Degradation of Lignin in Wheat Straw during Growth of the Oyster Mushroom (*Pleurotus ostreatus*) Using Off-line Thermochemolysis with Tetramethylammonium Hydroxide and Solid-State ^{13}C NMR

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The oyster mushroom (*Pleurotus ostreatus*) is widely cultivated on wheat straw (*Triticum aestivum*); however, there is a need to better understand the relationship between the chemical composition of the compost and mushroom growth. Wheat straw was degraded over a period of 63 days by *P. ostreatus* during which time it was sampled at weekly intervals. Off-line thermochemolysis with tetramethylammonium hydroxide and solid-state ^{13}C NMR were then used in the molecular characterization of the undegraded wheat straw and the degraded samples. The degraded wheat straw samples had a lower proportion of syringyl- to guaiacyl-derived moieties and cinnamyl- to guaiacyl-derived moieties than the undegraded control. There were increases in both guaiacyl and syringyl acid to aldehyde ratios with composting time, which showed that side-chain oxidation has been mediated by *P. ostreatus*. The ^{13}C NMR spectra confirmed the increase in carboxyl content but indicated that the overall lignin and methoxyl contents remained relatively constant, although some nonsystematic variations were observed. The spectra also showed a decrease in amorphous noncellulosic polysaccharides in relation to the crystalline cellulose upon degradation.

Keywords: *Lignin; carbohydrate; oyster mushroom; Pleurotus ostreatus; thermochemolysis; solid-state ^{13}C NMR; degradation; wheat straw; Triticum aestivum*

INTRODUCTION

Wheat straw (*Triticum aestivum*) is the major lignin-containing ingredient of mushroom compost (*1*). Species from the genus *Pleurotus* are known to selectively degrade lignin in preference to the polysaccharides in wheat straw (*2*, *3*). It has also been shown that the edible oyster mushroom (*Pleurotus ostreatus*) preferentially removed lignin in contrast to the nonselective degradation of lignocellulose by *Phanerochaete chrysosporium* during the treatment of cotton stalks with these two particular white rot fungi (*3*). Cupric oxide depolymerization of wheat straw biodegraded by *Pleurotus eryngii* showed that there was a decrease in the relative amount of phenolic lignin units following fungal attack without any significant change in the amounts of phenolic cinnamic acids (*4*).

In light of the relationship between the chemical composition of compost and mushroom growth (*1*) there is a need to track the molecular transformations in lignin during mushroom production. However, instead of applying oxidative treatments and spectroscopic techniques to isolated wheat straw lignin preparations (*5*), we have carried out the molecular characterization of whole straw samples, using thermochemolysis in the presence of tetramethylammonium hydroxide (hereafter simply termed thermochemolysis) as well as solid-state nuclear magnetic resonance (NMR). Thermochemolysis is an analytical method that has been applied to the characterization of lignin residues in geochemical samples (*6*) and has also been used with a ^{13}C-labeled methyl group in the study of fungally degraded woods (*7*). Off-line thermochemolysis at 300 °C yields the same lignin products in similar proportions as on-line pyrolysis at 600 °C in the presence of tetramethylammonium hydroxide. One significant advantage of the off-line method, when compared to on-line pyrolysis, is that internal standards can be added and the reaction products quantified (*8*).

Decomposition of lignin, due to the base-catalyzed reactions caused by tetramethylammonium hydroxide during thermochemolysis, occurs only where propyl−aryl ether linkages are adjacent to hydroxyl groups on the alkyl side chain (*9*). Methylation of hydroxyl groups results in the formation of mono-, di-, or trimethoxybenzenes with either a one-, two-, or three-carbon side chain, which can be subsequently analyzed by gas chromatography−mass spectrometry (GC-MS) (*10*). Increases in the relative acid/aldehyde intensity ratios of 3,4-dimethoxybenzoic acid methyl ester to 3,4-dimethoxybenzaldehyde $(Ad/Al)_G$ and 3,4,5-trimethoxybenzoic acid methyl ester to 3,4,5-trimethoxybenzaldehyde $(Ad/Al)_S$ provide an excellent indicator of oxidative fungal decay (*6*, *11*). Summation of mono-, di-, and trimethoxybenzene derivatives provides a measure of the amounts of

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labile ether-linked lignin and cinnamic acid units if normalized to 100 mg of organic carbon (Λ) (*6*, *12*).

In this study we demonstrate how thermochemolysis and ^{13}C solid-state NMR can be used to monitor changes in the molecular composition of wheat straw as a function of cultivation time during the commercial production of *P. ostreatus*.

MATERIALS AND METHODS

Cultivation of *P. ostreatus*. Wheat straw (*T. aestivum*) was cut into lengths of between 5 and 10 cm; the moisture content of the substrate was maintained at ~75% (w/w) by the addition of water. The substrate was compacted into plastic bags and pasteurized using steam; the temperature was maintained at 62.5 ± 2.5 °C for 4 h and then kept at 52.5 ± 2.5 °C for 3 days. After the substrate had cooled to room temperature, 1−5% (relative to the wet weight of the substrate) of spawn was mixed thoroughly with the substrate. The bags were sealed and kept in the dark at 24.5 ± 1.5 °C for 4 weeks, after which time the ambient temperature was lowered to between 12 and 18 °C. The surface of the wheat straw was illuminated to ~100 lx and sprayed with clean water to keep an air humidity of 90%. After 1 day, the temperature was raised to between 18 and 25 °C to achieve fruiting body growth. Following this increase in ambient temperature, ~170 g (dry weight) of wheat straw was removed at 0, 7, 14, 21, 28, 35, 42, 49, 56, and 63 days. Each of these samples was freeze-dried and then powdered to pass through a 63 μm sieve.

General. Organic solvents were distilled using an Oldershaw column. The authentic standards 4-methoxyacetophenone (P5), 3,4-dimethoxybenzaldehyde (G4), 3,4-dimethoxyacetophenone (G5), 3,4,5-trimethoxybenzaldehyde (S4), and 3,4,5-trimethoxyacetophenone (S5) as well as the GC internal standard *n*-eicosane were obtained from Sigma-Aldrich Chemical Co. (Gillingham, Dorset, U.K.). The compound labels (G, S, and P) denote structures shown in Figure 2.

Elemental Analysis. The organic carbon, hydrogen, and nitrogen contents of wheat straw samples were determined in quadruplicate using a Carlo Erba 1106 elemental analyzer. Ash content was determined gravimetrically following combustion of the powdered wheat straw at 650 °C for 18 h (*13*). Blanks and samples were interchanged to account for possible instrumental drift.

Off-line Thermochemolysis. For each experiment borosilicate glass tubing (o.d. = 5 mm, i.d. = 4 mm) was sealed at one end with a natural gas/oxygen flame to give a vessel length of 13 cm. Each vessel was rinsed with dichloromethane and oven-dried for 12 h at ~75 °C. Sample (0.5 mg) was placed in each of these reaction vessels with 100 μL of tetramethylammonium hydroxide solution (25% in methanol). These preparations were left overnight in a vacuum desiccator in the presence of P_2O_5 to facilitate thorough mixing prior to the removal of methanol under vacuum. The dried mixtures were sealed under vacuum and heated in an oven at a temperature of 250 °C for 30 min. The temperature inside the vessels was constantly monitored (±0.5 °C) before, during, and after each experiment using a chromel−alumel thermocouple, and the measurements were monitored on a chart recorder. The low thermal inertia of the glass vessels thus enabled precise temperature control throughout the experiments. After cooling, the reaction vessels were opened and the inner surfaces of the tube were washed five times with 1 mL of dichloromethane. The combined extracts were dried under a stream of N_2 and dissolved in 100 μL of dichloromethane. A known weight of the internal standard *n*-eicosane was added from a stock solution using an automatic microconstriction pipette.

Gas Chromatography—Mass Spectrometry. GC-MS was performed using a Hewlett-Packard 5890 II gas chromatograph (GC) directly coupled with a Hewlett-Packard 5972 single-quadrupole mass spectrometer (ionization energy, 70 eV; mass range, *m*/*z* 50−550 amu; cycle time, 1 s) with helium as carrier gas. The GC was fitted with a fused silica capillary column (30 m × 0.2 mm i.d.) coated with a 5% phenylmethylsilicone bonded stationary phase (HP-5, film thickness, 0.25 μm). The GC was temperature programmed from 60 to 150 °C at 15 °C min^{-1}, from 150 to 215 °C at 4 °C min^{-1}, and from 215 to 300 °C at 30 °C min^{-1} and held isothermally at 300 °C for 5 min.

The relative response factors (RRF) of the authentic standards P5, G4, G5, S4, and S5 were each measured relative to the internal standard *n*-eicosane and can be written as

$$\mathrm{RRF} = [\mathrm{area}\ (x)/\mathrm{wt}\ (x)][\mathrm{wt}\ (n\text{-eicosane})/\mathrm{area}\ (n\text{-eicosane})] \quad (1)$$

where wt (*x*) and wt (*n*-eicosane) are the known weights of the authentic standard and area (*x*) and area (*n*-eicosane) are the peak areas of the authentic standard and *n*-eicosane. The unknown weight of individual pyrolysis products is written as

$$\mathrm{wt}\ (x) = \mathrm{RRF}[\mathrm{wt}\ (n\text{-eicosane})][\mathrm{area}\ (x)/\mathrm{area}\ (n\text{-eicosane})] \quad (2)$$

where wt (*x*) is the unknown weight of an individual thermochemolysis product in eq 2, wt (*n*-eicosane) is the known weight of *n*-eicosane, and area (*x*) and area (*n*-eicosane) are the peak areas of the product and *n*-eicosane, respectively. The amounts of thermochemolysis products other than those authentic standards listed above were semiquantitative in that an average RRF of 3.2 was applied.

Thermochemolysis Parameters. Acid/aldehyde $(Ad/Al)_G$ parameters were measured using peak areas of 3,4-dimethoxybenzoic acid, methyl ester (G6), and G4 [$(Ad/Al)_G$ = G6/G4] as well as 3,4,5-trimethoxybenzoic acid, methyl ester (S6), and S4 [$(Ad/Al)_S$ = S6/S4]. The syringyl/guaiacyl ratio (S/G) was calculated by dividing the sum of the peak areas from syringyl derivatives (S4−S15) by the sum of the peak areas from their guaiacyl counterparts (G4−G15). The Γ parameter was determined from the ratio of G6 to the sum of the peak areas of the threo and erythro forms of 1-(3,4-dimethoxyphenyl)-1,2,3-trimethoxypropane (G14 and G15). The compound labels (G, S, and P) denote structures shown in Figure 2. The Λ values were calculated as the sum of the amounts of all lignin-derived normalized to 100 mg of carbon in the sample (*12*, *14*).

Solid-State ^{13}C Nuclear Magnetic Resonance. Solid-state ^{13}C NMR spectra were obtained for native and degraded wheat straw samples using a Bruker DSX200 instrument equipped with double-bearing probes for cross-polarization (CP) and magic angle spinning (MAS). The resonance frequency for ^{13}C was 50 MHz, and the sample was spun at the magic angle with a speed of 6.0 kHz. Typically, 30000 scans were accumulated with high-power ^1H decoupling for the CP experiments. For CP, the contact time was 1.5 ms and the relaxation delay was 1.5 s. Dipolar dephasing experiments were carried out using dephasing periods of 50 and 100 μs. All spectra were obtained at ambient temperature and processed using a line broadening factor of 50 Hz. Chemical shifts were calibrated using an external sample of tetrakistrimethylsilane (TKS).

RESULTS

Changes in the elemental composition of wheat straw occurred with mushroom growth (Table 1). The control wheat straw had a nitrogen content of 0.78 ± 0.14% (w/w), whereas fungally decayed wheat straw had nitrogen contents ≥1.0% (w/w) with a maximum value equal to 1.53 ± 0.02% after incubation for 56 days. Although the nitrogen content of the degraded wheat straw was greater than that of the undegraded control, absolute amounts of nitrogen remained low relative to the carbon, hydrogen, and oxygen contents (Table 1). The carbon content decreased from 45.68 ± 0.07% (w/w) in the undegraded wheat straw to 37.62 ± 0.07% (w/w)

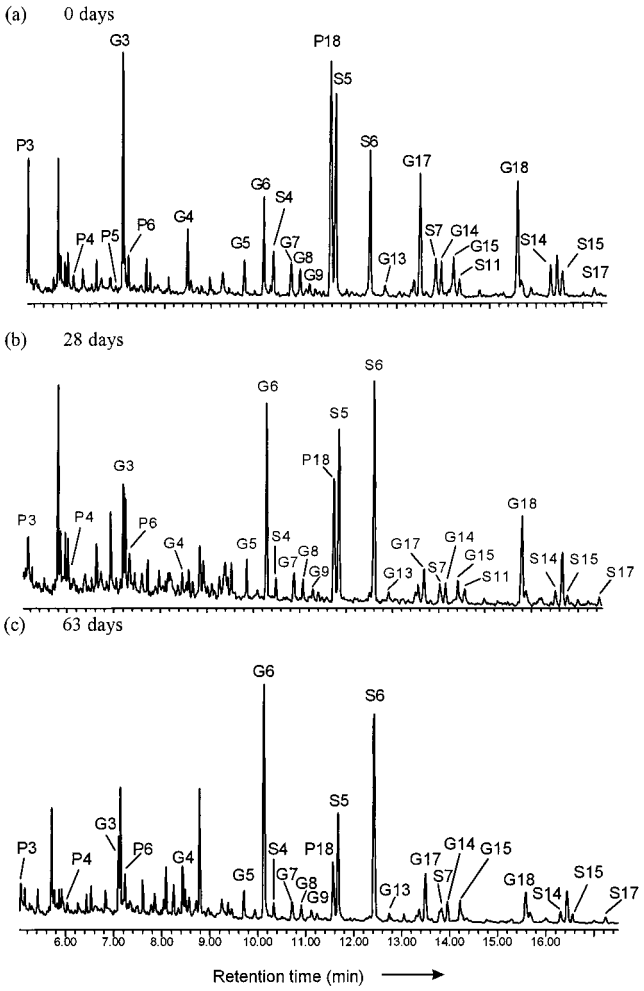

Figure 1. Partial traces for the total ion current (TIC) of the thermochemolysis products from (a) undegraded control wheat straw, (b) wheat straw degraded for 28 days by *P. ostreatus*, and (c) wheat straw degraded for 63 days by *P. ostreatus*. Labels denote structures shown in Figure 2.

Table 1. Elemental Composition of Wheat Straw Degraded by *P. ostreatus*

time (days)	% N	% C	% H	% O
0	0.78 ± 0.14	45.68 ± 0.07	5.77 ± 0.12	47.77
7	1.00 ± 0.06	36.38 ± 0.21	5.69 ± 0.15	56.92
14	1.18 ± 0.12	37.31 ± 0.41	5.83 ± 0.31	55.67
21	1.18 ± 0.07	38.60 ± 0.10	5.67 ± 0.33	54.55
28	1.34 ± 0.02	38.58 ± 0.21	6.13 ± 0.21	53.95
35	1.11 ± 0.03	37.27 ± 0.18	5.54 ± 0.08	56.08
42	1.40 ± 0.05	36.24 ± 0.06	5.57 ± 0.12	56.79
49	1.33 ± 0.06	34.05 ± 0.05	5.63 ± 0.11	58.99
56	1.53 ± 0.02	36.02 ± 0.11	5.64 ± 0.13	56.81
63	1.49 ± 0.06	37.62 ± 0.07	5.77 ± 0.13	55.12

following 63 days of growth of *P. ostreatus*. All decayed wheat straws had lower carbon contents than that of their undegraded counterpart; however, no systematic decrease in carbon content was observed for incubation periods >7 days. Within experimental variation the hydrogen content of wheat straw appeared to be invariable upon fungal degradation (Table 1). In contrast, *P. ostreatus* cultures accumulated oxygen, the content of which increased from 47.77% (w/w) in native wheat straw to 55.12% (w/w) after 63 days (Table 1). The C/N ratio of native wheat straw was 58; this generally decreased with mushroom growth to give a C/N of 25.4 after 63 days.

Figure 1a shows the major thermochemolysis products from the undegraded wheat straw, which were

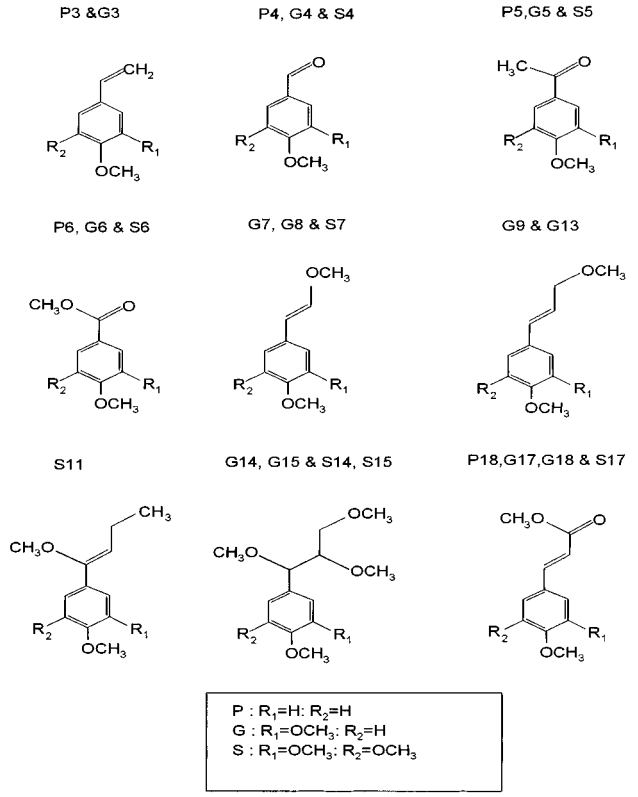

Figure 2. Structures of major thermochemolysis products following treatment of the control and biodegraded samples of wheat straw with tetramethylammonium hydroxide.

identified as 4-methoxystyrene (P3), 3,4-dimethoxystyrene (G3), *trans*-3-(4-methoxyphenyl) acrylic acid, methyl ester (P18), S5; S6; and *cis*- and *trans*-3-(3,4-dimethoxyphenyl) acrylic acid, methyl ester (G17 and G18). Other important products included 4-methoxybenzaldehyde (P4), 4-methoxybenzoic acid, methyl ester (P6), G14, and G15 as well as the threo and erythro forms of 1-(3,4,5-trimethoxyphenyl)-1,2,3-trimethoxypropane (S14 and S15) (Figure 1a). After 28 days of decay by *P. ostreatus*, the two main thermochemolysis products were G6 and S6. The amounts of G3, S5, P18, G17, and G18 decreased in intensity relative to the other products (Figure 1b). A further increase in incubation time of 63 days resulted in such a decrease in the relative abundance of P18 that it was now no longer a major product (Figure 1c).

The ratio of dimethoxyphenol to monomethoxyphenol derivatives at the different stages of mushroom cultivation is shown in Figure 3a. The S/G value of the control was 1.20, which is similar to previous measurements made on lignin from wheat straw using alkaline nitrobenzene oxidation (*1*) and ^{13}C NMR (*15*). The S/G values then decreased to 0.95 and 0.82 after cultivation for 7 and 63 days, respectively (Figure 3a). The C/G ratio of the undegraded wheat straw was 0.93, and then after 7 days of growth of *P. ostreatus*, this decreased to 0.47 (Figure 3b). With longer incubation times (14−63 days) this value decreased only slightly (Figure 3b). In contrast, the $(Ad/Al)_G$, $(Ad/Al)_S$, and Γ parameters increased with cultivation time (Figures 4 and 5). The $(Ad/Al)_G$ and the $(Ad/Al)_S$ values of the undegraded wheat straw were 1.95 and 3.5, respectively; after 56 days, the $(Ad/Al)_G$ was 10.5 and the $(Ad/Al)_S$ was 12 (Figure 4). The Γ parameter increased by about an order of magnitude with cultivation time up to and including 42 days prior

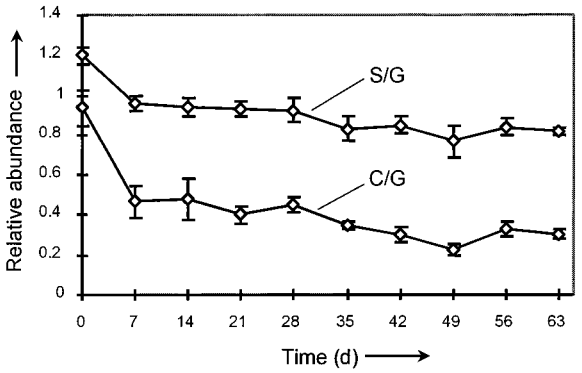

Figure 3. Time-dependent changes in (a) syringyl/guaiacyl derivatives (S/G) and (b) cinnamyl/guaiacyl derivatives (C/G) during the fungal degradation of wheat straw by *P. ostreatus*.

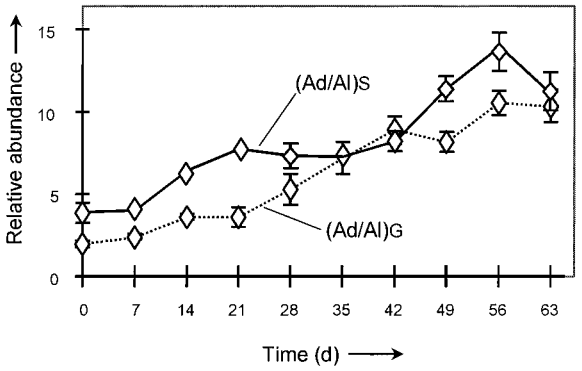

Figure 4. Time-dependent changes in (a) 3,4-dimethoxybenzoic acid methyl ester/3,4-dimethoxybenzaldehyde $(Ad/Al)_G$ and (b) 3,4,5-trimethoxybenzoic acid methyl ester/3,4,5-trimethoxybenzaldehyde $(Ad/Al)_S$ during the fungal degradation of wheat straw by *P. ostreatus*.

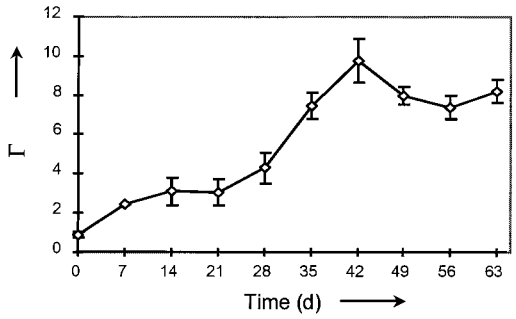

Figure 5. Time-dependent changes in 3,4-dimethoxybenzoic acid methyl ester relative to the summed amounts of threo and erythro forms of 1-(3,4-dimethoxyphenyl)-1,2,3-trimethoxypropane (Γ) in wheat straw during the fungal degradation by *P. ostreatus*.

to a small decrease between 42 and 56 days of growth (Figure 5). The amount of lignin and cinnamic acid derivatives, as expressed by Λ value, for native wheat straw was 4.6. This decreased to a Λ value of 3.5 after 7 days and then slowly decreased to give a final value of 2.7 after 63 days of biodegradation (Figure 6), which is equivalent to an overall 41% decrease from the undegraded straw.

The normal CPMAS ^{13}C NMR spectra of the control (undegraded) wheat straw and its counterparts following *P. ostreatus* growth (28 and 63 days) are presented in Figure 7, and the corresponding carbon distributions are listed in Table 2. The estimated error in the carbon distributions is ±5% of the intensity for the smaller bands measured (<15% of the total carbon) and ±2%

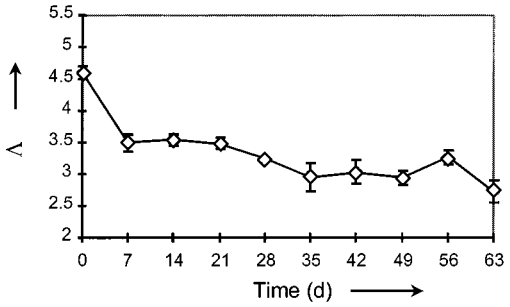

Figure 6. Time-dependent changes of carbon normalized yields of methylated lignin phenols and cinnamic acids (Λ) released by thermochemolysis of native and *P. ostreatus*-degraded wheat straw.

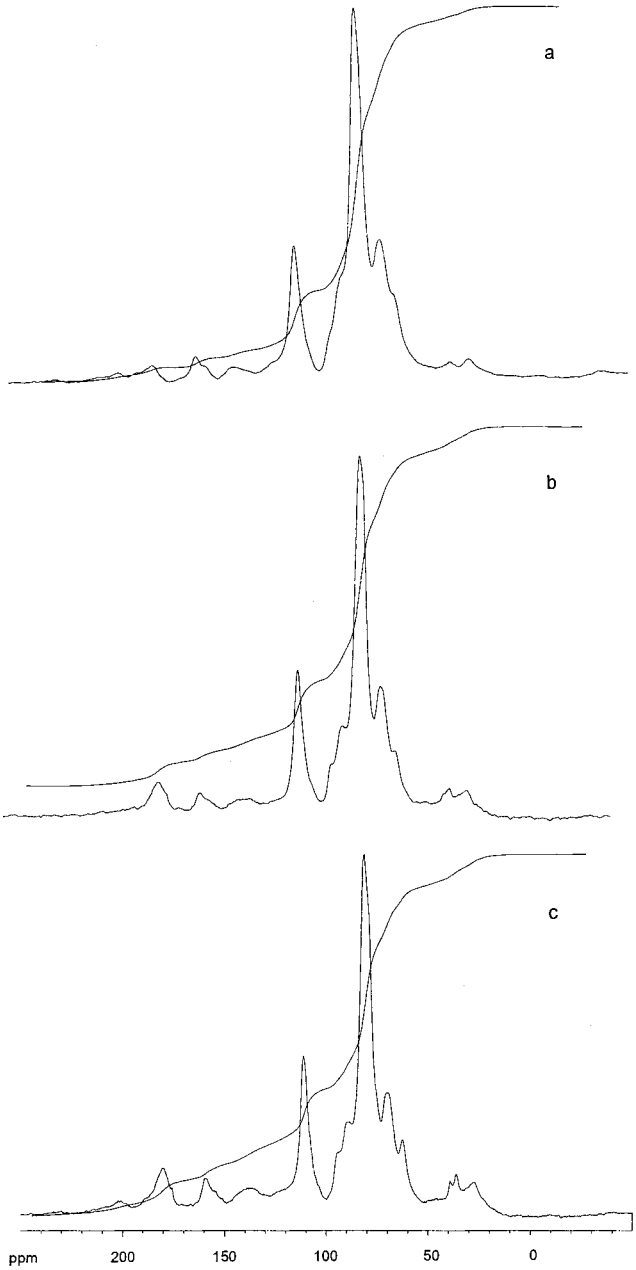

Figure 7. Solid-state ^{13}C CP/MAS NMR spectra for (a) undegraded control wheat straw, (b) wheat straw degraded for 28 days by *P. ostreatus*, and (c) wheat straw degraded for 63 days by *P. ostreatus*.

for the dominant cellulose band. Figure 8 shows the corresponding dipolar dephased spectra obtained with

Table 2. Distribution of Carbon in Native and *P. ostreatus*-Degraded Wheat Straw as a Percentage of the Total Carbons from ^{13}C NMR Spectra

	% carbon						
sample time (days)	acetyl	lipid, protein	methoxyl	carbohydrate (C-2,C-3, C-4,C 5, and C-6 of cellulose and xylans)	carbohydrate (C-1 of cellulose and xylans) and aliphatic lignin	aromatic lignin	carboxyl/ carbonyl
0	1.9	1.4	4.6	65.0	9.8	12.4	4.9
7	1.7	2.2	2.8	64.0	10.0	14.4	4.9
14	2.8	2.4	3.1	63.8	10.8	11.8	5.3
21	2.1	1.9	2.6	64.9	10.5	11.9	5.1
28	2.6	2.4	2.6	63.6	10.7	11.4	6.7
35	2.6	2.8	2.0	63.7	10.6	11.5	6.8
42	3.2	3.1	2.9	61.9	9.4	11.8	7.7
49	4.2	2.9	2.6	61.5	9.9	11.5	7.4
56	3.4	4.0	3.2	59.7	9.9	11.3	8.5
63	2.4	3.1	2.8	59.4	9.4	14.1	8.8

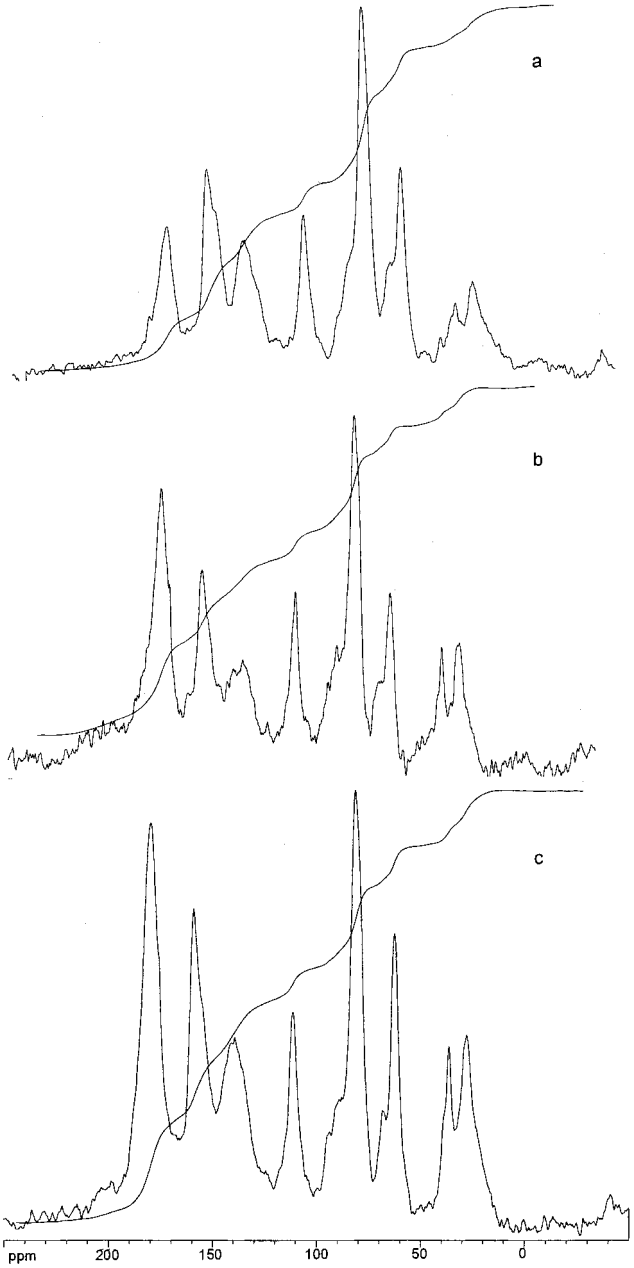

Figure 8. Solid-state ^{13}C CP/MAS/DD NMR spectra obtained with a dephasing time of 50 μs for (a) undegraded control wheat straw, (b) wheat straw degraded for 28 days by *P. ostreatus*, and (c) wheat straw degraded for 63 days by *P. ostreatus*.

a dephasing time of 50 μs. Dipolar dephased spectra obtained using a dephasing period of 100 μs are presented for the control (Figure 9a) and the degraded wheat straw following 63 days of incubation with *P. ostreatus* (Figure 9b). The methoxyl bands in the dipolar dephased spectra are well resolved from the carbohydrate resonances, which are significantly reduced in intensity compared to the normal CP spectra. All of the spectra are presented with integrals across the whole chemical shift range. Resonances from cellulose and xylans dominate the 60−110 ppm region (Figure 7a). The distinct broad resonance at 72 ppm is from C-2, C-3, and C-5 carbons of cellulose as well as carbons from xylans (*16*, *17*). Other significant cellulose peaks are at 65 ppm (C-6), 106 ppm (C-1), and 84 and 89 ppm (both C-4) (Figure 7a). However, there are signals at 75.7, 81.4, and 63.6 ppm in the ^{13}C NMR spectra of acetylated milled wood lignins from wheat straw, which have been assigned to the different carbon atoms α, β, and γ in β-O-4 structures, respectively (*15*). The areas of the

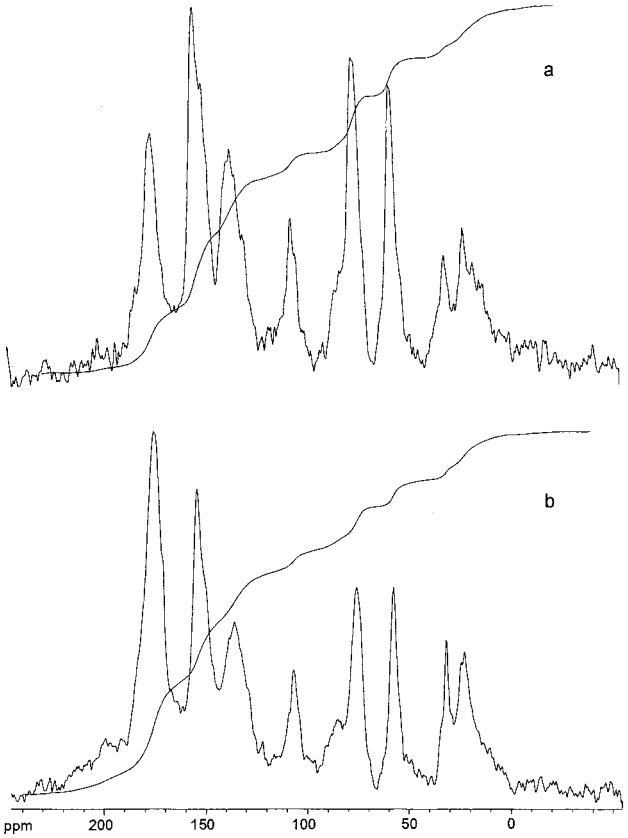

Figure 9. Solid-state ^{13}C CP/MAS/DD NMR spectra obtained with a dephasing time of 100 μs for (a) undegraded control wheat straw and (b) wheat straw degraded for 63 days by *P. ostreatus*.

different carbon resonances at 65, 72, 84, 89, and 106 ppm provide estimates of the relative amounts of cellulose and xylans (*17*) (Table 2). Undegraded wheat straw was composed of 74.8% of carbohydrate carbon, similar amounts were observed for incubation times up to and including 49 days (Table 2). At 56 and 63 days the amounts of carbohydrate carbon were 69.6 and 68.8%, respectively (Table 2). However, the resolution of the carbohydrate resonances improves with increasing time (Figure 7), and this probably reflects the greater resistance of crystalline cellulose to degradation in relation to amorphous cellulose and xylans.

The resonance at ~174 ppm can indicate the presence of amide carbons in proteins as well as acetate groups, which are known to be present in hemicellulose, and carbonyls in lignin (*18*−*20*). Increasing intensity in the carboxyl/carbonyl region (160−200 ppm) was observed during mushroom growth. Amounts of carboxyl/carbonyl carbon increased from 4.9% of the total carbon (mole percent) in undegraded wheat straw to 8.8 mol % after 63 days of mushroom growth (Table 2). However, because the N/C atomic ratio increased only from 0.015 to 0.033, this corresponds to an increase from 1.5 to 3.3 mol % carboxyl/carbonyl carbon given there is one carbonyl carbon per amide nitrogen. Therefore, it can be estimated that the carboxyl/carbonyl carbon content increased from 3.4 to 5.5 mol % due to oxidative degradation, which is clearly significant.

^{13}C NMR of Bermuda grass yielded a resonance at ~31.9 ppm, which indicated aliphatic CH_2 groups that may be present in proteins and lipids (*21*). Because wheat straw is composed of 4.7−5.7% dry weight protein, the resonance at 33 ppm is probably associated primarily with protein (*5*, *22*). The relative amount of methylene carbon in native wheat straw was 1.4%, which increased after 7 days to an average of 2.7% over the incubation period (Table 2).

Both native and degraded wheat straw exhibit resonances between 130 and 100 ppm from carbons that are H substituted, which occur downfield from the signals between 153 and 134 ppm and are assigned to carbons substituted by oxygen or other carbon atoms (Figure 9a) (*15*, *16*, *23*). The peak at 153 ppm is attributed to C3 and C5 carbons in syringyl aromatic units, which are O-alkylated. The total aromatic lignin content for native wheat straw was 12.4%, which is similar to the aromatic contents measured for wheat straw degraded for times up to and including 63 days, which suggests that there is no overall loss in lignin upon mushroom growth (Table 2).

The well-resolved peak in the dipolar dephased spectra (Figures 8 and 9) at 56 ppm is assigned mainly to methoxyl carbons from lignin, but glucuronic acid in xylan could also contribute to this signal (*19*). Some variation in the relative intensity of the methoxyl peak to the aromatic carbon lignin peaks is evident (Figures 8 and 9). However, the variations are not significant, bearing in mind the complex spin dynamics involved in dipolar dephasing by which the methoxyl resonance is decaying more rapidly than that of the aromatic carbons and the relative rates probably vary from sample to sample (compare Figures 8a,b and 9a,b).

DISCUSSION

The systematic decrease in C/N atomic ratios from 68.3 in native wheat straw to 29.5 after 63 days of decay confirmed that the treated wheat straw residues had been chemically altered relative to the undegraded analogue (Table 1). Fungal biomass has a C/N ratio of ~8, and here the undegraded wheat straw has a C/N ratio, if atomic weights are not factored in, of 58.6 (*24*). The decrease in C/N atomic ratios could be due to increasing amounts of fungal mycelium within the wheat straw. Similarly, C/N ratios decreased from 27.2 in fresh cattle manure to 8.7 after 147 days of composting (*25*), and nitrogen contents increased from 0.2% in wheat straw to 1.4% after 76 days of growth of *Agaricus bisporus*.

Thermochemolysis products from the control sample consisted of P, G, and S derivatives; the presence of all three phenylpropane structures is consistent with lignin derived from wheat straw (*15*). Previous work on the ^{13}C NMR spectroscopy of wheat straw lignin extracts showed that *p*-coumaric acid was mainly ester-linked to lignin, whereas 35−75% of the recovered ferulic acid was ether-linked to milled straw lignin or enzyme lignin (*26*). In this study the cinnamic acid derivatives, P18 and G18, could therefore arise by breaking either ether or ester bonds during thermochemolysis (Figure 1a). The decrease in the C/G ratio with increasing cultivation time is explained by the loss of P18 and G18 relative to the other thermochemolysis products (Figure 3b).

The solid-state ^{13}C NMR spectra of ^{13}C-enriched lignin in wheat straw from coniferin and an unenriched counterpart share resonances between 75 and 94 ppm, which may be assigned to β carbon atoms in β-O-4-type structures. The signal intensity of these units suggested that between 65 and 74% of lignin monomers in wheat straw lignin are linked by β-O-4 bonds (*27*). The mechanism of thermochemolysis in the presence of tetramethylammonium hydroxide is selective for cleavage of β-O-4 bonds when there is an aliphatic α-hydroxyl group on the alkyl side chain (*9*). However, ~18−28% of lignin monomers in wheat straw are linked via β-5, $\beta-\beta$, and β-1 bonds (*27*), which are not broken during thermochemolysis. Therefore, the thermochemolysis products from wheat straw are only partially representative of lignin, where there was an apparent 41% decrease in combined lignin and cinnamic acid derivatives (Λ values) after 63 days of decay. As the solid-state ^{13}C NMR evidence has indicated that this does not represent an overall loss of lignin with fungal degradation (Table 2), instead the decreasing Λ values with increasing cultivation time can be better explained by one or both a decrease in frequency of labile ether bonds, due to recondensation reactions during fungal decay, and a decrease in cinnamic acid content.

The decreases in the S/G ratio of the wheat straw lignins with cultivation time (Figure 3a) may result from syringyl units being more susceptible to degradation because they are involved in fewer aryl−aryl bonds and have a lower redox potential than the guaiacyl units (*28*). Thermochemolysis is selective toward cleavage of aryl−alkyl ether linkages, so we do not assert that the decreases in S/G values occur throughout lignin but suggest that the preferential decay of syringyl compared to guaiacyl moieties occurred in lignin units linked by β-O-4 bonds. These decreases contrast with observations from earlier studies. Analytical pyrolysis (Py-GC-MS) of beech wood treated with *P. ostreatus* for 98 days revealed very little difference in the relative yield of guaiacyl- and syringyl-type phenols upon biodegradation (*29*). Another study, using both copper oxide depolymerization and Py-GC-MS, showed that wheat straw

decayed for 80 days by *P. eryngii* also showed little change in the S/G ratio (*4*). These suggest that, in light of the present work, both the nature of the starting material and the species of *Pleurotus* used in the biodegradation process are important controls on the relative extents of decay of syringyl and guaiacyl moieties.

The large increases in $(Ad/Al)_G$ and $(Ad/Al)_S$ (Figure 4a,b) suggested that *P. ostreatus* had induced oxidative cleavage of the Cα−Cβ bonds located on the alkyl side chain in a manner similar to other white rot fungi (*30*). Aromatic aldehyde derivatives are formed from alcohols by fungally mediated oxidative cleavage of Cα−Cβ bonds at the Cα position and then the aldehydes are subsequently oxidized to carboxylic acids (*31*−*33*).

Thermochemolysis products G14 and G15 are derived from lignin moieties with a complete methylated glycerol side chain. Comparison of the abundances of the thermochemolysis products from native and degraded wheat straw showed a decrease of G14 and G15 relative to other thermochemolysis products with a cleaved side chain such as G6 (Figure 1a−c). This side-chain-cracking reaction can be tracked by plotting Γ as a function of incubation time (Figure 5), which increases by an order of magnitude, indicating extensive side-chain oxidation and associated degradation, after 42 days of mushroom growth. For incubation times in excess of 42 days, either this process is then inhibited or the rate of loss of the short-chain lignin units becomes similar to the rate of side-chain cleavage.

^{13}C NMR spectra of the undegraded control and the degraded samples show that cellulose and xylans were the major structural components of the wheat straw throughout the 63 days of cultivation (Table 2). Other workers have observed that in the spectra of highly crystalline celluloses a sharp resonance is observed at 89 ppm, whereas broad signals are reported from amorphous noncellulosic polysachcharides (*17*, *19*). The improved resolution of polysaccharide resonances, with increasing cultivation time, including the shoulder at 89 ppm upon decay (Figure 7a−c), is consistent with the removal of amorphous noncellulosic polysaccharides and persistence of relatively crystalline cellulose in the fungally decayed residues. A similar rapid degradation of the noncellulosic polysaccharides in wheat straw was reported following biodegradation by *P. ostreatus* (*22*). The moderate decrease in carbohydrate content with time may be explained by the incorporation of fungal biomass, namely, mycelium of *P. ostreatus*, within the cell walls of the residual wheat straw. Incorporation of fungal polysaccharides could mask the full extent of cellullose and xylan degradation. This notion is supported by the concomitant increases in nitrogen content and increasing signal intensity of the resonance at 174 ppm from the amide carbon in proteins with increasing fungal attack (Tables 1 and 2) and is consistent with the possibility that invading fungal proteins from *P. ostreatus* account for the decrease in C/N ratio. Similarly, the increase in the resonance at ~33 ppm due to protein and lipid content (*21*) could also be due to the mixing of fungal biomass with wheat straw. The concomitant increase in carboxyl/carbonyl groups with time could be due to an increase in aromatic acids from the oxidative cleavage at Cα−Cβ bonds in the lignin side chains. This is supported by the increases in $(Ad/Al)_G$ and $(Ad/Al)_S$ observed in residual wheat straw samples analyzed by thermochemolysis (Figure 3a,b).

Off-line thermochemolysis is a useful analytical technique for monitoring chemical changes in commercial mushroom composts. The primary structure of the biopolymer lignin is substantially degraded during commercial growth of *P. ostreatus*. The main transformations to the lignin structure include preferential decay of syringyl compared to guaiacyl moieties, oxidative cleavage between Cα−Cβ bonds in lignin side chains, and degradation of the cinnamic acids. Although solid-state ^{13}C NMR analysis is an effective tool for obtaining structural information on native wheat straw, upon fungal degradation spectral interpretation is complicated by the presence of resonances from polysaccharides and proteins in the fungal mycelia.

ABBREVIATIONS USED

$(Ad/Al)_G$, 3,4-dimethoxybenzoic acid, methyl ester/3,4-dimethoxybenzaldehyde; $(Ad/Al)_S$, 3,4,5-trimethoxybenzoic acid, methyl ester/3,4,5-trimethoxybenzaldehyde; Γ, 3,4-dimethoxybenzoic acid, methyl ester/sum of threo and erthyro forms of 1-(3,4-dimethoxyphenyl)-1,2,3-trimethoxypropane; Λ, sum of lignin and cinnamic acid derivatives normalized to 100 mg of organic carbon in sample; (S/G), syringyl/guaiacyl; (C/G), cinnamyl/guaiacyl.

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