**IR spectroscopy in white rot decayed beech**

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**Abstract**

Small steaks of beech wood were exposed to white rot fungus (*Trametes versicolor*) for period of 84 days to investigate chemical alteration in decayed wood by Infra Red (IR) spectroscopy. Decayed samples were analyzed by using Attenuated Total Reflection (ATR) Infrared Spectroscopy as a rapid method with two week intervals. Analyses showed that chemical alteration in wood begins after second week of exposure. Appearing new peaks indicates chemical modification of cell walls during days 28 to 70 of exposing to fungus and disappearance of the peaks at day 84 indicates removal of the cell wall constituents. This research also showed that ATR spectroscopy is a very applicable and a rapid method in studying wood biodegradation.

**Keywords:** Infrared spectroscopy, white-rot decay, *Trametes versicolor*

**Introduction**

By now, several types of biodegradations have been recognized in wood. They are categorized as fungal decays, bacterial degradation and insect attacks. Fungal decays are the most important and widespread types of decay in wood. Fungal wood decays are recognized as white-, brown- and soft-rot (Zabell & Morrell, 1992; Eriksson et al., 1990). White-, brown-, and soft-rot fungi attack wood based on their enzymatic systems. When their enzymes penetrate into wood cell wall, alter its chemistry and breakdown the cell wall polymers into ingestible constituents. Brown-rot fungi selectively decay cell wall polysaccharides, with limited lignin degradation. The decay system in this type is based on both chemical and enzymatic attacks (Highley & Dashek, 1998; Eriksson et al., 1990). Chemical changes following brown-rot decay have been studied by different authors. White-rot fungi have the capacity to degrade lignin as well as other wood cell wall components, although the rate at which they do so is different. Selective (or preferential) and simultaneous white-rots are categorized based on removal of cell wall constituents. Selective white-rots degrade hemicelluloses and lignin, resulting in defibrillation through dissolution of middle lamella. In contrast, simultaneous or non-selective fungi remove lignin, hemicelluloses and cellulose at similar rates, resulting in homogenous cell wall decay. The influence of white rot decay on wood chemistry has been studied by various methods (Eriksson et al., 1990; Blanchette et al., 1985; Martínez et al., 2001). Soft-rot fungi remove all cell wall constituents, resulting collapse of cell walls. Two types of soft-rot fungi have been distinguished. Type-I, is erosive and attacks all cell wall layers simultaneously and remove all cell wall polymers in similar rates; and type-II, attacks mostly polysaccharides in secondary cell wall layers selectively, resulting some growing typical cavities. Chemistry of soft-rot fungi has been investigated by Levy & Peterson (1965), Savory & Pinion (1958) and Nilsson et al. (1989).

Different methods and techniques have been applied to monitor fungal decays, such as gravimetric analysis or loss of weight (Wälchli, 1970), loss of dynamic modulus of elasticity (Mohebby & Militz, 2002; Mohebby, 2003; Macheck, 1998a&b), Technical microscopies e.g. light, polarized, electron microscopies (Anagnost, 1998), NMR spectroscopy, FTIR

All methods mentioned above are tedious and time consuming. FTIR spectroscopy is a rapid way to recognize wood chemistry qualitatively as well as decay in wood (Pandey, 1999; Pandey and Theagarjan, 1997). This technique was recently used by Pandey & Pitman (2003) to present fungal decay in hard- and soft-wood. The reported method is easy and rapid. However it requires preparation of small pellets with KBr. In this research another method has been used as a very rapid and easier technique than the FTIR spectroscopy to monitor white-rot decay in wood. Attenuated Total Reflection (ATR) Infrared Spectroscopy is applicable with very small quantities of wood powder or solid samples (few milligrams) without any previous preparation.

**Material and Methods**

**Samples:** Small beech (Fagus sylvatica) blocks (5×5×20 mm) were prepared and dry weights of 10 mini-blocks were determined and autoclaved at 121ºC for 20 minuets.

**Microorganisms:** Strains of typical white rot (Trametes versicolor) fungus, which was maintaining at 4ºC on peptone yeast extract slants (per liter: 20g glucose, 5g peptone, 2g yeast extract, 1g KH2PO4, 0.5g MgSO4 · 5H2O, 15g agar) used as test fungal strains.

**Medium:** Glucose malt-extract (ME) plates (per liter: 15g agar, 10g glucose, 4.5g malt-extract) were prepared and autoclaved at 121ºC for 20 minutes. The ME plates were incubated at 27ºC and 70% relative humidity. Agar plugs (3mm diameter) were punched from the leading edge of the mycelium grown on ME plates and used as inoculums for the experiments.

The test mini-blocks were introduced to the plates carefully and incubated at 27ºC and 70% relative humidity for 84 days. Samplings were carried out every two week intervals.

**Mass loss determination:** Samples were cleaned carefully and dried at 103±2ºC to determine their dry weights after fungal attack. The mass losses of individual samples were calculated (Eq. 1) and used to determine mean percentage of mass losses for each sampling duration.

\[
M_L (\%) = \frac{(W_i - W_f)}{W_i} \times 100
\]

Where \(M_L\) stands for percentage of mass loss, \(W_i\) and \(W_f\) indicate initial and final weights of samples before and after decay, respectively.

**IR spectroscopy:** Attenuated Total Reflection (ATR) Infrared Spectroscopy has an advantage over other methods. It is very easy and rapid method due to using solid material (as solid wood or powdered). For this purpose, dried samples were milled and passed through mesh 40. IR spectra were collected directly from wood powder on detector prism. Spectra were recorded using a Bruker Vectra 22 FTIR Spectrometer equipped with a DuraSampleIR II™ detector. All spectra were taken at a spectral resolution of 4cm\(^{-1}\) between wave number ranges of 5000-600cm\(^{-1}\). Sample and background scans were measured with 60 scans. Background spectra were collected using an empty collector. A rubber band method was used for baseline correction. The band for CO\(_2\) was removed to make a suitable baseline correction (Mohebby, 2003). Collection of data and correction were carried out by OPUS software.

**Results and discussion**
The mean mass loss for beech wood exposed to *T. versicolor* is shown in Fig. 1. The main loss was determined after fourth week of exposure. The greatest extents of decay were measured at days 70 and 84 with about 51 and 60% of mass losses respectively.

![Fig. 1- Mass loss in white rot decayed beech wood](image1.png)

An IR spectrum of un-decayed beech wood is shown in Fig. 2. A strong OH bond vibration is seen at 3500-3300 cm\(^{-1}\) (1) relating to absorbed water in wood and a prominent C-H stretching vibration around 2881 cm\(^{-1}\) (2). There are many well defined peaks in fingerprint region of 1800-600 cm\(^{-1}\). The assigned peaks in the mentioned region for beech wood are un-conjugated C=O stretching in xylan at 1724 cm\(^{-1}\) (3); conjugated C-O stretching at 1587 cm\(^{-1}\) (4); aromatic skeletal vibration at 1500 cm\(^{-1}\) (5); C-H deformation in lignin and carbohydrates at wave numbers 1450 cm\(^{-1}\) (6) and 1417 cm\(^{-1}\) (7); C-H deformation in cellulose and hemicelluloses at 1363 cm\(^{-1}\) (8); C-H vibration in cellulose and C\(_1\)-O vibration in syringyl derivatives at 1319 cm\(^{-1}\) (9); syringyl ring and C-O stretching in lignin and xylan at wave number 1226 cm\(^{-1}\) (10); C-O-C vibration in cellulose and hemicellulose at 1151 cm\(^{-1}\) (11); aromatic skeletal and C-O stretch at 1116 cm\(^{-1}\) (12); C-O stretch in cellulose and...
hemicelluloses at wave number 1024 cm\(^{-1}\) (13) and C-H deformation in cellulose at 892 cm\(^{-1}\) (14).

**Fig. 3- IR spectra of decayed beech wood at various periods of white rot attack**

The IR spectra of white rot decayed beech wood are shown in Fig. 3. It indicates changes of peaks in finger print region at different stages of decay. Comparison between the peaks from different stages of attack with the un-decayed wood reveals appearance of new peaks after 28 days of exposure to fungus, which are prominent indications of new bands obtained from altered cell wall constituents due to reaction with fungal enzymes. Subtraction of peaks obtained from attacked wood after 70 and 84 days of exposure to fungus from the un-decayed beech clearly shows appearance of new peaks in finger print region that are some times unknown (Figs. 4 & 5). In both figures, the baselines indicate no differences between the un-decayed and the decayed wood; while the peaks under the baselines show increase of bands and above the baselines indicate prominent decrease of the bands. The spectra from 70days exposed wood reveal appearance of new peaks around 1800-1650 cm\(^{-1}\) and 1550-1500 cm\(^{-1}\), the finger print regions for hemicelluloses (especially xylan) and aromatic derivatives of lignin respectively. The increases of the peaks indicate that new bands are occurred due to breakdown of lignin and hemicellulose polymers. During fungal attack, different types of hydrolyzing enzymes could be librated to alter and break linkages in the cell wall components and release them as small molecules. During this period, many chemical changes should be occurred in those constituents that fungus could assimilate them as carbon sources.
Fig 4- Subtracted IR spectra of white rot decayed beech wood after 70 days

Fig 5 shows that the underneath peaks are removed and a wide range of peaks are appeared above the baseline after 84 days of exposure. The peaks above the baseline reveal removal of chemical constituents from the cell walls. White rot fungus could remove almost main part of hemicelluloses, lignin and partially cellulose according to the spectra and assigned peaks (table 1). The results revealed that *T. versicolor* is a non-selective white rot fungus due to removal of all types of the cell wall constituents.

Chemical analyses and microscopic studies have shown that white rot fungus *Coriolus (Trametes) versicolor* is a non-selective or simultaneous type and removes lignin, hemicelluloses and cellulose (Anagnost, 1998; Eriksson et al., 1990; Highley & Dashek; 1998; Mohebby, 2003). Pandey & Pitman (2003) studied chemical changes in beech wood decayed by *C. versicolor* by FTIR spectroscopy. They showed decrease in intensities of lignin, carbohydrate bands, preferentially hemicelluloses. Their results indicated that *C. versicolor* decays the hemicelluloses more preferential than the cellulose.
Faix et al. (1993) studied chemical alteration in beech wood decayed by *C. versicolor* by FTIR and recorded complex bands between 1200-900 cm\(^{-1}\) due mainly to polysaccharides and the hemicellulose bands at 1740 cm\(^{-1}\) with small changes. However, decay results revealed increased intensity at 1646 cm\(^{-1}\) band due to conjugated carbonyl groups originating from lignin. While the aromatic skeletal vibration band at 1596 cm\(^{-1}\) decreased in the intensities.

Comparison between the results of IR bands after 70 days of exposure supports results of Faix et al. (1993) due to a sequential decay of the cell wall constituents initiating from lignin, hemicellulose to cellulose respectively.

**Conclusion**

ATR spectroscopy was used to examine chemical alterations in beech wood decayed by *T. versicolor* qualitatively. The used technique here showed that *T. versicolor* decays the cell wall components non-selectively. The results support IR spectroscopies of previous authors. According to the results, ATR spectroscopy is an easy and applicable technique to give rapid results from decayed wood over the time.
Table 1. Assigned peaks of white rot decayed beech after 84 days of exposure

<table>
<thead>
<tr>
<th>Wave number (cm(^{-1}))</th>
<th>Assignments</th>
<th>Peak situation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1724</td>
<td>C=O un-conjugated groups in lignin and carboxylic acid esters in hemicelluloses(^1)</td>
<td>-</td>
</tr>
<tr>
<td>1587</td>
<td>Conjugated C-O(^2)</td>
<td>-</td>
</tr>
<tr>
<td>1500</td>
<td>Aromatic skeletal; Benzene ring vibration in lignin(^3)</td>
<td>-</td>
</tr>
<tr>
<td>1450</td>
<td>C-H deformation in lignin &amp; carbohydrates; CH3, CH2, benzene ring vibration in lignin(^4)</td>
<td>-</td>
</tr>
<tr>
<td>1417</td>
<td>C-H deformation in lignin &amp; carbohydrates; Aromatic skeletal vibrations combined with C-H in plane deformation(^5)</td>
<td>-</td>
</tr>
<tr>
<td>1363</td>
<td>C-H deformation in cellulose and hemicelluloses; C-H bending vibration in cellulose and hemicelluloses(^6)</td>
<td>-</td>
</tr>
<tr>
<td>1319</td>
<td>C-H vibration in cellulose; C(_1)-O in syringyl derivatives(^7)</td>
<td>-</td>
</tr>
<tr>
<td>1226</td>
<td>Syringyl ring; C-O stretch in lignin and xylan(^8)</td>
<td>-</td>
</tr>
<tr>
<td>1151</td>
<td>C-O-C vibration in cellulose &amp; hemicelluloses(^9)</td>
<td>+</td>
</tr>
<tr>
<td>1116</td>
<td>Aromatic skeletal; C-C stretch; O-H association band in cellulose and hemicelluloses(^10)</td>
<td>-</td>
</tr>
<tr>
<td>1024</td>
<td>C-O stretch in cellulose &amp; hemicelluloses; C-O of primary alcohol(^11)</td>
<td>-</td>
</tr>
<tr>
<td>892</td>
<td>C-H deformation in cellulose ; C(_1) group frequency in cellulose and hemicellulose(^12)</td>
<td>-</td>
</tr>
</tbody>
</table>

1. Sundell et al., 2000; Takahashi et al., 1989
3. Pandey & Theagarjan, 1997; Schultz & Glasser, 1986; Pandey, 1999
4. Pandey & Theagarjan, 1997; Kimura et al., 1992
5. Sundell et al., 2000; Pandey & Theagarjan, 1997; Pandey, 1999; Rodrigues et al., 1998
7. Pandey & Theagarjan, 1997; Pandey, 1999; Pandey & Pitman, 2003
9. Pandey & Theagarjan, 1997; Rodrigues et al., 1998
10. Schultz & Glasser, 1986; Pandey, 1999
11. Pandey, 1999; Pandey & Theagarjan, 1997
12. Pandey & Theagarjan, 1997; Rodrigues et al., 1998

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