Investigation on the Attack of *Trichoderma viride* on Wheat Straw Composites Manufactured with Methylene Diphenyl Diisocyanate

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Trichoderma viride can infect wheat straw composites (WSC), thus affecting the quality of boards. This study investigated the change in color of the composite and its chemical composition after the straw was infested with mold with for 4, 8, or 12 weeks. Fourier transform infrared spectroscopy (FTIR) and high-performance liquid chromatography (HPLC) were used to analyze chemical structural changes in the WSC after the infestation. The infested surface and core layers were examined and analyzed. The infection of *T. viride* on the WSC can darken its color. After 12 weeks of cultural infestation, 19.6% of cellulose, 27.2% of xylan, 9.3% of lignin, and 31.9% of ethanol extracts were degraded. The degradation on WSC by *T. viride* was 9 times and 14 times more than the degradations of pine and poplar wood, respectively. *T. viride* attacked WSB differently on its surface and center layers. More lignin in the WSB surface layer was degraded. In contrast, cellulose and xylan were degraded to a greater degree in the center.

Keywords: Trichoderma viride; Wheat straw composites; Mold

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INTRODUCTION

Wheat straw composite (WSC) is a fiber-based panel using wheat straw as the raw materials and methylene diphenyl diisocyanate (DMI) as the adhesive. It is made through a series of processes including crushing, drying, screening, sizing, hot pressing, and sanding. WSC is environmentally friendly, unlike other composites that contain significant amounts of formaldehyde (Bowyer and Stockmann 2001; Wang *et al.* 2002). WSC has a high ratio of strength to weight and excellent nail holding abilities, allowing it to be used as material for furniture and interior decorations. WSC made with oriented fibers can be used for construction materials including wallboard, floor underlayment, roof sheathing, and concrete forms. WSC is a good insulator and can be used as the lining material in ceilings and walls. It is also a good packaging material (Li and Yuan 2005). However, compared with wood-based panels, WSC is more vulnerable to microbiological damage. Specifically, the potential damage caused by mold and mildew is greater than in wood-based panel.

Trichoderma viride is a mold widely found in nature. It usually grows on the surface of wood, seeds, and plant residues. It secretes cellulase (Berghem and Pettersson 1973; Shoemaker and Brown 1978; Beldman *et al*. 1985; Zhou *et al.* 2008), xylanase (Ujiie *et al.* 1991; Gomes *et al*. 1992), and lignin-degrading enzymes (Flores *et al.* 2010). The enzymes degrade the cellulose, hemicellulose, and lignin components of wheat straw (Zayed and

Meyer 1996; Wang *et al*. 2009; Iqbal *et al.* 2011) and reduce the performance of WSC. Zhang *et al*. (2018) reported that the density of WSC infested by *T. viride* decreased.

High performance liquid chromatography (HPLC) and Fourier transform infrared spectroscopy (FT-IR) can be used to characterize the chemical constituents of materials. HPLC can determine the level of 8-hydroxyquinoline copper in wood products, as well as the structure of free phenolic acids after decay (Chen *et al*. 2015). HPLC can accurately and quickly to detect 8-hydroxyquinoline copper fungicide in fibrous materials (Chi and Yan 2008). Lignin that is partially degraded by white rot fungus can be detected. Fan *et al*. (2014) used FT-IR to determine the content of cellulose in wood. Pandey and Pitman (2003) used FT-IR to study the chemical changes in wood after decay by brown rot and white rot fungi. The Browning process gradually reduced the carbohydrate content and quality of the wood. Rodrigues *et al*. (1998) analyzed the lignin content in *Eucalyptus globulus* Labill wood using FT-IR with a very high level of confidence coefficient.

Trichoderma viride is a dark green parasitic mold. It is a useful fungus for industry and biocontrol. Particularly, it feeds on the cellulose in straw and produces a wide variety of enzymes, such as cellulases and chitinases. The germination rate of *Trichoderma viride* spores is highest at 15 °C to 30 °C, and the mycelium grows at temperatures between 4 °C and 42 °C. The most suitable temperature for growing is 25 °C to 30 °C. Spores germinate faster at a relative humidity (RH) of 95% and rarely germinate at a RH below 85%. Therefore, it is easy to breed *Trichoderma viride* under conditions of high temperature, high humidity, and poor ventilation in an acidic medium.

WSC is an environmental friendly material, the main components of WSC could be degraded by T. *viride*, and the properties and performance would be affected. The objective of this research was to investigate color changes on the surface and in the core of WSC infested by *T. viride*. Changes in the chemical composition and concentration of chemicals in WSC caused by *T. viride* were analyzed using FT-IR and HPLC methods. The results can provide better understanding of the properties of WSC infested by *T. viride*.

EXPERIMENTAL

Materials

WSC was manufactured by Novofiber Inc., Yangling, China. The equipment was a 122 cm (4 ft) continuous hot press manufactured by Diefenbach Machinery Equipment Co., Ltd. (Eppingen, Germany). The wheat (*Triticum aestivum* L.) straw was collected from Shaanxi province, China. The straw was cut to less than 50 mm in length. The crushed wheat straw was less than 5 mm wide and 0.3 to 0.4 mm thick. The straw was combined with 7% methylene diphenyl diisocyanate (MDI) and pressed at 180 °C and a maximum pressure of 4.5 MPa for 480 s. The WSC panels were 2440 mm by 1220 mm and 12 mm thick with a density of 600 kg/m^3. The overall production process consisted of the straw bales, cutting to length, size classification, drying, adding adhesive, mat formation, pressing, cooling, trimming, sanding, and packaging.

Methods

Preparation and inoculation of WSC samples

The surfaces of the WSC panels were sanded to remove any dust and demolding agents. Sixty samples with dimensions of 50 mm x 50 mm x 12 mm were cut from the panels. *T. viride* were inoculated on the surface of potato dextrose agar (PDA) through the

coating plate method in 130 mm square Petri dishes. These samples were cultured for one week at 28 °C and 85% RH. Two sterilized glass rods (3 mm in diameter) were placed in parallel on the cultured colonies, and the samples were placed on the glass rods. Four samples were placed in each Petri dish (as shown in Fig. 1). The Petri dishes were incubated at 28 °C and 85% RH. Twenty samples were removed at 4, 8, and 12 weeks to measure the color and chemical composition.

Fig. 1. Four inoculated samples were placed in each Petri dish

Observation and verification of mycelium growing inside the samples

The samples were cut through the thickness to expose the core, and the distribution of the inner mycelium was observed with a stereo microscope. The ocular and objective lenses had 10x and 4x magnifications, respectively. A small trace of dust was scraped from the infested sample with sterilized tweezers and placed on the surface of sterilized PDA medium in a Petri dish. The samples were incubated at 28 °C and 85% RH for 7 days after which the growth of mycelium was observed.

Chemical composition analysis of WSC after infestation

The WSC samples infested for various periods of time were divided into two groups. One group was used to analyze the chemical composition of the whole sample, and the other group was used to separately analyze surface layers and core. A handheld knife was used to separate 2 mm surface layers from the core.

The cellulose, hemicellulose, and lignin contents of the WSC were determined as previously described (Sluiter *et al.* 2008). Accordingly, HPLC was used to measure the monosaccharides in the hydrolysate from the surface and core samples, from which their cellulose and hemicellulose contents were calculated. The cellulose content was calculated based on the amount of glucose and the hemicellulose based on the amount of xylose. Xylan is the main component of hemicellulose in the Gramineae straw (Mamman *et al.* 2008). The standards were glucose and xylose (Sigma, 99% Analytical reagent). The lignin content was calculated according to the residue weight. The HPLC flow phase was 5 mmol/L sulfuric acid (pH = 2) and flow rate was 0.5 mL/min. The column temperature was set at 45 °C.

Surface and core samples were ground to 40- to 60-mesh powder. Approximately 2 g to 3 g of the powder were placed in a muffle furnace and burned at 575 ± 25 °C for 4

h, then cooled for half an hour in a desiccator, and weighed. After weighing, the crucible was returned to the muffle furnace for 5 to 10 min and cooled and weighed again. This was repeated until the weight was stable.

The content of ash *X* (%) was calculated using Eq. 1,

$$\boldsymbol{X} = \frac{(\boldsymbol{M_2} - \boldsymbol{M_1})}{\boldsymbol{M}} \quad (1)$$

where M_1 is the weight (g) of the crucible, M_2 is the weight *(g)* of the crucible containing slag after it is burned, and *M* is the weight (g) of the original sample.

Approximately 0.5 g of the 40- to 60-mesh powder was extracted with ethanol in a 500 mL Soxhlet extractor at 85 °C for 15 h. Three replicates were performed. The ethanol soluble extractive content (*X*) was calculated using Eq. 2,

$$\boldsymbol{X} = \frac{(\boldsymbol{M_1} - \boldsymbol{M_2})}{\boldsymbol{M_1}} \quad (2)$$

where M_1 is the weight (g) of the powder before extraction and M_2 is the dry weight (g) after extraction.

FTIR analysis

The chemical compositions of the WSC samples were analyzed using the KBr plate method of FTIR spectroscopy (Nicolet, S10, Waltham, MA, USA) for each incubation time. The scanned wavelength range was from 4000 cm^{-1} to 400 cm^{-1} with 64 scans.

RESULTS AND DISCUSSION

WSC Color Change after Mold Infestation

The surface and internal colors changed due to the growth of *T. viride* mycelium. The brightness of the sample surfaces gradually faded and darkened (Fig. 2). Samples sliced at different depths revealed internal darkening (Fig. 3). A darker color was observed closer to the surfaces and edges of the samples indicating that *T. viride* easily attacked the WSC through its surfaces and edges.

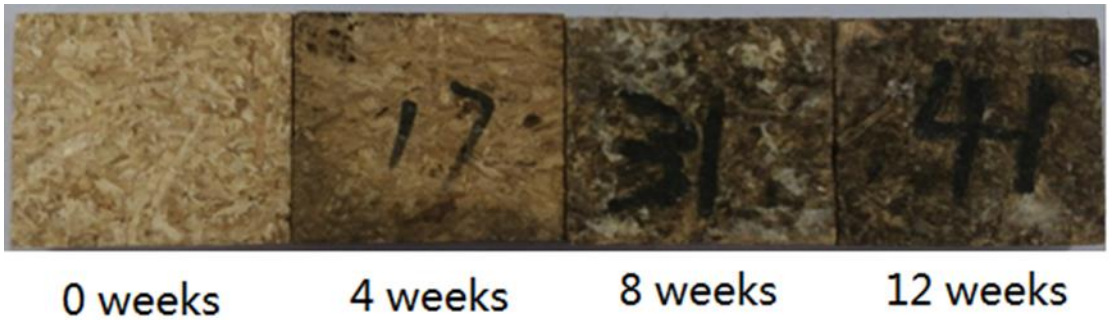

Fig. 2. The surface colors of WSC infected by *T. viride* for various durations

Hyphae of *T. viride* from the core of the WSC panels are shown in Fig. 4. Ten small pieces of samples cores were taken with sterilized tweezers and inoculated on PDA medium. Green colonies appeared near each sample after one week of culturing (Fig. 5.), indicating that the inner part of the WSC samples had been invaded by *T. viride*, which contained the hyphae of *T. viride*.

a) 10 mm from the edge **b)** Center location

Fig. 3. The color images of WSC before and after infestation at the different locations

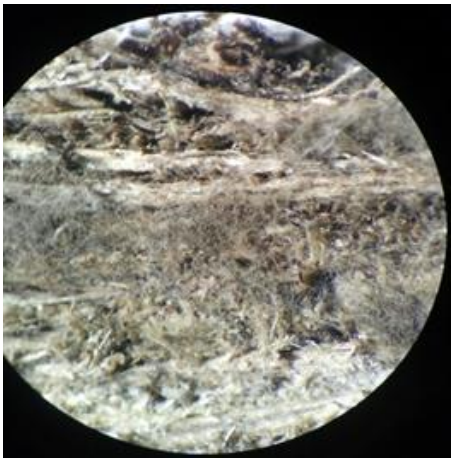

Fig. 4. Mycelium in wheat straw boards observed by stereomicroscope with 10× eye lens and 4× objective lens

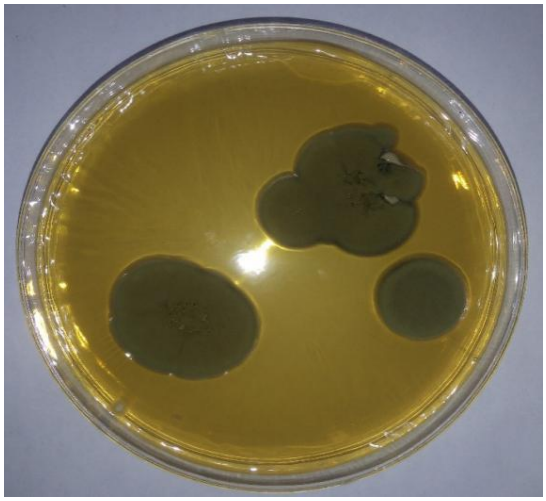

Fig. 5. Cultivation of mycelium found in wheat straw board

Chemical Composition of Whole Samples

The content of each component as a percent of sample mass (W_1) is shown in Table 1. The cellulose, lignin, and ash contents increased with time because the xylans and extractives were more readily degraded by *T. viride*.

The content of each component as a percent of the original sample weight (W_2) is shown in Table 2. These were calculated using Eq. 3 (Jin and Tai 1989),

$$W_2 = W_1 \times (1 - \alpha) \quad (3)$$

where α is the weight loss as a fraction calculated using Eq. 4.

$$\alpha = \frac{W_{T1} - W_{T2}}{W_{T1}} \times 100\% \quad (4)$$

where W_{T1} is the total weight loss of the sample before decay and W_{T2} is the total weight loss of the sample after decay.

Table 1. Chemical Composition of whole-board samples by Infection Time

Infection Time/week	Cellulose Content (%)	Xylan Content (%)	Lignin Content (%)	Extractive (%)	Ash Content (%)
0	27.60 ± 0.10	16.73 ± 0.13	18.40 ± 0.09	5.72 ± 0.13	7.72 ± 0.08
4	29.15 ± 1.70	15.92 ± 1.32	18.38 ± 0.14	5.43 ± 0.31	8.71 ± 0.11
8	29.49 ± 0.01	16.15 ± 0.21	20.00 ± 0.12	5.15 ± 0.25	9.19 ± 0.06
12	28.05 ± 0.14	15.38 ± 0.41	21.09 ± 0.10	4.92 ± 0.14	9.82 ± 0.04

The extractives were resins, pigments, and tannins. Wheat straw also contains monosaccharides, starches, proteins, alkaloids, and some inorganic compounds. These substances are good food sources for microorganisms, and this is the plausible reason why their mass loss rate was greater than that of cellulose, xylan, and lignin. The main components of ash were siliceous and waxy, which could not be consumed by mold so the ash percentage increased.

Table 2. Chemical Composition Converted to and Original-Weight Basis

Infection Time/week	Cellulose Content (%)	Xylan Content (%)	Lignin Content (%)	Extractive (%)	Ash Content (%)
0	27.60 ± 0.10	16.73 ± 0.13	18.40 ± 0.09	5.72 ± 0.33	7.72 ± 0.08
4	27.08 ± 1.58	14.78 ± 1.23	17.07 ± 0.13	5.04 ± 0.29	8.09 ± 0.11
8	24.79 ± 0.01	13.57 ± 0.18	16.81 ± 0.10	4.33 ± 0.38	7.73 ± 0.05
12	22.20 ± 0.11	12.17 ± 0.32	16.69 ± 0.08	3.90 ± 0.11	7.77 ± 0.03
4-week degradation rate	1.87	11.61	7.25	11.90	
8-week degradation rate	10.17	18.86	8.66	24.32	
12-week degradation rate	19.56	27.22	9.30	31.90	

During the first 4 weeks of the *T. viride* infection, the extractive content decreased from 5.7% to 5.0%, an 11.9% change. The xylan content decreased from 16.7% to 14.8%, an 11.6% change. The lignin content changed 7.2%, decreasing from 18.4% to 17.1%. The cellulose content decreased from 27.6% to 27.1% so that 1.9% of cellulose was degraded. From week 4 to week 12, the degradation rate of cellulose was greater, while the degradation rate of lignin was significantly less. At the end of the 12-week experiment, cellulose, xylose, lignin, and extractives were reduced by 19.6%, 27.2%, 9.3%, and 31.9%, respectively. The ash content remained the same. To further support the results, Yan *et al.* (2008) also reported that *T. viride* attacks the WSC.

Zhang *et al.* (2018) obtained mass losses of 2.2% and 1.5% for *Pinus massoniana* Lamb and *Populus tomentosa,* respectively when infected by *T. viride* for 12 weeks. This compares to 11.2% and 20.4% for WSC in this study. The mass loss rates for the WSC panels were 9 and 14 times higher than pine and poplar wood, respectively. The resistance of WSC to *T. viride* was much lower than that of wood due to its high extract content.

Chemical composition changes on WSC surfaces

Chemical composition changes on the surface layers are showed in Tables 3 and 4. The surface cellulose, xylanose, lignin, and extractive contents decreased. After 12 weeks of infection, 9.41% of the cellulose, 14.74% of the xylan, and 22.45% of the lignin were degraded. The amount of cellulose increased and then decreased, indicating that the composition change of the surface was different from that of the main part of the plate. The reason for this finding might also be that enzymes secreted by *T. viride* destroyed the structure of the wheat straw cells, causing more substances to be extracted.

Table 3. Chemical Composition of Sample Surfaces by Infection Time

Infection Time/week	Cellulose Content (%)	Xylan Content (%)	Lignin Content (%)	Extractive (%)	Ash Content (%)
0	25.72 ± 0.50	15.33 ± 0.51	20.22 ± 0.12	5.60 ± 0.60	7.08 ± 0.02
4	28.80 ± 2.60	16.23 ± 1.96	17.89 ± 0.28	6.83 ± 0.45	8.13 ± 0.24
8	28.94 ± 0.27	16.20 ± 0.30	19.45 ± 0.01	6.77 ± 0.32	8.35 ± 0.06
12	29.44 ± 1.79	16.52 ± 1.80	19.81 ± 0.20	5.77 ± 0.17	9.21 ± 0.02

Table 4. Chemical Composition of Sample surfaces converted to an original-weight basis

Infection Time/week	Cellulose Content (%)	Xylan Content (%)	Lignin Content (%)	Extractive (%)	Ash Content (%)
0	25.72 ± 0.36	15.33 ± 0.36	20.22 ± 0.12	5.60 ± 0.60	7.08 ± 0.02
4	26.75 ± 1.71	15.08 ± 1.29	16.62 ± 0.26	6.34 ± 0.41	7.55 ± 0.22
8	24.32 ± 0.16	13.61 ± 0.18	16.35 ± 0.01	5.69 ± 0.26	7.02 ± 0.05
12	23.30 ± 1.00	13.07 ± 1.01	15.68 ± 0.16	4.57 ± 0.13	7.29 ± 0.01

Chemical composition changes in the WSC interior layers

Chemical composition changes in the WSC interiors are presented in Tables 5 and 6. The cellulose, xylanose, and extractive contents decreased, while the content of lignin increased at week 4. As such, it is evident that more than just the surface of the WSC was attacked by *T. viride*. After 12 weeks, 28.4% of the cellulose and 37.8% of the xylose were

degraded, and the lignin content was still higher than that of the initial content. The cellulose and xylose degraded more in the core than at the surface. The differences seen between the surface layer and core layer might be related to the higher surface density of the WSC and a lower oxygen content in the core.

Table 5. Chemical Compositions of Sample Interiors by times

Infection Time/week	Cellulose Content (%)	Xylan Content (%)	Lignin Content (%)	Extractive (%)	Ash Content (%)
0	29.47 ± 0.21	18.13 ± 0.88	16.58 ± 0.06	5.84 ± 0.05	8.36 ± 0.14
4	29.51 ± 2.01	15.60 ± 1.77	18.86 ± 0.00	4.02 ± 0.18	9.30 ± 0.02
8	30.05 ± 0.30	16.10 ± 0.30	20.54 ± 0.26	3.53 ± 1.21	10.03 ± 0.05
12	26.66 ± 1.40	14.25 ± 0.65	22.38 ± 0.00	4.08 ± 0.45	10.44 ± 0.10

Table 6. Chemical Compositions Sample Cores Converted to an Original-weight Basis

Infection duration (week)	Cellulose Content (%) (average) (standard deviation).	Xylan Content (%)	Lignin Content (%)	Extractive (%)	Ash Content (%)
Non-infection	29.47 ± 0.15	18.13 ± 0.62	16.58 ± 0.06	5.84 ± 0.05	8.36 ± 0.14
4	27.41 ± 1.45	14.49 ± 1.17	17.52 ± 0.01	3.73 ± 0.17	8.64 ± 0.01
8	25.26 ± 0.18	13.53 ± 0.18	17.27 ± 0.22	2.97 ± 0.82	8.43 ± 0.04
12	21.10 ± 0.78	11.28 ± 0.37	17.71 ± 0.01	3.22 ± 0.35	8.26 ± 0.08

Infrared spectroscopy analysis of the WSCs after infestation

Figure 6 shows the infrared spectra of the WSC surfaces at the different infection times.

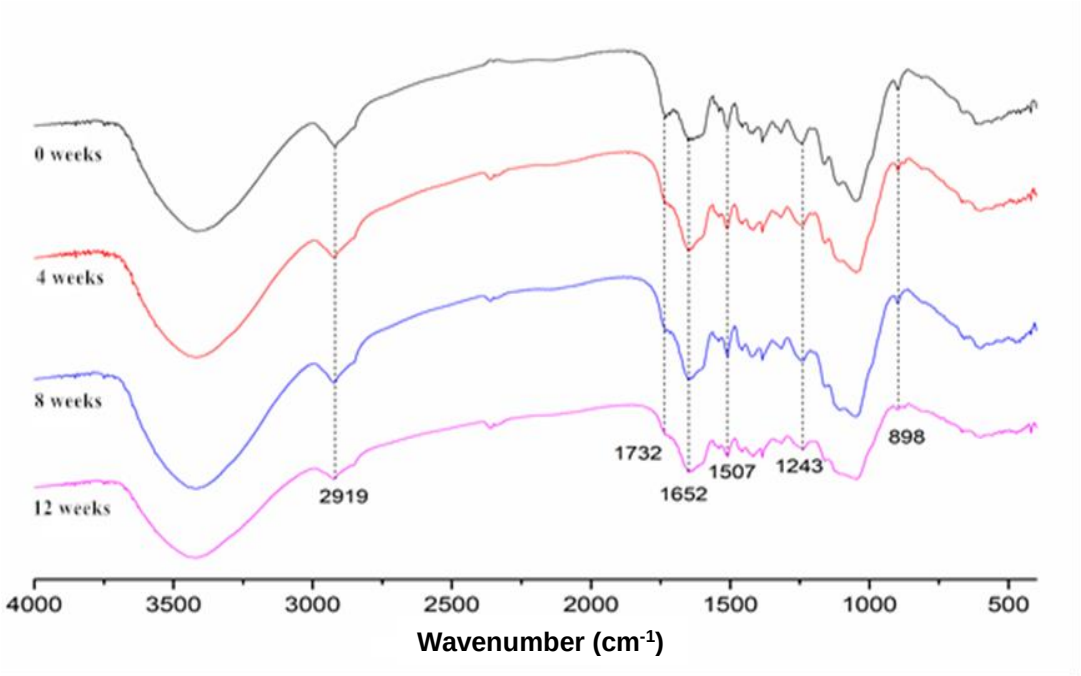

Fig. 6. FT-IR spectrogram of the WSC surfaces

The peaks are attributed to the bonds shown in Table 7 (Pandey and Pitman 2003). The characteristic peaks of cellulose were 2919 cm^{-1}, 1425 cm^{-1}, 1370 cm^{-1}, and 898 cm^{-1}. Increasing duration resulted in smaller peaks at 2919 cm^{-1} and 898 cm^{-1}. This indicates that the cellulose content decreased and the WSCs were actually degraded by *T. viride*.

Table 7. FT-IR Absorption Peak Location and Assignment of Wheat Straw Board

Wavenumber (cm^{-1})	Group Characteristic Peak Attribution
2919	C-H bond stretching vibration in methyl and methylene
1732	The C-O bond stretching vibration of lignosaccharide acetyl
1652	The stretching vibration of conjugate carbonyl C=O bond in lignin
1507	The stretching vibration of the benzene ring skeleton
1243	Stretching vibration of lignin phenol ether bond C-O-C
898	Characteristics of cellulose β-chain

The characteristic peak of hemicellulose is 1730 cm^{-1}, which indicates the stretching vibration of the C=O bonds on the acetyl and carboxyl groups. The absorption peak at 1732 cm^{-1} decreased with infection time. This shows that the hemicellulose content gradually decreased as the infection time increased. The hemicellulose in the WSCs was also degraded by *T. viride*.

Due to the complex composition of lignin and its lack of a clear characteristic peak, the tentative assignment of vibrational bands is somewhat challenging. Researchers have studied the absorption peaks of several typical lignin samples (Faix 1991; Rodrigues *et al.* 1998; Mohammed-Ziegler *et al.* 2004). The stretching vibration of a conjugated carbonyl group in lignin is 1652 cm^{-1}. Similarly, the stretching vibration of a benzene skeleton is 1507 cm^{-1}. Lastly, 1243 cm^{-1} is the stretching vibration of lignin phenol ether bond. These peaks were reduced in various degrees indicating that *T. viride* could degrade lignin. It can be concluded that cellulose, hemicellulose and lignin of WSC could be degraded by *T. viride*. The results were consisted with the results of HPLC method.

CONCLUSIONS

1. The infestation of *T. viride* darkens the surface of a WSC and change increases as the infestation proceeds.
2. *T. viride* not only attacks cellulose and hemicellulose in the WSC, but it attacks lignin and ethanol-soluble extractives, as well, to a greater extent. The density of WSCs decreased. After 12 weeks of infestation, 19.6% of cellulose, 27.2% of xylan, 9.3% of lignin, and 31.9% of the extractives were degraded. *T. viride* affects WSC more seriously than wood. The degradation of WSC by *T. viride* was 9 to 14 times greater than reported for pine and poplar, respectively.
3. *T. viride* attacks WSCs differently on the surface compared to the interior. More lignin was degraded in the surface layer, while more cellulose and xylan degradation occurred in the interior. *T. viride* proliferates to the interior of the WSCs in four weeks. The edge of the WSC was invaded by *T. viride*, followed by the interior of the WSC.

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