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Assessing corn stover composition and sources of variability via NIRS

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Abstract Corn stover, the above-ground, non-grain portion of the crop, is a large, currently available source of biomass that potentially could be collected as a biofuels feedstock. Biomass conversion process economics are directly affected by the overall biochemical conversion yield, which is assumed to be proportional to the carbohydrate content of the feedstock materials used in the process. Variability in the feedstock carbohydrate levels affects the maximum theoretical biofuels yield and may influence the optimum pretreatment or saccharification conditions. The aim of this study is to assess the extent to which commercial hybrid corn stover composition varies and begin to partition the variation among genetic, environmental, or annual influences. A rapid compositional analysis method using near-infrared spectroscopy/partial least squares multivariate modeling (NIR/PLS) was used to evaluate compositional variation among 508 commercial hybrid corn stover samples collected from 47 sites in eight Corn Belt states after the 2001, 2002, and 2003 harvests. The major components of the corn stover, reported as average (standard deviation) % dry weight, whole biomass basis, were glucan 31.9 (2.0), xylan 18.9 (1.3), solubles composite 17.9 (4.1), and lignin (corrected for protein) 13.3 (1.1). We observed wide variability in the major corn stover components. Much of the variation observed in the structural components (on a whole biomass basis) is due to the large variation found in the soluble components. Analysis of variance (ANOVA) showed that the harvest year had the strongest effect on corn stover compositional variation, followed by location and then variety. The NIR/PLS rapid analysis method used here is well suited to testing large numbers of samples, as tested in this study, and will support feedstock improvement and biofuels process research.

Keywords *Zea mays* L. · Corn stover · Maize stover · Biomass · Crop residue · Biomass conversion feedstock · Lignocellulosic biorefinery · Compositional analysis · Compositional variability · Near infrared reflectance spectroscopy · NIRS · Biorefinery · Biofuels

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Introduction

In 2008, the United States (US) produced 9.0 billion gallons of ethanol for transportation fuel (RFA 2009).

Nearly all of this ethanol was produced from corn, utilizing the edible, starch-rich corn kernels as the feedstock. The conversion of corn kernels to fuel has been controversial on a "food vs. fuel" and "net energy basis" (Dale 2008). Plant-based, lignocellulosic biomass is suggested as a renewable feedstock for conversion to transportation fuels, materials, and power in biorefineries (Ragauskas et al. 2006). Recent studies estimate US biomass resources at as much as 1.3 billion tons per year, enough material to satisfy 30% of current US transportation fuel demand (Perlack et al. 2005). Corn stover, the above-ground, non-grain portion of the crop, is the largest currently available agricultural residue that potentially could be collected as a biofuels feedstock (Kadam and McMillan 2003; Graham et al. 2007). Modifications to corn breeding methods or manipulation of the corn germplasm may further increase corn stover biomass yields over current trends (Dhugga 2007).

Since corn stover is a large, available, fairly concentrated, non-food biomass source, it has been a model feedstock for biofuels process development research at the National Renewable Energy Laboratory (NREL) (Decker et al. 2007). Biochemical conversion of biomass to biofuels entails collecting and transporting the feedstock to the biorefinery, pretreating the biomass using thermochemical means, hydrolyzing the polysaccharide constituents with enzymes, fermenting the released simple sugars, and concentrating the fuel ethanol via distillation.

Biomass conversion process economics (Aden et al. 2002) are directly affected by the overall biochemical conversion yield, which is assumed to be proportional to the carbohydrate content of the feedstock materials used in the process. Most obviously, as polysaccharide content in a feedstock varies, theoretical maximum ethanol yields rise or fall. The aim of this study is to assess the extent to which commercial hybrid corn stover composition varies and begin to partition the variation among genetic, environmental, or annual influences. A rapid compositional analysis method using near-infrared spectroscopy/partial least squares multivariate modeling (NIR/PLS) was used to evaluate compositional variation among 508 commercial hybrid corn stover samples collected from 47 sites in eight Corn Belt states after the 2001, 2002, and 2003 harvests.

Experimental

Sources of corn stover samples

NREL analyzed 508 commercial hybrid corn stover samples collected from eight states across the US Corn Belt (Fig. 1). These were provided after comparative grain yield trials were run by the University of Minnesota Agricultural Experiment Station (MAES), the University of Wisconsin-Extension Madison, and Monsanto Corporation. NREL received 435 stover samples from these three organizations after the 2001 harvest. Only MAES sent additional samples (37 in 2002, 36 in 2003) of varieties that were replanted in similar trials for the following 2 years. The universities conduct annual side-by-side comparative grain yield trials that were open to the corn seed producers who market their products in those states. The purpose of these trials was to provide unbiased information to help farmers choose which corn varieties to plant the following spring. Through these trials, we were able to acquire stover samples from many genetically distinct hybrid corn varieties from across the US Corn Belt, with some varieties grown in the same locations over 3 years.

We held separate discussions with each potential stover supplier to identify appropriate sample sets for this work. Once the sample sets were identified, the suppliers were asked to provide 5–10 pounds of stover (10–20 whole stalks, minus cobs and grain) for each sample. Whole stalks were harvested at approximately 3–6 inches above the soil. Corn grain is not part of the stover and the starch in the grain would interfere with the cell-wall glucan analysis; thus, the grain and associated cobs were removed from the samples. The proportions of leaves and stalks, which have different compositions, are a potential source of variability in these samples. The 5–10 pound sample size was chosen to be large enough to minimize any unintentional sampling bias, but no attempt was made to control the anatomical representation in the samples collected. We did not attempt to specify the harvesting method or the timing of stover harvest, as these activities were completed after the main goals of our collaborators were satisfied. These samples were useful for us to broadly sample commercial hybrid corn stover variability, but they were not specifically selected to test the main factors thought to affect the variability. In this paper, we

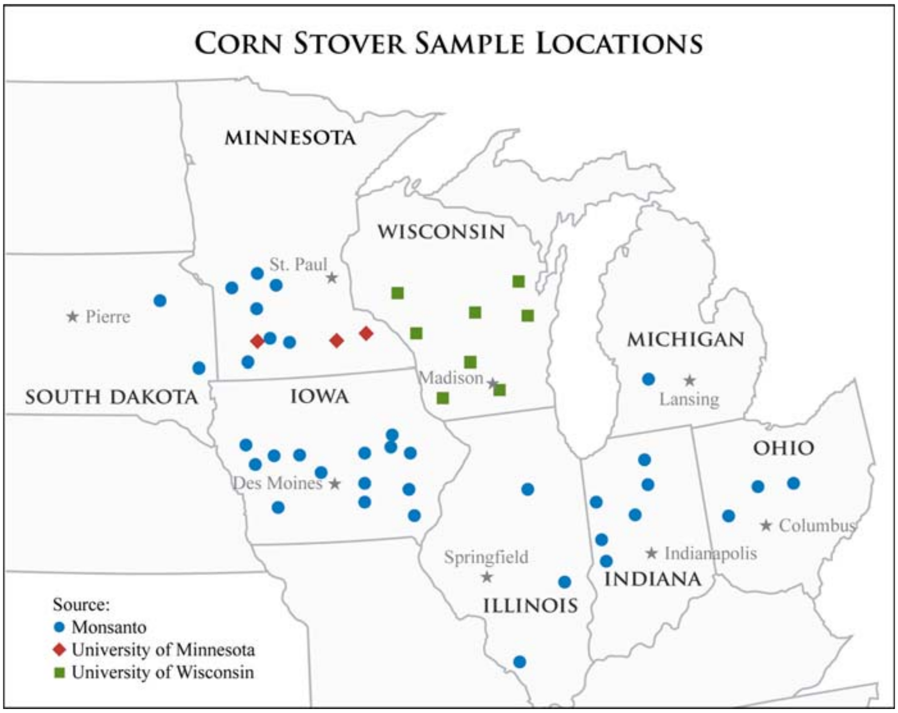

Fig. 1 Locations and sources of commercial, hybrid corn stover samples collected for this study

Table 1 Matrix of University of Minnesota hybrid corn stover samples spanning 3 years

U. Minn samples			Hybrid designation													
Year	Zip code	City	1	2	3	4	5	6	7	8	9	10	11	12	13	Total
2001	56152	Lamberton	1	1		1	1	2	1		2	1	1	1	1	13
2001	55964	Plainview	1	3	1	1	1	1	2	1	3	2	1	1	2	20
2001	56093	Waseca	1	2	2	2	2	2	2	2			1	2	2	20
2002	56152	Lamberton	1	1	1	1	1	1	1	1	1	1	1	1		12
2002	55964	Plainview	1	1	1	1	1	1		1	1	1	1	1	1	12
2002	56093	Waseca	1	1	1	1	1	1	1	1	1	1	1	1	1	13
2003	56152	Lamberton	3				3				3	3				12
2003	55964	Plainview	3				3				3	3				12
2003	56093	Waseca	3				3				3	3				12
		Total	15	9	6	7	16	8	7	6	17	15	6	7	7	126

The numbers of samples from a particular hybrid, location, and harvest year are shown. All sites are in the southern climatic zone of Minnesota

attempt to understand the underlying factors that affect compositional variability, from this sample set.

After the 2001 harvest, researchers at MAES provided 150 5–10 pound samples of stover from 52 commercial hybrids grown for grain yield trials at each of three locations in Minnesota—Lamberton, Waseca, and Plainview/Potsdam. These test plots are all located in the southern climate zone of the Minnesota and are indicated by diamonds on the map in Fig. 1. A subset of 53 samples from 13 of the 52 varieties (the varieties common to both the 2001 and 2002 sets) were scanned by NIR and their compositions predicted. A matrix of the Minnesota samples that we analyzed is presented in Table 1. Most varieties were replicated in all three locations in 2001. Samples were thoroughly dried to less than

20% moisture in a forced air oven at a temperature no higher than 50 °C prior to shipping. A more detailed description and the results of the "2001 Minnesota Hybrid Corn Performance Trials" have been published elsewhere (MAES 2002).

In 2002, MAES sent an additional 37 samples from the 13 hybrids that were re-tested out of the previous year's 52. The same three locations in the southern growing zone of Minnesota were used and the varieties were generally replicated in all locations (Table 1). The methods used and the results of the "2002 Minnesota Hybrid Corn Performance Trials" are published elsewhere (MAES 2003). In 2003, MAES sent 36 samples from only four out of the original 52 hybrids they tested that were common to both the 2001 and 2002 tests. Each of these varieties was sampled from triplicate plots in the same three locations in the southern growing zone of Minnesota. The methods used and the results of the "2003 Minnesota Hybrid Corn Performance Trials" are available online (MAES 2004).

Researchers from the University of Wisconsin-Extension Madison provided 194 5–10 pound samples of stover from 31 commercial hybrid varieties grown in 2001. Samples were obtained from eight locations in Wisconsin, representing the southern, south-central, and north-central climate zones of the state. These locations are indicated by squares on the map in Fig. 1, and a matrix of the Wisconsin samples is presented in Table 2. Hybrids were not planted at all locations, as they are typically grown only in favorable environments for that variety. The varieties were replicated at all sites within a given climate zone. Samples were thoroughly dried to less than 20% moisture in a forced air oven at a temperature no higher than 50 °C prior to shipping. A more detailed description and the results of the "2001 Wisconsin Corn Hybrid Performance Trials for Grain and Silage" can be found elsewhere (Lauer et al. 2001).

Many Monsanto researchers at 36 field stations provided 188, 5–10 pound stover samples grown in 2001 (Table 3). Samples were provided to NREL with coded variety identifications (i.e., samples "blind" to NREL) from 21 commercial hybrids grown at 36 different sites in seven states throughout the US Corn Belt. These locations are indicated by circles in Fig. 1. Hybrids were not replicated at all locations, as they are typically grown only in favorable environments for that variety. Most of the time, only single samples (i.e., few replicates) were provided for each variety at the different locations. Monsanto samples were sent directly from the fields to NREL via 2nd day shipping prior to complete drying. Every effort was made to dry the material immediately upon arrival at NREL. The results of the Monsanto yield trials are not publicly available.

Corn stover sample preparation methods

Samples were logged in as they arrived at NREL. Any samples that arrived wet were dried for several days in a warm, non-humid greenhouse to a relatively low moisture content (i.e., until they were not in danger of biological degradation). They were then transported to Hazen Research, Inc., (Golden, CO) where most samples were further dried in a forced air oven at 50 °C for 2–3 days. Since starch would interfere with the analysis of cell-wall glucan, we instructed Hazen to remove any cobs, and therefore corn kernels, from the samples. Hazen employed a yard waste shredder to coarsely shred each sample separately. Shredded samples were then milled with a rotary knife mill to pass through a 0.25-inch screen. Samples larger than 500 g total were mixed and divided using a riffle splitter to ensure that different aliquots of the sample were as physically (i.e., particle size distribution) and chemically uniform as possible. Samples were then packaged in labeled, one-gallon Ziploc bags (500 g aliquots) inside labeled plastic buckets with water-tight lids. Prepared samples were kept in a cool, dry biomass storage facility for long-term storage.

Wet chemical analysis of the stover 9 calibration samples

The Stover 9 model was calibrated with corn stover samples analyzed by standard NREL Laboratory Analytical Procedures (LAPs) (NREL 2009). These wet chemical compositional analysis procedures, originally adapted from wood and dietary fiber methods, have been expanded to better account for the more complex set of constituents found in corn stover and other herbaceous materials. Some of the main differences between corn stover and hardwoods, such as hybrid poplar, are the higher and more variable extractives content found in stover, the higher protein content of stover, and the higher ash content of stover.

Table 2 Matrix of 2001 University of Wisconsin hybrid corn stover samples analyzed in this study

U. Wisc samples			Hybrid designation																															
#	Zip code	Location	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Total
1	53546	Janesville, WI		3					1		1	3					3			1				3			1	3			1	1	3	24
2	53813	Lancaster, WI		2					1		1	3					3			1				3			1	2			1	1	3	22
3	53911	Arlington, WI		3					1		1	3					2			1				3			1	3			1	1	2	22
4	54630	Galesville, WI					1	6			1	1	3			1		1				1	3					5						23
5	54936	Fond du Lac, WI						6			1	1	3			1		1				1	3					5						22
6	54943	Hancock, WI					1	6			1	1	3			1		1				1	3					5						23
7	54165	Seymour, WI	1		1	1				1				5	3				5		1				1	3			3	3				28
8	54729	Chippewa Falls, W	1		1					1				6	3				7		1				1	3			3	3				30
		Total	2	8	2	1*	2	18	3	2	6	12	9	11	6	3	8	3	12	3	2	3	9	9	2	6	3	23	6	6	3	3	8	194

The number of samples from a particular hybrid and location are shown. Locations 1–3 are in the southern climatic zone, locations 4–6 are in the south-central zone and locations 7 and 8 are in the north-central zone of Wisconsin

* Sample not included in ANOVA analysis

To better account for the structural components in calibration stover samples, we performed a water extraction followed by an ethanol extraction. This removes components that may interfere with the subsequent analytical hydrolysis. The ethanol extract solution was dried and measured gravimetrically. Components within the water extract solution were measured and reported separately. We analyzed for sucrose in the calibration samples by analyzing the water extract solution for free glucose and sucrose. The free glucose and unmeasured fructose in the water extract was assumed to have been inverted from sucrose remaining in the plant tissue. We mathematically combined this glucose amount with an equal molar amount of fructose to determine an implied sucrose value. The free sucrose was measured directly and the total sucrose value was calculated when the implied sucrose (determined from glucose) and free sucrose were added together and reported. The amount of extractible inorganics (soil) in the calibration samples was determined by the difference between combustion ash analyses before and after the extractions. The remaining water soluble material, which represents an array of compounds, was reported by the model as "water extractible others". A detailed analysis of the components found in corn stover water extracts has been described elsewhere (Chen et al. 2007).

In the wet chemical compositional analysis of the calibration samples, the structural polysaccharides present in stover (largely cellulose and hemicellulose) were acid-hydrolyzed to monomers, analyzed separately by high-performance liquid chromatography (HPLC), and reported individually as anhydrosugars (glucan, xylan, galactan, arabinan, and mannan). Glucan is derived largely from cellulose and the other four sugars are derived largely from hemicellulose. The exact source of each of the sugars is not determined in this analysis, and each sugar is reported as an aggregate no matter the original source. Any starch in a sample would be counted as glucan and bias this value high. This would confound the compositional analysis since starch is not a structural polysaccharide. Acetic acid is measured directly in the analytical hydrolysate by HPLC and mathematically converted to the anhydro acetyl form. Protein is determined by multiplying the sample %N by 4.6. Lignin is determined gravimetrically and was corrected by subtracting the extracted biomass (i.e.

Table 3 Matrix of 2001 Monsanto hybrid corn stover samples analyzed in this study

Monsanto 2001Samples			Hybrid Desination																					
#	zip code	Location	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	total
1	43050	Mount Vernon, OH									1				1	1	1	1	1	1				7
2	43302	Marion, OH									1													1
3	45337	Laura, OH														1	1	1	1	1		1		6
4	46041	Frankfort, IN																1						1
5	46506	Bremen, IN									1													1
6	46975	Rochester, IN	Samples not given hybrid desination. Compositions included in summary statistics, but not ANOVA.																					9
7	47802	Vigo County, IN														1		1	1	1		1	1	6
8	47951	Kentland, IN											1		1		1		1		1	1		6
9	47991	Williamsport, IN											1			1						1		3
10	61740	Flanagan, IL										1	1	1	1		1			1	1	1		8
11	62432	Hidalgo, IL														1			1	1	1	1		5
12	62950	Jacob, IL																1	1	1			1	4
13	50158	Marshalltown, IA									1	1		1	2		1			1		1	1	9
14	50212	Ogden, IA										1	1		1						1			4
15	50220	Perry, IA											1		1			1						3
16	50251	Sully, IA												1	1	1	1	1			1	1	1	8
17	50701	Waterloo, IA									1			1			1	1			1	1		6
18	51019	Danbury, IA									1	1	1		1									4
19	51453	Lohrville, IA									1	1	1		1						1			5
20	51467	Westside, IA											1		1			1			1	1		5
21	51532	Elliott, IA														1		1	1	1	1			5
22	52314	Mt. Vernon, IA									1	1		1	1		1	1				1		7
23	52315	Newhall, IA									1			1	1			1			1	1		6
24	52353	Washington, IA													1	1	1	1	1	1		1	1	8
25	52577	Oskaloosa, IA													1	1		1	1	1	1	1	1	8
26	52625	Donnellson, IA														1	1		1	1	1	1	1	7
27	56110	Adrian, MN			1			1		1	1													4
28	56123	Currie, MN		1			1	1		1	1	1												6
29	56183	Westbrook, MN					1	1			1													3
30	56208	Appleton, MN	1																					1
31	56231	Danvers, MN	1	1																				2
32	56263	Milroy, MN									1													1
33	56271	Murdock, MN	1	1			1	1	1	1														6
34	57005	Corson, SD			1	1	1			1		1	1			1								7
35	57469	Redfield, SD	1	1	1		1	1		1		1												7
36	49335	Moline, MI	Samples not given hybrid desination. Compositions included in summary statistics, but not ANOVA.																					9
			4	4	3	1	5	5	1	5	13	9	9	6	15	11	10	14	10	11	12	15	7	188

The number of samples from a particular hybrid and location are shown. Samples are separated by state where varieties were grown

structural) protein value from the lignin, as the structural protein was assumed to condense with the lignin.

Stover 9 model description

A calibrated near-infrared spectroscopy/partial least squares (NIR/PLS) model, designated Stover 9, was used to predict the compositions of the corn stover samples. The Stover 9 rapid analysis NIR spectroscopic model for determining corn stover composition is described in another article in this issue (Wolfrum and Sluiter 2009) and is an update on the Stover 5c model described elsewhere (Hames et al. 2003).

The Stover 9 model was calibrated using wet chemical compositional analysis data on a diverse set of 77 corn stover samples. The samples span a broad range of constituent values and were collected from seven harvest years. We set up the PLS model to match the typical wet chemical compositional analysis errors, by choosing the number of principal components where the PLS cross validation errors match the LAP errors. So the NIR scanned samples predict as if they were analyzed using the more labor intensive LAPs. In the LAPs, differences greater than 1.5% for the carbohydrates and 1% for lignin are considered significant (Decker et al. 2007) (p. 1470). The accuracy of the predictions for the minor sugars (galactan, arabinan, and mannan) is limited by the low range of concentrations observed in the calibration samples. These components tend to predict close to the mean of the calibration set, and these predictions are less robust than the major sugars (i.e. glucan and xylan).

Rapid compositional analysis on corn stover samples using near-infrared spectroscopy

For NIR analysis, dry (<10% moisture), well-mixed, milled stover samples were scanned in a full-cup, natural products cell using a Foss 6500 forage analyzer with a sample transport module and a standard reflectance detector array. For each scan, a total of 32 spectra were collected and averaged to compensate for sample heterogeneity. The spectra were collected in the range of 400–2,500 nm. For quality control, a corn stover check cell was scanned with each day's scans and occasional samples were selected for re-analysis. We used WinISI 1.5 (Foss, Eden Prairie, MN) software to control the spectrometer, develop PLS equations and predict the compositions of the stover samples.

Stover 9 model output

The Stover 9 NIR/PLS model predicts 13 chemical components in each corn stover sample, based on a wet chemistry analyzed corn stover calibration sample set. All values are reported on a % dry weight, whole biomass basis (i.e. grams of each component per 100 g oven-dried, unextracted biomass). The stover 9 model reports three composite values (solubles, structurals, and component closure) that are calculated from the predicted constituent data instead of being predicted directly from spectroscopic data. The solubles composite value is the sum of the four NIR predicted soluble components: sucrose, extractible inorganic (soil), water soluble others and ethanol solubles. The structurals composite value sums nine predicted components: the structural polysaccharides (5 anhydro-sugars), lignin (corrected for protein), structural inorganics (ash), protein, and acetyl groups. The total component closure composite value is the sum of the solubles and structurals composite values, and should sum to approximately 100%. Using the solubles composite value, individual structural constituent values can be mathematically normalized to a % dry weight, solubles-free basis (i.e. grams of each component per 100 g oven-dried, extracted biomass).

The WinISI 1.5 software reports two QC statistics, global *H* and neighborhood *H*, with each sample analysis. These parameters describe how close a sample is to the calibration set mean (global *H*) or to the nearest calibration point (neighborhood *H*). These are reported in Mahalanobis distance units which are analogous to standard deviation units. A global *H* value less than three suggests a sample is spectroscopically similar to the calibration sample set mean and the model will predict the sample with the same errors as the wet chemistry methods. A neighborhood *H* value of less than 0.6 means there is a spectroscopically similar (near neighbor) sample in the calibration set, and the model should predict the sample well.

Data analysis

NIR predicted compositional data were imported to and analyzed using Excel 2003 (Microsoft, Redmond, WA). We used analysis of variance (ANOVA), using Design-Expert 7.1.1 (Stat-Ease, Minneapolis, MN), to partition the total variability among genetic, environmental, and annual factors, where appropriate. A full, general factorial design was used with the categorical factors: zip code (representing environmental factors), hybrid variety (representing genetic factors), and, if applicable, harvest year (representing annual factors). We chose a significance level of 95% in the ANOVA testing.

Results

Corn stover NIR compositions

Corn stover samples ($n = 508$) were collected from 47 locations located in eight US Corn Belt states from three harvest years (Fig. 1). The samples were catalogued, dried (if necessary), shredded, knife milled (below 0.25 inches), mixed, divided into 500 g sub-lots (if necessary), and stored. The dry (<10% moisture), well-mixed, milled stover samples were scanned by NIR, and the PLS model, Stover 9, was used to predict the stover compositions. The compositional ranges seen in these samples were within the sample ranges for the Stover 9 standard set, no trends were observed in the data sorted in scan order, the check cell data was consistent through the scans, and the replicated scan data looked normal (data not shown).

Table 4 Whole corn stover NIR compositional analysis summary statistical data

	% Dry weight (whole biomass basis)																Mahalanobis distance	
	Ethanol solubles	Sucrose	Extractible inorganics (soil)	Water extractible others	Solubles	Glucan	Xylan	Galactan	Arabinan	Mannan	Lignin (Corrected for protein)	Structural inorganics (ash)	Protein	Acetyl	Structurals	Component closure	Global *H*	Neighborhood *H*
Average	3.3	3.6	2.5	8.6	17.9	31.9	18.9	1.5	2.8	0.3	13.3	3.9	3.7	2.2	81.6	96.4	1.0	0.5
SD	0.4	2.1	0.5	2.3	4.1	2.0	1.3	0.2	0.3	0.1	1.1	0.9	0.8	0.3	3.6	1.6	0.3	0.1
Minimum	1.7	−1.0	0.0	1.4	5.7	26.5	14.8	0.8	1.6	0.0	11.2	0.8	1.1	0.9	70.4	90.7	0.4	0.2
Maximum	4.1	10.0	4.8	15.7	30.8	37.6	22.7	1.9	3.6	0.7	17.8	6.6	5.4	2.9	90.8	102.2	2.9	0.8
Range	2.4	11.0	4.8	14.2	25.0	11.0	7.9	1.1	2.0	0.7	6.6	5.8	4.3	2.0	20.4	11.5	2.6	0.7

We present the NIR compositional analysis statistics for the corn stover samples on a whole biomass, % dry weight basis in Table 4. The maximum global *H* value seen in the set was 2.9, and the average global *H* of the set was 1.0 Mahalanobis units, which suggests these corn stover samples are spectrally similar to the Stover 9 sample set calibration population. The maximum neighborhood *H* value was 0.8, though the average value was 0.5, which suggests some of the samples are spectrally distant from a nearby calibration sample and the data for these samples may be slightly less accurate. Taken together, these results suggest that the samples are spectrally similar to the calibration samples and that the model will predict these samples as if they were analyzed using wet chemical compositional analysis techniques.

The whole basis, total component closure averaged 96.4% dry weight (% dw) with a standard deviation (SD) of 1.6, suggests that nearly all of the corn stover component mass was properly measured and little double counting occurred. Uronic acids are a currently unmeasured component that may help raise the total component closure values closer to the ideal 100%. Total component closures ranged from 90.7 to 102.2% dw. The structural components averaged 81.6% dw, with a slightly larger SD of 3.6%. The major components of the corn stover, reported as average (standard deviation) % dry weight, whole biomass basis, were glucan 31.9 (2.0), xylan 18.9 (1.3), solubles composite 17.9 (4.1), and lignin (corrected for protein) 13.3 (1.1).

The soluble components in the corn stover samples ranged widely, from 5.7 to 30.8% dw with an average value of 17.9 (4.1)% dw. The largest contributor to the solubles composite was the water extractible others with an average of 8.6 (2.3)% dw. The average sucrose values varied widely with an average of 3.6 (2.1)% dw and a range of −1.0 to 10.0%. Three samples out of 508 (data not shown) had negative sucrose predictions with the minimum value being −1.0% dw. A negative sucrose value is not meaningful and should be interpreted as zero within the method error. Proportionately, the solubles ranged more widely than the structurals. This large range of variation in the solubles fraction is probably the result of harvesting stover at different levels of maturity at the different sites.

As the solubles were a major component of the variability seen in these samples, we also calculated

Table 5 Solubles-free corn stover NIR compositional analysis summary statistical data

	% Dry weight (solubles-free basis)									
	Glucan	Xylan	Galactan	Arabinan	Mannan	Lignin(corrected for protein)	Structural inorganics (ash)	Protein	Acetyl	Component closure
Average	38.9	23.0	1.8	3.4	0.4	16.2	4.8	4.5	2.6	95.7
SD	1.7	0.8	0.2	0.4	0.1	1.1	1.2	1.0	0.3	1.9
Minimum	34.1	20.2	1.1	2.0	0.0	13.8	1.0	1.3	1.1	89.6
Maximum	45.1	25.6	2.3	4.4	0.8	19.7	8.7	7.3	3.7	103.1
Range	11.0	5.4	1.2	2.4	0.8	5.9	7.7	6.0	2.6	13.6

the component compositions on a solubles-free, dry weight basis. This normalization was calculated by dividing each constituent value for structural components by the quantity (100%-% solubles) for that sample. We present these solubles-free data in Table 5. The solubles-free total component closure averaged 95.7 (1.9)% dw. The major components of the solubles-free stover were glucan 38.9 (1.7)% dw, xylan 23.0 (0.8)% dw, and lignin (16.2 (1.1)% dw). When the data are normalized to a solubles-free basis, the structural components generally had lower SD values than the corresponding values expressed on a whole biomass basis.

Histograms of major components

Frequency histograms of the major corn stover constituents on both a whole biomass and a solubles-free basis are shown in Fig. 2. They show broad yet fairly normal distributions, except for the lignin (corrected for protein) plot (Fig. 2c), which shows a distribution skewed toward higher lignin values. We plotted glucan, xylan, and lignin (corrected for protein) on a whole basis with the corresponding component plotted on a solubles-free basis. The solubles-free histograms are shifted to higher value, as expected, reflecting the different basis of the analyses. With the solubles removed from the sample, the remaining solubles-free components must proportionately increase to again sum to 100%. The whole, dry weight component closure (Fig. 2d) is plotted with the corresponding structurals composite value on a solubles-free basis. These both show average values near 100%, which suggests the NIR method and the normalization procedure are accounting for all components well on both a whole and solubles-free basis. The standard deviations for glucan and xylan are reduced on a solubles-free basis (Tables 4, 5). This is most noticeable in the narrower solubles-free xylan histogram (Fig. 2b). The lignin (corrected for protein) standard deviation is unchanged and the component closure standard deviation is higher on a solubles-free basis (Tables 4, 5).

Correlations among components

We present a table of correlations for all pairs of constituents on a whole, dry weight basis in Table 6. Only a few of the pairs show moderate correlations ($r > |0.65|$). Scatter plots of selected pairs of constituents, on a whole, dry weight basis, are presented in Fig. 3. A strong negative correlation ($r = -0.93$) existed between the soluble and structural composite values (Fig. 3a). Since these components necessarily sum to 100%, this correlation is to be expected. This trend also exists with similar negative correlations for glucan vs. solubles ($r = -0.75$) and xylan vs. solubles($r = -0.89$) (Table 6). Moderate positive correlations were seen for glucan vs. xylan ($r = 0.72$) (Fig. 3b) and glucan vs. lignin ($r = 0.75$) (Fig. 3c). A moderate, negative correlation was seen for glucan vs. protein ($r = -0.80$) (Fig. 3d) and lignin vs. protein ($r = -0.67$) (Table 6).

We list all the correlations of component pairs on a solubles-free basis in Table 7. Only two of the component pairs show moderately strong correlations ($r > |0.65|$) in the solubles-free data compared with the whole basis correlations. The glucan vs. xylan correlation seen on a whole basis disappeared when the components were compared on a solubles-free basis (Fig. 4a). This means that glucan and xylan content apparently vary independently in corn stover. The solubles-free glucan vs. lignin (Fig. 4b) and solubles-free glucan vs. protein (Fig. 4c) correlations

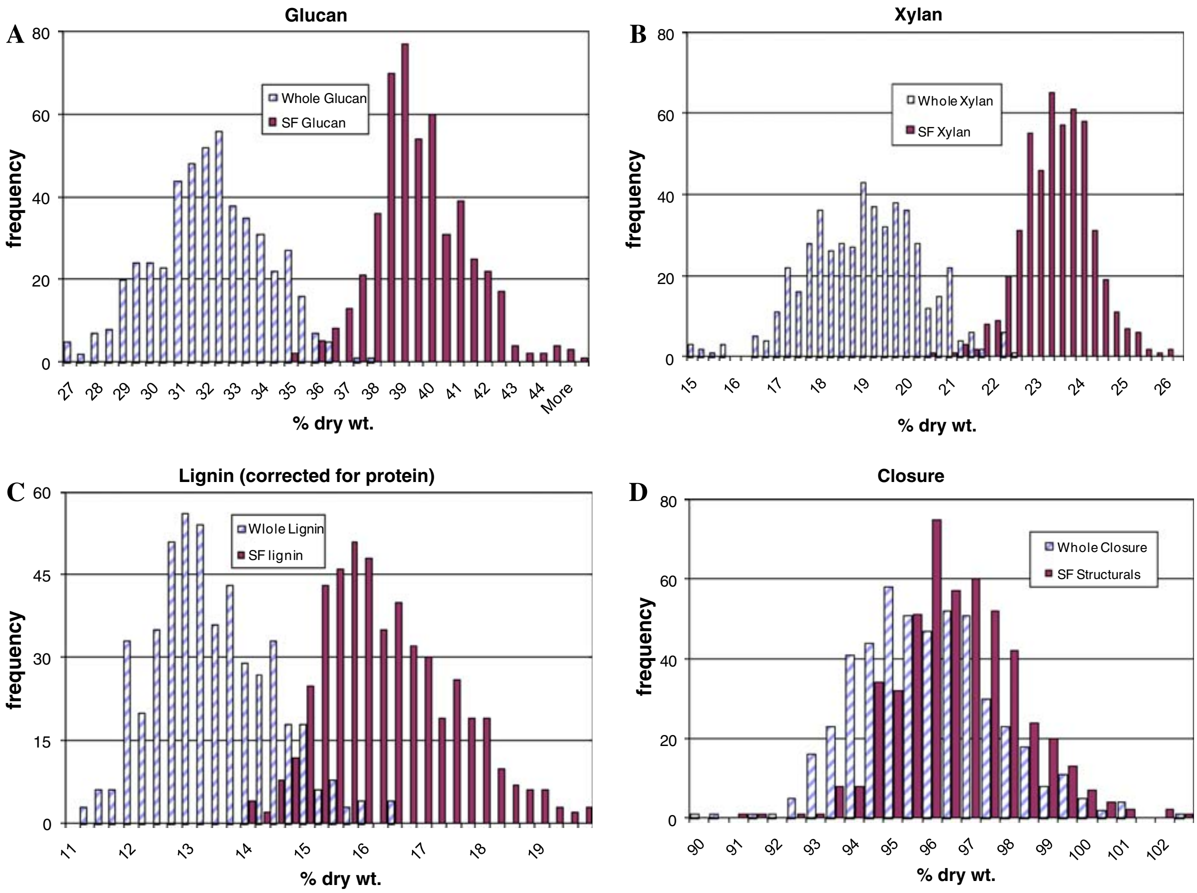

Fig. 2 Histograms of NIR predicted average corn stover constituents on a whole biomass, dry weight basis (*dashed bars*) and on a solubles-free (SF), dry weight basis (*solid bars*)

Table 6 Correlation coefficients (r) for component pairs on a whole, dry weight basis

	Xylan	Lignin	Protein	Structural inorganics	Acetyl	Solubles
Glucan	**0.72**	**0.75**	−**0.80**	−0.32	0.02	−**0.75**
Xylan		0.40	−0.52	0.02	0.25	−**0.89**
Lignin			−**0.67**	−0.34	0.06	−0.50
Protein				0.35	−0.11	0.38
Structural inorganics					0.26	−0.04
Acetyl						−0.16

Figures in bold show moderate ($r > |0.65|$) correlations

were similar, though weaker than those on a whole basis.

Analysis of variance

We used ANOVA to partition the variability seen in these samples according to genetic, environmental, or annual influences. We analyzed the three sample sets separately, as they were conducted independently using different experimental designs. General, full-factorial designs using two or three categorical factors—zip code (environment), variety (genetic), and, for the Minnesota samples only, harvest year (annual)—were developed and the NIR compositional

Fig. 3 Correlations between selected corn stover component pairs on a whole biomass, dry weight basis

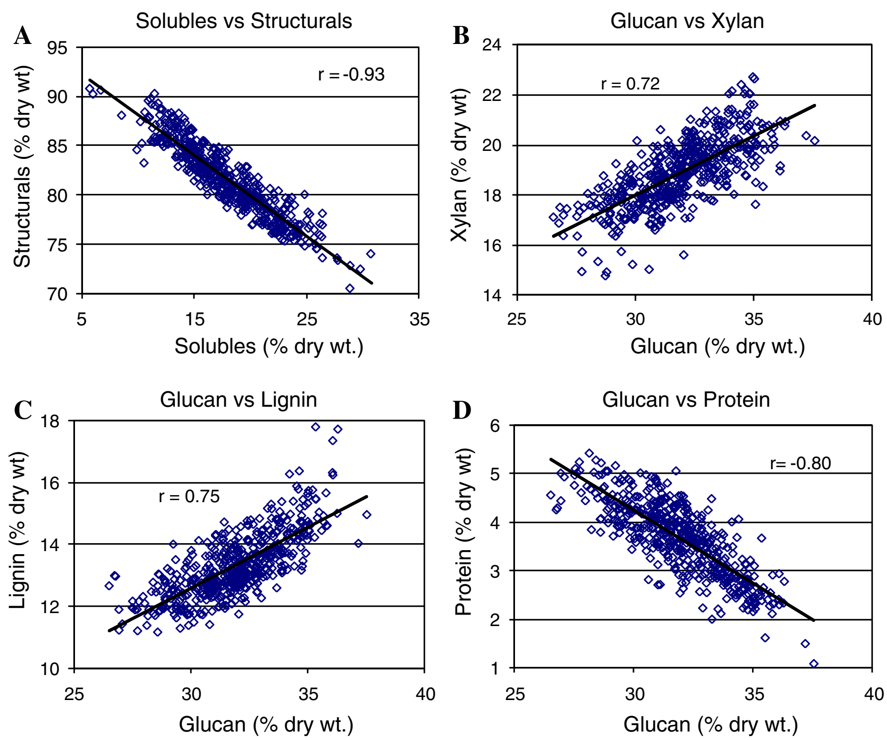

Table 7 Correlation coefficients (r) for component pairs on a solubles-free, dry weight basis

	Xylan	Lignin	Protein	Structural inorganics	Acetyl
Glucan	0.13	**0.66**	**−0.65**	−0.52	−0.13
Xylan		−0.15	−0.53	−0.12	0.08
Lignin			0.43	−0.37	0.01
Protein				0.43	0.10
Structural inorganics					0.20

Figures in bold show moderate ($r > |0.65|$) correlations

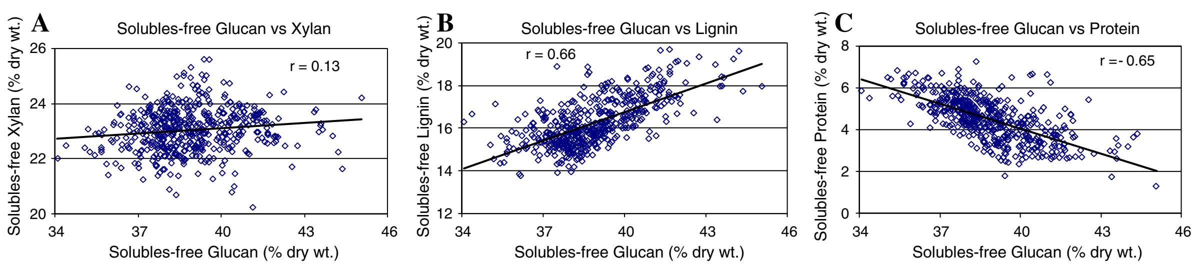

Fig. 4 Correlations between selected corn stover component pairs on a solubles-free, dry weight basis

data were used as the responses. These designs were not saturated, as all corn varieties were not grown at all locations but were sown in locations most favorable for growth (Tables 1, 2, 3). The Wisconsin and Monsanto data sets did not contain enough replication to evaluate the zip code/variety interaction.

Significant ($p < 0.0001$) main factor ANOVA models for glucan, xylan, lignin (corrected for protein), solubles, structurals, total component closure, solubles-free (sf) glucan, sf xylan, sf lignin (corrected for protein), and sf structurals were developed on the University of Wisconsin samples.

We discuss the whole basis glucan model below, though the results from the other component models give rise to similar conclusions. As seen in the ANOVA table (Table 8), both zip code ($p < 0.0001$) and variety ($p < 0.0001$) were significant factors in the glucan model. The model showed a barely significant ($p = 0.0412$) lack of fit, which suggests a higher order model may fit the data somewhat better. The mean square value for the zip code factor (20.79) explained more of the variance than the variety factor (3.01). This can be further seen in Fig. 5, which shows the model's predicted glucan values by zip code (left panel) or variety (right panel) with least significant difference bars around the predicted values. For both zip code and variety, most of the factors clustered near the grand average, with only a few factors being significantly higher or lower than the bulk of the samples. The glucan varied more based upon zip code than by variety. Analogous models were developed for the Monsanto samples, and similar glucan results were seen (Table 9), with zip code explaining more of the variance than variety. This model did not have a significant lack of fit, which means the model adequately fit the variance.

Significant ($p < 0.0001$) reduced, two-factor interaction ANOVA models were developed for the 3 years of sample data from the University of Minnesota. As seen in the ANOVA results (Table 10), all three main factors—variety ($p < 0.0001$), zip code (called location in the table) ($p = 0.0072$), and harvest year ($p < 0.0001$)—were significant. The variety by harvest year interaction was nearly significant ($p = 0.0573$), while the remaining interactions were not significant. This model also had a barely significant ($p = 0.0473$) lack of fit. The mean square value for the harvest year (24.97) explained most of the variability, followed by location (3.65), variety (2.88), and variety by harvest year interaction (1.22). As seen in Fig. 6, the harvest year main effect was shown by the consistently higher glucan for 2003 samples over 2001 and 2002 samples. The nearly significant variety by harvest year interaction can be seen as the glucan values were generally the same for 2001 and 2002, though a few varieties were higher in 2002 than 2001.

Discussion

In order to begin to replace petroleum with renewable transportation fuels, research is occurring to develop biomass to biofuels conversion processes. Biofuels can reduce imports of oil, develop rural economies, and reduce CO_2 emissions. Potential biofuels feedstocks, as mentioned in the Billion Ton study, include agricultural residues, wood residues, and dedicated energy crops (Perlack et al. 2005). The selection of feedstocks for biofuels processes will be made based on annual production mass per acre, the cost of production, the inputs needed to grow the crop, the feedstock composition, compositional variability, and other characteristics.

Current biofuels production is dominated by corn starch to ethanol processes. Corn stover is a lignocellulosic biomass source that does not compete with human food production capacity, and therefore it would likely be a less expensive feedstock for biofuels production than starch based feedstocks. Moreover, most of the cost of stover production should be covered by the grain portion of the crop. As many conversion plants will be needed to produce large volumes of biofuels, a wide geographic range will be employed to grow feedstocks. Differences in the feedstock compositions are expected to occur annually.

Corn stover is the largest source of agricultural residue currently available as a feedstock for biofuels production. It has been estimated that for every ton of

Table 8 Glucan ANOVA table for the 2001 University of Wisconsin hybrid corn stover samples

Source	Sum of squares	*df*	Mean square	*F* value	*p*-value prob > *F*
Model	341.92	36	9.50	16.30	<0.0001
A-Zip code	145.52	7	20.79	35.67	<0.0001
B-variety	87.41	29	3.01	5.17	<0.0001
Residual	96.75	166	0.58		
Lack of fit	39.10	52	0.75	1.49	0.0412
Pure error	57.65	114	0.51		
Cor total	438.66	202			

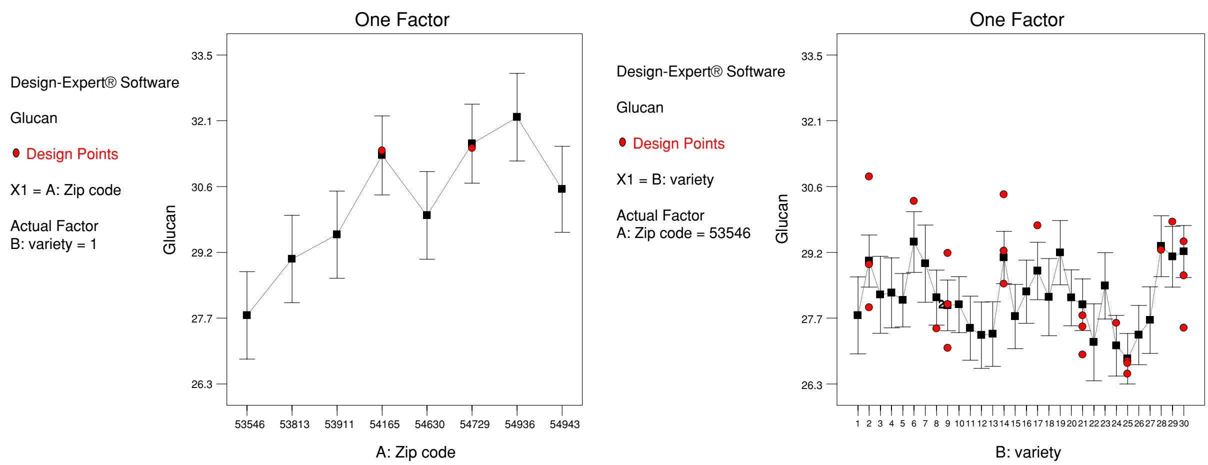

Fig. 5 ANOVA model predictions for University of Wisconsin commercial hybrid corn stover samples. The zip code model is shown in the left panel and the variety model in the right panel. Glucan values predicted by model (*squares*); least significant difference bars about predicted value and some of the design points (*circles*). Most factors cluster towards the grand average with a few factors significantly higher or lower than the average

Table 9 Glucan ANOVA table for the 2001 Monsanto hybrid corn stover samples

Source	Sum of squares	*df*	Mean square	*F* value	*p*-value prob > *F*
Model	500.67	46	10.88	8.26	<0.0001
A-zip code	428.66	27	15.88	12.05	<0.0001
B-variety	48.43	19	2.55	1.93	0.0176
Residual	150.20	114	1.32		
Lack of fit	149.96	113	1.33	5.52	0.3289
Pure error	0.24	1	0.24		
Cor total	650.86	160			

Table 10 Glucan ANOVA table for the 2001–2003 University of Minnesota hybrid corn stover samples

Source	Sum of squares	*df*	Mean square	*F* value	*p*-value prob > *F*
Model	132.18	31	4.26	6.06	<0.0001
A-variety	34.52	12	2.88	4.09	<0.0001
B-location	7.31	2	3.65	5.19	0.0072
C-harvest year	49.93	2	24.97	35.47	<0.0001
AC	18.24	15	1.22	1.73	0.0573
Residual	69.69	99	0.70		
Lack of fit	42.01	48	0.88	1.61	0.0473
Pure error	27.67	51	0.54		
Cor total	201.86	130			

corn grain one ton of corn stover is produced (Prihar and Stewart 1990). Experts estimate that between 105–117 million dry tons per year can be sustainably collected (Graham et al. 2007). However, the amount of sustainably harvestable corn stover varies locally, depending on soil and water conservation parameters (Wilhelm et al. 2004). Since corn will continue to be grown for its grain value, the stover can become an additional source of biofuels feedstock and provide corn growers an additional source of revenue.

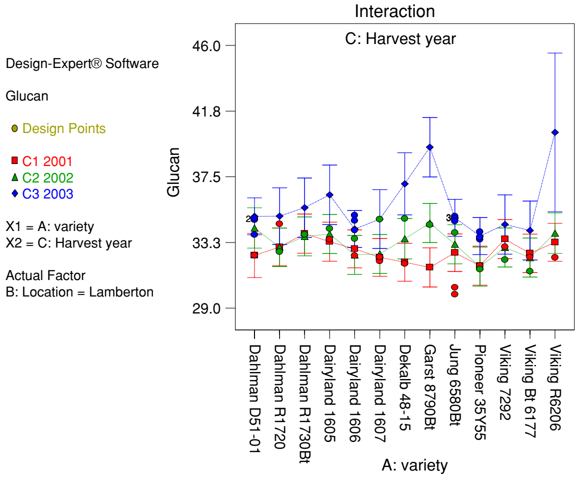

Fig. 6 ANOVA model predictions for University of Minnesota commercial hybrid corn stover samples. The variety/harvest year interaction is shown. Glucan values predicted by model (*squares*); least significant difference bars about predicted value and some of the design points (*circles*). The 2003 samples are generally higher than both the 2001 and 2002 samples

At NREL we seek to understand corn stover composition and variability to design economically viable processes for bioethanol production. Stover samples with higher carbohydrate amounts will be more valuable to a biochemical conversion process. Knowing the amount and type of carbohydrates available in a feedstock are important design criteria for a biochemical conversion process. Variability in the feedstock carbohydrate levels affects the maximum theoretical biofuels yield and may influence the optimum pretreatment or saccharification conditions. This information supports techno-economic modeling of biofuels production processes (Aden et al. 2002), modeling of stover collection (Hoskinson et al. 2007), and biofuels pretreatment research (Eggeman and Elander 2005). Here we used a NIR/PLS rapid analysis method to analyze 508 corn stover samples from across the US Corn Belt to assess their composition and compositional variability.

Stover composition

In this study we found an average, whole biomass basis composition of greater than 50% dw for glucan plus xylan, the two most abundant biomass sugars (Table 4). The remaining minor sugars (galactan, arabinan, and mannan) account for only an average of 4.6% dw. The relative importance of the different structural sugars depends on the fermentation organism used in the process, though the most efficient process would utilize all available sugars. The lignin is relatively inert in the biochemical conversion process, but it can be burned as boiler fuel to generate heat and electricity or converted to other high value products. The amount of lignin found in corn stover may make the process self sufficient in steam generation and may allow a bioconversion plant to export excess electricity as a co-product (Aden et al. 2002).

The compositional averages we report here may be skewed by the sampling protocol used in this study. We deliberately removed any grain, since it is not part of the stover. We also removed all cob material so as to eliminate effects of different amounts of cobs in different samples. Corn cobs are enriched in xylan (about 30% dw) relative to the other corn stover components (Decker et al. 2007) (p. 1467); thus, the stover values reported here have reduced xylan compositions. Differences in timing of stover harvest, in management of crop residue, cutting height, or other factors may have skewed the compositional results. It may be desirable to selectively harvest higher value fractions of corn stover to optimize collection systems (Hoskinson et al. 2007).

All the major components showed broad component distributions (Fig. 2). Thus, the average values reported here may not be relevant for any specific location and are not likely to be found in any single corn stover sample. Variation in the feedstock composition could affect any or all of the conversion process operations including pretreatment, saccharification, and fermentation. A conversion facility will need to be able to quickly check the feedstock composition in order to adjust process conditions to maximize yield and profitability. A biochemical conversion process will also need to be robust towards large variations in feedstock composition as seen here.

We found an average of 17.9% dw solubles in these corn stover samples (Table 4). These soluble components may complicate the valuing of feedstocks, as it is not certain what fraction of these solubles could be converted to products. They could be lost either before or after delivery to a biorefinery due to weathering or biological action in the field, during transit, or in storage. The soluble components may be extra corn stover mass to be transported or extra material paid for but not utilized in the process. The soluble components may be easily lost but also contain fermentable glucose and fructose, likely derived from sucrose (Chen et al. 2007). Any soluble carbohydrates that do arrive at a biorefinery may not survive pretreatment. Soluble sugars, especially fructose, are expected to degrade during pretreatment to form inhibitory compounds affecting the downstream saccharification or fermentation processes. These soluble sugars are a largely untapped source of potentially fermentable carbohydrates available for conversion to biofuels. We found an average of 3.9% dw of sucrose with a range of 0–10% dw (Table 4). A system that could capture and ferment these soluble sugars would have an increased product yield, though such a system may not be economical.

Much of the variation observed in the structural components on a whole biomass basis is due to the large variation found in the soluble components. We see this in the strong inverse relationship between solubles and structurals (Fig. 3a) and in the reduction in standard deviation in glucan and xylan from the whole compositional data to the solubles-free data (Tables 4, 5). The soluble components ranged more widely than the structural components (Table 4). The soluble components may be considered to be exogeneous to the structural cell wall components of corn stover. A negative trend between structurals and solubles was previously seen in a study by Wolfrum (Wolfrum et al. 2009) (in press for this issue). Pordesimo and colleagues reported on the compositional changes in standing corn stover composition from the late dent stage of development until 4 weeks after the suitable harvest time (Pordesimo et al. 2005). The solubles fraction changes the most and drops significantly after grain filling. The negative correlations between soluble and structural components may be caused by different degrees of weathering of the samples prior to our analysis. We did not control the harvest timing or technique for these samples, as they were "opportunity" samples available after other primary experiments.

The solubles-free compositions may be more relevant to process economics than whole dry weight biomass basis compositions. These solubles-free compositions better account for the stable carbohydrates available to a biofuels conversion process and may be a better basis on which to evaluate different feedstocks. In our analysis, we found an average 61.8% combined glucan and xylan in the solubles-free compositions (Table 5). These solubles-free carbohydrates are much more stable and likely to survive intact from harvest in the field, through possibly prolonged storage, and into a biorefinery process.

Constituent correlations

Most pairs of constituents in stover, on both a whole and solubles-free basis, show no trends in the scatter plots and are essentially independent of each other (Tables 6, 7). A few pairs of components show moderate correlations ($r > |0.65|$) that may reveal useful trends in the corn stover. The interesting positive correlation between glucan and xylan on a whole biomass basis (Fig. 3b) disappeared when the comparison was made on a solubles-free basis (Fig. 4a). Thus, the glucan and xylan compositions are apparently independent of each other on a solubles-free basis. The lignin/protein correlation also disappears in the solubles-free data, suggesting that one of these factors is more strongly influenced by the solubles. There were moderate ($r > |0.65|$) correlations still seen for sf-glucan versus sf-lignin and sf-protein. The positive trend of glucan with lignin suggests that when stover cell walls mature, both glucan and lignin contents increase. Therefore, unless selection pressures are brought to bear on these characteristics in breeding programs, it would seem that desirable high glucan stover samples will be coupled with less desirable higher lignin contents. The negative correlation between glucan and protein may be partially explained by the protein content remaining relatively constant among lower and higher glucan stover samples.

Sources of variability

Heterogeneity is an inherent characteristic of plants. Their chemical compositions vary as a function of morphology (e.g., stem:leaf ratio), anatomy (e.g., cell type ratios; maturity) and are a reflection of varying physiological roles and functional specializations of the cell types present in each tissue under a specific set of environmental and agronomic conditions. At the macroscopic level, corn stover is a mixture of stalks, leaves, leaf sheaths, tassels, cobs, and cob stems, each with its own chemical composition (Decker et al. 2007) (p. 1467). We attempted to reduce the compositional effect of anatomy by specifying 5–10 pound samples of whole corn stover without cobs or grain. This was hoped to reduce the effect of sampling different proportions of corn stover fractions on this analysis. Each sample was milled and thoroughly mixed to ensure that representative samples were NIR scanned. There are many sources of variability considered in this data including the anatomical fraction proportions, annual factors (harvest year), environmental factors (zip code), and genetics (corn hybrid varieties). Variability could also be introduced by different sample handling and storage schemes, though this is beyond the scope of this report. This analysis assumes that all fractions of corn stover would be collected as a feedstock. It may become desirable to collect selected corn stover fractions to optimize a biofuels process (Hoskinson et al. 2007).

Annual factor

The University of Minnesota provided the only sample set where we were able to test for harvest year effects plus interactions in addition to environmental and genetic main effects. The genetic and environmental influences are minimized in the University of Minnesota set by selecting the same array of commercial corn hybrids, grown in the same three locations in three successive growing seasons, by the same organization using the same agronomic practices. The ANOVA showed the harvest year factor to be the largest source of variance across the three sites and up to 13 varieties (Table 10). The harvest year/location mean square ratio is 6.84 and the harvest year/variety mean square ratio is 8.67, which signifies 6.84 and 8.67 times the variance is explained by the harvest year over the location and variety, respectively. The harvest year 2003 samples were significantly higher in glucan content compared to the 2001 and 2002 samples, which generally had similar glucan contents (Fig. 6). By controlling for all of the genetic variance between the years, as well as for a large fraction of the variance due to environmental factors, we believe we are looking primarily at the effect of differences in annual weather patterns. Still, differences in harvest timing or technique could confound this conclusion. Though the magnitude of the annual differences seen in 2003 was large, the inductive basis for this conclusion was small. The varieties chosen for the field trials changed each year and only 13 varieties from 2001 were replanted in 2002. Four varieties common to the 2001 and 2002 harvests were planted in 2003. The four varieties grown in 2003 may, by chance, have been unusual and affected this analysis. Only in this sample set were we able to analyze the two-factor interactions. The location/harvest year interaction was significant, which may be due to the strong effect of harvest year on the ANOVA.

Environmental factor

There are numerous environmental factors that could potentially influence the composition of an agricultural residue such as corn stover. Environmental factors can be broken into sub-groups such as uncontrollable physical factors and potentially more controllable agronomic practices. Uncontrollable physical factors include soil type (sand, clay, loam, etc.) and weather (temperature, precipitation, wind). More controllable agronomic factors include tillage method, planting date, planting density (seed/acre), soil pH and fertility, fertilizer type and amount, irrigation strategy, and herbicide and pesticide usage. Grain and stover harvesting practices to consider include the degree of corn senescence and drydown, the timing of the grain harvest, the method of grain and stover harvest, dual- or single-pass harvesting, and any precipitation or wind exposure prior to harvesting.

In this data set some of the grain was mechanically harvested from the field before collection of the stover. In other cases, the grain may have been harvested by hand (a much gentler process). Mechanical harvesting of grain, using a combine, breaks the stalks open and shatters the dried leaves as the tractor moves through the field. Therefore samples collected from combined corn may not be representative of the entire corn plant. If the stalks in a mechanically harvested field experienced rain between the grain and stover harvest times, a large fraction of any water-soluble solids present in the stover may have been washed out of the sample. Hand harvesting most likely better preserves the anatomical integrity of the stover. Differences in the timing of stover harvest relative to the grain harvest, as well as the harvest method itself, are considered to be environmental factors in this analysis of variance.

In all three ANOVA data sets, the zip code factor, as a proxy for environmental factors, was found to be significant and larger than the variety factor. The ratios between the zip code/variety mean squares for all three data sets are 6.91, 6.23, and 1.27 for the Wisconsin, Monsanto, and Minnesota models, respectively. This means that the zip code factor is explaining more of the variance than the variety factor. The Minnesota samples were all grown in one climatic zone which may explain why the variety and location explain similar amounts of variance. The Wisconsin samples were grown in three different climatic zones which may explain why location explains more variance than variety. It is unusual that the Monsanto location effect was as strong as the Wisconsin samples considering the larger geographic range covered by the Monsanto samples (Fig. 1). For the Wisconsin and Monsanto samples the environmental factor includes annual weather effects on the corn stover. The harvest year/location (zip code) mean square ratio in the Minnesota data is 6.84, and the annual weather effect is contained in the harvest year factor of this study.

Genetic factor

The corn crop in the US is extremely diverse in terms of the number of hybrid varieties that are marketed and planted each year. However, the genetic base of these hybrids is regarded as fairly narrow compared to the entire corn germplasm. As new varieties are released each year, older hybrids become obsolete, so the spectrum of corn hybrids being grown changes gradually from season to season. A given corn hybrid probably enjoys only about 3–5 years on the market before it is replaced by a better variety. Each one of these varieties is genetically distinct to some unknown degree and is marketed to farmers on the basis of predicted high performance (i.e., grain yield per acre) in specific geographic locations.

The variety factor was significant in all three data sets, though the smallest of the factors seen here. Only a few varieties were significantly different in composition than the rest of the varieties. Although variety is the smallest significant factor in this study, it is potentially the most controllable factor. The chemical composition of corn stover has traditionally not received much attention from corn breeders. Genetic factors that control the relative proportions of the anatomical fractions, the number of vascular bundles per stalk cross-section, or changes to the relative proportions of cellulose, hemicellulose, solubles, and lignin may be used to create new corn varieties with stover that is more amenable to processing for biofuels. Changes to the proportions of corn stover anatomical fractions or changes to the proportions of cellulose, hemicellulose, solubles, and lignin could help make corn varieties with stover that is more valuable to a biofuels process. Having a rapid method to determine corn stover composition can help breeders develop dual use corn varieties that are both high yield for grain and more easily and inexpensively converted to biofuels.

The ANOVA results seem consistent and reasonable, though the experimental designs were not saturated (Tables 1, 2, 3) and contain complex alias structures that could complicate their interpretation. What appear to be significant main effects in the ANOVA may be the result of higher order interactions. The main cause of this is biological in that all corn varieties could not be grown in all locations. Many of the models showed a significant lack of fit, which suggests that the models we chose were too simple to adequately describe the variance. The Wisconsin and Monsanto data sets did not have enough replication in order to test interactions among factors. We made extensive efforts to stabilize and preserve the samples in this study; still, it is possible that changes occurred to the samples that affected the compositional results.

Biomass conversion process economics are directly affected by the composition of the feedstock used in the process. It would be desirable to reduce or control the feedstock variability and increase the concentrations of the most valuable components for a particular process. Rapid analysis feedstock compositional methods can help in breeding systems and in valuing feedstocks for purchase. Breeders can use rapid methods to select varieties with desirable biofuels feedstock properties in addition to high yields. We might expect these characteristics to vary independently, but they may not. Stover samples that contain higher structural polysaccharides and lower solubles may command a higher feedstock price. Less desirable feedstock samples with high solubles content or samples that have been significantly degraded could be identified quickly in the field. Thus, a feedstock's conversion potential can be measured as a "useful carbohydrate" (i.e., structural carbohydrate) ton per acre metric, or biofuel gallon per acre metric rather than a dry ton per acre basis. At a biorefinery, rapid analysis can help with feedstock control, allowing for the matching of feedstock compositions to process conditions to optimize biofuels conversion efficiencies.

Conclusions

Corn stover samples ($n = 508$) from 47 locations in eight US Corn Belt states were analyzed by a rapid NIR/PLS method to determine average composition and compositional variability. The major components of the corn stover, reported as average (standard deviation) % dry weight, whole biomass basis, were glucan 31.9 (2.0), xylan 18.9 (1.3), solubles composite 17.9 (4.1), and lignin (corrected for protein) 13.3 (1.1). We observed wide variability in the major corn stover components. The soluble components strongly affected the amounts of structural constituents in these samples. The stover compositional variability observed was partitioned into, in descending order, harvest year, environment, and variety factors. The NIR/PLS rapid analysis method used here is well suited to testing large numbers of samples, as tested in this study, and will support feedstock improvement and biofuels process research.

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