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Quantifying Actual and Theoretical Ethanol Yields for Switchgrass Strains Using NIRS Analyses

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Abstract Quantifying actual and theoretical ethanol yields from biomass conversion processes such as simultaneous saccharification and fermentation (SSF) requires expensive, complex fermentation assays, and extensive compositional analyses of the biomass sample. Near-infrared reflectance spectroscopy (NIRS) is a non-destructive technology that can be used to obtain rapid, low-cost, high-throughput, and accurate estimates of agricultural product composition. In this study, broad-based NIRS calibrations were developed for switchgrass biomass that can be used to estimate over 20 components including cell wall and soluble sugars and also ethanol production and pentose sugars released as measured using a laboratory SSF procedure. With this information, an additional 13 complex feedstock traits can be determined including theoretical and actual ethanol yields from hexose fermentation. The NIRS calibrations were used to estimate feedstock composition and conversion information for biomass samples from a multi-year switchgrass (*Panicum virgatum* L.) biomass cultivar evaluation trial. There were significant differences among switchgrass strains for all biomass conversion and composition traits including actual ethanol yields, ETOHL (L Mg^{-1}) and theoretical ethanol yields, ETOHTL (L Mg^{-1}), based on cell wall and non-cell wall composition NIRS analyses. ETOHL means ranged from 98 to 115 L Mg^{-1} while ETOHTL means ranged from 203 to 222 L Mg^{-1}. Because of differences in both biomass yields and conversion efficiency, there were significant differences among strains for both actual (2,534–3,720 L ha^{-1}) and theoretical (4,878–7,888 L ha^{-1}) ethanol production per hectare. It should be feasible to improve ethanol yields per hectare by improving both biomass yield and conversion efficiency by using NIRS analyses to quantify differences among cultivars and management practices.

Keywords Switchgrass · Biomass · Ethanol · NIRS · Quality

For abbreviations used in this paper, please refer to Table 1.

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Introduction

High-yielding perennial grasses such as switchgrass (*Panicum virgatum* L.) have the potential to become valuable biomass energy crops that can be grown on marginal cropland. Agricultural products in general vary in composition and

other properties due to genetics, growth environment, crop management, harvest practices, and post-harvest storage and processing. Biomass produced for conversion to bioenergy will vary due to the same factors. The quality of agricultural products affects their use for food, animal feed, fiber, and industrial products. Likewise, biomass quality will affect its value for conversion to energy at a biorefinery. Maize (*Zea mays* L.) grain, which is more homogenous in properties and composition and is much simpler to process to ethanol than herbaceous biomass, is routinely assayed for quality at the biorefinery gate. Quantifying the actual and theoretical ethanol yields from biochemical conversion routes of biomass, such as simultaneous saccharification and fermentation (SSF), requires expensive and complex assays. Fast, inexpensive methods with high accuracy are needed for predicting maximum ethanol yields and actual conversion efficiencies by agronomists for optimizing feedstock quality via production management, by breeders and geneticists for developing cultivars or hybrids with improved processing capabilities and energy yields per ton, and by biorefineries for quickly measuring quality of each truck load of biomass at the biorefinery gate [1].

Near-infrared spectroscopy (NIRS) is widely applied in agriculture for determining the quality of forages, grains and grain products, oilseeds, coffee, tea, spices, fruits, vegetables, sugarcane, beverages, fats and oils, dairy products, eggs, meat, and other agricultural products [2]. NIRS is also widely used to quantify the composition of agricultural products because it meets the criteria of being accurate, reliable, rapid, non-destructive, and inexpensive. NIRS analysis is an analytical method where analytes are quantified in a sample based on the spectral characteristics of the sample. An analyte is defined here as a substance or chemical constituent of interest [3]. The amount of an analyte is predicted based on the sample's near-infrared reflectance spectra using equations fitted to a calibration set representative of the future samples. Laboratory analyses of the samples in the calibration set are conducted for each of the analytes of interest, the near-infrared reflectance spectra profiles of the samples are determined, and utilizing the laboratory and spectral data, prediction equations are developed and validated using mathematical and statistical procedures described by Shenk and Westerhaus [4], Westerhaus et al. [3] and Wold et al. [5, 6].

Most current commercialization efforts for converting lignocelluloses into liquid biofuels rely on biochemical processing. The complex plant cell wall structure requires initial pre-treatment of the biomass either mechanically, thermally, and possibly in the presence of chemical catalysts to expose the carbohydrates. Following pretreatment, the carbohydrates are enzymatically hydrolyzed and fermented into ethanol using microorganisms capable of fermenting both hexoses and pentoses. The pretreated biomass can be saccharified and fermented into ethanol [7, 8]. NIRS analysis procedures have been used previously to determine composition of biomass feedstocks [9–13] but to date, NIRS analyses has not been used to determine SSF biomass conversion efficiency. Sanderson et al. [9] determined the chemical composition of both woody and herbaceous biomass feedstocks including switchgrass samples and attempted to development NIRS calibrations for ash, lignin, uronic acid, cell wall sugars, C, H, N, O, and ethanol extractives which were not defined. Their results indicated that a broad-based NIRS calibration could be developed for use with an array of feedstocks but separate NIRS calibrations for different classes of biomass would result in improved accuracy and precision. Lorenz et al. [13] used NIRS analyses to predict the composition of maize (*Z. mays* L.) corn stover. The maize stover compositional values were then used to estimate theoretical ethanol yield of stover from an array of maize hybrids and lines by using the National Renewable Energy Laboratory's theoretical ethanol yield calculator. The maize hybrids and lines differed significantly for all evaluated traits including theoretical ethanol yields on a mass (L Mg^{-1}) and production area (L ha^{-1}) basis, thereby demonstrating that variability in corn stover composition directly affects potential ethanol production [13]. However, actual sugar or ethanol yields, which will be less than 100% efficient, were not determined experimentally. The theoretical ethanol yield calculator used by Lorenz et al. [13] does not account for variability in pretreatment effects, enzymatic hydrolysis, or ethanol fermentation yields as indirect consequence of changes in biomass composition and properties. Recently, Lorenzana et al. [14] reported maize stover cell wall composition and efficiency of cell wall glucose release by dilute acid/high temperature pretreatment and enzymatic hydrolysis of recombinant inbred lines using a NIRS calibration developed as part of their study, but again actual ethanol production was not measured.

This study had two primary objectives. The first was to develop broad-based NIRS calibrations for all major compositional components of switchgrass biomass and switchgrass biomass ethanol and released pentose yields from a laboratory SSF process using commercial cellulases and *Saccharomyces cerevisiae*. Composition information was needed to estimate theoretical maximum ethanol yields. The second objective was to determine if switchgrass cultivars and experimental strains adapted to the Midwest USA differ significantly in their actual and potential ethanol yields per ton and hectare. To address this objective, switchgrass samples from a multi-year biomass yield trial were analyzed using the NIRS prediction equations to obtain estimates of the biomass constituents and ethanol and released pentose yields. The

biomass yield data from the trial along with the ethanol conversion data were used to determine ethanol yields per hectare.

Materials and Methods

Development of NIRS Calibrations

The switchgrass biomass samples that were used to develop broad-based NIRS calibrations are representative of the potential range in switchgrass biomass quality that could be produced under different management and harvesting systems with currently available cultivars and experimental strains. The biomass samples were from the following studies and had been analyzed previously for forage quality traits.

(a) A 2-year study at Mead, NE and Ames, IA in which 6 N fertilizer rates (0, 60, 120, 180, 240, and 300 kg ha^{-1} N) and eight maturity stages at harvest, which ranged from boot stage harvest in late June to an end-of-growing season harvest after a killing frost, were evaluated [15].
(b) A study in which 20 upland and lowland cultivars or experimental strains were evaluated for biomass yield at five locations (Spooner and Arlington, WI, Mead, NE, Manhattan, KS, and Stillwater, OK) over a 2-year period [16] when harvest maturity ranged from R1 (late boot stage) to R5 (post-anthesis) as described by Moore et al. [17].
(c) A long-term C sequestration study near Mead, NE in which switchgrass samples were collected from two switchgrass cultivars grown with 3 N rates (0, 60, and 120 kg ha^{-1} N) and harvested either at anthesis or after a killing frost [18].
(d) Samples from a field trial near Mead, NE in which switchgrass hybrids were harvested at the RO (boot stage) to R3 maturity stage [19].
(e) Biomass samples from a half-sib family breeding nursery located near Mead, NE which were harvested at the R2 to R4 maturity stages.

Samples were prepared following the sample preparation guidelines outlined by Murray and Cowe [20]. Specifically, for each of the above studies, biomass samples were collected at harvest and dried in forced-air ovens at 60°C. The switchgrass biomass samples were ground through a 2-mm screen in a Wiley mill and then re-ground in a cyclone-type mill to pass a 1-mm screen. Ground samples were scanned using a Model 6500 near-infrared spectrometer (NIRSystems, Silver Springs, MD; now FOSS NIRSystems, Inc., Laurel, MD). A set of 482 samples were selected from the above experiments which represented the range of plant maturities, cultivars, ecotypes, fertility rates, and environments of the samples in the five experiments. NIRS software analyses procedures were used to select a calibration set of 112 samples from these experiments that represented the spectral diversity of the samples using procedures described by Shenk and Westerhaus [4] and Westerhaus et al. [3]. This was accomplished using the "Center" and "Select" modules of WINISI II[1] version 1.04 software from Infrasoft International LLC, State College, PA which is available through Foss NIRSystems Inc[1]. A switchgrass standard sample from the Lincoln ARS Forage Quality Laboratory was also included in the calibration set.

The calibration set of samples were analyzed for chemical composition, ethanol and pentose sugar yields following pretreatment and SSF, and forage quality traits on an oven dry weight basis. Dry matter concentration was determined on subsamples that were not used for other analyses by heating in a forced-air oven for 24 h at 105°C. Analytical methods and calculations and abbreviations for all traits are shown in Table 1. Depending on the specific analysis, each sample was analyzed in duplicate or triplicate and the laboratory mean value for that sample was used to develop the NIRS calibration. Additional details and discussion on the laboratory procedures are available in Dien [21].

Compositional Analysis

Total nitrogen (N) and carbon (C) concentration were determined in the University of Nebraska Agronomy and Horticultural Department's analytical laboratory by the LECO combustion method (Model FP 428 and FP 2000, LECO Corp., St. Joseph, MI) [22, 23]. Lipid content (EE) was determined by exhaustive extraction with diethyl ether [24]. Total mineral or ash content (ASH) was measured as loss of weight after combustion at 450°C for 16 h in a muffle furnace. Carbohydrates and lignin were determined using a sequential procedure as outlined by Dien et al. [25]. Soluble carbohydrates were extracted with 80% *v*/*v* ethanol at 60°C overnight [26]. The supernatant was analyzed by high-performance liquid chromatography (HPLC) for monosaccharides (glucose and fructose) and oligosaccharides (sucrose, stachyose, and raffinose) using a refractive index detector [25]. Fructans were not measured on the samples because no fructans were found in switchgrass by Dien et al. [25]. The alcohol-insoluble residue was treated with heat-stable α-amylase and amyloglucosidase in 0.1 M acetate buffer, pH 5, to release glucose from starch [27]. Sufficient 95% *v*/*v* ethanol was added to reach an alcohol concentration of 80%, after which the supernatant was removed and analyzed by HPLC for glucose released from starch. The remaining crude, alcohol-insoluble cell wall residue was subjected to a two-stage sulfuric acid hydrolysis using the Uppsala Total Dietary Fiber Method [27]. An aliquot from the first stage of the acid hydrolysis was

Table 1 Switchgrass biomass composition, and actual and potential ethanol yield traits determined by laboratory analysis or calculation, and references for the methods

Variable	Abbreviation	Units	Reference or equation
Composition variables			
Dry matter	DM	mg g^{-1}	Vogel et al. [34]
Carbon	C	mg g^{-1}	Watson and Isaac [23]
Nitrogen	N	mg g^{-1}	Watson and Isaac [23]
Extracted fat	EE	mg g^{-1}	Padmore [24]
Minerals (total ash)	ASH	mg g^{-1}	450°C muffle furnace for 6 h.
Klason lignin	KL	mg g^{-1}	Theander et al. [27]
Uronic acids[a]	UA	mg g^{-1}	Theander et al. [27], Ahmed and Labavitch [28]
Rhamnose [a]	RHA	mg g^{-1}	Theander et al. [27]
Fucose [a]	FUC	mg g^{-1}	Theander et al. [27]
Arabinose [a]	ARA	mg g^{-1}	Theander et al. [27]
Xylose [a]	XYL	mg g^{-1}	Theander et al. [27]
Mannose [a]	MAN	mg g^{-1}	Theander et al. [27]
Galactose [a]	GAL	mg g^{-1}	Theander et al. [27]
Glucose [a]	GLC	mg g^{-1}	Theander et al. [27]
p-Coumarate esters	PCA	mg g^{-1}	Jung and Shalita-Jones [30]
Esterified ferulates	FEST	mg g^{-1}	Jung and Shalita-Jones [30]
Etherified ferulates	FETH	mg g^{-1}	Iiyama et al. [29]
Cell wall concentration	CWC	mg g^{-1}	KL+UA+RHA+FUC+ARA+XYL+MAN+GAL+GLC+ PCA+FEST+FETH
ARA+XYL+Man+GAL	AXMG	mg g^{-1}	ARA+XYL+MAN+GAL
ARA+XYL	AX	mg g^{-1}	ARA+XYL
Sucrose	SUC	mg g^{-1}	Dien et al. [25]
Soluble glucose	GLCS	mg g^{-1}	Dien et al. [25]
Fructose	FRU	mg g^{-1}	Dien et al. [25]
Total soluble carbohydrates	SC	mg g^{-1}	SUC+GLCS+FRU
Starch	STA	mg g^{-1}	Dien et al. [25]
Non-structural carbohydrates (starch+SC)	NSC	mg g^{-1}	SC+STA
Total hexoses	HEX	mg g^{-1}	$((\mathrm{MAN}+\mathrm{GAL}+\mathrm{GLC})(180/162))+\mathrm{NSC}$
Total sugars	SUG	mg g^{-1}	$\mathrm{HEX}+((\mathrm{AX})(150/132))$
Ethanol and potential ethanol			
Ethanol/g dry forage	ETOH	mg g^{-1}	SSF[b], see "Materials and Methods" section for details.
Pentose sugars released/g dry forage	PENT	mg g^{-1}	SSF[b], see "Materials and Methods" section for details
Calculated ethanol traits			
Proportion of hexoses that are non-structural or soluble	PSOL	%	(NSC/HEX)100
Pentose proportion of total carbohydrates	PPEN	%	$(1-(\mathrm{HEX}/\mathrm{SUG}))100$
Theoretical ethanol from hexoses (excluding starch)	HEXE	mg g^{-1}	$[(\mathrm{MAN}+\mathrm{GAL}+\mathrm{GLC})0.57]+[0.51(\mathrm{GLCS}+\mathrm{FRU})]+[0.537\mathrm{SUC}]$
Estimated ethanol from non-structural carbohydrates[c]	NSCE	mg g^{-1}	$[0.51(\mathrm{GLCS}+\mathrm{FRU})+0.537\mathrm{SUC}+0.57\mathrm{STA})]0.9$
Cell wall ethanol	CWE	mg g^{-1}	ETOH-NSCE
Theoretical ethanol conversion efficiency from cell wall hexosans[d]	CWEP	%	$[\mathrm{CWE}/((\mathrm{MAN}+\mathrm{GAL}+\mathrm{GLC})0.57)0.9)]100$
Pentoses extraction efficiency	PENTP	%	$[0.88\mathrm{PENT}/(\mathrm{ARA}+\mathrm{XYL})]100$
Hexose ethanol extraction efficiency	HEXEP	%	(ETOH/HEXE)100
Forage quality composition			
Neutral detergent fiber	NDF	mg g^{-1}	Vogel et al. [34]
Acid detergent fiber	ADF	mg g^{-1}	Vogel et al. [34]
Acid detergent lignin	ADL	mg g^{-1}	ANKOM Technology -9/99

Table 1 (continued)

Variable	Abbreviation	Units	Reference or equation
In vitro dry matter digestibility	IVDMD	mg g^{-1}	Vogel et al. [34]
Crude protein	CP	mg g^{-1}	N×6.25

[a] Cell wall carbohydrates

[b] *SSF* simultaneous saccharification and fermentation using modified procedure of Dowe and McMillan [31] and quantified using HPLC as described by Dien et al. [32]

[c] Assumed that yeast converts all soluble sugars to ethanol with efficiency of 0.90

[d] Assumed that yeast could covert cell wall hexoses to ethanol with an efficiency of 0.90. To obtain estimates of 100% conversion efficiency, multiply by 1.11

analyzed for uronic acids [28] using glucuronic acid as the standard. Neutral sugars from the two-stage acid hydrolysis were analyzed as alditol-acetate derivatives by GC-FID. The acid-insoluble residue provided the Klason lignin concentration estimate after correction for ash.

Total (ester and ether linked) ferulates in the cell wall were extracted with 4 M NaOH for 3 h at 160°C from starch-free, alcohol-insoluble residues [29]. Ester-linked ferulates and *p*-coumarates were extracted from similar starch-free, alcohol insoluble residues with 2 M NaOH at 39°C for 24 h [30]. Ferulic and *p*-coumaric acid residues in the alkaline extracts were quantified by HPLC [30]. Ether-linked ferulate was calculated as the difference between total and ester-linked ferulic acid concentrations of each sample [29].

Pretreatment and Fermentation

Flask fermentations were conducted using a simultaneous saccharification and fermentation scheme based upon a modified method [31]. For each sample, 1.5 g of biomass was mixed with 8.5 ml of sulfuric acid solution (1.75% *w*/*v*) and reacted at 121°C for an hour using an autoclave. Samples were pretreated in bottles closed with screw cap lids to prevent water loss. Hydrolysates were adjusted to pH 4.5 by adding $Ca(OH)_2$ to neutralize the mineral acid and 1 M sodium citrate buffer stock diluted to 50 mM. Hydrolysates were supplemented with 1 ml of a 10×YP stock (200 g/L peptone and 100 g/L yeast extract). The hydrolysates were fermented using a simultaneous saccharification and fermentation scheme. Filter sterilized cellulase (5.0 FPU per dry gram biomass, GC220, Genencor Inc., Rochester, NY) and β-glucosidase (15 U per dry gram biomass, Novo188, Novozymes, Denmark) were added and the cultures were inoculated to an $O.D._{600}$ of 0.5 using *S. cerevisiae* D5A. The pre-fermentation culture was serially transferred twice, first on YP supplemented with 20 g/L glucose and next on YP supplemented with 50 g/L glucose. Each time the pre-culture was grown for 18 h at 35°C under agitation (150 rpm) in an incubator shaker. The final culture was concentrated to an $O.D._{600}$ of 50. The bottles were topped with screw cap holders with fitted silicone septa, which were pierced with 22 g stainless steel needles to allow for CO_2 exhaust. The fermentations were conducted at 35°C for 72 h while being mixed at 100 rpm using an incubator shaker. Ethanol, sugars, and organic acids were analyzed using a HPLC system equipped with an organic acids column (Bio-Rad Laboratories, CA) and a refractive index detector, as previously described [32]. All samples were analyzed in triplicate.

Forage Quality Analyses

Crude protein concentration was calculated as N×6.25 [33]. In vitro rumen dry matter digestibility (IVDMD), neutral detergent fiber, acid detergent fiber, and acid detergent lignin (ADL) were determined using the ANKOM Fiber Analyzer (ANKOM Technology Corp., Fairport, NY[1]) and the procedures described by Vogel et al. [34] and the ANKOM ADL procedure (ANKOM Technology -9/99, Method for Determining Acid Detergent Lignin in Beakers).

NIRS

The laboratory data were used to develop prediction equations using the procedures described by Westerhaus et al. [3]. The number of samples for which complete laboratory data was available was typically 111 or 112 but for some analytes the number of samples ranged from 102 to 108. The samples were divided into calibration and validation sets with the validation set having 17 or 18 samples depending upon the analyte. Validation samples were selected to represent the range of the entire sample set. The remaining samples were in the calibration group. Calibration equations for predicting unknowns were developed using the WinISI software modified PLS procedure. The calibration equations were tested using the WinISI software validation procedures by predicting analyte values of the validation set. Calibration equations were selected

using criteria described by Westerhaus et al. [4] and Shenk and Westerhaus [3]. Once the calibration equation was selected, the validation samples were included in the calibration set and calibration equations were redeveloped using the same math treatments and options as in the original calibrations. This additional calibration work was done to improve the precision and robustness of the calibrations. Calibrations statistics included the coefficient of determination (r^2), standard error of calibration (SEC), standard error of cross-validation (SECV), and standard error of prediction (SEP) values. The r^2 represents the proportion of the total variation among the samples for a trait that is explained by the calibration. The SEC is the standard error of the difference between the laboratory value and the NIRS-predicted value for the same sample. The SEP is the standard error of the difference between the laboratory value and the NIRS-predicted value of the validation set. SECV is the standard error of the difference between predicted and laboratory values when samples are sequentially removed from the calibration process. Acceptable NIRS calibration equations should have r^2 values greater than 0.80, and preferably greater than 0.90, with small SEC, SEP, and SECV values.

Switchgrass Biomass Cultivar Trial

The switchgrass cultivar and experimental strain evaluation trial was located in eastern Nebraska at the University of Nebraska's Agricultural Research and Development Center about 50 km west of Omaha, NE. The trial was a typical small plot trial with 21 cultivars or experimental strains. The trial was planted in 2002 and biomass was harvested in 2003, 2004, and 2005. The experimental design was a randomized complete block with six replicates. Seeded plots were 1.5×3.0 m and were separated on the ends by a 1.5-m wide alley that was also seeded to switchgrass. No fertilizer was applied the establishment year. In the post-establishment years, the study was fertilized in the spring with 112 kg ha^{-1} N (NH_4NO_3). Herbicides were used for weed control. The residual biomass on the plots from the previous year was removed before spring growth initiated by mowing or burning. Plots were harvested for biomass yield in mid-August when most cultivars or experimental strains in the nursery were headed. Maturity of the strains in the nursery ranged from R1 to R4 using the staging system of Moore et al. [17]. Plots were harvested with a plot flail harvester with a cutting height of 10 cm. The harvester cut a 0.91 m swath down the middle of each plot. Plots were sampled prior to harvest to obtain samples for biomass composition analysis and to determine dry matter concentration. Wet sample weights were added to plot yields to nullify the effect of sub-sample size on biomass yield. Yields are reported on a dry weight basis. The single harvest treatment and N fertilization rates were based on the results of Vogel et al. [15]. The harvested samples were dried, ground, and used for NIRS analysis with the procedures described above. Prior to predicting the composition of the samples, the NIRS spectra of the samples were analyzed for fitness to the calibration set of samples by using the Global H statistic to test for outliers [20]. All samples in the application set of samples had acceptable H values ($H<3.0$). The analytes listed in Table 1 were predicted for the samples from the yield test. The feedstock quality data and the yield data was used to calculate ethanol yield, theoretical ethanol yield, and potentially fermentable substrate yield (L Mg^{-1}) Mg and production per hectare (L ha^{-1}) using equations listed below. All data were analyzed using the PROC GLM procedure of PC-SAS for Windows [35]. Strain or cultivars were considered to be fixed effects. With perennials, mean performance over years is of primary importance; therefore, plot means over years were used in the statistical analyses.

Equations for Determining Yield (L) per Ton (Mg) and Production per Hectare (L Ha^{-1})

1. Specific volume of ethanol is 0.789 g mL^{-1} so 1/0.789 g mL^{-1}=1.267 mL g^{-1} ethanol. On a unit basis, mg g^{-1} = g kg^{-1} = kg Mg^{-1}.
2. Ethanol from glucan (mL g^{-1}) (or hexosan sugar) = [(180 g of glucose/162 g of glucan) × (0.51 g ethanol/g glucose)]/0.789 g ethanol per mL [21]. For equation 5 below, note that $((180/162) \times 0.51) = 0.57$.
3. Ethanol from xylan (or arabinan) (mL g^{-1}) = [(150 g xylose/132 g of xylan) × 0.51 g ethanol/g xylose)]/0.789 g ethanol per ml [21].

The trait and associated units listed in Table 1 can be inserted into the equations below. Sugars were expressed on an anhydrous basis. Actual ethanol yield via SSF was calculated using the ETOH result. Because the yeast that was used to ferment the released sugars cannot ferment pentose sugars, ETOH was only from reduced hexoses; 1 ha=10,000 m^2.

4. Ethanol yield from SSF released glucose from biomass:

$$\text{ETOHL}\left(\text{L Mg}^{-1}\right) = \text{ETOH} \times 1.267.$$

5. Theoretical ethanol yield from all biomass hexoses:

$$\begin{aligned}\text{HEXEL (L Mg}^{-1}) = (((&\text{MAN} + \text{GAL} + \text{GLC} + \text{STA}) \times 0.57)\\ &+((\text{GLCS} + \text{FRU}) \times 0.51) + (\text{SUC} \times 0.537))\\ &\times 1.267);\ \text{assuming 100\% conversion.}\end{aligned}$$

6. Ethanol yield from SSF released pentose sugars:

$$\text{PENTEL}\ (\text{L Mg}^{-1}) = \text{PENT} \times 0.51 \times 1.267 \times 0.8;\ \text{assuming 80\% conversion.}$$

7. Theoretical ethanol yield from pentose sugars:

$$\text{PENTETL}\ \left(\text{L Mg}^{-1}\right) = (\text{ARA} + \text{XYL}) \times 0.579 \times 1.267.$$

8. Total ethanol yield (ETOHTL) from SSF:

$$\text{ETOHTL}\ \left(\text{L Mg}^{-1}\right) = \text{ETOHL} + \text{PENTEL}.$$

9. Total ethanol production per ha from SSF:

$$\text{ETOHTLH}\ \left(\text{L ha}^{-1}\right) = \text{ETOHTL} \times \text{biomass production}\ \left(\text{Mg ha}^{-1}\right).$$

10. Total theoretical ethanol yield from all biomass sugars:

$$\text{ETOHTLT}\ \left(\text{L Mg}^{-1}\right) = \text{HEXEL} + \text{PENTETL}.$$

11. Total theoretical ethanol production from all biomass sugars:

$$\text{ETOHTLTH}\ \left(\text{L ha}^{-1}\right) = \text{ETOHTLT} \times \text{biomass production yield}\ \left(\text{Mg ha}^{-1}\right).$$

12. Conversion ratio of actual to theoretical ethanol on a liter-to-liter basis.

$$\text{ERATIO} = \text{ETOHTL}/\text{ETOHTLT}.$$

To convert to units used in production agriculture in the USA, the following conversion factors can be used: L ethanol $\text{ha}^{-1}/9.35$=gallons acre^{-1}; L Mg^{-1} ethanol × 0.24 = gallons *U*.S ton^{-1}; 1 ha=2.47 acres.

Results and Discussion

Switchgrass NIRS Calibrations

The calibration set used to develop the NIRS method represented a diverse population as evidenced by the large range in compositional properties (Table 2). Notable examples of properties with wide values include Klason lignin (92–256 mg g^{-1}), cell-wall associated glucans (190–320 mg g^{-1}), and xylan (129.75–238.36 mg g^{-1}). These components represent 62.3% of the average switchgrass biomass. Glucose and xylose content are particularly important because only carbohydrates can be biochemically converted into ethanol. Solubles sugars (e.g., glucose, fructose, and sucrose) were also significant and on average comprised 15% of the hexose sugars present in switchgrass. Most other major cell wall and soluble carbohydrates also exhibited significant variation. Exceptions included the more minor cell wall components, fucose (FUC) and rhamnose. Most samples did not have detectable levels of fucose. Variation in total carbon (C) was much smaller than for specific carbohydrates, illustrating that while total C variation is limited, plants display plasticity in the specific form it takes.

Differences in the partitioning of C between carbohydrates and lignin directly affect the theoretical ethanol yield, which is solely dependent upon total carbohydrates. The theoretical ethanol yield can be calculated for all biomass hexoses, pentoses, and all biomass sugars (see Eqs. 5, 7, and 10) from the carbohydrate composition. Likewise, the partitioning of C among different carbohydrates can affect the expected ethanol yield depending upon how efficiently the carbohydrates can be extracted (soluble vs. structural) and fermented (hexoses vs. pentoses). In this study, the hexoses were directly fermented to ETOH using a *S. cerevisiae* yeast strain selected for fermentation of cellulosic feedstocks. However, because *Saccharomyces* yeast does not ferment pentoses, xylose was measured directly in the spent fermentation broth. Approximately, a twofold difference was observed between low and high yielding switchgrass samples for ETOH (60.83–127.34 g mg^{-1}) and pentose (PENT, 116.61 to 255.37 g mg^{-1}) yields. Non-structural sugars (e.g., soluble and storage carbohydrates) are readily extractable and fermented in comparison to those associated with the cell wall. The contribution of ETOH from the non-structural carbohydrates (NSCE) was subtracted from total ETOH to give cell wall associated ethanol (CWE). NSCE was calculated by using an estimated 90% conversion efficiency for glucose and starch yeast fermentations. The CWE had a significantly lower mean value (66.88 g mg^{-1}) than ETOH (91.86 g mg^{-1}), indicating the significant affect of the more easily converted soluble and storage carbohydrates. Ethanol yield is affected by both the amount of carbohydrate present and the efficiency of their extraction and (for hexoses) conversion into ethanol. Conversion efficiency was directly measured by calculating the ratio of the ethanol or pentose yields and the theoretical yields. Pentoses were extracted by the dilute-acid pretreatment with 78.13% efficiency. By contract, the conversion efficiency of hexoses into ethanol was 50.2% and we estimate only 47.0% conversion efficiency for cell wall hexoses which are primarily from cellulose. The lower efficiencies for glucans compared to pentoses reflect in part the yield lost to ethanol production by

Table 2 Mean, standard deviation, and range values for switchgrass samples in the bioenergy NIRS calibration data set

Composition variables[a]	Units	Mean	Standard deviation	Minimum	Maximum
DM	mg g^{-1}	905	9	858	929
C	mg g^{-1}	438.7	6.4	417.2	450.1
N	mg g^{-1}	9.5	4.8	2.0	22.8
EE	mg g^{-1}	10.2	3.9	2.0	23.0
ASH	mg g^{-1}	76.9	15.7	1.1	118.3
KL	mg g^{-1}	166.1	32.6	92.0	256.3
UA	mg g^{-1}	15.8	1.8	12.03	23.1
RHA	mg g^{-1}	1.4	0.7	0.4	3.7
FUC	mg g^{-1}	0.0	0.1	0.00	0.5
ARA	mg g^{-1}	30.0	2.6	24.5	36.8
XYL	mg g^{-1}	190.8	22.9	129.8	238.4
MAN	mg g^{-1}	6.6	3.2	2.7	23.6
GAL	mg g^{-1}	9.5	1.5	4.25	14.7
GLC	mg g^{-1}	265.7	26.7	190.4	319.8
PCA	mg g^{-1}	5.76	1.6	2.2	11.0
FEST	mg g^{-1}	1.4	0.5	0.4	3.0
FETH	mg g^{-1}	0.8	0.6	0.0	2.4
CWC	mg g^{-1}	693.4	60.4	543.8	816.4
AXMG	mg g^{-1}	234.7	31.7	178.2	287.2
AX	mg g^{-1}	220.7	23.6	158.2	272.4
SUC	mg g^{-1}	27.5	13.7	2.08	54.8
GLCS	mg g^{-1}	8.3	4.9	0.3	21.4
FRU	mg g^{-1}	10.0	6.7	0.0	34.9
SC	mg g^{-1}	45.8	20.9	8.1	99.5
STA	mg g^{-1}	7.5	8.0	0.00	47.5
NSC	mg g^{-1}	54.8	26.2	10.2	119.7
HEX	mg g^{-1}	366.8	26.6	302.0	430.2
SUG	mg g^{-1}	617.8	47.1	507.1	703.5
PSOL	%	15	7	3	32
PPEN	%	41	2	34	46
Ethanol and potential ethanol					
ETOH	mg g^{-1}	91.96	14.9	60.8	127.3
PENT	mg g^{-1}	196.4	27.7	116.6	255.4
HEXE	mg g^{-1}	184.4	12.2	154.0	221.1
NSCE	mg g^{-1}	25.2	2.12	4.7	55.0
CWE	mg g^{-1}	66.9	10.3	38.7	90.7
CWEP	%	47.0	9.4	25.3	73.3
PENTP	%	78.1	6.4	58.2	93.1
HEXEP	%	50.2	9.05	33.3	73.6
Forage quality composition					
NDF	mg g^{-1}	712.7	44.0	587.7	807.9
ADF	mg g^{-1}	381.8	45.2	269.3	482.0
ADL	mg g^{-1}	54.6	12.9	10.9	84.34
IVDMD	mg g^{-1}	495.3	81.3	290.0	714.8
CP	mg g^{-1}	59.4	30.0	12.2	142.8

[a] Definitions for all abbreviations are listed in Table 1.

Table 3 Summary of switchgrass NIRS calibration statistics for bioenergy trait characterization sample set

Abbreviation	Equation type	Mean gkg^{-1}	*N*	SEC gkg^{-1}	r^2	SECV gkg^{-1}	SEP gkg^{-1}	RPD
			Biomass bioenergy variables					
C	Combined[a]	439.18	104	2.14	0.86	4.17		
	Cal & Val.[b]	439.02	88	3.65	0.62	5.07	4.49	1.42
N	Combined	9.46	107	0.42	0.99	0.61		
	Cal & Val.	9.20	89	0.52	0.99	0.63	0.72	6.66
EE	Combined.	10.26	111	1.82	0.78	2.80		
	Cal & Val.	10.08	94	2.19	0.69	3.46	3.30	1.18
ASH	Combined	77.45	107	5.54	0.84	8.60		
	Cal & Val.	76.40	87	10.13	0.35	10.50	8.89	1.77
KL	Combined	165.21	109	10.56	0.89	20.63		
	Cal & Val.	168.45	92	10.34	0.89	22.26	25.61	1.27
UA	Combined	15.73	108	0.75	0.76	0.87		
	Cal & Val.	15.67	91	0.73	0.74	0.88	1.03	1.74
RHA	Combined	1.44	107	0.24	0.86	0.27		
	Cal & Val.	1.45	89	0.26	0.83	0.31	0.24	2.91
FUC	Combined	0.10	12	0.01	0.96	0.07		
	Cal & Val.	0.10	9	0.03	0.60	0.06	0.08	1.25
ARA	Combined	29.85	109	1.03	0.85	1.32		
	Cal & Val.	29.99	92	1.02	0.85	1.33	1.31	1.98
XYL	Combined	191.00	110	6.00	0.93	8.41		
	Cal & Val.	192.24	93	6.65	0.91	9.18	8.45	2.71
MAN	Combined	6.53	105	0.47	0.98	0.87		
	Cal & Val.	6.65	88	0.51	0.97	1.02	1.02	3.13
GAL	Combined	9.55	107	0.38	0.93	0.61		
	Cal & Val.	9.54	91	0.30	0.95	0.64	1.23	1.21
GLC	Combined	265.90	107	5.70	0.95	7.82		
	Cal & Val.	267.45	89	6.12	0.95	8.11	11.61	2.3
PCA	Combined	5.66	108	0.36	0.95	0.49		
	Cal & Val.	5.69	90	0.36	0.95	0.51	0.73	2.19
FEST	Combined	1.42	110	0.16	0.91	0.21		
	Cal & Val.	1.41	91	0.15	0.92	0.22	0.27	1.85
FETH	Combined	0.82	98	0.24	0.78	0.38		
	Cal & Val.	0.78	82	0.24	0.74	0.37	0.54	1.11
CWC	Combined	693.60	110	17.90	0.91	28.98		
	Cal & Val.	698.87	89	12.70	0.95	25.42	37.56	1.6
AXMG	Combined	237.18	109	6.32	0.92	8.76		
	Cal & Val.	238.52	92	6.69	0.91	9.38	11.05	2.87
AX	Combined	220.95	109	6.62	0.92	9.04		
	Cal & Val.	222.29	92	6.05	0.93	9.00	10.53	2.24
SUC	Combined	27.53	106	3.59	0.93	5.50		
	Cal & Val.	26.55	89	3.77	0.92	5.59	6.00	2.28
GLCS	Combined	8.14	108	1.71	0.87	2.25		
	Cal & Val.	8.15	93	2.05	0.80	2.69	3.19	1.53
FRU	Combined	10.13	108	2.02	0.91	2.77		
	Cal & Val.	10.30	91	2.05	0.91	3.03	3.21	2.08
SC	Combined	45.93	109	4.72	0.95	7.23		
	Cal & Val.	45.48	89	4.41	0.95	7.64	7.01[d]	2.98
STA	Combined	7.08	106	1.52	0.95	2.79		

Table 3 (continued)

Abbreviation	Equation type	Mean gkg^{-1}	*N*	SEC gkg^{-1}	r^2	SECV gkg^{-1}	SEP gkg^{-1}	RPD
	Cal & Val.	6.50	88	1.60	0.91	2.76	4.51	1.77
ETOH	Combined	91.91	102	3.50	0.94	4.96		
	Cal & Val.	91.62	86	3.98	0.92	5.68	6.48	2.3
PENT	Combined	196.22	104	6.47	0.95	10.40		
	Cal & Val.	199.14	86	7.41	0.92	10.59	12.35	2.24
NSC	Combined	54.17	101	3.31	0.98	5.05		
	Cal & Val.	54.57	84	4.45	0.97	6.18	6.97	3.76
HEX	Combined	367.14	103	7.36	0.92	10.33		
	Cal & Val.	367.69	86	7.65	0.90	10.39	15.88	1.67
SUG	Combined	618.56	102	11.36	0.94	13.92		
	Cal & Val.	621.04	85	12.54	0.92	14.53	28.40	1.65
PSOL	Combined	0.15	101	0.01	0.98	0.01		
	Cal & Val.	0.15	86	0.01	0.97	0.02	0.02	350
PPEN	Combined	0.41	103	0.01	0.94	0.01		
	Cal & Val.	0.41	86	0.01	0.87	0.01	0.01	200
HEXE	Combined	184.71	103	4.15	0.88	6.11		
	Cal & Val.	185.31	88	4.75	0.83	6.55	10.26	1.18
NSCE	Combined	25.51	107	1.80	0.98	2.58		
	Cal & Val.	25.28	91	1.89	0.97	3.14	2.68	0.79
CWE	Combined	66.18	105	4.73	0.79	6.17		
	Cal & Val.	66.18	87	4.81	0.76	6.27	5.91	1.74
CWEP	Combined	46.59	102	3.12	0.89	4.08		
	Cal & Val.	46.56	84	2.99	0.87	3.67	4.04	2.32
PENTP	Combined	78.13	105	3.55	0.69	4.10		
	Cal & Val.	78.41	86	3.52	0.64	4.06	4.37	1.46
HEXEP	Combined	50.38	104	2.59	0.91	3.40		
	Cal & Val.	50.11	86	2.60	0.91	3.27	3.18	2.84
		Forage quality traits						
IVDMD	Combined	494.76	109	12.45	0.98	22.68		
	Cal & Val	491.16	94	17.06	0.95	27.18	28.35	2.86
NDF	Combined	713.59	109	11.98	0.93	16.42		
	Cal & Val.	715.31	92	12.11	0.93	18.31	22.21	1.98
ADF	Combined	382.76	108	9.71	0.95	12.79		
	Cal & Val.	382.73	94	8.95	0.96	14.46	13.12	3.44
ADL	Combined	55.56	109	4.65	0.84	6.20		
	Cal & Val.	55.55	92	5.18	0.79	6.61	10.65	1.21
DM	Combined	906.09	103	3.33	0.77	4.07		
	Cal & Val.	905.64	89	3.09	0.82	4.16	5.74	1.56

SEC standard error of calibration, *RSQ* coefficient of determination, *SECV* standard error of cross validation, and *SEP* standard error of prediction, *RPD* ratio of SD/SEP

[a] The upper row (combined) for each variable contains the revised calibration equation on which both calibration and validation samples were included to improve the robustness of the prediction equations

[b] The lower row for each variable contains the calibration statistics and validations statistics (Cal & Val) for the calibration set of samples from which the validation set of samples were removed for use as validation samples

Table 4 Means and range values for biomass yield, forage composition, and energy yield traits of switchgrass cultivars and selected experimental strains from a biomass yield test grown in eastern Nebraska in 2002–2005

Entry	Traits								
	Yield, forage quality, lignin								
	Yield	IVDMD	NDF	ADF	ADL	N	EE	ASH	KL
	Mg ha^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}
Summer	13.0	506	731	403	55	9	9.9	65.6	157.2
Trailblazer	14.0	514	732	395	53	10	11.9	68.6	165.6
Shawnee	14.6	530	715	388	53	10	11.1	65.9	149.9
Kanlow	18.4	529	737	407	50	9	9.2	58.4	133.1
NE 2229	15.0	538	719	388	51	10	10.8	65.1	151.3
NE 2234	16.6	525	726	391	50	10	11.4	69.1	154.6
Mean	14.7	523	728	396	52	10	10.9	67.2	154.0
F	7.9*	5.38**	6.3*	4.6*	2.2*	3.9*	8.9*	7.8*	10.1*
LSD 0.05	0.8	8.2	5.6	5.5	1.7	0.5	0.5	2.6	4.7
Range min	11.7	506	709	384	50	9	9.2	58.4	133.1
Max	18.4	558	741	407	55	11	12.4	75.6	165.6
	Cell wall carbohydrates, total C								
	FUC	ARA	XYL	MAN	GAL	GLC	UA	RHA	C
	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}
Summer	0.1	29.9	208.2	4.9	8.5	281.9	15.9	1.2	445.4
Trailblazer	0.1	30.7	206.7	6.1	8.9	280.7	16.4	1.3	445.5
Shawnee	0.1	29.3	198.2	5.6	8.7	269.4	16.3	1.2	444.6
Kanlow	0.2	29.5	206.1	5.4	8.7	299.3	16.1	1.0	444.6
NE 2229	0.1	30.7	205.7	5.2	8.8	280.2	16.6	1.3	443.9
NE 2234	0.1	31.0	207.2	5.5	9.0	280.9	16.6	1.3	444.3
Mean	0.12	30.3	205.6	5.5	8.8	282.1	16.3	1.2	444.5
F	10.6*	7.8*	8.9	5.2*	3.8*	16.0*	5.4*	14.7*	4.4*
LSD 0.05	0.01	0.5	2.0	0.3	0.2	2.8	0.2	0.04	0.8
Range min	0.01	28.0	193.1	4.9	8.4	268.2	15.9	1.0	441.2
Max	0.02	31.4	208.9	6.5	9.3	299.3	16.8	1.4	445.8
	Other biomass constituents								
	PCA	FEST	FETH	SUC	GLCS	FRU	STA		
	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}		
Summer	6.31	1.46	1.16	27.4	8.1	6.1	7.3		
Trailblazer	5.45	1.30	1.11	21.6	6.3	5.4	6.7		
Shawnee	5.87	1.43	0.95	31.4	9.6	8.9	9.4		
Kanlow	8.83	2.53	0.76	27.1	6.3	6.4	3.0		
NE 2229	5.64	1.39	1.07	27.2	8.2	8.5	7.6		
NE 2234	5.64	1.43	1.09	22.1	6.5	6.3	4.8		
Mean	6.1	1.5	1.0	24.3	6.9	6.4	6.1		
F	76.9*	77.6*	8.2*	17.6*	6.9*	10.9*	8.1*		
LSD 0.05	0.2	0.1	0.1	1.5	0.7	0.6	1.0		
Range min	5.2	1.2	0.8	19.5	6.2	4.4	2.4		
Max	8.8	2.5	1.2	33.2	8.1	8.9	9.4		
	Biomass major components								
	CWC	AXMG	AX	SC	NSC	HEX	SUG	PSOL	PPEN
	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}
Summer	716.7	251.5	238.0	41.6	48.9	375.0	645.5	13.0	41.9

Table 4 (continued)

Entry	Traits								
Trailblazer	724.4	252.4	237.4	33.3	40.0	366.5	636.3	10.9	42.4
Shawnee	687.1	241.9	227.5	49.8	59.2	372.6	631.2	15.9	41.0
Kanlow	711.4	249.7	235.6	39.7	42.7	388.7	656.4	11.0	40.8
NE 2229	708.0	250.5	236.5	44.0	51.6	376.5	645.2	13.7	41.6
NE 2234	714.4	252.7	238.2	35.0	39.8	366.0	636.7	10.8	42.5
Mean	712.6	250.1	235.9	37.6	43.7	370.9	639.0	11.7	42.0
F	9.8*	10.1*	10.0*	14.5*	12.4*	8.6*	6.6*	14.2*	13.1*
LSD 0.05	6.7	2.2	2.1	2.4	3.1	3.7	4.9	0.8	0.3
Range min	669.1	234.6	221.1	29.7	34.6	363.9	622.5	9.4	40.4
Max	725.8	254.8	239.9	51.1	60.2	388.7	656.4	16.2	42.7
	Saccrification and fermentation products								
	ETOH	PENT	HEXE	NSCE	CWE	CWEP	PENTP	HEXEP	
	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	%	%	%	
Summer	82.9	210.1	190.3	23.5	59.4	39.3	77.7	43.7	
Trailblazer	81.8	209.4	186.1	19.2	62.5	41.3	77.6	44.0	
Shawnee	87.9	199.2	188.0	28.5	59.5	40.9	77.1	46.8	
Kanlow	80.4	197.3	199.6	20.4	60.0	37.4	73.7	40.4	
NE 2229	90.5	207.1	190.9	24.8	65.7	43.6	77.1	47.4	
NE 2234	83.9	207.2	186.8	19.1	64.8	42.9	76.5	45.0	
Mean	82.9	205.1	188.7	21.0	61.9	40.8	76.6	44.0	
F	13.7*	10.4*	14.8*	12.3*	8.3*	10.8*	8.1*	18.2*	
LSD 0.05	1.6	2.6	1.7	1.5	1.3	0.9	0.8	0.8	
Range min	77.5	192.5	184.6	16.6	57.7	37.4	72.9	40.4	
Max	90.5	210.9	199.6	28.9	65.7	43.6	78.6	48.0	
	Ethanol yield and theoretical yields per Mg and hectare								
	ETOHL	HEXEL	PENTEL	ETOHTL	ETOHTLH	PENTETL	ETOHTLT	ETOHTLTH	E-Ratio
	L Mg^{-1}	L Mg^{-1}	L Mg^{-1}	L Mg^{-1}	L ha^{-1}	L Mg^{-1}	L Mg^{-1}	L ha^{-1}	
Summer	105.1	246.4	108.6	213.7	2,762.8	174.6	421.0	5,479.5	50.8
Trailblazer	103.6	240.6	108.2	211.8	2,960.4	174.2	414.8	5,790.1	51.1
Shawnee	111.4	245.0	102.9	214.4	3,133.2	166.9	411.9	6,037.0	52.1
Kanlow	101.8	255.0	102.0	203.8	3,719.8	172.8	427.8	7,887.9	47.7
NE 2229	114.6	247.3	107.1	221.7	3,317.6	173.5	420.8	6,330.1	52.7
NE 2234	106.3	240.2	107.1	213.4	3,521.8	174.7	414.9	6,874.4	51.5
Mean	105	243.65	106.0	211.1	3,091.2	173.0	416.6	6,128.6	50.7
F	13.7*	8.2*	10.4*	9.6*	5.9*	10.0*	6.3*	8.6*	20.0
LSD 0.05	2.0	2.5	1.4	2.7	183.9	1.6	3.2	353.5	0.6
Range min	98.1	239.0	99.5	203.5	2,533.8	162.2	406.3	4,877.9	47.7
Max	114.6	255.0	109.0	221.7	3,719.8	176.0	427.8	7,887.9	52.7

See Table 1 for abbreviation definitions

** p=0.01, indicate significance

the yeast but primarily the more recalcitrant nature of the cellulose polymer vs. hemicelluloses.

Good-to-excellent prediction equations were obtained for most traits as indicated by the high r^2 and low SEC, SEP, and SECV values for the best predictive equations that were developed for each trait (Table 3). Initially, a subset of samples (approximately 20) were removed from the sample set to serve as validation samples. The remaining samples were used to develop the calibrations which were validated using the validation subset (see "Cal & Val" row for each

trait in Table 3). With these initial calibrations, the r^2 values were only less than 0.80 for ASH, EE, C, UA, FUC, FETH, CWE, pentose extraction efficiency (PENTP), and ADL. ASH is typically difficult to predict with NIRS. Based on our experience using NIRS for forage quality traits, it is more difficult to obtain r^2 values greater than 0.8 if the calibration set has limited variation for the calibration analyte, for analytes that are measured with low precision such as ADL, and for complex traits that include a biochemical reaction step in the analysis procedure such as IVDMD. As discussed previously, there was limited variation for total C in switchgrass biomass for which the range was 858–939 mg g^{-1}. CWE and PENTP are complex traits because of the SSF process. The SEP values were acceptable for most bioenergy traits, but exceeded 1.5% of the SEC by a limited extent for some traits (Table 3). To improve the accuracy of the final prediction equations, the validation samples were included in the calibration set and calibration equations were redeveloped using the same math treatments and options as in the original best calibrations (see rows "Combined" in Table 3). After including these additional samples, r^2 values improved to be greater than 0.8 for all traits except EE, UA, FETH, and CWE (all with r^2>0.76) and PENTP (r^2=0.69). For the major sugars, PCA, FEST, and SSF products (ETOH and PENT) the r^2 values were greater than 0.90. Combining the calibration and validations samples into a single combined calibration set also resulted in reduced SEC and SECV values for most traits. Another measure of the utility of calibrations is the ratio (RPD) of the standard deviation of the calibration samples (SD) to the standard error of prediction [36]. The RPD values greater than 2 indicate that the SEP is less than 0.5 the SD of the reference samples. The RPD ratios were typically greater than 2.2 for all major cell wall sugars, soluble sugars, ETOH, and PENT. The calibration results demonstrate that acceptable NIRS calibrations can be obtained for switchgrass biomass composition and SSF ethanol and soluble pentose yield.

Switchgrass Strain Differences for Actual and Theoretical Ethanol Yields

There were significant differences among the four check cultivars and experimental strains in the biomass yield trail for all traits in the strain evaluation trial including actual and theoretical ethanol yield per megagram and production per hectare (Table 4). The lowland cultivar, Kanlow had the highest biomass yields per hectare but its biomass also had the lowest SSF conversion efficiency. Ethanol yield from SSF (ETOHL) ranged from 98 to 115 L Mg^{-1}. Potential ethanol yield from released pentoses, assuming 80% conversion efficiency, ranged from 100 to 109 L Mg^{-1}. ETOHTL from

Table 5 Means and standard deviations (in parentheses) and correlations between calculated and NIRS predicted complex switchgrass biomass composition traits

Trait	Means (SD)		*r*
	Calculated from component values	NIRS-predicted values	
	g kg^{-1}	g kg^{-1}	
CWC	712.6 (22.16)	703.0 (22.7)	0.88**
AXMG	250.1 (9.8)	252.2 (9.7)	0.99**
AX	235.9 (10.1)	237.7 (7.7)	0.84**
SC	37.6 (10.7)	40.5 (11.5)	0.97**
NSC4	43.7 (13.0)	43.1 (14.4)	0.96**
HEX	370.9 (13.7)	374.6 (15.0)	0.95**
SUG	639.0 (14.5)	640.2 (15.6)	0.82**
HEXE	188.7 (7.3)	187.6 (7.0)	0.92**
NSCE	21.0 (6.3)	19.7 (7.0)	0.96**
CWE	61.9 (5.7)	64.1 (4.5)	0.77**
	%	%	
PSOL	11.7 (3.3)	11.7 (3.5)	0.99**
PPEN	41.9 (1.6)	42.1 (1.6)	0.93**
CWEP	40.9 (4.6)	40.4 (4.04)	0.95**
PENTP	76.6 (3.3)	76.8 (2.3)	0.88**
HEXEP	44.0 (4.6)	43.8 (4.3)	0.95**

N=378

** p=0.01, indicate significance

both glucose and released pentoses ranged from 204 to 222 L Mg^{-1}. An experimental upland strain (NE 2229) that was bred for improved forage yield and IVDMD had the largest ETOHL and ETOHTL values. With the assay that was used in this study, NE 2,229 produced over 12 L more ethanol per megagram from SSF glucose than the cultivar Kanlow.

Because of the differences in biomass yield per hectare and actual and theoretical ethanol yield per megagram among the cultivars and experimental strains, there were very large differences in ethanol production per hectare (Table 4). The range in SSF ethanol production (ETOHTLH) from the hexoses was 2,533–3,719 L ha^{-1} while the range in total theoretical ethanol production from all potential biomass sugars ranged from 4,877 to 7,887 L ha^{-1}. These large differences in ethanol production per hectare illustrate the need to optimize both biomass yield and conversion yield from biomass (L Mg^{-1}) in switchgrass production systems. None of the cultivars and strains in this trial was bred for improved SSF ethanol yield, but several including Shawnee and NE 2229 were bred for improved forage digestibility which had a positive effect on SSF ethanol yield. The results demonstrate that genetic differences exist among switchgrass for conversion efficiency and indicate that a focused breeding effort should result in additional improvements in conversion efficiency.

Plant breeders and agronomists need to analyze a large number of biomass samples to develop improved plants and management practices that result in increased ethanol yields and production per hectare. In the ARS switchgrass breeding programs at Lincoln and Madison, WI, 5,000–10,000 samples are generated per year per location for which composition and bioenergy conversion potential data is needed to efficiently bred for improved conversion efficiency. If done with conventional laboratory analyses, the wet laboratory work alone for the data listed in Table 4 for the switchgrass biomass yield test would have an approximate cost of over $110,000 (approximately $ 300 per sample × 21 entries × 6 replicates × 3 years) without including equipment costs. NIRS analysis costs including equipment and technical support would be about $5 per sample for a total of approximately $2,000 for this experiment. Good NIRS calibrations provide the capacity to conduct such bioenergy analyses economically and rapidly. Composition and conversion efficiency data obtained from NIRS analyses of biomass samples also will enable researchers to identify which biomass components have the largest effect on conversion efficiency and to determine which are the most amenable to genetic or cultural practice modification.

Biomass composition traits such as cell wall concentration, total sugars, or hexose extraction efficiency listed in Table 4 were determined by calculation using NIRS predicted constituents of these traits and the equations listed in Table 1. It is also feasible to develop NIRS calibrations for these calculated traits (Table 3). For the samples from the switchgrass yield test, we determined the concentration of these multi-constituent traits by both calculations from NIRS-predicted component data and direct NIRS prediction of the composite traits. The calculated and direct NIRS predicted means and standard errors of these complex biomass components were very similar and were highly correlated (Table 5). This comparison demonstrates the accuracy and precision of the NIRS calibrations that were developed for switchgrass biomass composition and also the power of NIRS technology to predict complex feedstock traits. As biomass conversion technology is developed, it should be possible to develop NIRS calibrations for biorefinery products and co-products.

Summary

NIRS calibrations were developed for switchgrass biomass that can be used to accurately estimate over 20 biomass components including cell wall and soluble sugars, and ethanol and released pentose sugars from a laboratory SSF procedure. With this information, it is feasible to calculate an addition 13 complex feedstock traits including theoretical ethanol yield from hexoses and SSF hexose ethanol conversion efficiency. Using this NIRS-derived biomass data, we demonstrated that switchgrass cultivars and experimental strains adapted to the Midwest USA differ significantly for all biomass composition traits analyzed, and actual and theoretical ethanol yield per megagram and per hectare. These calibrations and their improved future versions can be used in all aspects of switchgrass research including basic genetics, breeding, production, harvest, and storage. It should be feasible to develop switchgrass cultivars with improved ethanol yields per megagram and production per hectare. With good NIRS calibrations, it should be possible for switchgrass biomass feedstock to be marketed and utilized on the basis of its ethanol yield potential.

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