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Comparative study on enzymatic digestibility of switchgrass varieties and harvests processed by leading pretreatment technologies

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ABSTRACT

Feedstock quality of switchgrass for biofuel production depends on many factors such as morphological types, geographic origins, maturity, environmental and cultivation parameters, and storage. We report variability in compositions and enzymatic digestion efficiencies for three cultivars of switchgrass (Alamo, Dacotah and Shawnee), grown and harvested at different locations and seasons. Saccharification yields of switchgrass processed by different pretreatment technologies (AFEX, dilute sulfuric acid, liquid hot water, lime, and soaking in aqueous ammonia) are compared in regards to switchgrass genotypes and harvest seasons. Despite its higher cellulose content per dry mass, Dacotah switchgrass harvested after wintering consistently gave a lower saccharification yield than the other two varieties harvested in the fall. The recalcitrance of upland cultivars and over-wintered switchgrass may require more severe pretreatment conditions. We discuss the key features of different pretreatment technologies and differences in switchgrass cultivars and harvest seasons on hydrolysis performance for the applied pretreatment methods.

1. Introduction

Switchgrass is a plentiful, warm-season perennial grass adapted to a wide range of habitats across North America (Moser and Vogel, 1995). It is adapted to a wide range of soil conditions, tolerant to drought and poor soils, and thereby can grow on marginal lands with high dry biomass yields under low fertility conditions (Moser and Vogel, 1995; Paine et al., 1996; Sanderson et al., 1996). These characteristics make switchgrass a potentially promising biofuel energy crop.

Switchgrass can be converted into biofuels through biochemical processes such as hydrolysis and fermentation (Wiselogel et al., 1994; McLaughlin et al., 1999). Its composition determines the quality of biofuel produced and has different impacts on conversion processes (Sanderson and Wolf, 1995). High structural carbohydrate content, which includes cellulose and hemicellulose, is desirable to produce fermentable sugars from the switchgrass through biochemical conversion while high lignin content can adversely affect fermentation (Hayn et al., 1993; Casler and Boe, 2003; Kim and Holtzapple, 2006).

There are various factors affecting switchgrass biomass yield and composition: genotype (lowland vs. upland), ecotype (southern vs. northern), harvest time, fertilizer application, precipitation, storage method, and other environmental and cultivation conditions. In the literature, cultivar, location, and harvest time factors were found to be significant in affecting biomass yield and composition of switchgrass (Casler and Boe, 2003; Casler et al., 2004; Cassida et al., 2005). When grown in southern areas, lowland-southern varieties tend to have a higher dry mass yield potential than upland-northern cultivars, while the latter outperform in northern regions (Sanderson et al., 1999; Lemus et al., 2002; Casler et al., 2004; Cassida et al., 2005). Other than the dry biomass yield, composition of switchgrass is greatly affected by ecotype and latitude-of-origin. Lignocellulose content generally increases with latitude in upland-northern types, while it is the opposite for lowland types, indicating that latitude has

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a significant impact on lignocellulose yields of upland genotypes (Casler et al., 2004; Cassida et al., 2005). Casler et al. (2004) also showed that cellulose concentration increased with latitude in upland genotypes while it decreased sharply in lowland genotypes at latitudes above 36°. Moisture level of the switchgrass at harvest was generally higher for lowland southern types than upland northern types, while the latter contained greater amount of ash and nitrogen than the former (Cassida et al., 2005).

Seasonal harvest time is also an important factor that affects not only biomass yield, but also composition. The compositional change is strongly related to the quality of the feedstock for biofuel production (Adler et al., 2006). For example, high ash and moisture content may present a problem for combustion of switchgrass (Casler and Boe, 2003; Cassida et al., 2005). High cell-wall carbohydrate concentration, on the other hand, is considered to be beneficial in biofuel production. Delaying harvest time is known to reduce switchgrass biomass yield, ash, moisture, and soluble and storage carbohydrates concentrations, while increasing cell-wall carbohydrates and lignin concentrations as switchgrass matures during the growing season (Sanderson and Wolf, 1995; Casler and Boe, 2003; Adler et al., 2006). Spring harvest of the previous year's growth showed a decreased mineral concentration while lignocellulose content was increased as compared to fall harvest in various other energy crops (Burvall, 1997; Lewandowski and Heinz, 2003).

Only a few studies to date have examined the impact of switchgrass varieties and harvest seasons on biomass-to-fuels conversion processes (Adler et al., 2006; Boateng et al., 2006; Bals et al., 2010). Switchgrass has various cultivars and its quality can vary depending on the many factors described above. The objective of this study was to examine how the seasonal harvest time (fall vs. spring) and cultivars (upland vs. lowland) of switchgrass affected the biochemical conversion of switchgrass and to identify factors that influence the variations. Three cultivars (Dacotah, Shawnee and Alamo) were processed using the various pretreatment technologies developed and tested by the Biomass Refining Consortium for Applied Fundamentals and Innovation (CAFI). Compositions of the switchgrass which were harvested in different locations and harvest seasons were also compared. These comparisons are based on data obtained through identical experimental protocols and data analysis techniques using common supplies of switchgrass as previously reported by CAFI (Wyman et al., 2005; Mosier et al., 2005; Kim et al., 2009a).

2. Methods

2.1. Materials

Four different switchgrass feedstocks were provided by Ceres, Inc. (Thousand Oaks, CA). The switchgrass varieties examined in this study are Alamo (lowland variety, two different batches) and Shawnee and Dacotah (upland variety). Ecotype and harvest information of the switchgrass samples are summarized in Table 1. Small square bales of each Switchgrass type were stored in a building after harvest until they were dried to less than 10% moisture at 50 °C and knife- or ball-milled to 2–6 mm size.

Spezyme® CP (cellulase) was provided by Genencor, A Dinisco Division (Rochester, NY) and Novozyme 188 (β-glucosidase, Novo Nordisk, Novo Allé, Denmark) was purchased from Sigma (Cat. No. C6150). All other reagents and chemicals, unless otherwise noted, were purchased from Sigma–Aldrich (St. Louis, MO).

2.2. Substrate preparation

The switchgrass batches were pre-washed using 10 times volume per weight of hot DI water (80–90 °C) three times and dried in 45 °C oven. The washate from each washing step was collected to measure water-extractable soluble free sugars.

Table 1
Ecotype and harvest information of switchgrass feedstocks.

	Alamo 1	Alamo 2	Shawnee	Dacotah
Latitude-of-origin	29°N		38°N	46°N
Ecotype	Southern lowland		Northern upland	
Morphology	Thick stems		Thin stems	
Harvest location	Ardmore, OK 34°N (Elev. 870 ft)		Stillwater, OK 36°N (Elev. 960 ft)	Pierre, SD 44 (Elev. 1420 ft)
Plant date	June, 2005	June, 2007	June, 2005	December, 1999
Harvest date	December, 2006	November, 2007	December, 2006	May, 2008*

* Plot was allowed to stand over the winter.

Prior to pretreatment and enzymatic hydrolysis, the dried switchgrass samples were further milled through a 40 mesh screen (0.4 mm) using a Wiley mill (Thomas Scientific, Swedesboro, NJ) to provide uniform particle size between and within the switchgrass ecotypes. Materials for AFEX were milled through a 2 mm screen.

2.3. Compositional analysis

Composition of switchgrass samples was determined by following NREL (National Renewable Energy Laboratory) LAP standard analytical procedures (Ehrman, 1994a,b; Sluiter et al., 2006). Analyzed components were: glucan, xylan/galactan, arabinan, lignin, ash, protein, and acetyl. The liquid fraction of the pretreated switchgrass was analyzed for soluble mono-and oligosaccharides content by following LAP 014 (Sluiter et al., 2005). The nitrogen content for both unwashed and prewashed samples was determined using a Skalar Primacs SN Total Nitrogen Analyzer (Breda, The Netherlands). Total nitrogen in each sample was calculated compared to an EDTA calibration curve. The nitrogen values were multiplied by 6.25 to determine the crude protein content. The protein that was removed during extraction steps was subtracted from the total extractives content. Sugars were analyzed by HPLC as described in the HPLC analysis section of this paper. All measurements were made in triplicates. The values obtained were averaged and errors were calculated at the 95% confidence level using Microsoft Excel. The *F* test in single factor analysis of variance (ANOVA) was carried out in order to test the significance of the variability of the components. Statistical analysis was done by Data Analysis Tool pack in Microsoft Excel. The *p* values at least <0.05 were considered as significant.

2.4. Pretreatment

Conditions of each pretreatment method applied in this study are summarized in Table 2. The selected pretreatment conditions in Table 2 are the optimal switchgrass pretreatment conditions identified for each pretreatment technology. All pretreatment runs were made in triplicates. Further details are provided below.

2.4.1. Ammonia fiber expansion (AFEX)

All AFEX pretreatments were conducted in a 300 mL stainless steel (#316) Parr reactor. Prior to loading the biomass, the reactor was preheated to 130–140 °C. The desired amount of distilled water (2.0 g H_2O: g DM) was added to the biomass and then the material was placed in the reactor. The reactor was sealed and evacuated using a rotary vacuum pump. Meanwhile, the required amount of ammonia (with 4–6 g excess) was heated in a separate pressure vessel until the pressure reached 660–720 psig. The ammonia was then added to the reactor vessel (1.50 ± 0.05 g

Table 2
Optimal switchgrass pretreatment conditions of various pretreatment technologies.

Pretreatment Method	Substrate	Chemicals loading (per g dry biomass)	Temperature (°C)	Duration	Water in g per g dry biomass
AFEX	A	1.5 g NH_3	140	20 min	2 g H_2O
	S, D	1.5 g NH_3	150	30 min	2 g H_2O
DA	A, S, D	0.045 g H_2SO_4	160	10 min	8.9 g H_2O
LHW	A, S, D	6.7 g H_2O	200	10 min	6.7 g H_2O
Lime	A, S, D	1 g $Ca(OH)_2$	120	4 h	None
SAA	A, S	1.35 g NH_4OH	90	24 h	7.7 g H_2O
	D	1.35 g NH_4OH	160	1 h	7.7 g H_2O

A, Alamo; S, Shawnee; D, Dacotah.

NH_3: g DM) which marked the start of the residence time (20 min for Alamo switchgrass and 30 min for Shawnee and Dacotah switchgrass). The temperature was raised to the set point and maintained within ±10 °C of the set point for the entire residence time (heat-up ranged from 0 to 5 min). At the end of the residence time, the reactor was vented, allowing release of ammonia and water vapor. The reactor was then quenched in cool water for around 5 min. The biomass was removed from the reactor and placed in the fume hood overnight to allow the residual ammonia to evaporate. Alamo and Shawnee switchgrass pretreatments were performed in duplicate and the pretreated biomass from both experiments was combined prior to hydrolysis.

2.4.2. Dilute sulfuric acid (DA)

Prior to pretreatment, 50 g pre-washed switchgrass was presoaked overnight in 0.5 wt.% dilute acid at room temperature with a solid loading of 10 wt.% on dry basis. Pretreatments were run at 160 °C for 10 min in a 1 L Parr reactor made of Hastelloy C (Parr Instruments, Moline, IL) and heated in a 4-kW fluidized sand bath (model SBL-2D, Techne Co., Princeton, NJ). The biomass slurries were stirred at 200 rpm with 2 stacked pitched blade impellers with a diameter of 40 mm, pushing the material downwards. The temperature was monitored inside the reactor using a K-type thermocouple. The heat-up time for this system was about 2 min and was not included in the stated reaction time. After pretreatment, the reactor was quenched in a room temperature water bath until the temperature dropped to 80 °C. The slurry was vacuum filtered immediately through a glass fiber filter (Whatman®, Grade GF/A, diam. 11.0 cm) with the temperature being always higher than 60 °C. The resulting solids were then washed with room temperature deionized water until the filtrate pH reached above 6.

2.4.3. Liquid hot water (LHW)

The aqueous pretreatment of switchgrass consisted of mixing the substrate with DI water at 15% solids loading (w/w, g dry solids per g total) and heating at 200 °C for 10 min under pressure in order to keep the water in a liquid state. Reactions were conducted in 1 in. OD × 0.083 in. (2.54 cm × 2.1 mm) wall thickness, 316 stainless steel tubing capped at both ends with 1 in. (2.54 cm) Swagelok tube end fittings (Swagelok, Indianapolis, IN). Each tube was 4.5 in. (11.4 cm) in length and 45 mL in total volume. The sample volume was kept at 33.7 mL to give approximately 25% of head space for liquid expansion during heating to 200 °C. The reactor tube containing the slurry of switchgrass was heated by placing it in a Tecam® SBL-1 fluidized sand bath (Cole-Parmer, Vernon Hills, IL) set to 200 °C for 18 min, which included a 8 min heat-up and 10 min reaction time. After pretreatment, each tube was cooled by quenching in water for 10 min. The pretreated slurry was vacuum filtered using Whatman® No. 41 filter paper to remove the liquid fraction which was collected for further analysis. The retained solids on the filter paper were hot-washed as described later in this section. The pretreated solids and collected pretreatment liquid were stored in a freezer (−10 °C) until further analysis (Kim et al., 2009b).

2.4.4. Lime

The lime pretreatment was conducted in a pair of 304 stainless steel pipe reactors (5'' long, 1.5'' I.D.) with 1.5'' 304 stainless steel caps. The reactors were sealed using Teflon tape. Reactors were loaded with 8 g dry switchgrass and excess calcium hydroxide (1 g CaOH/g dry biomass) and water (15 g/g dry biomass). Constant 100 psi pure oxygen was supplied to a manifold through a flexible stainless steel hose attached to an oxygen tank. The reactors were connected to a swing arm to provide constant stirring and placed in a preheated temperature controlled oven at 120 °C. The reaction time was 4 h after which the reaction was quenched by removing the reactors from the oven and immediately placing them in an ice bath. Once cooled, the reactors were opened slowly to relieve pressure, and the contents were transferred to a 1 L plastic centrifuge bottle using DI water. The slurry was neutralized using 5 N HCl to a pH of approximately 4, then underwent several washings with DI water until the pH of the slurry rose to approximately 6. The final slurry was vacuum filtered and the filtered solids were collected for carbohydrate analysis. Moisture content and final weight of the solids was recorded to obtain pretreatment yield and the solids were stored in the freezer until compositional analysis and enzymatic hydrolysis was performed.

2.4.5. Soaking in aqueous ammonia (SAA)

Batch reactor was used for the SAA pretreatment of switchgrass. Biomass was soaked in a stainless steel reactor (1.375''IDx6''L). The reactor was loaded with 10 dry grams of switchgrass with 90 mL of 15% NH_4OH. The reactor was kept in a preheated temperature controlled oven at 160 °C for 60 min soaking time for Dacotah and at 90 °C for 24 h for Alamo and Shawnee. The heating time to reach the target temperature was about 20 min and that was not included in the stated reaction time. After pretreatment, the reactor was immediately removed from the oven and quenched to room temperature in water bath. The cooled slurry was vacuum filtered immediately through a filter paper (Whatman®, Grade 802 Fluted, size 32.0 cm). The vacuum filtered wet solid underwent further washing using deionized water until the pH reached approximately 6.

2.5. Enzymatic hydrolysis

Except for the AFEX treated switchgrass, all pretreated solids were washed with approximately 500 mL of hot DI water (80–90 °C) per 10 g dry solids. Moisture content of the hot-washed solids was measured. There was no further drying step. Enzymatic digestibility of the pretreated switchgrass was determined by following NREL standard protocol (LAP-009, Brown and Torget, 2005) with modifications described below. The pretreated switchgrass solids equivalent to 1 g glucan were transferred into a 250 mL Erlenmeyer flask and an aliquot of 100 mL 0.05 M, pH 4.8 sodium citrate buffer was added to give 1% glucan slurry. Two antibiotics, tetracycline (0.4 mL) and cyclohexamide (0.3 mL) were added to the mixture to prevent microbial growth during the hydrolysis. Enzymes were added at 15 FPU cellulase in Spezyme CP plus 30 CBU β-glucosidase in Novozyme 188 per g glucan of untreated raw switchgrass (equivalent to total 27 mg protein/g glucan in untreated biomass). The hydrolysis was carried out at 50 °C and an agitation rate of 150 rpm. Samples were taken at

1 h for initial rate, 24 and 168 h. Sugars and other components in the hydrolysate samples were analyzed by HPLC. Hydrolysis yields were calculated based on the glucan or xylan in pretreated/hot washed solids for all pretreatments except for AFEX which was hydrolyzed without the post-pretreatment washing step. All hydrolysis runs were in triplicates. Error bars represent 95% CI of a mean.

2.6. HPLC analysis

Hydrolysis samples were analyzed by Bio-Rad Aminex HPX-87H ion exchange column (300 mm × 7.8 mm, Bio-Rad Laboratories Inc., Hercules, CA) connected with a Milton Roy mini pump (Milton Roy Co., Ivyland, PA), Waters™ 717 plus autosampler, and Waters™ 2414 refractive index detector (Waters Corp., Milford, MA). The data was stored and processed using Empower™ 2 Chromatography Data Software (Waters Corp., Milford, MA). The mobile phase was 5 mM sulfuric acid in distilled, de-ionized water filtered to 0.2 μm. The mobile phase flow rate was 0.6 mL/min. The column temperature was maintained at 60 °C by an Eppendorf CH-30 Column Heater controlled by an Eppendorf TC-50 (Eppendorf, Westbury, NY).

3. Results and discussion

3.1. Compositional variability of switchgrass cultivars and harvests

The composition of three different cultivars of switchgrass, which included two different batches of a lowland cultivar (Alamo) and two different upland cultivars (Shawnee and Dacotah) are summarized in Table 3. Alamo and Shawnee were harvested in late fall while Dacotah was harvested in spring after the plot was allowed to stand over the winter. Only Dacotah had a composition that was significantly different ($p < 0.05$) from the other varieties, particularly for glucan, water extractable soluble sugars, protein, and lignin (Table 3B). Because Alamo batch 1 and 2 did not show any statistically significant compositional differences, further experiments employing pretreatment and enzymatic saccharification were conducted for Alamo (batch 1), Shawnee and Dacotah varieties only.

Switchgrass contains soluble components which are readily extractable in aqueous phase. These extractives include soluble sugars, such as sucrose, glucose, fructose, and xylose, and soluble lignin. Thammasouk et al. (1997) and Chen et al. (2010) have reported that the pre-extraction step is needed to improve the accuracy of measurement of macrocomponents (cellulose, xylan, and lignin) in lignocellulose. Klason lignin can especially be overestimated in the native material due to condensation/precipitation of extractives during the analysis of Klason lignin (Browning, 1967). The lower Klason lignin content of pre-extracted feedstocks more accurately reflects the true lignin content of the native material (Thammasouk et al., 1997). The water soluble free-sugars can also overestimate the structural carbohydrate content and enzymatic digestibility efficiencies of the structural carbohydrates. Unless removed from the native material, the water-extractable soluble sugars can interfere with comparisons among the cultivars. Thus, the switchgrass was pre-washed with hot-DI water to remove the soluble sugars before pretreatment was applied.

Contents of the water-extractable soluble free sugars are given in Table 3. It should be noted that the materials were pre-extracted with hot DI water to remove the extractives and the amounts of all other components were measured using the extractives-free switchgrass. The values were corrected to be based on the weight of the unextracted, initial dry material. Comparisons between the switchgrass samples showed that Dacotah contained significantly less water-extractables (<1% by dry weight) than the other three samples which contained more than 7% by dry weight. ANOVA analysis indicated that the water extractable sugar contents in the four switchgrass samples were statistically different at the $p < 0.05$ level, except for between Alamo batch 1 and Shawnee. Further analysis of the water-extractable sugars indicated that the extracted sugars were mainly sucrose, glucose, xylose and fructose (data not shown). Total structural carbohydrates, which include glucan, xylan, and arabinan, ranged from 54% to 61% by dry weight. While the structural carbohydrate content in the Alamo and Shawnee was not statistically different, Dacotah was found to contain significantly higher glucan than the other varieties. The difference was statistically significant at 5% level (Table 3B).

Table 3
Compositional analysis of the selected switchgrass cultivars. (A) Compositions of switchgrass by% dry weight of untreated, raw switchgrass (B) ANOVA test *p*-values of switchgrass composition comparisons at significance level of 0.05. Errors in 95% CI are less than 5% for all values.

	Alamo 1	Alamo 2	Shawnee	Dacotah
(A)				
Total structural carbohydrates (%)	53.8	56.3	54.0	60.9
Glucan (%)	(29.9)	(32.1)	(30.9)	(35.3)
Xylan (%)	(20.5)	(21.6)	(20.0)	(22.5)
Arabinan (%)	(3.4)	(2.7)	(3.1)	(3.1)
Water extractable soluble sugars (%)	9.6	6.9	8.2	0.8
Lignin (%)	18.8	19.5	19.7	22.6
Ash (%)	3.9	4.3	4.2	3.3
Acetyl (%)	2.2	2.8	2.9	3.6
Protein (%)	5.4	4.5	4.3	1.2
Mass closure (%)	93.7	94.3	93.3	92.3
	ANOVA test *p*-values			
	A1 vs. A2 vs. S			D vs. A1,A2,S
(B)				
Total structural carbohydrates	0.69			**0.009**
Glucan	0.33			**0.0003**
Xylan	0.58			0.13
Arabinan	0.32			0.40
Water extractable sugars	0.50			**0.001**
Lignin	0.12			**0.0001**
Ash	0.74			0.3
Acetyl	0.70			0.51
Protein	0.06			**0.0005**

The bold values are the *p*-values that are less than 0.05. The components with <0.05 *p*-values were considered to be significantly different between the cultivars.
A1, Alamo 1; A2, Alamo 2; S, Shawnee; D, Dacotah.

Compositional variability of the switchgrass varieties used in this study is graphically summarized in Fig. 1. The major components, including cellulose, xylan, lignin, water extractable sugars, protein, and ash contents, were compared depending on morphological type (lowland vs. upland) and harvest season (late fall vs. spring). As shown in Fig. 1, no statistically significant difference was found between Alamo and Shawnee, despite the fact they are different ecotypes based on the latitude-of-origin. Dacotah contains significantly more cellulose and lignin but less water extractable sugars and protein compared to both Alamo and Shawnee. The higher lignocellulose concentration in Dacotah could be partly due to its morphological type. Dacotah is a northern-upland variety of which latitude-of-origin and harvest location was the highest among the samples. In upland varieties, lignocellulose content generally increases with latitude, while the opposite is observed in lowland varieties. Alamo was grown at more northern latitude than its latitude-of-origin, which might have affected its compositions as well.

Delayed harvest probably lead to the greatest differences seen between the samples, which for switchgrass has shown to increase structural carbohydrates and lignin while reducing soluble sugars and ash (Casler and Boe, 2003; Sanderson and Wolf, 1995; Adler et al., 2006). Bals et al. (2010) reported that the October harvest

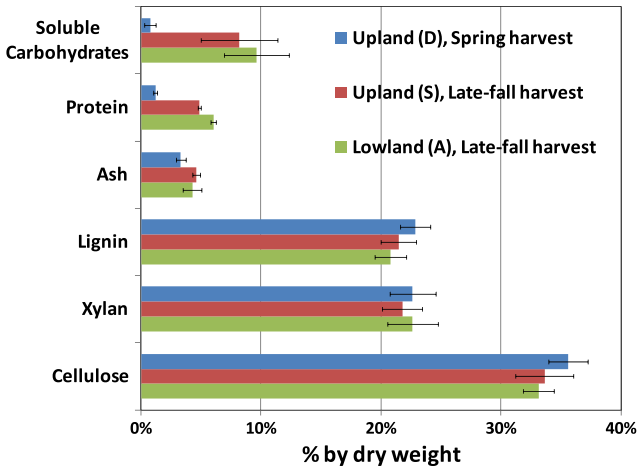

Fig. 1. Effect of ecotype and harvest season of switchgrass on composition. Error bars represent 95% CI. A, Alamo 1; S, Shawnee; D, Dacotah.

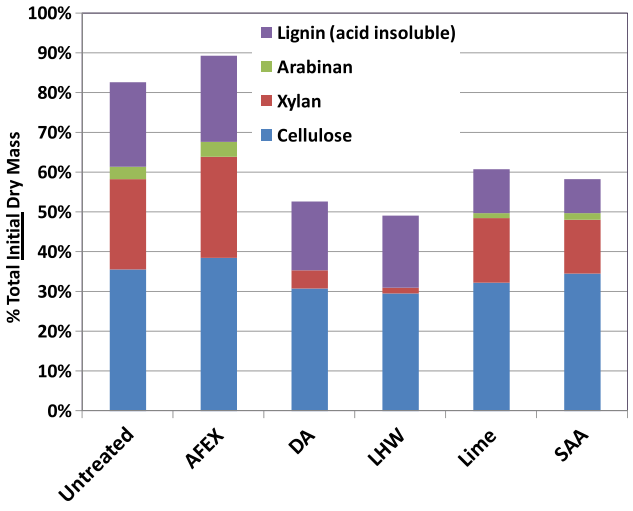

Fig. 2. Fate of solids during pretreatment of Dacotah switchgrass. Data is average of duplicate analysis.

of Cave-In-Rock switchgrass (CIR), an upland cultivar, contained more structural carbohydrates and lignin and less solubles and ash than the July harvest. In this study, only Dacotah switchgrass was harvested in spring, while all the other cultivars were harvested in late fall. As reported in various studies, ash and soluble and storage polysaccharides in switchgrass decrease, while Klason lignin and cellulose contents increase over the winter (Sanderson and Wolf, 1995; Casler and Boe, 2003; Lewandowski and Heinz, 2003; Adler et al., 2006). The non-cell wall carbohydrates found in switchgrass are mainly soluble sugars such as sucrose, glucose, and fructose and starch, which are susceptible to microbial degradation. Adler et al. (2006) explained in their study that the increased cell wall carbohydrates and lignin contents in switchgrass harvested in spring as compared to the fall harvests are attributed to starch-storing seeds being dropped off and leaching of soluble components such as sugars, protein, and organic acids over the winter. Bals et al. (2010) explained that the switchgrass mobilizes solubles and ash for storage in root system, which causes the reduced solubles and ash content for later harvests.

Other environmental factors, such as weather, precipitation, storage method and fertilization could also contribute to the compositional differences in switchgrass cultivars. Since storage method of switchgrass bales after harvest was identical for all four cultivars examined in this study, the effect of storage should be minimal. With limited information given on the other environmental factors, it is difficult to gauge the extent to which each factor might have played on the switchgrass compositions. The results, however, demonstrate the strong correlation between seasonal harvest time and switchgrass composition.

In summary, spring harvest of switchgrass exhibited a higher concentration of lignocellulose and a lower protein and water extractable soluble sugars than fall harvests. The compositional variability seemed to more depend on the harvest times rather than genotype or ecotype of the switchgrass cultivars.

3.2. Pretreatment of Switchgrass by leading pretreatment technologies

The fate of the main components in Dacotah switchgrass following each pretreatment is summarized in Fig. 2 for comparison between the pretreatment technologies. Except for AFEX, the pretreated slurry was washed as described in Section 2 and the remaining solids were analyzed for composition. Only Dacotah data is shown in Fig. 2, since the trend was very similar for Alamo and Shawnee. The compositions are given on the basis of % initial dry switchgrass before pretreatment. The total % mass balance is not 100% even for the untreated Dacotah, because only the major components (lignin and structural carbohydrates) are compared and other components, such as ash, protein, acetyl, and extractives are not included. The complete compositional data of untreated switchgrass is given in Table 3.

Liquid ammonia pretreatment improves rates and yields of lignocellulose hydrolysis through various modes of action: reducing lignin content, hydrolysis of hemicellulose, decrystallization and swelling of cellulose, and increasing surface area of biomass (Mosier et al., 2005; Teymouri et al., 2005; Kim and Lee, 2005; Gupta and Lee, 2009). Liquid ammonia is believed to act by ammonolysis of 4-*O*-methylglucuronic acid ester cross-linkages of hemicellulose and lignin, thereby making the treated biomass more accessible to enzymes (Lin et al., 1981). AFEX pretreatment involves treatment of biomass with liquid anhydrous ammonia at a moderate to high temperature (60–180 °C) and high pressure (250–700 psi) for a short period of time (5–30 min) (Teymouri et al., 2005; Balan et al., 2009). Similarly, SAA treats biomass with aqueous ammonia at a mild to moderate temperature (40–160 °C) for a long reaction time under atmospheric pressure, retaining most of the carbohydrates while removing lignin (Kim and Lee, 2005). As presented in Fig. 2, the structural carbohydrate and lignin contents were not changed significantly after the AFEX pretreatment. The amount of each component slightly increased as compared to the untreated switchgrass possibly due to measurement errors. Unlike AFEX, SAA removed 60% of lignin and 40% of xylan in initial switchgrass, while retaining over 95% of cellulose. The extent of hemicellulose and lignin removal was similar for Alamo and Shawnee.

Alkali pretreatment technology such as lime (calcium hydroxide) pretreatment improves digestibility of lignocellulose by removing lignin, acetyl, and the various uronic acid substitutions on hemicellulose (Chang and Holtzapple, 2000). Pretreatment conditions are very mild (80–120 °C) and generally requires hours of treatment time (Mosier et al., 2005). In the case of switchgrass, approximately 30% of hemicellulose and 60% of lignin was removed during the optimized lime pretreatment conditions.

Hemicellulose removal was the greatest for both dilute acid (DA) and liquid hot water (LHW) pretreatments among the pretreatment methods tested. Nearly 80–90% of initial xylan and arabinan were removed from the biomass during either DA or LHW pretreatment. Lignin removal was approximately 20% for both DA and LHW pretreatment. Dilute sulfuric acid acts as a catalyst to hydrolyze hemicellulose producing mainly monomeric sugars

upon pretreatment. Hydrolysis of hemicellulose is assisted by acetic and uronic acid substitutions released from hemicellulose during the liquid hot water pretreatment at neutral pH (pH 4–7) (Mosier et al., 2005). Unlike the DA pretreatment, the majority of the solubilized and hydrolyzed xylan is present in oligomeric forms in the LHW pretreated slurry. If the reaction condition is too severe during the acid-catalyzed pretreatment, the hydrolyzed xylan can be further reacted to form degradation products (Mosier et al., 2005; Kim et al., 2009b). Thus, the pretreatment conditions need to be carefully selected to minimize the loss of sugars to decomposition.

At least 85% of the initial cellulose was retained in solid phase for all pretreatments examined in this study. Except for the AFEX, all the other pretreatment technologies resulted in 40–50% solubilization of the initial switchgrass, each removing different components of switchgrass to varying extents. The extent of solubilization of switchgrass by each pretreatment was similar for all three switchgrass cultivars.

Compositions of pretreated switchgrass solids are compared in Fig. 3 for each pretreatment technology. The compositions in Fig. 3 are based on dry weight of the pretreated solids, except for untreated switchgrass which is based on initial dry solids. The higher lignocellulose and lower protein contents of Dacotah compared Alamo and Shawnee was preserved in AFEX treated solids since there was no solids loss during the pretreatment. For the other pretreatment technologies, the difference between the pretreated switchgrass varieties was less clear. This is partly due to the washing of the pretreated slurry to remove solubilized components, which in turn affects the composition of the remaining solids. The results suggest that the compositional differences between fall vs. spring harvest or upland vs. lowland variety are not preserved after pretreatment. The compositional differences become more dependent on the pretreatment technology and severity of pretreatment conditions applied for each type of the switchgrass.

3.3. Enzymatic hydrolysis of pretreated switchgrass varieties

Pretreated switchgrass varieties were enzymatically hydrolyzed following the standard enzymatic hydrolysis protocol which is carried out at 1% glucan loading by 15 FPU of Spezyme CP and 30 CBU Novozyme 188 per g glucan in untreated biomass. Yields of glucose and xylose sugars at 1, 24, and 168 h of the hydrolysis are summarized in Fig. 4. The 1 h hydrolysis yields are given to compare initial rates of the hydrolysis and the168 h hydrolysis results represent final yields of the sugars. Enzymatic digestibility of untreated switchgrass is given for comparison.

Untreated switchgrass varieties did not exhibit any significant differences in sugar yields. Without pretreatment, sugar yields were only 14–16% for glucose and 4% for xylose. All pretreatments improved both rates and yields of the hydrolysis. Dacotah switchgrass resulted in lower glucose yields than Alamo and Shawnee at a statistically significant level ($p < 0.05$). The difference in glucose yields between Dacotah and the other two switchgrass varieties seemed greatest at the initial stage of the hydrolysis and started to decrease as the hydrolysis progressed. Glucose yield from the pretreated Dacotah at 1 h of hydrolysis was only half of that from the other two varieties pretreated at the same conditions, especially for AFEX, DA, and LHW pretreatments. Despite that Dacotah was pretreated at a more severe condition than Alamo for AFEX and SAA pretreatment (Table 2), Dacotah gave a lower sugar yield than Alamo. Glucose yield from pretreated Dacotah after 168 h was 5–20% lower than the other two varieties. Alamo consistently resulted in the highest glucose yield among the samples for all pretreatment technologies. It should be noted that Alamo is a lowland variety while the other two are upland cultivars. Unlike the glucose yields, there was no consistent trend in xylose yields between the pretreated switchgrass cultivars. Xylose yield was the lowest for Dacotah for AFEX, DA, LHW and SAA treatments, while Shawnee resulted in the lowest xylose yield for lime pretreatment. In general the results indicate that spring harvested Dacotah is more recalcitrant to pretreatment and enzymatic conversion than fall-harvests of the other two varieties. Although the higher cellulose content in Dacotah harvested in the spring slightly increases its potential biofuel yield, that potential is more difficult to realize due to its higher recalcitrance. The differences in enzymatic digestibility between the fall-harvest of lowland Alamo and upland Shawnee were much less than the difference found between the Shawnee and Dacotah which are both upland ecotypes with different harvest seasons. The enzymatic hydrolysis results suggest that not only compositions but also reactivity of switchgrass to pretreatment and enzymatic hydrolysis is strongly dependent on harvest season, with later harvests being more difficult to hydrolyze than early harvests.

Bals et al. (2010) also reported the lower sugar yields from later harvests than from early harvests of switchgrass. In their study they showed that October harvest of CIR switchgrass required more severe pretreatment conditions than July harvest to achieve the similar extent of hydrolysis. Average glucose released for the October harvest was approximately 100 g/kg biomass lower than

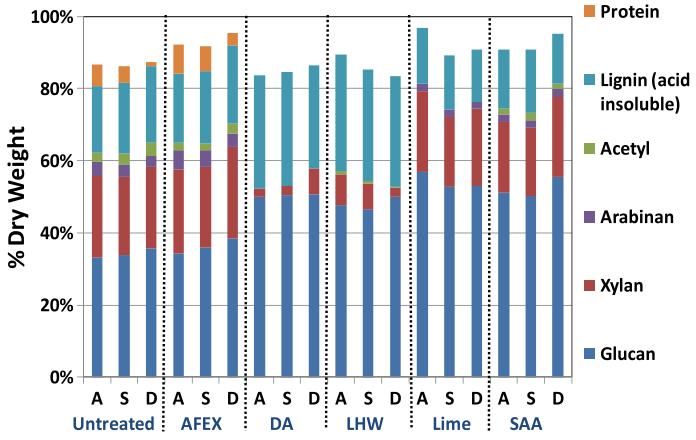

Fig. 3. Composition of switchgrass varieties pretreated by leading pretreatment technologies. Untreated switchgrass composition is based on initial dry weight. Pretreated switchgrass composition is based on pretreated solids dry weight. Pretreatment conditions are given in Table 2. Data is average of duplicate analysis. A, Alamo 1; S, Shawnee; D, Dacotah.

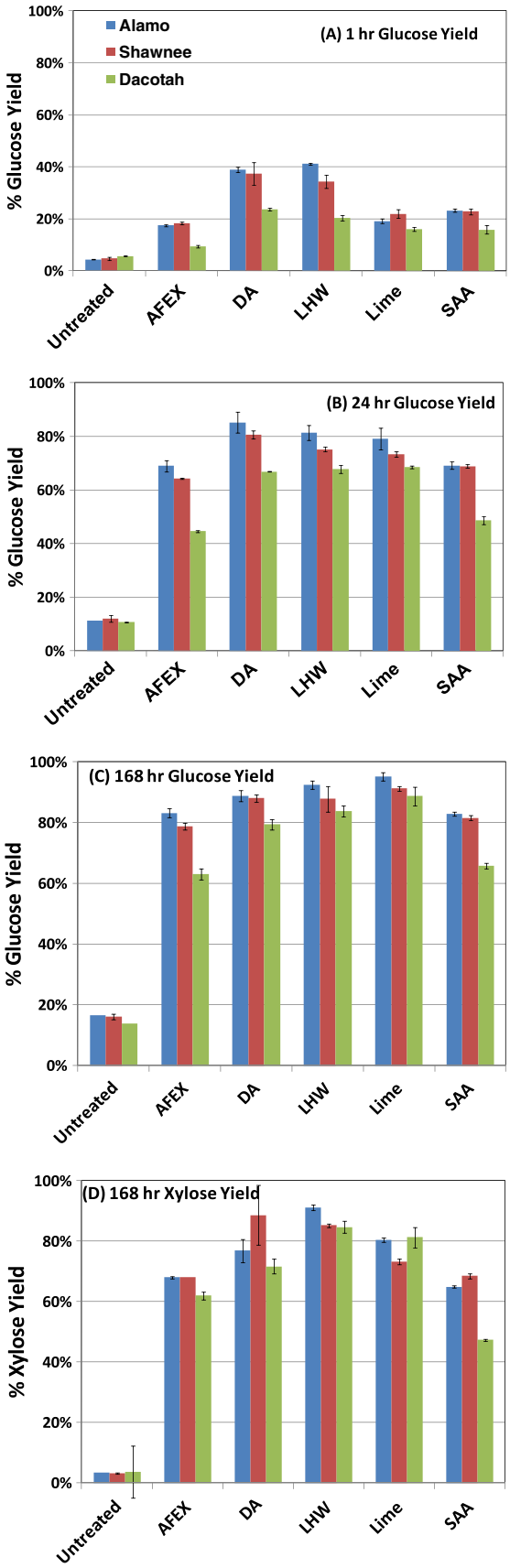

Fig. 4. Enzymatic digestibility of switchgrass pretreated by various pretreatment technologies. Pretreatment conditions are given in Table 2. Yields based on glucan or xylan in pretreated/hot washed solids for all pretreatments except for AFEX. Error bars represent 95% CI.

the July harvest of CIR. On the other hand, October harvest of lowland Alamo released more glucose than upland CIR on average, which is consistent with the results in this study. One of the reasons suggested for this observation was that the early harvests contain more soluble sugars than later harvests, requiring lower pretreatment conditions to preserve the soluble sugars. Since the switchgrass samples in this study were pre-extracted by water to remove all soluble sugars before pretreatment and hydrolysis, the effect of soluble sugars on determining pretreatment conditions and the digestibility of the pretreated switchgrass was excluded. As suggested by Bals et al. (2010), a lower lignin content may be one of the reasons for the greater sugar yields observed in the early harvests of the switchgrass. Another factor would be that the drying of the switchgrass over winter may negatively affect the recalcitrance of the cellulose. Switchgrass plots over the winter dry out and become more brittle as the plots undergo dry and cold weather (Adler et al., 2006). Pretreatment efficiency and enzyme accessibility of switchgrass might have decreased due to collapse of plant cell walls from the drying over the winter. What factors are responsible for these observations and how they affect on biochemical conversion of the different types of switchgrass and harvests are still elusive and deserve a further study.

Recommended optimal harvest date is varied depending on the types of biofuel to be produced and conversion technology. Some authors in prior literature recommended delaying switchgrass harvest for thermochemical conversion of switchgrass due to increasing dry mass and lignocellulose concentration and decreasing ash and nitrogen contents for the later harvests (Casler and Boe, 2003; Adler et al., 2006). Spring harvests are also expected to minimize impact on wildlife habitat value (Adler et al., 2006). The study by Bals et al. (2010) suggests that the benefit for early harvest is greater than later harvest due to lower costs for pretreatment and higher ethanol yields of early harvests. Consistent to their findings, this study also confirms that delaying harvest till spring has detrimental effects on biochemical conversion of cellulose to fermentable sugars. It should be noted, however, harvesting switchgrass too early of growing stages have adverse effects on stands in long-terms (Casler and Boe, 2003). Generally, the early harvests also contain more moisture than later harvest which may contribute to add up the overall transportation and storage costs. Thus, optimal harvest strategies should be determined carefully, considering various factors, such as types of conversion processes, water content of harvests, transportation to conversion facility, storage methods, long-term biomass yields, etc., which also have implications for overall biorefining processes. A further study in a more controlled experimental design would be critical to separately gauge the significance of these variables on biochemical conversion of switchgrass to biofuels. An integrated approach to analyze the correlation between the factors and biofuel production from switchgrass would be critical to cost- and energy-efficient utilization of switchgrass for biofuel production.

4. Conclusions

For all pretreatment technologies studied (AFEX, dilute acid, liquid hot water, lime, and soaking in aqueous ammonia) saccharification yield response was strongly correlated with harvest season of the switchgrass, even when non-structural sugars were excluded. The contributions of eco/genotypes of switchgrass to sugar yields were less obvious than the harvest season. The patterns of saccharification yields to harvest season was similar regardless of the pretreatment methods applied, though to a varying extent. Harvest time was a more important factor than ecological or morphological type of the switchgrass in determin-

ing the quality of the switchgrass feedstock for biofuel production.

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