance maximum near 3450 cm\(^{-1}\).21 Corresponding first OT bands would be expected—if a factor of OT: fundamental vibration = 1.93 is applied—to be located near 6660 cm\(^{-1}\). Therefore, the band near 6660 cm\(^{-1}\) is due to the first OT of a O(2)–H(2) stretching vibration of cellulose exhibiting H-bonding of intermediate strength. These groups were strongly reduced during degradation. Also, O–H groups of amorphous polysaccharides (6690 cm\(^{-1}\)) were reduced.

The PC3 loadings confirm the orientation of the O–H and C–H vibrations already discussed before. Maxima (i.e. parallel O–H overtone vibrations) were expected and found at 6680 cm\(^{-1}\), 6470 cm\(^{-1}\), intramolecular H-bonds of glucomannan and cellulose. Moreover, additional information on the orientation of lignin in brown-rotted wood could be gained by means of PCA, because spectra had not been subjected to any normalisation before multivariate analysis. Aromatic C–H vibrations exhibited a parallel orientation. By analysing characteristic mid-IR fingerprint bands, Åkerholm and Salmén15 proposed that aromatic rings of lignin tend to be oriented along the direction of cellulose microfibrils. If an overall parallel orientation of these moieties is present in S2 wood cell walls, the absorbance of the 5980 cm\(^{-1}\) band is expected to be higher in 0° than in 90° polarisation mode, because aromatic C–H groups of G-lignin (mainly C(2)–H(2), C(5)–H(5) and C(6)–H(6)) are oriented at a 30° angle in relation to the C4–C1–α–β–γ phenylpropane axis of lignin moieties. The positive loading band of the aromatic C–H vibration therefore proves the preferred 0° orientation of lignin in wood. However, some observations in the second-derivative spectra and PCA loadings led to the conclusion that, among the structural polymers cellulose, glucomannan and lignin, lignin shows by far the least molecular orientation. Amplitude minima of second-derivative spectra [Figure 2(al–cl)] assigned to intramolecular H-bonded O(3)–H(3)··· O(5) of glucomannan and cellulose (6700 cm\(^{-1}\) and 6480 cm\(^{-1}\)) are much less intense than that of lignin (5980 cm\(^{-1}\)). However, the PC3 loading spectrum [Figure 5] shows the converse relationship; here, the positive loading band near 5990 cm\(^{-1}\) assigned to lignin is weaker than the loading bands at 6680 cm\(^{-1}\) and 6470 cm\(^{-1}\), which are assigned to the parallel oriented intramolecular H-bonds of glucomannan and cellulose.

The first OT of the phenolic O–H stretching vibration band (PC3 loading at 6874 cm\(^{-1}\)), however, did not show a strong orientation in either the 0° or 90° direction. The lack of molecular orientation of the phenolic groups may be due to the fact that their formation during brown rot can be either a result of the cleavage of aryl ethers or of demethoxylation. The former would lead to a newly formed phenolic group in position 4 of the aromatic ring, the latter to a phenolic group in position 3. If these O–H groups underwent intramolecular hydrogen bonding forming a five-membered ring, as proposed by Kubo and Kadla,27 this would lead to a perpendicular orientation of the O–H vibration in the former case and to a parallel oriented O–H vibration in the latter case. The results of polarised FT-NIR spectroscopy provide no distinct orientation of this band and, therefore, suggest that both reactions take place during brown rot.

A negative loading band of the main conformation \(\Gamma_1\) of intermolecularly-bound O(4)–H(4) was found at 6612 cm\(^{-1}\) confirming again the perpendicular orientation of this band. A more complex situation, however, was again found between 6540 cm\(^{-1}\) and 6260 cm\(^{-1}\). It was indicated before that this band region assigned to H-bonding in cellulose crystals is far from being exclusively 0° oriented. A local maximum indicating 0° orientation was found at 6295 cm\(^{-1}\). Maréchal and Chanzy21 reported three conformations of the O(6)–H(6) group of cellulose with different abilities of its oxygen to accept H-bonding of the O(2)–H(2) group. They proposed that in cellulose \(\Gamma_2\) at least two-thirds of O(6)–H(6) groups exhibit only intermolecular H-bonding [Conformation I, 6612 cm\(^{-1}\)] and that in less than 10% of the O(6)–H(6) groups, the oxygen is the acceptor of the H-bond of O(2)–H(2) [Conformation III]. This strong H-bond, the strongest H-bond reported in their cellulose study, would be 0° oriented and may be the source of the 0° band at 6295 cm\(^{-1}\). The PC3 loadings [Figure 5] and the difference spectra [Figure 3(c)] discussed before suggest that this parallel H-bond contributes only to a minor extent to the second-derivative
amplitude region near 6300 cm\(^{-1}\), because it is only found as a local extremum within the very broad band.

Perpendicular bands were found in the PC3 loading spectrum [Figure 5] at 6340 cm\(^{-1}\) and 6260 cm\(^{-1}\). The former may be due to the strong intermolecular H-bond [O(6)–H(6)–O(3')] of cellulose I\(_\text{I}\) conformation III.\(^{21}\) This assignment, however, would not completely correspond to the fundamental vibration that has been reported for this O–H group at 3305 cm\(^{-1}\) if the factor first FT-NIR spectral band at 1.93 was applied. However, the mid-IR band at 3280 cm\(^{-1}\) that was also assigned to O(6)–H(6)–O(3') of cellulose\(^{26}\) could be the corresponding fundamental to the first FT band at 6340 cm\(^{-1}\). Below 3280 cm\(^{-1}\), no 90° oriented bands were found in polarised FT-mid-IR spectra of the same wood samples. In ATR-FT-mid-IR spectra, however, the most characteristic difference of the O–H stretching fundamental vibrations [second derivatives] between the Cladophora and cotton cellulose samples concerned two strongly H-bonded O–H groups. The I\(_\text{I}\)-enriched cotton sample exhibited a characteristic band at 3270 cm\(^{-1}\), whereas the I\(_\text{A}\)-enriched Cladophora cellulose had a smaller band at 3270 cm\(^{-1}\) (I\(_b\)) and a strong band at 3234 cm\(^{-1}\) (I\(_b\) mid-IR spectra will be presented elsewhere). Corresponding first OTs are expected to be located near 6340 cm\(^{-1}\) and 6270 cm\(^{-1}\) that are therefore assigned as strongly H-bonded O–H groups of cellulose I\(_b\) (6340 cm\(^{-1}\)) and cellulose I\(_b\) (6270 cm\(^{-1}\)).

Furthermore, the preferred 90° orientation of O–H vibrations of amorphous polysaccharides [found here at 6794 cm\(^{-1}\)] and of the C–H vibrations [5809 cm\(^{-1}\), 5670 cm\(^{-1}\), 5560 cm\(^{-1}\)] and of the combination band at 6430 cm\(^{-1}\) were also confirmed in the PC3 loadings that showed minima at the respective wavenumbers.

**Principal component analysis of O–H stretching vibrations first overtone**

To further resolve polarised effects of brown-rot degradation and the orientation of O–H bonds in spruce wood, PCAs of second derivatives of spectra recorded in parallel and perpendicular mode were calculated separately in the O–H stretching vibrations first 0° region (7044–6049 cm\(^{-1}\)). As before, the bulk variance was due to different intensities of the raw spectra. In both cases, the variance describing brown-rot degradation of spruce was found on PC2. In 0° mode, PC2 described 6% of the variance; in 90° mode it was 7%. The scores of the second-derivative spectra that were highly correlated (r = 0.95) reflected the situation of the loadings of the joint PCA [Figure 5(a)] and are therefore not shown here. Degraded samples scored negatively on PC2 of both PCAs. PC2 loadings [Figure 6] were both dominated by positive phenol loading bands (6900 cm\(^{-1}\)) of similar intensities supporting the lack of orientation of these groups that had been formed during brown rot. Negative loading bands of free and weakly H-bonded O–H groups [6990 cm\(^{-1}\) and 6774 cm\(^{-1}\)] were more intense in 90° polarisation, indicating the preferred degradation of polysaccharides whose main chains are oriented parallel to the tracheid direction in preference to those with rather perpendicular orientation. The weakly H-bonded O[2]–H[2] of cellulose and the main conformation of O[6]–H[6]–O[3'] with corresponding absorbance maxima near 6660 cm\(^{-1}\) and 6612 cm\(^{-1}\) overlap in the PC2 loading resulting in a minimum of the PC2 loading spectrum (90°) at 6630 cm\(^{-1}\). All these bands were lost during degradation. Additional perpendicular bands that were lost during degradation became visible here near 6520 cm\(^{-1}\) and 6430 cm\(^{-1}\). These weak bands could be due to either intermolecular H-bonding of non-cellulosic polysaccharides or within wood in general or due to intermolecular H-bonding of the cellulose I\(_b\) crystal allomorph that is present to a minor extent in spruce. The reference spectrum shows distinct amplitude minima at these positions [Figure 4(b)].

Second-derivative spectra of spruce wood and all celluloses presented here have a minimum near 6290 cm\(^{-1}\) [Figure 4]. This minimum had been assigned to the H-bonds of the cellulose I\(_b\) phase\(^{1}\) and to crystalline cellulose in general\(^{6}\) and was expected to exhibit mainly 90° orientation. However, in the course of this polarised FT-NIR study of wood, it was found several times [Figures 3(c) and 5(b)] that a number of bands overlap between 6350 cm\(^{-1}\) and 6230 cm\(^{-1}\). In 90° polarisation a PCA loading at 6270 cm\(^{-1}\) was observed that probably corresponds to the 6280 cm\(^{-1}\) PC2 loading band in Figure 5(b).

Loadings of the PCA calculated from the 0° spectra [Figure 6] show the expected intramolecularly H-bonded O–H groups O[3]–H[3]–O[5] of glucomannan [6670 cm\(^{-1}\)] and cellulose [6480 cm\(^{-1}\)] but also reveal additional parallel bands at 6280 cm\(^{-1}\) and 6240 cm\(^{-1}\), first 0T of stretching vibration of putative strongly intramolecularly H-bonded OH groups of polysaccharides, bands that are reduced during brown rot.
These bands may correspond to bands of 0° oriented fundamental O–H vibrations in spruce wood at 3246 cm\(^{-1}\) and 3234 cm\(^{-1}\) that were found in polarised FT-mid-IR spectra (second-derivative) of the same samples; a broad band with an amplitude minimum at 3234 cm\(^{-1}\) was also found in the second derivative of the ATR-FT-mid-IR spectrum of Cladophora cellulose (not shown here).

**Conclusions**

In the course of this study, polarised transmission FT-NIR spectroscopy was introduced as a tool to investigate polysaccharide degradation and lignin modification during brown rot of soft wood. Although it is well known that polysaccharides are degraded by these wood decay fungi, the changes in the various polysaccharide bands allowed conclusions to be drawn on the mode of action of these microorganisms. It could be concluded from NIR spectra that, of the main wood polysaccharides, amorphous cellulose and glucomannan, which are both oriented along the direction of the tracheids, are most susceptible to degradation. The loss of orientation in the corresponding NIR region of the weakly H-bonded OH groups near 6990 cm\(^{-1}\) could serve as a sensitive marker for early brown-rot processes of wood. On the other hand, cellulose structures exhibiting intramolecular H-bonds between O(2)–H(2) and O(6), which represent only a minor proportion of the O(2)–H(2) groups,\(^{21}\) are least susceptible to degradation. Furthermore, we were able to follow the formation of phenolic groups in lignin during brown rot and conclude that the cleavage of aryl ethers and demethoxylation takes place to a similar extent.

The basis of this study was polarised NIR spectroscopy of spruce wood. Therefore, an additional outcome was the detailed information on the orientation of O–H and C–H groups of this material. With this method, FT-NIR band assignments from the literature were confirmed and some of them were defined more precisely. Furthermore, for a number of additional O–H stretching vibrations first OT bands in spruce wood assignments were suggested. The parallel orientation of the phenylpropane units of lignin in wood cell walls was confirmed.

However, a number of assignments of first OT bands in the O–H and C–H stretching vibrations region of wood spectra still remain unsolved. This applies, in particular, to the region of bands near 6300 cm\(^{-1}\), where our studies indicated several sources of vibration. Based on ATR-FT-mid-IR spectra corresponding first OTs are expected to be located near 6340 cm\(^{-1}\) and 6270 cm\(^{-1}\) that are therefore assigned as strongly H-bonded O–H groups of cellulose \(\nu_\text{o} (6340 \text{ cm}^{-1})\) and cellulose \(\nu_\text{v} (6270 \text{ cm}^{-1})\). Further research is also required to assign the various C–H stretching vibrations first OT bands of the wood polysaccharides and of lignin between 5900 cm\(^{-1}\) and 5500 cm\(^{-1}\) to distinct C–H-containing groups of these macromolecules. Furthermore, much information on fungal wood degradation, and of spruce wood in general, is hidden in the combination bands (below 5500 cm\(^{-1}\)), which we have not discussed in this work.

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**References**


