# Production and Characterization of Natural Fiber-Reinforced Thermoplastic Composites Using Wheat Straw Modified with the Fungus *Pleurotus ostreatus*

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**ABSTRACT:** Wheat straw is an abundantly available and potentially valuable biomass that is currently under-exploited. In this study, the feasibility of using wheat straw as a filler in high-density polyethylene (HDPE)-based composites was explored. Straw was treated with the white-rot fungus *Pleurotus ostreatus* with the aim of improving adhesion between straw and plastic, and thereby the mechanical properties of the composite. Results indicate that the use of sterilized straw is necessary to inhibit the growth of indigenous organisms that preclude, likely through competition, removal of lignin, and hence, improved bonding between straw and plastic. Light and transmission electron microscopy revealed cell wall modification in sterilized, inoculated straw. Reduced thermal stability of treated straw did not negatively affect the production of injection-molded straw–plastic composites (SPC). Comparable interfacial adhesion, based on activation energies obtained in dynamic mechanical analysis, was observed in untreated and treated straw- and pine-based thermoplastic composites. The results of this study indicate that wheat straw represents a promising alternative to wood fillers in the production of thermoplastic composites. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 102: 5191–5201, 2006

**Key words:** composites; degradation; fibers; modification; thermoplastics

## INTRODUCTION

Wheat straw (*Triticum aestivum* L.) is an abundant source of plant fiber which is produced in large quantities every year. The total worldwide production of cereal straw is estimated to exceed 2900 million tons per year.[1] At present, this potentially valuable resource is greatly under-exploited. Applications for straw encompass the housing and building, composites manufacture,[2–6] thermal insulation,[7] and energy[8] sectors of our economy.

Modification of straw (e.g., through physical, chemical, or biological means) has been generally considered necessary prior to use in the manufacture of natural fiber-reinforced thermoplastic composites. Biological treatment is expected to cause a limited degradation of the waxy cuticle, lignin, and hemicelluloses in the straw without much cellulose removal.[9–11] Degradation of these selected components of wheat straw is expected to (1) improve adhesion between the straw and plastic, and thereby the mechanical properties of a composite material; and (2) possibly reduce the amount of polyethylene required in the production of high-density polyethylene (HDPE)-based composite materials, and hence, the cost of the composite product. Lignin removal or modification by white-rot fungi or their derived enzymes has previously been applied in pulp production,[12,13] environmental bioremediation,[14] bioremediation of preservative-treated waste wood,[15–17] particle and fiberboard manufacturing,[18,19] and upgrading animal feeds.[20–22]

Sterilization of the straw prior to inoculation is potentially an important component in achieving sufficient growth of *Pleurotus ostreatus* for degradation of the waxy cuticle, lignin, and hemicelluloses. Yadav[23] demonstrated that processing of straw under farm (nonsterile) conditions resulted in growth of contaminating fungi,

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low specific growth rate of the inoculated fungus, and poor substrate utilization. In addition, it has been shown that sterilization of wood chips prior to application of fungal inoculum in biopulping is necessary to achieve consistent results when using fungi for delignification.[24]

One principal governing limitation of the selective removal of lignocellulosic material prior to use in a natural fiber-reinforced thermoplastic composite is the potential loss of thermostability of the degraded product. The primary criterion used in the selection of a thermoplastic for production of a wood- or straw-plastic composite (SPC) is that the melting or softening temperature of the thermoplastic is less than the thermal degradation temperature of the wood- or straw-filler ($\sim 210^{\circ}C$ for wood and undegraded wheat straw).[25] This thermal criterion restricts the polymer class of potential thermoplastics to polyolefins.[25] Treatment of wheat straw with a white-rot fungus which selectively degrades lignin, the thermally most stable component in lignocellulosic materials,[26] may reduce the thermal stability of the degraded product below 210°C, thus precluding the use of polyethylene, and polyolefins in general, in the production of a thermoplastic composite.

The overall goal of this study was to explore the feasibility of using fungal-modified wheat straw as filler in HDPE-based composites. The specific objectives of this study were to:

1. Characterize physical, chemical, thermal, and morphological properties of wheat straw following treatment with *P. ostreatus*;
2. Evaluate the influence of straw sterilization on the effectiveness of straw degradation by *P. ostreatus*;
3. Prepare injection-molded SPCs from fungal-modified and nonmodified wheat straw; and
4. Perform dynamic mechanical analysis (DMA) on SPC and determine activation energies for α-transition of HDPE in SPC.

## EXPERIMENTAL

### Wheat straw

A hard red spring variety of wheat straw (*Triticum aestivum* var. Westbred 936), obtained from Grant 4-D Farms (Rupert, ID) during the year 2000 cropping season, was used in all experiments. Straw stems were mechanically separated and stored indoors at $21^{\circ}C \pm 2^{\circ}C$ and 13% moisture content (MC) until used.[27] Overall straw stem length was less than $\sim 10$ cm with typical values ranging from 5 and 10 cm. Some of the straw used in the experiments was chopped through a screen with a 1.78-cm hole-diameter using a Nelmor-chopper.

### Preparation of fungal inoculum

*Pleurotus ostreatus* ATCC 32783 was chosen based on its lignin-degrading activity.[28] Fungal stock cultures were maintained at Utah State University on agar slants containing 41 g yeast-malt (YM) agar (Becton, Dickinson and Company, Sparks, MD) per liter of water. Fresh slants were prepared and inoculated every 2 weeks. Slants were incubated at room temperature with caps loosely attached. Mycelia from two- to three-week-old slants were used to inoculate 100 mL of liquid starter cultures in 500-mL Erlenmeyer flasks equipped with cotton plugs. For liquid starter cultures, YM broth (Becton, Dickinson and Company) containing 21 g of medium per liter of water was prepared, autoclaved, and 100× stock mineral solution was added (10 mL per L of YM broth). Stock mineral solution (100×) consisted of 3.0 g of $MgSO_4 \cdot 7H_2O$, 0.5 g of $MnSO_4 \cdot H_2O$, 1.0 g of NaCl, 0.1 g of $FeSO_4 \cdot 7H_2O$, 0.1 g of $CoSO_4$, 0.1 g of $CaCl_2 \cdot 2H_2O$, 0.1 g of $ZnSO_4 \cdot 7H_2O$, 0.01 g of $NaMoO_4 \cdot 2H_2O$, 0.01 g of $CuSO_4 \cdot 5H_2O$, 0.01 g of $AlK(SO_4)_2 \cdot 12H_2O$, 0.01 g of $H_3BO_3$ per liter of water (all chemicals were of standard laboratory grade). To this solution, 1.5 g/L nitrilotriacetic acid (NTA) was added and the pH adjusted to 6.5 with KOH. The stock solution was filter-sterilized and stored at 4°C.

Starter cultures were incubated for 2–3 days at room temperature in a metabolic shaker (New Brunswick Scientific Series 25, Edison, NJ) at $\sim 200$ rpm. After sufficient growth, the starter cultures were aseptically transferred to 2.8-L Fernbach flasks containing 1.5 L of liquid culture medium (same as for starter cultures) and incubated as previously described for 3–4 days. Fungal pellets were separated from medium by centrifugation (Sorvall RC-5B) at $\sim 10{,}000 \times g$ for 10–15 min and transferred to sterile 500-mL bottles with sufficient spent medium to submerge the pellets. These bottles were shipped under refrigeration to Washington State University and stored at 4°C until use (no longer than 2 weeks from date of arrival).

### Fungal inoculation, incubation, and processing of wheat straw

Wheat straw was treated with different amounts of fungal inoculum and sterile distilled water (Table I). For each treatment, with the exception of nos. 12, 13, and 14, 100 g of wheat straw was placed in each of the three 53.3 cm × 21.0 cm × 12.1 cm mushroom spawn bags (Myco Supply, Pittsburgh, PA), equipped with a 0.3-μm filter patch which allowed gas exchange while simultaneously precluding the passage of contaminants during incubation. To prepare the fungal inoculum for application to the wheat straw, 500 mL of inoculum in spent medium was transferred into a sterile blender (Waring, Torrington, CT), and 50 mL sterile distilled water was added to aid homogenization. The solution was homogenized in the blender for 30 s at low speed setting. Sixty milliliters of sterile distilled water was added to each bag to obtain a straw MC of $\sim 70\%$ (based on dry weight). The desired amount of

**TABLE I**
**Experimental Matrix Used to Identify Conditions that Promote the Growth of *P. ostreatus*[a]**

| Treatment no. | Sterile distilled water (mL per bag) | Inoculum (mL per bag) | Straw autoclaved | Straw chopped (0.7-in. screen) |
|---|---|---|---|---|
| 1 | 60 | 30 | No | No |
| 2 | 60 | 30 | No | Yes |
| 3 | 60 | 30 | Yes | No |
| 4 | 60 | 60 | No | No |
| 5 | 60 | 120 | No | No |
| 6 | 60 | 120 | Yes | Yes |
| 7 | 0 | 120 | No | No |
| 8[b] | 20 | 60 | No | No |
| 9[b] | 50 | 30 | No | No |
| 10[b] | 65 | 15 | No | No |
| 11 | 60 | n.a.[c] | No | No |
| 12 | n.a.[d] | n.a.[d] | No | No |
| 13 | 0 | 0 | No | No |
| 14 | 0 | 0 | Yes | No |

[a] Each treatment was performed in triplicates. All treatments were incubated in mushroom spawn bags, except for nos. 12, 13, and 14.
[b] Sterile distilled water and inoculum were mixed prior to application on straw. In all other treatments, water and inoculum were added consecutively to the straw.
[c] 120 mL of growth media was added instead of inoculum.
[d] Treatment no. 12 consisted of 6% sodium hypochlorite (bleach). For a description of application, please see text.

inoculum, containing 8.9 ± 0.8 mg fungal dry weight per mL culture, was then added to each bag using a sterile pipette. The bags were sealed using an electronic impulse sealer and well shaken. Fungal dry weight was determined by adding 30 mL of remaining homogenized inoculum to each of three dried and weighed 50-mL centrifuge tubes. The tubes were spun for 20 min at 6°C and 35,300×$g$ in a centrifuge (Beckman J2-HS, Fullerton, CA), weighed to the nearest 0.001 g, and the supernatant was decanted. Following oven-drying at 80°C for 12 h, the tubes were reweighed and fungal dry weights calculated.

Some of the straw was chopped and/or autoclaved prior to inoculation (Table I). Prior to autoclaving, straw (100 g) was placed in aluminum pans and covered with aluminum foil. The pans were then autoclaved for 40 min at 121°C and transferred to a laminar flow cabinet to dry overnight. The straw was transferred from the pans into spawn bags and autoclaved for 20 min at 121°C. The bags were again placed in the laminar flow cabinet and immediately inoculated following the procedure described above.

A control, consisting of 60 mL sterile distilled water and 120 mL growth medium, was included in the experiments (treatment no. 11, Table I). A bleach (6% sodium hypochlorite) treatment (no. 12) was also included to determine its effectiveness in straw delignification. For the bleach treatment, 100 g of straw was added to each of the four aluminum pans. One gallon of distilled water and 150 mL bleach were added to each pan. The straw in the pans was submerged in this solution for 5 h, rinsed with distilled water twice, and oven-dried.

All bags were incubated at 24°C and 65% relative humidity for 3 months. Following weight loss and MC measurements (described below), the straw was ground in a laboratory mill (Thomas-Wiley®, Model 4, Thomas Scientific, Swedesboro, NJ) equipped with a 1-mm screen and stored at 4°C until use in either analyses of straw properties or production of SPCs.

### Analysis of selected properties of wheat straw

#### Weight loss and MC

At the end of incubation, the straw was weighed, oven-dried until constant weight was reached, and reweighed to determine dry weight and MC. MC was calculated using the following equation:

$$\mathrm{MC} = \frac{W_w - W_D}{W_D} \times 100\ (\%)$$

where $W_w$ is the wet weight of straw (g) and $W_d$ is the dry weight of straw (g).

The effectiveness of fungal degradation was estimated as the weight loss (on a dry-weight basis) over the 3-month incubation period for treatment nos. 1–11.

#### Light and transmission electron microscopy

Light and transmission electron microscopy were performed on sterilized-inoculated straw specimens from the mushroom spawn bags and untreated control samples. Inoculated wheat straw and control specimens were sliced into small pieces (1–2 mm long segments)

and immersed overnight in a chilled (4°C) fixative solution containing 0.5% (v/v) glutaraldehyde (Ted Pella, Redding, CA), 2% (v/v) paraformaldehyde (Ted Pella), and 50 m*M* PIPES buffer (Research Organics, Cleveland, OH), pH 7.3. Specimens were then dehydrated in a graded ethanol series, infiltrated with LR White resin (Ted Pella), and allowed to polymerize overnight at 50°C. Semithin sections (0.5–1 μm) for light microscopy were cut with glass knives, and stained with 1% (w/v) Safranin O (Ted Pella) or 1% (w/v) toluidine blue (Ted Pella), and viewed at 1000× on an Olympus BH compound light microscope (Olympus Optical Co., Tokyo, Japan). Digital images were collected with a Pulnix CCD camera (JAI Pulnix, Sunnyvale, CA). Thin sectioning for transmission electron microscopy was accomplished using a diamond knife and an ultramicrotome (Ultracut R, Leica Microsystems, Vienna, Austria). Silver sections were collected on uncoated 300-mesh nickel grids. Sections were stained for 12 min with a uranyl acetate (UA)-$KMnO_4$ solution consisting of three parts 2% aqueous UA (Ted Pella), and one part 1% $KMnO_4$ (Mallinckrodt, St. Louis, MO), mixed and filtered immediately prior to staining. Stained sections were observed with an electron microscope (Jeol JEM 1200EX, JEOL, Tokyo, Japan) at the Washington State University Electron Microscopy Center, and photographed with Kodak electron microscopy film.

### Thermogravimetric analysis

Ground straw samples were dried in a vacuum oven at room temperature overnight to obtain a straw MC between 5% and 7%. Thermogravimetric analysis (TGA) was then performed using a simultaneous thermal analyzer (Rheometric Scientific STA 625, Piscataway, NJ). Straw samples of ~4 mg weight were heated in an aluminum pan (Rheometric Scientific L7168 2mm) to 580°C at a heating rate of 60°C/min. The maximum temperature (580°C) was held for 20 min, followed by cooling to 30°C at a rate of 60°C/min. Each sample was run in duplicates. After each TGA run, the data obtained were converted from the Rheometric software (RSI Orchestrator, Version V6.5.5) into an Excel (Microsoft) file, and weight losses were calculated based on the original sample weights with correction for the buoyancy effect of the air.

### Chemical analyses

Carbohydrate and lignin analyses were performed on ~ 350 mg of oven-dried material of treatment nos. 4, 5, 6, 11, 12, 13, and 14 from the spawn bags (Table I). Individual monosaccharides (araban, xylan, mannan, galactan, and glucan) were separated on a glass column packed with 3% cyanopropyl silicone (SP-2340; Supelco, Bellefonte, PA) in a gas chromatograph (Hewlett–Packard 5890, Series II, Wilmington, DE) at 205°C following sulfuric acid hydrolysis according to TAPPI Method T 249 cm-85. One sample of each treatment was analyzed in duplicate chromatographic runs. Acid-insoluble Klason lignin for one sample of each treatment was determined according to Effland.[29]

## Production of SPCs

Treatment nos. 5, 6, 13, 14, and a control consisting of 60-mesh Southern yellow pine flour were compounded and processed into thermoplastic composites. Sixty percent (by weight) of individual fiber treatments and 40% (by weight) of HDPE powder (Equistar Chemical LB010000, Houston, TX) were thoroughly mixed by shaking in a plastic bag and then compounded into pellets at 180°C and 20 rpm in a Haake PolyLab System (Rheocord 300p and Rheomix 600p, Thermo Haake, Karlsruhe, Germany), equipped with roller rotors. Processing time was ~ 5 min for each batch. Pellets were added to a screw-driven capillary extrusion rheometer (Acer 2000, Rheometric Scientific), equipped with a noncommercial die, soaked for 30 min at 180°C, and injection-molded into samples (nominal dimensions: 1.4 mm thickness, 6 mm width, 45 mm length) for DMA. Density of DMA specimens prepared from treatment nos. 5, 13, and pine flour was $1.34 \pm 0.02$ g/cm$^3$ whereas density of samples based on treatment nos. 6 and 14 were $1.39 \pm 0.01$ and $0.93 \pm 0.04$ g/cm$^3$, respectively.

## DMA of SPCs

DMA can provide valuable molecular and morphological information about a material in the solid state by subjecting it to dynamic loads over a broad range of temperature and frequency.[30] DMA was conducted in dual cantilever mode in a Rheometrics RSA II solids analyzer. Initially, dynamic strain sweep tests from $10^{-4}$ to $10^{-3}$ were run at −50°C, 25°C, and 100°C to ensure linearity throughout the test. Dynamic temperature scans from −50°C to 100°C were conducted at sequential frequencies of 0.1, 1, and 10 Hz and a strain of $10^{-4}$. In all experiments, the heating rate was 2°C/min, and the soak time was 1 min.

The activation energy for the α-transition of SPC was calculated using the Arrhenius equation:[31]

$$k = A_e \exp\left[-\frac{E_a}{RT}\right]$$

where $k$ is the rate constant or test frequency; $A_e$ is the frequency factor; $R$ is the ideal gas constant, 8.314 J/mol K; $T$ is the temperature (K); and $E_a$ is the activation energy. The peak temperatures of $E''$ at different frequencies were calculated using software (RSI Orchestrator, version V6.5.5).

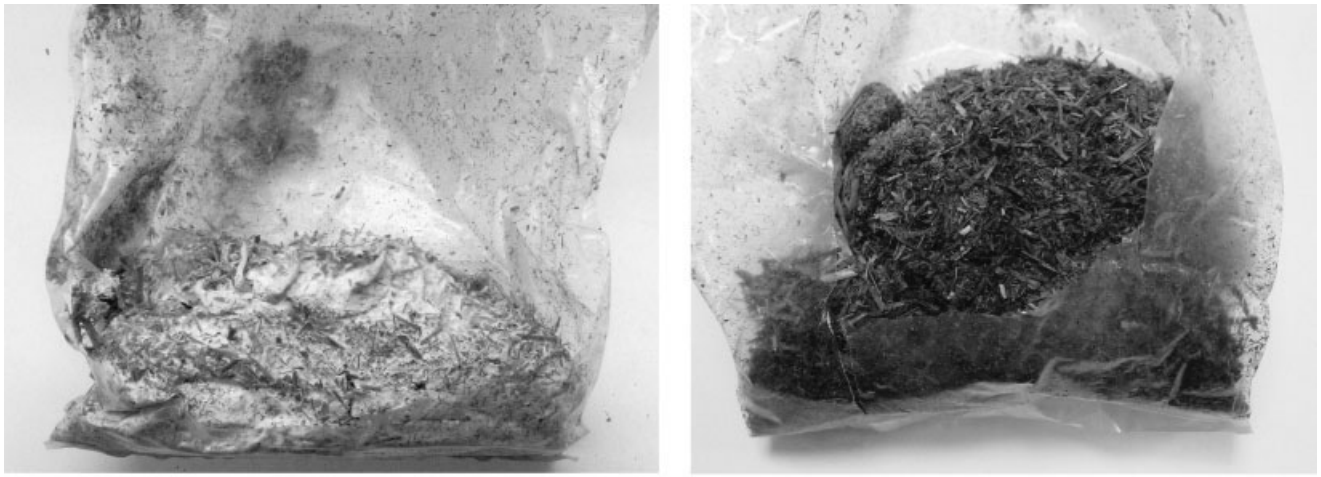


**Figure 1** Bags containing wheat straw, following 3 months of incubation with *P. ostreatus*. (a) Straw sterilized prior to inoculation. (b) Straw not sterilized prior to inoculation.

Statistical analysis was conducted to determine whether the differences in activation energy required for α-transition were significant at a 95% confidence level for SPC based on treated and untreated straw. A Tukey–Kramer multiple-comparison ANOVA test was performed with Number Cruncher Statistical Software (Kaysville, Utah).

## RESULTS AND DISCUSSION

### Visual observations of fungal colonization on wheat straw

It was determined by visual observation that *P. ostreatus* successfully colonized straw only when sterilized straw was used. After ~ 2 weeks of incubation, abundant fluffy, white *P. ostreatus* mycelium was observed in the spawn bags containing sterilized straw [Fig. 1(A)]. It was observed in additional experiments that colonization of sterilized straw was only successful when the straw had been chopped prior to sterilization and inoculation. In all treatments in which unsterilized straw was used, the straw was colonized by a plethora of microfungi [Fig. 1(B)]. No attempt was made to isolate and identify the fungi present, since this was beyond the scope of the project.

### Analysis of selected properties of wheat straw

#### Weight loss and MC

Weight loss was observed in treatment nos. 1–11 (Fig. 2). The relatively high weight losses in treatment nos. 5, 6, and 11 correspond to the three treatments that had

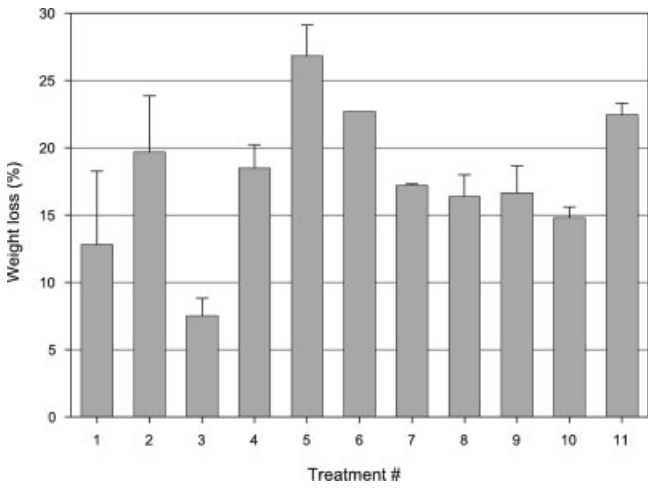


**Figure 2** Weight losses of wheat straw obtained with treatment nos. 1–11 (see Table I for description of treatments; weight loss of treatment no. 6 was determined with one replicate only, and hence, no error bar shown). Error bars represent the standard deviation for triplicates of each treatment.

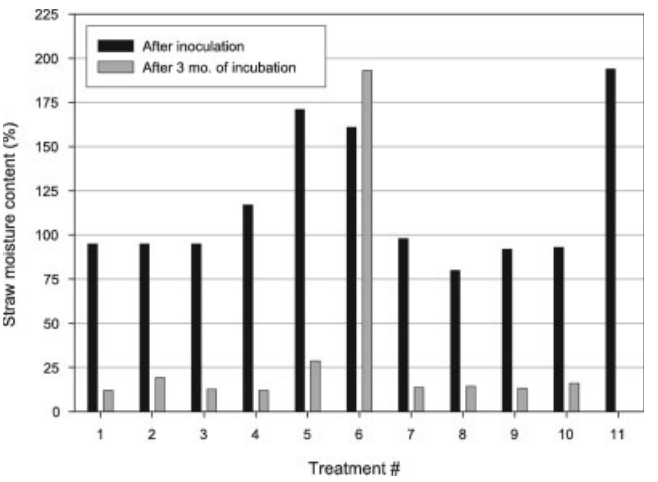


**Figure 3** Moisture content (MC) of straw obtained with treatment nos. 1–11 following inoculation and 3 months of incubation (see Table I for description of treatments; MC following incubation was not determined for treatment no. 11).

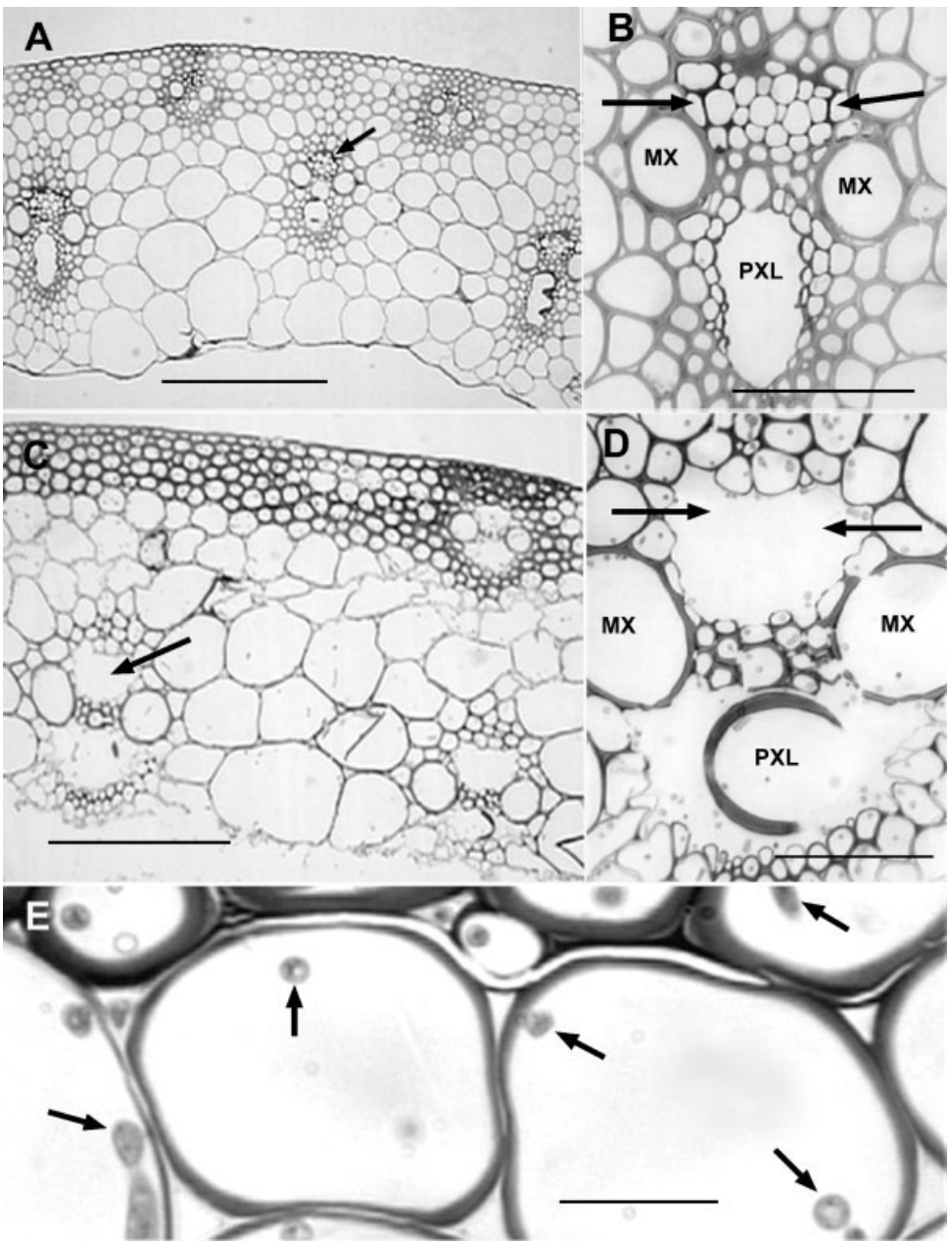


**Figure 4** Light microscope images comparing *Pleurotus ostreatus* treated and untreated straw samples for the distribution of *P. ostreatus* hyphae and tissue-level effects of fungal degradation. (A) Rehydrated control wheat straw cross section showing rounded, apparently structurally well preserved sclerified parenchyma cells. Arrow indicates the region of conducting phloem cells (sieve tube members and companion cells) within a vascular bundle. Bar = 200 μm. (B) A higher magnification of a vascular bundle from a control sample. Nacreous cell walls of the conducting phloem remain intact (arrows). MX, metaxylem vessel element; PXL, protoxylem lacuna. Bar = 50 μm. (C) Cross section of a wheat stem degraded with *P. ostreatus* showing less rounded cells than the control samples, possibly indicating widespread weakening of cell walls associated with fungal degradation. Hyphal filaments can be seen in nearly every cell of all tissues present. Arrows indicate spaces in vascular bundles where the residual cell walls of conducting phloem cells have been removed my fungal treatment. Bar = 200 μm. (D) A higher magnification of a vascular bundle in a degraded wheat stem. Arrows indicate a space where fungal enzymes have removed the nacreous walls of conducting phloem cells. Bar = 50 μm. (E) A higher magnification showing *P. ostreatus* hyphae (arrows) within sclerified parenchyma cells of degraded wheat straw. Bar = 10 μm.

comparatively high straw MC (more than 150%) after inoculation (Fig. 3), suggesting that MC (either as a threshold value or a continuum) influenced degradation. Weight loss of straw treated with growth medium (no. 11, Fig. 2) was overall as high or higher than weight loss of unsterilized straw inoculated with *P. ostreatus* (no. 5, Fig. 2); hence, inoculation of unsterilized straw with *P. ostreatus* was more likely not an

effective component in the observed weight loss. Although weight loss was observed in treatment nos. 1–11, weight loss values are not necessarily indicative of the absolute effectiveness of degradation; it was impossible to fully separate fungal mycelia and other biomass from the straw following incubation, thus potentially producing an underestimate of the effectiveness of degradation.

## Microscopy

Light and electron microscopy revealed that *P. ostreatus* colonized all types of wheat stem tissues in sterilized, inoculated straw. Light microscopy revealed that the wheat stems of the control sample showed little evidence of cellular distortion caused by drying and rehydration of the straw, indicating that the cell walls of the sclerefied storage parenchyma retained sufficient rigidity to resist collapse during dehydration [Fig. 4(A)]. In contrast, the equivalent cells of the *P. ostreatus* treated samples appeared less rounded, and often distorted [Fig. 4(C)]. In addition, the nacreous cell walls of the conducting phloem cells were completely removed from all vascular bundles in all treated samples examined, indicating that fungal colonization was widespread throughout the samples [compare Fig. 4(B,D)]. Figure 4(E) shows hyphae within sclerified parenchyma cells of fungal-treated wheat straw at higher magnification.

Electron microscopy revealed evidence of cell wall modification by fungal enzymes. Control samples [Fig. 5(A–D)] showed that cell walls of sclerenchyma fibers [Fig. 5(A,C)] and sclerefied parenchyma [Fig. 5(B,D)] contained numerous dark-staining fine striations representing differential deposition of cell wall polymers, possibly lignin. These cell walls were smooth along the surface bordering the cell lumen, and the cells contained little residual cellular debris. In contrast, the cell walls in wheat stems treated with *P. ostreatus* (Fig. 6) appeared more uniformly stained, with very few fine striations, indicating a possible loss of cell wall polymers with exposure to fungal enzymes. Numerous fungal hyphae were seen within the lumen of all cell types examined. The cells also contained a granular slime sheath that coated both the fungal hyphae and the cell walls of the rehydrated wheat stems [Fig. 6(A–D)]. A hyphal sheath, consisting of a thin glucan layer, was previously

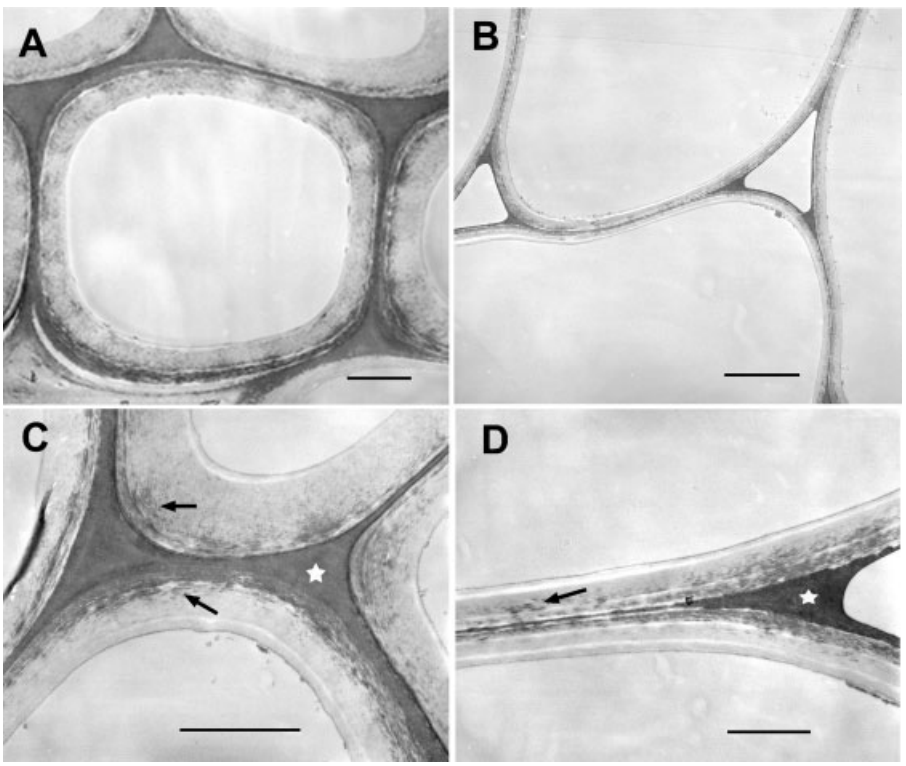


**Figure 5** Transmission electron micrographs showing the intact cell wall structure and cell wall striations present in rehydrated control wheat stems. (A) Low magnification cross section of a sclerenchyma fiber showing a smooth cell wall surface at the cell lumen, numerous fine striations in the secondary wall, and a darkly staining middle lamella. Bar = 2 μm. (B) Low magnification cross section showing the cell walls of several sclerified storage parenchyma cells. Cell walls are clearly defined with a smooth interior surface. Bar = 5 μm. (C) A higher magnification of sclerenchyma fiber cell walls. Note the darkly staining middle lamella (star) and the fine cell wall striations (arrow). Bar = 1 μm. (D) Higher magnification of sclerified parenchyma cell walls. Note the smooth cell wall boundaries, the fine striations within the secondary walls, and the darkly staining middle lamella. Bar = 1 μm.

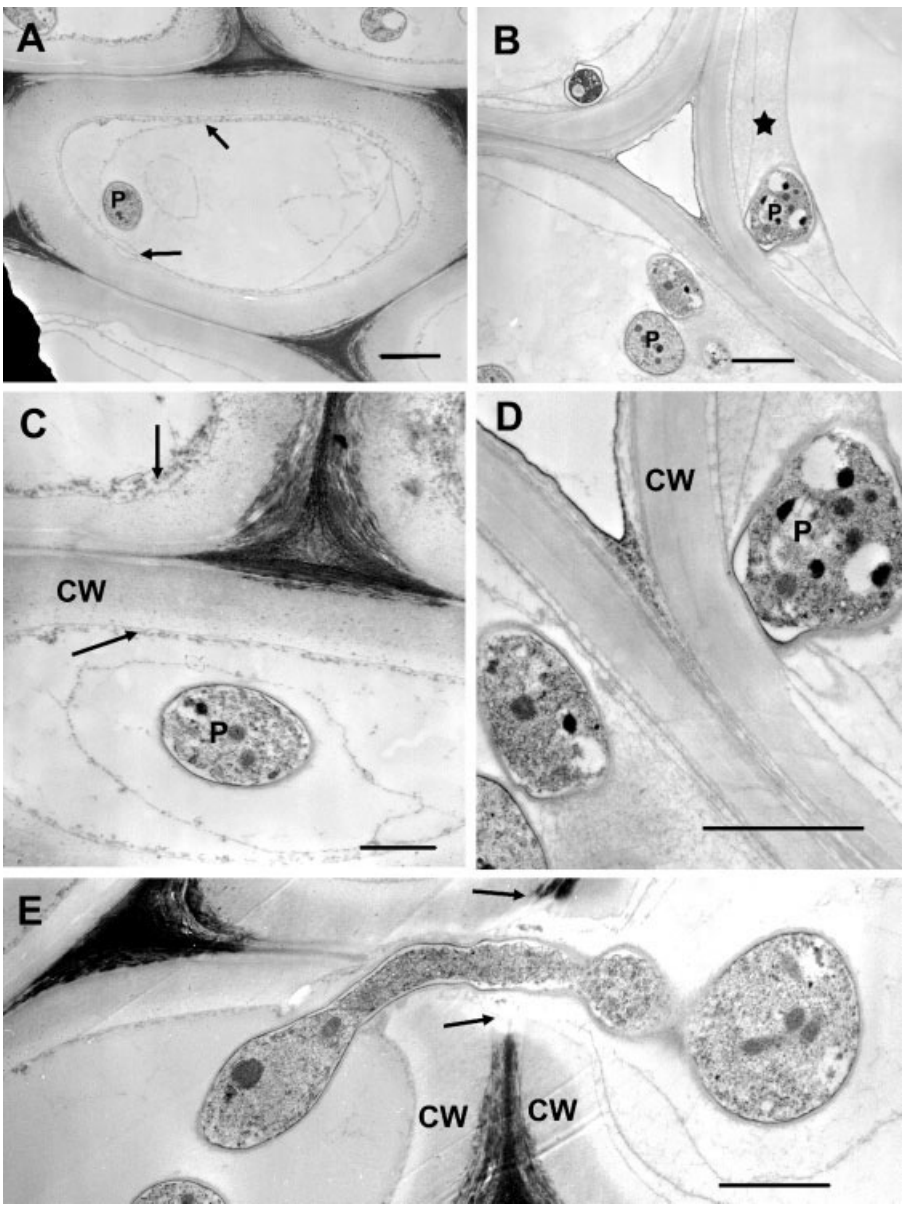


**Figure 6** Transmission electron micrographs showing the modified cell wall structure of *Pleurotus ostreatus* treated wheat stems. (A) Low magnification of sclerenchyma fibers containing *Pleurotus* hyphae (P) showing a cell wall with relatively few cell wall striations and with a granular appearing material coating the interior surface (arrows). Bar = 2 μm. (B) Low magnification showing walls of sclerified parenchyma cells. Each cell contains numerous *Pleurotus* hyphal filaments (P) surrounded by a coating material (star) apparently secreted by the fungi. Bar = 2 μm. (C) Higher magnification of sclerenchyma fibers containing *Pleurotus* hyphae (P). A granular material coats the interior surface of the cells (arrows) and the cell walls contain relatively few fine striations. Bar = 1 μm. (D) Higher magnification of the sclerefied parenchyma shown in (B). Note the secreted material surrounding the hyphae (P), the cell walls (CW) with few striations, and relatively light staining of the middle lamella. Bar = 2 μm. (E) A hyphal filament traversing thick cell walls (CW) of sclerenchyma fibers. Note the lack of staining of the middle lamella near the region of cell wall penetration (arrows), indicating removal of pectin by fungal enzymes. Bar = 2 μm.

observed for a closely related fungal species, *P. eryngii*.[32] Hyphal sheaths have been observed around many wood decay fungi[33–38] and sapstaining fungi.[39–42] The hyphal sheath may protect extracellular enzymes from inactivation and retain them close to the fungal hyphae. Optimal pH levels of 7–9 have been reported for xylanases and pectinases, thus indicating that the extracellular sheath may also serve to maintain an environment favorable to enzyme activity since wood is acidic, with a typical pH of 4–5.[40]

As a final observation, hyphae of *P. ostreatus* were often seen traversing cell walls [Fig. 6(E)]. The pathways between cells may have begun as natural pits, or as cracks caused by cell wall drying, but the cell wall at these sites show evident cell wall modification from fungal enzyme activity.

## Thermogravimetric analysis

TGA was used in this study to determine whether a change in the chemical composition due to fungal degradation of the straw had an effect on its thermal stability. TGA is a technique for measuring the weight loss of a substance as a function of temperature. It has previously been employed to identify changes in the components of mushroom and straw compost[43–46] and to evaluate fungal degradation of wood.[47] TGA showed that the highest weight losses of straw occurred in the temperature range between 200°C and 350°C, followed by a less active pyrolysis stage which ranged from 350°C to 600°C [Fig. 7(A,B)]. Initial substrate weight losses in the low temperature range of up to 100°C can be attributed to dehydration. These results, overall, are comparable to findings by Sharma[43] who investigated straw degraded by *P. ostreatus* after 20 and 40 days of incubation.

In the temperature ranges between 260°C and 330°C and between 380°C and 580°C, the relative weight losses of sterilized straw inoculated with *P. ostreatus* were higher than those of unsterilized-inoculated straw [Fig. 7(A)], suggesting that a higher level of fungal degradation was achieved with sterilized straw. However, the straw thermal behavior may have been influenced by the large amount of fungal biomass present in the sterilized and treated straw.

It is apparent that the sterilization process *per se* reduced the thermal stability of the straw [Fig. 7(B)]. The thermal stability of the straw, and hence the extent of degradation, was not influenced by size modification, induced through chopping to 1.8 cm, prior to fungal treatment [Fig. 7(C)]. Importantly, the observed reduced thermal stability of sterilized-treated straw did not noticeably affect the production of SPCs based on this filler material.

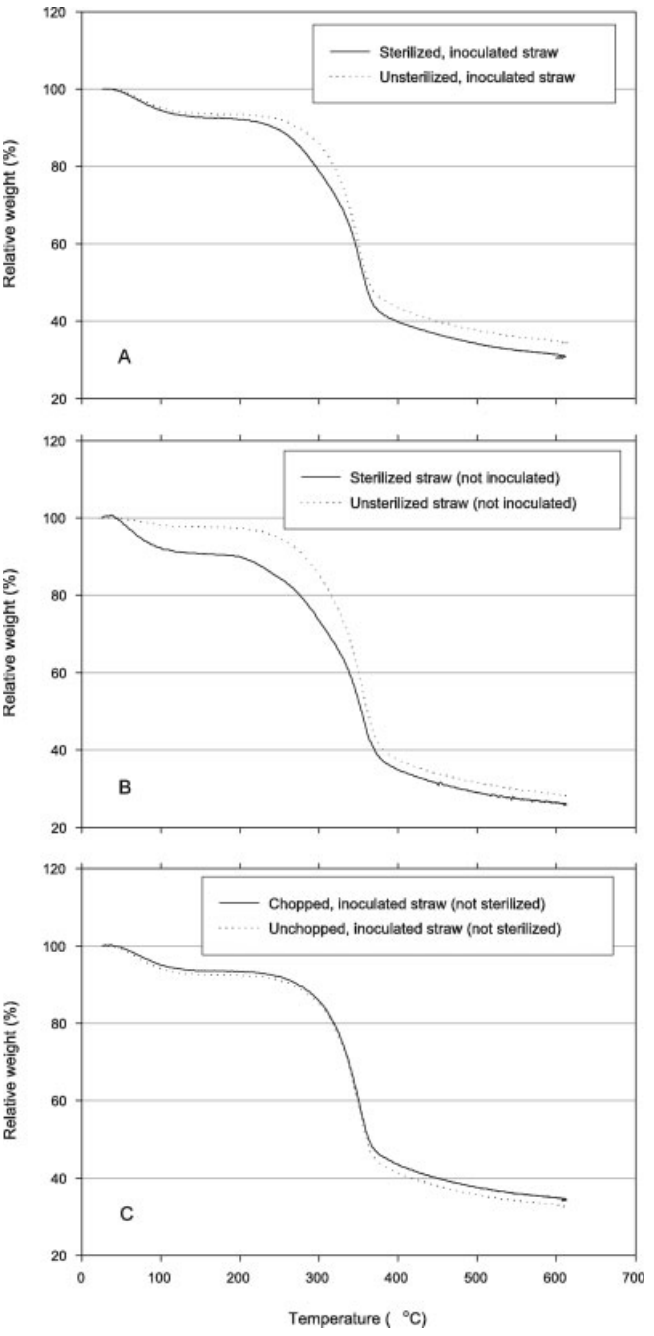


**Figure 7** Thermal stability of straw treatments illustrating (A) effect of sterilization prior to inoculation with *P. ostreatus*; (B) effect of sterilization; and (C) effect of straw stem length.

## Chemical analysis

Results from the carbohydrate analyses (Table II) were used to estimate the amounts of hemicelluloses and cellulose in straw. Glucan is the dominant carbohydrate which mostly makes up the cellulose. However, a portion of the glucan is combined with mannan in the hemicellulose fraction. The ratio between glucan and mannan is 1 : 2. The hemicellulose content was therefore estimated as the sum of all nonglucose sugars plus ½ of the mannan fraction for each sample (Table III). Cellulose content was estimated as the sum of the glucan value minus ½ of the mannan fraction. The dominant nonglucose carbohydrate in hardwoods as well as in wheat straw is xylan. The total sum of the hemicellulose, cellulose, and lignin fractions is less than 100% (Table III) due to extractives, crude protein, ash, and silica present in the straw.

Unsterilized, untreated wheat straw contained 21.3% lignin and therefore less lignin than most wood species.[26] The amount of lignin in straw determined in this study is comparable to results by Muller[48] who found 18.2% lignin in the internodes of summer wheat and 20.2% lignin in winter wheat. Harper and Lynch[49] also determined lignin content in different anatomical parts of wheat straw and found 14.2% lignin in internodes and 16.7% in node cores.

Overall, the lowest amount of hemicellulose was found in sterilized, noninoculated straw. This can be explained with the autoclaving procedure which is

**TABLE II**
**Carbohydrate Composition of Straw at the End of a 3-Month Incubation Under Different Treatment Scenarios**

| Treatment | Weight of specified compound/weight of oven-dried straw (%)[a] | | | | |
|---|---|---|---|---|---|
| | Arabinan | Xylan | Mannan | Galactan | Glucan |
| Not sterilized, not inoculated | 3.0 | 18.8 | 1.2 | 0.8 | 32.2 |
| Sterilized, not inoculated | 2.7 | 14.4 | 1.3 | 0.8 | 27.4 |
| Sterilized, inoculated (no. 6) | 2.4 | 17.6 | 2.1 | 0.7 | 33.7 |
| Not sterilized, inoculated (no. 4) | 2.5 | 18.4 | 1.3 | 0.8 | 31.1 |
| Growth media (no. 11) | 2.6 | 18.0 | 1.2 | 1.1 | 31.6 |
| Bleach (no. 12) | 3.3 | 17.5 | 1.1 | 0.9 | 31.7 |

[a] Each value represents the average of two chromatographic runs of one sample.

comparable to steam-explosion treatment, albeit performed at lower pressure. Steam-explosion has been documented to solubilize large amounts of sugars in wood[50] and wheat straw.[51] In fact, some monosaccharides were likely lost in all straw samples during the autoclaving step which is part of the sulfuric acid hydrolysis.

The consistent values of hemicellulose and cellulose across all biological treatments (Table III) would suggest minimal degradation of these compounds with and without inoculation with *P. ostreatus*. In addition, the reduction in lignin in the sterilized, inoculated straw (treatment no. 6, Table III) would suggest possible degradation from *P. ostreatus*. However, a sufficient number of replicates were not processed in the sugar and lignin analyses to permit a statistical analysis of differences between treatments (Table III). Hence, any apparent differences cannot be established as statistically significant.

### DMA of SPCs

Results from DMA provide conflicting evidence on the significance of *P. ostreatus* treatment in improving the straw–HDPE interphase. Values for storage modulus were highest for SPC manufactured with sterilized and treated straw, indicating an improvement in the straw–HDPE interphase over an SPC based on unsterilized straw (data not shown). However, activation energies of SPC based on treated and untreated straw were similar, irrespective of sterilization (data not shown). Generally, in composites, high activation energies are associated with large degrees of interactions between polymer matrix and filler. Hence, higher activation energy would be expected for treated straw than untreated for the results to be consistent with the storage modulus data. In either case, comparable interfacial adhesion, based on activation energies, was observed in untreated and treated straw- and pine-based thermoplastic composites.

## CONCLUSIONS

The following conclusions were drawn from various analyses:

1. On the basis of visual observations, inoculation of unsterilized wheat straw with *P. ostreatus* did not result in dominance of *P. ostreatus* on the straw under the conditions applied in the present study. Conversely, when straw was sterilized prior to fungal inoculation, *P. ostreatus* dominated over existing microorganisms.
2. Using light and transmission electron microscopy, cell wall modification in sterilized and ino-

**TABLE III**
**Cellulose, Hemicellulose, and Lignin Composition of Straw at the End of a 3-Month Incubation under Different Treatment Scenarios**

| Treatment | Weight of specified compound/weight of oven-dried straw (%) | | |
|---|---|---|---|
| | Hemicellulose[a] | Cellulose[a] | Lignin[b] |
| Not sterilized, not inoculated | 24.3 | 31.6 | 21.3 |
| Sterilized, not inoculated | 19.9 | 26.8 | 22.8 |
| Sterilized, inoculated (no. 6) | 23.8 | 32.7 | 17.4 |
| Not sterilized, inoculated (no. 4) | 23.6 | 30.5 | 25.9 |
| Growth media (no. 11) | 23.5 | 31.0 | 25.4 |
| Bleach (no. 12) | 23.4 | 31.1 | 22.6 |

[a] Each value represents the average of two chromatographic runs of one sample.
[b] Each value represents the result from a single sample.

culated straw was observed. Hyphae of *P. ostreatus* were present throughout the wheat stem tissues of sterilized and inoculated straw. The nacreous cell walls of the conducting phloem cells were completely removed from all vascular bundles in all specimens examined. Fungal hyphae were surrounded with a granular slime sheath which may protect extracellular enzymes from inactivation.

3. Sterilized, inoculated straw was less thermally stable than unsterilized, inoculated straw. Reduced thermal stability of treated straw did not negatively affect the production of SPCs.
4. The consistent values of hemicellulose and cellulose across all biological treatments would suggest minimal degradation of these compounds with and without inoculation with *P. ostreatus*. In addition, the reduction in lignin in the sterilize, inoculated straw would suggest possible degradation from *P. ostreatus*. However, a sufficient number of replicates were not processed in the sugar and lignin analyses to establish statistical significance of the observed trends.
5. DMA indicated that similar interfacial adhesion was obtained for straw- and pine-based thermoplastic composites.
6. The results obtained in this study demonstrate that wheat straw represents a promising alternative to wood fillers in the production of wood plastic composites (WPC).

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## References

1. Sun, X. F.; Sun, R. C.; Tomkinson, J.; Baird, M. S. Polym Degrad Stab 2004, 83, 47.
2. White, N. M.; Ansell, M. P. J Mater Sci 1983, 18, 1549.
3. Sauter, S. L. In Proceedings of the 30th Washington State University International Particleboard/Composite Materials Symposium; Washington State University: Pullman, WA, 1996; p 197.
4. Simonsen, J. Constr Build Mater 1996, 10, 435.
5. Zhang, Y.; Lu, X.; Pizzi, A.; Delmotte, L. Holz Roh Werkst 2003, 61, 49.
6. Boquillon, N.; Elbez, G.; Schönfeld, U. J Wood Sci 2004, 50, 230.
7. Strivastava, A. C.; Gupta, R. Biol Wastes 1990, 33, 63.
8. Kaltschmitt, M.; Reinhardt, G. A.; Stelzer, T. Biomass Bioenergy 1997, 12, 121.
9. Valmaseda, M.; Almendros, G.; Martinez, A. T.; Biomass Bioenergy 1991, 1, 261.
10. Moyson, E.; Verachtert, H. Appl Microbiol Biotechnol 1991, 36, 421.
11. Gamble, G. R.; Akin, D. E.; Makkar, H. P. S.; Becker, K. Appl Environ Microbiol 1996, 62, 3600.
12. Messner, K.; Srebotnik, E. FEMS Microbiol Rev 1994, 13, 351.
13. Camarero, S.; Barrasa, J. M.; Pelayo, M.; Martínez, A. T. J Pulp Pap Sci 1998, 24, 197.
14. Aust, S. D. Microb Ecol 1990, 20, 197.
15. Lamar, R. T.; Dietrich, D. M. J Ind Microbiol 1992, 9, 181.
16. Lee, D.-H.; Takahashi, M.; Tsunoda, K. Holzforschung 1992, 46, 81.
17. Lee, D.-H.; Takahashi, M.; Tsunoda, K. Holzforschung 1992, 46, 467.
18. Kharazipour, A.; Bergmann, K.; Nonninger, K.; Hüttermann, A. J Adhes Sci Technol 1998, 12, 1045.
19. Felby, C.; Hassingboe, J.; Lund, M. Enzyme Microb Technol 2002, 31, 736.
20. Zadrazil, F. Eur J Appl Microbiol 1977, 4, 273.
21. Akin, D. E.; Sethuraman, A.; Morrison, W. H., III; Martin, S. A.; Eriksson, K.-E. Appl Environ Microbiol 1993, 59, 4274.
22. Hatakka, A. I.; Mohammadi, O. K.; Lundell, T. K. Food Biotechnol 1989, 3, 45.
23. Yadav, J. S. Biotechnol Bioeng 1988, 31, 414.
24. Scott, G. M.; Akhtar, M. In Lignin, Humic Substances and Coal; Hofrichter, M., Steinbüchel, A.; Eds.; Wiley-VCH: Weinheim, 2003; Biopolymers; Steinbüchel, A., Ed.; Vol. 1, Chapter 6.
25. Wolcott, M. P.; Englund, K. In Proceedings of the International Particleboard/Composite Materials Symposium; Washington State University: Pullman, WA, 1999; p 103.
26. Fengel, D.; Wegener, G. Wood: Chemistry, Ultrastructure, Reactions; Walter de Gruyter: Berlin, 1989.
27. Houghton, T. P.; Thompson, D. N.; Hess, J. R.; Lacey, J. A.; Wolcott, M. P.; Schirp, A.; Englund, K.; Dostal, D.; Loge, F. Appl Biochem Biotechnol 2004, 113–116, 71.
28. Haider, K.; Trojanowski, J. Arch Microbiol 1975, 105, 33.
29. Effland, M. J. Tappi J 1977, 60, 143.
30. Saini, D. R.; Shenoy, A. V. Polym Commun 1985, 26, 50.
31. Turi, E. A. Thermal Characterization of Polymeric Materials, Vol. 1; Academic Press: San Diego, CA, 2nd ed.; 1997.
32. Barrasa, J. M.; Gutiérrez, A.; Escaso, V.; Guillén, F.; Martinez, M. J.; Martinez, A. T. Appl Environ Microbiol 1998, 64, 325.
33. Palmer, J. G.; Murmanis, L.; Highley, T. L. Mycologia 1983, 75, 995.
34. Palmer, J. G.; Murmanis, L.; Highley, T. L. Mycologia 1983, 75, 1005.
35. Highley, T. L.; Palmer, J. G.; Murmanis, L. L. Holzforschung 1983, 37, 179.
36. Ruel, K.; Joseleau, J.-P. Appl Environ Microbiol 1991, 57, 374.
37. Green, F., III; Clausen, C. A.; Larsen, M. J.; Highley, T. L. Can J Microbiol 1992, 38, 898.
38. Jellison, J.; Chen, Y.; Fekete, F. A. Holzforschung 1997, 51, 503.
39. Schmid, R.; Liese, W. Phytopathol Z 1965, 54, 275.
40. Gharibian, S.; Hoffert, C.; Abraham, L. D.; Breuil, C. New Phytol 1996, 133, 673.
41. Ouellette, G. B.; Chamberland, H.; Goulet, A.; Lachapelle, M.; Lafontaine, J.-G. Can J Microbiol 1999, 45, 582.
42. Schirp, A. Ph.D. Thesis, Department of Biological Sciences, University of Waikato, Hamilton, New Zealand, 2002.
43. Sharma, H. S. S. Thermochim Acta 1990, 173, 241.
44. Sharma, H. S. S. Thermochim Acta 1996, 285, 211.
45. Blanco, M.-J.; Almendros, G. J Agri Food Chem 1994, 42, 2454.
46. Blanco, M.-J.; Almendros, G. Plant Soil 1997, 196, 15.
47. Beall, F. C.; Merrill, W.; Baldwin, R. C.; Wang, J.-H. Wood Fiber 1976, 8, 159.
48. Muller, F. M. Tappi J 1960, 43, 209A.
49. Harper, S. H. T.; Lynch, J. M. J Sci Food Agri 1981, 32, 1057.
50. Saddler, J. N.; Brownell, H. H.; Clermont, L. P.; Levitin, N. Biotechnol Bioeng 1982, 24, 1389.
51. Vallander, L.; Eriksson, K.-E. Biotechnol Bioeng 1985, 27, 650.