# FTIR and Spectroscopy and Color Change of Wood for Assesment and Monitoring of Sofwood Degradation by White-rot Fungus

*by* Sunardi Sunardi

**Submission date:** 31-May-2020 02:31PM (UTC+0700)
**Submission ID:** 1335039772
**File name:** Sunardi_IC3PE-1_rev.pdf (359.74K)
**Word count:** 2815
**Character count:** 14652

# FTIR Spectroscopy and Color Change of Wood for Assessment and Monitoring of Softwood Degradation by White-rot Fungus *Porodaedalea pini*

Sunardi[1,a], Wiwin Tyas Istikowati[2], Futoshi Ishiguri[3], Shinso Yokota[3]

[1]*Chemistry Department, Faculty of Mathematics and Natural Science, Lambung Mangkurat University Jl. A Yani Km. 36 Banjarbaru, South Kalimantan, Indonesia*

[2]*Faculty of Forestry, Lambung Mangkurat University Jl. A Yani Km. 36 Banjarbaru, South Kalimantan, Indonesia*

[3]*Faculty of Agriculture, Utsunomiya University, Utsunomiya, Tochigi 321-8505, Japan*

Corresponding author: sunardi@ulm.ac.id; masunardi@gmail.com

***Abstract***. *Picea jezoensis* wood block was degraded by a white-rot fungus (*Porodaedalea pini*) under laboratory condition for different periods up to 120 days. Chemical composition changes of the wood caused by white-rot fungus degradation were evaluated by Fourier Transform infrared (FTIR) spectroscopy. Additionally, discoloration of the wood meal was analyzed using colorimeter by calculating CIELab values. The results showed that the constant value in the relative changes of lignin and carbohydrates band ratio, indicating that *P. pini* degrades lignin and carbohydrates non-selectively, with a slight preference for lignin. Fungus degradation of wood resulted in rapid color changes (L* value) that correlate well with degradation of lignin. This study also showed that FTIR spectroscopy is a very simple and fast method fore valuating wood degradation.

## INTRODUCTION

The chemical components of lignocellulose (hardwood, softwood, and nonwood) mainly consist of three natural polymers: cellulose, hemicellulose, and lignin [1-3]. The biological degradation of wood has drawn attention of researchers for many years. They are classified as insect attacks, bacterial, and fungal degradation. Fungal degradation of wood is the most important and extensive type of wood degradation. This process should not be regarded only as a negative process, because it can also has positive effects on nutrient cycling processes in nature and it can be applied in biotechnological processes [4]. Wood degradation by fungal enzymatic processes has been reproduced under laboratory conditions and applied to industrial scale to produces bioethanol [5] and bio-pulp [4].

The ability of fungi to decay the chemical components of wood such as cellulose, lignin, and hemicellulose is based primarily on the action of lignocellulolytic enzymes that was produced [6]. *Porodaedalea pini* is white-rot fungi species belongs to Hymenochaetaceae that broadly distributed in Europe, East Asia, and North America [7]. In the previous research, we reported that *P. pini* produce various ligninocellulolytic enzymes [1, 8].

Fourier transform infrared (FTIR) spectroscopy has often been used to evaluate wood chemical components alteration of fungal degradation of wood. This method is nondestructive, fast and simple sample preparation and also only using a small amount of samples [9]. The aim of the present study was to investigated and monitored the fungal degradation process of the softwood (*P. jezoensis*) by white-rot fungus *P. pini* by means using FTIR spectra and color changes. *P. jezoensis* wood blocks were degraded to different stages throughout subjecting wood samples to *P. pini* for different intervals time (0 to 120 days). The relative changes in the lignin/carbohydrate characteristic bands and infrared crystallinity indexes of wood after degradation were investigated. Color change during the degradation process was indicated by the change of CIELab'color system value also were evaluated. Wood discoloration was attributed to the modification of the chromophores and chemical component change of wood [10].

## EXPERIMENTAL

### Microorganisms and Culture Conditions

The white-rot fungus, *Porodaedalea pini* strain WD1174 were used in this study were obtained from Forestry and Forest Product Research Institute (FFPRI), Tsukuba, Ibaraki, Japan. The cultures were maintained on potato-dextrose-agar (PDA) medium in petridishes an incubated at 26 ± 2°C °C for 27 days. To study the wood degradation, sterilized polypropylene bottle with 100 mL medium of malt extract (1.5%), agar (2%),peptone (0.3%) and glucose (4%) was inoculated by a disk of 7 mm in diameter of mycelia which was punched out using a cork borer from PDA plates. The culture on the medium in the bottle was placed in the incubation room at 26 ± 2°C and 70 ± 2% relative humidity until fully cover the medium surface.

### *P. jezoensis Wood Samples*

Small spruce heartwood (*P. jezoensis*) samples (20 x 20 x 10 mm) were prepared from wood samples, oven-dried at 60 °C and then weighted. Samples were sterilized with propylene oxide gas for 4h, then adjusted the moisture content of samples to 50-70% using sterilized distilled water. The samples were exposed to *P. pini* in the polypropylene bottles and then it was stored in the incubation room at 26 ± 2°C under a dark condition for 30, 60, 90 and 120 days. After degradation process, the samples were washed and brushed carefully to remove the mycelia.

### Fourier Transform Infrared Spectroscopy

FTIR spectra of wood samples before and after degradation process were recorded on a Shimadzu FTIR-8201 PC using KBr disks containing about 1% dried mill of wood samples in the wavenumber range of 4000-400 $cm^{-1}$. Each sample was grounded and pelletized with KBr. The characteristic peaks of the wood were ascribed with the assist of the literatures. Changes in the lignin and carbohydrate content were calculated the peak height ratio of the lignin and carbohydrate groups ($I_{1512}/I_{1735}$, $I_{1512}/I_{1373}$, *and* $I_{1512}/I_{894}$). The FTIR spectra also were used for calculation of the lateral order index (LOI, $A_{1427}/A_{894}$), Total Crystallinity Index (TCl, $A_{1373}/A_{2924}$) [11], and hydrogen bond intensity (HBI) [12].

### Wood Color Measurement

Wood color of the dried mill was measured by using a colorimeter (CR-400, Konica Minolta, Japan). The CIELab color system (L* (a lightness factor), a*( redness factor), b*( yellowness factor)) was employed to evaluate the change of wood color. The color deviation (ΔE*ab) were determined using the L*, a*, b* values as a function of degradation period:

$$\Delta E^*ab = [(L_2^* - L_1^*))_2 + (a_2^* - a_1^*)_2 + (b_2^* - b_1^*)_2]_{1/2}$$

where $L_2^*$, $a_2^*$, and $b_2^*$ represent the values before degradation process and $L_1^*$, $a_1^*$, and $b_1^*$ represent the values after degradation process [10].

## RESULTS AND DISCUSSION

### Visual Observation of Wood Degradation

By visual observation, it has been established that the growing periods of *P. pini* fungus on the surface of wood was about 30 d (Fig. 1). Wood blocks were covered completely by fungus mycelia at 60 days (Fig. 1). These mycelia mass have a different color (whitish) with mycelia mass on the medium surface before wood blocks cultivation (reddish-brown color). On the 60 days cultivation period, the color of the fungus mycelia on the surface changed to be red-brown. After wood blocks brushed and cleaned from the mycelia, the surface of wood showed

darkness, and at the advanced stage of degradation several white spots on the top surface and black flecks on the bottom surface were observed (Fig. 2).

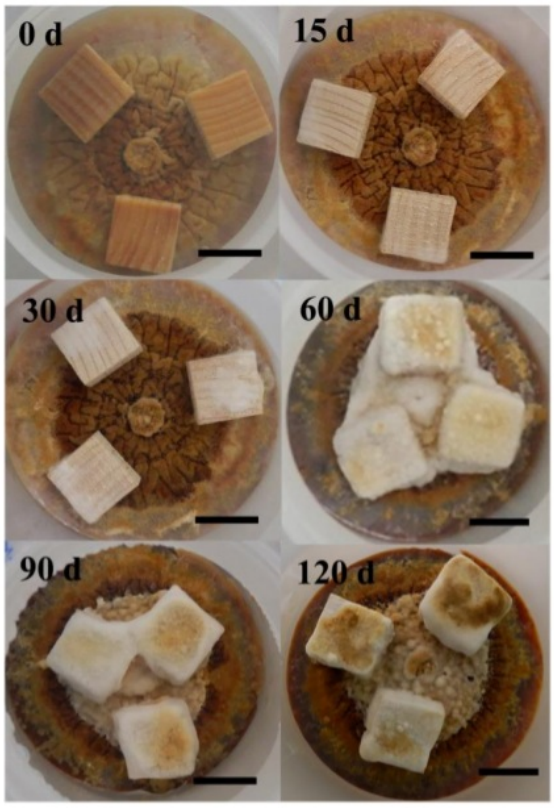


**FIGURE 1.** Photographs of mycelia growth of *P. pini* during degradation test. Bars size = 2 cm.

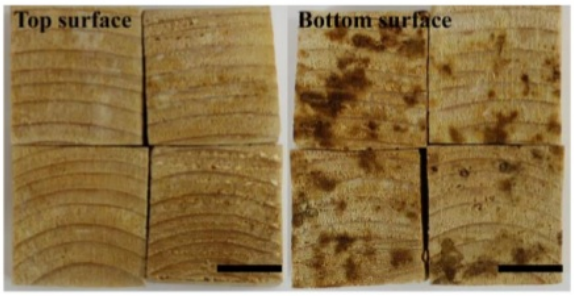


**FIGURE 2.** Photographs of *P. jezoensis* wood blocks after degradation for 90 days. Bars size = 1 cm.

## FTIR Spectroscopy

The Fourier transform infrared (FTIR) spectra were used to evaluate changes in the chemical components (cellulose, hemicellulose, and lignin) of wood before and after degradation. Fig. 3 showed that the spectra of *P.*

*jezoensis* wood before and after degradation process were similar with slight differences in peak intensity in fingerprint regions can be detected in various wavenumber. Despite the difficulty of the spectra caused by peak overlapping, many of the definite peak, which were considered as the characteristic bands of wood before and after degradation were summarized in Table. 1.

A change in the peak intensity was observed at the initial stage (30 days) of degradation. A comparison of the nondegraded and degraded wood samples shown a considerable increase of the peak intensity after 30 d of wood degradation. After 60 days of degradation periods, many peaks intensity of wood degraded shown a considerable decrease and after that gradually increase until 120 d.

The peak at 894 $cm^{-1}$ due to C-H deformation is characteristic of the glycosidic bond β-(1➔4) cellulose. The range between 1200 $cm^{-1}$ and 1000 $cm^{-1}$ which attained a maximum at 1033 $cm^{-1}$ and 1056 $cm^{-1}$ due to C-O stretching is region of cellulose and hemicellulose. The band at 1158 $cm^{-1}$ due to C-O-C stretching vibration is characteristic of other glycosidic bonds of polysaccharides, particularly mannan. The band located at 1265 $cm^{-1}$, which was assigned to the C-O stretch in the guaiacyl ring of the lignin components. The bands located at 1427 $cm^{-1}$ and 1512 $cm^{-1}$ , which were assigned to aromatic skeletal vibration of lignin. Moreover, bands at 2900 $cm^{-1}$ and 2924 $cm^{-1}$ are due to asymmetric stretching of C-H from $CH_2$ and $CH_3$ from cellulose structure.

**TABLE 1**. Important FTIR spectra of softwood and wood samples

| Wavenumber ($cm^{-1}$) | | Assignment | Peak condition |
|---|---|---|---|
| Sampel | Softwood [10] | | |
| 894 | 897 | C-H deformation; glucose ring streching (cellulose, hemicellulose, pectin) | + |
| 1033 | 1024 | C-O streching (hemicellulose and cellulose) | - |
| 1056 | | C-O streching (hemicellulose and cellulose) | - |
| 1158 | 1153 | C-O-C vibration in the structure of cellulose | |
| 1265 | 1261 | C-O stretching of guaiacyl in the lignin | + |
| 1373 | 1371 | C-H deformation vibration (cellulose); O-H bending vibration (lignin) | |
| 1427 | 1421 | $CH_2$ bending vibration (cellulose); aromatic skeletal vibration (lignin) | |
| 1458 | 1465 | C-H deformation; C-$H_3$ and -$CH_2$- bending vibration (lignin) | + |
| 1512 | 1509 | C=C streching vibration in aromatic structure (lignin) | + |
| 1635 | 1653 | C=O streching of conjugated/aromatic ketone (lignin) | + |
| 1735 | 1730 | C=O streching of acetyl and carbonyl groups (lignin and hemicellulose) | + |
| 2900, 2924 | 2890; 2919 | C-H streching in methylene and methyl group (cellulose) | + |
| 3410 | 3373 | O-H streching (hydrogen-bonded) | - |

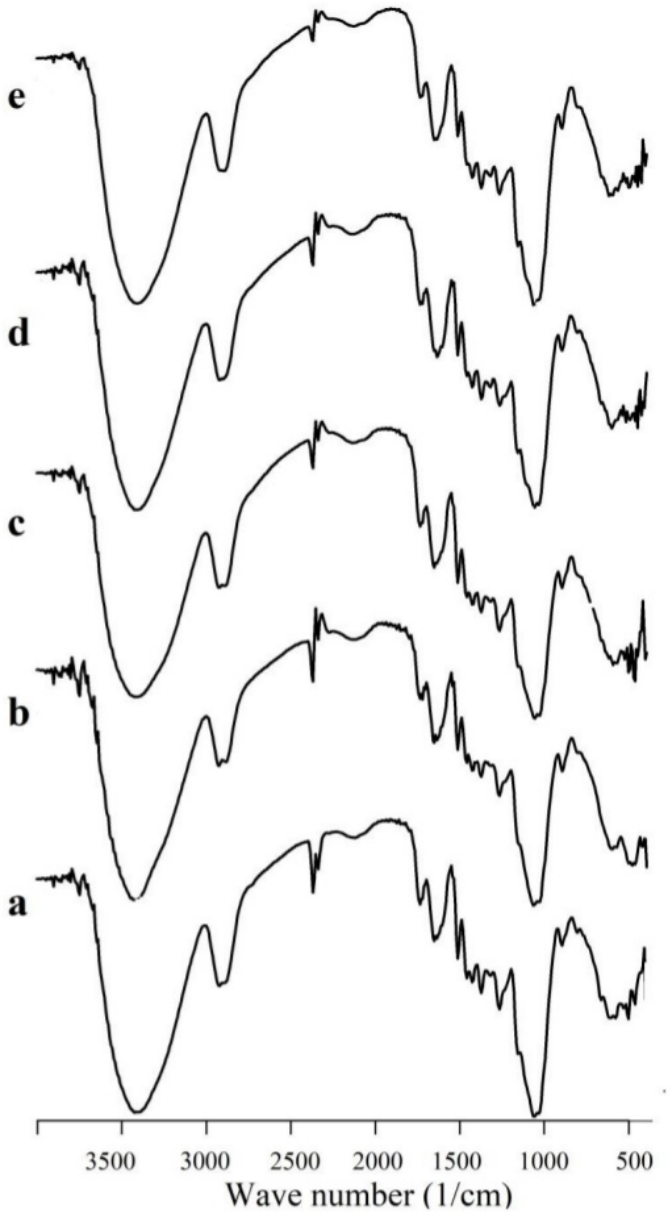


**FIGURE 3.** Fourier transform infrared spectra of *P. jezoensis* (a) nondegraded wood; (b) degraded for 30 days; (c) degraded for 60 days; (d) degraded for 90 days; (e) degraded for 120 days

Table 2 showed the peak height ratios of the bands that relate to lignin and carbohydrate groups ($I_{1512}/I_{1735}$, $I_{1512}/I_{1373}$, *and* $I_{1512}/I_{894}$ ) as a function of degradation periods. During the degradation process, the value of these peaks relatively constant. It can be seen that *P. jezoensis* wood degradation by *P. pini* resulted in a considerable content in the lignin/carbohydrate content that is a performer of the simultan destructive of lignin and carbohydrates

during the degradation process. Sunardi et al. [1] has reported the similar results on the simultan degradation of *P. jezoensis* by *P. pini* fungus using chemical components of wood determination methods.

**TABLE 2**. Relative changes in the lignin and carbohydrate band ratio

| Degradation periode (days) | $I_{1512}/I_{1735}$ | $I_{1512}/I_{1373}$ | $I_{1512}/I_{894}$ |
|---|---|---|---|
| 0 | 0.89 | 1.09 | 0.97 |
| 30 | 0.92 | 1.05 | 1.04 |
| 60 | 0.83 | 1.12 | 1.02 |
| 90 | 0.89 | 1.13 | 0.99 |
| 120 | 0.89 | 1.19 | 0.98 |

In this study, we calculated the value of Total Crystallinity Index and the Lateral Order Index from FTIR spectra according to Nelson and O`Connor [11] from the absorption ratios of $A_{1377}/A_{2922}$ and $A_{1426}/A_{898}$, respectively (Table 3). The TCl and LOI values indicate that the crystalline cellulose relatively constant with a slight increase after 120 days of degradation. Based on the data, it is suggested that, the amorphous and crystalline cellulose components of wood were degraded by white-rot fungus *P. pini* simultaneously. In the previous report [1,8], white-rot fungus *P. pini* produce endoglucanase and exoglucanase enzymes in high activites. It is known that endoglucanase preferentially attacks the amorphous region of cellulose, and exoglucanase attacks the crystalline region [13]. In this research, therefore, both endoglucanase and exoglucanase enzyme may act synergistically to degrade amorphous and crystalline cellulose. The hydrogen bond intensity (HBI) of cellulose in the wood (before and after degradation) fluctuated at the values from 1.25 to 1.46. The HBI values confirm different transformation of cellulose during degradation process of *P. jezoensis* by white-rot fungus *P. pini*.

**TABLE 3**. FTIR crystallinity index and hydrogen bond index data of wood

| Degradation periode (days) | TCl $A_{1377}/A_{2924}$ | LOI $A_{1427}/A_{894}$ | HBI $A_{3410}/A_{1320}$ |
|---|---|---|---|
| 0 | 1.00 | 1.07 | 1.33 |
| 30 | 1.01 | 1.00 | 1.25 |
| 60 | 1.06 | 1.05 | 1.32 |
| 90 | 1.03 | 1.09 | 1.34 |
| 120 | 1.03 | 1.14 | 1.46 |

## Color Changes During Degradation

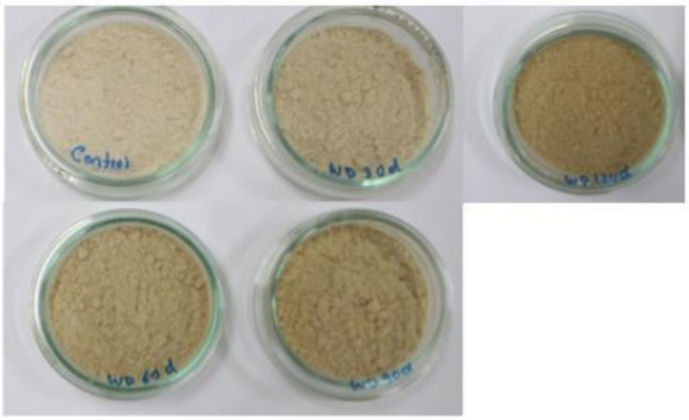


**FIGURE 4.** Photographs of dried mill *P. jezoensis* wood during degradation test

Fig. 4 showed the photograph of dried powder wood sample of *P. jezoensis* after degradation for 30, 60, 90, and 120 days. By visual observation it has been established that the dried powder wood have different color and gradually became reddish-brown during the degradation process. The change of dried wood color during degradation quantitatively was evaluated using CIELab color system. Table 4 showed that the wood color changes due to degradation by *P. pini using* CIELab color system. The L* values gradually decreased with increase of decay period, whereas a* and b* value gradually increased. In addition, the increase of Δb* value was greater that that of Δa*. As the results, the wood color became darker-reddish-yellowish, being ΔE*ab value was 10.08 in 120 days block (Table 4).

**TABLE 4.** CIELab system value and color differences of wood after degradation

| Degradation period (days) | L* | ΔL* | a* | Δa* | b* | Δb* | ΔE*ab |
|---|---|---|---|---|---|---|---|
| 0 | 85.13 ± 0.6 d | - | 2.30 ± 0.2 a | - | 16.05 ± 0.3 a | - | - |
| 30 | 81.73 ± 0.3 c | -3.40 | 2.68 ± 0.1 b | 0.38 | 17.16 ± 0.2 b | 1.11 | 3.60 |
| 60 | 77.89 ± 0.5 b | -7.24 | 3.75 ± 0.0 c | 1.45 | 18.73 ± 0.2 c | 2.68 | 7.86 |
| 90 | 77.37 ± 0.5 b | -7.76 | 4.30 ± 0.1 d | 2.00 | 21.10 ± 0.2 e | 5.06 | 9.48 |
| 120 | 76.33 ± 0.3 a | -8.80 | 4.42 ± 0.1 d | 2.12 | 20.48 ± 0.3 d | 4.44 | 10.08 |

Note: Value represent means of five repetitions ± standard deviations. The different letter shows the significant differences values by the 95% confidence level based on Tukey's HSD test.

In this present study, the ΔE*ab values exceeded the value of 3, which considered the limit for the visibility to the naked eye [14] and indicate appreciable color changes according to the standard of color change in the CIELab system. In several wood species were found that the redness color (a*) value and darkness (L*) value parameter related with the phenolics and extractive content of wood, while the yellowness (b*) value parameter correlate with the reaction or degradation of cell wall chemical components (cellulose, hemicelluloses, and lignin) to be small molecule [15-16]. According to [17], white rot fungi produce polyphenolases catalyzing the secondary oxidation of amino acid in the presence of phenol, are able to form the red pigment during the wood degradation.

## CONCLUSION

The wood degradation process of *P. jezoensis* by white-rot fungus *P. pini* was confirmed and monitored by FTIR spectroscopy and color changes. The FTIR spectroscopy analysis provided evidence of the chemical components changes of degraded wood. The discoloration of degraded wood related with components modification of the wood during biodegradation process. This result also confirmed that the biodegradation of *P. jezoensis* by white-rot fungus *P. pini* are non selectively process. This fungus simultaneously degrades cellulose (amorphous and crystalline part) and lignin in *P. jezoensis* wood. FTIR spectra and color changes of wood degraded by white-rot fungus supported the chemical components change of wood and can be considered as simple, fast and inexpensive methods for monitoring wood degradation.

## REFERENCES


1. Sunardi, J. Tanabe, F. Ishiguri, J. Ohshima, K. Iizuka, and S. Yokota, *Int. Biodeter. Biodegr.* **110**, 108-112 (2016).
2. W.T. Istikowati, H. Aiso, Sunardi, B. Sutiya, F. Ishiguri, J. Ohshima, K. Iizuka, and S. Yokota, *J. Wood Chem. Technol.* **36**, 250-258 (2016).
3. Sunardi, N.M. Febriani, and A.B. Junaidi, *AIP Conference Proceeding* **1868**, 020008 (2017).
4. S. Malherbe and T.E. Cloete, *Rev. Environ. Sci. Bio.* **1**: 105-114 (2002).
5. I. Kamei, Y. Hirota, and S. Meguro, *Bioresour. Technol.* **126**: 137-141 (2012).
6. P. Baldrian and V. Valášková, *FEMS Microbiol. Rev.* **32**: 501-521 (2008).
7. M. Tomšovský, P. Sedlák, and L. Jankovský, Mycol. Prog. **9**: 225-233 (2010).
8. Sunardi, A. Nakamura, F. Ishiguri, and S. Yokota, *Asian J. Chem.* **30**: 317-322 (2018).

9. S. Durmaz, O. Ozgenc, I.H. Boyaci, U.I. Yildiz, and E. Erisir, *Vib. Spectrosc.* **85**: 202-207 (2016).
10. D. Rosu, C-A. Teaca, R. Bodirlau, and L. Rosu, *J. Photochem. Photobiol.B: Biol.* **99**: 144-149 (2010).
11. M.L. Nelson and R.T. O`Connor, *J. Appl. Polym. Sci.* **8**: 1311-1324 (1964).
12. A.M.A. Nada, S. Kamel and M. El-Sakhawy, *Polym. Degrad. Stab.* **70**: 347-355 (2000).
13. M. Dashtban, H. Schraft, and W. Qin, *Intl. J. Biol. Sci.* **5**, 578-595 (2009).
14. D.N.S. Hon and N. Minemura, "Color and discoloration," in *Wood and cellulosic chemistry*, edited by D.N.S. Hon and N. Shiraishi (Marcel Dekker, New York, 2001), pp. 385-442.
15. N. Gierlinger, D. Jacques, M. Grabner, R. Wimmer, M. Schwanninger, P. Rozenberg, and L.E. Páques, *Trees* **18**,102-108 (2004).
16. R. Moya, R.S. Fallas, P.J. Bonilla, and C. Tenorio, *Molecules* **17**, 3639-3652 (2012).
17. L. Schánèl, *Experientia* **22**, 517-518 (1966).

# FTIR and Spectroscopy and Color Change of Wood for Assesment and Monitoring of Sofwood Degradation by White-rot Fungus

ORIGINALITY REPORT

| 9% | 4% | 7% | 3% |
|---|---|---|---|
| SIMILARITY INDEX | INTERNET SOURCES | PUBLICATIONS | STUDENT PAPERS |

PRIMARY SOURCES

| | | |
|---|---|---|
| 1 | Tomczak, Fabio, Bernard Riedl, and Pierre Blanchet. "Plasma Deposition of ZnO Thin Film on Sugar Maple: The Effect on Contact Angle", Advances in Contact Angle Wettability and Adhesion Mittal/Advances, 2013.<br>Publication | 1% |
| 2 | silvafennica.fi<br>Internet Source | 1% |
| 3 | Qomariyatus Sholihah, Aprizal Satria Hanafi, Ahmad Alim Bachri, Rahmi Fauzia. "Ergonomics Awareness as Efforts to Increase Knowledge and Prevention of Musculoskeletal Disorders on Fishermen", Aquatic Procedia, 2016<br>Publication | 1% |
| 4 | www.dissertations.wsu.edu<br>Internet Source | 1% |
| 5 | Submitted to Brunel University<br>Student Paper | 1% |

| | | |
|---|---|---|
| 6 | Submitted to Indian Institute of Technology, Kharagpure<br>Student Paper | 1% |
| 7 | Kacik, F.. "Chemical changes of beech wood due to CO"2 laser irradiation", Journal of Photochemistry & Photobiology, A: Chemistry, 20110705<br>Publication | 1% |
| 8 | V. S. Karande, A. K. Bharimalla, G. B. Hadge, S. T. Mhaske, N. Vigneshwaran. "Nanofibrillation of cotton fibers by disc refiner and its characterization", Fibers and Polymers, 2011<br>Publication | <1% |
| 9 | S. Nami Kartal, Evren Terzi, Tsuyoshi Yoshimura, Rachel Arango, Carol A. Clausen, Frederick Green. "Preliminary evaluation of storax and its constituents: Fungal decay, mold and termite resistance", International Biodeterioration & Biodegradation, 2012<br>Publication | <1% |
| 10 | www.mdpi.com<br>Internet Source | <1% |
| 11 | www.research-collection.ethz.ch<br>Internet Source | <1% |
| 12 | Ozlem Ozgenc, Sefa Durmaz, Bedri Serdar, Ismail H. Boyaci, Haslet Eksi-Kocak, Murat | <1% |

Öztürk. "Characterization of fossil Sequoioxylon wood using analytical instrumental techniques", Vibrational Spectroscopy, 2018
Publication

---

13 N. Gierlinger, M. Schwanninger, R. Wimmer. " Characteristics and Classification of Fourier-Transform near Infrared Spectra of the Heartwood of Different Larch Species ( sp.) ", Journal of Near Infrared Spectroscopy, 2017 <1%
Publication

---

14 Huang, X.. "A spectrocolorimetric and chemical study on color modification of heat-treated wood during artificial weathering", Applied Surface Science, 20120501 <1%
Publication

---

15 Attila Kiss, Krisztina Takács, András Nagy, Magdolna Nagy-Gasztonyi et al. "In vivo and in vitro model studies on noodles prepared with antioxidant-rich pseudocereals", Journal of Food Measurement and Characterization, 2019 <1%
Publication

---

16 Dae Hee Park, Jong Jin Park, Ibukunoluwa Fola Olawuyi, Won Young Lee. "Quality of White mushroom (Agaricus bisporus) under argon- and nitrogen-based controlled atmosphere storage", Scientia Horticulturae, 2020 <1%
Publication

---

| | | | |
|---|---|---|---|
| Exclude quotes | On | Exclude matches | Off |
| Exclude bibliography | On | | |