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ORIGINAL

Bio-oil from base-catalyzed depolymerization of organosolv lignin as an antifungal agent for wood

Patrícia Soares Bilhalva Dos Santos[1] · **Xabier Erdocia**[1] · **Darci Alberto Gatto**[2] · **Jalel Labidi**[1]

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Abstract The development of value-added lignin products from industrial residues of pulping is still a challenge. The present study was aimed to use oil obtained from organosolv lignin by base-catalyzed depolymerization at 300 °C for 40 min as a wood-protecting agent. First, the bio-oil was diluted to 1 wt% into an acetone and water solution, and physicochemical properties such as density, pH and viscosity were analyzed. After that, *Pinus* sp. sapwood samples were impregnated with the diluted oil solution (WAO). The treated wood samples were characterized in terms of color variation, hardness (Shore D) tests, scanning electron microscope, pyrolysis–gas chromatography/mass spectrometry, infrared spectroscopy through attenuated total reflectance (ATR-IR) and mass loss after fungal attack. The inhibition effect of the oil solution was tested against *Trametes versicolor* (l. Ex fries) Pilat. The application of the oil solution in the wood improved significantly its fungal resistance as the mass loss was considerably lower in the treated samples.

Introduction

One of the most important properties of wood as an outdoor construction material is its biological durability (Calonego et al. 2010), since weathering exposure causes modifications to the surface, where the development of micropores allows the entrance of fungi (Tolvaj and Faix 1995). Wood protection increases its life span and thus slows down the demand for fresh wood and deforestation (Paes et al. 2001). The traditional

✉ Jalel Labidi
jalel.labidi@ehu.es

[1] Environmental and Chemical Engineering Department, University of the Basque Country, Plaza de Europa 1, 20018 San Sebastián, Spain

[2] College of Materials Engineering (PPGCEM), Federal University of Pelotas, Félix da Cunha 809, Pelotas, RS 96010-000, Brazil

wood protection methods use chemicals that are considered toxic to the environment and can harm human health (Singh and Singh 2012). According to Temiz et al. (2008), tall oil derivatives showed positive effects against fungi decay, but did not fulfill the requirements for acceptable wood preservatives. Tung oil performed better than linseed oil or rustikal oil (Humar and Lesar 2013). Oils from various sources were also tested in this context (Heräjärvi et al. 2014).

Lignin as part of the cell wall has a natural bio-protective effect (Humphreys and Chapple 2002). This aromatic polymer is an antioxidant with a high potential for implementation in wood protection processes (Wang and Rinaldi 2013). According to Chirkova et al. (2011), the action of lignin protective agents can be attributed to the presence of phenolic compounds. Warsta et al. (2012) state excellent prospects for lignin-based products due to several physicochemical factors, such as the presence of aromatic structures that promote good mechanical properties, compatibility with industrial chemicals and ample possibilities of alternative chemical transformations.

According to Pelaez-Samaniego et al. (2013), via chemical and thermochemical processes, wood and other biomass are promising raw materials for the production of chemicals and fuels. Several studies are being conducted to obtain phenolic compounds (aldehydes, alcohols and acids) from lignin through base-catalyzed depolymerization (BCD). This method consists of the oxidation of lignin ethers (Toledano et al. 2012; Erdocia et al. 2014).

Lignin can be extracted from wood and other lignocellulosic materials by different methods. Among them, organosolv extraction method shows better utilization of raw materials to obtain value-added products (lignin and hemicellulose) and quality cellulose pulp (Dos Santos et al. 2014); in addition, the process has lower environmental impact because it enables the recovery of used organic solvent (Quesada-Medina et al. 2010).

The present study aims to evaluate wood protection with bio-oil by base-catalyzed depolymerization obtained from an organosolv lignin. Lignin coming from the black liquor is currently reused as an energy source in the cellulose industry (Dos Santos et al. 2014). It is believed that the solid residue (char and lignin) generated after the depolymerization could be used as energy source because, according to Erdocia et al. (2014), the Mw of the residual lignin after BCD is similar to that of natural lignin. It is important to note that this depolymerization method yields compounds with higher added value through the production of bio-oil and therefore reduces initial solid waste by approximately 40 %, a reason to consider this method to improve the economics of pulp industry.

The antifungal potential of the bio-oil treated wood was tested against the *Trametes versicolor* fungus; color variation was determined with a colorimeter. Effects of decay on bio-oil treated wood were characterized by scanning electron microscopy (SEM), hardness (Shore D) tests, pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS), infrared spectroscopy through attenuated total reflectance (ATR-IR) and mass loss. To the authors' knowledge, such use of bio-oil obtained from lignin base-catalyzed depolymerization has not been reported in the literature.

Materials and methods

Organosolv delignification

The organosolv lignin was obtained from gray ironbark (*Eucalyptus paniculata* Sm) harvested from a homogeneous population located in Southern Brazil (29°57′17″S 51°37′31″W). Wood samples were obtained from four different trees; they were cut from each tree (DBH height, 1.30 m) and then placed in a climatic chamber at 20 °C and 65 % of relative humidity. Then, all wood samples were mixed and milled by a Wiley mill (40 mesh size) to prepare them for the organosolv treatment. Delignification reaction was performed using organosolv process described by Dos Santos et al. (2014). The raw material was treated with the organosolv process in a 4 L stirred reactor equipped with a temperature control system. A 60 % ethanol–water solution (v/v) served as cooking liquor; the solid/liquid ratio was 1/10, and the cooking temperature was 180 °C for 90 min. After removal of the solid residue (pulp), the used liquor was mixed with two volumes of acidified water with sulfuric acid at pH 2. The precipitated lignin was separated by filtration, washed with acidified water and then vacuum-dried at 50 °C to constant weight.

Base-catalyzed depolymerization (BCD)

Base-catalyzed depolymerization (BCD) was used to obtain bio-oil from organosolv lignin; the lignin obtained from the previous stage was dissolved in 4 % NaOH (solid-to-liquid ratio 1/20) and introduced in a pressure batch −5500 Parr microreactor equipped with a 4848 Reactor controller (Toledano et al. 2012). Reaction conditions were 300 °C and 40 min under constant stirring. Under these conditions, lignin is depolymerized and gives rise to a mixture of phenolic components (bio-oil) and other products (char and residual lignin). The reaction mixture was then acidified with HCl to pH 1, and the residual lignin and char were precipitated and separated from the liquid by vacuum filtration. The yields of phenol monomer and solid products (char and lignin) were evaluated. The aqueous liquid fraction was liquid–liquid extracted with ethyl acetate to remove phenolic monomers, and the ethyl acetate was removed by rotary evaporation.

Bio-oil characterization

The bio-oil was analyzed by GC–MS in an ethyl acetate solution; instrument Agilent GC [7890A-MS (5975C inert MSD with Triple-Axis Detector); capillary column HP-5MS, (5 %-phenyl)-methylpolysiloxane (60 m × 0.25 mm)]; and calibration with phenol, *o*-cresol, *m*-cresol, *p*-cresol, guaiacol, catechol, syringol, acetovanillone, veratrol, 4-hydroxybenzoic acid and 4-hydroxy-3-methoxyphenylacetone (Sigma-Aldrich).

Wood treatment

After evaluation of the bio-oil composition, the preservative solution (WAO) was prepared. It consists of solution containing 1 % bio-oil, 5 % acetone and 94 % of water. The prepared WAO was used to treat sapwood samples of *Pinus* sp. (DBH height 1.30 m) with dimensions of 2.5 × 2.5 × 0.9 cm^3 after acclimatization in a climatic chamber at 20 °C and 65 % RH. Treatment was done in desiccators by two approaches (full cell) according to a modified ASTM D 1413-99 (ASTM International 2008a) method. Treatment (T1): The wood was in direct contact with the preservative solution for 60 min under vacuum of 0.8–1.0 bars. Treatment (T2): Initial vacuum at 0.8–1.0 bar for 30 min, then after the injection of the preservative solution, the vacuum was applied again to 0.8–1.0 bars for 60 min. Weight percent gains (WPG in %) of the samples due to the treatment with WAO are calculated by Eq. 1.

$$\mathrm{WPG} = (\mathrm{Wm} - \mathrm{Wu})/\mathrm{Wu}) \times 100 \quad (1)$$

where Wm = dried weight of sample after treatment and Wu = dried weight of untreated samples.

Evaluation of treated wood

The evaluation of treated wood is very important for the determination of wood uses. The antifungal activity to fungus *T. versicolor* for the different treatments with WAO was evaluated after a period of 4 and 48 weeks. The period of 48 weeks is critical for the analysis due to the increasing degradation of the samples exposed to the fungus. The antifungal activities were estimated by the modified accelerated resistance test against *T. versicolor* (Linnaeus ex Fries) Pilat. This fungus was used because according to Aguiar and Ferraz (2011), it causes white-rot and is more selective to lignin degradation and therefore appropriate for evaluating the efficiency of this product in the wood.

The treated specimens were tested for resistance to biological attack according to ASTM D 2017-94 (ASTM International 2008b). All wood specimens were sterilized in an autoclave at 121 °C for 20 min prior to fungal exposure. Six samples for treatment were introduced, and the control was also performed on six untreated *Pinus* sp. specimens. The specimens were incubated for two different periods (4 and 48 weeks) at 27 ± 1.1 °C, 72 ± 2 % RH.

Three different types of wood were exposed to fungi attack: untreated wood (UT) and two treated woods by different full cell methods, treatment (T1) and treatment (T2).

After fungi attack, the mycelium was removed and the specimens were weighed to determine their moisture contents at the end of fungal exposure. The specimens were then dried at 103 °C, and their final weight was recorded. Afterward, the percentage weight losses were calculated for individual test blocks, from the conditioned weights before and after exposure to the decay fungi.

Mass losses (ML) after fungus exposure were evaluated, and the samples were classified according to the average ML as: ML 0–10 % (highly resistant), ML 11–24 % (resistant), ML 25–44 % (moderately resistant) and ML >45 % (slightly resistant or nonresistant).

The samples were examined using a scanning electron microscope (JEOL 6610LV) at an accelerating voltage of 5–15 kV, low-vacuum (LV) detector, with multi-segment model BSED (backscattered electrons) and embedded EDS (environmental secondary detector) system (silicon drift detector technology).

The tissue segments from the radial section of wood samples were removed and coated with a highly conductive film of gold to obtain images with 500×–1000× magnifications.

The ATR-IR by direct transmittance in a single-reflection was carried out. The equipment was configured for 32 scans in a range of 4000–750 cm^{-1} with a resolution of 4 cm^{-1}. The ATR-IR was evaluated in the samples only after fungal exposure (for period of 48 weeks).

The pyrolysis was carried out using a CDS analytical Pyrobrobe 5150. The pyrolysis temperature was set at 650 °C for 15 s with a heating rate of 2 °C ms^{-1}. Then the products were analyzed in a GC (7890A)-MS (5975C inert MSD with Triple-Axis Detector) Agilent equipped with a capillary column HP-5MS ((5 %-phenyl)-methylpolysiloxane, 30 m × 0.25 mm). The oven program started at 50 °C and was held for 2 min at this temperature. Then it was raised to 120 °C at 10 °C/min and held 5 min, raised to 280 °C at 10 °C/min, held 8 min and finally raised to 300 °C at 10 °C/min and held 10 min.

The variation of color was determined with a colorimeter (CR-400 Minolta Chroma Meter)* according to the ISO 7724 Standard (ISO 1984). The equipment was adjusted with a D65 light source. The parameters L^* (lightness), a^* (green–red chromatic coordinate) and b^* (blue-yellow chromatic coordinate) were determined. Moreover, ΔE (color differences) were measured by Eq. 2:

$$\Delta E = \left(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}\right)^{1/2} \quad (2)$$

The Shore D hardness of *Pinus* sp. samples was tested on the tangential lateral sides according to ASTM D 2240-00 (ASTM International 2008c) standard, and five replicates were made per sample on six samples for each treatment method using a durometer and expressed as Shore D hardness.

Data were analyzed by descriptive statistics and analysis of variance (ANOVA). If the null hypothesis was rejected, the average values were compared with Tukey test at 5 % of probability of error ($P < 0.05$).

Results and discussion

Bio-oil yield of base-catalyzed depolymerization (BCD)

The yield of lignin extraction from the species gray ironbark (*Eucalyptus paniculata* Sm) obtained in a previous work by Dos Santos et al. (2014) was about 19.7 wt%.

The yield of bio-oil from BCD of this lignin was 21.9 wt%, and of solid products (char and lignin), it was 58.3 %wt. Extraction yields (%) and solid waste bio-oil are expressed as a percentage of dry organosolv lignin content introduced into the reactor. The yield and composition results obtained are comparable to those found by Toledano et al. (2012), 19.7 % for the oil by base-catalyzed depolymerization (BCD) from organosolv lignin of the species *Olea europea.*

Bio-oil characterization

Table 1 illustrates the phenolic bio-oil composition of products detected by GC–MS. Bio-oil is usually formed by different chemical compounds such as phenols, ketones, acids, aldehydes, alcohols, ethers and esters. However, the method used in the analysis of the GC–MS was focused on phenolic compounds and was calibrated with phenol, *o*-cresol, *m*-cresol, *p*-cresol, guaiacol, catechol, syringol, acetovanillone, veratrol, 4-hydroxybenzoic acid and 4-hydroxy-3-methoxyphenylacetone. The yields of phenolic monomers were calculated as percentage of the obtained compounds, referred to oil weight obtained in each case (w/w).

It could be observed that the syringol concentration in the oil was very low, because syringyl groups are more susceptible to BCD method than the guaiacyl groups (Erdocia et al. 2014). Catechol with a concentration of 1.4 % was one of the dominant components of bio-oil produced by BCD. According to IARC (1999), approximately 50 % of catechol produced in the world is used for insecticides and antiseptic applications. The phenol content in the oil was around 0.4 %. As stated by Suzuki et al. (1997), all phenolic compounds have a preservative effect. Mohan et al. (2008) confirmed that most phenolic compounds have disinfectant properties. According to the requirement of Directive 2001/90/EC (Directive 2001), creosotes should not contain more than 0.005 % (50 ppm) of benzo(a)pyrene(BaP) and 3 % of water-extractable phenols, or otherwise they are not allowed to be commercialized.

Wood treatment

The aqueous preservative solution, WAO, presented a pH of 5.3, viscosity of 0.86 $mm^2\ s^{-2}$ and density of 0.98 g ml^{-1}. The two treatment methods utilized presented significant differences in WPG values of solution preservative (Table 2).

Table 1 Composition of products detected by GC–MS in the bio-oil (BCD) from organosolv lignin

Compound	% (W/W)
Phenol	0.391
o-Cresol	0.056
m-p-Cresol	0.171
Guaiacol	0.110
Catechol	1.371
4-Methylcatechol	0.155
Syringol	0.044

Table 2 Description of the rate of absorption of preservative in samples and mass loss of the samples in relation to the performed treatments

Time (weeks)	0	4		48	
Treatment	Weight percent gain (WPG, %)	Weight loss (%)	Indicated class of resistance	Weight loss (%)	Indicated class of resistance
Untreated	–	2.7 (1.2)ab	Highly resistant	43.1 (1.8)b	Moderately resistant
Treatment 1	20.51 (1.48)a	1.2 (0.2)b	Highly resistant	26.1 (1.5)a	Moderately resistant
Treatment 2	25.32 (1.25)b	0.9 (0.3)a	Highly resistant	23.3 (1.8)a	Resistant

The values in parentheses are SD; mean values in the same column followed by the same letter are not statistically different at level of 5 % by the Tukey test

The weight percentage gain of treatment (T1) was lower compared to treatment (T2). According to Tondi et al. (2012), the measure of retention by weight is an important evaluation parameter of impregnation as this characteristic affects all tests.

In Table 2, the results of the weight loss (%) of WAO-treated wood at different times of exposure to the fungus (*T. versicolor*) are shown. It can be observed that for the period of 4 weeks, the treatment T2 was more effective against the tested fungus. However, after 48 weeks, the results were not statistically different for both treatments.

The results reported in Table 2 show that treatments 1 and 2 reduced the weight loss by 39.44 and 45.94 %, respectively, compared to the untreated samples after exposure to the fungus (48 weeks).

The visual effects of *T. versicolor* treatment of *Pinus* sp. wood are presented in Fig. 1, where the untreated samples are visibly degraded compared with treated wood.

Scanning electron microscopy (SEM) study

Figure 2 shows the SEM analysis, where the degradation of wood can be seen in radial direction by *T. versicolor* (white-rot fungus) of untreated (UT) and treated wood samples (T1–T2).

In the case of the untreated wood (Fig. 2—UT), there are signs of cell and cell wall degradation (see the arrows) by *T. versicolor*, a white-rot fungus, which simultaneously degrades lignin and hemicelluloses, leaving the cells punched either with holes or with thinned secondary walls (Singh et al. 2013).

In Fig. 2—T1, the images of the treated wood show broken cells, where the cell wall was not fully preserved but the presence of bordered pits in the cell wall can be observed (see the arrows).

In Fig. 2—T2, little breakage is visible in the cells, because the cell wall is preserved (see the arrows). This result also suggests that treatment 2 strengthens wood cell walls, accounting for the increase in hardness.

Fig. 1 Biological attack of *Trametes versicolor* in *Pinus* sp. wood, treated with aqueous solution preservative (WAO) from bio-oil BCD and untreated samples

According to Klüppel and Mai (2013), the changes in wood properties can only be efficient with the material located in the cell wall rather than in the lumens; for this reason, most methods are primarily aimed at filling the cell wall microspores.

Infrared spectroscopy (ATR-IR)

As shown in Fig. 3a, spectra are similar for the samples with slight variations in some regions such as at 3330 cm^{-1} referred to OH stretching according to Delmotte et al. (2008). A slight increase can be observed around 2905 cm^{-1} in the T2 samples referred to intensified C–H stretching vibration. This can be attributed to the higher fatty acid content present in the timber, since some fatty acid compounds present in the composition contain methyl and methylene groups (Poletto et al. 2012). In sample T2, a slight increase at 1731 cm^{-1} referred to C=O stretching can be observed.

The spectra in Fig. 3b show that there is a slight increase when wood is treated with WAO in the bands 1592 and 1505 cm^{-1} which are related to aromatic skeletal vibrations and (C=O) stretching, for the sample of T1 and T2 due to the presence of phenolic monomers in the WAO.

However, untreated wood (UT) exposed to the fungus and control wood were very similar. The bands at 1426 cm^{-1} for the aromatic skeleton of lignin (Chen et al. 2014) also increased in the treated woods.

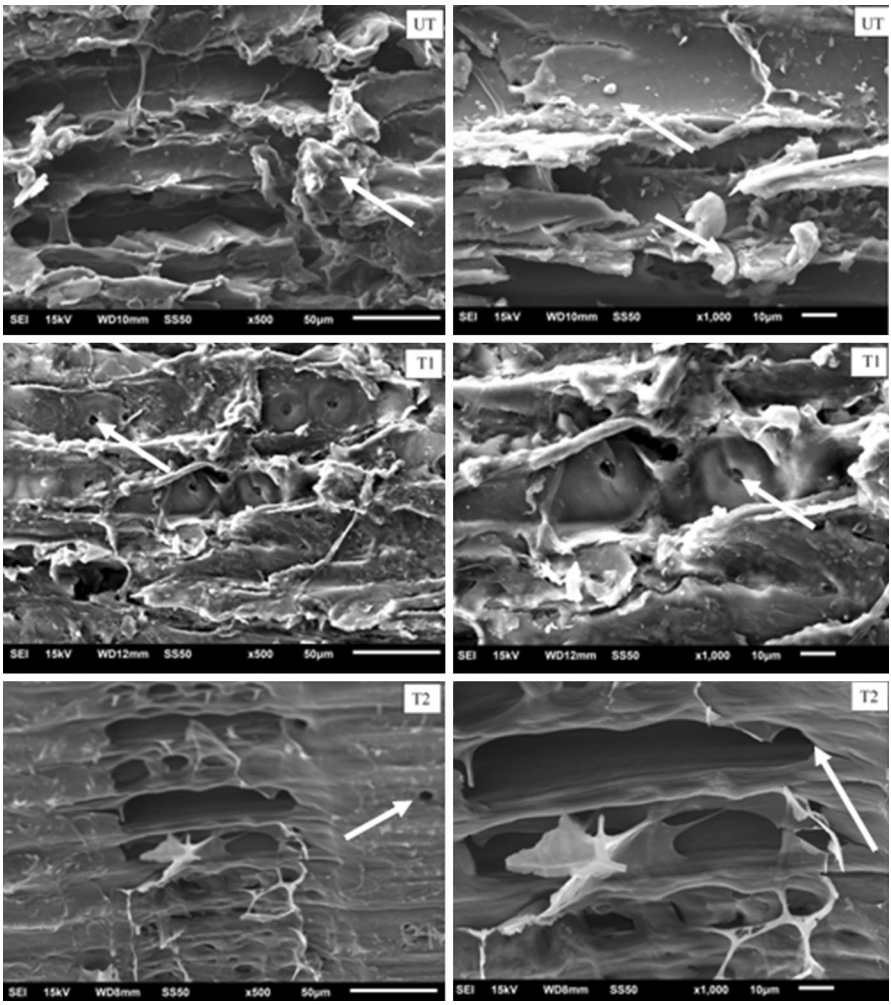

Fig. 2 Photomicrographs of scanning electron microscope (SEM) of the wood *Pinus* sp. after degradation by *T. versicolor* for 48 weeks. *UT* untreated, *T1* treatment 1, *T2* treatment 2 (×500 and ×1000)

Finally, the peak 1268 cm^{-1} that corresponds to the syringyl and guaiacyl condensed vibration is more intense in treated woods.

In this study, the intensity of the indicated peaks of the treated samples (T1 and T2) remains similar to those of the control sample (C) demonstrating the effectiveness of the protection treatment performed in this study with preservative of WAO. According to Pandey and Pitman (2003), weight loss of wood is reflected in the decrease in the intensity of these bands, resulting from the degradation of polysaccharides present in the wood.

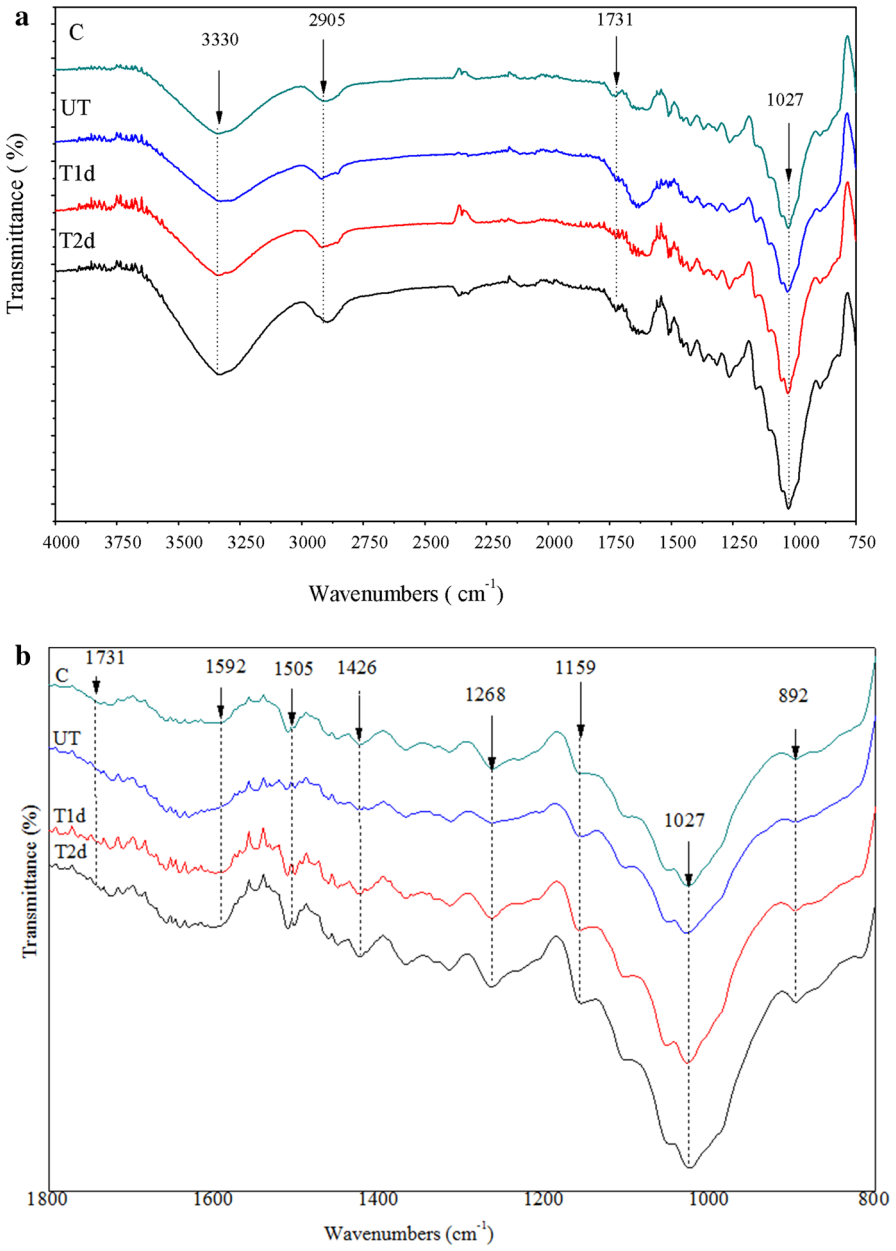

Fig. 3 Infrared spectroscopy (ATR-IR) for *Pinus* sp. wood not treated and treated with the aqueous solution preservative (WAO) from bio-oil BCD, after fungal exposure (for period of 48 weeks), compared to the control wood (not exposed to the fungus). **a** ATR-IR spectra from 4000 to 750 cm^{-1} **b** ATR-IR spectra from 1800 to 800 cm^{-1}

Table 3 Relative concentration of the compounds identified by pyrolysis-GC/MS in the samples treated with aqueous solution preservative (WAO) from bio-oil BCD

Peak	Name of compound (library/ID)	Retention time (min)	Relative concentration (%)			
			C	UT	T1d	T2d
1	Diethylhydroxylamine	3.942	1.094	0.800	1.029	0.387
2	1-Methylcyclopropanemethanol	4.120	1.412	ND	ND	0.437
3	Furfural	4.940	2.222	1.583	1.932	0.756
4	Phenol	7.401	1.169	1.081	1.168	1.388
5	D-Limonene	8.274	0.362	2.251	2.096	0.727
6	Phenol	9.273	3.103	6.970	4.447	1.635
7	Cyclohexane	9.812	1.547	0.691	0.956	0.251
8	Benzoic acid	10.577	1.208	0.600	0.285	0.158
9	Octanoic acid	10.676	1.378	0.800	1.043	0.272
10	Phenol	11.329	4.505	3.718	3.055	1.411
11	1.2-Benzenediol	14.020	2.184	0.720	0.581	0.251
12	2-Methoxy-4-vinylphenol	14.967	4.569	2.919	4.556	1.62
13	Vanillin	17.169	2.707	1.704	1.421	0.649
14	Phenol	18.218	4.175	2.115	3.031	1.297
15	Beta-D-glucopyranose	18.721	ND	1.010	1.196	ND
16	Benzoic acid	19.446	0.463	1.432	0.556	0.110
17	Dodecanoic acid	20.084	ND	1.538	0.638	0.147
18	Hexadecanoic acid	24.583	ND	1.037	0.252	0.790
19	*n*-Hexadecanoic acid	24.943	0.549	5.282	4.390	12.030
20	2-Hexadecanone	26.367	ND	1.011	ND	0.877
21	Octadecanoic acid	26.550	ND	2.994	9.365	6.157

ND not detectable

Pyrolysis

Table 3 shows the chromatograms of Py-GC/MS analysis of all wood samples which were exposed to fungus *T. versicolor* for 48 weeks. They were compared to the control wood (C) which was not exposed to fungus and not treated with WAO.

The fungal interference can be seen with the presence and magnitude of some peaks in the wood samples exposed to the fungus. It is supposed that the differences are due to degradation of certain compounds by fungi during development. According to Tamburini et al. (2014), the Py-GC/MS is a good tool to determine wood chemical composition because it uses small amounts of samples, does not require prior treatment and gets the results in a short time.

As given in Table 3, the 21 most representative peaks were selected. The majority of pyrolysis products obtained from the untreated samples were also found in wood treated with WAO.

It was possible to observe the formation of beta-D-glucopyranose, dodecanoic acid, hexadecanoic acid and 2-hexadecanone (peaks 15, 17, 18 and 20) during the

exposure of wood to fungus *T. versicolor*. Due to the absence of these acids in the wood control (not exposed to the fungus), it could be concluded that these compounds were produced by the degradation of wood components by the fungus. However, untreated wood exposed to the fungus showed higher values of these compounds compared to wood treated with T1 and T2 due to more degradation of untreated wood.

Finally, the fatty acids (peaks 19 and 21) showed increases in T2. The n-hexadecanoic acid was the most abundant fatty acid in all the pyrograms, mainly in the sample T2, where it was approximately 20 times higher than in control sample (C). This behavior was confirmed by the band at 2900 cm^{-1} in the FTR-IR analysis, in which the highest intensity was observed in T2.

Color test

This study evaluates the change in color of pinewood before and after different treatments with WAO, but also after the degradation by the fungus *T. versicolor* for the two different exposure periods (4 and 48 weeks).

For untreated wood, colorimetric assay was not conducted for periods of 48 weeks (IV) of exposure to the fungus, because of the degradation of the wood that affects the results of the analysis of color patterns.

Wood color was measured on the $L^*a^*b^*$ system. Changes in colorimetric parameters were calculated by the difference between targeted and control samples of the different treatments (T1 and T2). Both treatments (T1 and T2) presented modified parameters of color (a^*, b^* and L^*) in relation to the untreated wood (UT-I), as shown in Fig. 4 (T1-I and T2-I).

Both treated samples were darker (L^*) than the untreated wood (UT-I), while there was an increase in L^* value in both treatments (T1-IV and T2-IV) with 48 weeks of exposure to the fungus.

After treatment with WAO solution, the samples showed an increase in the intensity of the colorimetric parameters a^*, which refers to the green–red pigment, from 6.5–7.2 to 9.4–10.3. This parameter was more intense (11.7–12.9) after 4 weeks of exposure to fungi and remained stable after 48 weeks.

The yellowing (b^*) was intensified in the samples after treatment, and in relation to exposure (48 weeks); the fungus caused on average an increase in (b^*).

Values of color variation (ΔE^*) are defined as the difference in $L^*a^*b^*$ color space between two distinct points (Table 4).

Very high color variation (ΔE^*) was observed between the samples treated with WAO (T1 and T2) and the untreated samples: 11.0 and 7.78, respectively. A positive value of ΔE^* means a greater amount of color variation throughout the test.

Colorimetric parameters are sensitive for detecting any modification of the wood such as the modifications caused by treatment with WAO and modifications caused by fungus attack.

Furthermore, it is possible to observe the influence of the exposure time to the fungus on color variation of the wood. The longer the exposure time is, the greater the color variation (ΔE^*) is. The highest value of color change (36.6) was obtained

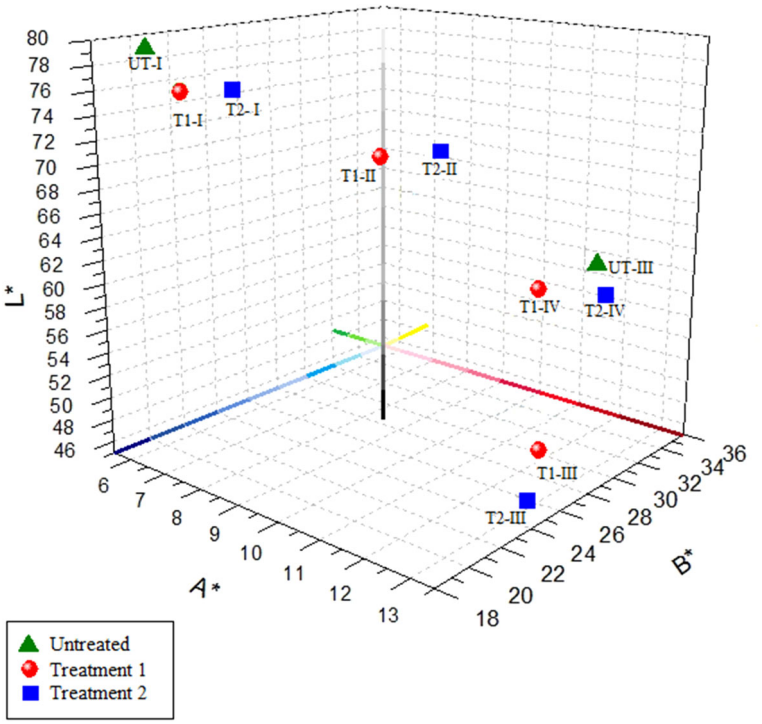

Fig. 4 Effect of impregnation treatment with aqueous solution preservative (WAO) from bio-oil BCD and fungus attack on the color of the sample surfaces. I—in nature, II—treated, III—in contact with the fungus for 4 weeks, IV—treated in contact with the fungus for 48 weeks

for untreated samples after exposure to the fungus (48 weeks) relative to the control sample.

Hardness (Shore D)

The objective was to evaluate the use of hardness (Shore D) tests to characterize the bio-deterioration of wood treated with WAO, subjected to attack by decay fungi *T. versicolor* (white-rot). It is possible to observe in Table 5 that the fungal attack statistically reduced the average value of hardness of the untreated samples compared to the control sample (C) not treated and not attacked by fungi. Treatment 2 had statistically higher average value of hardness (Shore D) of the *Pinus* wood even after fungal attack. Demonstrating that the fungus did not alter the hardness of treated samples, the hardness was improved as compared to untreated samples.

The results of this study showed no significant difference between the average hardness of *Pinus* sp. wood per rotting fungus; a similar observation for Brinell hardness was reported by Green et al. (2004). In this sense, it can be considered that the Shore D hardness method was sensitive to deterioration of samples exposed to the fungus *T. versicolor*. However, it is important to note that treatment increased

Table 4 Effect of different treatments and fungus attack on color parameters of *Pinus* sp. samples

Treatment	Colorimetric parameters											
	I						II					
	L^*	a^*	b^*	ΔE^*	C^*	h	L^*	a^*	b^*	ΔE^*	C^*	h
Untreated	79.3 (1.0)	6.0 (0.5)	19.7 (1.3)	–	20.7 (1.3)	72.9 (1.1)	–	–	–	–	–	–
T1	75.1 (3.5)	6.5 (1.2)	20.7 (1.7)	–	21.7 (1.9)	72.8 (2.0)	70.73 (2.6)	9.4 (0.9)	25.6 (0.7)	11.0 (1.0)	26.8 (0.6)	70.4 (1.9)
T2	75.9 (2.4)	7.2 (1.3)	22.1 (1.7)	–	23.2 (1.9)	71.9 (2.4)	70.9 (3.1)	10.3 (1.1)	27.1 (0.7)	7.8 (0.9)	29.0 (0.8)	69.1 (2.0)
Treatment	Colorimetric parameters											
	III						IV					
	L^*	a^*	b^*	ΔE^*	C^*	h	L^*	a^*	b^*	ΔE^*	C^*	h
Untreated	61.6 (3.9)	11.7 (2.4)	34.7 (3.0)	23.8 (4.4)	36.6 (3.6)	71.5 (2.2)	–	–	–	–	–	–
T1	49.1 (4.9)	12.9 (1.6)	26.4 (1.8)	21.9 (2.1)	29.4 (1.8)	64.0 (3.0)	58.2 (0.1)	11.1 (0.5)	31.9 (1.1)	13.5 (2.2)	33.7 1.2)	70.8 (0.1)
T2	48.2 (3.3)	12.8 (1.1)	25.9 (0.3)	24.2 (4.0)	28.9 (0.5)	63.7 (2.1)	59.4 (4.5)	12.0 (2.4)	34.1 (0.5)	12.2 (1.2)	36.2 (0.3)	70.6 (3.9)

Values in parentheses are SD

I—Mean values of five repetitions in wood control

II—Mean values of five repetitions of wood treated with solution WAO, before exposure to the fungus *Trametes versicolor*

III—Mean values of five repetitions of wood treated with solution WAO, after exposure to the fungus *Trametes versicolor* for 4 weeks

IV—Mean values of five repetitions of wood treated with solution WAO, after exposure to the fungus *Trametes versicolor* for 48 weeks

Table 5 Effect of different treatments with aqueous solution preservative (WAO) from bio-oil BCD and fungus attack on Shore hardness of the samples

Treatment	Shore
Untreated (UT)	32.88 (4.47)a
T1d	50.08 (1.96)bc
T2d	52.54 (2.62)c
Control (C)	48.40 (1.47)b

Values in parentheses are SD; mean values in the same column followed by the same letter are not statistically different at level of 5 % by the Tukey test

the hardness, because even after degradation, the treated sample (T2d) showed higher hardness values than control samples (C).

Conclusion

Durability of *Pinus* sp. wood was increased with the applied treatment of aqueous solution preservative (WAO) obtained from lignin depolymerization. Treatments 1 and 2 reduced the weight loss (39.4 and 45.9 %, respectively) compared to the untreated samples after exposure to the fungus *T. versicolor* (48 weeks).

FTIR showed that the peaks related to aromatic structures were more intense in the treated woods due to the phenolic nature of the WAO.

The interference of fungus *T. versicolor* in the wood studied can be seen in the pyrolysis analysis, the formation of some compounds (dodecanoic acid and hexadecanoic acid) that were not present in control samples. The increase in decay resistance in the sample T2 may have occurred by the increase in fatty acids observed in Py-GC/MS analysis.

The two treatments with the aqueous solution preservative (WAO) showed red and yellow color variations observed by the naked eye in comparison with the same samples without treatment.

The most effective treatment was treatment 2, as the resulting samples could be classified as decay resistant when exposed to the fungus *T. versicolor* for 48 weeks. Treatment 2 increased the hardness (Shore D) of the wood even after degradation.

These results are very preliminary, but all treated samples presented better results than untreated samples, leading to the conclusion that the use of aqueous solution preservative (WAO) from organosolv lignin depolymerization as a wood preservative is very promising.

It is extremely important to perform other types of analysis (such as resistance to leaching and evaporation and resistance to flame) in future work to observe possible applications and limitations of using WAO.

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