2012

Effect of surfactants on the pretreatment of sugarcane bagasse with dilute ammonia

Shuo Cao
Louisiana State University and Agricultural and Mechanical College, scao2@tigers.lsu.edu

EFFECT OF SURFACTANTS ON THE PRETREATMENT OF SUGARCANE BAGASSE WITH DILUTE AMMONIA

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Department of Food Science

by
Shuo Cao
B.S., Henan University, 2006
December 2012

DEDICATION

This work is dedicated to my dearest parents Linpu Cao and Yanxia Guo. Their love, support and encouragement throughout these years have allowed me to achieve and complete the goals in my life. This dedication is also extended to my host family Randy and Mona Hayden. Without them, this research would not have been accomplished.

ACKNOWLEDGEMENTS

I would like to express my deepest appreciation to my advisor Dr. Giovanna M. Aita for her support, guidance and motivation throughout this research.

Sincere appreciation is also extended to my committee members Dr. John Finley and Dr. Joan King from the Department of Food Science at Louisiana State University Agricultural Center, Baton Rouge, LA.

A special thank you goes to Dr. Eizi Morikawa from the Center for Advanced Microstructures and Devices (CAMD) at Louisiana State University, Baton Rouge, LA for his help with FTIR measurements; Ms. Ying Xiao from the Socolofsky Microscopy Center at Louisiana State University, Baton Rouge, LA for her assistance with the preparation of SEM images; and Ms. Wanda S. LeBlanc from the X-Ray Diffraction and Geochemistry Laboratory in the Department of Geology and Geophysics at Louisiana State University, Baton Rouge, LA for assisting me with the use of XRD.

I also would like to thank the graduate students from the Department of Food Science and everyone at the Audubon Sugar Institute.

TABLE OF CONTENTS

DEDICATION i

ACKNOWLEDGEMENTS iii

LIST OF TABLES vii

LIST OF FIGURES viii

LIST OF ABBREVIATIONS x

ABSTRACT xi

CHAPTER 1. LITERATURE REVIEW 1
1.1 Second Generation Biofuel 1
1.2 Lignocellulose 2
1.3 Biomass Pretreatment 3
1.3.1 Biological pretreatment 4
1.3.2 Physical pretreatment 4
1.3.3 Thermochemical pretreatment 4
1.4. Non-Ionic Surfactants 6
1.4.1. Tween 80 7
1.4.2. Tween 20 8
1.4.3. PEGs 8
1.5. Biomass Enzymatic Hydrolysis and Ethanol Fermentation 9
1.5.1 Enzymatic hydrolysis 9
1.5.2 Ethanol fermentation 9
1.6. Goal of this Study 10

CHAPTER 2. COMBINED EFFECT OF SURFACTANTS ON DILUTE AMMONIA TREATED SUGARCANE BAGASSE 11
2.1. Introduction 11
2.2 Methods and Materials 13
2.2.1 Substrate 13
2.2.2 Non-ionic surfactant-dilute ammonia pretreatment 14
2.2.3 Chemical composition of sugarcane bagasse 14
2.2.4 Enzyme hydrolysis and fermentation 14
2.2.5 Chemical analysis of hydrolysis and fermentation samples 15

2.2.6 Scanning electron microscopy (SEM) analysis 17
2.2.7 Fourier transform infrared spectroscopy (FTIR) analysis 17
2.2.8 X-ray powder diffraction (XRD) analysis 17
2.2.9 Thermogravimetric (TGA) analysis 18
2.3. Results and Discussion 18
2.3.1 Effect of the non-ionic surfactant-dilute ammonia pretreatment on the biomass chemical composition 18
2.3.2 Enzymatic digestibility of the non-ionic surfactant-dilute ammonia pretreated sugarcane bagasse 21
2.3.3 Ethanol yields of the non-ionic surfactant-dilute ammonia pretreated sugarcane bagasse 25
2.3.4 Analytical studies 26
2.3.5 SEM images 26
2.3.6 FTIR analysis 28
2.3.7 XRD analysis 30
2.3.8 TGA analysis 32
2.4. Conclusions 33

CHAPTER 3. EFFECT OF TWEEN 80 ON THE PRETREATMENT AND ENZYMATIC HYDROLYSIS OF DILUTE AMMONIA PRETREATED SUGARCANE BAGASSE 35
3.1 Introduction 35
3.2 Methods and Materials 37
3.2.1 Substrate 37
3.2.2 Tween 80-dilute ammonia pretreatment 37
3.2.3 Chemical composition of pretreated sugarcane bagasse 38
3.2.4 Enzyme hydrolysis 38
3.2.5 Chemical analysis of hydrolysis samples 39
3.2.6 FTIR analysis 40
3.2.7 XRD analysis 40
3.2.8 TGA analysis 41
3.3 Results and Discussion 41
3.3.1 Effect of the Tween 80-dilute ammonia pretreatment on the biomass chemical composition 41
3.3.2 Enzymatic digestibility of the Tween 80-dilute ammonia pretreated sugarcane bagasse 43
3.3.3 FTIR analysis 46
3.3.4 XRD analysis 47
3.3.5 TGA analysis 50
3.4 Conclusions 51

CHAPTER 4. SCALE UP OF TWEEN 80-DILUTE AMMONIA PRETREATMENT OF SUGARCANE BAGASSE 53
4.1 Introduction 53
4.2 Methods and Materials 54

4.2.1 Substrate 54
4.2.2 Tween 80-dilute ammonia pretreatment 54
4.2.3 Chemical composition of pretreated sugarcane bagasse 55
4.2.4 Enzymatic hydrolysis 55
4.2.5 Chemical analysis of hydrolysis samples 55
4.2.6 FTIR analysis 57
4.2.7 XRD analysis 57
4.3 Results and Discussion 57
4.3.1 Effect of scale-up Tween 80-dilute ammonia pretreatment on the biomass chemical composition 57
4.3.2 Enzymatic digestibility of scale-up Tween 80-dilute ammonia treated sugarcane bagasse 59
4.3.3 FTIR analysis 62
4.3.4 XRD analysis 63
4.4 Conclusions 65

SUMMARY AND FUTURE WORK 66

REFERENCES 67

VITA 79

LIST OF TABLES

Table 1. 1. Typical lignocellulosic biomass compositions (% dry basis) 3

Table 2. 1. Chemical composition of surfactant-dilute ammonia pretreated sugarcane bagasse (g/100g dry biomass). 21

Table 2. 2. Crystallinity data of surfactant-dilute ammonia pretreated sugarcane bagasse and controls..... 30

Table 3. 1. Chemical composition of Tween 80-dilute ammonia pretreated sugarcane bagasse and controls (g/100g dry biomass)..... 42

Table 3. 2. Crystallinity data of Tween 80-dilute ammonia pretreated sugarcane bagasse and controls..... 47

Table 3. 3. Onset temperatures for Tween 80-dilute ammonia pretreated sugarcane bagasse and controls..... 51

Table 4. 1. Chemical composition of 3% Tween 80-dilute ammonia pretreated sugarcane bagasse at 4.7% and 10.5% solids loading (g/100g dry biomass). 59

Table 4. 2. Glucose concentration for 3% Tween 80-dilute ammonia pretreated sugarcane bagasse at 4.7% and 10.5% solids loadings in a 4 L or 20 L bioreactor. 62

Table 4. 3. Crystallinity data for 3% Tween 80-dilute ammonia pretreated sugarcane bagasse at 4.7% and 10.5% solids loadings in a 4 L or 20 L bioreactor. 63

LIST OF FIGURES

Figure 1. 1. Structures of Tween 20, Tween 80 and PEGs. 7

Figure 2. 1. Enzymatic Digestibilities and Ethanol Yields for Surfactant-Dilute Ammonia Pretreated Bagasse and Controls.....24

Figure 2. 2. Sugarcane Bagasse SEM Images..... 27

Figure 2. 3. FTIR Spectra for Non-Ionic Surfactant-Dilute Ammonia Pretreated Sugarcane Bagasse and Controls (untreated, water treated, ammonia treated). 29

Figure 2. 4. XRD Spectra for Non-Ionic Surfactant-Dilute Ammonia Pretreated Sugarcane Bagasse and Controls (untreated, water treated, ammonia treated). 31

Figure 2. 5. TGA Curves for Non-ionic Surfactant-Dilute Ammonia Pretreated Sugarcane Bagasse and Controls (untreated, water treated, ammonia treated). 33

Figure 3. 1. Endwise Degradation of Polysaccharides (Fengel and Wegener, 1984)……43

Figure 3. 2. Percent Cellulose Digestibility for Tween 80-Dilute Ammonia Pretreated Sugarcane Bagasse. 44

Figure 3. 3. Percent Hemicellulose Digestibility for Tween 80-Dilute Ammonia Pretreated Sugarcane Bagasse. 45

Figure 3. 4. Percent Cellulose Digestibility for 1.5%, 3% and 5% Tween 80-Dilute Ammonia Pretreated Sugarcane Bagasse.. 45

Figure 3. 5. XRD Spectra for Tween 80-Dilute Ammonia Pretreated Sugarcane Bagasse and Controls..... 48

Figure 4. 1. Percent Cellulose Digestibility for 3% Tween 80-Dilute Ammonia Pretreated Sugarcane Bagasse…….61

Figure 4. 2. Percent Hemicellulose Digestibility for 3% Tween 80-Dilute Ammonia Pretreated Sugarcane Bagasse..... 61

Figure 4. 3. XRD Spectra for 3% Tween 80-Dilute Ammonia Pretreated Sugarcane Bagasse in a 4 L or 20 L Bioreactor 64

LIST OF ABBREVIATIONS

CBU: The enzyme amount, which converts1 mole of cellubiose to 2 mole of glucose in 1 min

CrI: Total crystallinity index based on XRD analysis.

D_5A: A strain of *Saccharomyces cerevisiae*.

FPU: Filter-paper units. It is defined as the amount of enzyme releasing 1 μmole of reducing sugar from filter paper per ml per min.

FTIR: Fourier transform infrared spectroscopy.

HLB: hydrophile-lipophile balance.

HPLC: High performance liquid chromatography.

LOI: Lateral order index.

NREL: National Renewable Energy Laboratory.

PEG: Polyethylene glycol.

SEM: Scanning electron microscopy.

TCI: Total crystallinity index based on FTIR analysis.

TGA: Thermogravimetric analysis.

Tween: Polysorbate.

XRD: X-ray diffraction.

ABSTRACT

Lignocellulosic biomass is composed of cellulose, hemicellulose and lignin which are not readily available for conversion in their native form. It is widely accepted that lignin acts as the "glue" that binds cellulose and hemicellulose, giving rigidity and resistance to lignocellulose. The use of non-ionic surfactants during pretreatment can help alter the structure of lignocellulosic biomass to improve cellulose digestibility and ethanol yields.

Tween 80, Tween 20, PEG 4000, or PEG 6000 was used with ammonium hydroxide for the pretreatment of sugarcane bagasse. The pretreatment was carried out by mixing sugarcane bagasse, ammonium hydroxide (28% v/v) and water at a ratio of 1: 0.5: 20, adding 3% (w/w) surfactant based on the weight of dry biomass, and heating the mixture to 160 ℃ for 1 h. The final concentration of ammonium hydroxide was 0.65% w/w at 4.7% solids loading. Chemical compositions were determined before and after pretreatment. Fibers were hydrolyzed using commercial enzymes, Spezyme CP and Novozyme 188. Fermentable sugars and ethanol concentrations were analyzed by HPLC. The results indicated that PEG 4000 and Tween 80 gave the highest cellulose digestibilities (62%, 66%) and ethanol yields (73%, 69 %), respectively.

Tween 80 was selected over PEG 4000 because of its low cost. The effect of two concentrations of ammonium hydroxide 0.26% w/w (1: 0.2: 20, biomass: ammonium hydroxide: water ratio) and 0.65% w/w (1: 0.5: 20, biomass: ammonium hydroxide: water ratio), and Tween 80 (1.5%, 3% and 5% w/w, based on the weight of dry biomass) were evaluated during pretreatment at 4.7% solids loading. The greatest lignin removal (37%), cellulose digestibility (66%) and hemicellulose digestibility (43%) were observed at 1: 0.5: 20 ratio supplemented with 3% (w/w) Tween 80. These pretreatment parameters were selected for scale-up experiments at a higher solids loading (10.5%) in a 20 L bioreactor. The greatest lignin removal (55%), cellulose

digestibility (72%) and hemicellulose digestibility (57%) were observed with 3% Tween 80-dilute ammonia pretreatment at 1: 0.5: 8 ratio with a final concentration of ammonium hydroxide of 1.47% w/w. Morphological changes in the structure of non-ionic surfactant-dilute ammonia pretreated sugarcane bagasse were observed.

CHAPTER 1. LITERATURE REVIEW

1.1 Second Generation Biofuel

Fossil resources are no longer regarded as sustainable and have become questionable from the economical, ecological and environmental point of view (i.e., greenhouse gas emission, air and water pollution, and declining petroleum reserves). Therefore, the production and use of fuels and chemicals derived from plants or organic wastes have received great attention. First generation biofuels can help reduce some greenhouse gas emissions and improve domestic energy security. The production of first generation biofuels is done at a commercial scale today. Ethanol is produced from corn starch in the United States or from sugarcane in Brazil, with almost 50 billion liters produced annually worldwide (Naik et al., 2010). The most important disadvantage of first generation biofuels is the competition with the human food supply for water, land and other resources (Searchinger et al., 2008). In comparison, second generation biofuels, also known as advanced biofuels, are generated from lignocellulosic biomass. Lignocellulosic biomass can be woody crops, grassy crops or agricultural waste products. The processing technologies for second generation fuels are relatively immature with pilot plants supplying less than 0.1% of the world biofuel production (Sims et al., 2010). A report by the U.S. Department of Energy (DOE) and the U.S. Department of Agriculture (USDA) suggested that the United States has the potential to produce more than 1.3 billion dry tons of lignocellulosic biomass per year, including agricultural (933 million tons/year) and forest resources (368 million tons/year) (DOE, 2005). One billion dry tons of biomass theoretically equals to about 80-130 billion gallons of cellulosic ethanol. The U.S. DOE has set a target to produce about 60 billion gallons of biofuels from biomass to replace 30% of transportation fuels by 2030 (TheWhiteHouse, 2006). Also, it was reported that technological progress could enable biofuels to become competitive

with petroleum, providing benefits to the U.S. economy by 2022 (USDA, 2010). There is great potential for further cost reductions and production increases of cellulosic fuels. However, significant investment in research and development are still needed to address the challenges seen with second generation fuels.

1.2 Lignocellulose

Lignocellulose consists of the residual non-food parts of current crops, such as stems, leaves and husks that are left behind once the food crop has been extracted, as well as other crops that are not used for food purposes (non-food crops), such as switchgrass, sorghum, energy cane, jatropha, whole crop maize, Miscanthus and cereals that bear little grain, and also industry wastes such as woodchips, skins and pulp from fruit pressing (Inderwildi and King, 2009). Lignocellulosic biomass typically contains 50-80% (dry basis) carbohydrates that are polymers of C5 and C6 sugar units. Most carbohydrates can be processed either chemically or biologically to yield biofuels such as ethanol (Zheng et al., 2009). Second generation biofuel technology uses the convertible sugars (cellulose and hemicellulose) locked by lignin. Lignin, cellulose and hemicellulose are the three major components in lignocellulosic biomass. They are linked into a carbohydrate-lignin complex which is highly resistant to biochemical processes. A summary of lignocellulosic biomass compositions is given in Table 1. 1.

Cellulose (40–60% of the dry biomass) is a linear polymer. The length of a cellulose molecule is determined by the number of glucan units in the polymer, referred to as the degree of polymerization. The degree of polymerization of cellulose depends on the type of plants and typically is estimated to be from 200 to 27,000 glucan units (Taherzadeh and Karimi, 2007). Cellulose from wood pulp has typical chain lengths between 300 and 1,700 units. Cotton and

other plant fibers as well as bacterial cellulose have chain lengths ranging from 800 to 10,000 units (Klemm et al., 2005).

Hemicellulose (20–40% of the dry biomass) consists of short highly branched chains of various C5 and C6 sugars with xylose as the main sugar. Other sugars include arabinose, galactose, glucose, and mannose. Also found in the sugar polymer are smaller amounts of acetyl groups. Hemicellulose, because of its branched and amorphous nature, is relatively easy to hydrolyze as compared to cellulose (Hamelinck et al., 2005).

Lignin (10–25% of the dry biomass) is a large complex polymer of phenylpropane and methoxy groups, a non-carbohydrate polyphenolic substance that encrusts the cell walls and cements the cells together. It is degradable by only few organisms into higher value products such as organic acids, phenols and vanillin. Lignin is extremely resistant to chemical and enzymatic degradation (Palmqvist and Hahn-Hagerdal, 1999).

Table 1. 1. Typical lignocellulosic biomass compositions (% dry basis) (Aita and Salvi, 2009; Hamelinck et al., 2005)

Components	Glucan	Xylan	Lignin	Other*
Pine	44.55	6.30	27.67	21.48
Corn stover	36.40	21.40	17.20	25.00
Switchgrass	31.98	21.09	18.13	28.80
Sugarcane bagasse	40.20	21.50	24.20	14.10
Energy cane bagasse	43.00	24.00	22.00	11.00
Sweet sorghum bagasse	44.50	27.70	22.00	5.80
Wheat straw	37.60	21.70	18.00	22.70

*Protein, ash, nonstructural sugars and extractives.

1.3 Biomass Pretreatment

Cellulose and hemicellulose must be broken down into monomeric sugars (fermentable sugars) prior to fermentation. This bioconversion process involves the following main steps: (1) pretreatment to breakdown the carbohydrate-lignin complex into cellulose, hemicellulose and

lignin; (2) enzyme hydrolysis to convert cellulose and hemicellulose into hexose and pentose sugars; (3) addition of microbial strain or strains to ferment hexose and/or pentose sugars to ethanol; and (4) recovery and distillation of ethanol to meet fuel standards (Aita et al., 2011). Of these four steps, pretreatment is the key step because it alters the carbohydrate-lignin structure to make enzymes accessible to both cellulose and hemicellulose (Yang and Wyman, 2008). Biomass pretreatment represents one of the main economic costs in the process. It has been described as the second most expensive unit cost in the conversion of lignocellulose to ethanol preceded by the cost of biomass (Mosier et al., 2005).

Several pretreatment technologies have been developed. They can be classified as biological, physical and thermo-chemical platforms. Combinations of these technologies have been also developed.

1.3.1 Biological pretreatment

Biological pretreatment methods employ brown, white and/or soft-rot fungi which mostly degrade lignin and hemicellulose and some cellulose (Sanchez, 2009). The main drawback is the low hydrolysis rate as compared to other technologies (Sun and Cheng, 2002a).

1.3.2 Physical pretreatment

Physical pretreatment methods mechanically alter cellulose crystallinity (i.e., chipping, grinding, and/or milling) by reducing the size of biomass. Physical pretreatment technologies require high energy input and are often used in combination with other pretreatment technologies.

1.3.3 Thermochemical pretreatment

1.3.3.1 Acid pretreatment

Acid pretreatment can be used either as concentrated or diluted acids (i.e., sulfuric acid and acetic acid) to solubilize the hemicellulose and make the cellulose more accessible to

enzymes. Equipment corrosion and acid recovery are main drawbacks when using concentrated acid pretreatments. Also, high operational and maintenance costs reduce the interest of using concentrated acid pretreatments at commercial scales (Wyman, 1996).

1.3.3.2 Ionic liquids

Ionic liquids are organic salts that can be used for the pretreatment of cellulosic biomass. However, toxicity of some ionic liquids to enzymes and fermentative microorganisms has been reported (Yang and Wyman, 2008; Zhao et al., 2009). In addition, the economics of ionic liquid pretreatments need to be improved before they can be applied at an industrial scale.

1.3.3.3 Alkali pretreatment

Alkali pretreatment is one of the promising technologies for grassy feedstocks. Alkali pretreatment increases cellulose digestibility and are very effective in lignin removal, exhibiting minor cellulose and hemicellulose solubilization as compared to acid processes (Carvalheiro et al., 2008). They are more effective on agricultural residues than on woody materials (Kumar and Wyman, 2009). Sodium, potassium, calcium, and ammonium hydroxides are suitable alkaline pretreatments. Ammonium hydroxide can cause swelling, decrease the degree of polymerization and crystallinity of cellulose, which causes the structure of lignin to disrupt.

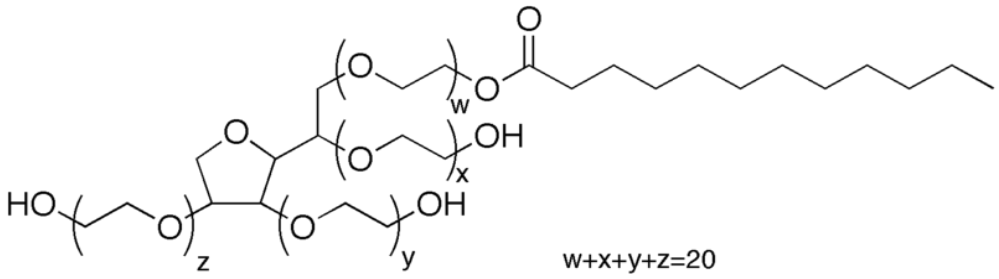
1.3.3.4 Ammonia fiber expansion (AFEX) pretreatment

AFEX pretreatment is a process in which lignocellulosic biomass is exposed to liquid ammonia at high temperature and pressure for a short period of time, followed by a fast release in pressure. The structure of the material is changed after pretreatment, resulting in increased water holding capacity and higher digestibility (Galbe and Zacchi, 2007). However, AFEX pretreatment is not an efficient technology for high lignin content materials since this process almost does not remove lignin (Kumar et al., 2009a).

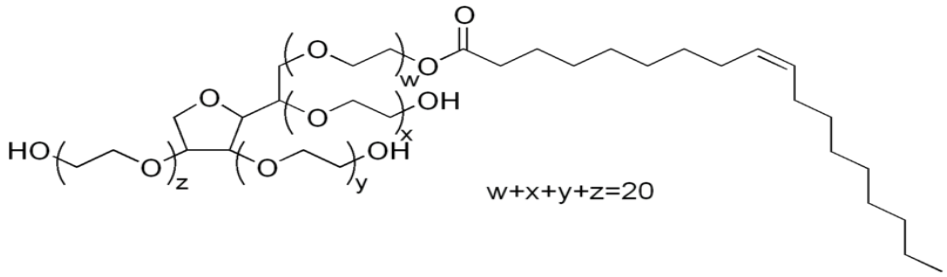
1.4. Non-Ionic Surfactants

Surfactants have both hydrophobic and hydrophilic properties which can decrease the surface tension between two liquid phases to help improve the removal of hydrophobic compounds (Escalante et al., 2005), and modify the structure and surface of biomass, making it more hydrophilic (Qing et al., 2010). The above mentioned properties make surfactants potential candidates as pretreatment additives. It was reported that the non-ionic surfactant Tween 20 successively extracted hydrophobic degradation products from lignin and hemicellulose, thereby enhancing lignin removal during pretreatment (Kurakake et al., 1994). Furthermore, addition of surfactants during enzymatic hydrolysis of lignocellulosic biomass have been reported to aid in the conversion of cellulose into soluble sugars thus reducing the use of enzymes and overall processing costs (Eriksson et al., 2002). Non-ionic surfactants, Tween 20 and Tween 80, have been found to be most effective (Eriksson et al., 2002). Several concepts have been developed to explain the mechanism of how surfactants enhance enzymatic digestibility: (1) surfactants alter the substrate structure and make it more accessible to enzymes (Helle et al., 1993; Kaar and Holtzapple, 1998); (2) surfactants stabilize enzymes and prevent their denaturation during hydrolysis (Kaar and Holtzapple, 1998; Kim et al., 1982); (3) surfactants increase positive interactions between substrates and enzymes (Eriksson et al., 2002; Kaar and Holtzapple, 1998); and (4) surfactants reduce non-productive adsorption of enzymes to lignin (Eriksson et al., 2002). However, a mechanism that can consistently explain how surfactants improve enzymatic hydrolysis has yet to be developed. One of the most popular explanations is that surfactants can reduce non-productive enzyme binding to lignin and other molecules involved in cellulase activity (Eriksson et al., 2002; Qing et al., 2010). Their effect on pretreatment varies with the hydrophile-lipophile balance (HLB) values of the surfactant. Surfactants having high HLB values have been reported to be more useful in extracting hydrophobic degradation products

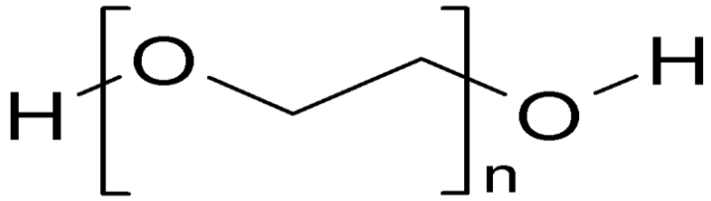
from lignin and hemicellulose (Kurakake et al., 1994). Tween 20 (HLB 16.7), Tween 80 (HLB 15.0), PEG 4000 (HLB 18.5), and PEG 6000 (HLB 19.0) are four non-ionic surfactants that have been used during pretreatment and enzymatic hydrolysis of lignocellulosic biomass. Their structures are depicted in Figure 1. 1.



Tween 20



Tween 80



Polyethylene Glycol (PEG)

Figure 1. 1. Structures of Tween 20, Tween 80 and PEGs.

1.4.1. Tween 80

Tween 80 (polysorbate 80) is derived from polyethoxylated sorbitan and oleic acid. The hydrophilic-lipophilic balance (HLB) of Tween 80 is 15, which means it is highly water soluble. Surfactants with high HLB values are useful in extracting hydrophobic degradation products from lignin and hemicellulose (Kurakake et al., 1994). Addition of Tween 80 during enzymatic

hydrolysis of biomass can significantly reduce enzyme concentrations and help with the recycling of enzymes (Tu and Saddler, 2010). It was reported that Tween 80 increased lignin removal by about 52% in acid treated biomass (140 °C, 1% H_2SO_4) and by 114% in water treated biomass (220 °C) (Qing et al., 2010). Tween 80 has been reported to decrease non-productive binding of enzymes to biomass (Börjesson et al., 2007b; Qing et al., 2010).

1.4.2. Tween 20

Tween 20 is a polyoxyethylene derivative of sorbitan monolaurate. Tween 20 is distinguished from the other members in the polysorbate range by the length of the polyoxyethylene chain and the fatty acid ester moiety. Similar to Tween 80, Tween 20 is also a high HLB value (16.7) non-ionic surfactant. Research has shown that addition of Tween 20 during the pretreatment of wheat straw with dilute acid improved both enzymatic hydrolysis and fermentation of pretreated biomass (Qi et al., 2010). Similar observations have been reported during the pretreatment and enzymatic hydrolysis of recycled newspaper (Kim et al., 2007). Tween 20 has been reported to enhance enzymatic hydrolysis of corn stover at low enzyme loadings (5 FPU cellulase/g dry biomass) (Kaar and Holtzapple, 1998).

1.4.3. PEGs

Polyethylene glycol (PEG) is a polyether compound with repeated structures of $HO\text{-}CH_2\text{-}(CH_2\text{-}O\text{-}CH_2\text{-})_n\text{-}CH_2\text{-}OH$. They are also high HLB value non-ionic surfactants. Generally, PEGs are more expensive than Tweens with a market price of about U.S. $1.5/kg as compared to $0.25/kg for Tweens (Tu and Saddler, 2010). Mohsenzadeh et al. (2012) reported that the combined use of PEG-2000 with NaOH during pretreatment of softwood spruce and hardwood birch improved delignification, enzyme hydrolysis and fermentation yields. Similar findings have been reported by Ladisch et al. (1983) and Chen et al. (2005).

1.5. Biomass Enzymatic Hydrolysis and Ethanol Fermentation

1.5.1 Enzymatic hydrolysis

Cellulases and xylanases are the enzymes commonly used during biomass hydrolysis. Both fungi and bacteria can produce cellulases. However, most commercially available cellulases come from fungi, such as *Trichoderma reesei* and *Aspergillus niger*. There are three types of cellulases, endo-glucanase, exo-glucanase and β-glucosidase. Endo-glucanase acts randomly on the amorphous sites on the cellulosic fiber. Exo-glucanase removes cellobiose units from the non-reducing ends of cellulose chains. β-glucosidase converts cellobiose into two glucose molecules. Enzymatic degradation of hemicellulose mainly requires endo-xylanases and β-xylosidases (Aita and Kim, 2010). Other accessory enzymes include arabinofuranosidase, glucuronidase, acetylxylan esterase, ferulic acid esterase, and coumaric acid esterase. Enzymatic hydrolysis can be affected by substrate and end-product concentration, enzyme activity and hydrolysis conditions (Talebnia et al., 2010). Cellulases have an optimum temperature of 45-50 ℃ and pH of 4-5 (Galbe and Zacchi, 2002). Cellulase and xylanase enzyme loadings in hydrolysis depend on the substrate type.

1.5.2 Ethanol fermentation

Saccharomyces cerevisiae is one of the most commonly used organisms for ethanol fermentation. Separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) are two popular methods applied during enzymatic hydrolysis and fermentation of pretreated lignocellulosic biomass. When enzymatic hydrolysis and fermentation are performed sequentially, it is referred to as SHF. A solids loading of 10 % (w/w) are defined as the most adequate for SHF considering mixing difficulties and accumulation of inhibitors in the medium (Aita and Kim, 2010). The two process steps can be performed simultaneously as in the case of SSF. Major drawbacks in SSF include incompatible hydrolysis (45 to 50 ℃) and

fermentation (30 ℃) temperatures, ethanol tolerance of microorganisms, and enzyme inhibition by ethanol (Aita and Kim, 2010). High ethanol yields have been reported with SHF and SSF (Öhgren et al., 2007; Saha and Cotta, 2006).

1.6. Goal of this Study

This study aimed at (1) evaluating four non-ionic surfactants as catalysts during the pretreatment of sugarcane bagasse with dilute ammonium hydroxide and evaluating their effect on the chemical composition of biomass, biomass structure, enzymatic hydrolysis, and ethanol yields; (2) selecting the best non-ionic surfactant and evaluating its effect on enzymatic hydrolysis yields at various pretreatment parameters; and (3) scaling up the best combination of pretreatment parameters and evaluating its effect on biomass structure and sugar yields.

CHAPTER 2. COMBINED EFFECT OF SURFACTANTS ON DILUTE AMMONIA TREATED SUGARCANE BAGASSE

2.1. Introduction

Lignocellulose is a type of biomass and refers to any plant material produced by photosynthesis. Lignocellulosic biomass is considered as a potential resource for the production of second generation ethanol, which can provide enough transportation fuel without threatening the food supply and biodiversity. Cellulose, hemicellulose and lignin are the three main components linked into a carbohydrate complex that is highly resistant to biochemical conversion. Cellulose and hemicellulose must be broken down into monomeric sugars (fermentable sugars) before their fermentation into ethanol (Aita et al., 2011). This process can be summarized into four steps: (1) pretreatment, to breakdown the carbohydrate-lignin complex into cellulose, hemicellulose and lignin; (2) enzyme hydrolysis, to convert cellulose and hemicellulose into hexose and pentose sugars; (3) fermentation, to ferment hexose and/or pentose sugars to ethanol; and (4) ethanol recovery (Aita et al., 2011). Of these four steps, pretreatment is a key step because it helps denature the highly complex carbohydrate-lignin structure. An effective pretreatment can lower the downstream unit operation cost (Yang and Wyman, 2008).

Pretreatment with caustic is applied to disrupt the structure of lignin and to increase the susceptibility of enzymes during hydrolysis. Caustic alters the carbohydrate-lignin complex in biomass by effectively disrupting the ester bonds between lignin and hemicellulose, commonly found in grasses, thus allowing for a great portion of the hemicellulose to be selectively removed (Chundawat et al., 2011). Similarly, the lignin and cellulose complex is also altered causing the β-1,4-glucosidic bonds in cellulose to be more susceptible to enzymatic hydrolysis (Rivers and

Emert, 1988; Salvi et al., 2010). Several pretreatment methods have been developed (i.e., biological, mechanical, acid, alkaline, ionic liquids), but most of these methods suffer from relatively low sugar yields, high processing costs or great investment risks (Sanchez, 2009; Sun and Cheng, 2002a; Wyman, 1996; Yang and Wyman, 2008; Zhao et al., 2009). Dilute ammonia pretreatment has shown great success in the delignification of grassy feedstocks by cleaving the C-O-C bonds and other ether and ester bonds present in the carbohydrate-lignin complex (Aita et al., 2011). Dilute ammonia is effective in lignin removal, exhibiting less cellulose and hemicellulose solubilization than acid or hydrothermal processes (Carvalheiro et al., 2008). Alkaline pretreatment improves cellulose digestibility by increasing lignin removal thus improving enzyme hydrolysis and fermentation yields by decreasing the non-productive binding of lignin to enzymes (Eriksson et al., 2002; Qing et al., 2010). Aqueous ammonia has higher selectivity than other alkaline salts, it is non-polluting and non-corrosive, recoverable and widely used; therefore, making it a valuable pretreatment additive (Kim et al., 2003).

Recently, several studies have indicated that the addition of non-ionic surfactants during pretreatment can enhance delignification and improve enzyme hydrolysis (Börjesson et al., 2007a; Börjesson et al., 2007b; Kristensena et al., 2007; Kurakake et al., 1994; Ouyang et al., 2010; Qi et al., 2010; Qing et al., 2010). Surfactants have both hydrophobic and hydrophilic properties that can decrease surface tension to help remove hydrophobic compounds (Escalante et al., 2005). It was reported that surfactants successively removed hydrophobic degradation products from lignin and hemicellulose thus enhancing delignification during pretreatment (Kurakake et al., 1994). Several mechanisms have been proposed to explain the effect of surfactants on enzymes. Surfactants can enhance enzymatic digestibility by (1) changing the substrate structure to make it more accessible to enzymes (Helle et al., 1993; Kaar and

Holtzapple, 1998); (2) stabilizing enzymes to prevent denaturation (Kaar and Holtzapple, 1998; Kim et al., 1982); (3) increasing positive interactions between substrates and enzymes (Eriksson et al., 2002; Kaar and Holtzapple, 1998); and (4) reducing enzyme non-productive binding to lignin and other molecules involved in cellulase activity (Eriksson et al., 2002; Qing et al., 2010). However, a mechanism that can consistently explain how surfactants improve enzymatic hydrolysis has yet to be developed. Non-ionic surfactants such as Tween 20, Tween 80, PEG 4000, and PEG 6000 have been found to be effective in reducing the amount of lignin remaining in the pretreated material and in accelerating the enzymatic hydrolysis by increasing cellulose accessibility (Börjesson et al., 2007a; Börjesson et al., 2007b; Kim et al., 2007; Kristensena et al., 2007). Kurakake et al. (1994) reported that surfactants having higher HLB (hydrophile-lipophile balance) values were useful for the extraction of hydrophobic degradation products from lignin and hemicellulose (Kurakake et al., 1994). Tween 20 (HLB, 16.7), Tween 80 (HLB, 15.0), PEG 4000 (HLB, 18.5), and PEG 6000 (HLB, 19.0) are four non-ionic surfactants with high HLB values with potential use as catalysts during pretreatment and enzymatic hydrolysis.

The goal of this study was to investigate the combined effect of non-ionic surfactants (Tween 80, Tween 20, Polyethylene glycol 6000 or Polyethylene glycol 4000) and dilute ammonium hydroxide on the pretreatment of sugarcane bagasse in terms of changes in biomass chemical composition, cellulose digestibility and ethanol yield as compared to pretreatment with dilute ammonia only.

2.2 Methods and Materials

2.2.1 Substrate

Sugarcane bagasse was collected from Louisiana sugar mills during the grinding season (September through December, 2011) and stored in 50 gal drums at -20 ℃.

2.2.2 Non-ionic surfactant-dilute ammonia pretreatment

Tween 80 (Sigma-Aldrich, St. Luis, MO), Tween 20 (Sigma-Aldrich, St. Luis, MO), PEG 4000 (Sigma-Aldrich, St. Luis, MO) or PEG 6000 (Sigma-Aldrich, St. Luis, MO) was used in combination with ammonium hydroxide for the pretreatment of sugarcane bagasse. The pretreatment was run by mixing sugarcane bagasse, ammonium hydroxide (28% v/v solution) and water at a ratio of 1: 0.5: 20, adding 3% (w/w) surfactant based on the weight of dry biomass, and heating the mixture to 160 ℃ in a 4 L bioreactor for 1 h. Untreated, water pretreated and ammonia pretreated bagasse were used as controls. The final concentration of ammonium hydroxide was 0.65% w/w. Pretreatments were carried out at 4.7% w/w solids loading.

2.2.3 Chemical composition of sugarcane bagasse

Surfactant-dilute ammonia pretreated sugarcane bagasse and controls (untreated, water pretreated, and dilute ammonia pretreated sugarcane bagasse) were analyzed for glucan, xylan, lignin, arabinan, mannan, ethanol extractives, and ash content using NREL's Laboratory Analytical Procedures (LAPs #42618, 42619, 42620, 42621,42622). NREL reference material (8491 sugarcane bagasse) was analyzed as an internal sample to ensure the accuracy of the procedures.

2.2.4 Enzyme hydrolysis and fermentation

Surfactant-dilute ammonia pretreated, ammonia pretreated and water pretreated bagasse were hydrolyzed using a combination of commercially available cellulose degrading enzymes, Spezyme CP (Genencor, Danisco US Inc., Rochester, NY) and Novozyme 188 (Sigma–Aldrich, Inc., St. Luis, MO). Spezyme CP (cellulases) and Novozyme 188 (cellobiases) were used at 15 FPU/g glucan, and at 15 CBU/g glucan (half strength), respectively. A second test at a higher enzyme loading of 30 FPU Spezyme CP and 30 CBU Novozyme 188/g glucan (full strength) was conducted to asses any improvements in both hydrolysis and fermentation yields.

Approximately, 10 g dry weight of surfactant-dilute ammonia pretreated, ammonia pretreated or water pretreated bagasse were each loaded into 250 ml Erlenmeyer flasks following NREL's hydrolysis and fermentation protocols (LAP TP-510-42630). Additionally, 1 g yeast extract (Becton Dickinson and Company, Sparks, MD), 2 g peptone (Becton Dickinson and Company, Sparks, MD), 5 g citrate buffer (1 M stock solution, pH 4.8), and water were added to each flask to a final weight of 100 g. The pH of each mixture was adjusted to 4.8 with concentrated hydrochloric acid. All flasks were sterilized at 121 ℃ for 30 min. Enzymes were added and all flasks were incubated at 55 ℃ in a shaker incubator (Amerex Instruments Inc., Lafayette, CA) for 24 h at 200 rpm. Samples (2 ml) were taken prior to the addition of enzymes (time 0 h) and post enzyme hydrolysis (time 24 h). Flasks were inoculated with *Saccharomyces cerevisiae* (D_5A) to a final concentration of 1 X 10^7 CFU/ml and incubated at 30 ℃ in a shaker incubator at 200 rpm for an additional two days. Samples were withdrawn at 48 h and 72 h. Studies were conducted at least in duplicate.

2.2.5 Chemical analysis of hydrolysis and fermentation samples

All samples (0 h, 24 h, 48 h, and 72 h) were analyzed for sugars (glucose, cellobiose, arabinose, and xylose), ethanol and organic acids (lactic, acetic and formic). Samples were centrifuged (12,000 X g), filtered (0.2 μm Syringe Filters, Nagle Company, NY) and diluted accordingly. Sugars were analyzed by high performance liquid chromatography (HPLC) (Agilent 1200 Series) with a BioRad Aminex HPX-87P (P), lead form, 300 mm X 7.8 mm (ID), 9 μm column and a differential refractive index detector (G1362A Agilent). Ethanol was analyzed by HPLC (Agilent 1200 Series) with a potassium column (BioRad Aminex HPX-87K, 300 mm X 7.8 mm (ID)) and Refactive Index detector. Lactic acid, acetic acid and formic acid were analyzed by HPLC (Agilent 1100 Series) with a hydrogen column (BioRad Aminex HPX-87H,

300 mm X 7.8 mm (ID)) and a DAD detector. Percent theoretical cellulose, hemicellulose and ethanol yields were calculated using the equations as described by NREL's laboratory procedures (LAP#42630) and are as follow:

$$\%\text{Theoretical Cellulose Yield} = \frac{[\text{Glucose}] + 1.053[\text{Cellobiose}]}{1{:}111\, f\,[\text{Biomass}]} \times 100\% \quad (1)$$

$$\%\text{Theoretical Hemicellulose Yield} = \frac{0.9[\text{Xylose}] + 0.9[\text{Arabinose}]}{1.136\, f\,[\text{Biomass}]} \times 100\% \quad (2)$$

$$\%\text{Theoretical Ethanol Yield} = \frac{[\text{EtOH}]_F - [\text{EtOH}]_o}{0.51(f\,[\text{Biomass}]1.111)} \times 100\% \quad (3)$$

Where each item is defined below,

[Glucose]	residual glucose concentration (g/L)
[Cellobiose]	residual cellobiose concentration (g/L)
1.053	multiplication factor that converts cellobiose to equivalent glucose
[Biomass]	dry biomass concentration at the beginning of the fermentation (g/L)
f	cellulose or hemicellulose fraction in dry biomass (g/g)
[Xylose]	residual xylose concentration (g/L)
[Arabinose]	residual arabinose concentration (g/L)
$[\text{EtOH}]_f$	ethanol concentration at the end of the fermentation (g/L) minus any ethanol produced from the enzyme and medium
$[\text{EtOH}]_o$	ethanol concentration at the beginning of the fermentation (g/L) which should be zero

(TABLE continued)

0.51	conversion factor for glucose to ethanol based on stoichiometric biochemistry of yeast
0.9	multiplication factor that converts xylose and arabinose to equivalent glucose
1.111	converts cellulose to equivalent glucose
1.136	converts hemicellulose to equivalent xylose

2.2.6 Scanning electron microscopy (SEM) analysis

A JEOL JSM-6610LV scanning electron microscope (JEOL, USA Inc., Peabody, MA) at 10 keV was used to observe any morphological changes in biomass before and after pretreatment. Prior to imaging, the samples were sputter-coated with platinum twice to make the fibers conductive, avoiding degradation and build-up of charge on the specimen.

2.2.7 Fourier transform infrared spectroscopy (FTIR) analysis

A Thermo Scientific Nicolet Nexus 670 FTIR Spectrometer and Smart iTR with a diamond window (Thermo Fisher Scientific Inc., Waltham, MA) was used. About 5 mg of sample was loaded each time and the background spectrum was automatically suppressed before each sample was run. Spectrum was set at 600–4000 cm^{-1} with a resolution of 4 cm^{-1} and at 64 scans per sample.

2.2.8 X-ray powder diffraction (XRD) analysis

Crystallinity index (CrI) of all samples was determined by XRD using a Siemens D-5000 diffractometer (Bruker, Germany) and Cu-K radiation generated at 30 kV and 20 mA. Samples were scanned from 5 ° to 40 ° with 0.05 ° step size per 3 seconds. Diffract AT V3.1. was the software used to run the XRD. The crystallinity index was determined by the equation below (Segal et al., 1959):

$$CrI = (I_{002} - I_{am})/I_{002}$$

Where, I_{002} is the scattered intensity at the main peak for cellulose type I; I_{am} is the scattered intensity due to the amorphous portion evaluated as the minimum intensity between the main and secondary peaks.

2.2.9 Thermogravimetric (TGA) analysis

TGA curves were obtained from a TGA/SDTA851^e thermobalance (Mettler Toledo**,** Columbus, OH) using 150 μL aluminum crucibles. About 20 mg of sample was used on each run. The experiments were carried out under a continuous nitrogen flow of 20 ml/min with a temperature ramp set at 5 ℃/min. Sample weight loss was recorded from 34 ℃ to 700 ℃. Experiments were performed in duplicate.

2.3. Results and Discussion

2.3.1 Effect of the non-ionic surfactant-dilute ammonia pretreatment on the biomass chemical composition

The chemical composition of sugarcane bagasse before and after pretreatment with or without the addition of a surfactant is summarized in Table 2. 1. The chemical composition of untreated sugarcane bagasse (41% cellulose, 24% hemicellulose and 23% lignin) was found to be within the range of already published data (Aguilara et al., 2002; Aita and Salvi, 2009; Rabelo et al., 2009). Significant lignin removal was observed after the combined pretreatment of sugarcane bagasse with ammonia and a non-ionic surfactant. Delignification of 18%, 37%, 32%, and 47% (g/100 g dry biomass) was observed with Tween 20-dilute ammonia pretreatment, Tween 80-dilute ammonia pretreatment, PEG 6000-dilute ammonia pretreatment, and PEG 4000-dilute ammonia pretreatment, respectively, as compared to 14% for dilute ammonia pretreated sugarcane bagasse and 4% for water pretreated sugarcane bagasse. Lignin can be soluble in alkaline, acid or neutral environments at high temperatures. Surfactants having a hydrophilic and a hydrophobic side can bind to both lignin (hydrophobic side) and water (hydrophilic side)

pulling the lignin into the water, and enhancing its solubility; hence, the higher percent delignification observed in the combined surfactant-dilute ammonia pretreatments. The effect of surfactants on pretreatment varied with the HLB (hydrophile-lipophile balance) values of each surfactant. Hydrophilic surfactants having high HLB values have been reported to be useful in extracting hydrophobic degradation products from lignin and hemicellulose (Kurakake et al., 1994). Tween 20 (HLB, 16.7), Tween 80 (HLB, 15.0), PEG 4000 (HLB, 18.5), and PEG 6000 (HLB, 19.0) have high HLB values, thus making them highly water soluble. The fact that the addition of Tween 20 during pretreatment can help solubilize lignin has also been reported elsewhere (Seo et al., 2011). According to Kurakake et al. (1994), Tween 20 which has a higher HLB value than Tween 80 removed 22% and 27% lignin in beech bagasse by upper critical-temperature solvent pretreatment with 3.33% wt surfactant at 170 ℃ and 190 ℃, respectively. The higher lignin removal (37%) observed with Tween 80 in this study can be attributed to the thickness of the adsorbed surfactant layers on the hydrophobic substance. It was reported that the thickness of absorption of aqueous polyoxyethylene (Tween) surfactants with hydrophobic surfaces increased with increasing length of the alkyl moiety (Graca et al., 2007). As Tween 80 possesses one unsaturated carbon–carbon double bond in the alkyl moiety, on physical grounds one expects unsaturation to force the molecule to lie more nearly parallel to the hydrophobic substrate. This is probably why the thickness of Tween 80's layer at full surface coverage was lower than that of Tween 60 and Tween 20 as reported by Graca et al. (2007). Based on these observations, if more Tween 20 is absorbed onto the bagasse surface, less Tween 20 would solubilize in water to enhance lignin removal, leading to less delignification. The opposite would be true for Tween 80, where its hydrophobic side would combine with lignin causing it to pull more lignin into the water, thus resulting in higher delignification.

Qing et al. (2010) reported 25.6% and 16.7% lignin removal in corn stover that was soaked in a 3% w/w Tween 80 solution prior to pretreatment with dilute acid (140 ℃, 1% sulfuric acid for 40 min) and water only (220 ℃, 30 min), respectively. PEG 4000-dilute acid pretreatment and water only pretreatment showed 17% and 9% lignin removal, respectively (Qing et al., 2010). In our study, PEG 6000-dilute ammonia and PEG 4000-dilute ammonia pretreatments showed higher or equal delignification to Tween 80-dilute ammonia pretreated sugarcane bagasse. It is known that Tween series absorb to the surface of biomass by H-bonding with oxygen ethylene of the hydrophilic head and by hydrophobic interaction with the hydrophobic tails (Paria and Khilar, 2004). Hydrophobic interactions have been found to be the dominating binding force in PEG series surfactants-lignin interactions (Börjesson et al., 2007a). According to Gierer (1986), acidic groups and hydrophobic sites present on the surface of lignocellulosic biomass are the absorption sites for Tween 20 and Tween 80. Tween series absorption to lignin is affected by the properties of lignocellulose such as lignin concentration, number of acidic groups present and pH of the solution (i.e., dissociation of acidic groups on lignocellulose) (Seo et al., 2011). In the Tween-dilute ammonia pretreatments, the high pH of the solution neutralized the acidic groups resulting in less available absorption sites for Tween 20 and Tween 80. Thus, higher delignification was observed with the PEGs-dilute ammonia pretreatments.

The level of cellulose and hemicellulose (glucan and xylan) is directly related to the yields of fermentable sugars and ethanol, thus minimizing carbohydrates loss during the pretreatment process is crucial (Kim et al., 2007; Kim et al., 2003). For all the surfactant-dilute ammonia pretreatments, an average of 44% hemicellulose loss was observed as compared to 25% and 19% for dilute ammonia pretreated sugarcane bagasse and water pretreated sugarcane

bagasse, respectively. Hemicellulose can be dissolved in an acid or an alkaline environment (Balaban, 1999; Lawther et al., 1996). Removal of hemicellulose increases the mean pore size of the substrate which enhances the probability of cellulose to become hydrolyzed by enzymes (Chandra et al., 2007). Approximately, 95%, 87%, 82%, and 86% of the cellulose was retained in the biomass post treatment with Tween 20-dilute ammonia, Tween 80-dilute ammonia, PEG 6000-dilute ammonia, and PEG 4000-dilute ammonia, respectively.

Table 2. 1. Chemical composition of surfactant-dilute ammonia pretreated sugarcane bagasse (g/100g dry biomass).

Biomass component	Untreated	Water	Dilute ammonia	Tween 20 dilute ammonia	Tween 80 dilute ammonia	PEG 6000 dilute ammonia	PEG 4000 dilute ammonia
Ash	6.39±0.28	4.03±0.32	2.16±0.10	5.40±0.28	3.62±0.49	4.68±0.42	4.39±0.60
Extractives	2.64±0	3.61±0.11	1.88±0.07	4.09±0.11	2.26±0.14	3.72±0.50	3.28±0.17
Lignin	22.69±0.12	21.77±0.10	19.58±1.02	18.58±0.53	14.37±1.79	15.35±0.47	12.03±0.16
Glucan	40.71±0.20	40.50±0.02	40.25±0.14	38.85±0.93	35.32±1.80	33.35±1.22	35.01±1.53
Xylan	24.95±1.26	20.24±0.00	18.67±1.20	13.96±0.03	15.06±1.20	11.37±1.05	16.02±1.03
Arabinan	2.83±0.05	0.88±0.31	2.32±0.30	2.16±0.09	1.91±0.25	1.79±0.12	1.48±0.35
Mannan	ND	ND	ND	ND	ND	ND	ND
Solids remaining	100	92.24±0.71	86.56±1.91	83.03±2.07	72.39±4.41	70.60±3.77	72.39±2.50

ND: None detected.

2.3.2 Enzymatic digestibility of the non-ionic surfactant-dilute ammonia pretreated sugarcane bagasse

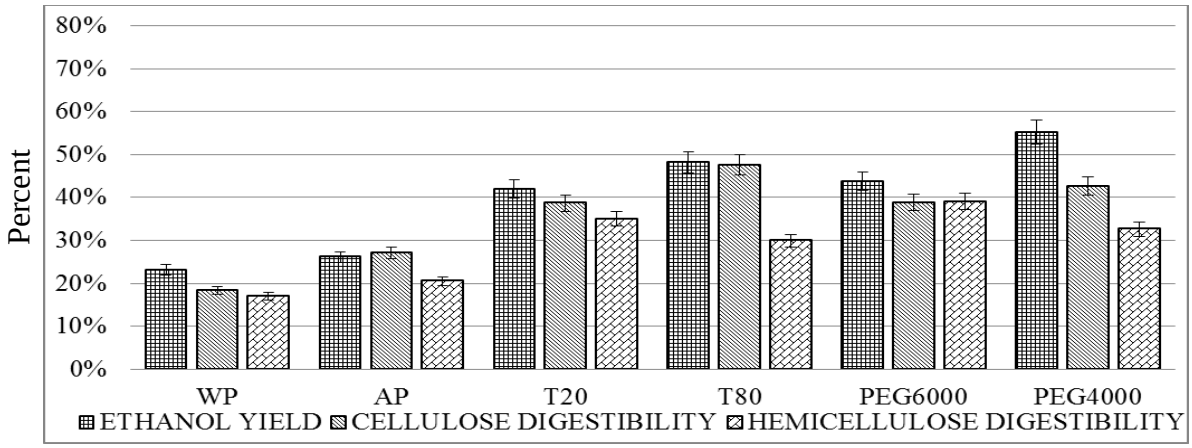
Cellulose digestibility, hemicellulose digestibility and ethanol yields for surfactant-dilute ammonia pretreatments of sugarcane bagasse and controls are summarized in Figure 2. 1. The low hemicellulose yields observed were attributed to the fact that the enzyme cocktail used contained mostly cellulase-degrading enzymes. Enhanced cellulose and hemicellulose digestibility yields were observed in surfactant-dilute ammonia pretreated sugarcane bagasse as compared to controls. Tween 80-dilute ammonia pretreatment resulted in the highest cellulose yield. The cellulose digestibility of Tween 80-dilute ammonia pretreated sugarcane bagasse was

48% at the combined enzyme loading of 15 FPU/g glucan and 15 CBU/g glucan (half strength), which represents a 159% and 76% yield increase as compared to water pretreated bagasse and dilute ammonia pretreated bagasse, respectively. At the enzyme loading of 30 FPU/g glucan and 30 CBU/g glucan (full strength), Tween 80-dilute ammonia pretreated sugarcane bagasse resulted in a 66% cellulose digestibility, which represents a 151% and 81% increase as compared to water pretreated bagasse and dilute ammonia pretreated bagasse, respectively. Qing et al. (2010) reported 90% cellulose digestibility for pretreated corn stover (140 ℃, 1% sulfuric acid for 40 min) with 3% w/w Tween 80 and 83.1% cellulose digestibility for Tween 80-water pretreated corn stover (220 ℃, 30 min) at the enzyme loading of 60 FPU/g glucan supplemented with 120 CBU/g glucan after 96 h enzyme hydrolysis. Lower cellulose digestibilities (88.1%, 78.2%) were observed at an enzyme loading of 10 FPU/g glucan and 20 CBU/g glucan post 96 h hydrolysis, respectively. A total of 33 ± 0.2 g of glucose/100 g dry biomass were released with Tween 80-dilute ammonia pretreated sugarcane bagasse at full strength enzyme loading; whereas, only 14 ± 0.5 g and 9 ± 0.8 g were detected in dilute ammonia pretreated and water pretreated biomass, respectively. Aita et al. (2011) reported a total of 34 ± 1.4 g of glucose/100 g dry biomass with dilute ammonia pretreated bagasse (160 ℃ , 1h, 1: 0.5: 8 (biomass : ammonium hydroxide : water ratio)) at 30 FPU of Spezyme CP/g of glucan and 32 CBU of Novozyme 188/g of glucan. However, Tween 80-dilute ammonia pretreatment required less ammonium hydroxide and water, and resulted in almost the same yield of glucose/100 g dry biomass as that reported by Aita et al. (2011). Surfactants allow for more delignification resulting in less non-productive enzymes binding to lignin and more hydrophilic biomass surface interactions, thereby making it easier for enzymes dissolved in water to access the biomass surface (Eriksson et al., 2002).

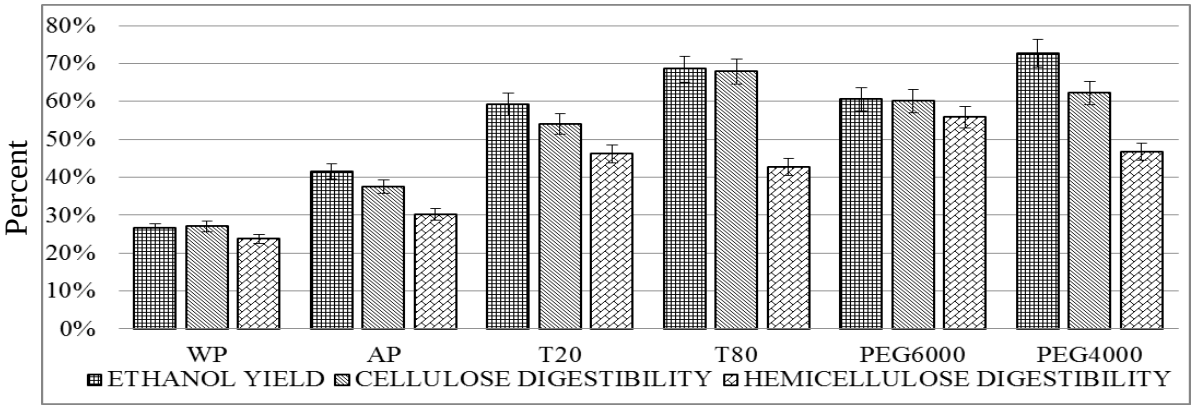
Cellulose digestibilities of Tween 20-dilute ammonia pretreated sugarcane bagasse were 39% and 54% at half strength and at full strength enzyme loadings, respectively. An increase in digestibility yields of 44% and 42% was observed when compared to dilute ammonia pretreatment. Significant increase in glucan conversion has been observed in dilute sulfuric acid pretreated wheat straw (121 ℃, 90 min) with Tween 20. The highest glucose yield (70%) was obtained from 2% sulfuric acid and 1% Tween 20 at 20 FPU/g glucan and 40 CBU/g glucan after 72 h hydrolysis (Qi et al., 2010). Eriksson et al. (2002) reported a slightly lower cellulose digestibility for Tween 20-steam pretreated spruce (46%) impregnated with 2.4% SO_2 (w/w, based on the water content) for 20 min at room temperature followed by a second pretreatment with steam at 215 ℃ for 3 min than that with Tween 80 (49%) added during enzymatic hydrolysis. Lower cellulose digestibility of Tween 20-dilute ammonia pretreated sugarcane bagasse than that of Tween 80 was also observed in this work. This result may be attributed to less lignin removal in Tween 20-dilute ammonia pretreatment, leading to more non-productive enzyme binding.

PEG 6000-dilute ammonia and PEG 4000-dilute ammonia pretreatments showed an increase in cellulose digestibility (58%, 41%; 63%, 59%) as compared to dilute ammonia pretreatment at half and full strength enzyme loadings, respectively. Surfactants containing ethylene oxide (EO) chains have been shown to enhance the enzymatic conversion of lignocellulose (Eriksson et al., 2002; Kaar and Holtzapple, 1998; Zhang et al., 2011). In two enzymes systems, PEG 4000 with 91 EO units had the strongest ability to enhance enzymatic hydrolysis (Ouyang et al., 2010). This observation was confirmed in our study as PEG 4000-dilute ammonia pretreatment had higher glucan yields (43%, 62%) than dilute ammonia pretreatment (27%, 38%) at half and at full strength enzyme loadings. Although PEGs-dilute

ammonia pretreated sugarcane bagasse resulted in higher or equal lignin removal than Tween 80-dilute ammonia pretreated sugarcane bagasse, cellulose digestibilities of PEGs-dilute ammonia pretreated bagasse were lower than Tween 80-dilute ammonia pretreated bagasse. Zhang et al. (2011) indicated that cellulase's long time exposure to PEG 4000 could cause structural changes of certain amino acids (tyrosine, tryptophan, glutamic acid, and aspartic acid) present in cellulases thus reducing the capacity of enzymes to bind and degrade the lignocellulose.



a. Enzymatic Digestibility and Ethanol Yields at 15 FPU/g Glucan and at 15 CBU/g Glucan.



b. Enzymatic Digestibility and Ethanol Yields at 30 FPU/g Glucan and at 30 CBU/g Glucan.

Figure 2. 1. Enzymatic Digestibilities and Ethanol Yields for Surfactant-Dilute Ammonia Pretreated Bagasse and Controls. Water pretreated (WP), dilute ammonia pretreated (AP), Tween 20-dilute ammonia pretreated (T20), Tween 80-dilute ammonia pretreated (T80), PEG 6000-dilute ammonia pretreated (PEG 6000), and PEG 4000-dilute ammonia (PEG 4000) pretreated sugarcane bagasse at (a) enzyme loading of 15 FPU/g glucan and at 15 CBU/g glucan, and (b) enzyme loading of 30 FPU/g glucan and at 30 CBU/g glucan.

2.3.3 Ethanol yields of the non-ionic surfactant-dilute ammonia pretreated sugarcane bagasse

Saccharomyces cerevisiae (D_5A) was used in this study for the conversion of glucose to ethanol. The ethanol concentration of Tween 80-dilute ammonia pretreated sugarcane bagasse after 48 h fermentation was 13 ± 0.1 g and 18 ± 1.6 g/100 g of dry biomass with theoretical yields of 48% and 69% at half and full strength enzyme loadings, respectively (Figure 2. 1). Ethanol concentration for water pretreated and ammonia pretreated sugarcane bagasse averaged 7 ± 0.2 g and 10 ± 0.6 g/100 g of dry biomass with theoretical yields of 27% and 42% at full enzyme strength, respectively. An ethanol concentration of 16 ± 0.1 g and 20 ± 1.1 g/100 g of dry biomass with theoretical yields of 55% and 73% were observed for PEG 4000-dilute ammonia pretreated sugarcane bagasse at half strength and at full strength enzyme loadings, respectively. Tween 80- dilute ammonia pretreated sugarcane bagasse had higher cellulose conversion than PEG 4000-dilute ammonia pretreated sugarcane bagasse, but the ethanol concentration of PEG 4000-dilute ammonia pretreatment was higher than that of Tween 80-dilute ammonia pretreatment. PEG 4000 has been reported to be a better plasticizer and pore former than Tween 80 in controlling drug release from microporous membrane-coated matrix tablets (Mishra and Mishra, 2010). It is possible that during the first 48 h of fermentation, PEG 4000 resulted in more pore formation on the yeast cell membrane as compared to Tween 80, resulting in higher ethanol yields. Yun et al. (2012) reported that pore formation capability of PEG 6000 is stronger than that of Tween 80. However, in our study the yields observed with PEG 6000 were not that different from those observed with Tween 80. PEG 6000-dilute ammonia pretreated bagasse resulted in 12 ± 0.5 g and 16 ± 0.2 g ethanol/100 g dry biomass with 44% and 61% ethanol yields as compared to Tween 80's ethanol concentrations of 13 ± 0.1 g and 18 ± 1.6 g/100 g of dry biomass at half and at full strength enzyme loadings, respectively. The ethanol concentration of Tween 20-dilute ammonia pretreated bagasse was 11 ± 0.1 g and 15

± 0.8 g/100 g dry biomass with 42% and 59% ethanol yields at half and at full strengths, respectively. It was reported that wheat straw pretreated with dilute sulfuric acid (121 ℃, 2% w/v, sulfuric acid, 90 min) and with 1% w/v Tween 20 resulted in 59% ethanol yield after 72 h fermentation, and 0.51 g ethanol/g glucose (Qi et al., 2010).

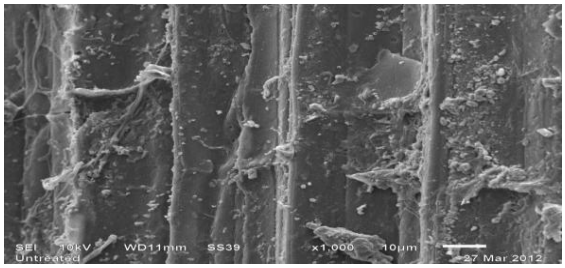
2.3.4 Analytical studies

Organic acids such as acetic acid (0.5-9 g/l), lactic acid (10-40 g/l) and formic acid (0.5-2.7 g/L) can inhibit fermentation by interfering with cell maintenance of *S. cerevisiae* (Maiorella et al., 1983). The concentration of acetic acid was lower than 0.04 g/l and those of lactic acid and formic acid were undetected. The above mentioned organic acids concentrations are lower than the ones considered to be inhibitory to yeast. Therefore, the concentration of organic acids in this study was insufficient to inhibit fermentation.

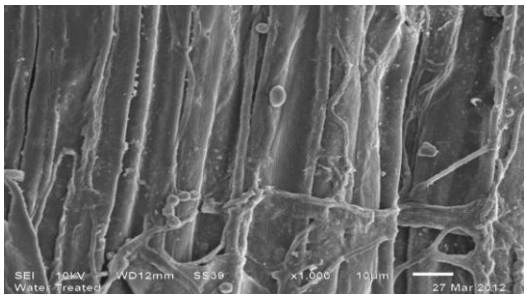
2.3.5 SEM images

SEM images of untreated, water pretreated, dilute ammonia pretreated, and surfactant-dilute ammonia pretreated sugarcane bagasse are shown in Figure 2. 2. Untreated and water pretreated bagasse showed compact and rigid fibril structures (Figure 2. 2 a, b). Dilute ammonia pretreated sugarcane bagasse (Figure 2. 2 c) resulted in some swelling and scaling due to the breakdown of ester bonds in the carbohydrate-lignin complex by ammonolysis reaction (Salvi et al., 2010). All surfactant-ammonia pretreated bagasse samples showed more swelling and scaling when compared to untreated, water pretreated, and dilute ammonia pretreated bagasse (Figure 2. 2 d to g). The more exposed the cell wall structure the greater the accessibility of hydrolytic enzymes to the biomass surface, thus facilitating the hydrolysis of lignocellulosic biomass. Tween 80-dilute ammonia pretreated sugarcane bagasse showed the most swelling and scaling of the biomass structures among surfactants. This observation is in accord with Tween 80-dilute

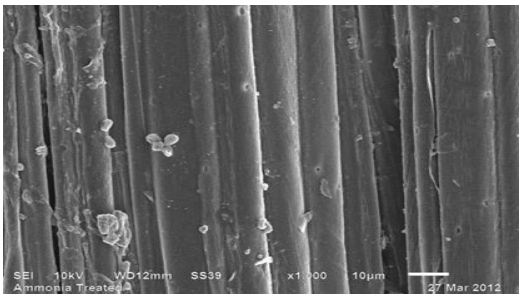
ammonia pretreatment resulting in the second highest lignin removal (37%) and the highest cellulose digestibility (66%).



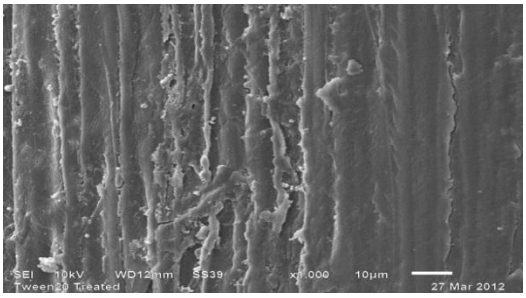
a. Untreated



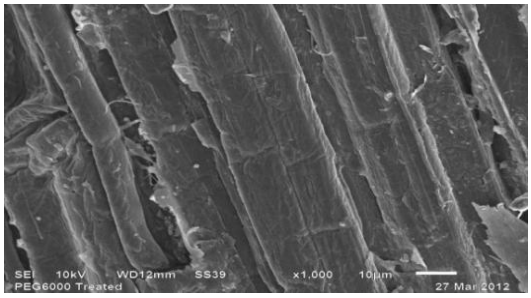
b. Water pretreated



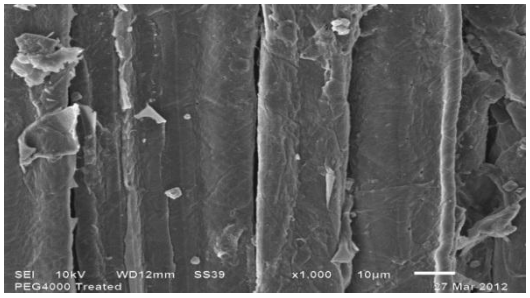
c. Dilute ammonia pretreated



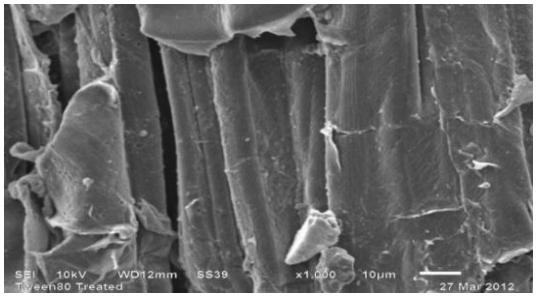
d. Tween 20-dilute ammonia pretreated



e. PEG 6000-dilute ammonia pretreated



f. PEG 4000-dilute ammonia pretreated



g. Tween 80-dilute ammonia pretreated

Figure 2. 2. Sugarcane Bagasse SEM Images. (a) untreated, (b) water pretreated, (c) dilute ammonia pretreated, (d) Tween 20-dilute ammonia pretreated, (e) PEG 6000-dilute ammonia pretreated, (f) PEG 4000-dilute ammonia pretreated, and (g) Tween 80-dilute ammonia pretreated. All images were taken at 1, 000 X magnification.

2.3.6 FTIR analysis

FTIR analysis was conducted to examine the cellulose structure of untreated, controls (water and dilute ammonia) and surfactant-dilute ammonia pretreated bagasse samples (Figure 2. 3). Two infrared ratios related to cellulose structure were calculated: (1) α 1423 cm^{-1}/ α 897 cm^{-1}, the ratio of peak areas at 1423 cm^{-1} and 897 cm^{-1} ,which is referred to as lateral order index (LOI) (Hurtubise and Krassig, 1960); and (2) α 1372 cm^{-1}/ α 2900 cm^{-1}, the ratio of peak areas at 1372 and 2900 cm^{-1}, which is known as the total crystallinity index (TCI) (Nelson and O'Connor, 1964a). The absorption band at 1423 cm^{-1} relates to the CH_2 scissoring motion (Nelson and O'Connor, 1964b). The absorption band at 897 cm^{-1} assigned as C-O-C stretching is characteristic of β-anomers or β-linked glucose polymers (Nelson and O'Connor, 1964b). Native cellulose in higher plants is found in the form of cellulose type I, which is highly crystal. The ratios can be the evidence of the fraction of cellulose type I present in the cellulosic material (Kuo and Lee, 2009; Oh et al., 2005). In other words, low LOI values are indicative of less cellulose type I present. Cellulose type I is transformed to cellulose type II, type III or amorphous cellulose during pretreatment. The ratio of the absorption bands at 1372 cm^{-1} (C-H bending) and 2900 cm^{-1} (C-H stretching) were chosen as being suitable for identifying both cellulose type I and cellulose type II structures (Nelson and O'Connor, 1964a). Therefore, higher TCI values are indicative of biomass with a higher crystallinity and a more ordered structure of cellulose (cellulose Type I) (Spiridon et al., 2011; Yang et al., 2007; Zhao et al., 2010). LOI and TCI values (Table 2. 2) for all surfactant-dilute ammonia pretreated bagasse decreased as compared to untreated (1.2157 ± 0.0754, 0.4415 ± 0.0032), water pretreated (1.1321 ± 0.0126, 0.4241 ± 0.0192) and dilute ammonia (1.0403 ± 0.0595, 0.3851 ± 0.0058) pretreated sugarcane bagasse, respectively (Table 2. 2). Tween 80-dilute ammonia pretreated bagasse resulted in the lowest LOI (0.5969 ± 0.0411) and TCI (0.2380 ± 0.0143) values, followed by PEG 4000-dilute

ammonia pretreated sugarcane bagasse (0.6288±0.0280, 0.2613±0.0096), respectively. Tween 20-dilute ammonia (0.9512±0.0042, 0.3571±0.0171) and PEG 6000-dilute ammonia (0.6897±0.0645, 0.2802±0.0012) pretreated bagasse resulted in higher LOI and TCI values as compared to the other surfactants. Similar findings have been reported in sugarcane bagasse pretreated by thermal degradation (Yoon et al., 2011; Zhao et al., 2010). A decrease in crystallinity index (CrI) calculated by FTIR was obtained in the *Pinus radiata* and *Eucalyptus globulus* after exposure to two brown rot fungi, *Gloephylum trabeum* and *Laetoporeus sulphureus* (Monrroy et al., 2011). Nada et al. (2009) reported a decrease in LOI values for cotton linters pretreated with 1 N NaOH under reflux for 1.5 h.

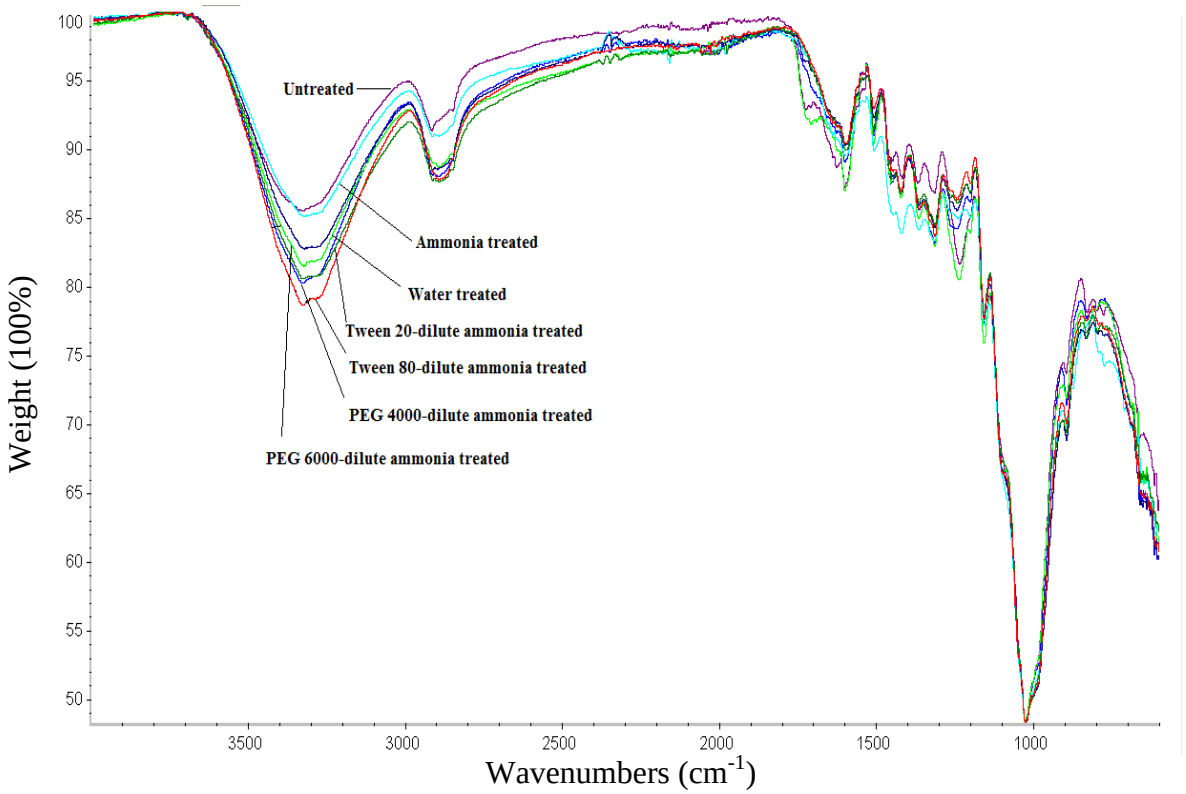


Figure 2. 3. FTIR Spectra for Non-Ionic Surfactant-Dilute Ammonia Pretreated Sugarcane Bagasse and Controls (untreated, water treated, ammonia treated).

Table 2. 2. Crystallinity data of surfactant-dilute ammonia pretreated sugarcane bagasse and controls.

	Untreated	Water	Dilute ammonia	Tween 20 dilute ammonia	Tween 80 dilute ammonia	PEG 4000 dilute ammonia	PEG 6000 dilute ammonia
LOI	1.2157±0.0754	1.1321±0.0126	1.0403±0.0595	0.9512±0.0042	0.5969±0.0411	0.6288±0.0280	0.6897±0.0645
TCI	0.4415±0.0032	0.4241±0.0192	0.3851±0.0058	0.3571±0.0171	0.2380±0.0143	0.2613±0.0096	0.2802±0.0012
CrI	0.4000±0.0092	0.4576±0.0001	0.5765±0.0049	0.7879±0.0001	0.9211±0.0063	0.8842±0.0183	0.8089±0.0001

LOI: lateral index based on FTIR analysis.
TCI: total crystallinity index based on FTIR analysis.
CrI: crystallinity index based on XRD analysis.

2.3.7 XRD analysis

XRD is one of the simplest and most widely used methods for measuring crystallinity index (CrI). CrI was used to further examine the crystallinity of cellulose since determination of CrI by FTIR spectroscopy gives only relative values from both crystalline and amorphous regions. The CrI calculated from an FTIR spectrum is often compared with those from XRD and/or NMR measurements (Park et al., 2010). Two typical diffraction peaks, (101) and (002) lattice, were observed at 2θ=22° and 16°, which correspond to planes of crystalline cellulose type I. The lowest intensity between (101) and (002) lattice was observed at 18 °, which corresponds to the scattered intensity due to the amorphous portion. A new narrow peak around 26 ° was reported due to hydroxyapatite crystals (Wan et al., 2006), with 002 diffraction (Kaushik et al., 2010). The CrI of various sugarcane bagasse samples was determined based on the XRD patterns for quantitative comparison and are depicted in Table 2.2. All XRD spectra are depicted in Figure 2. 4. An increase in CrI was observed with surfactant-dilute ammonia pretreated samples. No significant difference was observed between the CrI for water pretreated and untreated samples. The highest CrI (0.9211±0.0063) was observed with Tween 80-dilute ammonia pretreated sugarcane bagasse. In lignocellulosic biomass, the CrI measures the relative amount of

crystal cellulose in the total solid. Surfactant-dilute ammonia pretreatment removed lignin and hemicellulose which are amorphous, resulting in an increased CrI. According to Chang and Holtzapple (2000), pretreatment of poplar wood with lime resulted in lignin removal and an increase in CrI. XRD data of sugarcane bagasse steam pretreated in the presence of CO_2 and SO_2 showed that pretreated materials have higher CrI values when compared with untreated materials, due to the partial removal of hemicellulose after pretreatment (Corrales et al., 2012). Kim et al. (2003) indicated that the increased CrI in corn stover pretreated by aqueous ammonia was primarily due to the removal of amorphous substances (lignin and hemicellulose), not due to changes in the basic crystalline structure of the cellulose. Corn stover and poplar pretreated by ammonia fiber expansion (AFEX), ammonia recycled percolation (ARP), controlled pH, dilute acid, flow through, lime, and SO_2 technologies have shown an increase in CrI (Kumar et al., 2009b).

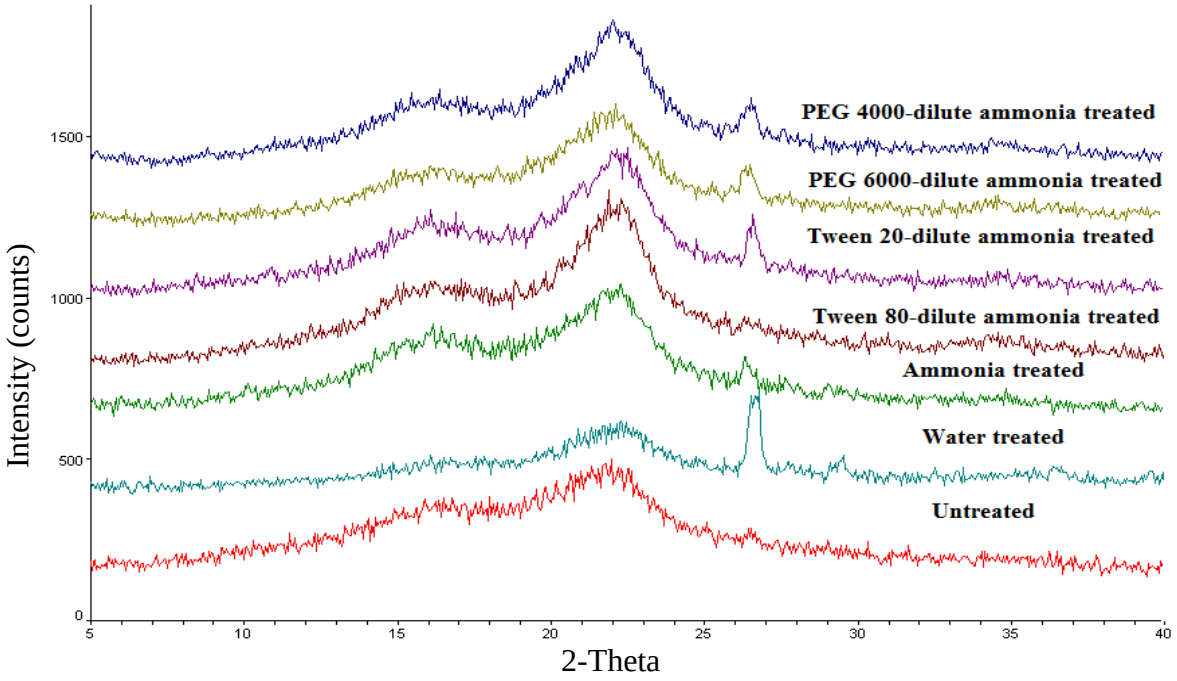


Figure 2. 4. XRD Spectra for Non-Ionic Surfactant-Dilute Ammonia Pretreated Sugarcane Bagasse and Controls (untreated, water treated, ammonia treated).

2.3.8 TGA analysis

TGA can be used to determine the thermal stability of bagasse by monitoring the weight change as the sample is heated. TGA curves are summarized in Figure 2. 5. Maximum rates of weight loss were observed between 260 ℃ and 360 ℃ for sugarcane bagasse before and after pretreatment. Decomposition of lignin, cellulose and hemicellulose in untreated sugarcane bagasse was observed at temperatures between 250 ℃ to 380 ℃. Ruzene et al. (2009) observed maximum rates of weight loss between 260 ℃ and 360 ℃ for sugarcane bagasse bleached with sodium chlorite. Significant weight loss for sugarcane bagasse pretreated with alkali and acetic acid in a two-stage process has been reported between 230 ℃ to 390 ℃ (Zhao et al., 2010).

Tween 80-dilute ammonia and PEG 4000-dilute ammonia pretreated sugarcane bagasse had the highest onset of degradation temperature (310.33 ±0.20 ℃ and 312.65 ±0.60 ℃) due to the large amounts of lignin removed (Ruzene et al., 2009; Zhao et al., 2010). Onset degradation temperatures for PEG 6000 and Tween 20 were 307.90 ± 0.31 ℃ and 302.61 ± 1.45 ℃, respectively. High onset of degradation temperature means high thermal stability. Biomass with high lignin content translates to low thermal stability and low onset temperature which was observed in untreated (263.48 ± 2.02 ℃), water pretreated (272.24 ± 1.35 ℃) and dilute ammonia pretreated (298.13 ± 0.71 ℃) sugarcane bagasse. Lignocellulosic biomass after chemical or biological treatment should have higher onset of degradation temperatures than untreated biomass because of lignin and hemicellulose removal leading to high thermal stability (Ruzene et al., 2009; Xiao et al., 2011; Yang et al., 2010). Untreated sugarcane bagasse resulted in more weight loss between 200 ℃ and 300 ℃. This was probably due to the higher hemicellulose content as compared to pretreated biomass. Pyrolysis of hemicellulose begins at

lower temperatures (220 ℃ to 300 ℃) than cellulose (315 ℃ to 400 ℃). Lignin begins to decompose as its weight loss takes place at 160 ℃ (Wanga et al., 2008; Yang et al., 2007).

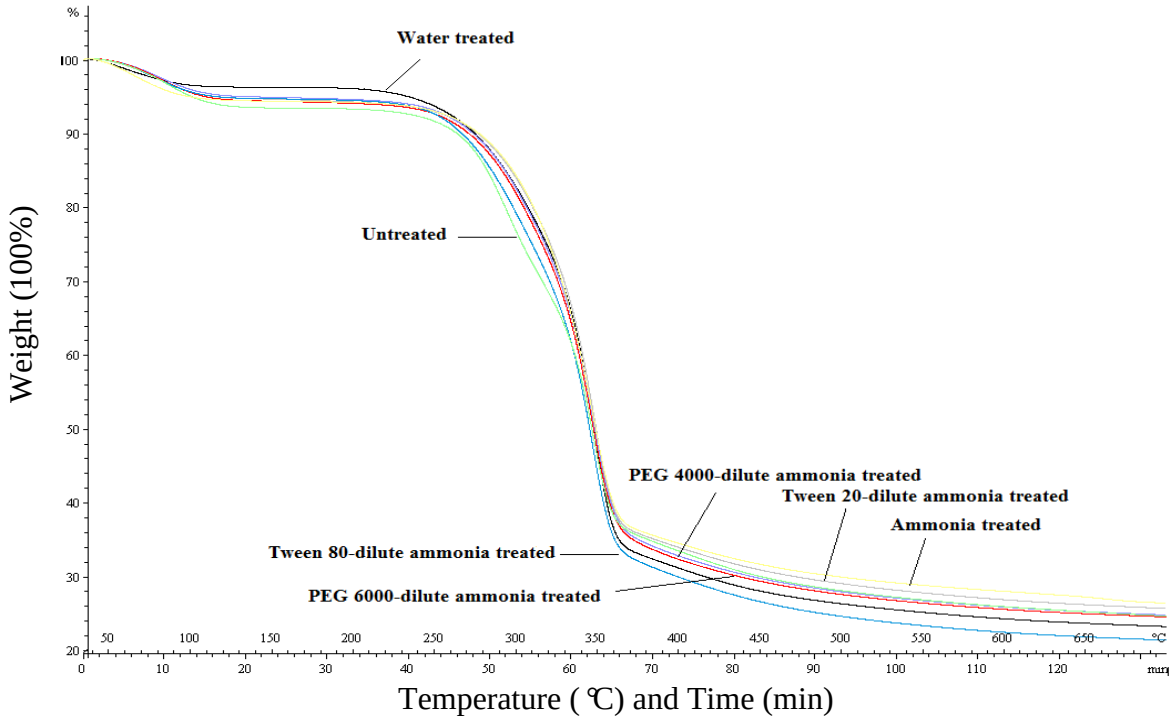


Figure 2. 5. TGA Curves for Non-ionic Surfactant-Dilute Ammonia Pretreated Sugarcane Bagasse and Controls (untreated, water treated, ammonia treated).

2.4. Conclusions

The combined effect of non-ionic surfactants with ammonium hydroxide during pretreatment enhanced the removal of lignin and retained most of the cellulose, especially in pretreatments with Tween 80 (37%, 87%) and PEG 4000 (47%, 86%), respectively.

A total of 33 ± 0.2 g of glucose/100 g dry biomass were released with Tween 80-dilute ammonia pretreated sugarcane bagasse. However, PEG 4000-dilute ammonia pretreated sugarcane bagasse had the highest ethanol concentrations of 16 ± 0.1 g and 20 ± 1.1 g/100 g of dry biomass with theoretical yields of 55% and 73% at half and at full strength enzyme loadings, respectively. SEM images revealed severe disruption of the biomass structure. FTIR showed a

decrease of LOI and TCI values due to the transformation of highly crystal cellulose type I to lower crystal cellulose type II, III and amorphous cellulose. XRD resulted in an increase in CrI because of the removal of amorphous substrates such as lignin and hemicellulose. An increase in thermal stability was observed by TGA for all surfactant-dilute ammonia pretreated biomass.

CHAPTER 3. EFFECT OF TWEEN 80 ON THE PRETREATMENT AND ENZYMATIC HYDROLYSIS OF DILUTE AMMONIA PRETREATED SUGARCANE BAGASSE

3.1 Introduction

Humans are facing severe energy security and environmental challenges. Biomass is a promising renewable energy resource that can provide alternative transportation fuels such as bioethanol or biodiesel in the short-term (Hamelinck et al., 2005; Sun and Cheng, 2002b). The production of first generation liquid biofuels (mainly from food crops, like sugarcane, corn and oil seeds) is well understood (Luiz Carlos Basso, 2011; Searchinger et al., 2008). However, crops for first generation biofuels compete with the human food supply, for water, land, and other resources (Searchinger et al., 2008). Second generation biofuels produced from non-food biomass can offer a solution to these problems. Lignocellulosic biomass include agricultural by-products (cereal straw, sugarcane bagasse, forest residues), wastes (organic components of municipal solid wastes), and dedicated feedstocks (purpose-grown vegetative grasses, short rotation forests and other energy crops) (Reddy and Yang, 2005; Salvi et al., 2010). Lignocellulosic biomass is composed of carbohydrate polymers (cellulose and hemicellulose) and lignin which are not readily available in their native form. Ethanol production from lignocellulosic biomass comprises four main steps which include pretreatment, enzyme hydrolysis, fermentation and ethanol recovery, and refining (Aita et al., 2011). Of these four steps, pretreatment is a key step as it alters the carbohydrate-lignin structure thus making enzymes more accessible to the sugar polymers for later conversion into fuels and chemicals. An effective pretreatment can lower downstream unit operation costs. About 18% of the total processing cost comes from pretreatment in biological production of cellulosic ethanol, more than for any of the other steps (Yang and Wyman, 2008).

A dominant concern in second generation fuels is achieving high sugar yields with low cost pretreatment processes and cellulosic enzymes. Ammonia being a selective reagent for lignin, non-corrosive and a relatively less expensive chemical, is an appropriate choice for pretreatment (Kim et al., 2003). Dilute ammonia pretreatment has shown great success in delignification. It removes lignin by cleaving C-O-C bonds and other ether and ester bonds in the carbohydrate-lignin complex (Aita et al., 2011). Greater removal of lignin can improve both enzyme hydrolysis and fermentation. Also, ammonia-based pretreatments can protect the glucan from decomposition as compared to acid-based pretreatment technologies.

Another promising approach to lower the cost of pretreatment is the addition of surfactants, especially non-ionic surfactants. Recently several studies have indicated that the addition of surfactants, especially non-ionic surfactants, during pretreatment or enzymatic hydrolysis can enhance delignification, improve enzyme hydrolysis and lower enzyme loadings (Börjesson et al., 2007a; Börjesson et al., 2007b; Qing et al., 2010; Seo et al., 2011). Several explanations have been proposed to describe the mechanism of how surfactants enhance delignification and enzymatic digestibility (Eriksson et al., 2002; Helle et al., 1993; Kaar and Holtzapple, 1998; Kim et al., 1982). However, a mechanism that can consistently explain how surfactants work has yet to be proposed. One of the most popular explanations for enhancement of enzymatic hydrolysis by surfactants is that surfactants can reduce non-productive enzyme binding to lignin during hydrolysis (Eriksson et al., 2002; Qing et al., 2010).

Polyethylene glycol (PEG) and polyoxyethylene (Tween series) are two promising surfactants use to improve pretreatment and enzymatic hydrolysis. However, Zhang et al. (2011) indicated that cellulase's long time exposure to PEG 4000 could reduce the capacity of the enzyme to bind and degrade the lignocellulose due to conformational changes in the amino acids

present in cellulase. Tween 80 (polysorbate 80) is derived from polyethoxylated sorbitan and oleic acid. The hydrophilic-lipophilic balance (HLB) of Tween 80 is 15, which means it is highly water soluble. Surfactants with high HLB values are useful for the extraction of hydrophobic degradation products from lignin and hemicellulose (Kurakake et al., 1994). Tween 80 can increase lignin removal during pretreatment and enhance enzymatic hydrolysis (Eriksson et al., 2002; Qing et al., 2010). Also, addition of Tween 80 during enzymatic hydrolysis of pretreated biomass can significantly reduce the total enzyme cost and help with the recycling of enzymes (Tu and Saddler, 2010). Previous research have indicated that Tween 80 in combination with ammonium hydroxide gave the best overall performance based on its effect on biomass structure, sugar yield (33 ± 0.2 g of glucose/100 g dry biomass) and ethanol yield (18 ± 1.6 g/100 g dry biomass) as compared to Tween 20, PEG 4000 and PEG 6000. Tween 80 was chosen as the best surfactant for further research because of its encouraging delignification, sugar yield and market value (U.S. $0.25/kg) (Tu and Saddler, 2010).

The goal of this study was to investigate the effect of various pretreatment conditions on the chemical composition, cellulose and hemicellulose digestibilities of Tween 80-dilute ammonia pretreated sugarcane bagasse.

3.2 Methods and Materials

3.2.1 Substrate

Sugarcane bagasse was collected from Louisiana sugar mills during the grinding season (September-December) and stored in 50 gal drums at -20 ℃.

3.2.2 Tween 80-dilute ammonia pretreatment

Tween 80 was used in combination with ammonium hydroxide for the pretreatment of sugarcane bagasse. Biomass pretreatment was carried out by mixing sugarcane bagasse,

ammonium hydroxide (28% v/v solution) and water at a ratio of 1: 0.2: 20 (biomass: ammonium hydroxide: water) or 1: 0.5: 20, with final ammonium hydroxide concentrations of 0.26% w/w or 0.65% w/w, respectively, at a biomass loading of 4.7% w/w. The mixture was supplemented with 1.5%, 3% or 5% (w/w) Tween 80 (based on the weight of dry biomass) and then heated to 160 ℃ for 1 h in a 4 L bioreactor. Untreated bagasse and bagasse pretreated with water only at 1: 20 (biomass: water) ratio were run as controls. Additional pretreatment controls were run with dilute ammonia at 1: 0.2: 20 ratio or at 1: 0.5: 20 ratio without the addition of Tween 80. Controls were carried out with Tween 80 only at 1.5%, 3% or 5% (w/w, based on the weight of dry biomass) at 1: 20 (biomass: water). All controls were run at a 4.7% solids loading.

3.2.3 Chemical composition of pretreated sugarcane bagasse

Samples were analyzed for glucan, xylan, lignin, arabinan, mannan, ethanol extractives, and ash content using NREL's Laboratory Analytical Procedures (LAPs #42618, 42619, 42620, 42621, 42622). NREL reference material (8491 sugarcane bagasse) was analyzed as an internal sample to ensure the accuracy of the procedures.

3.2.4 Enzyme hydrolysis

Tween 80-dilute ammonia pretreated, Tween 80 pretreated, dilute ammonia pretreated and water pretreated were hydrolyzed using a combination of commercially available cellulose degrading enzymes, Spezyme CP (Genencor, Danisco US Inc., Rochester, NY) and Novozyme 188 (Sigma–Aldrich, Inc., St. Luis, MO). Spezyme CP (cellulases) and Novozyme 188 (cellobiases) were used at 30 FPU/g glucan and at 30 CBU/g glucan, respectively. Approximately, 10 g dry weight of Tween 80-dliute ammonia pretreated, Tween 80 pretreated, ammonia pretreated, or water pretreated bagasse were each loaded into 250 mL Erlenmeyer flasks following NREL's hydrolysis and fermentation protocols (LAP TP-510-42630).

Additionally, 5 g citrate buffer (1 M stock solution, pH 4.8) and water were added to each flask to a final weight of 100 g. The pH of each mixture was adjusted to 4.8 with concentrated hydrochloric acid. All flasks were sterilized at 121 ℃ for 30 min and cooled to 30 ℃ prior to the addition of enzymes. Enzymes were added and all flasks were incubated at 55 ℃ in a shaker incubator (Amerex Instruments Inc., Lafayette, CA) for 72 h at 200 rpm. Samples (2 ml) were taken prior to the addition of enzymes (time 0 h) and post enzyme hydrolysis (time 24 h, 48 h and 72h). Studies were conducted at least in duplicate.

3.2.5 Chemical analysis of hydrolysis samples

All samples (0 h, 24 h, 48 h, and 72 h) were analyzed for sugars (glucose, cellobiose, arabinose, and xylose). Samples were centrifuged (12000 X g), filtered (0.2 μm Syringe Filters, Nagle Company, NY) and diluted accordingly. Sugars were analyzed by high performance liquid chromatography (HPLC) (Agilent 1200 Series) with a BioRad Aminex HPX-87P (P), lead form, 300 mm X 7.8 mm (ID), 9 μm column and a differential refractive index detector (G1362A Agilent). Percent theoretical cellulose and hemicellulose yields were calculated using the equations provided by NREL's laboratory procedures (LAP#42630) and are as follow:

$$\%\text{Theoretical Cellulose Yield} = \frac{[\text{Glucose}] + 1{:}053[\text{Cellobiose}]}{1{:}111\, f\,[\text{Biomass}]} \times 100\% \quad (1)$$

$$\%\text{Theoretical Hemicellulose Yield} = \frac{0.9[\text{Xylose}] + 0.9[\text{Arabinose}]}{1.136\, f\,[\text{Biomass}]} \times 100\% \quad (2)$$

Where each item is defined below,

[Glucose]	residual glucose concentration (g/L)

(TABLE continued)

[Cellobiose]	residual cellobiose concentration (g/L)
1.053	multiplication factor that converts cellobiose to equivalent glucose
[Biomass]	dry biomass concentration at the beginning of the fermentation (g/L)
f	cellulose or hemicellulose fraction in dry biomass (g/g)
[Xylose]	residual xylose concentration (g/L)
[Arabinose]	residual arabinose concentration (g/L)
0.9	multiplication factor that converts xylose and arabinose to equivalent glucose
1.111	converts cellulose to equivalent glucose
1.136	converts hemicellulose to equivalent xylose

3.2.6 FTIR analysis

A Thermo Scientific Nicolet Nexus 670 FT-IR Spectrometer and Smart iTR with a diamond window (Thermo Fisher Scientific Inc., Waltham, MA) was used to perform FTIR spectroscopy. About 5 mg of sample was loaded each time and the background spectrum was automatically suppressed before each sample was run. Spectrum was set at 600–4000 cm^{-1} with a resolution of 4 cm^{-1} and 64 scans per sample.

3.2.7 XRD analysis

Crystallinity index (CrI) of all samples was determined by XRD using a Siemens D-5000 diffractometer (Bruker, Germany) and Cu-K radiation generated at 30 kV and 20 mA. Samples were scanned from 5 ° to 40 ° with 0.05 ° step size per 3 seconds. The software used to run the XRD was Diffract AT V3.1. The crystallinity index was determined as the portion of crystalline material in the sample as indicated in the equation below (Segal et al., 1959):

$$CrI = (I_{002} - I_{am})/I_{002}$$

Where, I_{002} is the scattered intensity at the main peak for cellulose type I; I_{am} is the scattered intensity due to the amorphous portion evaluated as the minimum intensity between the main and secondary peaks.

3.2.8 TGA analysis

TGA curves were obtained from a TGA/SDTA851^e thermobalance (Mettler Toledo**,** Columbus, OH) using 150 μL aluminum crucibles. About 20 mg of sample was loaded for each run. The experiments were carried out under a continuous nitrogen flow of 20 ml/min with a temperature ramp set at 5 ℃/min. Sample weight loss was recorded from 34 ℃ to 700 ℃. Experiments were performed in duplicate.

3.3 Results and Discussion

3.3.1 Effect of the Tween 80-dilute ammonia pretreatment on the biomass chemical composition

The chemical composition of all pretreated samples and controls are summarized in Table 3. 1. Alkaline pretreatment causes swelling of biomass leading to a decrease in the degree of polymerization and crystallinity, an increase in internal surface area, disruption of the lignin structure, and separation of structural linkages between lignin and carbohydrates (Fan et al., 1987). For the dilute ammonia controls, the most lignin (14%) was removed by pretreatment at 1: 0.5: 20 (biomass: ammonium hydroxide: water) ratio. A 23% lignin removal was observed for 3% Tween 80 as compared to 6.8% and 12% for 1.5% Tween 80 and 5% Tween 80 pretreatments at 1: 0: 20 ratio (controls), respectively. The most delignification (37%) was observed when 3% Tween 80 was used with biomass, ammonium hydroxide and water at 1: 0.5: 20 ratio. More than 20% glucan was lost with controls containing Tween 80 only pretreatments. The most important reactions responsible for the loss of polysaccharides and reduction of the chain length of cellulose in alkaline pretreatments are peeling of end groups (I and III) (Figure 3. 1). These

reactions result in the formation of alkali stable end groups like alcohols or carboxyl groups, and the alkaline hydrolysis of glycosidic bonds and acetyl groups (Fengel and Wegener, 1984). Xylan can be selectively removed in an alkaline environment with aqueous potassium hydroxide (Hendriks and Zeeman, 2009). About 30% of the xylan was lost in all dilute ammonium hydroxide only pretreatments. The mechanism is believed to be saponification of intermolecular ester bonds crosslinking with hemicellulose and lignin (Zheng et al., 2009).

Table 3. 1. Chemical composition of Tween 80-dilute ammonia pretreated sugarcane bagasse and controls (g/100g dry biomass).

Pretreatments (Biomass: Ammonium Hydroxide: Water ratio)	Ash	Extractive	Lignin	Arabinan	Xylan	Glucan	Mannan	Solids remaining
Controls								
Untreated	6.39±0.28	2.64±0	22.69±0.12	2.83±0.05	24.95±1.26	40.71±0.20	ND	100
1:0:20	4.03±0.32	3.61±0.11	21.77±0.10	0.88±0.31	20.24±0.00	40.50±1.02	ND	92.24±0.71
1:0.2*:20	9.75±0.28	4.01±0.02	23.13±1.38	1.87±0.25	15.68±0.78	34.91±0.15	ND	89.35±0.61
1:0.5**:20	2.16±0.10	1.88±0.07	19.58±1.02	2.32±0.30	18.67±1.20	40.25±0.14	ND	86.56±1.91
1.5% Tween 80, 1:0:20	2.95±0.82	3.70±0.64	21.15±0.35	1.18±0.55	12.80±1.10	32.76±0.67	ND	74.54±1.53
3% Tween 80, 1:0:20	5.12±0.26	4.30±0.41	17.47±0.51	0.62±0.22	11.91±0.31	27.37±0.07	ND	66.8±1.32
5% Tween 80, 1:0:20	12.69±0.80	5.30±0.37	19.92±0.79	0.63±0.29	11.14±1.74	25.17±1.64	ND	74.85±2.14
Experimental								
1.5% Tween 80, 1:0.5**:20	6.46±0.51	3.16±0.77	18.87±0.28	1.35±0.99	13.05±0.62	30.36±0.43	ND	73.25±1.62
3% Tween 80, 1:0.5**:20	3.62±0.49	2.26±0.14	14.37±1.79	1.91±0.25	15.06±1.20	35.32±1.80	ND	72.39±4.41
5% Tween 80, 1:0.5**:20	7.09±0.32	4.27±0.00	20.72±0.56	1.48±0.09	12.62±1.02	40.03±1.34	ND	86.20±1.12
1.5% Tween 80, 1:0.2*:20	7.77±0.91	3.92±0.29	20.82±0.24	1.48±0.62	13.40±0.58	30.10±0.85	ND	77.49±1.51
3% Tween 80, 1:0.2*:20	9.14±0.60	5.41±0.11	17.59±1.03	1.71±0.05	10.83±1.24	28.96±0.02	ND	73.65±1.14
5% Tween 80, 1:0.2*:20	6.09±0.46	3.91±0.07	22.29±1.47	1.58±0.23	13.92±1.03	35.10±0.16	ND	82.89±2.35

ND=None detected.
*= Ammonium hydroxide at a final concentration of 0.26% w/w at 4.7% solids loading.
**= Ammonium hydroxide at a final concentration of 0.65% w/w at 4.7% solids loading.

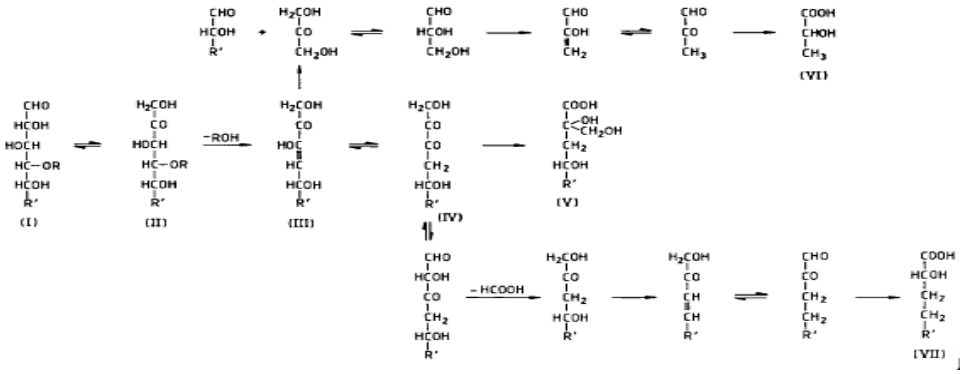


Figure 3. 1. Endwise Degradation of Polysaccharides (Fengel and Wegener, 1984).

3.3.2 Enzymatic digestibility of the Tween 80-dilute ammonia pretreated sugarcane bagasse

Cellulose digestibility and hemicellulose digestibility at an enzyme loading of 30 FPU Spezyme CP and 30 CBU Novozyme 188/g glucan of Tween 80-dilute ammonia pretreated sugarcane bagasse and controls are summarized in Figure 3. 2 and Figure 3. 3. Pretreatment controls at 1: 0.5: 20 ratio showed higher cellulose digestibility (37%) than those at 1: 0.2: 20 (24%). However, the highest cellulose digestibility (66%) was observed with 3% Tween 80-dilute ammonia pretreated sugarcane bagasse at 1: 0.5: 20 ratio. Cellulose digestibility increased by 78% and by 120% as compared to the controls dilute ammonia pretreated bagasse (1: 0.5: 20 ratio) and 3% Tween 80 pretreated sugarcane bagasse (1: 0: 20 ratio), respectively. A cellulose digestibility of less than 30% was observed for all pretreatments with Tween 80 only (control). Furthermore, the addition of 3% Tween 80 during pretreatment with or without ammonium hydroxide resulted in higher cellulose digestibility than those observed for 1.5% and 5% Tween 80 additions (Figure 3. 4). Kaar and Holtzapple (1998) obtained similar results with the addition of Tween 80 during hydrolysis of corn stover, where the largest increase in conversion was seen with surfactant additions between 0.05 and 0.1 g/g dry biomass. The lower amount of lignin remaining in bagasse after pretreatment is indicative of a more severe destruction of the lignocellulosic matrix, which improves enzyme accessibility to cellulose (Rivers and Emert, 1988). According to Qing et al. (2010), the use of Tween 80 during pretreatment can modify the

surface properties of the lignin remaining on the pretreated solids and improve cellulase effectiveness and accessibility to the surface of biomass. Also, it was reported that lignin in pretreated lignocellulosic biomass was responsible for a large portion of protein adsorption during enzymatic hydrolysis, and blocking such non-productive adsorption of cellulases to lignin could enhance cellulose conversion (Eriksson et al., 2002; Yang and Wyman, 2006).

The highest hemicellulose digestibility (59%) was observed with 5% Tween 80 at 1: 0.5: 20 ratio as compared to 43% hemicellulose digestibility for 3% Tween 80-dilute ammonia pretreated bagasse at 1: 0.5: 20 ratio. Higher hydrolysis could be expected if xylanases were added into the mix. All Tween 80-dilute ammonia pretreatments showed higher hemicellulose digestibility than the controls. The results are similar to those observed for cellulose digestibility, which are in agreement with lignin solubilization by ammonia and Tween 80 (Kumar et al., 2009a; Kurakake et al., 1994) .

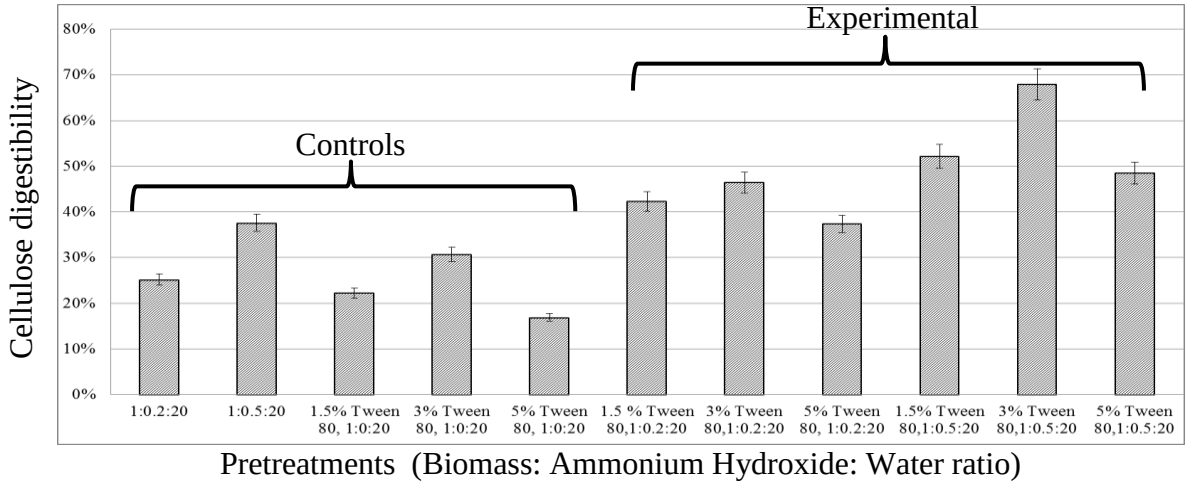


Figure 3. 2. Percent Cellulose Digestibility for Tween 80-Dilute Ammonia Pretreated Sugarcane Bagasse. Experimental runs were carried out at 1: 0.2: 20 (biomass: ammonium hydroxide: water) ratio and at 1: 0.5: 20 ratio with 1.5%, 3% or 5% Tween 80. Final ammonium hydroxide concentrations were 0.26% w/w and 0.65% w/w, respectively. Ammonium hydroxide controls (1: 0.2: 20; 1: 0.5: 20) were run without the addition of surfactant. Tween 80 controls (1: 0: 20) were run at 1.5%, 3% and 5% (w/w dry biomass) without the addition of ammonium hydroxide. All pretreatments were carried out at 4.7% solids loading. Enzymatic hydrolysis was carried out at enzyme loadings of 30 FPU/g glucan and at 30 CBU/g glucan.

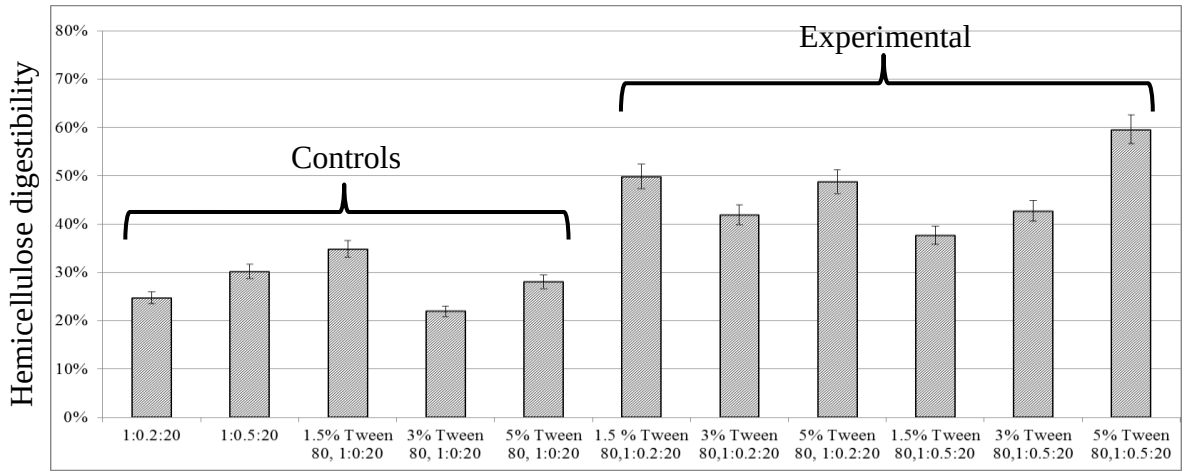


Figure 3. 3. Percent Hemicellulose Digestibility for Tween 80-Dilute Ammonia Pretreated Sugarcane Bagasse. Experimental runs were carried out at 1: 0.2: 20 (biomass: ammonium hydroxide: water) ratio and at 1: 0.5: 20 ratio with 1.5%, 3% or 5% Tween 80. Final ammonium hydroxide concentrations were 0.26% w/w and 0.65% w/w, respectively. Ammonium hydroxide controls (1: 0.2: 20; 1: 0.5: 20) were run without the addition of surfactant. Tween 80 controls (1: 0: 20) were run at 1.5%, 3% and 5% (w/w dry biomass) without the addition of ammonium hydroxide. All pretreatments were carried out at 4.7% solids loading. Enzymatic hydrolysis was carried out at enzyme loadings of 30 FPU/g glucan and at 30 CBU/g glucan.

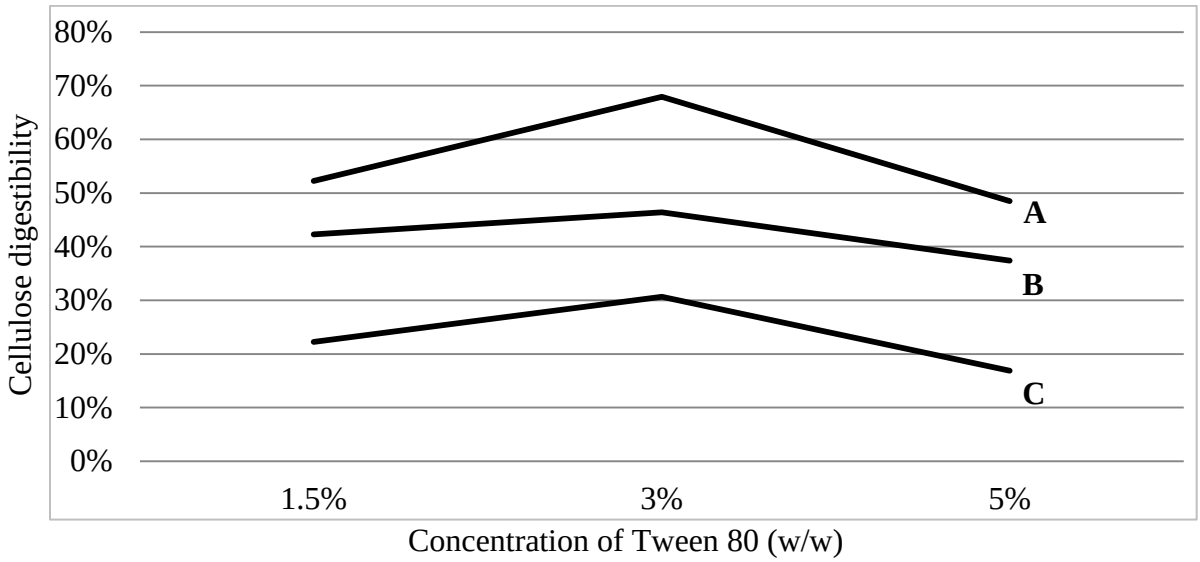


Figure 3. 4. Percent Cellulose Digestibility for 1.5%, 3% and 5% Tween 80-Dilute Ammonia Pretreated Sugarcane Bagasse. (A) 1.5%, 3% and 5% Tween 80-dilute ammonia pretreated sugarcane bagasse at 1: 0.5: 20 (biomass: ammonium hydroxide: water) ratio; (B) 1.5%, 3% and 5% Tween 80-dilute ammonia pretreated sugarcane bagasse at 1: 0.2: 20 ratio; and (C) 1.5%, 3% and 5% Tween 80 only pretreated sugarcane bagasse at 1: 0: 20 ratio (controls). Final ammonium hydroxide concentrations were 0.26% w/w and 0.65% w/w. All pretreatments were carried out at 4.7% solids loading. Enzymatic hydrolysis was carried out at enzyme loadings of 30 FPU/g glucan and at 30 CBU/g glucan.

3.3.3 FTIR analysis

Crystallinity data is summarized in Table 3. 2. LOI was calculated by the ratio of peak areas at 1423 cm^{-1} and 897 cm^{-1} and it indicates the fraction of cellulose type I (which is highly crystal) in the cellulosic material (Oh et al., 2005). The total crystallinity index (TCI) can be calculated by the ratio of peak areas at 1372 cm^{-1} and 2900 cm^{-1}, and it stands for the total cellulose in biomass, including cellulose type I, type II and type III (Nelson and O'Connor, 1964a). Therefore, lower LOI and TCI values indicate that more cellulose type I has been transformed to cellulose type II, type III (a more disorderly structure of cellulose) and amorphous cellulose during pretreatment (Deguchi et al., 2006). Lower LOI and TCI values were observed with Tween 80-dilute ammonia pretreated sugarcane bagasse as compared to controls. The lowest LOI (0.5969 ± 0.0004) and TCI (0.2380 ± 0.0003) values were obtained with 3% Tween 80-dilute ammonia pretreated bagasse at 1: 0.5: 20 (biomass: ammonium hydroxide: water) ratio as compared to untreated (1.2690 ± 0.0145, 0.4392 ± 0.0072), water pretreated (1.1411 ± 0.0019, 0.4105 ± 0.0027), ammonia pretreated (1.0824 ± 0.0014, 0.3892 ± 0.0005), and 3% Tween 80 only pretreated (1.0267 ± 0.0004, 0.2893 ± 0.0021) sugarcane bagasse, respectively. These observations are consistent with both lignin removal (Table 3. 1) and enzymatic hydrolysis data (Figure 3. 2, Figure 3. 3) reported earlier. Laureano-Perez et al. (2005) reported that AFEX pretreated biomass resulted in an increase in the OH and amorphous cellulose peaks as compared to untreated samples due to biomass depolymerization and disruption of the crystalline structure. Furthermore, AFEX pretreated biomass showed a reduction or disappearance of the carbonyl peak, indicating a decrease in the number of bonds associated with hemicellulose (hydrolysis of hemicellulose) and a disruption in the lignin polymer.

Table 3. 2. Crystallinity data of Tween 80-dilute ammonia pretreated sugarcane bagasse and controls.

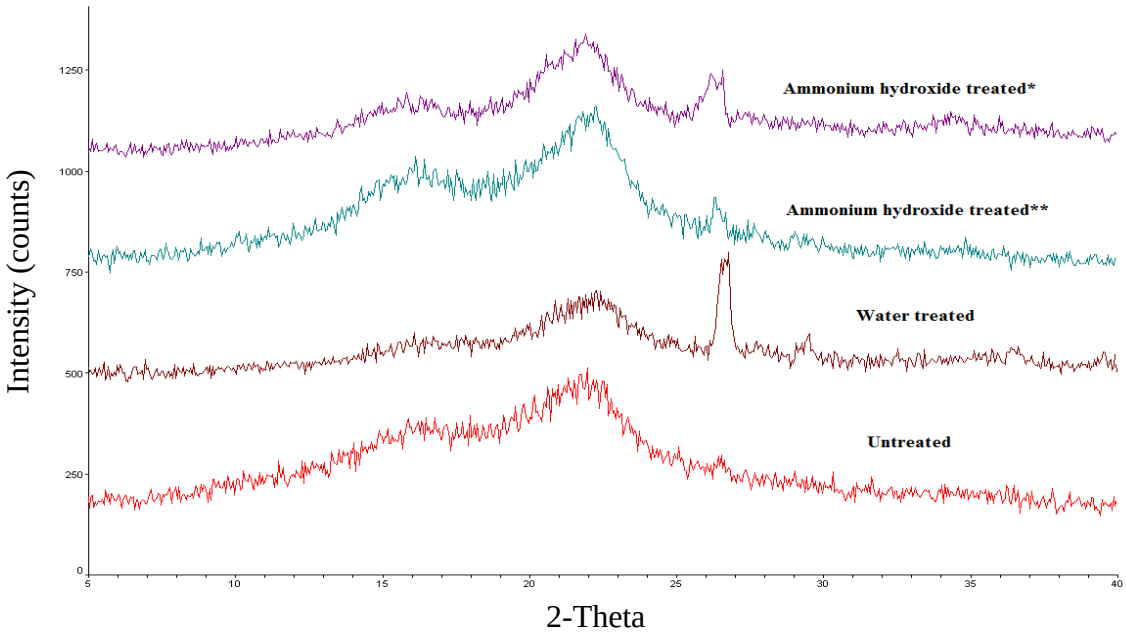
Pretreatments (Biomass: Ammonium Hydroxide: Water ratio)	LOI	TCI	CrI
Controls			
Untreated	1.2690 ±0.0145	0.4392 ±0.0072	0.4000 ±0.0092
1:0:20	1.1411 ±0.0019	0.4105 ±0.0027	0.4576 ±0.0001
1:0.2*:20	1.0873 ±0.0023	0.4291 ±0.0053	0.5000 ±0.0226
1:0.5**:20	1.0824 ±0.0014	0.3892 ±0.0005	0.5765 ±0.0049
1.5% Tween 80 1:0:20	1.1042 ±0.0710	0.3733 ±0.0003	0.4444 ±0.0113
3% Tween 80 1:0:20	1.0267 ±0.0004	0.2893 ±0.0021	0.7586 ±0.0029
5% Tween 80 1:0:20	0.9828 ±0.0066	0.2580 ±0.0024	0.6429 ±0.0136
Experimental			
1.5% Tween 80 1:0.5**:20	0.8595 ±0.0151	0.2928 ±0.0013	0.8710 ±0.0110
3% Tween 80 1:0.5**:20	0.5969 ±0.0004	0.2380 ±0.0003	0.9211 ±0.0063
5% Tween 80 1:0.5**:20	1.0330 ±0.0014	0.2369 ±0.0006	0.7442 ±0.0040
1.5% Tween 80 1:0.2*:20	0.9914 ±0.0006	0.3204 ±0.0001	0.7089 ±0.0089
3% Tween 80 1:0.2*:20	0.9529 ±0.0034	0.2520 ±0.0003	0.8718 ±0.0023
5% Tween 80 1:0.2*:20	0.9978 ±0.0020	0.2651 ±0.0009	0.5417 ±0.0061

*= Ammonium hydroxide at a final concentration of 0.26% w/w at 4.7% solids loading.
**= Ammonium hydroxide at a final concentration of 0.65% w/w at 4.7% solids loading.

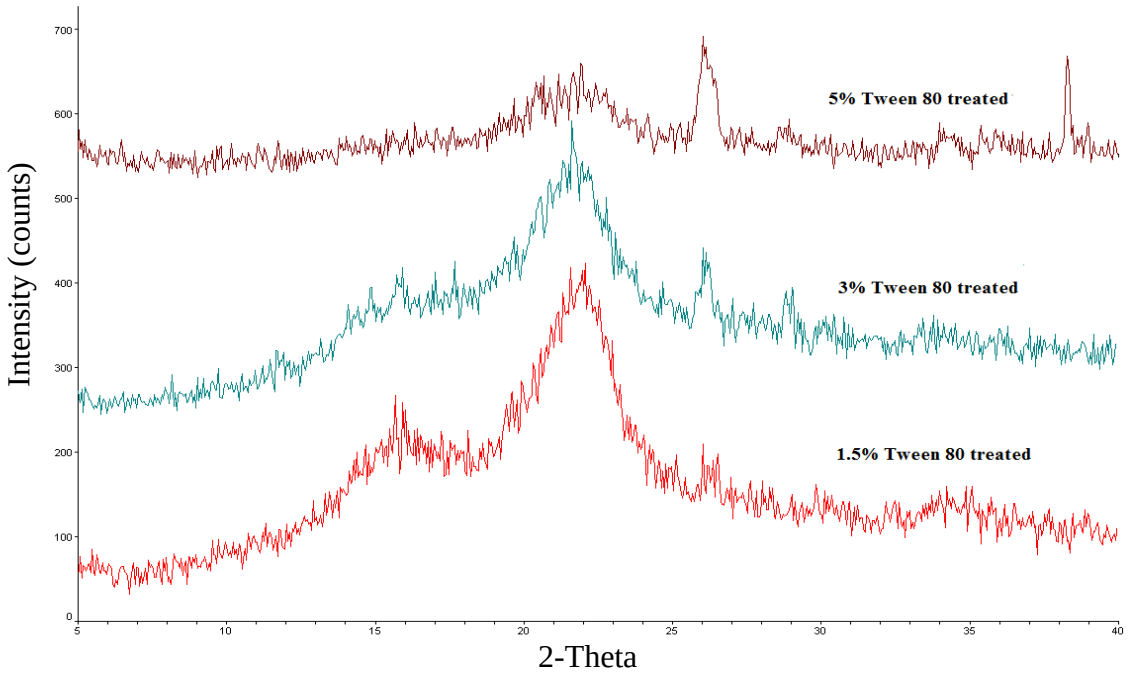
3.3.4 XRD analysis

XRD is often used to determine the crystallinity index of cellulose combined with FTIR (Park et al., 2010). Two typical diffraction peaks, (101) and (002) lattice, were observed at 2θ = 22 ° and 16 °, which correspond to planes of crystalline cellulose type I. The lowest intensity between (101) and (002) lattice was observed at 18 °, which corresponds to the scattered intensity due to the amorphous portion. A new narrow peak around 26 ° was reported due to hydroxyapatite crystals (Wan et al., 2006), with 002 diffraction (Kaushik et al., 2010). XRD spectra is shown in Figure 3. 5. CrI measures the relative amount of crystalline cellulose in the

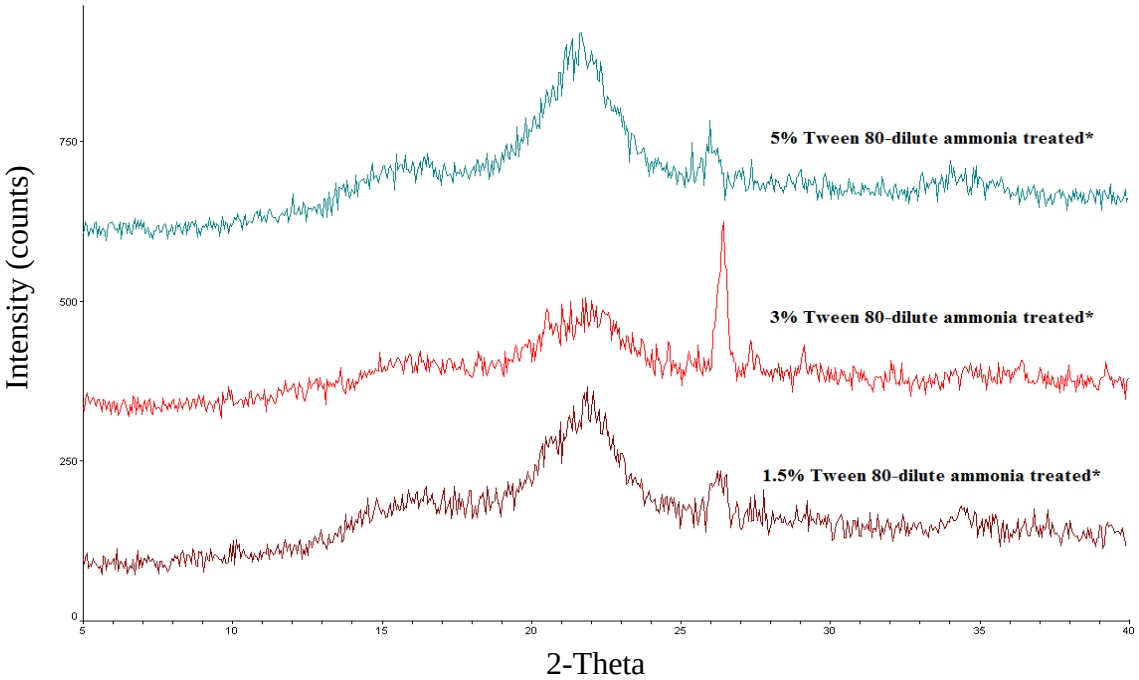
total solid. Tween 80-dilute ammonia pretreatment removed lignin and hemicellulose resulting in an increased CrI. An increase trend in CrI was observed for all Tween 80-dilute ammonia pretreated bagasse as compared to controls (Table 3. 2). The highest CrI (0.9211 ±0.0063) was observed with 3% Tween 80-dilute ammonia pretreated bagasse at 1: 0.5: 20 (biomass: ammonium hydroxide: water) ratio as this pretreatment removed the most lignin. Changes in CrI may be due to the removal of amorphous substances (lignin and hemicellulose), not due to changes in the basic crystalline structure of the cellulose (Corrales et al., 2012; Kim et al., 2003). Significant removal of lignin and some of the hemicellulose was confirmed by chemical composition data (Table 3. 1) as reported earlier. Similar observations were reported in Miscanthus pretreated with a solution of sodium chlorite and acetic acid at 70 ℃ for 1 h. The lignin content in pretreated biomass decreased from 24.4% to 8.6%, while the CrI values of delignified Miscanthus increased as compared to the untreated biomass (Yoshida et al., 2008).



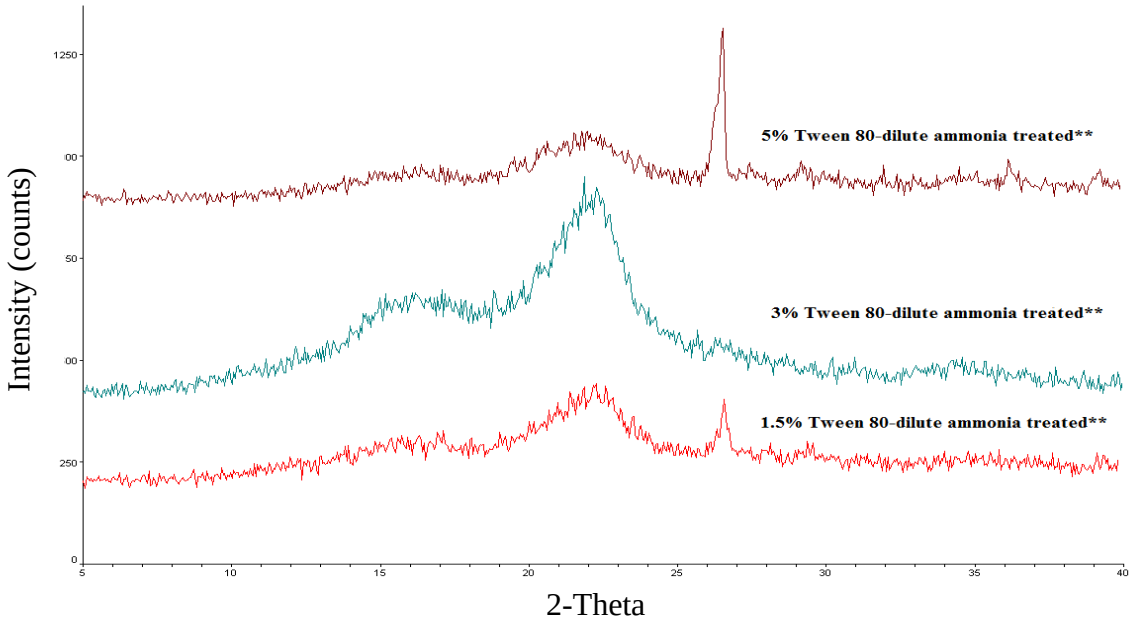
a. XRD Spectra for Controls. (A) Untreated, water treated and ammonia treated controls. Controls for ammonia treated bagasse were run at 1: 0.2: 20 (biomass: ammonium hydroxide: water) ratio or at 1: 0.5: 20 ratio with a final ammonium hydroxide concentration of 0.26% w/w* or 0.65% w/w**, respectively.



b. XRD Spectra for Tween 80 Controls Pretreated Sugarcane Bagasse. 1.5%, 3% and 5% Tween 80 pretreatments were run at 1: 0: 20 (biomass: ammonium hydroxide: water) ratio without the addition of ammonium hydroxide.



c. XRD Spectra for Tween 80-dilute ammonia experimental runs. 1.5%, 3% and 5% Tween 80-dilute ammonia pretreatments were carried out at 1: 0.2: 20 ratio with a final ammonium hydroxide concentration of 0.26% w/w*.



d. XRD Spectra for Tween 80-dilute ammonia experimental runs. 1.5%, 3% and 5% Tween 80-dilute ammonia pretreatments were carried out at 1: 0.5: 20 ratio with a final ammonium hydroxide concentration of 0.65% w/w**.

Figure 3. 5. XRD Spectra for Tween 80-Dilute Ammonia Pretreated Sugarcane Bagasse and Controls. (A) Untreated, water treated and ammonia treated controls. Controls for ammonia treated bagasse were run at 1: 0.2: 20 (biomass: ammonium hydroxide: water) ratio or at 1: 0.5: 20 ratio with a final ammonium hydroxide concentration of 0.26% w/w* or 0.65% w/w**, respectively. (B) Tween 80 controls. 1.5%, 3% and 5% Tween 80 pretreatments were run at 1: 0: 20 (biomass: ammonium hydroxide: water) ratio without the addition of ammonium hydroxide. (C) Tween 80-dilute ammonia experimental runs. 1.5%, 3% and 5% Tween 80-dilute ammonia pretreatments were carried out at 1: 0.2: 20 ratio with a final ammonium hydroxide concentration of 0.26% w/w*. (D) Tween 80-dilute ammonia experimental runs. 1.5%, 3% and 5% Tween 80-dilute ammonia pretreatments were carried out at 1: 0.5: 20 ratio with a final ammonium hydroxide concentration of 0.65% w/w**.

3.3.5 TGA analysis

TGA can be used to determine thermal stability by monitoring weight loss as temperature increases gradually. As for biomass, thermal stability is mostly related to the lignin content because lignin begins to decompose at 160 ℃, which is lower than the melting points of cellulose and hemicellulose (Wanga et al., 2008; Yang et al., 2007). Less lignin content results in higher onset temperature. Compared to controls, Tween 80-dilute ammonia pretreated sugarcane bagasse resulted in an increase of the onset temperature, which is consistent with the removal of

lignin and hemicellulose reported earlier. The highest onset temperature (311±0.20 ℃) was observed with 3% (w/w) Tween 80-dilute ammonia pretreated sugarcane bagasse at 1: 0.5: 20 ratio. This observation is in agreement with the removal of large amounts of lignin as previously reported.

Table 3. 3. Onset temperatures for Tween 80-dilute ammonia pretreated sugarcane bagasse and controls.

Pretreatment (Biomass: Ammonium Hydroxide: Water ratio)	Onset Temperature (℃)
Controls	
Untreated	263±1.45
1:0:20	272±1.04
1:0.2*:20	282±0.75
1:0.5**:20	298±0.83
1.5% Tween 80,1:0:20	291±0.90
3% Tween 80,1:0:20	301±0.48
5% Tween 80,1:0:20	298±0.05
Experimental	
1.5% Tween 80,1:0.2*:20	293±0.15
3% Tween 80,1:0.2*:20	306±0.18
5% Tween 80,1:0.2*:20	280±0.08
1.5% Tween 80,1:0.5**:20	302±0.17
3% Tween 80,1:0.5**:20	311±0.20
5% Tween 80,1:0.5**:20	303±0.23

*= Ammonium hydroxide at a final concentration of 0.26% w/w at 4.7% solids loading.
**= Ammonium hydroxide at a final concentration of 0.65% w/w at 4.7% solids loading.

3.4 Conclusions

Combined 3% Tween 80-dilute ammonia pretreatment at 1: 0.5: 20 (biomass: ammonium hydroxide: water) ratio resulted in higher delignification (37%), higher percent cellulose digestibility (66%), higher CrI (0.9211±0.0063), and lower LOI (0.5969±0.0004) and TCI (0.2380±0.0003) values than controls and pretreatments with varying ammonium hydroxide, and

Tween 80 concentrations. Tween 80 has both hydrophobic and hydrophilic properties that could decrease the surface tension between two liquid phases and improve delignification. Also, applying Tween 80 during pretreatment of biomass could modify both the surface properties of lignin remaining on the pretreated solids and improve cellulase accessibility to the biomass surface. Tween 80 has a low cost and, as a stable surfactant, has great potential in being used as a catalyst in the presence of ammonium hydroxide during pretreatment of lignocellulosic biomass.

CHAPTER 4. SCALE UP OF TWEEN 80-DILUTE AMMONIA PRETREATMENT OF SUGARCANE BAGASSE

4.1 Introduction

The potential of bioethanol as a future transportation fuel has been extensively reviewed (Lynd et al., 1991; Lynd et al., 1996; Von Blottnitz and Curran, 2007). One of the most promising methods for biomass conversion is enzymatic hydrolysis following biomass pretreatment (Kumar et al., 2009a; Zheng et al., 2009). Pretreatment helps improve enzymatic hydrolysis by breaking down the biomass structure, removing lignin, and freeing cellulose and hemicellulose (Chundawat et al., 2011). Several pretreatment methods have been reported (Ballesteros et al., 2008; Salvi et al., 2010; Yoon et al., 2011). However, the combination of dilute ammonia pretreatment with non-ionic surfactants has not been extensively explored.

Bioethanol from sugarcane bagasse in Brazil (Pandey et al., 2000) and from corn stover in the U.S. (Sheehan et al., 2004) are the two dominating sources for biofuel in the world. After laboratory scale optimization, evaluation of pretreatment at high solids loading at a pilot scale should be evaluated. The term "solids loading" here is defined by the amount of dry material that enters the process divided by the total mass of material and water added to the material. Studies utilizing low solids loadings (<5% solids, w/w) are numerous and helpful. However, in order to improve fuel yields, pretreatments at high solids loadings are preferred (Modenbach and Nokes, 2012). The main advantage of high solids loading is high efficiency. Larger amounts of biomass available in the reaction results in the production of higher sugar concentrations, which leads to an increase in biofuel concentrations (Hodge et al., 2008; Modenbach and Nokes, 2012; Roche et al., 2009). The conversion process is more environmentally friendly, as less wastewater could be produced under certain processing conditions (Stickel et al., 2009; Um and Hanley, 2008). It

should be noted that the water absorption capacity is a function of the lignocellulosic material, and significant water can be brought into the process, just through the selection of a particular type of material (Modenbach and Nokes, 2012). However, some conversion processes have been developed to reduce process water and wastewater by recovering and recycling liquid streams (Mohagheghi and Schell, 2010; Stenberg et al., 1998). Energy usage of high solids loading pretreatments for heating, cooling, mixing, and ethanol distillation can be reduced as more biomass becomes available and less water is consumed, which renders the overall conversion process more energy efficient (Modenbach and Nokes, 2012).

The goal of this study was to scale up 3% Tween 80-dilute ammonia pretreatment from a 4 L reactor to a 20 L reactor and to evaluate the effect of increased solids loading on the chemical composition, enzymatic hydrolysis and cellulose crystallinity of the pretreated biomass.

4.2 Methods and Materials

4.2.1 Substrate

Sugarcane bagasse was collected from Louisiana sugar mills during the grinding season (September-December) and stored in 50 gal drums at -20 ℃.

4.2.2 Tween 80-dilute ammonia pretreatment

Pretreatment was run by mixing sugarcane bagasse, ammonium hydroxide (28% v/v solution) and water at a ratio of 1: 0.5: 8 or 10.5% (w/w, dry biomass) solids loading and at 1: 0.5: 20 or 4.7% (w/w, dry biomass) solids loading, with 3% (w/w) Tween 80 (based on the weight of dry biomass). The final concentrations of ammonium hydroxide were 1.47% w/w and 0.65% w/w, respectively. The mixture was heated to 160 ℃ for 1 h in a 4 L mixing bioreactor or a 20 L tumbling bioreactor. Controls were run with water or ammonium hydroxide at 4.7% solids loading. The final concentration of ammonium hydroxide (control) was 0.65% w/w.

4.2.3 Chemical composition of pretreated sugarcane bagasse

Tween 80-dilute ammonia pretreated sugarcane bagasse and controls (untreated, water treated, and dilute ammonia treated sugarcane bagasse) were analyzed for glucan, xylan, lignin, arabinan, mannan, ethanol extractives, and ash content using NREL's Laboratory Analytical Procedures (LAPs #42618, 42619, 42620, 42621,42622). NREL reference material (8491 sugarcane bagasse) was analyzed as an internal sample to ensure the accuracy of the procedures.

4.2.4 Enzymatic hydrolysis

Tween 80-dilute ammonia pretreated, dilute ammonia pretreated, and water pretreated sugarcane bagasse were hydrolyzed using a combination of commercially available cellulose degrading enzymes, Spezyme CP (Genencor, Danisco US Inc., Rochester, NY) and Novozyme 188 (Sigma–Aldrich, Inc., St. Luis, MO). Spezyme CP (cellulases) and Novozyme 188 (cellobiases) were used at 30 FPU Spezyme CP and 30 CBU Novozyme 188/g glucan. Approximately, 10 g dry weight of Tween 80-dilute ammonia pretreated, dilute ammonia pretreated, or water pretreated were each loaded into 250 ml Erlenmeyer flasks following NREL's hydrolysis and fermentation protocols (LAP TP-510-42630). The pH of each mixture was adjusted to 4.8 with concentrated hydrochloric acid. All flasks were sterilized at 121 ℃ for 30 min. Enzymes were added and all flasks were incubated at 55 ℃ in a shaker incubator (Amerex Instruments Inc., Lafayette, CA) for 24 h at 200 rpm. Samples (2 ml) were taken prior to the addition of enzymes (time 0 h) and post enzyme hydrolysis (time 24 h).

4.2.5 Chemical analysis of hydrolysis samples

All samples (0 h, 24 h, 48 h, and 72 h) were analyzed for sugars (glucose, cellobiose, arabinose, and xylose). Samples were centrifuged (12,000 X g), filtered (0.2 μm Syringe Filters, Nagle Company, NY) and diluted accordingly. Sugars were analyzed by high performance liquid chromatography (HPLC) (Agilent 1200 Series) with a BioRad Aminex HPX-87P (P), lead form,

300 mm X 7.8 mm (ID), 9 μm column and a differential refractive index detector (G1362A Agilent). Percent theoretical cellulose and hemicellulose were calculated using the equations as described by NREL's laboratory procedures (LAP#42630) and are as follow:

$$\%\text{Theoretical Cellulose Yield} = \frac{[\text{Glucose}] + 1{:}053[\text{Cellobiose}]}{1{:}111\, f\, [\text{Biomass}]} \times 100\% \quad (1)$$

$$\%\text{Theoretical Hemicellulose Yield} = \frac{0.9[\text{Xylose}] + 0.9[\text{Arabinose}]}{1.136\, f\, [\text{Biomass}]} \times 100\% \quad (2)$$

Where each item is defined below,

[Glucose]	residual glucose concentration (g/L)
[Cellobiose]	residual cellobiose concentration (g/L)
1.053	multiplication factor that converts cellobiose to equivalent glucose
[Biomass]	dry biomass concentration at the beginning of the fermentation (g/L)
f	cellulose or hemicellulose fraction in dry biomass (g/g)
[Xylose]	residual xylose concentration (g/L)
[Arabinose]	residual arabinose concentration (g/L)
0.9	multiplication factor that converts xylose and arabinose to equivalent glucose
1.111	converts cellulose to equivalent glucose
1.136	converts hemicellulose to equivalent xylose

4.2.6 FTIR analysis

A Thermo Scientific Nicolet Nexus 670 FTIR Spectrometer and Smart iTR with a diamond window (Thermo Fisher Scientific Inc., Waltham, MA) was used. About 5 mg of sample was loaded each time and the background spectrum was automatically suppressed before each sample was run. Spectrum was set at 600–4000 cm^{-1} with a resolution of 4 cm^{-1} and at 64 scans per sample.

4.2.7 XRD analysis

Crystallinity index (CrI) of all samples was determined by XRD using a Siemens D-5000 diffractometer (Bruker, Germany) and Cu-K radiation generated at 30 kV and 20 mA. Samples were scanned from 5 ° to 40 ° with 0.05 ° step size per 3 seconds. Diffract AT V3.1. was the software used to run the XRD. The crystallinity index was determined as the portion of crystalline material in the sample as indicated in the equation below (Segal et al., 1959):

$$CrI = (I_{002} - I_{am})/I_{002}$$

Where, I_{002} is the scattered intensity at the main peak for cellulose type I; I_{am} is the scattered intensity due to the amorphous portion evaluated as the minimum intensity between the main and secondary peaks.

4.3 Results and Discussion

4.3.1 Effect of scale-up Tween 80-dilute ammonia pretreatment on the biomass chemical composition

The chemical composition analysis of pretreated sugarcane bagasse is summarized in Table 4. 1. Significant lignin removal was observed in all Tween 80-dilute ammonia pretreatments. The highest lignin removal (55%) was observed with 3% Tween 80-dilute ammonia treated bagasse at 1: 0.5: 8 (biomass: ammonium hydroxide: water) ratio in the 20 L bioreactor. The second highest delignification (43%) was observed with 3% Tween 80-dilute

ammonia treated bagasse at 1: 0.5: 8 ratio in the 4 L bioreactor. Lower water input (1: 0.5: 8) resulted in higher solids loading (10.5% w/w) and concentration of ammonium hydroxide (1.47% w/w) in the system. High concentration of ammonia can effectively increase the surface area by swelling the biomass particles and increasing enzyme accessibility to carbohydrates, while reducing the degree of polymerization and crystallinity of the cellulose fraction (Kumar et al., 2009a). The hemicellulose fraction can be partially hydrolyzed under strong alkaline conditions (Kim et al., 2009). The bonds between the lignin and carbohydrates are broken resulting in some of the lignin being solubilized (Galbe and Zacchi, 2007; Hendriks and Zeeman, 2009; Jørgensen et al., 2007). Furthermore, higher lignin removal was observed with the 20 L tumbling bioreactor than with the 4 L spindle bioreactor. Researches have indicated that reactor designs can significantly affect the pretreatment results due to heat and mass transfer phenomenon (Jorgensen et al., 2007; Modenbach and Nokes, 2012). Chen et al. (2009) reported a 12.2% delignification with corn stover treated with 1.5% sulfuric acid at 106-108 ℃ for 6 h at 9.1% solids loading. Compared to acid pretreatments, alkali pretreatments (i.e., lime, aqueous ammonia and sodium hydroxide) remove more lignin from the raw material because of the solubilization of lignin in alkaline solution (Chen et al., 2009; Kim et al., 2009). Pretreatment with sodium hydroxide has shown lignin removal of 73.9% at 11% solids loading (Chen et al., 2009).

Approximately, 77% glucan and 65% xylan were retained after pretreatment of biomass with 1.47% ammonium hydroxide at 10.5% solids loading in the 20 L bioreactor. The highest amount of glucan (88%) and xylan (69%) retained in the biomass were observed with biomass pretreated with 0.65% ammonium hydroxide at 4.7% solids loading. Average xylan loss was about 35%. The loss of xylan is expected as alkali pretreatments can partially hydrolyze

hemicellulose (Garlock et al., 2011). As pretreatment severity increases (due to an increase in temperature and time) and more xylan is removed from the biomass, the solubilized xylo-oligomers are simultaneously hydrolyzed from higher to lower degrees of polymerization, eventually resulting in monomeric xylose and degradation products (Kabel et al., 2007). At high temperature and alkali conditions, biomass surface area is increased (Kumar et al., 2009a) as well as the formation of alkali stable end groups like alcohol or carboxyl groups (Fengel and Wegener, 1984), which leads to more glucan loss (Gupta, 2008). Therefore, a higher glucan loss was observed in the more ammonia-concentrated pretreatments.

Table 4. 1. Chemical composition of 3% Tween 80-dilute ammonia pretreated sugarcane bagasse at 4.7% and 10.5% solids loading (g/100g dry biomass).

Biomass component	Untreated	Water	Dilute ammonia	Tween 80-dilute ammonia 4.7%* 20 L	Tween 80-dilute ammonia 4.7%* 4 L	Tween 80-dilute ammonia 10.5%** 20 L	Tween 80-dilute ammonia 10.5%** 4 L
Ash	6.39±0.28	4.03±0.32	2.16±0.10	1.73±0.14	3.62±0.49	5.74±0.57	4.08±0.43
Extractives	2.64±0	3.61±0.11	1.88±0.07	7.69±0.81	2.26±0.14	5.79±0.95	11.12±1.52
Lignin	22.69±0.12	21.77±0.10	19.58±1.02	13.14±0.47	14.37±1.79	10.11±0.55	12.93±0.84
Glucan	40.71±0.20	40.50±0.02	40.25±0.14	36.11±0.29	35.32±1.80	31.49±1.37	33.76±1.77
Xylan	24.95±1.26	20.24±0.00	18.67±1.20	16.91±1.52	15.06±1.20	16.27±0.17	16.24±0.33
Arabinan	2.83±0.05	0.88±0.31	2.32±0.30	1.01±0.21	1.91±0.25	0.89±0.02	1.02±0.52
Mannan	ND	ND	ND	ND	ND	ND	ND
Solids remaining	100	92.24±0.71	86.56±1.91	76.59±0.97	72.39±4.41	70.28±3.91	79.15±2.42

ND: None detected.
*= Ammonium hydroxide at a final concentration of 0.65 % w/w.
**= Ammonium hydroxide at a final concentration of 1.47% w/w.

4.3.2 Enzymatic digestibility of scale-up Tween 80-dilute ammonia treated sugarcane bagasse

The enzymatic digestibility of pretreated sugarcane bagasse is summarized in Figure 4. 1 and Figure 4. 2. Pretreatments with 3% Tween 80-dilute ammonia at 10.5% solids loading resulted in cellulose digestibilities of 72% and 70% in the 20 L and 4 L bioreactors, respectively. This represents a 167% and 159% increase as compared to water only pretreatment; and a 89% and 84% increase as compared to dilute ammonia only pretreatment, respectively. Percent

enzymatic hydrolysis yields at 10.5% solids loading were higher than those observed at 4.7% solids loading, especially in the 20 L bioreactor, which were 72% and 64%, respectively. High solids loading pretreatments have less energy input for water heating, which can increase thermal efficiency (Modenbach and Nokes, 2012), and result in more biomass being available for chemical hydrolysis. This leads to higher delignification through solubilization of lignin in the ammonia solution than pretreatment at lower solids loading (Kim et al., 2009). Less lignin remaining on the pretreated biomass results in less non-productive binding of enzymes, which favors cellulase activity (Eriksson et al., 2002), thus resulting in higher enzymatic hydrolysis yields as shown in our study. Average glucose concentrations for 3% Tween 80-dilute ammonia pretreated bagasse at 10.5% solids loading and 4.7% solids loading in a 20 L bioreactor were 35.88 g/l and 33.85 g/l, respectively, while the average glucose concentration for 3% Tween 80-dilute ammonia pretreated bagasse in the 4 L bioreactor were 33.06 g/l and 33.22 g/l, respectively (Table 4. 2). Lee et al. (2011) reported 25 g/l of fermentable sugars from acid treated corn cobs (under vacuum with a solution of 30 g/l of oxalic acid in a 500 L reactor for 20 min at room temperature at 1: 6, solid: liquid ratio) and 2.74 g/l glucose from the hydrolysate after washing the pretreated biomass. Maas et al. (2008) reported a glucose concentration of 15.4 g/l for lime pretreated wheat straw after 8 h hydrolysis by a cocktail of commercially available enzymes. Tween 80-dilute ammonia pretreated bagasse in 4 L or 20 L at 4.7% and 10.5% solids loading resulted in higher hemicellulose digestibility (42.7%, 52.6%; 60.3%, 56.9%), respectively, than water (23.8%) and dilute ammonia (30.2%) pretreatment controls. Overall, the hemicellulose yields observed were lower than the cellulose yields. The low hemicellulose yields observed were attributed to the fact that the enzyme cocktail used contained mostly cellulase-degrading enzymes.

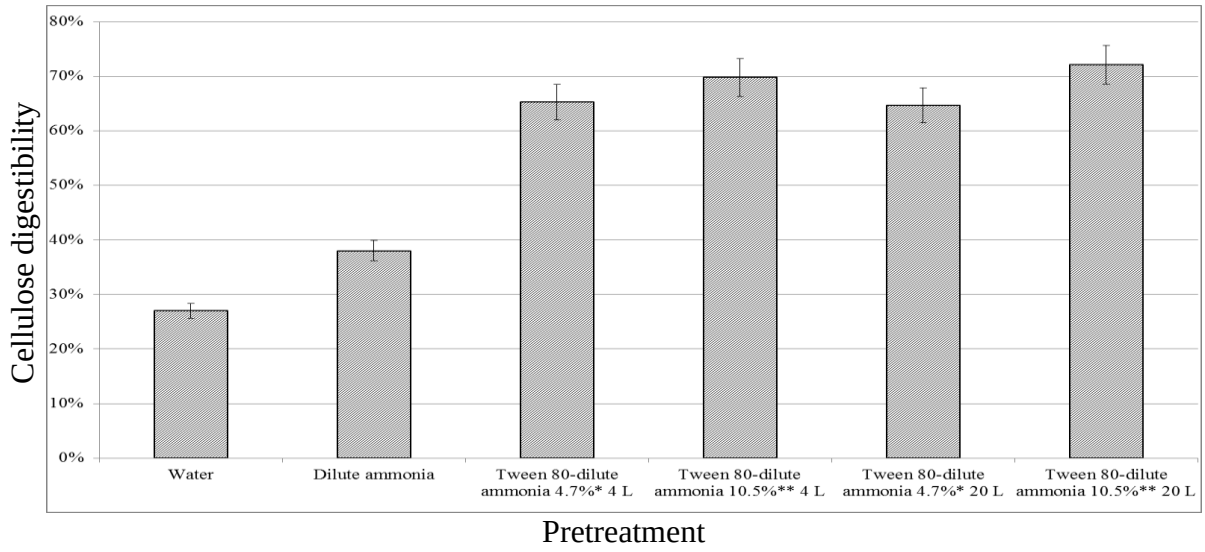


Figure 4. 1. Percent Cellulose Digestibility for 3% Tween 80-Dilute Ammonia Pretreated Sugarcane Bagasse. Runs were at 10.5%** w/w and 4.7%* w/w solids loadings in a 4 or 20 L bioreactor, with 3% (w/w) Tween 80. Final concentrations of ammonium hydroxide were 1.47% w/w and 0.65% w/w at 10.5%** w/w and 4.7%* w/w solids loadings, respectively. Water treated control was at 4.7%* solids loading. Dilute ammonia treated control was at 4.7%* solids loading with a final concentration of ammonium hydroxide of 0.65% w/w. Controls were without Tween 80. Enzymatic hydrolysis was at enzyme loadings of 30 FPU/g glucan and at 30 CBU/g glucan.

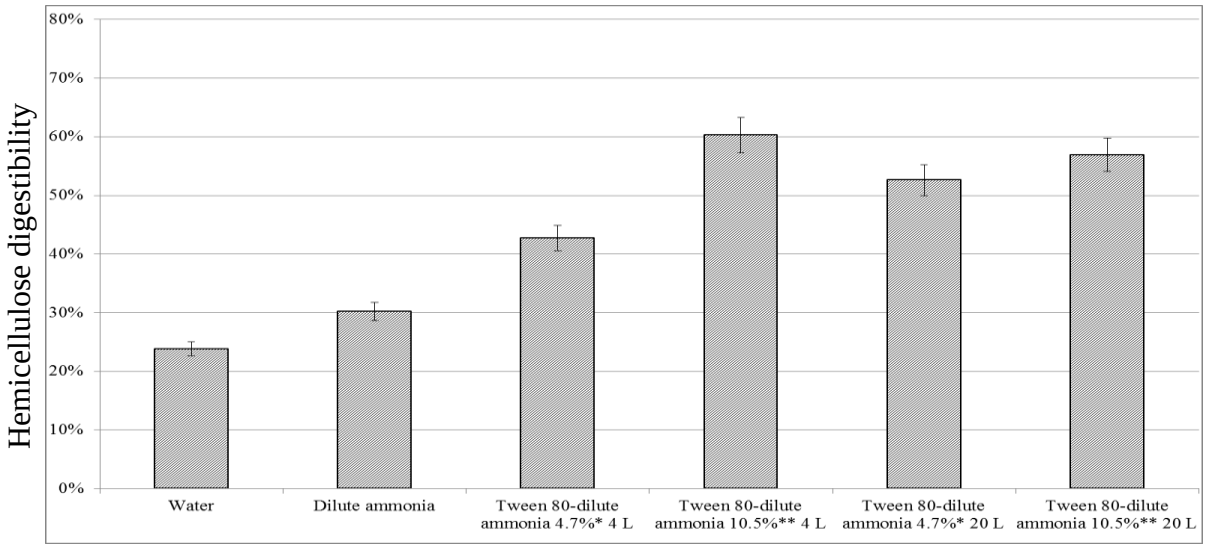


Figure 4. 2. Percent Hemicellulose Digestibility for 3% Tween 80-Dilute Ammonia Pretreated Sugarcane Bagasse. Runs were at 10.5%** w/w and 4.7%* w/w solids loadings in a 4 or 20 L bioreactor, with 3% (w/w) Tween 80. Final concentrations of ammonium hydroxide were 1.47% w/w and 0.65% w/w at 10.5%** w/w and 4.7%* w/w solids loadings, respectively. Water treated control was at 4.7%* solids loading. Dilute ammonia treated control was at 4.7%* solids loading with a final concentration of ammonium hydroxide of 0.65% w/w. Controls were without Tween 80. Enzymatic hydrolysis was at enzyme loadings of 30 FPU/g glucan and at 30 CBU/g glucan.

Table 4. 2. Glucose concentration for 3% Tween 80-dilute ammonia pretreated sugarcane bagasse at 4.7% and 10.5% solids loadings in a 4 L or 20 L bioreactor.

Pretreatment (Biomass: Ammonium Hydroxide: Water ratio)	Glucose concentration (g/l)
Controls	
1:0:20	14.53 ±0.12
1:0.5:20*	18.15 ±0.07
Experimental	
3% Tween 80 1:0.5:8**, 4 L	33.06 ±0.18
3% Tween 80 1:0.5:20*, 4 L	33.22 ±0.09
3% Tween 80 1:0.5:8**, 20 L	35.88 ±0.17
3% Tween 80 1:0.5:20*, 20 L	33.85 ±0.03

*= 4.7% w/w (dry biomass) solids loading with 0.65% w/w ammonium hydroxide concentration. **=10.5% w/w (dry biomass) solids loading with 1.47% w/w ammonium hydroxide concentration.

4.3.3 FTIR analysis

FTIR results for 3% Tween 80-dilute ammonia pretreated sugarcane bagasse and controls are summarized in the Table 4. 2. Pretreatment at 10.5% solids loading in a 20 L bioreactor had the lowest LOI (0.5000 ±0.0001) and TCI (0.1637 ±0.0000) values as compared to untreated (1.2157 ±0.0754, 0.4415 ±0.0032), water pretreated (1.1321 ±0.0126, 0.4241 ±0.0192) and ammonia pretreated (1.0403 ±0.0595, 0.3851 ±0.0058) bagasse. This trend is similar to the ones reported in Chapters 2 and 3. Lower LOI indicates less cellulose type I, which is highly crystal, in the treated biomass. Cellulose type I can be changed into cellulose type II, type III or amorphous cellulose at high temperature and pressure (Deguchi et al., 2006). Pretreatment with liquid ammonia, or certain amines such as ethylene diamine allow for changes in the structure of cellulose to take place and this transformation can lower the crystallinity of cellulose (Pe ́rez and Mazeau, 2004). High TCI values are indicative of a more ordered structure of cellulose in the biomass. Yoshida et al. (2008) reported an increase in sugar yields as the crystallinity of the

substrate decreased. Kristensen et al. (2008) reported lower LOI values in steam treated wheat straw (0.52) as compared to untreated wheat straw (0.56) at solids loading ranging from 25% to 32%.

Table 4. 3. Crystallinity data for 3% Tween 80-dilute ammonia pretreated sugarcane bagasse at 4.7% and 10.5% solids loadings in a 4 L or 20 L bioreactor.

Pretreatment (Biomass: Ammonium Hydroxide: Water ratio)	LOI	TCI	CrI
Controls			
Untreated	1.2157 ±0.0754	0.4415 ±0.0032	0.4000 ±0.0092
1:0:20	1.1321 ±0.0126	0.4241 ±0.0192	0.4576 ±0.0001
1:0.5:20*	1.0403 ±0.0595	0.3851 ±0.0058	0.5765 ±0.0049
Experimental			
3% Tween 80 1:0.5:8**, 4 L	0.5110 ±0.0013	0.2573 ±0.0000	0.9606 ±0.0027
3% Tween 80 1:0.5:20*, 4 L	0.5969 ±0.0007	0.2380 ±0.0003	0.9211 ±0.0001
3% Tween 80 1:0.5:8**, 20 L	0.5000 ±0.0001	0.1637 ±0.0000	0.9150 ±0.0004
3% Tween 80 1:0.5:20*, 20 L	0.5340 ±0.0038	0.2432 ±0.0001	0.8712 ±0.0058

*= 4.7% w/w (dry biomass) solids loading with 0.65% w/w ammonium hydroxide concentration. **=10.5% w/w (dry biomass) solids loading with 1.47% w/w ammonium hydroxide concentration.

4.3.4 XRD analysis

The CrI of various sugarcane bagasse samples was determined based on the XRD patterns for quantitative comparison and are depicted in Table 4. 2. All XRD spectra are depicted in Figure 4. 3. Two typical diffraction peaks, (101) and (002) lattice, were observed at 2θ = 22° and 16 °, which correspond to planes of crystalline cellulose type I. The lowest intensity between (101) and (002) lattice was observed at 18 °, which corresponds to the scattered intensity due to the amorphous portion. A new narrow peak around 26 ° was reported due to hydroxyapatite crystals (Wan et al., 2006), with 002 diffraction (Kaushik et al., 2010). CrI measures the relative amount of crystalline cellulose in the total solid. The total amount of crystalline cellulose

increases with the removal of lignin and hemicellulose, which are amorphous substances. An increase in CrI was observed with all 3% Tween 80-dilute ammonia pretreated samples as compared to untreated sugarcane bagasse (0.4000 ±0.0092). The highest CrI (0.9606 ±0.0027) was observed with 3% Tween 80-dilute ammonia pretreatment at 10.5% solids loading in a 4 L bioreactor as compared to water only or dilute ammonia pretreated bagasse (0.4576 ±0.0001, 0.5765 ±0.0049). A 0.9150 ±0.0004 CrI was observed in bagasse pretreated with 3% Tween 80 and dilute ammonia in a 20 L bioreactor. It was observed that at 10.5% w/w solids loading the CrI values were higher than the values observed at 4.7% w/w solids loading. Similar findings have been reported in poplar wood pretreated with peracetic acid (Zhu et al., 2008), in corn stover pretreated with lime (Kumar et al., 2009b), and in sweet sorghum bagasse and sugarcane bagasse pretreated with hot water (Wang et al., 2012).

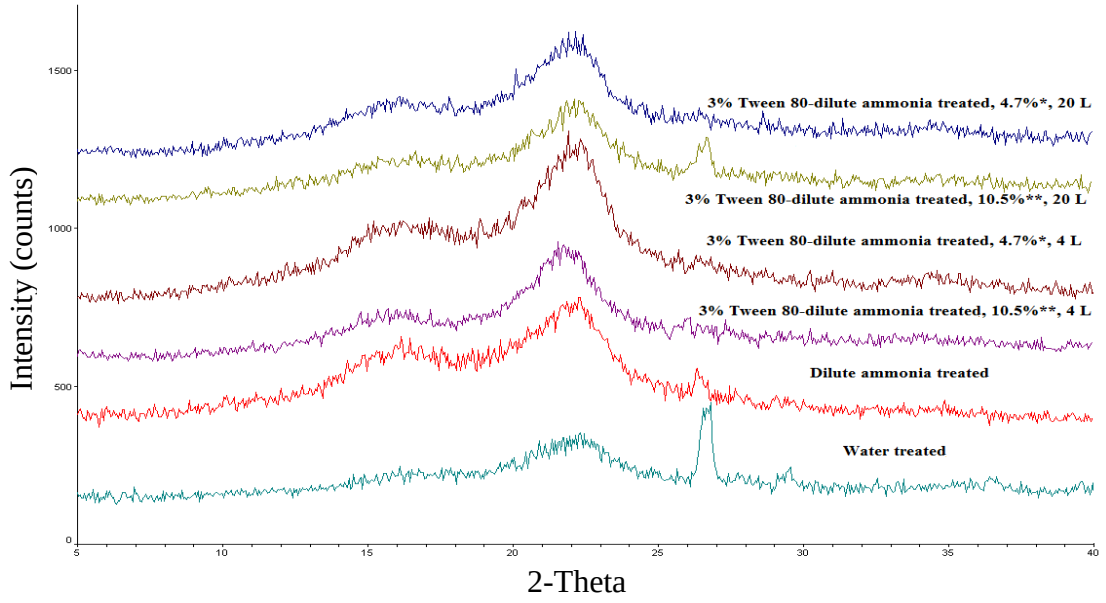


Figure 4. 3. XRD Spectra for 3% Tween 80-Dilute Ammonia Pretreated Sugarcane Bagasse in a 4 L or 20 L Bioreactor. Runs were at 4.7%* w/w and 10.5%** w/w solids loadings in a 4 or 20 L bioreactor, with 3% (w/w) Tween 80 (based on the weight of dry biomass). Final concentrations of ammonium hydroxide were 0.65% w/w and 1.74% w/w at 4.7%* w/w and 10.5%** w/w solids loadings, respectively. Water treated control was at 4.7% solids loading. Dilute ammonia treated control was at 4.7% solids loading with a final concentration of ammonium hydroxide of 0.65% w/w. Controls were run without the addition of Tween 80.

4.4 Conclusions

Scale up from a 4 L to a 20 L reactor of 3% Tween 80-dilute ammonia pretreatment at 10.5% solids loading removed the most lignin (55%) and retained 77% of the glucan as compared to the same pretreatment conditions at a lower solids loading. The average loss of xylan was about 35%. A 72% and 70% cellulose digestibility was achieved with sugarcane bagasse pretreated at 10.5% solids loading as compared to 65% at 4.7% solids loading in a 20 L or 4 L bioreactor, respectively.

Average glucose concentrations for 3% Tween 80-dilute ammonia pretreated bagasse at 10.5% and 4.7% solids loadings in a 20 L bioreactor were 35.88 g/l and 33.85 g/l, respectively, while average glucose concentrations for 3% Tween 80-dilute ammonia pretreated bagasse in a 4 L bioreactor were 33.06 g/l and 33.22 g/l, respectively. A decrease in both LOI and TCI values of pretreated samples were observed due to changes in the structure of cellulose. Changes in crystallinity were also observed because of lignin and hemicellulose removal as confirmed by biomass chemical composition analysis, FTIR and XRD analysis.

SUMMARY AND FUTURE WORK

Pretreatment of Tween 80 with dilute ammonia was the best combination among all non-ionic surfactants (Tween 20, PEG 4000, PEG 6000) tested because of its potential for delignification (37%), cellulose digestibility (66%) and low market value ($0.25/kg). Addition of non-ionic surfactants increases delignification during ammonia pretreatment by making the surface of lignin more hydrophilic thus enhancing lignin's solubility. It appeared that the addition of non-ionic surfactants during pretreatment improved enzymatic hydrolysis by enhancing enzyme activity and disrupting the carbohydrate-lignin complex. Non-ionic surfactants are also good pore formers on the yeast membrane thus enhancing the release of ethanol. Furthermore, non-ionic surfactants altered the crystalline structure of cellulose in sugarcane bagasse as confirmed by FTIR and XRD analysis.

Pretreatment with 3% Tween 80 at 0.65% ammonium hydroxide resulted in better delignification, enzymatic digestibility and biomass structural changes than pretreatment with 0.26% ammonium hydroxide at 4.7% solids loading. Pilot scale (20 L) runs of 3% Tween 80-dilute ammonia pretreatment with 1.47% ammonium hydroxide at 10.5% solids loading resulted in higher delignification (55%) and glucose yields (72%) than those observed for laboratory (4 L) runs. Changes in the structure of cellulose were observed by FTIR and XRD analysis.

Future research work will evaluate the effect of lower temperatures and shorter retention times during Tween 80-dilute ammonia pretreatment of sugarcane bagasse and other energy crops and their effect on biomass chemical composition, biomass structure, enzyme hydrolysis, and ethanol yields. Research will also focus on the use of xylanases along with cellulases during enzymatic hydrolysis to boost sugar yields. Mass and energy balances will be calculated as well as processing costs.

REFERENCES

Aguilara, R., Ramírez, J.A., Garrotec, G., Vázquez, M. 2002. Kinetic study of the acid hydrolysis of sugarcane bagasse. *Journal of Food Engineering*, **55**(4), 309-318.

Aita, G., Kim, M. 2010. Pretreatment Technologies for the Conversion of Lignocellulosic Materials to Bioethanol. in: *Sustainability of the Sugar and Sugar−Ethanol Industries*, (Ed.) G. Eggleston, Vol. 1058, American Chemical Society. ACS Symposium Series, pp. 117-145.

Aita, G.A., Salvi, D.A., Walker, M.S. 2011. Enzyme hydrolysis and ethanol fermentation of dilute ammonia pretreated energy cane. *Bioresource Technology*, **102**(6), 4444-8.

Aita, G.M., Salvi, D. 2009. Lignocellulose: a source for fuels and chemicals. *Louisiana Agriculture Magazine*, **52**(4), 12-13.

Balaban, M., Ucar, G. 1999. The effect of the duration of alkali treatment on the solubility of polyoses. *Turk Journal of Agriculture and Forestry*, **23**, 667-671.

Ballesteros, I., Ballesteros, M., Manzanares, P., Negro, M.J., Oliva, J.M., Sáez, F. 2008. Dilute sulfuric acid pretreatment of cardoon for ethanol production. *Biochemical Engineering Journal*, **42**(1), 84-91.

Börjesson, J., Engqvist, M., Sipos, B., Tjerneld, F. 2007a. Effect of poly(ethylene glycol) on enzymatic hydrolysis and adsorption of cellulase enzymes to pretreated lignocellulose. *Enzyme and Microbial Technology*, **41**(1-2), 186-195.

Börjesson, J., Peterson, R., Tjerneld, F. 2007b. Enhanced enzymatic conversion of softwood lignocellulose by poly(ethylene glycol) addition. *Enzyme and Microbial Technology*, **40**(4), 754-762.

Carvalheiro, F., Duarte, L.C., Gírio, F.M. 2008. Hemicellulose biorefineries a review on biomass pretreatments. *Journal of Scientific and Industrial Research*, **67**, 849-864.

Chandra, R.P., Bura, R., Mabee, W.E., Berlin, A., Pan, X., Saddler, J.N. 2007. Substrate pretreatment: the key to effective enzymatic hydrolysis of lignocellulosics? *Advances in Biochemical Engineering/Biotechnololgy*, **108**, 67-93.

Chang, V.S., Holtzapple, M.T. 2000. Fundamental factors affecting biomass enzymatic reactivity. *Appl Biochem Biotechnol*, **84-86**, 5-37.

Chen, J., Spear, S.K., Huddleston, J.G., Rogers, R.D. 2005. Polyethylene glycol and solutions of polyethylene glycol as green reaction media. *Green Chemistry*, **7**(2), 64.

Chen, M., Zhao, J., Xia, L. 2009. Comparison of four different chemical pretreatments of corn stover for enhancing enzymatic digestibility. *Biomass and Bioenergy*, **33**(10), 1381-1385.

Chundawat, S.P., Beckham, G.T., Himmel, M.E., Dale, B.E. 2011. Deconstruction of lignocellulosic biomass to fuels and chemicals. *Annual Review Chemical and Biomolecular Engineering*, **2**, 121-45.

Corrales, R.C., Mendes, F.M., Perrone, C.C., Sant Anna, C., de Souza, W., Abud, Y., Bon, E.P., Ferreira-Leitao, V. 2012. Structural evaluation of sugar cane bagasse steam pretreated in the presence of CO2 and SO2. *Biotechnology of Biofuels*, **5**(1), 36.

Deguchi, S., Tsujii, K., Horikoshi, K. 2006. Cooking cellulose in hot and compressed water. *Chem Commun (Camb)*(31), 3293-5.

DOE. 2005. A billion-ton feedstocks supply for a bioenergy and bioproducts industry. http://feedstockreview.ornl.gov/pdf/billion_ton_vision.pdf.

Eriksson, T., Börjesson, J., Tjerneld, F. 2002. Mechanism of surfactant effect in enzymatic hydrolysis of lignocellulose. *Enzyme and Microbial Technology*, **31**(3), 353-364.

Escalante, M., Rodriguez-Malaver, A.J., Araujo, E., Gonzalez, A.M., Rojas, O.J., Penaloza, N., Bullon, J., Lara, M.A., Dmitrieva, N., Perez-Perez, E. 2005. Effect of surfactants on Fenton's reagent-mediated degradation of Kraft black liquor. *Journal of Environmental Biology*, **26**(4), 709-18.

Fan, L., Gharpuray, M.M., Lee, Y.H. 1987. *Cellulose hydrolysis*. Springer-Verlag.

Fengel, D., Wegener, G. 1984. *Wood : chemistry, ultrastructure, reactions*. W. de Gruyter, Berlin ; New York.

Galbe, M., Zacchi, G. 2007. Pretreatment of lignocellulosic materials for efficient bioethanol production. *Advances in Biochemical Engineering/Biotechnology*, **108**, 41-65.

Galbe, M., Zacchi, G. 2002. A review of the production of ethanol from softwood. *Applied Microbiology and Biotechnology*, **59**(6), 618-28.

Garlock, R.J., Balan, V., Dale, B.E., Pallapolu, V.R., Lee, Y.Y., Kim, Y., Mosier, N.S., Ladisch, M.R., Holtzapple, M.T., Falls, M., Sierra-Ramirez, R., Shi, J., Ebrik, M.A., Redmond, T., Yang, B., Wyman, C.E., Donohoe, B.S., Vinzant, T.B., Elander, R.T., Hames, B., Thomas, S., Warner, R.E. 2011. Comparative material balances around pretreatment technologies for the conversion of switchgrass to soluble sugars. *Bioresource Technology*, **102**(24), 11063-11071.

Gierer, J. 1986. Chemistry of delignification. *Wood Science and Technology*, **20**(1), 1-33.

Graca, M., Bongaerts, J.H., Stokes, J.R., Granick, S. 2007. Friction and adsorption of aqueous polyoxyethylene (Tween) surfactants at hydrophobic surfaces. *Journal of Colloid and Interface Science*, **315**(2), 662-670.

Gupta, R. 2008. Alkaline pretreatment of biomass for ethanol production and understanding the factors influencing the cellulose hydrolysis. in: *Chemical Engineering*, Vol. Doctor of Philosophy, Auburn University. http://etd.auburn.edu/etd/handle/10415/1034, pp. 260.

Hamelinck, C.N., Hooijdonk, G.v., Faaij, A.P.C. 2005. Ethanol from lignocellulosic biomass: techno-economic performance in short-, middle- and long-term. *Biomass and Bioenergy*, **28**(4), 384-410.

Helle, S.S., Duff, S.J., Cooper, D.G. 1993. Effect of surfactants on cellulose hydrolysis. *Biotechnology and Bioengineering*, **42**(5), 611-617.

Hendriks, A.T., Zeeman, G. 2009. Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresource Technology*, **100**(1), 10-8.

Hodge, D.B., Karim, M.N., Schell, D.J., McMillan, J.D. 2008. Soluble and insoluble solids contributions to high-solids enzymatic hydrolysis of lignocellulose. *Bioresource Technology*, **99**(18), 8940-8948.

Hurtubise, F.G., Krassig, H. 1960. Classification of fine structural characteristics in cellulose by infrared spectroscopy - use of potassium bromide pellet technique. *Analytical Chemistry*, **32**(2), 177-181.

Inderwildi, O.R., King, D.A. 2009. Quo vadis biofuels? *Energy and Environmental Science*, **2**(4), 343.

Jørgensen, H., Kristensen, J.B., Felby, C. 2007. Enzymatic conversion of lignocellulose into fermentable sugars: challenges and opportunities. *Biofuels, Bioproducts and Biorefining*, **1**(2), 119-134.

Jorgensen, H., Vibe-Pedersen, J., Larsen, J., Felby, C. 2007. Liquefaction of lignocellulose at high-solids concentrations. *Biotechnology and Bioengineering*, **96**(5), 862-870.

Kaar, W.E., Holtzapple, M.T. 1998. Benefits from tween during enzymic hydrolysis of corn stover. *Biotechnology and Bioengineering*, **59**(4), 419-427.

Kabel, M.A., Bos, G., Zeevalking, J., Voragen, A.G., Schols, H.A. 2007. Effect of pretreatment severity on xylan solubility and enzymatic breakdown of the remaining cellulose from wheat straw. *Bioresource Technology*, **98**(10), 2034-2042.

Kaushik, A., Singh, M., Verma, G. 2010. Green nanocomposites based on thermoplastic starch and steam exploded cellulose nanofibrils from wheat straw. *Carbohydrate Polymers*, **82**(2), 337-345.

Kim, H.J., Kim, S.B., Kim, C.J. 2007. The Effects of nonionic surfactants on the pretreatment and enzymatic hydrolysis of recycled newspaper. *Biotechnology and Bioprocess Engineering*, **12**(2), 147-151.

Kim, M.H., Lee, S.B., Ryu, D.D.Y. 1982. Surface deactivation of cellulase and its prevention. *Enzyme and Microbial Technology*, **4**(2), 99-103.

Kim, T.H., Kim, J.S., Sunwoo, C., Lee, Y.Y. 2003. Pretreatment of corn stover by aqueous ammonia. *Bioresource Technology*, **90**(1), 39-47.

Kim, T.H., Nghiem, N.P., Hicks, K.B. 2009. Pretreatment and fractionation of corn stover by soaking in ethanol and aqueous ammonia. *Applied Biochemistry and Biotechnology*, **153**(1-3), 171-179.

Klemm, D., Heublein, B., Fink, H.P., Bohn, A. 2005. Cellulose: fascinating biopolymer and sustainable raw material. *Angewandte Chemie International Edition*, **44**(22), 3358-3393.

Kristensena, J.B., Börjessonb, J., Bruuna, M.H., Tjerneldb, F., Jørgensena, H. 2007. Use of surface active additives in enzymatic hydrolysis of wheat straw lignocellulose. *Enzyme and Microbial Technology*, **40**(4), 888-895.

Kumar, P., Barrett, D.M., Delwiche, M.J., Stroeve, P. 2009a. Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. *Industrial and Engineering Chemistry Research*, **48**(8), 3713-3729.

Kumar, R., Mago, G., Balan, V., Wyman, C.E. 2009b. Physical and chemical characterizations of corn stover and poplar solids resulting from leading pretreatment technologies. *Bioresource Technology*, **100**(17), 3948-3962.

Kumar, R., Wyman, C.E. 2009. Effects of cellulase and xylanase enzymes on the deconstruction of solids from pretreatment of poplar by leading technologies. *Biotechnology Progress*, **25**(2), 302-314.

Kuo, C.-H., Lee, C.-K. 2009. Enhancement of enzymatic saccharification of cellulose by cellulose dissolution pretreatments. *Carbohydrate Polymers*, **77**(1), 41-46.

Kurakake, M., Ooshima, H., Kato, J., Harano, Y. 1994. Pretreatment of bagasse by nonionic surfactant for the enzymatic hydrolysis. *Bioresource Technology*, **49**(3), 247-251.

Ladisch, M.R., Lin, K.W., Voloch, M., Tsao, G.T. 1983. Process considerations in the enzymatic hydrolysis of biomass. *Enzyme and Microbial Technology*, **5**(2), 82-102.

Laureano-Perez, L., Teymouri, F., Alizadeh, H., Dale, B.E. 2005. Understanding factors that limit enzymatic hydrolysis of biomass: characterization of pretreated corn stover. *Applied Biochemistry and Biotechnology*, **121-124**, 1081-1099.

Lawther, J.M., Sun, R., Banks, W.B. 1996. Effects of extraction conditions and alkali type on yield and composition of wheat straw hemicellulose. *Journal of Applied Polymer Science*, **60**(11), 1827-1837.

Lee, J.W., Houtman, C.J., Kim, H.Y., Choi, I.G., Jeffries, T.W. 2011. Scale-up study of oxalic acid pretreatment of agricultural lignocellulosic biomass for the production of bioethanol. *Bioresource Technology*, **102**(16), 7451-7456.

Luiz Carlos Basso, T.O.B.a.S.N.R. 2011. Ethanol Production in Brazil: The Industrial Process and Its Impact on Yeast Fermentation, Biofuel Production-Recent Developments and

Prospects. in: *Biofuel Production-Recent Developments and Prospects*, (Ed.) D.M.A.D.S. Bernardes, InTech. http://www.intechopen.com/books/indexing/biofuel-production-recent-developments-and-prospects/ethanol-production-in-brazil-the-industrial-process-and-its-impact-on-yeast-fermentation, pp. 596.

Lynd, L.R., Cushman, J.H., Roberta J, N., Wyman, C.E. 1991. Fuel ethanol from cellulosic biomass. *Science*, **251**, 1318-1323.

Lynd, L.R., Elander, R.T., Wyman, C.E. 1996. Likely features and costs of mature biomass ethanol technlogy. *Applied Biochemistry and Biotechnology*, **57/58**, 741-761.

Maas, R.H., Bakker, R.R., Boersma, A.R., Bisschops, I., Pels, J.R., de Jong, E., Weusthuis, R.A., Reith, H. 2008. Pilot-scale conversion of lime-treated wheat straw into bioethanol: quality assessment of bioethanol and valorization of side streams by anaerobic digestion and combustion. *Biotechnology for Biofuels*, **1**(1), 14.

Maiorella, B., Blanch, H.W., Wilke, C.R. 1983. By-product inhibition effects on ethanolic fermentation by *Saccharomyces cerevisiae*. *Biotechnology and Bioengineering*, **25**(1), 103-121.

Mishra, M., Mishra, B. 2010. Design and evaluation of microporous membrane coated matrix tablets for a highly water soluble drug. *Chemical and pharmaceutical bulletin (Tokyo)*, **58**(7), 995-1000.

Modenbach, A.A., Nokes, S.E. 2012. The use of high-solids loadings in biomass pretreatment--a review. *Biotechnology and Bioengineering*, **109**(6), 1430-1442.

Mohagheghi, A., Schell, D.J. 2010. Impact of recycling stillage on conversion of dilute sulfuric acid pretreated corn stover to ethanol. *Biotechnology and Bioengineering*, **105**(5), 992-996.

Mohsenzadeh, A., Jeihanipour, A., Karimi, K., Taherzadeh, M.J. 2012. Alkali pretreatment of softwood spruce and hardwood birch by NaOH/thiourea, NaOH/urea, NaOH/urea/thiourea, and NaOH/PEG to improve ethanol and biogas production. *Journal of Chemical Technology and Biotechnology*, **87**(8), 1209-1214.

Monrroy, M., Ortega, I., Ramirez, M., Baeza, J., Freer, J. 2011. Structural change in wood by brown rot fungi and effect on enzymatic hydrolysis. *Enzyme and Microbial Technology*, **49**(5), 472-477.

Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y.Y., Holtzapple, M., Ladisch, M. 2005. Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource Technology*, **96**(6), 673-686.

Nada, A.M.A., Mongy, S.A.E.-., El-Sayed, E.S.A. 2009. Effect of different treatments on cellulose toward carboxylation and its application for metal ion absorption. *BioResources*, **4**(1), 80-93.

Naik, S.N., Goud, V.V., Rout, P.K., Dalai, A.K. 2010. Production of first and second generation biofuels: A comprehensive review. *Renewable and Sustainable Energy Reviews*, **14**(2), 578-597.

Nelson, M.L., O'Connor, R.T. 1964a. Relation of certain infrared bands to cellulose crystallinity and crystal lattice type. Part II. A new infrared ratio for estimation of crystallinity in celluloses I and II. *Journal of Applied Polymer Science*, **8**(3), 1325-1341.

Nelson, M.L., O'Connor, R.T. 1964b. Relation of certain infrared bands to cellulose crystallinity and crystal latticed type. Part I. Spectra of lattice types I, II, III and of amorphous cellulose. *Journal of Applied Polymer Science*, **8**(3), 1311–1324.

Oh, S.Y., Yoo, D.I., Shin, Y., Kim, H.C., Kim, H.Y., Chung, Y.S., Park, W.H., Youk, J.H. 2005. Crystalline structure analysis of cellulose treated with sodium hydroxide and carbon dioxide by means of X-ray diffraction and FTIR spectroscopy. *Carbohydrate Research*, **340**(15), 2376-2391.

Öhgren, K., Bura, R., Lesnicki, G., Saddler, J., Zacchi, G. 2007. A comparison between simultaneous saccharification and fermentation and separate hydrolysis and fermentation using steam-pretreated corn stover. *Process Biochemistry*, **42**(5), 834-839.

Ouyang, J., Dong, Z., Song, X., Lee, X., Chen, M., Yong, Q. 2010. Improved enzymatic hydrolysis of microcrystalline cellulose (Avicel PH101) by polyethylene glycol addition. *Bioresource Technology*, **101**(17), 6685-6691.

Palmqvist, E., Hahn-Hagerdal, B. 1999. Fermentation of lignocellulosic hydrolysates. II: inhibitors and mechanisms of inhibition. *Bioresource Technology*, **74**(1), 25-33.

Pandey, A., Soccol, C.R., Nigam, P., Soccol, V.T. 2000. Biotechnological potential of agro-industrial residues. I: sugarcane bagasse. *Bioresource Technology*, **74**(1), 69-80.

Paria, S., Khilar, K.C. 2004. A review on experimental studies of surfactant adsorption at the hydrophilic solid-water interface. *Advances in Colloid and Interface Science*, **110**(3), 75-95.

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. *Biotechnology for Biofuels*, **3**, 10.

Pe ŕez, S., Mazeau, K. 2004. Conformations, Structures, and Morphologies of Celluloses. in: *Polysaccharides: Structural Diversity and Functional Versatility, Second Edition*, (Ed.) S. Dumitriu, CRC Press.

Qi, B., Chen, X., Wan, Y. 2010. Pretreatment of wheat straw by nonionic surfactant-assisted dilute acid for enhancing enzymatic hydrolysis and ethanol production. *Bioresource Technology*, **101**(13), 4875-4883.

Qing, Q., Yang, B., Wyman, C.E. 2010. Impact of surfactants on pretreatment of corn stover. *Bioresource Technology*, **101**(15), 5941-5951.

Rabelo, S.C., Maciel Filho, R., Costa, A.C. 2009. Lime pretreatment of sugarcane bagasse for bioethanol production. *Applied Biochemistry and Biotechnology*, **153**(1-3), 139-150.

Reddy, N., Yang, Y. 2005. Biofibers from agricultural byproducts for industrial applications. *Trends in Biotechnology*, **23**(1), 22-7.

Rivers, D.B., Emert, G.H. 1988. Factors affecting the enzymatic hydrolysis of municipal-solid-waste components. *Biotechnology and Bioengineering*, **31**(3), 278-281.

Roche, C.M., Dibble, C.J., Knutsen, J.S., Stickel, J.J., Liberatore, M.W. 2009. Particle concentration and yield stress of biomass slurries during enzymatic hydrolysis at high-solids loadings. *Biotechnology and Bioengineering*, **104**(2), 290-300.

Ruzene, D.S., Silva, D.P., Vicente, A.A., Teixeira, J.A., de Amorim, M.T., Goncalves, A.R. 2009. Cellulosic films obtained from the treatment of sugarcane bagasse fibers with N-methylmorpholine-N-oxide (NMMO). *Applied Biochemistry and Biotechnology*, **154**(1-3), 38-47.

Saha, B.C., Cotta, M.A. 2006. Ethanol production from alkaline peroxide pretreated enzymatically saccharified wheat straw. *Biotechnology Progress*, **22**(2), 449-453.

Salvi, D.A., Aita, G.M., Robert, D., Bazan, V. 2010. Dilute ammonia pretreatment of sorghum and its effectiveness on enzyme hydrolysis and ethanol fermentation. *Applied Biochemistry and Biotechnology*, **161**(1-8), 67-74.

Sanchez, C. 2009. Lignocellulosic residues: biodegradation and bioconversion by fungi. *Biotechnology Advances*, **27**(2), 185-194.

Searchinger, T., Heimlich, R., Houghton, R.A., Dong, F., Elobeid, A., Fabiosa, J., Tokgoz, S., Hayes, D., Yu, T.H. 2008. Use of U.S. croplands for biofuels increases greenhouse gases through emissions from land-use change. *Science*, **319**(5867), 1238-1240.

Segal, L., Creely, J.J., Jr, A.E.M., Conrad, C.M. 1959. An empirical method for estimating the degree of crystallinity of native cellulose using the X-ray diffractometer. *Textile Research Journal*, **29**(10), 786-794.

Seo, D.J., Fujita, H., Sakoda, A. 2011. Structural changes of lignocelluloses by a nonionic surfactant, Tween 20, and their effects on cellulase adsorption and saccharification. *Bioresource Technology*, **102**(20), 9605-9612.

Sheehan, J., Aden, A., Paustian, K., Killian, K., Brenner, J., Walsh, M., Nelson, R. 2004. Energy and environmental aspects of using corn stover for fuel ethanol. *Journal of Industrial Ecology*, **7**(3-4), 117-146.

Sims, R.E., Mabee, W., Saddler, J.N., Taylor, M. 2010. An overview of second generation biofuel technologies. *Bioresource Technology*, **101**(6), 1570-1580.

Spiridon, I., Teaca, C.A., Bodîrlău, R. 2011. Structural changes evidenced by FTIR spectroscopy in cellulose materials after pretreatment with ionic liquid and enzymatic hydrolysis. *BioResources*, **6**(1), 400-413.

Stenberg, K., Tengborg, C., Galbe, M., Zacchi, G., Palmqvist, E., Hahn-Hagerdal, B. 1998. Recycling of process streams in ethanol production from softwoods based on enzymatic hydrolysis. *Applied Biochemistry and Biotechnology*, **70-72**, 697-708.

Stickel, J.J., Knutsen, J.S., Liberatore, M.W., Luu, W., Bousfield, D.W., Klingenberg, D.J., Scott, C.T., Root, T.W., Ehrhardt, M.R., Monz, T.O. 2009. Rheology measurements of a biomass slurry: an inter-laboratory study. *Rheologica Acta*, **48**(9), 1005-1015.

Sun, Y., Cheng, J. 2002a. Hydrolysis of lignocellulosic materials for ethanol production: a review. *Bioresource Technology*, **83**(1), 1-11.

Sun, Y., Cheng, J. 2002b. Hydrolysis of lignocellulosic materials for ethanol production： a review. *Bioresource Technology*, **83**(1), 1-11.

Taherzadeh, M.J., Karimi, K. 2007. Acid based hydrolysis processes for ethanol from lignocellulosic materials: a review. *BioResources*, **2**(3), 472-499.

Talebnia, F., Karakashev, D., Angelidaki, I. 2010. Production of bioethanol from wheat straw: An overview on pretreatment, hydrolysis and fermentation. *Bioresource Technology*, **101**(13), 4744-4753.

TheWhiteHouse. 2006. The White House National Economic Council (2006) Advanced Energy Initiative. http://georgewbush-whitehouse.archives.gov/ceq/advanced-energy.html.

Tu, M., Saddler, J.N. 2010. Potential enzyme cost reduction with the addition of surfactant during the hydrolysis of pretreated softwood. *Applied Biochemistry and Biotechnology*, **161**(1-8), 274-287.

Um, B.H., Hanley, T.R. 2008. A comparison of simple rheological parameters and simulation data for Zymomonas mobilis fermentation broths with high substrate loading in a 3-L bioreactor. *Applied Biochemistry and Biotechnology*, **145**(1-3), 29-38.

USDA. 2010. Effects of Increased Biofuels on the U.S. Economy in 2022. http://www.ers.usda.gov/Publications/ERR102/ERR102.pdf.

Von Blottnitz, H., Curran, M.A. 2007. A review of assessments conducted on bio-ethanol as a transportation fuel from a net energy, greenhouse gas, and environmental life cycle perspective. *Journal of Cleaner Production*, **15**(7), 607-619.

Wan, Y., Hong, L., Jia, S., Huang, Y., Zhu, Y., Wang, Y., Jiang, H. 2006. Synthesis and characterization of hydroxyapatite–bacterial cellulose nanocomposites. *Composites Science and Technology*, **66**(11-12), 1825-1832.

Wang, W., zhuang, X., Yuan, Z., Yu, Q., Qi, W., Wang, Q., Tan, X. 2012. Effect of structural changes on enzymatic hydrolysis of eucalyptus, sweet sorghum bagasse and sugarcane bagasse after liquid hot water pretreatment. *BioResources*, **7**(2), 2469-2482.

Wanga, G., Lia, W., Lia, B., Chen, H. 2008. TG study on pyrolysis of biomass and its three components under syngas. *Fuel*, **87**(4-5), 552-558.

Wyman, C. 1996. *Handbook on Bioethanol: Production and Utilization*. Taylor and Francis.

Xiao, L.p., Sun, Z.J., Shi, Z.J., Xu, F., Sun, R.C. 2011. Hot water pretreated woody biomass. *BioResources*, **6**(2), 1576-1598.

Yang, B., Wyman, C.E. 2006. BSA treatment to enhance enzymatic hydrolysis of cellulose in lignin containing substrates. *Biotechnology and Bioengineering*, **94**(4), 611-617.

Yang, B., Wyman, C.E. 2008. Pretreatment: the key to unlocking low-cost cellulosic ethanol. *Biofuels, Bioproducts and Biorefining*, **2**(1), 26-40.

Yang, H., Yan, R., Chen, H., Lee, D.H., Zheng, C. 2007. Characteristics of hemicellulose, cellulose and lignin pyrolysis. *Fuel*, **86**(12-13), 1781-1788.

Yang, X., Zeng, Y., Zhang, X. 2010. Influence of biopretreatment of the character of corn stover lignin as shown by thermogravimetric and chemical structural analysis. *BioResources*, **5**(1), 488-498.

Yoon, L.W., Ngoh, G.C., May Chua, A.S., Hashim, M.A. 2011. Comparison of ionic liquid, acid and alkali pretreatments for sugarcane bagasse enzymatic saccharification. *Journal of Chemical Technology and Biotechnology*, **86**(10), 1342-1348.

Yoshida, M., Liu, Y., Uchida, S., Kawarada, K., Ukagami, Y., Ichinose, H., Kaneko, S., Fukuda, K. 2008. Effects of cellulose crystallinity, hemicellulose, and lignin on the enzymatic hydrolysis of miscanthus sinensis to monosaccharides. *Bioscience, Biotechnology, and Biochemistry*, **72**(3), 805-810.

Yun, Y., Zhang, P., Zhu, M., Liu, C., Wang, L., Chen, C., Li, J. 2012. Preparation and characterization of poly(phthalazinone ether sulfone) hollow fiber ultrafiltration membranes. *Langmuir* **28**(28), 10627–10627.

Zhang, Y., Zhang, Y., Tang, L. 2011. Effect of PEG4000 on cellulase catalysis in the lignocellulose saccharification processes. *Journal of Chemical Technology and Biotechnology*, **86**(1), 115-120.

Zhao, H., Jones, C.L., Baker, G.A., Xia, S., Olubajo, O., Person, V.N. 2009. Regenerating cellulose from ionic liquids for an accelerated enzymatic hydrolysis. *Journal of Biotechnology*, **139**(1), 47-54.

Zhao, X., Heide, E.v.d., Zhang, T., Liu, D. 2010. Delignification of sugarcane bagasse with alkali and peractic acid and characterization of the pulp. *BioResources*, **5**(3), 1565-1580.

Zheng, Y., Pan, Z., Zhang, R. 2009. Overview of biomass pretreatment for cellulosic ethanol production. *International Journal of Agricultural and Biological Engineering*, **2**(3), 51-68.

Zhu, L., O'Dwyer, J.P., Chang, V.S., Granda, C.B., Holtzapple, M.T. 2008. Structural features affecting biomass enzymatic digestibility. *Bioresource Technology*, **99**(9), 3817-3828.

VITA

Shuo Cao was born in Puyang, China. She attended Henan University from 2006 to 2010 where she received a Bachelor of Engineering degree in Bioengineering. In August 2010, she began her master program in the Department of Food Science at Louisiana State University Agricultural Center in Baton Rouge, LA. In December 2012, she will receive the degree of Master of Science in Food Science.