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### Variability in composition of individual botanical fractions of *Miscanthus × giganteus* and their blends

Daniel A. Williams<sup>ab</sup>, Mary-Grace C. Danao<sup>ab</sup>, Kent D. Rausch<sup>a</sup>, Marvin R. Paulsen<sup>a</sup> & Vijay Singh<sup>a</sup>

<sup>a</sup> Department of Agricultural and Biological Engineering, University of Illinois, Urbana, IL 61820, USA

<sup>b</sup> Energy Biosciences Institute, University of Illinois, Urbana, IL 61820, USA

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## Variability in composition of individual botanical fractions of *Miscanthus* × *giganteus* and their blends

Daniel A. Williams<sup>a,b</sup>, Mary-Grace C. Danao<sup>b</sup>, Kent D. Rausch<sup>a</sup>, Marvin R. Paulsen<sup>b</sup> and Vijay Singh<sup>a</sup>

<sup>a</sup>Department of Agricultural and Biological Engineering, University of Illinois, Urbana, IL 61820, USA; <sup>b</sup>Energy Biosciences Institute, University of Illinois, Urbana, IL 61820, USA

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Biorefineries require a consistent biomass feedstock to ensure optimal processing efficiency and a convenient method to evaluate biomass delivered at the factory gate. The combination of fractionation, selective blending of biomass streams, and densification is one strategy of ensuring feedstock with uniform quality attributes. In this study, dry mass fraction and compositional variability of botanical fractions of *Miscanthus* × *giganteus* harvested post-senescence were determined. Fourier transform near infrared (FT-NIR) spectroscopy was used to monitor composition of blends. Results showed that the majority of miscanthus stalks were composed of rind (48–63%) followed by sheath (17–28%), nodes (10–16%), and pith (8–13%). Rind had the highest glucan content (46.1%) while lignin content was highest in node (25.5%) and rind fractions (24.2%). Ash content was highest in blade fractions (8.9%) and was less than 1% in rind, pith, and nodes. Variability in composition was reflected in the 4250–4350 cm<sup>-1</sup> and 5500–6000 cm<sup>-1</sup> regions of the botanical fractions' FT-NIR spectra. Principal components analysis (PCA) of FT-NIR spectra of rind-blade blends were useful in screening blends based on structural carbohydrates, lignin and ash contents demonstrating the use of NIR spectroscopy in monitoring feedstock formulation.

**Keywords:** blending; feedstock; formulation; near infrared spectroscopy; principal components analysis

### Introduction

Since the 1980s, the US government has encouraged research using herbaceous feedstocks for conversion to ethanol for use as transportation fuel. [14] These warm season perennial grasses, as feedstocks for bioenergy production, produce large quantities of biomass with minimal inputs. [18] Grasses are mostly composed of cellulose, hemicellulose, and lignin, which typically account for 40, 20, and 20% (w/w) of their composition, respectively, with the balance composed of organic acids, ash, and extractives. [21] The plant can be separated into its root system, stalks, and leaves during harvest and further broken down in the laboratory into tissue types – the dermal, vascular, and fundamental tissues. The relative amounts of each tissue type vary depending on the function of that plant component, and thus lead to variations in composition. These variations, along with compositional variation that arise due to species variability, growing conditions, and different methods of harvest, collection, transportation, and storage, will need to be overcome in order to supply bioprocessing facilities with a steady stream of lignocellulosic feedstock with uniform composition throughout the year. Most bioconversion technologies have a narrow tolerance range for the physical characteristics (e.g., particle size, moisture content, structural carbohydrates content, ash content, etc.) of the feedstock. [17]

So how can a bioprocessing facility produce a consistent product using lignocellulosic feedstocks with variable composition? One approach is to understand what

practices cause these variations and to attempt to minimize these variations. For example, the process of cutting biomass and laying it on the ground before collecting it introduces ash (e.g., soil) and other contaminants that can affect the overall chemical composition. [11] Size reduction of biomass is typically rendered by mechanical means (e.g., cutting, shredding, milling, compression), making it convenient for incorporation in harvest or postharvest handling. Comminution is an energy intensive process with potentially significant feedstock cost implications and it affects decisions during biomass storage; [1,10] transportation; [7] and bioconversion. [12,30,32] Shah et al. [23] found that dry matter loss (DML) for corn stover bales stored outdoors with tarp and breathable film covers could be as high as 17%, with more than half of the total DML occurring early during the storage period. A quality-based valuation of biomass feedstocks based on moisture, carbohydrate, ash, and other components will need to be implemented so that farmers and suppliers have incentives to implement best management practices which preserve biomass feedstock quality. [11]

A second approach is to employ proven techniques from the corn wet milling and dry milling process industries. Both industries have been fractionating corn to make ethanol and valuable coproducts for several decades. Fractionating corn into its component parts (germ, fiber, and endosperm) allows for the removal of oil and fiber in the kernel, leaving a higher percentage of starch for ethanol production or other products. Fractionating

\*Corresponding author. Email: gdanao@illinois.edu

reduces the volume of material entering a processing plant, thereby increasing throughput and overall plant efficiency. A fractionation process may be applied as an additional step to pretreatment and handling of lignocellulosic biomass. For example, biochemical conversion processes are sensitive to the ratio of C5 to C6 sugars and the accessibility of these sugars for conversion are important in optimizing pretreatment and fermentation conditions. Ash content is important to control as it displaces valuable carbohydrates and, in thermochemical conversion, can impair catalysts and contributes to slag formation within the combustion process. Therefore, fractionation technologies to remove botanical fractions or particle sizes with high ash contents may be employed to reduce compositional variability, enhance bioconversion efficiency, and recycle minerals back to the soil. Fractions with high lignin contents may be diverted to thermochemical conversion streams because of lignin's higher heating value compared to structural carbohydrates. [8] Fractions with low lignin content and high structural carbohydrate content are desired for biochemical conversion because the structural carbohydrates can be converted to fermentable sugars and lignin interferes with pretreatment. [3] Leaves and other thin or fragile tissues may require a less severe pretreatment or no pretreatment at all. [19] Individual botanical fractions may also be blended to deliver feedstock to meet a set of bioconversion specifications. [24]

To monitor the blending process, composition must be measured cost effectively in near real time. Biomass composition is typically determined using conventional wet chemistry assays, but these assays tend to be time consuming, destructive, and involve extensive sample preparation, specialized laboratory equipment, and well-trained personnel. An alternative to current wet chemistry assay methods is to utilize near infrared spectroscopy (NIRS) coupled with multivariate analysis. NIRS has been used in the agricultural and food industries for years, from analysis of moisture and protein content in cereal grains and oilseeds, [16] to the compositional determination of biomass. Sanderson et al. [21] demonstrated individual carbohydrates can be estimated in woody and herbaceous feedstocks such as straw, corn stover, poplar, etc. using NIRS spectroscopy and partial least squares regression (PLSR). Hames et al. [6] reported NIRS calibration models for corn stover feedstock and dilute acid pretreated corn stover. Pordesimo et al. [20] later used the corn stover feedstock model to investigate the variability of stover composition with crop maturity at harvest. They took samples from corn plants from approximately two weeks before the corn grain reached physiological maturity to approximately one month after the grain was at a suitable moisture content for harvesting. Their results showed large decreases in the extractives content of the samples, with increases in both xylan and lignin content. The corn stover feedstock model was also used by Hoskinson et al. [8] to provide compositional data for a study investigating the variation in quality and quantity of corn stover available under different harvesting scenarios. PLSR models of NIRS spectra were used to evaluate compositional

variation and sources of variability in 508 commercial hybrid corn stover samples collected from 47 sites in eight Corn Belt states after the 2001, 2002, and 2003 harvests. [28] Similarly, Haffner et al. [5] demonstrated the use of PLSR models of NIRS spectra of 241 *Miscanthus × giganteus* samples harvested from seven sites in Illinois for fast monitoring of miscanthus in plant breeding studies. NIRS spectra can also be used to classify materials. Ye et al. [31] fractionated corn stover into botanical fractions (node, leaf, rind, pith, sheath, and husk) and scanned them with an Fourier transform near infrared (FT-NIR) spectrophotometer. For each fraction, spectral data was taken and a principal components analysis (PCA) was conducted followed by soft independent modeling class analogy (SIMCA) modeling. Results showed that the model developed could classify 60 additional botanical fractions correctly. NIRS can be used as a tool to measure variability in composition and potential sugar yields of individual fractions, to ensure blending consistency, and for tracking quality at the factory gate. The objectives of this study were to determine the mass fraction and composition of the botanical fractions of *Miscanthus × giganteus* and to demonstrate the use of NIRS and principal component analysis (PCA) to monitor the composition of blended fractions of miscanthus.

## Experimental

### Sample preparation

*Miscanthus × giganteus* stalks ( $n = 4$ ) from six stands were harvested by hand in January 2013 from the Energy Biosciences Institute (EBI) Energy Farm at the University of Illinois in Urbana, IL, USA (40°3'50.55" N, 88°11'26.9" W). Each stand was treated as a replication. Since the stalks were cut during the senescent stage, some of the leaves, or blades, had already fallen off and were not included in the mass fraction analysis. However, blade samples near the stands were collected so that blade composition could be determined. The stalks were taken to the laboratory where they were manually separated into stalks and blades (Figure 1). The stalks were cut to separate nodes from internodes. The internodes were further fractionated by manually peeling off the sheath; splitting the stalk in half, longitudinally; and scraping the pith from the rind.

Triplicate subsamples (approximately 1 g each) of each botanical fraction from each stand were used to determine moisture content by oven drying at 103°C for 24 h. [2] Moisture content was used to determine the dry mass fraction of botanical fractions. The rest of the samples were dried at 60°C for 72 h for subsequent FT-NIR and composition analyses. After drying, the samples were ground using a cutting mill (Model No. SM 2000, Retsch, Inc., Haan, Germany) fitted with a 2 mm sieve, poured into resealable plastic bags, and stored at room temperature until blending, NIRS scanning, and compositional analyses. Blends of rind and blade fractions (10–90% w/w) were

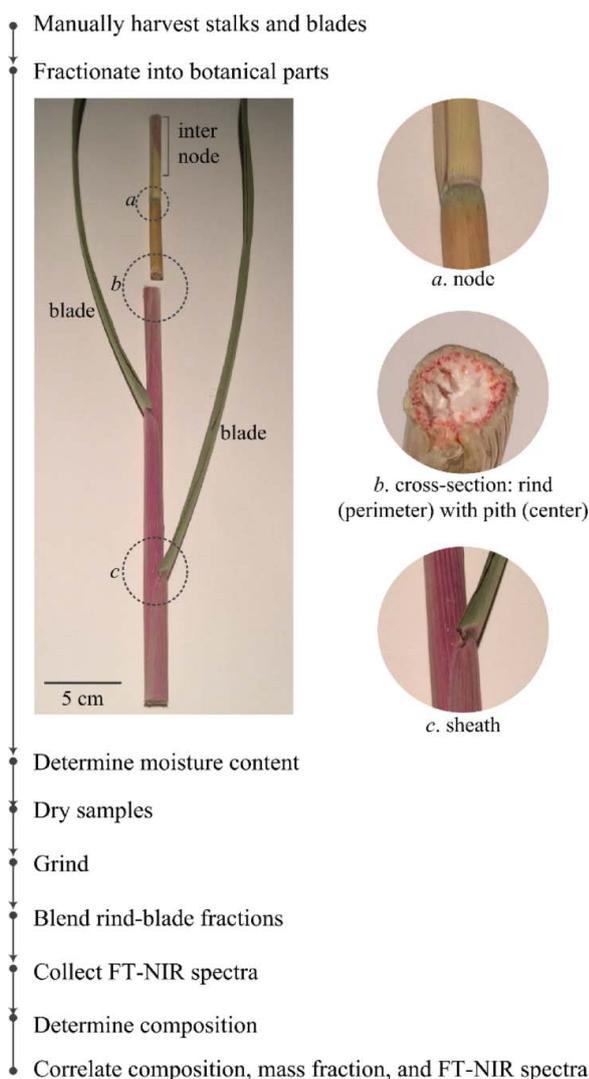


Figure 1. Overview of test procedures and botanical fractions of a miscanthus stalk (pre-senescence).

prepared to demonstrate the wide range of structural carbohydrates achievable in formulating miscanthus feedstock.

### FT-NIR scanning

Prior to compositional analyses, a Fourier transform near infrared (FT-NIR) spectrophotometer (Spectrum™ One NTS, Perkin Elmer, Waltham, MA USA) was used to scan all samples from 4000 to 10000  $\text{cm}^{-1}$  with a spectral resolution of 4  $\text{cm}^{-1}$ . A 2–5 g subsample of an individual botanical fraction or blended fraction was poured into a near infrared reflectance accessory (NIRA) cup, which was placed on a flat, level surface. To ensure consistent packing density, a spatula was run across the top of the NIRA cup to level the sample surface and scrape off the excess. The NIRA cup was placed on the spectrophotometer set to record a spectra from an average of 32 scans. Afterwards, the cup was manually rotated 30° to 45° clockwise, and re-scanned for a total of six recorded spectra for each subsample. This procedure was repeated for

two more subsamples. All recorded spectra were averaged and used in subsequent PCA analysis.

### Compositional analysis and statistical tests

Chemical composition analyses of the individual botanical fractions were conducted in the Bioprocess Engineering Laboratory at the University of Illinois following standard procedures developed by National Renewable Energy Laboratory (NREL). [25,26,27] The samples were wrapped in a filter bag (XT4, Ankom Technology, Macedon, NY) and extracted by distilled water and 95% ethanol in a Soxhlet extractor for 6 and 16 h, respectively. The weight loss after 24 h of oven drying at 45°C was determined as extractives. The dried extractive-free samples were analyzed for carbohydrate content, acid soluble and insoluble lignin. Analysis was performed using the two step acid hydrolysis method. Samples were hydrolyzed in 72% (w/w) sulfuric acid at 30°C for 2 h and diluted into 4% sulfuric acid before heating in an autoclave (121°C) for 1 h. The autoclaved solutions were filtered through crucibles. One aliquot of filtrate was neutralized with calcium carbonate to a pH value between 5 and 6. Afterwards, the filtrate was centrifuged at 500-g (IEC CL30, Thermo Fisher Scientific, Inc., Waltham, MA) for 3 min to remove fine solids and further filtered using a 0.2  $\mu\text{m}$  filter and placed into 200  $\mu\text{L}$  HPLC vials. HPLC analysis was conducted using an ion exclusion column (Aminex HPX-87P, Bio-Rad, Hercules, CA) maintained at 85°C, with water as the eluent flowing at 0.6 ml/min. Glucose, xylose, arabinose, and galactose concentrations were measured using HPLC with a refractive index detector (Model 2414, Waters Corporation, Milford, MA). HPLC detection limits of sugar are 0.001% (w/v). [13]

A second aliquot of filtrate was used for acid soluble lignin measurement, which was conducted using a spectrophotometer (Evolution Array, Thermo Scientific, Waltham, MA) set at 280 nm and using 4% sulfuric acid as the reference (blank). The crucibles with acid insoluble portion were dried at 105°C for 4 h to determine dry weights followed by ashing at 575°C for 4 h. [13]

Structural carbohydrates (sum of glucan and xylan contents), lignin and ash content measurements were conducted in duplicate while moisture and extractives measurements were conducted in triplicate. The compositional means of the botanical fractions were determined, compared and tested for significance at  $\alpha = 0.05$  using a one-way analysis of variance and Tukey's test in the SAS [22] (Version 9.3, SAS Institute, Inc., Cary, NC, USA).

For blended rind-blade fractions, lignin was estimated using the composition means resulting from the spectrophotometric assay and the mass fractions each of rind and blade in the blend in a mass balance equation:

$$L_f = w_r L_r + w_b L_b$$

where  $L_f$ ,  $L_r$ , and  $L_b$  are the lignin contents (%) of the blended sample, rind component, and blade component,

respectively; and  $w_r$  and  $w_b$  are the mass fractions of the rind and blade in the blend, respectively. The same mass balance equation was used to estimate glucan, xylan and ash contents (%) of the blends using the composition means of the individual botanical fractions determined via HPLC and ashing.

### Spectral processing and PCA

All NIRS spectra were preprocessed using mean-centering and multiplicative scatter correction (MSC) in Unscrambler® X [29] (Version 10.1, Camo Software, Inc., Woodbridge, NJ USA). MSC was used to remove scatter or interferences resulting from baseline shifts and particle size distribution of the nonhomogeneous ground samples. Preprocessed spectra were examined for differences among individual botanical fractions. For blended rind-blade fractions, preprocessing was followed by PCA to reduce multiplicity of the spectral data into more manageable components, with the intent that the first few principal components would capture the spectral variation resulting from the wide range of composition of the blends.

## Results and discussion

### Mass fraction of botanical fractions

The majority of the miscanthus stalks were composed of rind, the hard outer portion of the stalk, with a dry mass fraction of 48–63%, with a mean and standard deviation of  $56 \pm 4.9\%$  (Figure 2). The remaining botanical fractions had the following mass fractions in decreasing ranked order: sheath 17–28%; nodes 10–16%; and pith 8–13%. Since the stalk samples were harvested during the senescent stage, the blades had already fallen off and were not included in the mass fraction analysis.

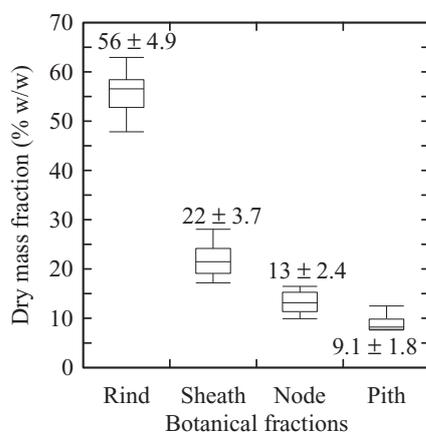


Figure 2. Dry mass fraction of botanical fractions of *Miscanthus × giganteus*. Median-based box plots represent the minimum, maximum, and interquartile range with the mean  $\pm$  one standard deviation stated. Miscanthus samples were harvested during the senescent stage and, thus, blades were not included in the analysis.

### Chemical composition of botanical fractions

Across botanical fractions, ranges for each component were as follows: glucan 32.2–46.1%; xylan 20.9–25.3%; arabinan 0.0–6.1%; lignin 18.7–25.5%; and ash 0.4–8.9% (Figure 3). While large variations were seen among individual sugars, the total structural carbohydrate levels for rind, pith, and sheath were not different from each other, showing that if glucan content decreased, the xylan and arabinan contents increased proportionally. However, nodes and blades had the lowest estimated carbohydrate levels at 62.0 and 59.8%, respectively, compared to rind, pith and sheath. Overall, variations in composition within each botanical fraction were low.

The compositions of the botanical fractions of miscanthus were comparable to those reported for corn stover and switchgrass with blades having the lowest and rind with the highest glucan contents. [15] In switchgrass, leaves had the lowest glucan content while internodes had the highest. For the hemicellulose components, the pith fraction for miscanthus, husk fraction for corn stover, and nodes fraction for switchgrass had the highest xylan contents. Variations in xylan content of up to 13.5% were observed in corn stover and 6.0% in switchgrass fractions. Miscanthus was comparable to switchgrass having the largest absolute difference, 4.4%, in xylan content found in the rind and pith. Similarly, arabinan contents were low in the rind, pith and nodes of corn stover and miscanthus and in the internodes of switchgrass; while high arabinan levels were found in the leaves fraction of miscanthus, husk fraction of corn stover, and nodes fraction of switchgrass. Variations in arabinan contents for all three feedstocks were below 5%.

Miscanthus blades had the lowest lignin content at 18.7% but, in the blades of corn stover and of switchgrass, lignin contents were as high as 24.0 and 25.1%, respectively. [15] In miscanthus, nodes had the highest lignin content at 25.5%, which was comparable in lignin contents to the nodes of corn stover (23.6%) and switchgrass (21.8%, on average depending on cultivars). Variations in lignin content of miscanthus (e.g., between nodes and blades a 6.8% difference was measured) and switchgrass (which exhibited 4.8% variation) were comparable. Comparatively, corn stover variation at 14.3% was high.

Lastly, in terms of ash content, the three feedstocks were comparable; the rind, node, and pith fractions had the lowest ash content while the blades had the highest ash levels. Again, the largest absolute difference (8.5%) in ash contents was between nodes and blades of miscanthus. This time, ash variation was more comparable to corn stover (7.6%) than to switchgrass (3.8%).

These measurements showed that as a lignocellulosic feedstock, variations in miscanthus composition were comparable to that of corn stover composition and not as consistent as switchgrass composition. This could be attributed to composition data being available for more botanical fractions and hence, specialized tissues, of miscanthus (rind, pith, sheath, node, and blade) and corn stover (rind, pith, sheath, node, blade and husk) than

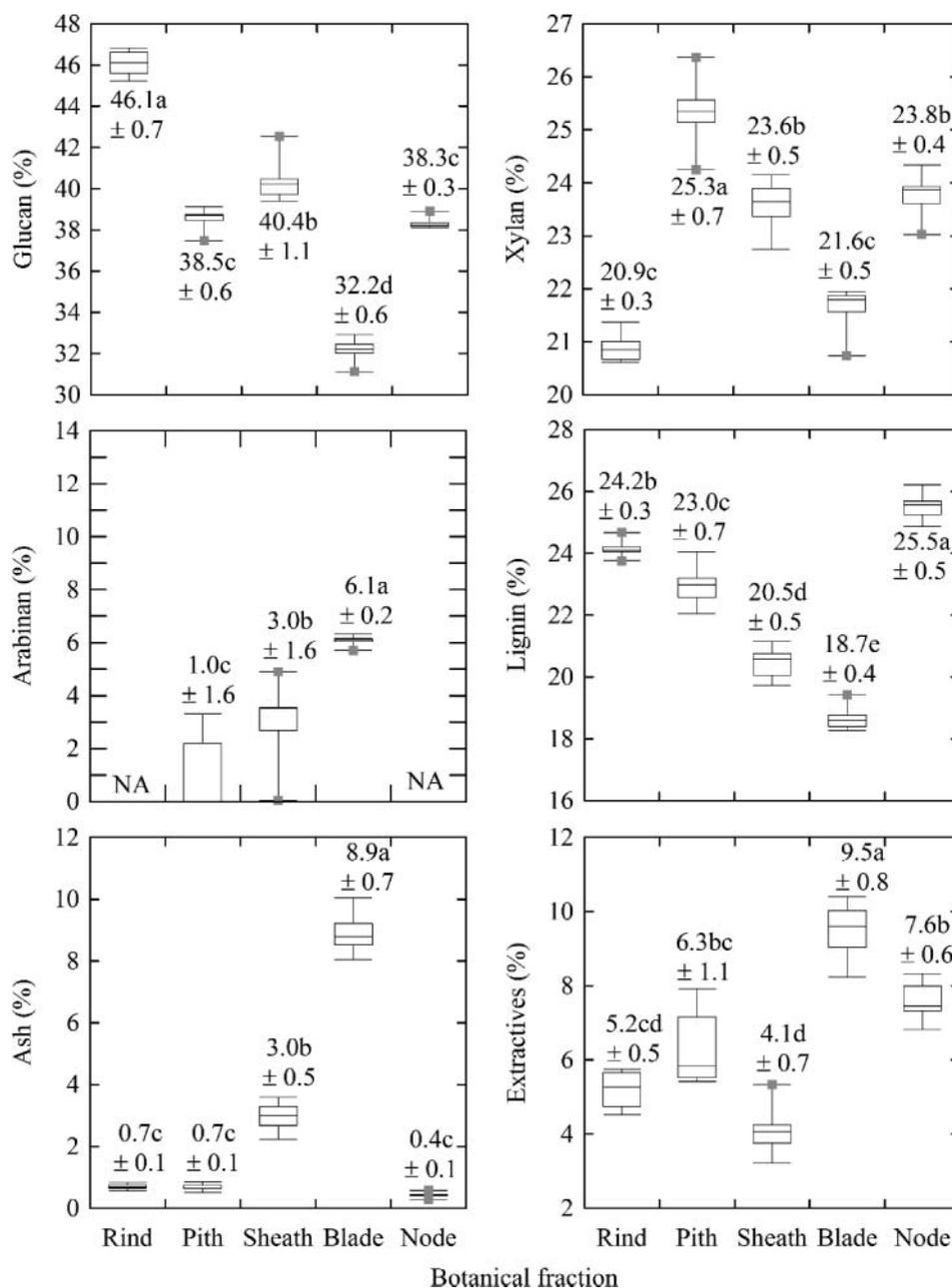


Figure 3. Composition of botanical fractions of *Miscanthus × giganteus*. Median-based box plots represent the minimum, maximum, interquartile range (IQR), and outliers (■) which were data lying at a minimum 1.5·IQR distance from the median. Component mean and one standard deviation values are provided in each plot; in some cases, component was not measured in the botanical fraction and was denoted 'NA'. Mean values followed by the same lowercase letter in the same plot were not different from each other ( $p > 0.05$ ).

switchgrass (internode, node, and blade). [15] Overall, the carbohydrate levels in miscanthus ranged from 54 (blade fraction) to 67% (rind fraction) were comparable to those reported for: corn stover 39–66%; corncobs 49–65%; and wheat 41–57%. [4,20]

#### FT-NIR spectra of botanical fractions and PCA of rind-blade blends

Variability in composition of botanical fractions was also reflected in their FT-NIR spectra (Figure 4). Variations can be seen among fractions with different absorption intensities across all wavenumbers. The leaf structures,

blade and sheath, exhibited additional peaks in the combination region from 4000–5000  $\text{cm}^{-1}$ , specifically at 4255 and 4323  $\text{cm}^{-1}$ , which could be attributed to differences in tissue types between the blades and stalks and lower lignin and ash contents of the leaf structures.

With blade and rind fractions having large differences in spectral responses, blends with different mass fractions of blade and rind were expected to yield the largest variation in composition of blends. These blends were scanned and their NIRS spectra analyzed using PCA. The NIRS spectra of the blends showed the same trends as pure botanical fractions with absorption variations across all wavenumbers (Figure 5). Distinct absorbance peaks at

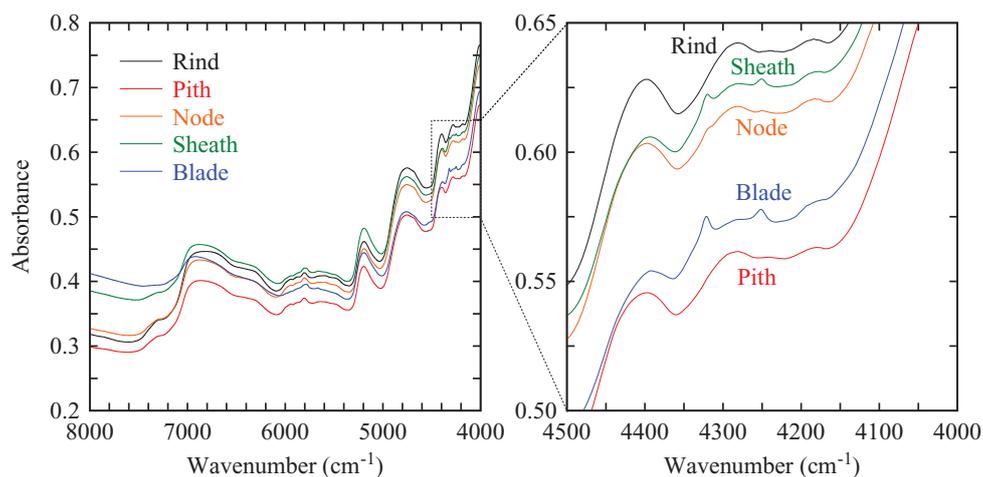


Figure 4. Raw NIR spectra of botanical fractions.

4255 and 4323  $\text{cm}^{-1}$  for blades and sheath decreased as the blade mass fraction,  $w_b$ , decreased. A plot of the first principal component (PC1) of the PCA against the third principal component (PC3) showed PC1 was sensitive to these two components (Figure 6). Based on mean values for glucan, lignin, and ash from wet chemistry assays of the individual fractions of miscanthus, glucan content increased from 32 to 45% as PC1 decreased; likewise, lignin content increased from 19.5 to 23.5% as ash content decreased from 8.0 to 1.5%. This result demonstrated the potential use of NIRS to screen for feedstocks with desired composition, or quality, attributes and to monitor biomass fractionation and blending processes in the formulation of advanced biofuel feedstock. It is expected that blends of other botanical fractions of miscanthus would yield a variation in composition that can be monitored using NIRS and that variation will become wider with the inclusion of samples from different stages of maturity and conditions for growing, harvesting, handling and preprocessing, and storage. Additional work to determine the effects of moisture content and particle size on the NIRS screening method need to be conducted to make the approach practical and efficient.

### Conclusions

Miscanthus harvested at the post-senescent stage exhibited the following dry mass fraction in decreasing order: rind, sheath, node, and pith. Blades were excluded from the mass fraction analysis as they had already fallen off. Across botanical fractions, composition ranged from: glucan 32.2–46.1%; xylan 20.9–25.3%; arabinan 0.0–6.1%; lignin 18.7–25.5%; and ash 0.4%–8.9%. Overall, the estimated structural carbohydrates content of rind 67.0%, pith 63.8%, and sheath 63.9% fractions were comparable. The compositional variations within each botanical fraction were low but variations across some botanical fractions were significant. Blade had the lowest glucan, lowest lignin, and highest ash contents making them different from the other botanical fractions. Compared to other lignocellulosic feedstocks, variations in miscanthus composition were comparable to that of corn stover composition and not as consistent as switchgrass composition. The FT-NIR spectra of individual botanical fractions and blends of the rind and blade fractions exhibited these compositional variations. These results demonstrated that FT-NIR spectroscopy may be used to identify or classify individual botanical fractions and to monitor

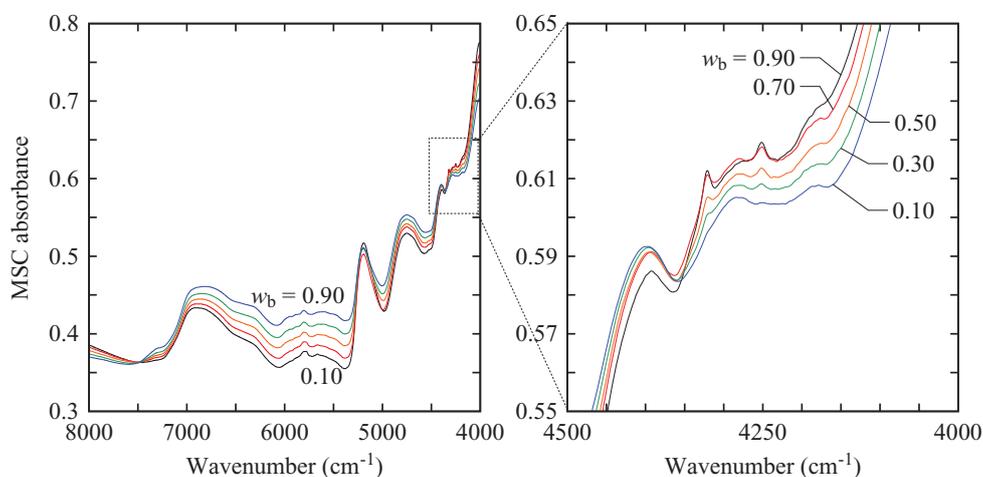


Figure 5. MSC spectra of five rind-blade blends showing variations in composition and distinct absorption bands in the 4250–4350  $\text{cm}^{-1}$  and 5500–6000  $\text{cm}^{-1}$  ranges as blade mass fraction,  $w_b$ , increased.

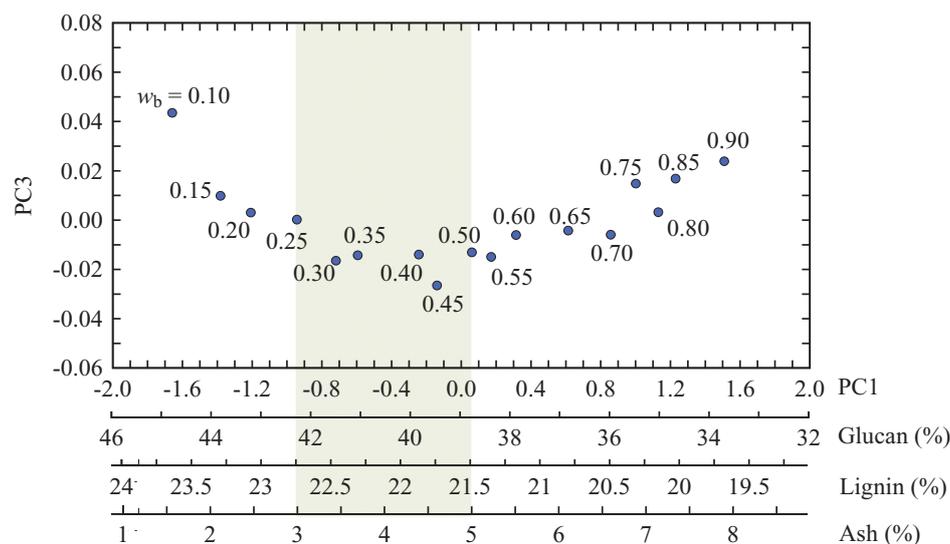


Figure 6. Blends of rind and blade fractions can be differentiated across the first principal component (PC1) of the PCA of the NIR spectra. Data are labeled according to the blade fraction ( $w_b$ ) and the rind fraction,  $w_r = 1 - w_b$ . Samples that fall within the shaded region have estimated structural carbohydrates content between 60.4 and 63.7%; lignin content between 21.5 and 22.8%; and ash content between 3 and 5%. Structural carbohydrates were estimated to be the sum of glucan and xylan contents.

composition of mixed or blended fractions to a desired set of specifications.

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### ORCID

Mary-Grace C. Danao <http://orcid.org/0000-0001-7898-5004>  
 Marvin R. Paulsen <http://orcid.org/0000-0002-7355-9337>

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