

## Assessment of Early Stage Fungal Decay of Wood

### by FT-NIR-spectroscopy

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### ABSTRACT

*The most efficient wood-rotting fungi are basidiomycetes and ascomycetes. In general, microbial decay processes go along with a loss of wood quality. For example, brown rot decay leads to a rapid decrease in wood strength already in early stages of growth. On the other hand, a projected biodegradation may enhance the quality when sound wood or surface properties of wood are modified by means of biotechnology. The well-known standard methods to assess changes in infected wood are time-consuming, and therefore unacceptable for efficient process-control and quality-assurance. We could show, that fungal infestation of hardwood (Fagus sylvatica L.) and softwood (Picea abies L. Karst) can be assessed by means of FT-NIR spectroscopy in combination with uni- and multivariate data analysis. The rapid method is shown to be suitable for these purposes, presumed the regression models are well chosen. The results of degradation experiments of wood blocks, veneers, and wood shavings demonstrated that different types and stages of decay could be distinguished and lignin content after decay could be estimated from FT-NIR spectra collected from the degraded wood surfaces and milled wood. Spectral data were subjected to principal component analysis (PCA) and uni- and multivariate regression models were calculated. Mass loss after degradation and lignin content could be estimated from degraded wood surfaces and it was possible to estimate the selectivity of white rot fungi for the preferential degradation of lignin.*

### INTRODUCTION

Wood is colonized and degraded by a variety of micro-organisms. Wood quality suffers from microbial decay processes. Conventional methods are not suitable to assess early infestation of wood on the one hand and to evaluate the potential of basidiomycetes for lignocellulose biotechnology processes such as bio-pulping (Akhtar et al. 1997) on the other hand. Selective white rot fungi remove more lignin than polysaccharides in early decay stages which makes them interesting as a biotechnological tool for lignocellulose processing industries. The pretreatment of lignocellulosic materials with white rot fungi to facilitate the disintegration of wood in the refining processes during thermomechanical pulp production or to increase the accessibility of wood polysaccharides to cell wall degrading enzymes to yield monomeric sugars for bioethanol production are two examples of many that have been investigated (Akhtar et al. 1997; Amirta et al. 2006). Alternatives to rapidly assess fungal action on lignocellulose in early stages are needed. Fourier transform near-infrared (FT-NIR) reflectance spectroscopy has been proven a powerful tool to estimate both chemical and physical parameters of wood (Tsuchikawa 2007). NIR absorption bands arise from overtones and combination bands caused by vibrations of C-O, O-H, C-H, and N-H groups, which have their fundamental molecular vibrations in the mid-IR (MIR) region, but also information about wood density (Thygesen 1994) is reflected in FT-NIR spectra. Kelley et al. (2002) were able to predict mass loss of brown rotted softwood from reflectance spectra of wood meals.

This paper focuses on qualitative and quantitative analysis of FT-NIR spectra from wood surfaces and wood meals to assess the changes in the composition of wood caused by the action of the fungi (brown

rot and white rot). For that purpose our recent work is reviewed, but also new examples of the analysis of the spectral data are presented.

## MATERIALS AND METHODS

### Fungi

The origin of the cultures is the collection of the Institute of Chemical Engineering, TU Vienna. White rot (WR) fungi: *Bjerkandera adusta*, *Ceriporiopsis subvermispora* CBS 347.63 and FPL 105.752, *Hypoxylon fragiforme*, *Oxyporus latemarginatus*, *Phanerochaete chrysosporium*, *Phlebia brevispora*, *Phlebia radiata*, *Phlebia tremellosa*, *Trametes cervina*, *Trametes versicolor* ZIM L017 and CTB 863 A, *Tyromyces chioneus* DSM 5242. Brown rot (BR) fungi: *Coniophora puteana* CBS 237.91, *Gloeophyllum trabeum*, *Poria placenta* MAD 698. The cultures were maintained on malt extract agar (MEA, Fluka) slants, and pre-cultivated for two weeks on MEA plates before use.

### Incubation of veneers, blocks, and shavings

Veneers (30 mm x 50 mm x 1.6 mm) were steam sterilised for 15 min and soaked in 2% (w/v) corn steep liquor (CSL, Agrana, Austria) containing suspended fungal mycelium (one malt extract agar plate overgrown by the fungus was mixed with 150 ml 2% (w/v) sterile CSL in a Waring blender for 30 s at full speed). Then they were put in agar dishes (9 cm Ø) with 25 ml water agar (1.5%) and beech tooth picks as spacers to allow growth of the fungi throughout the entire wood surface in a moisture-saturated atmosphere. The Petri dishes were incubated at 28°C for up to ten weeks. Steam sterilised spruce wood blocks (1 cm x 1 cm x 3 cm) were inoculated following the procedure of EN 113 and incubated for up to 20 weeks at 28°C. Fresh spruce wood shavings (3 g, particle size 0.4 – 2 mm, 29.0 % total lignin content, 100 % initial moisture content) were steam sterilised at 121 °C for 15 min and inoculated with 3 ml of mycelium suspension (1 MEA plate / 70 ml 1.5 % (w/v) corn steep liquor, 200 % moisture content). Samples were incubated for up to 14 days at 28°C.

### Sample preparation for FT-NIR analyses

After incubation, the fungal mycelia were removed from the surface of the decayed veneers and the samples were dried for one week at 50°C. Then, they were milled (Retsch Ultra Centrifugal Mill ZM 1000, fixed ring sieve, 80 µm holes) and oven-dried at 50°C for another week. Finally, the milled wood samples were extracted according to Schwanninger and Hinterstoisser (2002), except cyclohexane was used instead of benzene (Fengel and Przyklenk 1983), and dried for another week at 50°C. Control samples (non-treated and steam-sterilised) were dried, milled, and extracted in the same way.

### FT-NIR spectroscopy and data analysis

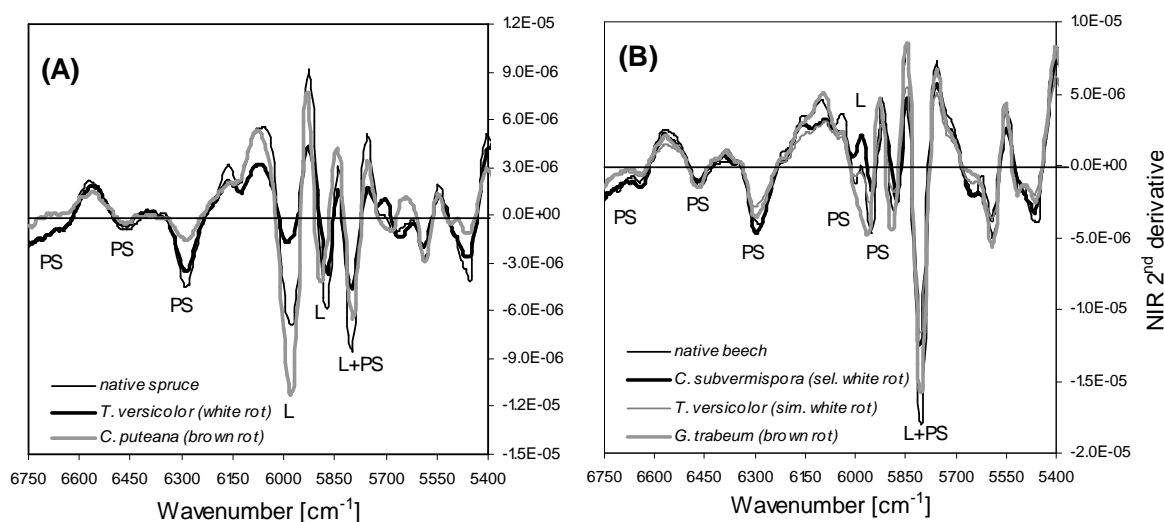
FT-NIR reflectance spectra were recorded at ambient temperature using a fibre-probe connected to a Bruker FT-IR spectrometer (Equinox 55) (Schwanninger et al. 2004). Ten to twenty spectra from areas (9-10 mm<sup>2</sup>) of the solid samples at random positions on the front and backside of the veneers and four replicate spectra of milled samples and extracted milled samples were recorded (Fackler et al. 2007b). Spectra were processed by means of 17-points smoothing filter and a second order polynomial to obtain 2<sup>nd</sup> derivatives (Savitzky and Golay 1964) with OPUS software (version 5.5, [www.brukeroptics.de](http://www.brukeroptics.de)). Partial least squares regression (PLSR) models of average spectra were calculated and optimised with the software OPUS Quant 2. Principal component analyses (PCA) of average spectra were carried out after mean-centering the data with the software The Unscrambler™ (www.camo.com).

## RESULTS AND DISCUSSION

### NIR Spectra

Figures 1A, B show 2<sup>nd</sup> derivatives of FT-NIR reflectance spectra from spruce and beech wood surfaces before and after degradation by white and brown rot fungi. Calculation of the 2<sup>nd</sup> derivatives helped accentuating differences between the spectra and served as data pre-treatment for PCA. Changing lignin contents of spruce wood caused by degrading fungi can be estimated within the wavenumber region from 6080 to 5800 cm<sup>-1</sup> (Fig. 1A). Bands deriving from the aromatic ring (C-H) and C-H vibrations of methyl and methylene groups are visible in this region (Shenk et al. 2001).

Lower lignin contents – e.g. caused by selectively delignifying fungi – lead to lower amplitude values of the 2<sup>nd</sup> derivative spectra. Particularly the amplitude minimum near 5980 cm<sup>-1</sup> correlates with the lignin content of milled spruce wood (Schwanninger et al. 2004) and spruce wood surfaces (Fackler et al. 2007c). In beech wood spectra (Fig. 1B) C-H deriving bands of xylan superimpose this region. They show minima near 6000, 5955, 5880, and 5800 cm<sup>-1</sup>. Thus, in hardwoods, a local maximum from lignin occurs between two minima (6000 and 5955 cm<sup>-1</sup>). Changes of lignin contents can be estimated from this spectral region. Fungal wood decay leads to changes of the polysaccharide contents which are reflected between 6750 and 6230 cm<sup>-1</sup>. The bands have mainly been assigned to the 1<sup>st</sup> overtone of the fundamental OH-stretching vibrations of cellulose (Tsuchikawa and Siesler 2003a,b).



**Figure 1.** 2<sup>nd</sup> derivatives of characteristic FT-NIR reflectance spectra of wood surfaces after prolonged fungal decay. The region between 6750 and 5400 cm<sup>-1</sup> that was used for PCA is shown. Lignin and polysaccharides assigned bands are marked as (L) and (PS), respectively: (A) Spruce wood after 20 weeks degradation by *T. versicolor* and *C. puteana*. (B) Beech wood after 10 weeks degradation by *C. subvermispora*, *T. versicolor*, and *G. trabeum*.

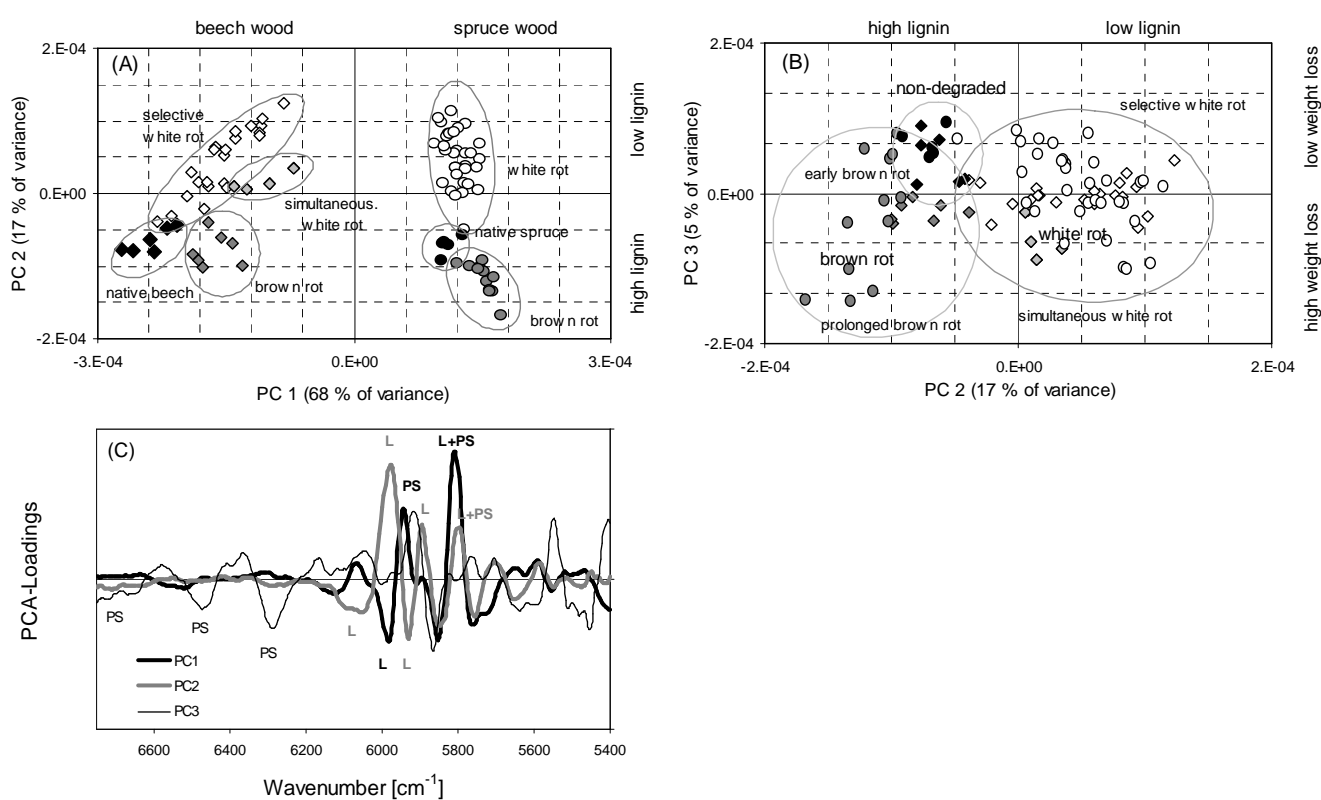
### Multivariate data analysis: PCA

Principal component analysis allows the projection of multi-dimensional data onto a few orthogonal features, called principal components (PCs), constructed as a linear combination of the original variables – e.g. the data points of FT-NIR spectra – to maximise the description of the data variance. The aim is to reveal the sharpest low-dimensional projection (i.e. the most informative projection) to find clusters of similar samples. The 2<sup>nd</sup> derivatives of veneer surface spectra between 6750 and 5400 cm<sup>-1</sup> have been subjected to PCA (92 samples, 350 data-points per sample, Fig. 2). This region includes bands assigned to the 1<sup>st</sup> overtones of molecular structures of carbohydrates (semi-crystalline cellulose: 6718 cm<sup>-1</sup>, crystalline cellulose: 6450 cm<sup>-1</sup> and 6287 cm<sup>-1</sup> (Tsuchikawa and Siesler 2003 a,b). Lignin dominates the region between 6100 and 5750 cm<sup>-1</sup>. Beech and spruce wood samples are separated along the PC1 axis (68% of the variance). PC2 (17% of the variance) separates the samples in white and brown rot degraded ones. The PC1-PC2 (Fig. 2A) plot reveals two main clusters (beech and spruce wood) which are divided into four and three sub-clusters: selective white rot, simultaneous white rot (found only in beech), brown rot, and native samples. The PC1 loading spectrum (Fig. 2C) shows the highest positive loadings at bands assigned to xylan 5940 and 5805 cm<sup>-1</sup> and negative loadings at 5980 (lignin) and 5850 cm<sup>-1</sup> (cellulose). Thus, beech wood samples with lower lignin contents (lower than the average) and higher xylan contents score negatively on PC1, spruce samples score positively. Degraded beech wood samples score less negatively on PC1, indicating that xylan, which dominates the PC1 loadings, was degraded by each of the investigated decay fungi. The PC2 loading spectrum is dominated by lignin derived bands: positive loadings at 5975, 5900; and 5775 cm<sup>-1</sup>; and negative loadings at 6040, 5930, 5844, and 5750 cm<sup>-1</sup> reflect more or less the shape of an inversed second derivative lignin spectrum. Higher lignin contents are reflected in lower amplitude

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values near  $5980\text{ cm}^{-1}$  (Fig. 1A). Thus, wood samples subjected to brown rot with higher lignin contents score negatively and selective white rotted samples score positively on PC2.

PC3 (5% of the data variance) separates the samples according to the weight loss of wood caused by the decay fungi. Polysaccharides dominated bands prevail the PC3 loadings spectrum ( $6750\text{ cm}^{-1}$ : amorphous cellulose, hemicelluloses,  $6450\text{ cm}^{-1}$  semi-crystalline cellulose,  $6287\text{ cm}^{-1}$  crystalline cellulose). Non-degraded samples with high polysaccharides contents score positively on PC3, samples with lower polysaccharides contents score negatively. Obviously, the degradation of wood polysaccharides contributes to a higher extent to weight loss than the degradation of lignin. The cluster with non-degraded samples overlaps with that of brown rotted samples, indicating that early brown rot cannot be reliably detected. Within the white rot cluster, wood samples separate between those that had been subjected to selective white rot fungi that score positively on PC2 and PC3, and simultaneously white rotted samples that score positively on PC2 and negatively on PC3. The PC2-PC3 scores plot further demonstrates that white rot fungi in general are able to degrade lignin of spruce wood more selectively than that of beech wood. These findings were confirmed by quantitative analysis of the same NIR spectra (Fackler et al. 2007a).



**Figure 2.** PCA of spruce wood (circles) and beech wood (diamonds) surface spectra (second derivative) calculated between  $6750$  and  $5400\text{ cm}^{-1}$ . (A) Scores plot PC1-PC2; (B) Scores plot PC2-PC3; (C) Loading spectra PC1, PC2, PC3 [loading bands assigned to polysaccharides (PS), and lignin (L)].

### Quantitative analysis - regression models to predict lignin content and weight loss

FT-NIR data were used to calculate regression models in order to predict the lignin content and weight loss of fungal degraded spruce and beech wood. To obtain reference data, lignin was determined according to (Schwanninger and Hinterstoisser 2002), weight loss was determined gravimetrically. The regression models are summarised in Table 1: Linear models were developed to estimate the lignin content of milled spruce wood and spruce wood surfaces using the amplitude minima of the  $2^{\text{nd}}$  derivative spectra near  $5980\text{ cm}^{-1}$ . PLS regression models in the spectral region characteristic for lignin ( $6080 - 5800\text{ cm}^{-1}$ ) were calculated for beech wood. The regression models to predict the weight loss are mainly based on two characteristics reflected in FT-NIR spectra: 1.) changes of the wood composition that have been discussed in the above chapter PCA, and 2.) reduced wood density as a consequence of fungal action that leads to less intense FT-NIR reflectance spectra.

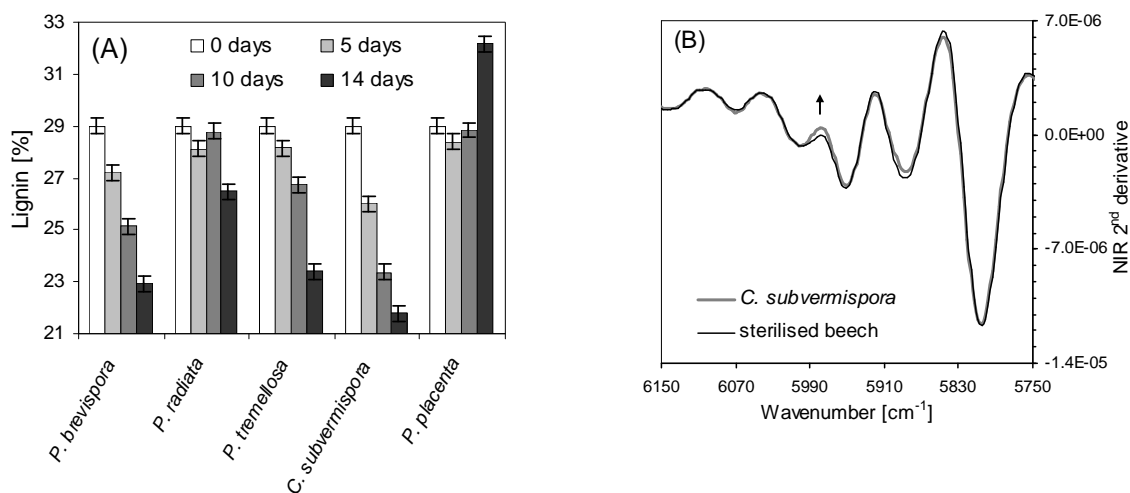
**Table 1. Regression models to predict weight loss and lignin content of degraded spruce and beech wood calculated from FT-NIR spectra.**

Component	Sample Form	R <sup>2</sup>	RMSECV <sup>1</sup> [%]	SD <sup>2</sup> [%]	Reference
Lignin	Milled spruce wood	0.95	--	0.3	Schwanninger et al. 2004
	Surface of spruce wood	0.95	--	0.3	Fackler et al. 2007c
	Milled beech wood	0.81	1.0	--	Fackler et al. 2007b
	Extractives free milled beech wood	0.96	0.6	--	Fackler et al. 2007b
	Surface of beech wood	0.81	1.0	--	Fackler et al. 2007b
Weight loss	Surface of spruce wood	0.93	4.4	--	Fackler et al. 2007c
	Surface of beech wood	0.81	5.1	--	Fackler et al. 2007b,c

<sup>1</sup> root mean square error of cross validation<sup>2</sup> standard deviation

### Early fungal degradation of wood

Delignification of spruce wood shavings during solid state fermentations with white rot fungi were determined with the FT-NIR method: differences in lignin contents between consecutive fermentation days could be elucidated (Fackler et al. 2006). Fig. 3A shows the lignin contents of the shavings after treatment with four strains of selective white rot fungi and one brown rot fungus (*P. placenta*) after 5, 10 and 14 days. Whereas most white rot fungi were able to delignify the wood shavings significantly within 5 days, it took 14 days until wood shavings treated with the brown rot fungus *P. placenta* showed the expected increase of the lignin content caused by selective degradation of polysaccharides. Selective delignification of beech shavings by *C. subvermispora* can be reliably detected within 10 days, indicated by an increase of the local maximum near 5980 cm<sup>-1</sup> reflecting a reduction of the lignin content by 1 % point (Fackler et al. 2007a).



**Figure 2. (A) Lignin content of fungal treated spruce wood shavings determined with the FT-NIR method. (B) 2<sup>nd</sup> derivative spectra of milled beech wood shavings that had been treated for 10 days with a selective white rot fungus.**

### CONCLUSIONS

FT-NIR spectroscopy serves as a quick and reliable analytical tool to assess fungal degradation of wood caused by basidiomycetes. The method makes use of chemical and physical changes that occur in spruce and beech wood, while white and brown rot fungi grow on it. Particularly, the changing lignin content caused by fungal action turned out to be useful for this purpose. Several regression models to estimate its content were developed. On the one hand, FT-NIR spectroscopy can be used to monitor the kinetics of fungal action during short-term modification of wood shavings, and to evaluate the delignification selectivity of novel white rot strains for industrial purposes. On the other hand, early white rot degradation of wood can be detected within several days. Thus FT-NIR spectroscopy is appropriate as a quality control tool to assess fungal wood decay in wood processing industries and has great potential for process control in lignocellulose biotechnology.

### ACKNOWLEDGEMENTS

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