



Comparison of big bluestem with other native grasses: Chemical composition and biofuel yield



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ABSTRACT

Multiple entry selections of big bluestems and three native C4 grass species, including switchgrass, miscanthus, and Conservation Reserve Program (CRP) mixture grass, were evaluated for their chemical composition and ethanol yields via diluted sulfuric acid pretreatment following simultaneous saccharification and fermentation (SSF). Big bluestem and switchgrass had a similar glucan content that was significantly higher than miscanthus and CRP grass. Big bluestem had the highest average mass recovery (55.56%) after acid pretreatment, and miscanthus had the lowest mass recovery (46.3%). A positive correlation was observed between glucan recovery and mass recovery. No significant difference in average efficiency of SSF was observed among four native grasses, but ethanol yields from big bluestem entries, which averaged 26.2%, were consistently greater than the other three grasses. The highest ethanol yield among the 10 entries was from big bluestem cultivar KAW (27.7%). Approximately 0.26 kg ethanol with 9.4 g/L concentration can be produced from 1 kg of big bluestem biomass under current processing conditions. A negative relationship exists between lignin content and the efficiency of SSF with $R = -0.80$, and a positive relationship exists between ethanol yield and glucan content with $R = 0.71$.

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1. Introduction

The development of biofuels from lignocellulosic biomass could reduce our dependence on fossil fuel resources, reduce greenhouse gas emissions, and reduce competition between food and fuel [1]. Perennial herbaceous energy crops are abundant sources of lignocellulosic biomass, but they are not commonly recognized as important as traditional agricultural residues. In fact, perennial herbaceous energy crops may offer many economic benefits, including high yield, ability to grow easily in an annual cycle without pesticides or fertilizers and with low energy input, ability to increase wildlife biodiversity, ability to increase soil quality, ability to reduce soil nutrient losses and to promote nutrient recycling from municipal and agricultural wastes, ability to

sequester soil carbon, and ability to mitigate greenhouse gas emissions [2]. The United States (US) Department of Energy (DOE) established the Herbaceous Energy Crops Research Program (HECP) in 1984 to develop data and information leading to commercially viable systems for production of herbaceous biomass for fuels and energy feedstocks [3]. Thirty-five potential herbaceous crops, 18 of which are perennial grasses such as big bluestem, switchgrass, and Conservation Reserve Program (CRP) mixture grass, were initially studied in the HECP [4].

Big bluestem (*Andropogon gerardii*), an ecologically dominant warm-season (C4) perennial native grass that comprises as much as 80% of plant biomass in the Midwestern prairies of North America, has been reported that the average and range of cellulose content, hemicellulose content, and biomass yield were 37.2% with a range of 33.5–49.8%, 23% with a range of 17.7–31.5%, and 7 Mg/ha with a range of 3.2–11.4 Mg/ha, respectively. The potential ethanol per hectare unit was calculated by multiplying yield data (kg/ha) bases on cellulose content (% of dry biomass), yielding a factor of 1.11 to

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account for weight gain during hydrolysis because of the addition of a water molecule. During glucose to ethanol fermentation, the resulting kilogram glucose per hectare data was multiplied by 0.5114 to account for the weight loss of two carbon dioxide molecules, and multiplied by 1.2764 to convert ethanol weight to volume (kilogram to liter). The average estimated ethanol yield of big bluestem calculated from a previous study was 1886 L/ha [5]. Big bluestem productivity is relatively high due to efficient utilization of nutrients; research has shown that big bluestem produces twice the biomass per unit of applied nitrogen compared with switchgrass or indiangrass [6]. Big bluestem also establishes easily from seed and spreads vigorously by vegetative growth of underground rhizomes with a robust root system [7]. In addition to low input costs and other economic considerations, bluestem prairie carries the advantage of serving a range of purposes in the ecosystem because it provides wildlife habitat, cattle grazing, and hay and pasturelands [8].

Switchgrass (*Panicum virgatum*) is another native C4 perennial grass on North America prairies that achieves biomass yield similar to or slightly higher than big bluestem [9]. Switchgrass has been selected as a “model” high-potential energy crop by Oak Ridge National Laboratory (ORNL) [4]. Switchgrass had the highest yields in DOE research trials in the mid-1980s, and breeding work was subsequently focused on switchgrass to the exclusion of other options [10]. Switchgrass has potential as a renewable fuel source but will necessitate large infrastructural changes, and even at maximum output, such systems could not provide the energy currently derived from fossil fuels [11]. Previous reviews have summarized switchgrass's potential as an energy crop in terms of historical study, biological and agronomical aspects, biofuel production via sugar and thermal platforms, and other utilizations and constraints [4,10,11].

Miscanthus (*Miscanthus sinensis*), originating from Asia, is a perennial non-wood rhizomatous tall grass native to subtropical and tropical regions. Miscanthus was first cultivated in Europe in the 1930s, when it was introduced from Japan [12]. Miscanthus has been used as a biofuel feedstock in Europe since the early 1980s and recently in North America for productivity trials [12–14]. Miscanthus, the European “model” herbaceous energy crop, was initially studied as a fuel source for steam and power generation. Research showed biomass yields of established miscanthus stands from 38.1 to 60.8 Mg/ha [12–14]. Miscanthus offers many advantages, such as low fertilizer and pesticide requirements, and some limitations, which are high establishment costs, poor overwintering, and insufficient water supply at some sites [12]. Miscanthus was recently identified as a promising energy crop for the Midwest, with yields exceeding those of switchgrass, the DOE model species, in U.S. side-by-side replicated field trials [15].

The Conservation Reserve Program (CRP) is a cost-share and rental payment program under the United States Department of Agriculture (USDA) [16] that is administered by the USDA Farm Service Agency (FSA) to prevent soil erosion and enhance groundwater recharge from highly erodible lands. CRP grasses comprise native perennial grasses such as big bluestem, indiangrass, little bluestem, switchgrass, sideoats grama, silver bluestem, sand lovegrass, bundleflower, sunflower, and Old World bluestem [17]. The percentage of each species within the grass mixture varies by location. CRP grass has a great biomass yield potential, with 38–63 million dry metric tons anticipated every year [18]. Research on CRP grass mixture has focused primarily on its impact on soil quality [19]. Linnebur recently studied the potential of CRP grass for biofuel production, focusing on effects of torrefaction as a pre-treatment method on chemical and elemental compositions, thermal properties, and energy density of CRP biomass [20].

Although research data for big bluestem are less available than for switchgrass and miscanthus, natural pure stands of big bluestem are more common than switchgrass in Midwestern tallgrass prairies. In general, big bluestem is more palatable than hay and grass in the latter part of the season, so producers may prefer big bluestem as a long-term option [21]. Some landowners also consider switchgrass excessively invasive [22]. Production of ethanol and value-added chemicals via consolidated bioprocessing (a direct fermentation process) have indicated that big bluestem is a superior feedstock to switchgrass and eastern gamagrass [23]. Another advantage of big bluestem is that it can produce twice the biomass per unit of applied nitrogen than switchgrass or indiangrass [7]. In addition, big bluestem is the dominant species in the second year, whereas switchgrass dominates in the first establishment year [24], thus reinforcing that big bluestem increased significantly when grown in monoculture or with indiangrass and switchgrass in the second year [25]. Madakadze et al. reported that in southwestern Quebec, Canada, the list of average lignocellulose content ranked from high to low was cordgrass, big bluestem, switchgrass, sandreed, and indiangrass [26]. Waramit et al. reported that big bluestem tends to contain higher cellulose concentrations than switchgrass [27].

Our research has shown that big bluestem has favorable bioconversion characteristics and comparable bio-oil yield through hydrothermal conversion [28–30]. Few direct comparisons of the potential biofuel yield of big bluestem with other herbaceous perennial biomasses are available. Therefore, the objectives of this research were to compare the chemical composition of big bluestem and three other promising native herbaceous perennial biomass including switchgrass, miscanthus, and CRP grass, to study their potential on ethanol yield through sulfuric acid pretreatment following simultaneous saccharification and fermentation (SSF), and to provide useful insights for bioenergy industry and biomass producers.

2. Materials and methods

2.1. Materials

Three big bluestem ecotypes, including Cedar Bluffs (CDB), Top of the World (TOW), 12Mile (12 M), and the KAW cultivar, which are widely planted to restore marginal lands, were harvested in October 2013 from reciprocal garden plots at the Plant Materials Center in Manhattan, KS. Four switchgrass genotypes (three genotypes, SWG 2007-1, SWG 2007-2, and SWG 2007-3, were from Oklahoma State University's switchgrass breeding program and were provided by Dr. Yanqi Wu) and one switchgrass native (SWNT) were used in this study. The switchgrass field was established in 2010 by transplanting seedlings and thereafter harvesting annually in fall (October–November). The miscanthus field was established in 2009. Plant biomass samples for switchgrass and miscanthus were harvested from the Kansas State University Agronomy Farm in Manhattan, KS, in late October 2013 and used for further analyses. CRP grass was generously provided by an agricultural farm at Bison, KS. The grass samples were ground into powder using a Retsch cutting mill (Haan, Germany) with a 1-mm sieve. All chemicals used for this research were purchased from Sigma Chemical Co. (St. Louis, MO).

2.2. Composition analysis

Moisture content of ground biomass samples was determined by drying approximately 2 g of each sample in a forced-air oven at 105 °C for 4 h [31]. Extractives, glucan, and lignin contents of the biomass samples were determined by following NREL laboratory

analytical procedures [31]. Lignin, the major non-carbohydrate component, is the sum of acid-insoluble and acid-soluble lignin. Glucan after enzymatic hydrolysis was determined by a high-performance liquid chromatograph (HPLC) (Shimadzu, Kyoto, Japan) equipped with an RCM monosaccharide column (300 × 7.8 mm) (Phenomenex, Torrance, CA) and a refractive index detector (RID10A, Shimadzu, Kyoto, Japan). The mobile phase was 0.6 mL min⁻¹ of double-distilled water, and the oven temperature was 80 °C.

2.3. Sulfuric acid pretreatment

Pretreatment was conducted in a reactor (Swagelok, Kansas City Valve & Fitting Co., Kansas City, KS) made from 316 L stainless steel with a measured internal volume of 75 mL (outside diameter of 38.1 mm, length of 125 mm, and wall thickness of 2.4 mm). The ground grass sample was mixed with 1.5% w/v diluted sulfuric acid to load 8.0% (w/v, 4 g dry mass in 50 mL solution) solid content.

A sand bath (Techne, Inc., Princeton, NJ) with a temperature controller was used as the heating source. After the sand temperature was increased to 160 °C, the reactor was submerged in boiling sand for 40 min, then immediately transferred to room-temperature water to decrease the internal temperature below 50 °C in 2 min. All slurry removed from the reactor was washed with hot distilled water and separated by filtration. The supernatant was collected into a 100-mL volumetric flask. Part of the supernatant was analyzed by HPLC, as described above. A portion of the solid mass after filtration was used for enzymatic hydrolysis, and the remaining portion and the liquid part were used for moisture and glucan content determination. Glucan recovered as solids in pretreatment residues was defined as in eq. (1):

$$\text{Glucan recovery (\%)} = \frac{m_{\text{pretreatment}}}{m_{\text{original}}} \times 100\% \quad (1)$$

where $m_{\text{pretreatment}}$ (g) is the weight of glucan after acid pretreatment, and m_{original} (g) is the weight of glucan in the original biomass.

2.4. Enzymatic hydrolysis

Enzymatic hydrolysis was carried out with the pretreated sample at 4% solids concentration (grams dry weight per 100 mL) in 50 mM sodium acetate buffer solution (pH 5.00) and 0.02% (w/v) sodium azide to prevent microbial growth. Enzyme loading (Accellerase 1500, containing glucan and β-glucosidase, provided by Dupont Genencor Science, Wilmington, DL) was 1 mL g⁻¹. Flasks mixed with sample, buffer solution, and enzyme were incubated in a water bath at a constant temperature of 50 °C and agitation of 140 rpm. Total sugar analysis was conducted at the end of hydrolysis (72 h) on supernatants by HPLC as previously described. Efficiency of enzymatic hydrolysis (EEH) was calculated by eq. (2):

$$\text{EEH (\%)} = \frac{c \times V \times 0.9}{m_{\text{glucan}}} \times 100\% \quad (2)$$

where c is the concentration (g/L) of glucan after 72 h of enzymatic hydrolysis determined by HPLC analysis, V is the total volume (L), and m_{glucan} is the weight of glucan before enzymatic hydrolysis (g). The factor 0.9 is the glucan-to-glucose content conversion factor.

2.5. Simultaneous saccharification and fermentation

An identical enzyme and buffer system with 4% solids concentration were used in SSF as in enzymatic hydrolysis without antibiotics. Activation of dry yeast (Ethanol Red, Lesaffre Yeast Corp., Milwaukee, WI) was conducted by adding 1.0 g of dry yeast to 19 mL of preculture broth (containing 20 g glucose, 5.0 g peptone, 3.0 g yeast extracts, 1.0 g KH₂PO₄, and 0.5 g MgSO₄·7H₂O per liter) and shaking at 200 rpm in an incubator at 38 °C for 25–30 min. The activated yeast culture had a cell concentration of ≈ 1 × 10⁹ cells/mL, ensuring that the inoculated system contained a yeast concentration of ≈ 1 × 10⁷ cells/mL. SSF was conducted in an incubator shaker (Model I2400, New Brunswick Scientific Inc., Edison, NJ) at 38 °C and 150 rpm. At time intervals of 0, 3, 8, 24, 48, and 72 h, 0.5 mL were removed from each flask. The sample was centrifuged and diluted 10 times, and the supernatant was filtered for sugar analysis and ethanol yield by HPLC. Efficiency of SSF (ESSF) and total ethanol yield were calculated using eqs. (3) and (4):

$$\text{ESSF (\%)} = \frac{c \times V}{m_{\text{glucan}} \times 1.11 \times 0.511} \times 100\% \quad (3)$$

where c is the concentration (g/L) of ethanol after 72 h of SSF determined by HPLC analysis, V is the total solution volume (L), and m_{glucan} is the weight of glucan before enzymatic hydrolysis (g). Factor 1.11 and 0.511 are the glucan-to-glucose content conversion factor and the mass coefficient of glucose to ethanol, respectively.

$$\text{Ethanol yield (\%)} = \frac{\text{Weight of ethanol}}{\text{Weight of feedstock}} \times 100\% \quad (4)$$

2.6. Statistical analysis

All data were reported as the average of duplicates. Analysis of variance (ANOVA) and Tukey's studentized range (HSD) test were analyzed using SAS (SAS Institute, Inc., Cary, NC). In general, fully balanced ANOVA tests were performed following the general linear models (GLM) procedure. Correlations were determined using Pearson's correlation.

3. Results and discussion

3.1. Chemical composition comparison between big bluestem and other native grasses

Structural polysaccharides and lignin comprise the major chemical composition of native grasses. Structural polysaccharides contain cellulose and hemicellulose; cellulose is represented by glucan, whereas hemicellulose primarily constitutes xylan and arabinan. Lignin is a complex phenolic polymer. Based on Tukey's HSD test ($P < 0.05$), the chemical composition of the 10 entries from four native grass species varied significantly, with the exception of arabinan content, as shown in Table 1. Chemical composition ranged from 31.3 to 39.9% for glucan, 19.5–28.7% for xylan, 3.8–4.5% for arabinan, and 14.1–15.5% for lignin. The low lignin content of grasses is desirable for enzymatic hydrolysis and ethanol fermentation, but lignin content is higher (20–30%) in woody biomass such as pine, poplar, spruce, and willow [32]. Of the 10 entries, big bluestem native cultivar KAW had the highest glucan content (39.9%) and the highest xylan content, and it had the second-highest lignin content of the three other big bluestem entries. Western ecotype CDB had the highest glucan content among the three big bluestem ecotypes, thus confirming our previous research regarding the effect of ecotype on the chemical composition of big bluestem [28]. Switchgrass genotype SWG 2007-2 had

Table 1
Summary of glucan, xylan, arabinan, and lignin contents of 10 native grass entries.

Entry	Glucan content (%)	Xylan content (%)	Arabinan content (%)	Lignin content (%)
Big bluestem				
CDB	38.1 ± 0.1 g ^a	23.2 ± 0.1 bc	4.1 ± 0.1 a	15.2 ± 0.1 abc
TOW	35.0 ± 0.2 b	22.3 ± 0.1 b	3.8 ± 0.1 a	14.7 ± 0.9 abc
12M	35.9 ± 0.1 cd	22.7 ± 0.2 bc	4.5 ± 0.1 a	14.1 ± 0.1 a
KAW	39.9 ± 0.4 h	25.8 ± 0.2 eg	4.1 ± 0.1 a	15.0 ± 0.1 abc
Switchgrass				
SWG 2007-1	36.9 ± 0.2 ef	25.0 ± 0.4 de	4.0 ± 0.1 a	15.9 ± 0.1 c
SWG 2007-2	37.1 ± 0.2 f	28.7 ± 0.1 h	4.4 ± 0.1 a	15.2 ± 0.1 abc
SWG 2007-318	36.2 ± 0.4 de	26.5 ± 0.9 g	4.2 ± 0.5 a	15.3 ± 0.2 bc
SWG-native	35.3 ± 0.1 bc	27.0 ± 0.2 g	4.4 ± 0.1 a	15.5 ± 0.1 bc
Miscanthus				
Miscanthus	31.3 ± 0.1 a	24.1 ± 0.5 cd	3.9 ± 0.3 a	14.4 ± 0.3 ab
CRP grass				
CRP grass	31.5 ± 0.1 a	19.5 ± 0.3 a	4.2 ± 0.3 a	14.9 ± 0.1 abc

^a Means with the same letter within the same group are not significantly different at $P < 0.05$.

the highest xylan content (28.7%), and the switchgrass native had the highest lignin content (15.5%) of all entries. The CRP grass mixture had lower glucan, xylan, and lignin content than other grasses. Our hypothesis was that although big bluestem and switchgrass comprise a majority of CRP grass mixture, other constituent grasses may cause significant differences in CRP grass chemical composition. Average glucan, xylan, and lignin contents of four native grass species are compared in Fig. 1. Big bluestem and switchgrass had similar glucan content, which was significantly higher than miscanthus and CRP grass. Big bluestem, switchgrass, and miscanthus had similar xylan content, which was significantly higher than CRP grass. Lignin content for switchgrass was significantly higher than big bluestem. In this study, the chemical composition of big bluestem was consistent with previous research [28,33], but switchgrass had similar polysaccharides content and lower lignin content than previous research [28,29]. Cell wall composition may differ due to method of analysis, climate, harvest date, and crop cultivation practices.

3.2. Diluted acid pretreatment

Diluted sulfuric acid pretreatment of big bluestem and other grasses was conducted at 1.5% acid concentration at 160 °C for

Table 2
Summary of glucan, xylan, arabinan, and lignin contents of 10 native grass entries after 1.5% sulfuric acid pretreatment.

Entry	Glucan content (%)	Xylan content (%)	Arabinan content (%)	Lignin content (%)
Big bluestem				
CDB	62.9 ± 0.2 ef ^a	<1.0	4.2 ± 0.5 a	31.9 ± 1.2 a
TOW	59.6 ± 0.4 cd	<1.0	4.2 ± 0.9 a	33.2 ± 0.1 a
12M	57.9 ± 0.2 bc	<1.0	3.9 ± 0.9 a	34.0 ± 0.2 ab
KAW	63.8 ± 0.3 f	<1.0	4.3 ± 0.4 a	32.5 ± 0.5 a
Switchgrass				
SWG 2007-1	58.5 ± 0.2 c	<1.0	4.8 ± 0.1 a	37.2 ± 0.4 cd
SWG 2007-2	61.1 ± 0.5 de	<1.0	5.2 ± 0.6 a	35.7 ± 1.0 bc
SWG 2007-3	59.7 ± 0.4 cd	<1.0	4.7 ± 0.1 a	36.7 ± 0.3 c
SWG native	55.7 ± 0.8 ab	<1.0	3.7 ± 0.2 a	37.2 ± 0.3 cd
Miscanthus				
Miscanthus	56.1 ± 1.0 ab	<1.0	4.2 ± 0.2 a	36.8 ± 0.6 c
CRP grass				
CRP grass	54.0 ± 1.0 a	<1.0	4.3 ± 1.1 a	39.5 ± 0.6 d

^a Means with the same letter within the same group are not significantly different at $P < 0.05$.

40 min. This pretreatment condition was optimized by different acid concentrations (0%, 1.0%, 1.5%, and 2.0%) in our previous study [34]. Acid pretreatment significantly increased glucan and lignin contents compared with grasses without acid pretreatment (Table 2). Diluted acid pretreatment notably did not significantly affect the trend of glucan and lignin content of the 10 entries in this study, indicating that all entries responded similarly to diluted acid pretreatment; however, almost all xylan and most arabinan were hydrolyzed during diluted acid pretreatment, corresponding to less than 1% xylan content and decreased arabinan content (Table 2). The decrease of xylan and arabinan contributed to an increase in glucan and lignin contents in the treated biomass. Hemicellulose content of grasses after pretreatment decreased to 4% from initial content of 24–33%, suggesting that diluted acid pretreatment helps release cellulose for enzymatic hydrolysis because hemicellulose is recognized as a shielding factor in cellulose digestion [35,36]. Average mass recovery and glucan recovery for four native grass species are shown in Fig. 2. Big bluestem had the highest mass recovery (55.6%), and miscanthus had the lowest mass recovery (46.3%). Glucan recovery showed a trend similar to that of mass recovery and a positive relationship, with a coefficient of determination of $R^2 = 0.76$ (Fig. 3). Big bluestem yielded the highest glucan recovery of up to 90.2%, suggesting that big bluestem had

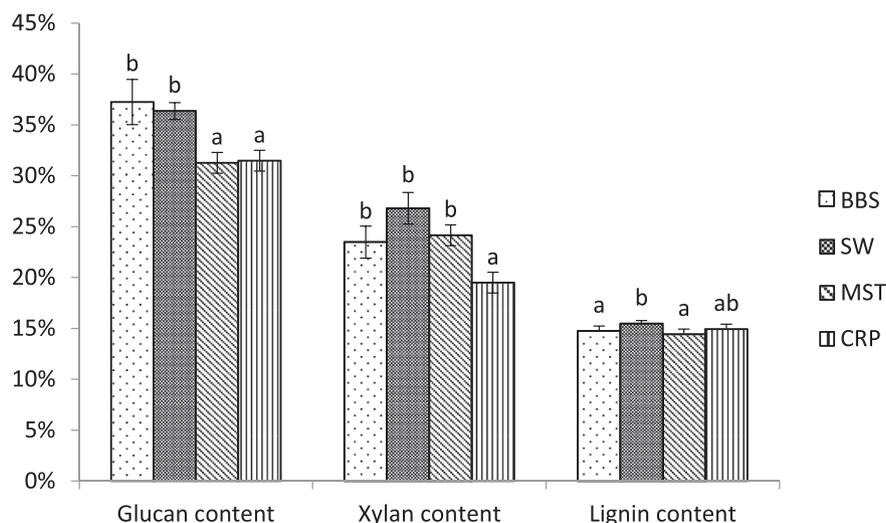


Fig. 1. Comparison of average glucan, xylan, and lignin contents of four native grass species. The bars with the same letter within the same group are not significantly different at $P < 0.05$.

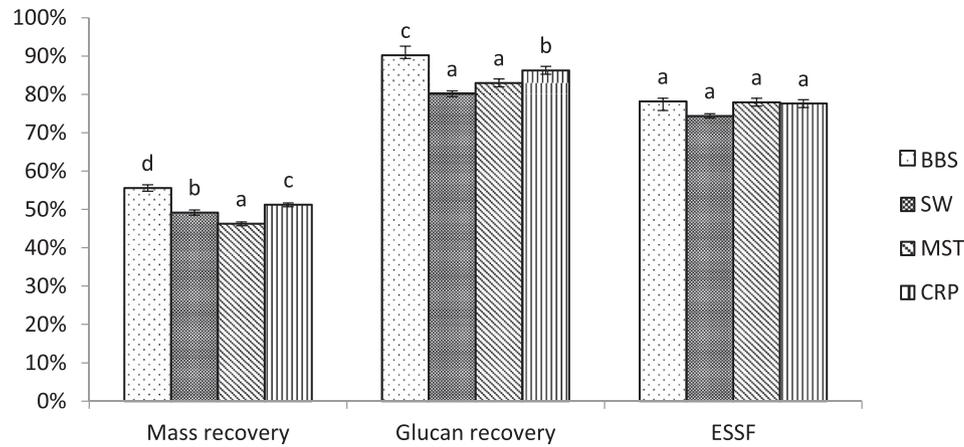


Fig. 2. Comparison of average mass recovery, glucan recovery, and efficiency of simultaneous saccharification and fermentation (ESSF) of four native grass species. Bars with the same letter within the same group are not significantly different at $P < 0.05$.

the best pretreatment efficiency, corresponding to less than 10% glucan degradation.

3.3. Simultaneous saccharification and fermentation

Time course of EEH and ESSF of big bluestem cultivar KAW is shown in Fig. 4. Two curves followed a classical hydrolysis and fermentation pattern in which glucan conversion rapidly increased in the first 24 h and reached its maximum after 72 h. However, the ESSF curve lagged behind the EEH curve throughout the entire process because SSF started after glucose was released from cellulose through enzymatic hydrolysis. Moreover, maximum ESSF of big bluestem cultivar KAW was 79%, which is less than its EEH (90%) because some by-products (e.g., glycerol) were formed during the fermentation process. By-product formation during ethanol fermentation was also reported by Yazdani and Gonzalez [37]. Ethanol and glucose time profiles for big bluestem cultivar KAW during the SSF process is shown in Fig. 5. Glucose concentration in fermentation broth increased during the first 7 h, then decreased when the yeast began to uptake glucose at a higher rate and almost completely consumed it throughout the SSF process. No significant statistical difference in average ESSF existed among the four native grasses, although big bluestem had a higher ESSF of 78.2% (Fig. 2). A possible explanation is that the effect of diluted acid pretreatment was too strong, and that removing most of the obstacle of biomass structure neutralized differences in the grasses. Previous studies

reported slightly higher ESSF for switchgrass (87–90%) and miscanthus (72–91.2%) compared with this study [38–40]. The effect of lignin content on ESSF is shown in Fig. 6. The negative relationship between lignin content and ESSF was significant at $P < 0.05$ with $R^2 = 0.63$, indicating that recalcitrance of cellulose increased as lignin content increased. The lignin content explained 63% of the variation in ESSF (Fig. 6). Accordingly, reducing lignin content can benefit ethanol yield. Previous study has also reported that reducing lignin content can improve conversion efficiencies of lignocellulosic biomass to sugars and ethanol [41]. Average ethanol yield of big bluestem, switchgrass, miscanthus, and CRP grass were $26.2 \pm 1.3\%$, $21.7 \pm 0.6\%$, $20.2 \pm 1.0\%$, and $21.1 \pm 1.0\%$ of dry mass, respectively. Big bluestem entries were consistently greater than the three other grasses. Big bluestem cultivar KAW had the highest ethanol yield (27.7%) among the 10 entries, corresponding to the highest glucan content. Comparison of ethanol yield among the entries is shown in Table 3. Big bluestem-KAW and the average of big bluestem had significantly higher ethanol yields than the other three grasses. In addition, published results have shown that big bluestem has a higher ethanol yield than other biomasses such as corn stover (7–19%) [42–44], wheat straw (6–20%) [45,46], rice straw (11%) [47], sweet sorghum (19%) [48], aspen (10%) [49], and spruce (8%) [50]. A positive relationship has been found between glucan content and ethanol yield (slope = 0.71 and $P < 0.01$) (Fig. 7). The relatively low coefficient of determination ($R^2 = 0.50$) of the linear regression in Fig. 7 indicates that ethanol yield was likely

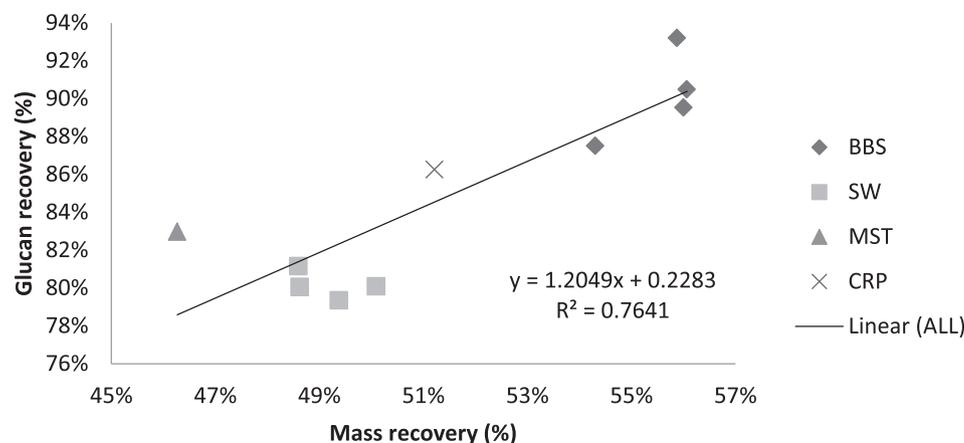


Fig. 3. Relations between mass recovery (%) and glucan recovery (%) after diluted acid pretreatment.

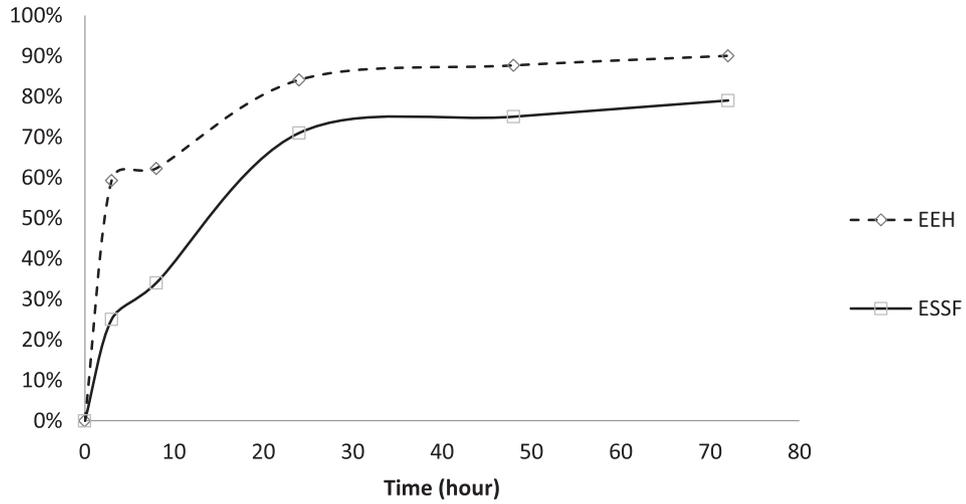


Fig. 4. Time course of efficiency of enzymatic hydrolysis (EEH) and efficiency of simultaneous saccharification and fermentation (ESSF) of big bluestem cultivar KAW.

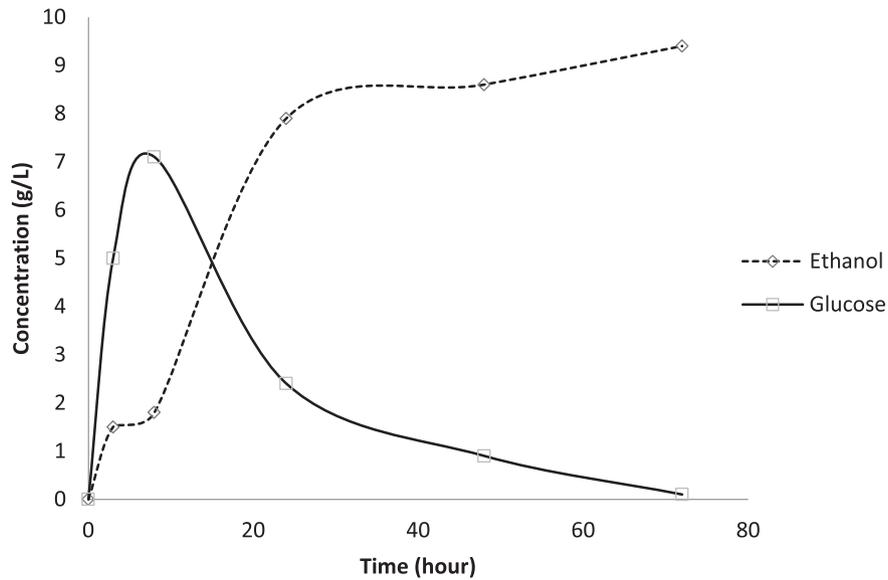


Fig. 5. Ethanol yield and glucose consumption time profile for big bluestem cultivar KAW simultaneous saccharification and fermentation process.

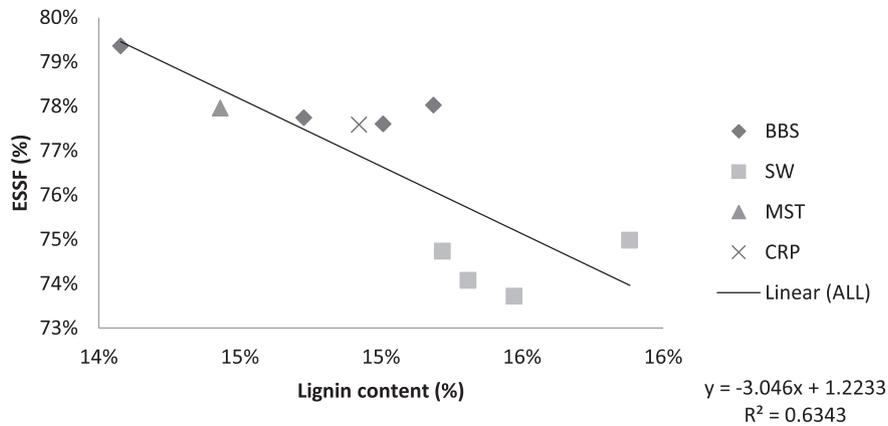


Fig. 6. Relationship between lignin content (%) and efficiency of simultaneous saccharification and fermentation (ESSF) (%).

Table 3
Comparison of ethanol yield of big bluestem with switchgrass, miscanthus, and CRP grass.

Specie	Ethanol yield (% dry biomass)
Best big bluestem-KAW	27.7 ± 1.1 b ^a
Switchgrass	21.7 ± 0.6 a, A ^b
Miscanthus	20.2 ± 1.0 a, A
CRP grass	21.1 ± 1.0 a, A
Big bluestem-average	26.2 ± 1.3 b B

^a Lowercase letters (a and b) indicate whether the means of ethanol yield of the best-yielding big bluestem-KAW, switchgrass, miscanthus and CRP grass are significantly different at $P < 0.05$.

^b Uppercase letters (A and B) were used to indicate the difference among the average ethanol yield for all big bluestems, switchgrass, miscanthus, and CRP grass. Means with the same letter are not significantly different at $P < 0.05$.

affected by other factors besides the glucan content of feedstocks, such as pretreatment methods, enzyme loading, etc. Fig. 8 shows detailed mass balance analysis of big bluestem with diluted sulfuric acid pretreatment and SSF. Approximately 0.26 kg ethanol with 9.4 g/L concentration can be produced from 1 kg big bluestem under current processing conditions. To the best of our knowledge, this is the first dataset that provides fundamental information

regarding big bluestem's potential as feedstock for ethanol production and a head-to-head comparison with other native grasses, specifically widely used switchgrass.

4. Conclusions

Big bluestem and switchgrass contain similar glucan content that is significantly higher than miscanthus and CRP grass. Big bluestem has lower lignin content than switchgrass. A positive correlation between glucan recovery and mass recoveries was observed. Fermentation results did not show a significant difference in average ESSF among four native grasses; however, ethanol yields of big bluestem entries (26.2%) were consistently greater than the three other grasses. The highest ethanol yield among the 10 entries was in big bluestem cultivar KAW (27.7%). A negative relationship exists between lignin content and ESSF with $R^2 = 0.63$, and a positive relationship exists between ethanol yield and glucan content with $R^2 = 0.50$. Approximately 0.26 kg ethanol with 9.4 g/L concentration can be produced from 1 kg big bluestem under current processing conditions. Results indicate that big bluestem could serve as a suitable energy grass in the Midwest with similar or better glucan content and ethanol yield compared with other native C4 grasses.

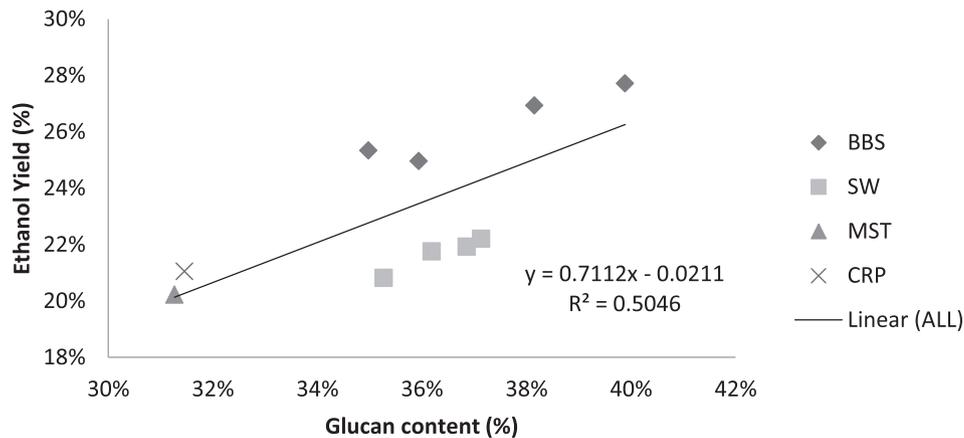


Fig. 7. Relationship between glucan content (%) and ethanol yield (%).

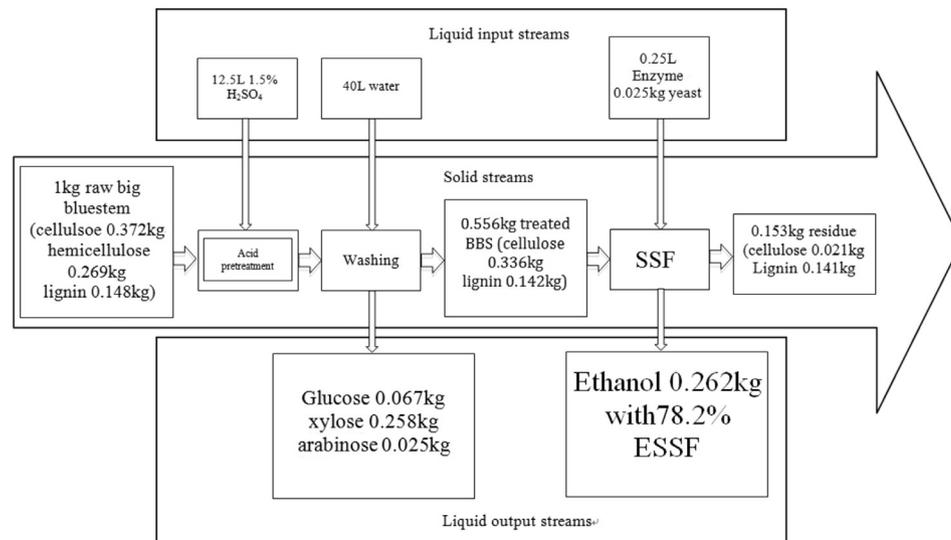


Fig. 8. Mass balance analysis of big bluestem processing for ethanol production.

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