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0273-2289/03/105-108/0423/$20.00

Preliminary Investigation of Fungal Bioprocessing of Wheat Straw for Production of Straw-Thermoplastic Composites

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Abstract

Straw utilization for composites is limited by poor resin and polymer penetration, and excessive resin consumption owing to the straw cuticle, fines, and lignin-hemicellulose matrix. White-rot fungi degrade these components of straw and could, therefore, potentially be used to improve resin penetration and resin binding without the use of physical or chemical pretreatments. Although long treatment times and large footprints the limit use of fungal treatments on a large scale, distributed fungal pretreatments could alleviate land requirements. In this article, we present progress toward the development of a passive fungal straw upgrading system utilizing white-rot fungi.

Index Entries: Fungal upgrading; white-rot fungi; wheat straw; *Pleurotus ostreatus*; straw composite.

Introduction

The principal barriers to straw utilization for straw-thermoplastic composites are resin penetration and resin consumption. Resin penetration is limited by the physical barrier presented by the cuticle and underlying epidermis on the external surface of the straw, and by the lignin-hemicellulose matrix in the internal vascular layer. Resin consumption is increased because the resin does not bind well to the cuticle, and because fines, created when the nodes and leaves are ground, have high surface areas and require much more resin. Since straw thermoplastics can contain as much as 50–70 wt% straw, resin binding and performance are of the utmost importance, as has been shown in wood-plastic composites *(1,2)*.

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The physical barriers to resin penetration are the same physical barriers that limit access of cellulase enzymes to the cellulose fibers when producing glucose from straw cellulose for fermentation to ethanol or for production of fuels and chemicals. Much of the research on removal of the lignin-hemicellulose barrier to date has been on conversion of the cellulose to glucose, since glucose can easily be fermented to ethanol using common yeasts *(3)*. Dilute-acid hydrolysis of the cellulose to glucose lowers fermentable carbohydrate yields due to thermal decomposition of xylose to furfural and glucose to hydroxymethylfurfural *(4)*. Thus, much work has been done on the use of cellulase enzymes, since they are specific for cellulose, form only glucose, and the hydrolysis is performed at mild temperatures. However, cellulases are relatively large enzymes and cannot fit through most of the spaces in the intact vascular layer of straw *(5,6)*. Thus, physical, chemical, and thermal pretreatments are employed to increase the penetration of cellulases into this lignocellulose matrix *(3,5,7)*. Many pretreatments have been developed, including acids *(8,9)*, alkalis *(10,11)*, organosolvents *(12)*, steam explosion *(10,13)*, and physical treatments. Although effective, the pretreatments are costly, negatively affecting the economics of utilization. Pretreatments with white-rot fungi, which have been shown to completely degrade lignocellulose, increase glucose yields without significant capital or energy-intensive steps *(14)*. The principal drawbacks to centralized white-rot fungal pretreatments are that the process footprints are large and that treatment times are often 8 wk or longer, much too long for use at large industrial facilities *(14)*.

Since lignin and hemicellulose limit resin penetration just as they limit cellulase penetration into the matrix, these pretreatments would be expected to improve resin penetration as well. Indeed, steam explosion has been investigated to remove lignin and hemicellulose to allow better resin penetration and adhesion *(15)*. While significant improvement in interfacial adhesion was seen in steam exploded broom fibers, the extensive physical damage to the fibers imparted by the steam-explosion process eliminated any mechanical property improvements. Of course, steam explosion pretreatment, whether for better resin penetration or for better penetration of cellulases, requires significant capital equipment and energy input. Thus, an inexpensive, low-capital, low-energy-input treatment, such as a fungal pretreatment, that removes the cuticle, lignin, and hemicellulose would be ideal. Physical removal of straw components that form fines *(16)* would also help to reduce resin consumption. Combining physical removal of fines *(16)* and fungal treatment into a distributed process that could be done on-site at a small scale would minimize the land area required as well as capital and energy inputs.

White-rot fungi remove lignin using extracellular peroxidase enzymes, attacking the lignocellulose matrix by growing into the cell walls, where they secrete extracellular cellulases, hemicellulases, and peroxidases *(17)*. Ligninolytic enzymes are produced in secondary metabolism under conditions of carbon or nitrogen deprivation *(17)*. The

degraded lignin is not used as a growth substrate but is removed to open up the matrix to cellulases and hemicellulases so that over time near-complete degradation is possible *(18)*. While cellulose and hemicellulose are the principal growth substrates for white-rot fungi *(17)*, some white-rot fungi, including several *Pleurotus* species, attack straw lignin and hemicellulose without much cellulose removal *(19,20)*. There is also evidence of degradation of the cuticle during degradation by white-rot fungi *(21)*. Once through the cuticle, the fungi possess the necessary cellulases and hemicellulases to degrade the epidermal layer, allowing access to the vascular layer from the outer surface of the residue. Thus, in a single degradation step, white-rot fungi could potentially upgrade the straw to a more desirable feedstock for straw-thermoplastic composites. That is, the upgraded straw product should have a higher cellulose content, and partial degradation of the vascular hemicellulose and lignin, the cuticle layer, and the epidermis should allow better penetration by resins used in thermoplastic extrusion processes.

This article describes preliminary results from ongoing work at the Idaho National Engineering and Environmental Laboratory (INEEL) to bracket the process parameters necessary to reproducibly operate a passive, distributed, fungi-based straw "bioupgrading" system. These data will be used by INEEL, together with Washington State University, to devise and build a pilot-scale fungal bioupgrading system suitable for use in both centralized and distributed systems. Included in this work are the preliminary results of ongoing exploratory tests conducted at INEEL to bracket the "best" conditions of inoculum and moisture for fungal upgrading of the straw. Although preliminary, the results show that by limiting nitrogen and providing sufficient inoculum, it is possible to operate a selective fungal degradation system without prior sterilization of the wheat straw. The pilot-scale design, as well as testing of the fungal-treated straw in composite materials, is being implemented at Washington State University.

Materials and Methods

Wheat Straw

Wheat straw (Westbred 936), 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. Twenty large bales of Westbred 936 (1.2×2.4 m [4×8 ft] bales) were produced and stored in a stack at the side of the field at Grant 4-D Farms. Only the protected center bales from the interior of the stack were used for the studies. This was done to minimize the effects of exogenous nitrogen sources (i.e., bales touching the soil) and water (i.e., bales on the outer periphery of the stack), which were important variables because we intended to limit nitrogen and vary water in the fungal degradation tests. To better handle the straw for the laboratory studies, the large bales were rebaled as needed into smaller 0.61×1.2 m (2×4 ft)

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

	Composition (wt%)[a]
Glucan	37.2
Xylan	22.1
Galactan	1.2
Mannan	3.0
Arabinan	1.6
Lignin with extractives	18.9
Ash	10.1
Total[b]	89.7

[a]Based on 100% dry wt of material.
[b]Remaining fraction attributed to unknown contents of uronic acids, protein, and so on and to recovery errors in analysis techniques.

bales containing about 22.7 kg (50 lb) each and placed in covered storage. To remove the plant components that are the sources of high-surface-area fines (leaves, sheaths, nodes, and fines), the straw was rethreshed before use as described by Hess et al. *(16)*. Only the separated straw stems were used in the laboratory studies. The composition of the untreated straw stem fraction, determined as described under Compositional Analysis, is shown in Table 1.

Cultures and Maintenance

Pleurotus 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 *(22,23)*. It was obtained from the Northern Regional Research Laboratory (NRRL) (Peoria, IL). Stock cultures were maintained on agar slants at room temperature prepared at 20 g/L of YM agar (Difco, Detroit, MI) and containing 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 ammonium molybdate tetrahydrate— and were subcultured every 2 wk. Stock mycelial inocula were produced 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 just 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, and stored at 4°C until use, typically 2 to 3 wk or less.

Nitrogen Consumption Experiments

Since it would be uneconomical to sterilize the straw for use in distributed systems, unsterilized straw is preferred, and thus the inoculated *P. ostreatus* must be able to outcompete or overtake the indigenous microbial population. White-rot fungi are late colonizers in nature and dominate under conditions of nitrogen limitation *(24)*. Thus, minimizing the amount of nitrogen carried over to the straw in the inoculum could allow the inoculated fungi to more quickly overtake the indigenous microbes, minimizing or eliminating the need for sterilization. The nitrogen consumption tests were designed to estimate the initial nitrogen level required to produce a suitable amount of biomass for inoculating the straw while minimizing the amount of nitrogen remaining in the culture medium.

In these experiments, approx 500 mL of wet fungal pellets of *P. ostreatus* were transferred to a sterile blender, and 500 mL of medium was added. Media tested included YM broth with trace minerals (as described earlier), and a nitrogen-limited medium containing 20 g/L of glucose, 1.0–3.5 g/L of yeast extract, and trace minerals (as described earlier). The mixture was 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 fresh nitrogen-limited medium in sterile shake flasks and incubated for 14 d at 30°C, 135 rpm. Replicate flasks were sacrificed periodically, and the fungal pellets were washed with distilled water and separated from the liquid by centrifuging for 10 min at 26,892*g*. Fungal biomass was measured gravimetrically after drying for 48 h at 105°C. Total Kjeldahl nitrogen (TKN) was measured as previously described *(25)*.

Column Inoculum Preparation

The most suitable conditions for minimization of nitrogen remaining in the culture and production of biomass in the inoculum were used (*see* Results). The yeast extract concentration used in the nitrogen-limited medium for column inoculum production was 3.0 g/L. The column inoculum cultures were cultured and harvested in a manner identical to that used for the nitrogen consumption experiments just described, except that the cultures were harvested between d 5 and 7, and the mycelial pellets were homogenized in the spent medium, without centrifugation.

Column Experiments

Because we intended to standardize the inoculum, it was desirable to know the exact amount of fungal inoculum added to the straw. In addition, it was desirable to minimize heterogeneity in the treated straw by initially evenly distributing the fungus over the entire surface of the straw. To accomplish this, *P. ostreatus* was added to the straw by spraying a liquid suspension of homogenized mycelia onto the straw with mixing.

Assuming the availability of a viable stabilized homogenized mycelial inoculum, this would also be a more cost-effective method for inoculating straw piles in the field than using a tumbler to mix solid inoculum into untreated straw. However, because spraying is not a commonly used inoculation method, there was no obvious initial range of fungal inoculum to test. Since 10 wt% of solid inoculum is often used in soil bioremediation to inoculate with white-rot fungi using the solid inoculation method (Dr. S. D. Aust, December 2001, personal communication, Utah State University), we arbitrarily assumed that the solid inoculum contained 1% of its weight as dry fungal biomass and chose the range of 0–1 wt% dry fungal biomass for the initial column experiments.

The fungal pellets in the inoculum cultures, produced immediately before inoculation of the straw columns, were 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 standard hand-pump garden sprayer for addition to the straw stems. No extraordinary measures were taken beyond this point to maintain sterility, except for using initially sterile equipment.

Air-dried straw stems (150 g dry wt) were spread onto a clean, dry tray in a thin layer, and the inoculum was sprayed onto the stems, with frequent mixing of both the inoculum and the stems. Enough 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 air across the tray of inoculated straw to evaporate excess water, 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 at 105°C to determine the actual biomass concentration of the slurry. The initial moisture range to be tested was chosen based on empirical evidence from compost biofiltration and soil bioremediation using white-rot fungi. First, a gravimetric moisture range of up to 70% (41% on a wet basis) has worked very well in compost biofiltration experiments degrading volatile organic compounds (VOCs) at the INEEL *(26,27)*. Second, it has been shown that white-rot fungi seem to grow and compete best with indigenous microbes in soil at moisture levels at or below 0.5 g of H_2O/g dry soil *(28)*. Thus, since it was desired to have the inoculated fungus successfully compete with the indigenous microbes, the initial gravimetric moisture range was chosen to bracket these values, at 0.40–0.70 g of H_2O/g of dry stems (40–70% on a dry basis).

The inoculated straw was added to clean, initially sterile glass columns fabricated from glass process pipe. The airtight columns were comprised a 12-in. section of 3in. id Pyrex process pipe with 2in. id reducers at each end (ACE Glass, Vineland, NJ), and Teflon® end caps. 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 15.5 psig and a flow rate sufficient to turn over the air in the system once per day. Approximately 2.5 g dry wt of straw was sampled from the top and bottom of each column initially and approximately every 3 to 4 wk thereafter. The samples were dried at 105°C overnight and ground to 60 mesh in a Wiley mill for compositional analysis.

Compositional Analysis

Carbohydrate and lignin compositions of untreated and treated straw samples were determined by quantitative saccharification using the method of Saeman et al. *(29)*. Two aliquots of each sample were analyzed per column by quantitative saccharification for each set of 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, and lignin was calculated by weight difference as Klason lignin with extractives and ash, as previously described *(6)*.

Determination of Dominance of Inoculated Fungus

Pleurotus species have been shown to be somewhat selective for hemicellulose and lignin rather than cellulose *(22,30)*, including *P. ostreatus (22,23)*. Because a mixed culture of indigenous microbes can degrade the most easily accessible fractions of both cellulose and hemicellulose, the indigenous microbes would not be expected to show significant selectivity for specific polysaccharides. Thus, the mass balances of cellulose and hemicellulose could potentially be used to determine whether the inoculated *P. ostreatus* was dominant, by comparing the relative amounts of degradation of cellulose and hemicellulose. Thus, a column that showed similar degradation of both cellulose and hemicellulose (cellulose/hemicellulose ratio similar to that for undegraded straw) was said to be dominated by indigenous microbes. Conversely, a column that showed significantly greater hemicellulose degradation than cellulose degradation (significantly increased ratio of cellulose/hemicellulose) was said to be dominated by the inoculated *P. ostreatus*. The performance measures chosen for the inoculum competition column tests were the relative changes in cellulose and hemicellulose after degradation (expressed as the cellulose/hemicellulose ratio), as well as overall hemicellulose removal. Thus, cellulose, hemicellulose, lignin/extractives, and ash were measured, and the mass balances from d 0 to the sample date were compared. It is true that lignin removal could be used separately or together with cellulose/hemicellulose ratio as an indication of *P. ostreatus* activity. However, routine measurement of extractives would be necessary to discern changes in lignin and extractives. Since the cellulose/hemicellulose ratio was believed to be a good indicator of the relative activity of the inoculated white rot fungus vs the indigenous microbes, it was decided

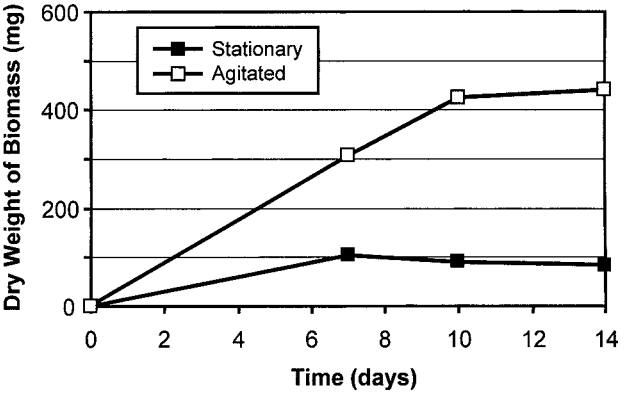

Fig. 1. Comparison of *P. ostreatus* biomass production in stationary and agitated cultures. This test used the YM broth/trace minerals medium.

not to measure the extractives for treated straw samples until the final conditions were chosen. Thus, lignin is expressed as "lignin with extractives" in this article. The extractives will be determined by soxhlet extraction for the final conditions chosen.

Results and Discussion

Inoculum Production and Nutrient Carryover

Since it would be uneconomical to sterilize the straw for use in distributed systems in the existing infrastructure, and also on such a large scale, it is imperative that the system treat only unsterilized straw. The inoculum preparation tests were done to estimate the initial nitrogen level required to produce a suitable amount of biomass for inoculating the straw while minimizing the amount of nitrogen remaining in the culture medium. These tests were necessary because the slow-growing white-rot fungi are late colonizers in nature and express their ligninolytic systems under conditions of nitrogen limitation *(24)*. Thus, minimizing the amount of nitrogen that carries over to the straw in the fungal inoculum could allow the inoculated fungus to more quickly overtake the microbes already present in the straw, and thereby minimize or eliminate the need for sterilization of the straw.

Experiments were performed initially using the YM broth/trace minerals medium, with inoculum amounts ranging from 0.1 to 5.0 OD. Tests were run to compare the production of fungal biomass in stationary vs agitated cultures (*see* Fig. 1). As expected, biomass production in the agitated cultures far exceeded that in the stationary cultures, by more than four times. The TKN of the cultures was monitored over the course of the experiments, but it did not change significantly even when the cultures entered secondary metabolism (data not shown). This indicated that the cultures were not nitrogen limited.

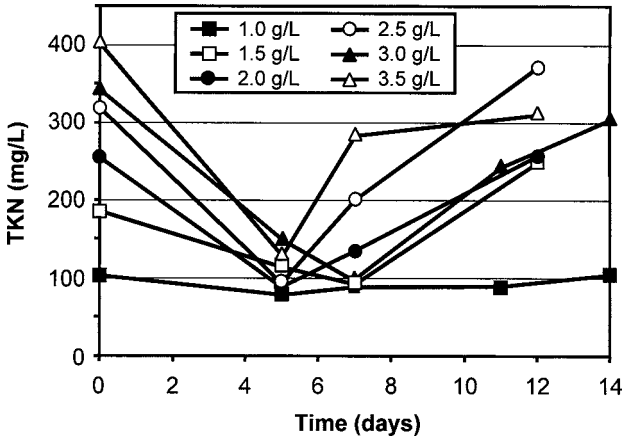

Fig. 2. Effect of yeast extract addition (which contains about 10 wt% nitrogen) on minimum nitrogen level attainable in inoculum production cultures.

Cultures were then tested in the nitrogen-limited medium, inoculating homogenized mycelia to 1.0 OD. The minimum nitrogen concentration observed in the cultures was about 80–100 ppm of TKN (Fig. 2). The first sample was taken on d 5 to ensure that a sample would be obtained before the minimum nitrogen concentration was reached (onset of stationary phase), because the original agitated YM broth–grown cultures entered secondary metabolism on or about d 7–10, depending on the amount of inoculum (not shown). As shown in Fig. 2, the minimum nitrogen levels were observed in the first sample on day 5, after which the TKN of the culture fluid increased. The increase in medium nitrogen indicates either exportation of nitrogen from the cells to the medium (as extracellular enzymes), or significant cell death. The shift to earlier onset of stationary phase is consistent with the switch to a more severe nutrient limitation in the new medium. Biomass production did not increase significantly going from 3.0 to 3.5 g/L of yeast extract in the culture, and thus it was decided to produce inoculum for the column tests using 3.0 g/L of yeast extract in the nitrogen-limited medium. Use of this medium resulted in the production of 5300–6700 mg/L of *P. ostreatus* mycelia in 5 d (data not shown).

Column Tests

Exploratory experiments were performed to estimate the minimum amount of inoculum necessary to overtake the indigenous microbes in the straw in 12 wk or less. For these tests, the amount of fungal inoculum was varied from 0 to 11 mg of homogenized *P. ostreatus* mycelia/g of air-dried straw stems. The moisture content used for these experiments was 60–70% on a dry basis (0.6–0.7 g of H_2O/g dry stems), based on prior experience at INEEL using compost biofilters to degrade VOCs *(26,27)*. A comparison of the degradation patterns for each amount of inoculum is shown in Fig. 3.

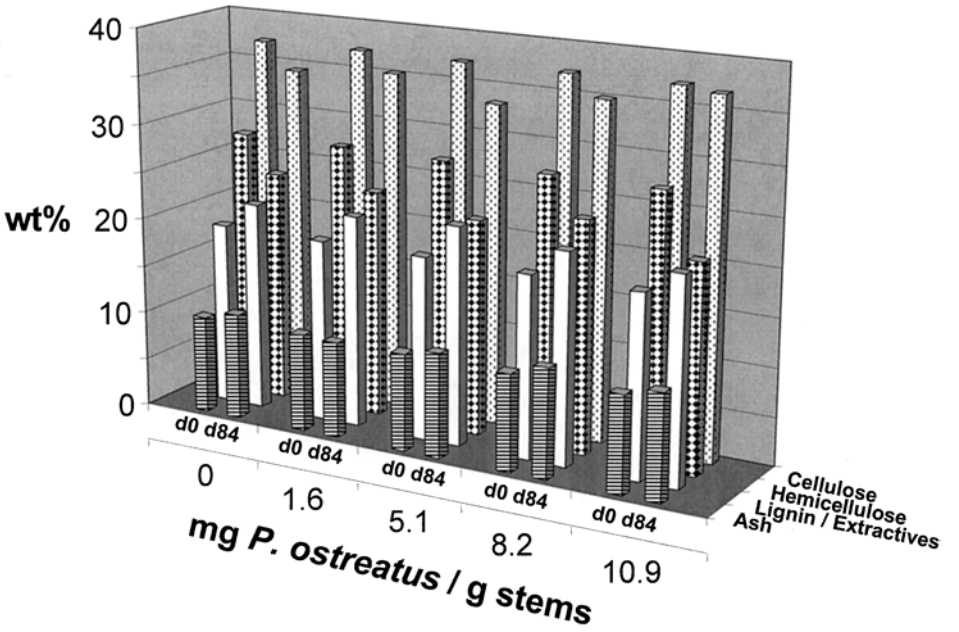

Fig. 3. Straw stem compositions at d 0 and 84 as function of fungal inoculum amounts from 0 to 10.9 mg of *P. ostreatus*/g stems.

Few differences were seen between the control (0 mg of *P. ostreatus*/g) and inoculated columns containing <5.1 mg/g. Above 5.1 mg/g, relatively less cellulose was degraded with increasing amount of inoculum. At 10.9 mg/g, little cellulose was degraded, suggesting dominance of the cultures by *P. ostreatus*. Lignin/extractives contents increased, suggesting that lignin was not degraded; however, it is likely that the extractives increased and lignin decreased, as has been shown previously *(20)*.

The next tests performed in the columns were designed to bracket the moisture range necessary for the fungal degradation to proceed. Since hemicellulose is generally the most easily degraded fraction of the stems, hemicellulose degradation was used as the performance measure for these tests. The effect of 40, 55, and 70% moisture on the degradation of hemicellulose at various times over 12 wk of treatment is shown in Fig. 4 for both the uninoculated control and for the 10.9 mg/g columns. Moisture had little effect on straw stem degradation in control columns below 70% (0.7 g of H_2O/g dry stems) (*see* Fig. 4A). In columns inoculated with *P. ostreatus*, there was a slight effect of moisture below 70% (Fig. 4B), but only after 60 d of culture. Although this effect was not significant at 55% moisture, there was a linear trend of increased hemicellulose removal at 84 d, going from 40 to 70% moisture (Fig. 4B). In all experiments, including those using >5.1 mg/g of inoculum, the first 3 wk of degradation gave nearly identical results, indicating that even though dominance by

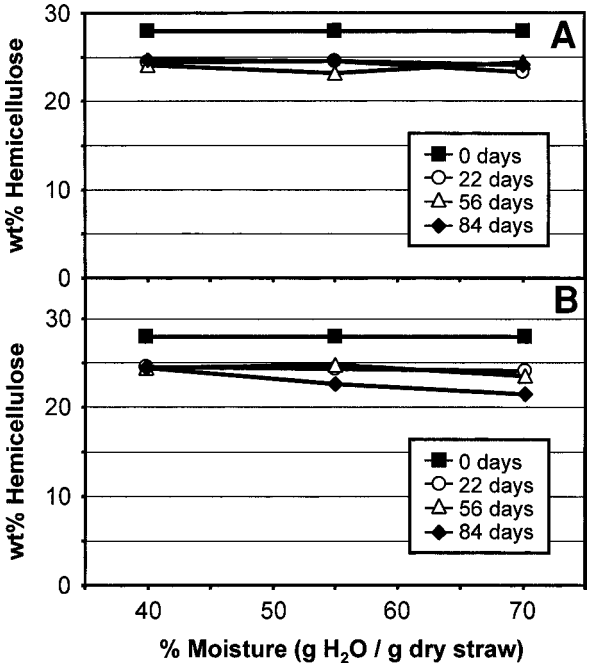

Fig. 4. Effect of moisture content on degradation of hemicellulose at various times: (**A**) control (0 mg *P. ostreatus*/g stems); (**B**) 10.9 mg *P. ostreatus*/g stems.

P. ostreatus was observed at 84 d using higher amounts of inoculum, the initial 3 wk of the culture was still dominated by the indigenous organisms. This may be due to nitrogen levels being too high during this period, or to a significant lag time in *P. ostreatus* growth during its acclimation to straw stems as a substrate for growth. In any event, it seems clear that even higher moisture contents will give increased degradation of hemicellulose in the column tests; hence, we have begun testing gravimetric moisture contents from 90 to 150%.

The last set of column tests was designed to determine the amount of inoculum necessary to obtain suitable feedstock for production of straw thermoplastic composites, while obtaining this composition in 12 wk or less. It is expected that removal of hemicellulose will allow better resin penetration and adhesion, since similar results have been obtained using steam-exploded broom fibers *(15)*. The fiber damage imparted by steam explosion would not be expected to be present in straw stems treated with fungi, especially by a fungus that does not degrade significant cellulose *(22)*. However, for this to occur the inoculated *P. ostreatus* must overtake the indigenous microbes. Since *P. ostreatus* is selective for hemicellulose and lignin degradation vs cellulose degradation, a clear signal that the inoculated *P. ostreatus* has overtaken the indigenous organisms would be a significant increase in the cellulose/hemicellulose ratio. Therefore, the

Table 2
Cellulose/Hemicellulose Ratios at 0 and 84 d
for the 70%-Moisture Column Treatments[a]

Inoculum (mg *P. ostreatus*/g stems)	Cellulose/ hemicellulose	Organism(s) assumed dominant	Removal of hemicellulose in 84 d (%)
Day 0	1.33 ± 0.08	—	—
0	1.43 ± 0.05	Indigenous	13.7
1.6	1.49 ± 0.05	Indigenous	15.1
5.1	1.53 ± 0.09	Indigenous	21.7
8.2	1.46 ± 0.09	Indigenous	14.1
10.9	1.75 ± 0.11	*P. ostreatus*	24.8

[a]A significantly increased cellulose/hemicellulose ratio vs the d 0 ratio indicates selective degradation of hemicellulose vs cellulose, and thus degradation by *P. ostreatus* that is relatively greater than that by indigenous organisms.

laboratory performance measures for these experiments were set as increased removal of hemicellulose, together with an increased ratio of cellulose/hemicellulose. The composite material properties will dictate the final amount of inoculum and treatment time chosen.

The cellulose/hemicellulose ratios and percentage of hemicellulose removal data at 0 and 84 d for the 70% moisture column treatments are given in Table 2. In only the 10.9 mg/g case was the cellulose/hemicellulose ratio increased significantly. This indicates that the amount of *P. ostreatus* inoculum should be set at 10.9 mg/g or greater. Thus, the range of inoculum amount for the next experiments will be 11–100 mg/g or higher. Nearly 60% removal of hemicellulose in 12 wk has been shown in the literature using *Pleurotus* species, albeit in initially-sterile straw *(20)*. At 10.9 mg/g of inoculum, we achieved a nearly 25% reduction in hemicellulose with little loss of cellulose. Thus, increased amounts of inoculum over 10.9 mg/g are expected to increase the amount of degradation in the same time period; experiments utilizing 21, 31, and 41 mg of *P. ostreatus*/g of straw stems are under way at the INEEL.

Conclusions

A minimum of 80–100 ppm of nitrogen was carried over to the straw stems in the mycelial inoculum. Above inoculum levels of 5.1 mg of *P. ostreatus*/g dry stems, the inoculated fungus has overtaken the indigenous microbes in nonsterile stems by 3 to 4 wk into the treatment, at the conditions tested. Moisture content was shown to have little effect on degradation below about 70% gravimetric moisture content. An inoculum amount of 10.9 mg of *P. ostreatus*/g stems was shown to be sufficient to effect the desired degradation trends, but not in the desired time frame of 12 wk or less. Higher amounts of inoculum or inoculum that is

preacclimated to straw may be required for this to occur. Future work includes testing up to 150% moisture and 100 mg/g of fungal inoculum.

Acknowledgments

We thank the University of Idaho, Aberdeen Research and Extension Center for use of its plot-harvesting equipment for the mechanical straw stem separation; Dr. Stephen Aust and Paul Swaner at Utah State University for maintaining and supplying the fungal cultures for inoculum production; and Karen Miller, Neal Yancey, and Jeff Parry at the INEEL. This project is administered by the Idaho Department of Water Resources Energy Division. This work is supported in part by the US Department of Energy, Assistant Secretary for Energy Efficiency and Renewable Energy (EE) under DOE Idaho Operations Office Contract DE-AC07-99ID13727. Additional support is provided by the Idaho Wheat Commission, Grant 4-D Farms, and Energy Products of Idaho.

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