

**SELECTED PROPERTIES OF BEECH WOOD DEGRADED BY
THE WHITE-ROT FUNGUS *TRAMETES VERSICOLOR*.**

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ABSTRACT

The aim of this paper was a study of alterations in the selected physical properties and chemical composition of beech wood in the course of short and medium-term degradation by the erosive white-rot fungus *Trametes versicolor*. The fungus caused a gradual weight loss and a drop in density of the tested specimens which was almost proportional to the time of decay. The composition of beech wood was changing with the time of bio-degradation, and a deep delignification and a moderate removal of cellulose in the later degradation stages were observed. The above alterations were reflected in the rate of wood/water surface interactions, permeability, and whitening of wood with the time of degradation. Remarkable is an extreme increase in the relative rate constant of surface swelling of beech wood, even after a short degradation accompanied by only a 4 % weight loss.

KEY WORDS: beech wood, *Trametes versicolor*, weight loss, density, composition, swelling, colour, VIS and FTIR spectra

INTRODUCTION

The influence of wood destroying fungi and micro-organisms on wood and lignocellulosics has its pros. and cons.. Wood destroying pathogens play an important role in the conversion of plants residua into humic moieties - an organic portion of the soil. On the other hand, these organisms reduce apparently physico-mechanical properties of wood, which is an undesirable phenomenon in practice.

Intentional degradation of wood chips by the white-rot fungi and the ascomycete *Ophiostoma piliferum*, however may positively influence their properties from the viewpoint of their processing in the pulp and paper industry. Ascomycete *O. piliferum* degrades the extractives in wood, thus

increasing its permeability and improving impregnation of the chips with pulping media (Wall et al. 1996). The action of white-rot fungi is more complex and results in partial delignification, decomposition of hemicelluloses and lipophilic extractives in wood. Quantitative and qualitative chemical changes combined with the structural alterations of the cell walls in wood may modify positively its properties important from the viewpoint of pulping processes (Messner and Srebotnik 1994, Fisher et al. 1996, Solár et al. 2005). In many cases, increase in the kinetics of delignification of chips pre-treated by white-rot fungi, reduction of pulp Kappa number and better mechanical properties of the pulp were reported (Messner and Srebotnik 1994, Solár et al. 2001b).

The aim of this contribution was to estimate the alterations in selected physical and some chemical characteristics of beech wood due to its degradation by erosive strain of the white-rot fungus *Trametes versicolor*. The obtained results may be interesting from the viewpoint of pulping processes, drying and impregnation of the bio-degraded wood by different media.

MATERIAL AND METHODS

For intentional bio-degradations of beech wood a 60 cm long section of the tree trunk was taken from its middle part. From this section a prism with dimensions of 3x3x 60cm, (longer dimension parallel with grain) was prepared. The position of the prism was approximately 6 cm from the section circumference. The age of the tree was 82 years. The dimensions of specimens prepared from the prism were 3x3x1 cm. The shorter dimension was parallel, and the longer ones were perpendicular to grain. From the specimens a comparable series, each comprising 5 pieces, were prepared. A criterion of selection was number of annual rings, density and position of the specimens in the prism. A small number of the specimens in a series was due to some long lasting analyses of the selected physical properties of wood. From this reason, this contribution was focused on the estimation of trends rather than on the precise data from a numerous series of the compared specimens.

Biodegradation

The erosive white-rot fungus *Trametes versicolor*, strain CTB 863 A was used for degradations. The fungus was grown on malt – agar soil. The degradations were carried out in Kolle's flasks at 26 °C during 15, 30 and 60 days. The activity of the fungus was stopped by a 2-hour immersion of the specimens into 99 % methanol. A two-step drying of the specimens was performed; the first step - careful drying in an open space was followed by drying in a dessicator over P₂O₅, both steps were carried out at ambient temperature.

Methods of measurements and analyses

- weight loss of the specimens due to bio-degradation was determined as a difference of their weight in absolutely dry state, prior to and after degradations,
- density of the specimens was determined in their absolutely dry states,
- coefficients of permeability in axial direction were determined by using the method of Regináč et al. (1977),
- kinetics of facial swelling was determined according to the contact method with PC processing of the data (Solár et al. 2006),
- colour of wood samples was estimated with the spectrometer Conica Minolta CM-2600 D in the range of 360 – 740 nm with a resolution of 10 nm, a width of the band was 10 nm, and

a precision of the reflex registration 0.01 %. From each series comprising 5 specimens totally the 60 shots were taken. The spectra were evaluated in co-ordinates of the colour space „CIELAB“, and as “differential spectra”. The differential spectra represented a difference between the spectra of bio-degraded and sound beech wood,

- extractives of medium polarity were determined by extraction with benzene/ethanol (2:1 v/v) mixture in a Soxhlet apparatus; time of extraction was 8 h,
- cellulose was determined by using the method of Kürschner and Hoffer (K-H), and 3 delignification steps were performed,
- lignin in wood and residual lignin in the cellulose preparations were determined by TAPPI Standards T-13m method,
- nitrobenzene oxidation of extractive-free wood (4.0 ml of 2M NaOH and 0.25 ml of C₆H₅NO₂ on 200 mg of wood meal) were performed at 180 °; time of oxidation was 2.5 h; the finals after separation steps were analysed by HPLC, UV detector with optimised wavelength was used,
- total hydrolysis of extractive-free wood meals was performed by the method of Seaman et al. (1954) and monosaccharides were determined by GLC of their aldnitril-acetates,
- FTIR spectra were obtained by Nicolet Magna 750 spectrometer using KBr technique; difference spectra were expressed as a difference between the spectra of bio-degraded and sound wood in their absolutely dry states.

Note: three specimens from each series were used for monitoring of the swelling kinetics and chemical analyses of the degraded wood. The criterion for the selection was the weight loss close to that of the complete series of the corresponding specimens. Chemical analyses in detail are described in a book (Kačík and Solár 1999).

RESULTS AND DISCUSSION

General data and chemical analyses

Tab. 1 presents the weight loss of the examined testing specimens of sound and degraded beech wood by *Trametes versicolor*.

Tab. 1: Weight loss and density of beech wood specimens degraded by *T. versicolor*

Statistical data	Sound wood	15 - day degradation	30 – day degradation	60 - day degradation
Weight loss (%)	0	3.96	11.87	26.35
n	-	5	5	5
v (%)	-	5.38	7.17	11.40
Density (g.cm ⁻³)	0.6629	0.6180	0.5487	0.4594
n	5	5	5	5
v (%)	0.950	1.032	2.630	7.970

As it follows from Tab. 1, the intensity of degradation of beech wood samples showed a decreasing tendency with the time of fungal action.

A drop in the beech wood density was of similar trend as its weight loss, and the variability of the presented data was relatively low.

Reduction in density of the bio-degraded beech wood points out indirectly its increasing porosity with the time of degradation.

In Tab. 2, the basic chemical analyses of sound and bio-degraded beech wood are given.

Tab. 2: Weight loss (Δm), contents of BA extract, lignin and cellulose in sound beech wood and that degraded by the fungus *T. versicolor* (%)

Loss/component	Sound wood	15-day degradation	30-day degradation	60-day degradation
Δm	-	4.09	12.28	23.63
BA extract	0.91	0.71	0.96	1.09
Lignin	22.43 (22.23)	20.31 (19.34)	19.98 (17.36)	19.05 (14.39)
Cellulose	51.96 (51.48)	53.57 (51.01)	54.26 (47.14)	55.00 (41.55)

* data in brackets represent the contents of wood components expressed to the weight of specimens before degradation, considering their weight loss and BA extract; due to insignificant amounts of residual lignin (0.28 - 0.31%) in the K-H cellulose preparations, the contents of this polysaccharide in wood were not corrected for lignin.

The data in Tab. 2 confirmed the erosive character of the used strain of white-rot fungus. Except for a deep delignification of beech wood, representing 21.9 and 35.3 % of the removed lignin within 30 and 60 days of degradation, the fungus also removed 10.4 or 19.9 % of cellulose, respectively, in the same periods. Degradation of cellulose, similarly as in the case of lignin, proceeded since the early stages of the rot, however at a much lower rate, and the cellulose contents in the extractive-free wood were increased relatively with the time of degradation.

The data concerning the alterations in the polysaccharide portion of sound and bio-degraded beech wood obtained by GLC of the corresponding hydrolysates are in Tab. 3.

Tab. 3: "Anhydro-saccharides" in sound and bio-degraded extractive-free beech wood (in %)

Component	Sound wood	15-day degradation	60- day degradation
Glucan*	47.04 (45.56)	46.98 (44.16)	51.06 (38.57)
Mannan	0.87 (0.86)	0.83 (0.79)	0.89 (0.67)
Xylan	15.07 (14.93)	16.26 (15.49)	18.03 (13.62)
Arabinan	1.17 (1.16)	1.20 (1.14)	1.70 (1.28)
Rhamnose side units	0.75 (0.74)	0.72 (0.69)	0.64 (0.48)
Galactan	traces	traces	traces

* lower values of "glucan" in wood as compared to the corresponding contents of K-H cellulose may result from the presence of residual non-cellulosic polysaccharides and lignin in the preparations (see Tab. 2); data in parentheses express contents of polysaccharides recalculated to weight of wood prior to bio-degradation

As it follows from Tab. 3, the contents of glucan, xylan and arabinan in extractive free bio-degraded beech wood were increasing relatively with the time of fungal action. The content of mannan remained almost unchanged, and only a drop in the rhamnose side units present in hemicelluloses was found. The contents of polysaccharides expressed to weight of wood prior to bio-degradations confirmed, however, an increasing rate of degradation of cellulose, rhamnose side units and of glucomannans with the progressing fungal attack. Xylanes and arabinans turned out to be relatively resistant towards the white-rot fungus..

The increased rate of decomposition of cellulose and some hemicelluloses in the later stages of the rot may partly result from the depleted sources of accessible less cross-linked portions

of lignin rich in syringyl units in the cell walls (Solár et al. 2000) while the fractions of lignin in the middle lamellae with the increased concentration of guaiacyl structures (Fergus 1968, Fengel and Wegener 1984) are more resistant to ligninases (Yokota et al. 1988).

The data resulting from HPLC of products of NB oxidation of “in vitro” lignin in the extractive-free wood meals of beech wood are presented in Tab. 4.

Tab. 4: Yield of products of nitrobenzene oxidations of lignin in sound and bio-degraded extractive-free beech wood by *T. versicolor* (in % on lignin in wood)

Oxidation product	Sound wood	15-day degradation	60- day degradation
3,4-dihydroxybenzaldehyde	0.245	0.245	0.203
p-hydroxybenzaldehyde	0.223	0.239	0.212
Vanillic acid	1.329	1.314	1.4616
Syringic acid	3.509	3.661	3.729
Vanilline	13.722	13.931	14.381
Syringylaldehyde	34.206	34.173	32.221
Total	53.234	53.563	52.208
S/G ratio	2.493	2.453	2.241
S/G ratio incl. corresponding acids*	2.506	2.482	2.268

* ratio of Σ syringyl aldehyde + syringic acid to Σ vanilline + vanillic acid

HPLC of nitrobenzene oxidation products of sound and the bio-degraded beech wood pointed out a slight differences in the total yields of oxidation products of lignin. A mild, 2 % reduction in the total yield of NB oxidation products from lignin in the sample after a 60-day degradation results probably from condensation of lignin via coupling of the phenoxi- and derived quinonemethide radicals arising in the process of fungal degradation. In this case, however, the influence of relative enrichment of lignin for more cross-linked structures on the yield of finals should be considered.

Reduced S/G ratio in lignin, apparent after 60 days of beech wood bio-degradation, indicates the preferential degradation of more abundant and less cross-linked syringyl structures in the cell wall lignin.

Physical properties

Fig. 1 illustrates the values of coefficients of axial permeability of sound and bio-degraded beech wood for water as polar, and for n-hexane as a non-polar medium.

Fig. 1 points out in general an increase in the coefficient of axial permeability of beech wood with the time of bio-degradation when a non-polar liquid n-hexane was used as a medium. This phenomenon reflects an increased porosity of the bio-degraded material. The low coefficient of permeability of sound wood may result from a negligible change in the lumina cross-sectional area of the vessels due to contact with a non-polar medium during the measurements*.

*Note: Similarly, measurements of the diffusion coefficients of sound wood with non-polar media instead of water yielded moderately reduced values axial and increased ones in perpendicular directions (Stamm 1946).

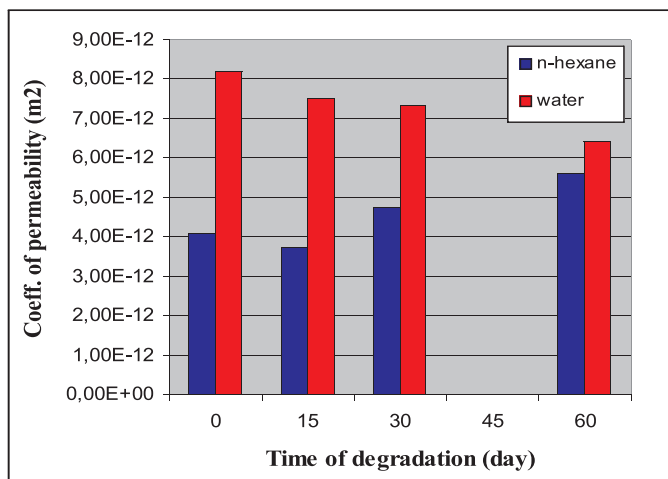


Fig. 1: Influence of polarity of media on the coefficients of axial permeability of sound and by *T. versicolor* degraded beech wood ($n = 3 - 5$; $w_{in.} = 4.5\%$; $v_{coeff. max.} 70\%$)

On the other hand, the coefficient of axial permeability of sound wood for water was higher, and was of a decreasing trend with the time of degradation. Such an observation follows possibly from a different mode of swelling of the cell walls in the vessels of sound and bio-degraded wood. We are of the opinion that swelling of the cell walls of vessels in the bio-degraded wood tends also into the lumina, thus reducing their cross-section area. This assumption is based on statistical evaluation of the cross-sections area of the lumina in the vessels of sound and bio-degraded hornbeam wood by the white-rot fungus *Phanerochaete chrysosporium* (MANOVA, a number of the observed lumina varied from 81 and 140) in the process of saturation by the different polar media (Solár et al. 2003). From Fig. 1 it follows, that the influence of polarity of the liquid on the permeability of sound and bio-degraded wood may differ, and may often be beyond any assumptions.

Monitoring of the surface swelling of beech wood showed at an extreme increase in the kinetics of interactions of the bio-degraded wood with the polar medium – water (Fig. 2).

As it follows from Fig. 2, degradation of beech wood by *T. versicolor* influenced markedly the kinetics of its facial swelling for more than two orders. The highest rate constant of this process was determined for the specimens degraded for 30 days. The time of degradation prolonged to 60 days resulted in a 30 % reduction of the constant found for the specimens degraded for 30 days. Remarkable is, that even a short-term 15-day bio-degradation of beech wood with only a 4 % weight loss influenced extremely the rate constant of its facial swelling. The increase in the rate of wood/water interactions results possibly from the qualitative and quantitative alterations of wood components including its structural changes on microscopic and macroscopic levels.

Compared to sound wood, the final values of facial swelling of bio-degraded wood after 24 h were lower, which is in accordance with the diminished density of the bio-degraded wood.

Bio-degradation of beech wood by *T. versicolor* altered also its optical properties. Axis a^* in the CIELAB colour space comprises the colour of surface in the range from green (-100 to 0 %) to red (0 to 100 %), and axis b^* expresses the colour range from blue (-100 to 0 %) to yellow (0 až 100 %). Co-ordinate L^* represents lightness of wood surface in per cent of a standard (lightness of 100 %).

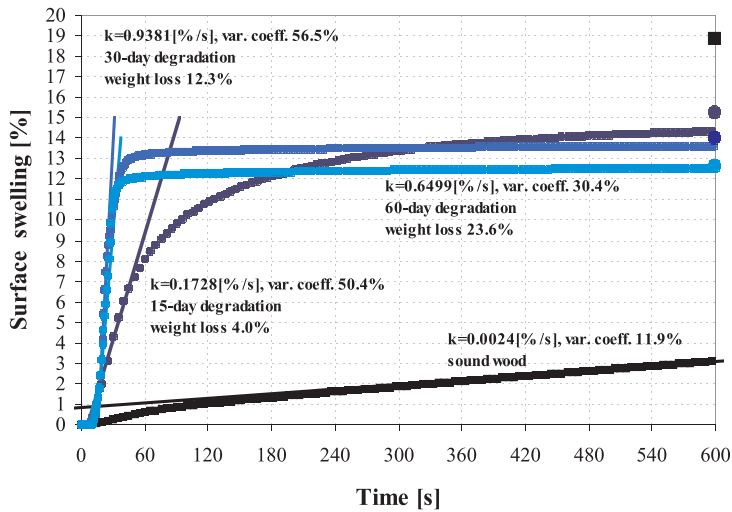


Fig. 2: Rate and relative rate constants of the first phase of surface swelling of beech wood as a function of time of degradation with *T. versicolor*; number of specimens $n = 3$; $w_{in} = 4,2\%$; medium: distilled water; $t = 20\text{ }^{\circ}\text{C}$ (marks on the right axis y of the chart are the final values of swelling after a 24-hour monitoring)

Figs. 3 and 4 show the alterations in colour and lightness of beech wood due to short-and medium term bio-degradation by white-rot fungus, expressed in the CIELAB scale.

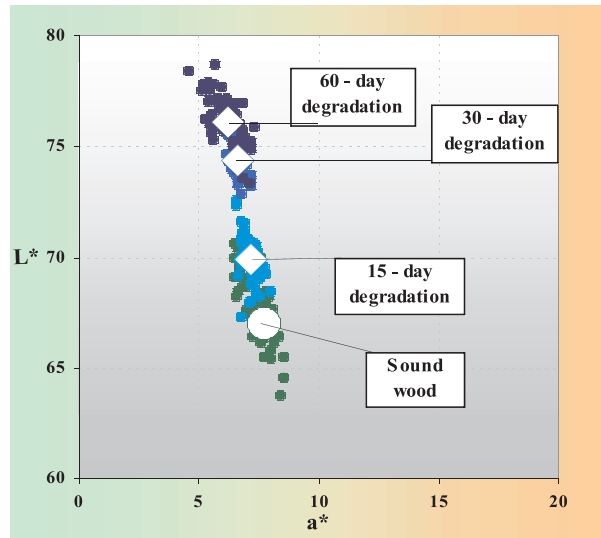


Fig. 3: Changes in lightness (L^*) and color of the surface of sound and degraded beech wood by *T. versicolor* in co-ordinate a^* (var. coeff. in L^* : sound wood - 2.66 %, 15 days degr. -1.45 %, 30 days degr. -1.10 % and 60 days degr. 1.57 %; var. coeff. in a^* : sound wood - 8.53 %, 15 days degr. - 4.42 %, 30 days degr. - 4.21 and 60 days degr. 10.47 %)

Fig. 3 points out an apparently rising lightness of the surface of test specimens with the time of bio-degradation. At the same time, a gradual shift in the colour from red to green can be seen.

A similar phenomenon (increased lightness: co-ordinate L^* , and an apparent shift in the colour of wood in co-ordinate b^*) can be observed in Fig. 4.

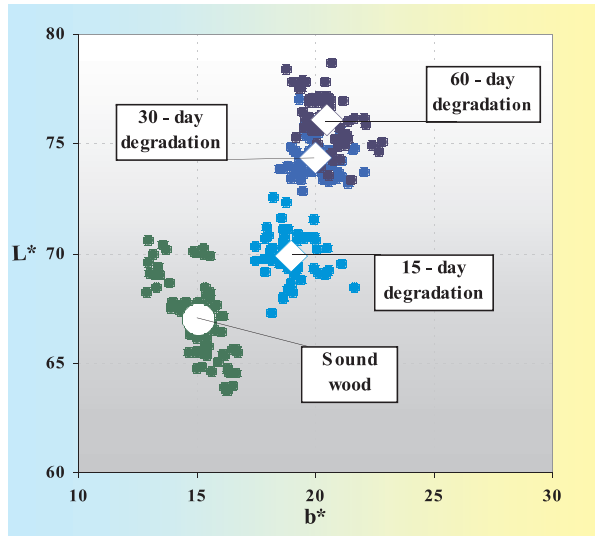


Fig. 4: Changes in lightness (L^*) and colour of the surface of sound and degraded beech wood by *T. versicolor* in direction of axis b^* (var. coeff. L^* : sound wood - 2.66 %, 15 days degr. -1.45 %, 30 days degr. -1.10 % and 60 days degr. 1.57 %; var. coeff. b^* : sound wood - 6.64 %, 15 days degr. -4.70 %, 30 days degr. -3.84 % and 60 days degr. 4.37 %)

Fig. 4 indicates both an increase in the lightness of beech wood with the time of bio-degradation and a marked shift (up to 5.5 % of the range of axis b^* in the CIELAB scale) in its colour from blue to yellow one.

The extent of shifts in colour and lightness of wood is not quite proportional to time of fungal action and is moderately diminished in later periods of degradation (30 and 60 days).

In Fig. 5, the differential spectra of the sound and bio-degraded beech wood in the range of wavelength 360-740 nm are presented.

An increase in the light reflection with maximum λ_{\max} of 600-610 nm in the differential spectra refers to decreasing intensity of light absorption by wood with the time of decay. This phenomenon probably follows from the gradually reduced content of lignin (bio-polymer active in UV, VIS and IR regions) in the degraded wood and the enzymatic alterations of lignin. The exception from the compared spectra represents the spectrum of wood bio-degraded for 15 days with a characteristic minor minimum at 410 nm. Its appearance may result from formation of intermediate chromophoric structures, mostly in lignin (quinones, biphenyl and conjugated phenolic structures with α -carbonyl), in the initial stages of enzymatic delignification of wood.

Samples of the extractive-free sawdust of beech wood were also analysed by the FTIR. These spectra represent the overlapped spectra of all wood constituents and their evaluation may

provide a distorted information. The possible advantage, however, is the estimation of alterations of “in vitro” components of wood occurring in the process of bio-degradation.

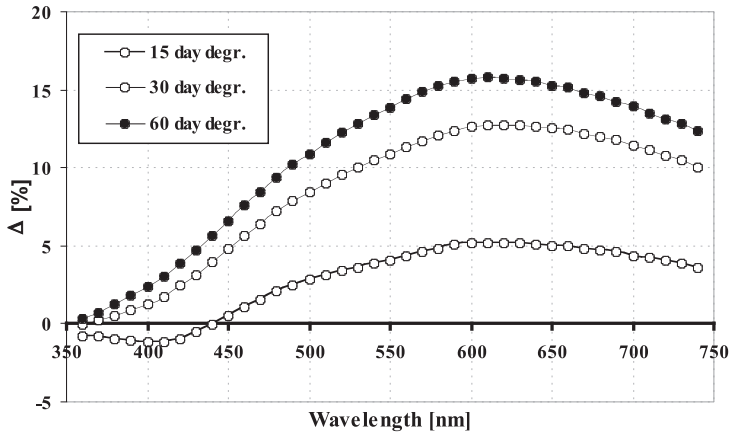


Fig. 5: Differential reflection spectra of beech wood degraded by the fungus *T. versicolor*

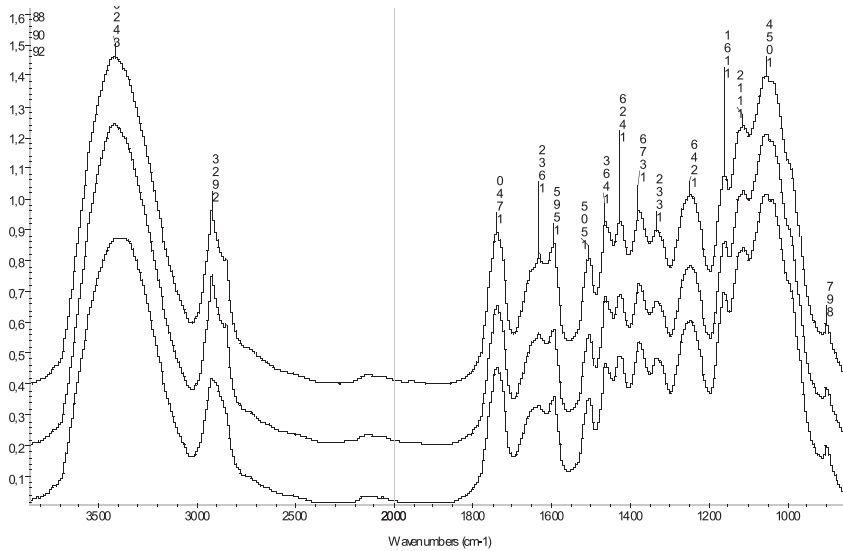


Fig. 6: FTIR spectra of sound and bio-degraded beech wood by *T. versicolor* (Samples: 88 – sound wood; 90 – 15 days degraded wood; 92 – 60 days degraded wood)

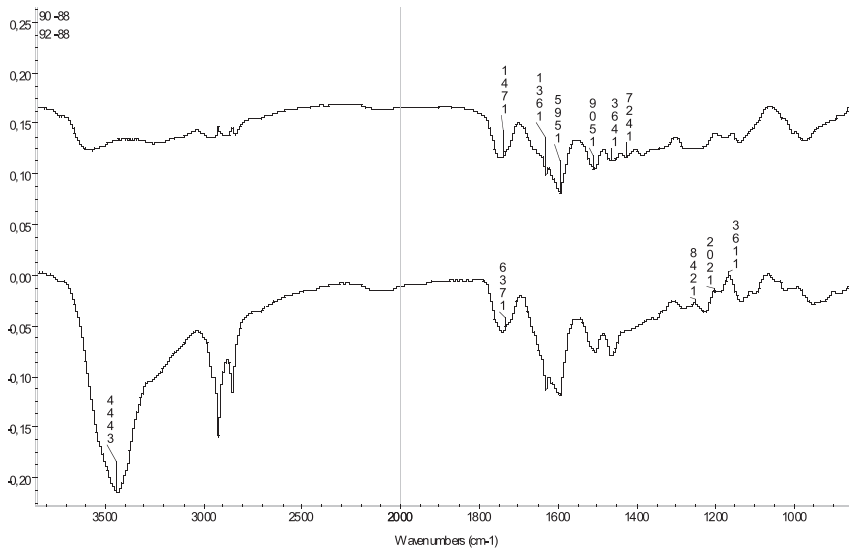


Fig. 7: Differential FTIR spectra of bio-degraded beech wood by *T. versicolor* (90 – 88: spectrum of beech wood degraded for 15 days; 92 – 88: spectrum of beech wood degraded for 60 days)

In the spectra of bio-degraded samples of wood (Fig. 6) a reduction in the absorbances of the bands at 3420 cm^{-1} (assoc. hydroxyl groups) and at $2923 - 2852\text{ cm}^{-1}$ (C-H vibr. - stretching and bending modes in $-\text{CH}_2-$ and $-\text{CH}_3$ groups) are apparent. These alterations are tiny for a 15-day bio-degraded wood and deeper for wood degraded for 60-days. Reduction in the absorbance of these bands with the time of fungal attack follows from the proceeding degradation of polysaccharides and demethylation of lignin.

Minima in the differential spectra at 1740 cm^{-1} (β -keto group, CO in carboxyls of esters and carboxylic acids) hint at the almost equal reduction in the contents of the side chains of 4-O methyl glucuronic acid in xylenes in both degraded wood sample (Fig. 7).

A diminished absorbance of the band at 1632 cm^{-1} in the spectra of bio-degraded wood may follow from reduced contents of α -CO groups conjugated with p-hydroxi substituted aromatic ring and o-quinoid structures in lignin.

A suppressed absorbance of the band at 1595 cm^{-1} (aromatic ring stretch. assoc. with C-O stretch. mode in lignin, carboxylate ion C=O stretch.) in the spectra of bio-degraded samples and the corresponding minima in difference spectra point out a partial decomposition of aromatic nuclei in lignin and degradation of 4-O-methylglucurono-xylenes, however, also cannot be excluded.

Absorbance of the band at 1505 cm^{-1} (vibr. of arom. nuclei), declining with the time of bio-degradation, indicates a partial decomposition of lignin in the degraded samples.

A band at 1463 cm^{-1} (arom. ring vibr. and C-H def.) does not change in the process of bio-degradation apparently (Fig. 7). A slight increase in the minima in difference spectra of the bio-degraded samples with the time of decay might indicate a partial decomposition of syringyl units in lignin.

Bio-degradation of beech wood did not cause apparent changes in the absorbances of the bands at 1426 and 1375 cm^{-1} , the explanation might be a speculative one- a relative increase in the

lignin content of wood and degradation of this moiety on the other hand. The former band can be attributed to aromatic skeletal vibrations, the latter one comprises symmetric C-H and phenolic O-H deform. and C-O stretch. modes.

A slight increase of a small, sharp band at 1161 cm^{-1} (guaiacyl ring breath. with C-O stretch. and C-O stretch. mode in tert. alcohols) and a maximum in the difference spectrum of wood bio-degraded for 60 days indicate enrichment of lignin for guaiacyl structures.

Note: for interpretation of absorption bands in the FTIR spectra the following sources were used: Sarkanen and Ludwig 1971; Kováč and Leško 1980; Fengel and Wegener 1984; Faix and Banhoff 1988).

CONCLUSIONS

Comparison of experimental data concerning the selected chemical and physical properties of sound and degraded beech wood by the erosive white-rot fungus *Trametes versicolor* resulted in deriving the following conclusions:

- degradation of beech wood by white-rot fungus *T. versicolor* was accompanied with weight loss and a drop in its density - proportional to time of degradation,
- bio-degradation resulted in faster removal of lignin than of cellulose from beech wood; hemicelluloses were more stable, except for modification in their structure (splitting off the side units of 4-O-methylglucuronic acid and L-rhamnose),
- a short, 15-day bio-degradation removed 15 % of lignin from beech wood and did not cause any marked structural changes of this component expressed in syringyl to guaiacyl nuclei ratio,
- a medium-term 60-day bio-degradation leading to a 35 % removal of lignin from wood was accompanied by a relative increase of guaiacyl nuclei representation in lignin,
- FTIR spectra confirmed all the above chemical alterations of beech wood degraded by *T. versicolor*, including the loss of chromophores in the short-term experiments,
- modified structure and increased contents of polar carbohydrates in the bio-degraded wood resulted in its reduced axial permeability for a polar liquid, and increased this property for a non-polar liquid,
- bio-degradation increased extremely the rate of beech wood surface swelling in water; This phenomenon may affect negatively the dimensional stability of wood on the interface of sound and degraded material and lead to crack formation during drying or impregnation of dry material with polar liquids,
- bio-degradation of beech wood with *T. versicolor* changed apparently its optical properties, and with the amount of lignin removed - an increase in the lightness of wood was registered.

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