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Fungal Upgrading of Wheat Straw for Straw-Thermoplastics Production

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Abstract

Combining biologic pretreatment with storage is an innovative approach for improving feedstock characteristics and cost, but the magnitude of responses of such systems to upsets is unknown. Unsterile wheat straw stems were upgraded for 12 wk with *Pleurotus ostreatus* at constant temperature to estimate the variation in final compositions with variations in initial moisture and inoculum. Degradation rates and conversions increased with both moisture and inoculum. A regression analysis indicated that system performance was quite stable with respect to inoculum and moisture content after 6 wk of treatment. Scale-up by 150× indicated that system stability and final straw composition are sensitive to inoculum source, history, and inoculation method. Comparative testing of straw-thermoplastic composites produced from upgraded stems is under way.

Index Entries: Fungal upgrading; engineered storage; biological preprocessing; *Pleurotus ostreatus*; straw composite.

Introduction

Agricultural crop residues are a valuable renewable biomass resource. In 1999, American farmers harvested 53,909,000 acres of wheat *(1)*. The straw from this acreage of wheat represents >50 million t annually. Currently, some of the straw is harvested (baled) for use as livestock bedding or low-grade animal feed. However, these low-value uses provide only a

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minimal return. Nationally, only about 3.2% of the economic return on wheat is from straw *(1)*. Producers have long recognized the potential economic and environmental benefits of producing forage, bioenergy, and bioproducts from excess wheat straw residue. However, because of the low bulk density of straw and the loss of fermentable sugars to microbial activity during storage, there are harvest, transportation, storage, and preprocessing methods and logistics issues that must be worked out before the excess straw can be economically utilized on a national scale.

The U.S. Department of Energy and U.S. Department of Agriculture recently began a concentrated national effort under the Biomass Research and Development Act of 2000 to develop and demonstrate working biorefineries in the near term. The "vision" and "roadmap" documents for near-term utilization of agricultural residues to produce fuels, chemicals, and bioproducts have recently been completed and focus primarily on corn stover and cereal straws as the feedstocks *(2,3)*. Objectives and research pathways identified in the roadmap document for stover and straw preprocessing and storage issues include the following *(3)*:

1. Cost-Effective Pre-Delivery Treatment Processes—The development and testing of cost-effective pre-conversion treatment processes to increase energy- and chemical-density of raw materials at the point of harvest.
2. Best Practices for Harvesting and Storage—The biomass/agricultural communities must identify, develop, test, and implement best practices for cost-effective and environmentally sound pre-treatment, collection, storage, and transport of plant and animal residue-based feedstocks. This should lead to improved plant and animal residue recovery, more effective separation, improved handling and storage technologies/procedures, and reduced environmental impacts.

Thus, several issues related to preprocessing and storage have been identified as important research and development priorities for the near term. An innovative and potentially useful approach to addressing these issues would be to combine preprocessing and storage into a single system. In this way, energy use and infrastructure could be reduced by modifying the feedstock while it is waiting to be utilized. These modifications could be biological or chemical in nature. In the case of biological treatments, for such a system to be workable, it would be necessary for microbes carrying out the desired modifications to outcompete indigenous microorganisms vying for the same resources.

Straw utilization for composites is limited by poor resin and polymer penetration, and excessive resin consumption owing to the straw cuticle, fines, and the lignin-hemicellulose matrix *(4)*. Some white-rot fungi, including *Pleurotus ostreatus*, degrade the cuticle and selectively degrade lignin and hemicellulose, leaving behind relatively more cellulose *(4)*. Thus, treatments by these fungi could potentially be used to improve resin penetration and resin binding without the use of physical or chemical pretreat-

ments. Although long treatment times and large footprints limit the use of fungal treatments on a large scale, distributed fungal pretreatments could alleviate land requirements.

In a previous study *(4)*, we presented the results of a preliminary investigation to determine whether *P. ostreatus* could be competitive with indigenous organisms in unsterilized wheat straw stems. A detailed description of the potential benefits of preparing straw-thermoplastic composites from wheat straw stems upgraded by selective degradation by a white-rot fungus was provided in that study *(4)*. In general, the potential benefits focus primarily on the reduction of fines (reduced external surface area) via a selective harvest method *(5)*, and removal of amorphous matrix components by *P. ostreatus* to increase internal surface area and allow better penetration of composite formulation components into the lignocellulose matrix *(4)*. Our previous study was conducted with the aim of moving toward the development of a passive, potentially distributed fungal upgrading system to improve feedstock characteristics for production of straw-thermoplastic composites *(4)*. As envisioned, the system would be constructed and operated similarly to passive composting systems and could be operated for 12 wk or longer in a distributed or centralized manner, depending on land use requirements. Such a system fits within the frameworks of both engineered storage systems and pre-conversion processing.

In the preliminary study it was found that above about 11 mg of *P. ostreatus*/g of stems and 0.77 g of H_2O/g of stems, the inoculated *P. ostreatus* was generally competitive with indigenous microbes *(4)*, which is consistent with a previous report showing good competitiveness of *Pleurotus* sp. with soil microorganisms *(6)*. In the present article, we describe completed laboratory studies conducted at the Idaho National Engineering and Environmental Laboratory (INEEL) that were tasked with determining acceptable moisture and inoculum ranges for pilot-scale fungal upgrading tests. Inoculated *P. ostreatus* was found to more completely dominate degradation of the straw stems as inoculum size and moisture content increased, but to be less selective with respect to polysaccharide degradation. Inoculum and moisture levels of 40 mg of *P. ostreatus*/g of stems and 1.6 g of H_2O/g of stems, respectively, allowed successful competition of the inoculum with indigenous organisms and gave acceptable amounts of degradation of xylan and glucan (on a total degradation basis). Statistical analysis of the data was conducted to predict the variability of final compositions in response to ±30% variations in initial moisture and inoculum levels. Minimal variations in final composition would be desirable to ensure consistent product composition in outdoor systems having few environmental controls. In addition, we present the experimental design for the composite formulation/extrusion testing, as well as initial results from several extrusion tests conducted at the Wood Materials & Engineering Laboratory at Washington State University (WSU). In the near term, these data will be used to devise and test a pilot-scale fungal upgrading windrow system at WSU for demonstration of larger-scale operation and extrusion.

Table 1
Composition of Westbred 936 Straw Stem Fraction Used in Fungal Treatment Studies[a]

Component	Wt %[b]
Glucan	37.2 ± 0.8
Xylan	22.1 ± 0.5
Galactan	1.2 ± 0.8
Arabinan	3.0 ± 0.4
Mannan	1.6 ± 0.4
Lignin with extractives	18.9 ± 0.1
Ash	10.1 ± 0.0
Other [c]	5.8 ± 2.1
SUM	100.0 ± 0.2

[a] Uncertainties given are the SDs for four independent replicate measurements.
[b] Based on 100% dry wt of material.
[c] Remaining fraction attributed to unknown uronic acids, proteins, and so on, and to recovery errors in analysis techniques.

Materials and Methods

Wheat Straw

Westbred 936 wheat straw, a hard red spring variety, was obtained from Grant 4-D Farms (Rupert, ID). All the straw utilized was produced during the year 2000 cropping season and rebaled and stored as previously described *(4)*. Straw stems utilized for these tests were mechanically separated as previously described *(5)* during the fall of 2000 and were stored indoors at 21 ± 2°C and 13% moisture until used (all tests were begun by the fall of 2002). The composition of the untreated straw stem fraction, determined as described under Compositional Analyses, is shown in Table 1.

Cultures and Maintenance

P. ostreatus NRRL 2366 was chosen for use in the fungal degradation tests based on its ability to selectively degrade the noncellulose components of wheat straw *(7,8)*. It was obtained from the Northern Regional Research Laboratory (Peoria, IL). Stock cultures were maintained at Utah State University at room temperature on agar slants containing 20 g/L of YM agar (Difco, Detroit, MI) and the following trace minerals: 0.02 g/L of $FeSO_4 \cdot 7H_2O$, 0.004 g/L of $CuSO_4 \cdot 5H_2O$, 0.002 g/L of $ZnSO_4 \cdot 7H_2O$, 0.002 g/L of $MnSO_4 \cdot H_2O$, and 0.001 g/L of $(NH_4)_2MoO_4 \cdot 4H_2O$. Stock cultures were subcultured every 2 wk. Stock mycelial inocula were produced at Utah State University as follows: Fungal mycelia were transferred from the maintenance slants to 100 mL of 20 g/L YM broth (Difco) using a sterile loop and grown in agitated culture for 2 to 3 d at room temperature

and 180 rpm. This culture was transferred to a sterile Fernbach flask containing 1 L of 20 g/L YM broth with trace minerals as already described, and agitated for 4 d at room temperature and 180 rpm. The fungal pellets were harvested by light centrifugation (380*g*) in sterile centrifuge bottles, transferred to sterile bottles with sufficient spent medium to submerge the pellets, shipped under refrigeration to the INEEL, and stored at 4°C until use (typically 2 to 3 wk or less).

Small-Column Experiments

Unsterile straw stems were used in all experiments because sterilization of large quantities of straw for construction of large windrows would be uneconomical and impractical. For this strategy to be effective, it was necessary that the inoculated fungus be able to compete with the indigenous organisms in the straw. Since white-rot fungi dominate in nature under conditions of nitrogen deprivation *(9)*, experiments were previously performed *(4)* to determine inoculum production conditions necessary to limit nitrogen addition during inoculation of the straw with the fungus. C/N ratios in the media tested ranged from about 29 to 89 and were adjusted by adding yeast extract to 20 g/L glucose solutions. The nitrogen-limited medium finally utilized for straw stem inoculum production contained 3.0 g/L of yeast extract, for a C/N of 32.6. Mycelial inocula for inoculation of straw stems were produced in this nitrogen-limited medium, using as inoculum the fungal pellets produced at Utah State University in YM broth *(4)*.

Approximately 500 mL of wet fungal pellets of *P. ostreatus* grown at Utah State University in YM broth were transferred to a sterile blender and blended for 2 min, producing a slurry of finely chopped mycelial fragments. The optical density (OD) at 550 nm was determined for dilutions of this slurry using a standard UV/VIS spectrophotometer. The undiluted slurry was then inoculated to 1.0 OD into the fresh nitrogen-limited medium in sterile shake flasks and incubated for 5–7 d at 30°C, 135 rpm. The fungal pellets in the inoculum cultures were then transferred with the spent medium to a sterile blender and blended for 2 min. The OD at 550 nm was determined for dilutions of this slurry, and the concentration of biomass was estimated from a previously measured calibration. The undiluted slurry was transferred to a sterile hand-pump garden sprayer for addition to the straw stems. No extraordinary measures were taken beyond this point to maintain sterility, except the use of initially sterile equipment.

Air-dried straw stems (150 g dry wt at about 9–13 wt% moisture) were weighed onto a clean, dry, tared tray and spread in a thin (5-cm) layer. The homogenized mycelial inoculum slurry was then sprayed onto the stems, with frequent mixing of both the inoculum and the stems. Sufficient inoculum was added to reach the desired initial level of fungal inoculum in the stems. Periodically during addition of inoculum, a fan was used to blow nonsterile air across the tray of inoculated straw to evaporate excess water,

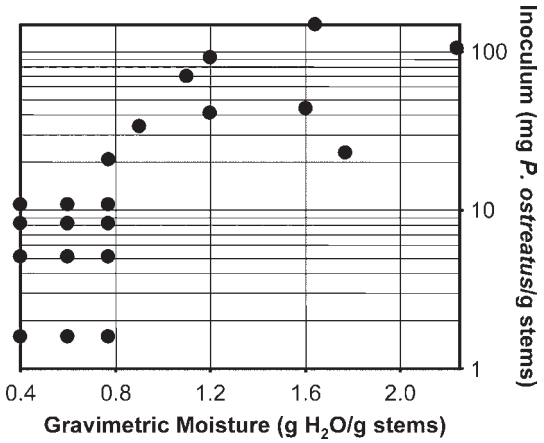

Fig. 1. Combinations of inoculum amount and moisture content tested.

with frequent mixing of the straw. After the desired amount of inoculum was added, additional sterile distilled water was sprayed onto the straw as needed to reach the desired initial moisture content for the particular experiment. A separate sample of the well-mixed inoculum slurry was then added to a tared bottle and dried to constant weight at 105°C to determine the actual biomass concentration of the slurry. In addition, several small samples of the inoculated stems were transferred to tared bottles and dried to constant weight at 105°C to determine the actual moisture content of the inoculated stems.

Combinations of inoculum amounts and moisture contents tested in this exploratory study are shown in Fig. 1; controls lacking inoculum were also conducted at 0.4–0.77 g of H_2O/g of stems and are not shown in Fig. 1. The first tests performed *(4)* are represented by the 12 points in the lower left-hand corner of Fig. 1. When these tests indicated that higher inoculum was needed for better selectivity and that higher moisture was needed for faster degradation *(4)*, parameter testing moved to the combinations plotted in the upper middle and right-hand corner of Fig. 1.

The inoculated straw was added to clean, initially sterile columns fabricated from glass process pipe as previously described *(4)*. The columns were prepared in triplicate with approx 50 g dry wt of inoculated stems in each column. The loaded columns were supplied with humidified oil-free instrument air at 193 kPa and a flow rate sufficient to turn over the air in the system once per day (about 10 mL/min). Approximately 2.5 g (dry wt) of straw was sampled from the top and bottom of each column initially and approx every 3 to 4 wk thereafter for 12 wk. The samples were combined, dried to constant weight overnight at 105°C, and ground to 60 mesh in a Wiley mill for compositional analyses.

Large-Scale Treatment in Drums

Straw stems were first treated for 6 wk in small columns with *P. ostreatus* at 40 mg of *P. ostreatus*/g of stems and 1.6 g of H_2O/g of dry stems, as described. These stems were then inoculated 1:10 into fresh stems, the moisture content was adjusted to 1.6 g of H_2O/g of dry stems, and the mixture was added to the drums for treatment. Because of the large amount of nitrogen-limited inoculum needed for the initial small-column step, the nitrogen-limited inoculum was produced in a slightly different manner than described above. This mycelial inoculum was produced at Utah State University as previously, but the mycelia from the maintenance slants were transferred directly into the nitrogen-limited medium without first being enriched in YM broth. Thus, both of the enrichment steps in the preparation of this mycelial inoculum were carried out in the nitrogen-limited medium. The fungal pellets produced in this manner were harvested as before, shipped under refrigeration to the INEEL, and stored at 4°C until use (up to 4 wk).

After 6 wk the treated stems were removed from the glass columns and mixed by hand at 1:10 (w/w) with fresh, air-dried, uninoculated straw stems. Random samples of the 6-wk-degraded stems were dried, ground to 60 mesh, and analyzed for composition as described under Compositional Analyses. While it was not known how much inoculum would be necessary for the altered inoculation method, a 10 wt% inoculation of wood chips containing an active culture of the desired white-rot fungus has been successful in soil bioremediation *(10)*. The moisture content was brought to 1.60 g of H_2O/g of stems by spraying distilled water with a pressurized garden sprayer onto the fresh stems as they were mixed with the treated straw from the glass columns. The inoculated stems were then packed into 208.5-L drums at about 7.5 kg dry wt of inoculated straw per drum. Before loading the drums, a 56-cm-diameter perforated steel disk was placed in the bottom of each drum and elevated to about 5.7 cm above the bottom of the drum using screws. Humidified oil-free instrument air at 127.6 kPa was supplied at 400–500 mL/min to the bottom of each drum beneath the perforated disk; the pressure drop over each drum was about 41.4 kPa. The air exited the system separately through the centers of the lids of each drum through in-line 16-cm^2 Whatman HEPA-Vent Filters with a porosity of 0.3 μm (Whatman, Newton, MA). After 6 or 12 wk of treatment, the drums were opened and several samples were removed from various locations within the straw beds. The samples were dried, ground to 60 mesh, and analyzed for composition as described under Compositional Analyses. The drums were then resealed and shipped to the Wood Materials and Engineering Laboratory at WSU for analyses of various composite formulations and extrusion testing. Untreated straw was also sent to WSU for these analyses. For the composite testing, the straw samples were referred to as Neat (untreated), Degrade1 (treated for 6 wk), and Degrade2 (treated for 12 wk).

Compositional Analyses

Ash content was determined as follows: At least 1 g of dry stems, ground to 60 mesh, was ashed in a muffle furnace at 650°C for 18–24 h. Ash content was calculated by weight difference. Carbohydrate and lignin compositions of untreated and treated straw samples were determined by quantitative saccharification using the method of Saeman et al. *(11)*. Two aliquots of each sample were analyzed by quantitative saccharification for each of the three replicate columns at each condition, for a total of 12 independent measurements of each composition. Carbohydrate analyses were done by high-performance liquid chromatography using a Bio-Rad HPX-87P carbohydrate column as previously described *(12)*. The acid-insoluble fraction from the quantitative saccharification was ashed at 650°C, and Klason lignin with extractives was calculated by weight difference. The amounts of glucan and xylan degraded per 100 g of initial weight were calculated by mass balance assuming that the sum of lignin, ash, and extractives remained constant. The percentage conversions of glucan and xylan (ΔG and ΔX, respectively) were then calculated by dividing by the initial basis weight of each and multiplying by 100.

Preliminary Extrusion Tests

To evaluate how the formulation components affect extrusion product performance, a fractional factorial design was created for statistical analysis. The fractional design is shown for the Neat and treated straw composite testing in Table 2. As already noted, Degrade1 and Degrade2 represent wheat straw that was inoculated with *P. ostreatus* and incubated for 6 and 12 wk, respectively. The values in Table 2 are percentages required to make a 2-kg batch for extrusion.

Composite formulations were prepared as follows: The straw samples as received from INEEL were ground to 0.69 mm in a hammer mill and oven dried to 1.1% moisture. The dried straw samples were then blended with various amounts of high-density polyethylene (HDPE), lubricants, and maleated polyethylene blends (MAPE) (*see* Table 2). The mixed formulations were then extruded with a 35-mm Cincinnati Milacron® Model CMT 35 counterrotating conical twin screw extruder (Cincinnati Milacron, Batavia, OH), which produced a 9.525×38.1 mm^2 solid cross-section. Flexural strength, density, and water sorption were measured for the extruded samples according to ASTM Standard Methods *(13,14)*.

Results and Discussion

The experimental data presented herein are the result of exploratory research aimed at bracketing the necessary moisture and inoculum loads for effective pilot-scale distributed upgrading of wheat straw stems for production of straw-thermoplastic composites *(4,15)*. An exploratory approach was chosen for these tests because full-scale outdoor systems having few environmental controls would be difficult if not impossible to closely control. Both temperature and moisture levels vary owing to variations in heat,

Table 2
Design of Experiment for Wheat Straw Extrusion[a]

Run no.	Straw stem treatment	Straw stems (wt%)	HDPE (wt%)	MAPE (wt %)	Lubricant (wt %)
3	Degrade1	60	36.25	3	0.75
5	Degrade1	55	42	0	3
8	Degrade1	75	22	0	3
9	Degrade1	70	26.75	1	2.25
10	Degrade1	55	42	0	3
14	Degrade1	60	38.25	1	0.75
24	Degrade1	55	41	4	0
25	Degrade1	55	38	4	3
26	Degrade1	75	18	4	3
27	Degrade1	60	36.25	3	0.75
29	Degrade1	55	45	0	0
30	Degrade1	60	38.25	1	0.75
32	Degrade1	75	18	4	3
33	Degrade1	75	21	4	0
1	Degrade2	55	38	4	3
4	Degrade2	75	21	4	0
6	Degrade2	65	35	0	0
7	Degrade2	55	42	0	3
11	Degrade2	55	41	4	0
13	Degrade2	75	22	0	3
15	Degrade2	55	45	0	0
16	Degrade2	65	35	0	0
20	Degrade2	55	38	4	3
31	Degrade2	75	18	4	3
2	Neat	75	18	4	3
12	Neat	55	38	4	3
17	Neat	55	41	4	0
18	Neat	55	45	0	0
19	Neat	65	32	0	3
21	Neat	55	42	0	3
22	Neat	65	31.5	2	1.5
23	Neat	65	31.5	2	1.5
28	Neat	75	25	0	0
34	Neat	65	31	4	0

[a]Five replicates were run for each formulation.

humidity, and wind. Precise real-time control of temperature and moisture in such a system would be very expensive, and thus counter to the goal of using distributed systems. We therefore decided to choose conditions of moisture and inoculum that provided average fungal degradation of the stems and rapid competitiveness of the inoculated fungus at a single temperature. We then conducted statistical analyses of the data in order to predict the expected variability of final composition in response to variations in initial moisture and inoculum.

Proxy Variable for Degradation Tests

Tests were designed to determine moisture levels and inoculum amounts that maximized hemicellulose degradation. In preliminary testing *(4)* it was shown that 10.9 mg of *P. ostreatus*/g of dry stems was sufficient to allow successful competition of the inoculated fungus with indigenous microbes. However, 10.9 mg of *P. ostreatus*/g of dry stems and a gravimetric moisture content of 0.77 g of H_2O/g of straw were too low to effect significant degradation of the straw in 12 wk *(15)*. In those tests a proxy variable, the ratio of cellulose and hemicellulose compositions (*C/H*), was used to indicate the relative change in composition occurring from indigenous microbes and by *P. ostreatus*. This ratio was used because *P. ostreatus* has been shown to be somewhat selective for hemicellulose and lignin degradation vs cellulose degradation *(7,8)*, while the indigenous microbes were shown in uninoculated controls to be nonselective for one polysaccharide or the other *(4)*.

Owing in part to the low and thus more uncertain measurements of the nonxylan carbohydrate components of hemicellulose (galactan, arabinan, and mannan), we decided to change the proxy variable to one exhibiting less variability as the result of measurement uncertainty. Two additional proxy ratios were assessed: the ratio of glucan and xylan compositions (*G/X*) and an estimate of the relative degradation of xylan vs glucan ($\Delta X/\Delta G$). This "degradation ratio" was calculated from the estimated conversions, which assumed little change in the sum of ash, lignin, and extractives. Implicit in this is an assumption of minimal mineralization of lignin to CO_2, and thus losses of lignin are assumed to be from depolymerization to extractives; extractives would increase, keeping the sum of lignin and extractives constant. This allowed a closed mass balance to be estimated and the amounts of xylan and glucan degraded to be calculated on an initial weight basis.

A comparison of these ratios for the preliminary testing at 0–10.9 mg of *P. ostreatus*/g of stems and 0.77 g of H_2O/g of stems is shown in Table 3. The majority of the glucan/xylan-based ratios had lower standard deviations than the corresponding cellulose/hemicellulose-based ratios, which was expected because xylan represents a greater fraction of the straw stems than galactan, arabinan, and mannan (Table 1). The relative changes in the three proxy variables were consistent among the data. For example, when *C/H* and *G/X* did not change significantly, indicating nonselective degradation of glucan and xylan, the estimated degradation ratio $\Delta X/\Delta G$ was about 1. Similarly, when *C/H* and *G/X* exhibited only a small increase, $\Delta X/\Delta G$ was only slightly larger than 1, while larger increases in *C/H* and *G/X* corresponded with large increases in $\Delta X/\Delta G$. This may provide some support for the assumption used to estimate $\Delta X/\Delta G$. The proxy ratio chosen for use was the degradation ratio $\Delta X/\Delta G$; with this change, the con-clusions of the preliminary study were unchanged, that is, *P. ostreatus* was shown to outcompete the indigenous organisms by 56 d. In addition, from 0 to 22 d the $\Delta X/\Delta G$ ratios did not change significantly from 1.0, suggesting that under

Table 3
Comparison of Cellulose/Hemicellulose, Glucan/Xylan, and Xylan Degradation/Glucan Degradation Ratios for Preliminary Tests *(4)* at 0.77 g of H_2O/g of Dry Stems [a]

	Amount of Inoculum (mg of *P. ostreatus*/g of dry stems)				
Day/ratio [b]	0	1.6	5.1	8.2	10.9
Day 0					
C/H	1.33 ± 0.08	1.33 ± 0.08	1.33 ± 0.08	1.33 ± 0.08	1.33 ± 0.08
G/X	1.69 ± 0.06	1.69 ± 0.06	1.69 ± 0.06	1.69 ± 0.06	1.69 ± 0.06
$\Delta X/\Delta G$	NA [c]	NA	NA	NA	NA
Day 22					
C/H	1.47 ± 0.04	1.45 ± 0.04	1.44 ± 0.05	1.37 ± 0.08	1.43 ± 0.05
G/X	1.71 ± 0.06	1.72 ± 0.06	1.69 ± 0.06	1.72 ± 0.06	1.68 ± 0.05
$\Delta X/\Delta G$	1.11 ± 0.14	1.11 ± 0.07	1.02 ± 0.06	1.14 ± 0.04	0.95 ± 0.08
Day 56					
C/H	1.36 ± 0.03	1.43 ± 0.06	1.52 ± 0.04	1.55 ± 0.06	1.50 ± 0.07
G/X	1.64 ± 0.03	1.73 ± 0.04	1.80 ± 0.12	1.84 ± 0.13	1.87 ± 0.07
$\Delta X/\Delta G$	0.85 ± 0.11	1.19 ± 0.04	1.26 ± 0.07	1.18 ± 0.08	1.78 ± 0.42
Day 84					
C/H	1.43 ± 0.05	1.49 ± 0.05	1.53 ± 0.09	1.46 ± 0.09	1.75 ± 0.11
G/X	1.70 ± 0.02	1.73 ± 0.01	1.77 ± 0.05	1.73 ± 0.03	1.82 ± 0.07
$\Delta X/\Delta G$	1.04 ± 0.02	1.15 ± 0.05	1.17 ± 0.13	1.12 ± 0.03	1.52 ± 0.10

[a] Uncertainties given are the SDs for 12 independent replicate measurements.
[b] *C/H*, cellulose/hemicellulose composition ratio; *G/X*, glucan/xylan composition ratio; $\Delta X/\Delta G$, xylan/glucan degradation ratio (this ratio was calculated assuming little change in the sum of ash, lignin, and extractives).
[c] NA, not applicable since on d 0 no degradation had occurred.

the conditions tested, in the initial 22 d of culture *P. ostreatus* did not dominate degradation of the stems.

Effect of Process Variables on Xylan and Glucan Removal and Selectivity

The time courses of the polysaccharide, lignin with extractives, and ash contents for experiments conducted at 44.0 mg of *P. ostreatus*/g of stems and 1.60 g of H_2O/g of stems are presented in Fig. 2. Time courses such as these were used to estimate xylan and glucan conversions for separate replicate samples, which were then averaged. The xylan and glucan conversions and $\Delta X/\Delta G$ ratios for 23.0–149 mg of *P. ostreatus*/g of stems and 1.10–2.24 g of H_2O/g of stems are presented in Table 4; refer to Fig. 1 for the complete set of inoculum and moisture combinations tested. Of the moisture and inoculum combinations shown in Fig. 1, the (moisture, inoculum) combinations (0.77, 21.0), (0.90, 34.0), and (1.20, 41.0) were performed early in the study and displayed visually uneven growth of

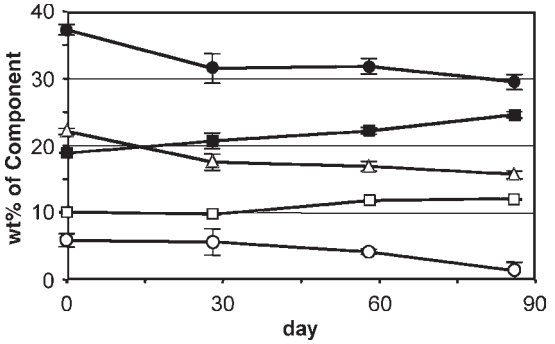

Fig. 2. Time courses of straw stem components for upgrading stems using 44 mg of *P. ostreatus*/g of stems at a moisture content of 1.6 g of H_2O/g of stems. (●) glucan; (△) xylan; (■) lignin with extractives; (□) ash; (○) sum of galactan, arabinan, and mannan.

Table 4
Xylan and Glucan Conversions for Upgrading of Wheat Straw Stems Using *P. ostreatus* at 23.0–149 mg/g of Stems and Moisture Contents of 1.10–2.24 g of H_2O/g of Stems[a]

Inoculum (mg *P. ostreatus*/g stems)	Moisture (g H_2O/g stems)	ΔX (%)	ΔG (%)	$\Delta X/\Delta G$
4 wk				
23.0	1.77	23.4 ± 3.0	16.4 ± 3.2	1.44 ± 0.09
44.0	1.60	24.3 ± 7.6	19.2 ± 8.4	1.31 ± 0.18
70.0	1.10	19.6 ± 1.8	15.3 ± 1.6	1.28 ± 0.01
92.0	1.20	25.2 ± 4.0	18.2 ± 4.2	1.40 ± 0.12
105	2.24	26.4 ± 1.0	19.0 ± 0.6	1.39 ± 0.01
149	1.64	22.9 ± 0.2	17.9 ± 1.0	1.28 ± 0.06
8 weeks				
23.0	1.77	29.7 ± 0.9	21.8 ± 3.1	1.38 ± 0.15
44.0	1.60	34.5 ± 3.5	27.1 ± 3.7	1.28 ± 0.05
70.0	1.10	35.4 ± 0.9	26.6 ± 1.3	1.33 ± 0.05
92.0	1.20	32.3 ± 3.2	25.8 ± 3.1	1.25 ± 0.03
105	2.24	38.8 ± 0.5	32.2 ± 0.5	1.21 ± 0.01
149	1.64	39.7 ± 0.5	33.6 ± 3.0	1.19 ± 0.09
12 weeks				
23.0	1.77	36.3 ± 1.7	29.3 ± 2.7	1.25 ± 0.06
44.0	1.60	43.8 ± 2.6	37.3 ± 3.1	1.18 ± 0.03
70.0	1.10	37.0 ± 2.7	32.5 ± 3.6	1.14 ± 0.04
92.0	1.20	46.7 ± 3.1	38.3 ± 3.0	1.22 ± 0.04
105	2.24	48.3 ± 4.3	41.2 ± 5.4	1.18 ± 0.05
149	1.64	42.9 ± 2.6	37.9 ± 2.2	1.13 ± 0.05

[a] Uncertainties given are the SDs for 12 independent replicate measurements.

fungus in the stems, indicating inhomogeneous distribution of the mycelia over the straw stem surface. Samples taken from the tops and bottoms of these columns also displayed widely variable degradation results, indicating poor distribution of the inoculum. The methods were modified, and the uneven distribution of fungal mycelia on the straw was minimized in future experiments. These three data sets were thus not considered further in analyses of the data. The $\Delta X/\Delta G$ ratios for the preliminary tests conducted at 0.0–10.9 mg of *P. ostreatus*/g of stems and 0.40–0.77 g of H_2O/g of stems are given in Table 3, while the xylan and glucan conversions for those preliminary experiments are available elsewhere *(15)*.

Effect of Treatment Time

Treatment time is an important process variable because large process footprints such as those required for this type of treatment require large amounts of land, and thus long treatment times can have a negative influence of the economics of the process, depending on land use requirements *(16)*. In addition, depending on the sensitivity of the treatment process to initial and transient conditions, widely variable product compositions produced in time-sensitive degradation processes could have a large effect on the next step of the manufacturing process. Longer treatments gave progressively smaller gains in xylan removal and larger gains in glucan removal (Table 4). This observation is consistent with the fungus first utilizing the easily degraded hemicellulose and amorphous cellulose fractions, beginning with the hemicellulose.

In 4 wk, the maximum conversions observed were about 27% xylan and 19% glucan. At 8 wk, the maximum conversions observed were about 40% xylan and 34% glucan, while at 12 wk they were about 48% xylan and 41% glucan. At very low inoculum levels, the initial degradation (at 4 wk) was generally nonselective and thus primarily owing to indigenous organisms (Table 3). Above 10.9 mg of *P. ostreatus*/g of stems, the early degradation was much more selective for xylan vs glucan, indicating significant activity of the inoculated fungus. Maximum selectivities for xylan removal ($\Delta X/\Delta G$) for inoculum levels exceeding 10.9 mg of *P. ostreatus*/g of stems were observed earlier in the treatments, with all moisture and inoculum combinations showing similar selectivities after 12 wk of treatment.

Effect of Moisture and Inoculum

Higher conversions of xylan and glucan were seen with increases in both moisture content and inoculum size (Table 4), but no correlation was observed between the conversions and the relative amounts of inoculum and moisture (ratio of inoculum to moisture content; not shown). Thus, it is unlikely that these two parameters comprise an interaction effect that is important to the operation of the system. Lower moisture contents gave lower overall amounts of degradation, but seemingly better selectivities for xylan degradation although coefficients of variation for conversions were

higher at low moisture contents owing to the smaller changes in overall composition. Higher moisture gave better overall degradation but poorer selectivity for xylan degradation. Selective xylan degradation may not have as great an effect on the properties of straw-thermoplastic composites as may overall degradation. In the present study, selectivity for xylan removal was a convenient proxy measure of relative activity of the inoculated fungus to indigenous microbes. However, there are other uses for treated straw feedstock, such as for production of fermentable sugars for fuels and chemicals, in which selective xylan removal would be useful. If achieving high selectivity for xylan degradation is important to the final use of the feedstock, lower moisture levels would be preferred. Finally, higher inoculum was found to give faster overall degradation, which was expected.

Regression and Sensitivity Analyses of Degradation Data

Although the tests were performed in an exploratory manner and thus neither a complete factorial design nor a complete fraction of a factorial design was completed, a significant amount of revealing information was collected in the tests. Since the goal was to bracket allowable moisture and inoculum ranges, statistical analyses of the xylan and glucan degradation data were conducted by regression analysis and used to explore system sensitivity to initial moisture and inoculum contents.

Regression Analyses

The conversion data for all tests were combined into a single data set represented by 234 data points varying in inoculum amount (I, mg of *P. ostreatus*/g of stems), gravimetric moisture content (M, g of H_2O/g of stems), and treatment time (t, d). A power series expansion of the three variables through the second-order terms was fitted using linear regression; the expansion included the terms I, M, t, IM, It, Mt, I^2, M^2, and t^2, with an intercept of zero. Note that this equation has no basis in theory and was chosen simply because its shape was appropriate. The primary goal of the regression analyses was to obtain statistically valid equations for both ΔX and ΔG, and to use these relationships to estimate the sensitivity of the system to inoculum, moisture, and treatment time.

The xylan conversion (ΔX) and glucan conversion (ΔG) data were fitted separately to the power series expansion, resulting in r^2 values of 0.925 and 0.910, respectively. However, an analysis of variance indicated that the terms M, IM, M^2 in both analyses were statistically insignificant and thus unnecessary to fit the data. The data were refitted after dropping those terms, resulting in statistically valid fits with r^2 values of 0.924 and 0.909. The results of the regression analyses are presented for both fits in Table 5, and comparisons of the measured and predicted values of ΔX and ΔG are shown in Figs. 3 and 4, respectively. Relatively good fits to the data were obtained, indicating that the data were internally consistent and that the system behaved in a predictable manner. The fits were more accurate at higher values of inoculum and moisture, caused by the higher

Table 5
Regression Models for Xylan and Glucan Conversions

	Regression Results[a]	
	$\Delta X = \alpha_x I + \beta_x t + \gamma_x It + \delta_x Mt + \varepsilon_x I^2 + \phi_x t^2$	$\Delta G = \alpha_g I + \beta_g t + \gamma_g It + \delta_g Mt + \varepsilon_g I^2 + \phi_g t^2$
DOF[b]	233	233
r^2	0.924	0.909
α_i	0.106	0.0551
β_i	0.514	0.408
γ_i	1.68×10^{-3}	1.43×10^{-3}
δ_i	0.106	0.0770
ε_i	-8.19×10^{-4}	-4.45×10^{-4}
ϕ_i	-4.11×10^{-3}	-2.89×10^{-3}

[a] Variable definitions: ΔX (xylan conversion, %); ΔG (glucan conversion, %); I (inoculum amount, mg of *P. ostreatus*/g of stems); M (moisture content, g of H_2O/g of stems); t (time, d).
[b] DOF, degrees of freedom for the regression analysis.

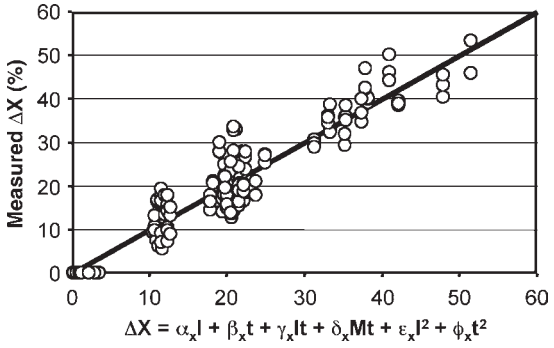

Fig. 3. Comparison of predicted and measured xylan conversions for fungal upgrading tests. The line shown has a slope of 1.0 and represents a perfect fit to the data.

amount of variability in degradation data at lower inoculum levels and moisture contents. The predicted conversions of xylan (ΔX), glucan (ΔG), and the ratio $\Delta X/\Delta G$ with time are shown for the ultimately selected treatment conditions (40 mg of *P. ostreatus*/g of stems, 1.60 g of H_2O/g of stems) in Fig. 5. The percentages of degradation for the nearest experimentally observed combination (44.0 mg of *P. ostreatus*/g of stems, 1.60 g of H_2O/g of stems) were under-predicted by 5–10% at later treatment times (not shown). It is clear from Fig. 5 that the time-rate of degradation had decreased substantially by 12 wk and thus harvesting at 10 or 14 wk would make little difference in the final composition. In addition, the selectivity for xylan degradation over glucan degradation is predicted to be initially about 2.0 and then to decrease with time to about 1.2. This suggests that shorter treatment times would be preferred with this organism if a more selective degradation is desired, although the initially high rate of decline of $\Delta X/\Delta G$ is most likely an artifact of higher measurement uncertainties in the data at lower moisture and inoculum.

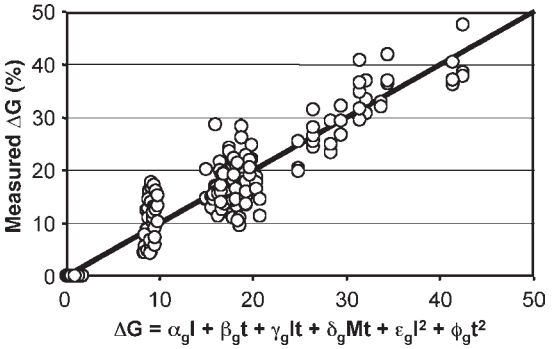

Fig. 4. Comparison of predicted and measured glucan conversions for fungal upgrading tests. The line shown has a slope of 1.0 and represents a perfect fit to the data.

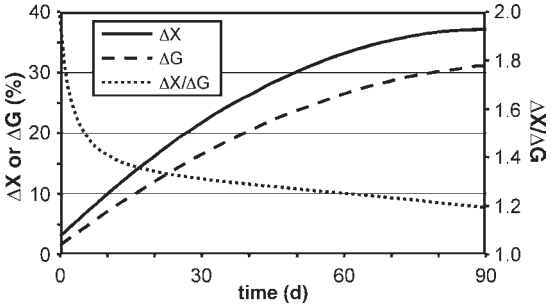

Fig. 5. Predicted time courses of xylan conversion (Δ*X*, %), glucan conversion (Δ*G*, %), and the degradation ratio (Δ*X*/Δ*G*) for 40 mg of *P. ostreatus*/g of stems and 1.60 g of H_2O/g of stems.

Sensitivity Analyses and System Stability

Sensitivity analyses were conducted using the regression models to test the sensitivity of the treatment method at 21 ± 2°C. First, the inoculum was varied ±30% in the regression model (28.0–52.0 mg of *P. ostreatus*/g of stems) at constant moisture. Second, the moisture was separately varied ±30% (1.12–2.08 g of H_2O/g of stems) at constant inoculum. The upper and lower bounds chosen represent very large variations in both inoculum and moisture. The predictions are plotted vs time in Fig. 6 for xylan degradation. The xylan degradation ranges at 12 wk for varied inoculum and moisture were 34.7–39.3 and 32.5–41.6% degraded, respectively. Similarly, the glucan degradation ranges at 12 wk for varied inoculum and moisture were 29.2–32.8 and 27.7–34.4% degraded, respectively (not shown). When varying only one parameter, the final compositions are predicted to be relatively insensitive to inoculum size, with the largest deviation of at most ±5% degradation at 12 wk. For moisture the system was predicted to be more sensitive, but it was less sensitive at shorter degradation times.

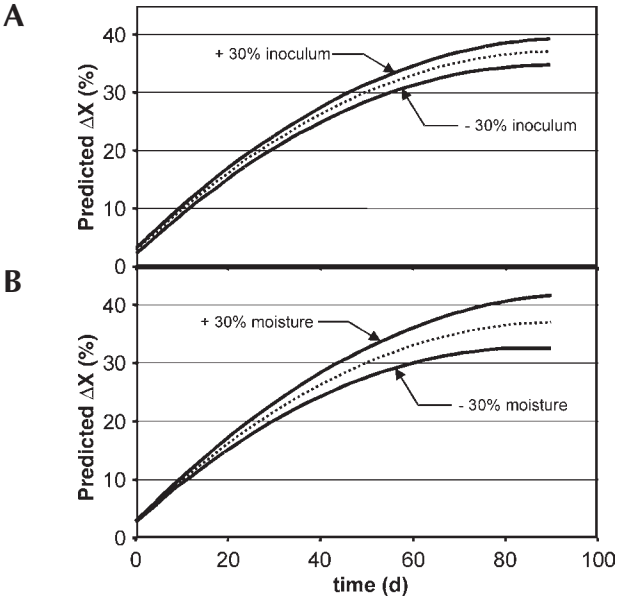

Fig. 6. Inoculum and moisture sensitivity analyses for fungal treatment of straw stems at 21 ± 2°C. **(A)** ±30% variation in inoculum at 1.6 g of H_2O/g of stems. The dotted line is the prediction for the midpoint of 40 mg of *P. ostreatus*/g of stems. **(B)** ±30% variation in gravimetric moisture content at 40 mg of *P. ostreatus*/g of stems. The dotted line is the prediction for the midpoint of 1.6 g of H_2O/g of stems.

This indicates that initial moisture is the more critical parameter to control, and also that the system is less sensitive to initial moisture at shorter treatment times. Shorter treatment times could be used without compromising final compositions by increasing initial inoculum size, depending on final costs.

Additional sensitivity analyses were conducted by simultaneously varying both inoculum amount and moisture content (±30% for each). This analysis predicted maximum ranges at 6 and 12 wk of 24.5–31.9 and 30.1–43.8% xylan degraded, and 19.4–24.7 and 25.9–36.1% glucan degraded, respectively. This corresponds to $\Delta X/\Delta G$ ranges of 1.27–1.29 and 1.16–1.21 at 6 and 12 wk, respectively, and indicates that the expected $\Delta X/\Delta G$ does not vary as widely as would be suggested by combining the individual sensitivity analyses. In addition, shorter treatment times were again favored for minimum variation in the treated straw stem compositions.

In terms of selectivity and reduced sensitivity to initial moisture and inoculum, the results indicate that shorter treatment times are preferred, especially if moisture is either not controlled or poorly controlled. The regression models were next used to generate topographic plots of ΔX, ΔG, and $\Delta X/\Delta G$ at the various combinations of inoculum and moisture.

The results are plotted in Figs. 7–9 for the regression model predictions after 6 wk of treatment. For locations of the parameter combinations used in this study on these plots, refer to Fig. 1 (which uses the same axes). Note that the parameter combinations (21.0, 0.77), (34.0, 0.90), and (41.0, 1.20) were not included in the statistical analyses because of poor distribution of the fungal inoculum onto the straw stems. The topographic plot of percentage xylan degraded after 6 wk of treatment is shown in Fig. 7. The diamond represents the conditions chosen for preparation of treated stems for the extrusion testing. The region of only 15–20% xylan degradation roughly corresponds to the region in which the inoculated *P. ostreatus* was observed to be unable to outcompete the indigenous microbes, since about 15% xylan degradation was observed to occur without inoculum. Increased xylan removal is predicted as both moisture and inoculum increase. There are, however, wide ranges of parameter combinations that will give the same amount of xylan degradation, indicating a fairly insensitive system in terms of overall xylan degradation after 6 wk of treatment. The curvature of the dividing curve between 25–30 and 30–35% xylan degradation, and above 100 mg of *P. ostreatus*/g of stems, seems odd in that it curves back toward the inoculum axis. However, this was experimentally observed by comparing the results of the (149, 1.67) and (105, 2.24) parameter combinations (*see* Fig. 1). Since these experiments were independently replicated, the behavior appears to be real.

The topographic plot of percentage glucan degraded after 6 wk of treatment is shown in Fig. 8. Again, the diamond represents the conditions chosen for preparation of treated stems for the extrusion testing. The region of only 10–15% glucan degradation closely corresponds to the experimentally observed region in which the inoculated *P. ostreatus* was unable to outcompete the indigenous microbes. Increased glucan removal is predicted as both moisture and inoculum increase. As shown for xylan, there are again wide ranges of parameter combinations that give the same amount of glucan degradation, indicating that the system is also fairly insensitive in terms of overall glucan degradation after 6 wk of treatment.

Finally, the topographic plot of $\Delta X/\Delta G$ after 6 wk of treatment is shown in Fig. 9. The diamond again represents the conditions chosen for preparation of treated stems for the extrusion testing. The region of $\Delta X/\Delta G$ of 1.20–1.25 encompasses the region in which it was experimentally observed that $\Delta X/\Delta G$ was about 1.0. A ratio of $\Delta X/\Delta G$ of 1.0 indicates nonselective polysaccharide degradation and was taken as an indication of poor competition of the inoculated fungus with the indigenous microbes. This reinforces the observations that at low moisture and inoculum, the regression model predictions are less accurate. Figure 9 also shows that $\Delta X/\Delta G$ of 1.25–1.30 is predicted after 6 wk of treatment over a very large percentage of the possible moisture and inoculum combinations. Thus, the system is very stable with respect to selectivity of polysaccharide degradation within the parameter ranges tested.

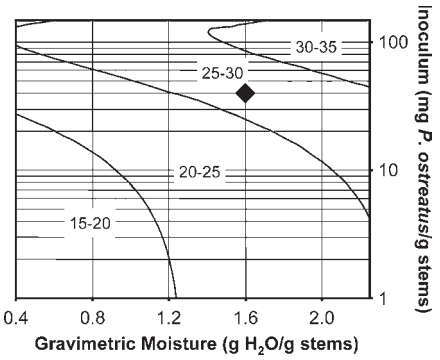

Fig. 7. Predicted topographic plot of xylan conversion (ΔX, %) with inoculum amount and moisture content. The ranges presented on the plot are ranges of xylan conversion predicted in each region. The diamond marks the conditions chosen for preparation of treated stems for the extrusion tests.

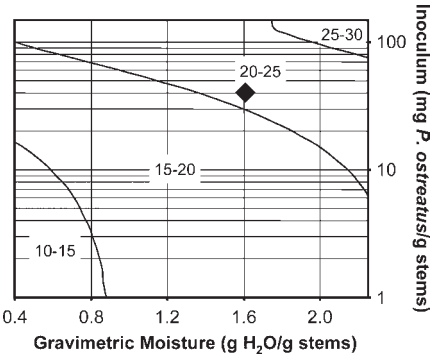

Fig. 8. Predicted topographic plot of glucan conversion (ΔG, %) with inoculum amount and moisture content. The ranges presented on the plot are ranges of glucan conversion predicted in each region. The diamond marks the conditions chosen for preparation of treated stems for the extrusion tests.

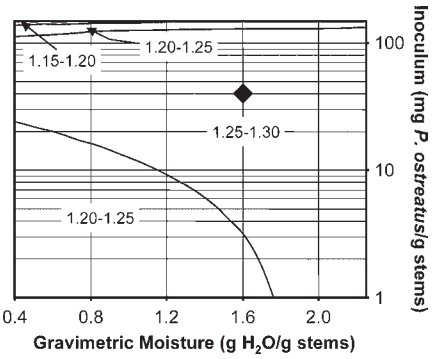

Fig. 9. Predicted topographic plot of degradation ratio ($\Delta X/\Delta G$) with inoculum amount and moisture content. The ranges presented on the plot are ranges of $\Delta X/\Delta G$ predicted in each region. The diamond marks the conditions chosen for preparation of treated stems for the extrusion tests.

Table 6
Xylan and Glucan Conversions and Degradation Ratios Estimated for Upgrading of Wheat Straw Stems Using *P. ostreatus* at 40.0 mg/g of Stems and a Moisture Content of 1.60 g of H_2O/g of Stems in Scaled-Up Columns [a]

	Percentage degraded			
	Degrade1		Degrade2	
Component degraded	Small Columns [b]	55-gal Drums	Small Columns [b]	55-gal Drums
ΔX (%)	29.8 ± 1.0	11.9 ± 0.9	28.1 ± 1.0	24.2 ± 4.9
ΔG (%)	18.3 ± 1.4	13.4 ± 1.5	18.5 ± 1.2	26.8 ± 3.4
$\Delta X/\Delta G$ [b]	1.64 ± 0.09	0.89 ± 0.03	1.52 ± 0.05	0.90 ± 0.08

[a] Uncertainties given are the SDs for eight independent replicate measurements.
[b] Columns were inoculated at the indicated concentrations of *P. ostreatus* and moisture concentration and grown for 6 wk, and the degraded stems were used to inoculate drums at a 1:10 weight ratio.

Scaled-Up Preparation of Stems for Extrusion Tests

The larger-scale columns, while necessary to produce treated straw stems for use in the preparation of composite formulations for extrusion testing, were also a good test of the sensitivity of the system to scale up to larger columns and to changes in inoculum source and inoculation method. After 6 wk of treatment in the small columns used to inoculate the drums, 28–30% xylan was degraded while about 18.5% of glucan was degraded (Table 6). This gave $\Delta X/\Delta G$ ratios of 1.52–1.64. For comparison, at 40.0 mg of *P. ostreatus*/g of stems and 1.60 g of H_2O/g of stems, the regression models predict 27.2% xylan degradation and 21.1% glucan degradation at 6 wk, for a $\Delta X/\Delta G$ of 1.29. Clearly, *P. ostreatus* was dominant in the small columns used to prepare enrichment inoculum for the barrels. However, the $\Delta X/\Delta G$ values were well outside the range predicted by the regression models. Apparently, the nitrogen-limited inoculum produced by Utah State University and shipped to INEEL for these columns was either more active or better acclimated to the nitrogen-limited conditions in the straw stems. This effect was repeatable, indicating that the history of the inoculum used may have a significant effect on $\Delta X/\Delta G$, although the actual glucan and xylan conversions were not far from the predicted values. The inoculum produced for the small columns used to inoculate the drums was produced differently than the inoculum used to inoculate the small columns in the moisture and inoculum tests—it was better acclimated to nitrogen-limited conditions. This is because the mycelia were transferred directly into the nitrogen-limited medium (C/N of 32.6) without first being grown in the carbon-limited YM broth (C/N of 7.7). Since both enrichment steps in the production of the mycelial inoculum were carried out in the nitrogen-limited medium, this likely resulted in a mycelial inoculum that was better acclimated to low-nitrogen conditions when

it was added to the straw stems, which have a C/N of about 80 *(17)*. Thus, system performance is sensitive to inoculum source and history.

The altered inoculation method also resulted in a different degradation pattern than that observed in the small-column tests. After 6 and 12 wk of treatment in the drums, only 11.9 and 24.2% of the xylan was degraded, respectively (Table 6). Glucan degradation was similarly reduced, with only 13.4 and 26.8% of the glucan degraded at 6 and 12 wk, respectively. This equates to $\Delta X/\Delta G$ values of 0.89 and 0.90 at 6 and 12 wk, respectively. Thus, selective degradation did not occur in the larger-scale columns, which indicates that *P. ostreatus* was not dominant. In fact, less degradation occurred in the barrels after 6 wk of degradation than was either observed or predicted in the small columns. It is likely that the low levels of degradation observed in the drums were owing to slower colonization of the fresh stems by the *P. ostreatus* growing in the solid enrichment inoculum. In the small-column tests, the indigenous microbes were shown to degrade about 15–20% of the polysaccharides in 12 wk in the absence of *P. ostreatus (4,15)*, which likely represents the most easily accessible glucan and xylan fractions. If *P. ostreatus* were to colonize the straw more slowly from the solid enrichment inoculum, the primary effect on the degradation system would be to extend the treatment time necessary to reach the desired levels of xylan and/or glucan degradation in the final product. Thus, inoculating the fresh stems with partially degraded stems before introduction to the drums was an ineffective inoculation method when compared with inoculating by spraying homogenized mycelia onto the stem surfaces. The nonselective degradation pattern in the drums may or may not be a detriment to the physical properties of straw-thermoplastic composites produced from Degrade1 and Degrade2 straw stems, since selectively degraded stems have not yet been compared with nonselectively degraded stems.

Preliminary Extrusion Tests

The Neat-, Degrade1-, and Degrade2-treated straw stems were run on the extruder. Measurement of several properties of the extruded composite formulations is under way (*see* Table 2). An example of the preliminary data, the data for runs 2, 31, and 32, is presented in Table 7. In these runs, composite formulations were prepared by mixing Neat, Degrade1, or Degrade2 stems at 75 wt% with 18 wt% HDPE, 4 wt% MAPE, and 3 wt% lubricant. Few statistical differences were seen in the measured values of density, modulus of elasticity (MOE), and modulus of rupture (MOR) in Table 7. This indicates that at this combination of formulation components, fungal treatment of the stems in the drums (which resulted only in nonselective degradation) may not have improved the stems for composite production. However, little can be learned by comparing only three treatments taken from such a large fractional factorial design. Thus, the data will not be compared further here but will be compared with respect to density, MOE, and MOR in the context of the complete factorial design in a future article.

Table 7
Preliminary Measurements of Density, MOE, and MOR for Neat, Degrade1, and Degrade2 Straw Stems Extruded at 75 wt% Straw Stems, 18 wt% HDPE, 4 wt% MAPE, and 3 wt% Lubricant[a]

	Straw stem source		
Property	Neat	Degrade1	Degrade2
Density (kg/m^3)	1190 ± 6	1200 ± 7	1230 ± 25
MOE (MPa)	3400 ± 35	3210 ± 24	3070 ± 19
MOR (MPa)	19.9 ± 1.7	18.0 ± 1.5	18.2 ± 2.0

[a] Uncertainties given are the SDs for five independent replicate measurements of each property.

Conclusions

Initial rates of xylan and glucan removal from wheat straw stems by *P. ostreatus* were in general higher than later in the tests. In tests in which *P. ostreatus* was inoculated to levels high enough to allow successful competition with indigenous microbes, early degradation of the xylan and glucan was nonselective, suggesting that there is a lag time before *P. ostreatus* can overtake the culture. Higher inoculum levels resulted in faster overall degradation of the stems but decreased the selectivity for xylan degradation. Increasing the moisture content also resulted in higher degradation rates of both glucan and xylan but also decreased the selectivity for xylan degradation. The regression analysis indicated that the data were internally consistent and predictable. Predictions were in general more accurate at higher values of inoculum and moisture and indicated that at 12 wk the system is insensitive to inoculum size and somewhat more sensitive to moisture content. The system is predicted to be less sensitive to moisture at 6 wk than at 12 wk. This together with decreasing selectivity of polysaccharide degradation with treatment time indicates that the system is less sensitive to initial conditions at shorter treatment times. Scale-up from the laboratory columns to drums indicated that the system is sensitive to both inoculum source/history and inoculation method. Comparative testing of straw-thermoplastic composites produced from the untreated and nonselectively degraded stems is under way.

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