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Chemical profiles of switchgrass

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ABSTRACT

Chemical analysis studies were conducted for four populations of switchgrass (Alamo, Kanlow, GA993, and GA992), *Panicum virgatum* L., which were partitioned into leaves, internodes, and nodes. The variations in carbohydrate compositions, lignin and extractives content, higher heating value (HHV), and the syringyl:guaiacyl ratio of switchgrass were determined. The experimental results indicated that bulk chemical profiles for the four populations of switchgrass were comparable. However, the results from three morphological components of switchgrass, leaves, internodes and nodes, provided a significant diversity among the analytical results studied.

1. Introduction

In light of insufficient long-term supply of petroleum resources, increased global populations, and global climate change, society has begun to develop sustainable fuels, energy and chemicals from renewable bioresources (Ragauskas et al., 2006). The US federal government has proposed "the 20 in 10 Plan" to reduce 20% gasoline consumption by 2017, which requires more than sevenfold increase in alternative fuels production (Twenty In Ten, 2007). In addition, the production of 79.5 billion liters cellulosic bioethanol is required by the Renewable Fuel Standard (RFS) by 2022 (US CRS Report, 2009). These future demands of cellulosic biofuels will rely on cellulosic bioresources such as forests, perennial grasses, wood and agricultural residuals (Antizar-Ladislao and Turrion-Gomez, 2008; Galbe and Zacchi, 2007; Pu et al., 2008). A promising feedstock for these biofuel requirements is switchgrass which is a native warm-season, C4 perennial grass with high production yield and a wide geographical adaption in Centre and North America (Bouton, 2007; Mclaughlin and Kszos, 2005).

One of the key technologies currently required in the production of cellulosic biofuels is pretreatment which is needed so as to increase enzyme digestibility of biomass. Pretreatment technologies reduce recalcitrance by removing lignin, hemicelluloses, and lignin–carbohydrate complexes, modifying cellulose crystallinity and the morphology of the cell wall (Galbe and Zacchi, 2007). Understanding the physical and chemical properties of switchgrass is essential for optimizing pretreatment technologies for this bioresource. In the present study, the chemical and physical properties of four switchgrass samples (i.e., Alamo, Kanlow, GA993, and GA992) and their morphological components (leaves, internodes, and nodes) were studied. Their impacts on conversion technologies for biofuels are also discussed.

2. Methods

2.1. Chemicals

All chemicals were purchased from VWR and used as received.

2.2. Samples

Four populations of switchgrass, Alamo, Kanlow, GA992 and GA993, were seeded in 2000 at the University of Georgia plant sciences farm near Watkinsville, GA (33°52′N; 83°32′W) USA, on coarse sandy loam (fine, kaolinitic, thermic typic kanhapludults). GA993 is a population selected from Alamo. GA992 is another population selected from Alamo and Kanlow. The plants were fertilized by applying 56 kg ha^{-1} N each spring until 2005, harvested and removed from the plots each autumn. No harvest was taken in 2006,

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but in February 2007, the residual stems were clipped 20 mm above ground level and left on the field. In 2007, the regular harvest method was applied to the switchgrass. In August 2008, two replicates (R3 and R6) of these four populations of switchgrass were harvested. Once harvested, the switchgrass samples were air-dried until the moisture content was <10% of dry weight. The leaves (including blade and sheath), stem (or internodes), and nodes portions of switchgrass were manually separated and ground in Wiley mill to pass a 5 mm screen. Samples were then dried in a vacuum desiccator over P_2O_5 for 3 days to yield a final 5% moisture content.

2.3. Monosaccharide and lignin content analysis

Switchgrass samples (160–170 mg, OD) were hydrolyzed with 72% H_2SO_4 (1.5 ml) for 1 h at 30 °C. The hydrolysates were diluted to 4% H_2SO_4 with DI water and a second hydrolysis was carried out at 121 °C setting for 1 h in an autoclave. The hydrolysis solution was quickly cooled to room temperature and filtered through a porcelain crucible and the residue was used to determine Klason lignin content. The acid soluble lignin content was determined by UV absorption of filtrate at 205 nm (Dence, 1992). Hence, the lignin contents reported were the sum of Klason and acid soluble lignin content. The filtrate was analyzed by Dionex chromatography, high performance anion exchange chromatography with pulsed amperometric detection (HPAEC–PAD) for monosaccharide analysis (Pronto, 1998).

2.4. Extractives analysis

Extractives content was measured according to TAPPI method T204 om-85 (TAPPI Test Methods, 1992–1993a). Extractives content of switchgrass samples was based on the weight difference between the oven-dried original and extracted samples. Extractives content analyzed in the present study was accomplished using successive extraction with hot-water and benzene/alcohol.

2.5. Ash analysis

The ash content of the extracted switchgrass samples was analyzed according to TAPPI procedure T211 om-85 (TAPPI Test Methods, 1992–1993b).

2.6. Heating value of combustion

The higher heating value (HHV) for the switchgrass samples was measured in an adiabatic oxygen bomb calorimeter according to TAPPI method T684 om-06 (TAPPI Test Methods, 2006).

2.7. Pyrolysis molecular-beam mass spectrometry

The ground switchgrass samples were analyzed at National Renewable Energy Laboratory (Golden, CO) for their syringyl:guaiacyl ratios by pyrolysis molecular-beam mass spectrometry (Py-MBMS) (Evan and Milne, 1987; Sykes et al., 2008). The Py-MBMS analysis employs a quartz tube (2.5 cm inside diameter) pyrolysis furnace coupled with a custom-built pyrolysis-molecular-beam mass spectrometer using an Extrel Model TQMS C50 mass spectrometer for pyrolysis vapor analysis. The ground samples (~20 mg) were pyrolyzed at 500 °C temperature and 5 l/min helium flow into the mass spectrometer. The molecular beam was achieved through the first vacuum stage of 10^{-3} torr and a second vacuum stage of 10^{-5} torr and collimated with the proper placement of a slit into a quadrupole mass spectrometer. The molecular beam formed intercepts a low-energy electron beam (22.5 eV) into a quadrupole mass spectrometer, yielding a positive ion mass spectrum. Mass spectra obtained were averaged and background was removed on a Merlin automation data system version 2.0. A typical Py-MBMS spectrum can be found in Agblevor's article (Agblevor et al., 1994). Syringyl to guaiacyl (S:G ratio) were estimated by summing the syringyl peaks intensity (154, 167, 168, 182, 194, 208, 210) divided by guaiacyl peaks intensity (124, 137, 138, 150, 164, 178) (Sykes et al., 2008). The mass peak assignment associated with Py-MBMS spectrometry in the present study is summarized in Table 1.

Table 1
Mass spectrum peak assignments associated with Py-MBMS for switchgrass (Evan and Milne, 1987). Abbreviation: m/z = mass:charge ratio of fragments extracted. Major lignin peak assignments: syringyl (S) and guaiacyl (G).

m/z	Mass spectrum peak assignment	Lignin assignment
124	Guaiacol	G
137[a]	Ethylguaiacol, homovanillin, coniferyl alcohol	G
138	Methylguaiacol	G
150	Vinylguaiacol	G
152	4-Ethylguaiacol, vanillin	G
154	Syringol	S
164	Allyl-+propenyl guaiacol	G
167[a]	Ethylsyringol, syringylacetone, propiosyringone	S
168	4-Methyl-2,6-dimethoxyphenol	S
178	Coniferyl aldehyde	G
180	Coniferyl alcohol, syringylethene	S,G
182	Syringaldehyde	S
194	4-Propenylsyringol	S
208	Sinapyl aldehyde	S
210	Sinapyl alcohol	S

[a] Fragment ion.

2.8. Data analysis

All data were reported as a mean value from two replicates. Multiple comparisons were performed using analysis of variance (ANOVA) assuming entries as a fixed effects and replicates as random effects. A least significant difference (LSD) was obtained with 95% significant difference ($P < 0.05$) among the four populations of switchgrass or three morphological portions.

3. Results and discussion

3.1. Biomass raw materials

Law et al. (2001) reported that three morphological portions of switchgrass (Cave-in-Rock), leaves, seedhead, and stem (including leaves sheath), were characterized by different chemical and physical properties. In their study, these morphological portions of switchgrass were compared for their chemical properties including lignin, holocellulose, hot-water solubility, benzene/ethanol solubility and fibrous qualities. In brief, leaves were distinguished from stems by significant differences in chemical characteristics, though both had similar fiber quality. In the present study, four populations of switchgrass with two replicates were studied for their chemical and physical properties. The initial and ground portions of switchgrass included leaves, internodes, and nodes. Among the four switchgrass examined, the percentage dry mass of three portions of switchgrass and production yield was similar (Table 2). On average, these four populations of switchgrass contained 27.0% internodes, 3.7% nodes, and 69.3% leaves based on dry mass.

3.2. Heat combustion value

The higher heating value (HHV) of bioresource components can be correlated with their chemical composition according to Hallac et al. (2009). In the study of acid catalyzed liquefaction of bagasse

Table 2
Percentages of three morphological portions for four populations of switchgrass.

Populations	% Internodes[a]	% Nodes[a]	% Leaves[a]	Leaves/internode ratio[a]
Alamo	26.8	3.7	69.5	2.7
Kanlow	25.9	3.2	71.0	2.7
GA993	27.9	4.2	67.9	2.4
GA992	27.4	3.6	68.9	2.5
LSD (5%)	9.3	0.7	8.9	1.2
Mean	27.0	3.7	69.3	2.6

[a] All data were reported as a mean value from two replicates.

in ethylene glycol, the HHV ranging from 11.04 to 39.59 MJ kg^{-1} was positively correlated to the carbon and hydrogen elemental content and negatively related to the oxygen elemental content for bagasse and its liquefaction products (Zhang et al., 2007). These results indicated that an increase in carbon content and lower oxygen content leads to a higher HHV. Understanding combustion values and their relationship to chemical composition could be an important parameter for future biopower applications of switchgrass. Higher heating value (HHV) of non-extracted internodes portions for all switchgrass samples were measured in a bomb calorimeter. The results indicated that the four switchgrass populations have similar HHV with an average 18.8 ± 0.2 MJ kg^{-1}. These heating combustion values were very close to other feedstocks. For example, the heating value of switchgrass was just 1.34–2.43 MJ kg^{-1} lower than that of softwood. While it was 0.85–1.2 MJ kg^{-1} lower than that of hardwood according to a recent publication (Hallac et al., 2009).

3.3. Extractives content of four populations of switchgrass

The switchgrass samples were successively extracted with hot-water followed by benzene/alcohol. The extractives content of each step was shown in Table 3. This data indicated that these samples had significant hot-water extractives with mass yields ranging from 17.0% to 20.8%. A subsequent benzene/ethanol extraction provided gravimetric yields from 2.6% to 12.3%. In general, the extractives content were similar among the whole-plant of switchgrass samples studied (Table 3). However, there was a significant difference on extractives content among the three fractions of each switchgrass sample with the leaves containing the highest amount of extractives (Table 3). The average value of hot-water extraction for internodes was 15.9%, and this was about 4.3% greater than that of nodes. Hot-water extractives from leaves contained a large amount of extractives, almost 3.5% greater than that of internodes and 7.9% greater than that of nodes. During benzene/alcohol extraction step, there was no significant difference between internodes and nodes. Benzene/alcohol extraction of leaves portion contained about 6% greater extractives than the other portions. Compared to Law's results on the extractives content from Cave-in-Rock switchgrass (Table 3), the leaves portion of the present switchgrass contained similar amounts of hot-water extractives (19.4% vs. 20.1) and 6% lower benzene/alcohol extractives content than Cave-in-Rock (10.2% vs. 4.2% on average) (Law et al., 2001). The extractives content of the internodes portions of the present switchgrass contained slightly greater hot-water extractives and benzene/alcohol extractives content than that of Cave-in Rock switchgrass.

3.4. Chemical compositions of switchgrass

Understanding the chemical compositions of switchgrass is an important issue for future utilization of switchgrass for biofuels production. The original switchgrass samples, without extraction, were analyzed by acid hydrolysis for carbohydrates, lignin (including Klason lignin and acid soluble lignin) and ash content analysis. The results showed that Alamo had the lowest lignin content (1% lower than the others), while there was no significant difference for the other chemical analysis preformed for the bulk switchgrass plant samples (Table 4). On average, the average chemical compo-

Table 4
Chemical compositions of three morphological portions for four populations of switchgrass.

Populations[a]	Arabinose[b]	Galactose[b]	Glucose[b]	Xylose[b]	Lignin[b]	Ash[b]
Alamo (S)	2.1	0.6	43.7	22.8	18.5	2.1
Kanlow (S)	2.3	0.6	43.7	24.2	19.1	2.5
GA 993 (S)	2.2	0.7	46.1	24.5	20.0	1.6
GA 992 (S)	2.3	0.7	43.8	24.6	19.9	1.5
LSD (5%)	0.4	0.1	4.5	3.5	1.0	0.4
Alamo (N)	3.2	0.9	35.7	23.7	22.2	2.3
Kanlow (N)	3.5	1.0	35.6	24.4	22.6	2.5
GA 993 (N)	3.3	0.9	40.1	26.8	22.7	1.8
GA 992 (N)	3.5	0.9	37.9	26.0	23.7	1.8
LSD (5%)	0.7	0.2	5.9	4.2	0.6	0.4
Alamo (L)	4.6	1.5	37.2	23.2	22.3	4.6
Kanlow (L)	3.8	1.5	35.2	22.6	23.0	4.6
GA 993 (L)	4.4	1.6	34.3	20.8	23.7	4.6
GA 992 (L)	4.6	1.6	35.8	22.4	23.3	4.4
LSD (5%)	0.6	0.2	1.64	2.4	1.1	0.4
Alamo (W)	3.8	1.2	38.8	23.1	21.2	3.8
Kanlow (W)	3.4	1.3	37.4	23.1	22.6	4.0
GA993 (W)	3.7	1.3	37.8	22.1	22.4	3.6
GA992 (W)	3.9	1.3	38.0	23.1	22.0	3.5
LSD (5%)	0.4	0.2	1.5	2.2	0.5	0.4

[a] S: internodes portion; N: nodes portion; L: leaves portion; W: whole-plant.
[b] Based on OD weight of switchgrass. All data were reported as a mean value from two replicates.

Table 3
Extractives content of three morphological portions for four populations of switchgrass and Cave-in-Rock switchgrass.

Morphological portions	Extraction	Alamo[a]	Kanlow[a]	GA993[a]	GA992[a]	5%LSD	Cave-in-Rock[b]
Internodes	Hot-water	16.0	17.0	14.9	15.7	3.8	12.4
	Benzene/Alcohol	5.3	3.8	4.3	5.4	3.4	1.7
Nodes	Hot-water	12.0	12.5	9.3	12.4	5.2	–
	Benzene/Alcohol	5.1	2.6	5.4	4.0	7.8	–
Leaves	Hot-water	19.7	18.2	20.8	18.8	3.1	20.1
	Benzene/Alcohol	12.3	10.2	8.7	9.9	7.9	4.2
Whole-plant	Hot-water	18.4	17.7	18.6	17.7	2.7	–
	Benzene/Alcohol	10.2	8.4	9.7	8.5	6.5	–
Mean values of three morphological portions for four populations of switchgrass	Extraction	Internodes	Nodes	Leaves		LSD (5%)	
	Hot-water	15.9	11.5	19.4		2.7	–
	Benzene/Alcohol	4.7	4.3	11.2		2.5	–

[a] All data were reported as a mean value from two replicates.
[b] Law et al. (2001).

Table 5
Comparison of average chemical compositions between three morphological portions of switchgrass and other published results.

	Arabinose	Galactose	Glucose	Xylose	Lignin	Ash
Internodes[a]	2.2	0.7	44.3	24.0	19.6	1.9
Nodes[a]	3.4	0.9	37.3	25.2	22.7	2.1
Leaves[a]	4.4	1.6	35.6	22.3	23.0	4.6
LSD (5%)	0.6	0.1	3.6	2.6	1.5	0.8
Whole-plant[a]	3.7	1.3	38.0	22.8	22.1	3.7
Switchgrass[b]	3.2	1.1	34.3	20.9	17.5[e]	–
Corn stove[c]	5.5	2.9	36.8	22.2	23.1	–
Switchgrass[d]	3.6	2.1	34.8	23.4	21.4[e]	7.1
Fescue[d]	3.0	1.1	39.8	23.2	18.1[e]	6.7

[a] All data were reported as a mean value from two replicates.
[b] Agblevor et al. (1994).
[c] Galbe and Zacchi (2007).
[d] Thammasouk et al. (1997).
[e] Klason lignin content.

Table 6
S/G ratio of three morphological portions for four populations of switchgrass from Py-MBMS analysis.

Populations	S/G ratio				S/G ratio in whole-plant[a,b]
	Internodes[a]	Nodes[a]	Leaves[a]	5%LSD	
Alamo	0.71	0.61	0.46	0.05	0.52
Kanlow	0.67	0.62	0.46	0.03	0.52
GA993	0.69	0.58	0.47	0.04	0.54
GA992	0.67	0.60	0.46	0.05	0.52
Average	0.68	0.60	0.46	0.03	0.52

[a] All data were reported as a mean value from two replicates.
[b] LSD (5%): 0.04.

nent in the bulk switchgrass samples was 3.7% arabinose, 1.3% galactose, 38.0% glucose, 22.8% xylose, 22.1% lignin, and 3.7% ash content (Table 5).

The chemical composition for the nodes, leaves and internodes of switchgrass were analyzed for all switchgrass samples (Table 4). Statistically, there was no significant difference for carbohydrate content among the four populations of switchgrass. However, Alamo and Kanlow contained about 1.5% greater of lignin content than GA993 and GA992 in internodes portions. The internodes and nodes portions of Alamo and Kanlow contained 0.5–1% greater ash content than that of GA993 and GA992. The results also showed that three portions of the four switchgrass contained significantly different chemical composition (Table 5). For example, the internodes portions contained greater amounts of glucose (8.7% more) and less hemicellulose sugars, such as arabinose (1.1% less), galactose (0.9% less), and xylose content (1.8% less), than that from nodes and leaves portion of switchgrass. The average lignin content for leaves portion of switchgrass had the highest lignin content (i.e., 3.4% more than that of internodes) (Table 5). However, the leaves portion of switchgrass contained about 2.5% lower ash content on average than internodes and nodes portion of switchgrass.

Table 5 compares the chemical compositions from various bioresources against the switchgrass samples in the present study. Compared to other published results for switchgrass, the whole-plant of switchgrass contained similar amount of carbohydrate and lignin content, but 3% less ash content on average (Thammasouk et al., 1997). In comparison to other herbaceous feedstocks, our results were similar in chemical composition (Agblevor et al., 1994; Galbe and Zacchi, 2007; Thammasouk et al., 1997).

3.5. Py-MBMS analysis of switchgrass

Molecular-beam mass spectroscopy was conducted for all switchgrass samples as summarized in Table 6. The results indicated that S/G ratio for bulk switchgrass samples was similar. However, the S/G ratio varied widely among nodes, internodes and the leave portions of switchgrass. The internodes portion had the highest amount of S/G ratio (average 0.68), while leaves portion contained the lowest amount of S/G ratio (average 0.46). The S/G ratio for internodes portion (average 0.68) was very close to the literature S/G ratio (0.7) for *Miscanthus* lignin analyzed by NMR and thioacidolysis (Villaverde et al., 2009). The observed switchgrass values differ significantly from the typical S/G ratio found for poplar which typically range from 1.3 to 2.2 (Robinson and Mansfield, 2009). Chang and Sarkanen (1973) demonstrated that the greater the S/G ratio the faster the delignification rate for kraft pulping of hardwoods. The S/G ratio has also been reported to be an indicator for morphological portion of plant (Gorshkova et al., 2000). The results of that study indicate that the fiber-rich portion was characterized with an elevated S/G ratio. A recent publication by Davison et al. (2006) has documented that both the lignin content and the S/G ratio contribute to the release of xylose from acid pretreatment. Likewise, Corredor et al. (2009) has reported that forage sorghums with a low syringyl/guaiacyl value was more readily enzymatically hydrolyzed after an acidic pretreatment. The S/G values seen in Table 6 suggest a potential range of switchgrass reactivity during pretreatment and subsequent enzymatic deconstruction.

4. Conclusion

According to the results of chemical and structural analysis, the four populations switchgrass characterized in this study had similar bulk chemical properties. The most significant differences among these switchgrass were ash and lignin content. Alamo contained the lowest lignin content. However, the chemical and structural results among the three portions of switchgrass, leaves, internodes, and nodes, were significantly different. In fact, leaves contained the highest amount of arabinose, galactose, lignin, and ash content. In addition, leaves also had the lowest S/G ratio and glucose content. The content of the lignin and glucose among the three portions of switchgrass differed by 3.4% and 8.7%, respectively. Anderson stated that the leaves portion tends to be digested more easily than internodes portion of grass with Depol 740 ferulic acid esterase and cellulase (Anderson and Akin, 2008). In the present study, the switchgrass samples had an average 69.3% leaves portion which could provide an opportunity to utilize the whole switchgrass plant for bioethanol production. Future development of pretreatment technologies to convert switchgrass into biofuels will benefit from being able to tailor process chemistries to the differences noted in this report.

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