THE EFFECT OF ECOTYPE AND PLANTING LOCATION ON PROPERTIES AND BIOFUELS YIELD OF BIG BLUESTEM

by

KE ZHANG

B.E., Central South University, China, 2005
M.S., Central South University, China, 2008

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Biological and Agricultural Engineering
College of Engineering

KANSAS STATE UNIVERSITY
Manhattan, Kansas

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Abstract

Renewable fuels derived from lignocellulosic biomass could reduce our dependence on fossil fuel resources and reduce greenhouse gas emissions. Big bluestem is an ecological-dominant warm-season (C4) perennial native grass that comprises as much as 80% of the plant biomass in prairies in the Midwestern grasslands of North America. Its high cellulosic content and low agricultural input recently have made big bluestem a promising feedstock for ethanol production. The overall goals of this study are to evaluate the potential of big bluestem in terms of ethanol production comparing with other native grasses by diluted sulfuric acid pretreatment and simultaneous saccharification and fermentation and to understand the effects of ecotype and planting location on the chemical and elemental compositions and thermal properties as well as fermentable sugar yield of big bluestem along the Great Plains precipitation gradient. A total conversion efficiency of 79.2% and an ethanol concentration of 9.4 g/L were achieved after 72 h fermentation. About 0.262 kg (~0.332 Liters) ethanol could be produced from one kilogram dry mass of big bluestem under the present condition.

Planting location had significant effects on chemical and elemental as well as specific heat, thermogravimetric parameters, high heating value and glucan mass yield. Ecotype had significant effects on glucan, xylan, lignin, and ash contents, and C, O, and H elemental fractions as well as specific heat, high heating value and glucan mass yield, whereas planting location significantly affected all measured variables. The ecotype-location interaction had significant effects on glucan, lignin, hydrogen contents and specific heat. Up to 97%, 88% and 80% of the variation in compositions can be explained by annual precipitation, growing degree days and potential evapotranspiration in 2010 respectively. Among all environmental factors, potential evapotranspiration had the most significant effect on thermal properties. Planting location had a stronger influence than ecotype and interaction between location and ecotype. Precipitation in 2010 possibly played a more significant role in divergence of glucan mass yield of the big bluestem.

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Table of Contents

List of Figures

List of Tables

Acknowledgements

Time really does fly when you're concentrating. This will be the last part I write for my doctoral dissertation. While that won't mean any change in my dedication to research, it does mean that one chapter of my career is drawing to a close.

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Dedication

To my wife Yusi Xu, my daughter Kelsey Zhang

To my beloved parents

Chapter 1 - Introduction

1.1 Abstract

Big bluestem (*Andropogon gerardii*) is an ecological-dominant warm-season (C4) perennial native grass that comprises as much as 80% of the plant biomass in prairies in the Midwestern grasslands of North America. The species was adopted as a forage crop. Its high cellulosic content and low agricultural inputs recently have made big bluestem a promising feedstock for ethanol production and bio-oil. The objective of this paper is to review the big bluestem as a bioenergy crop with respect to biological and conversion aspects. Biological aspect of big bluestem includes distribution and adaptation, ecotypes and varieties currently studied as well as production management and disease and pest control. Conversion aspects include discussion of the conversion of big bluestem biomass to bio-ethanol and bio-oil. Estimated ethanol yield of big bluestem is about 1886 L/ha, comparable to previously reported herbaceous biomass. Various constraints and potentials of big bluestem as an energy crop are analyzed in the final section of this paper. A critical need exist for plant breeding research which focuses on modifying big bluestem composition in order to minimize recalcitrance to bioconversion, as well as increasing biomass yields.

1.2 Background

1.2.1 Energy shortage and environmental benefits

Renewable fuels derived from biomass could reduce our dependence on fossil fuel resources and reduce greenhouse gas emissions (Dien et al., 2006). First-generation biofuel, produced from starch-based and sugar-based biomass, could not be sustainable due the competition with food crops and land availability (Tilman et al., 2006). Therefore, lignocellulosic biomass, including dedicated energy crops such as big bluestem, switchgrass, forest residues, and agricultural residues, could effectively impact biofuel production because they require low production inputs and less competition with food production.

The Biomass Research and Development Technical Advisory Committee (formed to advise the US Department of Energy (DOE) and US Department of Agriculture on program priorities as part of the USA Biomass Research and Development Act of 2000 set a national goal

for biomass to supply 5% of total industrial and electric generation energy demand, 20% of transportation fuel consumption, and 25% of biobased chemicals and materials by 2030 (Perlack et al., 2005), requiring an annual supply of 907 million Mg (1 billion dry tons) of biomass. Approximately one-third of this biomass is projected to oraginate from perennial crops such as big bluestem and switchgrass. Achievement of this goal requires significant technological advances in plant breeding, biology, and agronomy, and conversion technology (Koonin, 2006; Ragauskas et al., 2006).

The conversion of perennial grasses may offer environmental benefits, including increased soil quality, reduced losses of soil nutrients, recycling nutrients from municipal and agricultural wastes, soil carbon sequestration, and mitigating greenhouse gas emissions (Adler et al., 2007; Farrell et al., 2006; Sanderson et al., 2004).

1.2.2 Historical study of big bluestem as a bioenergy crop in the United States

The Herbaceous Energy Crops Research Program (HECP), one of three research programs supported by DOE pertaining on energy feedstocks supported, was established in 1984. This program focuses on evaluating the best non-woody species and geographical regions for energy crops. The overall goal of the HECP was to develop data and information that will lead to commercially viable systems for producing herbaceous biomass for fuels and energy feedstocks. Oak Ridge National Laboratory (ORNL) did the field management study and reported that big bluestem can be economically produced on various sites and incorporated into conventional farming operations. Six universities (Cornell University, Virginia Polytechnic Institute and State University, Auburn University, Purdue University, Iowa State University, and North Dakota State University) and one private company (Geophyta) were selected to participate in the evaluation of 35 potential herbaceous crops, 18 of which were perennial grasses projects, including big bluestem (Table 1.1) (L. Wright, 2007). At Purdue University, big bluestem and several other herbaceous crops were tested under five levels of fertilizer in both normal and droughty conditions from 1985 to 1989 at four sites. Big bluestem showed relatively similar yield to other perennial crops with 6.8-9.7 Mg/ha (Cherney et al., 1990). Anderson et al. (Anderson et al., 1996) from Iowa State University reported six herbaceous crops with mono-crop, double-crop, rotating-crop, and inter-crop combinations at various fertilizer levels at two sites (good cropland and marginal cropland) from 1988 to 1992. Big bluestem showed better

established ability than reed canarygrass in margin land in 1988 and biomass yield ranged from 5.5 to 29.7 Mg/ha. With a yield of 29.7 Mg/ha, the big bluestem yield was the highest among all of grasses evaluated and was almost five times higher than switchgrass at some conditions. Anderson et al. also conducted economic potential analysis that showed big bluestem competed well with other perennial crops, with an average specified cost of 33.13 dollar/dry Mg^{-1} in good cropland and 35.33 dollar/dry Mg^{-1} in margin land (Anderson et al., 1994)., In the early 1990's, the, USDA Natural Resources Conservation Service in Mississippi and southern Georgia conducted limited research on big bluestem as energy crops(Kszos et al., 2000). In addition, a grass breeding program led by Viands (approximately 16,000 individual plants transplanted from germinated seeds collected from the Northeast) was implemented to develop big bluestem varieties and other grasses for dedicated bioenergy feedstocks in terms of stand, pure live seed (PLS), seed tag label quick germination, seeding rate, and morphology parameters (Northern New York Agricultural Development Program, 2009).

Table 1.1 Species screened by Herbaceous Crops Program 1986-1992.

Species	Institution and year of project start						
	VA Tech	**Auburn**	**Geophyta**	**Cornell**	**Purdue**	**ISU[1]**	**NDSU[2]**
	1985	1985	1985	1985	1985	1988	1988
Grasses: Perennial							
Big bluestem (w)					X	X	
Bahiagrass (w)		X					
Bermudagrass (w)		X[4]					
Crested wheatgrass (c)							X
CRP mixture of grasses (c/w)							X
Eastern gamagrass (c)				X			
Energy Cane (w)		X					X
Intermediate wheatgrass (c)							
Johnsongrass (w)		X[4]					
Napiergrass (w)							
Redtop (c)				X			
Reed canarygrass (c)			X	X	X[5]	X	X
Smooth bromegrass (c)							X
Switchgrass (w)	X	X	X	X	X	X	X
Tall Fescue (c)	X	X	X		X[5]		
Timothy (c) /redtop (c)/ clover			X	X			
Weeping lovegrass (w)	X				X		
Wheatgrass mixture (c)							X
Grasses: Annual							
Corn (w)		X	X		X	X	
Pearl millet (w)		X[4]					
Foxtail millet (w)							X
Rye (c)		X[3]	X[3]		X[3]	X[3]	
Sorghum, Forage (w)			X			X	
Sorghum, Sweet (w)		X[4]			X	X	
Sorghum x sudangrass (w)	X				X	X	
Sudangrass (w)				X			
Legumes: Annual							
Soybeans						X	
Legumes: Perennial							
Alfalfa			X	X[6]	X	X[7]	X[6]
Birdsfoot trefoil	X		X		X	X	
Crownvetch	X						
Flatpea	X			X			
Serecia lespedeza	X	X			X		
Sweet clover				X			
Other							
Forage brassica				X			
Kale				X			
Meadow (mixed grasses & legumes)				X			

Note: Grasses are designated as cool season (c) or warm season (w) crops.

[1] ISU = Iowa State University.

[2] NDSU = North Dakota State University.

[3] Rye was always interseeded among other species or as the cool season species in a double crop system – most often with sorghums.

[4] These crops were frequently the base species in a double-cropping or intercropping systems.

[5] Reed canary grass and tall fescue were grown alone and interseeded with sorghum.

[6] Alfalfa was intercropped with bromegrass at Cornell and NDSU, and grown alone at NDSU.

[7] Alfalfa was intercropped with sorghum and sorghum x sudangrass.

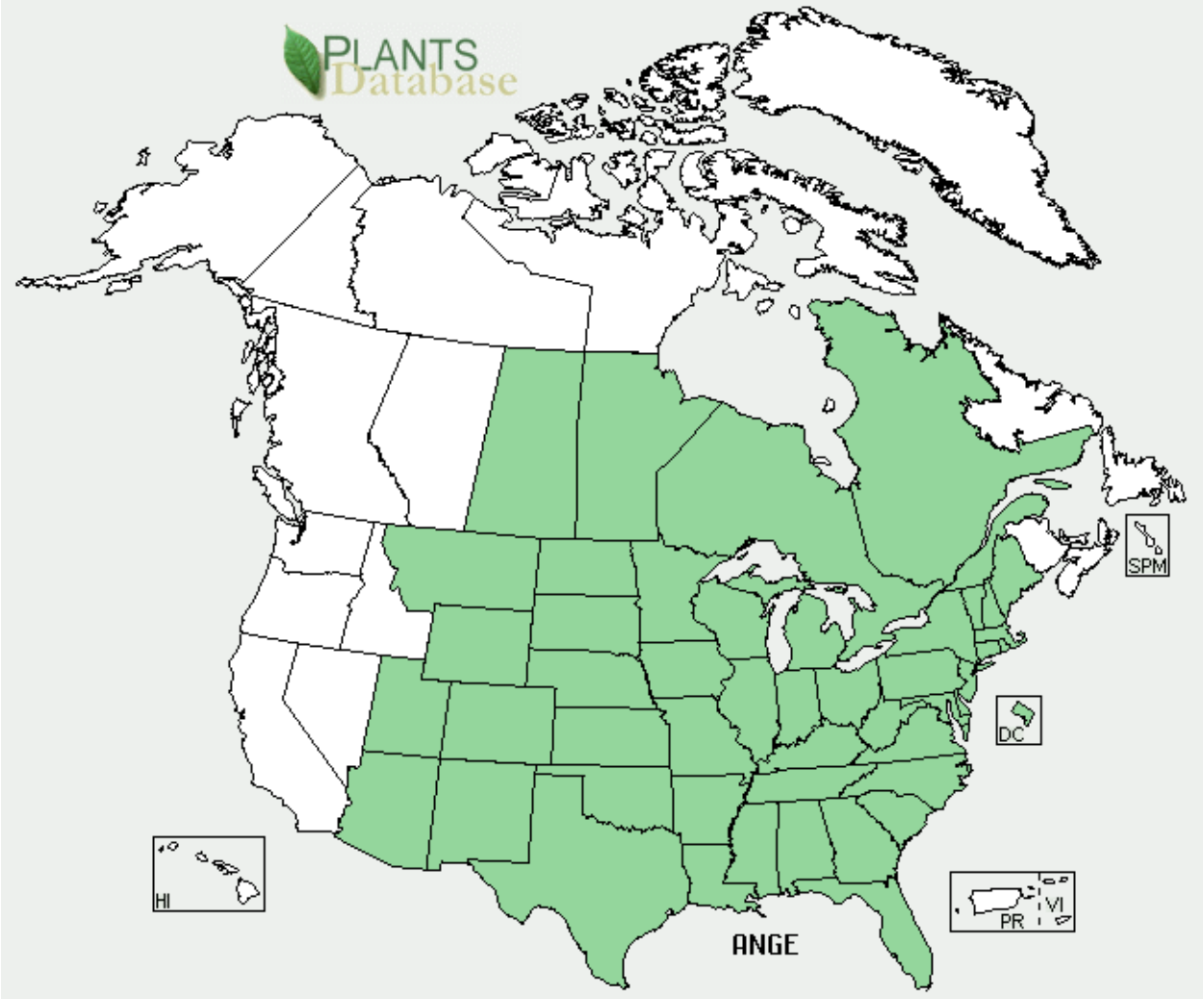
1.3 The biology of big bluestem

1.3.1 Description, distribution and adaptation

Big bluestem (*Andropogon gerardii*) is an ecological-dominant warm-season (C4) perennial native grass that comprises as much as 80% of the plant biomass in prairies in the Midwestern grasslands of North America (F. W. Gould and Shaw, 1983; A. K. Knapp et al., 1998). Big bluestem is often glaucous; clums robust, often in large tufts, sometimes with short rhizomes, 1 to 2 m tall, usually sparingly branching toward the summit; lower sheaths and blades sometimes villous, occasionally densely so, the blades flat, elongate, mostly 5 to 10 mm. wide, the margins very scabrous; racemes on the long-exserted terminal peduncle mostly 3 to 6, fewer on the branches, 5 to 10 cm. long, usually purplish, sometimes yellowish; rachis straight, the joints and pedicels stiffly ciliate on one or both margins, the joints hispid at base; sessile spikelet 7 to 10 mm. long, the first glume slightly sulcate, usually scabrous, the awn geniculate and tightly twisted below, 1 to 2 cm. long; pedicellate spikelet not reduced, or but slightly so, awnless, staminate. Roots of big bluestem may reach depths of two to four feet at the end of the establishment year. Roots of well-established plants may reach depths of seven to eight feet (Owsley).

Big bluestem is widely distributed among the United States, Canada, and Mexico, as shown in Figure 1. It also adapts well to low nutrient and moisture content soil. A recent analysis indicated that more than 25 million hectares of land classified by the USDA as rangeland/grassland within land capability class 3–6 soils (more marginal/less productive soils) could be utilized for bioenergy crop production in select states in the central Great Plains (Kansas, Nebraska, Oklahoma, and South Dakota) (USDA, 2010a). Big bluestem is adaptable in most native prairie ecosystems and represents three times the biomass as switchgrass in midwestern grasslands (Epstein et al., 1998). Big bluestem productivity is high due to efficient nutrition utilization; it produces twice the biomass per applied nitrogen compared to switchgrass and indiangrass (L. C. Johnson and Matchett, 2001), it establishes easily from seed, and it spreads vigorously by vegetative growth of underground rhizomes with a robust root system (Perry and Baltensperger, 1979). In addition to economic considerations, bluestem prairie serves a range of purposes in the ecosystem by providing wildlife habitat, cattle grazing, and hay and pasturelands (Fargione et al., 2009).

Figure 1.1 Big bluestem distribution from USDA-NRCS PLANTS Database.



1.3.2 Ecotypes and varieties currently studied

As big bluestem evolved across North America, nature selection at each locale produced a hypothetically unique gene to adapt specific environmental conditions. In addition, breeders collected seeds and reproduced them to evaluate the particular genetic and morphological characteristics as well biomass yield, quality, and adaptability. These strains with uniformity are registered as cultivar, ecotypes, or varieties (Table 1.2).

Waller and Lewis (Waller and Lewis, 1979) studied the relationship between latitude of origin and variety yield. Southern varieties of big bluestem that migrate north had higher biomass yield due to extended photoperiods and longer vegetative. Previous studies of ecotype have provided extensive information regarding agriculture, plant breeding, and restoration ecology (Hufford and Mazer, 2003), as well as broadening understanding of plant population structure and dynamics (Gray, 2012). Mintenko evaluated different ecotypes of native grasses in terms of turfgrass potential under three mowing heights across the northern Great Plains region (Mintenko et al., 2002). Etterson correlated climate change with the evolutionary potential of prairie legume by investigating three populations in three environments across a broad latitudinal range in the Great Plains (Etterson, 2004). The big bluestem ecosystem has been extensively studied for decades as it relates to the effect of climate on grass growth; controls on community structure; ecological responses to grazing, burning, and mowing; and restoration effectiveness (Epstein et al., 1998; Fay et al., 2003; He et al., 1992; Jackson et al., 2010; A. Knapp et al., 2001; Silletti and Knapp, 2001). In addition, big bluestem has been a model species for prairie ecology for nearly one hundred years. Ecotypes of A. *gerardii* were originally described nearly 50 years ago (McMillan, 1959). In McMillan's study, six big bluestem ecotypes along a latitudinal gradient in the United States were planted in Austin, Texas. He found that the northern ecotypes produced fewer flowering culms than the southern ecotypes (McMillan, 1964), and southern ecotypes had earliest spring activity, latest flowering, and latest dormancy (McMillan, 1965). Recently, Johnson et al. (in prep) investigated the genotypic and environmental contribution to observed phenotypic variation of big bluestem using a reciprocal common garden design across the precipitation gradient at three sites. The westernmost ecotype exhibited drought-adapted features with dwarfed stature and significantly reduced canopy area, thus reducing transpiration

water loss. Their data demonstrate strong planting site and ecotype effects as well as interaction between ecotype and planting site (Johnson et al., in prep) . Delucia et al. (1992) studied the effects of soil temperature on big bluestem growth. At 25℃ soil temperature, total big bluestem biomass and relative growth rate (RGR) were maximized. At higher and lower temperatures, both biomass yield and RGR decreased. Soil temperature does not have significant effect on leaf area ratio.

Table 1.2 Summaries of cultivar, variety, and ecotype of big bluestem.

Variety	Origin of materials
Bison	Central North Dakota
Bonilla	East central South Dakota
Rountree	West central Iowa
Kaw	East central Kansas
Pawnee	Pawnee County, Nebraska
Hampton Germplasm	Arkansas, Missouri, and Oklahoma
OZ-70 Germplasm	Arkansas, Missouri, and Oklahoma
Refuge Germplasm	Arkansas
Northern MO Germplasm	North Missouri
PI 9083274	Logan County, Arkansas
PI 483446	South central Kansas and east Oklahoma
Bonanza	Derived from Pawnee
Goldmine	Derived from Kaw
Niagara	Elma, New York
Goldstrike	Nebraska
Champ	Nebraska and Iowa
Earl	Texas
Chet	Texas
Fults	Fult's Hill, Illinois
Walters	Walters, Illinois
DeSoto	DeSoto, Illinois
12miles	Illinois
Carnahan	Carnahan, Kansas
Konza	Konza, Kansas
Tallgrass	Tallgrass, Kansas
Top of the World	Top of the World, Kansas
Webster	Webster, Kansas
Saline	Saline, Kansas
Cedar Bluff	Cedar Bluff, Kansas

1.3.3 Establishment and management

Big bluestem seeds are typically collected in September and October when the seed head no longer has a creamy center. The collected seeds are dried for two to four weeks. At 50℉ and 50% humidity, seeds can be stored up to seven months. Moist soil should be used fill germination trays or pots and compacted at the bottom. Germination uniformity may improve in cold stratification (40℉, 35% humidity). After the seeds are sown by hand, a thin layer of soil should cover seeds, and then the soil should be kept evenly moist during germination. Seeds should not be fertilized. At alternating day/night temperatures (set at 75/65℉) and 12-14 daylight hours (may be extended artificially), a greenhouse is established. Seedlings should then be transplanted into plug cells, This stage does not require moist soil. Plugs should be moved to cold frame in early to late spring. When the plant and soil can be completely pulled from the pot as one unit, seedlings are ready for outplanting, which can occur from late May to early October.

From late winter to early spring, seeds should be sown directly outside with several irrigations. Emergence will occur in four weeks. Big bluestem established in a new area depending on seedling performance when the seed emerges. Masters (Masters, 1997) reported that big bluestem stands were successfully established in three out of four environments evaluated with seedings at 110 PLS/m^2, and in all environments with seedlings at 220 to 440 PLS/m^2 in the Central Great Plains of North America. Big bluestem stands developed appropriately in 1987 and 1988 when seeded 14 and 6 plants/m^2 respectively, on areas treated with Atrazine (Lawrence et al., 1995). On two sites, subsequent big bluestem yields were at least 1.2 Mg/ha greater in areas treated with Metolachlor than in untreated areas. Atrazine increased big bluestem yields by 1.2 and 2.4 Mg/ha^1 at two study sites (Masters, 1997). A small big bluestem research plot demonstrated that biomass yield was not affected by stands when stand frequencies the year after establishment were 40% or greater (Vogel, 1987). A cool-season and warm-season forage grasses seedling morphology study found that the *Andropogoneae* species took three to five days less than other warm-season grasses and three to 15 days less than cool-season grasses to reach third-leaf emergence (Newman and Moser, 1988). Compared to 25℃ and 30℃, big bluestem seedling growth dramatically reduces at 20℃. Crabgrass, switchgrass, caucasion bluestem, and indiangrass seedling weight at 28 days was higher than the big bluestem seedling in averaged across temperatures (Hsu et al., 1985).

In mid-summer to late fall, plants are ready to harvest. The growth rate of rhizomatic regeneration increases in the following years even though the first-season growth is often slow. Fire disturbance causes underground rhizomes to resprout and if fire occurs during the summer (active growth stage), the regeneration will slow. Since rhizomes have winter-stores of carbohydrates, the regeneration following a springtime fire is much more vigorous.

1.3.4 Fertilizer

In a big bluestem study conducted in Iowa, Kaw big bluestem produced over 7 tons/ha when 150 kg N/ha was applied, compared to 4 tons/ha of dry matter with no applied N (Hall et al., 1982). In Minnesota, Owsley reported that big bluestem can produce 2 - 3.5 tons of forage per acre under moderate fertility (Owsley). In 1978 and 1979, Earl big bluestem yielded 3.3 tons/ac and 5.4 tons/ac of dry matter at El Reno Experiment Station, Oklahoma. During those same years, another line of big bluestem in that location yielded 4.9 tons/ac and 7.1 tons/ac, respectively (Owsley). These results recommended big bluestem as a potential biofuel crop.

During drought conditions, big bluestem and other C4 grasses may experience decreased and increased total plant N allocation to shoots and to rhizomes. Soil N uptake and carbon assimilation are also limited by water availability. Drought-induced retranslocation may protect plant nitrogen from loss to herbivory, fire, and volatilization in these periods (Hayes, 1985). With the rate of nitrogen fertilization, reserve nitrogen presented positive relation. Due to over time the nitrogen accumulated, the 80lb nitrogen rate exceeded the needs, while nitrogen with the 40lb rate apparently obtained better forage production. Constituent reserves below those of unburned, non-fertilized pastures were not affected by Nitrogen per acre adversely.

1.3.5 Grazing

Big bluestem can survive substantial grazing. In Oklahoma, a study utilized big bluestem, little bluestem, indiangrass, and switchgrass to dominate pastures. Short duration rotation or two stocking rates and continuous grazing systems were evaluated. In September, total standing crop and dead standing in the rotation units was significantly higher than continuous grazing system. Cassels et al. reported that stocking rate of live and dead standing crops had significant effects on total standing (Cassels et al., 1995).

Currently, big bluestem is mainly used for grazing. The big bluestem losses due to grazing can be as much as two times greater than burning in the absence of grazing. However,

grazing conserves approximately 1g/m^2/yr-1 nitrogen that would have otherwise been lost as a result of combustion (Hobbs et al., 1991). Grazed big bluestem plants have significantly higher rates of photosynthesis than clipped or control plants (Wallace, 1990). The photosynthesis/transpiration ratio and stomatal sensitivity to humidity indicates that, in a higher light and lower moisture environment, leaves of grazed plants may have developed better than their clipped counterparts. Big bluestem can survive substantial grazing, but if the grazing continues closer than six to eight inches, other grass species will complete with it. In spring and summer, big bluestem is highly palatable to livestock, but it becomes coarse and less palatable during the fall and winter, thereby discourage livestock grazing.

In addition, big bluestem has been widely used for preventing wind erosion through providing aboveground protection (Fargione et al., 2009). Rhizomes of big bluestem are typically one to two inches below the soil surface in erosion control, although the main roots can extend down to 10 feet. White-tailed deer and bison graze vegetative parts, and then songbirds and prairie chickens consume the seeds (Fargione et al., 2009).

1.3.6 Disease and pests control

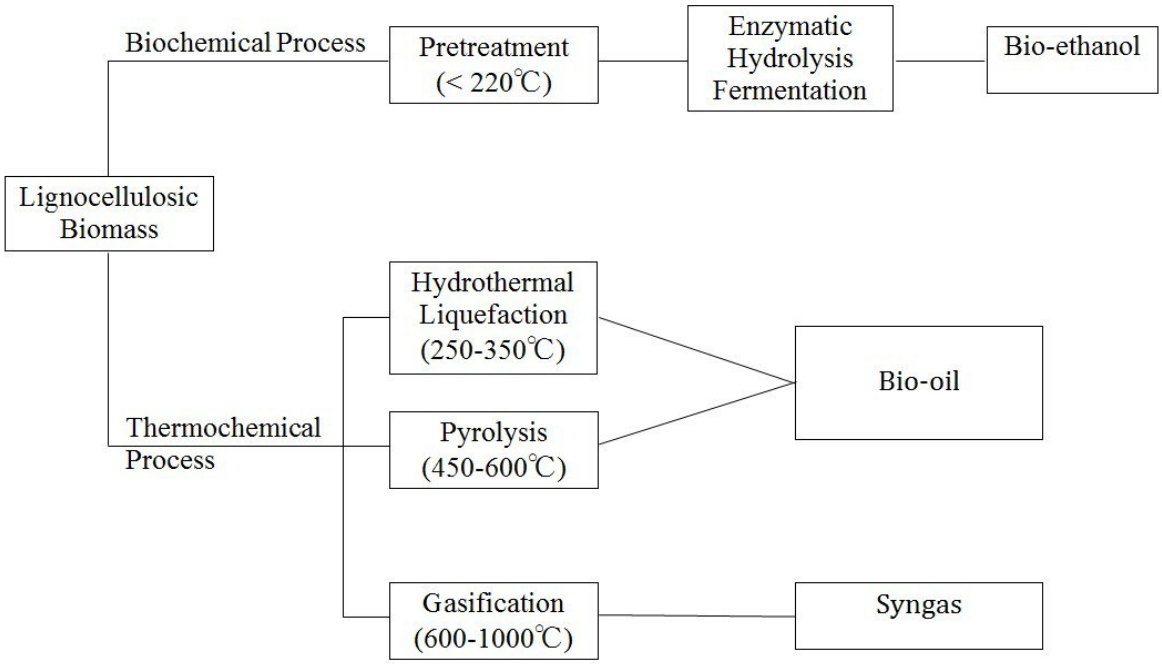
Several diseases adversely affect big bluestem. Kernel smut caused by *Sphacelotheca occidentalis* is characterized by gall-like structures that replace bluestem seeds. Culm smut caused by *Sorosporium provinciale* converts entire inflorescences into galls containing teliospores. Ergot of big bluestem is caused by *Claviceps purpurea*, and big bluestem leaf rust is caused by *Puccinia andropogonis*. Leaf spot of big bluestem can be attributed to *Phyllosticta andropogonivora* and *Ascochyte brachypodii*. One of the most serious pests of big bluestem is the bluestem seed midge *Contarinia wattsi*. This insect can reduce seed yields by more than 50%. No effective pest control currently exists for this insect; however, a wasp *Tetrastiches nebraskensis* parasitizes the midge in the Midwest (Vogel and Manglitz, 1989)

1.4 Conversion of big bluestem in to biofuels

Lignocellulosic biomass could be converted to biofuels through different processes, depending on raw material characteristics and the type of biofuels desired. As shown in Figure 1.2, two primary conversion processes are related to bio-ethanol, bio-oil, and syngas: biochemical process and thermochemical process. In thermal conversion technologies, direct combustion and co-firing with coal were first utilized for electricity production and were

responsible for over 97% of the world's bio-energy production (Demirbas, 2004). Pyrolysis has attracted the highest interest because it produces bio-oil which can be used as a fuel for transportation as well as for stable engines and converted into chemicals such as bio-lime nitrogen fertilizer (Czernik and Bridgwater, 2004). Biomass gasification has been extensively researched because of its increased efficiency compared to combustion. Torrefaction, another promising thermal process, improves quality terms of heat content, physical properties, and chemical composition for combustion and gasification applications (Shankar Tumuluru et al., 2011).

Figure 1.2 Major routes for biofuels conversion.



1.4.1 Conversion of big bluestem biomass to bio-ethanol

Bio-ethanol derived from lignocellulosic biomass via biochemical process includes the following stops: pretreatment, enzymatic hydrolysis, and fermentation(Christakopoulos et al., 1993). Bioethanol from lignocellulosic biomass is considered a viable option because it does not compete with starch-based crops for human food. However, the pretreatment is required to open

the microstructure of biomass for enzymatic hydrolysis which occurs at slightly higher temperature levels with biological catalysts.

1.4.1.1 Pretreatment process

Pretreatment is required prior to enzymatic hydrolysis in order to reduce cellulose crystallinity, increase biomass porosity, and improve enzyme accessibility (Sun and Cheng, 2002). Successful pretreatment must enhance enzyme efficiency, minimize carbohydrate losses, and inhibit by-product formation. Inhibitory compounds commonly found in hydrolysates include acetic acid, formic acid, levulinic acid, furaldehyde 2-furaldehyde (furfural), 5-hydroxymethyl-2-furaldehyde (HMF), vanillin, syringaldehyde, and conferyl aldehyde (Parawira and Tekere, 2011). The choice of pretreatment approaches depends on raw material characteristics and the final goal of the process. Physical, physico-chemical, chemical, and biological processes have been extensively studied for the pretreatment of lignocellulosic materials, and detailed descriptions of these processes have been described by Mosier et al. (Mosier et al., 2005), Sun and Cheng (2002), and Weil et al. (1994). The following sections briefly describe the major types of pretreatment that have been used for big bluestem research.

1.4.1.1.1 Physical pretreatment

Physical pretreatment of lignocellulosic biomass typically involves size comminution by milling, pelleting, and extrusion. The goal of milling is to reduce the crystallinity of cellulose fibers in the biomass. Size reduction of lignocelluloses is required in order to eliminate mass and heat transfer limitations during hydrolysis reactions (Schell and Harwood, 1994). The size of resulting materials is typically 10-30 mm after chipping and 0.2-2 mm after milling or grinding(Sun and Cheng, 2002).

Zhang et al. (in prepared) studied the effects of big bluestem size reduction via knife milling. The study reported that, for large particle sizes (8 mm), the cellulose recovery rate after pretreatment was 12% higher than smaller particles (1 mm). Moreover, big bluestem particles produced with a larger sieve had higher enzymatic hydrolysis efficiency, higher sugar yield, and less energy consumption than that of smaller particles. These results indicate that proper size reduction is desirable because it causes increased hydrolysis.

The extrusion process is a practical high-throughput physical pretreatment for large-scale operations for biomass conversion because of its cost-effective, fast, and simple process. The

main steps in this procedure consist of heating, mixing, and shearing the biomass material, resulting in physical and chemical modifications. These modifications include increases in surface area, specific surface area, pore size, and pore quantity, and a decrease in cellulose crystallinity, all of which facilitate enzyme access to cellulose. Screw speed and barrel temperature are the two most important factors responsible for disrupting the lignocellulose structure, causing defibrillation, and shortening the fibers, thus increasing carbohydrate accessibility to enzymatic attack. Moreover, extrusion does not produce any effluent, thereby resulting in no effluent disposal cost, no solid loss, and no safety issues. Karunanithy and Muthukumarappan (Karunanithy and Muthukumarappan, 2009) examined the effect of extruder parameters and moisture content of big bluestem on sugar recovery from enzymatic hydrolysis. Results indicated that maximum glucose (55.2%), xylose (92.8%), and combined sugar recovery (65.4%) was obtained at a screw speed of 100 rpm, a barrel temperature of 150°C, 15% moisture content, and a 3:1 compression ratio.

Pelleting is another physical pretreatment method used to agglomerate small particles into larger particles by mechanical or thermal processing. Pelleting of biomass involves size reduction of biomass feedstock, conditioning of the ground biomass by applying heat and/or moisture, and extrusion of ground biomass through a die (Colley et al., 2006; Lam et al., 2008; Larsson et al., 2008). Theerarattananoon et al. (2012) reported that the glucan content of big bluestem increased with increased die thickness and decreased with increased hammer mill screen size. Conversely, xylan content of t of big bluestem pellets decreased as die thickness increased and increased as hammer mill screen size increased. Among the three combinations of pelleting conditions, big bluestem pelleting using a die with thickness of 44.5 mm and a hammer mill screen size of 6.5 mm produced the highest sugar yield, the highest durability, and the greatest bulk density of biomass (Karnnalin Theerarattananoon et al., 2012).

1.4.1.1.2 Physico-chemical pretreatment

Two types of physico-chemical pretreatments discussed in literature are CO_2 explosion and extrusion combined with alkali. Carbon dioxide explosion is a biomass pretreatment that uses CO_2 as a supercritical fluid (SC- CO_2) (Yizhou Zheng et al., 1995). This technique was developed in order to adopt lower temperatures than those typically used in steam explosion and to reduce the cost compared to ammonia fiber explosion. Supercritical pretreatment conditions can effectively remove lignin, consequently increasing substrate digestibility. The addition of co-

solvents such as ethanol, water, and acetic acid can further improve the delignification process (Pasquini et al., 2005). Supercritical CO_2 has been employed primarily as an extraction solvent, but currently it is also considered for nonextractive, nonflammability, easy recovery after extraction, and environmental friendliness (Schacht et al., 2008). In aqueous solution, CO_2 forms carbonic acid, which favors biomass hydrolysis. CO_2 molecules are comparable in size to the molecules of water and ammonia; thus, they can penetrate in the small pores of lignocellulose. This mechanism is facilitated by high pressures. After the explosive release of CO_2 pressure, disruption of cellulose and hemicelluloses structure is observed and the accessible surface area for enzymatic attack increases. The employment of lower temperatures compared to temperatures used in other pretreatments prevents monosaccharide degradation and the formation of inhibitors. Luterbacher et al. (year) studied the glucose, hemicellulose sugars, and two degradation products from sugar yield in enzymatic hydrolysis after a biphasic mixture of an H_2O rich liquid (hydrothermal) phase and a CO_2 rich supercritical phase coexist pretreatment. They found that a high pressure (200 bar) CO_2-H_2O process was conducted at temperatures ranged from 150 to 250℃ and residence times from 20 s to 60 min. Pretreatment at 170℃ for 60 min produced the highest big bluestem glucose yields at 68% for 40% (wt%) solids (Luterbacher et al., 2010). CO_2 explosion has many advantages; this pretreatment method is not yet economically viable due to lower sugar yield and high capital cost for high pressure equipment.

Another interesting study about the optimization of extruder parameters in order to maximize enzymatic sugar recovery was devoted to the combined effect of alkali soaking and extrusion of big bluestem using a laboratory-scale single crew extruder at various barrel temperatures (45-225℃) and screw speeds (20-200 rpm). Optimum pretreatment condition was found at 90℃ barrel temperature, 155 rpm screw speed, 2.0% alkali (NaOH) concentration, and 4 mm particle size. The best glucose, xylose, and combined sugar recovery were 90.1%, 91.5%, and 89.9%, respectively (Chinnadurai and Muthukumarappan, 2011).

1.4.1.1.3 Chemical pretreatment

Chemical pretreatment involves the use of ozone, acids, alkali, organic solvents, and peroxides to degrade lignocelluloses in order to increase their susceptibility to enzymatic cellulose hydrolysis. Acid pretreatment is considered an effective pretreatment and has been extensively used in various biomasses which not only remove hemicellulose to high levels of

enzymatic hydrolysis but also convert solubilized hemicellulose into fermentable sugars (Zheng et al., 2007). However, this method has some limitations such as formation of degradation products, release of potential biomass fermentation inhibitors, and expensive construction (Leustean, 2009). Acid treatment commonly uses sulfuric acid, hydrochloric acid, nitric acid, or phosphoric acid, but, dilute sulfuric acid has been studied extensively because it is inexpensive and has proven effective up to 80% cellulose conversion efficiency (Zheng et al., 2007). Theerarattananoon et al. (year) examined dilute sulfuric pretreatment of pellet and un-pellet big bluestem for bioethanol production and obtained glucan hydrolysis efficiency ranged from 82% to 90%. Zhang et al. (In prepared) studied the effect of sulfuric acid concentration on pretreatment of big bluestem. The pretreatment was carried out at 160℃ for 40 min using four levels of diluted sulfuric acid (0, 1.0, 1.5, and 2.0% w/v) and 6.0% biomass loading (w/v, 3.05 g dry mass in 50 ml solution). Glucan yield was 17.8, 71.6, 74.2, and 69.6%, corresponding to biomass treated with acid concentrations of 0, 1.0, 1.5, and 2.0%, respectively. The 1.5 % acid concentration resulted in the highest glucan yield due to relatively high enzymatic hydrolysis efficiency and low glucan loss compared with other concentrations. Acid soaking plus microwave pretreatment at room temperature on big bluestem has been studied by Donepudi (2011) and maximum glucose and xylose recoveries of 72.9 and 31.2% were achieved at 0.7% acid level. He also carried out the similar pretreatments combining acid soaking with ultrasonic and obtained the maximum glucose recoveries of 27% at 0.7% acid concentration and 10 min processing time (Donepudi, 2011).

Alkaline treatment involves the use of sodium hydroxide, liquid ammonia, aqueous ammonia, lime or other alkalis for pretreatment of lignocelluloses. Alkali treatment has function of swelling biomass, decreasing polymerization, delignifying lignocelluloses, and increasing biomass surface area. In general, the alkaline pretreatment process has been used with lower temperature and longer periods, usually hours or days. Gould reported that glucose yield of big bluestem significantly increased from 0.131 to 0.361 (g/g staring material) after alkaline hydrogen peroxide pretreatment in 50 mL with 1% H_2O_2 for 24 h at 25°C with initial reaction pH of 11.5 (Gould, 1985). A combined alkali-microwave, alkali-ultrasound, and alkali-ozone pretreatment of big bluestem by Donepudi (2011) were examined at various alkali levels and processing times. Rajendran and Vivek (2009) used acid-catalyzed steam explosion and alkaline peroxidation to 1.5% hydrochloric acid hydrolysis for big bluestem treatment at 1200℃ for 30

min and acid to biomass ratio of 8:1. Then followed ethanol fermentation using *Saccharomyces cereviseae,* the maximum ethanol yield of 24.14 g/L and 76.9% fermentation efficiency were achieved. Another alkali pretreatment evaluation was studied by Guragain et al. in which maximum enzymatic hydrolysis yield and ethanol yield were observed to be 0.71 g/g and 95%, respectively (Guragain et al., 2013). Sills and Gossett (Sills and Gossett, 2012) reported using FTIR to predict saccharification from enzymatic hydrolysis of alkali-pretreated big bluestem. The pretreatments were conducted at four NaOH levels: 0, 5, 10, and 20g per 100g big bluestem with 5% (w/w) total solids concentration at 25℃ in batch reactors on a rotary shaker at 200 rpm for 24 hr for observing 12, 22, 51, 61% glucose conversion and 2, 12, 41, 53% xylose conversion, respectively.

Ozone treatment utilizes ozone gas to treat the lignocelluloses in order to delignify them. The process removes lignin and hemicelluloses without affecting much of the cellulose, while sparging the ozone gas (generated from the ozone generator) onto the biomass with some degree of moisture. The whole process occurs at normal temperature and pressure, but not all sparged ozone is utilized for the pretreatment; thus, unused ozone escapes to the atmosphere. This underutilization of ozone adds to the cost of pretreatment. Donepudi (2011) observed that acid and alkali pretreatment with ozone pretreatment yields higher sugar recovery for big bluestem than ozone treatment alone.

1.4.1.1.4 Biological pretreatment

Biological pretreatment is a selective degradation process by microorganisms. Previous studies reported that pretreatment of some biomass such as wood chips (Kumar et al., 2008), wheat straw (Filho et al., 1991), bermuda grass (Christov et al., 1997) and softwood (Duff and Murray, 1996) using white-rot fungi. Biological pretreatments require less energy and no safety issues are involved compared to other pretreatment processes. However, these pretreatment are less efficient and hard for large scale production. To date, biological pretreatment of big bluestem has not been reported in literature.

1.4.1.2 Enzymatic hydrolysis

Enzymatic hydrolysis facilitates the cleavage of bonds in order to deconstruct biomass into fermentable sugars. Enzymatic hydrolysis involves three main enzymes: β-1, 4-endoglucanases (EG), cellobiohydrolysases (CBH) or exo-glucanases, and β-glucosidases (BG).

EGs cleave to amorphous cellulose at internal sites of cellulose chains; CBHs degrade the crystalline structure of cellulose by attacking it at the chain ends and releasing cellobiose; and BGs, which are active only on cello-oligosaccharides and cellobiose, release glucose monomer units from the cellobiose (Kumar et al., 2008; Xu et al., 2009). Previous research reported that microorganisms (*Penicillium capsulatum, Talaromyces emersonii, and Aureobasidium pullulans*) can degrade hemicellulose while exhibiting greater efficiency than cellulose because hemicellulose does not have tight crystalline structures (Christov et al., 1997; Filho et al., 1991). Yang et al. (2011) summarized the current understanding of key features of pretreated biomass and glycosyl hydrolases that influence sugar release and suggested opportunities to further advance understanding of lignocellulosic bioconversion by newly advanced technologies such as genomics, proteomics, and microscopy.

1.4.1.2.1 Enzymatic hydrolysis of cellulose

Cellulases are typically used in the enzymatic hydrolysis of cellulose. Enzymatic hydrolysis requires mild conditions (4.5 pH and approximately 50°C). It is different to conventional hydrolysis using alkaline reagents or concentrated acid. Duff and Murray reported that the best potential for commercial scale use is fungal cellulases (Duff and Murray, 1996), while cellulases are produced by bacterial species such as *Clostridium*, *Cellulomonas*, and *Bacillus* (Bisaria, 1998). As a complex system of three enzymes, cellulases act synergistically to hydrolyze cellulose. The three enzyme components are β-glucosidase (EC 3.2.1.21), 1,4- β -d-glucan cellobiohydrolyase (EC 3.2.1.91), and 1,4-β-d-glucan glucanohydrolase (EC 3.2.1.3) (Ladisch et al., 1983; Wright et al., 1988). These enzymes are respectively referred to as endoglucanase, exoglucanase, and cellobiase.

To form glucose, cellobiose, and cellotriose, endoglucanase randomly cleaves cellulose chains. Cellobiose units are released by exoglucanase attacks the non-reducing end of cellulose and cleaves cellobiose units into fermentable glucose units. Since cellobiose accumulation results in cellulase inhibition, most fungal cellulases which must be supplemented exhibit limited β-glucosidase activity (Ryu and Mandels, 1980). A cellulase dosage of 10 FPU (filter paper units) per gram of biomass enables high glucose yields in 48–72 h; therefore, it is often used in studies (Gregg and Saddler, 1996). Table 1.3 summarizes previous results of big bluestem in regards to dosage and hydrolysis conditions of enzymatic hydrolysis.

Table 1.3 Cellulose activates and hydrolysis conditions from previous big bluestem studies.

Pretreatment	Enzyme activity	Conditions	Result/yield	Reference
Extrusion+alkali	Cellulase:15 FPU, glucosidase:60 CBU	50℃, 150rpm, 72h	90.1% glucose conversion, 91.5% xylose conversion	(Chinnadurai and Muthukumarappan, 2011)
Alkali	Cellulase:15 FPU, glucosidase:25 CBU	2.5% TS, 50℃, 130rpm, 48h	61% glucose conversion, 53% xylose conversion	(Sills and Gossett, 2012)
Alkali	Cellic CTec2 and Cellic HTec2 in rate of 9:1	6% TS, 50℃, 150rpm, 48h	0.71% sugar yield	(Guragain et al., 2013)
Acid	Accellerase 1500	6% TS, 50℃, 140rpm, 96h	78.6% glucose conversion	(Karnnalin Theerarattananoon et al., 2012)
CO_2-H_2O	Cellulase:15 FPU, glucosidase:30 CBU	1% TS, 50℃, 144h	68% glucose yield	(Luterbacher et al., 2010)
Microwave	Cellulase:15 FPU, glucosidase:60 CBU	50℃,150rpm, 72h	30.1% glucose conversion, 7.7% xylose conversion	(Donepudi, 2011)
Ultrasound	Cellulase:15 FPU, glucosidase:60 CBU	50℃,150rpm, 72h	38.2% glucose conversion, 4.5% xylose conversion	(Donepudi, 2011)
Ozone	Cellulase:15 FPU, glucosidase:60 CBU	50℃,150rpm, 72h	17.7% glucose conversion, 8.1% xylose conversion	(Donepudi, 2011)

1.4.1.2.2 Enzymatic hydrolysis of hemicelluloses

Three main enzymes in the complete hydrolysis of xylan are endo-β-1-4-xylanase which primarily targets internal β-1-4 bonds between xylose units, exoxylanase which releases xylobiose units, and β-xylosidase which releases xylose from xylobiose and short chain xylooligosachharides (Saha and Bothast, 1999). Several ancillary enzymes tare responsible for cleaving side-groups, although depolymerization primarily involves α –glucuronidase, α-L-arabinofuranosidase, acetylxylan esterase, p-coumaric acid esterase, and ferulic acid esterase (Saha and Bothast, 1999).

Penicillium capsulatum and *Talaromyces emersonii* with complete enzyme systems have been used for degradation of xylan (Tuohy et al., 1991). Other microorganisms, such as

Aureobasidium pullulans and several Fusarium species (Chiang et al., 1981; Hahn-Hägerdal et al., 1994), have been reported as sources for hemicellulose-degrading enzymes (Christov et al., 1997). Also, Bachmann and McCarthy reported that, in cellulase systems, synergism is exhibited in xylan-degrading systems (Bachmann and McCarthy, 1991). Since xylan does not form tight crystalline structures, accessibility to the substrate is easier, while the number of enzymes required for xylan hydrolysis is much greater than for cellulose hydrolysis (Gilbert and Hazlewood, 1993). No comprehensive effort using hemicellulose-degrading enzymes to optimize hydrolysis of big bluestem has been reported to date.

1.4.1.3 Fermentation

Supernatant from enzymatic hdrolysis of lignocelluloses can contain hexoses and pentoses if cellulose and hemicellulose are hydrolyzed. Depending on the lignocellulose source, the hydrosylate typically consists of glucose, xylose, arabinose, galactose, mannose, fucose, and rhamnose (Saha, 2003). Glucose and xylose are the dominant sugars in the mixture. *Saccharomyces cerevisiae* and *Zymomonas mobilis* are capable of efficiently fermenting glucose into ethanol but are unable to ferment xylose. Other yeasts, such as *Pachysolen tannophilus*, *Pichia stipiti*s, and *Candida shehate,* can ferment xylose into ethanol (Wang et al., 1980). Du preez (1994) and Hahn-Hagerdal et al. (1994) noted the difficulties associated with commercial use of xylose-fermenting yeasts, including low ethanol tolerance, difficulty in optimization of fermentation parameters, and slow rate of fermentation. An alternative approach is to convert xylose into an isomer called xylulose using xylose isomerase (Chiang et al., 1981; Gong et al., 1981; Jeffries, 1981). Xylulose can then be fermented by traditional yeasts. However, Saha (Saha) stated that this approach is not cost-effective and that development of genetically engineered microorganisms capable of fermenting hexoses and pentoses into ethanol should be a priority. *S. cerevisiae* is of particular interest in this regard, and recent reviews detail efforts to improve pentose fermentation using this microorganism (Chu and Lee, 2007; Hahn-Hägerdal et al., 2007).

In addition to separate hydrolysis and fermentation (SHF), other approaches include direct microbial conversion (DMC) and simultaneous saccharification and fermentation (SSF). DMC utilizes microorganisms that simultaneously produce cellulase to hydrolyze cellulose and ferment the resulting sugars into ethanol. *Clostridium thermocellum* and *Clostridium thermosaccharoliticum* have been used in DMC studies (Wyman, 1994), but significant by-product formation and low ethanol tolerance are limitations to this approach. In SSF, enzymatic

hydrolysis and fermentation take place in the same vessel. Rationale for this approach is that since cellulase activity is inhibited by glucose, rapid fermentation into ethanol increases the rate and efficiency of the overall process.

An in vitro ruminal (IVR) digestion assay for estimation of ethanol production was first tested with switchgrass, big bluestem, and eastern gamagrass (Weimer et al., 2005). This method greatly reduces processing time and expense for evaluating the potential ethanol yield fermentability of feedstocks. Eastern gamagrass gave the best fit in a linear regression between gas production from IVR and ethanol production (R^2= 0.824). This method along with the traditional IVDMD and in vitro organic matter digestibility (IVOMD) methods were used in a second study that evaluated eastern gamagrass, big bluestem, and sand bluestem at multiple locations over three years (Weimer and Springer, 2007). They reported that big bluestem had higher fermentability than eastern gamagrass or sand bluestem, but eastern gamagrass yield (6.0-7.9 Mg ha^{-1}) was higher than big bluestem (3.9-4.5 Mg ha^{-1}) and sand bluestem (5.9–6.4 Mg ha^{-1}). However, significant environmental effects on fermentability were present, as well as significant varietal differences (Weimer and Springer, 2007).

1.4.2 Conversion of big bluestem to bio-oil via hydrothermal liquefaction

Hydrothermal liquefaction (HTL) is a thermo-chemical conversion technique which uses liquid sub-critical water as a reaction medium for the conversion of organic matters to bio-oil, gases, char, and water-soluble matters in a heated, pressurized, and oxygen-absent enclosure (Ocfemia et al., 2006). HTL is conducted under elevated pressure (50 to 200 atm) and low temperature (200°C to 400°C) to keep water in either liquid or supercritical state. Water serves as both reaction medium and reactant offer several advantages: 1) no need to dry biomass; 2) high energy and separation efficiency; 3) high throughputs; 4) completely sterilized products from any pathogens, including bio-toxins, bacteria, or viruses; and 5) reduced mass transfer resistance in hydrothermal conditions (Peterson et al., 2008). When water in HTL is at supercritical condition and still in a liquid state, it has a range of exotic properties. At conditions close to the critical point, water has several very advantageous properties, including low viscosity and high solubility of organic substances, thereby making subcritical water an excellent medium for fast, homogeneous, efficient reactions (Franck, 1983; Krammer and Vogel, 2000; Kruse and Dinjus, 2007). Moreover, researchers have reported that many reactions have a high

activation volume at subcritical conditions because a high dielectric medium of subcritical water lowers the activation energy of a reaction for a transition state of higher polarity than the initial state (Kubiatko and Vaculova, 2011; Savage, 1999).

The primary product of HTL is an oily, organic liquid called bio-oil (or heavy oil), solid residue (or bio-char), aqueous products (or bio-crude or light oil), and gases. Bio-oil is a viscous, corrosive, unstable mixture of a large number of oxygenated molecules, depending on the pyrolysis process and biomass feedstock. Due to high oxygen content, the bio-oil heating value is less than half of petroleum liquid. Bio-oil must be upgraded before use as liquid fuel (Demirbas, 2011). They may serve as starting material for valuable petroleum-based fuels (e.g., gasoline and diesel) and products such as polymers, aromatics, lubricants, and asphalt (Peterson et al., 2008). Aqueous phase (light oil) reforming processes have been successfully utilized for converting biomass-derived water soluble carbohydrates to liquid alkanes and hydrogen (Huber et al., 2005). The main gaseous products are carbon dioxide and carbon monoxide. In Akalın's study, the major components from HTL of cornelian cherry stones were (Z, Z)-9, 12-octadecadienoic acid, phenols, and furfurals. Among major identified compounds, the relative concentration of (Z, Z)-9, 12-octadecadienoic acid was the highest, which is main fatty acids product such as palmitic acid (n-hexadecanoic acid) and linoleic acid ((Z, Z)-9,12-octadecadienoic acid) in bio-oils from hydrothermal liquefaction of biomass (Akalın et al., 2012; Garrote et al., 2007; Liu et al., 2006; Quitain et al., 2003; Valdez et al., 2011).

Bio-cured, the aqueous fraction of products after HTL, can be converted to liquid fuel, hydrogen, or chemicals (Czernik and Bridgwater, 2004). Karagoz et al. (2005) reported that bio-oils from the hydrothermal treatment of cellulose consisted of furan derivatives, whereas lignin-derived oil contained phenolic compounds (Karagöz et al., 2005). Previous research showed bio-oils derived from corn stover via hydrothermal liquefaction contained e phenol, guaiacol, 4-ethyl-phenol, 2-methoxy-4-methyl-phenol, 4-ethylguaiacol, 2,6-dimethoxyphenol, 1,2,4-trimethoxybenzene, 5-tert-butylpyrogallol, 1,10-propylidenebis-benzene, 1-(4-hydroxy-3,5-dimethoxyphenyl)-ethanone, acetic acid, 1-hydroxy-2-propanone, furfural, 3-methyl-2-cyclopenten-1-one, 2,5-hexanedione, and desaspidinol (Zhang et al., 2008). Bio-char is similar to that of coal with less fibrous structure and high calorific value, making it an excellent candidate for solid fuel. Bio-char is highly resistant to decomposition upon land application and has a number of positive effects relating to soil fertility (Bruun and Luxhoi, 2008). In addition, the

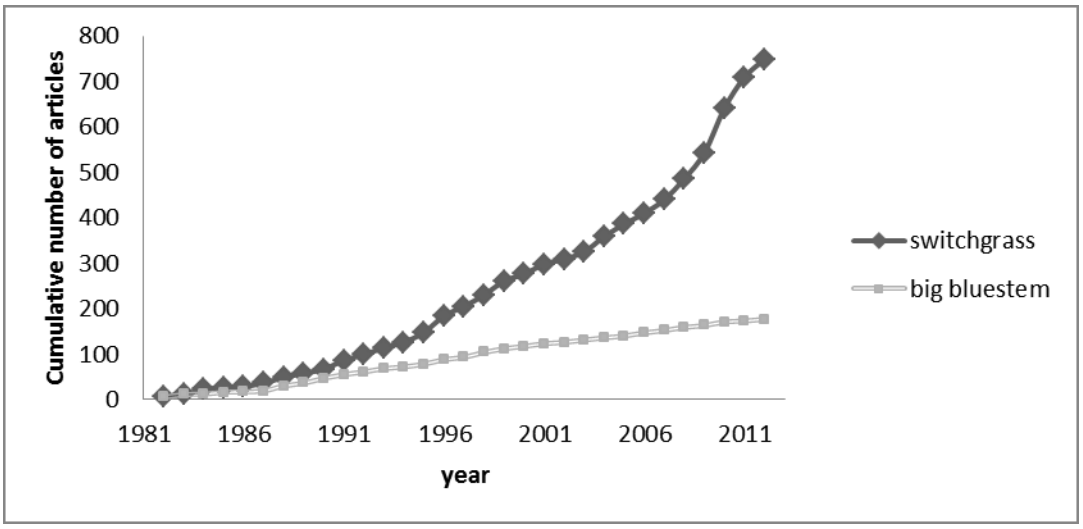
application of bio-char as an effective adsorbent has been studied extensively. The sorption properties of activated carbons are extremely versatile and can be used to remove a variety of inorganic and organic contaminants, such as heavy metals (Babel and Kurniawan, 2003), arsenates (Mohan and Pittman Jr, 2007), organic dyes (Crini, 2006), and many other toxic substances (Radovic, 2004) from water.

Gan et al. (2012) conducted HTL of big bluestem into bio-oil using sodium hydroxide as catalyst at 1,100 psi and 280℃. The study reported bio-oil yields of big bluestem were in the range of 19.5–27.2%. The bio-oil carbon content ranged from 69.8% to 77.9%, and oxygen content ranged from 14.0% to 22.0%. They also analyzed the effect of ecotype and planting location on bio-oil yield as well as carbon and oxygen content in the bio-oil. The general conclusion of this study indicated that bio-oil yield of big bluestem HTC was significantly affected by both ecotype and planting location, but planting location was most influential. In addition, they found that bio-oil C and O contents were primarily significantly affected by ecotype. They suggested that big bluestem and switchgrass have similar potential for bio-oil production via HTL. Another fast pyrolysis biorefinery of big bluestem study reported that bio-oil yield was 71% and the most important constituents (hydroxyacetaldehyde, phenol, and anhydroglucose yields) were 13, 5, and 9%, respectively. Both yields of big blustem were approximately 50% higher than switchgrass due to its low potassium content (Bergan et al., 2006). Tiffany et al. (2006) analyzed economical feasibility and indicated that the returns on investment from big bluestem (US$19.38 Mg^{-1}) exceed switchgrass (US$10.47 Mg^{-1}).

1.4.3 Potential and future outlook of big bluestem for biofuels

Figure 1.3 shows the cumulative number of articles on switchgrass and big bluestem listed in AGRICOLA literature database from 1981 to 2011. The figure indicates that a majority of previous studies investing herbaceous grasses as energy crop have focused only on analysis of switchgrass during the past two decades. However, compared to switchgrass, big bluestem as native and potential energy crop gained much less attention based on one-third of the cumulative number of related articles selected as a model species and funding support from DOE after an admittedly very limited screening effort (Parrish and Fike, 2005; Wright and Turhollow, 2010).

Figure 1.3 Cumulative number of articles on switchgrass and big bluestem listed in AGRICOLA literature database.



Several factors suggest sole reliance on switchgrass, however, Natural pure stands of big bluestem are more common than switchgrass in the tallgrass prairie of Midwest. Big bluestem is generally more palatable as hay and grass in the latter part of the season, so producers concerned about longterm options may prefer it (King and Coriolis, 1999). Some landowners also consider switchgrass excessively invasive. Production of ethanol and value-added chemicals via consolidated bioprocessing (a direct fermentation process) indicated that big bluestem is a superior feedstock over switchgrass and estern gamagrass (Weimer and Springer, 2007). Another advantage of big bluestem is that it can produce twice the biomass per unit of applied nitrogen than switchgrass or indiangrass (Perry and Baltensperger, 1979). In addition, big bluestem is the dominant species in the second year after switchgrass dominates in the first establishment year (Tilman et al., 2001). Thus reinforcing that the proportion of big bluestem significantly increased when grown in monoculture or with indiangrass and switchgrass at second year (Hong et al., 2013). Madakadze et al. (year) reported that the average lignocellulose content ranked cordgrass > big bluestem > switchgrass > sandreed > indinagrass in southwestern Quebec, Canada (Madakadze et al., 1998). Waramit et al. reported that big bluestem tends to contain higher cellulose concentrations than switchgrass (Waramit et al., 2011).

Table 1.4 summarizes the average cellulose content, hemicellulose content, and biomass yield of big bluestem from previous studies. The average and range of cellulose content,

hemicellulose content, and biomass yield were 37.2% with a range of 33.5 to 49.8%, 23% with a range of 17.7 to 31.5%, and 7 Mg/ha with a range of 3.2 to 11.4 Mg/ha. 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 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). Table 1.5 compares potential ethanol yields of big bluestem and other selected biomass. In general, perennial warm-season grass ethanol yields were lower than annual crop yields because latter had higher biomass yield. The estimated ethanol yield of big bluestem calculated from a previous study was 1886 L/ha, which is comparable to those previously reported herbaceous biomasses (switchgrass, miscanthus, and eastern gamagrass). Total estimated ethanol yields followed similar trends to total biomass yields. In addition, big bluestem had similar production costs compared to switchgrass and lower costs compared to alfalfa and reed canarygrass (Spinelli and Hartsough, 2001). While comparing the cost of bio-oil processing, big bluestem was determined to be less expensive than switchgrass and to produce more bio-oil from pyrolysis. The returns on investment from big bluestem (US$19.38 Mg^{-1}) also exceeded switchgrass (US$10.47 Mg^{-1}) (Tiffany et al., 2006). Although big bluestem is a promising feedstock for bioethanol production and thermal energy conversion, there are some constraints remained. Utilization of hemicellulose, which accounts for approximately 20-25% of big bluestem, must be improved. Another issue limiting wide acceptance is difficulty in planting and establishment. Seed requires processing in order to remove hairs or specialty drills in order to be planted. Research on plant breeding should focus on modifying the composition of big bluestem in order to minimize recalcitrance to bioconversion in addition to increasing biomass yields.

Table 1.4 Average cellulose content, hemicellulose content, biomass yield, and calculated potential ethanol yield of big bluestem from previous studies.

Cellulose content (%)	Hemicellulose content (%)	Yield (Mg/ha)	Reference
NA	NA	6.1	(J. Propheter et al., 2010)
NA	NA	8.5	(Stork et al., 2009)
NA	NA	4.5	(J. L. Propheter and Staggenborg, 2010)
NA	NA	8.5	(Cherney et al., 1990)
NA	NA	8.3	(Anderson et al., 1996)
NA	NA	5.5	(Meyer et al., 1994)
NA	NA	11.4	(J. F. Johnson and Gresham, 2013)
NA	NA	3.2	(Dokyoung Lee et al., 2009)
34.7	29.2	NA	(Jefferson et al., 2004)
33.1	17.7	NA	(Donepudi, 2011)
34.5	27.0	NA	(K Zhang et al., 2012)
37.9	21.1	NA	(Sills and Gossett, 2012)
40.1	21.6	NA	(Karnnalin Theerarattananoon et al., 2012)
37.6	19.9	NA	(Luterbacher et al., 2010)
35.0	18.2	NA	(Karunanithy and Muthukumarappan, 2009)
35.6	20.2	NA	(Guragain et al., 2013)
49.8	31.5	NA	(Bergan et al., 2006)
33.5	23.7	NA	(D. Johnson et al., 1995)
37.2	23.0	7.0	Average of previous study

Table 1.5 Comparison of potential ethanol yields of big bluestem and other selected biomass.

Biomass	Potential ethanol yields(L/ha)	Reference
Big bluestem calculated from previous study	1886	NA
Kaw big bluestem [1]	1893	(J. Propheter et al., 2010)
Big bluestem [2]	2602	(Stork et al., 2009)
Kanlow switchgrass [1]	2070	(J. Propheter et al., 2010)
Switchgrass [2]	3289	(Stork et al., 2009)
Miscanthus[1]	2499	(J. Propheter et al., 2010)
Eastern gamagrass [2]	3019	(Stork et al., 2009)
Photoperiod-sensitive sorghum [1]	7637	(J. Propheter et al., 2010)
Sweet sorghum [3]	9920	(J. Propheter et al., 2010)
Dual-purpose forage sorghum [4]	6516	(J. Propheter et al., 2010)
Brown midrib sorghum [4]	4591	(J. Propheter et al., 2010)
Rotated corn [4]	7737	(J. Propheter et al., 2010)
Continuous corn [4]	7087	(J. Propheter et al., 2010)

[1] Ethanol yields from stover components only.

[2] Ethanol yields from the average of all entries for a same species.

[3] Ethanol yields from grain, bagasse and leaves, and extracted fermentable carbohydrates combined.

[4] Ethanol yields from stover and grain components combined.

Chapter 2 - Big bluestem glucose content and yield from enzymatic of hydrolysis as affected by ecotype and planting location along the precipitation gradient of the Great Plains[1]

2.1 Abstract

Three big bluestem ecotypes from central Kansas (Cedar Bluffs and Webster populations), eastern Kansas (Konza and Top of the World populations), and Illinois (12Mile and Fults populations), as well as the Kaw cultivar, were harvested from four reciprocal garden planting locations (Colby, Hays, and Manhattan, KS; and Carbondale, IL) and evaluated for their chemical (glucan, xylan, arabinan, lignin and ash) and elemental (carbon, oxygen, hydrogen, nitrogen and sulfur) compositions. The objective of this research was to study the effects of ecotype and planting location on the chemical and elemental compositions of big bluestem along the Great Plains precipitation gradient (~1200 to 400 mm mean annual precipitation). All the populations revealed a large variation in cellulose (31.8–36.5%), hemicellulose (24.96–29.74%), lignin (14.4–18.0%), carbon (47.3–51.3%), and nitrogen (4.91–6.44%). Planting location had significant effects on both chemical and elemental compositions of big bluestem. Ecotype had significant effects on glucan, xylan, lignin, and ash contents as well as on carbon, oxygen, and hydrogen elemental fractions. In addition, the interaction between ecotype and planting location had significant effects on glucan, lignin, and hydrogen. Planting location had a greater effect on chemical and elemental compositions than the ecotype and interaction between location and ecotype. The total sugar content of the big bluestem (regardless of ecotype) increased as the Great Plains precipitation gradient increased from west to east. Annual precipitation, growing degree days and potential evapotranspiration in 2010 explained up to 97%, 88% and 80% of the variation in compositions respectively.

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[1] This chapter has been published as a peer-reviewed research paper in the Journal of *Industrial Crops and Products.* 2012. 40:210-218.

2.2 Introduction

With the rapid increase in worldwide consumption of nonrenewable fossil fuels, the production of renewable fuels from biomass is attracting more research attention. Renewable fuels derived from biomass could reduce our dependence on fossil fuel resources and reduce greenhouse gas emissions (Dien et al., 2006). First-generation biofuel, produced from starch-based and sugar-based biomass, is limited because of competition with food crops and other land demands (Tilman et al., 2006). Thus, lignocellulosic biomass, including dedicated energy crops such as switchgrass, big bluestem, forest residues, and agricultural residues, could play an important role in biofuel production because of low production inputs and potentially low competition with food production. A recent analysis indicated that over 25 million hectares of land classified by the USDA as rangeland/grassland within land capability class 3–6 soils (more marginal/less productive soils) could be utilized for bioenergy crop production in select states in the central Great Plains (Kansas, Nebraska, Oklahoma, and South Dakota) (USDA, 2010b).

Big bluestem (*Andropogon gerardii*) is a dominant warm-season (C4) perennial native grass that comprises as much as 80% of the plant biomass in prairies in the midwestern grasslands of North America (F. W. Gould and Shaw, 1983; A. K. Knapp et al., 1998). This research helps lay the foundation for the potential development of big bluestem as a bioenergy feedstock on these range/grasslands. Although big bluestem has been studied extensively for decades in terms of the effect of climate on grass growth; controls on community structure; ecological responses to grazing, burning, and mowing; and restoration effectiveness(Epstein et al., 1998; Fay et al., 2003; He et al., 1992; Jackson et al., 2010; A. Knapp et al., 2001; Silletti and Knapp, 2001), the potential use of bluestem for bioenergy has not been evaluated adequately. Ecotypes of *A. gerardii* were originally described nearly 50 years ago (McMillan, 1959), but variables related to biofuel potential across the precipitation gradient of tallgrass prairie have not been broadly characterized. This study will utilize the sharp precipitation gradient across the Great Plains (1200 to 400 mm mean annual precipitation [MAP]) and reciprocal garden research plots to investigate the biofuel potential of *A. gerardii* ecotypes and how such potential is affected by planting location across the Great Plains.

Big bluestem is adaptable in most native prairie ecosystems and can represent as much as three times the biomass as switchgrass in midwestern grasslands (Epstein et al., 1998). Big bluestem productivity is high due to efficient nutrition utilization; it produces twice the biomass

per applied nitrogen compared with switchgrass and indiangrass (L. C. Johnson and Matchett, 2001), establishes easily from seed, and spreads vigorously by vegetative growth of underground rhizomes with a robust root system (Perry and Baltensperger, 1979). In addition to economic considerations, bluestem prairie serves a range of purposes in the ecosystem because it provides wildlife habitat, cattle grazing, and hay and pasturelands (Fargione et al., 2009).

Previous research has been carried out to evaluate big bluestem for conversion to ethanol. Weimer et al. (2007) studied big bluestem for ethanol production through consolidated bioprocessing. Jung and Vogel (Jung and Vogel, 1992) reported that big bluestem leaves contained more neutral detergent fiber and relatively higher levels of cellulose and lignin at the vegetative stage than switchgrass, resulting in a greater *in vitro* fermentability than switchgrass. Alexander (Alexander et al., 2008) demonstrated that big bluestem produced 39% and 16% more mass than Shawnee and Cave-in-Rock switchgrass, respectively and big bluestem had larger yields and lower amounts of ash than switchgrass due to the higher nitrogen utilization efficiency.

In this research, three big bluestem ecotypes (Central Kansas [CKS], Eastern Kansas [EKS], and Illinois [IL], with two populations comprising each ecotype) and the widely planted Kaw cultivar (KAW) were harvested from each of four reciprocal garden planting locations (Colby, Hays, and Manhattan, KS; and Carbondale IL). This reciprocal design allows us to study the effect of ecotype and planting location on chemical and elemental composition. Results from this research will provide basic data that will potentially enable more efficient plant breeding for bioenergy production by providing scientific knowledge about the role of the genetic and environmental factors that influence the development of big bluestem varieties for use as a bioenergy crop. The plants analyzed here also were part of a big bluestem ecotype experiment to examine the cline in phenotypic variation (biomass, phenology, canopy characteristics) across the Great Plains precipitation gradient (~1200 to 400 mm mean annual precipitation) and the relative role of environment and ecotype in affecting the phenotype.

2.3 Materials and methods

2.3.1 Materials.

Three big bluestem ecotypes, CKS (Cedar Bluffs [CDB] and Webster [WEB] populations), EKS (Konza [KON] and Top of the World [TOW] populations), and IL (12Mile [12M] and Fults [FUL] populations), and the KAW cultivar, which is widely planted to restore

marginal lands, were harvested from reciprocal garden plots in four planting locations (Colby, Hays, and Manhattan, KS; and Carbondale, IL) in 2010. Among the four locations, the Colby planting site was used to test the threshold of drought tolerance and the possibility for planting in the drier locations of the Great Plains. Two populations from each ecotype were evaluated for their chemical and elemental compositions. Glucan, xylan, arabinan, lignin and ash made up a major chemical composition of biomass. Elemental compostion was reported as carbon, oxygen, hydrogen, nitrogen and sulfur. The big bluestem 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).

2.3.1.1 Seed Collection.

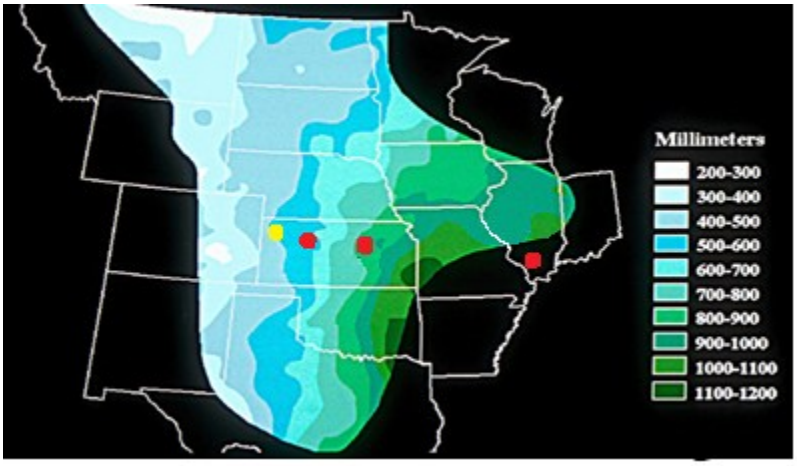
Seeds for the populations and ecotypes were collected by hand from pristine ungrazed prairie in the fall of 2008. Figure 2.1 and Table 2.1 show the GPS coordinates of seed collection sites (latitude and longitude) for the seeds that were later harvested from grown plants. For each ecotype region (central Kansas, eastern Kansas, and Illinois), four populations were collected within 50 miles of the reciprocal garden planting locations. Two populations per ecotype were analyzed in this paper. Populations were at least 10 miles distant from one another. In fall 2008, a subset of seeds from all populations was germinated and grown in 4 x 4 in pots in the greenhouse using standard greenhouse potting mix (Metro-Mix 510; Sun Gro Horticulture, Vancouver, BC, Canada). For KAW, we obtained seed from the USDA Plant Materials Center, Manhattan, KS. We included KAW because it is widely used for restoration planting in Conservation Reserve Program lands throughout the Great Plains.

2.3.1.2 Planting Locations.

These plants were later installed at the reciprocal garden sites (Colby, Hays, and Manhattan, KS; and Carbondale, IL) in August 2009. Table 2.2 shows environmental conditions and short-term and long-term weather patterns at the reciprocal garden planting sites. Mean annual precipitation showed a striking contrast across the four locations. To test the limits of the tolerance of big bluestem, the plants were installed in Colby. At each planting location, all 12 populations (3 ecotypes x 4 populations per ecotype) were replicated in 10 blocks. For this study we used only two of the 4 populations per ecotype. Plants were assigned randomly to blocks,

spaced 50 cm apart, and planted into shadecloth to control weeds. The KAW cultivar and sand bluestem (data not included here) were also included, making 14 plants per block.

Figure 2.1 Reciprocal gardens across the precipitation gradient. Yellow dot is Colby satellite site. Seeds were collected from native prairie with 50 miles of each planting site. The isoclines represent the precipitation gradient in terms of mean annual precipitation (modified from Burke) across the central grasslands of the United States. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)



2.3.1.3 Plant Harvest.

The plants were part of a large bluestem ecotype variation experiment to examine the phenotypic variation across the Great Plains precipitation gradient (Johnson et al., in preparation) and the role of environment and ecotype in affecting the phenotype. These plants were extensively characterized in terms of canopy area, height, and phenology in the summer of 2010 (Johnson et al., in preparation) and harvested by hand in October 2010. The harvested plant biomass (foliage, inflorescence, stalks) was dried at 60 °C for at least 1 week before being stored at room temperature.

Table 2.1 Collection sites for *A.gerardii* populations

Ecotype	Population collection site	County	Latitude (N)	Longitude (W)	Elevation (m)
CKS, Hays	Webster Reservoir (WEB)	Rooks	39^{o} 24’	99^{o} 32'	606
	Cedar Bluffs Reservoir (CDB)	Trego	38^{o} 45’	99^{o} 46'	688
EKS, Manhattan	Konza Prairie (KON)	Riley/Geary	39^{o} 05’	96^{o} 36'	366
	Top of the World Park (TOW)	Riley	39^{o} 13’	96^{o} 37'	379
IL, Carbondale	Twelve Mile (12M)	Effingham, Fayette, and Marion	38°46'	88°50'	NA
	Fults (FUL)	Monroe	37°58’	89°48'	215

Table 2.2 The location of the reciprocal garden in the four planting sites.

Environment conditions	Reciprocal Garden Planting Site			
	Colby, KS Northwest Kansas Agricultural Research Center	Hays, KS Agricultural Research Center–Hays	Manhattan, KS USDA Plant Materials Center	Carbondale, Illinois Southern Illinois University Agronomy Center
Annual precipitation, 2010 (cm)	44.57	50.11	67.82	66.95
Mean annual precipitation since 1961 (cm)	50.47	58.22	87.15	116.73
Precipitation of driest year, cm (year)	28.37 (1967)	36.27 (1988)	39.16 (1966)	66.95 (2010)
Growing degree days average since 1961	3167	3799	4156	4087
Growing degree days, 2010	3461	4193	4105	4474
Potential evapotranspiration (cm)	144	139	127	99
Aridity index (PET^a-PPT^b)	97	81	41	-18
Soil type	Silt-loam	Roxbury silt-loam	Sandy-loam	Stoy silt-loam

2.3.2 Analytical methods.

2.3.2.1 Chemical Composition Analysis.

Moisture content of ground big bluestem samples was determined by drying about 2 g of each sample in a forced-air oven at 105 °C for 4 h (Sluiter, Hames, Hyman, et al., 2008). Extractives and chemical composition of the big bluestem were determined by following NREL laboratory analytical procedures (Sluiter, Hames, Hyman, et al., 2008; Sluiter, Hames, Ruiz, et al., 2008). Structural carbohydrates in biomass were reported as percentages of glucan and xylan. Lignin, the major non-carbohydrate component, is the sum of acid-insoluble and acid-soluble

lignin. Glucose, xylose, mannose, and arabinose in acid-hydrolyzed samples were determined by analyzing the supernatant from acid-hydrolysis using an 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. The supernatants of acid-hydrolyzed samples were neutralized with $CaCO_3$ to pH 6 before being filtered through 0.2 μm hydrophilic PTFE syringe filters (Millipore, Billerica, MA). The monosaccharide was analyzed by using an HPLC with a Rezex RPM-monosaccharide column (300 × 7.8 mm; Phenomenex, CA) and a refractive index detector (RID-10A, Shimadzu, MD). The column was eluted with double-distilled water at a flow rate of 0.6 mL/min. The temperature of the chromatograph column was maintained at 80 °C.

2.3.2.2 Elemental Analysis.

The elemental composition of the big bluestem samples was measured with CHNS/O Elemental Analyzer (PerkinElmer 2400 Series II, PerkinElmer Inc., Waltham, MA). About 2 to 3 mg (accurate to 0.001mg) of the ground sample with fine uniform particle size was weighed into tin capsules using a PerkinElmer AD-6 Autobalance (PerkinElmer Inc., Waltham, MA). The ground sample was packed with foil, introduced into the combustion chamber through a funnel, and burned under a pure oxygen atmosphere. The gases (CO_2, N_2, SO_2, and H_2O) from combustion were separated in a quartz column containing copper wires detected by a thermoconductometer detector. Elemental compositions are reported as a percentage of initial dry weight (w/w, db).

2.3.2.3 Statistical Analysis.

Chemical and elemental compositions of big bluestem samples are 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.

2.4 Results and discussion

Both ecotype and planting location had significant effects on chemical and elemental compositions of the big bluestem ($P < 0.05$), except the effect of ecotype on xylan + arabinan,

nitrogen, and sulfur contents. The chemical composition of the seven big bluestem populations and 3 ecotypes from four planting locations varied significantly when specific constituents were considered (Table 2.3). For all of the big bluestem samples, the average and range of the chemical composition across planting locations and ecotypes are 34.5% ±2.4 from 29.6–39.5% for glucan, 23.6% ±2.0 from 19.2–26.8% for xylan, 3.5% ±0.7 from 2.1–4.8% for arabinan, 16.8% ±1.8 from 12.0–19.3% for lignin, and 4.3% ±0.7 from 3.1–5.6% for ash. The range of the chemical constituents in glucan, xylan, and ash contents (Table 2.3) are similar to those reported by previous research (Jefferson et al., 2004; Titgemeyer et al., 1996; C. Wyman, 1996); however, big bluestem had lower lignin content compared with other lignincellulosic biomass (Table 2.4) such as sorghum biomass (Zhao et al., 2009), corn stover (Lloyd and Wyman, 2005; Zeng et al., 2007; Zhao et al., 2009; Zhu et al., 2006), and wheat straw (Saha et al., 2005; F. Sun and Chen, 2008; Zhu et al., 2006). This may make pretreatment and enzymatic hydrolysis of structural polysaccharides in the bioconversion processes easier for big bluestem.

The elemental composition analysis is important for calculating biomass heat content, performing mass and heat balances in the bioconversion process, and predicting potential pollution problems during biomass thermal processes. Table 5 shows the elemental carbon (C), hydrogen (H), oxygen (O), sulfur (S), and nitrogen (N) contents in the big bluestem samples. For all of the big bluestem samples, the average and range of the elemental composition across planting locations and ecotypes are 49.1% ±1.4 (range of 47.1–51.4%) for C, 5.9% ±0.3 (range of 4.9–6.5%) for H, 43.3% ±1.6 (range of 40.7–46.1%) for O, 0.84% ±0.2 (range of 0.61–1.27%) for N, and 0.92% ±0.1 (range of 0.78–0.98% for S. Results showed that big bluestems had a desirable molar ratio of H/C, with average of 1.44 and a range of 1.23–1.52, which can result in less smoke and water-vapor formation and thereby reduced energy loss during gasification processes (Bridgeman et al., 2008). The comparison of elemental composition of big bluestem with other lignocellulosic biomass is shown in Table 2.6. Big bluestem contains relatively higher carbon content than other grasses and crop residues, which potentially translates into relatively higher heat content for big bluestem. The results show that big bluestem could potentially serve as suitable energy grass in the Midwest with similar or better chemical and elemental compositions compared with other biomass crops and grasses.

Table 2.3 Chemical composition of big bluestem by population and planting site.

Population-location	Chemical composition (%, db)					
	Glucan	Xylan	Arabinan	Xylan+ Arabinan	Lignin	Ash
CDB (CKS)-Colby	32.5±0.1	21.4±0.1	3.73±0.01	25.1±0.1	14.8±0.4	3.97±0.32
WEB (CKS)-Colby	32.8±0.1	22.3±0.2	3.20±0.01	25.5±0.1	15.2±0.1	3.91±0.14
KON (EKS)-Colby	29.6±0.1	20.7±0.11	3.77±0.02	24.5±0.1	13.3±0.2	5.33±0.90
TOW (EKS)-Colby	30.8±0.2	20.7±0.2	4.10±0.01	24.78±0.2	13.9±0.1	4.97±0.94
12M(ILL)-Colby	29.6±0.2	19.2±0.1	3.84±0.06	23.01±0.2	12.0±0.1	5.06±0.25
FUL (ILL)-Colby	32.6±0.2	22.0±0.2	3.97±0.10	26.0±0.01	14.9±0.1	3.18±0.06
KAW (CULTIVAR)-Colby	34.9±0.1	23.7±0.3	2.12±0.01	25.8±0.3	16.3±0.1	3.89±0.55
CDB (CKS)-Hays	36.1±0.1	22.2±0.6	3.15±0.70	25.4±1.3	18.6±0.2	3.79±0.03
WEB (CKS)-Hays	35.2±0.5	23.2±0.3	2.66±0.42	25.9±0.7	17.7±0.1	3.41±0.36
KON (EKS)-Hays	33.3±0.2	21.9±0.2	3.02±0.29	24.9±0.1	17.4±0.1	4.25±0.26
TOW (EKS)-Hays	32.7±0.2	21.3±0.5	3.48±0.41	24.8±0.9	17.2±0.1	5.60±0.24
12M (ILL)-Hays	31.8±0.5	22.6±0.5	3.97±0.06	26.6±0.5	14.2±0.3	3.12±0.24
FUL (ILL)-Hays	32.8±0.4	21.7±0.7	3.02±0.43	24.8±1.1	16.8±0.1	3.62±0.31
KAW (CULTIVAR)-Hays	34. 9±0.1	22.5±0.1	2.54±0.23	25.1±0.4	16.9±0.2	3.60±0.23
CDB (CKS)-Manhattan	36.7±0.6	23.9±0.3	3.28±0.21	27.2±0.1	18.7±0.2	4.66±0.40
WEB (CKS)-Manhattan	34.6±0.4	25.3±0.3	3.10±0.01	28.4±0.3	18.8±0.1	3.92±0.26
KON (EKS)-Manhattan	35.9±0.4	25.1±0.5	3.33±0.50	28.4±0.1	17.6±0.4	4.52±0.55
TOW (EKS)-Manhattan	35.1±0.3	25.5±0.8	3.02±0.16	28.5±0.7	18.2±0.6	4.77±0.30
12M (ILL)-Manhattan	34.0±0.2	23.4±0.2	3.87±0.17	27.3±0.4	15.2±0.2	4.93±0.49
FUL (ILL)-Manhattan	37.1±0.2	26.6±0.1	2.90±0.32	29.5±0.4	17.3±0.3	3.18±0.19
KAW (CULTIVAR)–Manhattan	38.3±0.6	24.7±0.4	2.28±0.03	27.0±0.4	17.6±0.1	4.51±0.01
CDB (CKS)-Carbondale	35.6±0.1	24.6±0.4	3.86±0.11	28.5±0.3	17.7±0.2	4.83±0.18
WEB (CKS)-Carbondale	36.3±0.4	26.1±0.2	4.73±0.05	30.8±0.2	18.2±0.1	4.29±0.02
KON (EKS)-Carbondale	36.2±0.2	25.4±0.1	4.74±0.15	30.1±0.2	17.6±0.2	5.58±0.34
TOW (EKS)-Carbondale	36.2±0.1	25.8±0.3	4.75±0.03	30.5±0.3	18.5±0.5	4.90±0.03
12M (ILL)-Carbondale	35.1±0.4	24.3±1.2	3.78±0.41	28.1±1.6	16.7±0.1	4.82±0.16
FUL (ILL)-Carbondale	36.6±0.5	26.4±0.2	4.25±0.04	30.7±0.2	18.2±0.2	4.64±0.10
KAW (CULTIVAR)-Carbondale	39.5±0.4	26.8±0.1	2.70±0.14	29.5±0.1	19.4±0.2	4.30±0.04
Average	34.5 ±2.4	23.6 ±2.0	3.5 ±0.7	27.0 ±2.1	16.9 ±1.8	4.3±0.7

Table 2.4 Comparison of the chemical composition of different types of biomass[a]

Type of biomass	Chemical composition (%, db)			
	Glucan	Xylan+Arabinan	Lignin	Ash
Big bluestem-this study	34.5	27.0	16.8	4.3
Corn stover	38	26	19	6
Soybean	33	14	-	6
Wheat straw	38	29	15	6
Rye straw	31	25	-	6
Barley straw	42	28	-	11
Switchgrass	37	29	19	6
Indiangrass	39	29	-	8
Little bluestem	35	31	-	7
Prairie cordgrass	41	33	-	6
Miscanthus	43	24	19	2
Intermediate wheatgrass	35	29	-	6
Reed canarygrass	24	36	-	8
Smooth bromegrass	32	36	-	8
Timothy	28	30	-	6
Tall fescue	25	25	14	11
Alfalfa	27	12	-	9
Forage sorghum	34	17	16	5
Sweet sorghum	23	14	11	5
Pearl millet	25	35	-	9
Sudangrass	33	27	-	12

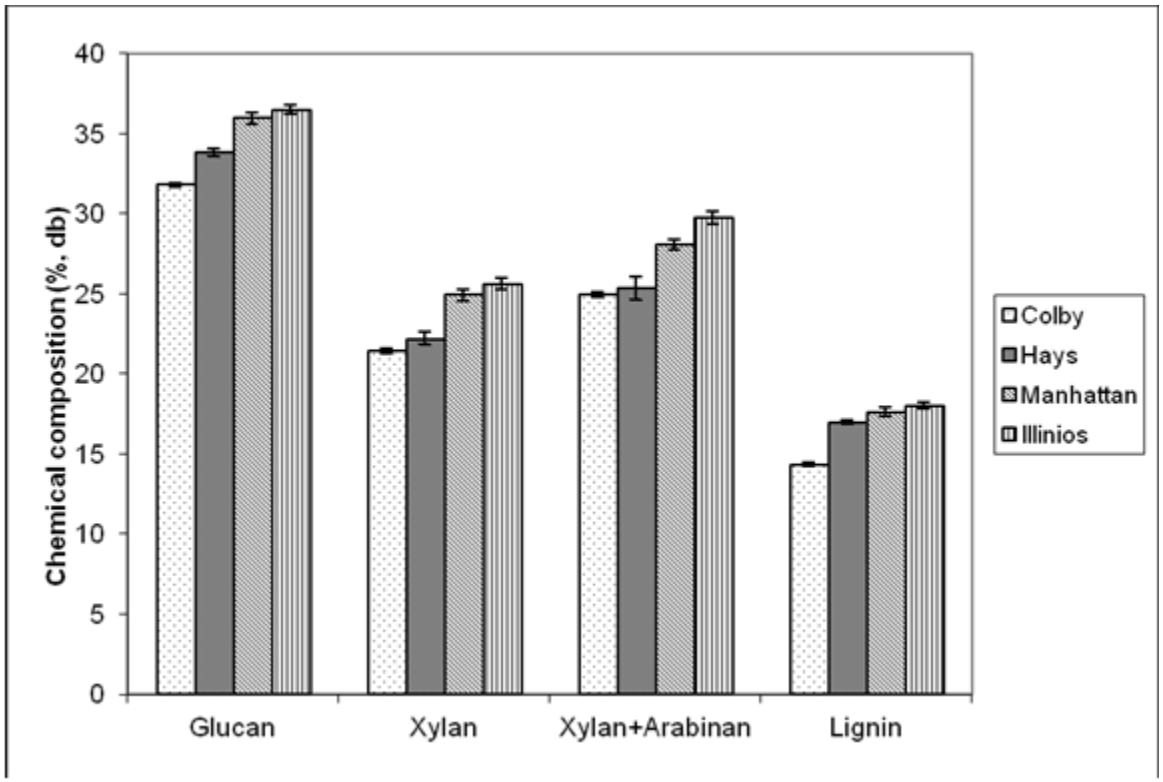
[a] Data source: Lee et al., 2007 (J. Lee et al., 2007)

2.4.1 Effects of Planting Location on Chemical Composition.

Figure 2.2 shows the effects of planting location on the chemical composition of big bluestem. Big bluestem populations planted in Illinois generally had higher cellulose (glucan) contents, with an average of 36.5% compared with the average of populations planted in Colby, KS (31.8%); Hays KS (33.8%); and Manhattan, KS (36.0%). The average cellulose content of big bluestem planted in Illinois was 4.7% higher than those from Colby in western Kansas, indicating that the same big bluestem populations would yield ≈15% more cellulose if planted in Illinois instead of western Kansas. Table 2.7 shows the linear regression results between

composition and environmental factors associated with the planting locations. The 2010 annual precipitation explained 37–97% of the variation in biomass composition based on coefficients of determination (R^2). In addition to the sharp difference in precipitation from the westernmost planting location (Colby) to the easternmost planting location (Illinois), the difference in potential evapotranspiration between east and west is also responsible for composition differences. The 2010 growing degree days explained 17–88% of the variation in chemical concentrations. The potential evapotranspiration explained 55–80% of the variation in biomass composition (Table 2.7). The higher precipitation gradient in Illinois is almost one and a half times higher than Colby, which provides a better environment for biomass accumulation. A similar tendency was also observed for hemicellulose (xylan and arabinan). The highest and the lowest hemicellulose contents in the four planting locations, respectively, are Illinois with an average of 29.7% and Colby with an average of 25.0% (Figure 2.2). The difference in hemicellulose content was about 19% among the four locations. The total structural polysaccharides content of big bluestem planted in Illinois was about 15% higher than that planted in Colby; however, this increase was associated with higher lignin content. The average lignin contents of all planting locations exhibited a decreasing trend with the ecotype from east to west. In fact, 2010 growing degree days and 2010 precipitation explained 88% and 74% of the variation in lignin concentrations, respectively (Table 2.7). Big bluestem in Colby had average of 14.4% lignin, which is significantly lower than samples planted in Illinois, with average of 18.0% (Figure 2.2). Taking into account the adverse effects of lignin in hydrolysis, further research is needed to determine the sugar yield and fermentation efficiency of all samples to determine the overall location effects. The range of ash contents among 28 samples was quite different in four locations. Ash contents of big bluestem from Illinois (with an average of 4.8%; data not shown) were higher than those populations in the other three planting locations in Kansas. Results suggest that big bluestem planted in Kansas with lower ash content would be best suited for the thermoconversion of biomass to biofuel (Monti et al., 2008).

Figure 2.2 Effects of planting location on chemical composition of big bluestem.

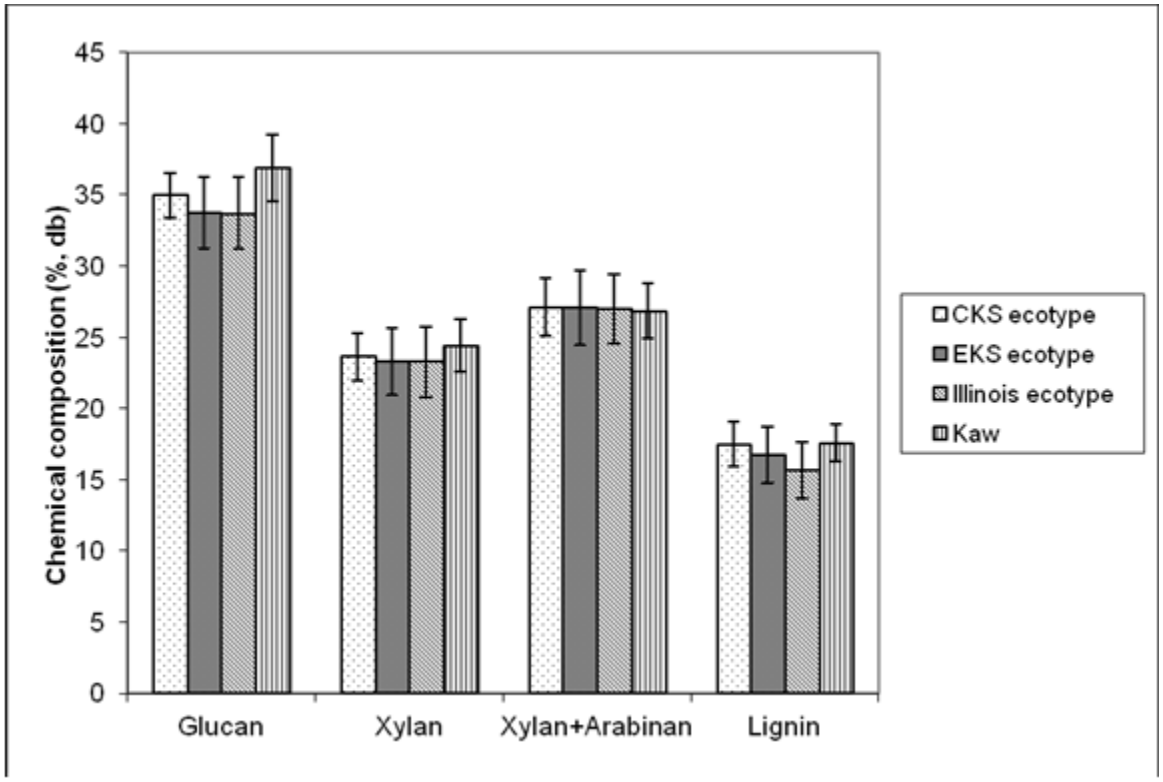


2.4.2 Effects of Ecotype on Chemical Composition.

The composition results also showed a significant variation among the different ecotypes at $P < 0.001$ and F values from 3.36 to 28.5, except xylan+arabinan, with $P = 0.935$ and $F = 0.14$ (Table 2.8). Based on F value, ecotype had more significant effects on glucan and lignin, with F values of 28.5 and 16.2, respectively. Hays ecotype and KAW had significantly higher glucan contents than East KS and Illinois ecotypes. KAW had the highest glucan content among all the ecotypes (Figure 2.3). This could be explained by the fact that the KAW cultivar, as the native released cultivar, was selected and bred for carbohydrate accumulation. Of these 28 samples, the highest carbohydrates content was found in KAW at the Illinois location, which indicates combined effects of ecotype and planting location. Although xylan content differs significantly

among the different ecotypes, the average values of xylan of the different ecotypes are similar (Figure 2.3), indicating no clear effect of ecotype on the average xylan contents within the ecotypes from west to east. This result is probably because glucan and xylan contents were not solely affected by ecotype. The highest and lowest lignin contents of big bluestem were Central KS ecotype and Illinois ecotype, respectively. Results suggest that the Central KS ecotype showed higher lignin content (17.5%) than the Illinois ecotype (15.7%) because of adaptation to drought necessitated by a dry growing environment. The high lignin content may result in relatively lower efficiency of degradation in bioconversion.

Figure 2.3 Effects of ecotype on chemical composition of big bluestem.



2.4.3 Effects of Interactions between Location and Ecotype on Chemical Composition.

Variations in the glucan, xylan, xylan+arabinan, lignin, and ash contents among the 28 samples were analyzed by two-way ANOVA for examining the genetic and environmental effects on chemical composition of the big bluestem. In general, ANOVA analysis revealed that ecotype and location had significant effects on chemical composition including glucan, xylan, lignin, and ash contents as well as xylan + arabinan content (Table 2.8). Location had larger *F* values (7.2–73.6) than ecotype (0.14–28.5) and interactions (1.12–3.59), showing that location effects were always highly significant with larger *F* values, at times approaching two orders of magnitude larger; however, significant interactions between location and ecotype have been found only for glucan, with $P < 0.002$ and an *F* value of 3.59, and lignin, with $P = 0.018$ and an *F* value of 2.64, indicating that the glucan and lignin contents of big bluestem were significantly affected by the combined effects of ecotype and growing locations.

2.4.4 Effects of Ecotype and Planting Location on Elemental Composition.

Table 2.5 shows the carbon, hydrogen, nitrogen, oxygen, and sulfur fractions and H/C ratio of the big bluestem samples. The range of elemental fractions is 47.1–51.4% for carbon, 4.93–6.45% for hydrogen, 40.7–46.1% for oxygen, 0.61–1.27% for nitrogen and 0.78–0.98% for sulfur. The variations of the elements are 10.1% for carbon, 30.8% for hydrogen, 13.2% for oxygen, 108% for nitrogen, and 25.6% for sulfur. The average ratio of H/C is 1.44 with variation of 23.6%. Two-way ANOVA analysis shows through larger *F* values that location had more effects than ecotype and ecotype-location interaction on elemental composition of big bluestem (Table 2.8). Location had significant effects on all of the elemental fractions, with *F* values from 12.0 to 80.8 and $P < 0.001$. Ecotype had significant effects on carbon, oxygen, and hydrogen with *F* values from 2.94–11.50 and *P* values from 0.001–0.044. Ecotype-location interaction had a significant effect only on carbon content. The linear regression results between composition and environmental factors showed that precipitation explained 37–79% of variation in elemental fractions based on coefficients of determination (R^2) from 0.37–0.79 in growing year 2010 (Table 2.7). Growing degree days and the potential evapotranspiration also explained a large variation in the elemental composition of the big bluestem samples.

Table 2.5 Elemental composition of big bluestem and ratio of hydrogen to oxygen (H/C) as affected by population and planting site.

Population-location	Elemental composition (%)					H/C[a]
	C	H	O	N	S	
CDB (CKS)-Colby	48.6±0.1	5.56±0.05	44.3±0.1	0.64±0.01	0.85±0.01	1.37
WEB (CKS)-Colby	47.2±0.1	5.75±0.06	45.5±0.1	0.69±0.01	0.88±0.04	1.47
KON (EKS)-Colby	47.4±0.1	5.57±0.01	45.6±0.1	0.69±0.01	0.83±0.04	1.41
TOW (EKS)-Colby	47.9±0.1	5.62±0.01	45.1±0.1	0.61±0.01	0.83±0.03	1.40
12M (ILL)-Colby	47.6±0.1	4.93±0.03	46.1±0.1	0.57±0.01	0.78±0.02	1.23
FUL (ILL)-Colby	48.5±0.1	5.66±0.03	44.2±0.1	0.72±0.01	0.89±0.01	1.40
KAW (CULTIVAR)-Colby	49.8±0.1	5.36±0.01	43.4±0.1	0.52±0.01	0.83±0.02	1.28
CDB (CKS)-Hays	47.8±0.1	6.01±0.02	44.3±0.1	1.02±0.02	0.97±0.02	1.51
WEB (CKS)-Hays	47.5±0.1	5.75±0.04	44.9±0.1	0.92±0.01	0.88±0.01	1.45
KON (EKS)-Hays	47.4±0.0	5.88±0.02	44.7±0.1	1.12±0.02	0.93±0.01	1.49
TOW (EKS)-Hays	47.7±0.1	5.88±0.02	44.5±0.1	1.00±0.01	0.93±0.01	1.47
12M (ILL)-Hays	47.1±0.1	5.76±0.02	45.6±0.1	0.69±0.02	0.87±0.02	1.46
FUL (ILL)-Hays	48.8±0.1	6.11±0.01	43.1±0.1	1.07±0.03	0.98±0.01	1.50
KAW (CULTIVAR)-Hays	49.0±0.1	6.13±0.04	43.2±0.1	0.73±0.03	0.98±0.01	1.49
CDB (CKS)-Manhattan	49.3±0.1	6.03±0.03	42.7±0.1	1.15±0.07	0.92±0.01	1.47
WEB (CKS)-Manhattan	49.8±0.1	5.95±0.01	42.6±0.1	0.77±0.04	0.93±0.01	1.43
KON (EKS)-Manhattan	47.9±0.1	5.86±0.01	44.4±0.1	0.86±0.01	0.93±0.03	1.46
TOW (EKS)-Manhattan	49.47±0.1	5.97±0.02	43.0±0.1	0.73±0.02	0.93±0.02	1.45
12M (ILL)-Manhattan	49.3±0.1	5.94±0.03	42.6±0.1	1.14±0.03	0.96±0.01	1.43
FUL (ILL)-Manhattan	50.0±0.1	6.23±0.01	42.1±0.1	0.66±0.01	0.96±0.01	1.49
KAW (CULTIVAR)-Manhattan	49.6±0.1	5.90±0.02	42.8±0.1	0.75±0.01	0.94±0.04	1.43
CDB (CKS)-Carbondale	50.7±0.1	6.30±0.02	41.1±0.1	0.93±0.01	0.97±0.02	1.49
WEB (CKS)-Carbondale	50.8±0.1	6.45±0.01	41.1±0.1	0.76±0.02	0.97±0.02	1.52
KON (EKS)-Carbondale	50.1±0.1	6.16±0.01	41.5±0.1	1.27±0.03	0.95±0.01	1.47
TOW (EKS)-Carbondale	51.4±0.1	6.11±0.01	40.8±0.1	0.84±0.01	0.94±0.02	1.42
12M (ILL)-Carbondale	50.5±0.1	5.85±0.01	41.8±0.1	0.88±0.04	0.94±0.03	1.38
FUL (ILL)-Carbondale	51.3±0.1	6.03±0.01	40.8±0.1	0.95±0.01	0.94±0.01	1.41
KAW (CULTIVAR)-Carbondale	51.3±0.1	6.15±0.01	40.7±0.1	0.87±0.01	0.96±0.01	1.43
Average	49.1±1.4	5.9 ±0.3	43.3 ±1.6	0.84 ±0.2	0.92 ±0.1	1.44±0.1

[a] $H/C=\frac{H\%/1}{C\%/12}$

Because the carbon content is the most important factor related to its bioconversion yield and heat content, the histogram showed a parabolic trend with ecotype from west to east, indicating that the middle-location ecotype (EKS ecotype) had the lowest carbon content of the three ecotypes (Figure 2.4). In general, the carbon content of the big bluestem (average of 50.8%) planted in Illinois is higher than its counterparts planted in the Kansas locations (average of 49.2% for Manhattan, 47.7% for Hay, and 47.8% for Colby). Decreased longitude of planting location resulted in increased carbon content, which was similar to the trend of environmental effect on chemical composition. Also noteworthy is that the big bluestem in Colby had significantly lower nitrogen content (average of 0.65%) compared with other locations (average of 0.9%) (Figure 2.5). Low nitrogen fraction in biomass could be an advantage for the combustion process with low NO_x emission (Obernberger and Thek, 2004). However, planting location had no clear effect on hydrogen and sulfur (Figure 2.6).

Figure 2.4 Effects of planting location on carbon content of big bluestem.

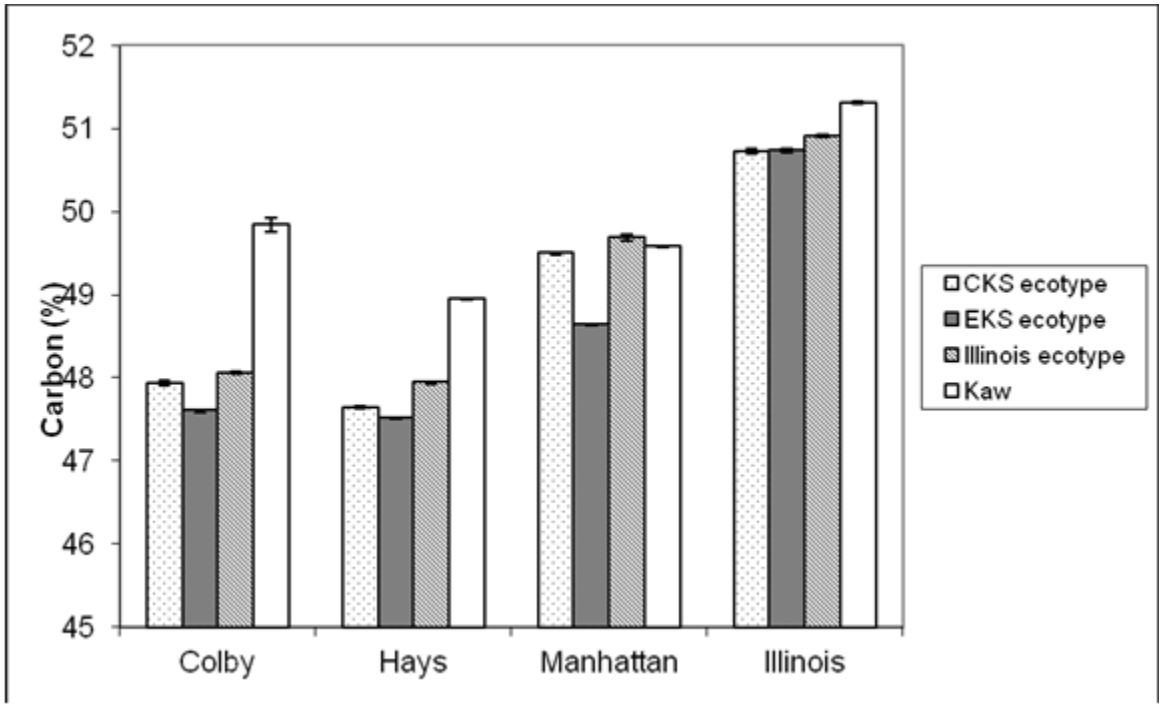


Figure 2.5 Effects of planting location on nitrogen content of big bluestem.

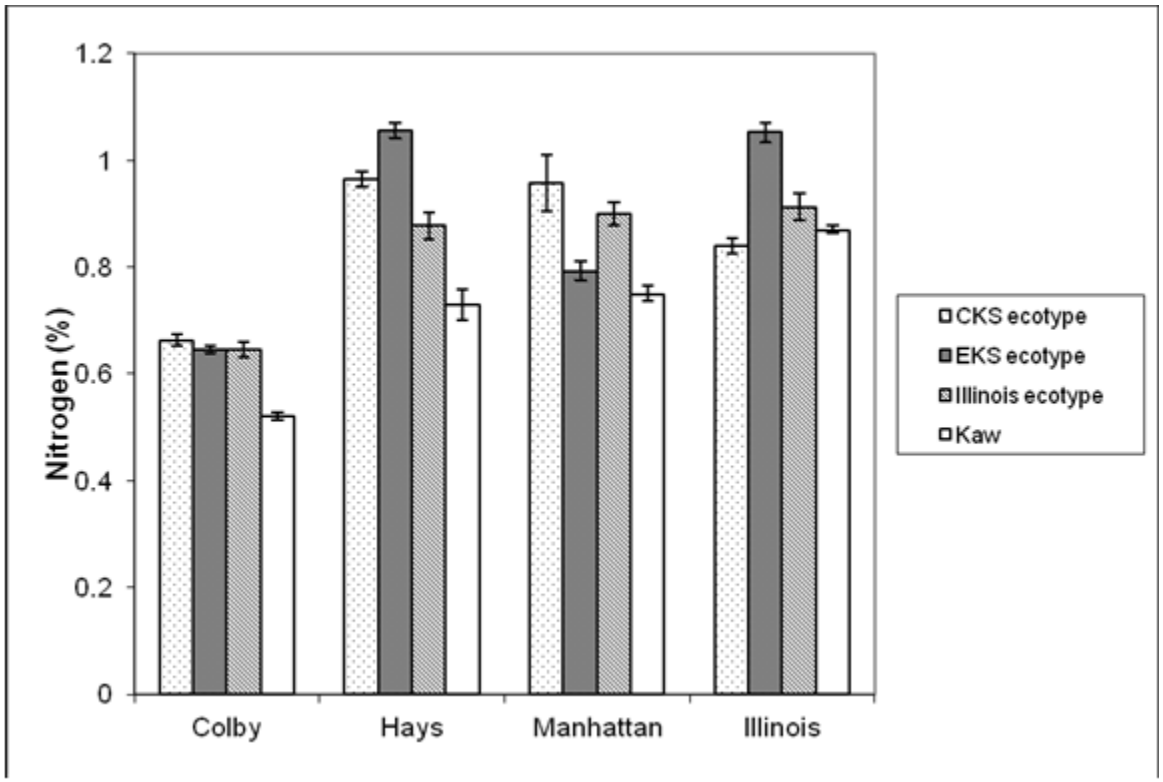


Figure 2.6 Effect of ecotype on carbon, oxygen contents hydrogen, nitrogen, and sulfur.

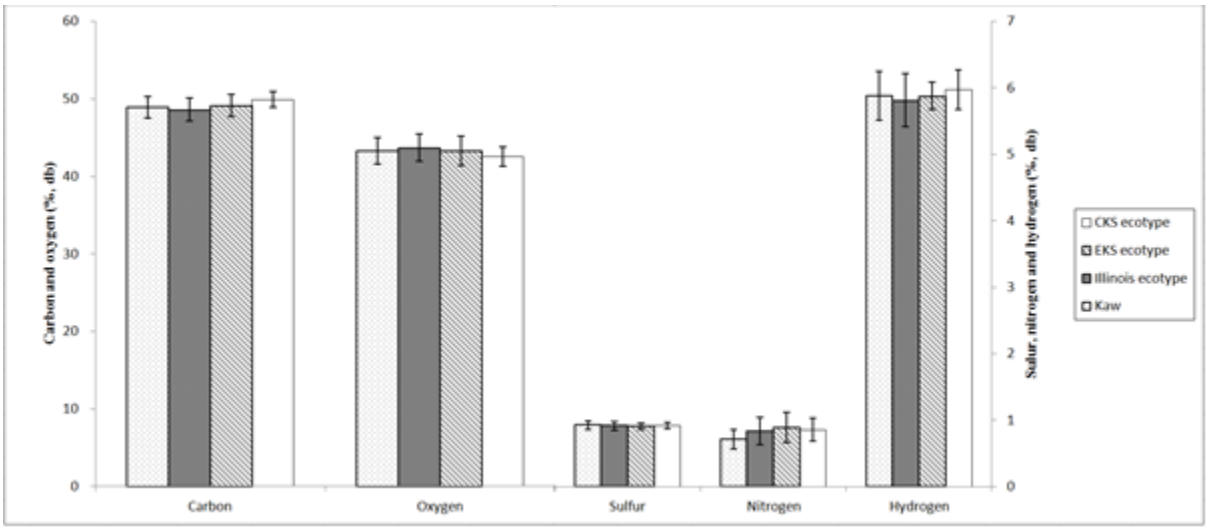


Table 2.6 Comparison of the elemental composition of different types of biomass[a].

Type of biomass	Elemental composition (%)					H/C [b]
	C	H	O	N	S	
Big bluestem—this study	49.1	5.9	43.3	0.84	0.92	1.44
Bagasse (sugarcane)	44.8	5.3	39.6	0.38	0.01	1.42
Barley straw	45.7	6.1	38.3	0.4	0.1	1.60
Cotton stalk	13.6	5.8	43.9	-	-	5.12
Corn stover	43.7	5.6	43.3	0.61	0.01	1.54
Pine (bark)	52.3	5.8	38.8	0.2	-	1.33
Popular (hybrids)	48.5	5.9	43.7	0.47	0.01	1.46
Redwood	53.5	5.9	40.3	0.1	-	1.32
Rice straw	41.8	4.6	36.6	0.7	0.08	1.32
Switchgrass	47.5	5.8	42.4	0.74	0.08	1.47
Wheat straw	43.2	5.0	39.4	0.61	0.11	1.39

[a] Data source: Cheng 2010(Cheng, 2010)

[b] $\text{H/C}=\frac{H\%/1}{C\%/12}$

Table 2.7 Effects of environmental conditions on chemical composition and elemental fractions of big bluestem analyzed by linear regression models.

Composition (% db)	PPT[a] 2010 (cm)	PPT[a] since 1961 (cm)	GDD[b] avg. (cm)	GDD[b] 2010 (cm)	PET[c] (cm)	Aridity index
Glucan	0.94	0.84	0.93	0.8	0.72	0.81
Xylan	0.97	0.93	0.78	0.66	0.8	0.88
Xylan+ Arabinan	0.88	0.99	0.63	0.67	0.91	0.96
Lignin	0.74	0.63	0.96	0.88	0.55	0.63
Ash	0.37	0.65	0.08	0.17	0.67	0.64
Carbon	0.7	0.96	0.42	0.57	0.95	0.96
Hydrogen	0.69	0.69	0.9	0.96	0.64	0.69
Oxygen	0.79	0.99	0.61	0.76	0.98	0.99
Nitrogen	0.37	0.34	0.74	0.82	0.32	0.36
Sulfur	0.61	0.52	0.91	0.87	0.46	0.52

[a] PPT: Precipitation

[b] GOD: Growing degree days

[c] PET: Potential evapotranspiration

Table 2.8 Effects of ecotype (E), location (L), and interaction between ecotype and planting location on the chemical and elemental composition of big bluestem.

Composition /elements (%)	Source of variation	Location	Ecotype	L×E
Glucan	*F*	73.56	28.51	3.59
	P	<0.001	<0.001	0.002
Xylan	*F*	58.98	3.36	1.811
	P	<0.001	0.028	0.096
Xylan+ Arabinan	*F*	63.70	0.14	1.12
	P	<0.001	0.935	0.369
Lignin	*F*	48.98	16.23	2.61
	P	<0.001	<0.001	0.018
Ash	*F*	7.23	9.62	1.39
	P	<0.001	<0.001	0.224
Carbon	*F*	80.77	11.50	1.69
	P	<0.001	<0.001	0.123
Oxygen	*F*	86.66	5.98	1.67
	P	<0.001	0.002	0.129
Hydrogen	*F*	45.27	2.94	3.24
	P	<0.001	0.044	0.005
Nitrogen	*F*	12.02	2.60	1.13
	P	<0.001	0.065	0.359
Sulfur	*F*	29.52	0.46	1.15
	P	<0.001	0.706	0.347

Chapter 3 - Thermal properties of big bluestem as affected by ecotype and planting location along the precipitation gradient of the Great Plains[2]

3.1 Abstract

The objective of this research was to study the effect of ecotype and planting location on thermal properties of big bluestem. Three big bluestem ecotypes (CKS, EKS, ILL) and a cultivar (KAW) were harvested in 2010 from four locations (Colby, Hays, and Manhattan, KS; and Carbondale, IL) and were evaluated for their thermal properties, including specific heat, thermal conductivity, thermal stability, high heating value, and proximate contents. All populations revealed a large variation in specific heat (2.35–2.62 kJ/kg/K), thermal conductivity (77.85–99.06 $\times 10^{-3}$ W/m/K), thermogravimetric analysis as weight loss during the heating process (71–73%), and high heating value (17.64–18.67 MJ/kg). Specific heat of the big bluestem samples was significantly affected by planting location, ecotype, and interaction between location and ecotype. Planting location had stronger influence on specific heat than ecotype. Specific heat increased as temperature increased, and a linear correlation model for specific heat prediction was developed as a function of temperature. Ecotype, planting location, and the interaction of ecotype and planting location did not have a significant effect on thermal conductivity; however, density and particle size showed a completely opposite relationship on thermal conductivity. With the exception of weight loss, planting location alone had a significant effect on thermogravimetric parameters of big bluestem. Both planting location and ecotype significantly affected high heating value. Among all environmental factors, potential evapotranspiration had the most significant effect on thermal properties.

3.2 Introduction

Renewable energy has received growing attention as people have become more conscious of the fossil fuel shortage and greenhouse gas emissions have been related to global warming

[2] This chapter has been published as a peer-reviewed research paper in the Journal of *Energy.* 2014. 64: 164-171.

(Bioenergy, 1998). In 2010, renewable energy resources supplied 8% of the nation's total energy consumption and up to 8.05 quadrillion Btu (increased from 6% in 2009). Biomass led the other renewable energy resources (such as wind, solar, geothermal, and hydroelectric) by contributing 53% of the nation's renewable energy supply in 2010 (Bioenergy, 1998). Biomass resources include various natural and derived materials, such as woody and herbaceous species, wood wastes, bagasse, agricultural and industrial residues, waste paper, municipal solid waste, sawdust, biosolids, grass, waste from food processing, animal wastes, aquatic plants, algae, etc. (Yaman, 2004). Big bluestem (*Andropogon gerardii*) is regarded as second-generation biomass and recently has been proposed as promising energy crops because their growth requires few agricultural inputs (fertilizer and pesticides). In the United States, big bluestem dominates the tallgrass prairie of North America and is a major component of prairie biomass (Knapp et al., 1998; Weaver, 1968). Moreover, tolerance to heat and drought have enabled big bluestem to fill the deficiency in grasslands in the Midwestern U.S. when cool-season grasses (C3) are unproductive (Barnes et al., 1995; Moore and Anderson, 2000). Recently Nature article reported that successional herbaceous vegetation, such as big bluestem and alfalfa, on marginal lands in tem Midwestern U.S. states can not only provide greenhouse gas emissions mitigation, but also produce substantial proportion of future biofuel energy target (Gelfand et al., 2013).

Thermal, biological, and physical processes are the three major technologies that help make use of a wide variety of biomass. In thermal conversion technologies, direct combustion and co-firing with coal were first utilized for electricity production and once were responsible for over 97% of the world's bio-energy production (Demirbas, 2004). Pyrolysis has attracted the highest interest because it produces bio-oil, which can be used as a fuel in stable engines and converted into chemicals such as bio-lime nitrogen fertilizer (Czernik and Bridgwater, 2004). Biomass gasification has been researched extensively due to its higher efficiencies compared with combustion, and fast pyrolysis is still at a relatively early stage of development (Bridgwater, 2003). Torrefaction, another promising thermal process, improves the quality of biomass in terms of heat content, physical properties, and chemical composition for combustion and gasification applications (Shankar Tumuluru et al., 2011).

Understanding, predicting, and controlling these thermal processes and designing processing equipment require knowledge of the thermal properties of biomass, such as specific heat, thermal conductivity, thermal stability, high heating value, and proximate contents.

Specific heat of a substance (kJ/kg/K) is defined as the amount of heat required to increase the temperature of a unit of mass by one degree. Specific heat affects the total energy required for thermal conversion of biomass into biofuels. The specific heat of biomasses depends largely on their composition; using the specific heat of each component of a mixture and the mass fraction is usually sufficient to predict the specific heat of the mixture (Rao et al., 2010). Although the method based on the specific heat of the components in the mixture is most widely used to predict specific heat because of its simplicity, experimentally determined value is usually higher than predicted value (Rahman, 2008). Koch utilized differential scanning calorimetry (DSC) as a convenient technique for measuring the specific heat of wood and bark of 72 spruce pine trees (Koch, 1968).

Thermal conductivity of a material (W/m K) is a measure of its ability to conduct heat. Thermal conductivity of biomass depends mostly on composition and the characteristics of the biomass that affect the heat flow paths through the material. Mohsenin reviewed thermal conductivity measurement techniques for both steady-state and transient-sate transfers (Mohsenin, 1980). The heated probe method is simple, fast, and requires only a small sample, and it has been widely used for thermal conductivity determination (Murakami et al., 1996). Thermal stability, the ability of a material to resist changes in physical shape or size as its temperature changes, is essential to understand and predict the reactions and kinetics during biomass thermal conversion. Thermogravimetric analysis (TGA) is the usual technique for determining thermal stability by quantitative measurement of weight changes (loss/gain) associated with thermally induced transition as a function of temperature or time (Dranca and Vyazovkin, 2009). High heating value (MJ/kg) is an important thermal parameter to characterize the amount of energy produced by the combustion of a unit mass of a material. Proximate analysis is a simple and rapid procedure for defining the substance energy content and determining how clean and efficient the substance is for the purpose.

Our recent research on big bluestem showed that planting location and ecotype as well as interaction of planting location and ecotype had significant effects on chemical and elemental composition of big bluestem (Zhang et al., 2012). In addition, our study also showed that bio-oil yield from big bluestem through hydrothermal conversion was significantly affected by both ecotype and planting location (Gan et al., 2012). However, we found no research on the thermal properties of big bluestem, especially the effects of ecotype and planting location on the thermal

properties of big bluestem. Therefore, the objectives of this research were to characterize the thermal properties of big bluestem and to study the effects of ecotype and planting location on thermal properties of big bluestem and thus, fill a critical gap in fundamental knowledge of thermal properties of a valuable bioenergy grass.

3.3 Materials and methods

3.3.1 Materials.

Three big bluestem ecotypes, CKS (Cedar Bluffs [CDB]), EKS (Konza [KON]), and ILL (12Mile [12M]), and the KAW cultivar, which is widely planted to restore marginal lands, were harvested from reciprocal garden plots in four planting locations (Colby, Hays, and Manhattan, KS; and Carbondale, IL) in 2010. Among the four locations, the Colby planting site was used to test the threshold of drought tolerance and the possibility for planting in drier Great Plains locations. Two populations from each ecotype were evaluated for thermal properties. Details of seed collection and planting location have been described previously (Ke Zhang et al., 2014). The big bluestem samples were ground into powder using a Retsch SM2000 cutting mill (Haan, Germany) with a 1.0-, 2.0-, and 4.0-mm sieve, respectively. For thermal conductivity measurement, only 2.0- and 4.0-mm particle sizes were used. After grinding, each sample was fully mixed in sealed plastic storage bags. To eliminate any error that might be caused by water, samples were dried at 45 ^{o}C for 24 h before measuring specific heat, thermal conductivity, high heating value, and proximate content.

3.3.2 Specific heat by differential scanning calorimetry. (DSC)

Specific heat of big bluestem was measured with DSC Q200 V24.4 instrument (TA Instruments, New Castle, DE) calibrated with indium and zinc. An empty sealed pan was used as a reference for every measurement. Three-step scans were carried out in this study. The first scan was conducted with an empty hermetic pan to determine the baseline background heat flow, which was subtracted from subsequent measurements. Next, sapphire was weighed and sealed in a pan for determination value of E. E was the calibration constant and calculated by using Equation 3.1:

$$E = \frac{C_{ps} \times H_r \times M}{H \times 60} \qquad (3.1)$$

where C_{ps} is specific heat of sapphire, which was standard and obtained from the literature (kJ/kg/K); H_r is heating rate, which was 10 in this study (K/min); M is sapphire mass (mg); H is measured heating value (mW); and 60 is conversion constant (min to sec).

For the sample run, an empty pan and a pan with a 5-mg sample were placed into the DSC. The specific heat of the sample is calculated by transposition equation and substituting E. Large-volume stainless steel pans were used. All measurements were held at 323 K for 10 min, scanned from 323 K to 473 K at a heating rate of 10 K/min, and then held at 473 K for another 10 min. The sample was characterized in an inert environment by using nitrogen with a gas flow rate of 50 ml/min.

3.3.3 Thermal conductivity by probe method.

Figure 3.1 shows a diagram of the experimental apparatus for measurement of thermal conductivity using the heated needle probe. The container is filled with biomass with a confined particle size and sample density. The straight needle probe (60 mm; Thermal logic, Washington, USA) containing a heater wires as heat source and a thermocouple as a temperature-measuring device is inserted at the center of the container. The container's diameter is 30 mm to ensure measurement time (3 min) shorter than the time allowing heat transfer to reach the wall of the container. After the initial temperature is equilibrated at room temperature, the heating wire is activated and heated at a constant rate of energy input supplied by a pair of AAA batteries. Then, the temperature rise over time is measured with thermocouple and analyzed by Data Acquisition System NI USB-9161(National Instruments). For the mathematical analysis, thermal conductivity can be expressed by Equation 3.2:

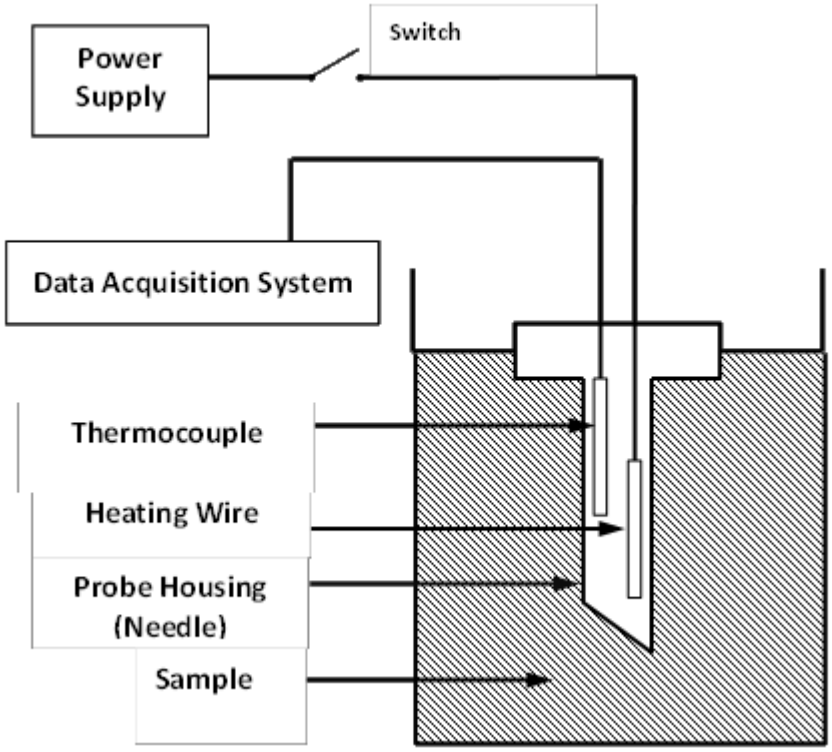
$$k = \frac{q \ln\left(\frac{t}{t_0}\right)}{4\pi\left(T - T_0\right)} \qquad (3.2)$$

where k and q are the thermal conductivity (W/m/K) and heater power dissipated per unit length (W/m), respectively. T_0 and t_0 are temperature (K) and time (s) at initial condition. T and t represent temperature (K) and time (s) since the probe heated is energized. The heat power q is calculated using Equation 3.3:

$$q = \frac{E_{ref}^2}{R_{ref}^2}(R_m) \qquad (3.3)$$

where *Rm* is 1041.5Ω for the probe heater and E_{ref} (1Ω) and R_{ref} (0.04V) are voltage and resistance in the reference resistor, respectively.

Figure 3.1 The diagram of apparatus for thermal conductivity measurement using the probe method.



3.3.4 Thermogravimetric analysis.

The determinations were performed with the Perkin–Elmer Pyris1 thermogravimetric analysis (TGA) instrument (Norwalk, CT) to record the sample mass change with temperature over the course of the paralysis reaction. Initial TGA measurement was carried out at a heating rate of 10 °C/min up to 900 °C to establish the temperature range required for the following investigations. About 8 mg of each sample obtained from a tensile bar was placed in the pan and heated from 30 to 600 °C at a heating rate of 30 °C/min in a nitrogen environment.

3.3.5 High heating value.

Gross energy contents were determined by means of a calorimeter (IKA-Calorimeter C 200, IKA-Werke GmbH and Co. KG, Staufen, Germany) with a benzoic acid standard. About 1.00 g of each pelleted sample was put into an adiabatic bomb and burned to ash. All samples were ground by a miller with a 1.0-mm sieve. Powder samples were compacted into pellets for measurement to reduce error caused by incomplete combustion resulting from dry, loose samples blown away during sudden release of volatiles. In addition, heating value was calculated based on the elemental composition of the biomass sampled.

3.3.6 Proximate analysis.

Ash content was determined according to ASTM D-1102-84, "Standard test method for ash in wood" (ASTM., 2001). Volatile matter determination was made in accordance to ASTM E872-82, "Standard test method for volatile matter in the analysis of particulate wood fuels" (ASTM., 2006). Fixed carbon was determined by subtracting the summation of the moisture, volatile matter and ash contents from the total sample mass.

3.3.7 Statistical analysis.

The reported thermal property values of big bluestem samples are the average of at least two replicates. Data were analyzed with analysis of variance (ANOVA) and Tukey's studentized range (HSD) test in SAS (SAS Institute, Inc., Cary, NC). In general, fully balanced ANOVA tests were performed by following the general linear models (GLM) procedure. Correlations were determined using stepwise multiple regressions and multiple linear regression analysis. In addition to investigating effects of environmental conditions on thermal properties of big bluestem, we performed multiple univariate linear regression analysis. For all weather data, we used the National Oceanic and Atmospheric Administration database and historical records dating back to 1961 (see (K Zhang et al., 2012) for further details).

3.4 Results and discussion

3.4.1 Specific heat

Figure 3.2 shows the typical DSC plots of big bluestem, in which heat flow was investigated as a function of temperature. The maximum temperature of DSC measurement was

set at 473K to prevent explosion of the sealed pan from emissivity of sample pyrolysis. A previous study did not recommend DSC analysis without the lid, although this approach can heat to higher temperature and was more approximate for biomass pyrolysis and gasification (Wolfinger et al., 2001). The green curve is the baseline with the sealed pan at a heating rate of 10K/min and was subtracted from following measurements. The red curve and blue curve represent the biomass sample and reference (sapphire) under the same conditions, respectively. The heat flow curve includes the heats evolved during decomposition and reaction of the pyrolysis sample material. Therefore, during an endothermic heat effect, specific heat values increase, whereas specific heat decreases during an exothermic reaction (Strezov et al., 2007). The heat flow curve of the sample shows a decreased trend after 420K, although three curves exhibit the positive relationships between heat flow and temperature from 323K to 420K. Because the samples were previously dried to eliminate error from water, this endothermic reaction was associated with decomposition of hemicellulose (Yang et al., 2007). Cultivar KAW planted in IL exhibited the most pronounced endothermic peak in this range due to its relatively higher specific heat value and hemicellulose content. The typical relationship between the specific heat and temperature of big bluestem is illustrated in Figure 3.3. The value of specific heat increased linearly from 1.73 to 2.24 kJ/kg/K as temperature increased from 323K to 420K and followed a second-order polynomial in the whole range. This result suggests that the specific heat of big bluestem increased with heating until the component because to decompose. The average value of the specific heat of big bluestem at 420K is 2.46 kJ/kg/K (Table 3.1), which is in line with the previously reported range of 2.30 to 2.60 kJ/kg/K for wood and grass particle biomasses (Larfeldt et al., 2000). Stepwise multiple regression analysis was conducted to relate specific heat to temperature with factors K and K^2. The best equation accounted for 85.1% of variation with a standard error of the estimate of 0.012 expressed as Equation 3.4:

$$Specific\ heat\ of\ big\ bluestem = -0.1581T^2 + 124.64T - 22281 \qquad (3.4)$$

Figure 3.2 Typical calorimeter plots for specific heat determination of big bluestem (green curve shows the baseline, and red and blue curves represent the DSC curve of big bluestem and a reference).

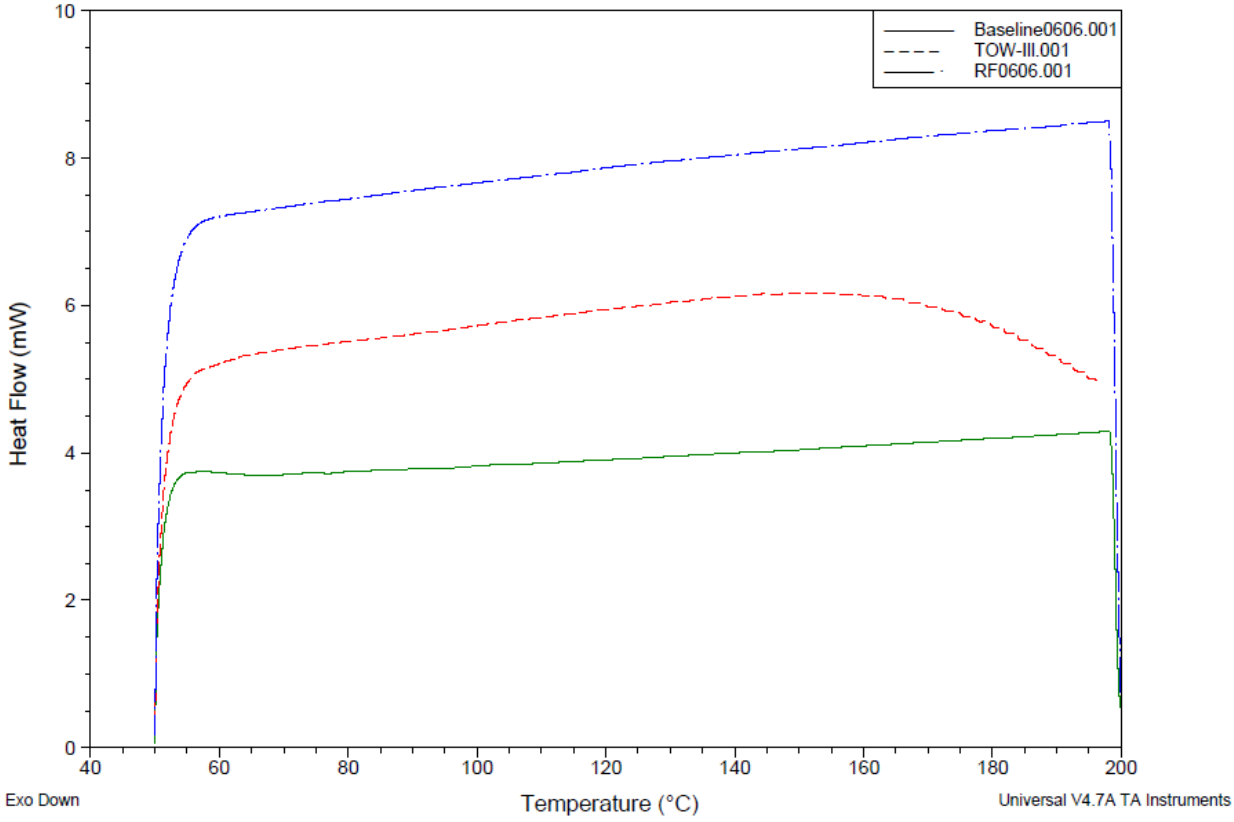
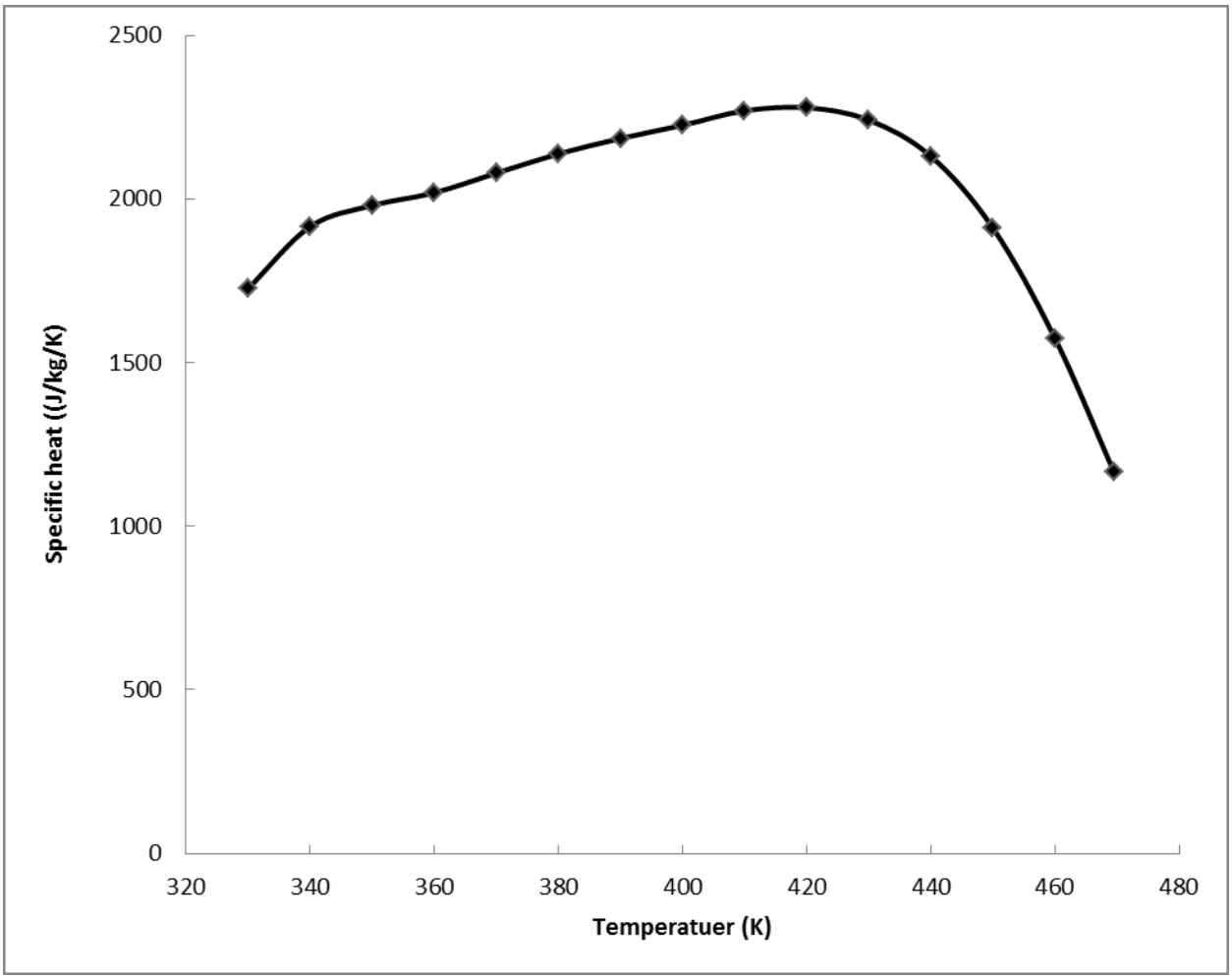


Figure 3.3 Specific heat of big bluestem as a function of temperature.



Equation 3.4 agreed well with the work of Gupta et al. (Gupta et al., 2003), which indicated that the specific heat of biomass increased by 40% and basically followed a linear pattern before 430 K then followed a second-order polynomial for the entire pyrolysis process (Figure 3.3).

The bars on left side of Figure 3.4 show the effects of planting location on the specific heat of big bluestem. Big bluestem populations planted in Illinois generally had a higher specific heat at 420K, with an average of 2.62 kJ/kg/K compared with averages of 2.34 kJ/kg/K, 2.40 kJ/kg/K, and 2.35 kJ/kg/K for populations planted in Colby, Hays, and Manhattan, KS, respectively. The average specific heat of big bluestem planted in Illinois was 0.25 kJ/kg/K, which is higher than those from Kansas and indicates that the same big bluestem populations would have around 10.4% higher value of specific heat if planted in Illinois instead of Kansas.

The effect of ecotype on specific heat is shown on right side of Figure 3.4. CKS, EKS, and ILL ecotypes had statistically similar average specific heat values regardless of planting location, but native cultivar KAW had significantly higher specific heat value than other ecotypes, as indicated by the different letters analyzed by Tukey's HSD test. This result was in agreement with previous studies (Gan et al., 2012; Zhang et al., 2012). Table 3.2 shows that the interaction between planting location and ecotype also had significant effect on specific heat of big bluestem ($p < 0.05$); however, the interaction effect was much less than that of planting location and ecotype and had a smaller *F*-value (11.3). It is noted that the role of the ecotype was always a greater source of variation than location and the interaction between ecotype and location for specific heat value based on three orders of magnitude larger *F*-value (Table 3.2).

Figure 3.4 Comparisons of average specific heat of big bluestem as affected by planting location and ecotype. Different letters (a and b) above the standard deviation bars indicate they are statistically different at a 95% confidence level.

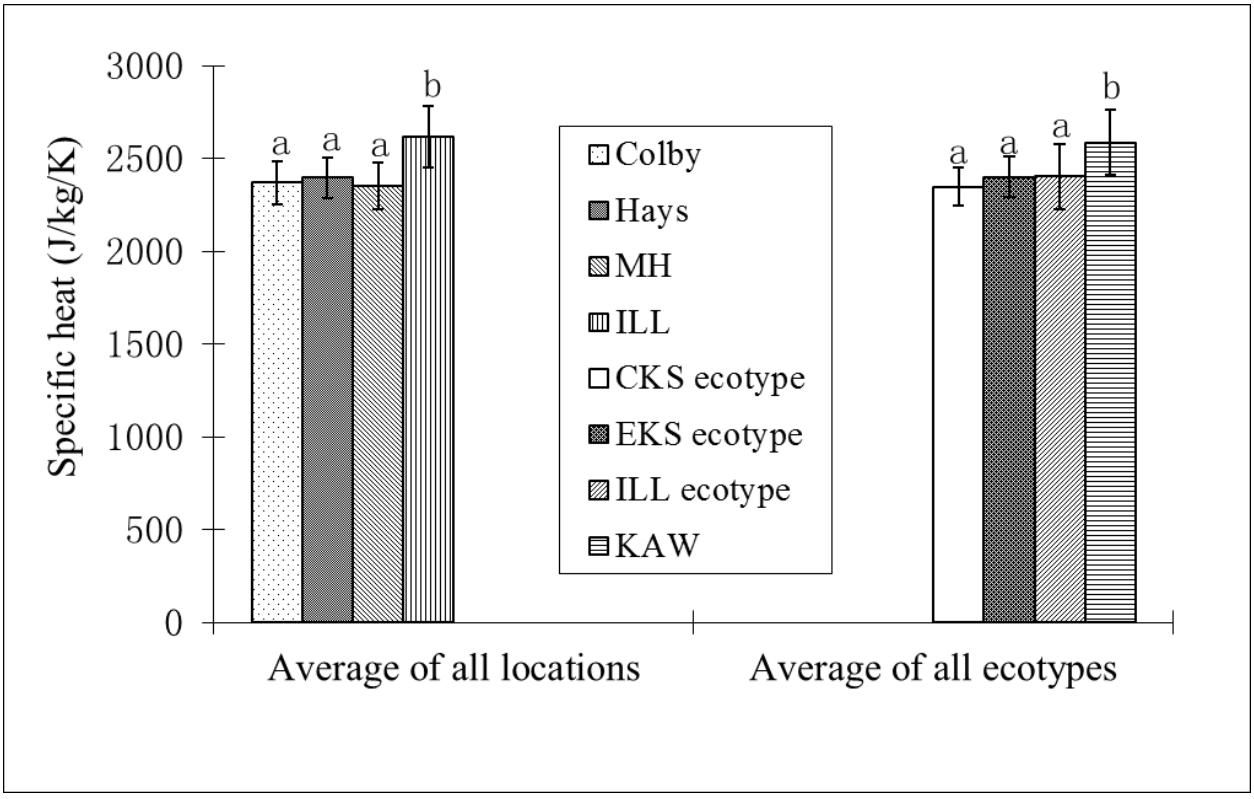


Table 3.1 Specific heat, thermal conductivity, and thermogravimetric parameters by planting location and ecotype.

Sample	Specific heat (kJ/kg/K)	Thermal conductivity (mW/m/K)	T_{onset} (°C)	T_{end}(°C)	T_{max}(°C)	Weight loss (%)
CKS-Colby	2.23±0.05	79.28±2.02	296.1±1.2	404.9±7.2	357.6±7.5	71.53±0.1
EKS-Colby	2.33±0.05	80.49±2.83	291.8±2.6	410.3±2.8	363.9±1.7	71.35±0.1
ILL-Colby	2.42±0.05	90.43±1.77	296.2±7.3	406.3±0.7	356.2±2.1	71.70±0.4
KAW-Colby	2.50±0.06	87.74±1.94	301.2±0.2	412.1±.02	364.1±0.1	74.33±0.5
CKS-Hays	2.29±0.06	89.02±3.96	292.4±0.8	405.9±1.8	363.0±1.0	71.13±0.6
EKS-Hays	2.32±0.05	82.00±2.38	291.7±1.1	407.6±7.7	356.6±0.5	71.25±2.7
ILL-Hays	2.42±0.02	85.48±1.15	291.5±5.4	410.2±4.4	364.6±5.3	72.08±2.2
KAW-Hays	2.54±0.02	82.96±2.23	290.4±0.5	407.3±0.4	364.1±0.1	72.38±0.5
CKS-MH	2.43±0.04	85.49±3.55	299.2±1.7	404.1±1.7	353.2±0.7	70.95±0.2
EKS-MH	2.38±0.03	84.05±0.80	302.9±2.9	407.3±3.5	358.4±2.5	70.18±2.0
ILL-MH	2.15±0.03	83.31±3.07	299.7±4.8	409.5±4.5	366.2±0.7	72.95±1.1
KAW-MH	2.44±0.06	90.04±3.78	304.1±0.1	411.4±0.5	362.1±0.1	72.28±0.4
CKS-ILL	2.44±0.06	82.22±3.071	299.5±2.5	401.2±2.8	354.9±0.6	73.63±0.3
EKS-ILL	2.56±0.04	87.76±3.11	300.9±4.4	399.0±3.3	352.8±3.8	73.28±1.1
ILL-ILL	2.62±0.04	87.58±0.04	299.5±5.8	394.6±0.1	353.4±0.8	72.63±0.3
KAW-ILL	2.85±0.04	96.39±3.78	298.5±0.7	405.1±0.1	359.3±0.4	74.38±0.5

Table 3.2 Effects of location, ecotype, and interaction between planting location and ecotype on the thermal properties of big bluestem.

Thermal properties	Source of variation	Location	Ecotype	Location × ecotype
Specific heat	*F*	65.87	85656.45	11.292
	P	<0.001	<0.001	<0.001
Thermal conductivity	*F*	1.00	92.41	0.936
	P	0.419	0.792	0.522
T_{onset}	*F*	13.11	6.07	1.094
	P	<0.001	0.673	0.419
T_{end}	*F*	10.64	36.14	1.382
	P	<0.001	0.066	0.274
T_{max}	*F*	10.05	34.16	1.433
	P	0.001	0.063	0.205
Weight loss	*F*	4.71	5.15	0.996
	P	0.015	0.025	0.481
High heating value	*F*	43.02	419671.84	1.258
	P	<0.001	<0.001	0.330
Volatile matter	*F*	313.13	8.11	33.679
	P	<0.001	<0.001	<0.001
Fixed carbon	*F*	50.16	0.40	1.531
	P	<0.001	0.009	0.219
Ash	*F*	1426.49	5.57	85.550
	P	<0.001	<0.001	<0.001

3.4.2. Thermal conductivity

Table 3.3 summarizes the calculated thermal conductivity of big bluestem at three different densities and particle sizes at 298K. As density increased from 300 to 360 kg/m^3, the value of thermal conductivity of big bluestem with particle sizes of 1 mm, 2 mm, and 3 mm increased by 32, 44, and 49%, respectively. This result is probably explained by the fact that

compression decreases the proportion of air held in samples, and air has lower thermal conductivity (24 $\times 10^{-3}$ W/m/K) than biomass (~ 85×10^{-3} W/m/K). The dependence of biomass thermal conductivity on sample density was also reported by previous research (Siritheerasas et al., 2007). Moreover, a strong negative correlation between thermal conductivity and particle size was reported by Hankalin et al. (2009). Biomass with smaller particle size has a higher thermal conductivity, which results in a better heat transfer and/or mass transfer in thermal processing. Thus, biomass with small particle size is considered advantageous to thermal conduct efficiency and has a higher production yield in the thermal conversion process (Li et al., 2004).

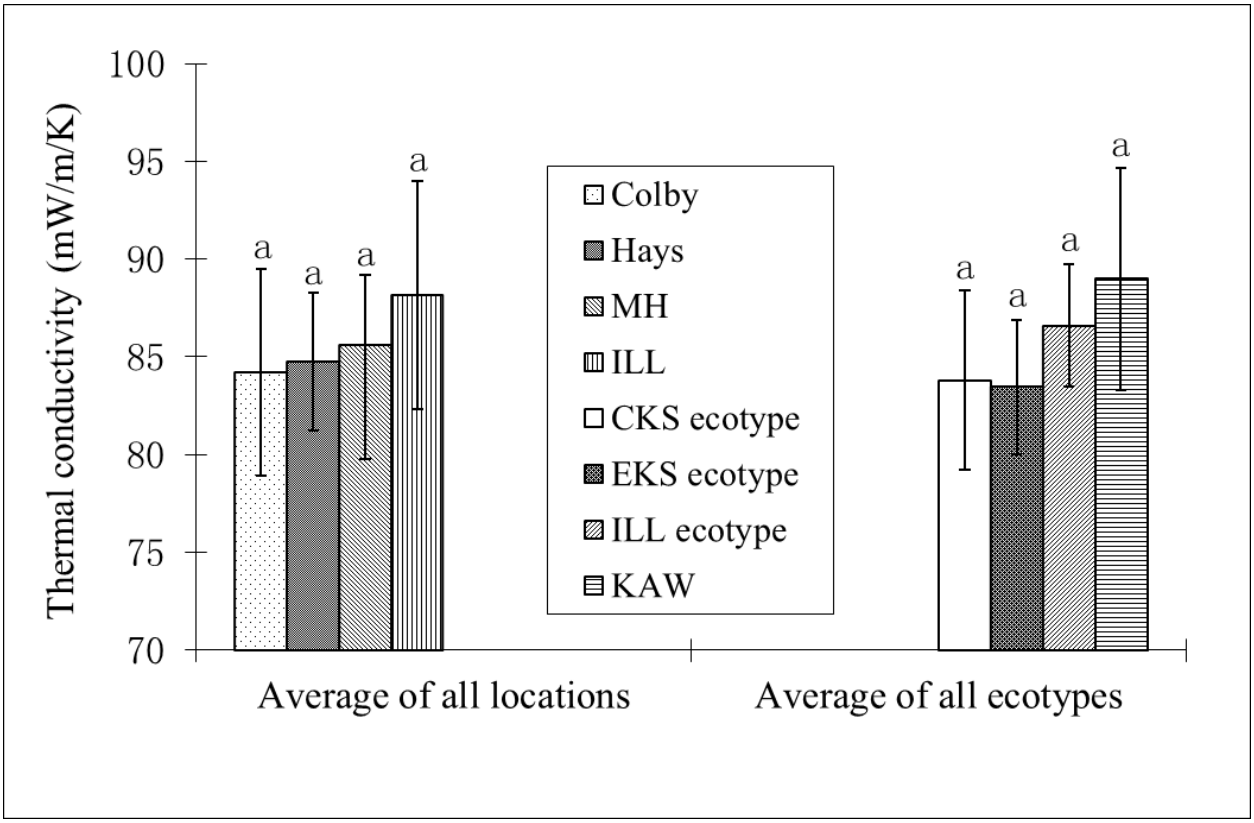
Table 3.3 Effects of density and particle size on thermal conductivity of big bluestem.

Density (kg/m^3)	Thermal conductivity (mW/m/K$\times 10^{-3}$)		
	Particle size (mm)		
	1.0	2.0	3.0
300	63.58	57.89	53.69
330	79.90	75.95	72.74
360	84.08	83.21	80.04

To study the effects of planting location and ecotype on thermal conductivity of big bluestem, the probe method was conducted with the 1-mm sample in 360 kg/m^3 at 298K. Thermal conductivity values were in the range of 77.85–99.06 $\times 10^{-3}$ W/m/K at room temperature, depending on planting location and ecotype (Table 3.1). This result was higher than the thermal conductivity of woody and grass biomass (46.12–76.23 $\times 10^{-3}$ W/m/K) reported by Yang et al. (2007) and is partly explained by the fact that the thermal conductivity was measured on samples with higher sample density in this study. In Figure 3.5, although there was no significant difference in mean value of thermal conductivity among the planting locations and ecotypes, the thermal conductivity of the Illinois location (average 88.49 $\times 10^{-3}$ W/m/K) and KAW (average 86.70 $\times 10^{-3}$ W/m/K) was higher than those of the Colby location (average 74.999 $\times 10^{-3}$ W/m/K) and CKS ecotype (average 78.55 $\times 10^{-3}$ W/m/K). It is suggested that location and ecotype did not have significant effects on the average thermal conductivity from west to east, probably because thermal conductivity was not affected solely by location and ecotype. Table 3.5 showed the high correlation (R^2=0.99, 0.98, and 0.95) between potential evapotranspiration, aridity index,

precipitation since 1961, and thermal conductivity, indicating that these three environmental factors may play a significant role in thermal conductivity values of big bluestem.

Figure 3.5 Comparisons of average thermal conductivity of big bluestem as affected by planting location and ecotype. Different letters (a and b) above the standard deviation bars indicate they are statistically different at a 95% confidence level.

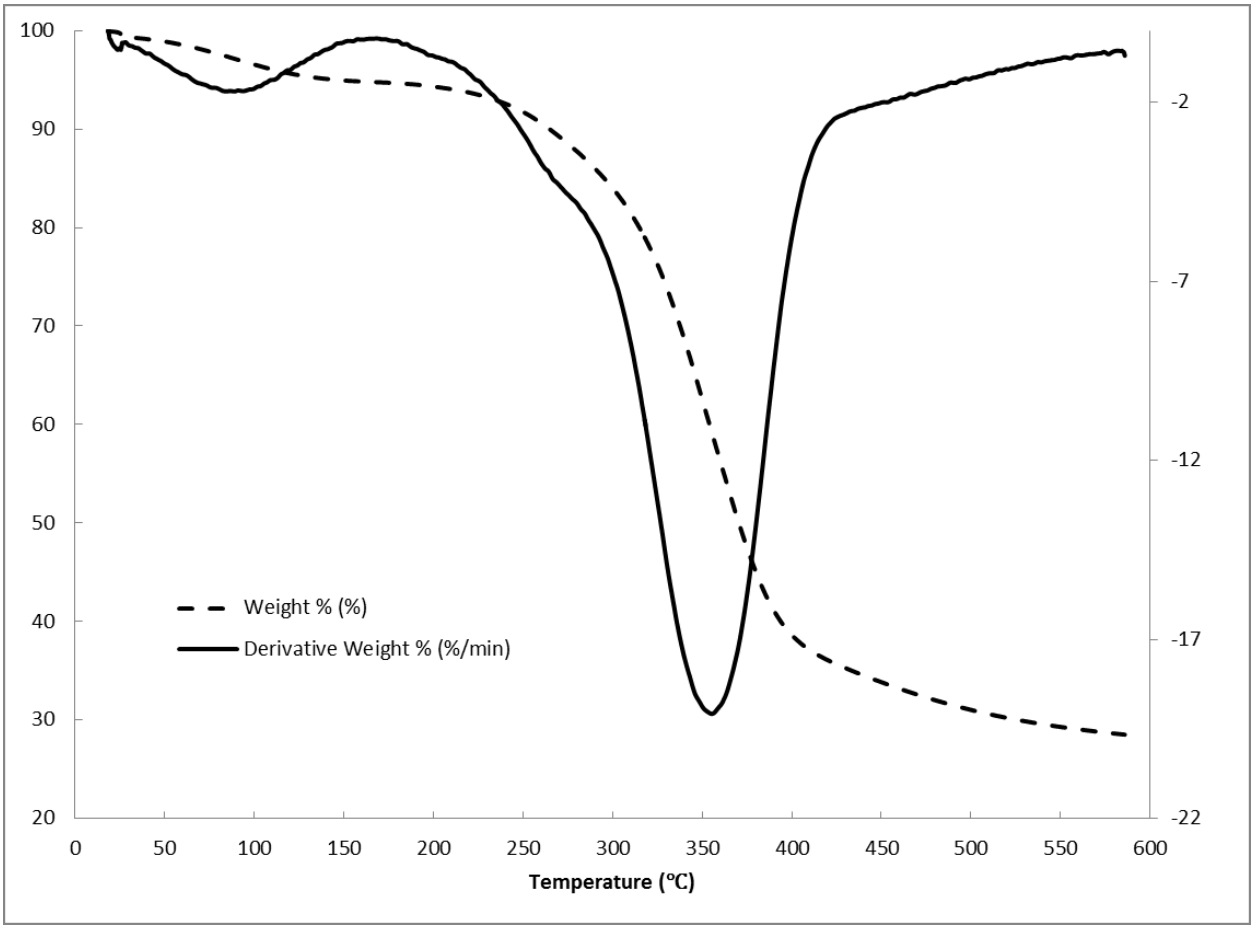


3.4.3 Thermogravimetric analysis

The thermogravimetric data were summarized in Table 3.1. Figure 3.6 shows the typical TGA and DTG curves of big bluestem. In general, all big bluestem samples revealed similar curves: samples lost 10% of their weight within the temperature range of 30 to 250 ℃, and significant weight loss occurred between 250 ℃ and 400 ℃. The weight loss of big bluestems growing in Kansas and those from Illinois were 71 and 73%, respectively, after achieving thermogram plateaus during heating. This result suggested that big bluestem growing in Illinois contained a higher amount of thermal decomposition contents (cellulose, hemicellulose, and

lignin) compared with samples from the Kansas growing locations. The weight loss of big bluestem was significantly affected by both planting location and ecotype (Table 3.2). This result followed a similar trend in previous chemical composition analysis. In other words, big bluestem from Illinois and KAW ecotypes had higher carbohydrate and lignin contents with weight loss than their counterparts.

Figure 3.6 Thermal gravimetric and derivative thermal gravimetric analysis of big bluestem.



For DTG profiles, the rate of weight loss versus temperature of big bluestem, all curves exhibited three-stage thermal decomposition behaviors. The first stage occurred at peak temperature around 80 to 85 ℃, which was related to the evaporation of water in the samples prior to the bulk of the weight loss. The second peak, decomposition of hemicellulose, was relatively small and located at 195 to 255 ℃, which is partly overlapped by the biggest peak. The

third stage appeared from 200 to 510 ℃, with 65% weight loss and peak temperature around 355 to 365 ℃, indicating thermal decomposition of the main constituents of big bluestem, including cellulose and lignin. Hemicellulose was easy to decompose at a low temperature of 200 to 315 ℃ because of its random, amorphous, and rich branch structure (Yang et al., 2007). The thermal decomposition of cellulose occurred at temperatures ranging from 275 to 350 ℃ (Chen and Kuo, 2010). Among the three constituents, lignin was the most difficult to decompose under the whole temperature range from 200 to 600 ℃ because its complicated oxygen functional groups have different thermal stabilities (Brebu and Vasile, 2010). Based on statistical results in Table 3.2, although no significant difference in averages of T_{one}, T_{end}, and T_{max} were observed among ecotype and interaction and ecotype, those thermogravimetric parameters of big bluestem were significantly affected by planting location ($p < 0.05$). These results suggested that planting location rather than ecotype and interaction influenced the thermal stability of big bluestem. Furthermore, the weight loss of big bluestem gradually increased to about 3% as planting location changed from west to east. This result qualitatively confirms the variation trend of contents of carbohydrates and lignin in previous research (Zhang et al., 2012). Multiple univariate linear regression analysis was conducted to find significant associations of environmental predictors with thermogravimetric parameters, and the results showed 89% of the variation in the end time of thermal decomposition (T_{end}), 86% of the variation in the maximum time of thermal decomposition (T_{max}), and 71% of the variation in the weight loss were explained by potential evapotranspiration. Precipitation in 2010 also explained a large variation in the onset time of thermal decomposition (T_{onset}) of the big bluestem samples with coefficients of determination ($R^2 = 0.70$) in growing year 2010 (Table 3.5).

3.4.4 High heating value and proximate analysis.

Heating value is the most important parameter in characterizing a substance as combustible, and it is widely used in the determination of a number of additional thermal properties such as enthalpy of formation and adiabatic reaction temperature. High heating value (HHV), representing the heat of combustion relative to liquid water as the product, was measured with an adiabatic oxygen bomb calorimeter and compared with the predicted HHV based on ultimate and proximate analysis methods (Table 3.4). The proximate and ultimate analyses of biomass are essential for their efficient and clean utilization, whereas the HHV of these materials

determine the quantitative energy content of these fuels. The HHV of big bluestem ranged from 17.28 to 19.05 MJ/kg. Prediction model 1 (Dulong-Bertholot equation) overpredicts the HHV of samples and appears to have bigger bias error to experimental value. Nevertheless, the deviation of the prediction model 2 based on simple and rapid proximate analysis falls in a narrower range with the least residual sum of squares (RSS), indicating proximate analysis is good choice in terms of the accuracy of predicting HHV (Table 3.4). According to the correlations among HHV and proximate analysis components (volatile matter, fixed carbon, and ash) in this study, the resulting equation 3.5 has been derived from multiple linear regression analysis with factors (volatile matter and fixed carbon) using a least squares fitting program. This equation accounted for 85.5% of variation with a standard error of the estimate of 254.68:

$$High\ heating\ value = -1109.78 + 207.46 volatile\ matter + 210.82 fixed\ carbon \qquad (3.5)$$

In general, higher fixed carbon and volatile matter contents resulted in a higher HHV. Big bluestem from Illinois had significantly higher HHV (average of 18.67 MJ/kg) than those from Kansas (average of 17.64 MJ/kg for Colby, KS; average of 17.73 MJ/kg for Hays, KS; and average of 18.07 MJ/kg for Manhattan, KS) (Table 3.4). In addition to location, the effect of ecotype on HHV showed the same trend as on specific heating of big bluestem. KAW had significantly higher HHV than other ecotypes, which might be partly attributed to the higher carbohydrate contents of native cultivar. In general, HHV of big bluestem was significantly affected by location ($p < 0.01$) and ecotype ($p < 0.01$), with the latter being more influential (as shown by a large F-value). The interaction effect of ecotype and location on HHV was statistically insignificant ($p > 0.05$).

Table 3.4 High heating value and proximate analysis of big bluestem.

	HHV (MJ/kg)	Prediction 1[a](MJ/kg)	Prediction 2[b](MJ/kg)	Volatile matter (%)	Fixed carbon (%)	Ash (%)
CKS-Colby	18.02±7.36	17.92	17.73	74.27±0.2	17.57±0.2	8.16±0.1
EKS-Colby	17.45±40.35	17.28	17.70	73.07±0.2	17.51±0.1	9.41±0.3
ILL-Colby	17.28±7.59	16.29	17.54	74.84±0.2	17.40±0.1	7.76±0.1
KAW-Colby	18.39±23.11	18.14	18.43	76.72±0.3	17.02±0.4	6.26±0.1
CKS-Hays	17.90±6.52	18.26	17.51	73.29±0.3	17.94±0.4	8.77±0.2
EKS-Hays	17.60±21.30	17.86	17.20	71.20±0.1	17.50±0.3	11.31±0.2
ILL-Hays	17.98±28.40	17.47	17.59	73.40±0.3	17.58±0.3	9.02 ±0.1
KAW-Hays	17.96±2.87	18.92	18.63	74.85±0.3	17.54±0.2	7.61±0.1
CKS-MH	18.29±37.63	19.08	17.76	73.50±0.4	17.41±0.7	9.10±0.3
EKS-MH	18.02±101.65	18.92	17.59	73.41±0.1	17.59±0.3	9.01±0.1
ILL-MH	18.38±23.17	19.61	17.92	75.55±0.2	17.53±0.1	6.92±0.2
KAW-MH	18.29±39.00	18.92	18.56	74.48±0.1	17.28±0.1	8.24±0.1
CKS-ILL	18.57±45.56	20.14	17.93	75.67±0.1	18.88±0.1	5.45±0.1
EKS-ILL	18.51±17.16	19.69	17.81	76.48±0.1	19.06±0.2	4.46±0.1
ILL-ILL	18.61±2.18	19.29	17.66	75.75±0.1	19.21±0.1	5.04 ±0.1
KAW-ILL	19.05±6.14	20.16	18.45	77.51±0.13	18.10±0.1	4.39±0.1
RSS [c]	NA	11.4	4.0	NA	NA	NA

[a] HHV = 339 C+1214(H-O/8) 226 H+105 S (Selvig and Gibson, 1945)

[b] HHV = 353.6FC + 155.9VM-7.8ASH (Parikh et al., 2005)

[c] RSS (residual sum of squares) = $\sum_{i=1}^{n}\left(HHV_{predicted} - HHV_{measurement}\right)^2$

Table 3.4 shows the results of the proximate analysis calculated on a dry basis. The volatile matter content of big bluestem ranged from 68.75 to 74.65%. The volatile matter was exclusive of moisture vapor and consists of permanent gases such as CH_4, CO_2, and CO forming the bio-oil after condensation (Stahl et al., 2004). Fixed carbon, producing a char and burning as a solid material in the combustion system, ranged from 16.75 to 19.24%. Ash, the inorganic residues, ranged from 4.35 to 11.46%. Variations in the fixed carbon, volatile, and ash contents

among the 16 samples were analyzed by two-way ANOVA to examine the genetic and environmental effects on proximate analysis of the big bluestem. With the exception of the interaction effect on the fixed carbon, significant effects of planting location, ecotype, and an interaction between location and ecotype were observed on the volatile matter, fixed carbon, and ash contents. Location had larger *F* values (50.16–1426.49) than ecotype (0.40–8.11) and interactions (1.53–85.55), showing that location effects were always highly significant, with much larger *F* values on proximate analysis of big bluestem, an order of magnitude larger than *F* values of ecotype and their interaction for volatile matter and fixed carbon contents, and approaching more than three orders of magnitude larger for ash content (Table 3.5). Table 3.5 shows environmental factors, including mean annual precipitation since 1961, potential evapotranspiration, and aridity index variation, which had a significant effect on the heat value with large coefficients of determination (R^2>0.90). The mean annual precipitation since 1961 in Illinois is twice as high as that in Colby, which provides a better environment for big bluestem with high heating value. For proximate analysis, variables were also associated with environmental predictors; the potential evapotranspiration explained 64, 86, and 79% of the variation in volatile matter content, fixed carbon content, and ash content, respectively. It is suggested that the potential evapotranspiration played a greater role in divergence of the high heating value and proximate analysis variables of the big bluestem.

Table 3.5 Effects of environmental conditions on thermal properties of big bluestem analyzed by the multiple univariate linear regression analysis.

Thermal properties	PPT[a] 2010 (cm)	PPT[a] since 1961 (cm)	GDD[b] since 1961 (cm)	GDD[b] 2010 (cm)	PET[c] (cm)	Aridity index
Specific heat	0.21	0.62	0.14	0.44	0.80	0.70
Thermal conductivity	0.61	0.95	0.47	0.63	0.99	0.98
T_{onset}	0.70	0.56	0.30	0.07	0.42	0.49
T_{end}	0.32	0.75	0.19	0.43	0.89	0.81
T_{max}	0.38	0.77	0.15	0.25	0.86	0.80
Weight loss	0.14	0.54	0.05	0.23	0.71	0.61
High heating value	0.69	0.98	0.50	0.59	0.99	0.99
Volatile matter	0.22	0.56	0.03	0.07	0.64	0.58
Fixed carbon	0.28	0.70	0.23	0.55	0.86	0.77
Ash	0.26	0.67	0.08	0.20	0.79	0.71

3.5 Conclusions

Specific heat of big bluestem was significantly affected by planting location, ecotype, and interaction between location and ecotype, but ecotype was the dominant factor. A positive correlation model was developed between specific heat and temperature. There was no significant effect on thermal conductivity of big bluestem. A positive relationship between thermal conductivity and density and a negative relationship between the particle size and thermal conductivity were observed. With the exception of weight loss, planting location alone had a significant effect on thermogravimetric parameters of big bluestem. As a general conclusion, both planting location and ecotype significantly affected high heating value, but the latter was more influential. Potential evapotranspiration was the most significant environmental factor affecting all thermal properties of big bluestem selected for this study.

Chapter 4 - Glucan yield from enzymatic hydrolysis of big bluestem as affected by ecotype and planting location along the precipitation gradient of the Great Plains

4.1 Abstract

Three big bluestem ecotypes from central Kansas (Cedar Bluffs and Webster populations), eastern Kansas (Konza and Top of the World populations), and Illinois (12Mile and Fults populations), as well as the Kaw cultivar, were harvested from four reciprocal garden planting locations (Colby, Hays, and Manhattan, KS; and Carbondale, Ill.) and used to study effects of ecotype and planting location on glucan content and glucan yield from enzymatic hydrolysis along the Great Plains precipitation gradient (~1200 to 400 mm mean annual precipitation). The populations varied widely in glucan content (31.8–36.5%), lignin content (14.4–18.0%), mass recovery (52.0–59.7%) and glucan recovery (79.0–87.50%) after acid treatment, enzymatic hydrolysis efficiency (EEH) (84.6–88.9%), and glucan mass yield (20.8–29.3%). Planting location had significant effects on all variables evaluated. Ecotype had significant effects on glucan recovery, EEH, and glucan mass yield. In addition, interaction between ecotype and planting location also had significant effects on glucan mass yield. Planting location had a stronger influence than ecotype and interaction between location and ecotype. Total glucan mass yield of big bluestem (regardless of ecotype) increased as the Great Plains precipitation gradient increased from west to east. Annual precipitation, growing degree days, and potential evapotranspiration in 2010 accounted for 90%, 85% and 78% of the variation in glucan mass yield.

4.2 Introduction

Currently, nonrenewable fossil fuel reserves are being depleted at an increasing rate and negative effects of greenhouse gas emissions are accelerating global warming (Bioenergy, 1998). Production of renewable fuels derived from lignocellulosic biomass with low carbon dioxide emissions is attracting increased research attention (Dien et al., 2006). Thus, lignocellulosic biomass, including dedicated energy crops such as switchgrass, big bluestem, forest residues, and agricultural residues, could play an essential role in replacing fossil fuels because of low production inputs and potentially low competition with food production. In 2010, the USDA

reported that lignocellulosic biomass could be planted as a dedicated bioenergy crop in select states in the central Great Plains (Kansas, Nebraska, Oklahoma, and South Dakota) in which over 25 million hectares of land are classified by the USDA as rangeland/grassland within land capability class 3–6 soils (more marginal/less productive soils) (Han et al., 2014).

Big bluestem (*Andropogon gerardii*) is a dominant warm-season (C4) perennial native grass comprising as much as 80% of plant biomass in prairies in Midwest grasslands of North America (Gould and Shaw, 1983; Knapp et al., 1998). In addition to its abundant supply, big bluestem is considered a potential bioenergy crop because its growth requires few agricultural inputs (fertilizer and pesticides) and has better tolerance to heat and drought. These advantages have enabled big bluestem to thrive in Midwestern grasslands, when cool-season grasses (C3) are unproductive (Lawrence et al., 1995; Moore and Anderson, 2000; Moser and Vogel, 1995; Perry and Baltensperger, 1979). Big bluestem has been selected and studied extensively for decades in an effort to understand the effect of climate on grass growth; controls on community structure; ecological responses to grazing, burning, and mowing; and restoration effectiveness (Epstein et al., 1998; Fay et al., 2003; He et al., 1992; Knapp et al., 2001; Silletti and Knapp, 2001). However, potential use of bluestem for bioenergy has not been evaluated adequately. Ecotypes of *A. gerardii* were originally described nearly 50 years ago (McMillan, 1959), but variables related to biofuel potential across the precipitation gradient of tallgrass prairie have not been broadly characterized. In this research, the effect of sharp precipitation gradient across the Great Plains (1200 to 400 mm mean annual precipitation) on biofuel potential of *A. gerardii* was studied.

Conversion of lignocellulosic biomass into ethanol includes three major steps: pretreatment, enzymatic hydrolysis, and fermentation (Christakopoulos et al., 1993). Pretreatment is an essential process using chemical and/or physical agents to break down natural recalcitrance of lignin frame and improve interaction between glucan and cellulases during enzymatic hydrolysis (Kádár et al., 2007; Saha and Bothast, 1999). Although conversion of biomass into biofuel has been studied intensively, few studies have been published on the conversion of big bluestem to fermentable glucan. Extrusion was used as pretreatment method to increase glucan yield from big bluestem with 71.3% glucan recovery, combined alkali soaking and extrusion to improve the conversion rate to 90.1% (Chinnadurai and Muthukumarappan, 2011; Karunanithy and Muthukumarappan, 2009). Weimer et al. studied the potential of big

bluestem for ethanol production by consolidated bioprocessing. The objective of this research was to study effects of ecotype and planting location on glucan content in the biomass and glucan yield from enzymatic hydrolysis along the Great Plains precipitation gradient (~1200 to 400 mm mean annual precipitation).

4.3 Materials and methods

4.3.1 Materials.

Three big bluestem ecotypes, CKS (Cedar Bluffs [CDB] and Webster [WEB] populations), EKS (Konza [KON] and Top of the World [TOW] populations), and IL (12Mile [12M] and Fults [FUL] populations), and the KAW cultivar, which is widely planted to restore marginal lands, were harvested from reciprocal garden plots in four planting locations (Colby, Hays, and Manhattan, KS; and Carbondale, IL) in 2010. Among the four locations, the Colby planting site was used to test the threshold of drought tolerance and the possibility for planting in drier locations of the Great Plains. Two populations from each ecotype were evaluated for glucan content and yield.

Seeds for the populations and ecotypes were collected by hand from pristine ungrazed prairie in the fall of 2008. Plants were installed at the reciprocal garden sites (Colby, Hays, and Manhattan, KS; and Carbondale, IL) in August 2009. At each planting location, all 12 populations (3 ecotypes x 4 populations per ecotype) were replicated in 10 blocks. We used only two of the four populations per ecotype for this study. Plants were assigned randomly to blocks, spaced 50 cm apart, and planted into shadecloth to control weeds. The KAW cultivar and sand bluestem (data not included here) were also included, making 14 plants per block. Details of seed collection, planting location, and harvest have been described previously by Zhang et al (year). No nitrogen fertilizers and pesticides were applied to the planting plots. Table 3.1 shows the characteristics of the soils for four planting locations.

The plants were part of a large bluestem ecotype variation experiment to examine phenotypic variation across the Great Plains precipitation gradient and the role of environment and ecotype in affecting the phenotype. These plants were characterized extensively in terms of canopy area, height, and phenology in the summer of 2010 (Johnson et al., in preparation) and harvested by hand in October 2010. The harvested plant biomass (foliage, inflorescence, stalks) was dried at 60 °C for at least one week before being stored at room temperature. The big

bluestem 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.).

Table 4.1 Selected seasonal enviromenental variables and characteristics of the soils for the four planting sites.

Environment conditions	Reciprocal garden planting site			
	Colby, KS Northwest Kansas Agricultural Research Center	Hays, KS Agricultural Research Center–Hays	Manhattan, KS USDA Plant Materials Center	Carbondale, Illinois Southern Illinois University Agronomy Center
Annual precipitation, 2010 (cm)	44.57	50.11	67.82	66.95
Mean annual precipitation since 1961 (cm)	50.47	58.22	87.15	116.73
Precipitation of driest year, cm (year)	28.37 (1967)	36.27 (1988)	39.16 (1966)	66.95 (2010)
Growing season PPT (Apr1-Aug31,2010)	39.21	48.84	67.23	47.01
Growing degree days average since 1961	3167	3799	4156	4087
Growing degree days, 2010	3461	4193	4105	4474
Potential evapotranspiration (cm)	144	139	127	99
Aridity index (PET[a]-PPT[b])	97	81	41	-18
Ave temp (℃)	10.5	12	13	13.3
Elevation (m)	972	603	315	127
Soil type	Silt-clay-loam	Silt-loam	Silt-loam	Silt-loam
Soil classification	Aridic Haplustolls	Arguistoll	Udifluvent	Fragiaquic Hapludalf
pH	NA	6.0	7.5	5.0
CEC [c](meq/100g)	24.6	25.1	8.5	13.7
% sand	8.5	21.5	41.0	7.5
% silt	60.0	58.5	51.0	78.5
% clay	31.5	20.0	8.0	14.0
%C ± SE	1.01 ± 0.013	1.88 ± 0.039	0.71 ± 0.011	2.67 ± 0.179
%N ± SE	0.10 ± 0.001	0.17 ± 0.004	0.06 ± 0.001	0.21 ± 0.007

[a] PET: Potential evapotranspiration.

[b] PPT: Precipitation.

[c] CEC: Cation exchange capacity

4.3.2 Analytical Methods.

4.3.2.1 Composition Analysis.

Moisture content of ground big bluestem samples was determined by drying approximately 2 g of each sample in a forced-air oven at 105 °C for 4 h (Sluiter, Hames, Hyman, et al., 2008). Extractives, glucan, and lignin contents of the big bluestem were determined by following NREL laboratory analytical procedures (Sluiter, Hames, Hyman, et al., 2008; Sluiter, Hames, Ruiz, et al., 2008). 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 chromatography (HPLC, Shimadzu, Kyoto, Japan) equipped with an RCM monosaccharide column (300 × 7.8 mm; Phenomenex, Torrance, Calif.) 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.

4.3.2.2 Sulfuric Acid Pretreatment.

Pretreatment was conducted in a reactor (Swagelok, Kansas City Valve & Fitting Co., KS, USA) made from 316L 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 big bluestem was mixed with water and three concentration diluted sulfuric acid (0.5, 1.0 and 1.5% w/v) to load 6.0% (w/v, 3.05 g dry mass in 50 ml solution) solid content.

A sand bath (Techne, Inc., Princeton, N.J., USA) with a temperature controller was used to increase and control temperature. After the sand was increased to 160 ℃, the reactor was submerged in boiling sand for 40 min, then immediately transferred to room-temperature water to decrease the internal temperature to below 50 ℃ 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:

$$Glucan\ recovery(\%) = \frac{m_{pretreatment}}{m_{original}} \times 100\% \quad (4.1)$$

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

4.3.2.3 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. The enzyme loading (Accellerase 1500, containing glucan and β-glucosidase, generously provided by Dupont Genencor Science, Wilmington, Del., USA) 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 ℃ 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:

$$EEH(\%) = \frac{c \times V \times 0.9}{m_{EH}} \times 100\% \quad (4.2)$$

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

Taking into account the EEH of glucan based on both solid parts and the recovery of solid glucan after pretreatment, the combination glucan conversion rate is used to better compare the effect of different acid conditions on processes. This conversion rate was defined as the percentage of glucan in the solid part after pretreatment and EEH shown in:

$$Glucan\ yield(\%) = \frac{EEH \times glucan\ recovry}{100\%} \quad (4.3)$$

Glucan mass yield was calculated on the basis of 100g dry weight raw big bluestem as shown in the following formula 4.4:

$$Glucan\ mass\ yield(g/100g) = glucan\ content \times glucan\ yield \quad (4.4)$$

4.3.2.3 Statistical Analysis

Glucan content and yield of big bluestem samples 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, N.C.). In general, fully balanced ANOVA tests were performed following the general linear models (GLM) procedure.

4.4 Results and discussion

4.4.1 Glucan and Lignin Content of Big Bluestem.

The glucan content of seven big bluestem populations and three ecotypes, as well as cultivar KAW, from four planting locations showed significant diversity (Table 4.2). The average glucan and lignin contents across planting locations and ecotypes was 34.5± 2.4% (range of 29.6 to 39.5%) and 16.8±1.8% (range of 12.0 to 19.3%), respectively. The KAW cultivar planting in Illinois had the highest glucan content with 39.5%, which was 33% higher than the lowest glucan content obtained from KON in Colby. The range of glucan content of big bluestem was similar to reports by previous research, as shown in Table 4.3. However, big bluestem had a relatively lower lignin content (16.8%) compared with other lignocellulosic biomass such as corn stover (18.1%), forage sorghum (20.3%), sweet sorghum (18%), grain sorghum (18.1%), PS sorghum (19.2%), wheat straw (16.3%) and switchgrass (23.2%) (Chung et al., 2005; K Theerarattananoon et al., 2011; Karnnalin Theerarattananoon et al., 2012). This desirable trait makes pretreatment and enzymatic hydrolysis of structural polysaccharides in the bioconversion processes easier for big bluestem.

Table 4.2 Glucan and lignin contents as well as mass recovery, glucan recovery, efficiency of enzymatic hydrolysis, and glucan mass yield of big bluestem by population and planting site.

\	Glucan(%)	Lignin(%)	Mass recovery(%)	Glucan recovery(%)	EEH(%)	Glucan mass yield(%)
CDB (CKS)-Colby	32.5±0.1	14.8±0.4	52.3	82.2	86.9	23.2
WEB (CKS)-Colby	32.8±0.1	15.2±0.1	52.3	84.2	84.6	23.4
KON (EKS)-Colby	29.6±0.1	13.3±0.2	52.0	80.0	87.9	20.8
TOW (EKS)-Colby	30.8±0.2	13.9±0.1	52.5	80.7	87.4	21.7
12M(ILL)-Colby	29.6±0.2	12.0±0.1	53.5	85.7	86.0	21.8
FUL (ILL)-Colby	32.6±0.2	14.9±0.1	52.3	80.2	87.7	22.9
KAW (CULTIVAR)-Colby	34.9±0.1	16.3±0.1	54.0	79.0	86.6	23.9
CDB (CKS)-Hays	36.1±0.1	18.6±0.2	53.3	84.1	85.4	26.0
WEB (CKS)-Hays	35.2±0.5	17.7±0.1	53.3	84.2	84.9	25.2
KON (EKS)-Hays	33.3±0.2	17.4±0.1	53.5	80.5	87.4	23.4
TOW (EKS)-Hays	32.7±0.2	17.2±0.1	54.3	82.5	87.7	23.7
12M (ILL)-Hays	31.8±0.5	14.2±0.3	53.0	81.0	87.5	22.5
FUL (ILL)-Hays	32.8±0.4	16.8±0.1	54.8	79.7	87.5	22.9
KAW (CULTIVAR)-Hays	34. 9±0.1	16.9±0.2	54.1	80.8	87.0	24.5
CDB (CKS)-Manhattan	36.7±0.6	18.7±0.2	57.6	85.4	85.5	26.8
WEB (CKS)-Manhattan	34.6±0.4	18.8±0.1	56.4	84.3	87.4	25.5
KON (EKS)-Manhattan	35.9±0.4	17.6±0.4	58.9	85.7	88.7	27.3
TOW (EKS)-Manhattan	35.1±0.3	18.2±0.6	56.9	84.7	88.9	26.5
12M (ILL)-Manhattan	34.0±0.2	15.2±0.2	56.6	82.5	88.5	24.8
FUL (ILL)-Manhattan	37.1±0.2	17.3±0.3	56.8	84.6	88.9	27.9
KAW (CULTIVAR)–Manhattan	38.3±0.6	17.6±0.1	57.4	85.2	87.5	28.6
CDB (CKS)-Carbondale	35.6±0.1	17.7±0.2	58.9	86.6	86.9	26.8
WEB (CKS)-Carbondale	36.3±0.4	18.2±0.1	60.0	85.8	85.6	26.6
KON (EKS)-Carbondale	36.2±0.2	17.6±0.2	59.0	86.8	87.6	27.5
TOW (EKS)-Carbondale	36.2±0.1	18.5±0.5	59.2	87.2	88.5	28.0
12M (ILL)-Carbondale	35.1±0.4	16.7±0.1	59.7	87.5	87.1	26.8
FUL (ILL)-Carbondale	36.6±0.5	18.2±0.2	59.5	86.7	87.9	27.9
KAW (CULTIVAR)- Carbondale	39.5±0.4	19.4±0.2	58.8	85.5	86.7	29.3
Average	34.5 ±2.4	16.9 ±1.8	55.8	83.7	87.1	25.2

Table 4.3 Comparison of glucan content, mass recovery, glucan recovery, efficiency of enzymatic hydrolysis (EEH), and glucan mass yield of big bluestem and other selected lignocellulosic biomass.

Type of biomass	Glucan (%)	Lignin (%)	Mass recovery (%)	Glucan recovery (%)	EEH (%)	Glucan mass yield (g/100g)	Reference
Big bluestem-this study	34.5	16.8	55.8	83.7	87.1	25.2	NA
Corn stover	37.3	18.1	47.4	59.2	95	21.0	(K Theerarattananoon et al., 2011)
Forage sorghum	37.9	20.3	51.5	57.2	90	19.5	(K Theerarattananoon et al., 2011)
Sweet sorghum	34.2	18.0	44.7	47.2	97	15.7	(K Theerarattananoon et al., 2011)
Grain sorghum	37.8	18.1	51.7	56.6	81	17.3	(K Theerarattananoon et al., 2011)
BMR sorghum	40.5	15.5	45.0	42.8	97	16.8	(K Theerarattananoon et al., 2011)
PS sorghum	44.0	19.2	30.85	30.9	94	12.8	(K Theerarattananoon et al., 2011)
Wheat straw	41.2	16.3	58.9	79.9	91.2	30.0	(Karnnalin Theerarattananoon et al., 2012)
Switchgrass	32.2	23.2	52.6	81.3	90.3	23.6	(Chung et al., 2005)

4.4.2 Effects of Planting Location and Ecotype on Glucan Content.

Both ecotype and planting location had significant effects on glucan content of big bluestem ($p < 0.05$). Figure 1 shows the effects of planting location on glucan content. Different letters above the standard deviation bars indicate that means of glucan contents are significantly different based on LSD test ($p < 0.05$) at a 95% confidence level; e.g., glucan content of group b is significantly higher than glucan content of group a. Big bluestem populations planted in Illinois generally had higher glucan contents, with an average of 36.5% compared with the average of populations planted in Colby, KS (31.8%); Hays, KS (33.8%); and Manhattan, KS (36.0%). The average glucan content of big bluestem planted in Illinois was 4.7% higher than those from Colby in western Kansas, indicating that identical big bluestem populations would yield ≈15% more glucan if planted in Illinois instead of western Kansas. However, big bluestem planted in Manhattan, KS and Carbondale, IL did not yield significantly different glucan

contents, which might result from the fact that perception of Carbondale was substantially below normal in 2010 (Lee and Boe, 2005). Table 4.4 shows linear regression results between glucan content and environmental factors associated with the planting locations. The 2010 annual precipitation explained 94% of the variation in glucan content based on coefficients of determination (R^2). The difference in potential evapotranspiration between west and east is also responsible for glucan and glucan yield. Potential evapotranspiration explained 72% of the variation in glucan content (Table 4.4). The higher precipitation amount in Illinois is almost one and a half times higher than Colby, KS, which provides a better environment for biomass accumulation. In addition, the number of growing degree days in 2010 explained 80% of the variation in glucan content.

Table 4.4 Correlation coefficients between environmental conditions and glucan content, mass recovery, glucan recovery, efficiency of enzymatic hydrolysis (EEH), and glucan mass yield of big bluestem analyzed by linear regression models.

	PPT[a] 2010 (cm)	PPT avg since 1961 (cm)	GDD[b] avg. since 1961	GDD 2010	PET[c] (cm)	Aridity index
Glucan (%, db)	0.94	0.84	0.93	0.8	0.72	0.81
Mass recovery (%, db)	0.90	0.98	0.70	0.61	0.90	0.96
Glucan recovery (%, db)	0.84	0.99	0.58	0.52	0.93	0.97
EEH (%)	0.72	0.31	0.55	0.13	0.15	024
Glucan mass yield (%, db)	0.9	0.91	0.85	0.68	0.78	0.87

[a] PPT: Precipitation

[b] GDD: Growing degree days

[c] PET: Potential evapotranspiration

The glucan content also showed a significant variation among different ecotypes at $P <$ 0.001 and F values with 28.5 (Table 4.5). CKS ecotype and KAW had significantly higher glucan contents than eastern KS and Illinois ecotypes. KAW had the highest glucan content among all ecotypes (Figure 4.1), possibly due to the fact that the KAW cultivar, as the native released cultivar, was selected and bred for carbohydrate accumulation. Of these 28 samples, the highest glucan content was found in KAW at the Illinois location, indicating combined effects of

ecotype and planting location. No significant difference in glucan content between EKS and IL ecotypes across four planting locations was demonstrated, indicating no clear effect of ecotype on the average glucan content within ecotypes from west to east. This result is possibly because glucan contents were not solely affected by ecotype.

Table 4.5 Effects of ecotype (E), location (L), and interaction between ecotype and planting location (E×L) on glucan content, mass recovery, glucan recovery, efficiency of enzymatic hydrolysis (EEH), and glucan mass yield of big bluestem.

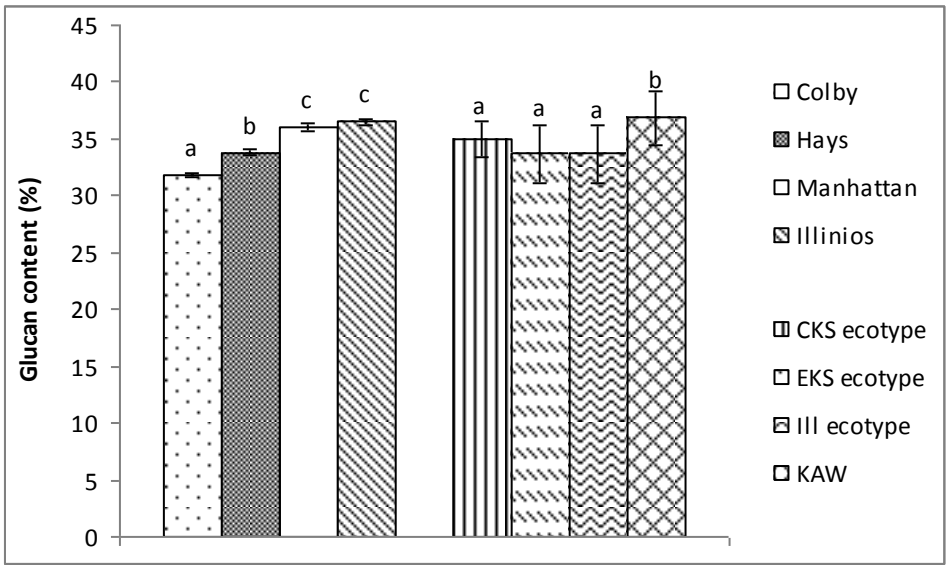
	Source of variation	Location	Ecotype	E×L
Glucan	F^a	73.56	28.51	3.59
	P^b	<0.001	<0.001	0.002
Mass recovery (%)	*F*	183.58	1.10	1.68
	P	<0.001	0.380	0.176
Glucan recovery (%)	*F*	31.47	3.45	2.47
	P	<0.001	0.042	0.055
EEH (%)	*F*	1.45	14.0	0.44
	P	0.21	<0.001	0.893
Glucan mass yield (%)	*F*	89.62	11.34	3.19
	P	<0.001	<0.001	0.021

[a] *F*-value: the ratio of two scaled sums of squares reflecting different sources of variability

[b] *P*-value:the probability of obtaining a test statistic at least as extreme as the one that was actually observed, assuming that the null hypothesis is true.

Variations in the glucan content among the 28 samples were analyzed by two-way ANOVA for examining genetic and environmental effects on glucan content and yield of big bluestem. In general, ANOVA analysis revealed that ecotype and location had significant effects on glucan content (Table 4.5). Location had a larger *F*-value (73.6) than ecotype (28.5) and interactions (3.59), showing that the role of location was always a greater source of variation than ecotype and the interaction between ecotype and location for glucan content based on a much larger *F*-value. The significant effects of ecotype and planting location on glucan and lignin contents potentially provide knowledge as to the role of genetic and environmental factors influencing development of big bluestem varieties for use as a bioenergy crop.

Figure 4.1 Effects of planting location and ecotype on glucan content of big bluestem. Error bars are standard deviations at 95% confidence level.

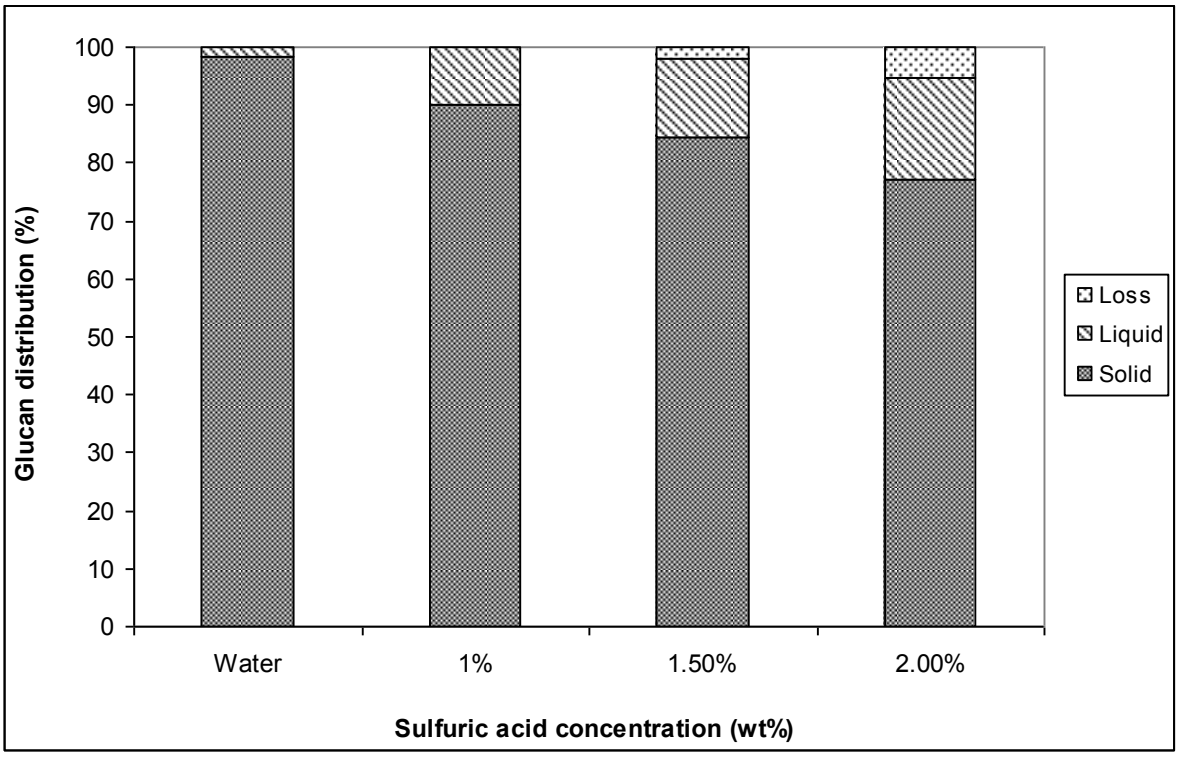


4.4.3 The Effect of Diluted Acid Concentration on Biomass Conversion

As the most widely cultivated and highest glucan content big bluestem ecotype growing in the Great Plains, KAW was selected as the reference for identification of pretreatment conditions that result in the highest total glucan mass yield from diluted acid pretreatment and following enzymatic hydrolysis. Three diluted sulfuric acid concentrations (1.0%. 1.5% and 2.0%) and water as a control were applied to investigate the effect of acid concentration on the big bluestem conversion rate. Figure 4.2 shows the percentage distribution of glucan in solid, liquid and loss with different concentrations of sulfuric acid and water pretreatment. Glucan percentage in solid dropped from 98.4 to 77.3% with an increase in acid concentration. Glucan loss increased as acid concentration increased from 0.1 to 5.2%, whereas glucan content in liquid increased from 1.6 to 17.6%, indicating that more severe pretreatment condition resulted in more glucan degradation. Similar results were reported by previous research (Xiang et al., 2004). More than 95% of hemicellulose (xylan plus arabinan) was removed from the solid at the same time (data not shown). Although acid pretreatment caused irreversible damage on the structure of big bluestem associated with cellulose loss, EEH increased from 79.7 to 90.0% after 72 hours hydrolysis as acid concentration increased from 1.0 to 2.0%, which was significantly higher than control samples pretreated with water (18.1%), as shown in Figure 4.3. Similar results were

reported by a previous study (Table 4.3), potentially because more severe pretreatment condition removing more of the hemicellulose and lignin barrier and making glucan more accessible during enzymatic hydrolysis. One notable result is that the pretreatment with 1.5% and 2.0% acid concentration yield the similar EEH (~ 90%).

Figure 4.2 Glucan distributed in solid, liquid, and loss parts as affected by sulfuric acid concentration.



The glucan yield is used to better evaluate the effect of different acid conditions on the final products by taking into account both the EEH of glucan based only on solid part and the percentage of the solid part of cellulose after pretreatment. Glucan yield was 17.8, 71.6, 74.2, and 69.6%, corresponding to biomass treated with water and acid concentrations of 1.0, 1.5, and 2.0%, respectively (Figure 4.4). The highest glucan yield was found at 1.5% but not at the highest acid concentration due to relatively high EEH and low glucan loss at 1.5% acid concentration compared with other concentrations (Figure 4.4). Therefore, 1.5% sulfuric acid was considered optimal and was used in subsequent experiments.

Figure 4.3 Effect of sulfuric acid concentration on efficiency of enzymatic hydrolysis (EEH). Error bars are standard deviations at 95% confidence level.

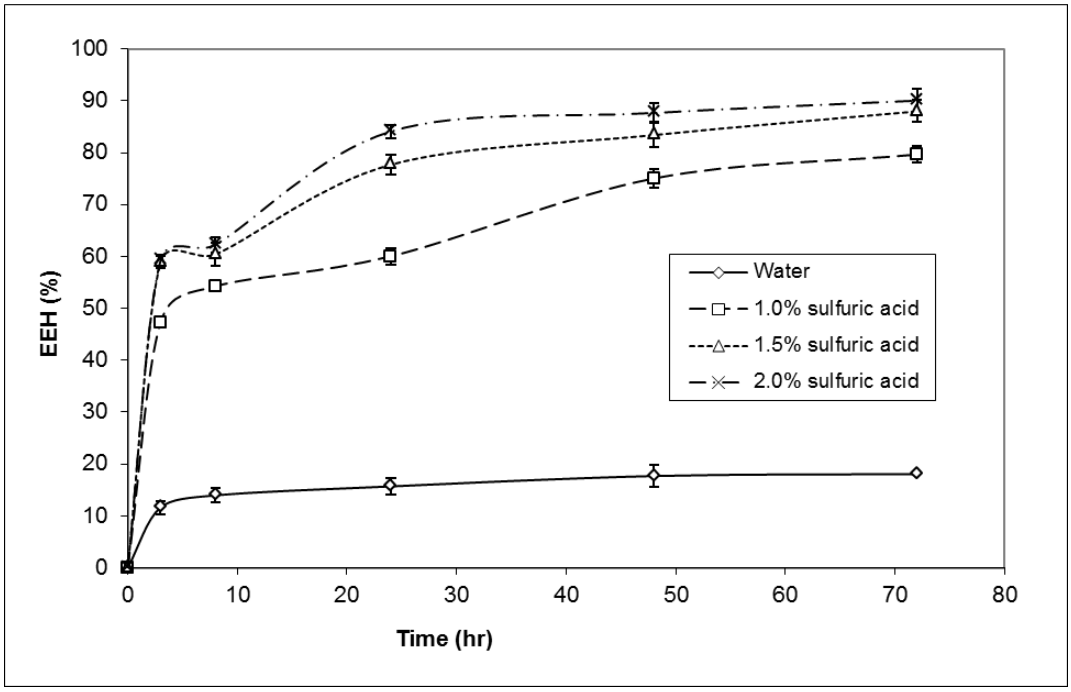
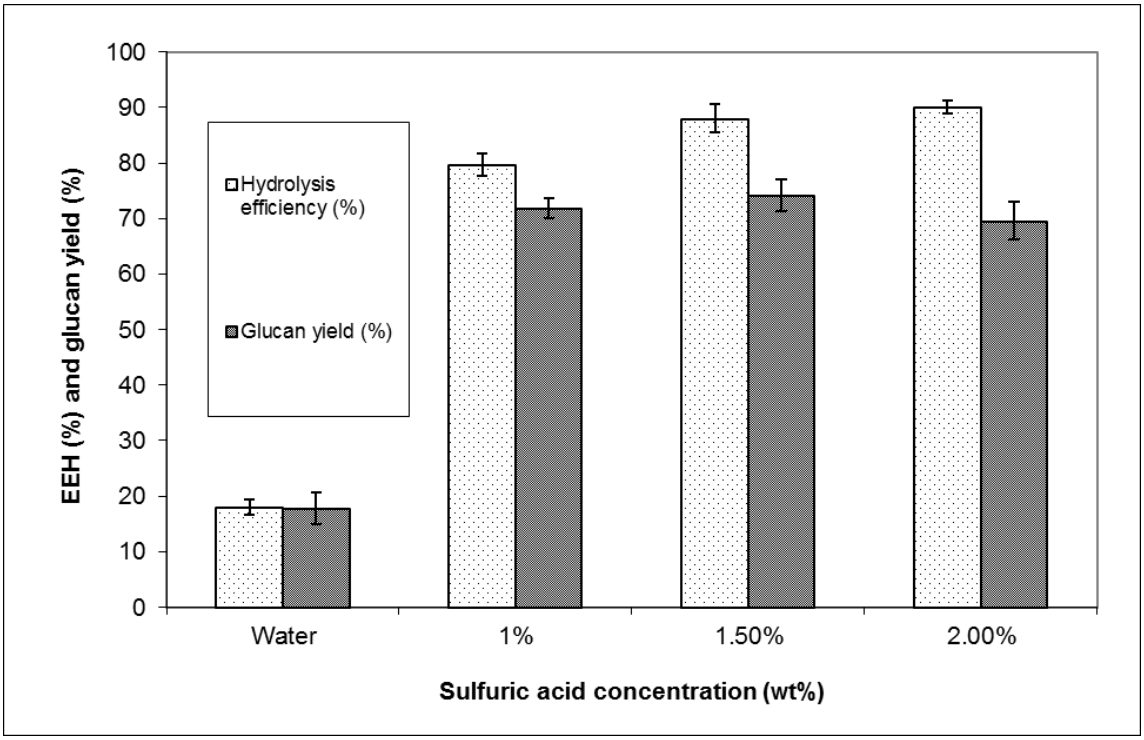


Figure 4.4 Effects of sulfuric acid concentration on efficiency of enzymatic hydrolysis (EEH) and glucan yield. Error bars are standard deviations at 95% confidence level.



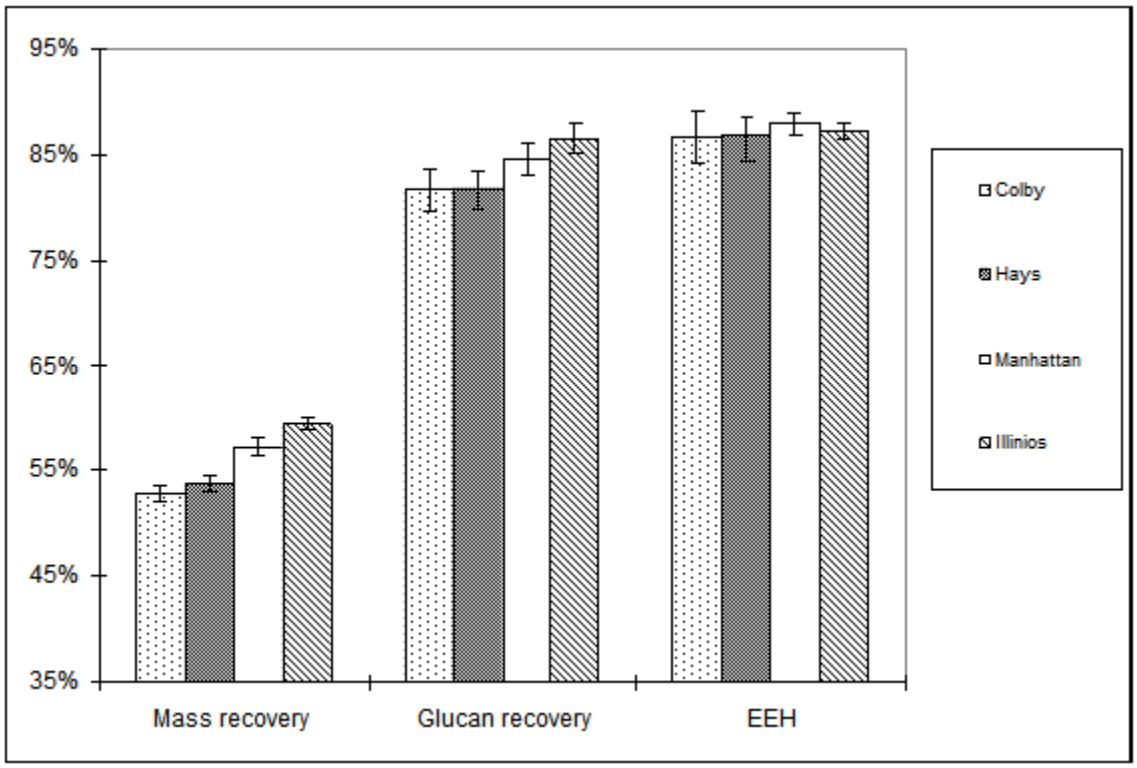
4.4.4 Effects of Planting Location and Ecotype on Pretreatment and Enzymatic Hydrolysis

Table 4.2 summarizes the mass and glucan recovery from the solid part after sulfuric acid pretreatment and efficiency of enzymatic hydrolysis, and cellulose content before and after processing from four planting locations and three ecotypes. For all big bluestem samples, the average and range across planting locations and ecotypes were 55.8% and from 52.0 to 60.0% for mass recovery, 83.7% and from 79.0 to 87.5% for glucan recovery, 87.1% and from 84.6 to 88.9% for EEH, and 25.2% and from 20.8 to 29.3% for glucan mass yield, respectively. Comparison of the glucan yield of big bluestem and other selected lignocellulosic biomass are listed in Table 4.3. Although the average of EEH of big bluestem was a bit lower than other lignocellulosic biomass reported by previous research, the average glucan recovery of big bluestem was much higher than other biomasses. In addition, as a result of sufficient glucan content of big bluestem compared with other biomasses, the glucan mass yield (final glucan production) of 25.2 g per 100 g of dry big bluestem had promising potential as other biomasses such as corn stover (21.0 g), forage sorghum (19.5 g), sweet sorghum (15.7 g), grain sorghum biomass (17.3 g), BMR sorghum (16.8 g), PS sorghum (12.8 g) wheat straw (30.0 g) and switchgrass (23.6g). Moreover, those biomasses used more severe pretreatment conditions with higher acid concentration and longer pretreatment times, suggesting that big bluestem has great potential as a dedicated bioenergy crop due to lower pretreatment requirements and higher glucan mass yield.

Based on ANOVA analysis summarized in Table 4.5, planting location had significant effects on mass recovery and glucan recovery of pretreatment as well as glucan mass yield (P value < 0.05). Those three parameters showed an increasing trend from west to east crossing location (Figure 4.5). Big bluestem populations planted in Illinois generally had a higher glucan mass yield, with an average of 27.5g per 100g dry big bluestem compared with averages of 22.5g, 24.0g, and 26.8g for populations planted in Colby, Hays, and Manhattan, KS, respectively, indicating that identical big bluestem populations would have approximately 22.2% higher glucan produced from pretreatment and enzymatic hydrolysis if planted in Illinois instead of Kansas (Figure 4.7). No significant effect of location was observed on EEH (P-value > 0.05). The possible explanation is that the effect of diluted acid pretreatment was so effective that it

removed almost the obstacle of biomass structure and neutralized the difference in structure from planting location. A similar trend was observed in mass recovery, glucan recovery and glucan content, however, so perhaps the positive relationship between glucan mass yield and latitude of the planting location simply emphasized that glucan content was the vital criteria affecting glucan mass yield.

Figure 4.5 Effects of planting location on mass recovery, glucan recovery, and efficiency of enzymatic hydrolysis (EEH). Error bars are standard deviations at 95% confidence level.



The effect of ecotype on mass recovery showed a similar trend for lignin content in which the highest and lowest lignin contents of big bluestem were found in CKS ecotype and IL ecotype, respectively (Figure 4.6). A possible reason is that lignin strongly contributes to recalcitrance during pretreatment. Although EEH differs significantly among various ecotypes, the average EEH values of different ecotypes are similar, indicating no clear effect of ecotype on average EEH within ecotypes from west to east. This result is probably because more severe pretreatment had a greater impact than ecotype. The effect of ecotype on glucan mass yield is shown in Figure 4.7. CKS, EKS, and ILL ecotypes had statistically similar average glucan mass yield regardless of planting location, but native cultivar KAW had significantly higher glucan

mass yield than other ecotypes, which was in agreement with previous studies (Gan et al., 2012; K Zhang et al., 2012). However, the trend was not consistent for individual ecotypes in each location. In other words, the effect of ecotype on glucan mass yield is dependent on the location. In Figure 7, KAW native cultivar had the highest glucan mass yield in Colby, Manhattan, KS and Carbonale, IL and the second highest in Hays, KS. Therefore, KAW is considered as an advantageous ecotype in term of glucan mass yield in sugar platform. EKS ecotype showed the highest glucan mass yield compared to CKS and IL ecotype in Manhattan, KS and Carbonale, IL location but had the lowest yield in Colby, KS. This phenomenon suggests that there was interaction between ecotype and location. Table 5 shows that interaction between planting location and ecotype had a significant effect only on glucan mass yield of big bluestem ($p < 0.05$); however, the interaction effect was much less than that of planting location and ecotype and had a smaller *F*-value (3.19). The role of the planting location was always a greater source of variation than ecotype and interaction between ecotype and location effects on glucan mass yield are based on much a larger *F*-value (Table 4.5). Among all big bluestem samples, KAW cultivar planting in Illinois was foremost in terms of final glucan mass yield of 29.3g per 100g dry big bluestem. Thus, KAW planting in Illinois could be selected and bred for a bioenergy crop. Multiple univariate linear regression analysis was conducted to find significant associations of environmental predictors with pretreatment and hydrolysis parameters, and results showed 90% of the variation in mass recovery, 84% of the variation in glucan recovery, 72% of the variation in EEH, and 90% of the variation in glucan mass yield was explained by precipitation in 2010 (Table 4.4).The number of growing degree days in 2010 and potential evapotranspiration can explain 85% and 78% of the variation in glucan mass yield, suggesting that 2010 precipitation possibly played a more significant role in divergence of glucan mass yield of the big bluestem.

Figure 4.6 Effects of ecotype on mass recovery, glucan recovery, and efficiency of enzymatic hydrolysis (EEH). Error bars are standard deviations at 95% confidence level.

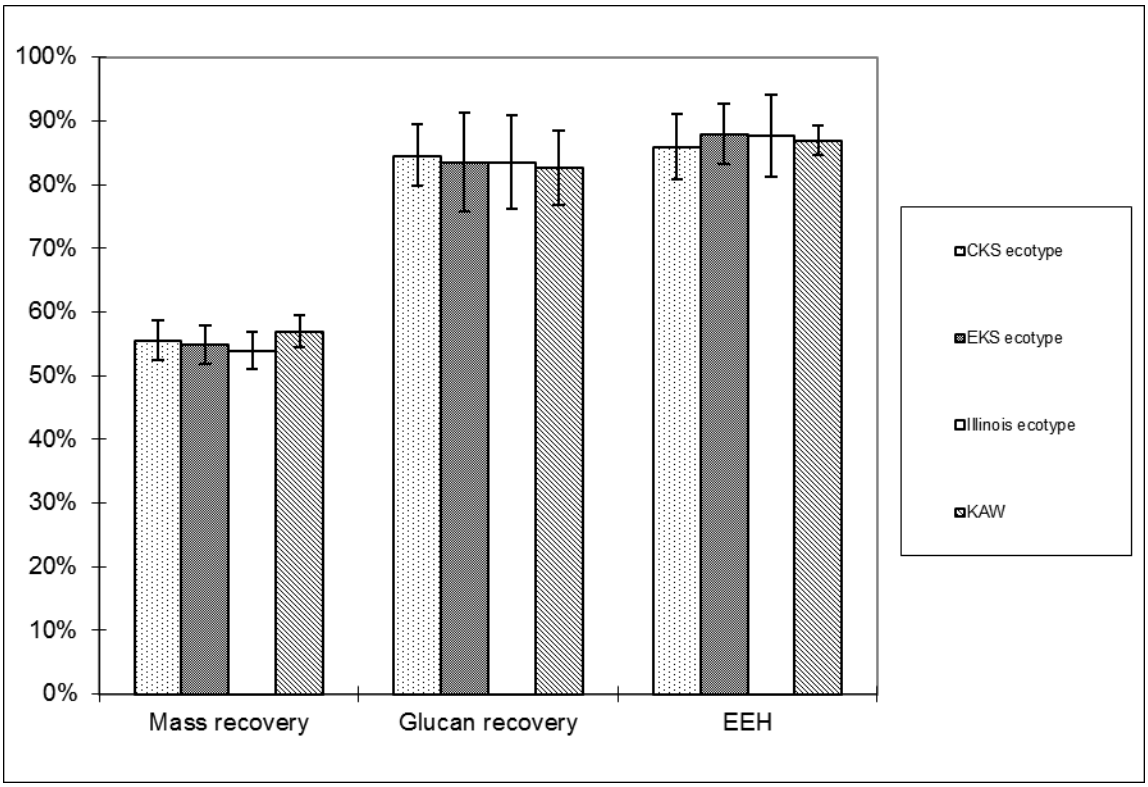
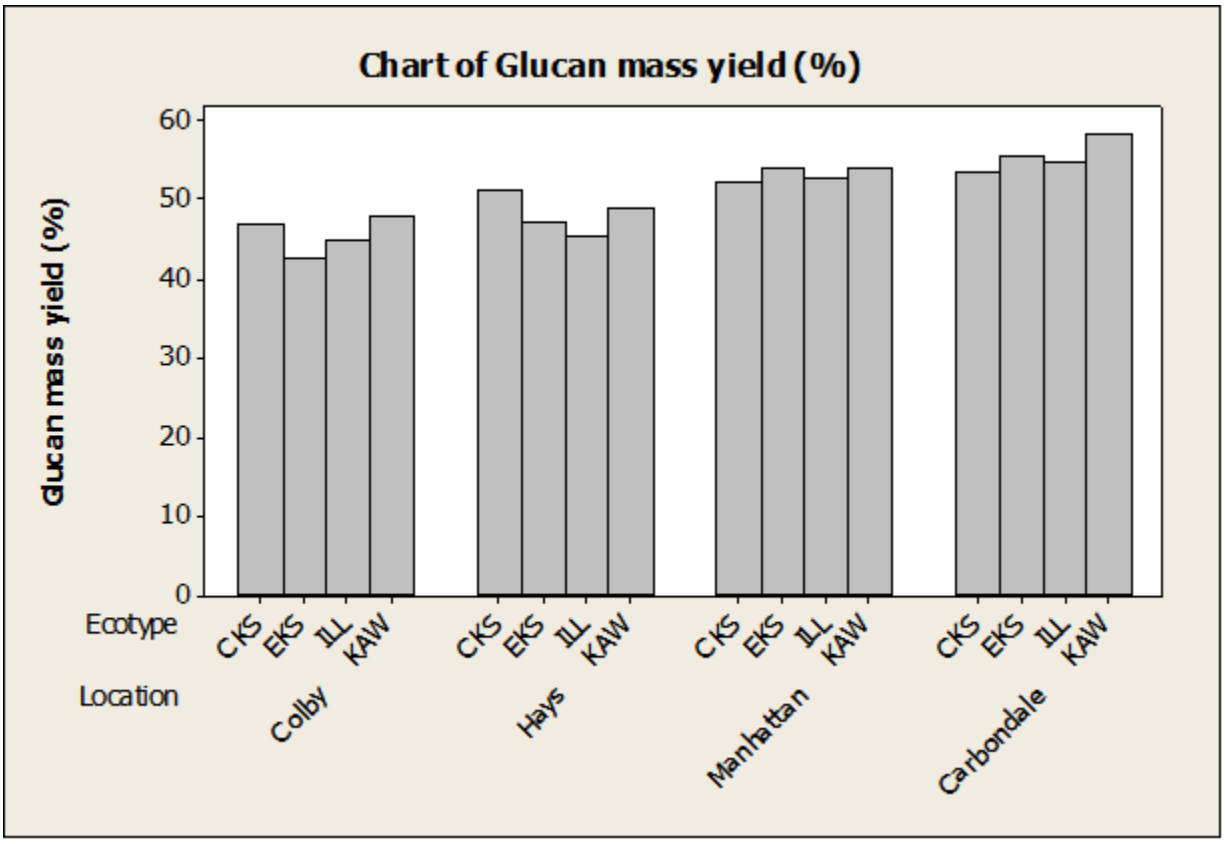


Figure 4.7 Effects of planting location and ecotype on glucan mass yield of big bluestem.



4.5 Conclusion

Planting location had significant effects on glucan content, mass recovery, glucan recovery, and glucan mass yield of big bluestem. Ecotype had significant effects on glucan content, glucan recovery, EEH, and glucan mass yield, whereas all measured variables except EEH were affected by planting location. The ecotype-location interaction had significant effects only on glucan content and glucan mass yield. In general, big bluestem planted in Illinois had a higher glucan content and glucan mass yield than populations planted in Kansas locations. Besides environmental effects, KAW was the best among all ecotypes in terms of glucan content and glucan mass yield. Up to 94%, 93%, and 93% of the variation in glucan content and yield can be explained by annual precipitation, number of growing degree days and potential evapotranspiration, respectively. Results showed that big bluestem could serve as a suitable energy grass in the Midwest with similar or better glucan content and glucan mass yield compared with other biomass crops and grasses.

Chapter 5 - Comparison of big bluestem with other native grasses on biofuel yield or production

5.1 Abstract

Big bluestem (BBS), an ecological-dominant warm-season (C4) perennial native grass, recently has been considered as a promising feedstock for biofuel production due to its high cellulosic content and low agricultural inputs. However, limited information exists regarding the comparison of big bluestem as a bioenergy crop with other herbaceous perennial biomass for biofuel potential. Therefore, multiple entry selections of big bluestem and three native C4 grasses species including switchgrass, miscanthus and 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, switchgrass and miscanthus also had similar xylan content that was significantly higher CRP grass. Big bluestem had more favorable lignin content compared to switchgrass. Big bluestem had the highest average mass recovery (55.56%) after acid pretreatment while miscanthus had the lowest mass recovery (46.3%). Positive correlation between glucan recovery and mass recoveries was observed. Efficiency of SSF curve lagged behind efficiency of the enzymatic hydrolysis (EEH) curve. No significant difference in average efficiency of SSF was observed among four native grasses, although big bluestem was the highest one (78.2%). However, ethanol yields from big bluestem entries were consistently greater than the other three grasses with average yield of 26.2%. The highest ethanol yield among ten entries was big bluestem cultivar (27.7%). Approximately 0.26kg ethanol with 9.4 g/L concentration can be produced form 1kg big bluestem 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.

5.2 Introduction

The development of biofuels from lignocellulosic biomass could reduce our dependence on fossil fuel resources and reduce greenhouse gas emissions, and reduce the competition of food vs. fuels (Dien et al., 2006; Tilman et al., 2006). Perennial herbaceous energy crops are classified

as an abundant lignocellulosic biomass, but they are not commonly recognized as traditional agricultural residues. However, perennial herbaceous energy crops may offer many economic benefits, including high yield, ability to grow easily with annual cycle, low energy input, ability to grow without pesticides or fertilizers, ability to increase wildlife biodiversity, increased soil quality, reduction of soil nutrients losses, nutrients recycling from municipal and agricultural wastes, soil carbon sequestration, and ability to mitigate greenhouse gas emissions (Adler et al., 2007; Farrell et al., 2006; Sanderson et al., 2004). The United States Department of Energy (DOE) established the Herbaceous Energy Crops Research Program (HECP) to develop data and information leading to commercially viable systems for production of herbaceous biomass for fuels and energy feedstocks since 1984 (Berger and Cushman, 1985). Thirty five potential herbaceous crops, 18 of which are perennial grasses projects, including big bluestem, switchgrass and Conservation Reserve Program (CRP) mixture grass, were initially studied in the HECP (Wright, 2007).

Big bluestem (*Andropogon gerardii*), an ecological-dominant warm-season (C4) perennial native grass that comprises as much as 80% of plant biomass in Midwestern prairies in North America and has been reported to achieve biomass yields ranging from 3.2 to 11.4 Mg/ha (Gould and Shaw, 1983; Johnson and Gresham, 2013; Knapp et al., 1998; Lee et al., 2009). Big bluestem productivity is high due to efficient nutrition utilization; it produces twice the biomass per applied nitrogen compared to switchgrass and indiangrass (Johnson and Matchett, 2001). Big bluestem also establishes easily from seed, and it spreads vigorously by vegetative growth of underground rhizomes with a robust root system (Perry and Baltensperger, 1979). In addition to economic considerations, bluestem prairie serves a range of purposes in the ecosystem by providing wildlife habitat, cattle grazing, and hay and pasturelands (Fargione et al., 2009). Swithgrass (*Panicum virgatum*) is another native C4 perennial grass on North America prairies that achieves biomass yield similar to or slightly more than big bluestem (Stork et al., 2009). Switchgrass has been selected as a "model" high-potential energy crop by Oak Ridge National Laboratory (ORNL) (Wright, 2007). Miscanthus, originating from Asia, is a perennial non-wood rhizomatous tall grass native to subtropical and tropical regions. Miscanthus has been used as a biofuel feedstock in Europe since the early 1980s and recently in North America for productivity trials (Brosse et al., 2012; Jørgensen, 1997; Lewandowski et al., 2000). Conservation Reserve Program (CRP) is a cost-share and rental payment program under the United States Department

of Agriculture (USDA), administered by the USDA Farm Service Agency (FSA) to prevent soil erosion and enhance ground water recharge from highly erodible lands. CRP grass mixture is comprised of native perennial grasses such as big bluestem, indiangrass, little bluestem, switchgrass, sideoats grama, silver bluestem, sand lovegrass, illinois bundleflower, maximillian sunflower, and old world bluestem (Venuto and Daniel, 2010). The percentage of each species within the grass mixture varies by location. CRP grass has a great biomass yield potential with 38 to 63 million dry metric tons anticipated every year (Perlack et al., 2005).

Research on CRP grass mixture has primarily focused on the impact on soil quality (Gebhart et al., 1994; Leddy et al., 1999; Randall et al., 1997). Recently, Linnebur studied the potential of CRP grass for biofuel production focusing on effects of torrefaction as a pretreatment method on chemical and elemental compositions, thermal properties, and energy density of the CRP biomass (Linnebur, 2013).A majority of previous studies investigating herbaceous grasses as energy crop have focused only on switchgrass as a "model" species (Parrish and Fike, 2005; Wright and Turhollow, 2010). Previous reviews have summarized information related to switchgrass as potential energy crop as it relates to historical study, biology and agronomy apspects, biofuels production via sugar and thermal platform as well as other utilization and constraints (Keshwani and Cheng, 2009; McLaughlin et al., 1999; Parrish and Fike, 2005; Rinehart, 2006; Sanderson et al., 2006; Wright, 2007). Miscanthus, a European "model" herbaceous energy crops, was initially studied as a fuel source for steam and power generation in Europe but has recently attracted attention in North America for productivity trials. Research showed that biomass yields ranged from 38.1 to 60.8 Mg/ha from established stands (Brosse et al., 2012; Heaton et al., 2008; Jørgensen, 1997; Lewandowski et al., 2000). Although research data for big bluestem is less than 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 long-term options (King and Coriolis, 1999). Some landowners also consider switchgrass excessively invasive. Production of ethanol and value-added chemicals via consolidated bioprocessing (a direct fermentation process) indicated that big bluestem is a superior feedstock over switchgrass and estern gamagrass (Weimer and Springer, 2007). Another advantage of big bluestem is that it can produce twice the biomass per unit of applied nitrogen than switchgrass or indiangrass (Perry and Baltensperger, 1979). In addition, big bluestem is the

dominant species in the second year while switchgrass dominates in the first establishment year (Tilman et al., 2001), thus reinforcing that big bluestem significantly increased when grown in monoculture or with indiangrass and switchgrass at second year (Hong et al., 2013). Madakadze et al. reported that, in southwestern Quebec, Canada, the list of average lignocellulose content ranked from high to low is cordgrass, big bluestem, switchgrass, sandreed, and indinagrass (Madakadze et al., 1998). Waramit et al. reported that big bluestem tends to contain higher cellulose concentrations than switchgrass (Waramit et al., 2011).

Our research showed that big bluestem has favorable characteristics related to bioconversion and comparable bio-oil yield through hydrothermal conversion (Gan et al., 2012; Zhang et al., 2012; Zhang et al., 2014). However, little information is available regarding head-to-head comparison of big bluestem with other herbaceous perennial biomass on the potential biofuel yield as bioenergy crop. Therefore, objectives of this research were to compare the chemical composition of big bluestem and three other promising native herbaceous perennial biomass (switchgrass, miscanthus and CRP grass), study their potential on ethanol yield through sulfuric acid pretreatment following simultaneous saccharification and fermentation (SSF), and providing useful insights for bioenergy industry and biomass producers

5.3 Materials and Methods

5.3.1 Materials

Three big bluestem ecotypes including Cedar Bluffs (CDB), Top of the World (TOW), and 12Mile (12M) and the KAW cultivar, which are widely planted to restore marginal lands, were harvested from reciprocal garden plots at Plant Materials Center in October 2013, in Manhattan, Kansas. Four switchgrass genotypes including SW16 (switchgrass plot16), SW17 (switchgrass plot17), SW18 (switchgrass plot18), and SWNT (switchgrass native) and miscanthus were harvested from the Agronomy Farm of Kansas State University in October 2013 in Manhattan Kansas. CRP grass was generously provided by agricultural farm at Bison, Kansas.

5.3.2 Analytical Methods

5.3.2.1 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 (Sluiter, et al., 2008). Extractives, glucan, and lignin contents of the biomass samples were determined by following NREL laboratory analytical procedures (Sluiter, et al., 2008; Sluiter, et al., 2008). 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 chromatography (HPLC, Shimadzu, Kyoto, Japan) equipped with an RCM monosaccharide column (300 × 7.8 mm; Phenomenex, Torrance, Calif.) 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.

5.3.2.2 Sulfuric acid pretreatment

Pretreatment was conducted in a reactor (Swagelok, Kansas City Valve & Fitting Co., KS, USA) made from 316L 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, 4g dry mass in 50 ml solution) solid content.

A sand bath (Techne, Inc., Princeton, N.J., USA) with a temperature controller was used to increase and control temperature. After the sand was increased to 160 ℃, the reactor was submerged in boiling sand for 40 min, then immediately transferred to room-temperature water to decrease the internal temperature to below 50 ℃ 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:

$$Glucan\ recovery(\%) = \frac{m_{pretreatment}}{m_{original}} \times 100\% \quad (5.1)$$

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.

5.3.2.3 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, Del., USA) 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 ℃ 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:

$$EEH(\%) = \frac{c \times V \times 0.9}{m_{glucan}} \times 100\% \quad (5.2)$$

where c is the concentration (g/L) of glucan after 72 hours 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.

5.3.2.4 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, USA) was conducted by adding 1.0 g of dry yeast into 19 mL of preculture broth (containing 20 g glucose, 5.0 g peptone, 3.0 g yeast extracts, 1.0 g KH_2PO4, and 0.5 g $MgSO_4 \cdot 7H_2O$ per liter) and shaking at 200 rpm in an incubator at 38℃ 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. The SSF was conducted in an incubator shaker (Model I2400, New Brunswick Scientific Inc., Edison, NJ, USA) at 38℃ and 150 rpm. At time intervals of 0, 3, 8, 24, 48, and 72h, 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 by HPLC. Efficiency of SSF (ESSF) and total ethanol yield were calculated using the following formulas (formula 5.3, 5.4):

$$ESSF(\%) = \frac{c \times V}{m_{glucan} \times 1.11 \times 0.511} \times 100\% \quad (5.3)$$

where c is the concentration (g/L) of ethanol after 72 hours SSF determined by HPLC analysis, V is the total solution volume (L), and m_{glucan} is the weight of glucan before enzymatic

hydrolysis (g). The factor 1.11 and 0.511 are the glucan to glucose content conversion factor and the mass coefficient of glucose to ethanol, respectively.

$$Ethanol\ yield\ (\%) = \frac{weight\ of\ ethanol}{weight\ of\ feedstock} \times 100\% \quad (5.4)$$

5.3.2.5 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, N.C.). In general, fully balanced ANOVA tests were performed following the general linear models (GLM) procedure. Correlations were determined using Pearson's correlation.

5.4 Results and Discussions

5.4.1Chemical composition comparison between big bluestem and other native grasses

Structural polysaccharides and lignin comprise a major chemical composition of native grasses. Structural polysaccharides contain cellulose and hemicellulose. Cellulose represented by glucan, while 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 ten entries from four native grasses species varied significantly with the exception arabinan content, as shown in Table 5.1. The ten entries of chemical composition ranged from 31.3 to 39.9% for glucan, 19.5 to 28.7% for xylan, 3.8 to 4.5% for arabinan, and 14.1 to 15.5% for lignin. Low lignin content of grasses is a desirable characteristic for enzymatic hydrolysis and ethanol fermentation whereas lignin is relatively higher (20-30%) in potential woody biomass such as pine, poplar, spruce, and willow (Pauly and Keegstra, 2008). Big bluestem native cultivar KAW had the highest glucan content (39.9%) among the ten entries, the highest xylan content, and second highest lignin content compared to the three other big bluestem entries. Western ecotype CDB had the highest glucan content among the three big bluestem ecotypes, thus comfirming our previous research regarding the effect of ecotype on the chemical composition of big bluestem (Zhang et al., 2012). Swithgrass plot17 had the highest xylan content (28.7%) and switchgrass native had the highest lignin content (15.5%) among the ten entries. CRP grass mixture had lower glucan, xylan and lignin content. The hypothesis was made that although big

bluestem and switchgrass comprise a majority of CRP grass mixture, other constituent grass may cause the significant differences to CRP grass chemical composition. The comparison of average glucan, xylan and lignin content of four native grasses species are compared in Figure 5.1. 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 significant higher than big bluestem, In this study, the chemical composition of big bluestem was consistent with previous research (Karunanithy and Muthukumarappan, 2009; Zhang et al., 2012), but switchgrass had a similar polysaccharides content and lower lignin content than previous research (Rinehart, 2006; Sanderson et al., 2006). Cell wall composition may differ due to method of analysis, climate and harvesting date, and crop cultivation practices.

Figure 5.1 Comparison of average glucan, xylan and lignin contents of four native grasses species, the same letter are not significantly different, based on Tukey's HSD test ($P < 0.05$).

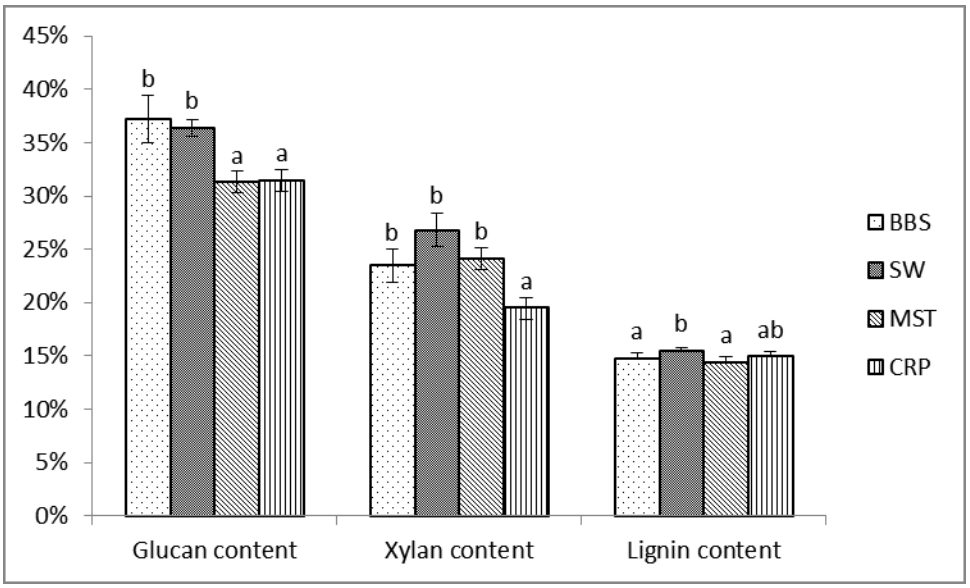


Table 5.1 Summary of glucan, xylan, arabinan, and lignin contents of ten native grasses entries.

Entry	Glucan content (%)	Xylan content (%)	Arabinan content (%)	Lignin content (%)
Big bluestem				
CDB	38.1±0.1 g	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				
SW16	36.9±0.2 ef	25.0±0.4 de	4.0±0.1 a	15.9±0.1 c
SW17	37.1±0.2 f	28.7±0.1 h	4.4±0.1 a	15.2±0.1 abc
SW18	36.2±0.4 de	26.5±0.9 g	4.2±0.5 a	15.3±0.2 bc
SWnative	35.3±0.1 bc	27.0±0.2 g	4.4±0.1 a	15.5±0.1 bc
Miscanthus	31.3±0.1 a	24.1±0.5 cd	3.9±0.3 a	14.4±0.3 ab
CRP grass	31.5±0.1 a	19.5±0.3 a	4.2±0.3 a	14.9±0.1 abc

The same letters are not significantly different, based on Tukey's HSD test ($P < 0.05$).

5.4.2 Diluted acid pretreatment

Diluted sulfuric acid pretreatment of big bluestem and other grasses was conducted at 1.5% acid concentration and 160℃ for 40 min. This pretreatment condition was optimized by different acid concentration (0%, 0.5%, 1.0%, 1.5% and 2.0) in our previous study (submitted paper). Acid pretreatment significantly increased glucan and lignin contents compared to the grasses without acid pretreatment (Table 5.2). Diluted acid pretreatment notably did not significantly affect the trend of glucan and lignin content among the ten entries, indicating that all entries responded diluted acid pretreatment under this condition in a similar way. However, almost all

xylan and most arabinan were hydrolyzed during diluted acid pretreatment, corresponding to less than1% xylan content and decreased arabinan content in Table 2. The decrease of xylan and arabinan contributed to an increase in glucan and lignin in treated biomass. Hemicellulose content of grasses after pretreatment decreased to 4% from raw material ranging 24-33%, suggesting that diluted acid pretreatment help to release cellulose for enzymatic hydrolysis because hemicellulose is recognized as shielding factor in cellulose digestion (R. Sun et al., 2010; Yoshida et al., 2008). Average mass recovery and glucan recovery for four native grasses species are shown in Figure 2.2. Big bluestem had the highest mass recovery (55.6%) and miscanthus had the lowest mass recovery (46.3%). Glucan recovery showed a similar trend to mass recoveries and a positive relationship with coefficient of determination (R^2=0.76) as shown in Figure 5.3. Big bluestem yielded highest glucan recovery up to 90.2%, suggesting that big bluestem had best pretreatment efficiency corresponding to only less than 10% glucan degradation.

Figure 5.2 Comparison of average mass recovery, glucan recovery and ESSF of four native grasses species, the same letter are not significantly different, based on Tukey's HSD test (P < 0.05).

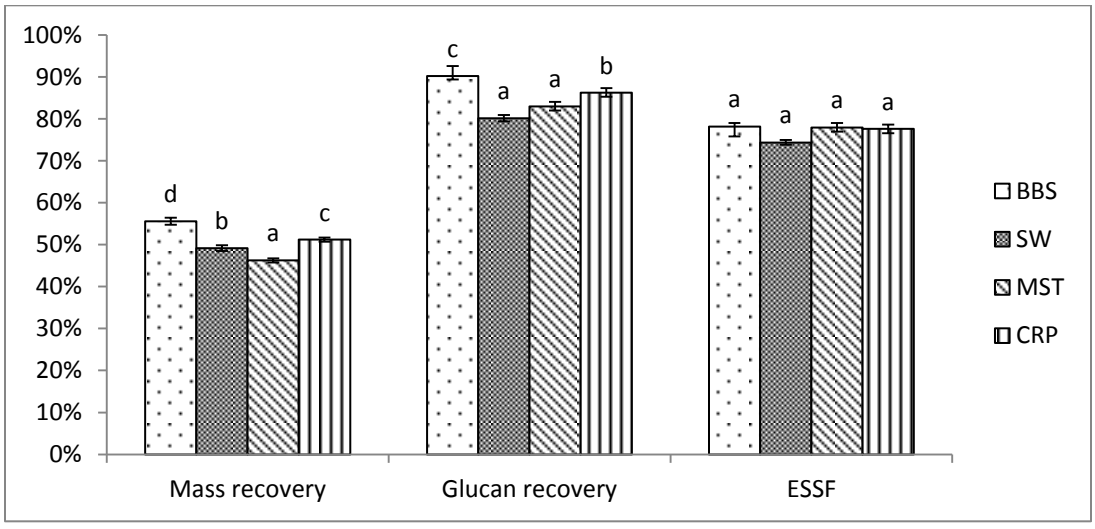
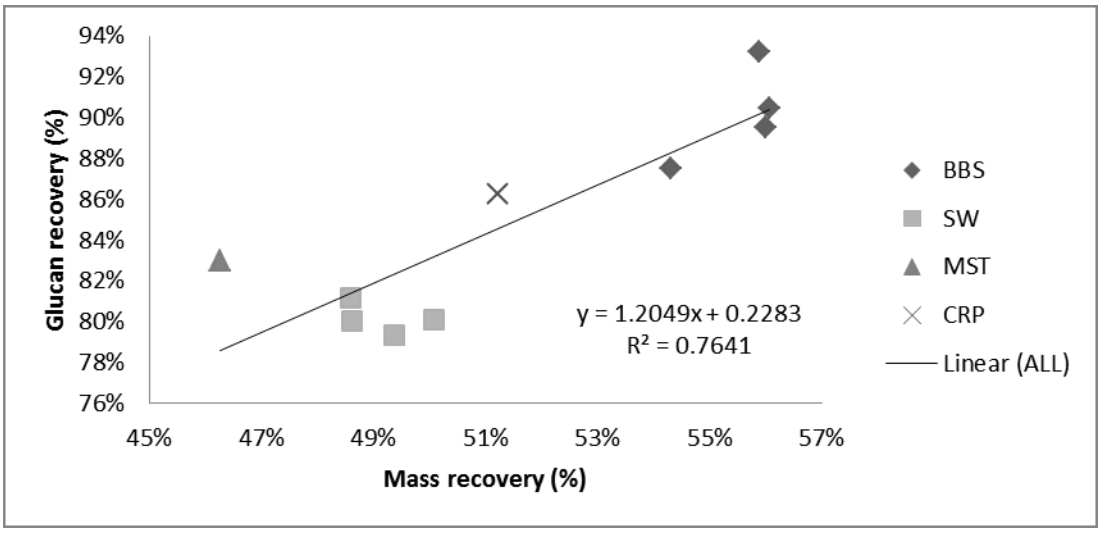


Table 5.2 Summary of glucan, xylan, arabinan, and lignin contents of ten native grasses entries after 1.5% sulfuric acid pretreatment.

Entry	Glucan content (%)	Xylan content (%)	Arabinan content (%)	Lignin content (%)
Big bluestem				
CDB	62.9±0.2 ef	<1	4.2±0.5 a	31.9±1.2 a
TOW	59.6±0.4 cd	<1	4.2±0.9 a	33.2±0.1 a
12M	57.9±0.2 bc	<1	3.9±0.9 a	34.0±0.2 ab
KAW	63.8±0.3 f	<1	4.3±0.4 a	32.5±0.5 a
Switchgrass				
SW16	58.5±0.2 c	<1	4.8±0.1 a	37.2±0.4 cd
SW17	61.1±0.5 de	<1	5.2±0.6 a	35.7±1.0 bc
SW18	59.7±0.4 cd	<1	4.7±0.1 a	36.7±0.3 c
SWnative	55.7±0.8 ab	<1	3.7±0.2 a	37.2±0.3 cd
Miscanthus	56.1±1.0 ab	<1	4.2±0.2 a	36.8±0.6 c
CRP grass	54.0±1.0 a	<1	4.3±1.1 a	39.5±0.6 d

The same letters are not significantly different, based on Tukey's HSD test ($P < 0.05$)

Figure 5.3 Relations between mass recovery (%) and glucan recovery (%) after diluted acid pretreatment.

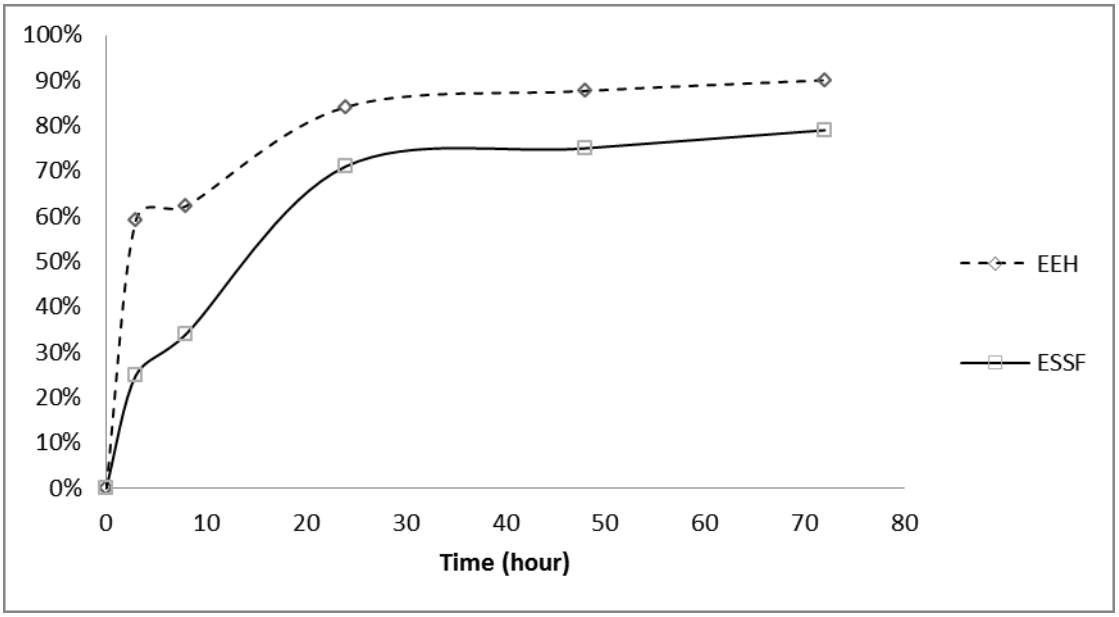


5.4.3 Simultaneous saccharification and fermentation

Time course of EEH and ESSF of big bluestem cultivar KAW is shown in Figure 5.4. Two curves followed a classical hydrolysis and fermentation pattern in which glucan conversion rapidly increased in the first 24 hours and reached the maximum after 72 hours. However, ESSF curve lagged behind 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 by-product glycerol was formed during the fermentation process. By-products formation during ethanol fermentation was also reported by Yazdani and Gonzalez (2007). Ethanol and glucose time profiles for big bluestem cultivar KAW during SSF process is shown in Figure 5.5. Glucose concentration in fermentation broth increased during the first seven hours then decreased once the yeast began uptake glucose at higher rate and almost completely consumed by yeast throughout SSF process. No significant statistic-difference in average ESSF existed among the four native grasses, although big bluestem had a higher ESSF of 78.2% (Figure 5.2). A possible explanation is that the effect of diluted acid pretreatment was too strong, consequently removing most of the obstacle of biomass structure and neutralizing the differences in the grasses. Previous studies reported slightly higher ESSF for swithgrass (87 - 90%) and miscanthus (72 - 91.2%) compared to this study (Chung et al., 2005; Kang et al., 2013; Li et al., 2013; Wyman et al., 1992). The

effect of lignin content on ESSF is shown in Fig. 6. The negative relationship between lignin content and ESSF was significant at 0.05 statistical level with R^2 of 0.63, indicating that recalcitrance of cellulose increased as lignin content increased. The lignin content explained 63% variation in ESSF (Figure 5.6). Average ethanol yield of big bluestem, switchgrass, miscanthus, and CRP grass were 26.2±1.3%, 21.7±0.6%, 20.2±1.0% and 21.1±1.0%, respectively. Big bluestem entries were consistently greater than the three other grasses.

Figure 5.4 Time course of EEH and ESSF of big bluestem cultivar KAW.



Big bluestem cultivar had the highest ethanol yield (27.7%) among the ten entries, correspond to highest glucan content. Comparison of ethanol yield among the ten entries was summarized in Table 5.3. Big bluestem-KAW and the average of big bluestem had significantly higher ethanol yields than the three other grasses. In addition, published results also showed big bluestem has a higher ethanol yield compared to other biomass, such as corn straw (2-10%) (Kim and Holtzapple, 2005; Öhgren et al., 2006; Varga et al., 2004), wheat straw (6-20%) (Ballesteros et al., 2006; Saha et al., 2005), rice straw (11%) (Sumphanwanich et al., 2008), sweet sorghum (19%) (Sipos et al., 2009), aspen (10%) (Asada et al., 2011) and spruce (8%) (Mirahmadi et al., 2010). A positive relation has been found between glucan content and ethanol yield (slop = 0.71 and $p < 0.01$), suggesting that a unit of glucan content increase correspondingly to 0.71% ethanol yield increase (Figure 5.7). The relatively low coefficient of determination ($R^2 = 0.50$) of the

linear regress in Figure 5.7 indicates that ethanol yield was likely affected by other factors besides the glucan content of feedstocks, such as pretreatment methods and enzyme dose. Figure 5.8 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 data set that provides fundamental information regarding big bluestem potential as feedstock for ethanol production and head-to-head comparison with other native grasses, specifically widely used switchgrass.

Figure 5.5 Ethanol yield and glucose consumption time profile for big bluestem cultivar KAW during SSF process.

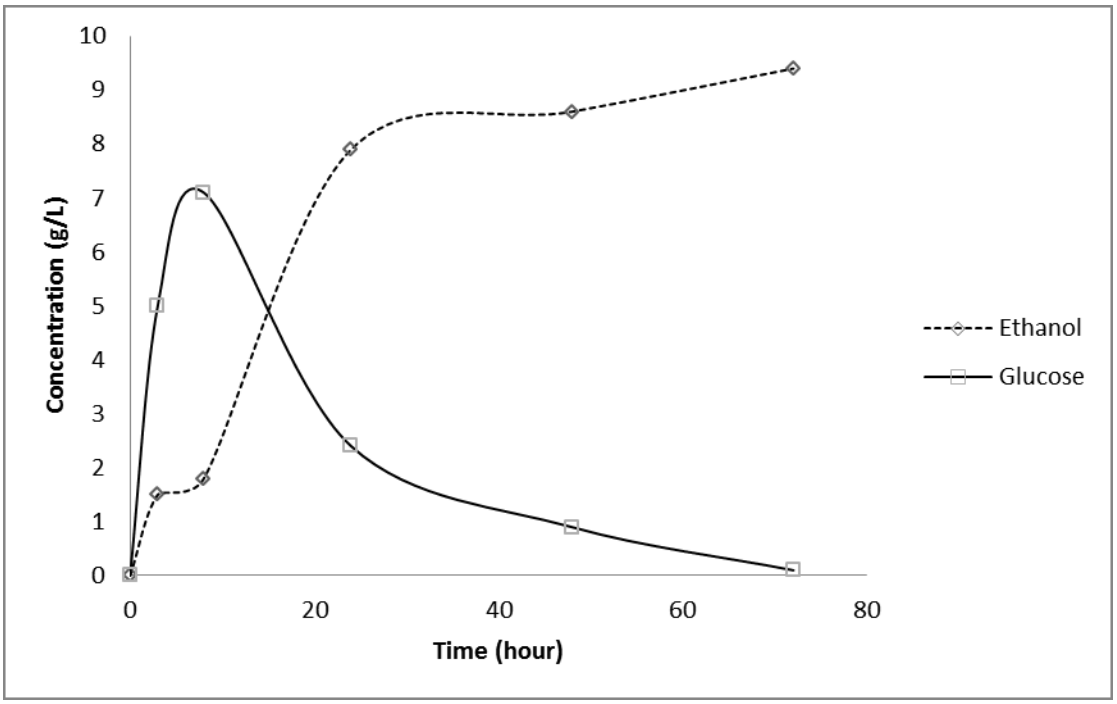


Figure 5.6 Relation between lignin content (%) and ESSF (%).

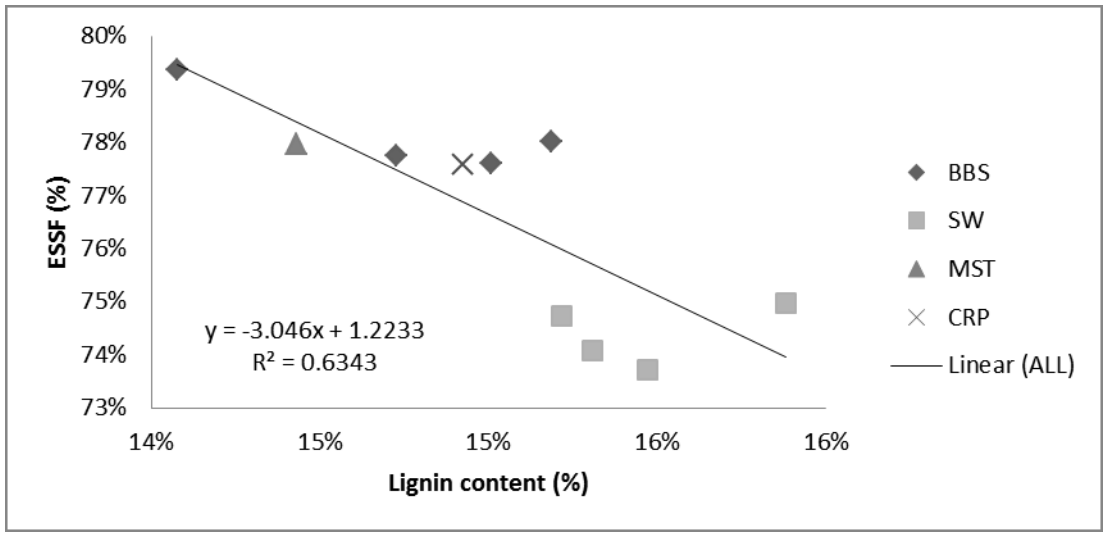


Figure 5.7 Relations between glucan content (%) and ethanol yield (%).

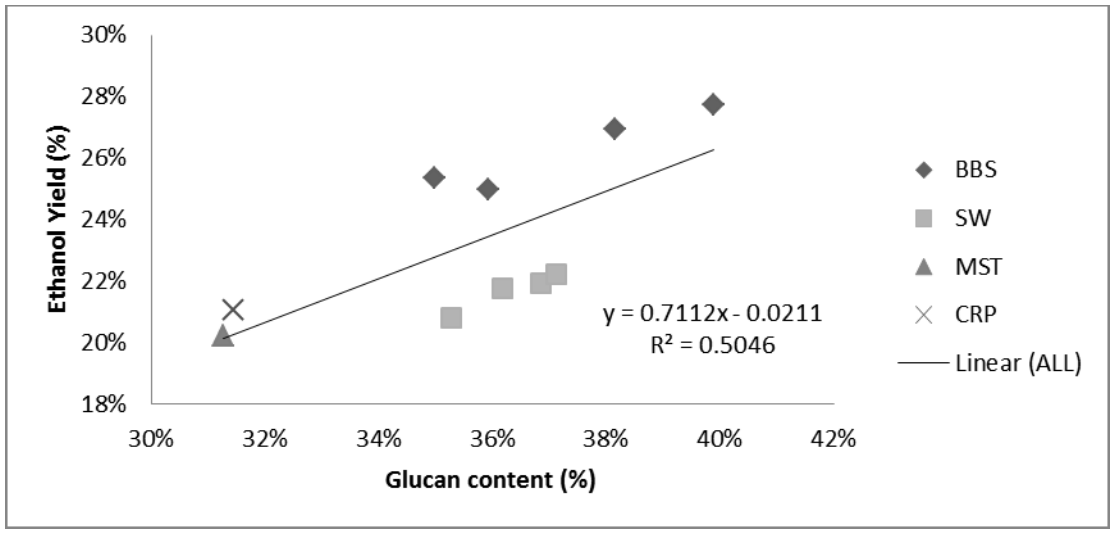
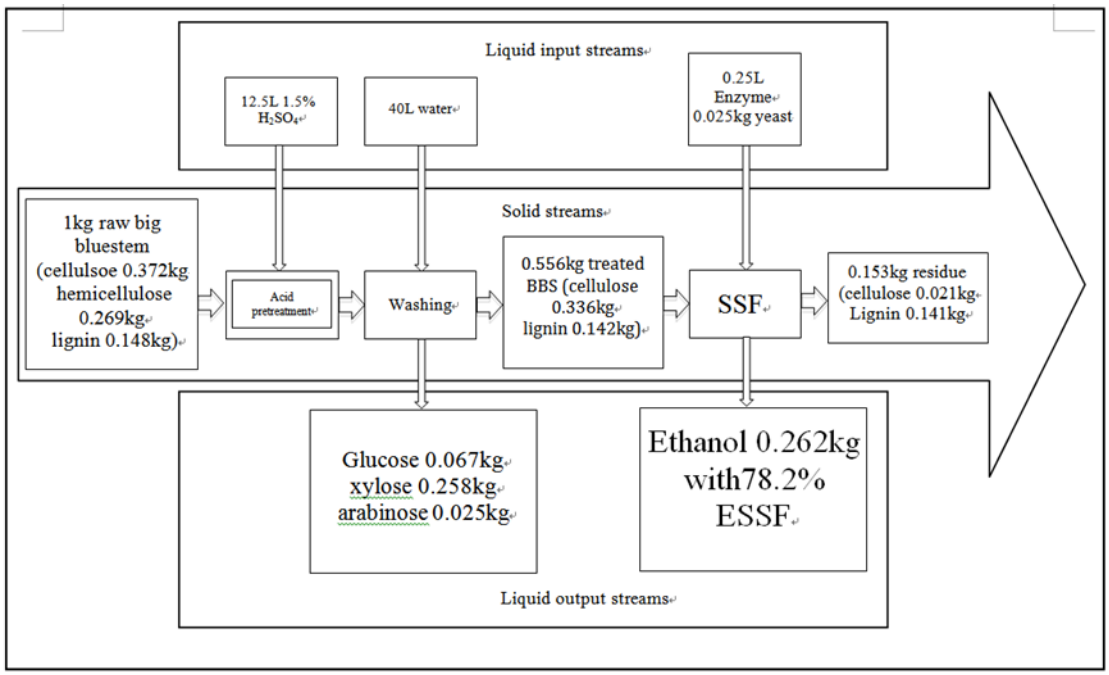


Table 5.3 Comparison of ethanol yield of big bluestem with switchgrass, miscanthus, and CRP grass.

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

Lowercase letters (a and b) indicate whether the means of ethanol yield the best yielding big bluestem-KAW, switchgrass, miscanthus and CRP grass are significantly different based on Tukey's HSD test ($p < 0.05$). 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. The same letter means they are not significantly different, whereas different letters mean they are significantly different in the order of b > a or B > A.

Figure 5.8 Mass balance analysis of big bluestem processing for ethanol production.



5.5 Conclusions

Big bluestem and switchgrass contain a similar glucan content that is significantly higher than miscanthus and CRP grass. Big bluestem has lower lignin content compared to switchgrass. A positive correlation between glucan recovery and mass recoveries was observed. The ESSF curve lagged behind the EEH curve, and no significant statistic-difference on average ESSF among four native grasses was noted. However, ethanol yields of big bluestem entries (26.2%) were consistently greater than the three other grasses. The highest ethanol yield among the ten entries was in big bluestem cultivar, which averaging 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 $R^2 = 0.5$. 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 a similar or better glucan content and ethanol yield compared with other native C4 grasses.

Chapter 6 - Conclusion and future work

6.1 Conclusions

Big bluestem, an ecological-dominant warm-season (C4) perennial native grass, recently has been considered as a promising feedstock for biofuel production due to its high cellulosic content and low agricultural input. However, the potential for biofuel production across the precipitation gradient of tallgrass prairie has not been broadly characterized. The goal of this research was to develop comprehensive understanding of big bluestem utilization for biofuel production via technical objectives that identify the potential impact of chemical composition, microstructure, physical properties, pretreatment methods, and bioconversion methods on bioconversion rate and biofuel yields from big bluestem ecotypes in planting locations across the Great Plains. Three big bluestem ecotypes from central Kansas (Cedar Bluffs and Webster populations), eastern Kansas (Konza and Top of the World populations), and Illinois (12Mile and Fults populations), as well as the Kaw cultivar, were harvested from four reciprocal garden planting locations (Colby, Hays, and Manhattan, KS; and Carbondale, IL) and evaluated for chemical and elemental composition (glucan, xylan, arabinan, lignin and ash) and elemental (carbon, oxygen, hydrogen, nitrogen and sulfur), Thermal properties (specific heat, thermal conductivity, thermogravimetric parameters and high heating value), sugars yield, and bioethanol yield as well as comparison with selected energy grasses.

Results from 28 big bluestem populations from four planting locations revealed a large variation in cellulose (31.8–36.5%), hemicellulose (24.96–29.74%), lignin (14.4–18.0%), carbon (47.3–51.3%), and nitrogen (4.91–6.44%). Planting location significantly affected chemical and elemental compositions of big bluestem, and ecotype had significantly affected glucan, xylan, lignin, and ash contents as well as carbon, oxygen, and hydrogen elemental fractions. In addition, interaction between ecotype and planting location had significantly affects glucan, lignin, and hydrogen. Planting location had a greater effect on chemical and elemental compositions than ecotype and interaction between location and ecotype. Total sugar content of the big bluestem (regardless of ecotype) increased as the Great Plains precipitation gradient increased from west to east. Annual precipitation, growing degree days and potential evapotranspiration in 2010 explained up to 97%, 88% and 80% of the variation in compositions, respectively.

Specific heat of big bluestem samples was significantly affected by planting location, ecotype, and interaction between location and ecotype, but planting location more strongly influenced specific heat than ecotype. Specific heat increased as temperature increased, and a linear correlation model for specific heat prediction was developed as a function of temperature. Ecotype, planting location, and the interaction of ecotype and planting location did not significantly affected thermal conductivity; however, density and particle size showed a completely opposite relationship on thermal conductivity. With the exception of weight loss, planting location significantly affected thermogravimetric parameters of big bluestem. Planting location and ecotype significantly affected high heating value. Among all environmental factors, potential evapotranspiration had the most significant effect on thermal properties.

Planting location significantly affected glucan content, mass recovery, glucan recovery, and glucan mass yield of big bluestem. Ecotype significantly affected glucan content, glucan recovery, EEH, and glucan mass yield, whereas all measured variables except EEH were affected by planting location. The ecotype-location interaction had significant effects only on glucan content and glucan mass yield. In general, big bluestem planted in Illinois had a higher glucan content and glucan mass yield than populations planted in Kansas locations. Besides environmental effects, KAW was the best among all ecotypes in terms of glucan content and glucan mass yield. 94%, 93%, and 93% of the variation in glucan content and yield can be explained by annual precipitation, number of growing degree days and potential evapotranspiration, respectively.

The biofuel potential of big bluestem was also compared to other energy grasses. Big bluestem and switchgrass had a 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. Big bluestem had more favorable lignin content comparing with switchgrass. Big bluestem had the highest average mass recovery (55.56%) after acid pretreatment while miscanthus had the lowest mass recovery (46.3%). A positive correlation between glucan recovery and mass recoveries was observed. Efficiency of the SSF curve lagged behind the EEH curve. No significant difference in average efficiency of SSF was observed among the four native grasses, although big bluestem was the highest one (78.2%). However, ethanol yields from big bluestem entries were consistently greater than the three other grasses with average yield of 26.2%. The highest ethanol yield among the ten entries was big bluestem

cultivar (27.7%). Approximate 0.26kg ethanol with 9.4 g/L concentration can be produced from 1kg big bluestem under current processing conditions. A negative relationship exists between lignin content and efficiency of SSF with R = -0.80 and a positive relationship exists between ethanol yield and glucan content with R = 0.71. Results showed that big bluestem could serve as a suitable energy grass in the Midwest with similar or better ethanol yield compared with other biomass crops and grasses.

6.2 Recommendation for future work

Most xylose and part of arabinose have been removed during diluted sulfuric acid pretreatment. The better pretreatment methods in terms of pentose reservation will be studied. On the other hand, the development of the pentose utilization would significantly improve the efficiency of diluted acid pretreatment.

Residues after fermentation could be utilized for many industrial productions, such as vanillin, DMSO, ethanol, xylitol sugar, and humic acid.

The relationship between significant phenotypic differentiations and biofuels related properties occurring naturally across a sharp precipitation gradient should be invested.

The effect of genetic variation on the potential of biofuels conversion of big bluestem should be invested.

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