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Biomass Characterization of Morphological Portions of Alamo Switchgrass

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Supporting Information

ABSTRACT: Comparative studies between the leaf and internode portions of switchgrass, *Panicum virgatum* L., were performed by compositional analysis and structural determination. GC-MS, ICP, and HPAEC-PAD were employed to analyze the chemical compositions of the fractionated switchgrass samples. Quantitative ^{13}C NMR and CP/MAS ^{13}C NMR techniques were employed to determine the structures of lignin and cellulose, respectively. These results indicated that the leaves and internodes differed chemically in the amounts of inorganic elements, hot-water extractives, benzene/ethanol extractives, carbohydrates, and lignin content. However, the ultrastructure of isolated cellulose was comparable between leaves and internodes. Ball-milled lignins isolated from leaves and internodes were found to have H/G/S ratios of 12.4/53.9/33.7 and 8.6/54.8/36.6, respectively.

KEYWORDS: morphological switchgrass, inorganic elements, extractives, NMR, cellulose, lignin

INTRODUCTION

Switchgrass, a warm-season perennial C-4 grass, has been intensively studied as a potential bioenergy crop in the United States for the past decade.[1] It is a desirable lignocellulosic feedstock for biofuel production because of several features, including a high production yield reported up to 14 tonne/acre, wide adaptation, positive environmental benefits, and a renewable root system.[1,2] There are two distinct ecotypes of switchgrass with various populations including lowland (e.g., Alamo and Kanlow) and upland varieties (e.g., Trailblazer, Blackwell, Cave-in-Rock, Pathfinder, and Caddo).[1] Morphologically, switchgrass includes a root system up to 3.5 m in length, stems made up of internodes, nodes, leaf sheaths up to 3 m height, leaves, and flowers.[3]

The chemical constituents of switchgrass have been reported to vary according to population, growth stage, and morphological portion sampled.[3−6] For instance, elemental analysis of Cave-in-Rock populations by Lemusa et al.[5] reported the lowest amounts of Cl, Mg, K, and Na, with other elements being comparable to those found in Alamo and Kanlow. Typically, switchgrass has three growth stages including vegetative, boot, and heading stages.[7] Jung et al. stated that the chemical constituents of switchgrass varied among the harvest period and morphological portions (leaves, stems including internodes, and leaf sheath).[7] A recent investigation by Sarath et al. also found variations in the chemical constituents, especially the lignin component, along the length of tillers of switchgrass.[6] Studies on four populations of switchgrass, Alamo, Kanlow, GA992, and GA993 (derived from Alamo and Kanlow), reported only a 2% variation on bulk lignin content.[8] On the other hand, the leaf, internode, and node portions of switchgrass were shown to vary with respect to the contents of carbohydrates, lignin, ash, and extractives as well as S/G ratio.[8] Extractives from switchgrass have been reported to consist of minerals, low molecular weight and oligomeric compounds, which were Soxhlet extracted from biomass using water and neutral organic solvents.[9,10] Many studies have shown that the quantities and composition of switchgrass extractives vary extensively depending upon the origin of samples, the process of their preparations, and the solvents used.[8−11] The content of extractives has also been shown to vary in morphological portions.[8,11] Low et al. reported that the extractives content of Cave-in-Rock switchgrass varied in the leaves, stem, and seedhead for hot-water and benzene/ethanol extractions.[11] Hu et al. also reported differences in the extractives content of hot water and benzene/ethanol in leaf, internode, and node portions of four populations of switchgrass.[8]

Potential applications of switchgrass have been documented in the literature, including pilot-scale cofiring with coal for biopower production,[12] syngas production,[13] and bioethanol production.[2] Future improvements in the applications of switchgrass for energy, chemicals, and liquid fuels will require a detailed knowledge of its chemical and physical properties.[14] It has been amply reported that biomass provides a sustainable, environmentally friendly means to produce biopower.[12,15] The combustion properties of biomass are significantly correlated to the C/H/O ratios of biomass.[12,15] In addition, biopower generation from herbaceous plants, including switchgrass, is known to be influenced by the presence of alkali metals contributing to the potential generation of sulfates, silicate, chlorides, and hydroxides, which can cause slogging and fouling problems during combustion.[15] Some of these process issues can be reduced by aqueous leaching of biomass to remove alkali metals from biomass.[15]

Another promising utilization of lignocellulosic feedstocks is the production of bioethanol. Practical conversion of lignocellulosic biomass into bioethanol via the biological approach requires

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a pretreatment step to reduce the recalcitrance of biomass to aid enzymatic deconstruction of cellulose into glucose and subsequent fermentation to ethanol.[16] Recent studies on pretreatment and saccharification of lignocellulosics indicate that the degree of polymerization (DP) and ultrastructure of cellulose are among the important factors that influence efficient enzymatic deconstruction of cellulose.[17−19] For instance, Samuel et al. investigated the ultrastructure changes of cellulose after dilute acid pretreatment of switchgrass using CP/MAS ^{13}C NMR.[19] These results indicated that dilute acid pretreatment reduced the percentage of amorphous cellulose and increased the crystallinity index of cellulose. During organosolv pretreatment and enzymatic deconstruction of *Buddleja davidii*, Hallac et al. monitored changes in plant cell walls and noted significant changes in the structure of cellulose.[18] These results suggested that organosolv pretreatment improved the yield of enzymatic hydrolysis through removal of lignin and hemicellulose and a reduction in the DP and crystallinity of cellulose.[18]

Alamo is a variant of lowland switchgrass that originated from Texas[1] with production yields of up to 14 tonne/acre and lower lignin content in comparison to other lowland types of switchgrass.[1,8] In the present study, the chemical structure of morphological portions of Alamo switchgrass was determined by fractionating the plant into sections and studying the plant cell wall chemistry in detail.

MATERIALS AND METHODS

Chemicals. All chemicals were purchased from VWR (Atlanta, GA) and used as received.

Sample Preparation. Alamo switchgrass was harvested 4 cm above ground on September 2009 by the Department of Plant Science at the University of Tennessee. The growing condition of this plant has been documented in the literature.[3] Once harvested, the switchgrass samples were air-dried, yielding a moisture content of 14.8%. Four morphological portions, leaves (including blade and sheath), internodes, nodes, and seedhead of switchgrass, were manually separated and ground with a Wiley mill to pass through a 0.841 mm screen. Leaf and internode portions of switchgrass were then additionally sieved to three fractions based on the particle size: <0.297, 0.297−0.707, and >0.707 mm.

Extractive Analysis. Switchgrass leaf and internode samples (∼2.00−3.00 g oven dry <0.71 mm) were Soxhlet extracted with water followed by benzene/ethanol (2:1, v/v) for 8 h each, using 6−10 cycles/h. The extractives content was determined gravimetrically according to the literature procedure.[14] The extracted biomass was air-dried for 1 day followed by vacuum oven-drying at 40 °C overnight. Standard deviation for the extractives content determinations was typically ≤1%.

The extractives were subsequently analyzed by gas chromatography−mass spectrometry (GC-MS). The extractives solution was collected and concentrated to 30.00 mL at 40 °C. The condensed extractives solution was then diluted to 100.00 mL. A sample of the aqueous solution (approximately 0.3 g) was weighed into an 8 × 40 mm autosample vial and dried in a centrivap concentrator equipped with a Centrivap cold trap (Labconco). A sample of the benzene/ethanol solution (1.00 mL) was evaporated to dryness under a stream of nitrogen at room temperature for 30 min. Heptadecanoic acid as an internal standard (1.00 mL, 4.0 mg/mL in methanol) was added to each sample vial and further evaporated to dryness under a stream of nitrogen. *N*-Methyl-*N*-*tert*-butyldimethylsilyltrifluoroacetamide (50.0 μL) was added as a derivatizing agent, and this mixture (1.0 μL) was injected into a GC-MS system using a HP 5890 II gas chromatograph equipped with Hewlett-Packard 5971A mass selective detector. The column used was a 6000 mm × 0.251 mm i.d., 0.25 μm, DB-5MS. GC-MS was operated under the following conditions: initial temperature, 150 °C; initial time, 10 min; ramp rate, 20 °C/min; final temperature, 280 °C; holding time, 33.5 min; injection temperature, 250 °C. The total ion peak area was used for quantification of individual compounds. The response factor for each individual compound was assumed as 1 for the calculations. Standard deviation for the determination of extractives compounds was typically ≤5%.

Sugar and Lignin Content Analyses. Sugar profiles and lignin content of the switchgrass samples were determined according to literature methods.[8] All results represent the mass percentages of original biomass. Standard deviation for sugars and lignin was ≤1.8%.

Ash Content and Trace Inorganic Elements Analyses. The ash content of switchgrass samples was measured by heating the sample at 525 °C for 3 h in a furnace. Acid-insoluble ash content was measured according to TAPPI method T 244.[20] ICP analysis was performed.[14] Total halogen analysis was performed by Huffman Laboratories, Inc., Golden, CO. Standard deviations were ≤0.5% for ash and acid-insoluble ash and ≤0.0022% for total halogen content.

Heating Value of Combustion. The higher heating value (HHV) of switchgrass samples was measured by combustion conducted in an adiabatic oxygen bomb calorimeter according to the literature.[14] Standard deviation for HHV was 0.2 MJ/kg.

Cellulose Isolation and Characterization. α-Cellulose and acid-hydrolyzed cellulose from switchgrass were used for molecular weight and structural NMR characterization, respectively. Both samples were prepared using holocellulose pulp prepared from milled switchgrass samples (0.297−0.707 mm) using a literature procedure.[14] The α-cellulose preparation was carried out using 17.5% NaOH solution to extract hemicellulose from holocellulose following literature methods.[21] The degree of polymerization (DP) of α-cellulose was determined by gel permeation chromatography (GPC) after tricarbanylation of α-cellulose.[14] In brief, α-cellulose (15.00 mg) was dried under vacuum at 40 °C for 24 h. Tricarbanylation of the dried α-cellulose was then performed using anhydrous pyridine (4.00 mL) and phenyl isocyanate (0.5 mL) at 70 °C for 48 h. Upon completion of the reaction, the mixture was quenched with methanol (1.00 mL) and precipitated in a methanol/water solution (7:3, v/v, 100.0 mL) followed by filtration through a membrane filter (pore size = 0.45 μm). It was then washed using a methanol/water solution (7:3, v/v, 30.00 mL × 3), followed by DI water (30.00 mL × 3). Tricarbanylated α-cellulose was air-dried for 24 h and then vacuum-dried at 40 °C for 24 h.

The molecular weight of the tricarbanylated α-cellulose solution was determined following a published procedure.[22] In brief, the tricarbanylated α-cellulose was dissolved in THF (1.00 mg/mL), filtered through a 0.45 μm filter, and injected into a GPC SECurity Agilent HPLC 1200 (a PSS-Polymer Standards Service, Warwick, RI). The columns used were four 300 mm × 7.8 mm i.d. Waters Styragel columns (HR1, HR2, HR4, HR6). An Agilent UV detector was used at 270 nm. Tetrahydrofuran was used as the mobile phase (1.00 mL/min). Data collection and processing were achieved using WinGPC Unity software (Build 6807). Molecular weight values (M_n and M_w) were determined using a calibration curve based on 6 narrow polystyrene standards ranging in molecular weight from 1.5×10^3 to 3.6×10^6 g/mol. The weight-average degree of polymerization (DP_w) was calculated by dividing the weight-average molecular weight of tricarbanilated α-cellulose (M_w) by 519. Standard deviations for GPC analysis of cellulose were determined to be 3.1×10^4 and 2.68×10^4 g/mol, and 60 for M_n, M_w, and DP, respectively.

The ultrastructure of cellulose was characterized by ^{13}C CP/MAS NMR and line fitting of the spectroscopic data as described by Pu et al.[17] The pure cellulose used for NMR characterization was isolated by refluxing a holocellulose sample (0.50 g of dry weight) in an HCl solution (50.00 mL, 2.5 M) for 4 h. NMR analysis was performed using a

Table 1. Morphological Portions and Extractives Content of Alamo Switchgrass

		extractives	
morphological portion	percentage	hot water (%)	benzene/ethanol after hot water (%)
leaf blade	23.1	18.5	4.2
leaf sheath	13.3		
internode	45.4	12.4	1.6
node	5.0	1.8	4.9
seedhead	13.2	24.3	0.8

Bruker Avance DMX-400 spectrometer operating at 100.59 MHz for ^{13}C nuclei. The isolated cellulose sample (~60% moisture) was packed in a 4 mm cylindrical ceramic MAS rotor and spun at 10 kHz. The CP/MAS NMR experiment was conducted using a 5 μs (90°) proton pulse, 1.5 ms contact pulse, 3 s recycle delay, and 8 k scans. NUTS NMR data processing software (Acorn NMR, Inc., Livermore, CA) was used for line fitting of the C-4 region of the cellulose spectra (79−92 ppm) (see the Supporting Information). The crystallinity index was determined by the integration ratio of the C-4 crystalline region of cellulose (δ 86−92) to the complete C-4 region of cellulose (δ 79−92).[19] Standard deviation associated with this measurement was ≤2.7%.

Lignin Isolation and Structural Characterization. Isolation of lignin from the leaf and internode portions of Alamo switchgrass was accomplished following a literature procedure with minor modification.[9] In brief, extracted switchgrass (20.00 g od leaves (<0.3 mm) or internodes (<0.3 mm)) samples were dried under vacuum at 40 °C for 24 h and milled in a 4 L porcelain jar with 1000.00 g of porcelain balls under N_2. The ball-milled switchgrass powder was then dried under vacuum at 40 °C for 24 h and extracted with a *p*-dioxane/water solution (96%, v/v, and 200 mL/20 g milled powder) for 24 h twice. The suspension of *p*-dioxane/water extract was combined after centrifugation at 1156*g* relative centrifuge field (RCF) for 10 min. The collected solids were freeze-dried to yield a crude lignin sample, which was dissolved with an acetic acid/water solution (9:1, v/v, 20 mL/g lignin), centrifuged, precipitated into water, and recovered after centrifugation at 1156*g* RCF for 10 min. The lignin was washed with water (200.00 mL × 2), freeze-dried, and then vacuum-dried at 40 °C for 24 h. This material was then dissolved in dichloroethane/ethanol (2:1, v/v and 10 mL/1.0 g lignin) and centrifuged to remove insolubles, and the solution was precipitated with the addition of diethyl ether (200.00 mL/20.0 mL solution). The precipitate was isolated by centrifugation and washed with diethyl ether followed by petroleum ether. The purified lignin was redissolved in an aqueous *p*-dioxane solution (50%, v/v) and freeze-dried to acquire the final lignin sample.

Lignin structure analysis was carried out using quantitative ^{13}C NMR recorded on a 400 MHz Bruker Avance/DMX NMR spectrometer using DMSO-d_6 as the solvent. The data were acquired with a 90° pulse, 11 s pulse delay at 50 °C, and 10240 scans. Manual phasing and baseline correction were performed on each spectrum.[9] Some contaminates have also been observed in the spectra of leaf and internode lignin, such as residue dioxane (δ 66.7), which could not be removed with extended purification.

RESULTS AND DISCUSSION

Chemical Compositions of Switchgrass. Biomass characterization of switchgrass is an important component in the efficient utilization and conversion of switchgrass into chemicals, fuels, and energy. Populations of switchgrass including Alamo have been studied for the chemical constituents in their morphological portions.[5,8] Previous characterization studies have shown that 20 switchgrass populations were comparable in their bulk chemical constituents among lowland and upland switchgrass samples.[5] The average contents of acid detergent lignin, cellulose, and hemicellulose were 6.3, 37.1, and 32.1% for these 20 switchgrass populations, respectively. Recent studies on four populations of switchgrass demonstrated that the morphological portions of switchgrass (leaves, internodes, and nodes) differed in cellulose content, lignin and extractives content, and syringyl/guaiacyl ratio.[8] In the present study, the morphological portions including leaves, internodes, nodes, and seedhead of Alamo switchgrass were prepared to study their chemical compositions.

The gravimetric percentages of these fractions are shown in Table 1. The mass percentages of these fractions included 36.4% leaves (23.1% for blade and 13.3% for sheath), 45.4% internodes, 5.0% nodes, and 13.2% seedhead. The gravimetric ratio of leaves to internodes was 0.80. These results showed that the percentage of leaf portion was 32.9% lower than the previous study on four populations of switchgrass (Table 1).The morphological portions of Alamo switchgrass, in the present study, were shown to have significant differences in extractives content. Leaves and internodes contained 22.7 and 14.0% extractives, respectively, which were 16.0 and 7.3% greater than the node portion and 2.4 and 11.1% lower than the seedhead portion. Similar results have also been reported recently for hot-water and benzene/ethanol extractives content on other lowland and upland switchgrass varieties.[8,10,11]

The chemical compounds, which were identified by GC-MS analysis in the extractives solution, were different in quality and quantity between leaves and internodes (Table 2).

In general, switchgrass extractives can be classified as aromatic compounds, carboxylic acid, sugars, alkanes, fatty acids, alcohols, and sterols.[9,10] To simplify subsequent analysis, the leaf and internode portions, which represent the major mass (81.8%) of the whole plant of switchgrass, were selected for further characterization. Table 2 shows the extractive compounds from hot-water and benzene/ethanol extractions of leaves and internodes from switchgrass. Several biologically active compounds were found in the extractives of the present study. For example, α-tocopherol, which has antioxidant properties,[23] is present in switchgrass leaves at a value of 85 (μg/g biomass) in the benzene/ethanol fraction. Sterols, which have broad medicinal applications,[24] were also observed in the benzene/ethanol extractives from the leaves portion (679 μg/g biomass). These biologically active compounds can be of interest as value-added products for future applications. The experimental results (Table 2) indicated that the internode portion has 12097 (μg/g biomass) more hot-water extractives but 3063 (μg/g biomass) less benzene/ethanol extractives than the leaf portion. The hot-water extractives from leaves and internodes of switchgrass were found to have several different chemical constituents. Ribose, fructose, xylose, sucrose, malic acid, and palmitic acid were detected in hot-water extractives of the internodes but not the leaves. The leaf hot-water extractives were found to have quinic acid and galactofuranose, whereas these compounds were not detected in the internode hot-water extractives. In addition, the benzene/ethanol leaf extractives had more extractives compounds detected by GC-MS analysis than the corresponding internodes extractives. The leaf benzene/ethanol extractives were shown to have glucose, α-tocopherol, monoglycerides, stigmasterol, and various carboxylic acid when compared to the corresponding leaf extractives.

Table 2. Hot-Water and Benzene/Ethanol Extractive Compounds of Leaf and Internode Portions of Alamo Switchgrass by GC-MS

	retention time (min)	leaves (μg/g biomass)	internodes (μg/g biomass)
hot-water extractive compounds			
malic acid/quinic acid	9.13/14.11	ND[a]/138	688/ND
C16:COOH/C18:COOH	15.94/17.92	ND/151	34/356
D-ribose/D-fructose	12.23/13.57	ND/ND	524/1608
galactofuranose/galactose	13.48/14.45	219/163	ND/ND
glucofuranose/glucosepyranose/glucose	13.90/14.53/15.28	111/ND/368	403/1911/1698
D-xylose/sucrose	15.62/24.49	ND/ND	404/5621
total detected (μg/g biomass) (hot-water extractives)		1150	13247
benzene/ethanol extractives compounds			
quinic acid/linolenic acid	14.11/17.73	48/384	ND/31
p-hydroxycinnamic acid/9,12-octadecadienoic acid	14.88/17.65	32/152	10/51
C12:COOH/C14:COOH	11.22/13.66	27/94	3/ND
C16:COOH/C18:COOH	15.85/17.92	332/81	ND/14
C20:COOH/C22:COOH	20.26/23.26	116/40	ND/ND
C23:COOH/C24:COOH	25.21/27.57	21/94	ND/ND
C25:COOH/C26:COOH	30.45/34.03	23/73	ND/ND
C27:COOH/C28:COOH	37.66/40.94	35/170	ND/ND
C30:COOH	48.89	276	ND
arabinose/D-ribose	10.75/11.34	ND/94	30/3
xylose/mannose	12.00/14.30	ND/ND	17/13
glucosepyranose/glucose	14.45/15.28	24/42/	ND/ND
cellotriose	24.07	ND	11
maltose/inositol	24.82/14.02	ND/ND	15/6
C24:OH/C32:OH	32.0/53.95	44/54	11/ND
α-tocopherol	38.62	85	ND
haptacosane/nonacosane	24.39/29.29	70/94	ND/ND
nonadecane/monopalmitglyceride	36.54/22.46	39/45	ND/ND
monooctadecanateglyceride	25.80, 26.05	71	ND
cholesterol/stigmasterol	39.30/44.11	85/230	17/ND
β-sitosterol/unidentified sterol	46.44/50.00	212/152	45/ND
total (μg/g biomass) (benzene/ethanol extractives)		3339	276

[a] ND, nondetectable.

As discussed previously, C, H, and O elements of biomass are strongly correlated with the higher heating value (HHV) of combustion for switchgrass,[12] whereas inorganic compounds detrimentally affect the HHV of biomass. For instance, a 1% increase of ash content results in 0.2 MJ/kg reduction of HHV.[25] For bioenergy and biopower application, it is essential to determine the mineral inorganic compound content and the HHV of switchgrass.

The leaf and internode portions of Alamo switchgrass were measured in terms of ash content, acid-insoluble ash content, and HHV as summarized in Table 3. These results indicated that the leaf portion of switchgrass has 39000 (mg/kg biomass) more ash content and 20000 (mg/kg biomass) more acid-insoluble ash content, and its HHV is 0.7 MJ/kg less than the internode portions of switchgrass. The HHV of native leaf and internode portions of switchgrass was similar to that of the stem portion of switchgrass (18.8 $\pm$ 0.2 MJ/kg).[8] HHV increased for postextracted leaves and internodes, presumably because of the removal of sugars and ash. This phenomenon was the reverse of previous studies on the extractives effect on HHV of softwood and hardwood,[14] which has a lower HHV after extraction. The trace inorganic contents for the leaf and internode portions of switchgrass were analyzed, and these data are summarized in Table 3. The results indicated that the leaf portion has significantly greater amounts of Ca, Mg, S, Si, and Mn than the internode portion. The total halogen content was 1068 mg/kg greater in the leaf portion than in the internode portion. After hot-water and benzene/ethanol extraction, there was a significant decrease in K, Mg, P, Mn, Na, and total halogen elements contents, whereas most other elements did not change significantly. This facile reduction in some inorganic elements provides an interesting opportunity to reduce the ash content of switchgrass.

Biomass composition of Alamo switchgrass was performed for the analyses of carbohydrate and lignin contents. The effect of particle size, morphological portions, and extraction on the chemical composition analysis was also investigated. The results showed that the composition of Alamo switchgrass varied from morphological portions: leaves, internodes, nodes, and seedhead. This analysis also showed the compositional analysis also varied slightly by particle size and extraction process (Table 4). In brief, the chemical composition of the leaf portions has much greater variation than the internode portion. Compared to the chemical composition of leaves, the composition of the internodes was only slightly affected by the particle size. In brief, the leaf portion

was significantly different from the internodes. These results were slightly different from the previous findings on four populations of switchgrass.[8] The leaf portion was found to have comparable glucan content and 4.4% less xylan content than four populations of switchgrass, whereas the internode portion had 2.7 and 2.4% greater glucan and lignin contents, respectively. The node fraction was found to have 6.9, 4.6, and 4.1% more glucan, xylan, and lignin contents, respectively. Differences in growing locale/season and age of harvesting of the switchgrass could contribute to these differences in part.[3,6,7]

Table 3. Mineral Inorganic Compounds, Ash Content, Acid-Insoluble Ash Content, and HHV of Leaves and Internodes of Alamo Switchgrass

ICP element	leaves (mg/kg)	leaves-extracted (mg/kg)	internodes (mg/kg)	internodes-extracted (mg/kg)
K	9550.0	25.7	6497.1	12.9
Ca	3715.0	4840.0	463.4	244.0
Mg	2635.0	284.8	443.4	91.3
P	2185.0	151.3	1299.9	49.6
S	1030.2	720.2	330.2	191.2
Si	616.5	550.3	221.3	247.0
Mn	188.1	54.8	51.7	16.0
Na	90.2	16.9	147.9	16.7
Fe	53.6	70.7	15.2	11.6
Zn	27.9	21.6	11.5	4.3
Cu	19.0	16.4	8.7	4.6
Al	13.0	19.3	1.6	2.2
Ba	8.2	8.6	5.1	3.0
Sr	7.6	8.5	2.0	1.1
As	<5.5	<5.9	<5.3	<5.7
Pb	<3.5	<3.7	<3.4	<3.6
Sn	<2.8	<3.1	<3.4	<3.1
B	2.6	1.4	0.2	0.2
Ni	2.2	1.1	1.1	0.8
Cr	0.6	0.6	0.3	0.3
Ti	0.4	0.6	0.1	0.2
Co	<0.4	<0.5	<0.4	<0.4
Cd	<0.3	<0.3	<0.3	<0.3
total detected (mg/kg)	20157.6	6806.3	9513.5	910.1
total halogen (mg/kg)	1665	12	597	10
ash	71000	41000	32000	16000
acid-insoluble ash	27000	ND[a]	700	ND
HHV (MJ/kg)	18.6	19.1	19.3	19.7

[a] ND, not determined.

Structure Characterization of Switchgrass Cellulose. The ultrastructure of cellulose is heterogeneous, made up of crystalline cellulose (I_α and I_β), para-crystalline cellulose, and cellulose at accessible and inaccessible surfaces.[26] These polymorphs can vary significantly in relative properties according to the sample origin. In the case of highly ordered cellulose originating from *Valonia*, para-crystalline and amorphous cellulose were reported in lower amounts than those typically reported for wood and cotton.[26] Pu et al. monitored the structural changes of kraft pulp cellulose during cellulase hydrolysis and demonstrated that cellulose I_α, para-crystalline, and amorphous regions of cellulose were more susceptible to cellulase deconstruction than cellulose I_β using solid state ^{13}C CP/MAS NMR experiment.[17] These results suggested that characterization of the structure of cellulose is an important consideration for bioethanol production.

A pure cellulose sample was prepared from the switchgrass using holocellulose pulping followed by a mild acid treatment to remove hemicelluloses. ^{13}C CP/MAS NMR spectroscopy was used for the ultrastructure characterization of cellulose (Figure 1). The most informative region is the C-4 region of cellulose at 79−92 ppm. Using nonlinear least-squares fitting of the ^{13}C CP/MAS NMR spectra, the relative amounts of cellulose I_α, cellulose I_β, para-crystalline cellulose, celluloses at accessible and inaccessible surfaces, and cellulose crystallinity index were determined. The assignments and relative proportion values are shown in Table 5 and suggested cellulose from leaves and internodes were similar in cellulose ultrastructure.

Solid state NMR for the leaf and internode cellulose showed 30% para-crystalline cellulose and 34% inaccessible fibril surface on average. The crystallinity index of switchgrass for leaves and internodes are similar, with an average value of 51%, which is comparable to a recent report[19] for bulk crystallinity index of Alamo switchgrass (44%).

Tricarbanylated α-cellulose prepared from switchgrass was used to determine the degree of polymerization by GPC, and these results are summarized in Table 6. Celluloses from leaves and internodes have similar weight-average molecular weight (M_w) values of 151.9×10^4 and 152.4×10^4 g/mol for leaves and internodes, respectively. The calculated DPs of cellulose were comparable, 2926 and 2935, for leaves and internodes, respectively.

Structure Characterization of Switchgrass Lignin. The collected spectra of ball-milled lignin from leaves and internodes are shown in Figure 2. Quantitative ^{13}C NMR spectroscopic data

Table 4. Chemical Compositions of Leaves and Internodes of Alamo Switchgrass

sample	particle size (mm)	Ara (%)	Gal (%)	Glu (%)	Xyl (%)	KL (%)	ASL[a] (%)
leaves	<0.71	2.9	1.5	30.7	15.2	19.5	3.5
internodes	<0.71	1.7	0.7	42.6	20.7	20.2	1.8
nodes	<0.71	2.8	1.0	40.5	26.8	24.7	2.1
seedhead	<0.71	2.9	1.5	36.6	19.8	23.3	4.1
leaves, extracted	<0.71	4.3	1.5	39.7	23.9	16.4	3.2
internodes, extracted	<0.71	2.0	0.6	47.2	25.5	21.1	1.6
nodes. extracted	<0.71	2.4	0.8	34.6	22.5	25.0	2.3
seedhead, extracted	<0.71	3.2	1.0	40.2	26.4	21.3	2.7
leaves, extracted	0.71−0.30	4.1	1.4	41.2	25.6	16.8	2.3
leaves, extracted	<0.30	4.0	1.5	35.0	18.7	17.9	2.1
internodes, extracted	0.71−0.30	2.1	0.6	48.0	26.1	21.8	1.5
internodes, extracted	<0.30	2.3	0.8	47.6	26.2	22.7	1.5

[a] Acid-insoluble fraction in cellulose.

analysis was carried out by integrating the signal intensity between 162.0 and 103 ppm and setting this value to six aromatic carbons after subtracting the integration value for the two vinyl carbons of ferulate and *p*-coumarate.[9]

Lignin structure assignments were accomplished according to recent studies as summarized in Table 7.[4,9] The methoxy group content was estimated on the basis of the relative integration range of 58−54 ppm. The results gave values of 0.95 and 0.99 per aromatic ring for leaf and internode lignins, respectively. These results were comparable to the recent investigation on the structure of lignin in switchgrass.[9] The integration of the acetyl methyl group signal (21−19 ppm) provided values of 0.18 and 0.19 per aromatic ring for the leaf and internode portions of Alamo switchgrass. An unconjugated-ester signal was observed at 175−168 ppm[9,27] and shown to be 0.48 and 0.40 per aromatic ring for leaves and internodes, respectively.

It has been suggested that the possible origin of the acetyl group in the spectra of isolated lignin is from acetylated xylan or lignin.[28] The result of sugar analysis indicated that isolated lignin contained 1.6% arabinose, 0.1% galactose, 0.9% glucose, 14.0% xylose, and 80.2% lignin. From the spectra, the C-1 xylan signal can be clearly assigned at 101.6 ppm.[29] The integration value for C1 of xylan was estimated on the relative integration range of 103−101 ppm. The results provided the values of 17.0 and 16.0 xylose units per 100 aromatic ring for leaf and internode lignins, respectively.

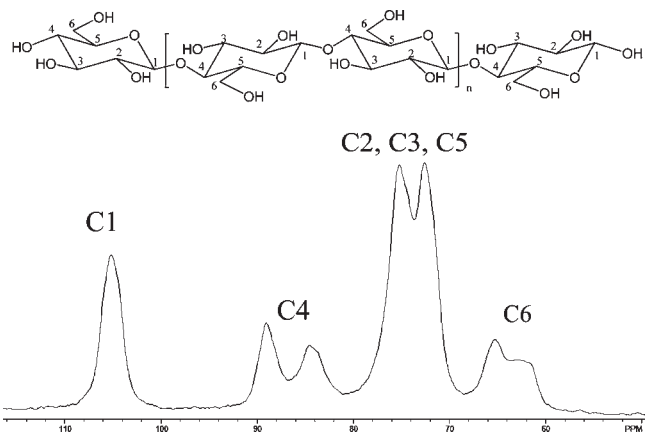

Figure 1. CP/MAS ^{13}C NMR spectrum of leaf cellulose of Alamo switchgrass.

The most valuable information obtained from the quantitative analysis of the ^{13}C NMR spectra from leaf and internode lignins was the relative amount of basic precursors present in the leaf and internode lignins. Lignin has been defined as a cross-linked complex polymer synthesized mainly through dehydrogenative polymerization of *p*-coumaryl alcohol (H), coniferyl alcohol (G), and sinapyl alcohol (S).[4] Studies on the lignin structure of C-4 perennial grasses have shown that *p*-coumaric and ferulic acid were also incorporated into lignin through ester or ether interlinkages.[9]

Table 7 shows that the NMR signals at 162−158 ppm were assigned for the C-4 carbon of *p*-coumaric acid (0.18 and 0.21 per aromatic ring for leaves and internodes).[9] The signal at 168−164 ppm was assigned for C-γ carbon of *p*-coumaric and ferulic acid (0.23 and 0.24 per aromatic ring for leaves and internodes).[9] These results suggested that leaf and internode lignins have similar amounts of *p*-coumaric and ferulic acid linked to the isolated lignin. The amount of ferulic acid can be calculated by subtraction of an integration value at 162−158 ppm from 168 to 164 ppm.[30] These results suggested that leaf and internode lignins have 0.05 and 0.03 per aromatic ring of ferulic acid, respectively. Compared to the recent study on the structure of lignin isolated from stem portion of four populations of switchgrass, the present structure of lignin was comparable in the amount of *p*-coumaric acid on average, but slightly greater in the amount of ferulic acid (0.02 per aromatic ring on average).[9] Another study on the structure of lignin by ^{13}C NMR indicated that dioxane lignin isolated from leaf sheath of a banana plant contained *p*-coumarate and ferulate, 0.07 and 0.05 per aromatic

Table 6. Molecular Weights of Cellulose and Lignin Isolated from Leaf and Internode Portions of Alamo Switchgrass

sample	M_w	M_n	DP_w
leaf cellulose[a]	151.87×10^4	15.41×10^4	2926
internode cellulose[a]	152.35×10^4	12.42×10^4	2935
leaf lignin[b]	5920	2299	
internode lignin[b]	4376	1848	

[a] Standard derivation for M_w 3.1×10^4g/mol, for M_n 2.68×10^4g/mol, and for DP_w 60. [b] Standard derivation for M_w 23 g/mol and M_n 17 g/mol.

Table 5. Assignments of Signals in the C-4 Region of the CP/MAS ^{13}C NMR Spectra of Isolated Cellulose from Leaves and Internodes of Alamo Switchgrass

				relative integrated intensity (%)	
assignment	chemical shift (ppm)	fwhh[a] (Hz)	line type	leaves	internodes
cellulose I_α	89.7	90	Lorentz	2	1
cellulose $I_{\alpha+\beta}$	89.0	91	Lorentz	12	12
para-crystalline cellulose	88.8	241	Gauss	30	29
cellulose I_β	88.1	135	Lorentz	3	3
accessible fibril surface	84.5	100	Gauss	9	12
inaccessible fibril surface	84.4	400	Gauss	36	33
accessible fibril surface	83.6	95	Gauss	8	9
crystallinity index (%)				51	50

[a] fwhh, full width at half-height.

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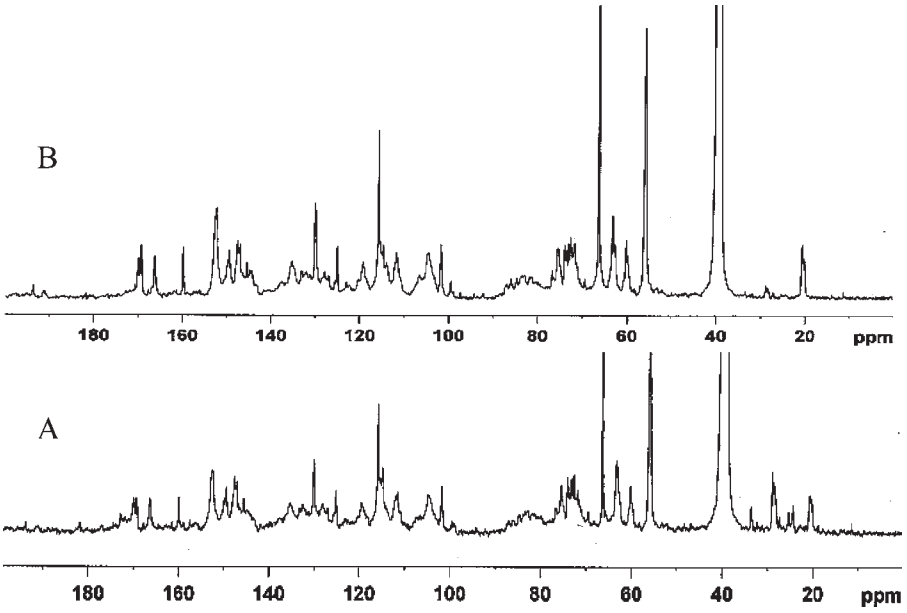

Figure 2. Quantitative ^{13}C NMR spectroscopy of leaf (A) and internode (B) lignins of Alamo switchgrass.

Table 7. Assignments and Integration Value of Quantitative ^{13}C NMR Spectra of Leaf and Internode Lignins

integration range	assignment	internode (/Ar)	leaf (/Ar)
195−193	Ar—CH═CH—CHO[14]	0.03	0.02
193−191	guaiacyl or syringyl benzaldehyde[27]	0.04	0.02
175−168	unconjugated COOR[27]	0.40	0.48
168−164	conjugated COOR[31]	0.24	0.23
162−158	C_4 *p*-coumaric acid[9,30]	0.21	0.18
158−156	C_4 H unit[27]	0.08	0.12
156−151	C_3 in 5−5′ ET, C_3/C_5 in S unit[27]	0.68	0.59
123−117	C_6 in G unit[14]	0.51	0.52
117−114	C_5 in G unit, C_3/C_5 in *p*-coumaric acid, C_5 in ferulic acid, β-carbon in *p*-coumaric and ferulic acid[4,9,14]	0.81	0.82
114−108	C_2 in G unit[14]	0.46	0.47
108−103	C_2/C_6 in S unit[14,27]	0.68	0.65
103−101	C1 in xylose[29]	0.16	0.17
90−78	α-CH in $\beta-\beta'$ and β-5, β-CH in β-*O*-4, C2/C5 in xylose[14,27]	0.70	0.77
61−58	C_γ in β-*O*-4 (G or S) without C_α═O[14]	0.32	0.30
58−54	methoxy[14,27]	0.99	0.95
21−19	CH_3 in acetyl group[28]	0.19	0.18
S/G ratio	$(I^a_{108-103}/2)/I_{114-108}$[14]	0.74	0.69

[a] *I*, integration value.

ring, respectively.[30] The amount of guaiacyl units for leaf and internode lignins can be calculated from the integration value at 123−117 ppm by subtracting the integration value for ferulic acid. This result suggested that leaf and internode lignins have 0.52 and 0.51 per aromatic ring of guaiacyl units respectively (G unit). The amount of *p*-hydroxyphenyl unit (H unit) was calculated using the integration value at 158−156 ppm. It was found to be 0.12 and 0.08 per aromatic ring for leaf and internode lignins, respectively. The amount of syringyl unit (S unit) was calculated from half the integration value at 108−103 ppm. The values were 0.32 and 0.34 per aromatic ring for leaf and internode lignins, but these values are tentative given the presence of the C-1 xylan signal. Given these results, the relative values of *p*-hydroxyphenyl/guaiacyl/syringyl unit (H/G/S) were calculated as 12.4/53.9/33.7 and 8.6/54.8/36.6 for leaf and internode lignins, respectively. The observed NMR S/G ratio including ferulic acid was 0.69 and 0.74 for leaf and internode lignins, respectively.

The major interlinkages of switchgrass, β-*O*-4, $\beta-\beta'$, β-5′, and ester interlinkages, have been observed in a previous study.[9] According to the assignments and integration values presented in Table 7, the relative amount of the major interlinkage, β-*O*-4 moieties, was calculated for lignin in leaf and internode portions of Alamo switchgrass. The result indicated that these interlinkages in switchgrass lignin were comparable (0.32/Ar and 0.30/Ar) for leaf and internode lignins.

The molecular weights of the acetylated ball-milled leaf and internode lignins, each containing polysacchardies, were measured by GPC. The results in Table 6 indicated that the molecular weight M_w of acetylated leaves ball-milled lignin was 35.3% greater than that of acetylated internode sample (5919.7 vs 4375.6 g/mol). A recent report on the molecular weight of ball-milled lignin from a bulk switchgrass sample was 5000 g/mol.[4] The difference in M_w of acetylated lignin can be influenced by the presence of a greater amount of xylan content in the isolated leaf and internode lignins. These results also suggested that the ball-milled lignins from leaves and internodes are comparable with the exception of molecular weight of their derivatized form in the present investigation.

Although the basic chemical constituents, such as extractives, mineral inorganic elements, carbohydrates, and lignin contents, were different between leaves and internodes of Alamo switchgrass, the chemical structures of the major components, cellulose and lignin, were comparable. These heterogeneous features[6] in morphological portions of switchgrass can provide potential benefits for future biofuel/biopower application.

■ ASSOCIATED CONTENT

Supporting Information. Cluster integration value of quantitative ^{13}C NMR spectra of leaf and internode lignins and spectral fitting for the C-4 region of the spectrum of leaves cellulose of Alamo switchgrass. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ DISCLOSURE

This work is part of the first author's requirements for the degree of Ph.D. at the Georgia Institute of Technology.

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■ ABBREVIATION USED

H/G/S, *p*-hydroxyphenyl/guaiacyl/syringyl unit; ICP, inductively coupled plasma; HPAEC-PAD, high-performance anion exchange chromatography with pulsed amperometric detection; CrI, crystallinity index; CP/MAS, cross-polarization/magic angle spinning; Ara, arabinan; Gal, galactan; Glu, glucan; Xyl, xylan; KL, Klason lignin; AIL, acid-insoluble lignin.

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