SELECTIVE HARVEST OF HIGHER VALUE WHEAT STRAW COMPONENTS

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Abstract

Each year, millions of tons of agricultural residues such as wheat straw are produced worldwide. In this paper, we describe ongoing efforts to solve technological, infrastructural, and economic challenges to using this straw for bioenergy and bioproducts. Among these challenges, silica in straw forms a low-melting point eutectic with potassium, causing excessive slagging deposits in boilers. The presence of chlorine causes corrosion beneath the slag deposits on boiler tubes. Silica fouls kilns and fines slow paper machines, limiting the usefulness of straw in linerboard production. Poor resin bonding to the waxy outer cuticle of the straw, poor resin penetration, and high resin consumption due to fines limit the use of straw for straw-thermoplastic composites and for straw particleboard. Poor cellulase penetration limits the use of straw for production of fuels (ethanol) and chemicals. Straw consists principally of stems, leaves, sheaths, nodes, awns, and chaff. Not all of these parts of straw residue are equally valuable. Leaves and sheaths are higher in silica, while chaff, leaves and nodes comprise the source of most of the fines. The predominantly fibrous straw stem, high in cellulose, is of greater value for bioenergy, biofuels, and bioproducts. Our approach to reducing silica content is to selectively harvest the straw stems using an in-field physical separation, leaving the remaining components in the field to build soil organic matter and contribute soil nutrients. To address resin issues and cellulase penetration, we are developing small, distributed windrow systems employing white rot fungi to upgrade the straw.

Keywords. Wheat straw, silica, harvest, white-rot, bioenergy, combustion, bioproduct, thermoplastic composite, crop residue

Introduction

Biomass conversion to fermentable sugars for production of fuels, chemicals, and value-added industrial products is well understood. These bioenergy strategies rely on inexpensive fermentable sugars for economic viability. Exploitation of the "whole crop," specifically, wheat straw or other plant material currently regarded as residue or waste, is a practical approach for obtaining a reliable and low-cost source of sugars. However, obstacles related to capital costs, energy consumption, waste streams, production logistics, and the quality of the biomass feedstock, while technically feasible, plague industrial-scale production of sugars from wheat straw. The collective impact of these obstacles negatively impacts the economic viability of crop residues as a cost-effective alternative source of commodity sugars.

While wheat straw is readily available in large quantities, non-stem fractions of the straw contain high levels of inorganic ash, which reduces sugar yields per ton of straw shipped, and also contributes to slagging and downtime when the straw is combusted or gasified in fluidized beds. Thus, some fractions of the straw are less desirable than others, and energy-efficient methods to fractionate and selectively harvest the stems must be developed.[1] In addition to in-field stem separation, it would be beneficial to further chemically fractionate the stems to increase the ease of use once shipped to the biorefinery or

manufacturing plant. The maximum benefit would be achieved with local (distributed) pretreatments, minimizing the handling and transporting of the undesirable components.

Much of the research on pretreatment of straw and lignocellulose in general has been focused on conversion of the cellulose to fermentable glucose using cellulase enzymes, which are used to convert the solid cellulose to fermentable glucose. Many pretreatments, including acids,[2,3] alkalis,[4,5] organosolvents,[6] steam explosion,[4,7] physical treatments,[8,9] and others,[10] have been developed. In general, these pretreatments remove or modify the lignin-hemicellulose barrier that limits access of the cellulase enzymes to the cellulose fibers. This is necessary because cellulases are relatively large enzymes and cannot fit through most of the spaces in the intact vascular layer of lignocellulose;[11] this is the same barrier that limits resin penetration for composites production. Although effective, all of the pretreatments listed above are costly. Pretreatment with white-rot fungi, some of which prefer hemicellulose and lignin degradation over cellulose degradation,[12,13] would increase glucose yields and allow better resin penetration without significant capital or energy intensive steps. The principal drawbacks to centralized white-rot fungal pretreatments are that the process footprints are large and that treatment times are often too long for use at large industrial facilities.

Earlier we described our work on in-field physical fractionation to reduce silica content by selectively harvesting the straw stems.[1] In this paper we describe a strategy to reduce the amount of undesirable residue components (silica, lignin, and hemicellulose) shipped to centralized biorefineries. This strategy varies somewhat depending on the end use, but generally consists of distributed harvest and processing steps.

Materials and Methods

Wheat straw

Westbred 936 wheat straw, hard red spring variety, was utilized for the studies. All straw utilized to date was produced during the year 2000 growing season. The straw was produced and baled by Grant 4-D Farms (Rupert, ID). Twenty large bales (4 ft × 8 ft bales) were produced and stored in a stack at the side of the field, and only the protected center bales were used. For the laboratory studies, these large bales were rebaled into smaller 2 ft × 4 ft bales containing about 50 lb each, and stored in covered storage.

Physical separation of straw

Wheat straw stem fractionation via physical separation has previously been described.[1] This separation involved the use of both a tined-cylinder separator and a plot combine. In addition, for some analyses small samples of intact senesced wheat plants were hand-separated into fractions.

Organisms and inoculum production

Pleurotus ostreatus NRRL 2366 was obtained from the Northern Regional Research Laboratory (NRRL, Peoria, IL). Stock cultures were maintained on YM agar slants (Difco, Detroit, MI) at room temperature, and were sub-cultured every 2 weeks. To produce stock mycelial inocula, fungal mycelia were transferred from the maintenance slants to 100 mL of YM broth (Difco) using a sterile loop, and were grown in agitated culture for 2-3 days at room temperature and 180 rpm. This culture was then transferred to a sterile Fernbach flask containing 1L of YM broth, and agitated for 4 days 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 2-3 weeks or less.

Fungal nitrogen consumption experiments

Experiments were conducted to determine optimum inoculum production conditions for minimum final medium concentration of nitrogen and maximum fungal biomass yield. Approximately 500 mL of wet fungal pellets were transferred to a sterile blender and 500 mL of nitrogen-limited medium containing 20 g/L glucose and 1.5 - 3.5 g/L yeast extract were added. The mixture was blended for 2 minutes, 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 days at 30 °C, 135 rpm. Replicate flasks were sacrificed periodically, and the fungal pellets were separated from the liquid phase by centrifugation for 10 min at 27,000 × *g*. Fungal mass was measured gravimetrically after drying for 48 hours at 105 °C. Total Kjeldahl Nitrogen (TKN) was measured as previously described.[14]

Column tests

No extraordinary measures were taken beyond the initial inoculum production steps to maintain sterility, except for using initially sterile equipment. Air-dried straw stems (150 g dry weight) were spread onto a tray and sprayed with homogenized fungal mycelia to the desired initial fungal concentration. Additional sterile water was then sprayed onto the straw to reach the desired initial moisture content. About 50 grams dry weight of inoculated stems were added to each of three initially-sterile triplicate glass columns. The columns were supplied with humidified air at 15.5 psig at a rate of one system volume per day. The columns were sampled initially and approximately every 3-4 weeks thereafter. The samples were dried at 105°C overnight and ground to 60 mesh in a Wiley mill for compositional analysis.

Analytical methods

Wheat straw compositions were determined using the Quantitative Saccharification Technique.[15] Carbohydrate concentrations in hydrolysates were measured by high performance liquid chromatography (HPLC) using a BioRad HPX-87P column with distilled water as eluent, as previously described.[11] Ash compositions were determined by ashing the straw samples to constant weight at 650 °C for 18-24 hours in a muffle furnace. Energy Dispersive Spectrometry (EDS),[16] at 10-20 KeV using a Phillips XL30ESEM, was used to determine the silicon, potassium and chlorine contents of the ash. EDS standards were prepared by weighing, mixing and ashing reagent grade silicates, oxides, chlorides, and standard Reference Materials. These were counted under the same conditions as the straw ash. Calibration curves were prepared and used to adjust the values from the internal quantitative program on the EDS system for matrix effects. Wet methods and Inductively Coupled Plasma (ICP) Spectrometry were used to verify the results.

Results and Discussion

Westbred 936 straw and stem compositions

The estimated biopolymer and ash compositions of the Westbred 936 whole straw and stem fraction used are shown in Table 1. These compositions are typical for wheat straw, except for a higher potassium content due to the prior crop rotation of potatoes, which require significant potassium fertilizer application.

Table 1. Compositions of the Westbred 936 whole straw and stem fraction used in this study.

Component	Wt% of Component[a] in	
	Westbred 936 Whole Straw	Westbred 936 Stem Fraction
Cellulose[b]	32.1	37.2
Hemicellulose[c]	26.9	27.9
Lignin with Extractives[d]	20.3	18.9
Other organic[e]	9.5	7.2
Ash	11.2	8.8
SiO_2	2.3	1.3
K	3.5	1.0
Cl	2.3	2.5
Other inorganic	0.9	5.0
SUM	100	100

a Based on 100% dry weight of material
b Cellulose estimated as total glucan
c Hemicellulose estimated as the sum of xylan, galactan, mannan, and arabinan
d Calculated by difference as Klason lignin with extractives
e Other organics attributed to protein, uronic acid, etc. contents and to recovery errors in the procedure

Field-scale fractionation of wheat straw

Replicate samples of Westbred 936 wheat straw were fractionated by hand to provide a benchmark against which to compare mechanical fractionation, and also by machine using the plot-harvesting equipment.[1] The ash and silica contents of the hand-fractionated straw fractions are shown in Figure 1. The ash and silica contents of the whole straw used for the hand fractionation were 9.0 and

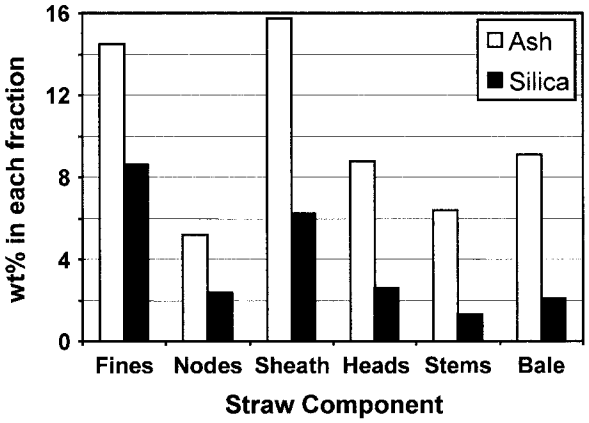

Figure 1. Distribution of ash and silica in hand-separated straw fractions of Westbred 936.

2.2 wt% ash and silica, respectively. The ash and silica contents the hand fractionated separated stems were 6.1 and 1.2 wt% ash and silica, respectively. Thus, hand fractionation reduced the ash content by 32% and the silica content by 45%. This is the benchmark to which the mechanical separation was compared.

The mechanical separation was performed on straw from a different field/bale,[1] resulting in slightly different ash and silica contents in the whole straw. The whole straw contained 11.2% ash and

2.6% silica. The mechanically fractionated stems contained 8.8% ash and 1.3% silica. Thus, the mechanical separation reduced ash content by 21% and silica content by 50%. The results indicate that the mechanical separation process reduced the silica content of the straw by selectively harvesting predominantly straw stems.

Fungal nitrogen consumption experiments

In a distributed infrastructure, it would clearly be uneconomical to sterilize the straw stems before treatment with fungi. Thus it is imperative that a distributed fungal treatment system be able to treat nonsterile straw. Such a system can be inoculated with straw containing growing fungal mycelia (solid inoculum), or by spraying a slurry of fungal mycelia onto the straw (liquid inoculum). For our experiments, we chose to employ the latter inoculation method because it allowed better control of the amount and distribution of fungal mycelia added to the straw. White-rot fungi dominate in nature under conditions of nitrogen limitation.[17] Thus, minimizing the amount of nitrogen that carries over to the straw in the fungal inoculum allows the inoculated fungi to overtake the microbes already present in the straw, and minimizes or eliminates the need for sterilization of the straw. Hence, the nutrient experiments were performed to determine the minimum amount of nitrogen that would be carried over to the straw stems in the fungal inoculum, using the spent medium as the liquid carrier stream for the homogenized mycelia.

Agitated cultures of *P. ostreatus* were used for these experiments rather than stationary cultures, because agitated cultures produced far more active biomass than the stationary cultures (not shown). Initial experiments utilized a carbon-limited YM Broth-based growth medium to determine workable levels of fungal biomass per unit volume that could then be homogenized and sprayed onto the straw stems. Once this level was estimated, a medium containing 2% glucose and trace minerals was used, with increasing levels of added yeast extract (the nitrogen source), to determine the extent and duration of fungal growth in the agitated inoculum production cultures. The optimal initial yeast extract concentration was in the range 1-3 g/L. Amounts of fungal biomass produced were too low below 1 g/L of initial nitrogen, while much above 3 g/L of initial nitrogen, the fungus became carbon limited. The cultures entered secondary metabolism between days 5 and 7 (Figure 2). At this point, production of

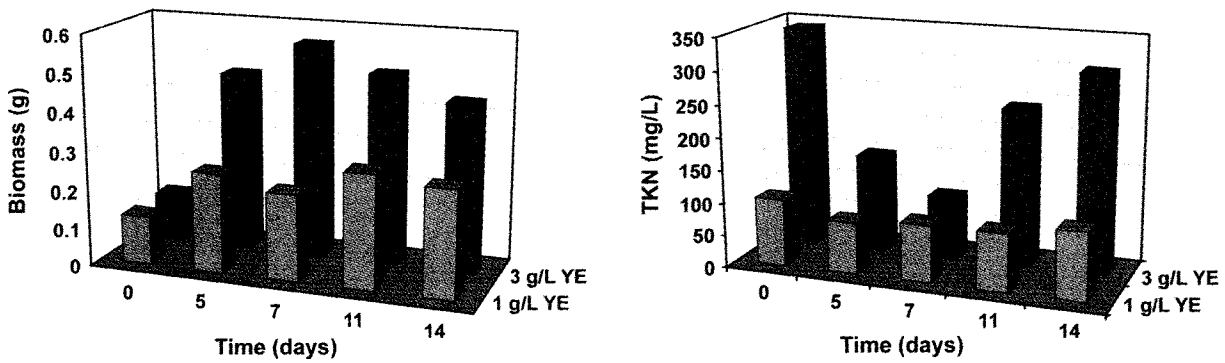

Figure 2. (Left Panel) Effect of initial nitrogen *P. ostreatus* growth on N-limited media; and **(Right Panel)** Variation of soluble nitrogen in *P. ostreatus* cultures grown on nitrogen-limited media.

extracellular enzymes, such as cellulases, hemicellulases, extracellular peroxidases, etc., began and increased the nitrogen level in the bulk liquid (Figure 2). The results indicated that about 80-100 mg/L of nitrogen (as TKN) was the lowest that can be carried over to the straw, and that the inoculum cultures should be harvested between days 5 and 7.

Column tests

Exploratory tests were designed to estimate the minimum fungal inoculum size necessary to overtake the indigenous microbes in the straw in 12 weeks or less. In these tests, fungal inoculum size was varied from 0 – 11 mg dry wt of mycelia per gram of stems, and moisture content was 60 – 77 % on a dry basis. Since *P. ostreatus* preferentially attacks hemicellulose and lignin over cellulose,[13] an increased cellulose / hemicellulose ratio versus the uninoculated control would indicate dominance of the culture by the inoculated *P. ostreatus*, assuming that the indigenous organisms show little or no selectivity in their degradation of the straw. After 84 days of treatment, the only case to show such an increase in the cellulose / hemicellulose ratio was at 10.9 mg *P. ostreatus* /g straw stems (Figure 3). This indicates that *P. ostreatus* has overtaken the culture and that the inoculum amount should be set at 10.9 mg/g or greater. Higher inoculum amounts could potentially be used to speed the process to less than 84 days.

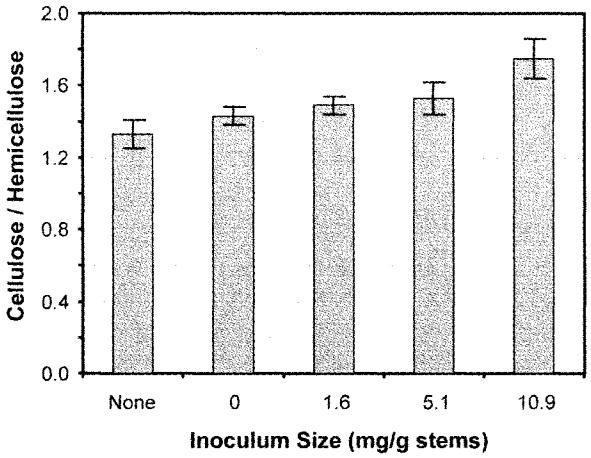

Figure 3. Cellulose/hemicellulose ratios at day 0 (None, or No Inoculum) and day 84 (all others). A significantly increased ratio compared to day 0 indicates domination of the culture by *P. ostreatus*.

Exploratory tests were also performed in the columns to determine the optimum moisture range necessary for the fungal degradation. Hemicellulose degradation was used as the performance measure for these tests. The effect of moisture on the degradation of hemicellulose at various times over 12 weeks of treatment is shown in Table 2 for the uninoculated control and for the 10.9 mg/g inoculated columns. In Table 2, the moisture contents are reported on a dry basis, i.e. grams of H_2O per gram of dry stems. These moisture contents represent the averages of three replicate columns; the variability among the replicates was 0.40 ± 0.14, 0.60 ± 0.09, and 0.77 ± 0.14 g H_2O/g dry straw. Moisture had little effect on straw stem degradation in the control columns (no fungus added). In the 10.9 mg/g columns, there was essentially no effect of moisture below 77% (0.77 g H_2O/g dry stems) until after 60 days had passed. There was a linear trend of increased hemicellulose removal going from 40 to 77% moisture. In all experiments the first 3 weeks gave nearly identical results, indicating that the faster-growing indigenous organisms dominated the initial 3 weeks. 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 moisture contents from 90 – 150% (0.9 - 1.5 g H_2O/g dry stems).

Table 2. The effect of moisture content on the degradation of hemicellulose at various times for the control (0 mg *P. ostreatus* / g stems) and for 1.6 – 10.9 mg *P. ostreatus* / g stems.

Inoculum (g fungus/g stems)	Moisture (g H_2O/g stems)	% Hemicellulose Remaining			
		Day 0	Day 22	Day 56	Day 84
0.0	0.40	100	87.7	86.4	88.3
	0.60	100	87.8	83.0	88.1
	0.77	100	83.5	87.6	86.3
1.6	0.40	100	88.7	88.5	88.9
	0.60	100	86.4	86.2	87.9
	0.77	100	88.7	88.7	84.8
5.1	0.40	100	87.3	86.1	73.9
	0.60	100	89.1	81.4	70.9
	0.77	100	86.4	80.0	78.2
10.9	0.40	100	87.8	87.3	87.6
	0.60	100	87.0	88.5	81.1
	0.77	100	86.1	84.2	75.2

Conclusions

Mechanical separation of straw stems using plot-harvesting equipment was as good as the separation achieved via fractionation of stems by hand. This indicates that, with the proper adjustments to the equipment settings, existing harvesting equipment can be modified to do this fractionation. Mechanical separation reduced the ash content of the harvested fraction by 21% and the silica content by 50%. Work on this task is continuing, and will include a field-scale stem separation test. In the fungal degradation tests, it was found that 80 – 100 ppm of organic nitrogen was the minimum amount that can be carried over to the straw without washing the mycelial pellets, when these cultures are used as inoculum for straw degradation tests. In the column tests, moisture content up to about 77% on a dry basis had little effect on degradation. An inoculum size of 10.9 mg *P. ostreatus* / g stems was sufficient to effect the desired degradation trends, but not in the desired time frame of 12 weeks or less. Higher inoculum amounts or inoculum that is pre-acclimated to straw may be required. Future work includes testing up to 150% moisture and 100 mg/g fungal inoculum, optimization of treatment duration, a field-scale test in passive windrows, and testing of composites made using *P. ostreatus*-treated stems.

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