

EVALUATION AND CHARACTERIZATION OF PELLETTED BIOMASS FROM  
SELECTED RESOURCES FOR ETHANOL PRODUCTION

by

KARNNALIN THEERARATTANANOON

B.S., University of Toronto, Canada, 2003  
M.S., University of Saskatchewan, Canada, 2006

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

2012

## **Abstract**

Lignocellulosic biomass such as agricultural residues tends to be a sustainable feedstock for production of biofuels and biobased products in the long term due to its high availability and relative low cost. However, conversion of lignocellulosic biomass to biofuels faces significant technical challenges. One of the major challenges is biomass logistics. The agricultural residues are often harvested during a limited harvest season and stored as bales with low bulk density, making them difficult to handle, transport, store, and use in their natural forms. Densification of biomass by pelleting process could significantly improve the bulk density of biomass and thus improve handling efficiency and reduce transportation and handling costs. The main focus of this research was to better understand the impacts of pelleting process as well as pelleting conditions on physical properties, chemical compositions, biomass structure, and fermentable sugar yield of sorghum stalk, corn stover, wheat straw, and big bluestem.

Results showed that pelleting process can increase biomass density up to 9-12 folds. Pelleting condition such as hammer mill screen size and ring-die pelleting mill die thickness had significant effects on bulk density, true density, and durability of biomass pellets. Although the pelleting process did not show significant effects on chemical composition of biomass before dilute-acid pretreatment process, glucan content of biomass pellets increased with the increase in ring-die pelleting mill die thickness and decreased with the increase in mill screen size after dilute-acid pretreatment. Opposite trend was observed for xylan content of biomass pellets as affected by pelleting conditions after dilute-acid pretreatment process. Biomass crystallinity increased after pelleting process, but not in a significant level. Softened surface region of biomass was removed after pelleting process, making the biomass more amendable to enzymatic attack. In this study, the optimum pelleting conditions were to grind the biomass feed using a 6.5-mm mill screen and to pellet biomass using a 44.5-mm ring-die pelleting mill die thickness. Under this optimum pelleting condition, the enzymatic conversion of cellulose of wheat straw pellets was the highest (94.1%), followed by corn stover pellet (93.1%), sorghum stalk pellet (92.1%), and big bluestem pellet (91.1%).

EVALUATION AND CHARACTERIZATION OF PELLETTED BIOMASS FROM  
SELECTED RESOURCES FOR ETHANOL PRODUCTION

by

KARNNALIN THEERARATTANANOON

B.S., University of Toronto, Canada, 2003  
M.S., University of Saskatchewan, Canada, 2006

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

2012

Approved by:

Major Professor  
Dr. Donghai Wang

# **Copyright**

KARNNALIN THEERARATTANANOON

2012

## **Abstract**

Lignocellulosic biomass such as agricultural residues tends to be a sustainable feedstock for production of biofuels and biobased products in the long term due to its high availability and relative low cost. However, conversion of lignocellulosic biomass to biofuels faces significant technical challenges. One of the major challenges is biomass logistics. The agricultural residues are often harvested during a limited harvest season and stored as bales with low bulk density, making them difficult to handle, transport, store, and use in their natural forms. Densification of biomass by pelleting process could significantly improve the bulk density of biomass and thus improve handling efficiency and reduce transportation and handling costs. The main focus of this research was to better understand the impacts of pelleting process as well as pelleting conditions on physical properties, chemical compositions, biomass structure, and fermentable sugar yield of sorghum stalk, corn stover, wheat straw, and big bluestem.

Results showed that pelleting process can increase biomass density up to 9-12 folds. Pelleting condition such as hammer mill screen size and ring-die pelleting mill die thickness had significant effects on bulk density, true density, and durability of biomass pellets. Although the pelleting process did not show significant effects on chemical composition of biomass before dilute-acid pretreatment process, glucan content of biomass pellets increased with the increase in ring-die pelleting mill die thickness and decreased with the increase in mill screen size after dilute-acid pretreatment. Opposite trend was observed for xylan content of biomass pellets as affected by pelleting conditions after dilute-acid pretreatment process. Biomass crystallinity increased after pelleting process, but not in a significant level. Softened surface region of biomass was removed after pelleting process, making the biomass more amendable to enzymatic attack. In this study, the optimum pelleting conditions were to grind the biomass feed using a 6.5-mm mill screen and to pellet biomass using a 44.5-mm ring-die pelleting mill die thickness. Under this optimum pelleting condition, the enzymatic conversion of cellulose of wheat straw pellets was the highest (94.1%), followed by corn stover pellet (93.1%), sorghum stalk pellet (92.1%), and big bluestem pellet (91.1%).

# Table of Contents

List of Figures .....	x
List of Tables.....	xiv
Acknowledgements .....	xv
CHAPTER 1 - INTRODUCTION .....	1
GENERAL BACKGROUND .....	1
RESEARCH OBJECTIVES.....	2
LITERATURE REVIEWS.....	3
Lignocellulosic Biomass .....	3
Biomass Feedstocks .....	5
Pelleting of Biomass.....	6
Bioconversion of Lignocellulosic Biomass to Bioethanol.....	7
Biomass Structural Analysis .....	12
Physical Properties of Biomass Pellets .....	14
LITERATURE CITED.....	16
CHAPTER 2 - EVALUATION AND CHARACTERIZATION OF SORGHUM BIOMASS AS FEEDSTOCK FOR SUGAR PRODUCTION .....	27
ABSTRACT .....	27
KEYWORDS: CELLULOSE CONVERSION, ENZYMATIC HYDROLYSIS, PRETREATMENT, SORGHUM BIOMASS. ....	27
INTRODUCTION.....	28
MATERIALS AND METHODS .....	29
Materials.....	29
Dilute Acid Pretreatment.....	30
Enzymatic Hydrolysis .....	31
Crystalline Structure Analysis Using X-Ray Diffraction.....	31
Chemical Structure Analysis Using FTIR.....	32
Morphological Structure Analysis Using Scanning Electron Microscopy .....	32

Analytical Methods .....	33
RESULTS AND DISCUSSION.....	33
Effect of Pretreatment Conditions on Yield of Fermentable Sugars.....	33
Enzymatic Hydrolysis .....	34
X-Ray Diffraction .....	35
Chemical Structure.....	35
Morphological Structure .....	37
CONCLUSIONS.....	37
ACKNOWLEDGEMENT .....	38
REFERENCES.....	38
APPENDIX - MATLAB CODE FOR SMOOTHING OF X-RAY DIFFRACTION SPECTRA .....	42
CHAPTER 3 - PHYSICAL PROPERTIES OF PELLETS MADE FROM SORGHUM STALK, CORN STOVER, WHEAT STRAW, AND BIG BLUESTEM .....	61
ABSTRACT .....	61
INTRODUCTION.....	62
MATERIALS AND METHODS .....	64
Sample Preparation .....	64
Pelleting.....	65
Equilibrium Moisture Content (EMC) and Equilibrium Relative Humidity (ERH) Relationship.....	65
Size, Bulk Density, and True Density of Pellets.....	66
Durability of Pellets .....	66
Data Analysis .....	67
RESULTS AND DISCUSSION.....	67
EMC-ERH Relationship.....	67
Effect of Moisture on Size and Density of Pellets .....	68
Effect of Moisture on Durability of Pellets.....	69
Effect of Die Size and Hammer Mill Screen Size on Density and Durability of Pellets .....	70
CONCLUSIONS.....	71
ACKNOWLEDGEMENT .....	72
REFERENCES.....	72

CHAPTER 4 - EFFECTS OF THE PELLETING CONDITIONS ON CHEMICAL COMPOSITION AND SUGAR YEILD OF CORN STOVER, BIG BLUESTEM, WHEAT STRAW, AND SORGHUM STALK PELLETS .....	82
ABSTRACT .....	82
INTRODUCTION.....	82
MATERIALS AND METHODS .....	84
Materials.....	84
Dilute Acid Pretreatment.....	86
Enzymatic Hydrolysis .....	86
Analytical Methods .....	87
RESULTS AND DISCUSSION.....	88
Physical Properties of Biomass Pellets .....	88
Effect of Pelleting Conditions and Pretreatment Process on the Chemical Composition of Biomass .....	89
Effect of Pelleting Process on Biomass Sugar Yield .....	90
CONCLUSIONS.....	91
ACKNOWLEDGEMENT .....	92
REFERENCES.....	92
 CHAPTER 5 - IMPACT OF PELLETING AND ACID PRETREATMENT ON BIOMASS STRUCTURE AND THERMAL PROPERTIES OF WHEAT STRAW, CORN STOVER, BIG BLUESTEM, AND SORGHUM STALK .....	 103
ABSTRACT .....	103
INTRODUCTION.....	104
MATERIALS AND METHODS .....	106
Materials.....	106
Biomass Pelleting.....	106
Pretreatment of Biomass .....	107
X-ray Diffraction Analysis.....	107
Fourier Transform Infrared Spectroscopy.....	107
Solid-State <sup>13</sup> C NMR Spectroscopy .....	108



Scanning Electron Microscopy .....	108
Thermogravimetric Analysis .....	108
RESULTS AND DISCUSSION .....	109
Crystalline Structure Analysis .....	109
FTIR Analysis .....	110
Solid-State <sup>13</sup> C NMR Analysis .....	111
Morphological Structure Analysis .....	113
Thermogravimetric Analysis .....	114
CONCLUSIONS .....	115
ACKNOWLEDGEMENT .....	116
REFERENCES .....	116
APPENDIX - MATLAB CODE FOR SMOOTHING OF X-RAY DIFFRACTION SPECTRA .....	120
CHAPTER 6 - CONCLUSIONS AND RECOMMENDATIONS .....	133
CONCLUSIONS .....	133
RECOMMENDATIONS .....	135

## List of Figures

Figure 1.1.1 Lignocellulose and Its Components (U.S. Department of Energy National Laboratory Operated by the University of California).....	24
Figure 1.2 Flow chart for the production of bioethanol from lignocellulosic biomass (Adapted from Balat and Balat, 2009) .....	25
Figure 1.3 Three types of reaction catalyzed by cellulase (Karmakar and Ray, 2011) .....	26
Figure 2.1 Effect of sulfuric acid concentration on glucose yield. ....	43
Figure 2.2 Percentage of enzymatic conversion of cellulose for corn stover and different types of sorghum biomass pretreated with 2% w/v sulfuric acid at 140°C for 30 min. ....	44
Figure 2.3 Percentage of enzymatic conversion of cellulose of corn stover and different types of sorghum biomass pretreated with 2% w/v sulfuric acid at 165°C for 10 min. ....	45
Figure 2.4 X-ray diffraction of untreated and treated sorghum stalks and corn stover: (a) BMR sorghum, (b) corn stover, (c) forage sorghum, (d) grain sorghum biomass, (e) photoperiod-sensitive sorghum, and (e) sweet sorghum bagasse. ....	46
Figure 2.5 FTIR spectra of untreated sorghum biomass and corn stover. ....	47
Figure 2.6 FTIR spectra of untreated sorghum biomass and corn stover in the fingerprint region (900-1800 cm <sup>-1</sup> ).....	48
Figure 2.7 FTIR spectra of sorghum biomass and corn stover in the fingerprint region (900-1800 cm <sup>-1</sup> ) after dilute acid pretreatment at 140°C for 30 min with 2% w/v acid.....	49
Figure 2.8 FTIR spectra of sorghum biomass and corn stover in the fingerprint region (900-1800 cm <sup>-1</sup> ) after dilute acid pretreatment at 165°C for 10 min with 2% w/v acid.....	50
Figure 2.9 FTIR spectra of sorghum biomass and corn stover in the fingerprint region (900-1800 cm <sup>-1</sup> ) after dilute acid pretreatment at 140°C for 30 min with 2% w/v acid and enzymatic hydrolysis. ....	51
Figure 2.10 FTIR spectra of sorghum biomass and corn stover in the fingerprint region (900-1800 cm <sup>-1</sup> ) after dilute acid pretreatment at 165°C for 10 min with 2% w/v acid and enzymatic hydrolysis.....	52
Figure 2.11 SEM images of untreated samples: (a) BMR sorghum (b) corn, (c) forage sorghum, (d) grain sorghum, (e) photoperiod-sensitive sorghum, and (f) sweet sorghum. ....	53

Figure 2.12 SEM images of samples pretreated at 140°C for 30 min with 2% sulfuric acid: (a) BMR sorghum (b) corn, (c) forage sorghum, (d) grain sorghum, (e) photoperiod-sensitive sorghum, and (f) sweet sorghum. ....	54
Figure 2.13 SEM images of samples pretreated at 165°C for 10 min with 2% sulfuric acid: (a) BMR sorghum (b) corn, (c) forage sorghum, (d) grain sorghum, (e) photoperiod-sensitive sorghum, and (f) sweet sorghum. ....	55
Figure 2.14 SEM images of samples after pretreatment at 140°C for 30 min with 2% sulfuric acid and enzymatic hydrolysis: (a) BMR sorghum (b) corn, (c) forage sorghum, (d) grain sorghum, (e) photoperiod-sensitive sorghum, and (f) sweet sorghum. ....	56
Figure 2.15 SEM images of samples after pretreatment at 165°C for 10 min with 2% sulfuric acid and enzymatic hydrolysis: (a) BMR sorghum (b) corn, (c) forage sorghum, (d) grain sorghum, (e) photoperiod-sensitive sorghum, and (f) sweet sorghum. ....	57
Figure 3.1 Equilibrium moisture content and equilibrium relative humidity of corn stover, sorghum stalk, big bluestem, and wheat straw pellets. ....	76
Figure 3.2 Effect of equilibrium moisture content on bulk density values of pellets of wheat straw, big bluestem (BB), corn stover, and photoperiod-sensitive sorghum stalk (PS). ....	77
Figure 3.3 Effect of equilibrium moisture content on true density values of pellets of wheat straw, big bluestem (BB), corn stover, and photoperiod-sensitive sorghum stalk (PS). ....	78
Figure 3.4 Effect of equilibrium moisture content on durability of pellets of wheat straw, big bluestem (BB), corn stover, and photoperiod-sensitive sorghum stalk (PS). ....	79
Figure 4.1 Extractive contents of wheat straw, corn stover, big bluestem, and sorghum stalk, as affected by hammer mill screen size and die thickness; pellet set 1 = 3.2 mm screen size, 31.8 mm die thickness, pellet set 2 = 3.2 mm screen size, 44.5 mm die thicknes. ....	95
Figure 4.2 Cellulose recovery of pretreated wheat straw, corn stover, big bluestem, and sorghum stalk, as affected by hammer mill screen size and die thickness; pellet set 1 = 3.2 mm screen size, 31.8 mm die thickness, pellet set 2 = 3.2 mm screen size, 44.5 mm die thickness, pellet set 3=6.5 mm screen size, 44.5 mm die thickness. ....	96
Figure 4.3 Maximum Enzymatic Conversion of Cellulose (%ECC) of wheat straw, corn stover, big bluestem, and sorghum stalk, as affected by hammer mill screen size and die thickness; pellet set 1 = 3.2 mm screen size, 31.8 mm die thickness, pellet set 2 = 3.2 mm screen size, 44.5 mm die thickness, pellet set 3 = 6.5 mm screen size, 44.5 mm die thickness. ....	97

Figure 5.1 Effect of pelleting parameters on CrI value of biomass; P1: pelleted biomass (3.2-mm mill screen size and 31.8-mm die thickness); P2: pelleted biomass (3.2-mm mill screen size and 44.5-mm die thickness); and P3: Pelleted biomass (6.5-mm mill screen size and 44.5-mm die thickness)..... 121

Figure 5.2 Effects of pelleting process and pretreatment process on CrI value of biomass. UN: unpelleted biomass with no pretreatment; UT: unpelleted biomass with pretreatment; PN: pelleted biomass with no pretreatment; and PT: pelleted biomass with pretreatment. .... 122

Figure 5.3 FTIR spectra of (a) untreated corn stover, (b) untreated big bluestem (BBS), (c) untreated wheat straw, and (d) untreated photoperiod-sensitive sorghum stalk (PS) densified under different hammer mill screen size (small-SS, and large-LS) and die thickness (small-SD, large-LD)..... 123

Figure 5.4 FTIR spectra in the fingerprint region (900-1800 cm<sup>-1</sup>) of (a) untreated corn stover, (b) untreated big bluestem (BBS), (c) untreated wheat straw, and (d) untreated photoperiod-sensitive sorghum stalk (PS) densified under different hammer mill screen size (small-SS, and large-LS) and die thickness (small-SD, large-LD)..... 124

Figure 5.5 FTIR spectra in the fingerprint region (900-1800 cm<sup>-1</sup>) of (a) pretreated corn stover, (b) pretreated big bluestem (BBS), (c) pretreated wheat straw, and (d) pretreated photoperiod-sensitive sorghum stalk (PS) densified under different hammer mill screen (small-SS, and large-LS) and die thickness (small-SD, large-LD). .... 125

Figure 5.6 <sup>13</sup>C NMR spectra of big bluestem: B0: untreated and unpelleted sample; B1: untreated and pelleted sample (pelleting condition: 3.2-mm hammer mill size and 31.8-mm die thickness); B2: untreated and pelleted sample (pelleting condition: 6.5-mm hammer mill size and 44.5-mm die thickness); B3: untreated and pelleted sample (pelleting condition: 3.2-mm hammer mill size and 44.5-mm die thickness); B3prt: pretreated and pelleted sample (pelleting condition is same as B3). .... 126

Figure 5.7 <sup>13</sup>C NMR spectra of corn stover: C0: untreated and unpelleted sample; C1: untreated and pelleted sample (pelleting condition: 3.2-mm hammer mill size and 31.8-mm die thickness); C2: untreated and pelleted sample (pelleting condition: 6.5-mm hammer mill size and 44.5-mm die thickness); C3: untreated and pelleted sample (pelleting condition: 3.2-mm hammer mill size and 44.5-mm die thickness); C3prt: pretreated and pelleted sample (pelleting condition is same as C3). .... 127

Figure 5.8 SEM images of untreated corn stover: (a) outer surface of unpelleted corn stover, (b) outer surface of pelleted corn stover, (c) cellulose bundles of pelleted corn stover, and (d) microfibril of pelleted corn stover.....	128
Figure 5.9 SEM images of corn stover after dilute-acid pretreatment process: (a) outer surface of unpelleted corn stover, (b) and (c) internal structure of pelleted corn stover, (d) annular rings of pelleted corn stover, and (e) holes in the cellulose wall of pelleted corn stover, and (f) microfibrils of pelleted corn stover.....	129
Figure 5.10 Thermal properties of the unpelleted and pelleted biomass samples: (a) corn stover, (b) big bluestem, (c) wheat straw, and (d) photoperiod-sensitive sorghum stalk. ....	130

## List of Tables

Table 2.1 Components in solid fractions of pretreated biomass .....	58
Table 2.2 Sugar yield in filtrate after dilute acid pretreatment .....	59
Table 2.3 Crystallinity index values for different types of biomass .....	59
Table 2.4 Assignment of FTIR absorption bands for sorghum biomass and corn stover .....	60
Table 3.1 Size of biomass pellets as affected by mill screen size, die thickness, and L/D ratio of the die .....	80
Table 3.2 Physical properties of biomass pellets as affected by mill screen size, die thickness, and L/D ratio of the die .....	81
Table 4.1 Pellet durability, bulk density, and true density as affected by mill screen size and die thickness .....	98
Table 4.2 Comparison of composition of unpelleted wheat straw, corn stover, big bluestem, and photoperiod-sensitive sorghum stalk .....	99
Table 4.3 Chemical composition of untreated biomass as affected by mill screen size and die thickness .....	100
Table 4.4 Chemical composition of biomass as affected by dilute-acid pretreatment (140 °C, 30 min) and pelleting process .....	101
Table 4.5 Sugar yield in filtrate of biomass after dilute acid pretreatment as affected by mill screen size and die thickness .....	102
Table 5.1 Assignment of FTIR absorption bands for sorghum biomass and corn stover .....	131
Table 5.2 Signal assignments for <sup>13</sup> C CP/MAS NMR spectra of big bluestem and corn stover .....	132

## **Acknowledgements**

I would like to express my deep appreciation to my advisor, Dr. Donghai Wang, for his encouragement, support, and guidance during my Ph.D. study. I am grateful for the opportunities that he has provided me. Thanks for supporting me in so many ways.

I would like to thank the other members of my supervisory committee, Dr. Scott Staggenborg, Dr. Richard Nelson and Dr. Wenqiao Yuan, for providing valuable comments, suggestion, and guidance. I also appreciate Dr. Praveen Vadlani for willing to serve as my outside chairperson.

Special thanks to Dr. Scott Staggenborg for providing biomass, Dr. Leland McKinney and Jonathan Wilson for providing biomass pellets. I also would like to thank Dr. Xiaorong Wu at Department of Biological and Agricultural Engineering, Dr. Keith Hohn and Mr. Myles Ikenberry at Department of Chemical Engineering, Mr. John Desper at Department of Chemistry and Mr. Kent Hampton at Department of Entomology for their technical assistance on HPLC, FTIR, X-ray diffraction, and SEM analyses.

I would like to express gratitude to Dr. Joseph Harner, Department Head, Dr. Naiqian Zhang, Graduate Program Director, Ms. Barb Moore, Mr. Randy Erickson, Mr. Darrell Oard, Ms. Lou Ann Claassen, and all other faculty members and staff in the Department of Biological and Agricultural Engineering, KSU for their help and cooperation in all the administrative and academic activities related to this research. I also would like to thank all the fellow students in the bioprocessing lab for their help on my research.

Special acknowledge to the Royal Thai Government for offering me financial support throughout my Ph.D. program.

Finally, I would like to thank my parents for their unconditional love and support.

# CHAPTER 1 - INTRODUCTION

## GENERAL BACKGROUND

Biomass-derived ethanol is one of the promising alternative energy sources for transportation fuel. It can be used as either a substitution for gasoline or as a blend with gasoline. Bioethanol is commonly blended with gasoline in concentrations of 10% bioethanol to 90% gasoline, known as E10 or gasohol (Balat and Balat, 2009). Benefits of using bioethanol include mitigation of greenhouse gas emissions and mediation of security and economic concerns related to oil imports and natural recourse limitation (Dien et al., 2009). According to a report from the Renewable fuels Association (RFA), the top five ethanol producers in 2010 were the United States (13.5 billion gallons per year), Brazil (6.92 billion gallons per year), European Union (1,176 million gallons per year), China (541 million gallons per year), and Canada (356 million gallons per year) (RFA, 2011).

Biomass-derived ethanol can be produced from sugar-based feedstocks such as sugar cane and sugar beets; starch-based feedstocks such as corn, sorghum, wheat, and potatoes; and lignocellulosic feedstocks such as crop residues, wood, and grasses. The United States currently consumes more than 140 billion gallons of transportation fuels annually. At present, ethanol is produced primarily from corn (~96%). Using 100% of the 2009 corn crop for ethanol production would produce only about 36 billion gallons of ethanol, which would only meet about 17% of the nation's needs. Furthermore, a dramatic increase in ethanol production, using current grain-starch-based technology, may be limited by the fact that grain production for ethanol will compete for limited agricultural land needed for food and feed production. As lignocellulosic biomass is abundant and relatively inexpensive, it appears to be a more sustainable feedstock for fuel ethanol production and offers major economic, environmental, and strategic benefits. The bioconversion of lignocellulosic biomass typically results in ethanol yield of about 110 to 270 l/t dry matter of agricultural residues and 125 to 300 l/t dry matter from forest residues (Sim et al., 2010). However, considerable technical improvements are still needed at current stage to make the lignocellulosic biomass-based bioethanol processes become efficient and economically feasible.

Lignocellulosic feedstocks for bioethanol production are often harvested during a limited harvest season and stored as bales with low bulk density. The low bulk density of biomass makes



it difficult to handle, transport, store, and use in its natural forms. It is a challenge to handle and transport these low bulk density biomass feedstocks to biofuel plants at a reasonable cost. Therefore, the biomass handling and transportation are one of key issues for the biomass supply system. One of the solutions to solve this issue is to densify the biomass feedstocks into pellets. If biomass is pelleted, it can be handled and transported with existing grain-handling equipment in the field, on the road, and at biorefinery plants. Any improvement in the density of biomass pellets will improve handling efficiency and reduce transportation and handling costs (Hess et al. 2007).

The pelleting process may result in physical and chemical property changes as well as chemical composition change, depending on processing conditions such as pressure, steam temperature, feeding rate, and type of binders. Although there are some published results related to biomass pelleting, they are mostly focused on economic analysis (Sokhansanj and Turhollow, 2004; Mani et al., 2005; Mani et al., 2006). Published literature focusing on the effect of the pelleting process on downstream processing such as pretreatment and fermentation is very limited. It is important to study the effects of pelleting factors such as steam temperature, pelleting pressure, type of binders, die size, die speed, and particle size of biomass on physical properties of pellets such as density, shape and size, microstructure and chemical composition, and fermentable sugars yield as well as final ethanol yield.

## **RESEARCH OBJECTIVES**

The overall mission of this research was to conduct a feasibility study of using pelleted biomass for cellulosic ethanol production. Specific objectives of this research are as follows:

- 1) To evaluate and characterize sorghum biomasses including grain sorghum biomass, forage sorghum, sweet sorghum bagasse, photoperiod-sensitive sorghum, and brown midrib (BMR) sorghum as feedstocks for sugar production.
- 2) To study the physical properties of pellets made from corn stover, sorghum stalk, wheat straw, and big bluestem.
- 3) To determine the effects of pelleting process on chemical composition and fermentable sugar yield of biomass pellets.

- 4) To investigate the impact of pelleting and dilute-acid pretreatment on biomass structure and thermal properties of wheat straw, corn stover, big bluestem, and photoperiod-sensitive sorghum stalk.

## **LITERATURE REVIEWS**

### **Lignocellulosic Biomass**

Lignocellulosic biomass includes forest residues such as wood; agricultural residues such as sugarcane, bagasse, corn stover, sorghum biomass, wheat straw, and rice straw; industrial residues such as pulp and paper processing waste and municipal solid wastes; and energy crops such as switchgrass (Kumar et al., 2009b). Lignocellulose consists primarily of plant cell wall materials. The approximate compositions of lignocellulose include 40-50% cellulose, 20-30% hemicellulose, and 15-20% lignin (Zaldivar et al., 2001; Saha, 2003). Other components in biomass are organic extractives which can be extracted using polar or nonpolar solvents. Some examples of organic extractives are proteins, waxes, fats, resins, gums, chlorophyll, phenolics, starches, essential oils, and saponins (Sluiter et al., 2008a; Yu et al., 2008). Chemical compositions of biomass vary by type of biomass. For example, corn stover was reported to be composed of 38-40% cellulose, 22-25% hemicellulose, and 17% lignin on a 100% dry matter basis (Mosier et al., 2005; Saha, 2003). Wheat straw was composed of 35-40% cellulose, 30-35% hemicelluloses, and 14-15% lignin (Sun et al., 1998). Sorghum biomass was composed of 30-45 % cellulose, 22-26% hemicellulose, and 17-20% Lignin (Salvi et al., 2010; Corredor et al., 2009; Reddy and Yang, 2007). The structure of lignocellulose and its component is shown in Figure 1.1.

### ***Cellulose***

Cellulose is a linear polymer composed of  $\beta$ -D-glucopyranose units, linked by  $\beta$ -1,4-glycosidic bonds. The smallest repetitive unit of cellulose is cellobiose, a compound of two glucose molecules (Zhang and Lynd, 2004; Kumar et al., 2008b). The number of glucose units in a cellulose chain is known as the degree of polymerization (DP). The average DP for native cellulose is on the order of 10,000 (Brown, 2003). Cellulose is responsible for the strength in biomass fiber due to its high degree of polymerization and crystallinity (Zhang and Lynd, 2004). In lignocellulosic biomass, cellulose exists in crystalline and amorphous forms, both of which

hydrolyze to six carbon sugars. The crystalline cellulose is more difficult to be digested by enzymes than the amorphous cellulose. The crystalline cellulose exists in the form of microfibrils, which are assemblies of several (1,4)  $\beta$ -D-glucan chains linked together by numerous hydrogen bonds in a lattice-like manner (Laureano-Perez et al., 2005).

### ***Hemicellulose***

Hemicellulose is a branch polymer that consists of various sugar units arranged in different proportions with different substitutions. The main sugar units include pentoses (L-arabinose, D-xylose), hexoses (D-glucose, D-mannose, D-galactose), sugar acids (D-glucuronic acid, 4-O-methyl-D-glucuronic acid, D-galacturonic acid), and O-methylated neural sugars (Sun, 2008; Kumar et al., 2008). The dominant carbohydrate components of hemicellulose are xylan and glucomannan. Hemicelluloses in softwood contain mainly glucomannans, while in hardwood contain mainly xylans (Kumar et al., 2008). Hemicellulose forms covalent bonds with lignin, hydrogen bonds with cellulose, and ester linkages with acetyl units and hydroxycinnamic acids. Hemicellulose and cellulose are polysaccharides that can be hydrolyzed to sugars, and eventually fermented to ethanol.

### ***Lignin***

Lignin is a complex network of aromatic compounds called phenylpropanoids (Laureano-Perez et al., 2005). The hydrophobic polymer of p-hydroxyphenyl, guaicyl, and syringyl residues in lignin fills in the spaces between the cellulose fibers and hemicellulose. Lignin performs an important role in strengthening cell walls by cross-linking polysaccharides and providing support to structural elements in the overall plant body. In addition, it helps the plant resist moisture and biological attack. Lignin is highly resistant to chemical cleavage and is thought to be the major barrier protecting cellulose fibers from cellulase enzymes during hydrolysis (Dien et al., 2009). Lignin is indigestible by enzymes and cannot be converted to ethanol (Sun, 2008; Zhang et al., 2009). The un-utilized residues of lignin can be used for phenolic compounds extraction for pharmaceutical purposes or it can be burned to produce electricity and heat (Kumar et al., 2008; Szulczyk et al., 2010).

## **Biomass Feedstocks**

### ***Big Bluestem***

Big bluestem (*Andropogon gerardii* Vitman) is a warm-season (C4) perennial, native grass grown in the eastern and central US (Weimer and Springer, 2007). It is about 6 to 8 feet tall, very leafy at the base, with some leaves carried up on the stem. It can be distinguished from other warm-season grasses by blue coloration at the base of the culm and purplish, 3-parted flower clusters that resemble a turkey's foot. It is tufted, forms sod, and has short, scaly rhizomes. Rhizomes are typically 1 to 2 inches below the soil surface, while the main root can extend downward to 10 feet (USDA, 2011).

Big bluestem is considered as a potential biomass energy crop due to its low input requirement, reduced management needs, high yield potential, and adaptability to a wide geographic range (Cherney et al., 1991; Prophet et al., 2010). It is best adapted to moist, sandy or clay loams but also occurs in dry or shallow soils.

### ***Corn Stover***

Corn (*Zea mays* L.) is a primary crop product in the United State. Most corn is used for animal feed. Corn can also be processed into food products (starch, sweeteners, corn oil, etc.) and industrial products such as fuel ethanol. In the United State, corn starch serves as the major feedstock for fuel ethanol production.

Corn is grown in most U.S. States, but more concentrated in the Heartland region (Illinois, Iowa, Indiana, eastern portions of South Dakota and Nebraska, western Kentucky and Ohio, and the northern two-thirds of Missouri). Currently, about 86 to 88 million acres of land in the United State are planted to corn (USDA, 2011). The amount of corn stover, stalk and leave residues generated after corn harvest, corresponds roughly to the same amount of the harvested corn. Although the amount of corn stover that can be removed is highly depending on soil type, crop yield, tillage scenario, and other geo-climatic factors corn stover is the major field crop residues in the United States with more than 238 million tons of corn stover are produced annually (Liu et al., 2010).

### ***Sorghum Stalk***

Sorghum is mostly used in animal diets and human food. It is among the most widely adaptable cereal grasses potentially useful for biomass and biofuel production. Sorghum is a tropical grass grown primarily in semiarid and drier parts of the world. In dryland conditions, sorghum could produce 33% more dry mass than corn. Currently, about 5 to 7 millions acres of land in the United State are planted to sorghum; resulting in about 58 million tons of sorghum biomass (stem and leaves) that can be used as lignocellulosic feedstocks for fuel ethanol production (USDA, 2011).

### ***Wheat Straw***

Wheat (*Triticum* spp.) is an important food source. Wheat grows up rapidly on crude land and contains abundant nutrient. Wheat ranks third among U.S. field crops in both planted acreage and gross farm receipts, behind corn and soybeans. Currently, about 53 to 59 millions acres of land in the United State are planted to wheat (USDA, 2011). Wheat straw is the main residue from the wheat crop. The average yield of wheat straw is 1.3 to 1.4 kg per kg of wheat grain, and thus results in a considerable amount of surplus straw (Sun and Tomkinson, 2004).

### **Pelleting of Biomass**

Crop residues such as corn stover, sorghum biomass, and wheat straw, and energy grass such as switchgrass, which are potential feedstocks for bioethanol production, are usually harvested during a limited harvest season and stored as bales with low bulk densities (Colley et al., 2006; Kaliyan et al., 2009). For example, the bulk density of switchgrass ranges from 24 to 111 kg/m<sup>3</sup>, from 40 to 80 kg/m<sup>3</sup> for corn stover, and from 49 to 266 kg/m<sup>3</sup> for wheat straw, depending on particle size and moisture content (Lam et al., 2008; Mani et al., 2006). This low bulk density characteristic of biomass makes it difficult to handle, transport, store, and utilize in its natural form. To improve the storability and reduce transportation costs of biomass, one of the effective solutions is to densify the biomass into pellets and briquettes (Kaliyan et al., 2009). Pelletization of biomass can greatly increase its bulk density from an initial value of 40 to 200 kg/m<sup>3</sup> to a final value of 600 to 800 kg/m<sup>3</sup> (Kaliyan et al., 2009). As an example, the bulk density of ground switchgrass was reported at 165.5 kg/m<sup>3</sup> and the bulk density of switchgrass pellets at 5-20 % (wet basis) moisture content ranged from 536 to 708 kg/m<sup>3</sup> (Colley et al., 2006). In addition to the increase in bulk density for biomass pellets, uniform size and shape of the pellet

make it easier to handle using existing handling and storage equipment for grains, with reduced moisture content and increased the long-term storage capability (Kaliyan et al., 2009; Holm et al., 2006). Densification of biomass basically turns the biomass into a more dense and durable form. Durability is the most important descriptor of the physical quality of the pellet, and it is defined as the ability of pellets to withstand destructive loads and forces during transport (Tabil and Sokhansanj, 1996).

Pelletizing of biomass involves grinding, conditioning by applying heat and/or moisture, and forcing the ground sample through die (Colley et al., 2006; Kaliyan et al., 2009; Larsson et al., 2008). Grinding or size reduction of biomass feedstock is accomplished by processing the biomass in one or two steps using grinders, choppers, and hammer mills. For agricultural residues received in bale form, size reduction is accomplished with a combination of bale grinders and hammer mills. The hammer mills are normally equipped with a screen size of 3.2 to 6.4 mm (Mani et al., 2006). The ground biomass is extruded through a round or occasionally a square cross-sectional die (Sokhansanj and Turhollow, 2004). Diameter of the die usually varies from 4 to 12 mm or even larger (Colley et al., 2006). Pellets are generally in cylindrical form, 6 to 8 mm in diameter and 10 to 12 mm long. After compaction, the pellets usually come out from the die at relative high temperatures, ranging from 70°C to 90 °C, as a result of the frictional heat generated during extrusion and material pre-heating. The pellets are cooled in a cooler to within 5 °C of the ambient temperature, and to within 0.5% of the original moisture content of the feed ahead of the conditioner. If pellets are not properly cooled, their durability may decrease due to stresses between the (cooled) outer layer and the (still) warmer center, which induces cracks in the pellets. After cooling, the pellets are conveyed from the cooler to storage areas using mechanical or pneumatic conveying systems (Mani et al., 2006).

### **Bioconversion of Lignocellulosic Biomass to Bioethanol**

Bioconversion of lignocellulosic biomass to bioethanol is difficult due to the resistant nature of biomass to breakdown, the costs for collection and storage of low density lignocellulosic feedstocks, the high cost of enzymes involved in the conversion of cellulose into fermentable sugars, and the need to find or genetically engineer microorganisms to efficiently ferment variety of sugars obtained when the hemicellulose and cellulose polymers are broken (Balat and Balat, 2009, Arantes and Saddler, 2011). Production of bioethanol from

lignocellulosic biomass through a biological route involves three major steps: pretreatment, enzymatic hydrolysis, and fermentation (Christakopoulos et al., 1993). The flow chart of the bioconversion process is shown in Figure 1.2. Pretreatment is a critical step. The purpose of pretreatment is to break up the lignin seal, pre-hydrolyze the hemicellulose, and disrupt the crystalline structure of the cellulose, thus allowing better access of cellulases to cellulose during enzymatic hydrolysis (Chandra et al., 2009; Corredor et al., 2008; Kadar et al., 2007; Sun and Cheng, 2002). The purpose of enzymatic hydrolysis is to hydrolyze cellulose into fermentable sugars. The liberated sugars will then be fermented into ethanol by fermentative microorganisms such as yeasts and bacteria during the fermentation process.

The success of using lignocellulosic biomass for bioethanol production greatly depends on the chemical and physical properties of the biomass, pretreatment method, optimization of the process conditions, and efficiency of the hydrolyzing enzymes and fermentation microorganisms (Corredor et al., 2008). Current researches focus on lowering pretreatment energy requirements, improvement of cellulose and hemicellulose conversion to sugar, conversion of lignin to value added products, the combined xylose and glucose fermentation, and efficient separation process for ethanol. Great research efforts have been conducted on pretreatment of corn stover (Wyman et al., 2005; Lau and Dale, 2009), sugarcane bagasse (Dawson and Boopathy, 2007), switchgrass (Dien et al., 2006; Alizadeh et al., 2005), wheat straw (Rosgaard et al., 2007; Kristensen et al., 2008), and hardwood and softwood biomasses (Soderstrom, 2002; Sassner and Zacchi, 2008).

### ***Pretreatment Process***

Due to the complex and highly compact structure of lignocellulosic feedstocks, hydrolysis of polysaccharides in these materials is not an easy task to accomplish. Pretreatment, an additional step prior to hydrolysis, is necessary to open up the structure and make it accessible for enzymatic attack. Pretreatment can be achieved through mechanical (size reduction through milling and extrusion processing), physical (steam treatment), thermal-chemical (dilute-acid treatment, concentrated-acid treatment, alkaline treatment, hydrogen peroxide treatment, hot water treatment, steam explosion, ammonia fiber explosion, and organic solvent treatments), and biological methods (microbial and enzyme degradation), or a combination of these methods (Sun and Cheng, 2002; Zhan et al., 2006; Corredor et al., 2008; Zheng et al., 2008). Each pretreatment method offers distinct advantages and disadvantages. Ideal pretreatment methods are cost effective and have as little carbohydrate degradation or loss and formation of inhibitory

substances as possible (Sun and Cheng, 2002). Among various pretreatment methods, thermo-chemical methods such as dilute acid, uncatalyzed steam explosion, pH-controlled hot water, treatment with lime, and treatment with ammonia are the most cost-effective and promising pretreatment methods (Mosier et al., 2005).

Major drawback of the thermo-chemical pretreatment methods is the formation of hydrolysate rich in substances that inhibit to yeast and enzyme. These inhibitory compounds are the degradation by-products from cellulose, hemicellulose, and lignin. They are toxic to the hydrolytic enzymes and fermenting micro-organism and thus lead to the decrease of ethanol yield and productivity during fermentation of lignocellulosic hydrolysates. The nature and concentrations of the final inhibitory compounds vary with the amount of solids in the reactors, conditions of pretreatment (time, pH, temperature, concentration of chemicals, etc) and the raw material used. Inhibitory compounds can be classified into three major groups; weak acids, furaldehydes, and phenolics. The inhibitory compounds commonly found in the 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).

In the uncatalyzed steam-explosion method, high-pressure steam is applied to biomass for a few minutes without the addition of chemicals. This process is terminated by decompression of the steam. Either low temperature and long residence time (e.g. 175 °C and 30min) or higher temperature and shorter residence time (e.g. 270 °C and 1 min) could be applied for an optimal pretreatment (Taherzadeh and Niklasson, 2004). The advantage of this process is the increase in surface area without decrystallizing the cellulose, and cellulose downstream is significantly improved. The pH-controlled hot water treatment is conducted at 200-300 °C under high pressure. The treatment can increase the biomass surface area and remove hemicellulose. The addition of bases such as potassium hydroxide is used to control the pH of hot water and thus minimize formation of degradation products. Treatment with lime usually requires several weeks to complete. It involves mixing lime with water and spraying it onto the biomass. Advantages of this method are removal of lignin, increase in biomass surface area, and removal of acetyl and uronic-acid fractions of hemicellulose. The ammonia fiber-explosion (AFEX) method uses anhydrous ammonia to treat biomass. In a typical treatment, one kg of dry biomass is exposed to 1-2 kg liquid ammonia at high temperature (e.g. 50-90 °C) and high pressure for a period of time



(e.g. 30 min) before the pressure is rapidly reduced (Taherzadeh and Niklasson, 2004). Advantages of this method are the increase in surface area of biomass, decrease in crystallinity of cellulose, partial solubilization of hemicellulose, and removal of lignin (Mosier et al., 2005; Leustean, 2009).

Despite some limitations on using dilute-acid pretreatment, including formation of degradation products, release of potential biomass fermentation inhibitors, washing and neutralization of acid before sugars proceed to fermentation, and the need for corrosion-resistant reactors (Mosier et al., 2005; Leustean, 2009), pretreatment with dilute acid is still considered an effective and relatively inexpensive pretreatment method for several types of biomass, which not only solubilize hemicellulose but also convert solubilized hemicellulose into fermentable sugars (Zheng et al., 2007). The finding that hemicellulose remained in pretreated solids hindered the access of cellulases to cellulosic substrates and thus decreased overall sugar yields, making the dilute-acid pretreatment method even more attractive (Bura et al., 2009). This method also eliminates use of hemicellulose enzymes during hydrolysis (Zaldivar et al., 2001; Saha et al., 2005). Zheng et al. (2007) concluded from their literature reviews that dilute-acid pretreatment can release more than 80% of sugars associated with the hemicellulose fraction and yield 80% enzymatic conversion of cellulose for most types of biomass materials. Sulfuric acid is the most extensively studied because of its low price and high efficiency (Mohammad and Niklasson, 2004).

### ***Hydrolysis Process***

Hydrolysis of lignocellulosic biomass is more complicated than that of pure cellulose due to the presence of nonglucan components such as lignin and hemicellulose. The hydrolysis process is normally carried out using acid or enzymes. Acid hydrolysis can be done by either using the dilute-acid process or concentrated-acid process. The most commonly used acids are sulfuric acid and hydrochloric acid (Li et al., 2008). Other acids being used are nitric acid and phosphoric acid. The dilute-acid process is usually conducted under high temperatures from 120 to 200 °C, high pressures from 15 psi to 75 psi, and reaction time between 30 min to 2 h by continuous process. The concentrated-acid process typically involves use of 60 to 90% sulfuric acid, mild temperature, and moderate pressure (Kumar et al., 2009b). In comparison to dilute-acid hydrolysis, concentrated-acid hydrolysis leads to less sugar degradation and gives higher sugar yields, up to >90% (Yu et al., 2008).

In comparison to enzymatic hydrolysis, concentrated-acid hydrolysis is less costly, gives higher sugar yield, and does not require proper pretreatment of biomass as enzymatic hydrolysis does; but it causes environmental pollution, sugar degradation, and formation of toxic compounds (furans, furfural, hydroxymethylfurfural, formic acid, acetic acid, etc.), which inhibit the subsequent fermentation process (Zheng et al., 2007; Kumar et al., 2009b; Li et al., 2008).

Enzymatic hydrolysis is a promising alternative that provides hydrolysates with a lower inhibitory impact on the fermentation step. Cellulases, enzymes involved in cellulose transformation, are divided into three major groups:  $\beta$ -1,4-endoglucanases (EG), cellobiohydrolases (CBH) or exo-glucanases, and  $\beta$ -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 (Xu et al., 2009; Wilson, 2009; Kumar et al., 2008). The three types of reaction catalyzed by cellulase are shown in Figure 1.3. Two major products from enzymatic hydrolysis of cellulose are glucose and cellobiose. The reaction mechanisms of cellulose hydrolysis include adsorption of enzymes onto the surface of cellulose and breakage of  $\beta$ -1,4 glucosidic bonds between glucose (Yeh et al., 2010). In general, biomass susceptibility to enzymatic hydrolysis is affected by enzyme accessibility and cellulose crystallinity, in which both factors vary by the lignocellulosic material used and the pretreatment method employed (McMillan, 1994).

In the United States, the Department of Energy (DOE) has been supporting industrial research to reduce the production cost of cellulases by offering grants to enzyme companies such as Genencor and Novozyme (Wilson, 2009). Currently, various commercial cellulases are available such as Celluclast 1.5L, Novozyme 188, Accellerase 1500<sup>TM</sup>, and Dyadic-Alternafuel200P.

### ***Fermentation Process***

Fermentation of lignocellulosic hydrolyzates is more difficult than the fermentation of sugar-based and starch-based feedstocks as it is mixed-sugars fermentation. The lignocellulosic hydrolyzates contain mainly six-carbon (C6) sugars and some of five carbon (C5) sugars. In addition, they contain a broader range of inhibitory compounds, where the composition and concentration of these compounds depend on the type of lignocellulosic materials, the chemical

used and nature of pretreatment, and the hydrolysis process (Zhang et al., 2009; Kadar et al., 2007; Taherzadeh and Niklasson, 2004). Different sugars in the hydrolyzates require different organisms for fermentation. The C6 sugars can be fermented to ethanol by many naturally occurring organisms with high yields, but the C5 sugars are more difficult to ferment to ethanol and usually at a relatively low yield. The C6 sugars include glucose, mannose, and galactose, which originate from glucan, mannan, and galactan, respectively. The C5 sugars are xylose and arabinose, which originate from xylan and arabinan, respectively (Szulczyk et al., 2010).

Ethanol fermentation can be carried out by different approaches such as separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) (Qi et al., 2010; Wilson, 2009). In SHF, the cellulose and hemicellulose are enzymatically hydrolyzed into fermentable sugars in one step, and then the sugars are fermented into ethanol in a second step. In SSF, the sugars are converted to ethanol by microorganisms as soon as they are produced from enzymatic hydrolysis. Both enzymatic hydrolysis and conversion of sugars to ethanol are taken place in the same vessel. The SSF approach is claimed to be the more efficient and cost-effective process compared to other approaches. Benefits of SSF approach include the reduced product inhibition on enzymatic hydrolysis, the reduced required enzyme loading, and the improved ethanol yield (Qi et al., 2010). However, the optimum temperatures for enzymatic hydrolysis and fermentation are quite different which affect enzyme activities and finally affect fermentation efficiency (Wilson et al., 2009). The optimum temperature for enzymatic hydrolysis of cellulose is around 50 °C, while the optimum temperature for yeast fermentation is typically around 33 °C (Larsen et al., 2008).

### **Biomass Structural Analysis**

Microstructure and properties of lignocellulosic biomass were found to influence the bioconversion rate and final ethanol yield. Surface areas accessible for cellulase binding, crystallinity, and morphology are main factors that affect pretreatment and hydrolysis.

#### ***Fourier Transform Spectroscopy***

Fourier transform infrared (FTIR) spectra are frequently used for investigating the structure of constituents and chemical changes in lignocellulosic biomass (Guo et al., 2008). This method has advantages over conventional chemical methods that are time consuming and can result in degradation of natural polymers (Pandey, 1999). In the FTIR spectra, cellulose-related

bands are usually seen around 900, 1098, 1162, 1370, and 1430  $\text{cm}^{-1}$ . Protein-related bands are seen at about 1549 and 1653  $\text{cm}^{-1}$ . The hemicellulose-related band is seen at 1732-1735  $\text{cm}^{-1}$  (Liu et al., 2005; Gastaldi et al., 1998). There are two types of lignin in the woods and grass species—guacyl and syringyl rings. The guacyl ring-related band and syringyl ring-related band are seen at about 1516-1517  $\text{cm}^{-1}$  and 1453-1456  $\text{cm}^{-1}$ , respectively (Corredor et al., 2009).

### ***X-ray Diffraction***

X-ray diffraction (XRD) is a tool to detect the presence of crystallinity in a biomass sample in terms of absorption peaks. XRD analysis has revealed that cellulose exists in various crystalline forms. The crystalline form is highly resistant to microbial and enzymatic degradation, while amorphous cellulose is hydrolysed much faster. During the pretreatment process, the cellulose crystalline structure is altered as a result from disruption of inter/intra hydrogen bonding of the cellulose chain (Kumar et al., 2009a). XRD can be also used to measure the crystallinity index—a parameter to estimate impacts of pretreatment and hydrolysis on the chemical structure of biomass. Zhang and Lynd (2004) mentioned in their review that pretreatment of biomass by either dilute acid or steam explosion was found to increase the CrI of lignocellulosic biomass. Unlike other pretreatment methods, ammonium fiber explosion (AFEX) pretreatment resulted in a decrease in CrI. The CrI value of lignocellulosic biomass was observed to decrease after hydrolysis as a result of decrystallization of the cellulose during hydrolysis (Corredor et al., 2009).

### ***Scanning Electron Microscope***

A scanning electron microscope (SEM) is a tool used to capture images of the surface of untreated and treated biomass. Found in corn stover, sorghum leaves and stems, and wheat straw, deposits observed on the surface of these untreated materials could be waxes, lignin, hemicellulose, and other binding materials. This deposit layer is mostly removed during pretreatment and thus results in a relatively smooth and clean surface of fiber bundles (Corredor et al., 2009). Based on the SEM results of wheat straw, cellulose still remained as the skeleton structure, despite chemical extraction of hemicellulose and lignin (Liu et al., 2005).

## **Physical Properties of Biomass Pellets**

For the design of storage, handling, and processing systems for conversion of biomass pellets to bioethanol, physical properties of the pellets must be known (Colley et al., 2006; Zhao et al., 2008). Physical properties of pellets include size, particle density (true density), bulk density, porosity, hardness, durability, moisture sorption rate, and equilibrium moisture content (Colley et al., 2006; Lam et al., 2008).

Moisture content of biomass feedstock is a key factor to determine the density and strength of the densified biomass. Several studies have reported that durability and strength of densified biomass increase with increasing moisture content until an optimum is reached (Kaliyan and Morey, 2009). Optimum moisture content of the feedstock is required to produce stable and durable pellets. For biomass to be pelletized, the optimum moisture content is usually in the range of 8-12%. Colley et al. (2006) studied the effects of moisture content on the physical characteristics of switchgrass pellets and showed that bulk density, particle density, durability, and hardness of switchgrass pellets were significantly affected by moisture content. The bulk density of switchgrass pellets decreased as the moisture content of the pellets increased. Maximum values of bulk density and particle densities of switchgrass pellets were 708 and 1462 kg/m<sup>3</sup>, respectively. The highest durability of the pellets (95.91%) was observed for pellets with 8.6% (wet basis) moisture content. Durability value is considered high when the computed value is above 80%, medium when between 70% and 80%, and low when below 70% (Colley et al., 2006). Low durability of pellets is not a desirable characteristic, as it can cause problems such as disturbance within pellet feeding systems, dust emissions, and an increased risk of fire explosion during pellet handling and storage. Particle size and particle-size distribution can also affect the physical properties of pellets. The study by Zhao et al. (2008) showed that bulk density of corn pellets decreased as particle size of the pellets increased. The same trend of particle size-bulk density relationship was also observed for wheat straw and switchgrass samples studied by Lam et al. (2008). Kaliyan et al. (2009) also showed that bulk density as well as durability of switchgrass briquettes, with and without steam conditions, decreased as particle size increased from 0.49 mm to 0.59 mm. Coarsely ground materials tend to give less durable pellets, possibly due to the natural fissure developed in the pellets that are susceptible to breakage (Mani et al., 2003; Kaliyan and Morey, 2009). Recommended particle size for good pellet quality is 0.5-0.7

mm. However, fine grinding is undesirable as it will increase the production cost (Kaliyan and Morey, 2009).

Biomass densification process variables such as temperature, applied pressure, hold time, size of the die, and die geometry (length-to-diameter or L/D ratio) were found to influence bulk density and durability of densified biomass (Larsson et al., 2008; Mani et al., 2006; Mani et al., 2003). Faborode (1990) conducted an analysis of extrusion compaction of fibrous agricultural residues for fuel applications and found that density and durability of pelleted biomass increased as the temperature of the extrusion press increased. Tabil and Sokhansanj (1996) observed an increase in durability of alfalfa pellets as a result of the increase in temperature of the pellets extruded out from the pellet mill. Grover and Mishra (1996) studied the effect of temperature and pressure conditions on the bulk density of briquettes of mustard stalk, sawdust, rice husk, and groundnut shell in a hydraulic press and found that as the temperature of hydraulic press increased, a better compaction and an increase in bulk density of the briquette biomass were observed.

Hill and Pulkinen (1988) found that bulk density and pellet durability decreased as the extrusion diameter increased from 0.25 inch to 0.5 inch, using a die with L/D of 8. When the experiment was repeated using a die with L/D of 10, bulk density and pellet durability improved even further. Kaliyan and Morey (2009) reported that the factors that increase pellet durability would also increase pellet density although the relationship between durability and density of biomass pellets was still unknown. The die with higher L/D ratios and smaller die size were found to produce more durable pellets. However, excessively long die or very small die diameter (high L/D value) would block the pellet mills (Hill and Pulkinen). Tabil and Sokhansanj (1996) found that the smaller die (6.1-mm diameter) plugged when moisture content was more than 10 % (w.b.) but larger die (7.8-mm diameter) could handle this moisture content. For the steam conditioning step, the bigger die is more preferable as it offers less resistance to the flow of grind particle through it and therefore is able to tolerate higher moisture than the smaller die (Tabil and Sokhansanj, 1996). Pellet durability was also influenced by the screen size of the hammer mill. Tabil and Sokhansanj (1996) observed the trend of an increase in alfalfa pellet durability when using a larger hammer mill screen. However, the difference in alfalfa pellet durability values when using different hammer mill screen size (3.2 mm and 6.5 mm) was not significant.

## LITERATURE CITED

- Alizadeh, H., Teymouri, F., Gilbert, T., and Dale, B. 2005. Pretreatment of switchgrass by ammonia fiber explosion (AFEX). *Appl. Biochem. Biotechnol.* 124 (1-3): 1133-1141.
- Arantes, V., and Saddler, J.N. 2011. Cellulose accessibility limits the effectiveness of minimum cellulose loading on the efficient hydrolysis of pretreated lignocellulosic substrates. *Biotechnology for Biofuels.* 4(3): 1-16.
- Balat, M., and Balat, H. 2009. Recent trends in global production and utilization of bio-ethanol fuel. *Applied Energy.* 86: 2273-2282.
- Brown, R.B. 2003. *Biorenewable resources: engineering new products from agriculture.* Iowa State Press, Ames.
- Bura, R., Chandra, R., and Saddler, J. 2009. Influence of xylan on the enzymatic hydrolysis of steam-pretreated corn stover and hybrid poplar. *Biotechnol. Prog.* 25(2): 315-322.
- Colley, Z., Fasina, O.O., Bransby, D., and Lee, Y.Y. 2006. Moisture effect on the physical characteristics of switchgrass pellets. *T ASAE.* 49(6): 1845-1851.
- Corredor, D.Y., Salazar, J.M., Hohn, K.L., Bean, S., Bean, B., and Wang, D. 2009. Evaluation and characterization of forage sorghum as feedstock for fermentable sugar production. *Appl. Biochem. Biotechnol.* 158: 164-179.
- Corredor, D.Y., Sun, X. S., Salazar, J. M., Hohn, K. L., and Wang, D. 2008. Enzymatic hydrolysis of soybean hulls using dilute-acid and modified steam-explosion pretreatments. *J. Biobased Mat. Bioenergy* 2(1): 1-8.
- Chandra, R. P., Ewanick, S.M., Chung, P.A., Au-Yeung, K., Rio, L.D., Mabee, W., and Saddler, J.N. 2009. Comparison of methods to assess the enzyme accessibility and hydrolysis of pretreated lignocellulosic substrates. *Biotechnol Lett.* 31(8): 1217-1222.
- Cherney, J.H., Johnson, K.D., Volenec, J.J., and Greene, D.K. 1991. Biomass potential of selected grass and legume crops. *Energy Source.* 13:283-292.
- Christakopoulos, P., Li, L.W., Kekos, D., and Macris, B.J. 1993. Direct conversion of sorghum carbohydrates to ethanol by a mixed microbial culture. *Bioresource Technol.* 45: 89-92.

- Dawson, L., and Boopathy, R. Q. 2007. Use of post-harvest sugarcane residue for ethanol production. *Bioresource Technol.* 98(9): 1695-1699.
- Dien, B. S., Jung, H.-J. G., Vogel, K. P., Casler, M. D., Lamb, J. F. S., Iten, L. B., Mitchell, R. B., and Sarath, G. 2006. Chemical composition and response to dilute-acid pretreatment and enzymatic saccharification of alfalfa, reed canarygrass, and switchgrass. *Biomass Bioenergy* 30(10): 880-891.
- Dien, B. S., Sarah, G., Pedersen, J.F., Sattler, S.E., Chen, H., Funnell-Harris, D.L., Nichols, N.N., and Cotta, M.A. 2009. Improved sugar conversion and ethanol yield for forage sorghum (*Sorghum bicolor* L. Moench) lines with reduced lignin contents. *Bioenergy.Res.* 2(3): 153-164.
- Faborode, M.O. 1990. Analysis of extrusion compaction of fibrous agricultural residues for fuel applications. *Biomass.* 21: 115-128.
- Gastaldi, G., Capretti, G., Focher, B., and Cosentino, C. 1998. Characterization and properties of cellulose isolated from the *Crambe abyssinica* hull. *Ind. Crop Prod.* 8(3): 205-218.
- Grover, P.D., and Mishra, S.K. 1996. Biomass briquetting: Technology and practices. FAO regional wood energy development programme in Asia, Bangkok, Thailand.
- Guo, G.-L., Chen, W.-H., Men, L.-C. and Hwang, W.-S. 2008. Characterization of dilute-acid pretreatment of silvergrass for ethanol production. *Bioresource Tech.* 99(14): 6046-6053.
- Hess, J.R., Wright, C.T., and Kenney, K.L. 2007. Cellulosic biomass feedstocks and logistics for ethanol production. *Biofuels, Bioproducts and Biorefining.* 1: 181–190.
- Hill, B., and Pulkinen, D.A. 1988. A study of factors affecting pellet durability and pelleting efficiency in the production of dehydrated alfalfa pellets. A special report. Saskatchewan Dehydrators Association, Tisdale, SK, Canada: 25.
- Holm, J.K., Henriksen, U.B., Hustad, J.E., and Sorensen, L.H. 2006. Toward an understanding of controlling parameters in softwood and hardwood pellet production. *Energy Fuels.* 20: 2686-2694.
- Kadar, Z., Maltha, S. F., Szengyel, Z., Reczey, K., and Laat, W.D. 2007. Ethanol fermentation of various pretreated and hydrolyzed substrate at low initial pH. *Appl. Biochem. Biotech.* 136-140: 847-858.



- Kaliyan, N., Morey, R.V., White, M.D., and Doering, A. 2009. Roll-press briquetting and pelleting of corn stover and switchgrass. *Trans. ASABE*. 52(2): 543-555.
- Karmakar, M., and Ray, R.R. 2011. Current trends in research and application of microbial cellulases. *Res. J. Microbiol.* 6(1):41-53.
- Kristensen, J., Thygesen, L., Felby, C., Jorgensen, H., and Elder, T. 2008. Cell-wall structural changes in wheat straw pretreated for bioethanol production. *Biotechnol. Biofuels* 1(5):1-9.
- Kumar, R., Mago, G., Balan, V., and Wyman, C. E. 2009a. Physical and chemical characterization of corn stover and poplar solids resulting from leading pretreatment technologies. *Bioresource Tech.* 100(17): 3948-3962.
- Kumar, R., Singh, S.P., Mishra, I.M., and Adhikari, D.K. 2009b. Recent advances in production of bioethanol from lignocellulosic biomass. *Chem. Eng. Technol.* 32(4): 517-526.
- Kumar, R., Singh, S., and Singh, O.V. 2008. Bioconversion of lignocellulosic biomass: Biochemical and molecular perspectives. *J. Ind. Microbiol. Biotechnol.* 35: 377-391.
- Lam, P.S., Sokhansanj, S., Bi, X., Lim, C.J., Naimi, L.J., Hoque, M., Mani, S., Womac, A.R., Ye, X.P., and Narayan, S. 2008. Bulk density of wet and dry wheat straw and switchgrass particles. *Appl. Eng. Agric.* 24(3): 351-358.
- Larsen, J., Petersen, M.O., Thirup, L., Li, H.W., and Iversen, F.K. 2008. The IBUS Process – Lignocellulosic bioethanol close to a commercial reality. *Chem. Eng. Technol.* 31(5):765-772.
- Larsson S.H., Thyrel, M., Geladi, P., and Lestander, T.A. 2008. High-quality biofuel pellet production from pre-compacted, low-density raw materials. *Bioresource Tech.* 99(15): 7176-7182.
- Lau, M. W. and Dale, B. E. 2009. Cellulosic ethanol production from AFEX-treated corn stover using *Saccharomyces cerevisiae* 424A(LNH-ST). *Proc. Natl. Acad. Sci. USA* 106(5):1368-1373.
- Laureano-Perez, L., Teymouri, F. Alizadeh, H., and Dale, B.E. 2005. Understanding factors that limit enzymatic hydrolysis of biomass. *Appl. Biochem. Biotechnol.* 124(1-3): 1081-1099.
- Leustean, I. 2009. Bioethanol from lignocellulosic materials. *Journal of Agroalimentary Processes and Technologies.* 15(1): 94-101.

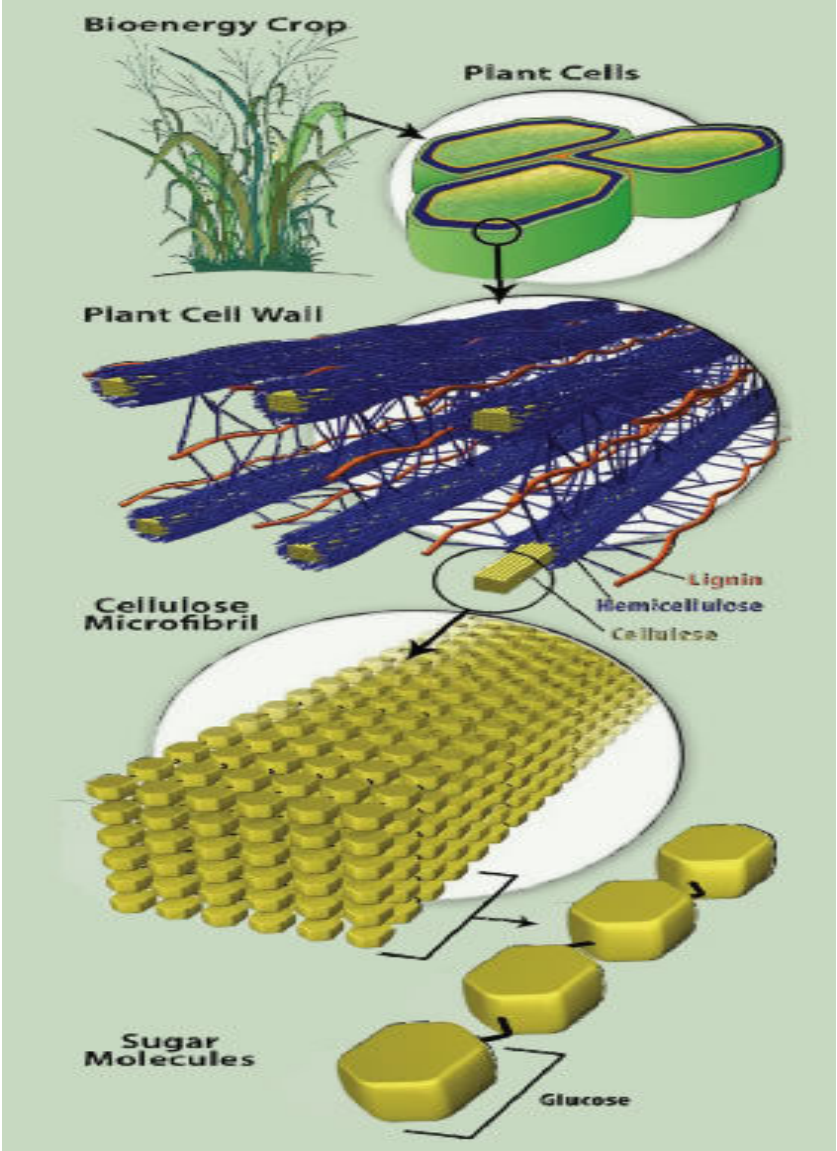
- Li, Wen-zhi, Xu, J., Wang, J., Yan, Yong-jie, Zhu, Xi-feng, Chen, Ming-qiang, and Tan, Zhi-cheng. 2008. Studies of monosaccharide production through lignocellulosic waste hydrolysis using double acids. *Energy Fuels*. 22: 2015-2021.
- Liu, L., Ye, X.P., Womac, A.R., and Sokhansanj, S. 2010. Variability of biomass chemical composition and rapid analysis using FT-NIR techniques. *Carbohydr. Polym.* 81:820-829.
- Liu, R., Yu, H., and Huang, Y. 2005. Structure and morphology of cellulose in wheat straw. *Cellulose*. 12(1): 25-34.
- Mani, S., Bi, X., and Sokhansanj, S. 2005. Environmental systems assessment of biomass densification process. CSAE Paper No.05081. Winnipeg, MB: CSAE/SCGR.
- Mani, S., Sokhansanj, S., Bi, X., and Turhollow, A. 2006. Economics of producing fuel pellets from biomass. *Appl. Eng. Agric.* 22(3): 421-426.
- Mani, S., Tabil, L.G., and Sokhansanj, S. 2003. An overview of compaction of biomass grinds. *Powder Handling and Processing*. 15(3): 160-168.
- McMillan, J. 1994. Pretreatment of lignocellulosic biomass. Pages 292-324 in: *Enzymatic conversion of biomass for fuels production*. M.E. Himmel, J.O. Baker, and R.P. Overend, Eds. American Chemical Society, Washington, DC.
- Mohammad, J. and Niklasson, C. 2004. Ethanol from lignocellulosic materials: pretreatment, acid and enzymatic hydrolyses, and fermentation. Page 49-68 in: *Lignocellulose biodegradation*. B.C. Saha, and K. Hayashi, Eds. American Chemical Society, Washington DC.
- Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y.Y., Holtzapple, M., and Ladisch, M. 2005. Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource Technol.* 96(6): 673-686.
- Pandey, K. K. 1999. A study of chemical structure of soft and hardwood and wood polymers by FTIR spectroscopy. *J. Appl. Polymer Sci.* 71(12): 1969-1975.

- Parawira, W., and Tekere, M. 2011. Biotechnological strategies to overcome inhibitors in lignocellulose hydrolysates for ethanol production: review. *Crit. Rev. Biotechnol. Sci.* 31(1): 20-31.
- Propheter, J.L., Staggenborg, S.A., Wu, X., and Wang, D. 2010. Performance of annual and perennial biofuel crops: yield during the first two years. *Agron. J.* 102(2):806-814.
- Qi, B., Chen, X., Wan, Y. 2010. Pretreatment of wheat straw by nonionic surfactant-assisted dilute acid for enhancing enzymatic hydrolysis and ethanol production. *Bioresour. Technol.* 101:4875-4883.
- Reddy, N., and Yang, Y. 2007. Structure and properties of natural cellulose fibers obtained from sorghum leaves and stems. *J. Agric. Food. Chem.* 55: 5569-5574.
- RFA. 2011. 2011 Ethanol Industry Outlook. Available at: [http://www.ethanolrfa.org/page/-/2011%20RFA%20Ethanol%20Industry%20Outlook .pdf?nocdn=1](http://www.ethanolrfa.org/page/-/2011%20RFA%20Ethanol%20Industry%20Outlook.pdf?nocdn=1). Accessed 23 March 2011.
- Rosgaard, L., Pedersen, S., and Meyer, A. 2007. Comparison of different pretreatment strategies for enzymatic hydrolysis of wheat and barley straw. *Appl. Biochem. Biotech.* 143(3): 284-296.
- Saha, B.C. 2003. Hemicellulose bioconversion. *J. Ind. Microbiol Biotechnol.* 30(5): 279-291.
- Salvi, D.A., Aita, G.M., Robert, D., and Bazan, V. 2010. Ethanol production from sorghum by a dilute-ammonia pretreatment. *J. Ind. Microbiol. Biotechnol.* 37: 27-34.
- Sassner, P., and Zacchi, G. 2008. Integration options for high-energy efficiency and improved economics in a wood-to-ethanol process. *Biotechnol. Biofuels* 1(4): 1-11.
- Sims, R.E.H., Mabee, W., Saddler J.N., and Taylor, M. 2010. An overview of second generation biofuel technologies. *Bioresour. Technol* 101: 1570-1580.
- Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., and Crocker, D. 2008a. Determination of structural carbohydrates and lignin in biomass. NREL/TP-510-42618. Golden, Colo.: National Renewable Energy Laboratory.

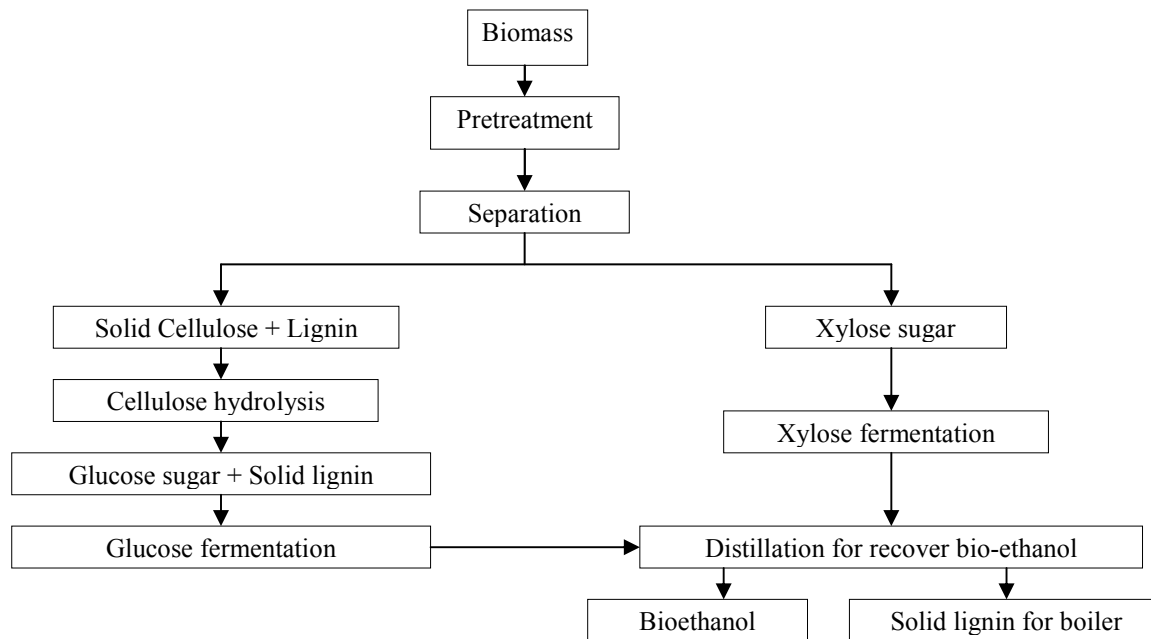
- Soderstrom, J. 2002. Stream pretreatment of softwood with SO<sub>2</sub> impregnation for ethanol production. *Appl. Biochem. Biotechnol.* 98: 5-21.
- Sokhansanj, S., and Turhollow, A.F. 2004. Biomass densification – cubing operations and costs for corn stover. *Appl. Eng. Agric.* 20(4): 495-499.
- Sun, Run-Cang. 2008. Detoxification of biomass for bioethanol. *Bioresources.* 4(2): 452-455.
- Sun, R., Fang, J. M., Rowland, P., and Bolton, J. 1998. Physicochemical and thermal characterization of wheat straw hemicellulose and cellulose. *J. Agric. Food Chem.* 46(7): 2804-2809.
- Sun, R., and Tomkinson, J. 2004. Separation and characterization of cellulose from wheat starw. *Separ Sci Technol.* 39(2):391-411.
- Sun, Y., and Cheng, J. 2002. Hydrolysis of lignocellulosic materials for ethanol production: A review. *Bioresource Technol.* 83(1): 1-11.
- Szulczyk, K.R., McCarl, B.A., and Cornforth, G. 2010. Market penetration of ethanol. *Renewable Sustainable Energy Rev.* 14:394-403.
- Tabil, L.G., and Sokhansanj, S. 1996. Process conditions affecting the physical quality of alfalfa pellets. *Appl. Eng. Agric.* 12(3): 345-350.
- Taherzadeh, M.J., and Niklasson, C. 2004. Ethanol from lignocellulosic materials: pretreatment, acid and enzymatic hydrolyses, and fermentation. *ACS Symp. Ser.* 889: 49-69.
- United State Department of Agriculture (USDA). 2011. Crop production: 2010 Summary. Available at: [http://usda.mannlib.cornell.edu/usda/current/CropProdSu/CropProdSu-01-12-2011\\_new\\_format.pdf](http://usda.mannlib.cornell.edu/usda/current/CropProdSu/CropProdSu-01-12-2011_new_format.pdf). Accessed March 2011.
- U.S. Department of Energy National Laboratory Operated by the University of California. The evolution road to biofuels. Available at: <http://www.lbl.gov/Publications/YOS/Feb/>. Accessed 24 March 2011.

- United State Department of Agriculture (USDA): Natural Resource Conservation Service. Plants Profile. Available at: <http://plants.usda.gov/java/profile?symbol= ANGE>. Accessed 21 April 2011.
- Weimer, P.J., and Springer, T.L. 2007. Fermentability of eastern gamagrass, big bluestem and sand bluestem grown across a wide variety of environments. *Bioresour. Technol.* 98: 1615-1621.
- Wilson, D.B. 2009. Celluases and biofuels. *Curr. Opin. Biotechnol.* 20:298-299.
- Wyman, C. E., Dale, B. E., Elander, R. T., Holtzapple, M., Ladisch, M. R., and Lee, Y.Y. 2005. Comparative sugar recovery data from laboratory-scale application of leading pretreatment technologies to corn stover. *Bioresource Technol.* 96(18): 2026-2032.
- Xu, F., Ding, H., and Tejirian, A. 2009. Detrimental effect of cellulose oxidation on cellulose hydrolysis by cellulase. *Enzyme Microb. Technol.* 45(3): 203-209.
- Yeh, An-I, Huang, Yi-Ching, and Chen, S.H. 2010. Effect of particle size on the rate of enzymatic hydrolysis of cellulose. *Carbohydr. Polym.* 79(1): 192-199.
- Yu, Y., Lou, X., and Wu, H. 2008. Some recent advances in hydrolysis of biomass in hot-compressed water and its comparisons with other hydrolysis methods. *Energy Fuels.* 22: 46-60.
- Zaldivar, J., Nielsen, J., and Olsson, L. 2001. Fuel ethanol production from lignocellulosic: A challenge for metabolic engineering and process integration. *Appl. Microbiol. Biotech.* 56(1-2): 17-34.
- Zhan, X., Wang, D., Bean, S. R., Mo, X., Sun, X. S., and Boyle, D. 2006. Ethanol production from supercritical-fluid-extrusion cooked sorghum. *Ind. Crops Prod.* 23(3): 304-310.
- Zhang, S., Marechal, F., Gassner, M., Perin-Levasseur, Z., Qi, W., Ren, Z., Yan, Y., and Favrat, D. 2009. Process modeling and integration of fuel ethanol production from lignocellulosic biomass based on double-acid hydrolysis. *Energy Fuels.* 23(3): 1759-1765.

- Zhang, Yi-Heng. P., and Lynd, L.R. 2004. Toward an aggregated understanding of enzymatic hydrolysis of cellulose: Noncomplexed cellulase systems. *Biotechnol. Bioeng.* 88(7): 797-824.
- Zhao B., Ileleji, K.E., and Ejeta, G. 2008. Physical property relationships of bulk corn stover particles. *Trans. ASABE.* 51(2): 581-590.
- Zheng, Y, Pan, Z, Zhang, R., Wang, D., and Jenkins, B. 2008. Non-ionic surfactants and non-catalytic protein treatment on enzymatic hydrolysis of pretreated creeping wild ryegrass. *Appl Biochem Biotechnol.* 146(1-3): 231-248.
- Zheng, Y., Zhongli, P., Zhang, R., Labavitch, J.M., Wang, D., Teter, S., and Jenkins, B.M. 2007. Evaluation of different biomass materials as feedstock for fermentable sugar production. *Appl. Biochem. Biotech.* 136-140(1-12): 423-436.

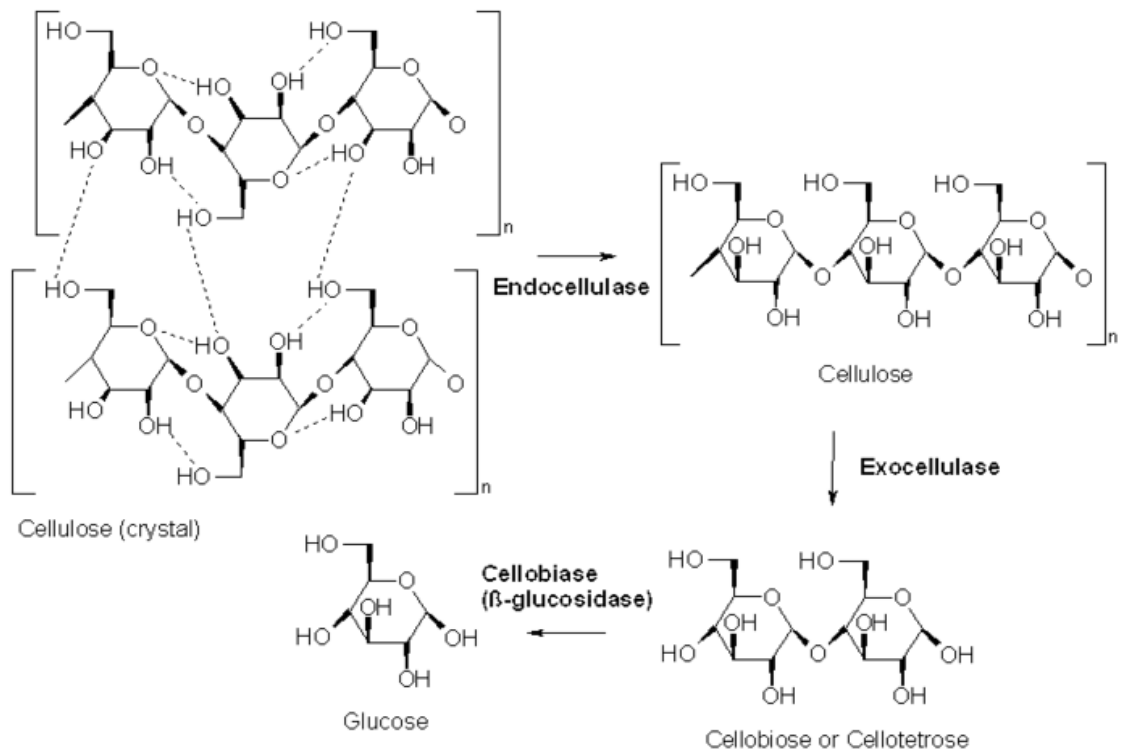


**Figure 1.1.1 Lignocellulose and Its Components (U.S. Department of Energy National Laboratory Operated by the University of California)**



**Figure 1.2** Flow chart for the production of bioethanol from lignocellulosic biomass  
(Adapted from Balat and Balat, 2009)





**Figure 1.3 Three types of reaction catalyzed by cellulase (Karmakar and Ray, 2011)**

# CHAPTER 2 - EVALUATION AND CHARACTERIZATION OF SORGHUM BIOMASS AS FEEDSTOCK FOR SUGAR PRODUCTION <sup>1</sup>

## ABSTRACT

Conversion of cellulosic biomass, such as agricultural residues, to biofuels offers significant economic, environmental, and strategic benefits. Sorghum is an important energy crops in the U.S. It is a renewable resource and is currently grown on about 10 million acres in the U.S. However, at present, there is a lack of scientific information and knowledge about the use of sorghum biomass for biofuel production. The objective of this research was to evaluate and characterize sorghum biomass as a feedstock for sugar production. Five types of sorghum biomass (brown midrib sorghum, forage sorghum, grain sorghum, photoperiod-sensitive sorghum, and sweet sorghum) were characterized and evaluated for sugar production. Pretreatment with dilute acid was used to increase yield of fermentable sugars. Effects of sulfuric acid concentration, treatment temperature, and residence time on yield of fermentable sugars were studied. Accellerase 1000 was used to hydrolyze cellulose into glucose at 50°C and pH 4.8 for 96 h. A high percentage of enzymatic conversion of cellulose (ECC) was observed for sorghum biomass that was pretreated under severe pretreatment temperature (85% to 98% ECC for biomass pretreated at 165°C for 10 min; 65% to 82% ECC for biomass pretreated at 140°C for 30 min). However, mass recovery and cellulose recovery of the solid fraction after pretreatment decreased under severe pretreatment conditions (70% to 85% cellulose recovery for sorghum biomass pretreated at 140°C for 30 min; 31% to 58% cellulose recovery for sorghum biomass pretreated at 165°C for 10 min).

**Keywords:** cellulose conversion, Enzymatic hydrolysis, <sup>1</sup> Pretreatment, Sorghum biomass.

---

<sup>1</sup> Results have been published. K. Theerarattananoon, X. Wu, S. Staggenborb, J. Propheter, R. Madl, and D. Wang. 2010. Evaluation and characterization of sorghum biomass as feedstock for sugar production. *T ASAE*. 53(2): 509-525

## INTRODUCTION

As the world population and economy expand, energy demand will increase (USCB, 2008; EIA, 2008). Energy consumption in the U.S. exceeds 100 quadrillion Btu per year, and 85% of this consumption is from fossil fuels (EIA, 2008). Fossil fuel production from the current major energy sources (coal, crude oil, and natural gas) will soon peak (Kharecha and Hansen, 2008), and the solution is to either develop new types of energy sources or produce substitute fuels using available alternative feedstocks. Conversion of lignocellulosic biomass into biofuels is a feasible option for substantial replacement of fossil fuels (Perlack et al., 2005). Lignocellulosic biofuels offer one of the best near-to-midterm alternatives for meeting our nation's transportation energy needs.

Production of bioethanol from lignocellulosic biomass through a biological route involves three major steps: pretreatment, enzymatic hydrolysis, and fermentation (Christakopoulos et al., 1993). Pretreatment is a critical step. The purpose of pretreatment is to break up the lignin seal, pre-hydrolyze the hemicellulose, and disrupt the crystalline structure of the cellulose, thus allowing cellulases better access to cellulose during enzymatic hydrolysis (Corredor et al., 2008; Kadar et al., 2007; Sun and Cheng, 2002). Pretreatment can be achieved through mechanical (size reduction through milling and extrusion processing), physical (steam treatment), thermal-chemical (dilute acid treatment, concentrated acid treatment, alkaline treatment, hydrogen peroxide treatment, hot water treatment, steam explosion, ammonia fiber explosion, and organic solvent treatments), and biological methods (microbial and enzyme degradation) or a combination of these methods (Sun and Cheng, 2002; Zhan et al., 2006; Corredor et al., 2008; Zheng et al., 2008). Ideal pretreatment methods are cost-effective and have as little carbohydrate degradation or loss and formation of inhibitory substances as possible (Sun and Cheng, 2002).

Despite of some limitations on using dilute acid pretreatment, including formation of degradation products, release of potential biomass fermentation inhibitors, washing and neutralization of acid before sugars proceed to fermentation, and the need for corrosion-resistant reactors (Mosier et al., 2005), pretreatment with dilute acid is still considered an effective and relatively inexpensive pretreatment method for several types of biomass, which not only solubilize hemicellulose but also convert solubilized hemicellulose into fermentable sugars. This

method also eliminates the use of hemicellulose enzymes during hydrolysis (Zaldivar et al., 2001; Saha et al., 2005).

The success of using lignocellulosic biomass for bioethanol production greatly depends on the chemical and physical properties of the biomass, pretreatment method, optimization of the process conditions, and efficiency of the hydrolyzing enzymes and fermentation microorganisms (Corredor et al., 2008). Great research efforts have been conducted on pretreatment of corn stover (Wyman et al., 2005; Lau and Dale, 2009), sugarcane bagasse (Dawson and Boopathy, 2007), switchgrass (Dien et al., 2006; Alizadeh et al., 2005), wheat straw (Rosgaard et al., 2007; Kristensen et al., 2008), and hardwood and softwood biomasses (Soderstrom et al., 2002; Sassner and Zacchi, 2008).

In the Midwest region of the U.S., sorghum is considered one of the promising lignocellulosic feedstocks for biofuel production because it is abundant in this region and its production ranks third among cereal crops (Linde et al., 2006; Zhan et al., 2006). Currently, not much work has been conducted on bioconversion of sorghum biomass for biofuels as compared to other types of biomass. Corredor et al. (2009) showed that up to 72% hexose yield and 94% pentose yield from forage sorghum stalk were obtained using modified steam explosion with 2% sulfuric acid at 140°C for 30 min and enzymatic hydrolysis with cellulase (15 FPU g<sup>-1</sup> cellulose) and β-glucosidase (50 CBU g<sup>-1</sup> cellulose). Salvi et al. (2010) conducted a study on ethanol production from sorghum fibers by a dilute ammonia pretreatment method and found that theoretical cellulose yield and hemicellulose yield for sorghum fibers pretreated by dilute ammonia and hydrolyzed by enzyme combination of Spezyme Cp (60 FPU g<sup>-1</sup> glucan) and Novozyme 188 (64 FPU g<sup>-1</sup> glucan) were 84% and 73%, respectively. The ethanol yield was 25 g per 100 g dry biomass.

The objective of this research was to evaluate and characterize sorghum biomasses from grain sorghum, forage sorghum, sweet sorghum, photoperiod-sensitive sorghum, and brown midrib (BMR) sorghum as feedstocks for sugar production.

## **MATERIALS AND METHODS**

### **Materials**

Sorghum biomass, including forage sorghum, photoperiod-sensitive sorghum, BMR sorghum, sweet sorghum, and grain sorghum, was harvested from Riley County, Kansas, and air

dried in an oven at 70°C to reduce the moisture content for long-term storage. The sorghum biomass was ground into powder with a Retsch cutting mill (Haan, Germany) with a 1.0 mm sieve. Sorghum biomass samples were stored at room temperature for future use. Corn stover grown in the same location was used as a control. All chemicals used for this research were purchased from Sigma Chemical Co. (St. Louis, Mo.). Accellerase 1000 (Danisco US, Inc., Genencor Division, Rochester, N.Y.) enzyme complex was used for hydrolyzing sorghum biomass into sugars. This enzyme complex contains multiple enzyme activities, mainly exoglucanase, endoglucanase (2500 CMC U g<sup>-1</sup> minimum), hemi-cellulase, and β-glucosidase (400 pNPG U g<sup>-1</sup> minimum). Exoglucanase activity is reported in carboxymethylcellulose (CMC U) activity units (one CMC U unit of activity liberates 1 μmol of reducing sugars in 1 min under specific assay conditions of 50°C and pH 4.8), and β-glucosidase is reported in pNPG units (one pNPG unit denotes 1 μmol of nitrophenol liberated from para-nitrophenyl-B-D-glucopyranoside in 10 min at 50°C and pH 4.8).

### **Dilute Acid Pretreatment**

Pretreatment was carried out in a pressure reactor (Parr Instrument Co., Moline, Ill.) with a 1 L reaction vessel. The ground sorghum biomass and corn stover were mixed with diluted sulfuric acid (2% w/v) to obtain 10% solid content (approximately 53 g in 500 mL diluted sulfuric acid solution). Effects of temperature and reaction time on sugar yield were studied (140°C for 30 min and 165°C for 10 min). Pretreated biomass was washed with hot distilled water and centrifuged four times to remove dissolved sugars and sulfuric acid. The supernatant was collected into a 2 L volumetric flask. A portion of the supernatant was neutralized with CaCO<sub>3</sub> and further analyzed for glucose and pentose content by using a high-performance liquid chromatograph (HPLC) with a Rezex RCM column (Phenomenex, Cal.). As hemicellulose is a polymer of hexose and pentose, glucose in the supernatant was considered to be from hydrolysis of both cellulose and hemicellulose, and pentose was counted as sugars released from hydrolysis of hemicellulose. Washed biomass samples were split into two portions. One portion was used for moisture content and chemical composition analyses; the other portion was used for subsequent enzymatic hydrolysis.

## Enzymatic Hydrolysis

Pretreated biomass samples were enzymatically hydrolyzed in solution with sodium acetate buffer (50 mM, pH 4.8) and 0.02% (w/v) sodium azide to prevent microbial growth during hydrolysis. The dry mass content of the hydrolysis slurries was 5% (w/v). Enzymatic hydrolysis was carried out in 125 mL flasks with 50 mL of slurry in a 50°C water bath shaker agitating at 140 rpm for 96 h. The enzyme loading (Accellerase 1000, Danisco US, Inc., Genencor Division, Rochester, N.Y.) was 1 mL g<sup>-1</sup> of cellulose. During enzymatic hydrolysis, the hydrolysis slurries were sampled periodically up to 96 h after the addition of enzyme by withdrawing 0.1 mL of slurry from each flask. Sample slurries were then mixed with 0.9 mL double-distilled water in 1.5 mL vials, and the vials were placed to boil in a water bath for 15 min to deactivate the enzyme. After enzyme inactivation, samples were centrifuged at 13,500 rpm for 15 min. The supernatants then were further diluted and filtered into 1.5 mL autosampler vials through 0.2 μm hydrophilic PTFE syringe filters (Millipore, Billerica, Mass.). Filtered samples were kept at 4°C before HPLC analysis.

The conversion efficiency of cellulose was expressed in terms of the percentage of cellulose enzymatically converted to glucose, i.e., enzymatic conversion of cellulose (ECC). ECC was calculated by comparing the glucose yield (g) after enzymatic hydrolysis with the initial glucose content (1.11 times the initial cellulose content) in the untreated biomass (Varga et al., 2004). The following formula was used to calculate ECC:

$$\text{ECC} = \frac{c \times V}{1.11 \times m} \times 100\% \quad (1)$$

where  $c$  is the concentration (g L<sup>-1</sup>) of D-glucose in the sampled hydrolysate determined by HPLC analysis,  $V$  is the total volume (L), and  $m$  is the weight of cellulose before enzymatic hydrolysis (g). The factor 1.11 is the cellulose to glucose conversion factor.

## Crystalline Structure Analysis Using X-Ray Diffraction

The crystalline structure of the sorghum biomass samples before and after pretreatment was analyzed by wide-angle x-ray diffraction (XRD) with a Bruker AXS D-8 diffractometer (AXS GmbH, Karlsruhe, Germany) operating at 40 kW, 40 mA. The radiation was copper Kα ( $\lambda = 1.54 \text{ \AA}$ ), and grade range was between 5° and 40° with a step size of 0.03°. Aperture, scatter, and detector slits each were 1°. The scan speed was set at 5° min<sup>-1</sup>. The presence of crystallinity

in a sample can be detected by absorption peaks. The crystallinity index (CrI) was calculated using the method of Segal et al. (1959) as follows:

$$\text{CrI} = \frac{I_{002} - I_{\text{amorphous}}}{I_{002}} \times 100 \quad (2)$$

where  $I_{002}$  is the intensity of the crystalline portion of biomass at about  $2\theta = 22.5^\circ$ , and  $I_{\text{amorphous}}$  is the peak for the amorphous portion at about  $2\theta = 16^\circ$ . In this study, the second highest peak after  $2\theta = 22.5^\circ$  was at  $2\theta = 16^\circ$  and was assumed to correspond to the amorphous region. However, the amorphous peak is reported to be around  $2\theta = 18.7^\circ$  in the literature. The diffractogram was smoothed using a smooth function in MATLAB (see the Appendix).

### **Chemical Structure Analysis Using FTIR**

Fourier transform infrared (FTIR) spectra are frequently used for investigating the structure of constituents and chemical changes in lignocellulosic biomass. Cellulose decrystallization is usually associated with reduced crystallinity. This suggests that crystallinity can be used to analyze sorghum biomass before and after acid pretreatment and enzymatic hydrolysis. FTIR measurements were performed using a Nexus 670 FT-IR spectrophotometer (Thermo-Nicolet Corp., Madison, Wisc.) equipped with a Smart Collector. Reagent KBr and samples were dried for 24 h at  $50^\circ\text{C}$  and then prepared by mixing 2 mg of sample with 200 mg of spectroscopy-grade KBr. All spectra were recorded in the absorbance mode in the wave number range of  $400\text{-}4000\text{ cm}^{-1}$  with a detection resolution of  $4\text{ cm}^{-1}$  and 32 scans per sample. OMNIC 6.1a software (Thermo-Nicolet Corp., Madison, Wisc.) was used to determine peak positions and intensities.

### **Morphological Structure Analysis Using Scanning Electron Microscopy**

Scanning electron microscopy (SEM) was used to measure the surface properties and microstructure of sorghum biomass before and after treatment. A Hitachi S-3500M SEM with an S-6542 absorbed-electron detector (Hitachinaka, Ibaraki, Japan) was used to exam the microstructure of sorghum biomass before and after treatment from 1.5K to 3K. Specimens were mounted on conductive adhesive tape, sputter coated with 4 nm of a 60% gold and 40% palladium mixture, and observed using a voltage of 15 to 20 kV.

## **Analytical Methods**

Moisture content of ground sorghum biomass and corn stover was determined by drying about 2 g of each sample in a forced-air oven at 105°C for 4 h (Sluiter et al., 2008b). Moisture content of pretreated wet samples was determined by drying approximately 2.5 g of sample in a forced-air oven at 49°C overnight and further drying at 105°C for a minimum of 4 h.

Extractives in dry, untreated biomass and chemical composition of untreated and pretreated biomass were determined by following NREL laboratory analytical procedures (Sluiter et al., 2005; Sluiter et al., 2008a). Structural carbohydrates in biomass were reported as percentages of glucan and xylan. Glucan is basically cellulose, and xylan is the major hemicellulose constituent. Lignin, the major noncarbohydrate 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 pretreated samples with an HPLC (Shimadzu, Kyoto, Japan) equipped with an RCM-monosaccharide column (300 × 7.8 mm; Phenomenex, Torrance, Cal.) and a refractive index detector (RID-10A, Shimadzu, Kyoto, Japan). The mobile phase was 0.6 mL min<sup>-1</sup> of double-distilled water, and the oven temperature was 80°C. The supernatants of pretreated samples were neutralized with CaCO<sub>3</sub> to pH 6 before being filtered through 0.2 μm hydrophilic PTFE syringe filters (Millipore, Billerica, Mass.).

The experiment for each biomass sample was replicated twice. Analysis of variance (ANOVA) and least-significant difference (LSD) at the 0.05 level were performed using SAS (2005 ver., SAS Institute, Inc., Cary, N.C.).

## **RESULTS AND DISCUSSION**

### **Effect of Pretreatment Conditions on Yield of Fermentable Sugars**

The effect of sulfuric acid concentration (1.0%, 1.5%, and 2.0%) on grain sorghum biomass conversion efficiency was studied at constant temperature and residence time (140°C for 30 min). Glucose yield increased as sulfuric acid concentration increased. Pretreatment with 2% sulfuric acid yielded the highest conversion efficiency of glucose (82% ECC at the 70th h of hydrolysis time) compared with lower concentrations of sulfuric acid (Figure 2.1). Therefore, 2% sulfuric acid was considered optimum and used in subsequent experiments.



When the untreated biomass is used as a reference point, glucan content in biomass increased significantly after dilute acid pretreatment, especially for pretreatment at mild temperature (Table 2.1). There was a significant increase in lignin content of biomass after dilute acid pretreatment. This phenomenon was more pronounced at higher pretreatment temperature, except for the case of grain sorghum and sweet sorghum. Most xylan (mainly hemicellulose) was hydrolyzed during dilute acid pretreatment, as seen from the significant decrease in xylan content of pretreated solid residues (Table 2.1).

Mass recovery and cellulose recovery for certain pretreatment conditions varied among biomass types. Under the pretreatment condition of 140°C for 30 min, cellulose recovery of various types of biomass ranged from 70% to 85%, whereas cellulose recovery biomass pretreated at 165°C for 10 min ranged from 31% to 58%. Mass recovery and cellulose recovery from the solid fraction after pretreatment decreased under more severe pretreatment conditions (165°C, 10 min; Table 2.1).

Content of hexose sugar (glucose) in the filtrate fraction of samples after pretreatment increased with the increase in pretreatment temperature (Table 2.2). Pentose content (xylose and arabinose) in the filtrate fraction decreased as pretreatment temperature increased. These results indicate that more glucose and less pentose were present in the liquid fraction after pretreatment at higher temperature. The decrease in pentose sugar was probably due to degradation of pentose at higher temperature.

### **Enzymatic Hydrolysis**

The maximum ECC of pretreated cellulose was between 65% and 82% for biomass pretreated at 140°C for 30 min (Figure 2.2) and between 85% and 98% for biomass pretreated at 165°C for 10 min (Figure 2.3). For biomass pretreated at 140°C for 30 min, corn stover yielded the highest ECC, followed by BMR sorghum, forage sorghum, photoperiod-sensitive sorghum, sweet sorghum, and grain sorghum. The ECC of corn stover (82.3%) was not much higher than that for BMR sorghum (80.5%). For biomass pretreated at 165°C for 10 min, BMR sorghum yielded the highest ECC, followed by sweet sorghum, corn stover, photoperiod-sensitive sorghum, forage sorghum, and grain sorghum. In this case, the ECC of corn stover (95%) was a bit lower than that of sweet sorghum (97%). The ECC of sorghum biomass was not much different from that of corn stover, regardless of pretreatment conditions, which indicates that

there is some potential for using sorghum stover in biofuel applications. Among different types of sorghum biomass, BMR sorghum yielded the highest ECC during enzymatic hydrolysis. This probably is because BMR sorghum has less lignin content than other types of sorghum, even less than corn, and a high ratio of cellulose to lignin. In general, biomass with less lignin is more digestible.

## **X-Ray Diffraction**

The crystallinity patterns of each biomass sample after pretreatment and after enzymatic hydrolysis look similar to their patterns before treatment (Figure 2.4). However, the intensity of crystallinity and amorphous peaks varied with treatment conditions as well as biomass types. Among different types of sorghum, photoperiod-sensitive sorghum had the highest intensity of both the crystalline peak and amorphous peak. Lower crystallinity has been associated with cellulose decrystallization as well as a high value of amorphous material. As shown in table 3, the CrI values for corn were higher than those for any type of sorghum. The increase of CrI values along with the significant increase of glucan content and the significant decrease of xylan content in pretreated solid residues (table 1) confirmed that dilute acid pretreatment was an efficient method for hydrolyzing the amorphous portion (hemicellulose) and disrupting the crystalline structure of the biomass. For most biomass samples, enzymatic hydrolysis resulted in a decrease of CrI values compared with untreated samples. However, the relationship between the crystallinity index of hydrolyzed biomass and its corresponding glucose conversion efficiency is not well defined. A high crystallinity index of biomass after hydrolysis does not mean that the biomass is difficult to enzymatically hydrolyze. For example, the CrI value of enzymatically hydrolyzed corn stover pretreated at 140°C was 45.88, which was relatively higher than the CrI of many sorghum samples, but its cellulose conversion after hydrolysis was the highest (82.3%) of all samples.

## **Chemical Structure**

The FTIR spectra of sorghum biomass and corn stover show several absorption bands that can be assigned to major structural components: hemicellulose, lignin, and cellulose. The assignment of FTIR absorption bands for sorghum biomass and corn stover is summarized in table 4. As shown in Figure 2.5, the 4000-1800  $\text{cm}^{-1}$  region of the absorbance spectra has only a few bands, which are attributed to the O-H group (at around 3340  $\text{cm}^{-1}$ ) and the C-H group (at

around  $2927\text{ cm}^{-1}$ ). These bands are pure, whereas other bands in the fingerprint region ( $1800\text{--}900\text{ cm}^{-1}$ ) are complex; this is a result of various vibration modes in carbohydrates and lignin (Gilbert et al., 1993; Pandey, 1999). Therefore, this investigation focused on the fingerprint region (Figures 2.6 to 2.10). In the fingerprint region, C-H bending modes appear at  $1435\text{--}1431\text{ cm}^{-1}$  (asymmetric) and  $1381\text{--}1373\text{ cm}^{-1}$  (symmetric), respectively, and the C-O of guaiacyl ring lies at  $1273\text{--}1271\text{ cm}^{-1}$ . Although all biomass spectra were similar, slight changes were observed from spectrum to spectrum. For example, there were no peaks at  $1714$ ,  $1660$ ,  $1273$ ,  $1207$ , and  $1088\text{ cm}^{-1}$  for the untreated biomass. The hemicellulose band appeared at  $1738\text{ cm}^{-1}$  for all original samples (Guo et al., 2008; Kumar et al., 2009; Pandey, 1999; Sun and Tomkinson, 2004). No hemicellulose band was observed after treatment and hydrolysis, indicating that hemicellulose was greatly hydrolyzed during the pretreatment process. The data in table 1, indicating that pretreated samples contained only about 2% to 4% xylose, further supported this claim. The chemical composition analysis of biomass (Table 2.2) supports the FTIR observations that the hemicellulose (xylan) content of biomass significantly decreases after pretreatment.

Lignin-related bands in the FTIR spectra were seen around  $1273$ ,  $1518$ ,  $1610$ , and  $1715\text{ cm}^{-1}$  (Kumar et al., 2009; Pandey, 1999; Sun et al., 1998). The band at  $1518\text{--}1514\text{ cm}^{-1}$ , attributed to the C=C of lignin, was observed for all untreated biomass and was strong in intensity for photoperiod-sensitive sorghum and sweet sorghum. This spectrum remained after pretreatment and was still seen after enzymatic hydrolysis. Detection of an absorption band at  $1715\text{ cm}^{-1}$ , due to the C=O stretching of the phenyl ester side chains of the lignin structure, in pretreated solid residues showed that the phenyl ester linkages between lignin and a few hemicelluloses had not been cleaved by dilute acid pretreatment. This finding supports the previous result for chemical composition analysis of biomass samples; dilute acid pretreatment can remove most of the xylan (hemicellulose) from biomass, while most of the glucan (cellulose) and lignin remain in the solid residues. The band at  $1606\text{--}1610\text{ cm}^{-1}$  is associated with the  $\alpha\text{-}\beta$  double bond of the propanoid side group in lignin-like structures (Corredor et al., 2009; Kumar et al., 2009; Pandey, 1999). For all samples, this band was defined after pretreatment but became weaker after enzymatic hydrolysis. Sorghum biomass and corn stover have two types of lignin: guaiacyl and syringyl rings. The band at  $1514\text{--}1518\text{ cm}^{-1}$  is associated with the guaiacyl ring in lignin (Corredor et al., 2009; Pandey, 1999; Sun et al., 1998). This band was observed in all untreated biomass samples and remained after pretreatment and enzymatic hydrolysis. The band

around  $1435\text{ cm}^{-1}$  is due to absorption of syringyl rings in lignin (Corredor et al., 2009; Gastaldi et al., 1998; Pandey, 1999). This band was observed in all untreated and treated samples. Among various types of sorghum after the pretreatment process, BMR sorghum, which had the lowest ratio of syringyl to guaiacyl rings in its lignin structure, yielded the highest ECC.

Cellulose-related bands in the FTIR spectra were seen around 904, 1381, 1435, 2927, and  $3340\text{ cm}^{-1}$  (Gastaldi et al., 1998; Gilbert et al., 1993; Kumar et al., 2009; Pandey, 1999; Sun et al., 1998). The band at  $1381\text{-}1373\text{ cm}^{-1}$  is due to C-H deformation (symmetric) of cellulose (Gastaldi et al., 1998; Gilbert et al., 1993; Pandey, 1999). This band was observed in all original samples at  $1381\text{ cm}^{-1}$ . After pretreatment, the band shifted to  $1373\text{ cm}^{-1}$  and decreased in intensity. The decrease in peak intensity was more pronounced after enzymatic hydrolysis. This decrease implies that cellulose is decrystallized because of the applied pretreatment and further hydrolyzed after enzymatic hydrolysis.

### **Morphological Structure**

Untreated samples seemed to have deposits on the outer surface (Figure 2.11). This surface layer can include waxes, hemicellulose, lignin, and other binding materials. The internal plant structure consists of vascular bundles and holes in the cellulose wall that are used for ventilation and metabolism. After dilute acid pretreatment, the surfaces were clean and smooth (Figures 2.12 and 2.13), a result of the removal of the outer surface layer by acid. Some annular rings and microfibrils were also observed. The diameter of individual cellulose microfibrils was about 7 to 9 microns. Pretreatment at higher temperature disrupted microfibrils much more and had a greater impact on particle size reduction than the lower temperature pretreatment condition. SEM images of pretreated biomass also revealed formation of some holes on the biomass surface and disruption of the biomass network consistent with hemicellulose removal during pretreatment. The compact outer layer was removed after enzymatic hydrolysis (Figures 2.14 and 2.15), revealing the holes as part of the internal structure of cellulose. The microfibrils are about 4 to 7 micron in width. Thus, enzymatic hydrolysis reduced and degraded cellulose, leaving a small, final solid that might require further degradation.

### **CONCLUSIONS**

Forage sorghum, photoperiod-sensitive sorghum, BMR sorghum, sweet sorghum, and grain sorghum biomasses were evaluated as potential feedstocks for biofuel production. FTIR,

SEM, and XRD were used to characterize the chemical structure, morphological structure, and crystallinity of the sorghum biomasses. At pretreatment conditions of 165°C for 10 min with dilute sulfuric acid solutions, the enzymatic conversion of cellulose ranged from 85% to 98%. BMR sorghum yielded the highest cellulose conversion rate (98%), followed by sweet sorghum, photoperiod-sensitive sorghum, forage sorghum, and grain sorghum. At pretreatment conditions of 140°C for 30 min, cellulose conversion rate ranged from 65% to 82%. BMR sorghum yielded the highest cellulose conversion rate (81%), followed by forage sorghum, photoperiod-sensitive sorghum, sweet sorghum, and grain sorghum. Pretreatment conditions had a significant effect on solid mass recovery and cellulose fraction recovery. Under pretreatment conditions of 140°C for 30 min and 165°C for 10 min, cellulose recoveries of sorghum biomass ranged from 70% to 85% and from 31% to 58%, respectively. Considering both sugar recovery and energy consumption, pretreatment of biomass at mild temperature is more favorable than pretreatment at high temperature. Structural analysis results showed that a low ratio of syringyl to guaiacyl rings in the lignin structure makes BMR sorghum easy to hydrolyze enzymatically.

## **ACKNOWLEDGEMENT**

This project was funded by the Sun Grant Program, U.S. Department of Transportation, Grant No. DTOS59-07-G-00056.

## **REFERENCES**

- Alizadeh, H., F. Teymouri, T. Gilbert, and B. Dale. 2005. Pretreatment of switchgrass by ammonia fiber explosion (AFEX). *Appl. Biochem. Biotech.* 124: 1133-1141.
- Christakopoulos, P., L.-W. Li, D. Kekos, and B. J. Macris. 1993. Direct conversion of sorghum carbohydrates to ethanol by a mixed microbial culture. *Bioresource Tech.* 45(2): 89-92.
- Corredor, D. Y., X. S. Sun, J. M. Salazar, K. L. Hohn, and D. Wang. 2008. Enzymatic hydrolysis of soybean hulls using dilute acid and modified steam-explosion pretreatments. *J. Biobased Mat. Bioenergy* 2(1): 1-8.
- Corredor, D. Y., J. M. Salazar, K. L. Hohn, and S. Bean, B. Bean, and D. Wang. 2009. Evaluation and characterization of forage sorghum as feedstock for fermentable sugar production. *Appl. Biochem. Biotech.* 158(1): 164-179.

- Dawson, L., and R. Q. Boopathy. 2007. Use of post-harvest sugarcane residue for ethanol production. *Bioresource Tech.* 98(9): 1695-1699.
- Dien, B. S., H.-J. G. Jung, K. P. Vogel, M. D. Casler, J. F. S. Lamb, L. B. Iten, R. B. Mitchell, and G. Sarath. 2006. Chemical composition and response to dilute-acid pretreatment and enzymatic saccharification of alfalfa, reed canarygrass, and switchgrass. *Biomass Bioenerg.* 30(10): 880-891.
- EIA. 2008. International energy outlook. Washington, D.C.: U.S. Department of Energy, Energy Information Administration. Available at:  
[http://tonto.eia.doe.gov/FTP/forecasting/0484\(2008\).pdf](http://tonto.eia.doe.gov/FTP/forecasting/0484(2008).pdf). Accessed 3 March 2010.
- Gastaldi, G., G. Capretti, B. Focher, and C. Cosentino. 1998. Characterization and properties of cellulose isolated from the *Crambe abyssinica* hull. *Ind. Crop Prod.* 8(3): 205-218
- Gilbert, C., S. Kokot, and U. Meyer. 1993. Application of DRIFT spectroscopy and chemometrics for the comparison of cotton fabrics. *Appl. Spectrosc.* 47(6): 741-748.
- Guo, G.-L., W.-H. Chen, W.-H. Chen, L.-C. Men, and W.-S. Hwang. 2008. Characterization of dilute acid pretreatment of silvergrass for ethanol production. *Bioresource Tech.* 99(14): 6046-6053
- Kadar, Z., S. F. Maltha, Z. Szengyel, K. Reczey, and W. D. Laats. 2007. Ethanol fermentation of various pretreated and hydrolyzed substrate at low initial pH. *Appl. Biochem. Biotech.* 136-140: 847-858.
- Kharecha, P. A., and J. E. Hansen. 2008. Implications of "peak oil" for atmospheric CO<sub>2</sub> and climate. *Global Biogeochem. Cycles* 22: GB3012.
- Kristensen, J., L. Thygesen, C. Felby, H. Jorgensen, and T. Elder. 2008. Cell-wall structural changes in wheat straw pretreated for bioethanol production. *Biotech. Biofuels* 1:5 (doi: 10.1186/1754-6834-1-5).
- Kumar, R., G. Mago, V. Balan, and C. E. Wyman. 2009. Physical and chemical characterization of corn stover and poplar solids resulting from leading pretreatment technologies. *Bioresource Tech.* 100(17): 3948-3962.
- Lau, M. W., and B. E. Dale. 2009. Cellulosic ethanol production from AFEX-treated corn stover using *Saccharomyces cerevisiae* 424A(LNH-ST). *Proc. Natl. Acad. Sci.* 106(5): 1368-1373.

- Linde, M., M. Galbe, and G. Zacchi. 2006. Steam pretreatment of acid-sprayed and acid-soaked barley straw for production of ethanol. *Appl. Biochem. Biotech.* 130: 546-562.
- Mosier, N., C. Wyman, B. Dale, R. Elander, Y. Y. Lee, M. Holtzapple, and M. Ladisch. 2005. Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource Tech.* 96(6): 673-686.
- Pandey, K. K. 1999. A study of chemical structure of soft and hardwood and wood polymers by FTIR spectroscopy. *J. Appl. Polymer Sci.* 71(12): 1969-1975.
- Perlack, R. D., L. L. Wright, R. L. Graham, B. J. Stokes, and D. C. Erbach. 2005. Biomass as feedstock for a bioenergy and bioproducts industry: The technical feasibility of a billion-ton annual supply. USDA and DOE Joint Report (April 2005). Oak Ridge, Tenn.: U.S. Department of Energy, Office of Scientific and Technical Information.
- Rosgaard, L., S. Pedersen, and A. Meyer. 2007. Comparison of different pretreatment strategies for enzymatic hydrolysis of wheat and barley straw. *Appl. Biochem. Biotech.* 143(3): 284-296.
- Saha, B. C., L. B. Iten, M. A. Cotta, and Y. V. Wu. 2005. Dilute acid pretreatment, enzymatic saccharification, and fermentation of wheat straw to ethanol. *Process Biochem.* 40(12): 3693-3700.
- Salvi, D. A., G. M. Aita, D. Robert, and V. Bazan. 2010. Ethanol production from sorghum by a dilute ammonia pretreatment. *J. Ind. Microbiol. Biotech.* 37(1): 27-34.
- Sassner, P., and G. Zacchi. 2008. Integration options for high energy efficiency and improved economics in a wood-to-ethanol process. *Biotech. Biofuels* 1:4 (doi: 10.1186/1754-6834-1-4).
- Segal, L., J. J. Creely, A. E. J. Martin, and C. M. Conrad. 1959. An empirical method for estimating the degree of crystallinity of native cellulose using the x-ray diffractometer. *Textile Res. J.* 29(10): 786-794.
- Sluiter, A., R. Ruiz, C. Scarlata, J. Sluiter, and D. Templeton. 2005. Determination of extractives in biomass. NREL/TP-510-42619. Golden, Colo.: National Renewable Energy Laboratory.
- Sluiter, A., B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, and D. Crocker. 2008a. Determination of structural carbohydrates and lignin in biomass. NREL/TP-510-42618. Golden, Colo.: National Renewable Energy Laboratory.

- Sluiter, A., B. Hames, D. Hyman, C. Payne, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, and J. Wolfe. 2008b Determination of total solids in biomass and total dissolved solids in liquid process samples. NREL/TP-510-42621. Golden, Colo.: National Renewable Energy Laboratory.
- Soderstrom, J., L. Pilcher, M. Galbe, and G. Zacchi. 2002. Two-step steam pretreatment of softwood with SO<sub>2</sub> impregnation for ethanol production. *Appl. Biochem. Biotech.* 98-100: 5-21.
- Sun, R., and J. Tomkinson. 2004. Separation and characterization of cellulose from wheat straw. *Sep. Sci. Tech.* 39(2): 391-411.
- Sun, R., J. M. Fang, P. Rowland, and J. Bolton. 1998. Physicochemical and thermal characterization of wheat straw hemicellulose and cellulose. *J. Agric. Food Chem.* 46(7): 2804-2809.
- Sun, Y., and J. Cheng. 2002. Hydrolysis of lignocellulosic materials for ethanol production: A review. *Bioresource Tech.* 83(1): 1-11.
- USCB. 2008. World Population Clock. August 2008. Washington, D.C.: U.S. Census Bureau. Available at: [www.prb.org/Articles/2008/worldpopulationclock2008.aspx](http://www.prb.org/Articles/2008/worldpopulationclock2008.aspx). Accessed 1 June 2009.
- Varga, E., K. Reczey, and G. Zacchi. 2004. Optimization of steam pretreatment of corn stover to enhance enzymatic digestibility. *Appl. Biochem. Biotech.* 114: 509-523.
- Wyman, C. E., B. E. Dale, R. T. Elander, M. Holtzapple, M. R. Ladisch, and Y. Y. Lee. 2005. Comparative sugar recovery data from laboratory scale application of leading pretreatment technologies to corn stover. *Bioresource Tech.* 96(18): 2026-2032.
- Zaldivar, J., J. Nielsen, and L. Olsson. 2001. Fuel ethanol production from lignocellulosic: A challenge for metabolic engineering and process integration. *Appl. Microbiol. Biotech.* 56: 17-34.
- Zhan, X., D. Wang, S. R. Bean, X. Mo, X. S. Sun, and D. Boyle. 2006. Ethanol production from supercritical-fluid-extrusion cooked sorghum. *Ind. Crops Prod.* 23(3): 304-310.
- Zheng, Y, Z. Pan, R. Zhang, D. Wang, and B. Jenkins. 2008. Non-ionic surfactants and non-catalytic protein treatment on enzymatic hydrolysis of pretreated creeping wild ryegrass. *Appl. Biochem. Biotech.* 146: 231-248.



## **APPENDIX - MATLAB Code for Smoothing of X-Ray Diffraction Spectra**

```
clc
```

```
clear
```

```
A = [imported y-values from Excel file];
```

```
B = [imported x-values from Excel file];
```

```
windowSize = 20;
```

```
b = ones(1,windowSize)/windowSize;
```

```
A1 = filter(b,1,A)
```

```
//copy the values of A1 (data after smoothing)
```

```
into a column of Excel file
```

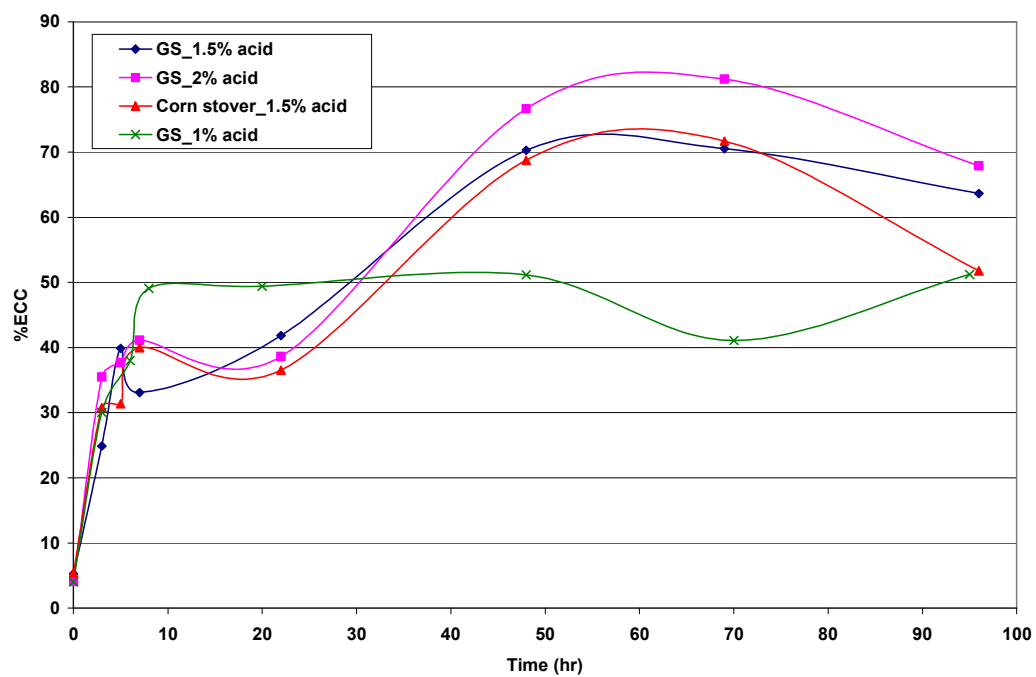
```
figure(1)
```

```
subplot(2,1,1)
```

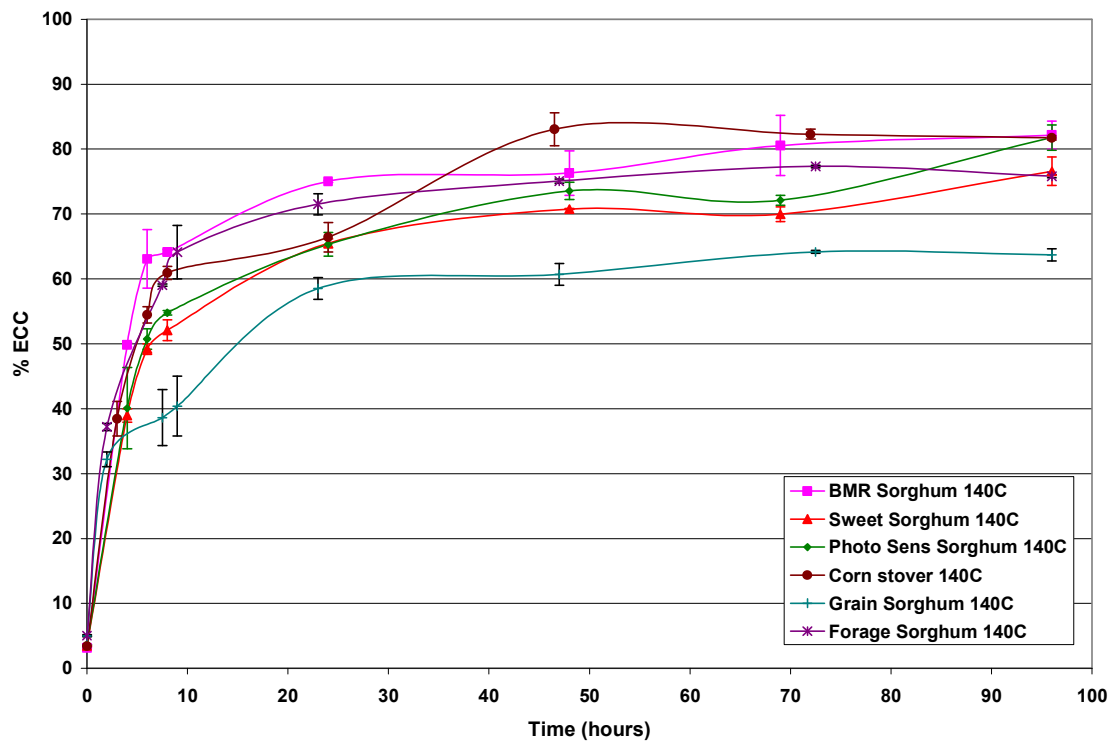
```
plot(B,A)
```

```
subplot(2,1,2)
```

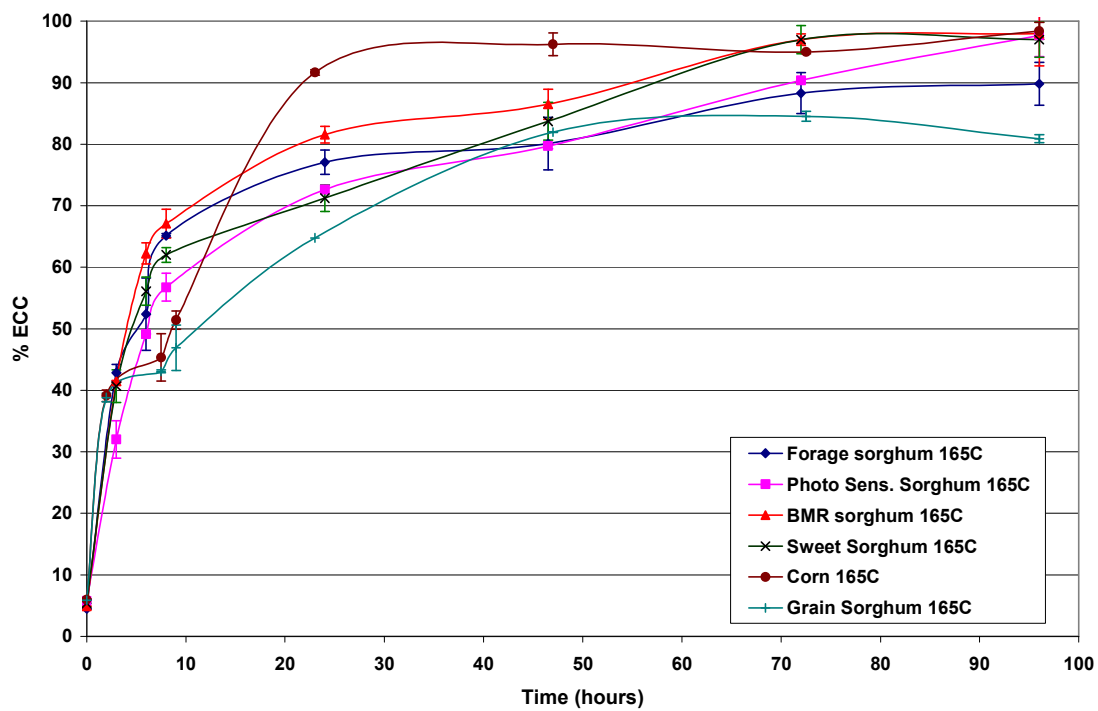
```
plot(B,A1)
```



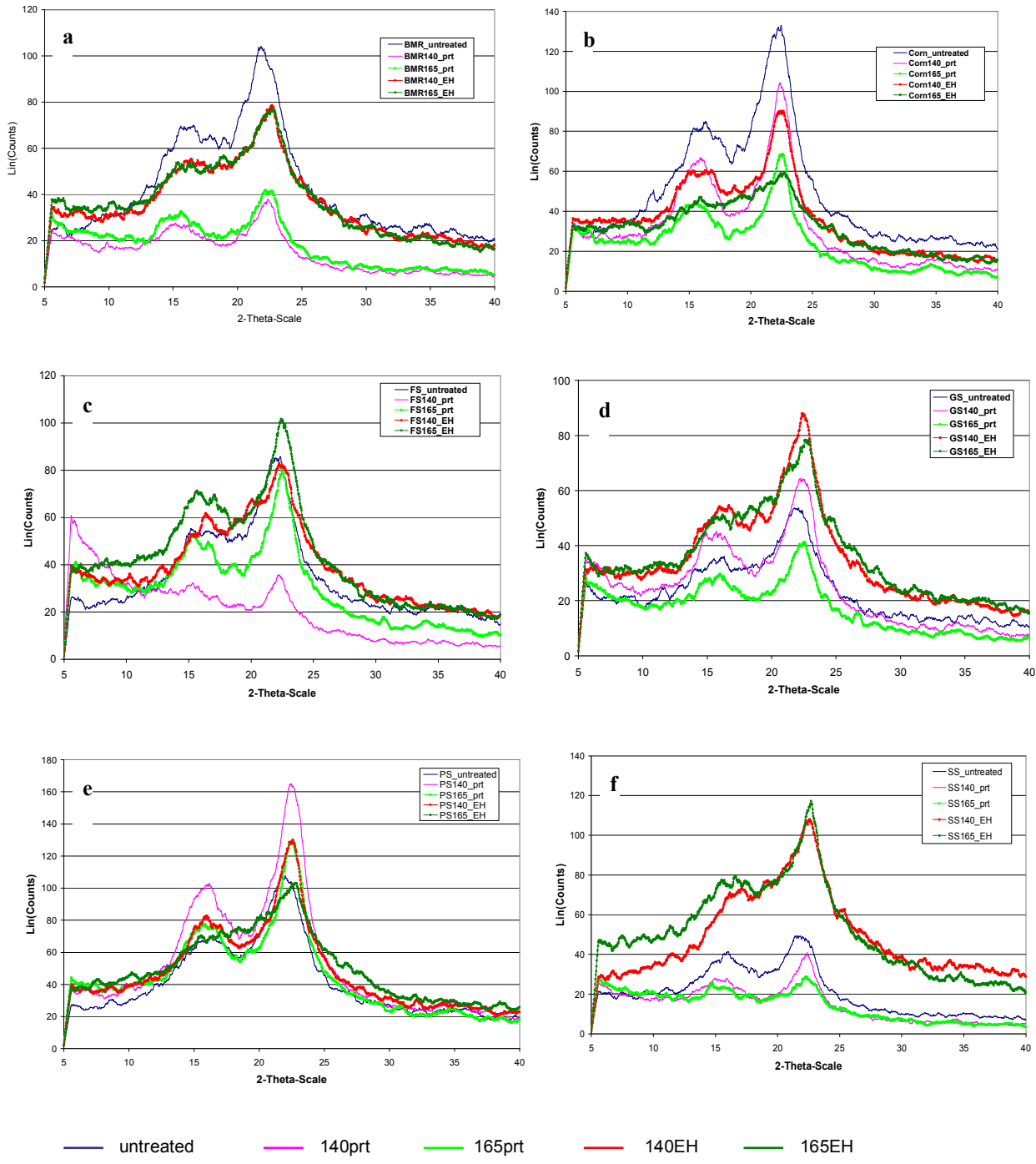
**Figure 2.1 Effect of sulfuric acid concentration on glucose yield.**



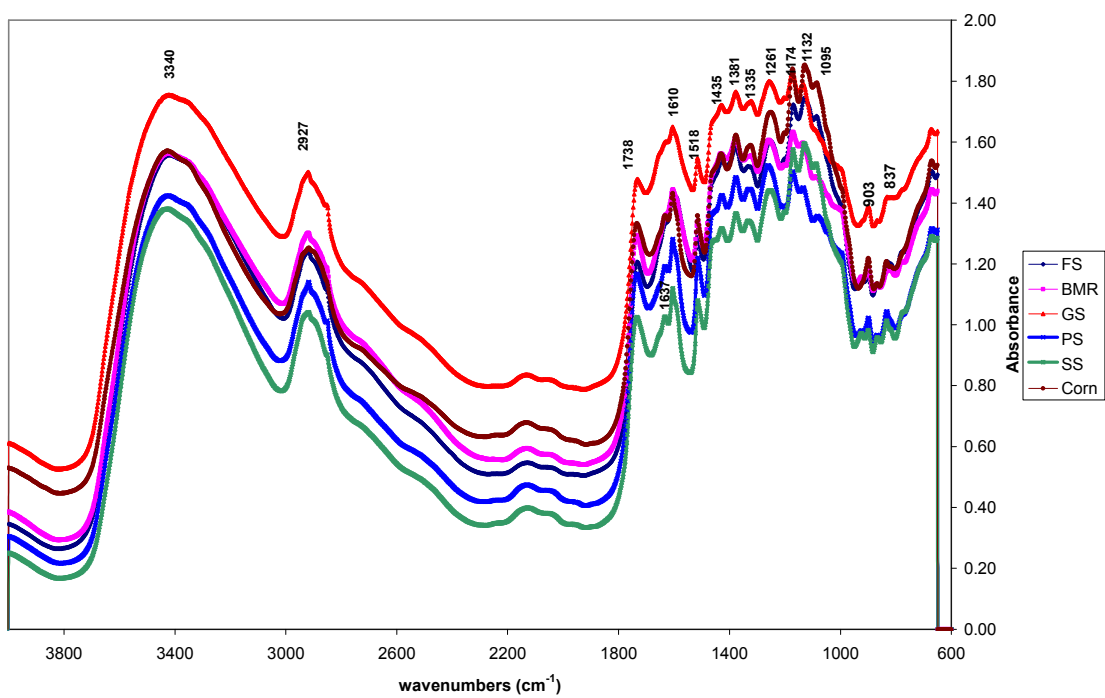
**Figure 2.2 Percentage of enzymatic conversion of cellulose for corn stover and different types of sorghum biomass pretreated with 2% w/v sulfuric acid at 140°C for 30 min.**



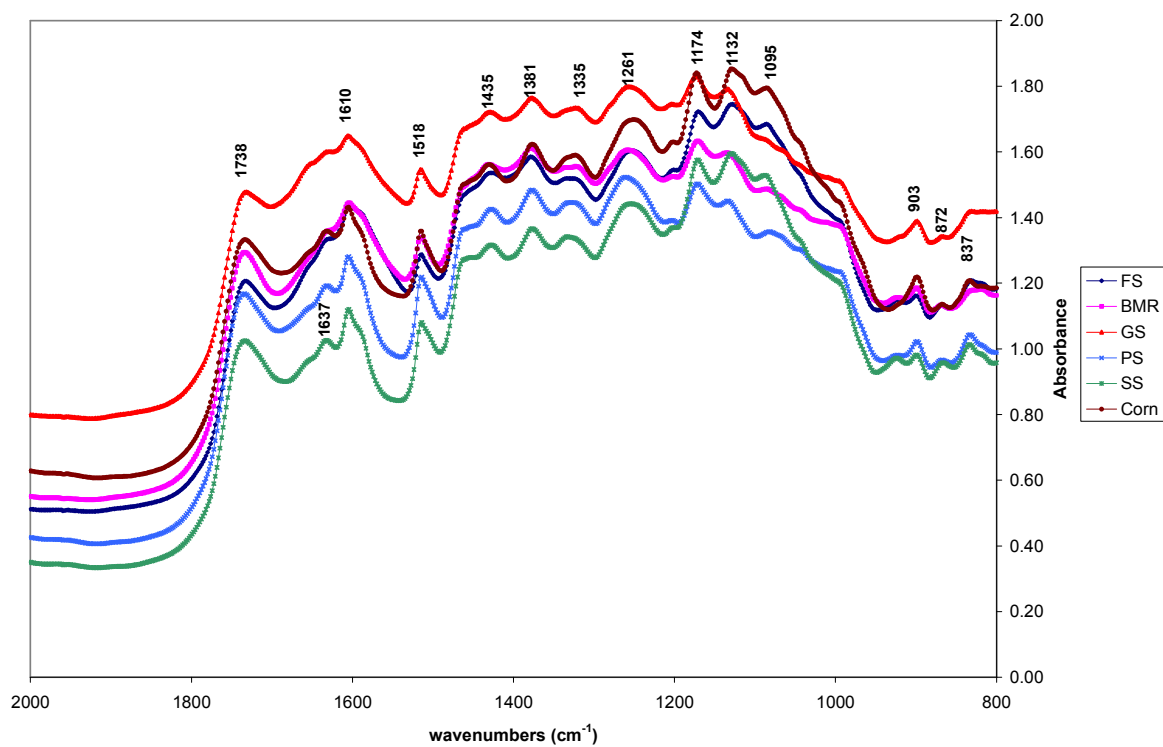
**Figure 2.3 Percentage of enzymatic conversion of cellulose of corn stover and different types of sorghum biomass pretreated with 2% w/v sulfuric acid at 165°C for 10 min.**



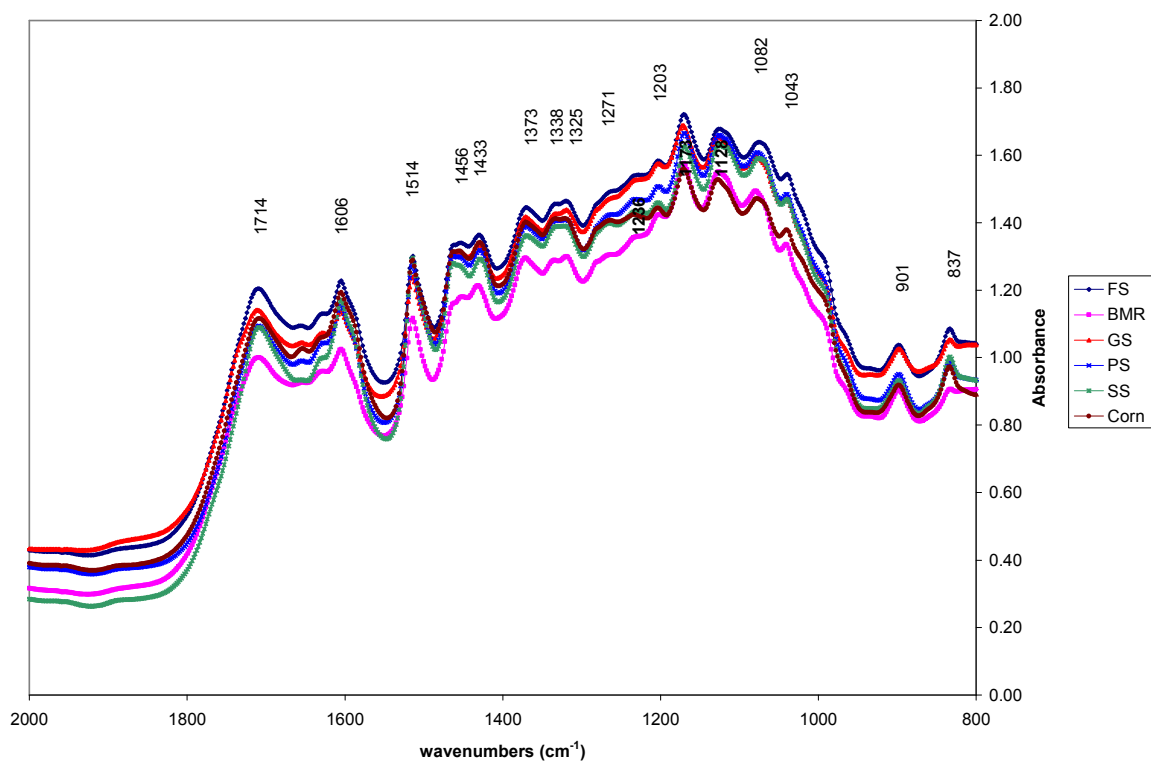
**Figure 2.4 X-ray diffraction of untreated and treated sorghum stalks and corn stover: (a) BMR sorghum, (b) corn stover, (c) forage sorghum, (d) grain sorghum biomass, (e) photoperiod-sensitive sorghum, and (f) sweet sorghum bagasse.**



**Figure 2.5 FTIR spectra of untreated sorghum biomass and corn stover.**

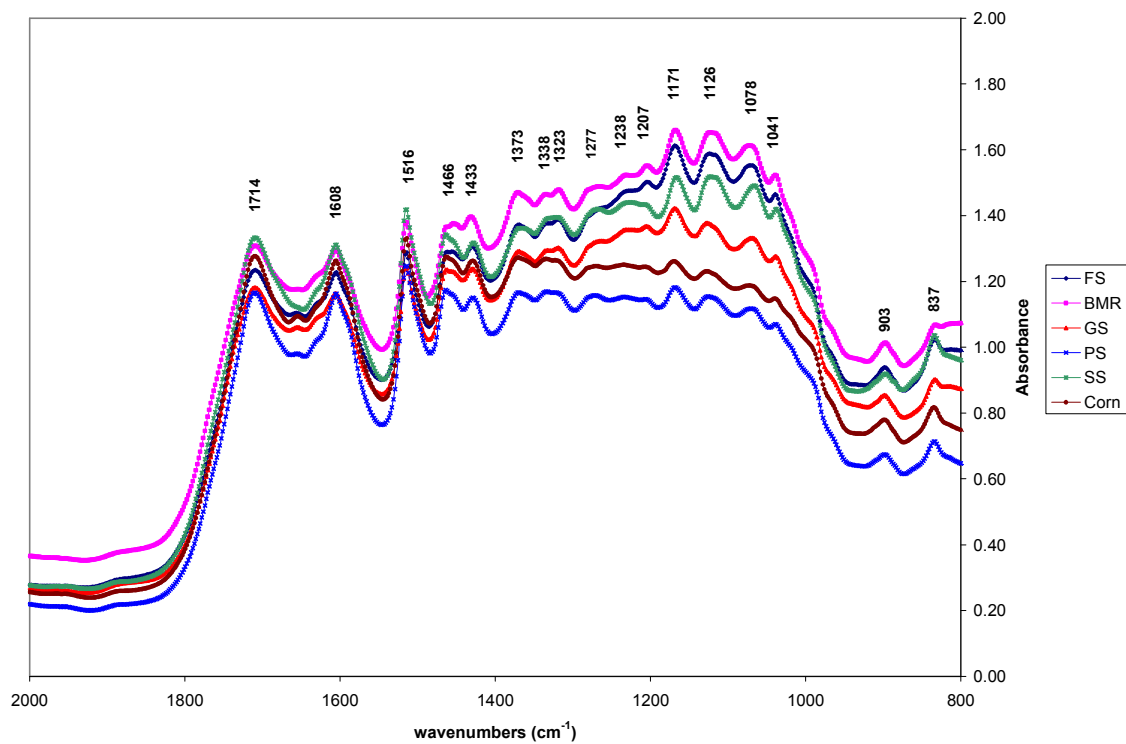


**Figure 2.6 FTIR spectra of untreated sorghum biomass and corn stover in the fingerprint region (900-1800 cm<sup>-1</sup>).**

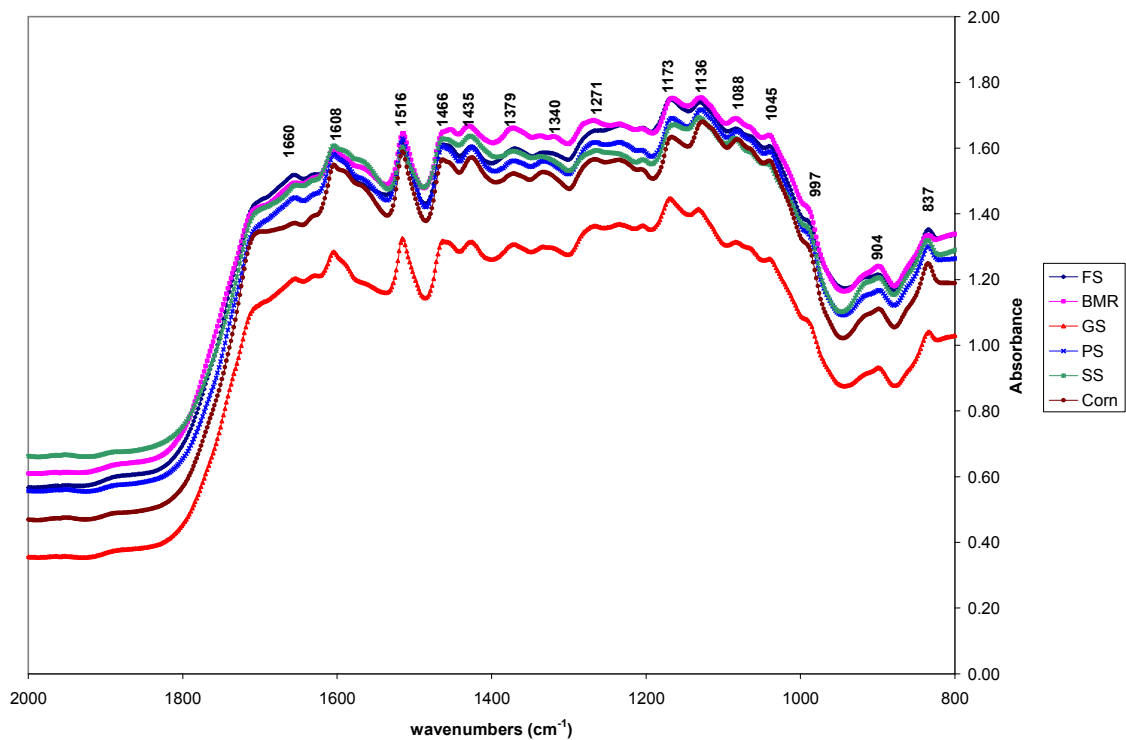


**Figure 2.7 FTIR spectra of sorghum biomass and corn stover in the fingerprint region (900-1800 cm<sup>-1</sup>) after dilute acid pretreatment at 140°C for 30 min with 2% w/v acid.**

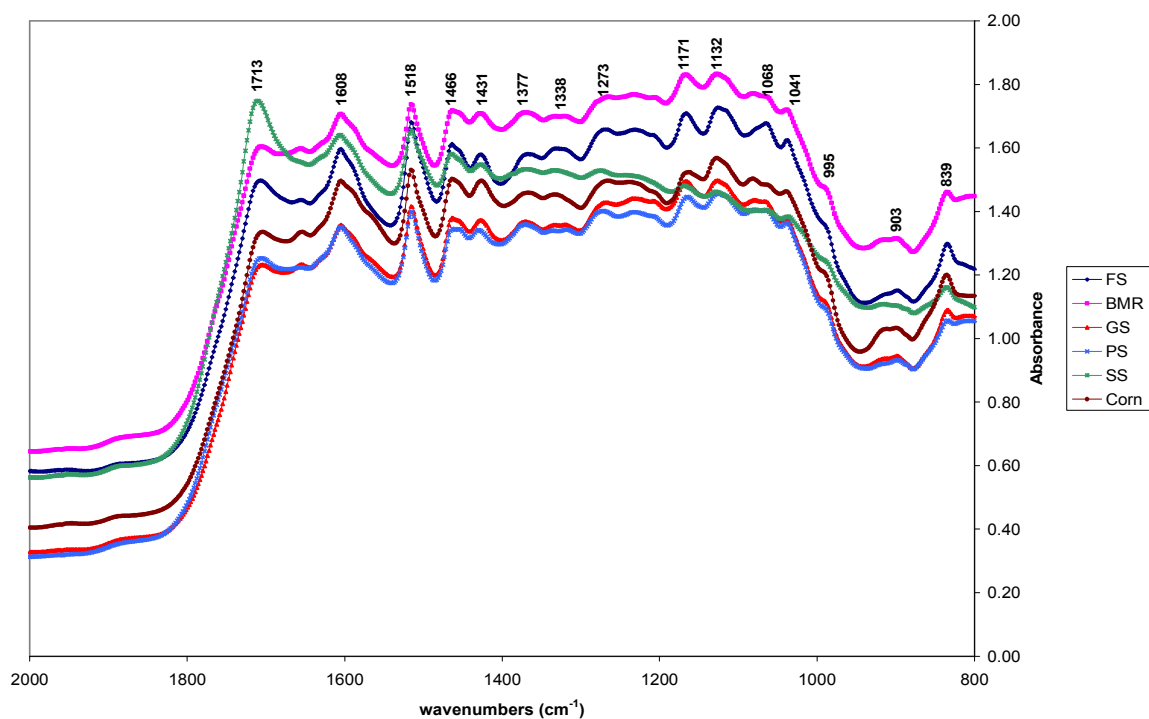




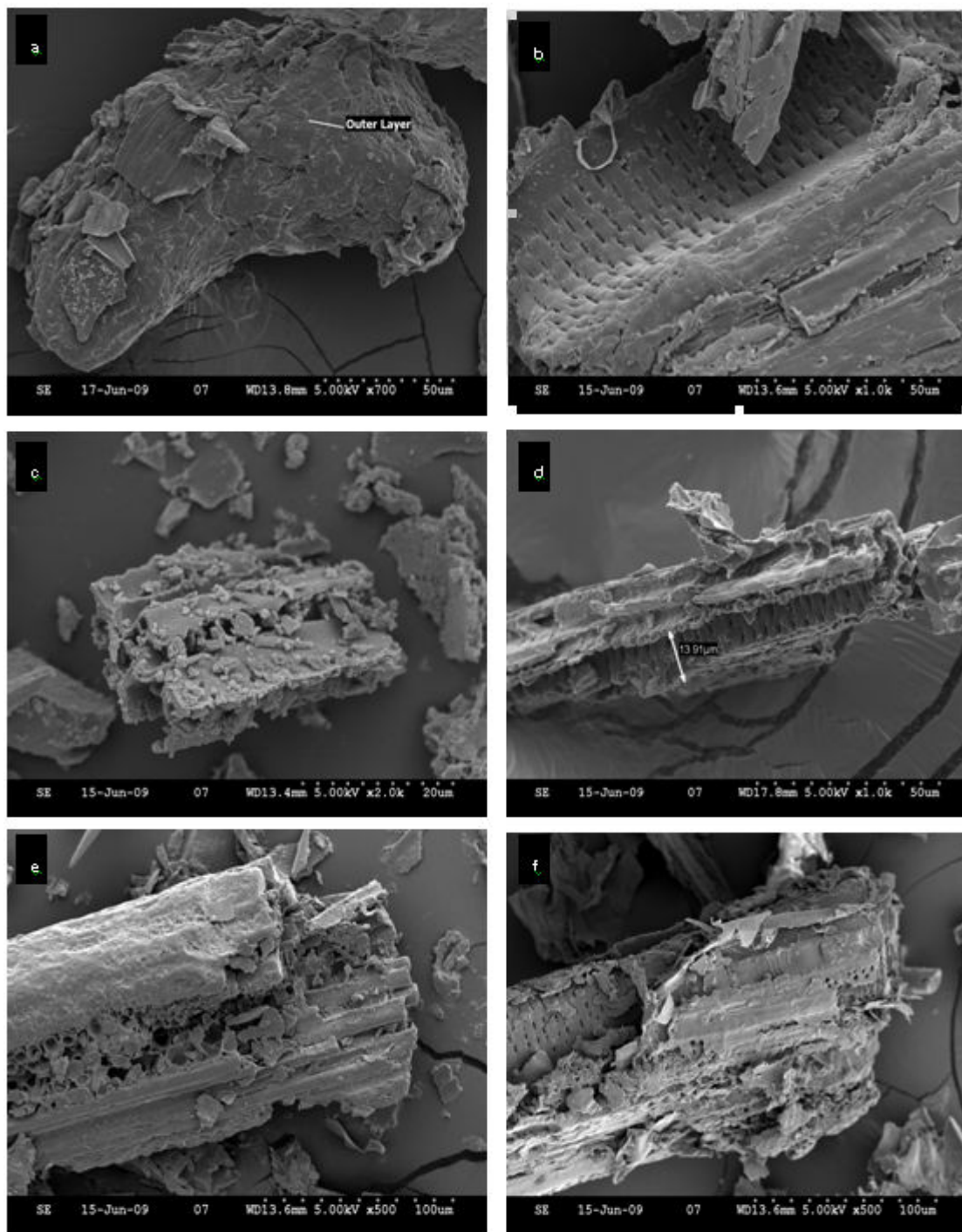
**Figure 2.8 FTIR spectra of sorghum biomass and corn stover in the fingerprint region (900-1800 cm<sup>-1</sup>) after dilute acid pretreatment at 165°C for 10 min with 2% w/v acid.**



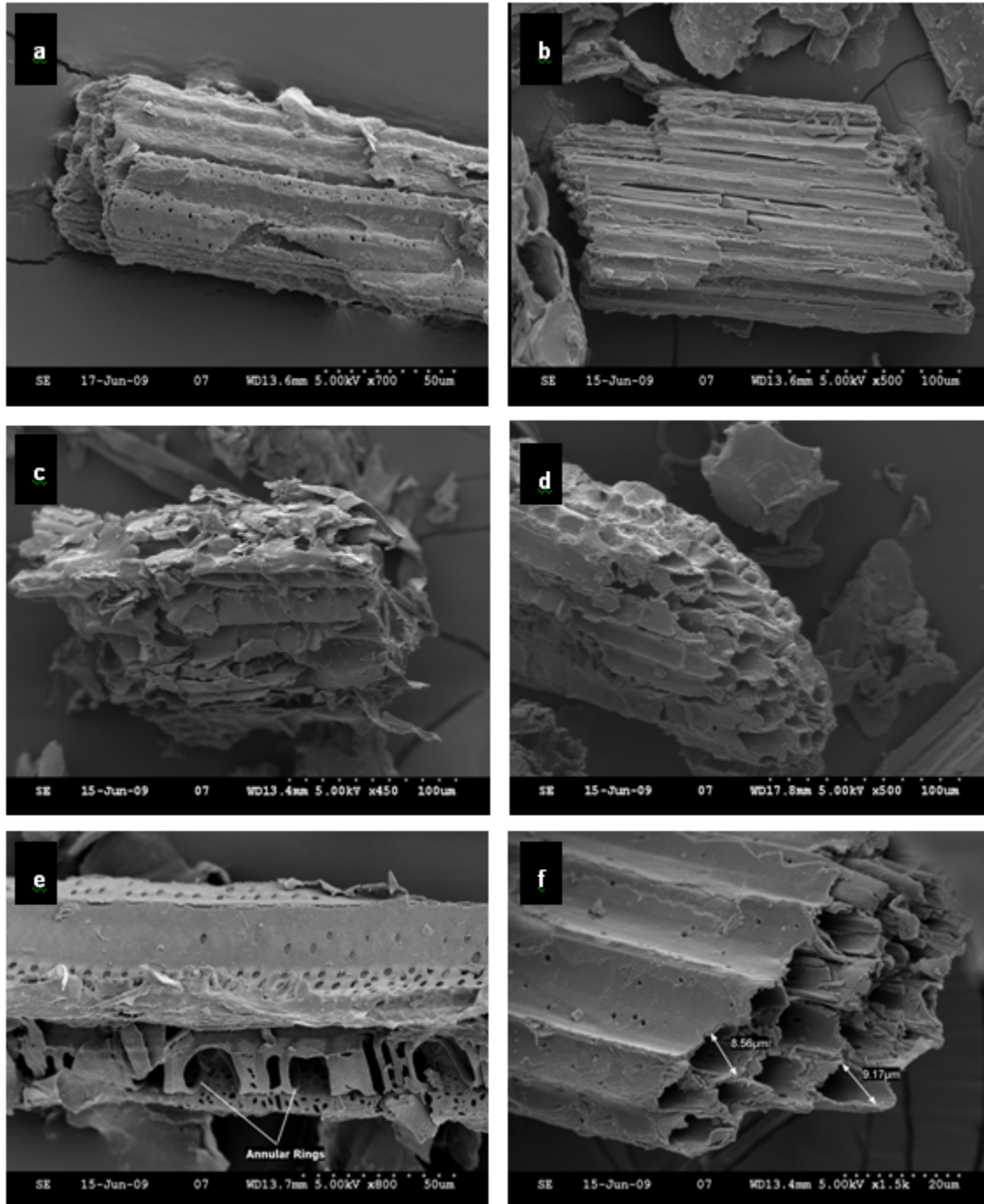
**Figure 2.9 FTIR spectra of sorghum biomass and corn stover in the fingerprint region (900-1800 cm<sup>-1</sup>) after dilute acid pretreatment at 140°C for 30 min with 2% w/v acid and enzymatic hydrolysis.**



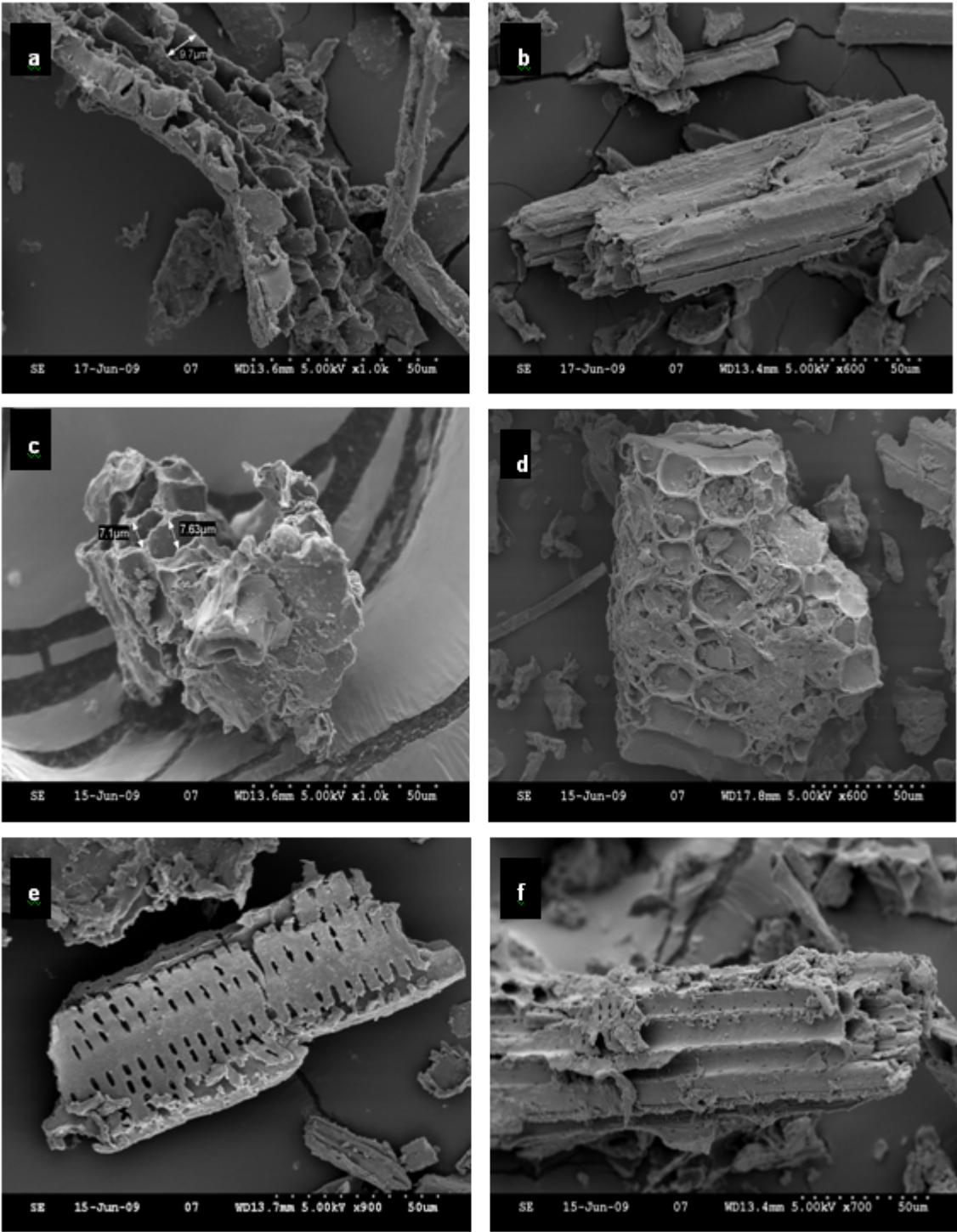
**Figure 2.10 FTIR spectra of sorghum biomass and corn stover in the fingerprint region (900-1800 cm<sup>-1</sup>) after dilute acid pretreatment at 165°C for 10 min with 2% w/v acid and enzymatic hydrolysis.**



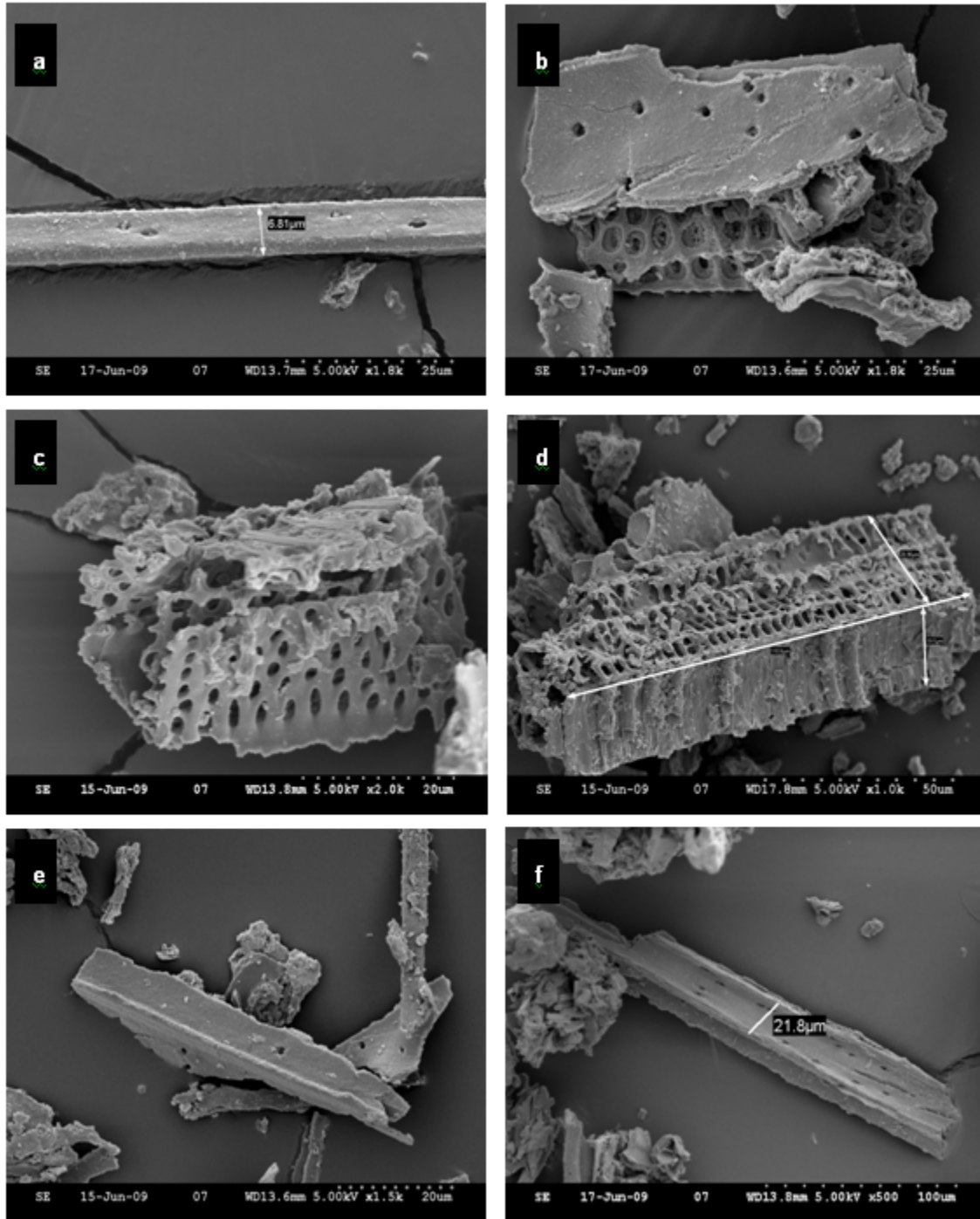
**Figure 2.11 SEM images of untreated samples: (a) BMR sorghum (b) corn, (c) forage sorghum, (d) grain sorghum, (e) photoperiod-sensitive sorghum, and (f) sweet sorghum.**



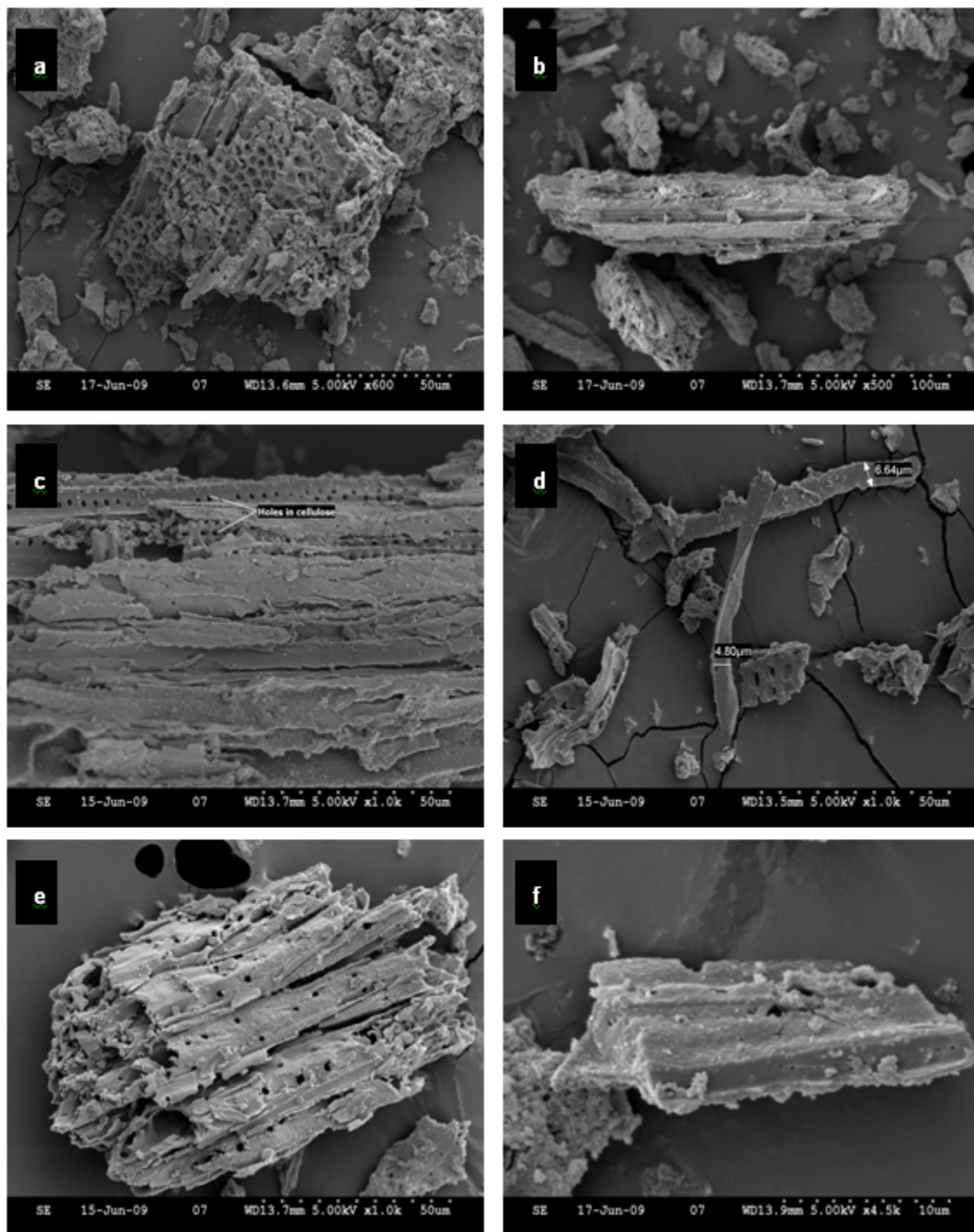
**Figure 2.12 SEM images of samples pretreated at 140°C for 30 min with 2% sulfuric acid: (a) BMR sorghum (b) corn, (c) forage sorghum, (d) grain sorghum, (e) photoperiod-sensitive sorghum, and (f) sweet sorghum.**



**Figure 2.13 SEM images of samples pretreated at 165°C for 10 min with 2% sulfuric acid: (a) BMR sorghum (b) corn, (c) forage sorghum, (d) grain sorghum, (e) photoperiod-sensitive sorghum, and (f) sweet sorghum.**



**Figure 2.14 SEM images of samples after pretreatment at 140°C for 30 min with 2% sulfuric acid and enzymatic hydrolysis: (a) BMR sorghum (b) corn, (c) forage sorghum, (d) grain sorghum, (e) photoperiod-sensitive sorghum, and (f) sweet sorghum.**



**Figure 2.15 SEM images of samples after pretreatment at 165°C for 10 min with 2% sulfuric acid and enzymatic hydrolysis: (a) BMR sorghum (b) corn, (c) forage sorghum, (d) grain sorghum, (e) photoperiod-sensitive sorghum, and (f) sweet sorghum.**



**Table 2.1 Components in solid fractions of pretreated biomass**

Sample	Pretreatment Conditions	Component in Solid Fractions (%) <sup>[a]</sup>					Mass Recovery (%)	Cellulose Recovery (%)
		Lignin	Glucan	Xylan	Ash			
Forage sorghum	Unpretreated	20.29 a	37.90 a	29.79 a	10.94 a			
	140°C/30 min	26.82 b	47.91 b	3.62 b	10.47 b	67.08	84.79	
	165°C/10 min	31.55 c	42.16 c	2.54 c	12.43 c	51.45	57.23	
Photoperiod-sensitive sorghum	Unpretreated	19.22 a	44.02 a	27.40 a	7.49 a			
	140°C/30 min	28.17 b	55.37 b	4.26 b	6.01 b	61.41	77.24	
	165°C/10 min	47.90 c	44.76 a	2.42 c	7.54 a	30.85	31.36	
BMR sorghum	Unpretreated	15.48 a	40.48 a	26.16 a	8.71 a			
	140°C/30 min	22.67 b	55.24 b	4.22 b	8.03 b	56.81	70.56	
	165°C/10 min	28.20 c	42.29 a	2.94 b	9.38 c	45.02	42.81	
Sweet sorghum	Unpretreated	18.03 a	34.24 a	26.81 a	4.27 a			
	140°C/30 min	33.82 b	54.15 b	2.64 b	4.58 b	46.38	73.35	
	165°C/10 min	32.30 b	36.17 a	2.41 b	5.43 c	44.65	47.17	
Grain sorghum	Unpretreated	18.11 a	37.84 a	24.60 a	10.87 a			
	140°C/30 min	28.21 b	44.49 b	4.34 b	12.06 ab	59.07	74.57	
	165°C/10 min	28.02 b	41.44 c	2.58 c	12.43 b	51.71	56.62	
Corn stover	Unpretreated	18.10 a	37.30 a	24.61 a	5.88 a			
	140°C/30 min	25.18 b	55.01 b	3.99 b	5.45 b	66.12	97.51	
	165°C/10 min	30.05 c	46.54 c	2.63 b	5.23 c	47.41	59.16	

<sup>[a]</sup> Means in the same biomass followed by different letters are significantly different at  $p < 0.05$ .

**Table 2.2 Sugar yield in filtrate after dilute acid pretreatment**

Samples	Pretreatment Conditions	Components in Filtrate Fractions <sup>[a]</sup>		
		Xylose	Arabinose	Glucose
Forage sorghum	140°C/30 min	14.82 a	2.75 a	18.28 a
	165°C/10 min	9.50 b	1.02 b	20.85 b
Photoperiod-sensitive sorghum	140°C/30 min	15.44 a	2.69 a	14.14 a
	165°C/10 min	8.10 b	2.07 b	21.42 b
BMR sorghum	140°C/30 min	16.68 a	3.22 a	15.77 a
	165°C/10 min	9.72 b	1.01 b	20.92 a
Sweet sorghum	140°C/30 min	17.86 a	2.41 a	21.20 a
	165°C/10 min	5.67 b	1.68 b	24.78 b
Grain sorghum	140°C/30 min	15.29 a	3.00 a	15.49 a
	165°C/10 min	9.94 b	2.47 a	18.06 b
Corn stover	140°C/30 min	17.54 a	3.30 a	12.90 a
	165°C/10 min	9.37 b	1.69 b	22.68 b

<sup>[a]</sup> g per 100 g of dry, untreated biomass. Means in the same biomass followed by different letters are significantly different at  $p < 0.05$ .

**Table 2.3 Crystallinity index values for different types of biomass <sup>[a]</sup>**

Sample	Untreated	140Prt	165Prt	140EH	165EH
BMR	37.04	46.51	45.39	37.00	32.90
Corn stover	47.82	63.32	60.68	45.88	25.83
Forage sorghum	40.99	56.47	52.91	35.72	38.30
Grain sorghum	39.53	49.49	49.63	45.95	34.19
P-S sorghum <sup>[b]</sup>	45.52	55.00	59.19	49.13	29.01
Sweet sorghum	32.58	56.06	33.92	36.57	38.55

<sup>[a]</sup> 140Prt = pretreated at 140°C for 30 min, 165Prt = pretreated at 165°C for 10 min, 140EH = enzymatic hydrolysis of biomass pretreated at 140°C for 30 min, and 165EH = enzymatic hydrolysis of biomass pretreated at 165°C for 10 min.

<sup>[b]</sup> Photoperiod-sensitive sorghum.

**Table 2.4 Assignment of FTIR absorption bands for sorghum biomass and corn stover**

Wavenumbers (cm <sup>-1</sup> )	Pattern in <sup>[a]</sup>	Assignment	Reference
3340	All	O-H stretching (indicates rupture of cellulose hydrogen bonds)	Gilbert et al., 1993; Kumar et al., 2009; Pandey, 1999
2927	All	C-H stretching (indicates rupture of methyl/methylene group of cellulose)	Gilbert et al., 1993; Kumar et al., 2009; Pandey, 1999
1738	Untreated	C=O ester; strong carbonyl groups in branched hemicellulose	Guo et al., 2008; Kumar et al., 2009; Pandey, 1999; Sun and Tomkinson., 2004
1714-1713	140Prt, 165Prt, and 165EH	C=O stretching (carboxylic acids/ester groups) from lignin	Kumar et al., 2009; Pandey, 1999
1660-1637	Untreated 140EH	Absorbed H <sub>2</sub> O, C=O with intramolecular hydrogen bond	Gilbert et al., 1993; Guo et al., 2008
1610-1606	All	Aromatic skeletal vibration + C=O stretching (related to lignin removal)	Kumar et al., 2009; Pandey, 1999
1518-1514	All	C=C (related to lignin removal) guaiacyl ring of lignin	Corredor et al., 2009; Pandey, 1999; Sun et al., 1998
1435-1431	All	C-H deformation (asymmetric) of cellulose; syringyl absorption of hardwood	Corredor et al., 2009; Gastaldi et al., 1998; Pandey, 1999
1381-1373	All	C-H deformation (symmetric) of cellulose	Gastaldi et al., 1998; Gilbert et al., 1993; Pandey, 1999
1340-1335	All	O-H in-plane deformation	Pandey, 1999
1273-1271	140Prt, 165Prt, 140EH, and 165EH	C-O of guaiacyl ring and C-O stretching	Pandey, 1999
1238-1236	140Prt and 165Prt	O-H in-plane deformation	Gilbert et al., 1993
1207-1203	140Prt and 165Prt	O-H in-plane deformation	Gilbert et al., 1993; Pandey, 1999
1136-1126	All	Antisymmetric C-O-C beta-1,4 glycosyl linkage of cellulose	Gilbert et al., 1993
1088	140EH	C-O of secondary alcohols	Pandey, 1999
904-901	All	Glucose ring stretch, C-H deformation (removal of amorphous cellulose)	Kumar et al., 2009; Pandey, 1999

<sup>[a]</sup> 140Prt = pretreated at 140°C for 30 min, 165Prt = pretreated at 165°C for 10 min, 140EH = enzymatic hydrolysis of biomass pretreated at 140°C for 30 min, and 165EH = enzymatic hydrolysis of biomass pretreated at 165°C for 10 min.

# CHAPTER 3 - PHYSICAL PROPERTIES OF PELLETS MADE FROM SORGHUM STALK, CORN STOVER, WHEAT STRAW, AND BIG BLUESTEM <sup>2</sup>

## ABSTRACT

Densification of biomass feedstocks can increase bulk density, improve storability, reduce transportation costs, and make these materials easier to handle using existing handling and storage equipment for grains. The objectives of this research were to study (1) the physical properties of pellets made from corn stover, sorghum stalk, wheat straw, and big bluestem (2) the effect of moisture content on bulk density, true density, and durability of the biomass pellets, and (3) the effect of hammer mill screen size and die thickness on bulk density, true density, and durability of the pellets. Biomass pelleting can significantly improve the bulk density from 47 to 60 kg/m<sup>3</sup> for biomass grinds to 360 to 500 kg/m<sup>3</sup> for biomass pellets. An increase in moisture level resulted in a decrease in density of the pellets. The effect of moisture content on durability of the pellets made from corn stover, wheat straw, and big bluestem showed a similar trend; the maximum durability value was 96.8% at the equilibrium moisture content (EMC) range of 9% (d.b.) to 14% (d.b.) for corn stover and wheat straw, and 9% (d.b.) to 11 % (d.b.) for big bluestem. A further increase in EMC value resulted in a decrease in pellet durability. For sorghum stalk pellets, the durability value increased initially with increased EMC and reached a maximum of 89.5% at EMC values between 14% (d.b) and 16% (d.b). Use of a larger hammer mill screen size (from 3.2 mm to 6.5 mm screen openings) resulted in significant increases of density and durability of biomass pellets, but not in significant levels. Use of a thicker die size (from 31.8 mm to 44.5 mm in thickness) resulted in significant increase of density and durability of biomass pellets.

Keywords: Bulk density, true density, durability, biomass pellets, equilibrium moisture content, equilibrium relative humidity

---

<sup>2</sup> Results have been published. K. Theerarattananoon, F. Xu, J. Wilson, R. Ballard, L. Mckinney, S. Staggenborg, P. Vadlani, Z.J. Pei, and D. Wang. 2011. Physical properties of pellets made from sorghum stalk, corn stover, wheat straw, and big bluestem. *Ind. Crop Prod.* 33: 325-332

## INTRODUCTION

Biomass-derived ethanol is used as an alternative fuel in the transportation sector. It can be used as a substitute for gasoline or blended with gasoline. Benefits of using biomass-derived ethanol include mitigation of greenhouse gas emissions and mediation of security and economic concerns related to oil imports (Dien et al., 2009). Typically, fuel ethanol is produced from sugar-based (e.g., sugar cane and sugar beet) and starch-based (e.g., corn, sorghum, wheat, potato) biomass feedstocks. Corn is the feedstock for more than 96% of current U.S. ethanol production, and sugar cane is the main feedstock for ethanol production in Brazil, the second largest ethanol producer behind the United States. Because sugar crops and grain crops are also in demand in other industries such as food and feed and given the increasing concerns about impact on land use, sources of these sugar-based and starch-based feedstocks will be limited. However, fuel ethanol can be produced from lignocellulosic biomass, which includes forest residues such as wood; agricultural residues such as sugarcane, bagasse, corn stover, sorghum biomass, wheat straw, and rice straw; industrial residues such as pulp and paper processing waste and municipal solid wastes; and energy crops such as switchgrass (Kumar et al., 2009). Lignocellulosic biomass is abundant, relatively inexpensive, and outside the human food chain; thus, it appears to be a more sustainable feedstock for ethanol production.

Forage crops normally have low bulk density when harvested. The two existing biomass harvesting systems consist of a separate shredding and windrowing (raking) followed by large rectangular baling, and a combination of shredding-windrowing followed by round baling (Sokhansanj and Turhollow, 2004). After baling, the biomass bales are transported by trucks to areas where they can be effectively processed and utilized. The bulky characteristic of biomass bales makes them difficult to transport over long distances and demands space for storage. Densification of biomass by pelleting can significantly increase the bulk density of biomass from 40-250 kg/m<sup>3</sup> up to 600-800 kg/m<sup>3</sup> (Kaliyan and Morey, 2009; Mani et al., 2003) and thus improve storability and reduce transportation costs (Kaliyan et al., 2009; Holm et al., 2006). In addition, the uniform size and shape make pelleted biomass easier to handle using existing handling and storage equipment. Information on physical properties of pellets is essential for proper selection of equipment and processing design for cellulosic ethanol production from biomass pellets (Colley et al., 2006; Zhao et al., 2008).

The physical properties of pellets include size, particle density (true density), bulk density, porosity, hardness, durability, moisture sorption rate, equilibrium moisture content, and other characteristics (Colley et al., 2006; Lam et al., 2008). Several studies have found that moisture content significantly affected the physical properties of densified biomass (Fasina and Sokhansanj, 1996; Fasina, 2008; Colley et al., 2006). Fasina and Sokhansanj (1996) studied the effect of moisture on durability of alfalfa pellets and found that pellet durability increased initially with absorbed moisture, reaching a maximum value of about 86% after absorbing about 3% to 5% (wet basis) of moisture. A further increase in absorbed moisture resulted in a decrease in durability value. Fasina (2008) studied the physical properties of peanut hull pellets and observed the same trend; peanut hull pellet durability initially increased with absorbed moisture content, reached a maximum value of 90.1% at a pellet moisture content of 9.1% (wet basis), and decreased with a further increase of absorbed moisture. Fasina (2008) also reported the moisture effect on bulk density and particle density of peanut hull pellets; an increase in moisture content of the peanut hull pellets resulted in a linear decrease in bulk density and particle density. Absorbed moisture also significantly affected physical properties of switchgrass pellets (Colley et al., 2006). As the moisture content of switchgrass pellets increased from 6.3% to 17.0% (wet basis), pellet diameter increased by 8%, length decreased by 17%, and bulk and particle densities decreased by 24% and 16% respectively.

The physical properties of biomass pellets also depends on pelleting process variables such as biomass particle size, die thickness, die geometry (length-to-diameter or L/D ratio), die speed, temperature, applied pressure, and hold time (Sun and Cheng, 2002; Larsson et al., 2007; Mani et al., 2006a; Mani et al., 2003). The study by Zhou et al. (2008) showed that corn stover density decreased with an increase in particle size. The same trend of particle size-density relationship was also observed for wheat straw and switchgrass samples studied by Lam et al. (2008). Kaliyan et al. (2009) showed that bulk density as well as durability of switchgrass briquettes, with and without steam conditions, decreased as switchgrass particle size increased from 0.49 mm to 0.59 mm. Coarsely ground materials tend to produce less durable pellets, possibly due to natural fissures that develop in the pellets making them susceptible to breakage (Mani et al., 2003; Kaliyan and Morey, 2009). Recommended particle size for good pellet quality is 0.5-0.7 mm. However, fine grinding is undesirable as it increases production cost (Kaliyan and Morey, 2009). Tabil and Sokhansanj (1996) observed an increase in durability of alfalfa pellets

as a result of increasing pellet temperature during extrusion from the pellet mill. Grover and Mishra (1996) studied the effect of temperature and pressure conditions on the bulk density of briquettes of mustard stalk, sawdust, rice husk, and groundnut shell in a hydraulic press and found that as the temperature of hydraulic press increased, a better compaction and an increase in bulk density of the briquette biomass were observed.

The objectives of this research were to study (1) the physical properties (size, bulk density, true density, and durability) of pellets made from corn stover, sorghum stalk, wheat straw, and big bluestem (2) the effect of moisture on bulk density, true density, and durability of the biomass pellets, and (3) the effect of pelleting process variables (die thickness and hammer mill screen size) on bulk density, true density, and durability of the biomass pellets.

## **MATERIALS AND METHODS**

### **Sample Preparation**

Corn stover, wheat straw, and big bluestem were obtained in the form of 1.8 × 1.2 × 1.8 m (6×4×4 ft) square bales. Photoperiod-sensitive sorghum (PS) stalk was obtained in the form of a round bale with a diameter of 1.83 m (6 ft). Photoperiod-sensitive sorghum was found to have a high biomass yield and high drought tolerance in dryland environments (Propheter et al., 2010). According to the study by Theerarattananoon et al. (2010), the high sugar yield (97%) of PS stalk obtained from using the dilute acid pretreatment (165 °C, 10 min) and enzymatic hydrolysis suggested that PS stalk has high potential for bioethanol production. The big bluestem was obtained as square bales from Star Seed in Beloit, Kansas in January 2009. Wheat, corn and sorghum biomasses were harvested by the Kansas State Agronomy Farm in November 2008 and December 2009 respectively. All biomass material was chopped to similar stem length (approximately 7-9 inches in length) by using a tub grinder (Model Haybuster H-1150 series, DaraTech Industries International, Inc., Jamestown, N.D.). The tub grinder was powered by a diesel engine which ground a large round bale in less than 30 seconds. All four biomass types were then transported to the Bioprocessing and Industrial Value Added Program (BIVAP) building located at 1980 Kimball Avenue in Manhattan, Kansas. Then the fine grindings of the chopped biomass were obtained by using a 7.4 kW (10 hp) hammer mill (Model 18-7-300, Schuttle-Buffalo Hammermill, Buffalo, N.Y.) with two different screens with 3.2 and 6.5 mm (1/8 and 3/8 in.) openings. Product was manually loaded onto a belt conveyor which fed into the

hammer mill. An air suction system and cyclone were attached to the hammer mill to remove the ground biomass. The ground biomass was kept in sealed paper bags at room temperature.

## **Pelleting**

Pelleting experiments were conducted by using a 22.1 kW (30 hp) ring-die pellet mill with 1.5 ton capacity (CPM Master model series 2000, California Pellet Mill (CPM) Co., San Francisco, Cal.). Pellets were made into three combinations as shown in Table 1. The pellet combinations were made based on two pelleting variables, including hammer mill screen size (3.2 mm and 6.5 mm screen openings) and die size (4.0 x 31.8 mm and 6.4 x 44.5 mm, hole diameter x effective thickness). The pellet mill main shaft was operated at 10,650 rpm. The feeder screw rate was 7 rpm. The pellets were made by extruding the biomass grinds through a round cross-sectional die.

Before pelleting, the moisture content of biomass grinds was adjusted to 10% (wet basis) by mixing tap water with the biomass grinds at room temperature for 2 min. No steam conditioning was used and no external binding agents were added in any of the pelleting experiments. All biomass grinds were at room temperature before entering the die. The temperature of biomass pellets exiting the die was between 74°C and 82°C. The increase in biomass temperature was due to frictional heating of the die during pelleting. The pellets were then cooled to room temperature by passing forced air through them.

## **Equilibrium Moisture Content (EMC) and Equilibrium Relative Humidity (ERH) Relationship**

Biomass materials can absorb or desorb moisture from the surrounding atmosphere during storage. Moisture content affects mechanical properties and durability of the pellets. The EMC-ERH relationship of biomass is useful in determining suitable conditions for biomass storage, such as proper design of storage silos and aeration systems (Fasina and Sokhansanj, 1996; Igathinathane et al., 2005). Biomass with reduced EMC can be stably stored over time. Furthermore, transportation costs for biomass can be reduced because less moisture is carried along with the biomass (Yan et al, 2009).

Moisture of the pellets was determined by drying about 25 g of each sample in a forced-air oven at 103°C for 24 h (ASABE standard S358.2, 2008). Moisture content was reported as a percentage on a dry basis. Equilibrium between the air and the biomass samples was obtained by



using an autocontrol environmental chamber (Model 518, Electro-tech systems Inc., Glenside, Penn.) to force air around the samples at a fixed relative air humidity and temperature. The air temperature used for all samples was 25°C. The working range for air relative humidity was 11% to 98%. The samples were weighed at intervals of 3 to 5 days until a constant weight was achieved. After completion of the equilibrating period, the EMC of the samples for each level of humidity was calculated according to the ASAE S358.2 (2003) method as follows:

$$EMC(\text{dry base \%}) = \frac{|W_1 - W_2|}{W_2} \times 100\%$$

where,  $W_1$  is the weight of the biomass until a constant weight (g) and  $W_2$  is the dry matter of the biomass (g). Physical properties of pellets (durability, bulk density, and true density) were determined immediately after the pellets were removed from the environmental chamber.

### **Size, Bulk Density, and True Density of Pellets**

Pellet size was measured with a digital caliper (0.01 mm resolution, model CD-56C, Mitutoyo Corp., Aurora, Ill.). Reported values are the average of 10 measurements for each biomass sample.

Bulk density of the pellets was determined according to ASABE Standard S269.4 (2007) for cubes, pellets, and crumbles. Pellets were poured into a cylindrical container from a certain height to facilitate free flow of the samples until the container overflowed. The excess material was removed by sliding a straight edge across the top of the container. Net weight of the pellets was obtained by subtracting the weight of the empty container from the combined weight of the pellets and container. Bulk density was calculated by dividing the mass by the container volume. Reported values are the average of three measurements for each type of pellet.

True density of the pellets was calculated by dividing the mass by the volume of individual pellets. The solid (rapeseed) displacement method was used to determine the volume of individual pellets. The pellets were put inside a cylindrical container and covered with rapeseed. Pellet volume was equal to the volume of rapeseed displaced. Reported values are the average of three measurements for each type of pellet.

### **Durability of Pellets**

Durability, defined as the ability of pellets to withstand destructive loads and force during transport, is the most important physical quality of a pellet (Tabil and Sokhansanj, 1996).

Durability is considered high when the computed value is above 80%, medium when the value is between 70% and 80%, and low when the value is below 70% (Colley et al., 2006). Low pellet durability is not desirable as it can cause problems such as disturbance within pellet feeding systems, dust emissions, and an increased risk of fire explosion during pellet handling and storage.

Pellet durability was determined according to ASABE standard S269.4 (2007). A sample of pellets was sieved on a No. 6 U.S. sieve with aperture size of 3.36 mm to remove fines. A 100-g sample of sieved pellets was tumbled at 50 rpm for 10 min. in a dust-tight enclosure. After tumbling, the sample was removed and sieved, and the percentage of whole pellets was calculated using the following formula:

$$\text{Durability (\%)} = \frac{\text{Mass of pellets after tumbling}}{\text{Mass of pellets before tumbling}} \times 100\%$$

### **Data Analysis**

Except for measurement of pellet size, all experiments for each biomass sample were carried out in triplicate. Data were analyzed by using the analysis of variance (ANOVA) and least-significant difference (LSD) at the 0.05 level procedures in SAS statistical software package (SAS Institute 2005, Cary, N.C.).

## **RESULTS AND DISCUSSION**

### **EMC-ERH Relationship**

Typically, the plot of EMC-ERH relationship for biological materials is sigmoidal in shape; EMC of biomass increases as ERH increases (Fasina, 2008). For example, the EMC-ERH plots of corn stalk, briquettes, and woody sawmill waste showed an increase in EMC from 4% to 12–16% as the ERH value increased from 47% to 78% (Singh, 2004). Similarly, results from the present study (Figure 3.1) showed that the EMC values of pellets of corn, wheat straw, big bluestem, and sorghum stalk were proportional to ERH and that EMC-ERH relationships were similar for all biomass samples.

## Effect of Moisture on Size and Density of Pellets

The pellets of wheat straw, big bluestem, corn stover, and sorghum stalk were in cylindrical form. The average diameter of these pellets was about 4.1 to 4.2 mm, and the average length ranged from 9 to 15 mm (Table 3.1).

Bulk density values of the four biomass grinds ranged from 46 to 60 kg/m<sup>3</sup> (table 2). Sorghum stalk grind had the highest bulk density value of 59.3 kg/m<sup>3</sup>. The bulk density values of biomass grinds obtained in this study were in the range of published data; bulk density of wheat straw and corn stover ranged from 24 to 52 kg/m<sup>3</sup> and from 40 to 80 kg/m<sup>3</sup> respectively, depending on particle size and moisture content (Lam et al., 2008; Mani et al., 2006b).

Pelletization of biomass could significantly increase bulk density values. For example, Colley et al. (2006) reported that bulk density of ground switchgrass was 165.5 kg/m<sup>3</sup> and that bulk density of switchgrass pellets at 5% to 20% (wet basis) moisture content ranged from 536 to 708 kg/m<sup>3</sup>. In the present study, the bulk density values of pellets of wheat straw, corn stover, big bluestem, and sorghum stalk ranged from 360 to 500 kg/m<sup>3</sup>, significantly (about 6 to 10 times) higher than the bulk density values before pelleting. Sorghum stalk and wheat straw pellets had the lowest and highest bulk density values respectively (Table 3.2). True density values of biomass pellets, which ranged from 430 to 617 kg/m<sup>3</sup>, were always higher than bulk density values because the volume of voids was excluded from the calculation.

Colley et al. (2006) studied the effects of moisture content on physical characteristics of switchgrass pellets and found that bulk density of switchgrass pellets decreased as moisture content of the pellets increased. Results from the present study followed the same trend; bulk density of the four types of biomass pellets (corn stover, sorghum stalk, wheat straw, and big bluestem) decreased with an increase in moisture content (expressed in terms of relative humidity; Figure 3.2). In other words, pellets with relatively high moisture content had a low bulk density value (Table 3.2). Sorghum stalk pellets had the highest moisture content and lowest bulk density, whereas wheat straw pellets had the lowest moisture content and highest bulk density. True density values of the biomass pellets also decreased with an increase in moisture content (Figure 3.3). Bulk density and true density of biomass pellets decreased with an increase in moisture content because pellets increased in volume when they absorbed more moisture. At any EMC value, big bluestem pellets had the highest bulk density and true density values, followed by pellets of corn stover, wheat straw, and sorghum stalk.

## **Effect of Moisture on Durability of Pellets**

Densification of biomass basically creates a more dense and durable product. Several studies have reported that the durability and strength of densified biomass increase with increasing moisture content until an optimum is reached (Kaliyan and Morey, 2009). Optimum moisture content of the feedstock is required to produce stable and durable pellets. The optimum moisture content for biomass to be pelletized is usually in the range of 8% to 12%. Colley et al. (2006) studied the effects of moisture content on the physical characteristics of switchgrass pellets and found that the pellets with 8.6% (wet basis) moisture content had the highest durability (95.9%). The effect of moisture content on the durability of biomass pellets in this study (corn stover, wheat straw, big bluestem, and sorghum stalk) is presented in Figure 3.4.

The maximum durability values of pellets of wheat straw, and corn stover were approximately 96.8% and remained steady in the EMC range of 9% (d.b.) to 14% (d.b.). In other words, an increase in moisture content from 9% (d.b.) to 14% (d.b.) did not affect pellet durability. However, increasing the moisture content beyond 14% (d.b.) reduced pellet durability. A similar trend in the durability-EMC relationship was observed for big bluestem pellets; the durability value of big bluestem pellet was highest at 96.8% in the EMC range of 9% (d.b.) to 11% (d.b.) and the value tended to decrease as moisture content increased beyond 11% (d.b.). This is in agreement with results from Tabil (1996), who studied the effect of high-humidity storage on alfalfa pellet durability and hardness. Tabil (1996) found that an increase in moisture content of the pellets from 6.3% (w.b.) to 10% (w.b.) did not affect pellet durability, but pellet durability decreased as moisture content of the pellets increased beyond 12% to 14% (w.b.).

Unlike pellets of corn stover, big bluestem, and wheat straw, the durability value of sorghum stalk pellets increased initially with EMC value and reached a maximum of 89.5% at EMC values between 14% (d.b.) and 16% (d.b.). Fasina (2008) conducted a study on physical properties of peanut hull pellets and found that the durability of the peanut hull pellets increased initially with moisture content; reached a maximum value of 90.3% at 9.1% (w.b.) moisture and decreased as the moisture content increased beyond 9.1% (w.b.). The initial increase in durability with an increase in moisture was probably due to the binding forces of the water molecules that strengthened the bonds between individual particles in the pellets. Further increases in moisture resulted in swelling and disintegration of the pellets. No capillary force was present to maintain

the pellet structure and thus left the pellets with cracks which made them susceptible to breakage (Singh, 2004; Fasina and Sokhansanj, 1996; Fasina, 2008).

### **Effect of Die Size and Hammer Mill Screen Size on Density and Durability of Pellets**

To investigate the effect of hammer mill screen size (or particle size of biomass grinds) on physical properties of biomass pellets, two hammer mill screens with the screen opening of 3.2 mm and 6.5 mm were used during fine grinding of biomass. These biomass grinds of corn stover, sorghum stalk, wheat straw, and big bluestem were then pelleted using the die with a thickness of 44.5 mm. The pellets of these biomass samples were in cylindrical form. According to Table 3.1, the average diameter of these pellets obtained from using a 3.2 mm hammer mill screen size was about 6.6-6.8 mm and the average length varied from 18-21 mm. The smaller size of pellets (6.4-6.7 mm in diameter, 14-16 mm in length) was obtained when using a larger hammer mill screen size of 6.5 mm.

To investigate the effect of die size on physical properties of biomass pellets, two dies with thickness of 31.8 mm and 44.5 mm were used during the biomass pelleting process. Prior to the pelleting step, all of the biomass samples (wheat straw, big bluestem, corn stover, and sorghum stalk) were ground through a 3.2 mm hammer mill screen. According to Table 3.1, the average diameter of pellets from a 31.8 mm die was about 4.1-4.3 mm and the average length varied from 9 to 15 mm. Larger pellets with average diameter of 6.5 -7.0 mm and average length of 16-22 mm were obtained when using a thicker die.

According to Table 3.2, all biomass pellets had a durability value above 80%, indicating that the pellets were very durable. Among the four types of biomass, sorghum stalk pellets had the lowest durability value. For the effect of biomass particle size on pellet durability, Kaliyan and Morey (2009) claimed that finely ground biomass tended to give more durable pellets than the coarsely ground as the coarsely ground biomass would act as predetermined breaking points in the pellets. However, Tabil and Sokhansanj (1996) observed the trend of an increase in alfalfa pellet durability when using a larger hammer mill screen. Due to the small difference in the screen size (3.2 mm and 6.5 mm) and the variability of durability, they concluded that the use of different hammer mill screen size resulted in no significant difference to durability of the alfalfa pellet. The result in this study followed the same trend as the study by Tabil and Sokhansanj (1996). In this study, using a larger hammer mill screen size (6.5 mm instead of 3.2 mm) resulted

in higher pellet durability values but not in a significant level for all four types of biomass (Table 3.2).

The use of a thicker die was found to significantly enhance durability of wheat straw pellet, corn stover pellet, and sorghum stalk pellet (Table 3.2). This result followed the same trend as the experimental result from the study by Behnke (1990). For big bluestem, the increase in pellet durability due to the use of thicker die was not significant.

Densification of biomass could result in a significant increase in the bulk density of biomass. According to Table 3.2, the bulk density of wheat straw, big bluestem, corn stover, and sorghum stalk significantly increased (about 9 to 12 fold) after the pelleting process. This increase in bulk density of the biomass could definitely improve ease of handling. Kaliyan and Morey (2009) reported that the factors that increase pellet durability would also increase pellet density although the relationship between durability and density of biomass pellets was still unknown. In this study, sorghum stalk pellet that had the lowest pellet durability also had the lowest bulk density and true density values as compared to the other types of biomass of any pelleting condition. Although not in a significant level, the use of a larger hammer mill screen size resulted in an increase in durability, bulk density and true density of the pellet. The use of a thicker die resulted in a significant increase in pellet durability and density. In another words, the effect of die size on durability and density of biomass pellet was more pronounced than the effect of hammer mill screen size (Table 3.2).

## **CONCLUSIONS**

In this study, we obtained data on the physical properties (i.e., bulk density, true density, and durability) of four varieties of biomass pellets (i.e., corn stover, wheat straw, big bluestem, and sorghum stalk). The pelleting process resulted in a significant increase in bulk density of these pellets, from 46 to 60 kg/m<sup>3</sup> to 360 to 500 kg/m<sup>3</sup>. The strong, uniformed-sized of biomass pellets with high bulk density makes them easier handle, transport, and store. In addition, the amount of space required for biomass storage will be reduced because of the increase in bulk density. Moisture content of pellets had some effect on their physical properties. As the moisture content of pellets increased, bulk density and true density values decreased because the pellets expanded. For pellets of corn stover and wheat straw, the increase in moisture content from 9% (d.b.) to 14% (d.b.) did not affect durability. However, increasing the moisture content beyond

14% (d.b.) reduced the durability of these pellets. For big bluestem pellet, the increase in moisture content from 9% (d.b.) to 11% (d.b.) did not affect durability but the durability decreased as the moisture content increased beyond 11% (d.b.). For sorghum stalk pellets, the durability value increased initially with moisture and reached a maximum value of 89.5% at 14% (d.b.) to 16% (d.b.) moisture content. Therefore, determination of optimum moisture content of the pellets, which varied by biomass type, was required for production of stable and durable pellets and for the selection of suitable storage conditions for the biomass pellets.

The physical properties of biomass pellets also varied by particle size of biomass and die thickness. More durable pellets were obtained when a larger hammer mill screen size was used during biomass (feed) grinding step. However, the effect of using a larger hammer mill screen size (6.5 mm screen openings instead of 3.2 mm) on pellet durability was not significant for all four types of selected biomass. Use of a thicker die (44.5 mm instead of 31.8 mm die thickness) resulted in a significant increase to pellet durability for wheat straw pellets, corn stover pellets, and sorghum stalk pellets. For big bluestem pellets, the effect of die thickness on pellet durability was not significant. Bulk density values of all four types of biomass were found to increase about 9 to 12 fold after pelleting process. Use of a larger hammer mill screen size resulted in an increase in bulk density and true density of pellets, but not to a significant level. An increase of die thickness produced a significant positive effect on both bulk density and true density of biomass pellets.

## **ACKNOWLEDGEMENT**

This project was funded by the Biomass Research and Development Initiative Competitive Grants Program (BRDI), USDA National Institute of Food and Agriculture, Grant No. 68-3A75-7-609.

## **REFERENCES**

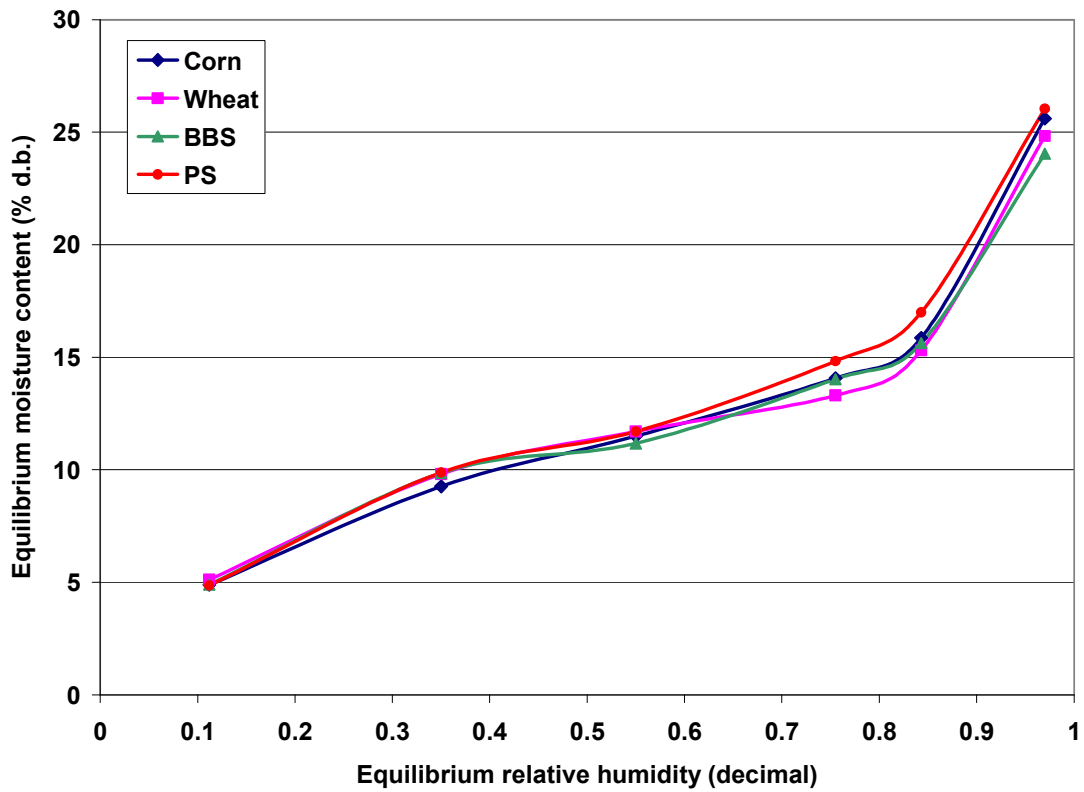
- ASABE Standard S269.4, 2007. Cubes, pellets, and crumbles: Definitions and methods for determining density, durability, and moisture content. ASAE. St. Joseph, Michigan.
- ASABE Standard S358.2, 2008. Moisture Measurement-forages. ASAE. St. Joseph, Michigan.

- Colley, Z., Fasina, O.O., Bransby, D., Lee, Y.Y., 2006. Moisture effect on the physical characteristics of switchgrass pellets. *T ASAE*. 49, 1845-1851.
- Dien, B. S., Jung, H.-J. G., Vogel, K. P., Casler, M. D., Lamb, J. F. S., Iten, L. B., Mitchell, R. B., Sarath, G., 2006. Chemical composition and response to dilute-acid pretreatment and enzymatic saccharification of alfalfa, reed canarygrass, and switchgrass. *Biomass Bioenerg.* 30, 880-891.
- Fasina, O.O., 2008. Physical properties of peanut hull pellets. *Bioresource Technol.* 99, 1259-1266.
- Fasina, O.O., Sokhansanj, S., 1996. Storage and handling characteristics of alfalfa pellets. *Powder Handling & Processing*. 8, 361-365.
- Grover, P.D., Mishra, S.K., 1996. Biomass briquetting: Technology and practices. FAO regional wood energy world development programme in Asia, Bangkok, Thailand.
- Holm, J.K., Henriksen, U.B., Hustad, J.E., Sorensen, L.H., 2006. Toward an understanding of controlling parameters in softwood and hardwood pellet production. *Energy & Fuels*. 20, 2686-2694.
- Igathinathane, C., Womac, A.R., Sokhansanj, S., Pordesimo, L.O., 2005. Sorption equilibrium moisture characteristics of selected corn stover components. *T ASAE*. 48, 1449-1460.
- Kaliyan, N., Morey, R.V., 2009. Factors affecting strength and durability of densified biomass products. *Biomass Bioenerg.* 33, 337-359.
- Kaliyan, N., Morey, R.V., White, M.D., Doering, A., 2009. Roll-press briquetting and pelleting of corn stover and switchgrass. *T ASAE*. 52, 543-555.
- Kumar, R., Singh, S.P., Mishra, I.M., Adhikari, D.K., 2009. Recent advances in production of bioethanol from lignocellulosic biomass. *Chem. Eng. Technol.* 32, 517-526.

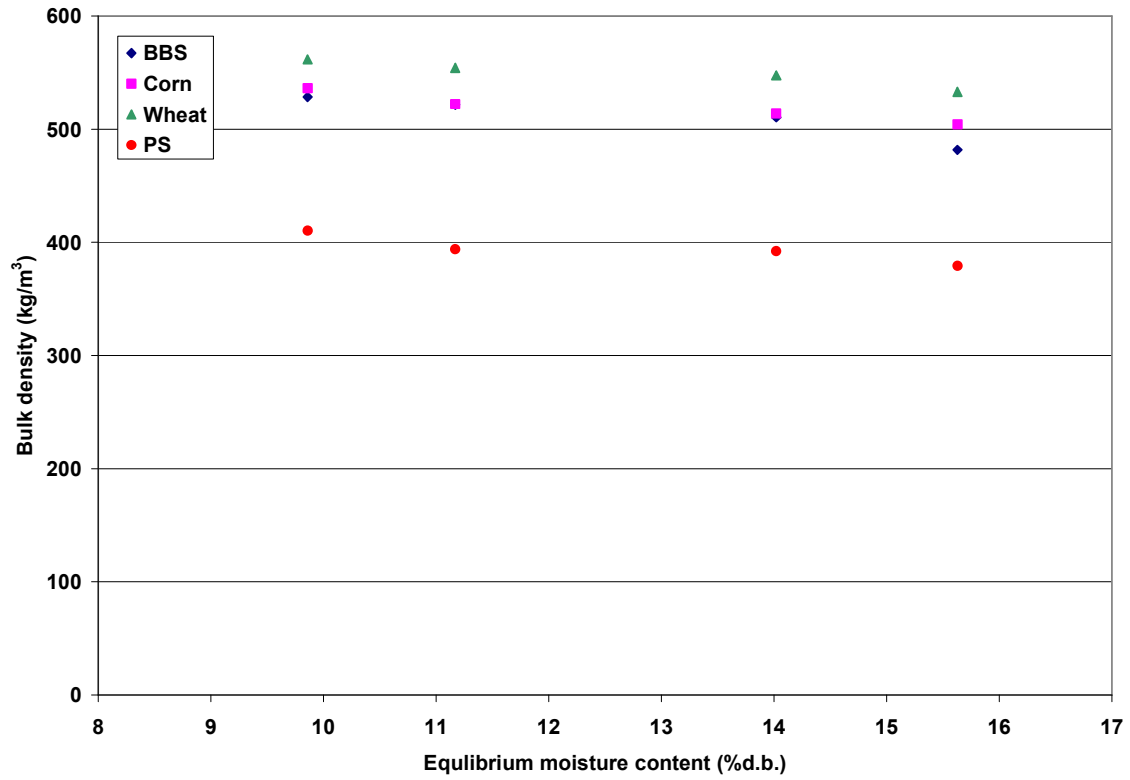


- Lam, P.S., Sokhansanj, S., Bi, X., Lim, C.J., Naimi, L.J., Hoque, M., Mani, S., Womac, A.R., Ye, X.P., Narayan, S., 2008. Bulk density of wet and dry wheat straw and switchgrass particles. *Appl. Eng. Agric.* 24, 351-358.
- Larsson, S.H., Thyrel, M., Geladi, P., Lestander, T.A., 2008. High-quality biofuel pellet production from pre-compacted, low-density raw materials. *Bioresource Tech.* 99, 7176-7182.
- Mani, S., Tabil, L.G., Sokhansanj, S., 2003. An overview of compaction of biomass grinds. *Powder Handling&Processing.* 15, 160-168.
- Mani, S., Sokhansanj, S., Bi, X., Turhollow, A. 2006a. Economics of producing fuel pellets from biomass. *Appl Eng Agric.* 22, 421-426
- Mani, S., Tabil, L.G., Sokhansanj, S., 2006b. Specific energy requirements for compacting corn stover. *Bioresource Technol.* 97, 1420-1426.
- Propheter, J.L., Staggenborg, S.A., We, X., Wang, D., 2010. Performance of annual and perennial biofuel crops: yield during the first two years. *Agron J.* 102, 806-814.
- Singh, R.N., 2004. Equilibrium moisture content of biomass briquettes. *Biomass Bioenerg.* 26, 251-253.
- Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Crocker, D., 2008. Determination of structural carbohydrates and lignin in biomass: NREL/TP-510-42618. National Renewable Energy Laboratory (NREL). Golden, Colorado.
- Sokhansanj, S., Turhollow, A.F., 2004. Biomass densification-cubing operations and costs for corn stover. *Appl. Eng. Agric.* 20, 495-499.
- Sun, Y., Cheng, J., 2002. Hydrolysis of lignocellulosic materials for ethanol production: A review. *Bioresource Tech* 83, 1-11.

- Tabil, L.G., 1996. Binding and pelleting characteristics of alfalfa. PhD diss. Department of Agricultural and Bioresource Engineering, University of Saskatchewan ,Saskatoon, Saskatchewan, Canada.
- Theerarattananoon, K., Wu, X., Staggenborg, S., Propheter, J., Madl, R., Wang, D., 2010. Evaluation and characterization of sorghum biomass as feedstock for sugar production. T ASAE. 53, 509-525.
- Yan, W., Acharjee, T.C., Coronella, C.J., Vasquez, V.R., 2009. Thermal pretreatment of lignocellulosic biomass. AIChE. 28, 435-440.
- Zhao B., Ileleji, K.E., Ejeta, G., 2008. Physical property relationships of bulk corn stover particles. T ASAE. 51,581-590.

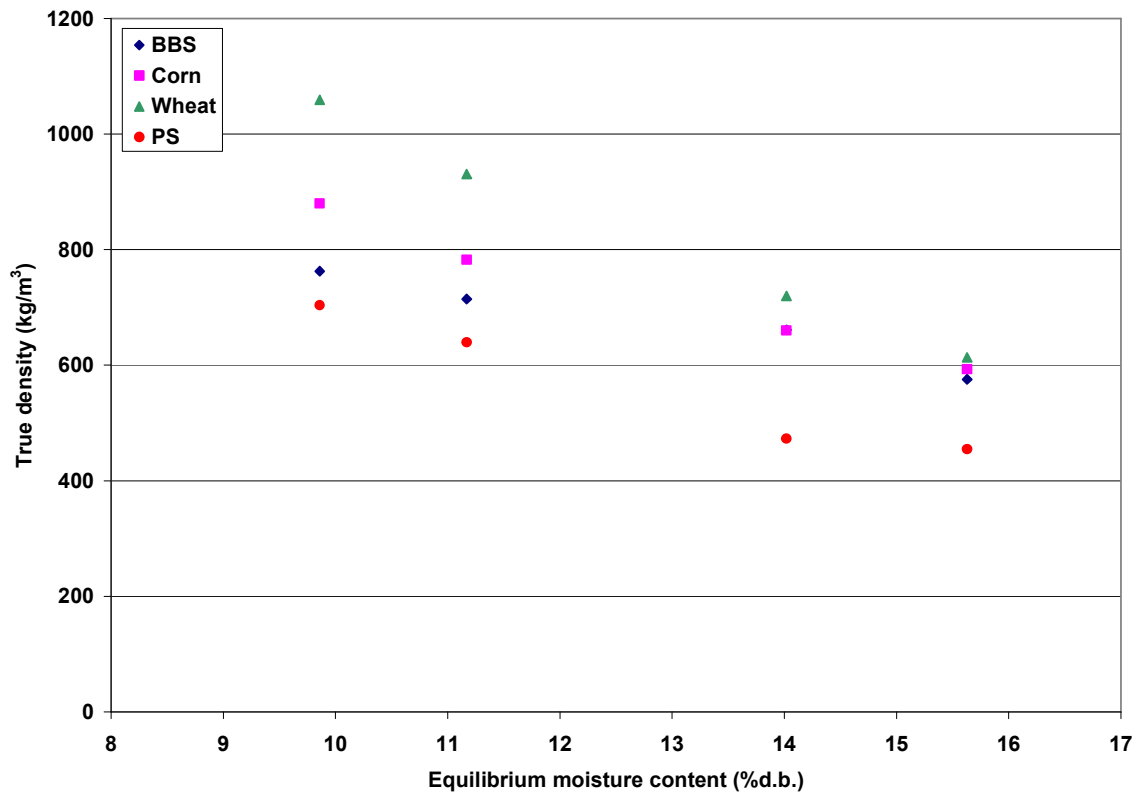


**Figure 3.1 Equilibrium moisture content and equilibrium relative humidity of corn stover, sorghum stalk, big bluestem, and wheat straw pellets. Pellets were made at pelleting condition of 3.2 mm screen size and 31.8 mm die thickness**

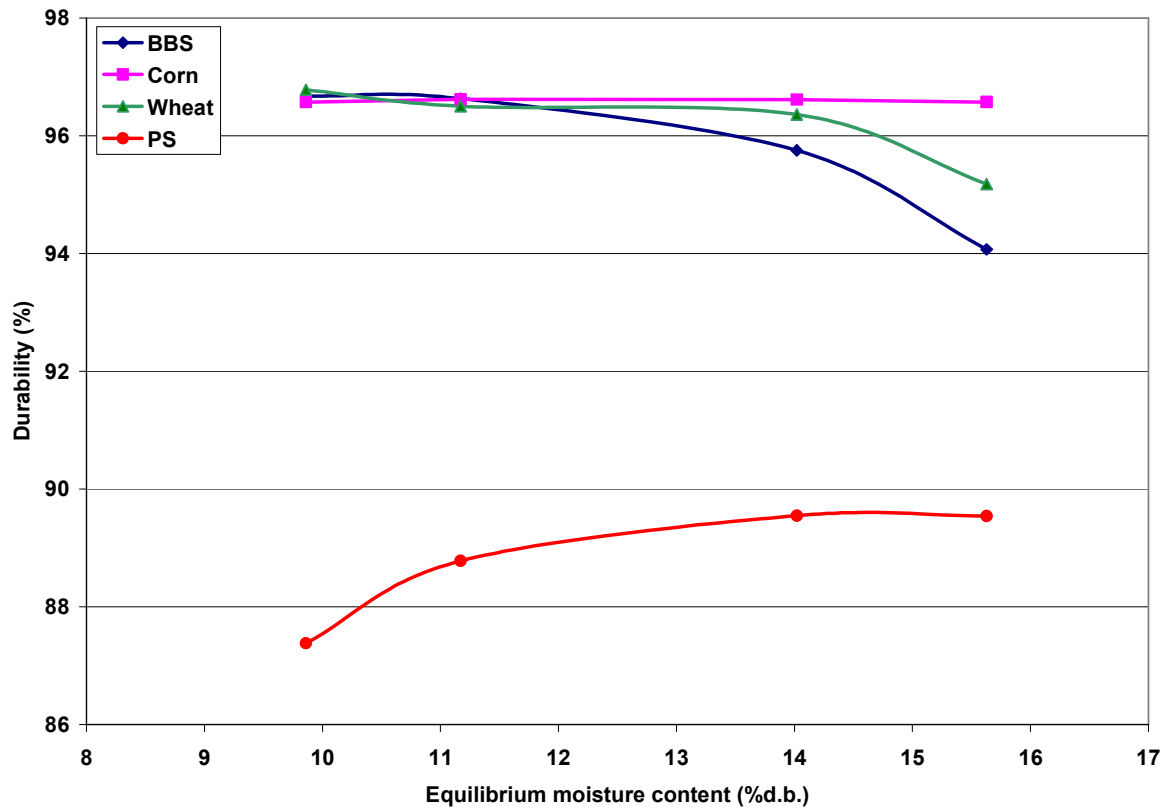


**Figure 3.2 Effect of equilibrium moisture content on bulk density values of pellets of wheat straw, big bluestem (BB), corn stover, and photoperiod-sensitive sorghum stalk (PS).**

**Pellets were made at pelleting condition of 3.2 mm screen size and 31.8 mm die thickness**



**Figure 3.3 Effect of equilibrium moisture content on true density values of pellets of wheat straw, big bluestem (BB), corn stover, and photoperiod-sensitive sorghum stalk (PS). Pellets were made at pelleting condition of 3.2 mm screen size and 31.8 mm die thickness**



**Figure 3.4 Effect of equilibrium moisture content on durability of pellets of wheat straw, big bluestem (BB), corn stover, and photoperiod-sensitive sorghum stalk (PS). Pellets were made at pelleting condition of 3.2 mm screen size and 31.8 mm die thickness**

**Table 3.1 Size of biomass pellets as affected by mill screen size, die thickness, and L/D ratio of the die**

Biomass Feedstock	Pelleting Conditions			Dimension of pellets (mean±SD)	
	Hammer Mill Screen Size (mm)	Die Thickness (mm)	L/D ratio	Diameter (mm)	Length (mm)
wheat straw pellet	3.2	31.8	8	4.24±0.14	12.9±1.59
	3.2	44.5	7	6.73±0.21	20.7±2.23
	6.5	44.5	7	6.47±0.06	14.7±2.67
big bluestem pellet	3.2	31.8	8	4.17±0.10	11.7±2.04
	3.2	44.5	7	6.62±0.13	18.4±2.28
	6.5	44.5	7	6.65±0.12	15.1±1.29
corn stover pellet	3.2	31.8	8	4.14±0.09	12.6±0.84
	3.2	44.5	7	6.65±0.08	20.2±1.94
	6.5	44.5	7	6.48±0.05	15.8±1.35
sorghum stalk pellet	3.2	31.8	8	4.12±0.06	13.3±1.86
	3.2	44.5	7	6.91±0.25	18.6±1.92
	6.5	44.5	7	6.51±0.15	15.6±1.49

**Table 3.2 Physical properties of biomass pellets as affected by mill screen size, die thickness, and L/D ratio of the die**

Biomass Feedstock	Pelleting Conditions			Properties of Pellets		
	Hammer Mill Screen Size (mm)	Die Thickness (mm)	L/D ratio	Bulk Density (kg/m <sup>3</sup> )	True Density (kg/m <sup>3</sup> )	Durability
chopped wheat straw	NA	NA	NA	47.7 a	NA	NA
wheat straw pellet	3.2	31.8	8	495.8 b	920.7 a	95.8 a
	3.2	44.5	7	554.1 c	1067.3 b	97.4 b
	6.5	44.5	7	649.2 d	1161.6 c	98.3 b
chopped big bluestem	NA	NA	NA	46.6 a	NA	NA
big bluestem pellet	3.2	31.8	8	467.4 b	772.6 a	95.7 a
	3.2	44.5	7	601.9 c	924.8 b	96.9 a
	6.5	44.5	7	618.0 d	1161.2 c	97.6 a
chopped corn stover	NA	NA	NA	50.9 a	NA	NA
corn stover pellet	3.2	31.8	8	469.1 b	895.3 a	96.4 a
	3.2	44.5	7	597.9 c	988.7 b	98.2 b
	6.5	44.5	7	624.6 d	1127.4 c	97.9 b
chopped sorghum stalk	NA	NA	NA	59.3 a	NA	NA
sorghum stalk pellet	3.2	31.8	8	365.2 b	597.2 a	85.7 a
	3.2	44.5	7	434.0 c	620.7 b	92.2 b
	6.5	44.5	7	478.6 d	713.8 c	93.5 b

<sup>[a]</sup> Means in the same biomass followed by different letters are significantly different at  $p < 0.05$



# **CHAPTER 4 - EFFECTS OF THE PELLETING CONDITIONS ON CHEMICAL COMPOSITION AND SUGAR YIELD OF CORN STOVER, BIG BLUESTEM, WHEAT STRAW, AND SORGHUM STALK PELLETS <sup>3</sup>**

## **ABSTRACT**

Pelleting of biomass can increase their bulk density and thus improve storability and reduce transportation costs. The objective of this research was to determine the effects of the pelleting conditions on chemical composition and fermentable sugars yield of the biomass. Corn stover, wheat straw, big bluestem and sorghum stalks were used for this study. Dilute sulfuric acid was used for biomass pretreatment. Accellerase 1500<sup>TM</sup> was used for cellulose hydrolysis. Effects of mill screen size, die thickness, and L/D ratio of die on chemical compositions and sugar yield were determined. Glucan content of the biomass was positively affected by die thickness and negatively affected by mill screen size. Opposite trend was observed for xylan content. Wheat straw pellets had the highest sugar yield (92.5% to 94.1%) and big bluestem pellets had the lowest sugar yield (83.6% to 91.1%). Optimum pelleting conditions is 6.5 mm screen size and 44.5 mm die thickness.

Keywords: Pellets, pretreatment, mill screen size, die thickness, sugar yield

## **INTRODUCTION**

More than 140 billion gallons of transportation fuels are consumed annually in the United States. Bioethanol is a promising alternative energy source for transportation fuel. Bioethanol's benefits include mitigation of greenhouse gas emissions and mediation of security and economic concerns related to oil imports [1]. The United States government has established the goal that biomass will supply 5% of the nation's power, 20% of its transportation fuels (~36 billion gallons) and 25% of its chemicals by 2030. A consistent supply of high-quality, low-cost feedstock is vital to achieving this goal and will help the biorefining industry become more

---

<sup>3</sup> Results have been published. Karnnalin Theerarattananoon, Feng Xu, Jonathan Wilson, Scott Staggenborg, Leland Mckinney, Praveen Vadlani, Zhijian Pei, and Donghai Wang. 2011. Effects of the pelleting conditions on chemical composition and sugar yield of corn stover, big bluestem, wheat straw, and sorghum stalk pellets. *Bioprocess and Biosystems Engineering*. Published online, 11 October 2011.

sustainable than the fossil-fuel systems it replaces. However, one of the most important challenges of establishing lignocellulosic biorefining as a self-sustainable enterprise is designing a logistics system that supplies feedstocks in a way that provides the needed quantity as well as quality of biomass while maintaining the economic and ecological viability of supply system infrastructures. The major problems with current biomass supply systems are the high costs of biomass harvest, transportation and storage, all of which can affect downstream bioconversion processes and biofuel yields.

Forage and agricultural residues typically are stored in bale form with low bulk density [2, 3]. For example, bulk densities for wheat straw, switchgrass, and corn stover are from 24 to 53 kg/m<sup>3</sup>, from 49 to 161 kg/m<sup>3</sup>, and from 40 to 80 kg/m<sup>3</sup>, respectively, depending on particle size and moisture content [4, 5]. The low bulk density of these biomasses makes them difficult to handle and transport over long distances. The use of forage handling equipment in the biomass energy system is severely limited by the low bulk densities of baled and ground biomass. Although grinding the biomass can increase bulk density compared with baled biomass, ground biomass remains difficult to store and handle at the biorefinery because its physical properties prevent it from flowing properly during unloading, storage and transfer operations. Flow characteristics are likely to force biorefineries to install additional conveyor systems; other costs associated with handling low bulk density feedstock include storage facilities to handle light material. The cost associated with these systems and facilities along with the labor needed to operate them could be reduced or eliminated if the bulk density of the feedstock could be increased prior to delivery. Densification is one way to maintain the economic and ecological viability of the biomass supply system infrastructure. The increase in bulk density together with the uniform size and shape of biomass pellets should result in improved handling efficiencies, storability and reduced transportation and handling costs [6]. Pelleted biomass has flowability characteristics similar to cereal grains and thus can be handled and transported with existing grain-handling equipment in the field, on the road, and at biorefinery plants [3, 7].

Pelleting is an agglomeration of small particles into larger particles by means of 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 the ground biomass through a die [2-3, 8]. Size reduction of the biomass feedstock is accomplished by processing the biomass in one or two steps using grinders, choppers, and

hammer mills. For agricultural residues received in bale form, size reduction is accomplished with a combination of bale grinders and hammer mills. Hammer mills are normally equipped with a screen size of 3.2 to 6.4 mm [9]. The ground biomass is extruded through a round or square cross-sectional die [8]. Diameter of the die usually varies from 4 to 12 mm or even larger [2]. After compaction, the pellets usually exit the die at high temperatures, ranging from 70 °C to 90 °C, as a result of the frictional heat generated during extrusion and material preheating. The pellets are cooled to within 5 °C of the ambient temperature and to within 0.5% of the original moisture content of the feed before the conditioner. If pellets are not properly cooled, their durability may decrease due to stresses between the outer layer and the warmer center, which induces cracks in the pellets.

Because pelleting of biomass involves heat and pressure and results in creation of heat during the extrusion process, these conditions may affect fermentable sugar yield or require additional preprocessing steps. Although some published results relate to biomass pelleting, they are mostly focused on economic analysis [9-11]. Published literature focusing on the effect of the pelleting process on downstream processing such as pretreatment and fermentation is limited; studying the effects of pelleting factors such as steam temperature, pelleting pressure, type of binders, die size, the L/D ratio of the die, and particle size of biomass on chemical compositions and fermentable sugar yield of the pellets is important to final ethanol yield and fermentation efficiency. Therefore, the objective of this research was to determine the effects of the pelleting process and pelleting factors on chemical composition and fermentable sugar yield of the biomass.

## **MATERIALS AND METHODS**

### **Materials**

Corn stover, wheat straw, and big bluestem packed in square bales (6 x 4 x 4 ft) and photoperiod-sensitive sorghum stalk packed in a round bale with a diameter of 6 ft were used for this research. Wheat, corn and sorghum were harvested at the Kansas State Agronomy Farm in November of 2008. The big bluestem bales were swathed and baled in Beloit, Kansas, by Doug Thiessen in January 2009. All biomass bales were chopped to a similar stem length (approximately 17 to 23 cm) by using a tub grinder (Model Haybuster H-1150 series, DaraTech

Industries International, Inc., Jamestown, N.D.). The tub grinder was powered by a diesel engine and ground a large round bale in under 30 sec. All four biomass types were then transported to the Bioprocessing and Industrial Value Added Program (BIVAP) building located at 1980 Kimball Avenue in Manhattan, KS. Fine grindings of the chopped biomass were obtained by using a 7.4 kW (10 hp) hammer mill (Model 18-7-300, Schutte-Buffalo Hammermill, Buffalo, N.Y.) with two different screens with 3.2 and 6.5 mm (1/8 and 3/8 in.) openings. The product was manually loaded onto a belt conveyor, which fed into the hammer mill. An air suction system and cyclone were attached to the hammer mill to remove the ground biomass, which was stored in sealed paper bags at room temperature.

Pelleting experiments were conducted using a 22.1 kW (30 hp) ring-die pellet mill with 1.5 ton capacity (CPM Master model series 2000, California Pellet Mill (CPM) Co., San Francisco, CA). Two die sizes (hole diameter x effective thickness) used in this study were 4.0 x 31.8 mm and 6.4 x 44.5 mm. To verify the effect of the pelleting process on downstream processing, the biomass before pelleting process was used as the control sample. Before pelleting, the moisture content of ground biomass was adjusted by mixing the mass of water equivalent to 10% room temperature water with the ground biomass for 2 min. No steam conditioning was done on and no external binding agents were added to any of the pelleting experiments. The temperature of biomass pellets exiting the die was 74 °C to 82 °C due to frictional heating of the die during pelleting. The pellets were cooled to room temperature through forced air.

All chemicals used for this research were purchased from Sigma Chemical Co. (St. Louis, MO). Accellerase 1500<sup>TM</sup> (formally known as Danisco, US Inc., Genencor Division, Rochester, N.Y.) enzyme complex was used for hydrolyzing sorghum biomass into sugars. This enzyme complex contains multiple enzyme activities; mainly exoglucanase, endoglucanase (2200-2800 CMC U/g (minimum), hemi-cellulase and beta-glucosidase (525-778 pNPG U/g (minimum)). Exoglucanase activity was reported in carboxymethylcellulose (CMC U) activity units. One CMC U unit of activity liberated 1  $\mu$ mol of reducing sugars in one minute under specific assay conditions of 50 °C and pH 4.8. Beta-glucosidase was reported in pNPG units. One pNPG unit denotes 1  $\mu$ mol of Nitrophenol liberated from para-nitrophenyl-B-D-glucopyranoside in 10 min at 50 °C and pH 4.8.

## **Dilute Acid Pretreatment**

Pretreatment was carried out in a Parr pressure reactor (Parr Instrument Company, Moline, IL) with a 1 L reaction vessel. The ground biomass samples were mixed with diluted sulfuric acid (2% w/v) to obtain 10% solid content (approximately 53 g in 500 mL diluted sulfuric acid solution). Biomass slurries were loaded in a reactor and treated at 140 °C for 30 min. For biomass pellets, the pellets were dissolved in the 2% (w/v) sulfuric acid solution to obtain biomass slurries before being loaded in the reactor. Pretreated biomass was washed with hot distilled water and centrifuged three times to remove dissolved sugars and sulfuric acid. The supernatants were collected into a 2 L volumetric flask. A portion of the supernatant was neutralized with CaCO<sub>3</sub> and further analyzed for glucose and pentose content by using a high-performance liquid chromatograph (HPLC) with a Rezex RCM column (Phenomenex, CA). More detail of HPLC was included in the analytical methods section. Because hemicellulose is a polymer of hexose and pentose, glucose in the supernatant was considered to be from hydrolysis of both cellulose and hemicellulose, and pentose was counted as sugars released from hydrolysis of hemicellulose. Washed biomass samples were split into two portions. One portion was used for moisture content and chemical composition analyses; the other portion was used for subsequent enzymatic hydrolysis.

## **Enzymatic Hydrolysis**

Pretreated biomass samples were enzymatically hydrolyzed in solution with sodium acetate buffer (50 mM, pH 4.8) and 0.02% (w/v) sodium azide to prevent the microbial growth during hydrolysis. The dry mass content of the hydrolysis slurries was 5% (w/v). Enzymatic hydrolysis was carried out in 125 mL flasks with 50 mL of slurry in a 50 °C water bath shaker agitating at 140 rpm for 96 h. The enzyme loading (Accellerase 1500<sup>TM</sup>, Genencor Inc., Rochester, N.Y.) was 1 mL/g of cellulose. During enzymatic hydrolysis, the hydrolysis slurries were sampled periodically up to 96 h after the addition of enzyme by withdrawing 0.1 mL of slurry from each flask. Sample slurries were then mixed with 0.9 mL double-distilled water in 1.5-mL vials, and vials were placed to boil in a water bath for 15 min to deactivate the enzyme. After enzyme inactivation, samples were centrifuged at 13,500 rpm for 15 min [12]. The supernatants were filtered into 1.5-mL autosampler vials through 0.2- $\mu$ m hydrophilic PTFE

syringe filters (Millipore, Billerica, Mass.). Filtered samples were kept at 4 °C before HPLC analysis.

The conversion efficiency of cellulose was expressed in terms of the percentage of cellulose enzymatically converted to glucose (i.e., enzymatic conversion of cellulose; ECC). It was calculated by comparing the glucose yield (g) after enzymatic hydrolysis with the initial glucose content (1.11 times the initial cellulose content) in the untreated biomass [13]. The following formula was used to calculate ECC:

$$ECC = \frac{c \times V}{1.11 \times m} \times 100 \%$$

where  $c$  is the concentration (g/L) of D-glucose in the sampled hydrolysate determined by HPLC analysis,  $V$  is the total volume (L) and  $m$  is the weight of cellulose before enzymatic hydrolysis (g). The factor 1.11 is the cellulose-to-glucose conversion factor.

## **Analytical Methods**

Moisture content of ground biomass was determined by drying approximately 2 g of each sample in a forced-air oven at 105 °C for 4 h [14]. Moisture content of pretreated wet samples was determined by drying approximately 2.5 g of sample in a forced-air oven at 49 °C overnight and further drying at 105 °C for a minimum of 4 h.

Extractives in dry, untreated biomass and chemical composition of untreated and pretreated biomass were determined by following NREL laboratory analytical procedures [15-16]. Structural carbohydrates in biomass were reported as percentages of glucan and xylan. Glucan is basically cellulose, and xylan is the major hemicellulose constituent. Lignin, the major noncarbohydrate 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 pretreated samples with an HPLC (Shimadzu, Kyoto, Japan) equipped with an RCM-monosaccharide column (300 × 7.8 mm; Phenomenex, Torrance, Cal., USA) and a refractive index detector (RID-10A, Shimadzu, Kyoto, Japan). The mobile phase was 0.6 mL/min of double-distilled water, and oven temperature was 80 °C. The supernatants of pretreated samples were neutralized with CaCO<sub>3</sub> to pH 6 before being filtered through 0.2-μm hydrophilic PTFE syringe filters (Millipore, Billerica, MA).

The experiment for each biomass sample was replicated twice. Analysis of variance (ANOVA) and least-significant difference (LSD) at the 0.05 level were conducted using SAS (SAS Institute 2005, Cary, NC).

## **RESULTS AND DISCUSSION**

### **Physical Properties of Biomass Pellets**

Physical properties (durability, bulk density and true density) of the biomass pellets as affected by pelleting variables (hammer mill screen size and ring-die pelleting mill die thickness) are shown in Table 1. The pelleting process significantly improved the bulk density of all four biomass samples up to 9 to 12 times (from 47 to 60 kg/m<sup>3</sup> for chopped biomass to 360 to 500 kg/m<sup>3</sup> for biomass pellets). Pelleting process variables, including hammer mill screen size and ring-die pelleting mill die thickness, were found to have some effects on the durability and density of the biomass. According to Table 1, bulk density values of biomass pellets were found to significantly increase when using a larger hammer mill screen size (6.5 mm instead of 3.2 mm). However, the hammer mill screen size did not cause any significant effect on both true density and durability of biomass pellets. Tabil and Sokhansanj (1996) also studied the effect of hammer mill screen size on durability of alfalfa pellets [17]. They found that the increase of hammer mill screen size from 3.2 mm to 6.5 mm resulted in no significant increase of pellet durability, possibly due to the small difference in the screen size and the variability of durability values.

Die thickness was found to have a positive effect on both bulk density and true density of the biomass pellets. Use of a thicker die (44.5 mm instead of 31.8 mm die thickness) also resulted in a significant increase of pellet durability. This phenomenon occurred for all four types of biomass pellets, except for sorghum stalk pellets that the effect of die size on their pellet durability was not in a significant level. Overall, the effect of die size on density and durability of biomass pellets was more pronounced than the effect of hammer mill screen size. In this study, the pelleting conditions with a thicker die (44.5 mm die thickness) and a larger hammer mill screen size (6.5 mm screen openings) increased the pellet durability and density. Pelleting of biomass, especially at the optimum pelleting conditions, would produce much denser biomass in a very durable pellet form as compared to baled or ground biomass, and thus helped to improve ease of handling and transportation of biomass samples,

## **Effect of Pelleting Conditions and Pretreatment Process on the Chemical Composition of Biomass**

The chemical compositions of each type of unpelleted and untreated biomass were similar to the reported values from previous studies [3, 12, 18-21] (Table 2). Pelleting variables (hammer mill screen size and ring-die pelleting mill die thickness) did not have significant effects on the chemical composition of untreated biomass samples, although their physical properties were affected by the pelleting variables as mentioned in the previous section (Table 3). This trend occurred for all four types of biomass. Extractives of biomass pellets were found to vary by the pelleting conditions and types of biomass (Fig.1). Among the four different types of biomass pellets, sorghum stalk has the highest extractives (26-30%), while the extractives for big bluestem and wheat straw are relatively low(16-19%). (Fig.1)..

Similar to the untreated biomass, lignin content of the pretreated biomass was not significantly affected by the hammer mill screen size and die thickness (Table 4). Pelleting process, except for the pelleting condition using mill screen size of 3.2 mm and die thickness of 31.8 mm, resulted in an increase of glucan content of pretreated biomass. The pelleting parameters also pronounced some effects on the glucan content of pretreated biomass pellets. Use of a thicker die (44.5 mm instead of 31.8 mm) resulted in a significant increase of glucan content of the pretreated biomass pellets. This trend was observed for all four types of biomass. Use of a larger hammer mill screen size (6.5 mm screen opening instead of 3.2 mm screen opening) resulted in a significant reduction of glucan content in the pretreated biomass. This trend was observed for all of the treated biomass pellets, except for big bluestem. Pelleting variables had the opposite effect on xylan content, which decreased when using a thicker die on ring-die pelleting mill but tended to increase when using a larger hammer mill screen (Table 4).

The cellulose recovery of biomass after pretreatment varied from both type of biomass and the pelleting conditions (Fig.2). For unpelleted biomass, corn stover had the highest cellulose recovery (80-84%). For corn stover and big bluestem, the pelleted biomass had a higher cellulose recovery than the unpelleted biomass. This trend was observed for all pelleting conditions. Unlike corn stover and big bluestem, the cellulose recovery of pelleted wheat straw appeared to be lower than that of unpelleted wheat straw. For sorghum stalk, pelleting did not have a significant effect on the cellulose recovery. A large hammer mill screen size yielded a higher cellulose recovery for wheat straw and corn stover and lower cellulose recovery for big bluestem



and sorghum stalk. A large die thickness resulted in a higher cellulose recovery for corn stover and big bluestem but a lower cellulose recovery for wheat straw and sorghum stalk. The pretreatment process was another factor that caused significant changes in the chemical compositions of biomass. Lignin content of biomass increased significantly from 16-19% to 33-36% after pretreatment (Tables 3 and 4); similarly, glucan content of biomass increased significantly from 41-46% to 52-59% after pretreatment. A significant decrease in xylan content (from 21-25% to 1.5-2.5%) was observed after pretreatment. With the dilute acid pretreatment, the hemicellulose was not only solubilized but also converted to fermentable sugar [22, 23].

The main components in filtrate fraction of both unpelleted and pelleted biomass after dilute acid pretreatment were xylose and glucose, which can be converted to fuel-ethanol via microbial fermentation (Table 5). Xylose and arabinose in the filtrate were pentose sugars that were solubilized from hemicellulose of biomass by the dilute acid during pretreatment process [23]. Besides pentose sugars, hemicellulose also was composed of glucose. Therefore, glucose in the filtrate fraction was considered to be from hydrolysis of both cellulose and hemicellulose.

### **Effect of Pelleting Process on Biomass Sugar Yield**

The enzymatic conversion of cellulose (%ECC) for all four types of biomass that was pelleted under different pelleting conditions was shown in Fig.3. The %ECC varied from 92% to 94.5% for wheat straw pellets, from 91% to 92.5% for sorghum stalk pellets, from 84% to 93.5% for corn stover pellets, and from 83.5% to 92% for big bluestem pellets. For all four types of biomass pellets, the use of a thicker die resulted in a significant increase of %ECC, especially for corn stover and big bluestem pellets. The significant increase of %ECC of biomass was mainly due to the significant increase in glucan content of the pretreated biomass when using a thicker die during the pelleting process. Glucan is the major component in biomass that can be hydrolyzed to fermentable sugar during enzymatic hydrolysis; a higher glucan content in the pretreated biomass results in higher %ECC or expected sugar yield of biomass. The use of a larger hammer mill screen also resulted in an increase of %ECC, but not at a significant level. In this study, the pelleting condition that resulted in the highest %ECC value of biomass was the hammer mill screen opening size of 6.5 mm and ring-die pelleting mill die thickness of 44.5 mm. At this optimum condition, the %ECC of biomass pellets was even higher than that of the corresponding unpelleted biomass (Fig.3). This result implies that pelleting of biomass can

improve the yield of glucose recovery from cellulose. Because the pelleting process involves pressing of the feed materials by the roll(s) through the die holes (open-ended cylindrical holes) made in the periphery of a die ring, the shear developed during extrusion disturbs the biomass structure and thus increases enzyme access to cellulose. During this process, the biomass materials are subjected to shearing, mixing and heating. The mechanical shear between the die surface and the biomass results in effective biomass deconstruction. The heat generated during the pelleting process made amorphous materials such as cellulose and lignin exhibit a second-order transition, whereas crystalline material such as sugar go through a first-order transition. Plasticization or thermal softening of the biomass materials enhances sugar release from the biomass [24]. As summarized by Zhan et al. (2006), the extrusion process could increase digestible starch fraction, reduce molecular weight of biomolecules, enhance creation of free sugars, and change the native structure of biomolecules [25]. Several studies even used the extrusion process as a physical pretreatment for biomass [24-27]. In this study, pelleting of biomass could be considered a preliminary pretreatment step and thus helps open the biomass structure before the biomass would actually be pretreated by dilute sulfuric acid. By comparing the four different types of biomass pellets, wheat straw pellets had the highest ECC value (94.1%), followed by corn stover pellets (93.1%), sorghum stalk pellets (92.1%), and big bluestem pellets (91.1%). Because the ECC values of pelleted biomass samples were equivalent to or even higher than the corresponding unpelleted samples and the pelleted biomass were easier to handle and transport compared with the unpelleted form, using pelleted biomass in fuel ethanol production has great potential.

## **CONCLUSIONS**

Hammer mill screen size and ring-die pelleting mill die thickness did not pronounce any significant effect on chemical composition of the untreated biomass pellets. The pretreatment process resulted in a significant increase of glucan content as well as lignin content, but significant decrease of xylan content, both in unpelleted and pelleted forms. For pretreated biomass pellets, the glucan content increased with the increase in die thickness and decreased with the increase in hammer mill screen size. On the contrary, xylan content of treated biomass pellets decreased as die thickness increased and increased as the hammer mill screen size increased. Among the three combinations of pelleting conditions used in this study, the pelleting

of biomass using a die with thickness of 44.5 mm and a hammer mill screen size of 6.5 mm gave the highest sugar yield as well as the highest durability and bulk density of biomass. Under this optimum pelleting condition, the maximum enzymatic conversion of cellulose (%ECC) of wheat straw pellet was the highest (94.1%), followed by corn stover pellet (93.1%), sorghum stalk pellet (92.1%), and big bluestem pellet (91.1%).

## **ACKNOWLEDGEMENT**

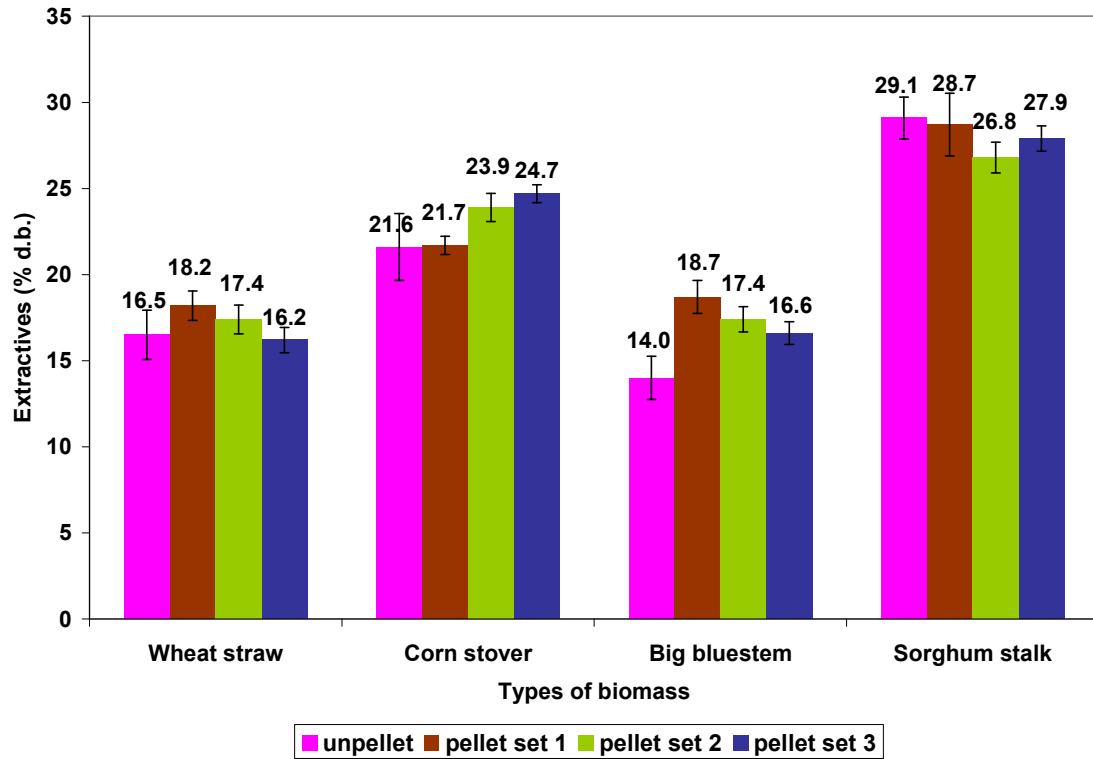
This project was funded by the Biomass Research and Development Initiative Competitive Grants Program (BRDI), USDA National Institute of Food and Agriculture, Grant No. 68-3A75-7-609. Contribution number 11-246-J from the Kansas Agricultural Experiment Station. This study was also partly supported by NSF award CMMI-0970112

## **REFERENCES**

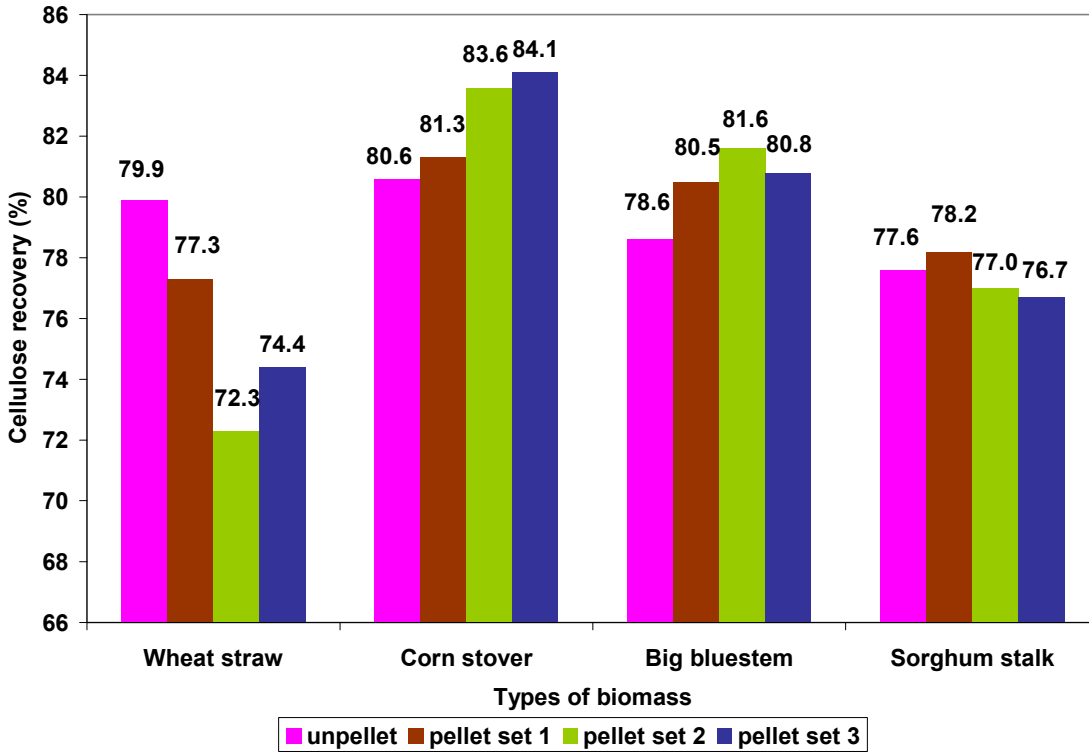
1. Dien BS, Jung H-JG, Vogel KP, Casler MD, Lamb JFS, Iten LB, Mitchell RB, Sarath G (2006) Chemical composition and response to dilute-acid pretreatment and enzymatic saccharification of alfalfa, reed canarygrass, and switchgrass. *Biomass Bioenerg* 30:880–891
2. Colley Z, Fasina OO, Bransby D, Lee YY (2006) Moisture effect on the physical characteristics of switchgrass pellets. *T ASAE* 49:1845–1851
3. Kaliyan N, Morey RV, White MD, Doering A (2009) Roll-press briquetting and pelleting of corn stover and switchgrass. *T ASAE* 52:543–555
4. Lam PS, Sokhansanj S, Bi X, Lim CJ, Naimi LJ, Hoque M, Mani S, Womac AR, Ye XP, Narayan S (2008) Bulk density of wet and dry wheat straw and switchgrass particles. *Appl Eng Agric* 24:351–358
5. Mani S, Tabil LG, Sokhansanj S (2006) Specific energy requirements for compacting corn stover. *Bioresource Technol* 97:1420–1426
6. Hess JR, Wright CT, Kenney K (2007) Cellulosic biomass feedstocks and logistics for ethanol production. *Biofuel Bioprod Bior* 1:181–190
7. Holm JK, Henriksen UB, Hustad JE, Sorensen LH (2006) Toward an understanding of controlling parameters in softwood and hardwood pellet production. *Energy & Fuels* 20:2686–2694

8. Larsson SH, Thyrel M, Geladi P, Lestander TA (2008) High-quality biofuel pellet production from pre-compacted, low-density raw materials. *Bioresource Tech* 99:7176–7182
9. Mani S, Sokhansanj S, Bi X, Turhollow A (2006) Economics of producing fuel pellets from biomass. *Appl Eng Agric* 22:421–426
10. Mani S, Bi X, Sokhansanj S (2005) Environmental systems assessment of biomass densification process. CSAE Paper No. 05081. Winnipeg, Manitoba.
11. Sokhansanj S, Turhollow A (2004) Biomass densification: cubing operations and costs. *Appl Eng Agric* 20:495–499
12. Theerarattananoon K, Wu X, Staggenborg S, Propheter J, Madl R, Wand D (2010) Evaluation and characterization of sorghum biomass as feedstock for sugar production. *T ASAE* 53:509–525
13. Varga E, Reczey K, Zacchi G (2004) Optimization of steam pretreatment of corn stover to enhance enzymatic digestibility. *Appl Biochem Biotech* 114:509–523
14. Sluiter A., Hames B, Hyman D, Payne C, Ruiz R, Scarlata C, Sluiter J, Templeton D, Wolfe J (2008) Determination of total solids in biomass and total dissolved solids in liquid process samples. NREL/TP-510-42621. National Renewable Energy Laboratory (NREL). Golden, Colorado
15. Sluiter A, Ruiz R, Scarlata C, Sluiter J, Templeton D (2005) Determination of extractives in biomass: NREL/TP-510-42619. National Renewable Energy Laboratory (NREL). Golden, Colorado
16. Sluiter A., Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D, Crocker D (2008) Determination of structural carbohydrates and lignin in biomass: NREL/TP-510-42618. National Renewable Energy Laboratory (NREL). Golden, Colorado
17. Tabil L, Sokhansanj S (1996) Process conditions affecting the physical quality of alfalfa pellets. *Appl Eng Agric* 12(3): 345-350
18. Mani S, Tabil LG, Sokhansanj S (2004) Evaluation of compaction equations applied to four biomass species. *Can. Biosyst. Eng* 46:355–361
19. Han M, Moon S, Kim Y, Kim Y, Chung B, Choi G (2009) Bioethanol production from ammonia percolated wheat straw. *Biotechnol. Bioprocess Eng* 14:606–611

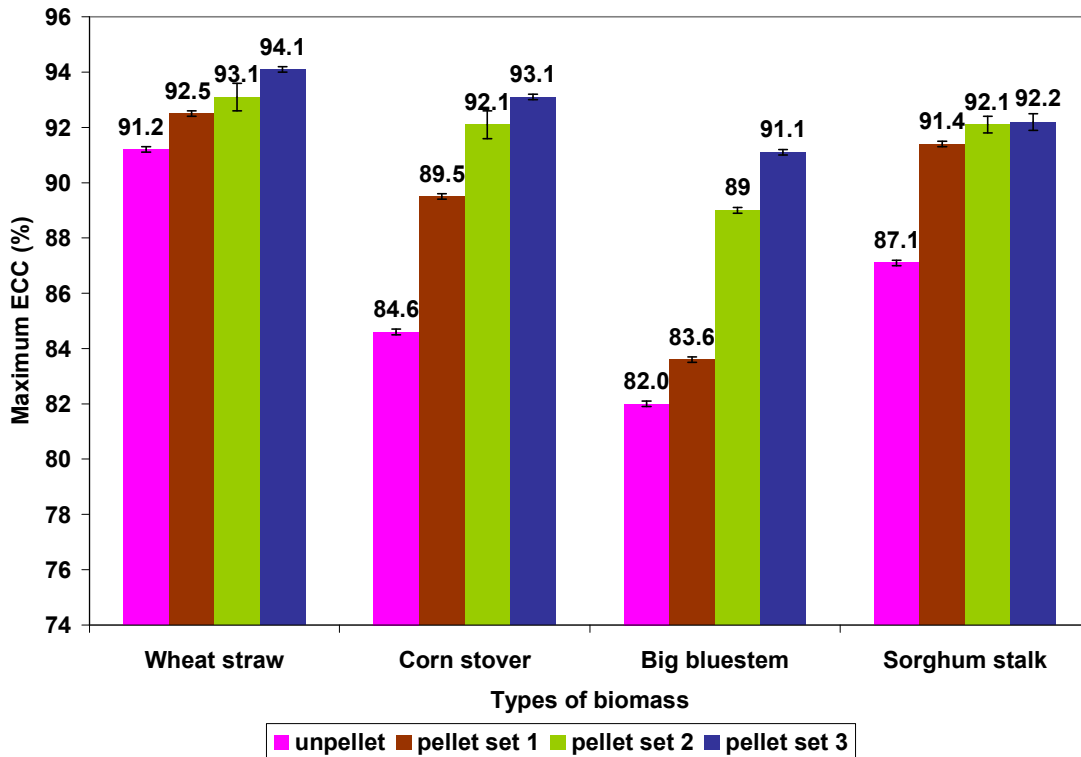
20. Yu Y, Lou X, Wu H (2008) Some recent advances in hydrolysis of biomass in hot-compressed water and its comparisons with other hydrolysis methods. *Energy & Fuels* 22:46–60
21. Luterbacher JS, Tester JW, Walker LP (2010) High-solids biphasic CO<sub>2</sub>-H<sub>2</sub>O pretreatment of lignocellulosic biomass. *Biotechnol. Bioeng* 107:451–460
22. Zaldivar J, Nielsen J, Olsson L (2001) Fuel ethanol production from lignocellulosic: A challenge for metabolic engineering and process integration. *Appl Microbiol Biotech* 56:17–34
23. Saha BC, Iten LB, Cotta MA, Wu YV (2005) Dilute acid pretreatment, enzymatic saccharification, and fermentation of wheat straw to ethanol. *Process Biochem* 40(12): 3693-3700
24. Karunanithy C, Muthukumarappan K (2010) Effect of extruder parameters and moisture content of switchgrass, prairie cord grass on sugar recovery from enzymatic hydrolysis. *Appl Biochem Biotechnol* 162:1785–1803
25. Zhan X, Wang D, Bean SR, Mo X, Sun XS, Boyle D (2006) Ethanol production from supercritical-fluid-extrusion cooked sorghum. *Ind Crop Prod* 23:304–310
26. Karunanithy C, Muthukumarappan K (2010) Effect of extruder parameters and moisture content of corn stover and big bluestem on sugar recovery from enzymatic hydrolysis. *Biol Eng.* 2(2):91–113
27. Dale BE, Weaver J, Byers FM (1999) Extrusion processing for Ammonia Fiber Explosion (AFEX). *Appl Biochem Biotech* 77–79:35–45



**Figure 4.1** Extractive contents of wheat straw, corn stover, big bluestem, and sorghum stalk, as affected by hammer mill screen size and die thickness; pellet set 1 = 3.2 mm screen size, 31.8 mm die thickness, pellet set 2 = 3.2 mm screen size, 44.5 mm die thicknes.



**Figure 4.2 Cellulose recovery of pretreated wheat straw, corn stover, big bluestem, and sorghum stalk, as affected by hammer mill screen size and die thickness; pellet set 1 = 3.2 mm screen size, 31.8 mm die thickness, pellet set 2 = 3.2 mm screen size, 44.5 mm die thickness, pellet set 3=6.5 mm screen size, 44.5 mm die thickness.**



**Figure 4.3 Maximum Enzymatic Conversion of Cellulose (%ECC) of wheat straw, corn stover, big bluestem, and sorghum stalk, as affected by hammer mill screen size and die thickness; pellet set 1 = 3.2 mm screen size, 31.8 mm die thickness, pellet set 2 = 3.2 mm screen size, 44.5 mm die thickness, pellet set 3 = 6.5 mm screen size, 44.5 mm die thickness.**



**Table 4.1 Pellet durability, bulk density, and true density as affected by mill screen size and die thickness**

Biomass feedstock	Pelleting conditions		Pellet durability (%)	Bulk density (kg/m <sup>3</sup> )	True density (kg/m <sup>3</sup> )
	Hammer mill screen size (mm)	Die thickness (mm)			
Chopped wheat straw	--	--	--	47.7 a	--
Wheat straw pellet	3.2	31.8	95.8 a	495.8 b	920.7 a
	3.2	44.5	97.4 b	554.1 c	1067.3 b
	6.5	44.5	98.3 b	649.2 d	1161.6 c
Chopped corn stover	--	--	--	50.9 a	--
Corn stover pellet	3.2	31.8	96.4 a	469.1 b	895.3 a
	3.2	44.5	98.2 b	597.9 c	988.7 b
	6.5	44.5	97.9 b	624.6 d	1127.4 c
Chopped big bluestem	--	--	--	46.6 a	--
Big bluestem pellet	3.2	31.8	95.7 a	467.4 b	772.6 a
	3.2	44.5	96.9 a	601.9 c	924.8 b
	6.5	44.5	97.6 a	618.0 d	1161.2 c
Chopped sorghum stalk	--	--	--	59.3 a	--
Sorghum stalk pellet	3.2	31.8	85.7 a	365.2 b	597.2 a
	3.2	44.5	92.2 b	434.0 c	620.7 b
	6.5	44.5	93.5 b	478.6 d	713.8 c

<sup>[a]</sup> Means in the same biomass followed by different letters are significantly different at p<0.05

**Table 4.2 Comparison of composition of unpelleted wheat straw, corn stover, big bluestem, and photoperiod-sensitive sorghum stalk**

Type of biomass	Chemical component	Chemical composition (%)		References
		This study <sup>[a]</sup>	Previous studies	
Wheat straw	Glucan	41.2 ± 0.2	37.6 - 44.5	[16, 18-19]
	Xylan	23.8 ± 0.1	22.9 - 24.3	
	Lignin	16.3 ± 0.1	7.6 - 21.3	
Corn stover	Glucan	42.2 ± 0.4	31.3 – 49.4	[3, 16, 19-20]
	Xylan	23.3 ± 0.3	21.8 – 26.2	
	Lignin	18.2 ± 0.1	3.1 – 18.2	
Big bluestem	Glucan	40.1 ± 0.6	37.6 ± 0.1	[21]
	Xylan	21.6 ± 0.1	19.9 ± 0.1	
	Lignin	18.7 ± 0.2	6.9	
Sorghum stalk	Glucan	41.7 ± 0.3	44.0	[20]
	Xylan	23.0 ± 0.1	27.4	
	Lignin	18.2 ± 0.1	19.2	

<sup>[a]</sup> Means in the same biomass followed by different letters are significantly different at  $p < 0.05$

**Table 4.3 Chemical composition of untreated biomass as affected by mill screen size and die thickness**

Biomass feedstock	Pelleting conditions		Chemical component of solid fractions (% db) <sup>[a]</sup>			
	Mill screen size (mm)	Die thickness (mm)	Lignin	Glucan	Xylan	Ash
Wheat straw	Unpelleted		16.3 a	41.2 a	23.8 a	3.11 a
	3.2	31.8	18.0 a	43.2 a	24.1 a	3.04 a
	3.2	44.5	17.5 a	44.8 a	22.8 a	4.13 a
	6.5	44.5	17.6 a	46.1 a	23.7 a	3.31 a
Corn stover	Unpelleted		18.2 a	42.2 a	23.3 a	2.13 a
	3.2	31.8	18.4 a	41.5 a	23.3 a	3.31 a
	3.2	44.5	18.9 a	42.6 a	22.9 a	3.29 a
	6.5	44.5	19.6 a	43.2 a	24.5 a	2.55 a
Big bluestem	Unpelleted		18.7 a	40.1 a	21.6 a	2.37 a
	3.2	31.8	18.0 a	40.9 a	23.1 a	2.81 a
	3.2	44.5	19.7 a	43.2 a	23.5 a	2.68 a
	6.5	44.5	19.8 a	42.7 a	24.1 a	2.50 a
Sorghum stalk	Unpelleted		18.2 a	41.7 a	23.0 a	2.02 a
	3.2	31.8	17.5 a	40.6 a	22.2 a	3.34 a
	3.2	44.5	18.3 a	42.3 a	22.5 a	3.24 a
	6.5	44.5	18.8 a	41.5 a	23.6 a	3.24 a

<sup>[a]</sup> Means in the same biomass followed by different letters are significantly different at  $p < 0.05$

**Table 4.4 Chemical composition of biomass as affected by dilute-acid pretreatment (140 °C, 30 min) and pelleting process**

Biomass feedstock	Pelleting conditions		Component in solid fractions (%) <sup>[a]</sup>				Mass recovery (%)
	Mill screen size (mm)	Die thickness (mm)	Lignin	Glucan	Xylan	Ash	
Wheat straw	Unpelleted		34.4 a	55.9 a	1.58 a	5.64 a	58.9
	3.2	31.8	34.1 a	52.9 b	1.95 b	5.48 a	63.1
	3.2	44.5	34.2 a	58.2 c	1.59 a	7.62 b	55.7
	6.5	44.5	34.8 a	55.6 a	1.82 b	8.29 c	61.7
Corn stover	Unpelleted		34.6 a	56.1 a	1.68 a	2.85 a	60.6
	3.2	31.8	34.0 a	52.5 a	2.36 b	4.87 b	64.2
	3.2	44.5	35.8 a	56.6 b	1.68 a	6.22 c	63.0
	6.5	44.5	35.4 a	54.1 b a	1.93 a	5.59 c b	67.1
Big bluestem	Unpelleted		34.9 a	54.5 a	1.64 a	2.84 a	57.9
	3.2	31.8	35.1 a	53.3 a	1.89 a	5.49 b	61.8
	3.2	44.5	35.3 a	58.5 b	1.59 a	4.12 c	60.2
	6.5	44.5	34.4 a	58.1 b	2.30 b	4.66 c b	59.4
Sorghum stalk	Unpelleted		33.3 a	53.4 a	1.53 a	3.25 a	60.6
	3.2	31.8	33.7 a	53.4 a	2.42 a	5.76 b	59.5
	3.2	44.5	37.0 a	58.0 b	1.62 a	5.73 b	56.2
	6.5	44.5	36.2 a	54.4 a	1.46 a	6.55 b	58.5

<sup>[a]</sup> Means in the same biomass followed by different letters are significantly different at  $p < 0.05$

**Table 4.5 Sugar yield in filtrate of biomass after dilute acid pretreatment as affected by mill screen size and die thickness**

Biomass feedstock	Pelleting conditions		Components in filtrate fractions (g/100 g of dry, untreated biomass)		
	Mill screen size	Die thickness (mm)	Xylose	Arabinose	Glucose
wheat straw	Unpelleted		14.4 ± 1.83	2.55 ± 0.28	8.28 ± 0.03
	3.2	31.8	17.1 ± 0.30	2.53 ± 0.05	9.80 ± 0.03
	3.2	44.5	17.2 ± 0.41	2.69 ± 0.04	12.4 ± 0.09
	6.5	44.5	16.3 ± 0.23	4.00 ± 0.08	11.8 ± 0.18
corn stover	Unpelleted		15.9 ± 0.09	2.50 ± 0.08	8.57 ± 0.07
	3.2	31.8	16.3 ± 0.27	2.86 ± 0.06	7.96 ± 0.46
	3.2	44.5	18.3 ± 1.43	2.59 ± 0.03	7.97 ± 0.12
	6.5	44.5	16.0 ± 0.66	4.36 ± 0.38	8.19 ± 0.06
big bluestem	Unpelleted		16.8 ± 0.07	2.98 ± 0.06	8.18 ± 0.11
	3.2	31.8	17.3 ± 3.79	2.69 ± 0.12	7.77 ± 1.73
	3.2	44.5	19.6 ± 0.39	2.69 ± 0.01	6.97 ± 0.22
	6.5	44.5	18.0 ± 0.55	4.50 ± 0.14	6.89 ± 0.49
sorghum stalk	Unpelleted		16.6 ± 0.10	2.49 ± 0.06	9.34 ± 0.02
	3.2	31.8	16.8 ± 1.70	2.34 ± 0.18	8.84 ± 0.80
	3.2	44.5	16.7 ± 0.04	1.41 ± 1.99	9.72 ± 0.10
	6.5	44.5	15.8 ± 0.89	4.78 ± 0.08	9.69 ± 0.39

# **CHAPTER 5 - IMPACT OF PELLETING AND ACID PRETREATMENT ON BIOMASS STRUCTURE AND THERMAL PROPERTIES OF WHEAT STRAW, CORN STOVER, BIG BLUESTEM, AND SORGHUM STALK <sup>4</sup>**

## **ABSTRACT**

Agricultural residues and energy crops are considered potential feedstocks for bioethanol production because of their high availability and energy potential as well as relatively low cost. Previous studies have shown that pelleting biomass feedstocks could increase their bulk density, thus increasing ease of handling and decreasing cost of handling and transportation; however, effects of the pelleting process on biomass structure have not yet been studied. The objective of this study was to investigate the impact of dilute-acid pretreatment and the pelleting process on biomass structure of cellulosic materials, including crystallinity index (CrI%) measured by the XRD method, structure of constituents and chemical changes determined by Fourier transform infrared spectroscopy (FTIR) and solid-state cross polarization/magic angle spinning (CP/MAS) <sup>13</sup>C NMR spectroscopy, morphological structure determined by Scanning Electron Microscope (SEM), and thermal properties determined by thermogravimetric analysis (TGA). Wheat straw, big bluestem, corn stover, and photoperiod-sensitive sorghum were used for this study. Pelleting did not have a significant effect on the pattern of FTIR spectra and solid-state <sup>13</sup>C NMR spectra of biomass. XRD analysis showed that biomass crystallinity increased after dilute-acid pretreatment and the pelleting process. Based on SEM analysis of biomass, dilute-acid pretreatment and pelleting enhanced the removal of the softened surface region of biomass. TGA analysis showed that the decomposition temperature of pelleted biomass was slightly higher than that of corresponding unpelleted biomass, indicating that the pelleted biomass was slightly more thermally stable than the unpelleted biomass..

Keywords: pelleting, biomass microstructure, crystallinity index, thermal property

---

<sup>4</sup> Results have been submitted for publication. K. Theerarattananoon, F. Xu, J. Wilson, S. Staggenborg, L. McKinney, P. Vadlani, Z.J. Pei, and D. Wang. Impact of the pelleting and acid pretreatment on biomass structure and thermal properties of wheat starw, corn stover, big bluestem, and sorghum stalk. *T ASAE*.

## INTRODUCTION

Agricultural residues such as corn stover, wheat straw, big bluestem, and sorghum stalk can serve as abundant and low-cost feedstocks for the production of fuel ethanol through pretreatment, hydrolysis, and fermentation. Use of crop residues adds value to crops and economically benefits farmers. Corn stover is the stalk and leaf residue left after grain harvest. In the United States, over 238 million tons of corn stover can be collected annually (Liu et al., 2010; Akin et al., 2006). Wheat straw is generated abundantly worldwide (529 million tons/yr); up to 15% of global wheat production is from North America (Buranov and Mazza, 2008). Big bluestem is a high-quality perennial warm-season grass that is widely distributed in the central United States (Weimer and Springer, 2007; Mitchell et al., 2001). Sorghum is a tropical grass that has high drought tolerance in a dryland environment (Propheter et al., 2010). According to 2010 data from the National Agricultural Statistics Service, sorghum is currently grown on 6 to 10 million acres in the United States (Feng et al., 2011). By comparing the dry mass production with corn, photoperiod-sensitive sorghum, which is a high-energy sorghum, can produce much more dry mass per acre than corn in dryland conditions, thus making it one of the potential feedstocks for bioethanol production (Feng et al., 2011).

In lignocellulosic biomass, cellulose chains typically consist of both ordered (crystalline) and less ordered (amorphous) regions (Park et al., 2010). Highly crystalline cellulose is less accessible to enzyme attack than amorphous cellulose; therefore, cellulose crystallinity affects the efficiency of enzyme contact with cellulose (Chang and Holzapple, 2000). Several techniques can be used to determine the degree of order (crystallinity) of cellulose, including solid-state  $^{13}\text{C}$  nuclear magnetic resonance (NMR), Raman spectroscopy, infrared (IR) spectroscopy, and X-ray diffraction (XRD) (Teeaar et al., 1987; Park et al., 2010). Biomass crystallinity is defined quantitatively as a weight fraction of crystalline material in the sample and is often known as the crystallinity index (CrI). Crystallinity index values are dependent on the perfection of the samples and the data evaluation method (Teeaar et al., 1987). The X-ray peak height diffraction method developed by Segal and coworkers (Segal et al., 1959) is the most popular method for estimating biomass CrI because it is the easiest method to use (Teeaar et al., 1987; Park et al., 2010). For the analysis of specific functional groups and the changes in chemical structure of biomass induced by processing, solid-state NMR spectroscopy and Fourier transform infrared spectroscopy are among the top choices. The advantage of solid-state NMR

spectroscopy is that the samples can be studied in their native forms without fractionation or isolation of components (Mao et al., 2010; Popescu et al., 2011).

Modification of biomass structure is achieved through various pretreatment methods and makes the biomass more amenable to enzymatic attack. Pelleting involves size reduction of biomass feedstock, conditioning of ground biomass by applying heat and/or moisture, and extrusion of the ground biomass through a die (Colley et al., 2006; Kaliyan et al., 2009). The size reduction of biomass and the extrusion step that occurs during biomass pelleting can be counted as pretreatment methods for lignocellulosic biomass. Particle size reduction by grinding can be referred to as a physical pretreatment method whereas the extrusion process is referred to as a thermo-mechanical pretreatment method. In their study on extrusion as a thermo-mechanical pretreatment for lignocellulosic ethanol, Lamsal et al. (2010) mentioned that the shear force developed during the extrusion process could serve to continuously remove the softened surface region of biomass and expose the interior to chemical and/or thermal action, resulting in a higher overall rate of decrystallization. Results from their studies also showed that the reduced sugar yield of wheat bran and soybean hull was affected by the biomass particle size and the extrusion parameters (screw speed Hz/maximum barrel temperature) (Lamsal et al., 2010). Studies by Karunanithy and Muthukumarappan (2010, 2011) showed that the biomass particle size and extruder parameters such as screw compression ratio, screw speed, and barrel temperature were important factors that affected the efficiency of extrusion pretreatment and thus sugar recovery of biomass. According to Yoo et al. (2011), the extrusion pretreatment had a unique impact on the morphology of biomass. They found that the cellulose crystallinity of soybean hull increased by 82% after extrusion pretreatment, although the change in its chemical composition was not significant due to crystallization of the amorphous phase of cellulose during the extrusion process.

Several other studies (Lamsal et al., 2010; Yoo et al., 2011; Karunanithy and Muthukumarappan, 2010; Karunanithy and Muthukumarappan, 2011) found that the extrusion pretreatment process and extruder parameters had effects on the morphology and fermentable sugar yield of lignocellulosic biomass. The previous study (Theerarattananoon et al., 2011) also showed that pelleting affected physical properties of the biomass; therefore, expanding the study on the impact of the pelleting process, pelleting parameters, and dilute acid pretreatment process on the morphology of biomass is important. The objective of this study was to investigate the



impact of pelleting process as well as dilute-acid pretreatment on biomass structure and thermal properties of cellulosic materials (corn stover, wheat straw, big bluestem, and photoperiod-sensitive sorghum stalk) using X-ray diffraction, Fourier transform infrared spectroscopy, solid-state cross polarization/magic angle spinning (CP/MAS)  $^{13}\text{C}$  NMR spectroscopy, scanning electron microscope, and thermogravimetric analysis.

## **MATERIALS AND METHODS**

### **Materials**

Big bluestem (BBS) was obtained from Star Seed in Beloit, Kansas, in January 2009. Wheat straw, corn stover, and photoperiod-sensitive sorghum (PS) stalk were harvested by the Kansas State University Agronomy Farm in November 2008 and December 2009. Sorghum stalk was obtained in the form of a round bale with a diameter of 1.83 m, and other biomass materials were obtained as square bales (1.8 x 1.2 x 1.8 m). The biomass materials were first chopped to similar stem length (7-9 in. in length) using a tub grinder (Model Haybuster H-1150 series, DaraTech Industries International, Inc., Jamestown, N.D.), then finely ground with a 7.4 kW (10 hp) hammer mill (Model 18-7-300, Schuttle-Buffalo Hammermill, Buffalo, N.Y.) with two different screen openings (3.2 mm and 6.5 mm).

### **Biomass Pelleting**

Densification of biomass materials was done with a 22.1 kW (30 hp) ring-die pellet mill with 1.5-ton capacity (CPM Master model series 2000, California Pellet Mill (CPM) Co., San Francisco, CA). Before pelleting, the biomass grinds were mixed with tap water for 2 min to adjust their moisture content to about 10% (wet basis). The biomass grinds were fed into the pellet mill through a 1,000-lb capacity surge bin above a conditioner and feeder screw. The feeder screw is capable of moving a specific volume of biomass at different RPM. For all runs in this experiment, the speed of the feeder screw was held constant at 45 RPM. Pellets were made in three combinations based on two pelleting variables, hammer mill screen size (3.2-mm and 6.5-mm screen openings) and die size (4.0 x 31.8 mm and 6.4 x 44.5 mm, hole diameter x effective thickness). The temperature of biomass pellets exiting the die was 74 °C to 82 °C due to frictional heating of the die during pelleting. The pellets were cooled to room temperature by forced air.

## Pretreatment of Biomass

Dilute acid pretreatment of the biomass materials, both in original and pelleted form, were conducted by mixing the ground biomass samples with dilute sulfuric acid (2% w/v) to obtain 10% solid content. Biomass slurries were then loaded in a Parr pressure reactor (Parr Instrument Company, Moline, IL) and treated at 140 °C for 30 min. Pretreated solid residues were washed with hot distilled water and centrifuged several times to remove dissolved sugar and sulfuric acid.

## X-ray Diffraction Analysis

Crystalline structure of the biomass samples before and after treatment was analyzed by wide-angle X-ray diffraction (XRD) with an ADP-3520 X-ray diffractometer (Philips Electronic Instrument Co., Houston, TX) operating at 35 kV, 20 mA; radiation was copper K $\alpha$  ( $\lambda = 1.54 \text{ \AA}$ ), and grade range was between 5 and 40° with a step size of 0.03°. Aperture, scatter, and detector slits each were 1°. The scan speed was set at 5°/min. Crystallinity in a sample can be detected by absorption peaks. Crystallinity index (CrI) was calculated using the method of Segal et al. (1959) as follows:

$$CrI = \frac{I_{002} - I_{amorphous}}{I_{002}} \times 100$$

where  $I_{002}$  is the intensity of the diffraction from the 002 plane at  $2\theta = 22.5^\circ$  and  $I_{amorphous}$  is the intensity of the background scatter at  $2\theta = 18.7^\circ$ . The diffractogram was smoothed using a smooth function in the MATLAB program (Appendix A).

## Fourier Transform Infrared Spectroscopy

Fourier transform infrared spectroscopy (FTIR) is normally used to investigate the structure of constituents and chemical changes in lignocellulosic biomass. Cellulose decrystallization usually is associated with reduced crystallinity, which suggests that crystallinity can be used to analyze biomass before and after treatment. FTIR measurements were performed using a PerkinElmer Spectrum 400 FT-IR spectrometer (PerkinElmer Inc., Waltham, MA). All spectra were recorded in the absorbance mode in the wave number range of 400-4000  $\text{cm}^{-1}$  with a detection resolution of 4  $\text{cm}^{-1}$  and 16 scans per sample.

## **Solid-State $^{13}\text{C}$ NMR Spectroscopy**

Solid-state  $^{13}\text{C}$  NMR spectroscopy is widely used in many studies for the analysis of chemical structure of biomass because it is non-destructive and can provide comprehensive structural information (Liitia et al., 2003; Wikberg et al., 2004; Mao et al., 2010; Popescu et al., 2011; Xiao et al., 2011). Corn stover and big bluestem were used as testing samples for the solid-state  $^{13}\text{C}$  NMR analysis to determine the effects of the dilute-acid pretreatment process, the pelleting process, and pelleting conditions on the chemical structure of biomass. Solid-state  $^{13}\text{C}$  NMR spectra were acquired on a Bruker Avance III 400 spectrometer (Bruker Biospin, Billerica, MA) operating at 400.1 MHz for  $^1\text{H}$  and 100.6 MHz for  $^{13}\text{C}$ . A 7-mm spin module in a 4-module multiple sample solids (MSS) probe (Revolution NMR, Ft. Collins, CO) was used. Spectrometer setup used 3-methylglutaric acid (MGA) as a secondary external chemical shift reference via the methyl peak at 18.84 ppm relative to TMS (Solid State Nuclear Magnetic Resonance (2006), 30(3-4), 125-129). Each sample was packed into a 7.0-mm zirconia rotor (Revolution NMR, Ft. Collins, CO). The rotors were closed with Kel-F end caps. Magic angle spinning used a sample spinning rate of 4 kHz. Cross polarization (CP) was used for all measurements using a contact time of 1.0 ms. A pulse delay of 2.0 seconds was used. The spectral width was 40 kHz and the acquisition time was 15 ms. Proton decoupling was performed with SPINAL-64 and a proton-decoupling field of 64 kHz. Each data set is the sum of 7200 transients.

## **Scanning Electron Microscopy**

Scanning electron microscopy (SEM) was used to measure the surface properties and microstructure of biomass before and after treatment. A Hitachi S-3500M SEM with an S-6542 absorbed-electron detector (Hitachinaka, Japan) was used to examine the microstructure of sorghum biomass before and after treatment from 1.5K to 3K. Specimens were mounted on conductive adhesive tape, sputter-coated with 4 nm of a 60% gold and 40% palladium mixture, and observed using a voltage of 15 to 20 kV.

## **Thermogravimetric Analysis**

Thermogravimetric analysis (TGA) was used to measure the thermal properties of biomass samples. The TGA was carried out on a Pyris 1 TGA Thermogravimetric Analyzer (The PerkinElmer Corporation, Norwalk, CT). Dynamic TG scans were conducted in a temperature

range from 30 to 800 °C at a heating rate of 20 °C/min. The experiments were carried out under a nitrogen purge at a constant rate of 20 mL/min to prevent oxidation of samples. Samples with approximately 5 to 6 mg of unpelleted and pelleted biomass were used.

## **RESULTS AND DISCUSSION**

### **Crystalline Structure Analysis**

Crystallinity index (CrI) could be used to estimate the impact of pretreatment on biomass crystallinity (Kumar et al., 2009). Crystallinity is counted as one of the factors (apart from lignin and hemicellulose content/distribution, particle size, and porosity of the cell wall) that influences the accessibility of cellulose microfibril to cellulase enzyme and thus determines the hydrolysis rate (Park et al., 2010). According to Fig. 1, pelleting parameters, including biomass particle size and die size, did not seem to have a significant effect on biomass crystallinity. Similar results were observed for all four types of biomass. Fig.2 shows the effect of the pelleting process (unpelleted biomass with no pretreatment (UN) vs. pelleted biomass with no pretreatment (PN)) and dilute acid pretreatment (UN vs. unpelleted biomass with pretreatment (UT)) on the biomass crystallinity. Biomass crystallinity obviously increased after the biomass was pretreated with dilute sulfuric acid. This phenomenon also occurred for pelleted biomass (Fig. 2, PN vs. pelleted biomass with pretreatment (PT)). The increase of biomass crystallinity after dilute acid pretreatment was due to the removal of hemicellulose, which is amorphous material, from the biomass network together with the disruption of hydrogen bonding of cellulose chain by dilute acid pretreatment, thus causing a relative increase in the amount of crystalline portion of biomass (Zaldivar et al., 2001; Liitia et al., 2003; Saha et al, 2005).

Proximate analysis of biomass showed no significant change in chemical compositions of biomass due to the pelleting process (data not shown). Because the biomass was subjected only to frictional heat generated during extrusion, the amount of heat was probably not intense enough to cause significant change in chemical compositions of biomass; however, the biomass crystallinity was found to increase slightly after the pelleting (extrusion) process (Fig. 2, UN vs. PN). A similar result was reported by Yoo et al. (2011), except that the changes in biomass crystallinity as affected by the pelleting process were not significant in this study. With no significant change in chemical compositions of biomass due to pelleting process, the slight increase in biomass crystallinity induced by the pelleting process was probably due to the

crystallization of the amorphous phase of cellulose. Another possible explanation is the change in lignin structure during pelleting process. Lignin has a low melting point of about 140 °C. When the biomass is heated, lignin turns soft and sometimes melts and exhibits thermosetting properties. During the pelleting process, biomass is subjected to frictional heat generated between the biomass and a die (Tabil and Sokhansanj, 1996), which results in softening of lignin in the biomass. Gilbert et al. (2009) conducted the dynamic mechanical thermal analysis (DMTA) of switchgrass pellets and found that the lignin in the pellets softened as the temperature increased to 70 °C. The further increase of temperature toward 100 °C caused evaporation of moisture and made the pellet harder and more brittle (Gilbert et al, 2009). The thermosetting behavior of lignin present in the pellets by heat generated during extrusion process can lead to loosening of the biomass' compact network and expose the crystalline cellulose more to the x-ray, as indicated by the slight increase of crystallinity index values after pelleting of biomass in this study.

### **FTIR Analysis**

The FTIR spectra of sorghum stalk, corn stover, big bluestem, and wheat straw (both unpelleted and pelleted) show several absorption bands that can be assigned to major structural components: cellulose, hemicellulose, and lignin. The assignment of FTIR absorption bands for these biomass samples are summarized in Table 1. As shown in Fig. 3, the 4000-1800  $\text{cm}^{-1}$  region of the absorbance spectra has only a few bands, which are attributed to the O-H group (at 3400-3200  $\text{cm}^{-1}$ ) and the C-H group (at around 2905-2850  $\text{cm}^{-1}$ ). These bands are pure, whereas other bands in the fingerprint region (1800-900  $\text{cm}^{-1}$ ) are complex; this is a result of various vibration modes in carbohydrates and lignin (Gilbert et al., 1993; Pandey, 1999). The effect of the pelleting process and pelleting variables (hammer mill screen size and die thickness) on the structure of constituents of biomass can be determined from the FTIR spectra of each type of biomass before and after treatment (Fig.4-5). No significant difference was observed in the pattern of FTIR spectra of biomass due to the pelleting process and pelleting variables. This result implied that the pelleting process and pelleting variables did not have much effect on the changes in structure of constituents of biomass samples.

In the fingerprint region, C-O of guaiacyl ring lies at 1273-1267  $\text{cm}^{-1}$ , and the C-H bending modes appear at 1450  $\text{cm}^{-1}$  and 1423-1417  $\text{cm}^{-1}$ , respectively. Although all biomass

spectra were similar, slight changes were observed from spectrum to spectrum. For example, there were no peaks at 1273-1267 and 1337-1329  $\text{cm}^{-1}$  for the untreated biomass (Fig.4). The hemicellulose band at 1740-1735  $\text{cm}^{-1}$ , attributed to C=O ester and strong carbonyl groups in branched hemicellulose (Guo et al., 2008; Kumar et al., 2009; Pandey, 1999; Sun and Tomkinson, 2004) appeared for all biomass samples before treatment but was not observed after dilute-acid pretreatment. The absence of this hemicellulose band after the pretreatment process indicated that the dilute acid pretreatment could effectively remove most of the xylan (hemicellulose) from biomass.

Cellulose-related bands in the FTIR spectra were seen around 900-895, 1100-1098, 1423-1417, 1450, 1650-1633, 2905-2850, and 3400-3200  $\text{cm}^{-1}$  (Gastaldi et al., 1998; Gilbert et al., 1993; Kumar et al., 2009; Pandey, 1999; Sun et al., 1998). The band at 900  $\text{cm}^{-1}$  was associated with the antisymmetric out-of-plane ring stretch of amorphous cellulose (Kumar et al., 2009; Pandey, 1999; Liu et al., 2005; Gilbert et al., 1993). The broadening of this band reflected a higher degree of disorder in the structure (Wang et al., 2006). In this study, the bands near 900  $\text{cm}^{-1}$  for all biomass samples retained their position after pretreatment and a shoulder at the higher frequency on the 900  $\text{cm}^{-1}$  also appeared, indicating the presence of two non-equivalent C-O-C bonds.

Lignin-related bands in the FTIR spectra were seen around 1273-1267, 1512-1509, and 1605-1590  $\text{cm}^{-1}$  (Kumar et al., 2009; Pandey, 1999; Sun et al., 1998). The band at 1512-1509  $\text{cm}^{-1}$ , attributed to the C=C guaiacyl ring of lignin, was observed for all untreated biomass. This spectrum remained after pretreatment. The band at 1605-1590  $\text{cm}^{-1}$  is associated with the  $\alpha$ - $\beta$  double bond of the propanoid side group in lignin-like structures (Corredor et al., 2009; Kumar et al., 2009; Pandey, 1999). This band was observed in all untreated and treated samples.

### **Solid-State $^{13}\text{C}$ NMR Analysis**

Similar to the FTIR analysis, the solid-state  $^{13}\text{C}$  NMR analysis is a qualitative technique that can be used to determine the chemical structure and composition between the biomass samples. Corn stover and big bluestem samples were selected for  $^{13}\text{C}$  NMR spectra (Fig. 6 and Fig. 7, respectively). For each type of biomass, the spectra of the unpelleted sample, the pelleted samples densified under different pelleting conditions, and the pretreated pelleted samples were compared and analyzed. The assignments and chemical shifts of the signals in  $^{13}\text{C}$  NMR spectra

of corn stover and big bluestem were summarized in Table 2. The assignments of each signal were referred from many studies (Mao et al., 2010; Popescu, et al., 2011; Xiao et al., 2011; Wikberg and Maunu, 2004).

### ***Cellulose***

Based on the  $^{13}\text{C}$  NMR spectra of big bluestem (Fig. 6) and corn stover (Fig. 7), distinct signals of crystalline and amorphous carbons of cellulose were detected; the signals at 105.4 ppm, 88.8 ppm, and 65.5 ppm were assigned to the C-1, C-4, and C-6 carbons of ordered cellulose, respectively, whereas the signals at 84.6 ppm and 63.0 ppm were assigned to the C-4 and C-6 carbons of disordered cellulose, respectively. The signals at 75.3-72.9 ppm corresponded to the C-2, C-3, and C-5 carbons of cellulose.

By comparing the spectra among big bluestem samples, including unpelleted big bluestem (B0) and pelleted big bluestem (pelleted under different pelleting conditions; B1, B2, B3), no significant changes in relative intensities of cellulose signals were observed. This phenomenon implied that the pelleting process and pelleting variables (die thickness and hammer mill screen size) did not have a strong effect on the cellulose structure. This trend also occurred for corn stover samples; however, relative intensities of cellulose signals for both corn stover and big bluestem samples increased after the pretreatment process. This result indicates that the pretreatment process did alter the cellulose structure of biomass.

### ***Hemiellulose***

The signals at 21.7 ppm and 171.2 ppm were assigned to methyl and carboxyl groups of the acetyl function of hemicellulose. These signals disappeared as the biomass was pretreated with dilute sulfuric acid, confirming the removal of hemicellulose by acid during the pretreatment process. On the other hand, no changes in the relative intensity of these signals were observed among the  $^{13}\text{C}$  NMR spectra of unpelleted biomass and pelleted biomass that was densified under different conditions, indicating that the pelleting process and pelleting conditions did not remove the hemicellulose, which is an amorphous fraction of biomass. The insignificant change in chemical composition of biomass after the pelleting process confirmed this finding.

## ***Lignin***

The signals for aromatic carbons of lignin in the  $^{13}\text{C}$  NMR spectra were shown in a region between 140 and 155 ppm. The main units in lignin are guaiacyl (G) and syringyl (S). The signal at 147.5 ppm corresponded to the C-3 and C-5 carbons in nonetherified S units and the C-3 carbons of G units. The signal at 153.3 ppm corresponded to the C-3 and C-5 carbons of S units and C-4 carbon of G units. Besides this region, the methoxyl groups in lignin gave a signal at 56.4 ppm. The relative intensity of lignin signals, especially for signals at 147.5 ppm and 56.4 ppm, increased after pretreatment of biomass, which is consistent with higher lignin in biomass residues after dilute acid pretreatment (data for biomass composition not shown); however, the relative intensities of lignin signals in the  $^{13}\text{C}$  NMR spectra of biomass before and after the pelleting process showed no significant difference.

## **Morphological Structure Analysis**

In the SEM image of the unpelleted corn stover sample before dilute-acid pretreatment (Fig 8a), the sample seemed to have deposits on its surface. This surface layer included waxes, lignin, hemicellulose, and other binding materials. After the pelleting process, this softened surface region of biomass was removed, revealing the clean and smooth surface with some small fine solid particles on the outer surface of pelleted biomass, as seen in Fig. 8b. Lamsal et al. (2010) claimed that improvement in the overall rate of decrystallization or sugar yield of biomass that was pretreated with a combination of thermo-mechanical (extrusion) and chemical (alkali and acid) pretreatment was due to the continuous removal of softened surface regions of the substrate by applied shear force in the extrusion process, thereby exposing the interior to chemical and/or thermal action. Fig. 8c shows the cellulose bundle of the pelleted corn stover. This cellulose bundle is composed of various ordered parallel microfibrils and is the backbone of the cell. The diameter of individual microfibril was about 21 microns (Fig. 8d). The length of these microfibrils was much longer, and some fibers were endless.

After dilute-acid pretreatment, fewer deposits were observed on the outer layer of corn stover, making the surface appear relatively clean and smooth (Fig. 9a, 9b). The biomass network was disrupted and the hemicellulose was removed during the dilute-acid pretreatment process, revealing plenty of holes in cellulose wall and annular rings that were parts of the biomass internal structure (Fig.9b-e). In addition, several microfibrils were separated, as can be



observed in Fig. 9f. The SEM image patterns affected by the treatment process of corn stover were similar to the patterns observed in sorghum stalk as reported in a previous study (Theerarattanananon et al., 2010). Overall, besides removal of the softened surface region of biomass, the pelleting process did not seem to cause significant changes in the morphological structure of biomass, because the SEM images of pelleted corn stover samples before and after dilute-acid pretreatment were about the same as those of unpelleted corn stover.

### **Thermogravimetric Analysis**

To understand the changes in thermal properties of biomass samples caused by the pelleting process, the thermogravimetric analysis (TGA) and first derivative thermogravimetric (DTG) of unpelleted and pelleted samples were measured. Figure 10 shows the TGA and DTG curves of corn stover, big bluestem, wheat straw, and sorghum stalk. Overall, the TGA distribution profiles (Fig. 10, left axis) of unpelleted and pelleted biomass samples were similar; the weight losses of samples that occurred within the TG's temperature of 30 to 250 °C were less than 10%, whereas a significant amount of weight loss from samples was observed as the TG's heating temperature increased from 250 to 400 °C. For all four types of biomass samples, the maximum weight loss of pelleted biomass was slightly less than that of the unpelleted biomass. The values of maximum weight loss were 70.9% (pelleted) and 75.7% (unpelleted) for corn stover, 75.1% (pelleted) and 76.5% (unpelleted) for big bluestem, 75.7% (pelleted) and 75.9% (unpelleted) for wheat straw, and 71.2% (pelleted) and 73.5% (unpelleted) for sorghum stalk.

According to the DTG (first derivative) profiles for the rate of weight loss of big bluestem and wheat straw (Fig.10b, 10c on right axis), the DTG curves of both unpelleted and pelleted biomass samples show two distinct peaks, indicating that the degradation can be explained by dividing the curves into two stages. The first stage, which appears at TG's temperature around 80 to 85 °C, represents the initial weight loss due to the elimination of absorbed or combined water in the sample (Tang et al., 2011). The second stage occurs from 210 to 530 °C, with the maximum decomposition rate at 350 to 370 °C, which is attributed to the decomposition of several components of biomass including hemicellulose, cellulose, and lignin. Hemicellulose was easily degradable and its decomposition temperature ranged from 220 to 315 °C (Yang et al., 2007). Cellulose decomposition occurred at 240 to 350 °C, producing anhydrocellulose and levoglucosan (Tang and Neill, 1964). Lignin decomposed slower and over

a broader temperature range (200 to 500 °C) than cellulose and hemicellulose because the various oxygen functional groups from its structure have different thermal stabilities (Brebou and Vasile, 2010). DTG profiles of unpelleted and pelleted corn stover samples show three distinct peaks (Fig. 10a). The first peak appeared at TG's temperature around 80 to 85 °C, which represents the initial weight loss due to the elimination of absorbed or combined water in the sample. The second peak was relatively small and occurred from 190 to 260 °C, which is attributed to decomposition of hemicellulose. The third peak was the largest peak and occurred from 270 to 530 °C, which is attributed to decomposition of lignin and cellulose. For sorghum stalk samples, the DTG profile of unpelleted sorghum stalk shows the same trend as the DTG profile of corn stover samples, but the DTG profile of pelleted sorghum stalk did not show the hemicellulose decomposition peak at 190 to 260 °C (Fig. 10d).

By referring to decomposition temperature as the point where the maximum weight loss rate of samples was attained, the decomposition temperatures of pelleted biomass seem to be slightly higher than those of corresponding unpelleted biomass; thus, pelleted biomass was a bit more thermally stable than the unpelleted biomass.

## **CONCLUSIONS**

Biomass crystallinity, measured by crystallinity index (CrI) values, of all four types of biomass materials obviously increased after dilute-acid pretreatment, indicating that the dilute-acid pretreatment process was effective in disrupting inter-intra hydrogen bonding of the cellulose chain and enhancing the breakdown of amorphous cellulose. A slight increase in the CrI values of biomass was observed after the pelleting process. Because there was no significant change in the chemical composition of biomass after the pelleting process, the increased biomass crystallinity was probably due to the crystallization of amorphous cellulose and the change in lignin structure when the biomass was subjected to intense mechanical shear and heat generated during extrusion.

Results from thermogravimetric analysis showed that the decomposition temperature of pelleted biomass was slightly higher than that of corresponding unpelleted biomass, implying that pelleted biomass was slightly more thermally stable than the unpelleted biomass. Results from the FTIR analysis showed no significant differences in the pattern of FTIR spectra of biomass due to the pelleting process and pelleting conditions. The same trend was observed for

the solid-state  $^{13}\text{C}$  NMR analysis of biomass; however, the  $^{13}\text{C}$  NMR analysis of biomass showed that the pretreatment process did alter the chemical structure of biomass, mainly due to degradation of hemicellulose. Morphological structure of biomass was found to be affected by both dilute-acid pretreatment and pelleting processes. Dilute-acid pretreatment and applied shear force in the pelleting process resulted in removal of the softened surface region of biomass. Pelleting parameters did not have a significant impact on the morphological structure of biomass.

In conclusion, pelleting parameters including die thickness and biomass particle size did not have a strong impact on the chemical structure and thermal property of biomass in this study. The pelleting process made the biomass more thermally stable. Both dilute acid pretreatment and pelleting processes caused an increase of biomass crystallinity and removal of the softened surface region of biomass. The modification of biomass structure by these treatment processes would make the biomass more amenable to enzymatic attack and thus improve fermentable sugar yield.

## **ACKNOWLEDGEMENT**

This project was funded by the Biomass Research and Development Initiative Competitive Grants Program (BRDI), USDA National Institute of Food and Agriculture, Grant No. 68-3A75-7-609. This is contribution number 12-162-J from the Kansas Agricultural Experiment Station. This study was also partly supported by NSF award CMMI-0970112. The NMR analysis was conducted at the Molecular Structures Group Laboratories, the University of Kansas, Grant No. NSF0060858.

## **REFERENCES**

- Akin, D.E., Morrison, W.H., Rigsby, L.L., Barton, F.E., Himmelsbach, D.S., Hicks, K.B., 2006. Corn stover fractions and bioenergy. *Appl. Biochem. Biotech.* 129-132, 104-116.
- Brebu, M., Vasile, C., 2010. Thermal degradation of lignin - a review. *Cellulose Chem. Technol.* 44(9), 353-363.
- Buranov, A.U., Mazza, G., 2008. Lignin in straw of herbaceous crops. *Ind. Crop Prod.* 28, 237-259.
- Chang, V.S., Holtzapfle, M.T., 2000. Fundamental factors affecting biomass enzymatic reactivity. *Applied Biochem. Biotechnol.* 84, 5-37.

- Colley, Z., Fasina, O.O., Bransby, D., Lee, Y.Y., 2006. Moisture effect on the physical characteristics of switchgrass pellets. *T ASAE*. 49, 1845-1851.
- Feng, X., Shi, Y.-C., Wu, X., Theerarattananoon, K., Staggenborg, S., Wang, D., 2011. Sulfuric acid pretreatment and enzymatic hydrolysis of photoperiod sensitive sorghum for ethanol production. *Bioprocess Biosyst Eng*. 34(4), 485-492.
- Gastaldi, G., Capretti, G., Focher, B., Cosentino, C., 1998. Characterization and properties of cellulose isolated from the *Crambe abyssinica* hull. *Ind. Crop Prod*. 8(3), 205-218.
- Gilbert, C., Kokot, S., Meyer, U., 1993. Application of DRIFT spectroscopy and chemometrics for the comparison of cotton fabrics. *Appl. Spectrosc*. 47(6), 741-748.
- Gilbert, P., Rye, C., Sharifi, V., Swithenbank, J., 2009. Effect of process parameters on pelletisation of herbaceous crops. *Fuel*. 88, 1491-1497.
- Guo, G.-L., Chen, W.-H., Men, L.-C., Hwang, W.-S., 2008. Characterization of dilute acid pretreatment of silvergrass for ethanol production. *Bioresource Tech*. 99(14), 6046-6053.
- Kaliyan, N., Morey, R.V., White, M.D., Doering, A., 2009. Roll-press briquetting and pelleting of corn stover and switchgrass. *T ASAE*. 52, 543-555.
- Karunanithy, C., Muthukumarrappan, K., 2010. Effect of extruder parameters and moisture content of switchgrass, prairie cord grass on sugar recovery from enzymatic hydrolysis. *Appl. Biochem. Biotechnol*. 162, 1785-1803.
- Karunanithy, C., Muthukumarrappan, K., 2011. Optimization of alkali, big bluestem particle size, and extruder parameters for maximum enzymatic sugar recovery using response surface methodology. *Bioresources*. 6(1), 762-790.
- Kumar, R., Mago, G., Balan, V., Wyman, C., 2009. Physical and chemical characterizations of corn stover and poplar solids resulting from leading pretreatment technologies. *Bioresource Tech*. 100, 3948-3962.
- Lamsal, B., Yoo, J., Brijwani, K., Alavi, S., 2010. Extraction as a thermo-mechanical pretreatment for lignocellulosic ethanol. *Biomass Bioenerg*. 34, 1703-1710.
- Lawther, J.M., Sun, R., Bank, W.B., 1995. Extraction, fractionation, and characterization of structural polysaccharide from wheat straw. *J. Agric. Food. Chem*. 43, 667-675.

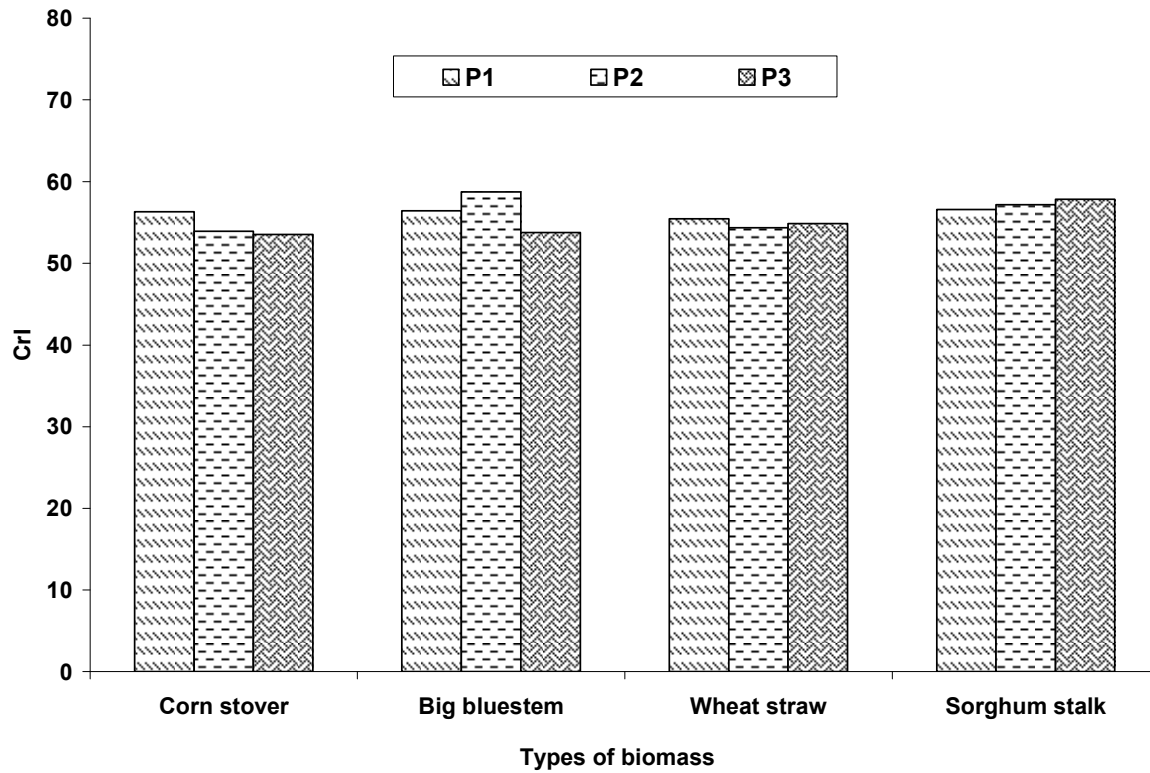
- Liitia, T., Maunu, S.L., Hortling, B., Tamminen, T., Pekkala, O., Varhimo, A., 2003. Cellulose crystallinity and ordering of hemicellulose in pine and birch pulps as revealed by solid-state NMR spectroscopic methods. *Cellulose*. 10, 307-316.
- Liu, L., Ye, X.P., Womac, A.R., Sokhansanj, S., 2010. Variability of biomass chemical composition and rapid analysis using FT-NIR techniques. *Carbohydrate Polymers*. 81, 820-829.
- Liu, R., Yu, H., Huang, Y., 2005. Structure and morphology of cellulose in wheat straw. *Cellulose*. 12, 25-34.
- Mao, J.-D., Holtman, K.M., Franqui-Villanueva, D., 2010. Chemical structures of corn stover and its residue after dilute acid prehydrolysis and enzymatic hydrolysis: insight into factors limiting enzymatic hydrolysis. *J. Agric. Food Chem.* 58, 11680-11687.
- Mitchell, R., Fritz, J., Moore, K., Moser, L., Vogel, K., Redfearn, D., Wester, D., 2001. Predicting forage quality in switchgrass and big bluestem. *Agron. J.* 93, 118-124.
- Pandey, K.K., 1999. A study of chemical structure of soft and hardwood and wood polymers by FTIR spectroscopy. *J. Appl. Polymer Sci.* 71(12), 1969-1975.
- Park, S., Baker, J.O., Himmel, M.E., Parilla, P.A., Johnson, D.K., 2010. Cellulose crystallinity index: measurement techniques and their impact on interpreting cellulase performance. *Biotechnol. Biofuels*. 3, 1-10.
- Popescu, C., Larsson, P.T., Vasile, C., 2011. Carbon-13 CP/MAS solid state NMR and X-ray diffraction spectroscopy studies on lime wood decayed by *Chaetomium globosum*. *Carbohydr Polym.* 83, 808-812.
- Propheter, J.L., Staggenborg, S.A., We, X., Wang, D., 2010. Performance of annual and perennial biofuel crops: yield during the first two years. *Agron.* 102, 806-814.
- Saha, B.C., Iten, B., Cotta, M.A., Wu, Y.V., 2005. Dilute acid pretreatment, enzymatic saccharification, and fermentation of wheat straw to ethanol. *Process Biochem.* 40(12), 3693-3700.
- Segal, L., Creely, J. J., Martin, A. E. J., Conrad, C. M., 1959. An empirical method for estimating the degree of crystallinity of native cellulose using the x-ray diffractometer. *Textile Res. J.* 29(10), 786-794.
- Sun, R., Fang, J. M., Rowland, P., Bolton, J., 1998. Physicochemical and thermal characterization of wheat straw hemicellulose and cellulose. *J. Agric. Food Chem.* 46(7), 2804-2809.

- Sun, R., Tomkinson, J., 2004. Separation and characterization of cellulose from wheat straw. *Sep. Sci. Tech.* 39(2), 391-411.
- Tabil, L., Sokhansanj, S., 1996. Process conditions affecting the physical quality of alfalfa pellets. *Appl. Eng. Agric.* 12(3), 345-350.
- Tang, J., Chen, K., Xu, J., Li, J., Zhao, C., 2011. Effects of dilute acid hydrolysis on composition and structure of cellulose in *Eulaliopsis banana*. *BioResources.* 6(2), 1069-1078.
- Tang, W.K., Neill, W.K., 1964. Effect of flame retardants on pyrolysis and combustion of alpha-cellulose. *J. Polym. Sci. Part C: Polym. Symp.* 6, 65-81.
- Teeaar, R., Serimaa, R., Paakkari, T., 1987. Crystallinity of cellulose, as determined by CP/MAS NMR and XRD methods. *Polymer Bulletin.* 17, 231-237.
- Theerarattananoon, K., Wu, X., Staggenborg, S., Propheter, J., Madl, R., Wang, D., 2010. Evaluation and characterization of sorghum biomass as feedstock for sugar production. *T ASAE.* 53, 509-525.
- Theerarattananoon, K., Xu, F., Wilson, J., Ballard, R., Mckinney, L., Staggenborg, S., Vadlani, P., Pei, Z.J., Wang, D., 2011. Physical properties of pellets made from sorghum stalk, corn stover, wheat straw, and big bluestem. *Ind. Crop Prod.* 33, 325-332.
- Wang, L., Zhang, Y., Gao, P., Shi, D., Liu, H., Gao, H., 2006. Changes in the structural properties and rate of hydrolysis of cotton fibers during extended enzymatic hydrolysis. *Biotechnol. Bioeng.* 93(3), 443-456.
- Weimer, P.J., Springer, T.L., 2007. Fermentability of eastern gamagrass, big bluestem, and sand bluestem grown across a wide variety of environments. *Bioresour. Technol.* 98, 1615-1621.
- Wikberg, H., Maunu, S.L., 2004. Characterisation of thermally modified hard- and softwoods by <sup>13</sup>C CPMAS NMR. *Carbohydr Polym.* 58, 461-466.
- Xiao, L.-P., Sun, Z.-J., Shi, Z.-J., Xu, F., Sun, R.-C., 2011. Impact of hot compressed water pretreatment on the structural changes of woody biomass for bioethanol production. *Bioresources.* 6(2), 1576-1598.
- Yan, L., Zhu, Q., 2003. Direct observation of the main cell wall components of straw by atomic force microscopy. *J. Appl. Polym. Sci.* 88, 2055-2059.

- Yang, H., Yan, R., Chen, H., Lee, D.H., Zheng, C., 2007. Characteristics of hemicellulose, cellulose and lignin pyrolysis. *Fuel*. 86, 1781-1788.
- Yoo, J., Alavi, S., Vadlani, P., Amanor-Boadu, V., 2011. Thermo-mechanical extrusion pretreatment for conversion of soybean hulls to fermentable sugars. *Bioresour. Technol.* 102, 7583-7590.
- Yu, Y., Lou, X., Wu, H., 2008. Some recent advances in hydrolysis of biomass in hot-compressed water and its comparisons with other hydrolysis methods. *Energy Fuels*. 22, 46-60.
- Zaldivar, J., Nielsen, J., Olsson, L., 2001. Fuel ethanol production from lignocellulosic: A challenge for metabolic engineering and process integration. *Appl. Microbiol. Biotech.* 56, 17-34.
- Zhu, L., O'Dwyer, J.P., Chang, V.S., Granda, C.B., Holtzapple, M.T., 2008. Structural features affecting biomass enzymatic digestibility. *Bioresour. Technol.* 99, 3817-3828.

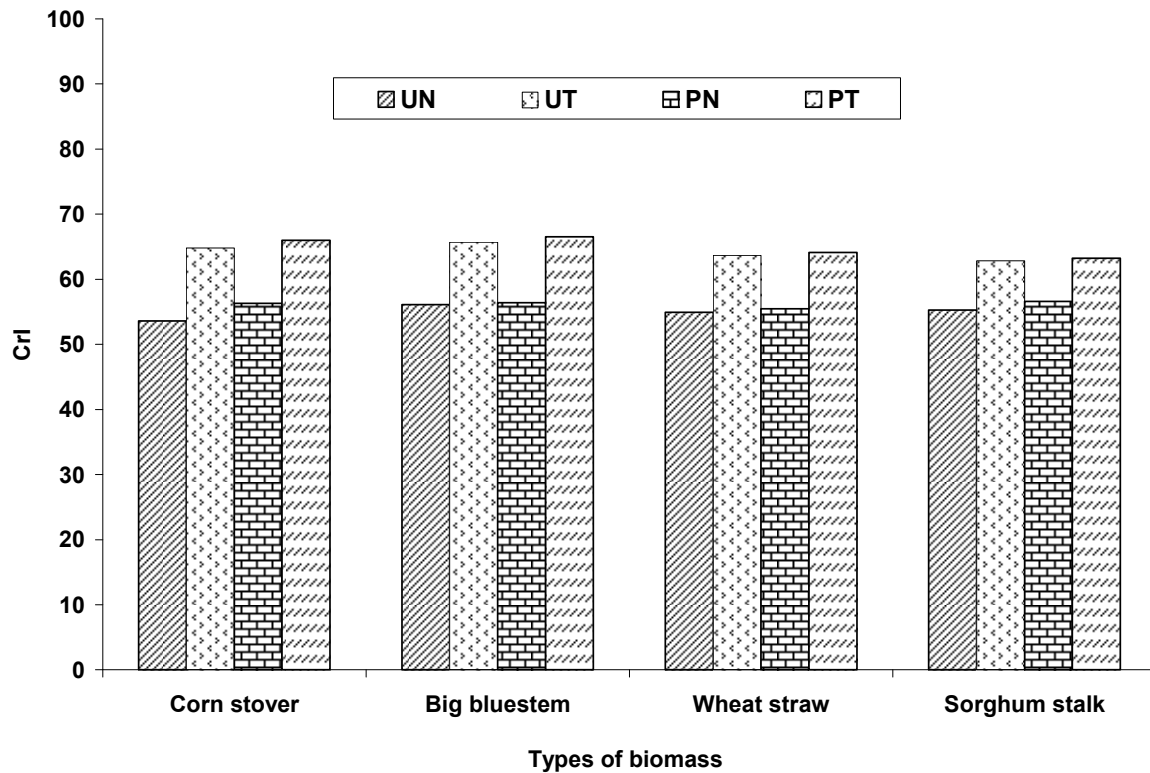
## **APPENDIX - MATLAB Code for Smoothing of X-Ray Diffraction Spectra**

```
clc
clear
A = [imported y-values from Excel file];
B = [imported x-values from Excel file];
windowsize = 20;
b = ones(1,windowsize)/windowsize;
A1 = filter(b,1,A)
//copy the values of A1 (data after smoothing)
into a column of Excel file
figure(1)
subplot(2,1,1)
plot(B,A)
subplot(2,1,2)
plot(B,A1)
```

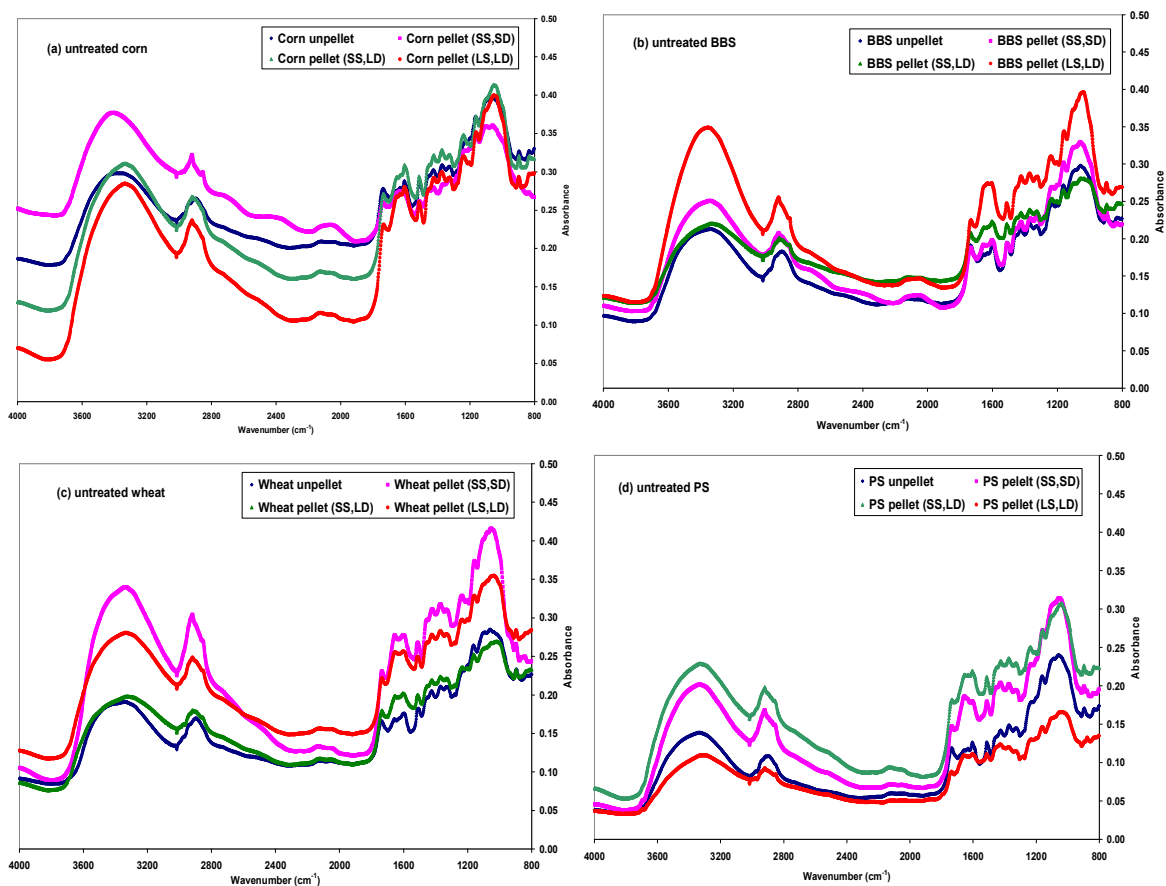


**Figure 5.1 Effect of pelleting parameters on CrI value of biomass; P1: pelleted biomass (3.2-mm mill screen size and 31.8-mm die thickness); P2: pelleted biomass (3.2-mm mill screen size and 44.5-mm die thickness); and P3: Pelleted biomass (6.5-mm mill screen size and 44.5-mm die thickness).**

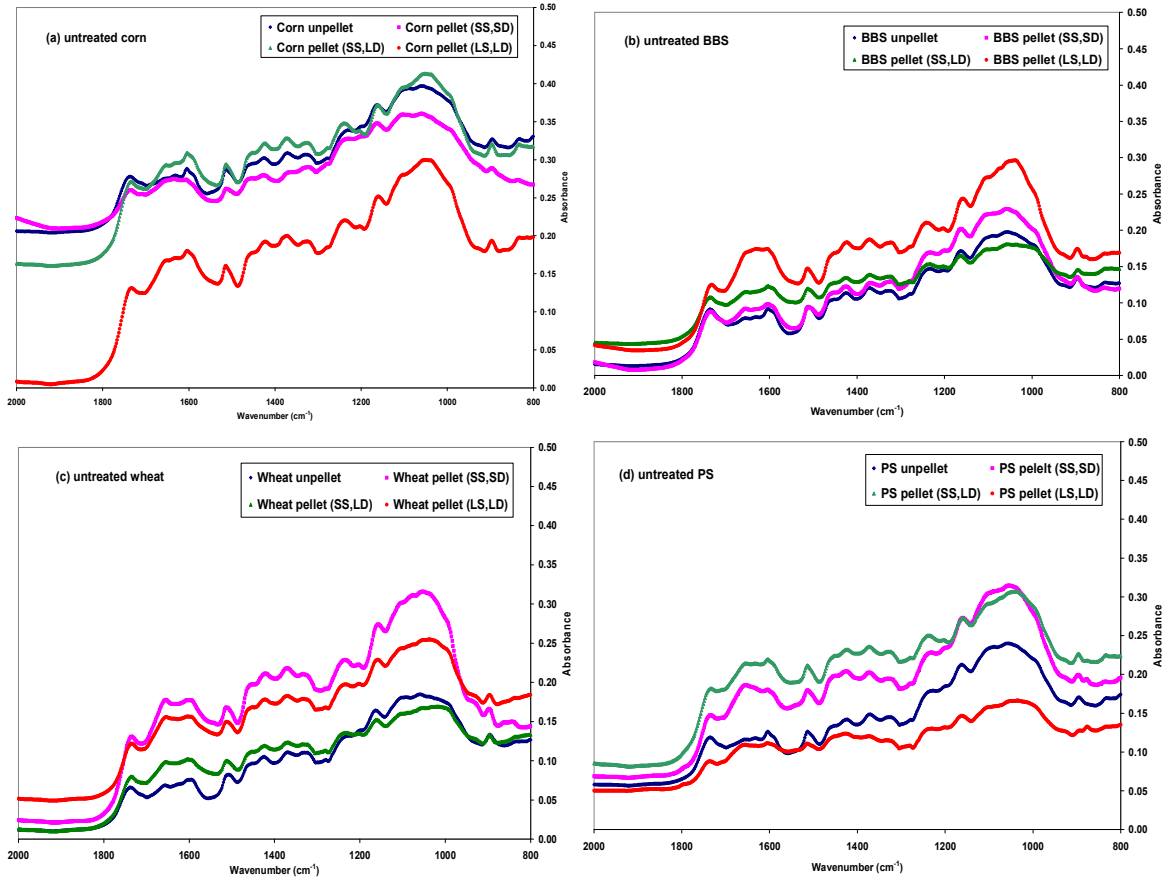




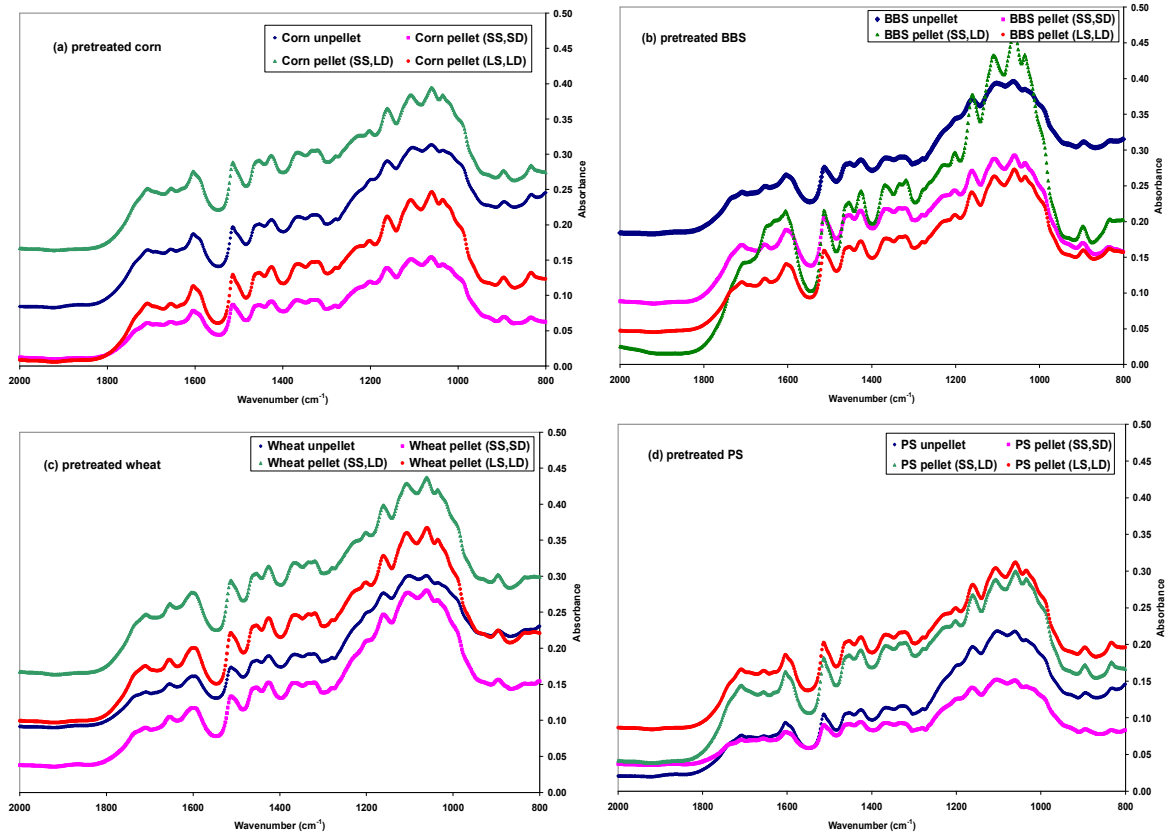
**Figure 5.2 Effects of pelleting process and pretreatment process on CrI value of biomass. UN: unpelleted biomass with no pretreatment; UT: unpelleted biomass with pretreatment; PN: pelleted biomass with no pretreatment; and PT: pelleted biomass with pretreatment.**



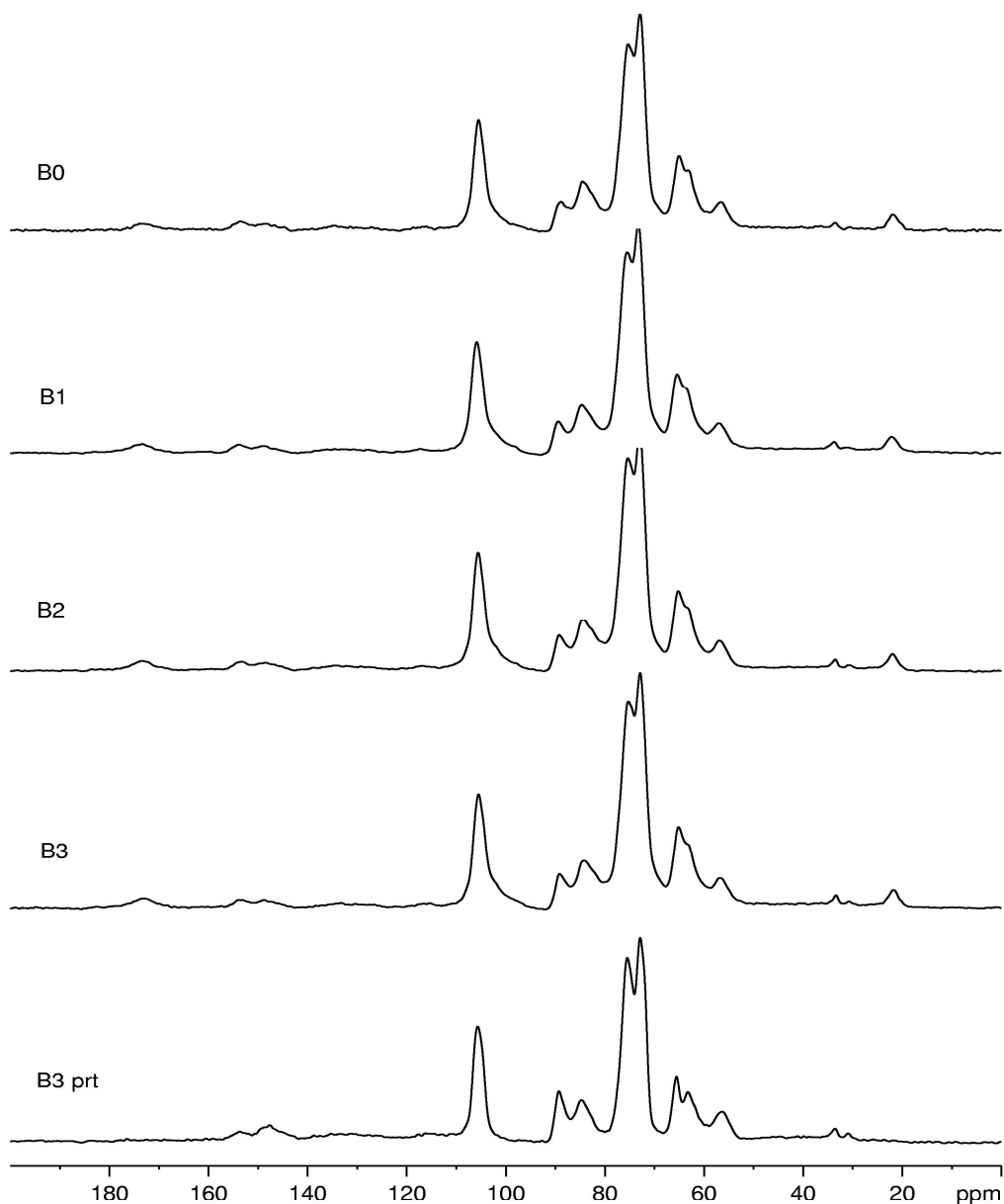
**Figure 5.3** FTIR spectra of (a) untreated corn stover, (b) untreated big bluestem (BBS), (c) untreated wheat straw, and (d) untreated photoperiod-sensitive sorghum stalk (PS) densified under different hammer mill screen size (small-SS, and large-LS) and die thickness (small-SD, large-LD).



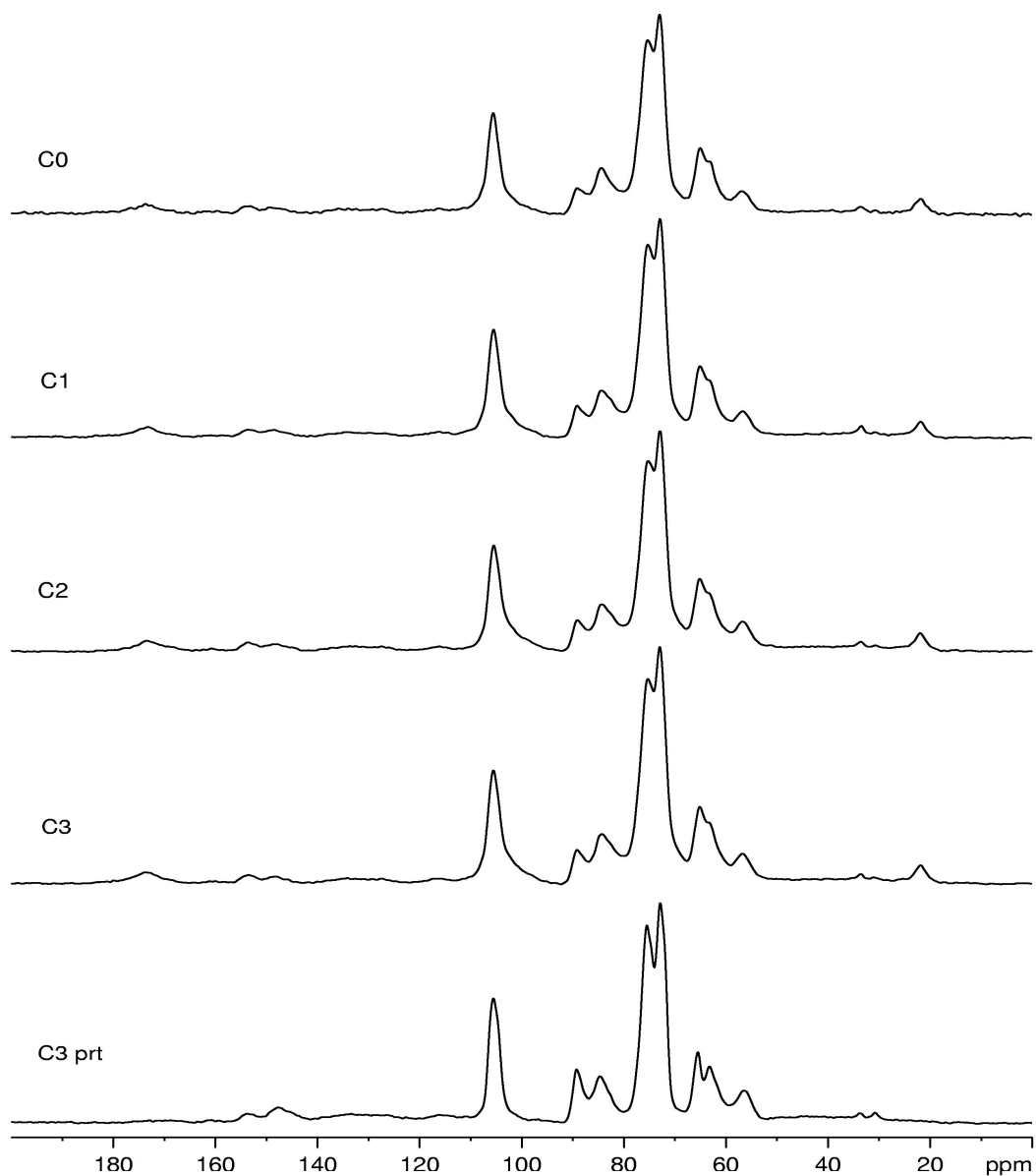
**Figure 5.4 FTIR spectra in the fingerprint region (900-1800 cm<sup>-1</sup>) of (a) untreated corn stover, (b) untreated big bluestem (BBS), (c) untreated wheat straw, and (d) untreated photoperiod-sensitive sorghum stalk (PS) densified under different hammer mill screen size (small-SS, and large-LS) and die thickness (small-SD, large-LD).**



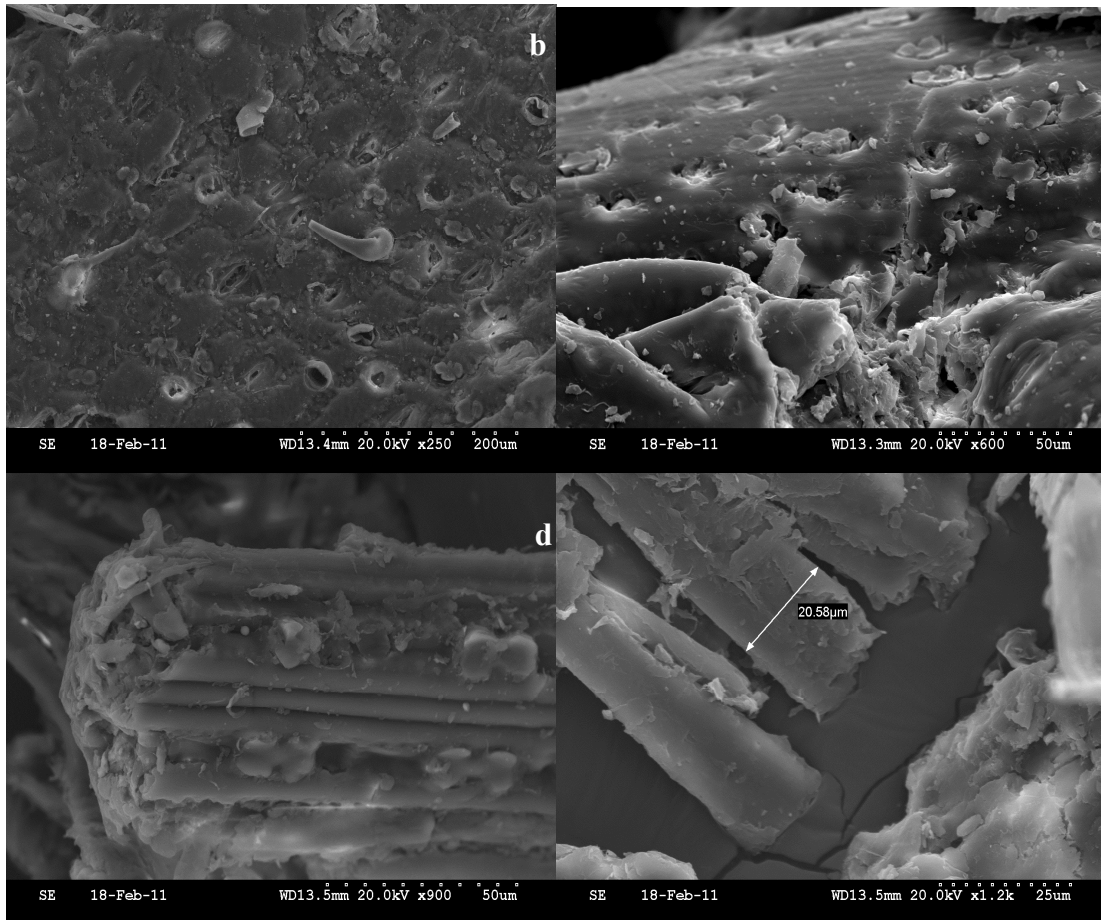
**Figure 5.5 FTIR spectra in the fingerprint region (900-1800 cm<sup>-1</sup>) of (a) pretreated corn stover, (b) pretreated big bluestem (BBS), (c) pretreated wheat straw, and (d) pretreated photoperiod-sensitive sorghum stalk (PS) densified under different hammer mill screen (small-SS, and large-LS) and die thickness (small-SD, large-LD).**



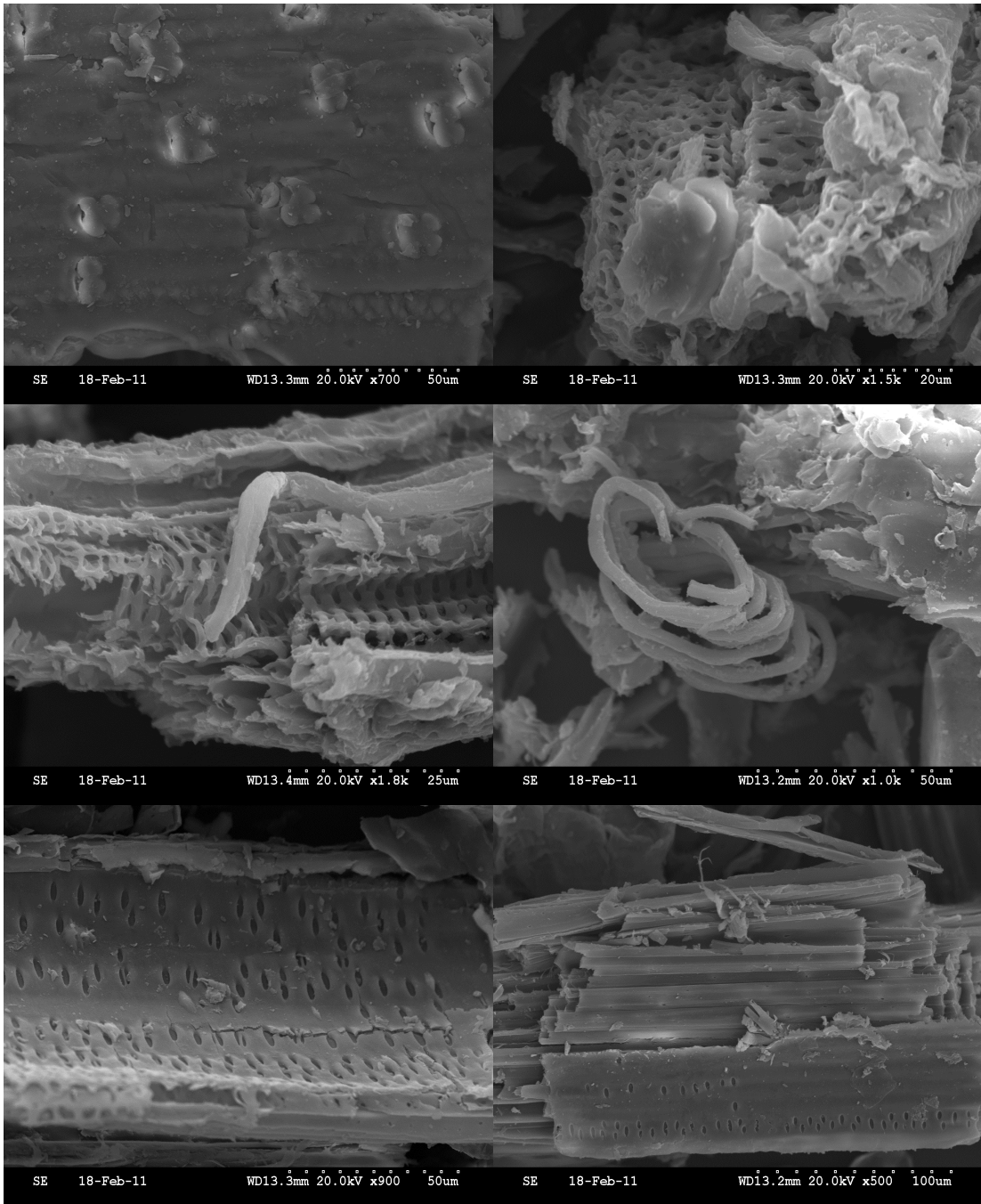
**Figure 5.6 <sup>13</sup>C NMR spectra of big bluestem: B0: untreated and unpelleted sample; B1: untreated and pelleted sample (pelleting condition: 3.2-mm hammer mill size and 31.8-mm die thickness); B2: untreated and pelleted sample (pelleting condition: 6.5-mm hammer mill size and 44.5-mm die thickness); B3: untreated and pelleted sample (pelleting condition: 3.2-mm hammer mill size and 44.5-mm die thickness); B3prt: pretreated and pelleted sample (pelleting condition is same as B3).**



**Figure 5.7**  $^{13}\text{C}$  NMR spectra of corn stover: C0: untreated and unpelleted sample; C1: untreated and pelleted sample (pelleting condition: 3.2-mm hammer mill size and 31.8-mm die thickness); C2: untreated and pelleted sample (pelleting condition: 6.5-mm hammer mill size and 44.5-mm die thickness); C3: untreated and pelleted sample (pelleting condition: 3.2-mm hammer mill size and 44.5-mm die thickness); C3prt: pretreated and pelleted sample (pelleting condition is same as C3).

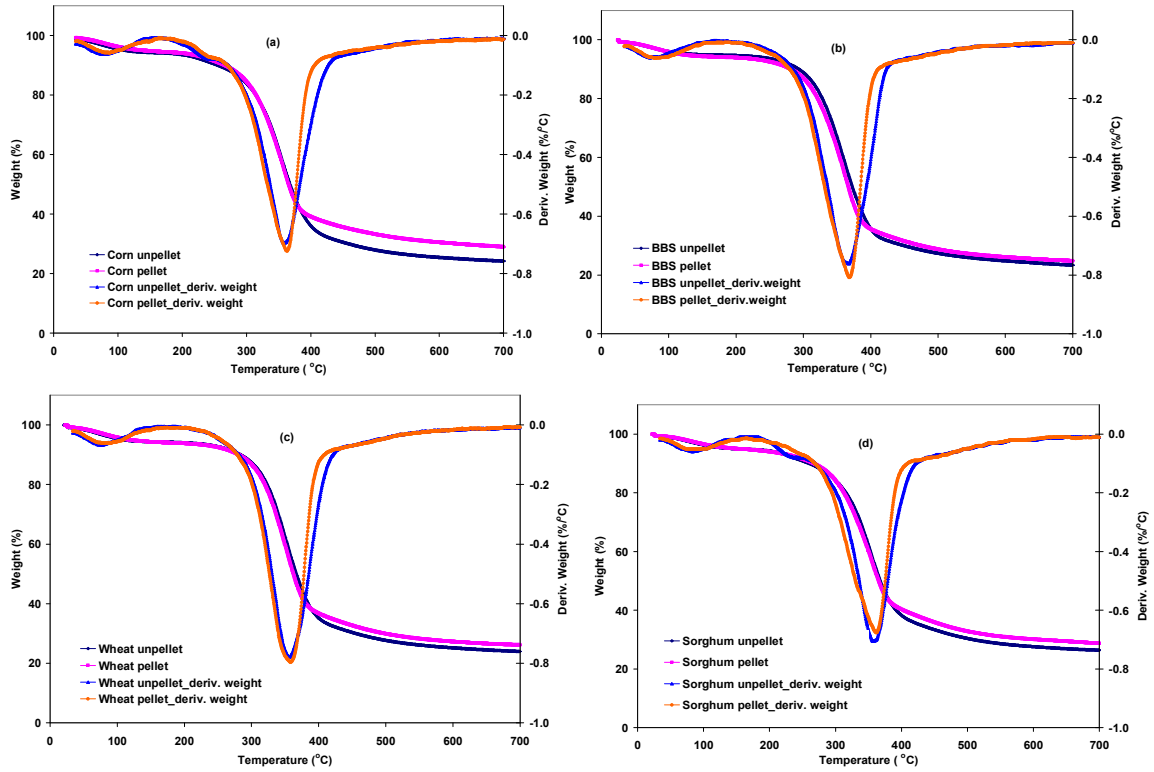


**Figure 5.8 SEM images of untreated corn stover: (a) outer surface of unpelleted corn stover, (b) outer surface of pelleted corn stover, (c) cellulose bundles of pelleted corn stover, and (d) microfibril of pelleted corn stover.**



**Figure 5.9 SEM images of corn stover after dilute-acid pretreatment process: (a) outer surface of unpelleted corn stover, (b) and (c) internal structure of pelleted corn stover, (d) annular rings of pelleted corn stover, and (e) holes in the cellulose wall of pelleted corn stover, and (f) microfibrils of pelleted corn stover.**





**Figure 5.10 Thermal properties of the unpelleted and pelleted biomass samples: (a) corn stover, (b) big bluestem, (c) wheat straw, and (d) photoperiod-sensitive sorghum stalk.**

**Table 5.1 Assignment of FTIR absorption bands for sorghum biomass and corn stover**

Wavenumbers (cm <sup>-1</sup> )	Pattern in <sup>[a]</sup>			Assignment	Reference	
	Unpelleted biomass	Biomass pellets				
		P1 <sup>[b]</sup>	P2 <sup>[c]</sup>	P3 <sup>[d]</sup>		
3400-3200	All	All	All	All	O-H stretching (indicates rupture of cellulose hydrogen bonds)	Pandey, 1999 Gastaldi et al, 1998 Gilbert et al., 1993
2905-2850	All	All	All	All	C-H stretching (indicates rupture of methyl/methylene group of cellulose)	Pandey, 1999 Gilbert et al., 1993
1740-1735	UT	UT	UT	UT	C=O ester; strong carbonyl groups in branched hemicellulose	Guo et al., 2008 Kumar et al., 2009 Pandey, 1999 Sun and Tomkinson., 2004
1650-1633	All	All	All	All	Absorbed H <sub>2</sub> O in cellulose, C=O with intramolecular hydrogen bond	Gilbert et al., 1993 Guo et al., 2008 Pandey, 1999 Liu et al., 2005
1605-1590	All	All	All	All	Aromatic skeletal vibration + C=O stretching (related to lignin removal)	Kumar et al., 2009 Pandey, 1999 Gastaldi et al, 1998
1512-1509	All	All	All	All	C=C (related to lignin removal) guaiacyl ring of lignin	Corredor et al., 2009 Pandey, 1999 Gastaldi et al., 1998 Sun et al., 1998
1450	All	All	All	All	C-H deformation (asymmetric) of cellulose	Gastaldi et al., 1998
1423-1417	All	All	All	All	C-H deformation (asymmetric) of cellulose, C-H bonds of the methoxyl groups	Gastaldi et al., 1998
1337-1329	Prt	Prt	Prt	Prt	O-H in-plane deformation, Syringil ring breathing deformation	Gastaldi et al., 1998 Gilbert et al., 1993 Pandey, 1999
1273-1267	Prt	Prt	Prt	Prt	C-O of guaiacyl ring and C-O stretching	Pandey, 1999 Gastaldi et al., 1998
1239-1232	UT	UT	UT	UT	O-H in-plane deformation	Pandey, 1999 Gilbert et al., 1993
1202-1198	All	All	All	All	O-H in-plane deformation	Gilbert et al., 1993 Pandey, 1999
1100-1098	All	All	All	All	Antisymmetric in-phase ring stretch of cellulose	Gilbert et al., 1993 Liu et al., 2005
1033-1026	All	All	All	All	C-H deformation in guaiacyl, C-O deformation in primary alcohol	Pandey 1999 Gastaldi et al, 1998 Gilbert et al., 1993
900-895	All	All	All	All	Antisymmetric out-of-plane ring stretch of amorphous cellulose	Kumar et al., 2009 Pandey, 1999 Liu et al., 2005 Gilbert et al., 1993

<sup>[a]</sup> UT = untreated (original) biomass, Prt = acid-pretreated biomass, EH = enzymatic hydrolysed biomass

<sup>[b]</sup> Pelleted by using a hammer mill screen size of 3.2 mm, die thickness of 31.8 mm

<sup>[c]</sup> Pelleted by using a hammer mill screen size of 3.2 mm, die thickness of 44.5 mm

<sup>[d]</sup> Pelleted by using a hammer mill screen size of 6.5 mm, die thickness of 44.5 mm

**Table 5.2 Signal assignments for  $^{13}\text{C}$  CP/MAS NMR spectra of big bluestem and corn stover**

Chemical shift (ppm)	Types of carbons
171.2	Carboxyl groups of hemicelluloses
153.3	Etherified S3, S5, G4 of lignin
147.5	Nonetherified S3, S5, G3 of lignin
132.8	Nonetherified S1, S4, G1 of lignin
115.9	G5 of lignin
105.4	C-1 of cellulose
88.8	C-4 of crystalline cellulose
84.6	C-4 of amorphous cellulose
75.3-72.9	C-2, C-3, and C-5 of cellulose
65.5	C-6 of crystalline cellulose
63.0	C-6 of amorphous cellulose
56.4	Methoxyl groups of lignin
21.7	$\text{CH}_3$ in acetyl groups of hemicelluloses

## CHAPTER 6 - CONCLUSIONS AND RECOMMENDATIONS

### CONCLUSIONS

Forage sorghum, photoperiod-sensitive sorghum, BMR sorghum, sweet sorghum, and grain sorghum biomasses were evaluated as potential feedstocks for bio-ethanol production. Fermentable sugar yields of these sorghum biomasses varied by type of sorghum biomass and pretreatment condition. The enzymatic conversion of cellulose to fermentable sugars increased when the biomass was pretreated with dilute sulfuric acid solutions at higher treatment temperature; the cellulose conversion rate ranged from 65% to 82% at pretreatment condition of 140 °C for 30 min and from 85% to 98% at pretreatment condition of 165 °C for 10 min. However, the recovery of solid mass and cellulose fraction decreased as pretreatment temperature increased. Considering both sugar recovery and energy consumption, pretreatment of biomass at mild temperature is more favorable than pretreatment at high temperature. Among five different types of sorghum biomasses, BMR sorghum yielded the highest cellulose conversion rate, due to a low ratio of syringyl to guaiacyl rings in the lignin structure that makes BMR sorghum easy to hydrolyze enzymatically.

Forage and agricultural residues and lignocellulosic materials that can be used as feedstocks for bio-ethanol production, typically are stored in bale form with low bulk density. Densification of these biomasses into pellet form can increase their bulk density and thus improves handling efficiency, storability and reduces transportation and handling costs. Four types of biomass including corn stover, sorghum biomass, wheat straw, and big bluestem were pelleted and evaluated to study the effects of pelleting process and pelleting conditions on their physical properties, chemical composition and fermentable sugar yield, and the biomass structure and thermal properties. The physical properties of biomass pellets, such as durability and density, were affected by moisture content of the pellets, particle size of biomass, and ring-die pelleting mill die thickness. As the moisture content of pellets increased, bulk density and true density values decreased because the pellets expanded. There existed optimum range of moisture content of pellets in which the pellet durability reached a maximum value. The optimum range of moisture content of pellets are 9% (d.b.) to 14% (d.b.) for corn stover and wheat straw pellets, 9% (d.b.) to 11% (d.b.) for big bluestem pellets, and 14% (d.b.) to 16% (d.b.) for sorghum stalk pellets. Bulk density values of biomass were found to increase about 9-12 fold after pelleting

process. Use of a larger hammer mill screen size (6.5 mm screen openings instead of 3.2 mm) during biomass (feed) grinding step resulted in the increase in bulk density, true density, and durability of biomass pellets, but not in a significant level. The larger ring-die pelleting mill die thickness (44.5 mm instead of 31.8 mm) had a positive effect on bulk density, true density, and durability of the corn stover, wheat straw, and sorghum biomass pellets. However, the effect of ring-die pelleting mill die thickness on pellet durability was not significant for big bluestem.

The impacts of pelleting process, pelleting variables (hammer mill screen size and ring-die pelleting mill die thickness), and dilute-acid pretreatment process on chemical composition, fermentable sugar yield, biomass structure, and thermal property of biomass were also investigated in this study. For untreated biomass, the pelleting process and pelleting variables did not have significant effects on chemical composition of the biomass. Dilute-acid pretreatment resulted in a significant increase of glucan and lignin content, but a significant decrease of xylan content of biomass for both in unpelleted and pelleted forms. This was mainly due to the removal of hemicellulose during dilute-acid pretreatment. The impact of pelleting conditions on chemical composition of biomass was only observed after dilute-acid pretreatment process; glucan content of pretreated biomass pellets increased with the increase in ring-die pelleting mill die thickness and decreased with the increase in hammer mill screen size. On the contrary, the xylan content of pretreated biomass pellets decreased as ring-die pelleting mill die thickness increased and increased as the hammer mill screen size increased. Lignin content of biomass was not affected by pelleting process and pelleting variables. Among the three combinations of pelleting conditions used in this study, biomass that was grinded using a hammer mill screen size of 6.5 mm and extruded using a ring-die pelleting mill die thickness of 44.5 mm had the highest sugar yield as well as the highest durability and bulk density values.

Biomass crystallinity was found to increase after dilute-acid pretreatment and pelleting process. However, the increase in biomass crystallinity induced by pelleting process was not as strong as by dilute-acid pretreatment. Since there was no significant change in the chemical composition of untreated biomass after pelleting process, the slight increase of biomass crystallinity after pelleting process was probably due to the crystallization of amorphous cellulose and the change in lignin structure when the biomass was subjected to mechanical shear and frictional heat generated during extrusion process. Both dilute-acid pretreatment and pelleting process induced the removal of softened surface region of biomass. Pelleting process made the

biomass more thermally stable. However, pelleting conditions used in this study did not have strong impact on the chemical structure and thermal property of biomass.

In overall, pelleting process significantly improved biomass bulk density, making the biomass easy to handle and transport. The mechanical shear and frictional heat evolved during pelleting process resulted in increased biomass crystallinity and removal of the softened surface region of biomass, and thus make the biomass more amendable to enzymatic attack, as confirmed by the increase in fermentable sugar yield of biomass. Pelleting parameters, including particle size of biomass and ring-die pelleting mill die thickness, had positive impact on physical properties of biomass (pellet durability and density). Although the impact of pelleting parameters on chemical structure and thermal property of biomass was not observed in this study, the pelleting parameters altered chemical composition of biomass pellets after dilute-acid pretreatment and thus somehow influenced the fermentable sugar yield of biomass. In this study, pelleting of biomass by using the hammer mill screen size of 6.5 mm and a ring-die pelleting mill die thickness of 44.5 mm gave the highest sugar yield as well as the highest durability and bulk density of biomass. Under this optimum pelleting condition, the maximum enzymatic conversion of cellulose of wheat straw pellets was the highest (94.1%), followed by corn stover pellet (93.1%), sorghum stalk pellet (92.1%), and big bluestem pellet (91.1%).

## **RECOMMENDATIONS**

Pelleting of biomass could improve handling efficiency and storability, and reduce transportation and handling costs. This study showed that the pelleting process and pelleting parameters (particle size of biomass feed and die thickness) had some impacts on the quality of biomass pellets as well as the chemical composition, fermentable sugar yield, and biomass structure of the pellets. However, there are also other factors such as moisture content of feed biomass, biomass feeding rate, binding agent, die specification that influence the quality and properties of the biomass pellets.

To improve quality of biomass pellets and evaluate their fermentable sugar yield corresponded to pelleting conditions, the following points are suggested for future study:

- 1. Expand the study range of particle size of feed biomass**

In our study, the feed biomass was grinded by using two different hammer mill screen sizes (3.2 mm and 6.5 mm). The result showed that the pellets made from biomass grinded using 6.5-mm mill screen had higher bulk density, true density, and durability values than that using a 3.2-mm mill screen, although this effect was not in significant level. Expanding our study by using various mill screen sizes for grinding the biomass prior to feeding it into pelleting mill would help us to better understand the effect of biomass particle size on pellet quality.

## **2. Vary moisture content of biomass feed**

The moisture content of biomass feed was adjusted to 10% (wet basis) before it was fed into the pelleting mill in our study. Moisture content of biomass will decrease after pelleting process due to friction heat generated during the extrusion of biomass through the die holes in the ring-die pelleting mill. Low moisture content of pellets is beneficial for storage and transportation of the pellets; high moisture biomass is susceptible to mold growth and spoilage. The critical moisture content for safe storage of most agricultural products is less than 15%. However, water in biomass acts as a natural binding agent during pelleting process and thus helps in improving the pellet quality (durability). Therefore, varying the moisture content of biomass feed will help us to determine the optimum moisture content of biomass feed that is beneficial for both storage/transportation and pellet quality aspects.

## **3. Optimum biomass feeding rate during pelleting process**

The feeder screw rate of biomass was kept constant at 7 rpm throughout all runs in this study. Varying the biomass feeding rate to determine the effect of feeding rate on pellet quality is suggested for future study.

## **4. Use of different binding agent during pelleting process**

In our study, water in biomass feed acts as natural binding agent during pelleting of biomass, holding the biomass particles together and thus produces durable pellets. Besides water, there are other binding agents available. For animal feeds, the commonly used binding agents for pelleting include calcium lignosulfonate, colloids, bentonite, starches, proteins, and calcium hydroxide (Tabil and Sokhansanj, 1996). Using different binding agent may result in

production of pellets with different quality. Therefore, investigating the effect of using different binding agent on pellet quality is suggested for future study.

**5. Determine the effect of storage time on pellet quality, moisture content and chemical composition of the pellets**

During storage period of biomass pellets, the pellet quality, moisture content and chemical composition might change from time to time. Monitoring the changes in pellet quality, moisture content and chemical compositions of pellets during storage period will be useful for further improvement of proper storage design.