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Rapid Characterization of Woody Biomass Digestibility and Chemical Composition Using Near-infrared Spectroscopy

Shen Hou and Laigeng Li*

Key Laboratory of Synthetic Biology, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, the Chinese Academy of Sciences, Shanghai 200032, China

* Corresponding author

Tel: +86 21 5492 4151; Fax:+86 21 5492 4015; E-mail: lgli@sippe.ac.cn

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Abstract

Rapid determination of the properties of lignocellulosic material is highly desirable for biomass production and utilization. In the present study, measurements of woody biomass digestibility and chemical composition using near-infrared reflectance (NIR) spectroscopy were calibrated. Poplar and eucalyptus materials were recorded in NIR spectrum as well as determined for their chemical compositions of Klason lignin, α-cellulose, holocellulose, lignin syringyl/guaiacyl (S/G) ratio and enzymatic digestibility. Fitting of the NIR information with chemical properties and digestibility by partial least-squares (PLS) regression generated a group of trained NIR models that were able to be used for rapid biomass measurement. Applying the models for woody biomass measurements led to a reliable evaluation of the chemical composition and digestibility, suggesting the feasibility of using NIR spectroscopy in the rapid characterization of biomass properties.

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Introduction

The wide distribution of forests and the large amount of woody biomass harvested each year has shown considerable potential as feedstock for the biofuel industry. Previous work demonstrated that the composition of biomass feedstock is a decisive factor in the biochemical conversion process, and can thereby have a significant impact on biofuel productivity (Sims et al. 2010). A number of studies concluded that the chemical composition of woody plants varies greatly across different parts of the plant anatomy and is highly influenced by a variety of genetic and environmental factors (McKendry 2002). As wood residue is an important feedstock for the cellulosic biofuel and fiber material industries, the poor uniformity of biomass feedstock poses a great challenge for the biofuel and other bioresource related industries.

Current methods for the chemical characterization of biomass feedstock are not applicable for large scale utilization because they are labor intensive and cannot provide analysis information in a time frame suitable for interfacing with on time processing systems. The time consuming techniques also greatly restrict large scale analysis of germplasms and plants in biomass study and production. Chemicals hazards related to wet chemical analysis is also a concern. A rapid and safe method to determine the variability of the physical and chemical properties of biomass feedstock, to provide a quick quantitative estimation of information such as Klason lignin, holocellulose, and α-cellulose content, would improve the efficiency of feedstock processing in an industrial environment and also provide great advantage for plant germplasm screening, genetic engineering and industrial crop cultivation.

Previous studies have placed a great deal of emphasis on the enzymatic hydrolysis process (Kumar et al. 2009), but little research has been reported regarding the digestibility of biomass based on its native chemical composition. Quantitative spectroscopy offers a fast and reliable alternative to traditional analytical methods for the determination of the chemical composition of biomass materials. Several spectroscopic techniques have been used to analyze biomass properties, including Fourier transform infrared (FT-IR) spectroscopes and near-infrared (NIR) spectroscopy (Sanderson et al. 1996; Allison et al. 2009). Applications of NIR spectroscopy within the agricultural and wood industries can be dated back to as early as the late 1950s (McClure 2003). But only recently have a few researchers revealed the potential of NIR spectroscopy as a technique for the rapid characterization of the composition of biomass materials (Tucker et al. 2000; Sivakesava et al. 2001; Lomborg et al. 2010).

The use of NIR reflectance-based modeling to determine composition of wood products has been reported (Tsuchikawa 2007). Usually, the NIR reflectance-based modeling requires an initial calibration process, of which both NIR spectra and composition data are collected from a calibration population that covers a typical range of interested materials. Distinct prediction models or a specific set of parameters are typically implemented for each plant species, but once the prediction model is established, the easily obtained NIR data will allow for the estimation of compositional traits within minutes.

The present research uses poplars and eucalyptus materials, which are the main resource for wood and pulping products as well as potential bioenergy production, to examine the relationship between enzymatic digestibility and the chemical content of wood samples. This research also inspects the feasibility of constructing a quantitative digestibility prediction model based on NIR spectroscopy. The goal of this paper is to propose an integrated method to rapidly screen the chemical composition as well as digestibility of woody biomass based on multivariate calibration models, which combine spectrometric data and traditional chemical analysis. The results reported here suggest that the NIR spectrometric analysis is able to provide a promising method for fast lignocellulosic biomass screening and quantitative characterization of biomass properties.

Results

Chemical analysis and enzymatic saccharification

A total of 279 samples were analyzed for their lignin, holocellulose and α-cellulose content as well as their lignin syringyl/guaiacyl (S/G) ratio. **Table 1** shows a summary of the chemical compositions of the analyzed samples. The determinations indicated variations in the range of 15.06–25.42% for lignin content, 66.79–80.91% for holocellulose content, 45.48–58.99% for α-cellulose content and 2.01–2.81 for lignin S/G ratio. These ranges were consistent with the chemical properties of poplar and eucalyptus wood materials described previously (Huang et al. 2007).

Digestion of the biomass samples was determined through enzymatic hydrolysis using acid-pretreated wood samples. The digestion efficiency was expressed in sugar release over the course of 72 h of hydrolysis. As shown in **Figure 1**, the results indicated that the digestion efficiency is highly related to biomass chemical properties – content of lignin, holocellulose, α-cellulose and lignin S/G ratio. Regression modeling was used to measure the relationship between chemical composition and digestibility. Results demonstrated that each of the four chemical properties had either a negative or positive correlation with digestion efficiency. Negative relationships were observed between lignin and sugar conversation during the 72 h digestion. In the samples with similar content of holocellulose, the saccharification process is inhibited by the percentage of α-cellulose. On the other hand, positive relationships of holocellulose content or lignin S/G ratio with sugar conversion were observed during hydrolysis. Together, these results demonstrated that lignin and α-cellulose inhibited digestion, while holocellulose and lignin S/G ratio benefited the process, suggesting that the composition of lignocellulosic biomass plays a critical role in affecting the efficiency of biomass conversion.

The rate of hydrolysis over a certain time period is another fundamental parameter to assess the conversion efficiency of biomass materials. Biomass materials with high or low contents of lignin (15 vs 25%), holocellulose (68 vs 80%), α-cellulose (45 vs 58%) and S/G ratio (2 vs 2.8) were selected for the study. The rate of hydrolysis in the course 1, 8 and 72 h of hydrolysis was

Table 1. Summary of the determined chemical properties of wood biomass

Compositions	Sample	Minimum	Maximum	Average	*SD*
Lignin (%)	279	15.06	25.42	20.55	2.41
Holocellulose (%)		66.79	80.91	74.83	2.51
α-cellulose (%)		45.48	58.99	52.16	3.39
S/G ratio		2.01	2.81	2.44	0.21

S/G, syringyl/guaiacyl.

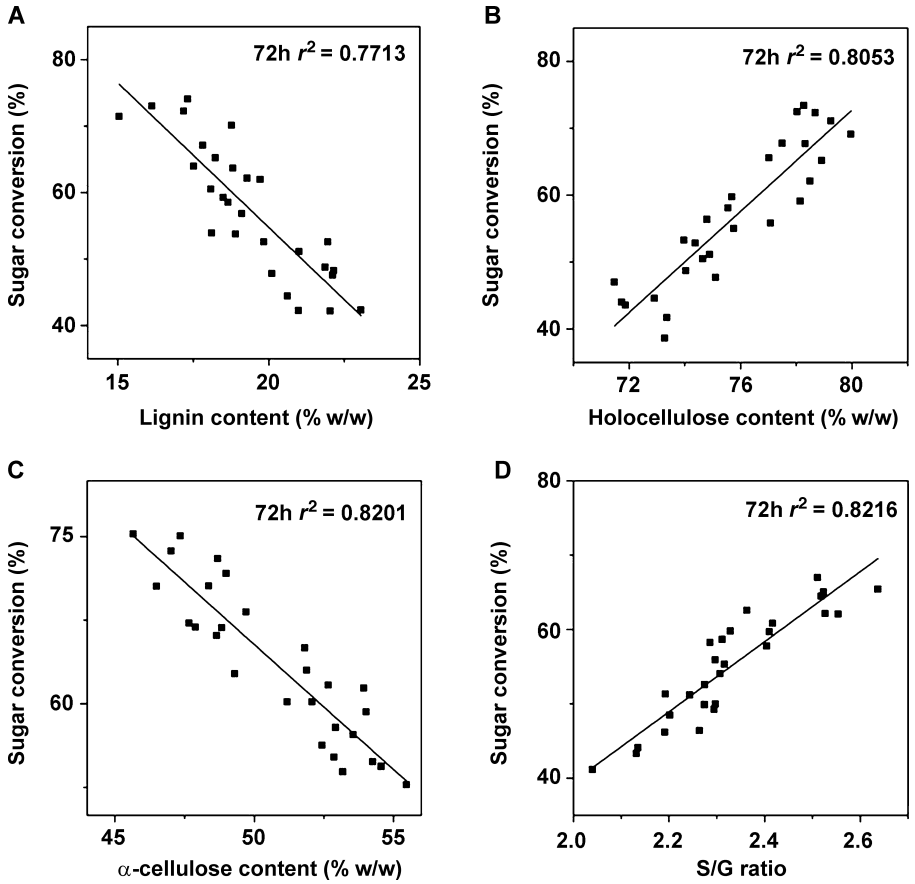

Figure 1. Correlations between digestibilities and chemical composition of biomass samples.

(A) Lignin content.
(B) Holocellulose content.
(C) α-cellulose content.
(D) Lignin syringyl/guaiacyl (S/G) ratio.

evaluated. As shown in **Figure 2**, the hydrolysis reached its maximum in 8 h under the experimental conditions. The contents of lignin, holocellulose, α-cellulose and S/G ratio, respectively, again showed their impact on the total sugar release, but displayed no clear effect on the hydrolysis rate in the studied intervals, suggesting that the biomass composition had stronger influence on total sugar release than on hydrolysis rate. Given that a limited number of the time intervals were examined in the present study, however, further detailed characterization is required to understand the more concise relationship between biomass composition and hydrolysis rate. Nevertheless, these results further confirmed that the composition of biomass plays a key role in controlling enzymatic hydrolysis after acid pretreatment.

Spectra data processing and PLS determination of biomass properties

A reflectance spectrum between 400 and 2 500 nm was recorded for the same wood mill samples. As shown in **Figure 3A**, the full spectrum of all 279 samples displayed a similar pattern but with obvious baseline discrepancy and reflectance peak shifts. The spectra data was processed as described in the methods to eliminate these experimental variations, which would obscure an effective calibration of the relationship between chemical properties and the spectrum. The transformed spectral dataset displayed minimal baseline discrepancy and characteristic peak uniformity (**Figure 3B**).

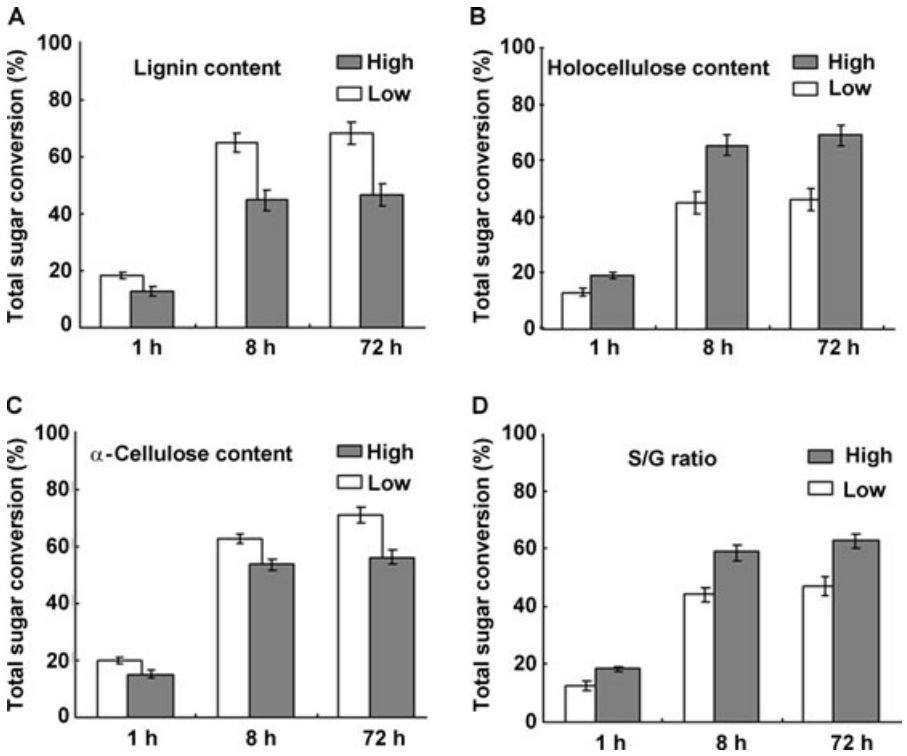

Figure 2. Evaluation of hydrolysis rate of the biomass samples with different chemical contents.

(A) High lignin content 25% versus low lignin content 15%.
(B) High holocellulose content 80% versus low holocellulose content 68%.
(C) High α-cellulose content 58% versus low α-cellulose content 45%.
(D) High syringyl/guaiacyl (S/G) ratio 2.8 versus low S/G ratio 2.

Partial least-squares (PLS) regression analysis was used for data modeling. The number of PLS vectors is a crucial factor affecting the accuracy of the model, as too many PLS vectors would lead to (over-fitting), while too few vectors would result in the loss of useful information (Kelley et al. 2004). In our study, a group of 30 PLS vectors was calculated initially. Fewer than 10 PLS vectors were eventually selected for the model to minimized prediction error as well as to avoid (over-fitting).

Partial least-squares regression was performed using the spectrum information of 279 samples coupled with the results of chemistry analysis of the lignin content of the calibration set. The lignin content of the samples ranged from 15.06 to 23.84% in poplar and from 16.68 to 25.42% in eucalyptus. In order to calibrate lignin content using the reflectance spectrum information, the regression models of the cross-validation were optimized with data processing methods as described in the methods. Results indicated that a PLS vector of 7 was optimal for predicting lignin content. The model yielded by the validation analysis satisfied with a R^2 of 0.983 1 and the root mean square of error of cross-validation (RMSECV) of 0.212 2. Meanwhile, a number of spectrum peaks at 1 152, 1 417, 1 668, 1 685, and 2 132 nm which corresponds with known aromatic absorption were also detected by coefficiency analysis. Together, these results suggest the established model was able to detect lignin content.

The content of holocellulose and α-cellulose was examined individually. The holocellulose content in poplar ranged from 72.18 to 80.91% and from 66.79 to 78.35% in eucalyptus. The α-cellulose content ranged from 45.48 to58.48% in poplar and form 45.53 to 58.99% in eucalyptus. For holocellulose, a model containing 8 PLS vectors generated the highest R^2 of 0.981 0, and the lowest RMSECV of 0.343 3. For α-cellulose, 8 PLS vectors were selected for the model, which generated a R^2 of 0.981 9 and RMSECV of 0.531 2. Coefficiency analysis revealed that a number of peaks in the spectra from 1 400 to 2 500 nm, including 1 490, 1 900, 1 930. 2 100, 2 276, 2 338, 2 482 nm, were assigned to cellulose-related signals. These results were in agreement with the results of previous studies (Jones et al. 2006; Huang et al. 2007).

To establish a PLS model to measure lignin S/G ratio, the chemical results of lignin S/G ratio in poplar (2.01 to 2.79) and in eucalyptus (2.20 to 2.81) were calibrated with the NIR

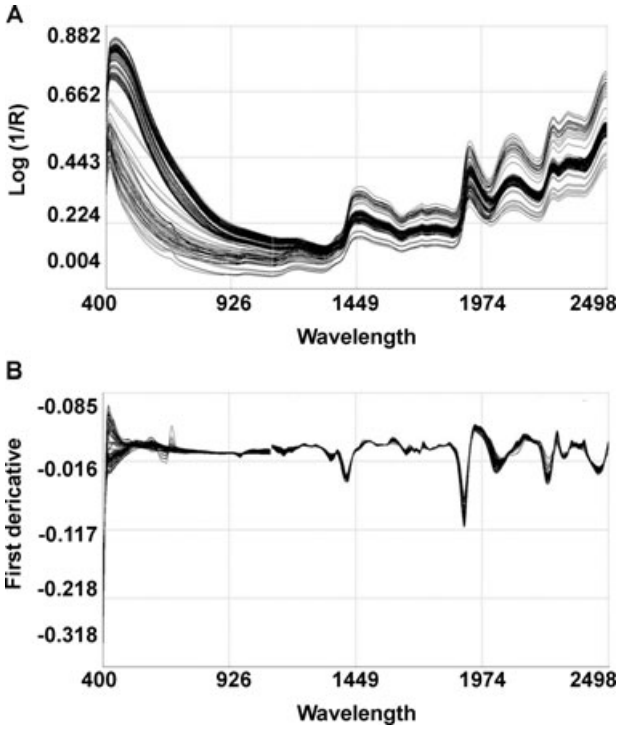

Figure 3. Near-infrared reflectance (NIR) reflectance spectrum information.

(A) NIR reflectance spectrum between 400 and 2500 nm recorded from biomass samples.
(B) Spectrum information transformed by derivatives.

spectrum information. We tested different numbers of PLS vectors and generated a group of testing models. The cross validation statistics indicated that the models had at best, a R^2 of 0.911 9 and RMSECV of 0.063 7. This suggests that the measurement of lignin S/G ratio via NIR would be relatively less accurate than that of other chemical properties. This could be due to the complex structure of lignin in the cell wall matrix and relatively inaccurate measurement of lignin S/G ratio by gas chromatography (Robinson and Mansfield 2009).

Additionally, a correlation between the monosaccharide compositions of the polymers and the NIR spectrum information was also investigated (data not shown). However, these attempts failed to establish a reliable model to measure these properties in the biomass.

Independent confirmation of the NIR model measurement for biomass chemical properties

To test the performance of the calibration models on field samples, a group of 38 poplar and eucalyptus samples were collected from various sources for independent verifications of the generated models. The acquisition of spectra data and the chemical analysis of the field samples were carried out using the same methods as for the calibration group. The content, mean and *SD* of the measured chemical values are summarized in **Table 2**. The data suggest that the samples used for the independent confirmation represented a diverse range of poplar and eucalyptus samples, which was covered by the calibration sample set.

Figure 4 shows the results of plotting the predicted versus actual values of the lignin (**Figure 4A**), holocellulose (**Figure 4B**), α-cellulose contents (**Figure 4C**), and S/G ratio (**Figure 4D**), of the verification samples. As summarized in **Table 3**, statistical analysis indicated that the correlation between the predicted and experimental measurements had as r^2 of 0.984 for lignin content, 0.988 for holocellulose, 0.971 for α-cellulose and 0.925 for lignin S/G ratio, respectively. These correlations satisfied the standard error of prediction (SEP) and SDR ratio (SD/RMSECV) values (**Table 3**) which were established for quality control as described in the methods. Therefore, these results demonstrated that it is feasible to use NIR analysis to establish computation models for measuring lignin, holocellulose and α-cellulose content and lignin S/G ratio for both poplars and eucalyptus biomass samples.

Quantitative prediction of digestibility

According to the above results, NIR reflectance-based models can satisfactorily predict the chemical compositions of biomass samples with relative accuracy. On the other hand, the above results also showed strong correlation between chemical properties and digestibility of biomass samples. This suggests that

Table 2. Summary of the chemical compositions by experimental measurement and near-infrared reflectance (NIR) prediction

Composition	Sample	Experimentally measured value				NIR predicted value			
		Min.	Max.	Average	*SD*	Min.	Max.	Average	*SD*
Lignin (%)	38	15.71	23.82	19.76	2.13	15.86	23.94	19.77	2.12
Holocellulose (%)		72.11	79.38	75.93	2.22	71.70	79.56	75.90	2.36
α-cellulose (%)		46.00	55.58	50.59	2.92	46.01	55.12	50.53	2.92
S/G ratio		2.10	2.75	2.42	0.19	2.12	2.84	2.41	0.21

S/G, syringyl/guaiacyl.

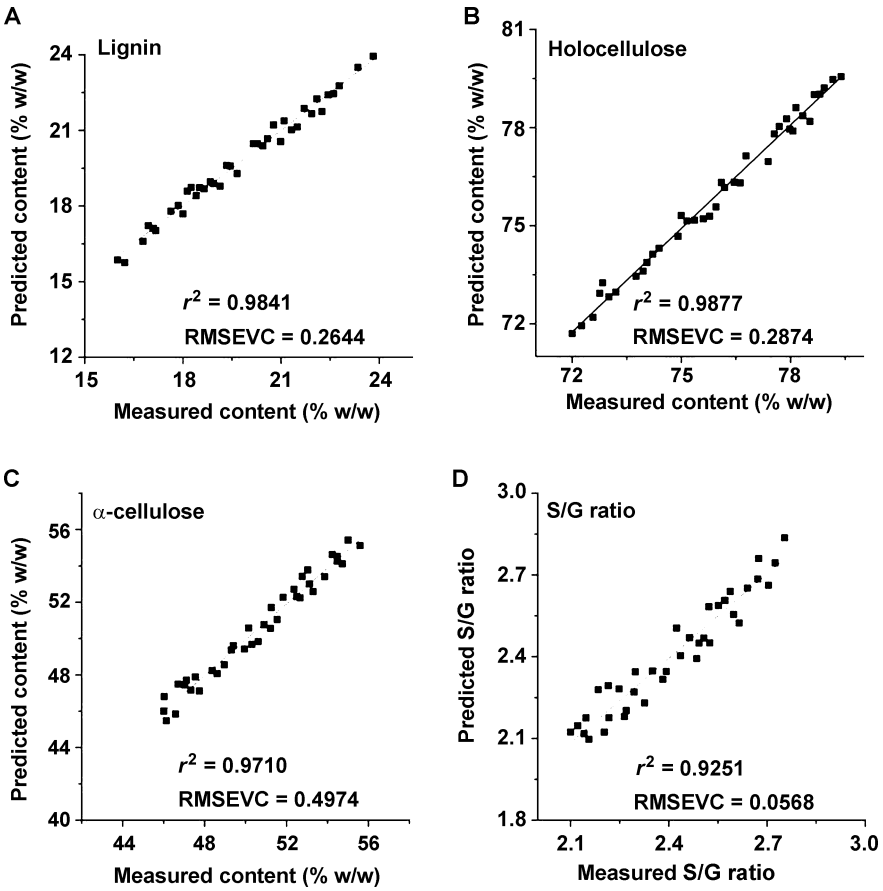

Figure 4. Correlation between experimentally measured chemical properties and the near-infrared reflectance (NIR) prediction.

(A) Lignin content.
(B) Holocellulose content.
(C) α-cellulose content.
(D) Syringyl/guaiacyl (S/G) ratio.

it is feasible to develop a calibration model to predict the conversion efficiency of biomass based information from its NIR spectrum. Thus, the same sample set that was used in the model calibration was also determined for its digestibility. The PLS statistical analysis of the relationship between the chemical properties, digestibility and NIR spectrum information led to four prediction models. Among them, the model with highest R^2 (0.912 9) and lowest RMSECV (2.225 3) was chosen for validation.

An independent verification of the prediction model for digestibility was performed with a group of field biomass samples. As shown in **Figure 5**, the predicted and experimentally measured values for digestion exhibited a strong correlation with a slope of 1.069 and R^2 of 0.912 9, demonstrating the feasibility of using an NIR model to make predictions for biomass digestibility.

Discussion

Lignocellulosic biomass is a complex of biopolymers, including lignin, cellulose and hemicellulose. The composition and structure of the polymers are crucial properties affecting plant growth and biomass utilization. A rapid characterization of the biomass properties is highly sought for biomass plant production as well as biomass energy processing. NIR technology has been developed for rapid determination of starch, oil, and protein content in wheat, rapeseed, soybean and other crops (Armenta et al. 2010; Hacisalihoglu et al. 2010). In recent years, attempts to evaluate biomass composition via NIR have been reported (Lomborg et al. 2010). Here we attempt to establish a rapid and convenient method for characterizing wood biomass through NIR analysis and modeling, which can be integrated into fast and high throughput processes.

Table 3. Statistics summary of the model evaluation and validation

Parameters	Content				
	Lignin	Holocellulose	α-cellulose	S/G	Digestibility
Correlation	0.984	0.988	0.971	0.925	0.913
SEP	0.264	0.287	0.497	0.0568	2.23
SDR	8.02	8.22	5.87	3.66	3.33

SDR, standard deviation of the root mean square of error of cross-validation; SEP, standard error of prediction; S/G, syringyl/guaiacyl.

In the present study, we demonstrated that NIR data can be applied to analyze the chemical content including, Kalson lignin, holocellulose, α-cellulose content and S/G ratio, as well as to predict the digestibility of biomass materials from poplar and eucalyptus trees. However, the NIR spectrum was unable to provide sufficient information to establish a model to measure the monosaccharide composition of the biomass polymers. The difficulty to predict monosaccharide compositions in wood may be due to two reasons. First, some monosaccharides have a low concentration in wood samples and their spectrum absorption is largely undetectable. Second, some monosaccharides are similar in chemical and physical properties and may display no distinguishable spectrum absorption in biomass.

Our results indicated that three major biomass biopolymers, lignin, cellulose and hemicellulose, impact biomass digestion to different degrees. Both lignin and α-cellulose inhibit sugar release from the biomass, while high holocellulose content and lignin S/G ratio benefited the release of sugar during digestion. In the past several years, modification of plant cell walls has been attempted in order to improve the efficiency of biomass utilization (Li et al. 2003; Sticklen 2006; Chen and Dixon 2007; Carroll and Somerville 2009). In those studies, lignin was selected as a target for modification. The present study, in addition to confirming lignin as a target of interest for biomass engineering, also provides a new line of quantitative evidence to understand how chemical composition affects conversion efficiency.

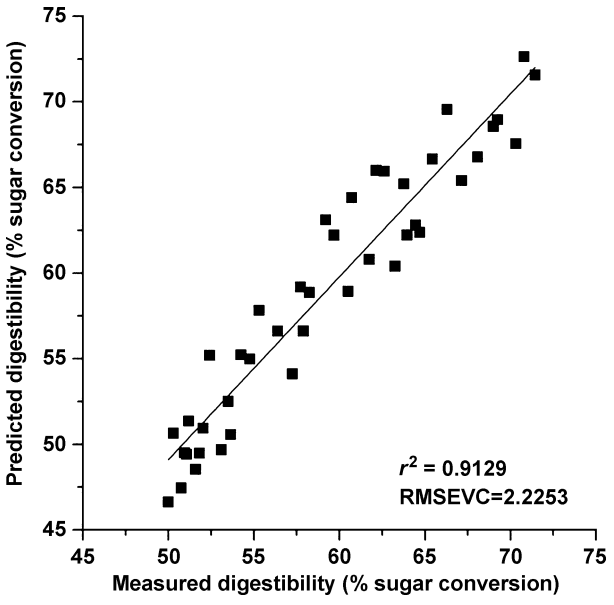

Figure 5. Correlation between experimentally measured digestibility and near-infrared reflectance (NIR) prediction.

Lignocellulosic biomass represents a most abundant renewable resource that can potentially provide a large portion of the future energy supply (Somerville 2006). However, only 2% of this resource is currently used by humans due to our limited knowledge about the chemistry and structures of the biomass (Pauly and Keegstra 2008). Efficient utilization of biomass resources particularly depends on how we can systematically characterize the properties of biomass plant in relation to their composition, structure, digestion, biosynthesis and modification. By demonstrating the feasibility of using quantitative models derived from NIR data to analyze the properties of biomass samples, our study presents a method that could lead to the establishment of faster, more convenient techniques for scientists as they search for the keys to achieve better biomass utilization.

Materials and Methods

Sample preparation

Wood samples were collected from poplar artificial forest plantations grown in Jiangsu, Shanghai and other places and from eucalyptus plantations grown in Guangdong and Yunnan provinces. Sampled trees included different varieties (clones), ages, and growing sites. Sample trees from various sources attributed to the variations in the chemical composition of lignocellulosic biomass. The samples were first dried according to an oven drying procedure (Hames et al. 2008). Then the samples were crushed with a knife mill and ground to pass 20–40 mesh screens. The ground sample was directly applied to the NIR reflectance spectrum and digestion analysis. As for chemical analysis, the wood mill was placed in a 50 mL capped test tube and extracted with acetone, which was replaced every 48 h for a total of six times. After each extraction, the acetone

was concentrated under reduced pressure, and the amount of nonvolatile extractives was determined gravimetrically (Sluiter et al. 2005). The extractive free wood mill was stored under −20 °C for further chemical composition analysis.

Kalson lignin content determination

The Kalson lignin content was measured by standard procedures (Sluiter et al. 2008). The total lignin content was reported as the combination of acid soluble lignin and acid-insoluble lignin.

S/G ratio measurement

S/G ratio of the wood samples was measured by a simplified thioacidolysis method (Robinson and Mansfield 2009). For each sample, 20 mg of ground, extract-free, oven-dried wood flour was weighed into a 2 mL glass vial with airtight screw-cap. The reaction mixture containing 2.5% boron trifluoride etherate and 10% (v/v) ethanethiol in dioxane was added to vials and the reaction was held at 100 °C for 4 h. The reaction was stopped by placing the reactions at −20 °C for 5 min. Tetracosane was then added as internal standard after the pH being adjusted to approximately 4. The reaction products were extracted from the aqueous mixture by methylene chloride. The organic phase was removed and dried with anhydrous sodium sulfate. Samples were derivatized by N,O-bis(trimethylsilyl) acetamide following the standard procedure (Sigma-Aldrich, St. Louis, MO, USA). This reaction product was analyzed by gas chromatography.

Holocellulose analysis

Holocellulose analysis was followed by a modified sodium chlorite procedure (Yokoyama et al. 2002). Holocellulose was isolated from the extractive-free wood mill prepared as described above. Approximately 300 mg of wood was suspended in 4 mL of deionized water in a 25 mL glass vial with an airtight cover. The reaction vial was then submerged in a water bath and maintained at 90 °C for preheating. The reaction was initiated by adding 4.5 mL of sodium chlorite solution. The reaction mixture had a final concentration of 5% (w/w) sodium chlorite and 5% (v/v) acetic acid. The temperature was held for 1 h, after which the reaction was cooled in a cold water bath, and filtered using a crucible. The isolated holocellulose was thoroughly washed with deionized water and dried and was then determined gravimetrically.

α-Cellulose analysis

A 100 mg sample of the oven dried holocellulose prepared above was weighed in a 15 mL vial and left to stand at room temperature for 30 min to allow moisture equilibration. The vial was added with 5 mL of 17.5% sodium hydroxide and left to react for an additional 30 min at room temperature. Then, the vial was added with the same volume of deionized water and placed on an ambient shaker for 60 min of reaction. After the fiber suspension was filtered using a crucible, it was first washed with deionized water and then with 1.0 M acetic acid solution three times. The residue was further thoroughly washed with deionized water and oven dried. The dried residue was weighted as α-cellulose content.

Enzymatic saccharification measurement

The enzymatic saccharification was measured to calculate the digestibility and hydrolysis rate of wood samples (Selig et al. 2008). The samples were measured with an acid pretreatment procedure to simulate the industrial biomass conversion process. Wood samples were milled to pass the 40 mesh screen. For each sample, 1 g of wood mill was weighted into a 20 mL glass scintillation vial with 10 mL 2% sulfuric acid. After the vial was left at 50 °C for 30 min, the reaction was heated to 121 °C for 1 h in an autoclave. The residue of the reaction was separated by centrifugation, washed and resuspended in 20 mL of 0.1 M sodium citrate buffer at pH 4.8. The mixture was preheated at 50 °C, then 60 filter paper units (FPU) cellulase (E.C. 3.2.1.4) from T. *viride* (Yakult Honsha, Tokyo, Janpan) was added. The hydrolysis rate was calculated by monitoring the sugar concentration in various reaction intervals. The total digestibility was determined after 72 h of hydrolysis. The cellulase activity under specific situations was determined following standard procedures (Adney and Baker 1996). The sugar concentration in the reaction was measured by dinitrosalicylic acid (DNS) method using glucose as standard.

NIR data acquisition

The NIR reflectance spectrum of wood samples was measured with a XDS Rapid Content Analyzer NIR spectrophotometer (Foss Analytical, Höganäs, Sweden) with spectrum collection software ISIscan. Approximately 1.5 g of each ground sample, prepared as described above was put in a non-NIR absorbing cuvette that fit into the sample holder. The spectrum covers a range of 400 to 2 500 nm with a resolution of 1 nm. Each spectrum is the average of 32 scans. Average reflectance spectra were recorded at 2 nm intervals and transformed into absorbance values by ISIscan.

Data processing

Spectral analysis was done on log (1/reflectance) data. The NIR data processing of PLS regressions were computed using the Win ISI4 (Foss Analytical, Höganäs, Sweden). In computation,

spectra data transformation, such as standard normal variate (SNV), Savitsky-Golay (SG) algorithm, first or second derivatives and first- or second-degree polynomials were applied to eliminate reflectance peak shift and baseline discrepancy. The computed prediction models were examined for their performance satisfaction using the cross-validation approach, which was judged by the root mean square of error of cross-validation (RMSECV) and coefficient of determination (R^2). In this study, a total of five models for biomass property determination were established. Each model is independently optimized according to cross-validation. On the other hand, the standard error of prediction (SEP) and the SDR ratio (SD/RMSECV) were used to examine the accuracy of the prediction of field samples. As suggested in a previous study, SDR value above 3.0 is considered sufficient for practical spectroscopy applications and the values between 5–10 are adequate for quality control (Peng and Chen 2008; Cozzolino 2009).

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References

Adney B, Baker J (1996) *Measurement of Cellulase Activities*. Biomass Analysis Technology Team Laboratory Analytical Procedure, National Renewable Energy Lab.

Allison GG, Morris C, Hodgson E, Jones J, Kubacki M, Barraclough T, Yates N, Shield I, Bridgwater AV, Donnison IS (2009) Measurement of key compositional parameters in two species of energy grass by Fourier transform infrared spectroscopy. *Biores. Technol.* **100**, 6428–6433.

Armenta S, Moros J, Garrigues S, De La Guardia M (2010) The use of near-infrared spectrometry in the olive oil industry. *Crit. Rev. Food Sci. Nutr.* **50**, 567–582.

Carroll A, Somerville C (2009) Cellulosic biofuels. *Annu. Rev. Plant Biol.* **60**, 165–182.

Chen F, Dixon RA (2007) Lignin modification improves fermentable sugar yields for biofuel production. *Nat. Biotechnol.* **25**, 759–761.

Cozzolino D (2009) Near infrared spectroscopy in natural products analysis. *Planta Med.* **75**, 746–756.

Hacisalihoglu G, Larbi B, Settles AM (2010) Near-infrared reflectance spectroscopy predicts protein, starch, and seed weight in intact seeds of common bean (*Phaseolus vulgaris* L.). *J. Agric. Food Chem.* **58**, 702–706.

Hames B, Ruiz R, Scarlata C, Sluiter A, Sluiter J, Templeton D (2008) *Preparation of Samples for Compositional Analysis*. Biomass Analysis Technology Team Laboratory Analytical Procedure, National Renewable Energy Lab.

Huang AM, Jiang ZH, Li GY (2007) Determination of holocellulose and lignin content in Chinese fir by near infrared spectroscopy. *Spectrosc. Spect. Anal.* **27**, 1328–1331.

Jones PD, Schimleck LR, Peter GF, Daniels RF, Clark A (2006) Nondestructive estimation of wood chemical composition of sections of radial wood strips by diffuse reflectance near infrared spectroscopy. *Wood Sci. Technol.* **40**, 709–720.

Kelley SS, Rials TG, Snell R, Groom LH, Sluiter A (2004) Use of near infrared spectroscopy to measure the chemical and mechanical properties of solid wood. *Wood Sci. Technol.* **38**, 257–276.

Kumar P, Barrett DM, Delwiche MJ, Stroeve P (2009) Methods for pretreatment of pignocellulosic biomass for efficient hydrolysis and biofuel production. *Ind. Eng. Chem. Res.* **48**, 3713–3729.

Li L, Zhou Y, Cheng X, Sun J, Marita JM, Ralph J, Chiang VL (2003) Combinatorial modification of multiple lignin traits in trees through multigene cotransformation. *Proc. Natl. Acad. Sci. USA* **100**, 4939–4944.

Lomborg CJ, Thomsen MH, Jensen ES, Esbensen KH (2010) Power plant intake quantification of wheat straw composition for 2nd generation bioethanol optimization – a near infrared spectroscopy (NIRS) feasibility study. *Biores. Technol.* **101**, 1199–1205.

McClure WF (2003) 204 years of near infrared technology: 1800–2003. *J. Near Infrared Spectrosc.* **11**, 487–518.

McKendry P (2002) Energy production from biomass (part 1): overview of biomass. *Biores. Technol.* **83**, 37–46.

Pauly M, Keegstra K (2008) Cell-wall carbohydrates and their modification as a resource for biofuels. *Plant J.* **54**, 559–568.

Peng XW, Chen HZ (2008) Rapid estimation of single cell oil content of solid-state fermented mass using near-infrared spectroscopy. *Biores. Technol.* **99**, 8869–8872.

Robinson AR, Mansfield SD (2009) Rapid analysis of poplar lignin monomer composition by a streamlined thioacidolysis procedure and near-infrared reflectance-based prediction modeling. *Plant J.* **58**, 706–714.

Sanderson MA, Agblevor F, Collins M, Johnson DK (1996) Compositional analysis of biomass feedstocks by near infrared reflectance spectroscopy. *Biomass Bioenergy* **11**, 365–370.

Selig M, Weiss N, Ji Y (2008) *Enzymatic Saccharification of Lignocellulosic Biomass*. Biomass Analysis Technology Team Laboratory Analytical Procedure, National Renewable Energy Lab.

Sims REH, Mabee W, Saddler JN, Taylor M (2010) An overview of second generation biofuel technologies. *Biores. Technol.* **101**, 1570–1580.

Sivakesava S, Irudayaraj J, Demirci A (2001) Monitoring a bioprocess for ethanol production using FT-MIR and FT-Raman spectroscopy. *J. Ind. Microbiol. Biotechnol.* **26**, 185–190.

Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D, Crocker D (2008) *Determination of Structural Carbohydrates and Lignin in Biomass*. Biomass Analysis Technology Team Laboratory Analytical Procedure, National Renewable Energy Lab.

Sluiter A, Ruiz R, Scarlata C, Sluiter J, Templeton D (2005) *Determination of Extractives in Biomass*. Biomass Analysis Technology Team Laboratory Analytical Procedure, National Renewable Energy Lab.

Somerville C (2006) The billion-ton biofuels vision. *Science* **312**, 1277.

Sticklen M (2006) Plant genetic engineering to improve biomass characteristics for biofuels. *Curr. Opin. Biotechnol.* **17**, 315–319.

Tsuchikawa S (2007) A review of recent near infrared research for wood and paper. *Appl. Spectrosc. Rev.* **42**, 43–71.

Tucker MP, Mitri RK, Eddy FP, Nguyen QA, Gedvilas LM, Webb JD (2000) Fourier transform infrared quantification of sugars in pretreated biomass liquors. *Appl. Biochem. Biotechnol.* **84–6**, 39–50.

Yokoyama T, Kadla JF, Chang HM (2002) Microanalytical method for the characterization of fiber components and morphology of woody plants. *J. Agric. Food Chem.* **50**, 1040–1044.

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