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Alternative pretreatment of sorghum bagasse for bio-ethanol production

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ALTERNATIVE PRETREATMENT OF SORGHUM BAGASSE FOR BIO-ETHANOL PRODUCTION

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DEDICATION

♥

To my beloved parents without whom

I would not have come so far...

ACKNOWLEDGMENTS

I would like to thank the Almighty for his blessings towards achieving this goal in my life and my family for their encouragement and unconditional support. Mom and Dad, I owe all my success to you. I would like to express my gratitude towards my parents, Mrs. Qin Chen and Mr. Guangqing Liu for their parental love and for making me the person that I am today.

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ABSTRACT

The majority of the world's energy needs are currently met through the use of fossil fuels. The possible depletion of fossil fuel resources and environmental concerns has prompted the search for alternative renewable and environmentally friendly energy resources. The goal of this research was to develop a microwave-assisted dilute ammonia pretreatment technology for the conversion of sweet sorghum into ethanol.

Conversion of biomass into ethanol requires a pretreatment step to open up the structure and decouple the cellulose, and hemicellulose and lignin in the biomass. In the first study, sorghum bagasse was pretreated with 28% ammonium hydroxide, and water at a ratio of 1:0.5:8 at different temperatures for 1h using microwave. Biomass treated at 160 ^{o}C for 1h with dilute ammonia removed 46% of the lignin while retaining 90% cellulose and 73% hemicelluloses.

Evaluation of microwave pretreatment of sorghum biomass based on enzymatic hydrolysis and fermentation results was carried out also. The best ethanol yields among all different pretreatment conditions were 22±1.1g/100 g dry biomass using the 1-2mm particle size under 130 ^{o}C for 1h. The raw bagasse averaged 10±0.9g ethanol/100 g dry biomass. The concentrations of glycerol, organic acids, and furfurals were below the inhibitory level.

A third study used Tween 80 in the pretreatment stage, which was supposed to enhance the performance of the pretreatment. Sorghum bagasse was pretreated with a combination of ammonium hydroxide and Tween 80 for 45min at 130^{o}C. For 3% Tween 80 concentration, the glucose obtained from the hydrolysis was 38.1 g per 100 g dry biomass, compared to 33.2 g per 100 g dry biomass for control. The ethanol yield was 19 g per 100 g dry biomass, a nearly 19% improvement over the control.

The final study was designed to test the hypothesis that by using microwave for pretreatment, the water requirements be dramatically reduced by single soaking and draining of the biomass prior to the pretreatment. The pretreatment was performed by mixing sorghum fibers and 28% ammonia hydroxide solution at a ratio of 1:0.5 and heating the mixture to 130^{o}C for 1 h. Ethanol yields were 17 g/100 g dry biomass.

CHAPTER 1
LITERATURE REVIEW

1.1 Liquid Fuels from Biomass

There is considerable interest in developing renewable energy resources to replace crude oil. This interest stems from a consequence of multiple factors that have set the stage for global energy crisis, uncertainty in the long-term availability of crude oil and environmental problems from the use of fossil fuels. Particularly, there is an increase concern about the extent of the United States (US) reliance on unstable regions of the world to meet its energy needs(Mosier et al., 2005). More recently, the global energy market instability due to complex geopolitical events contribute to fossil fuels supply, availability, and price volatility, rendering the finding of energy sources a strategic priority of the US government. The alternative fuel should be technically accessible, economically competitive and environmentally friendly (Meher et al., 2006). These multiple factors stimulate the interest in biofuels which can be obtained from renewable sources. While there exists all kinds of renewable energy (i.e. wind, solar, geothermal and waves) lignocellulosic biomass; however, is the only current renewable source of liquid fuels for transportation (Huber et al., 2006). The overall renewable biomass production in the US is estimated at approximately 1.3 billion metric tons per year without effect on food and fiber production for national consumption and export (Perlack et al., 2005). The good fuel substitutes should be both economically and environmentally sustainable, as well as minimize the impacts on the world food and agricultural land needs.

1.2 Pathways for the Generation of Liquid Fuels

There are several pathways to produce second generation fuels from biomass. The major pathways for bioethanol production are defined based on the nature of the conversion process and include the gasification pathway and the carbohydrate pathway. The latter can be further

divided into three pathways in terms of the specific types of carbohydrates at the starting point of the process(Keshwani and Cheng, 2009). These include the lignocellulose pathway, starch pathway and sucrose (glucose) pathway (Keshwani, 2009). Bioethanol from the starch and sucrose pathways is considered a first generation biofuel since the feedstocks used are typically food crops and the conversion process is relatively simple and direct. Bioethanol from the gasification pathway and the lignocellulose pathway is considered a second-generation biofuel since the feedstocks used are non-food based and the conversion process is in the development stage. Several challenges need to be overcome before bioethanol production from lignocellulosic biomass reaches its maximum potential. Lignocellulose is mainly comprised of cellulose, hemicelluose and lignin. The structure can be described as a skeleton of cellulose chains embedded in a cross-linked matrix of hemicellulose wrapped by a lignin crust(Keshwani and Cheng, 2009). The carbohydrates polymers in lignocellulosic biomass are not easily accessible for enzymatic hydrolysis. This recalcitrance is, to a large extent, because of cellulose and hemicelluloses are bounded in the lignin matrix of the biomass (Kim et al., 2010). Pretreatment or breakdown of lignocellulosic biomass is needed to reduce biomass recalcitrance and to make sugars available for conversion into fuels (Hahn-Hagerdal et al., 2006). Another challenge is reducing the cost of hydrolytic enzymes (cellulases) used in the lignocellulose pathway (Hahn-Hagerdal et al., 2006). The cost of cellulases is approximately 6 times the cost of amylases used in the starch pathway (Schubert, 2006). Enzymes cost for bioethanol production from the lignocellulose pathway is estimated to be $0.3 to $0.5 per gallon ethanol (DOE, 2007). The successful fermentation of five-carbon sugars is known as the final challenge of bioethanol production (Hahn-Hagerdal et al., 2006). Generally, five carbon sugars, which include xylose and arabinose, account for 20-30% of the carbohydrate fraction of lignocelluloses. In order to

make the overall process economically feasible, both five-carbon and six-carbon sugars need to be fermented into ethanol. Wild strains such as *Saccharomyces cerevisiae* and *Zymomonas mobilis* are not capable of fermenting five-carbon sugars. Genetically modified yeasts such as *Pachysolen tannophilus*, *Pichia stipitis* and *Candida shehate*, are capable of doing so at a slow rate of fermentation and have low ethanol tolerance (Saha, 2003).

1.3 Lignocellulose Pathway for Bioethanol Production

Lignocellulosic materials primarily consist of cellulose, hemicellulose and lignin that are closely bounded in a complex matrix. The skeleton of cellulose chains are embedded in a cross-linked matrix of hemicellulose surrounded by a crust of lignin (Keshwani, 2009). The interactions among cellulose, hemicellulose and lignin, as well as the barrier nature of lignin minimize the enzymatic accessibility to the sugar polymers. Table 1.1 shows that chemical compositions vary among the various types of lignocellulosic biomass. In average, biomass such as sorghum contains 48-49% cellulose, 20-26% hemicellulose and 19-20% lignin. Pretreatment is among the most, if not the most important step in the production of bioethanol from lignocellulosic biomass (Hahn-Hagerdal et al., 2006). The main purpose of pretreatment is to minimize the recalcitrance of lignocellulosic biomass to enzymatic hydrolysis. Pretreatment methods should improve enzyme accessibility by removing most of the lignin and/or hemicellulose, increase the porosity of biomass and reduce cellulose crystallinity (Hahn-Hagerdal et al., 2006). Additionally, an efficient pretreatment method should preserve the sugars as much as possible with minimum formation of inhibitors (Galbe and Zacchi, 2007). A number of pretreatment methods have been developed for improving hydrolysis of lignocellulosic biomaterials.

Table 1.1 Chemical composition of lignocellulosic biomass (percent dry basis) (Aita and Kim, 2010).

Feedstock	Cellulose (glucan)	Hemicellulose (xylan)	Lignin
Softwoods			
Douglas	50.0	2.4-3.4	28.3
Pine	44.6	5.3	27.7
Spruce	45	8.8	27.9
Hardwoods			
Black locust	41.6	17.7	26.7
Hybrid poplar	44.7	18.6	26.4
eucalyptus	49.5	13.1	27.7
Popuplus tristis	40-49.9	13-17.4	18.1-20
Crop Residues			
Corn cobs	45.0	35.0	15.0
Corn fiber	14.3	16.8-35.0	8.4
Corn stover	36.8-39	14.8-25.0	15.1-23.1
Cotton gin trash	20.0	4.6	17.6
Grasses (sugarcane, sorghum bagasse)	25-50	25-50	10-30
Rice straw	35-41	25-14.8	9.9-12.0
Rice hults	36.1	14.0	19.4
Wheat straw	30-38	21-50	20-23.4
Herbaceous Materials			
Bermuda grass	25.0	35.7	6.4
Switchgrass	31-32	20.4-25.2	14.5-18.1
Cellulose Wastes			
Newsprint	40-64.4	4.6-40	18.3-21
Paper	85-99	0.0	0-15

They are generally categorized as mechanical (e.g., milling, grinding), physico-chemical (e.g., auto-hydrolysis, liquid hot water, steam, supercritical fluids), chemical (e.g., alkali, acid, organic solvents, oxidizing agents) and biological (e.g., fungi) processes or combinations of these approaches. Most of these technologies suffer from relatively low sugar yields, severe reaction conditions, large capital investment, high processing costs, and great investment risks (Alvira et

al., 2010; Kumar et al., 2009). Acid pretreatment is able to hydrolyze the cellulose and hemicelluloses but at high capital cost because of the formation of inhibitors and equipment corrosion problems (Wyman, 1996). Oxidative pretreatment usually results in losses of cellulose and hemicellulose due to the oxidant used is non-selective (Hendriks and Zeeman, 2009). The use of organic solvents is too expensive to be employed for biomass though pure lignin could be obtained as by product by this method (Zhao et al., 2009). Biological pretreatment, which commonly refers to the use of the white-rot fungus to degrade lignocelluloses, in general, requires low energy input, low capital cost and mild environmental conditions. However, this technology is unattractive for an industrial process because of its slow rate (Aita and Kim, 2010; Hahn-Hagerdal et al., 2006). Alkaline based pretreatment demonstrate great success in the delignification of lignocelluloses. Ammonia breaks down the C-O-C bonds in lignin and the ether and ester bonds between lignin and hemicelluloses, as well as penetrates the crystalline structure in cellulose and causes swelling (Aita et al., 2010).

Most pretreatment methods require energy input in the form of heat and /or pressure. This heat and pressure is usually applied via high temperature steam either directly or indirectly. Microwave-based technologies reduce process energy requirements. Microwave heating is a uniform and selective process, with the ability to start and stop the process instantaneously (Datta, 2001). All these benefits along with microwave radiation have made its many applications in fields such as drying, heating, cooking, sterilization and microwave-assisted chemistry(Keshwani, 2009). As a result, it is reasonable to hypothesize that a microwave-based heating process could be used for the pretreatment of lignocelluloses. Microwaves can result in both thermal and non-thermal effects that can be applied to physical, chemical or biological processes. Microwave is an alternative heating method to conventional conduction and

convection heating. While the latter is based on superficial heat transfer; the former uses the ability to directly interact with the molecular structure of the heated object and of an applied electromagnetic field. The rapidly oscillating electric field component in the microwaves spins polar molecules (mostly water) and move back and forth disordered ions. Both mechanisms dissipate heat via molecular friction, and therefore, the heating is volumetric and rapid. When microwaves are used to treat lignocelluloses, it selectively heats the more polar part and creates a "hot spot" within the inhomogeneous materials. It is hypothesized that this unique heating feature results in an "explosion" effect among the particles, and improves the disruption of the recalcitrant structures of lignocellulose. In addition, the electromagnetic field used in microwave might create non-thermal effects of highly polarizing radiation, such as molecular mobility, field stabilization that also accelerate the destruction of the crystal structures. There is limited information related to the new pretreatment method using dielectric heating instead of conventional heating to improve the performance of pretreatment. Microwave pretreatment of rice straw and bagasse was initially reported by Ooshima et al.(Ooshima et al., 1984). Recently, microwave-assisted alkali/acid/H_2O_2 pretreatment of rice and wheat straw was investigated by Zhu et al. (Zhu et al., 2005). However, the sugar yields based on dry weight of untreated original materials was not provided; therefore, it is difficult to compare this technology with other pretreatment methods. In addition, the authors used an "open air" beaker to "boil" the straw-alkali solution so the volume loss due to evaporation may be significant.

The goal of this study was to investigate the effect of a microwave-assisted dilute-ammonia pretreatment on the structure and composition of sweet sorghum and to evaluate cellulose and fermentation yields of the pretreated biomass.

CHAPTER 2
COMPOSITIONAL CHANGES OF MICROWAVE-ASSISTED DILUTED AMMONIA PRETREATED SORGHUM

2.1 Introduction

Our society is currently extremely dependent on fossil fuels for its energy needs. As such, any events that threaten their availability influence the cost of petroleum supply. In addition, the negative impact of fossil fuels on the environment, particularly greenhouse gas emissions, has imposed a critical need on the society to identify and develop renewable fuel alternatives (Hahn-Hagerdal et al., 2006). The most promising renewable energy source for transportation currently identified by the U.S. government is ethanol produced from lignocellulosic materials. This source would replace the current generation of bioethanol produced from sugars or starch, which are currently imposing limits in terms of costs and, at the same time, place an upward pressure on the food market. A study supported by both DOE and USDA has indicated that the United States has sufficient land resources to produce over 1 billion dry tons of biomass annually, enough to displace at least 30% of the nation's current consumption of liquid transportation fuels (Hamelinck et al., 2005; Taherzadeh and Karimi, 2007; Taherzadeh and Karimi, 2007).

Demand for transportation fuels is expected to only increase in the US and around the world and the use of lignocellulosic biomass as biofuel feedstock represents one of the most suitable avenues for their production. Lignocellulosics that show potential for ethanol production include agricultural residues (i.e. corn stover, wheat straw, rice straw), agricultural byproducts (i.e. corn fiber, rice hull, sugarcane bagasse), and dedicated energy crops (i.e. switchgrass, sweet sorghum, high fiber sugarcane, Miscanthus) (Mosier et al., 2005).

The ability to make fuels and/or other value added products from lignocellulosics depends on the ease with which biomass is separated/broken down into cellulose, hemicellulose and lignin. Pretreatment of lignocellulose is a key step for the efficient utilization of biomass for ethanol production (Hahn-Hagerdal et al., 2006). Pretreatment softens and ruptures the cell wall and breaks down the bonds linking lignin to hemicellulose, while decreasing the crystallinity of cellulose. Both mechanisms improve downstream enzyme hydrolysis. A number of pretreatment methods have been developed for improving hydrolysis of lignocellulosic biomaterials. Most of these technologies suffer from relatively low sugar yields, severe reaction conditions, large capital investment, high processing costs, and great investment risks (Alvira et al., 2010; Kumar et al., 2009). Acid pretreatment is able to hydrolyze the cellulose and hemicelluloses but capital cost is high because of the formation of inhibitors and equipment corrosion problems(Wyman, 1996). Oxidative pretreatment usually results in losses of cellulose and hemicellulose due to the fact that all oxidants used are non-selective (Hendriks and Zeeman, 2009). Pretreatment with organic solvents is too expensive to be employed for biomass though pure lignin could be obtained as by product through this processing technology (Zhao et al., 2009). Biological pretreatment which commonly uses the white-rot fungus to degrade lignocelluloses requires low energy input, low capital cost and mild environmental conditions. However, it is otherwise unattractive at industrial scale because of the slow conversion rate (Aita and Kim, 2010; Hahn-Hagerdal et al., 2006). Alkaline based pretreatment demonstrate great success in delignifying lignocelluloses. In particular ammonia, which breaks down the C-O-C bonds in lignin and the ether and ester bonds between lignin and hemicelluloses, as well as it penetrates the crystalline structure in cellulose and causes swelling (Aita et al., 2010). Salvi et al. (Salvi et al., 2010) reported 24g ethanol per 100g dry sorghum bagasse by a dilute ammonia pretreatment method.

Dilute ammonia treatment removed 44% of the original lignin and 35% of the original xylan, and retained 90% of the glucan in the treated material. High glucan (cellulose) digestibility was observed in treated biomass due to increased surface area and porosity by using dilute ammonia pretreatment. Ammonia-based pretreatments with or without heat have demonstrated great success in the delignification of corn stover, barley bull and municipal wastes (Aita and Kim, 2010).

Microwave is an alternative heating method to conventional conduction and convection heating. Whereas the latter is based on superficial heat transfer; the former uses the ability of an applied electromagnetic field to directly interact with the molecular structure of the heated object. The rapidly oscillating electric field component in the microwaves spins polar molecules (mostly water) and move back and forth disordered ions with a frequency of 10^6-10^{10}. Both mechanisms dissipate heat via molecular friction, and therefore, the heating is volumetric and rapid. When microwaves are used to treat lignocelluloses, it selectively heats the more polar parts and creates a "hot spot" within the inhomogeneous materials. It is hypothesized that this unique heating feature results in an "explosion" effect among the particles, and improves the disruption of the recalcitrant structures of lignocellulose. In addition, the high electromagnetic might create non-thermal effects of highly polarizing radiation, such as molecular mobility, and field stabilization that also accelerate the destruction of the crystal structures. There is limited information related to the new pretreatment method using dielectric heating instead of conventional heating to improve this projects' performance. Microwave pretreatment of rice straw and bagasse was initially reported by Ooshima et al.(Ooshima et al., 1984). Recently, microwave-assisted alkali/acid/H_2O_2 pretreatment of rice and wheat straw was investigated by Zhu et al (Zhu et al., 2006). However, the sugar yields based on dry weight of untreated original materials was not

provided; therefore, it is difficult to compare this technology with other pretreatment methods. In addition, the authors used an "open air" beaker to "boil" the straw-alkali solution; the volume loss (due to evaporation) may be significant. The goal of this study was to investigate the effect that microwave-assisted dilute ammonia pretreatment has on the structure and composition of sweet sorghum lignocellulosic biomass.

2.2 Materials and Methods

- **Substrate**

Sorghum (*Sorghum bicolor (L.) Topper*) was harvested from the Hill Farm Research Station (Homer, LA) at the Lousisana State University Agricultural Center. Leaves, roots, and grains were removed and the stalks were crushed in a roller press (Farrel Company, Ansonia, CT) three times to extract the juice. The remaining fibers or bagasse were stored in sealed bags at -20^{o}C. Final moisture content was 20% (w/w). The milled sorghum bagasse was passed through ASTM E11 (body 8inch*2inch) sieves of mesh size of 18mm, 9.5mm, 6mm, 4mm, 2mm and 1mm using the Retsch Sieving machine and fractionated into three levels (9.5-18mm, 4-6mm and 1-2mm).

- **Microwave Assisted Dilute Ammonia Pretreatment**

Approximately, 15g dry weight each of sieved sorghum fibers (1-2mm, 4-6mm, and 9.5-18mm) were pretreated with ammonium hydroxide (28% v/v solution, Fisher Scientific, Pittsburgh, PA) and water at a ratio of 1:0.5:8, respectively, for 1 h at temperatures of 100 ,115, 130, 145, and 160 ℃ using a microwave heating system (Ethos, Milestone Inc, Monroe, CT). After pretreatment, the reactor was cooled down to 50 ^{o}C, the contents were released though a

stainless steel filter and washed with DI water three times. The pretreated biomass was dried to 10% moisture in a conventional oven at 40–45^{o}C for 24 hours, and stored in sealed plastic bags at 20^{o}C for further studies. Untreated and dilute ammonia treated (by conventional heating) samples were used as controls. All experiments were performed in duplicates.

- **Chemical Composition of Sweet Sorghum**

Untreated, dilute ammonia treated, and microwave-assisted dilute ammonia treated sorghum bagasse samples were analyzed for glucan, xylan, lignin, arabinan, mannan, 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.

- **Measurement of Biomass Porosity**

The accessibility of the hydrolytic enzymes to the biomass was quantified by measuring the porosity of biomass using Simons' Stain method (Simons, 1950). The numbers of both small and large pores are important factors in determining the accessibility of cellulases to the biomass (Tanaka et al., 1988). Two dyes, Direct Orange 15 (large and small molecules) and Direct Blue 1 (small molecules) competitively adsorb to the pores on biomass. A procedure adapted by Esteghlalian was used (Esteghlalian et al., 2001). The increase in the size of the biomass sample and isolation of the larger orange dye molecules via ultracentrifugation was made to improve the accuracy of the results (Keshwani and Cheng, 2009).The larger orange dye molecules were separated from the smaller orange dye molecules via centrifugation before the experiment. The large orange dye molecules can only penetrate the large pores in the biomass substituting the

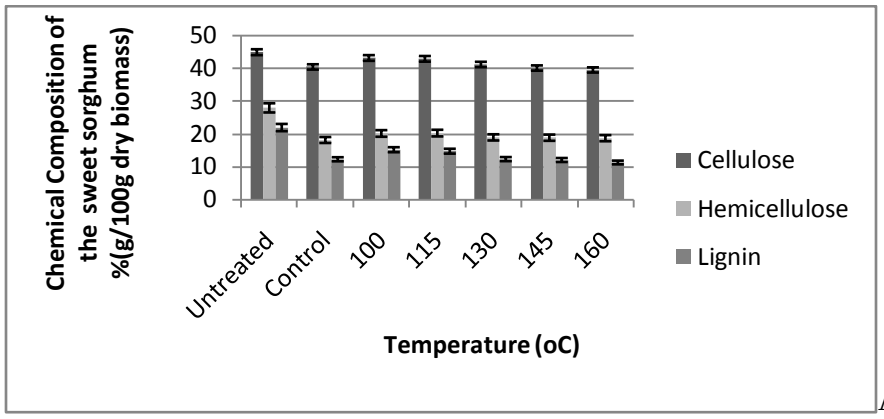
smaller blue dye molecules in these larger pores due to their higher affinity to the hydroxyl units of cellulose (Yu et al., 1995). Therefore, the amount of orange and blue dyes adsorbed by the biomass can be used to quantify the relative difference in porosity between biomass samples according to Keshwani and Cheng (Keshwani and Cheng, 2009).

2.3 Results and Discussion

- **Effect of Temperature and Particle size on Biomass Chemical Composition**

Lignin and sugar content in biomass are important factors that help assess the performance of pretreatment. Particularly, reducing lignin content can increase the enzyme accessibility to biomass substrate during hydrolysis (Salvi et al., 2009). Compositional changes of carbohydrates and lignin in sweet sorghum fibers treated with microwave assisted dilute ammonia pretreatment are shown in Figure 1. Untreated sorghum usually contains 45% cellulose (glucan), 28% hemicellulose (xylan) and 22% lignin, which is within the range of values published in literature. It was observed that lignin removal caused nearly 20% of the total mass loss. Approximately, 48 % of the initial lignin was removed during pretreatment at 160^{o}C, the highest temperature used in this study. A considerable amount of hemicellulose (34.5%) was also lost. Wyman et al. (2005) reported that ammonia pretreatment processes can remove some hemicellulose along with lignin. However, even under the most severe pretreatment conditions and for biomass of all three particle sizes; the amount of available cellulose was more than 90% of the original values, an indication of positive cellulose selectivity for this pretreatment method. Salvi et al. (Salvi et al., 2010) observed the removal of 44% lignin and was able to retain 90% cellulose after the pretreatment of sorghum bagasse with dilute ammonia by convetional heating. Aita et al. (Aita et al., 2010) reported the removal of 45% lignin, 30% hemicellulose and 9%

cellulose after the pretreatment of energy cane using dilute ammonia. Kim et al. (Kim et al., 2010) evaluated the effect of ammonia (0.03 to 0.3% w/w, ammonium hydroxide) on sugarcane



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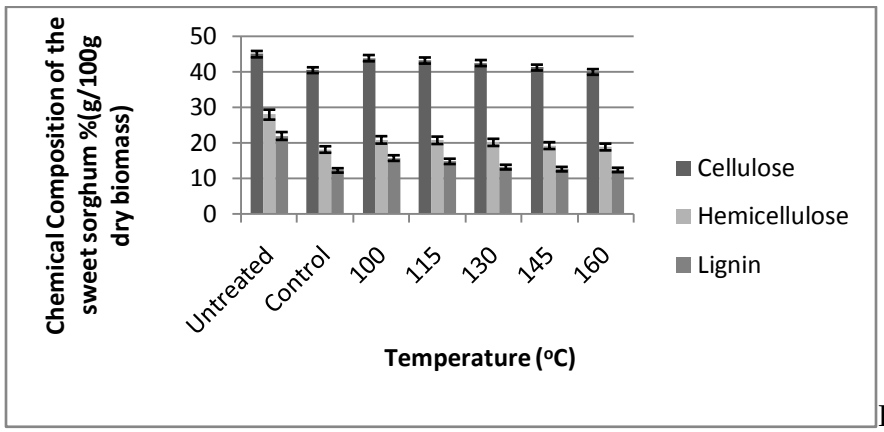

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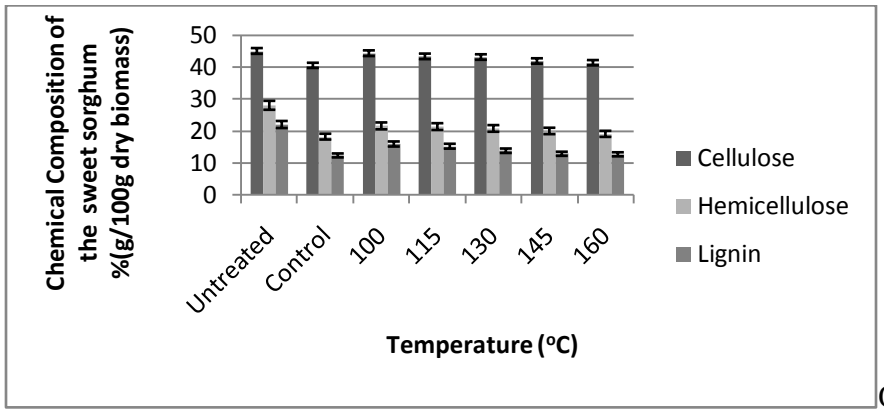

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Figure 2.1 Changes in the chemical composition of microwave-assisted dilute ammonia pretreated sweet sorghum fibers at various particle sizes (a: 1-2mm; b: 4-6mm; c: 9.5-18mm) and temperatures (130-160 ^{o}C). Control is sorghum bagasse pretreated with the same concentration of ammonia at 160 ^{o}C for 1 h by conventional heating.

bagasse stored at 30 °C at atmospheric pressure for 40 days without agitation. Maximum lignin removal (46%) and no cellulose loss were observed with biomass stored for 40 days with a 0.3% ammonia solution. The smaller the particle size, the more lignin was removed but so were the sugars, mostly due to the larger reacting surface of the fibers. It was also noticed that as temperature increased above 130 ^{o}C, more lignin was removed. Lignin dissolves at temperatures between 140 and 160^{o}C, and its softening range can be lowered by the existence of ammonia (Puri and Pearce, 1986). However, the loss of both cellulose and hemicelluloses also increases with increasing temperature.

It is important to find a proper pretreatment temperature where the process can remove most of the lignin while preserving the sugars. In our previous pretreatment studies with dilute ammonia at 160°C by conventional heating, 44% lignin and 35% hemicellulose were removed during the process and 90% of the cellulose was retained in sorghum bagasse (Salvi et al., 2010); whereas, 55% lignin, 30% hemicellulose, 9% cellulose were removed from energy cane bagasse (Aita et al., 2010). Microwave heating removed 4% more lignin than conventional heating and still retained 90% cellulose. Keshwani and Cheng (Keshwani and Cheng, 2009) pretreated switchgrass using microwave heating with 2% NaOH for 10 min at a power level of 250 W and observed 68% of lignin removal while retaining 82% of glucose. Similarly, pretreatment of Bermuda grass using microwave at a power level of 250 W with 1% NaOH for 10 min removed nearly 65% of the lignin and retained 87% of the glucose (Keshwani and Cheng, 2009). Zhu et al. (2006a) also reported that microwave-assisted alkali pretreatment of wheat straw removed more lignin and hemicellulose from lignocellulose with shorter pre-treatment time than the alkali one with conventional heating.

Particle size reduction increases the surface area to volume ratio and improves enzyme accessibility to the active binding sites. The milling of lignocellulose, however, is energy intensive, increases the pretreatment cost, and can make the process non-economical.(Hu et al., 2008). The highest biomass delignification (48.4%) was observed with particle size 1-2mm at 160 ^{o}C as shown in Figure 2.2

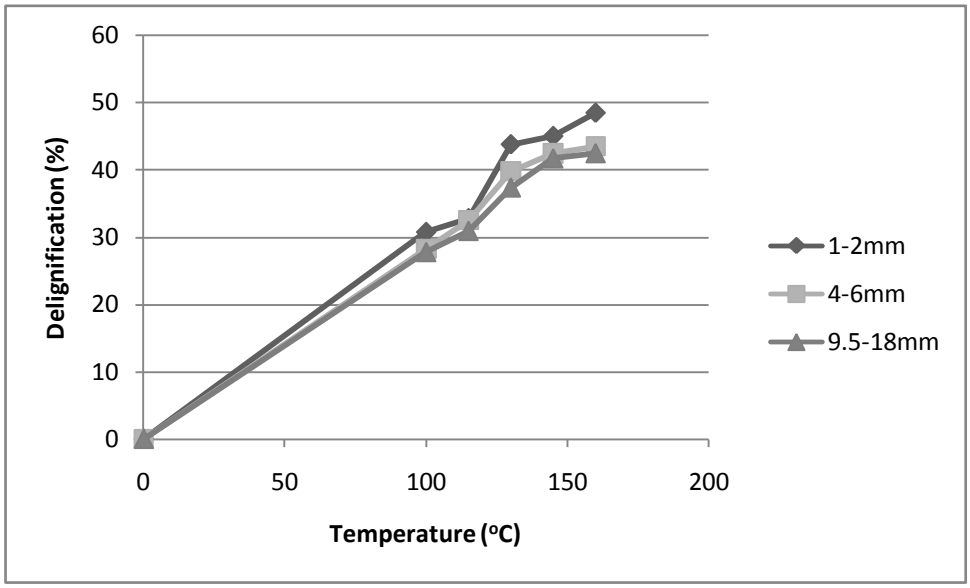

Figure 2.2 Percent Delignification of microwave assisted dilute ammonia treated sweet sorghum bagasse at various temperatures and particle sizes.

Although a higher lignin removal was observed with smaller particle size biomass, more sugars were lost because of the larger reacting surface area of biomass particle. Therefore, it is pivotal to consider both lignin removal and sugar degradation effects when selecting the best pretreatment condition for subsequent enzymatic hydrolysis and fermentation steps. Caulfield and Moore (1974) mentioned that decreased particle size and increased available surface affect crystallinity of the lignocelluloses. Hu et al. (2008) observed similar lignin removal with different particle sizes of switchgrass using NaOH assisted radio-frequency heating.

- **Effect of Temperature and Particle Size on Biomass Porosity**

An effective approach to evaluate biomass morphology change is by quantifying the porosity of pretreated sorghum samples using Simons' stain method. A recent study showed a correlation coefficient of 0.95 between adsorption of Simons' stain dye and enzymatic accessibility (Chandra et al., 2008). The extent of enzymatic accessibility improvement to the biomass fibers can be determined by the amounts of the smaller blue dye and the larger orange dye adsorbed by the lignocelluloses (Chandra et al., 2008; Esteghlalian et al., 2001). From Figure 2.3, it can be observed that the biomass with the smallest particle size at 160 ^{o}C can absorb the most both blue and orange dye, indicating the highest porosity. Since one of the purposes of pretreatment is the disruption of the biomass structure to increase porosity, a higher temperature and larger surface area will presumably result in a more severe pretreatment causing more disruption to the biomass structure and higher biomass porosity. For the different particle sizes, both the largest (9.5-18mm) and smallest particle (1-2mm) sizes showed higher amount of porosity than the middle size (4-6mm). After pretreatment, the middle size sorghum fibers formed clumps, which reduced the surface area available to ammonia. Due to the reduction of surface area of the middle particle size of sorghum fibers, the porosity created by the pretreatment may be lower than that of the other particle size fibers. Considering that the biomass fibers of smallest particle size had larger surface area than the fibers with largest particle size, it is reasonable to believe that biomass of particle size 1-2mm possesses higher porosity than the one of 9.5-18mm. Other studies have demonstrated that a strong alkali such as NaOH can create a high number of large pores due to more disruption to the biomass structure (Keshwani and Cheng, 2009). This may explain why the number of large pores in this study is low since the weak alkali-ammonia was employed for the pretreatment.

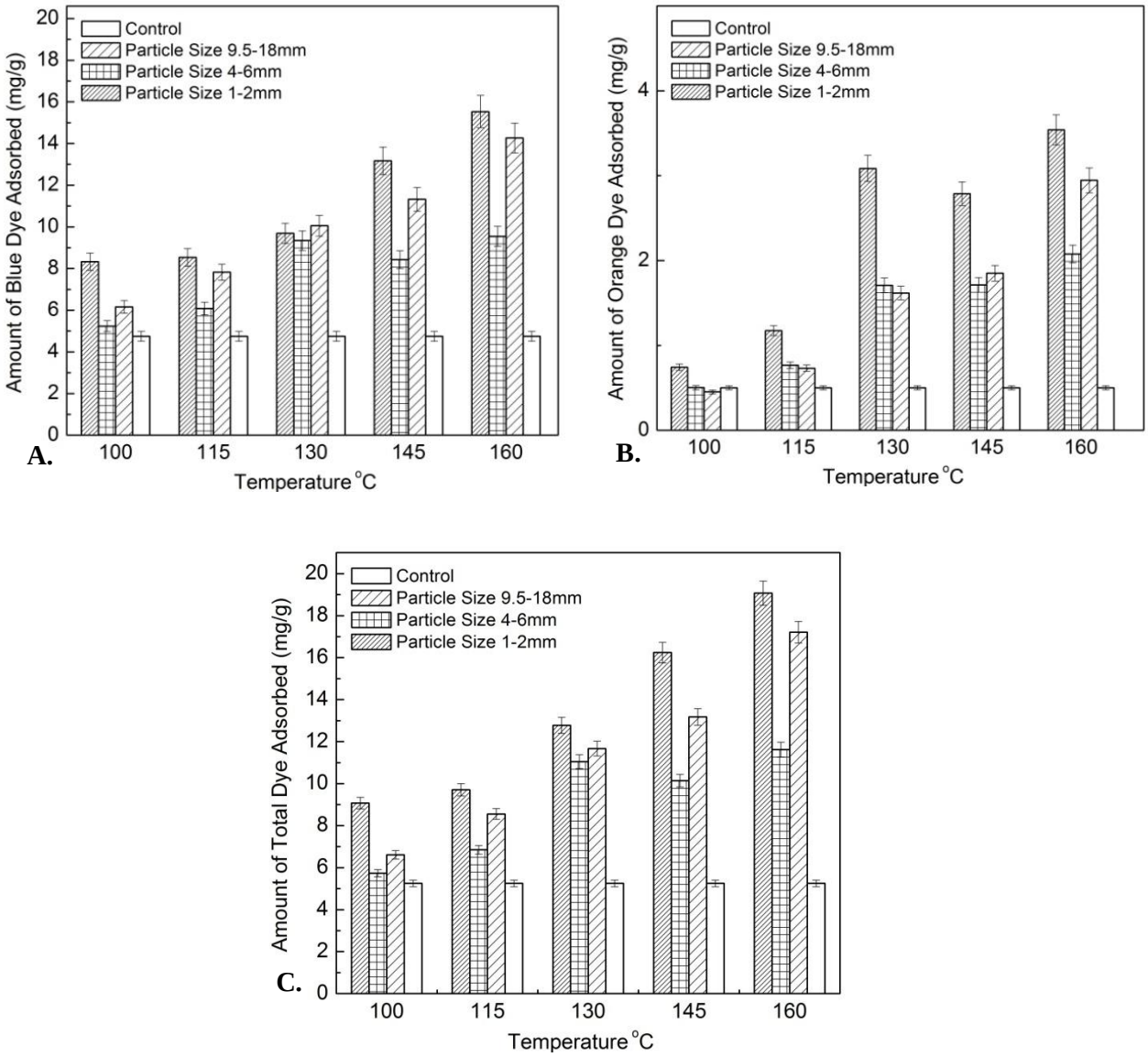

Figure 2.3 Adsorption of orange dye, blue dye and total dye by microwave-assisted dilute ammonia treated sweet sorghum. Control refers to untreated sorghum bagasse.

2.4 Conclusion

Microwave-assisted dilute ammonia pretreatment was successful in removing 48 % of the original lignin from sorghum fibers at comparatively low ammonia concentration, lower temperature, and in a relatively short reaction time compared to other pretreatment methods. This

pretreatment method increased the porosity of the biomass, which would enhance the enzymatic digestibility of sweet sorghum fiber. However, more in depth studies that include, enzymatic hydrolysis, fermentation, and a full scale up and the overall engineering and economic analysis of the conversion process are needed in order to establish the most suitable set of parameters that maximizes ethanol production.

CHAPTER 3
ETHANOL PRODUCTION FROM SWEET SORGHUM BY A MICROWAVE ASSISTED DILUTE AMMONIA PRETREATMENT

3.1 Introduction

Ethanol produced from lignocellulosic biomass has great potential to replace fossil fuels because of its renewability and suitability (Hahn-Hagerdal et al., 2006). Lignocellulosic biomass, such as agricultural residues, forest products and energy crops can be used for bio-ethanol production (Liu and Wyman, 2005). Lignocelluolsic biomass is composed of carbohydrate polymers (cellulose and hemicelluloses), and lignin with compositions varying amongst plant materials bound together in an intricated matrix (Aita and Kim, 2010). Pretreatment is the key step to break down and disrupt this matrix to allow for the accessibility of enzymes to cellulose and hemicellulose for the release of mono-sugars (Kim et al., 2003)

An effective pretreatment method is aimed at removing most of the lignin while at the same time, retaining most of the cellulose and hemicellulose (Aita and Kim, 2010). Currently, various pretreatment methods, such as steam explosion, supercritical fluids, alkaline hydrolysis, Ammonia fiber/freeze expansion, acid hydrolysis, organic solvents, have been widely investigated (Aita and Kim, 2010; Hahn-Hagerdal et al., 2006; Mosier et al., 2005). Additionally, dilute ammonia pretreatment of sorghum bagasse have been investigated in our previous study (Salvi et al., 2009). Ammonia, which is a relatively less expensive chemical, is a proper choice for pretreatment since it is a selective reagent for lignin (Salvi et al., 2010). Studies have been conducted using microwave assisted lime pretreatment of sweet sorghum bagasse for enzymatic saccharification. Most of these pretreatment methods utilize conventional heating. Dielectric heating, which includes microwave, ultrasound as well as radio frequency, is an alternative method for conventional heating. Microwave is an excellent heating

approach to replace conventional conduction and convection heating. The goal of this study was to investigate the effect of microwave-assisted dilute ammonia pretreatment on the enzymatic hydrolysis and ethanol fermentation of treated sorghum bagasse.

3.2 Materials and Methods

- **Biomass Preparation**

Sorghum (*Sorghum bicolor* (L.) Topper) was harvested from the Hill Farm Research Station (Homer, LA). Leaves, roots, and grains were removed and the stalks were crushed in a roller press (Farrel Company, Ansonia, CT) three times to extract the juice. The milled sorghum bagasse was passed through ASTM E11(body 8inch*2inch) sieves of 18 mm, 9,5mm, 6mm, 4mm, 2mm, 1mm using the Retsch Sieving machine and fractionated into three levels (9.5-18mm, 4-6mm and 1-2mm). Three kinds of particle sizes (1-2mm, 4-6mm, 9.5-18mm respectively) of sorghum fibers (15g dry weight) were pretreated with ammonium hydroxide (28% v/v solution, Fisher Scientific, Pittsburgh, PA) and water at a ratio of 1:0.5:8, respectively, for 1 h at temperatures ranging from 100℃ to 160 ℃ using microwave heating. Untreated and microwave-assisted dilute ammonia pretreated biomass were dried to 20% moisture in an oven at 40–45 ℃ overnight and stored for further studies. Untreated biomass was used as control. Composition analysis was conducted following NREL 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.

- **Inoculum Preparation**

Saccharomyces cerevisiae (D_5A) was obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were stored at -70^{o}C upon arrival. Yeast cells

were grown in YP media containing 1% (w/v) yeast extract (Becton– Dickinson and Company, Sparks, MD), 2% peptone (Becton–Dickinson and Company, Sparks, MD), and 3% glucose (Sigma–Aldrich, Inc., St. Louis, MO) at 30 ^{o}C for 16 h in a shaker incubator (Amerex Instruments Inc., Lafayette, CA) at 200 rpm. Approximately, 10 ml yeast solution was transferred to 2L YP media. The inoculated media was incubated at 30 ^{o}C for 24 h in a shaker incubator at 200 rpm. Cells were washed twice with deionized (DI) water and harvested by centrifugation at 8,000 rpm. Rinsed cells were resuspended in 50 ml DI water and stored at 4 ^{o}C. The final concentration was 1×10^{9} CFU/ml as confirmed on YP media by the pour plate method.

- **Enzymatic Hydrolysis**

An enzyme combination of Spezyme CP (Genencor, Danisco US Inc., Rochester, NY) containing cellulase and Novozyme 188 (Sigma–Aldrich, Inc., St. Louis, MO) containing β-glucosidase was evaluated for hydrolysis. The combination consisted of 60 FPU Spezyme CP/g of glucan and 64 CBU Novozyme 188/g of glucan. One-liter Erlenmeyer flasks were each loaded with 5 g (dry weight) biomass (microwave-assisted dilute ammonia treated or untreated), 0.5 g yeast extract, 1 g peptone, 25 g citrate buffer (1 M stock solution, pH 4.8), and water to bring the final weight to 500 g. The pH of each mixture was adjusted to 4.8 with concentrated hydrochloric acid. All flasks were autoclaved at 121^{o}C for 30 min. After autoclaving, flasks were cooled to 30 ^{o}C. Samples (5 ml) were taken prior to the addition of enzymes and labeled time 0. Enzymes were added and all flasks were incubated at 55 ^{o}C in a shaker incubator (Amerex Instruments Inc., Lafayette, CA) for 24 h at 200 rpm. Samples (5 ml) were withdrawn from each flask post enzyme hydrolysis (time 24 h). All samples were analyzed for sugars (glucose, cellobiose, arabinose, xylose), ethanol, glycerol, organic acids (lactic, acetic and formic), hydroxy-methyl-furfural (HMF), and furfurals. Experiments were run in triplicate.

- **Fermentation**

All flasks were cooled down to 30 ^{o}C post enzyme hydrolysis. Yeast cells (1 ml) from the stock solution were added to each flask and incubated at 30 ^{o}C in a shaker incubator at 200 rpm for an additional 2 days. Samples (15 ml) were withdrawn at 48 and 72 h and analyzed for sugars (glucose, cellobiose, arabinose, xylose), ethanol, glycerol, organic acids (lactic, acetic, and formic), HMF, and furfurals.

- **Analytical Procedures**
- **Sample Preparation**

Samples taken at time 0, 24, 48, and 72 h was centrifuged at 8,000 rpm and filtered (0.2 µm syringe filters, Nagle Company, New York City, NY). Dilutions of filtered solutions were made accordingly prior to chemical analysis.

- **Sugars and Glycerol Analysis**

Cellobiose, glucose, xylose, arabinose, and glycerol were analyzed by high-performance liquid chromatography (HPLC; Agilent 1200 series) with a BioRad Aminex HPX- 87P (Pl), lead form, 300 mm×7.8 mm (ID), 9 µm column, and a differential refractive index (DRI) detector (G1362A Agilent). The eluent solution was filtered water (0.2 µm) at a flow rate of 1 ml/min. Sample volume was 20 µl.

- **Ethanol Analysis**

Ethanol was analyzed by high-performance liquid chromatography (HPLC; Agilent 1200 series) with a BioRad Aminex HPX- 87K (Pl), lead form, 300 mm×4 mm (ID), 7 µm column,

and a differential refractive index (DRI) detector (G1362A Agilent). The column temperature was set at 85 ^{o}C. The eluent solution was filtered 0.01M K_2SO_4 buffer (0.2µm) at a flow rate of 0.6 ml/min. Sample volume was 20 µl.

- **Organic Acids Analysis**

Lactic acid, acetic acid, and formic acid were analyzed by HPLC (Metrohm Peak Ion Chromatography). The column used was a Dionex Ion Pac AS-11 HC anion exchange column with Anion Trap Ion Pac ATC-1. A 50 µl sample volume was used with a gradient method. The eluents used were 50 mM NaOH and DI water. Total run time was 54 min at flow rates ranging from 0.8 to 1.4 ml/min.

- **HMF and Furfural Analysis**

HMF and 2-furfural were analyzed using reverse-phase HPLC (Agilent 1100) with a C18 column, 150 mm×4 mm×5µm (Agilent Eclipse). A diode array detector was configured to collect absorbance at 280 and 330 nm. The gradient method had a flow rate of 1 ml/min, and a total run time of 15 min. Methanol and water were used as eluents at concentrations of 5% and 95% for 2 min, 30% and 70% for 3 min, 50% and 50% for 5 min, and 5% and 95% again for the last 5 min.

3.3 Results and Discussion

- **Effect of Particle Size on the Sugar Yield of Microwave- Assisted Dilute Ammonia Pretreated Sweet Sorghum**

Particle size is an important property of the substrate associated with total available area. The reduction of particle size can increase the effective surface area and improve enzyme accessibility to substrates (Chundawat et al., 2007; Mansfield et al., 1999). However, the milling of biomass requires energy input and more capital cost, and thus makes the process non-

economical. Therefore, it is important to select the proper particle size of biomass fibers which has enough surface area and requires less energy input for grinding. The untreated sorghum usually contains 45% cellulose (glucan), 28% hemicellulose (xylan) and 22% lignin, which is in the range of values published in literature. Microwave assisted dilute ammonia pretreatment of sorghum fibers, removed over 46% of the lignin while retaining more than 90% cellulose and 73% hemicellulose for all the different pretreatment conditions. The lignin removal was found to be similar across all particle sizes. Minimal effect of size reduction was observed for AFEX treated corn fibers (Moniruzzaman et al., 1997) and for hot water treated corn stover (Cullis et al., 2004). Further, Hu and Wen (Hu and Wen, 2008) observed that alkali pretreated switchgrass with particle size of less than 0.25mm resulted low sugar yields during enzymatic hydrolysis due to the degradation of sugars during pretreatment. .In our study, however, the smallest particle size resulted in the most glucose release among all different particle sizes. Previous studies have indicated that small particles would be conducive to the enhancement of the accessibility of enzymes to the substrate resulting in an increase in sugar yield. Chundawat et al (Chundawat et al., 2007) reported that size reduction along with water washing improved the hydrolysis of AFEX-treated corn stover.

- **Effect of Temperature on the Sugar Yield of Microwave Assisted Dilute Ammonia Pretreated Sweet Sorghum**

Changes in sugars and ethanol concentrations during Simultaneous Saccharification and Fermentation (SSF) process of microwave-assisted dilute ammonia and untreated sorghum bagasse are depicted in Figure 3.1. The highest glucose concentrations was 4.2 g glucose per 10 g dry biomass at 130^{o}C on samples with particle size of 1-2mm. Pretreatments at 145 ^{o}C and 160 ^{o}C resulted in 3.68g glucose /10g dry biomass and 3.61g glucose /10g dry biomass, respectively,

which suggest that some glucose was degraded due to the more harsh pretreatment conditions. Pretreatments at temperatures in the range of 200-230 ^{o}C generally result in higher sugar losses (Liu and Wyman, 2005). Redding et al. (2011) also reported lower sugar concentrations as pretreatment temperatures increased from 140-160^{o}C (Redding et al., 2011). Pretreatment temperatures below 130 ^{o}C resulted in lower sugar concentrations (3.2g glucose per 10g dry biomass). The lower glucose release can be attributed to the limited access of enzymes to the sugar polymers, which largely rely on the removal of lignin which dissolves at temperatures between 140 ^{o}C and 160 ^{o}C (Puri and Pearce, 1986). Therefore, pretreatment at 130 ^{o}C was selected as the optimal temperature to attain the most glucose release during enzyme hydrolysis.

During dilute ammonia pretreatment C-O-C bonds in lignin and other ether and ester bonds in the lignin carbonhydrate matrix are broken (Kim et al., 2003). The cleavage of the lignin-carbohydrate complex can result in pore formation and swelling of biomass, thus increasing surface area and subsequently improving enzyme accessibility (Kim et al., 2003; Mosier et al., 2005). In our previous study, 4.7g glucose/10g dry sorghum was achieved by pretreatment using the same ammonia concentration with conventional heating at 160^{o}C. The glucose yield is higher than microwave heating at 130 ^{o}C, the optimal temperature that was selected from this study. Two reasons may explain the relatively lower glucose yield using microwave heating than the one with conventional heating. The temperature of microwave heating pretreatment is lower than the conventional heating; additionally, two different mixing methods were used during pretreatment. A magnetic stirrer was used during microwave heating and tumbling for conventional heating. Hu and Wen (Hu and Wen, 2008) pretreated switchgrass by alkali microwave heating at 190 ^{o}C and achieved 3.15g glucose/10g dry biomass. Also, Keshwani and Cheng (Keshwani and Cheng, 2009) reported 2.8g glucose/10g dry switchgrass

and 2.5g glucose /10g dry Bermuda grass from enzymatic hydrolysis using microwave heating with 3% (w/w) NaOH pretreatment.

- **Ethanol Yield of Microwave and Dilute Ammonia Assisted – Pretreated Sweet Sorghum**

Ethanol concentration reached its highest peak at 48 h (Figure 3.1). No difference in ethanol concentration was observed at 72 h (data not shown). At the end of the fermentation process, the highest ethanol concentration among all parameters considered was 2.2g ethanol per 10g dry biomass at 130^{o}C and 1-2mm particle size. In our previous study, ethanol concentrations of 2.5g/10g dry biomass for sorghum (Salvi et al., 2009) and 2.3g/10g dry biomass for energy cane (Aita et al., 2010)were observed using conventional heating at 160 ^{o}C.

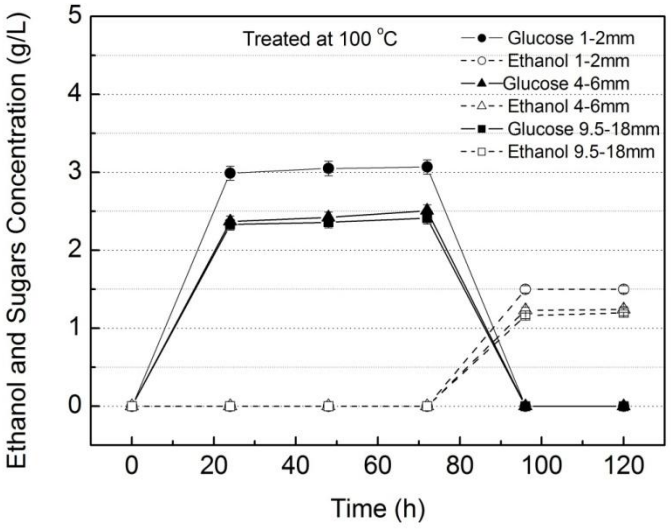

Figure 3.1 Enyzmatic hydrolysis and fermentation of sorghum pretreated at 100 ^{o}C at an enzyme concentrations of 60 FPU Spezyme CP and 64 CBU Novozyme 188/g glucan

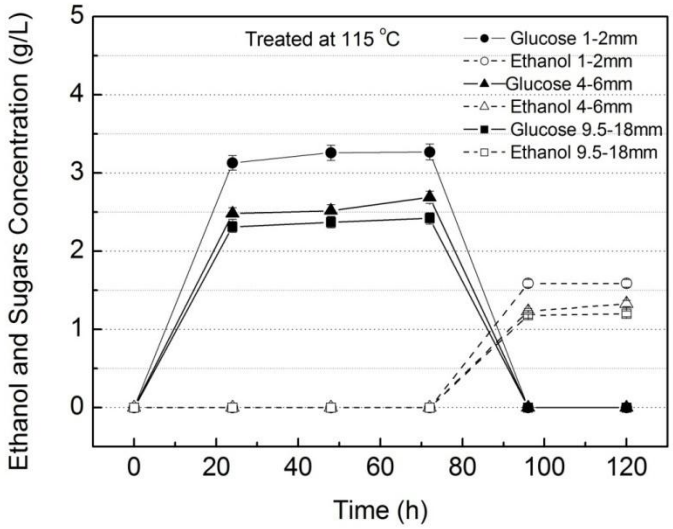

Figure 3.2 Enyzmatic hydrolysis and fermentation of sorghum pretreated at 115 ^{o}C at an enzyme concentrations of 60 FPU Spezyme CP and 64 CBU Novozyme 188/g glucan

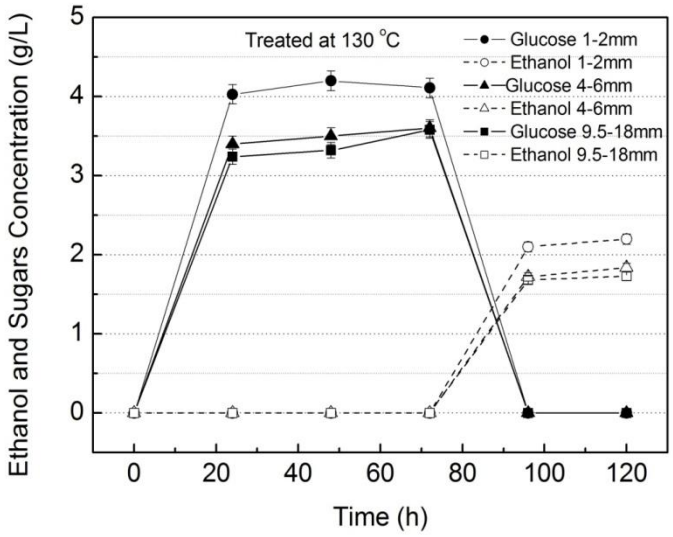

Figure 3.3 Enyzmatic hydrolysis and fermentation of sorghum pretreated at 130 ^{o}C at an enzyme concentrations of 60 FPU Spezyme CP and 64 CBU Novozyme 188/g glucan

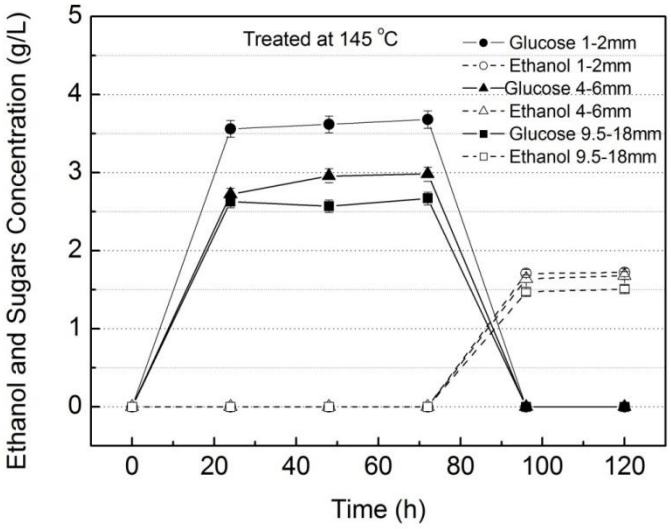

Figure 3.4 Enyzmatic hydrolysis and fermentation of sorghum pretreated at 145 °C at an enzyme concentrations of 60 FPU Spezyme CP and 64 CBU Novozyme 188/g glucan

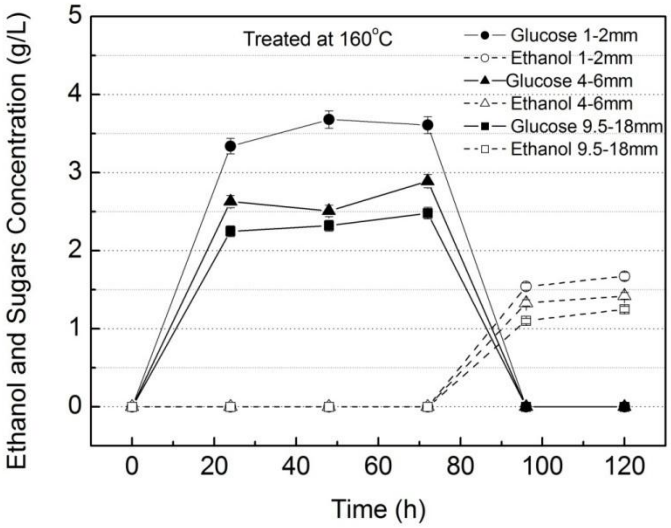

Figure 3.5 Enyzmatic hydrolysis and fermentation of sorghum pretreated at 160 oC at an enzyme concentrations of 60 FPU Spezyme CP and 64 CBU Novozyme 188/g glucan

The glucose release based on microwave pretreatment is lower than the conventional heating pretreatment. However, since two different mixing methods were employed during pretreatment, it is hard to compare the results from these two pretreatment methods.

Mamma et al. (1995) reported 1.15 g/10g dry sorghum ethanol yields using a mixed culture of *Fusarium oxysporum* and *Saccharomyces cerevisiae(Mamma et al., 1995)*. Another study reported ethanol yields of 1.6–2.58 g/10g dry sorghum by fermentation of soluble as well as insoluble sugars from sorghum juice and fiber (Mamma et al., 1995). Yu et al. (Yu et al., 2008) obtained higher ethanol yields (3.16 g ethanol/ 10g dry sorghum) by fermenting both 30% sulfuric acid-treated sorghum and sorghum juice. Both sorghum juice and fibers were fermented to ethanol using a mutant strain of baker yeast in their studies (Yu et al., 2008). Zhu et al. (2006b) was able to achieve ethanol yields of 3.11g ethanol/10g dry wheat straw using the microwave-assisted alkali pretreatment.

- **Energy Balance Studies**

Energy gain is also a very important parameter to evaluate the performance of different pretreatment conditions. If the energy gain is less than 0, it means the energy of the ethanol produced via this pretreatment condition is lower than the energy input of the pretreatment. On the contrary, if the energy of the ethanol produced is larger than the energy input from the pretreatment, then we could assume this particular pretreatment condition works. From Figure 3.6, we can see that the highest energy gain is 218 kJ at 130 ^{o}C using the 1-2mm particle size sorghum fibers, which is higher than the energy gain from conventional heating at 160 ^{o}C. It is noticed that when the pretreatment temperature at 145 ^{o}C and 160 ^{o}C, the energy gain were near 0. Therefore, 130^{o}C could be deemed as a critical temperature in this study. Particle size has an

evident effect on the energy gain too. The smaller the particle size, the more the energy we could obtain. Taking the energy input of grinding into account, similar results were observed as well. When the temperature was over 130 ^{o}C, the energy gain was very low and even less than 0 at 160^{o}C. The smaller particle size has a positive effect on the energy gain. The energy balance studies demonstrate that using microwave heating for ammonia pretreatment has an advantage over conventional heating.

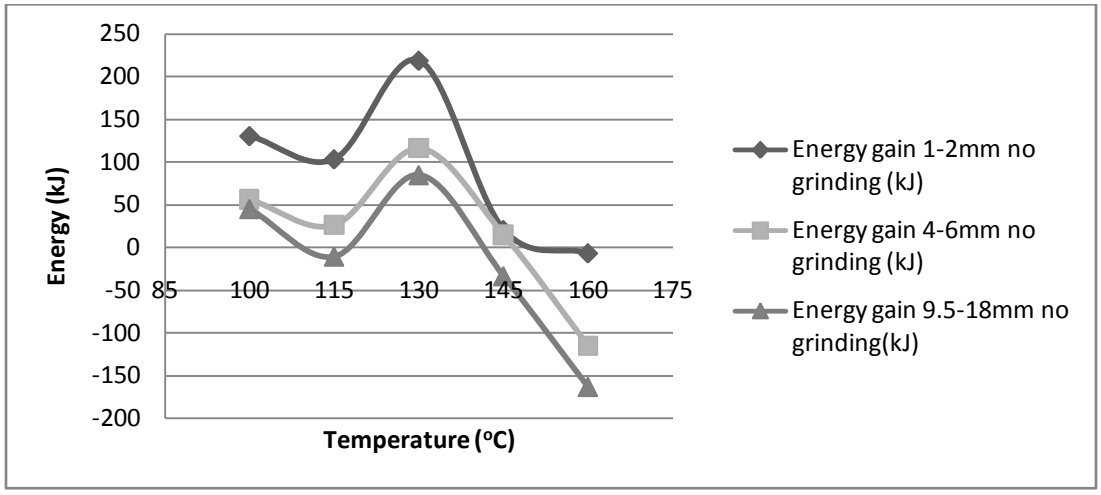

Fig 3.6 Energy gain by the pretreatment without the consideration of the energy input of grinding

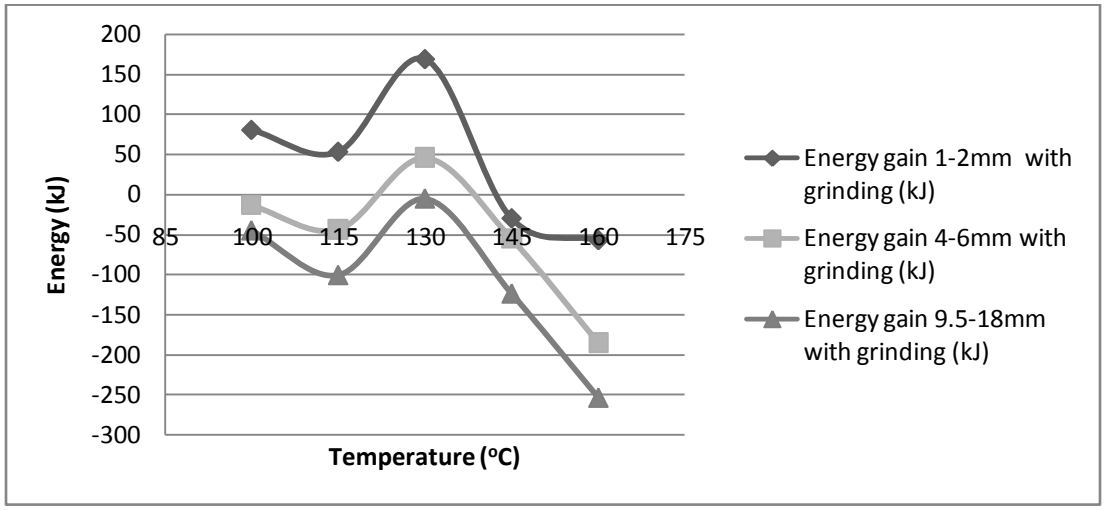

Fig 3.7 Energy gain by the pretreatment with the consideration of the energy input of grinding

- **Analytical Studies**

Maiorella et al. (1983) reported that concentrations of acetic acid (0.5–9 g/l), lactic acid (10–40 g/l), and formic acid (0.5–2.7 g/l)inhibited *Saccharomyces cerevisiae* by interfering with functions involved in cell maintenance (Maiorella et al., 1983). Glycerol at a concentration of 450 g/l alters the cell's osmotic pressure (Maiorella et al., 1983), and furfurals at concentrations of 3 g/l are considered antagonistic to cell growth (Palmqvist and Hahn-Hägerdal, 2000). In our study, concentrations of acetic acid (0.7 g/l), lactic acid (0.04 g/l) formic acid (0.14 g/l), glycerol (2.3 g/l), HMF and furfurals (<0.1g/L) were insufficient to produce any inhibitory effect.

3.4 Conclusions

Microwave assisted dilute ammonia treatment of sorghum biomass was evaluated based on the enzymatic hydrolysis and fermentation results. Glucose fermentation with *S.cerevisiae* resulted in 2.2g of ethanol/10g dry sorghum biomass using 1-2mm particle size of sorghum fibers under 130 ^{o}C for 1h. Glycerol, organic acids and furfurals concentrations were insignificant.

CHAPTER 4
PRETREATMENT OF SORGHUM BY NONIONIC SURFACTANT-ASSISTED DILUTE AMMONIA WITH MICROWAVE HEATING FOR ENHANCING ENZYMATIC HYDROLYSIS AND ETHANOL PRODUCTION

4.1 Introduction

The increased concern for the availability and cost of the petroleum supply and the negative impact of fossil fuels on the environment, particularly greenhouse gas emissions, has imposed a critical need on the society to identify and develop renewable fuel alternatives (Hahn-Hägerdal et al., 2006). As it was discussed in the previous chapters, ethanol production from lignocelluosic biomass is one of the most feasible pathways toward the production of renewable and sustainable liquid transportation fuels.

In our previous study, we investigated the effect of microwave assisted dilute ammonia pretreatment on the hydrolysis and fermentation of pretreated sorghum fibers. In order to improve the performance of microwave assisted dilute ammonia heating pretreatment process, we hypothesized that the addition of a non-ionic surfactant during pretreatment would improve hydrolysis and fermentation yields. Some studies has shown that the addition of a surfactant, such as Tween 80 or Tween 20 during enzymatic hydrolysis increases the conversion of cellulose to glucose as it improves enzyme stability, transforms the substrate structure and facilitate the interactions between enzyme and substrate (Castanon and Wilke, 1981; Eriksson et al., 2002). Additionally, Tween 80 has been demonstrated to improve lignin solubility and lignin removal during the pretreatment stage (Qing et al., 2010).

The lignin is widely known for its barrier nature to the efficient enzymatic hydrolysis since its unproductively adsorption of a large amount of the cellulase and the impeding enzyme

access to the substrate (Lu et al., 2002) Therefore, it would be beneficial to maximize lignin removal in order to enhance enzymatic hydrolysis yields (Qing et al., 2010).

There is limited information on the use of surfactant and dielectric heating to improve the performance of pretreatment. The goal of this study was to investigate the effect of surfactant-assisted dilute ammonia pretreatment by microwave heating on the hydrolysis of sweet sorghum.

4.2 Materials and Methods

- **Biomass Preparation**

Sorghum (*Sorghum bicolor* (L.) Topper) was processed by the same procedure as discussed in the previous chapter. Sorghum fibers (15g dry weight) were pretreated with ammonium hydroxide (28% v/v solution, Fisher Scientific) and water at a ratio of 1:0.5:8, combined with 0, 1.5% and 3% (w/w) Tween 80, for 45 min and 30min at 130 °C using microwave heating. The effect of particle size was not investigated in this chapter.

- **Measurement of Biomass Porosity**

The same procedure as Chapter 3

- **Inoculum Preparation**

The same procedure as Chapter 3

- **Enzymatic Hydrolysis**

The same procedure as Chapter 3

- **Fermentation**

The same procedure as Chapter 3

4.3 Results and Discussion

- **Effects of Surfactant Concentration on the Sugar Yield of Microwave Assisted Dilute Ammonia-Pretreated Sorghum Fibers**

It has been established for some time that the addition of surfactants to enzymatic hydrolysis of lignocelluloses can increase the conversion of polysaccharide fraction from lignocelluloses into fermentable sugars (Kumar and Wyman, 2009). The experiments were carried out to investigate if the use of surfactant could improve the enzymatic conversion of surfactant-assisted dilute ammonia pretreated sorghum bagasse. Pretreatment containing 3% Tween 80, resulted in maximum glucose and xylose yields post enzymatic hydrolysis (Figure 4.1). Glucose concentration was 3.81 g/L after 72 h hydrolysis. This yield corresponds to an improvement of 14.76 % in cellulose conversion as compared to 3.32g/L for the enzymatic hydrolysis of pretreated sorghum fibers without Tween 80. At 1.5% Tween 80, 3.61g/L glucose was released after 72 h hydrolysis, corresponding to 8.7% improvement over control. Similar effect has been reported on the enzymatic conversion of newspaper pretreated with only 0.5 % Tween 80 (Kim et al., 2006). The mechanism for this phenomenon has not been established (Kim et al., 2006; Qing et al., 2010). However, non-ionic surfactants, such as Tween 80, reduce the amount of cellulolytic enzymes adsorbed on the lignin fraction, leaving more free cellulases available for cellulose hydrolysis (Qing et al., 2010). The higher the surfactant concentration, the more sugars were released. At this time it was observed that the sorghum fibers released 2.9g/L glucose after 72h with the addition of 3% Tween 80; 2.7g/L glucose with the addition of 1.5% Tween 80 and 2.5g/L glucose without the addition of Tween 80 during pretreatment. The difference in glucose release from sorghum fibers pretreated for 30 min at the various Tween 80 concentrations was smaller than the fibers pretreated with Tween 80 for 45min. The amount of

xylose released was also affected by the different concentration of Tween 80 used during pretreatment.

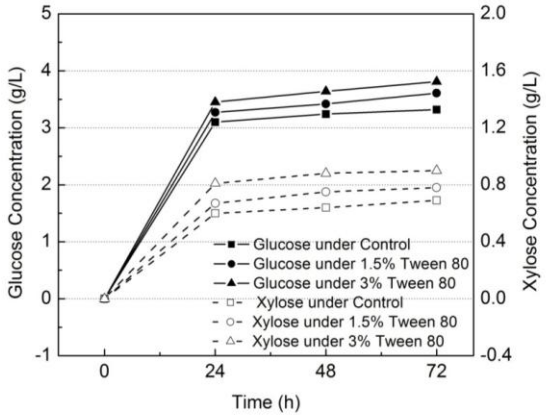

Fig 4.1a Pretreatment of sorghum fibers for 45mins. The control is the sorghum bagasse treated at the same conditions without Tween 80

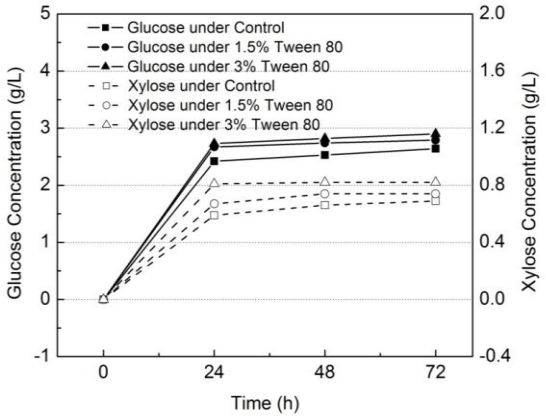

Fig 4.1b Pretreatment of sorghum fibers for 30mins. The control is the sorghum bagasse treated at the same conditions without Tween 80

- **Effects of Pretreatment Time on the Sugar Yield of Microwave-Assisted Dilute Ammonia Pretreated Sweet Sorghum**

Reaction time is an important parameter to be considered while evaluating pretreatment technologies. Long reaction times can destroy the crystalinity structure of the biomass enhancing enzymatic accessibility to the biomass and can also result in sugar degradation and increase energy consumption. The two pretreatment times (45 min and 30 min) selected in this study showed significant differences in sugar yields (Figure 4.2). Longer reaction times increased sugar yields. Increasing the reaction time from 30 to 45 min resulted in an increase in glucose concentration from 2.90 g/L to3.81g/L with the addition of 3% Tween 80. Xylose concentration increased from 0.7g/L to 0.9g/L, respectively. The surfactant effect on the release of arabinose and mannose, however, was negligible (data was not shown).

- **Effects of Surfactant Concentration and Pretreatment Time on the Ethanol Yield of Microwave-Assisted Dilute Ammonia Pretreated Sweet Sorghum**

Ethanol yields are directly depended on the glucose release during enzymatic hydrolysis. The maximum ethanol yield of 1.9g per 10g dry biomass was observed with the addition of 3% Tween 80 pretreatment of 45 min. Biomass treated with 1.5% Tween 80 produced 1.6g per 10g dry biomass. It demonstrates again that high concentration of surfactant and long pretreatment time is conducive to both the hydrolysis and fermentation process.

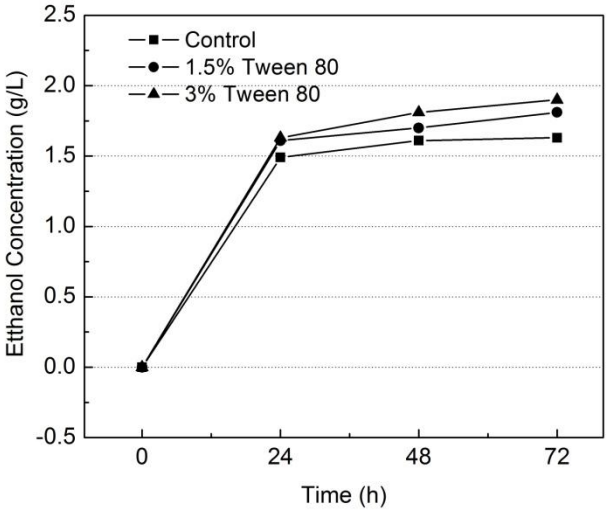

Fig 4.2.a Pretreatment of sorghum fibers for 45mins. The control is the sorghum bagasse treated at the same conditions without Tween 80

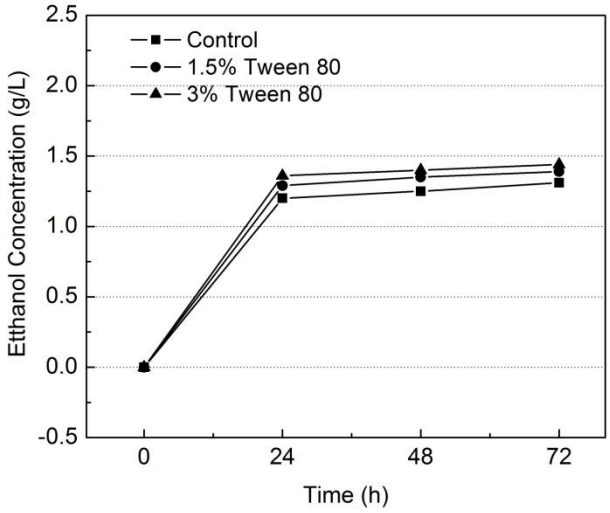

Fig 4.2.b Pretreat sorghum fibers for 30mins. The control is the sorghum bagasse treated at the same conditions without Tween 80

- **Biomass Porosity**

Most blue and orange dyes were absorbed on sorghum fibers treated at 130°C for 45min with 3% Tween 80 (Figure 4.3). Since one of the purposes of pretreatment is the disruption of the biomass structure to increase porosity, a higher temperature and larger surface area will result in a more severe pretreatment causing more disruption to the biomass structure.

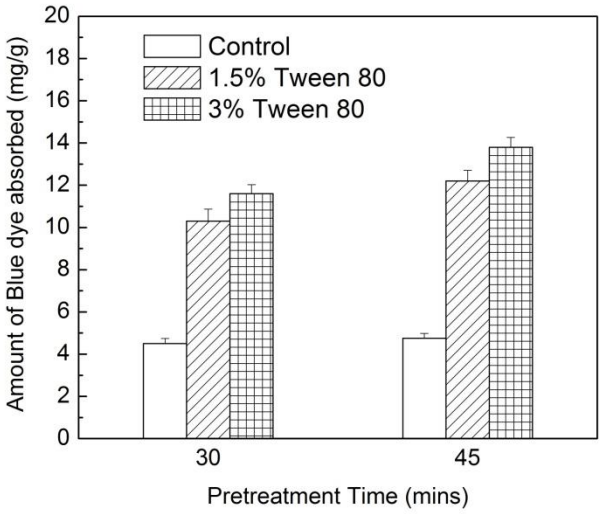

Figure 4.3a Amount of blue dye absorbed by biomass. The control is the sorghum bagasse treated at the same conditions without Tween 80

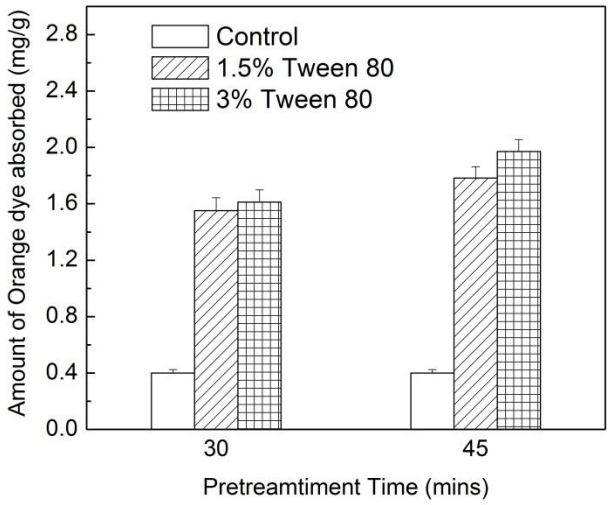

Figure 4.3b Amount of orange dye absorbed by biomass. The control is the sorghum bagasse treated at the same conditions without Tween 80

4.4 Conclusion

Pretreatment of sweet sorghum fibers with combined use of diluted ammonia and Tween 80 (0-3%) in the presence of microwave was investigated in an attempt to enhance the hydrolysis and fermentability of pretreated sorghum. The results show that sweet sorghum fibers pretreated for 45 min with 3% Tween 80 resulted in the release of 3.81g/L glucose as compared to sorghum fibers without the addition of Tween 80. Simon's stain method indicated a positive correlation between sorghum fibers porosity and concentration of Tween 80.

CHAPTER 5
ETHANOL PRODUCTION FROM SORGHUM BY A MICROWAVE ASSISTED DILUTE AMMONIA EXPLOSION PRETREATMENT

5.1 Introduction

The depletion of fossil fuels and the increased concerns about greenhouse gas emissions has led to a worldwide interest in developing renewable energy such as fuel ethanol (Lynd, 1996). First generation of green fuel is the ethanol produced from food resources, such as potato and corn. However, food-based ethanol will not be enough to satisfy the needs of society nowadays. Lignocellulosic biomass are very abundant resources, composed of carbohydrate polymers (cellulose and hemicelluloses), and lignin bound together in an intricated matrix with varying compositions amongst plant materials (Aita and Kim, 2010). The carbohydrate polymers can be degraded into six-carbon sugars (glucose and arabinose) and five-carbon sugars (xylose and mannose). Lignocellulosic biomass, such as agricultural residues, forest products and dedicated energy crops represents a renewable and sustainable resource for bio-ethanol production (Liu et al. 2008). Due to the recalcitrance nature of the lignocellulose, pretreatment is the key step to break down and disrupt this matrix to allow for the accessibility of enzymes to cellulose and hemicellulose for the release of sugars into their monomeric form (Kim et al., 2010). An effective pretreatment method aims at removing most of the lignin while at the same time, retaining most of the cellulose and hemicelluloses (Mosier et al., 2005). Currently, various pretreatment methods, such as steam explosion, supercritical fluids, alkaline hydrolysis, AFEX, acid hydrolysis, organic solvents, have been widely investigated (Liu and Wyman, 2005; Puri and Pearce, 1986; Redding et al., 2011; Wyman, 1996; Zhao et al., 2009). Most of these pretreatment methods utilize conventional heating. Dielectric heating, which includes microwave, and radio frequency, is an alternative method to conventional heating. Most methods,

including those based on microwave heating; require large amounts of water in the process. A radical improvement over current technologies would be if the amount of water in the pretreatment process could be reduced. Among the multiple advantages and benefits derived from this approach, include but are not limited to energy savings from water heating and subsequent simplification of downstream operations, including water treatment and disposal.

The fundamental of the microwave heating process have been discussed in previous chapters. Based on a fundamental approach, microwave heating could potentially offer these advantages from the perspective of reducing water consumption. In other fields, microwave explosion pretreatment as a novel technology for wood modification has been evaluated in recent years by several scientists. Li et al. (2010) reported the used of microwave explosion pretreatment of wood in a high intensive microwave electromagnetic field over a very short time. The moisture inside the biomass vaporizes quickly and the water steam creates a large internal vapor pressure within the wood cells. Under high internal pressure the weaker elements of wood structures, such as thin-walled cells and pit membranes, are ruptured to form many pathways for easy transportation of liquids and vapors through the lignocelluloses complex, increasing biomass permeability (Li et al., 2010).

In our previous study, microwave heating was used to pretreat sorghum fibers together with an ammonia solution. Ammonia, which is a relatively less expensive chemical, is a proper choice for pretreatment since it is a selective reagent for lignin.

Due to the existence of a large amount of water, the explosion effect of microwave may not happen in the heating process. In order to create the microwave explosion effect of the biomass, much lower water content was used compared to our previous study. The goal of this

study was to investigate the effect of microwave-assisted dilute ammonia explosion pretreatment on the structure and composition of sweet sorghum.

5.2 Materials and Methods

- **Biomass Preparation**

Sorghum (*Sorghum bicolor* (L.) Topper) was harvested from the Hill Farm Research Station (Homer, LA). Leaves, roots, and grains were removed and the stalks were crushed in a roller press (Farrel Company, Ansonia, CT) three times to extract the juice. Pretreatments were carried out at 130^{o}C. The effect of the particle size was not investigated for the explosion pretreatment method. Two pretreatment methods were used in this study: draining and spraying. For the draining pretreatment method, sorghum fibers (15g dry weight) were soaked with ammonium hydroxide (28% v/v solution) and water at a ratio of 1:0.5:8, respectively, for 1 h at 20^{o}C then drained for 1 h. Fibers were collected after draining and subjected to microwave heating at 130 ^{o}C for another hour. For the spraying method, sorghum fibers (15g dry weight) were sprayed by ammonium hydroxide (28% v/v) at a ratio of 1:0.5 respectively, kept in a sealed plastic bag for 1 h at 20 ^{o}C then subjected to microwave heating at 130 ^{o}C for an additional hour. Untreated and dilute ammonia pretreated biomass were dried overnight to 20% moisture in an oven at 40–45 ℃ and stored for further studies. Untreated biomass was used as control. Composition analysis was conducted following NREL 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.

- **Inoculum Preparation**

The same procedure as Chapter 3

- **Enzymatic Hydrolysis**

  The same procedure as Chapter 3

- **Fermentation**

  The same procedure as Chapter 3

5.3 Results and Discussion

- **Sugar Yield of Microwave-Assisted Dilute Ammonia Explosion of Sweet Sorghum**

Changes of sugars and ethanol concentrations during hydrolysis and fermentation are depicted in Figure 5.1It was observed that the spraying method resulted in 3.44 g glucose per 10g dry biomass as compared to 3.18g glucose per 10g dry biomass for the draining method post enzymatic hydrolysis. The glucose yields were lower than results (4.2 glucose /10 g dry biomass) obtained with microwave assisted dilute ammonia pretreatment at 130C for 1-2mm particle size sorghum fibers. However, the results obtained with both explosion pretreatment methods are similar to the sugar yields obtained from sorghum samples at 4-6mm particle size. Since the results from the spraying pretreatment method and draining methods were similar, the spraying method could be used without an input of excess water. The solid content in this study was at 67% in the chemical solution which could be deemed as no fluid conditions. Therefore, both the reactor volume required and the capital cost could be greatly reduced (Hu et al., 2008). Hu et al (2008) reported the release of 21 g glucose/100 g dry biomass in switchgrass pretreated by radio frequency-based dielectric heating at 50% solid content.

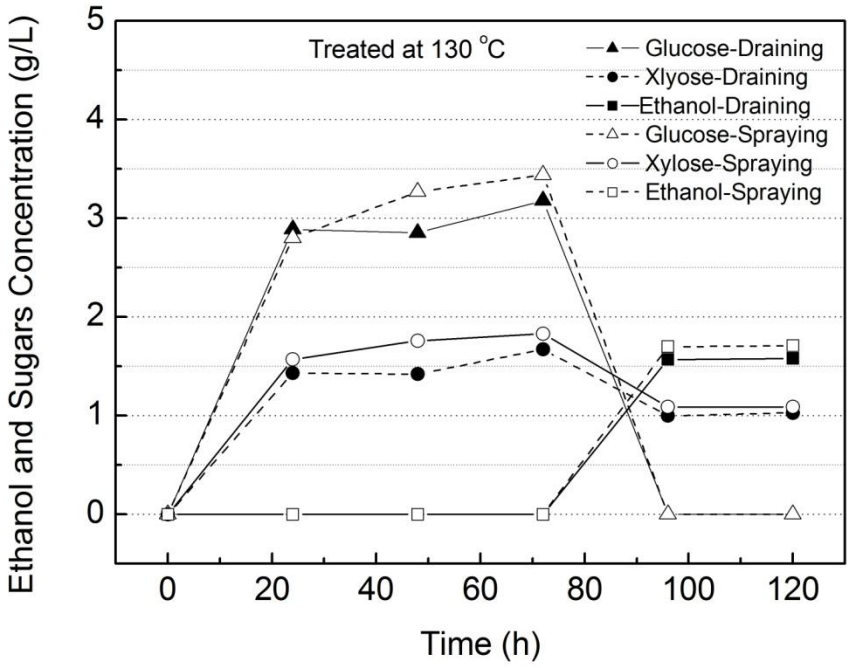

Figure 5.1 Sugars and ethanol concentrations of sorghum bagasse pretreated at 130 ^{o}C. Enzymes were used at a concentration of 60 FPU Spezyme CP and 64 CBU Novozyme 188/g glucan

- **Ethanol Yield of Microwave-Assisted Dilute Ammonia Explosion of Sweet Sorghum**

Ethanol concentration reached its highest peak at 48h (Figure 6) at the end of the fermentation process, the highest ethanol concentrations was 1.7g ethanol per 10g dry biomass using the spraying method and 1.6g ethanol per 10g dry biomass using the draining method. The difference between these two pretreatment methods indicates that soaking the biomass with the mixture of ammonia and water is not necessary for microwave explosion pretreatment. Therefore, the spraying method can save water to a certain extent as compared to microwave-assisted dilute ammonia pretreatment. Our prior study with dilute ammonia and conventional heating at 160 ^{o}C resulted in 2.5 g ethanol per 10 g dry energy cane bagasse (Aita et al., 2010)

and 2.3 g ethanol per 10 g dry sorghum bagasse (Salvi et al., 2009). The energy gain obtained from the spraying method is 444.2kJ for not heating with the eight parts of water, which is higher than the energy gain from the normal microwave heating pretreatment (218 kJ) and conventional heating (193 kJ).

5.4 Conclusion

The effects of two methods of microwave assisted dilute ammonia explosion treatment of sorghum bagasse were evaluated on enzymatic hydrolysis and fermentation yields. No evident difference between the spraying and draining method pretreatment was observed, indicating excess water is not necessary when using microwaves as the energy source for pretreatment. The glucose yields obtained from hydrolysis stage for spraying and draining method were 3.44 g and 3.18g per 10 g dry biomass, respectively. Glucose fermentation with *S.cerevisiae* resulted in 1.7g and 1.6 g of ethanol/10g dry sorghum biomass, respectively.

CHAPTER 6
SUMMARY AND FUTURE WORK

This project investigated the use of microwaves as energy source coupled with ammonium hydroxide as catalyst for the pretreatment of sorghum bagasse. In the course of this study, three different microwave assisted dilute ammonia pretreatment methods were evaluated.

For the first pretreatment method, three different particle size of sorghum bagasse were pretreated with ammonium hydroxide (28% v/v solution), and water at a ratio of 1: 0.5:8 at different temperatures for 1 h using microwave heating. From the various pretreatment conditions, the best parameters were based on improved sugar and ethanol yields. The best ethanol yields among all different pretreatment conditions were 22±1.1g/100 g dry biomass with 1-2mm particle size at 130°C for 1 h reaction time. Untreated sorghum bagasse averaged 10 g ethanol/100 g dry biomass.

The addition of a surfactant, Tween 80, at various concentrations during the pretreatment stage was performed in order to improve sugar and ethanol yields. Sorghum bagasse was pretreated with a combination of ammonium hydroxide and Tween 80 for 30 min and 45 min at 130^{o}C increased with increasing amounts of Tween 80. A concentration of 3% Tween 80 resulted in 38.1 g glucose per 100 g dry biomass, as compared to 33.2 g glucose per 100 g dry biomass for control (without surfactant) post enzymatic hydrolysis. Ethanol yield was 19 g per 100 g dry biomass, a nearly 19% improvement over the control. Pretreatment time was shortened by the addition of the surfactant.

The last pretreatment method was to evaluate the effect of microwave explosion on sorghum fibers. The pretreatment was performed by mixing sorghum fibers and 28% ammonia hydroxide solution by spraying or soaking at a ratio of 1:0.5 and heating the mixture to 130^{o}C for

1 h. Ethanol yields were 17 g/100 g dry biomass. Unterated bagasse averaged 10 g ethanol/100 g dry biomass. A significant amount of water and energy could be saved by this method.

- **Future Work**

Future work will focus on the scale up of microwave-based pretreatment of lignocellulosic biomass. Radio frequency heating as the energy source for pretreatment will be studied as well. We also would like to apply the microwave-base pretreatment techniques to other feedstocks, such as sugarcane bagasse, energy cane bagasse and other agricultural residues.

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APPENDIX: PROCEDURE FOR SIMONS' STAIN METHOD

1. Preparation of Staining Solution

1.1 Prepare 1% (w/v) solution of the low molecular weight blue dye (Direct Blue 1).

1.2 Prepare 1% (w/v) of the orange dye (Direct Orange 15). The orange dye contains two fractions based on molecular weight: high and low. For this procedure, the high molecular weight fraction must be isolated.

1.3 Pour 15 ml of the 1% orange dye solution into a 50 ml ultracentrifugation tube (fitted with a 100 K membrane). Spin at 4000 rpm for 10 min. At the end of the cycle, approximately 25-30% of the original volume should be retained.

1.4 Measure the density of the retentate and dilute appropriately to obtain a 0.2% w/v solution.

1.5 Prepare 1:1 staining solution mixture from the 1% blue dye and 0.2% orange dye.

2. Staining of the Biomass Sample

2.1 Weigh 50 mg of the biomass into a 125 ml Erlenmeyer flask and add 15 ml of the staining solution.

2.2 Incubate in a water bath set at 75 ℃ for 48 h.

2.3 After 48 h, filter each sample through a crucible. Make sure all solids are transferred from the flask to the crucible.

2.4 Wash the recovered solids with approximately 30 ml of cold DI water.

3. Stripping of Dye Molecules from Stained Biomass

3.1 Transfer the recovered solids into a 125 ml Erlenmeyer flask and add 40 ml of 25% pyridine stripping solution.

3.2 Incubate in a water bath set at 45 ℃ for 18 h.

3.3 After 18 h, filter each sample though a crucible and store approximately 10 ml of the filtrate for analysis.

4. Filtrate Analysis

4.1 Measure of the absorbance of the filtrate at 450 nm and 621.5 nm using 25% pyridine solution as a blank.

4.2 Use the following equations to calculate the concentration (g/L) of the blue and orange dyes in the filtrates:

$A_{450} = \varepsilon_O/450*L*C_O + \varepsilon_B/450*L*C_B$

$A_{621.5} = \varepsilon_O/621.5*L*C_O + \varepsilon_B/621.5*L*C_B$

In the above equations, A450 and A621.5 are the absorbances of the filtrate measured at 450 nm and 621.5 nm respectively, L is the width of the cuvette (1 cm) and ε is the extinction coefficient of each dye in the staining mixture at the respective wavelengths. From literature review, these values were determined to be: $\varepsilon_O/450 = 50.67$ L/g cm, $\varepsilon_B/450 = 1.97$ L/g cm, $\varepsilon_O/621.5 = 0.075$ L/g cm, $\varepsilon_B/621.5 = 15.65$ L/g cm.

5. Safety Considerations

5.1 Pyridine is a flammable chemical and should be stored in a cabinet designated for flammable chemicals.

5.2 Pyridine is a toxic chemical and must be handled using gloves and safety glasses.

VITA

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