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Rapid Biomass Analysis

*New Tools for Compositional Analysis of Corn Stover Feedstocks
and Process Intermediates from Ethanol Production*

**BONNIE R. HAMES,* STEVEN R. THOMAS, AMIE D. SLUITER,
CHRISTINE J. ROTH, AND DAVID W. TEMPLETON**

*National Bioenergy Center, National Renewable Energy Laboratory,
1617 Cole Blvd., Golden, CO 80401, E-mail: bonnie_hames@nrel.gov*

Abstract

New, rapid, and inexpensive methods that monitor the chemical composition of corn stover and corn stover-derived samples are a key element to enabling the commercialization of processes that convert stover to fuels and chemicals. These new techniques combine near infrared (NIR) spectroscopy and projection to latent structures (PLS) multivariate analysis to allow the compositional analysis of hundreds of samples in 1 d at a cost of about \$10 each. The new NIR/PLS rapid analysis methods can also be used to support a variety of research projects that would have been too costly to pursue by traditional methods.

Index Entry: Corn stover; near infrared spectroscopy; projection to latent structures; feedstock; multivariate analysis.

Introduction

Robust analytical methods are needed to support and enable biomass conversion processes because of the heterogeneity that is an inherent property of biomass. The chemical composition of a biomass feedstock varies as a function of many factors including plant genetics, growth environment, harvesting method, and storage. Many biomass feedstocks are residues of another process, which introduces the varying efficiency in the original process as an additional source of compositional variance. All of these sources of compositional variance are difficult to control; however, the composition of a given feedstock can be measured in real time and that information can be used to adjust process conditions for optimal conversion. The rapid, inexpensive compositional analysis methods described here are examples of new tools that will be needed for the commercialization of processes that convert biomass into fuels and valuable chemicals.

*Author to whom all correspondence and reprint requests should be addressed.

Standard wet chemical methods for the chemical characterization of biomass feedstocks and biomass-derived materials have been validated through the International Energy Agency and are available through the American Society for Testing and materials (ASTM) (see Website: www.astm.org/cgi-bin/SoftCart.exe/index.shtml?E+mystore) (1). However, these methods are not applicable in a commercial setting because they are very expensive (labor intensive) and cannot provide the analysis information in a time frame useful for process control. For example, a complete analysis using standard wet chemical methods costs \$800–\$2000 per sample and results are not available for days, sometimes weeks. By contrast, the new methods reported here can perform the same analysis for about \$10 per sample and the results are available in a time frame relevant for process control, meaning that the information can be used to make the process adjustments necessary for steady-state production. One approach to reducing the time and cost of compositional analysis is the development of rapid analysis methods that use multivariate analysis software to extract chemical information from easily obtained spectroscopic data. Rapid analysis methods match the precision and accuracy of their calibration methods so the savings are obtained without loss of precision or accuracy (2). New techniques, such as rapid analysis, are needed to provide analytical support for large-scale processes that convert biomass to fuels and chemicals.

As shown in Fig. 1, rapid biomass analysis can be useful at many stages of an industrial process. The methods described here involve the compositional analysis of biomass feedstocks and biomass-derived solids produced during ethanol production. These tools can be used to characterize feedstock as it enters the reactor. If necessary, rapid analysis can be used to guide feedstock blending. Monitoring chemical changes during the processing of biomass provides feed-forward and feed-backward information that can be used to ensure that the process maintains a steady state in spite of the feedstock variability. Finally, process residues and products can be evaluated to assess overall process efficiency. As more samples are analyzed, information can be obtained about the composition of an “ideal feedstock.” Field-mobile instruments can be calibrated for use as purchasing tools. Buyers can obtain compositional information about a biomass feedstock at the point of purchase. Feedstock prices can be based on quality instead of weight. Living plants, young plants, and perhaps even seeds can be evaluated and selected for desirable characteristics and production potential. This article describes the steps necessary to develop rapid biomass analysis for all of these applications.

Development of Rapid Analysis Method

Several steps are involved in the development of rapid analysis method, including gathering appropriate calibration samples, chemical characterization of the calibration samples, developing spectroscopic methods for the rapid technique, projection to latent structures (PLS) regression

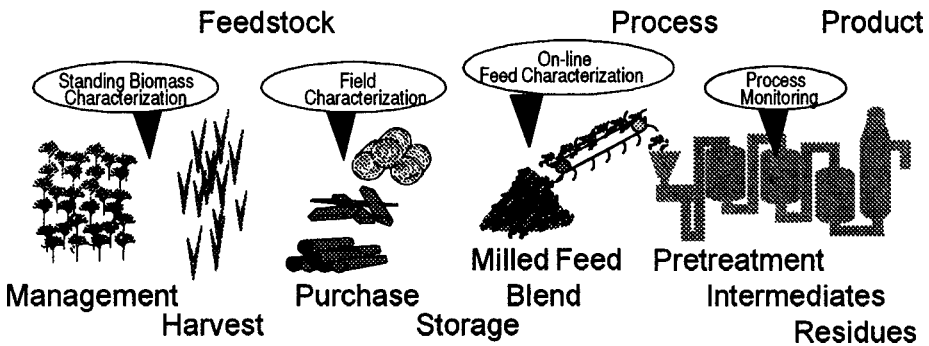


Fig. 1. Applications for rapid biomass analysis in biomass conversion processes.

and the validation of the PLS algorithm, and the development of quality assurance/quality control procedures including guidelines for appropriate application of the new methods.

Calibration Samples

The first step in developing a new method is the gathering of appropriate calibration samples. A minimum of 30 unique samples is needed for preliminary methods, and calibration sets for robust methods usually contain 100–300 well-characterized samples. Collecting and characterizing a good calibration set costs about \$300,000, which is by far the most expensive and time-consuming step in method development.

Calibration samples should have compositions similar to the samples to be analyzed. If possible, the calibration set should include samples that represent all known sources of compositional variance for that material. Another feature that is essential to a good calibration set is independent variance of the different constituents. Calibration sets should be checked for strong correlations among major constituents. Since the composition of plants is determined by physiology and plant viability, biomass feedstock samples with independently varying constituents are sometimes difficult to find. Several calibration samples can often be made from one feedstock by manually adding or removing specific plant tissues such as leaves or nodes. The extremes of a corn stover calibration set can be found within a single plant, in the various plant tissue types. For example, no whole corn stover feedstock will have as much protein as a sample consisting only of leaves. A research environment is ideal for the generation and collection of process calibration samples since reaction parameters are often skewed for experimental purposes. Larger-scale processes run at steady state, so development of a rapid analysis method to support these processes should begin during the reactor shake-down phase. Hundreds of samples may need to be evaluated to identify 30 unique samples for a preliminary calibration. The preliminary method can then be used to identify the best samples for expanding and improving the calibration set.

Rapid biomass analysis methods require calibration using robust and accurate methods. Corn stover is a whole-plant feedstock whose major constituents include cellulose, hemicellulose, lignin, protein, chlorophyll, and structural inorganics (silica). The hemicellulose in corn plants is a complex network consisting of an acetylated xylan backbone with branches incorporating galactose, arabinose, and uronic acids. The lignin in corn stover contains both syringyl and guaiacyl aromatic rings, and the side chains are enriched in ester groups. Standard methods for the determination of cellulose, hemicellulose, and lignin content in biomass are available from ASTM (*see Website: www.astm.org/cgi-bin/SoftCart.exe/index.shtml?E+mystore*). Standard methods optimized for the compositional analysis of corn stover are available from the National Renewable Energy Laboratory (NREL) (*see Website: www.ott.doe.gov/biofuels/analytical_methods.html*). Methods are available for the determination of total solids, structural inorganic matter, soil, nonstructural sugars, chlorophyll, protein, structural carbohydrates, acid-soluble lignin, and acid-insoluble lignin. Standard procedures are also posted for sample preparation and representative sampling of bulk feedstocks. Following these methods, a complete compositional analysis can be obtained with mass closures between 97 and 103%.

Rapid analysis methods look for patterns in spectroscopic data that correlate with changes in composition. A diverse calibration set with quality calibration data is required for an accurate and robust rapid analysis method.

Spectroscopic Methods

Quality spectroscopy is the second essential component of method development. Many different spectroscopic techniques can be used in the development of rapid analysis methods. For biomass samples, near infrared (NIR) spectroscopy offers several advantages. A wide variety of instruments are commercially available including rugged spectrometers designed for use outdoors or for process monitoring (*see Website: www.FOSS-Nirsystems.com and <http://asdi.com/index.htm>*). Sample handling devices have been developed specifically for the analysis of bulk biomass samples and biomass-derived liquids and slurries. Newer versions of field-mobile instruments include spectrometers that fit in a backpack and reduced wavelength versions that resemble digital cameras and run from palm pilots. These instruments collect spectroscopic data from wavelengths in the visible and NIR regions of the electromagnetic spectrum from 400 to 2500 nm. Spectra in these regions contain signals from C-H, O-H, N-H, and C=O bonds, so compositional information for cellulose, hemicellulose, protein, and lignin is contained in the NIR spectra of biomass samples. For biomass solids, NIR spectroscopy is usually done in reflectance mode. This is a surface technique capable of penetrating only about 150 μ into the biomass surface. To obtain a representative spectrum of biomass, samples must be ground to a uniform particle size or a large

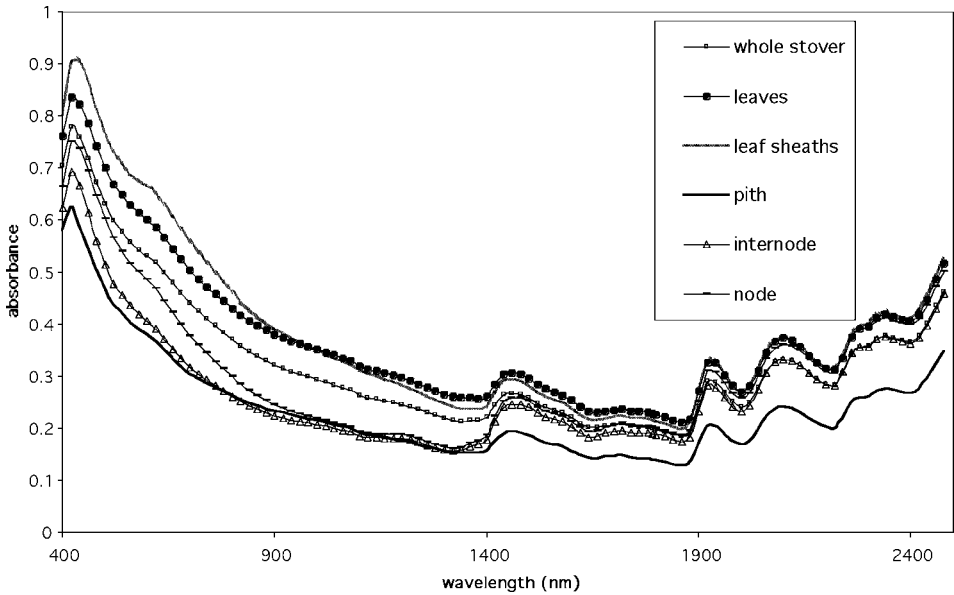


Fig. 2. NIR spectra of anatomic fractions of corn stover.

sample volume must be scanned during spectral collection. In most cases, 30–100 spectra are collected and averaged prior to PLS analysis. Reflectance spectroscopy separates the detector from the NIR light source, allowing sunlight to be used as the NIR source in field measurements. The spectroscopic technique selected must contain the desired information about the chemical composition of each sample. Since inorganic materials do not absorb IR light, nonstructural materials such as soil cannot be measured using NIR wavelengths. Structural inorganics can be measured indirectly if they perturb the absorbance of adjacent organic compounds. Figure 2 shows the NIR spectra of nine corn stover anatomic fractions. These spectra contain all of the necessary information to determine the chemical composition of these samples.

The selection of an appropriate spectroscopic method is the key to cost reduction and speed of analysis. The final rapid analysis method will extract chemical information from spectroscopic patterns. Once calibration is complete, compositional analysis becomes as fast and inexpensive as the spectroscopic method.

Multivariate Analysis

Multivariate analysis connects the compositional data with the spectroscopic data. The PLS (sometimes called partial least squares) method regresses the matrix of compositional information against NIR spectral data collected from 400 to 2500 nm. In simplified terms, PLS analysis solves hundreds of equations in thousands of variables to obtain a linear equation

that translates spectroscopic data into compositional information. Multivariate analysis methods were designed for complex systems like those found in biomass compositional analysis. Rapid analysis techniques using NIR spectroscopy are common in industrial applications monitoring the composition of animal feed, cheese, beer, and grain. The concept of NIR/PLS analysis of biomass is not novel. Only the application of this proven technology to biomass conversion and the development of calibrations specific for energy feedstocks is new. Method development in this area has great potential for high impact with low risk.

In support of the biomass conversion industries, rapid analysis methods are being developed at NREL using a variety of spectrometers and multivariate analysis software packages. Procedures are being developed for the standardization of spectra collected on different instruments and for the accurate transfer of PLS equations between instruments and software packages.

Rapid Analysis Methods for Corn Stover

The US Department of Energy has selected corn stover as a model feedstock for its enzyme sugar platform work. In support of enzyme sugar platform work, NREL has developed laboratory-based rapid analysis methods for the compositional analysis of corn stover feedstocks and corn stover-derived intermediates from a dilute-acid pretreatment process. In development are calibrations measuring the composition of liquid process intermediates and growing corn plants. The details and calibration statistics for those methods will be published separately. In this article, we describe applications of rapid analysis methods to genetic screening and bioprocess development.

Feedstock Method

The calibration set for the compositional analysis of corn stover feedstocks described here contains 47 samples representing five locations and three harvest years. The calibration set includes aged stover samples and hand-separated anatomic fractions. The calibration range and the SE of cross validation for each constituent are presented in Table 1. The accuracy of the NIR/PLS method is illustrated in Fig. 3, which compares the composition determined by wet chemistry with that estimated by the NIR/PLS equation. The diagonal line represents perfect agreement. The NIR/PLS data are from a full cross-validation model, which provides a conservative estimate of the accuracy of the final equation. The distribution of points parallel to the line represents the calibration range. The distribution of the points perpendicular to the line represents error in the NIR/PLS method. Figure 3 demonstrates that the NIR/PLS methods provides a complete compositional analysis for a wide range of samples with precision and accuracy that match the wet chemical methods used to obtain the calibration data.

Table 1
 Calibration Ranges and SEs of Cross Validation for NIR/PLS
 Method for Compositional Analysis of Corn Stover Feedstocks

Constituent	Min (% dry wt)	Max (% dry wt)	SECV (% dry wt) ^a
Glucan	26.9	48.0	1.445
Xylan	14.5	25.3	0.949
Lignin	10.8	27.4	1.120
Protein	0.0	9.2	0.828
St_inorg	0.0	13.6	1.029
Acetyl	0.0	4.5	0.200
Galactan	0.3	3.0	0.327
Arabinan	0.8	4.7	0.199
Mannan	0.0	1.5	0.136
Uronic_acids	2.2	4.4	0.1886

^aSE of cross validation.

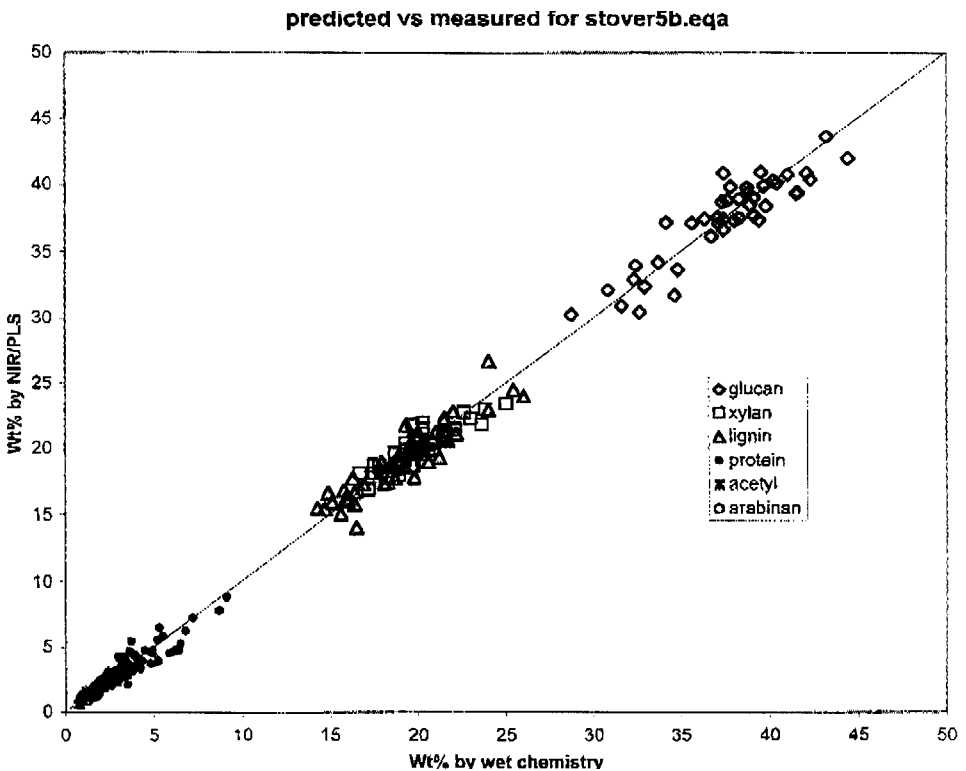


Fig. 3. Comparison of corn stover feedstock composition as determined by wet chemical and NIR/PLS methods.

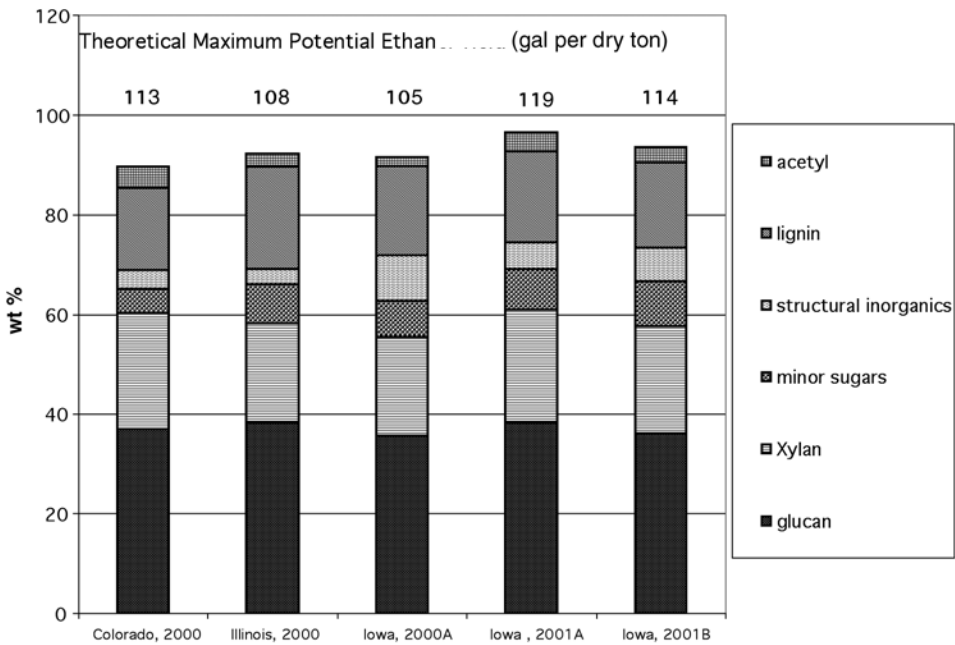


Fig. 4. Comparison of theoretical ethanol yields and composition of bulk corn stover feedstocks.

Application of Feedstock Method

In addition to significant savings in time and money, rapid analysis methods can be used to provide levels of information that were not previously available. For example, feedstock assessment and genetic studies require the screening of hundreds, sometimes thousands, of samples. Such studies would be too costly to pursue without the savings in time and cost provided by a rapid analysis method, which permits approx 200 samples to be analyzed from a bulk corn stover feedstock in 1 d at a cost of about \$2000. This enables large sample studies to be used to develop protocols for the representative sampling of fields, bales, totes, and bags of feedstock.

The ability to accurately sample a bulk feedstock and to analyze hundreds of samples for about \$10 each provides a new tool that is being used at NREL to assess the compositional variability of corn stover in the United States. as a function of variety, geographical location, harvest time, and collection method. Changes in feedstock composition during storage can also be monitored. Feedstock composition will be reported as a range of expected normal values. Significant differences have already been seen in the composition of bulk corn stover feedstocks. Figure 4 compares compositional information for three samples from the 2000 harvest and two from the 2001 harvest. The potential value of these bulk feedstocks for ethanol production is indicated by a theoretical maximum ethanol yield. The theoretical maximum yield assumes that 100% of the sugars are con-

Table 2
Calibration Ranges (% dry wt) and Standard Errors
of Cross Validation (SECV, % dry wt) for a NIR/PLS Method for
the Compositional Analysis of Pretreated Corn Stover Solids

Constituent	Range (% dry wt)	SECV (% dry wt) ^a
Glucan	39.6–68.9	1.549
Xylan	1.0–23.2	1.458
Lignin	22.1–33.5	1.506
Protein	1.1–4.9	0.479
Ash	0.5–16.6	1.467

^aSE of cross validation.

verted to ethanol and can be calculated from the carbohydrate content (for a calculator for theoretical ethanol yields, see Website: http://www.ott.doe.gov/biofuels/ethanol_calculator.html). The theoretical maximum ethanol yields for these feedstocks vary from 105 to 119 gal/dry ton. Clearly, weight alone is not a good measure of feedstock value. The differences shown in carbohydrates, lignin, inorganics, and acetyl content are of a magnitude that could significantly impact process economics. Compositional studies are under way to improve our understanding of feedstock value and compositional variance as a function of location; genetics; and cultivation, harvest, and storage methods.

The NIR/PLS rapid analysis method for feedstock characterization is also being used to screen thousands of corn plants to identify interesting cell-wall mutations. One such study quickly selected 44 individual plants from a population of 2000 for further DNA analysis (not shown). Each of these individuals had a xylan content that was significantly different from the general population. These individuals belonged to 11 families and represented the expected 1:4 ratio of an otherwise normal family. No unusual compositions were reported for members of the four inbred families used as negative controls. The rapid analysis method provided a means of selecting unusual samples based on their unusual cell-wall chemistry.

Field-based methods for the analysis of live plants are being developed for use in large-scale genetic screening projects. These methods will eliminate the costly and time-consuming milling and drying steps. The ability to identify interesting plants before flowering may improve the efficiency of cross-breeding experiments.

Process Intermediate Methods

The calibration set described here for the compositional analysis of solid process intermediates from the dilute-acid pretreatment of corn stover contains 96 samples produced using three bulk feedstocks. The calibration range and the SE of cross validation for each constituent are given in Table 2. The accuracy of the NIR/PLS method is illustrated in Fig. 5, which

compares the composition determined by wet chemistry with that estimated by the NIR/PLS equation. The diagonal line represents perfect agreement. Figure 5 demonstrates that the NIR/PLS method provides a complete compositional analysis for a wide range of samples with precision and accuracy that match the wet chemical methods used to obtain the calibration data.

Application of Process Intermediate Method

This NIR/PLS rapid biomass analysis method is being used to evaluate the performance of dilute-acid pretreatment of corn stover. Figure 6 shows compositional differences in pretreated samples of corn stover as measured by NIR/PLS on process samples. Rapid analysis allows process operators to determine when the sample is depleted in xylan and is ready for the next process step, enzymatic hydrolysis. Stopping pretreatment early will leave hemicellulose in the sample, which decreases enzyme efficiency (3). Continuing pretreatment beyond the point of xylan removal will result in the loss of fermentable sugars, which lowers potential ethanol yields.

Methods are being developed for the rapid compositional analysis of liquid and slurry process streams. When used together, the feedstock, solid process intermediate, and liquid/slurry methods can be used as feed-forward or feed-backward tools for process control.

Conclusion

For biomass analysis, rapid techniques based on NIR/PLS can provide significant savings in time and money with no loss of precision or accuracy relative to the calibration methods. Spectroscopy-based compositional analysis methods are applicable to a wide variety of biomass and biomass-derived materials. NREL is uniquely situated for the development of these methods for several reasons. First, years of experience in method development for biomass analysis and experience with a wide variety of biomass types provide the necessary data quality. Second, the availability at NREL of unique biomass samples produced in a research environment enhances calibration range and depth. These new rapid methods for biomass analysis can support and improve research and development by providing levels of information that would have been too costly to pursue using traditional wet chemistry-based compositional analysis methods. The development of rapid analysis methods for the compositional analysis of biomass and biomass-derived materials is a key element in enabling the commercialization of processes that produce fuels and chemicals from biomass.

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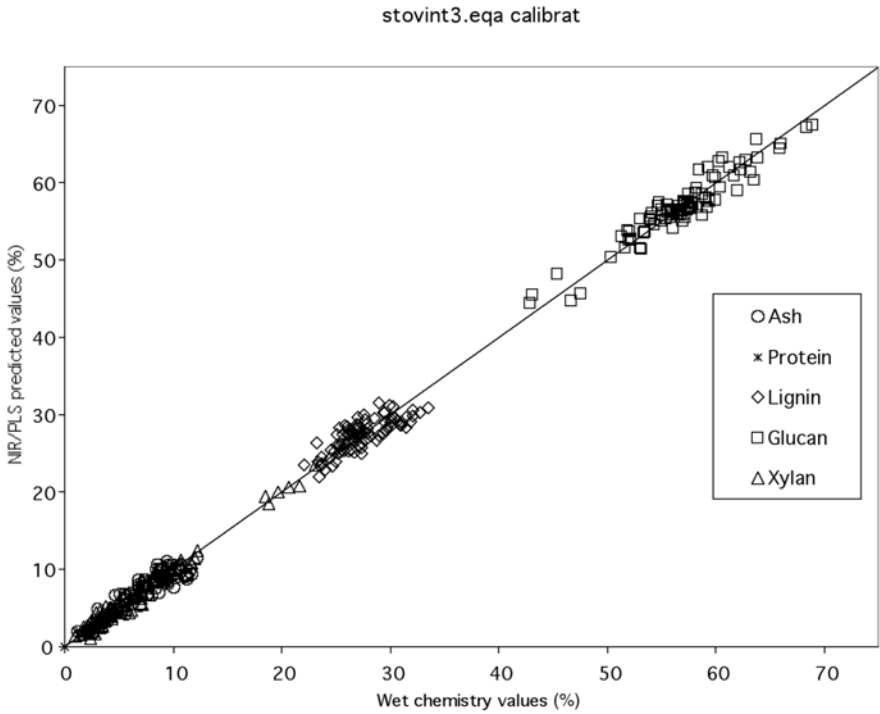


Fig. 5. Comparison of composition of pretreated corn stover solids as determined by wet chemical and NIR/PLS methods.

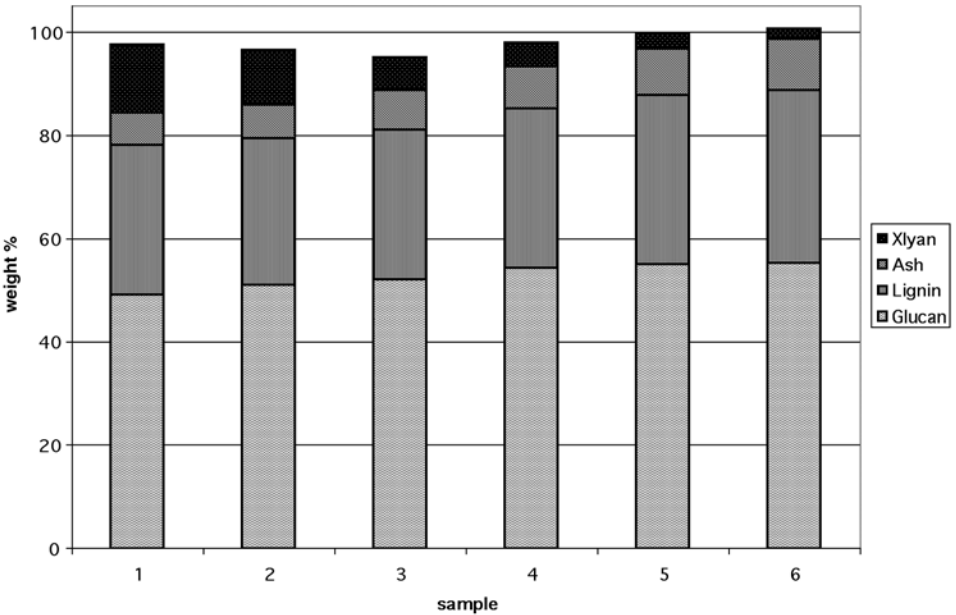


Fig. 6. Composition of pretreated corn stover samples demonstrating optimization of pretreatment for xylose removal.

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