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Analysis of properties to Distillers Dried Grains with Solubles (DDGS) and using destoner and low moisture anhydrous ammonia (LMAA) to utilize DDGS

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Analysis of properties to Distillers Dried Grainswith Solubles (DDGS) and using destoner and low moisture anhydrous ammonia (LMAA) to utilize DDGS

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DEDICATION

I dedicate this thesis to:

- My country — For blessing me with all the beauties in my life and for always protecting my right and showing me the correct way.

- My parents — The strongest persons I have ever met in my life; without them, I wouldn't have accomplished anything. Their unlimited love, sacrifices and supports throughout my life was the best encouragement for me.

- Kurt A Rosentrater — My most respect professor who has always been there as an exemplary scientist, is helping and teaching me how to show my ability and talent.

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ABSTRACT

With the rapid development of the ethanol industry, various research on distillers dried grains with solubles (DDGS) as a main co-product from the ethanol industry has been done in recent years. However, related research about basic properties to DDGS lacks of comprehensiveness. In addition, the efficient method and equipment to separate DDGS to high-valued fraction is still being investigated. Besides these, the possibility about using DDGS to produce ethanol is being explored, which is designed to own the advantages of comprehensiveness and systematicness.

This thesis is prepared in paper format, and is comprised of three manuscripts, as follow: the first part was to examine 18 samples from 10 plants in Midwest area and utilize standard laboratory methods to measure a series of properties. Final results showed moisture content of 8.69% (w.b.), water activity of 0.55, angle of repose of 48.04 º, geometric mean diameter (dgw) of 0.74 mm, geometric standard deviation (Sgw) of 1.72 mm, loose bulk density of 483.9 kg/m^3, packed bulk density of 568.5 kg/m^3, Hunter L of 56.71, Hunter a of 13.85, Hunter b of 46.51, shear strength of 0.0324 kg/cm^2. So it represents another step toward a complete baseline understanding of DDGS.

The second part was to use a destoner fractionation process for separating distillers dried grains with solubles (DDGS) into streams with various compositions. Results showed that destoner fractionation was somewhat efficient and effective. Runs with 8° angle and 27.5 percent air flow resulted in the highest value of protein and oil,

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which the light fraction had 28.15% protein, 10.50% oil, while the heavy fraction had 31.30% protein and 17.20% oil. Particle size distribution had a positive correlation coefficient (0.93) with oil parameters and a negative correlation coefficient (-0.96) with moisture parameters. Fiber had no relationship with particle size, and protein had a weak correlation coefficient with (-0.54) to particle size.

The third part described to use low-moisture anhydrous ammonia (LMAA) to pretreat DDGS and discussed varieties conditions to optimize the reaction. In previous research, low-moisture anhydrous ammonia (LMAA) pretreatment was investigated due to its high efficiency and less washing compared to other pretreatment methods. The final result showed that lower ammonia loading rate, higher moisture content, higher temperature and longer pretreatment time is evidently to improve the effect of ammonia to break lignocelluloses structure in DDGS, which can improve the hydrolysis of enzyme. Optimal LMAA conditions for DDGS were 80° C, 60% moisture content and 0.1 kg anhydrous NH_3/kg dry biomass with a 168h pretreatment time. Also comparing with other methods, LMAA to DDGS has a higher efficiency and environmental conservation, which is potentially fit for industry produce. In the future study, the financial analysis to this method will be done to discuss the possibility of LMAA in industry produce.

CHAPTER 1: INTRODUCTION

Objectives

The research objectives for this thesis were:

1. Investigate basic properties of contemporary DDGS, including moisture content, water activity, angle of repose, particle size, bulk density, color and shear strength, from ten dry grind corn ethanol facilities in the Midwest U.S.
2. Explore whether using a destoner is a reliable and useful method to separate DDGS into various compositions. In addition, using results from particle size, this study evaluated the relationships between particle size and chemical content, including protein, moisture, fat and fiber.
3. Explore whether using LMAA is a reliable and useful method to break down lignocellulose structure and pretreat DDGS, which can be used for enzymatic hydrolysis.

Thesis organization

This thesis contains one chapter of introduction, one chapter of literature review, three chapters of descriptive research procedures and results, one chapter of overall conclusions and future work, as well as cited references and acknowledgements.

The body of this thesis is divided into five chapters. Chapter 1 is an introduction that includes the objectives, organization of this thesis, and author's role. Chapter 2 is a literature review that includes properties of distillers dried grains with solubles (DDGS),

methods of separation to DDGS and pretreatment methods to DDGS. Chapter 3 entitled "Some properties of evolving distillers dried grains with solubles (DDGS) in 2012" is a research article modified from a manuscript to be submitted to the conference of ASABE 2013. In this work, several basic properties of contemporary DDGS from the Midwest U.S were investigated and compare the data with other research groups. Chapter 4 entitled "Fractionation of Distillers Dried Grains with Solubles (DDGS) through a Destoner" is a report of the trial tests of a new machine scheme in which uses detoner to separate DDGS into various fractions and evaluate the relationships between particle size and chemical content, including protein, moisture, fat and fiber. This report is modified from a manuscript to be submitted to the conference of ASABE 2013.The fifth chapter is a research paper in which a low-liquid pretreatment method of DDGS using aqueous ammonia is proposed. In this work, the various factors that might influence the pretreatment effectiveness including temperature, ammonia loading rate and pretreatment time, were evaluated and the potential relation between effect factors was also investigated via various statistics tests. This chapter is modified from a manuscript to be submitted to the conference of ASABE 2013.

Author's role

The author of this thesis has made a direct and substantial contribution to the work reported in this thesis. The author participated in conceiving and designing the study with major professor. The author was the main person who performed the lab procedures as well as the collection, analysis and interpretation of experimental data as

described in this thesis. The author was also responsible for writing the manuscripts based on the research approaches and the results obtained.

CHAPTER 2: LITERATURE REVIEW

This chapter will discuss three major topics, including 1) properties to DDGS 2) using destoner to separate DDGS 3) using the method of LMAA to pretreat DDGS for higher efficiency to enzymatic conversion.

2.1. Properties of Distillers Dried Grains with Solubles (DDGS)

2.1.1. Introduction

With pressure from possible shortage of fossil fuels, bioethanol as a fuel additive is gradually utilized to reach the demand for fuel (Schnepf and Yacobucci, 2013). Conversion corn to ethanol is an efficient method in the US ethanol industry, and has grown rapidly in recent years. In 2011 United States fuel ethanol production was the top producer in the world (RFA, 2012), which reached 13.9 billion U.S. liquid gallons (52.6 billion liters). According to Rosentrater and Muthukumarappan (2006), more than 95% US fuel ethanol plants are used corn as a major raw material to produce ethanol.

2.1.2. Distillers Dried Grains with Solubles (DDGS)

Dried Distillers Grains with Solubles (DDGS) is wet distillers grains (WDG) that has been dried with the concentrated thin stillage to 10~12 percent moisture. In the corn-based fuel manufacturing, bioethanol, distillers dried grains with solubles (DDGS) (or other co-products), and carbon dioxide are three main products. Among all products

from bioethanol industry, DDGS is an important ingredient, which is packaged and traded as a commodity feed product in US.

2.1.3. Basic Properties of DDGS

Common physical properties of DDGS include particle size, loose bulk density, packed bulk density, and angle of repose; these influence how much of the product can be stored in a given volume (Ileleji et al., 2008). In addition, moisture content, water activity and shear strength also affect the storability and material milling properties of DDGS. Because of the affection for reducing transportation costs and microbiological safety, moisture content is typically recommended to DDGS for feed products. Due to quantify the amount of “free” water for use by microorganism and chemical agents, water activity is a measurement of susceptibility to spoilage and deterioration during the storability and milling (Liu and Rosentrater, 2012). Shear strength is an indirect property to measure flowability parameter, which affects the strength of DDGS and flow problems when DDGS exposed to compressive stress during storability and milling (Ganesan et al, 2007). However, large variations in physical properties have been reported by different research groups over the years. (Shurson, 2005; Rosentrater, 2006; Ileleji et al., 2007).

2.1.3.1. Moisture Content

Most ethanol plants currently dry DDGS to a moisture level of approximately 10% to 12%. This moisture content is typically recommended for feed products, which is

due to reduce transportation costs and increase microbiologically safety (Liu and Rosentrater, 2012). Bhadra et al. (2009) obtained the result of moisture content with 4.32 - 8.89 (%, db), but Rosentrater (2006) obtained a higher value with 13.2 - 21.2 (%, db).

2.1.3.2. Water Activity

Water activity is a vapor pressure of water in a substance divided by that of pure water at the same temperature. Therefore, it is a measure of the energy status of the water in a system, and it directly affects the activity of microbes. Prezant et al. (2007) has shown that most bacteria are adapted for growing in an environment with a water activity of 0.9, mold is adapted to between 0.7 and 0.8, yeast is adapted more than 0.7, and very little microbial growth can occur if the water activity is below 0.65. Thus, water activity results are related to moisture content, and should be limiting to microbe growth. The samples in this study have a low water activity, which means a small probability of spoilage problems, DDGS should still be stored in bulk cautiously, in case of potential moisture migration from the environment, especially during the shipping. Rosentrater (2006) got a result of water activity with 0.53-0.63.

2.1.3.3. Angle of Repose

Angle of repose is defined as the angle that forms between a horizontal plane and the slope of a pile (at rest) that has been formed by dropping the bulk material from some elevation (Liu and Rosentrater, 2012). Angle of repose is a function of physical properties of the particles, including size, shape and porosity. Rosentrater (2006) got

angle of repose with 26.5^{o} - $34.\ 7^{o}$, but Bhadra et al. (2009) found a higher value with 35.94^{o} - $41.\ 60^{o}$.

2.1.3.4. Particle Size

Particle size distribution is a very important property, as it affects other properties including bulk density and angle of repose. Generally, the finer particle size directly leads to the greater surface area and more contact points, which means the smaller interstitial air spaces between particles. Consequently, it can cause greater compressibility, higher cohesive bulk strength and lower flowability (Liu and Rosentrater, 2012). By using a series of six selected sieves (Nos. 8, 12, 18, 35, 60, and 100), Liu (2008) measured surface color and moisture, protein, oil, ash and starch in both original samples and sieved fractions. That research indicated that there was a great variation in composition and color among DDGS from different plants. It may be feasible to fractionate DDGS for compositional enrichment based on particle size, which could be a vital addition to quality of DDGS. Clementson and Ileleji (2012) utilized three samples to measure morphological and chemical characteristics of DDGS produced by mixing three levels of condensed distillers soluble (CDS) with wet distillers grains and drying according to official methods (AOAC, 2002). Results showed that pore volume, particle porosity and effective bulk porosity decreased when CDS level increased. Furthermore, they observed that heterogeneity and particle segregation could cause sampling errors, and as a consequence nutrient and bulk density variability.

2.1.3.5. Bulk Density

Another key property is bulk density. Bulk density directly affects the cost for shipping of DDGS (Ileleji et al. 2008). Clementson and Ileleji (2010) designed a simulated apparatus to investigate the bulk density variability of DDGS during filling of railcar hoppers, and found that there was a significant difference between the initial and final measures of bulk density and particle size as the hoppers were emptied in both mass and funnel flow patterns, which was caused by particle size variations.

2.1.3.6. Color

Color is considered to be an indicator of nutritional quality by various research groups, which is related to amino acid digestibility (Batal and Dale, 2006; Fastinger and Mahan, 2006). Hunter L-a-b three-dimensional color space is the most common quality control parameter to test color of DDGS, which found that more yellow DDGS had a better quality (Liu and Rosentrater, 2012). Hunter L-a-b three-dimensional color space is organized in a cube form, which reflects the differences between points plotted in the color space correspond to visual differences between the colors plotted.

2.1.3.7. Shear Strength

Shear strength is the strength of a material or component against the type of yield or structural failure where the material or component fails in shear. It is an important property directly to reflect flowability of DDGS, which is often restricted by caking and bridging during storage and transportation. Jenike (1964) was the first to apply soil

mechanics techniques to measure the flow properties of powders. He developed a shear cell suitable for industrial powders, which can test shear strength and judge the flow properties of DDGS. Ganesan et al. (2009) got the result of shear strength with 0.03 kg/m^2.

2.1.4. Conclusion

Through some research has been done to study the properties of DDGS, production processes have been changing in recent years, and oil is now commonly removed. In order to understand the changes in the DDGS industry, new baseline data about these properties should be established, because they are essential for design of equipment, processing facilities, storage and material handling systems (Rosentrater, 2011). Thus the objective of this study was to investigate basic properties of contemporary DDGS, including moisture content, water activity, angle of repose, particle size, loose bulk density, packed bulk density, color and shear strength, from ten dry grind corn ethanol facilities in the Midwest U.S.

2.2. Methods of Separation to Distillers Dried Grains with Solubles (DDGS)

2.2.1. Introduction

There are three products generated from corn-based fuel manufacturing: bioethanol, distillers dried grains with solubles (DDGS) (or other co-products), and

carbon dioxide. Marketing of DDGS as an ingredient is directly related to sustainability of the dry grind plant, and is sold at a varying market price (US$85–300/ton) (Liu, 2008).

DDGS is mainly composed of protein, fiber, and fat, and is a dry mix of particulate materials. Due to various particle compositions, with high protein and high fiber particle, a method which can divide DDGS into high protein and high fiber fractions could contribute extra economic benefit (RFA, 2012). A high protein fraction will have a greater value as a feed to animals (Belyea et al., 2004), and a high fiber fraction has more potential for corn fiber gum or raw material for lignocellulose ethanol production (Singh et al., 2002). Besides these, DDGS has a moisture level of approximately 10% to 12%, lower water activity and shear strength.

2.2.2. Sieving

Sieving is a possible method to separate the various components of DDGS. Liu (2009) sieved four commercial samples of DDGS. Sieving was effective in producing fractions with varying compositions. As the particle size decreased, protein and ash contents increased, and total carbohydrate (CHO) decreased. Winnowing sieved fractions was also effective in shifting composition, particularly for larger particle classes. In addition, Srinivasan et al. (2005) found that sieving the DDGS into various size categories and then elutriating sieved fractions oflarger size classes at appropriate air flow velocities was more effective than sieving alone in separating fiber from DDGS. Srinivasan et al. (2009) designed an experiment to sieve DDGS at a rate of 0.25 kg/s (1 ton/h), which split DDGS into four fractions; the three largest sieve fractions were then

air classified using aspirators to separate fiber. Final results showed that nearly 12.4% by weight of DDGS was separated as fiber product, and two high protein products that had low fiber contents.

2.2.3. Aspiration

Aspiration is another method which has been attempted by researchers (Garcia and Rosentrater, 2008). They used three screenings and three air classifications as unit operations to separate a variety of sizes. After milling, an aspirator was used to process to the treated DDGS, which separated it into high and low terminal velocity fractions. The combination of the undersize fraction and the low terminal velocity fraction were substantially enriched in protein. The separation achieved by this process compared favorably to other reported processes, but was less complex.

2.2.4. Destoner

A destoner is a simple and efficient machine to remove stones and soil from grains. Its principle is to use air flow and shaking to separate. The stones stay on the top of the screen and the grains through it. As shown in Figure 2.1, air from pressure fans in the base is forced up through the deck. The uniform airflow vertically separates or stratifies the material, with lighter material in the upper strata flowing down the deck. Heavy particles such as stones, glass, metal etc., travel uphill and are discharges opposite the light material. The greatest advantage of a destoner is that it is convenient and fairly

inexpensive to operate, and thus might be appropriate for industrial production with better economic benefits (Heiland and Kozempel, 1988).

2.2.5. Conclusion

For separating DDGS to various particle compositions, fractionation as an efficient method, which can divide DDGS into high protein and high fiber fractions, could contribute extra economic benefit to usage of DDGS. Through some research has been done to study fractionation of DDGS, these methods have only met with limited, varying degrees of success. All these methods are ambiguous in efficiency and economies, which is far from the goal of sustainable industrial production.

2.3. Pretreatment Methods to Distillers Dried Grains with Solubles (DDGS)

2.3.1. Introduction

As a renewable energy resource, ethanol has the potential to partly replace gasoline as a fuel. Moreover, it is harmless to the environment at some degrees, which makes it a promising alternative to gasoline (Alinia et al., 2010). Cellulose is the most polymers of lignocellulose biomass, and is the principal source of fermented sugar to produce lignocellulose ethanol (Park et al., 2010). Among the four major steps of ethanol production from lignocellulosic biomass (pretreatment, enzymatic hydrolysis, fermentation, and ethanol separation), pretreatment is the most important step because the protective structure of hemicellulose and lignin defends the cellulose from hydrolysis

(Narayanaswamy et al., 2011). Therefore, an appropriate pretreatment method plays a crucial role in the whole ethanol production process.

Many pretreatment methods have been invented and investigated in recent years. Although a few methods are effective in some certain lignocellulose biomass, they may have little effect in other biomasses. The purpose of this study is to review published papers of pretreatment methods on different lignocellulosic biomass, and investigate how these methods have been utilized. The differences of various pretreatment methods have also been compared in this study.

2.3.2. Pretreatment Methods

This chapter reviewed several main pretreatment methods for lignocellulosic biomass in the recent 30 years: Supercritical Carbon Dioxide, Lime, Liquid Hot Water, Dilute Sulfuric Acid, Ammonia Fiber Explosion, Ammonia Fiber Expansion, Aqueous Ammonia and Low Moisture Anhydrous Ammonia. These pretreatment methods have been reviewed from published studies on various lignocellulose materials for ethanol production, and how these methods have been utilized. The methodological approach for this study was to compare pretreatment conditions, reducing sugar yield, enzymatic digestibility, and lignin removal.

2.3.2.1. Supercritical Carbon Dioxide

Supercritical carbon dioxide can be as an effective extraction solvent, due to the advantages of low cost, non-toxicity, non-flammability, easy recovery and

environmental acceptability (Zheng and Tsao, 1996). Eight papers about supercritical carbon dioxide (SC-CO_2) pretreatment were chosen from published articles in recent years, and these have shown that the SC-CO_2 method was effective on some lignocellulosic biomass. With a pretreated condition of 3500 psi and 150°C, 30% moisture content corn stover could obtain a 12% higher glucose yield than untreated corn stover (Narayanaswamy et al., 2011). Using SC-CO_2 to pretreat 73% moisture content aspen at 3100 psi and 165°C, sugar yield could achieve 84.7 ± 2.6% of theoretical maximum (Kim and Hong, 2001). Glucose yield from dry guayule was 77% of the theoretical yield, after pretreatment with SC-CO_2 at 4000 psi and 200°C (Srinivasan and Ju, 2010). With a condition of 80°C and 3600 psi, the concentration of fermentable sugar from 65% moisture sugarcane bagasse (expressed as g per kg of dry bagasse) was 380.0 g/kg with 74.2% of theoretical yield (Benazzi et al., 2013), which was very near the result of Santos et al (2011) with 72.0% of glucose theoretical yield; and at the similar condition, Srinivasan and Ju (2012) obtained a little lower result, with 56% of glucose theoretical yield. For wheat straw, Alinia et al. (2010) found that combined steam explosion and SC-CO_2 was more effective than the pretreatment of SC-CO_2 alone, with a sugar yield of the combined method of 234.6 g/kg higher, which was than 208.4g/kg (glucose/wheat straw) using SC-CO_2 alone. However, SC-CO_2 pretreatment may be inefficient with some biomass, such as rice straw, switchgrass and southern yellow pine. For example, Gao et al. (2010) merely achieved glucose yield of 32.4 ± 0.5% from pretreated rice straw; Kim and Hong (2001) only obtained 36.6 ± 1.97% of sugar theoretical yield from southern yellow pine. Meanwhile, Kim et al. (2001)

pointed out that without moisture content, the SC-CO_2 pretreatment is almost ineffective in removing the hemicellulose and lignin. When the moisture content reaches as high as 40-75%, a significant increase of glucose yield in the pretreated lignocelluloses is appeared. For the SC-CO_2 pretreatment method, the reason for its usage is due to the advantage of its economic value and environmental friendliness. What's more, CO_2 is easy to recover and recycle for further use. However, the cost of high pressure equipment may be barriers to the SC-CO_2 pretreatment method in large-scale production, which makes it too expensive for industrial application (Kim and Hong, 2001). No detailed economic costs have been discussed in the previous studies, but the influence of ultrasound power combined with SC-CO_2 treatment may be a previous future research direction at industrial plants (Benazzi et al., 2013).

2.3.2.2. Lime

Lime pretreatment, which is a mild alkaline pretreatment method, has been studied in recent years as well. In this study, 15 papers about lime pretreatment method were analyzed. Lime pretreatment has been used in various biomasses, such as corn stover, switchgrass, rice straw, sugarcane bagasse. For corn stover, the maximum glucose yield (91.3%) was achieved under the condition of 55°C and 28 days with aeration (Kim and Holtzapple, 2005). For switchgrass, with a condition of 50°C, 0.10 g Ca(OH)2 /g biomass and 100 ml water /g biomass wash intensity, glucose yield could reach 433.4 mg/g biomass, which increased 3.61 times compared to untreated switchgrass (Xu et al., 2010). When sugarcane bagasse was pretreated with 0.40 g/g lime

loading at 70°C for 65.6 h, the maximum glucose yield was 218.0 mg/g for screened bagasse (Rabelo et al., 2009). The glucose and xylose yield of rice straw pretreated with lime for 1h and 120°C could achieve 74% of the theoretical yield (Park et al., 2010). For poplar wood, with the condition of 21.7 bar (absolute) and 140 ºC for 2 h, glucan and xylan yield could achieve 95.5% and 21.7%, respectively (Sierra et al., 2009). In terms of coastal Bermuda grass (CBG), the maximum sugar yield was 78% of the theoretical yield, using an optimal lime loading condition of 0.1g/g of dry biomass at 100°C for 15 min (Wang and Cheng, 2011). Lime pretreatment was also efficient in other biomass, such as areca nut husk (Sasmal et al., 2012), Jatropha seed cakes (Liang et al., 2010), and rice hull (Saha and Cotta, 2008). Xu et al. (2011) found that lime could perform better when the NaOH was added at the beginning of the process. When switchgrass was pretreated under the condition of 0.10 g NaOH/g biomass and 0.02 g/g lime loading for 6 h, the total sugar yield reached 59.3% of the theoretical yield (Xu and Cheng, 2011). Kim et al. (2005) concluded that oxygen can enhance lime pretreatment because delignification can be improved in the presence of oxygen. Compared to acid pretreatment and hot water pretreatment, alkali may be able to result in better enzymatic saccharification (Park et al., 2010). As a relatively low-cost and safe reagent, lime may also form less fermentation inhibitions and require lower temperatures (Rabelo et al., 2013). However, the lignin removal results showed that the lime pretreatment method was not efficient compared with NaOH pretreatment (Wang and Cheng, 2011). For future work, Wang and Cheng (2011) recommended prehydrolysate analysis after lime pretreatment and the evaluation of fermentation potential from other biomass.

2.3.2.3. Liquid Hot Water

Liquid hot water (LHW), which is a pretreatment method using hot compressed water, has been proved to be efficient in separating hemicelluloses, cellulose and lignin (Wang et al., 2012). In this study, 15 papers discussing hot water pretreatment were reviewed. For corn stover, using a pH of 4.8, 190°C and 15 min, 90% cellulose in 16% corn stover slurry could be hydrolyzed to glucose, and ethanol could achieve nearly 88% of its theoretical yield (Mosier et al., 2005). However, using fungal degradation pretreatment alone on corn stover was more efficient than the combination of liquid hot water and fungal pretreatment (Wan and Li, 2011). For soybean straw, when pretreated at 210°C for 10 min, the maximum glucose yield was 70.76%. Wan et al. (2011) showed to the compared with NaOH soaking method, LHW was more efficient in increasing cellulose digestibility for soybean straw (Wan et al., 2011). Moreover, the LHW pretreatment method improved fungal degradation on soybean straw, which achieved 64.25% of theoretical glucose yield (Wan and Li, 2011). For sugarcane bagasse, with a condition of 160°C and 2 MPa, the reducing sugar yield achieved 78.5% of the theoretical (Yu et al., 2013). When wheat straw was pretreated at 230°C and SO2 concentration was equal to 0.024 g/mL, the total reducing sugar yield reached 93.9% (Liu et al., 2012). With the condition of 230°C and pretreatment severity equal to 4.71, the ethanol yield from miscanthus achieved 98.27% (Li et al., 2013). As to cattails, with the condition of 190°C for 15 min, the highest ethanol yield achieved was 88.7 ± 2.8% of the theoretical (Zhang et al., 2011). Liquid hot water pretreatment method was also applied to other lignocellulosic biomass, such as alfalfa (Screenath et al., 1999), oil palm

fronds (Goh et al., 2010), Populus tomentosa (Wang et al., 2012), and eucalyptus (Yu et al., 2009; Wei et al., 2013), which has also been proved effective in hydrolyzing hemicellulose. The advantages of the liquid hot water method are less corrosion problems (Wang et al., 2012), the potential to remove the majority of hemicellulose (Wei et al., 2013), low costs, and little or no inhibition in the fermentation process (Pérez et al., 2007). However, the energy input was much higher compared with the acid pretreatment method (Yu et al., 2013). For future work, Wang et al. (2012) suggested the development of a combination of fungal pretreatment and liquid hot water pretreatment to achieve higher ethanol yields, and Yu et al. (2013) recommended the development of combinations of liquid hot water pretreatment and aqueous ammonia in order to reduce energy inputs.

2.3.2.4. Acid

Acid pretreatment, one of the leading pretreatment processes, has been studied under commercial scale in recent years (Li et al., 2010). Various lignocellulosic biomasses have been pretreated with acid, such as corn stover, wheat straw, rice straw, sugarcane bagasse, rapeseed straw, cattails and olive tree. There were 13 papers about acid pretreatment analyzed in this study. For corn stover, when pretreated at 180°C for 96 h with acid 1% (w/w) using a percolation reactor, xylose was reported to have 70-75% recovery, while glucose had only 4.5% (Zhu et al., 2004). With conditions of 140°C for 40 min with sulfuric acid 1% (w/w), the glucose yield from corn stover achieved 82% (Lau et al., 2009). For wheat straw, when pretreated by dilute H2SO4 (0.75%, v/v)

at 45°C for 72 h, the maximum glucose yield achieved was 565 ± 10 mg/g (Saha et al., 2005). With conditions of 150°C for 30 min with sulfuric acid (50 mmol/L) and solid loading of 20-30%, the glucose yield from wheat straw could reach nearly 90% (Kootstra et al., 2009). As to sugarcane bagasse, the highest hemicellulose removal reached beyond 90% when bagasse was pretreated with mixed acid of sulfuric and acetic acid in the ratio of 1.5:10 or 1:10 (Rocha et al., 2011). In terms of rice straw, with the condition of 130°C for 20 min for ammonia percolation and 130°C for 40min for sulfuric acid pretreatment, the total reducing sugar achieved 89% (Kim et al., 2011). For pretreated olive trees, Cara et al. (2007) found that dilute acid pretreatment could increase glucose yield to 36.3% of raw material with sulphuric acid loading of 0.1% at 180°C (Cara et al., 2008). There were other lignocellulosic biomasses pretreated using dilute acid method as well, such as rapeseed straw (Castro et al., 2011), coastal Bermuda grass (2011), cattails (Zhang et al., 2011), sugar beet pulp (Zheng et al., 2013), and maple wood (Zhang et al., 2013). The studies published showed the effectiveness of the dilute acid pretreatment method. As the major pretreatment method, dilute acid has the potential of solubilizing hemicellulose, which could break down the chemical bonds in biomass (Li et al., 2010), and is relatively cheap (Cara et al., 2008). However, the use of acid may be inhibitory to sugar fermentation (Li et al., 2010). As estimated by Kootstra et al. (2009), the cost of sulfuric acid would be 8.8 US$ per metric ton wheat straw, assuming 5.17% (w/w) acid-to-straw ratio (Kootstra et al., 2009). Further studies are required to increase the ethanol production efficiency (Castro et al., 2011) and optimize the economics (Zhang et al., 2011).

2.3.2.5. Ammonia Fiber Explosion

Potential environmental problems and low recycling rate are the most serious disadvantages to acid pretreatment, which prevents it from being used extensively in industry. Therefore, more research groups prefer to use base as a treatment to avoid these problems. Ammonia fiber explosion (AFEX) was one of the first methods of using a base to pretreat corn stover. This approach uses immediate reduction of pressure after reacting at a relatively high temperature and short reaction period. AFEX has been utilized to pretreat various biomass, and resulted in 98% of the theoretical glucose yield by pretreating corn stover at 5 min, 90°C, 60% moisture content, and 1:1 ammonia loading to biomass (Teymouri, 2003; Teymouri et al., 2004; Teymouri et al., 2005). With further study using simultaneous saccharification and fermentation (SSF), the maximum ethanol was 96% of theoretical yield from pretreated corn stover (Teymouri et al., 2004; Teymouri et al., 2005). On this basis, AFEX was used for pretreating corn fiber and converted 83% of available glucan, 81% of the xylan and 68% of the arabinan after enzymatic hydrolysis (Hanchar et al., 2007). With a similar pretreatment condition, switchgrass obtained 85% of theoretical glucose yield (Bradshaw, 2005) and higher ethanol yield with 0.2g/g biomass (Alinia et al., 2010). In addition, AFEX was uesd to pretreat reed canary grass (Bradshaw, 2005) and coastal Bermuda grass (Lee et al., 2010), and had very similar results to the data of corn stover. One of the major advantages of AFEX pretreatment was nearly all of the ammonia could be recovered and reused, and residual ammonia could be used as nitrogen source for microbes (Teymouri et al., 2005). What's more, cellulose and hemicellulose were well preserved in the AFEX

process with a low rate degradation and higher sugar yield (Moniruzzaman et al., 1997). However, extra washing process was necessary for removing lignin and other cell wall extractives which remained after the pretreatment process (Chundawat et al, 2007). In addition, lower solubilization of hemicellulose and extra ammonia recycling systems needed were another two disadvantages for industry production (Eggeman and Elander, 2005). Very few studies have been done examine economic cost for the AFEX process, and only Wang et al (1998) did a cost estimate and sensitivity analyses, but without considering enzymatic hydrolysis and fermentation; the cost of AFEX was about $20 - $40 / ton of dry biomass treated. Future work should focus on developing improved methods to fully utilize all available sugars and enhance the purity and yields of glucose and pentose fractions, such as using more effective xylanase and using microorganisms capable of utilizing xylose to increase ethanol production yield (Teymouri, 2003).

2.3.2.6. Ammonia Fiber Expansion

By modifying the ammonia fiber explosion process, ammonia fiber expansion (AFEX) was invented in 2006, and used to pretreat more than 10 types of biomass in 5 years. Bals et al (2006) pretreated DDGS and obtained a glucose yield of 190g glucose/kg dry biomass, using pretreatment condition of 70°C with a loading rate of 0.8 anhydrous NH3/ kg dry biomass in 5 min. Lau et al.,(2008) then used simultaneous saccharification and fermentation (SSF) on pretreated DDGS, and found an ethanol productivity of 1.2 g/h/L. In addition, ammonia fiber expansion has been used to pretreat corn stover (Sendich et al., 2008; Lau et al., 2008; Lau et al., 2009; Garlock et al., 2009;

Lau, 2010; Lau and Dale, 2010; Gao et al., 2010) with an ethanol yield from 78.1 gal/ dry ton to 93.5 gal/ dry ton, and maximum hydrolysis theoretical yields of 74.2% glucan and 55.5% xylan. Besides these, miscanthus (Murnen et al., 2007), reed canary grass (Bradshaw et al., 2007), empty palm fruit bunch fiber (Lau et al., 2010), switchgrass (Bals et al., 2011), guayule (Chundawat et al., 2012) forage and sweet sorghum bagasse (Li et al., 2010) have been tested by ammonia fiber expansion. All have had similar or slightly lower ethanol yields than corn stover. Ammonia fiber expansion (AFEX) offers several advantages, including reduced production of inhibitory compounds and nutrient addition due to residual ammonia (Teymouri et al., 2005). Compared to ammonia fiber explosion, the modified method of ammonia fiber expansion has a milder reaction temperature and lower ammonia loading rate, which means more friendly environmental acceptability. In spite of decreasing the effect to the environment, ammonia fiber expansion still needs higher pressure in the pretreatment, which requires more stable and strong equipment, and causes a higher production cost. What's more, either ammonia fiber explosion or ammonia fiber expansion required extra ammonia recycling systems, which makes industry processing hard to decrease. In order to explore the possibility for ammonia fiber expansion in industry, Sendich et al (2008) and Bals et al (2011) did an economic analysis on the whole process. Sendich et al (2008) calculated the cost of ethanol production utilizing AFEX by using updated parameters and ammonia recovery configuration. These calculations indicated that the minimum ethanol selling price (MESP) could be reduced from $1.41/gal to $0.81/gal. Bals et al (2011) utilized a leading biorefinery model with four parameters: ammonia loading, water loading,

reaction temperature, and residence time, and determined that pretreatment conditions could change the costs of ethanol production by up to 35 cents per gallon of ethanol in an 850 ton/day refinery. Both of these models have their own limitations, such as unique type of biomass, not considering definitive between costs and revenues for a biorefinery, so more limited factors and more biomass should be considered and calculated with a new model in a future study.

2.3.2.7. Aqueous Ammonia

Due to disadvantages of AFEX, the methods of ammonia recycle percolation (ARP) and aqueous ammonia have been attempted by researchers in recent years. ARP has a maximum ethanol yield of 78% of theoretical maximum, using a condition of 185 °C and 1:10 of solid to corn stover (Gupta and Lee, 2009). However, high energy is still consumed, and 50% of hemicellulose is solubilized in ARP, which caused a lower maximum ethanol yield to be achieved. Aqueous ammonia can be used for swelling and delignification of various types of biomass, including corn stover (Chen et al., 2009), switchgrass (Isci et al., 2008; Himmelsbach et al., 2009), rice straw (Ko et al., 2009), wheat straw (Remond et al., 2010), oil palm empty fruit bunch fiber (Jun et al., 2011) and rapeseed straw (Kang et al., 2012), using reacting conditions of 1.0~30 wt.% of aqueous ammonia for 4 h to 10 days. The results showed that 60~70% of lignin can be removed and 100% cellulose and 85% hemicellulose can be retained in the solid, which give a better base to enzymatic activity and simultaneous saccharification fermentation. But the effectiveness is also dependent on the temperature, which means higher energy

consumption. Also, long treatment times and large washing steps limit utilization in industrial production.

2.3.2.8. Low Moisture Anhydrous Ammonia

In order to avoid the disadvantages listed above, low-moisture anhydrous ammonia (LMAA) process has been developed (Yoo et al., 2011), optimal conditions for corn stover reactions occur near 80 °C, 96 h and 50% moisture. The results show that SSF ethanol yield was 24.9 g/L and 89% of theoretical ethanol yield based on glucan and xylan. What's more, the amount of anhydrous ammonia is very low and easy to recycle, which means lower cost and a decrease environment effect.

2.3.3. Conclusion

Each pretreatment method has its own advantages and disadvantages. The SC-CO_2 pretreatment is friendly to environment; it doesn't discharge any harmful chemicals. But this method is limited to only a few lignocellulosic biomass materials because it is not strong enough (Narayanaswamy et al., 2011). Lime pretreatment is relatively cheap, and lime can be removed easily by neutralization. However, the effect of lime pretreatment does not reach the satisfactory efficiency. Hot liquid extraction is effective in partly hydrolyzing hemicellulose and breaking down the lignin and cellulose structure (Mosier et al., 2005). Dilute acid pretreatment offers good performance in terms of recovering hemicellulose, cellulose digestibility, and sugars, but suffers from its use of H2SO4. Ammonia is a better reagent than lime because it makes biomass delignified,

swells and preserves cellulose for a relative long time. Two types of AFEX can easily break the biomass structure and improve enzyme hydrolysis. But the cost of higher pressure and more stringent equipment decreases the financial efficiency in industrial production. APR has the advantages of an efficient delignification with 70%~95% lignin removal， swelling the biomass structure, and being easy to recycle. However, solubilized hemicellulose and higher energy consumption make it hard to apply in industrial production. In order to decrease the effect to environment, equipment, and financial cost and produce the highest glucose yield, low moisture anhydrous ammonia (LMAA) pretreatment has many advantages. LMAA pretreatment reduces the cost of water and ammonia, which effectively decreases energy cost. Meanwhile, the glucose yield achieved is higher than other pretreatment methods at optimal conditions.

Table 2.1 Summary of studies found in literature assessing effectiveness of biomass pretreatment.

Pretreatment	Biomass	Conditions	Results		References
			Time	Yield	
Supercritical Carbon Dioxide	Corn Stover	30% moisture content with SC-CO_2 pretreated at 3500 psi and 150 °C	60 min	Glucose Yield: 30g/100g	Narayanaswamy et al., 2011
	Switchgrass	SC-CO_2 pretreated at 3200 psi and 150 ºC	60 min	Glucose Yield: 14g/100g	
	Wheat Straw	For dry wheat straw, 190ºC and 30 min 12MPa; For wet wheat straw, 185ºC and 30 min 12MPa	30 min	Glucose Yield: 208.4 (g/kg)	Alinia et al., 2010
		Steam condition of 200ºC and 15 min; Supercritical CO_2 condition of 1700 psi, 190ºC and 60 min	60 min	Glucose Yield: 234.6 (g/kg)	
	Rice Straw	4300 psi and 110 ºC for	30 min	Glucose Yield: 32.4 ± 0.5%	Gao et al.,2010
	Aspen	Moisture content of 73% pretreated with SC-CO_2 at 3100 psi and 165°C	30 min	Glucose Yield: 79.4 ± 2.8%	Kim and Hong, 2001
	Southern Yellow Pine	Moisture content of 57% pretreated with SC-CO_2 at 3100 psi and 165°C	30 min	Glucose Yield: 36.6 ± 1.97%	
	Sugarcane bagasse	Moisture content of 65% pretreated at 80°C and 3600 psi	120 min	Sugar: 380 ± 9 g/kg	Benazzi et al., 2013
		Pretreated at 60°C and 2000 psi	60 min	Glucose Yield: 72%	ALF Santos et al, 2011
		Moisture content of 60% pretreated at 175°C and 3800 psi	30 min	Glucose Yield: 56%; pentose yields: 61%.	Srinivasan and Ju, 2012
	Dry Guayule	Pretreated at 4000 psi, 200 ºC, 60% moisture content	30 min	Glucose Yield: 77%	Srinivasan and Ju, 2010

Table 2.1 Summary of studies found in literature assessing effectiveness of biomass pretreatment (continued).

Lime	Corn Stover	Pretreated at 55 ºC for 4 weeks with 15 FPU/g cellulose, 0.073 g $Ca(OH)_2$	28 d	Glucose: 91.3% Xylose: 79.5%	Kim and Holtzapple, 2005
	Switchgrass	6h retention time with NaOH loading of 0.10g/g, lime loading of 0.02g/g, biomass wash intensity of 100 mL water/g	6 h	Glucose: 59.4%; Xylose: 57.3% Sugar: 59.3%	Xu and Cheng, 2010
		Pretreated at 50 ºC with 0.10 g $Ca(OH)_2$ /g raw biomass and 100 mL water/g raw biomass	24h	Glucose Yield: 433.4 (mg/g)	Xu et al., 2010
		Pretreated at 110 ºC with 0.248 g $Ca(OH)_2$ /g Alamo switchgrass and 6.89 bar O2	240 min	Glucan Yield: 85.9%; Xylan Yield: 52.2%	Falls and Holtzapple, 2011
		Pretreated at 120 ºC (Dacotah switchgrass) and 6.89 bar O_2	240 min + 72 h	Glucan Yield: 85.2%; Xylan Yield: 50.1%	Falls et al., 2011
	Rice Straw	Pretreated at 120ºC with lime loading of 20% for lime pretreatment; Hydrolyzed at 50ºC	1 h + 24 h	Ethanol Yield: 74%	Park et al., 2010
	Rice Hull	Pretreated at 121ºC with lime loading 100mg/g	1 h	Sugar: 154±1 (mg/g)	Saha and Cotta, 2008
	Sugarcane Bagasse	Pretreated at 70ºC for 65.6h with a lime loading of 0.40 g/g	65.6 h	Sugar: 367.2 (mg/g)	Rabelo et al., 2008
		Pretreated at 90ºC with a lime loading of 0.47 g/g	90 h	Ethanol: 164.1 (kg/ton)	Rabelo et al., 2013
	Coastal Bermuda grass	Pretreated with lime loading of 0.1 g/g of dry biomass at 100 ºC	15 min	Glucose Yield: 78%	Wang and Cheng, 2011
	Areca nut husk	Pretreated at 35ºC with a lime loading ratio of 0.5	60 min	Ethanol Yield: 0.43 (g/h/L)	Sasmal et al., 2012
	Jatropha seed cake	Pretreated with 0.1g lime and 9 mL water/g cake	3 h	Cellulose Yield: 68.9%	Liang et al., 2010
	Poplar wood	Pretreated at 140ºC with 21.7 bar absolute	2 h + 72 h	Glucan:95.5 % Xylan: 73.1%	Sierra et al., 2009
		Pretreated at 65ºC with oxygen	28 d	Glucose Yield: 76%	Sierra et al., 2010

Table 2.1 Summary of studies found in literature assessing effectiveness of biomass pretreatment (continued).

Liquid Hot Water	Corn stover	Pretreated at 190°C of a 16% slurry of corn stover	15 min	Ethanol Yield: 88%	Mosier et al., 2005
	Wheat Straw	Pretreated at 200°C with solid concentration of 5% or 10% (w/v)	40 min + 72 h	Glucose: 96% Sugar: 53%	Pérez et al., 2007
		Pretreated at 150°C with SO_2 concentration 0.024 g/mL	30 min	Glucose Yield: 93.9%	Liu et al., 2012
	Miscanthus	Pretreatment at 230°C (pretreatment severity equal to 4.71)	25 min + 24 h	Ethanol Yield: 98.27%	Li et al., 2013
	Eucalyptus	Pretreated at 180°C with wet disk milling	20 min	Glucose Yield: 88.12%; Xylose Yield: 91.26%	Wei et al., 2013
	Soybean Straw	Pretreated at 210°C	10 min	Glucose Yield: 64.55%	Wan et al., 2011
		Pretreated at 170°C, solid to liquid ratio = 1:10, with pressure maintained at 110 psi	3 min + 18 d	Xylose Yield: 92.83%	Wan and Li, 2011
		Liquid-solid ratio equal to 3g/g under 170°C for 60 min	60 min	Xylose Yield: 13.76 (g/L)	Vallejos et al., 2012
	Sugarcane Bagasse	Pretreated at 180°C, solid to liquid ratio = 1:20	30 min + 60 h	Glucose: 90.13%	Zhang et al., 2013
		Pretreated at 160°C and 2 MPa in the combination of LHW and aqueous ammonia	50 min + 72 h	Glucose Yield: 78.5%	Yu et al., 2013
	Populus Tomentosa	200°C under the combination of Lenzites betulina C5617	30 min	Glucose Yield: 60.26%	Wang et al., 2012
	Alfalfa	Raffinate treated with 4 % (w/v) cellulase; extract was under pH 5 with 1% pectinase and cellulase mixture at 50°C	96 h	Glucose Yield: 59-65 (g/L)	Screenath et al., 1999
	Cattails	Pretreated at 190°C with a cellulase loading of 60 FPU/g glucan in the presence of the yeast Saccharomyces cerevisiae	15 min + 48 h	Ethanol Yield: 77.6%	Zhang et al., 2011
	Oil palm frond	Pretreated at 178°C with the liquid to solid ratio of 9.6 and 10 bar	11.1 min + 48 h	Glucose Yield: 92.78%	Goh et al., 2010
	Eucalyptus Grandis	Pretreated with 5% w/v substrate at 500 rpm and 4.0 MPa: 180°C for first step, 200°C for second step	20 min + 20 min	Glucose Yield: 86.4% (1st) 96.6% (2^{nd})	Yu et al., 2009

Table 2.1 Summary of studies found in literature assessing effectiveness of biomass pretreatment (continued).

Dilute Sulfuric Acid	Corn Stover	Pretreated at 180 ºC with acid 1% w/w	96 h	Glucose Yield: 73%	Zhu et al., 2004
		Pretreated at 140 ºC with acid 1% w/w	40 min + 120 h	Glucose Yield: 82%	Lau et al., 2009
	Wheat Straw	Hydrolyzed at 45°C pH 5.0 with dilute H_2SO_4 (0.75%,v/v)	72 h	Sugar Yield: 565 ±10 (mg/g)	Saha et al., 2005
		Pretreated at 150 ºC and 20-30% solid loading	30 min + 24 h	Glucose Yield: 90%	Kootstra et al., 2009
	Switchgrass	Pretreated at 160 ºC with acid 1.2% w/w and solid loading of 3%	20 min + 72 h	Glucose Yield: 96%	Li et al., 2010
	Rice Straw	Pretreated at 130 ºC for 20 min for ammonia percolation, and 130ºC for 40 min for the whole pretreatment	40 min	Glucose Yield: 89%	Kim et al., 2011
	Sugarcane Bagasse	Pretreated at 200°C with 1:10 solid-to-liquid ratio for 60 min	60 min	Glucose Yield: 70%	Moraes Rocha et al., 2010
	Sugar beet pulp	Pretreated at 120°C with acid concentration of 0.66% and solid loading of 6%	30 min + 72 h	Glucose Yield: 62%	Zheng et al., 2013
	Rapeseed Straw	Pretreated at 200°C with 0.40% free acid concentration	27 min	Glucose: 65%	Castro et al., 2011
	Coastal Bermuda grass	Pretreated at 140°C with acid concentration of 1.2%	30 min + 72 h	Glucose Yield: 97%	Redding et al., 2011
	Cattails	Pretreated at 180ºC with a sulfuric acid concentration of 0.5% for 5 min	5 min	Ethanol: 90%	Zhang et al., 2011
	Maple wood	Pretreated at 160 ºC with sulphuric acid concentration of 0.5%	2.5 min + 72 h	Xylose: 84%	Zhang et al., 2013
	Olive Tree	Pretreated at 160 ºC with sulphuric acid concentration of 0.5%	2.5 min + 72 h	Xylose: 84%	Cara et al., 2008
Ammonia Fiber Explosion	Corn Stover	Pretreated at 90 °C, 60% moisture content, and 1:1 ammonia loading to biomass	5 min + 168 h;	Glucose Yield: 98%	Teymouri et al., 2004
		Pretreated at 90 °C, 60% moisture content, and 1:1 ammonia loading to biomass	5 min + 168 h	Glucose Yield: 97%;	Teymouri et al., 2005
	Corn Fiber	Pretreated at 90 °C, 60% moisture content, and 1:1 ammonia loading to biomass	30 min + 24 h;	Glucose Yield: 83%	Hanchar et al., 2007
	Switchgrass	Pretreated at 100 °C, 80% moisture content, and 1:1 ammonia loading to biomass	5 min + 168 h	Ethanol Yield: 0.2 (g/g biomass)	Alizadeh et al., 2005
		Pretreated at 120 °C, 60% moisture content, and 1.2:1 ammonia loading to biomass	5 min + 168 h	Glucose Yield :85%;	Bradshaw, 2005

Table 2.1 Summary of studies found in literature assessing effectiveness of biomass pretreatment (continued).

	Reed Canary grass	Pretreated at 100 °C, 60% moisture content, and 1.2:1 ammonia loading to biomass	5 min + 168 h	Glucose:106 %; Xylose:77%	Bradshaw, 2005
	Coastal Bermuda Grass	Pretreated at 100 °C, 60% moisture content, and 1:1 ammonia loading to biomass	30 min	Sugars: 94.8%	Lee et al., 2010
Ammonia Fiber Expansion	Corn Stover	Pretreated at 90 °C, 21 atm, and Biomass:NH_3:H_2O=1:0.3:0.25	5 min + 72 h	Ethanol:78.1 (gal/dry ton)	Sendich et al., 2008
		Pretreated at 90 oC, 60% moisture content, 1:1 kg ammonia/kg of dry matter; Hydrolyzed at pH 4.8, 50 °C, 200 rpm rotation	40 min + 168 h	Ethanol: 0.25 (g/h/L)	Lau et al., 2008
		Pretreated at l30°C, 60% moisture content, 1:1 kg ammonia/kg of dry matter	5 min + 72 h	Sugars: 14 (g/L).	Lau et al., 2009
		Pretreated at 90 oC, 60% moisture content, 1.5:1 kg ammonia/kg of dry matter; Hydrolyzed at pH 4.8, 50 °C, 200 rpm rotation	5 min + 72 h	Ethanol: 0.354 (L /kg)	Garlock et al., 2009
		Pretreated at 650 psi, initial and final temperatures were 130 ± 5°C and 110 ± 5°C	40 min + 120 h	Ethanol:267 (g/kg)	Lau, 2010
		Fermentation condition: pH 7.0, 37° C, 150 rpm	48 h	Ethanol:21.7 (g/L)	Lau and Dale, 2010
		Pretreated at l30°C, 60% moisture content, 1:1 kg ammonia/kg of dry matter	15 min + 24 h	Glucan:74.2 %; Xylan: 55.5%	Gao et al., 2010
	DDGS	Pretreated at 70° C, and 0.8 kg anhydrous ammonia/kg dry biomass	5 min + 168 h	Glucose: 190 (g/kg)	Bals et al., 2006
		Pretreated at 70^{o} C, 13.0% moisture content, and 0.8 kg anhydrous ammonia/kg dry biomass	40 min + 168 h	Ethanol: 1.2 (g/h/L)	Lau et al., 2008
	Miscanthus	Pretreated at 160 °C, 233% moisture, and 2:1 kg ammonia/kg of dry matter	5 min + 168 h	Glucan:96% ; Xylan: 81%	Murnen et al., 2007
	Reed Canary grass	Pretreated at l00 °C, 60% moisture content, and 1.2:1 kg ammonia/kg of dry matter	40 min + 72 h	Glucose:86 %; Xylose:78%	Bradshaw et al., 2007
	Palm Fiber	Pretreated at 135 °C, 45 min retention time, 1:1 NH3 to Dry Biomass	30~40 min + 72 h	Ethanol: 35.6 (g/L	Lau et al., 2010
	Switchgrass	Pretreated at 80°C, 40% moisture content, 0.9:1 kg ammonia/kg of dry biomass	20 min + 168 h	Glucose: 247 (g/kg)	Bals et al., 2010

Table 2.1 Summary of studies found in literature assessing effectiveness of biomass pretreatment (continued).

	Guayule	Pretreated at 150°C, 60% moisture content,1g NH3/g dry biomass	30 min + 168 h	Glucose Yield: 39%	Chundawat et al., 2012
	Forage	Pretreated at 140 °C, 120% moisture content and 2:1 ammonia loading to biomass	5 min + 72 h	Ethanol: 30.9 (g/L)	Li et al., 2010
	Sorghum Bagasse	Pretreated at 140 °C, 120% moisture content and 2:1 ammonia loading to biomass	5 min + 72 h	Ethanol: 42.3 (g/L)	
Ammonia Recycle Percolation	Corn Stover	Hydrolyzed with 15 FPU glucan 30 CBU of β-glycosidase at 38°C and 150 rpm	72 h + 168 h	Ethanol: 56%	Kim et al., 2005
		Pretreated at 30 °C, 50 wt.% of ammonia loading and 1:5 solid-to-liquid	28 d + 96 h	Ethanol: 73%	Li and Kim, 2011
	Hybrid poplar	Pretreated at 180 °C, 10 wt.% ammonia solution	30 min + 192 h	Enzymatic digestibility: 95%	Yoon, 1998
		Pretreated at 185 °C, 1:10 of solid:liquid	27.5 min + 72 h	Sugar: 78%	Gupta and Lee, 2009
Aqueous Ammonia	Corn Stover	Pretreated at 2% NaOH 120 °C; Hydrolyzed at cellulase loading of 20 FPU/g substrate and 8.0% substrate concentration	30 min + 48 h	Sugars: 81.2%	Chen et al., 2009
		Pretreated at 29.5 wt.% aqueous ammonium hydroxide, 10 mL/g biomass	10 d	Ethanol: 22 (g/L)	Isci et al., 2008
	Switchgrass	Pretreated at 27°C ,29.5% aqueous ammonium hydroxide, solid ratio of 5 L/kg	5 d	Ethanol: 73%	Himmelsbach et al., 2009
		Pretreated 4 kg of switchgrass with 20-L of aqueous ammonia	5 d	Ethanol: 74%	Himmelsbach et al., 2009
	Rice Straw	Pretreated at 69 °C, 10 h and an ammonia concentration of 21% (w/w)	30 min + 168 h	Glucan: 97.6 ± 3.2 (%)	Ko et al., 2009
	Wheat Straw	Pretreated with aqueous ammonia (30%, v/v),350 IU Tx-Xyl 11/g straw at 60°C	24h	Sugars: 53.6 ± 1.3%	Rémond et al., 2010
	Oil palm empty fruit bunches	Pretreated at 90 °C, 21% (w/w) aqueous ammonia	12h + 168h	Ethanol: 0.11 (g/h/L)	Jung et al., 2011
	Rapeseed Straw	Pretreated at 19.8% of ammonia, 69.0° C, and solid-to-liquid ratio of 1:10;	14.2 h	Glucose: 60.7%	Kang et al., 2012
LMAA	Corn Stover	80 °C, 50%-moisture sample;15 FPU /g-glucan, 30 CBU / g-glucan; 37°C, pH=7.0, anaerobic	96 h + 120 h	Ethanol: 24.9 (g/L)	Yoo et al., 2011

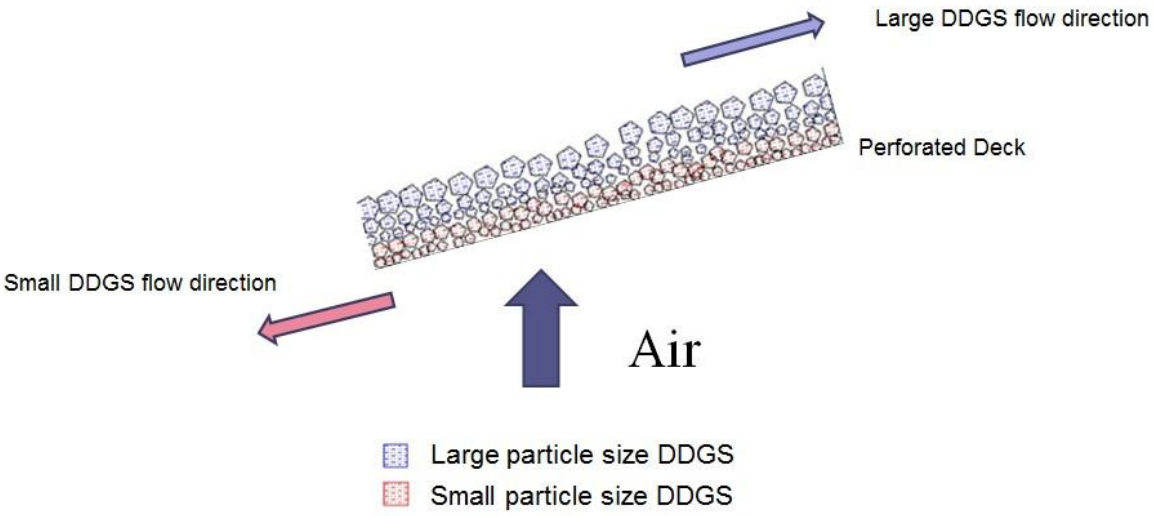

Figure 2.1 Principle of destoner to DDGS

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CHAPTER 3: Properties of Distillers Dried Grains with Solubles (DDGS)

3.1. Introduction

With pressure from shortage of fossil fuels, bioethanol as a fuel additive is gradually utilized to reach the demand for fuel (Schnepf and Yacobucci, 2013). Conversion corn to ethanol is the most efficient method in the US ethanol industry, and has grown rapidly in recent years. In 2011 United States fuel ethanol production was the top producer in the world (RFA, 2012), which reached 13.9 billion U.S. liquid gallons (52.6 billion liters). According to Rosentrater and Muthukumarappan (2006), more than 95% US fuel ethanol plants are used corn as a major raw material to produce ethanol.

In the corn-based fuel manufacturing, bioethanol, distillers dried grains with solubles (DDGS) (or other co-products), and carbon dioxide are three main products. Among all products from bioethanol industry, DDGS is an important ingredient, which is directly related to sustainability of dry grind plants, and is sold at a varying market price (US$85–140/ton) (Liu, 2008).

Common physical properties of DDGS include particle size, loose bulk density, packed bulk density, and angle of repose; these influence how much of the product can be stored in a given volume (Ileleji et al., 2008). In addition, moisture content, water activity and shear strength also affect the storability and material milling properties of

DDGS. However, large variations in physical properties have been reported by different research groups over the years. (Shurson, 2005; Rosentrater, 2006; Ileleji et al., 2007).

Through some research has been done to study the properties of DDGS, production processes have been changing in recent years, and oil is now commonly removed. In order to understand the changes in the DDGS industry, new baseline data about these properties should be established, because they are essential for design of equipment, processing facilities, storage and material handling systems (Rosentrater, 2011). Thus the objective of this study was to investigate basic properties of contemporary DDGS, including moisture content, water activity, angle of repose, particle size, bulk density, color and shear strength, from ten dry grind corn ethanol facilities in the Midwest U.S.

3.2. Materials and Methods

3.2.1. Materials

Sixteen DDGS samples were supplied by ten dry grind corn ethanol facilities located in the Midwest US, and labeled as 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10, to ensure anonymity. All samples were collected during the fall of 2011 and spring of 2012 (i.e., three unique samples per plant from two plants, two unique samples per plant from two plants, then one unique sample per plant from six plants), and were stored at room temperature (24±1 ºC) in sealed plastic storage bags. All properties were measured at room temperature (except moisture content) and studied with a completely randomized design.

3.2.2. Methods

Moisture content was determined following the standard Forage Analysis Procedure (NFTA, 2002), using a forced-convection laboratory oven (Thermo OGH & OMH180, Scientific Heratherm, Langenselbold, Germany) at 105 ºC for 3 h. Water activity was measured with a calibrated water activity meter (AquaLab series 3 TE, Decagon Devices, Pullman, Washington, USA). Angle of repose was measured by allowing DDGS to fall onto a 15.5 cm x 15.5 cm square plate in a Helle Shaw cell following the method described by Mohesenin (1980), and angle was measured by ImageJ software. Particle size was measured according to ANSI/ASAE S319.3 (ASABE, 2004), using U.S. sieve nos. 6 (3.36 mm), 8 (2.38 mm), 10 (2.00 mm), 14 (1.680 mm), 16 (1.19 mm), 20 (0.841 mm), 30 (0.595 mm), 40 (0.420 mm), 50 (0.297 mm), 70 (0.210 mm), Pan (0.044 mm). From the weight of DDGS collected on each sieve, the geometric mean diameter (d_{gw}) and the geometric standard deviation (S_{gw}) were calculated according to the standard. Bulk density of DDGS was measured using a filling hopper, stand, and 1 L cup (Seedburo 151, Seedburo Equipment Co, Chicago, IL, USA) with the method designed by USDA (1999). Color was measured using a spectrocolorimeter (LabScan XE 16807, Hunter Associates Laboratory, Reston, VA, USA), with the L-a-b opposable color scales (Hunter Associates Laboratory, Reston, VA, USA) (HAL, 2002). Shear strength was tested by a torvane shear device (26-2261, ELE International, Loveland, CO, USA) following the procedures described by Goossens (2004) and Zimbone et al. (1996).

3.3. Data Analysis

All collected data were analyzed with Microsoft Excel v. 2010 (Microsoft Corp, Redmond, WA), and SAS Enterprise 4.3 (SAS Institute, Cary, NC) software. Summary statistics, t-test (to test for differences within the processing plant), and ANOVA (to test for differences among processing plants) were tested for each property to determine whether significant differences existed, using a Type I (α) error rate of 0.05; if so, post-hoc LSD tests were conducted using a 95% confidence level to determine where those differences occurred.

3.4. Results and Discussion

Table 3.1 summaries the measured properties of the DDGS in this study, including minimum, maximum, mean values and standard deviations for each property, both for each individual plant and overall. Statistically significant differences were found from samples of the same plant, and among samples from different plants. Results show large variations in most properties, which are similar to other prior studies (Shurson, 2005; Ileleji et al., 2007; Rosentrater et al., 2006).

3.4.1. Moisture Content

As shown in Table 3.1, these samples ranged in moisture content from 6.66 to 10.48 % (w.b. - wet basis), with a mean of 8.69%. After converting to dry basis, the results ranged from 7.13% to 11.71% (d.b. – dry basis), with a mean of 9.52%. According to the results, these DDGS samples were well suited for storage because the

lowest limit of moisture content to most microbial growth in corn and related products is 13.5 % (d.b. – dry basis) (Beauchat, 1981). In addition, the moisture content data in this study are generally between the results of Rosentrater (2006) and Bhadra et al. (2009), and very similar to Kingsly et al. (2010) and Spiehs et al. (2002). The reasons for these differences probably are caused by the method of producing DDGS at the ethanol plants.

3.4.2. Water Activity

Overall, DDGS in this study had a low water activity, which ranged from 0.46 to 0.61. Water activity is a measure of the energy status of the water in a system, and it directly affects the activity of microbes. Prezant et al. (2007) has shown that most bacteria are adapted for growing in an environment with a water activity of 0.9, mold is adapted to between 0.7 and 0.8, yeast is adapted more than 0.7, and very little microbial growth can occur if the water activity is below 0.65. Thus, water activity results are related to moisture content, and should be limiting to microbe growth. The samples in this study have a low water activity, which means a small probability of spoilage problems, DDGS should still be stored in bulk cautiously, in case of potential moisture migration from the environment, especially during the shipping. These results are very similar to those found in previous work (Rosentrater 2006).

3.4.3. Angle of Repose

Angle of repose ranged from 35.48^{o} to 82.87^{o}, with a mean of 48.04^{o} (Figure 3.1 and Table 3.1). According to the LSD analysis, the results have an obvious separation

into two types of behaviors: a low value of about 40^{o} (including plant 1, 2, 3, 4, 5, 6 and 7); the other had a high value of about 75^{o} (including 8, 9 and 10). The results of the former were similar to Bhadra et al. (2006) and a little higher than Rosentrater (2006). The reason for the high value in the latter group may be influenced by particle size, composition of the DDGS particles, and the drying and cooling conditions, especially when sugar and fat molecules on the surface reach glass transition temperature, which affects the surface frictional properties such as stickiness and cohesion (Liu et al., 2011; Rosentrater, 2006).

3.4.4. Particle Size

Overall, geometric mean diameter (d_{gw}, mm) had a range from 0.34 to 1.28 mm, with a mean of 0.74 mm (Table 3.1). According to the LSD analysis, the results had an obvious separation into three types: the first group includes Plant 1 and 2, which had high values similar to the results of Clementson et al. (2009); the second group included Plant 3, 4, 5, 6 and 7, which had a mean value about 0.65, which was similar to the results of Liu (2008); the third group included Plant 8, 9 and 10, which had a low value, about 0.4, similar to Bhadra et al. (2012). Geometric standard deviation (S_{gw}, mm) ranged from 1.47 to 2.14 mm, with a mean of 1.72 mm (Table 3.1), which is very similar to the results of U.S. Grains (2008), and higher than Bhadra et al. (2009), Clementson et al. (2009) and Liu (2008). All these results show large variations in particle size distribution due to different plants.

3.4.5. Bulk Density

Loose bulk density ranged from 439.8 kg/m^3 to 570.6 kg/m^3, with a mean of 483.9 kg/m^3 (Table 3.1), which is similar to the results of Bhadra et al. (2009), and a little lower than Clementson et al. (2009) and Liu (2008). Packed bulk density ranged from 476.4 kg/m^3 to 666.6 kg/m^3, with a mean of 568.5 kg/m^3 (Table 3.1). According to the LSD analysis, most samples from different plants were significantly different from each other, which mean that there is a large variation across the different plants instead of bulk density.

3.4.6. Color

The DDGS color values in this study are shown in Table 3.1 as well. The range of Hunter – L (white-black axis) ranged from 51.77 to 61.29 with a mean of 56.70; the range of Hunter – a (red-green axis) was from 12.25 to 15.91, with mean of 13.85; the range of Hunter – b (blue-yellow axis) was from 41.63 to 51.60, with mean of 46.51. All these value were significantly higher than Rosentrater (2006) and Bhadra et al. (2007); Hunter – b was nearly 100% higher, which means more yellow and possibly better nutrient quality (Goihl, 1993 and Ergul et al., 2003). According to the LSD, most plants were significant different from each other, except the relationships among Plant 8, 9 and 10.

3.4.7. Shear Strength

Shear strength ranged from 0.022 kg/cm^2 to 0.050 kg/cm^2, with a mean of 0.032 kg/cm^2, which is similar to the data of Ganesan et al. (2007) and Ganesan et al. (2009).

According to the LSD, there were no significant differences in most samples, except Plant 1 which means that most samples had similar shear strength.

3.5. Conclusion

The goal of this research was to provide baseline property data for typical DDGS from Midwest from USA in 2011 and 2012. After experimental test, this study got the data of DDGS properties and compared with other researcher's results, which included moisture content, water activity, angle of repose, geometric mean diameter (d_{gw}), geometric standard deviation (S_{gw}), loose bulk density, packed bulk density, color content, shear strength. This research supplies up to date engineering data which is key to storing and handling DDGS, designing and utilizing equipment, and producing co-products from DDGS. Future work will focus on examining correlations between physical and chemical properties and explore the reasons why the differences occur in different samples.

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Table 3.1 Properties of distillers dried grains with solubles (DDGS). [a]

Property	Processing Plant	Number of Observations	Minimum	Maximum	Mean	Standard Deviation
Moisture Content (%, wb)	Overall	48	6.66	10.48	8.69	1.13
	1	9	7.72	8.90	8.37 bc	0.38
	2	9	6.66	7.21	6.99 a	0.20
	3	6	9.82	10.48	10.18 g	0.28
	4	6	7.70	10.32	9.63 fg	0.98
	5	3	8.16	8.86	8.61 cd	0.39
	6	3	9.01	9.63	9.33 def	0.31
	7	3	8.95	9.80	9.36 def	0.43
	8	3	8.34	9.60	8.90 ce	0.64
	9	3	9.04	9.60	9.27 def	0.29
	10	3	7.35	8.04	7.78 b	0.38
Water activity(-)	Overall	48	0.46	0.61	0.55	0.05
	1	9	0.54	0.56	0.55 a	0.01
	2	9	0.46	0.48	0.47 b	0.01
	3	6	0.59	0.60	0.60 c	0.01
	4	6	0.59	0.60	0.59 c	0.00
	5	3	0.53	0.53	0.53 d	0.00
	6	3	0.58	0.59	0.59 e	0.01
	7	3	0.58	0.58	0.58 ef	0.00
	8	3	0.57	0.58	0.58 f	0.00
	9	3	0.6	0.61	0.60 g	0.01
	10	3	0.56	0.56	0.56 h	0.00
Angle of Repose (º)	Overall	48	35.48	82.87	48.04	13.32
	1	9	38.44	44.54	42.03 ab	1.56
	2	9	37.89	43.42	41.31 b	1.20
	3	6	35.48	44.23	41.09 b	2.33
	4	6	41.32	47.91	43.92 a	2.05
	5	3	39.14	42.09	40.76 b	1.31
	6	3	39.52	42.97	41.14 b	1.23
	7	3	40.30	43.78	41.47 ab	1.38
	8	3	70.74	82.87	76.90 c	5.40
	9	3	65.32	81.78	73.06 c	5.91
	10	3	71.63	80.12	75.20 cd	2.95
Geometric mean diameter (d_{gw}, mm)	Overall	48	0.34	1.28	0.74	0.27
	1	9	0.74	0.92	0.82 a	0.06
	2	9	1.14	1.28	1.19 b	0.05
	3	6	0.59	0.78	0.65 c	0.08
	4	6	0.64	0.75	0.71 c	0.05
	5	3	0.63	0.73	0.68 c	0.05

Table 3.1 Properties of distillers dried grains with solubles (DDGS) (continued). [a]

	6	3	0.60	0.73	0.65 c	0.07
	7	3	0.58	0.69	0.64 c	0.06
	8	3	0.37	0.38	0.37 de	0.01
	9	3	0.34	0.34	0.34 d	0.01
	10	3	0.43	0.46	0.45 e	0.02
	Overall	48	1.47	2.14	1.72	0.15
	1	9	1.74	1.84	1.79 a	0.03
Geometric	2	9	1.47	1.51	1.49 b	0.01
standard	3	6	1.66	1.79	1.72 cd	0.05
deviation	4	6	1.66	1.75	1.72 cd	0.03
(S_{gw}, mm)	5	3	1.66	1.78	1.73 acd	0.07
	6	3	1.65	1.84	1.76 ac	0.10
	7	3	1.70	1.88	1.76 ac	0.10
	8	3	1.80	1.90	1.85 e	0.05
	9	3	2.08	2.14	2.10 f	0.03
	10	3	1.65	1.71	1.67d	0.03
	Overall	48	439.8	570.6	483.9	39.24
	1	9	543.4	570.6	555.5 a	11.20
Loose	2	9	439.8	446.0	442.7 b	2.27
Bulk	3	6	465.8	469.6	467.6 c	1.30
Density	4	6	462.4	470.8	467.0 c	3.42
(kg/m^3)	5	3	479.2	482.8	480.9 d	1.80
	6	3	497.1	501.4	499.0 e	2.18
	7	3	443.4	447.9	445.0 b	2.49
	8	3	497.0	505.0	500.1 e	4.29
	9	3	478.9	481.4	480.2 d	1.25
	10	3	471.0	477.7	473.3 ed	3.81
	Overall	48	476.4	666.6	568.5	58.35
	1	9	622.8	649.8	635.5 a	8.47
	2	9	476.4	506.2	491.1 b	8.96
Packed	3	6	524.6	542.6	532.4 c	8.03
Bulk	4	6	546.8	559.2	554.2 d	5.20
Density	5	3	500.4	550.6	533.5 c	28.64
(kg/m^3)	6	3	569.6	574.0	571.2 e	2.43
	7	3	525.8	529.6	528.2 c	2.09
	8	3	654.2	666.6	661.0 f	6.29
	9	3	619.4	626.0	622.5 a	3.31
	10	3	615.8	632.0	626.4 a	9.19
Color -	Overall	80	61.29	51.77	56.71	2.57
Hunter L	1	15	56.58	53.68	54.76 a	0.76
(-)	2	15	56.18	53.81	55.22 ab	0.84

Table 3.1 Properties of distillers dried grains with solubles (DDGS) (continued). [a]

	3	10	54.23	51.77	53.23 c	0.82
	4	10	59.22	56.98	58.17 d	0.64
	5	5	61.07	59.98	60.42 f	0.44
	6	5	60.43	58.26	59.39 e	0.92
	7	5	61.29	59.49	60.68 f	0.81
	8	5	59.81	59.49	58.96 de	0.99
	9	5	60.31	58.37	59.31 e	0.91
	10	5	56.06	55.45	55.79 b	0.23
Color - Hunter a (-)	Overall	80	15.91	12.25	13.85	0.92
	1	15	15.91	14.89	15.35 a	0.28
	2	15	13.95	13.09	13.45 bc	0.23
	3	10	13.43	12.88	13.18 d	0.21
	4	10	12.83	12.25	12.62 e	0.22
	5	5	15.12	14.63	14.89 f	0.19
	6	5	14.25	14.02	14.12 i	0.09
	7	5	13.50	13.16	13.30 bd	0.15
	8	5	13.64	13.16	13.59 cg	0.07
	9	5	14.01	13.49	13.78 gh	0.23
	10	5	14.52	13.62	13.92 hi	0.35
Color- Hunter b (-)	Overall	80	51.60	41.63	46.51	2.55
	1	15	49.55	47.59	48.24 a	0.56
	2	15	44.89	42.98	44.24 b	0.59
	3	10	43.07	41.63	42.28 c	0.46
	4	10	46.32	44.55	45.60 d	0.50
	5	5	51.60	50.55	51.11 e	0.38
	6	5	47.03	46.05	46.60 f	0.50
	7	5	50.39	48.75	49.74 g	0.60
	8	5	48.14	48.75	47.94 ah	0.25
	9	5	47.90	47.12	47.65 h	0.31
	10	5	49.16	47.57	48.01 ah	0.65
Shear Strength (kg/cm^2)	Overall	32	0.022	0.050	0.032	0.01
	1	6	0.040	0.050	0.045 a	0.01
	2	6	0.028	0.038	0.033 c	0.01
	3	4	0.026	0.034	0.030 bc	0.00
	4	4	0.024	0.032	0.028 c	0.01
	5	2	0.022	0.024	0.023 c	0.01
	6	2	0.022	0.026	0.024 bc	0.00
	7	2	0.032	0.036	0.034 bc	0.01
	8	2	0.030	0.032	0.031 b	0.00
	9	2	0.026	0.030	0.028 bc	0.01
	10	2	0.028	0.030	0.029 bc	0.00

[a] New values followed by the same letter within a given property are not significantly different among plants ($p < 0.05$)

Figure 3.1 Angle of repose of distillers dried grains with solubles (DDGS).

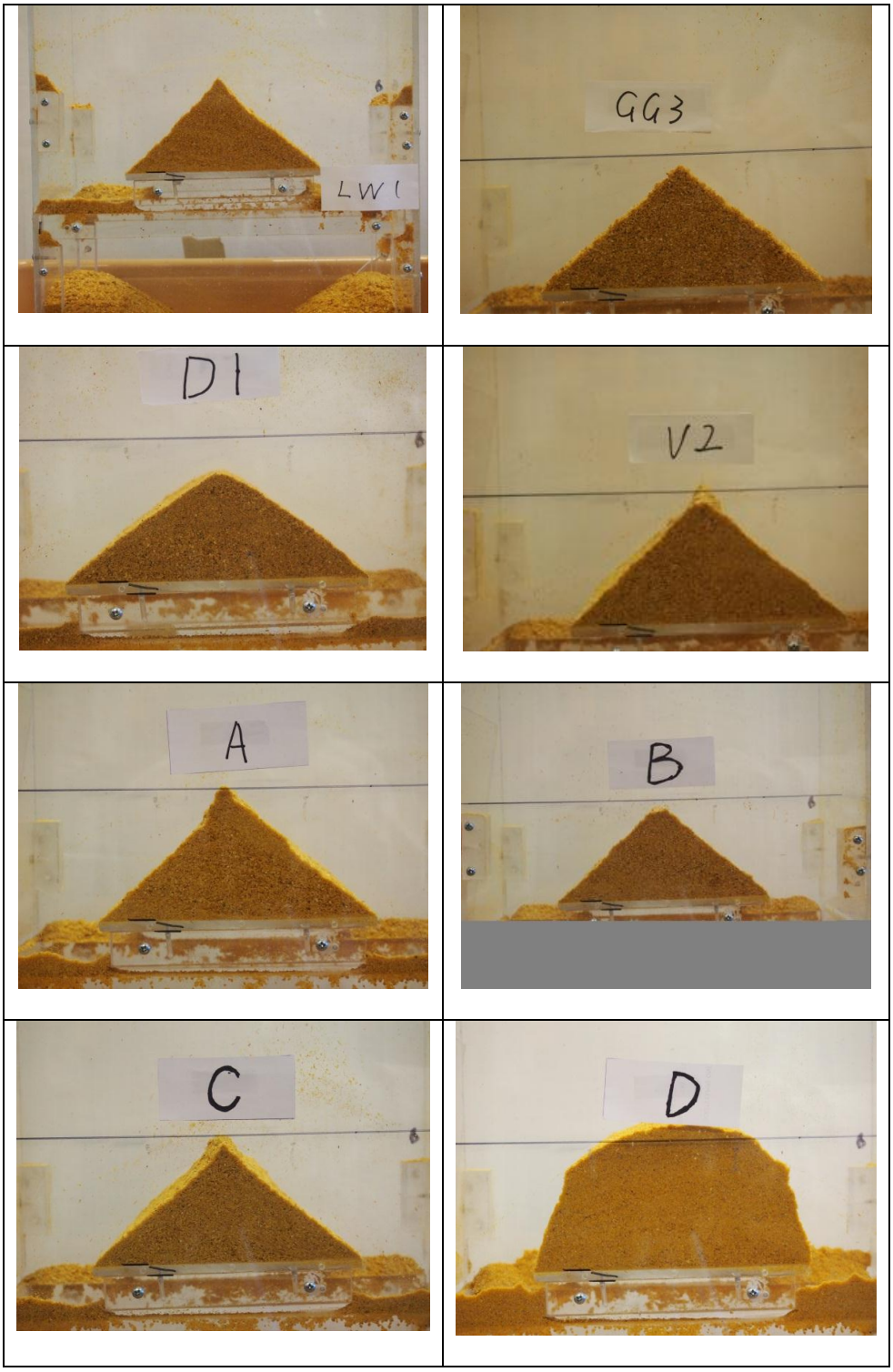

Figure 3.1 Angle of repose of distillers dried grains with solubles (DDGS) (continued).

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CHAPTER 4: Fractionation of Distillers Dried Grains with Solubles (DDGS) through a Destoner

4.1. Introduction

DDGS is mainly composed of protein, fiber, and fat, and is a dry mix of particulate materials. Due to various particle compositions, with high protein and high fiber particle, a method which can divide DDGS into high protein and high fiber fractions could contribute extra economic benefit (RFA, 2012). A high protein fraction will have a greater value as a feed to animals (Belyea et al., 2004), and a high fiber fraction has more potential for corn fiber gum or raw material for lignocellulose ethanol production (Singh et al., 2002).

A destoner is a simple and efficient machine to remove stones and soil from grains. Its principle is to use air flow and shaking to separate. The stones stay on the top of the screen and the grains through it. The greatest advantage of a destoner is that it is convenient and fairly inexpensive to operate, and thus might be appropriate for industrial production (Heiland and Kozempel, 1988).

Through some research has been done to study fractionation of DDGS, these methods have only met with limited, varying degrees of success. Thus, the main objective of this research was to explore whether using a destoner is a reliable and useful method to separate DDGS into various compositions. In addition, using results from

particle size, this study evaluated the relationships between particle size and chemical content, including protein, moisture, fat and fiber.

4.2. Materials and Methods

4.2.1. Materials

DDGS samples were supplied by one dry grind corn ethanol facility located in Iowa, were collected during the fall of 2011, and were stored at room temperature (24 ± 1oC) in sealed plastic storage bags. All composition contents were measured at room temperature and studied with a completely randomized design.

4.2.2. Methods

Particle size analysis was conducted using a sieve shaker (RX-86, W.S Tyler Incorporated, Mentor, OK, USA), according to standard procedure ANSI/ASAE S319.3 (ASABE 2004), using U.S. sieve nos. 6 (3.36 mm), 8 (2.38 mm), 10 (2.00 mm), 14 (1.68 mm), 16 (1.19 mm), 20 (0.841 mm), 30 (0.595 mm), 40 (0.420 mm), 50 (0.297 mm), 70(0.210mm) and Pan (<0.210 mm).

A pressure Destoner (G-2, Forsberg Incorporated, Thief River Falls, Minnesota, and U.S.A) was used to separate DDGS. A large scale test was conducted using air deflection angle in the range of 3o - 8o, and air flow rate in the range of 25% - 30%. Only in these ranges could be the destoner effectively separate DDGS particles (preliminary data at shown). The deck used on the destoner was steel, 60 mesh

(0.251mm). Nutrient analysis was measured using a calibrated NIR Analyzer (DA 7200, Instrumentvagen, Hagersten, Sweeden).

4.3. Data Analysis

All collected data were analyzed with Microsoft Excel v. 2010 (Microsoft Corp, Redmond, WA), and SAS State Version (SAS Institute, Cary, NC) software. Summary statistics, and ANOVA were used to test each property to determine whether significant differences existed away fractions using a Type I (α) error rate of 0.05; if so, post-hoc LSD tests were conducted using a 95% confidence level to determine where those differences occurred (Meier, 2006).

4.4. Results and Discussion

4.4.1. Optimal Condition

Table 4.1 presents composition analyses of DDGS treated by the destoner under the experimental conditions. The moisture of the treated DDGS fractions (w.b. - wet basis) varied from 8.20% to 11.25%, with an average of 9.76; protein recovered varied from 28.15% to 31.30%, with an average of 29.93%; oil recovered varied from 10.40% to 17.45%, with an average of 13.81%; fiber recovered varied from 6.95% to 7.20%, with an average of 7.10%.

Comparing to other methods of dry fractionation, the destoner fractionation had a higher value both in protein and fat than Wu and Stringfellow (1986), except for mesh size over sieve no. 80. Also, the results of protein in destoner fractionation from the light

fraction had a higher value than the aspirated fraction from the method of aspirating, but our heavy fraction was very similar; but in the oil composition, the destoner fractionation had an evident advantage both in the heavy and light fraction (Singh et al, 2002). However, comparing with the method of sieving and elutriation, sieving had a higher efficiency in selecting protein and fiber, but not in oil; elutriation had an evident lower value in protein and oil, but was a little higher in fiber (Srinivasan et al, 2005).

After comparing with other research results, our destoner fractionation may have a better separating rate in protein and oil, especially the latter; but it was not great in separating fiber. Based on an overall analysis of conditions in all fractions, when the destoner was set at an angle of 8° and air flow was 27.5%, the separation rate had the most economical combined efficiency.

4.4.2. Least Significant Difference Test (LSD)

In order to further explore the relationships between the heavy and light fractions, the fraction composition data were examined as shown in Figure 3.1, 3.2 and Fig 3.3. According to the figures, it is clearly shown that the heavy fraction had a higher value in oil and protein, lower value in moisture, and similar value in fiber to the unfractionated DDGS. It can be assumed that particle size was a possible cause of the differences in composition. In order to prove that assumption, least significant difference (LSD) was tested, with results shown in Table 4.4.

With the LSD analysis (Table 4.4), the results clearly show served trends: moisture and fiber didn’t have a significant difference between heavy and light; protein

had a weak significant difference, while oil had a strong significant difference between heavy and light fractions. Considering the limitation of the samples, these results show the variation in properties of composition from only one ethanol plant; these trends should be investigated using more samples from other plants in future study.

4.4.3. Correlation Tests

According to the LSD results, it proved that the compositions of different fractions were influenced by the individual particle size; this needed to be proved by a correlation test. In order to prove the assumption, DDGS samples were separated by sieving. Each sample was tested by NIR, and all the data were combined and analyzed (Table 4.5). Using correlation tests, the final result are shown in Table 4.7, which is helpful to find the linear correlation between each data point and all other respective points.

Table 4.7 clearly shows that moisture and particle size, moisture and oil had strong negative correlations. Also, oil and particle size had a strong positive correlation. An explanation to this result is that in these samples, intact germ, which contained highest amount of oil, was visible and naturally went to larger size fractions during sieving (Liu, 2008). Moisture is decided by water content, which solubility is opposite to oil, causing a negative correlation between oil and moisture. Fiber doesn't have a correlation with particle size, which similar to the results of Clementson and Ileleji (2012). The propensity of protein was weakly influenced by the particle sizes of various DDGS fractions, which was a similar result with other research groups (Liu, 2008 and

Clementson and Ileleji, 2012). An explanation of this result is that protein is equally distributed in the DDGS and doesn't affect the construction of intact germ, which is proportional to particle size (Liu, 2008).

4.5. Conclusion

The objectives of this research were to explore whether destoner fractionation was effecting in separating DDGS into components, and to examine the relationships between particle size and chemical content. The final results showed that destoner fractionation was efficient in a certain degree to separate oil fractions of DDGS, and 8° angle and 27.5% air flow had the highest value. Also, compared with other methods, destoner fractionation has advantages of relatively high efficiency and low cost, after considering the whole procedure. Particle size distribution had a positive correlation to oil, and a negative correlation to water. Fiber had no relationship with particle size, while protein had a weak correlation with particle size. Further fractionation should be explored reasons in future research.

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Table 4.1 Composition of DDGS separated by destoner according to angle and air flow [a].

Angle (°) / Air Flow (%)	Fraction	Moisture (%)	Protein (%)	Oil (%)	Fiber (%)
8 / 25	Light	10.35	29.75	12.40	7.20
		(0.07)	(0.07)	(0.00)	(0.00)
	Heavy	9.10	30.80	15.35	7.05
		(0.00)	(0.14)	(0.07)	(0.07)
8 / 27.5	Light	11.10	28.15[b]	10.50	7.20
		(0.00)	(0.07)	(0.14)	(0.00)
	Heavy	8.20[b]	31.30[b]	17.20	6.95
		(0.00)	(0.00)	(0.14)	(0.07)
8 / 30	Light	10.05	30.00	13.40	7.10
		(0.07)	(0.00)	(0.00)	(0.00)
	Heavy	8.85	30.55	15.70	7.00
		(0.07)	(0.07)	(0.14)	(0.00)
5 / 25	Light	10.50	29.70	11.60	7.20
		(0.00)	(0.14)	(0.14)	(0.00)
	Heavy	9.05	30.60	15.25	7.05
		(0.07)	(0.14)	(0.07)	(0.07)
5 / 27.5	Light	10.45	29.25	11.80	7.20
		(0.07)	(0.07)	(0.00)	(0.00)
	Heavy	8.60	30.15	16.35	6.95
		(0.00)	(0.07)	(0.07)	(0.07)
5 / 30	Light	10.70	28.50	11.15	7.20
		(0.00)	(0.14)	(0.07)	(0.00)
	Heavy	8.35	31.20	17.45[b]	6.95
		(0.07)	(0.14)	(0.07)	(0.07)
2 / 25	Light	10.45	29.80	11.70	7.10
		(0.07)	(0.14)	(0.14)	(0.00)
	Heavy	8.90	31.05	15.70	7.00
		(0.00)	(0.07)	(0.00)	(0.00)
2 / 27.5	Light	11.25[b]	28.45	10.40[b]	7.20
		(0.07)	(0.07)	(0.00)	(0.00)
	Heavy	9.15	30.40	15.55	7.05
		(0.07)	(0.00)	(0.07)	(0.07)
2 / 30	Light	10.80	29.85	12.45	7.15
		(0.00)	(0.07)	(0.07)	(0.07)
	Heavy	8.75	30.20	17.00	7.05
		(0.07)	(0.00)	(0.00)	(0.07)
Mean		9.76	29.93	13.81	7.10
Minimum		8.20	28.15	10.40	6.95
Maximum		11.25	31.30	17.45	7.20
Standard Deviation		1.015	0.929	2.452	0.097

[a] Values are reported as means of two batches and two replicates from each batch, and values in parentheses are standard deviation.

[b] Values in the highlighted cells are the lowest or highest value in moisture, protein and oil content

Table 4.2 Statistics analysis of properties of fractionated distillers dried grains with solubles (DDGS) by ANOVA test (Independent Variable: Airflow).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	35.52104494	8.88026123	2.92	0.0629
Error	13	39.47895506	3.03684270		
Corrected Total	17	75.00000000			

R-Square	Coeff Var	Root MSE	airflow Mean
0.473614	6.336923	1.742654	27.50000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
mc	1	0.25781473	0.25781473	0.08	0.7754
protein	1	8.32100214	8.32100214	2.74	0.1218
oil	1	24.96473628	24.96473628	8.22	0.0132
fiber	1	1.97749178	1.97749178	0.65	0.4342

Source	DF	Type III SS	Mean Square	F Value	Pr > F
mc	1	15.07564316	15.07564316	4.96	0.0442
protein	1	15.11808841	15.11808841	4.98	0.0439
oil	1	23.91690826	23.91690826	7.88	0.0148
fiber	1	1.97749178	1.97749178	0.65	0.4342

Table 4.3 Statistical analysis of properties of fractionated distillers dried grains with solubles (DDGS) by ANOVA test (Independent Variable: Angle).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	18.3501452	4.5875363	0.67	0.6273
Error	13	89.6498548	6.8961427		
Corrected Total	17	108.000000			

R-Square	Coeff Var	Root MSE	angle Mean
0.169909	52.52102	2.626051	5.000000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
mc	1	1.39894376	1.39894376	0.20	0.6598
protein	1	0.76860127	0.76860127	0.11	0.7438
oil	1	12.73655342	12.73655342	1.85	0.1973
fiber	1	3.44604675	3.44604675	0.50	0.4921

Source	DF	Type III SS	Mean Square	F Value	Pr > F
mc	1	17.82781731	17.82781731	2.59	0.1319
protein	1	0.00000903	0.00000903	0.00	0.9991
oil	1	11.80466032	11.80466032	1.71	0.2134
fiber	1	3.44604675	3.44604675	0.50	0.4921

Table 4.4 Statistical analysis of properties of fractionated distillers dried grains with solubles (DDGS) by LSD test. [a]

		Moisture	Protein	Oil	Fiber
Mean	Light	10.63	29.27	11.71	7.17
	Heavy	8.77	30.69	16.17	7.01
Standard Deviation	Light	0.37	0.70	0.93	0.05
	Heavy	0.33	0.41	0.83	0.06
Sum of Square		30.99	18.20	179.11	0.25
Mean Square		30.99	18.20	179.11	0.25
F Value		257.46	55.43	230.21	80.53
Pr>F		<.0001	<.0001	<.0001	<.0001
Alpha		0.05	0.05	0.05	0.05
Error Degrees of Freedom		34	34	34	34
Error Mean Square		0.120	0.328	0.778	0.003
Critical Value of t		2.032	2.032	2.032	2.032
Least Significant Difference		0.235	0.388	0.598	0.038

[a] Denotes that significant differences in a given property between fractions are present ($p < 0.05$)

Table 4.5 Composition of fractions of DDGS separated according to size. [a]

US Sieve Size No	Sieve Opening (mm)	Moisture (wb, %)	Protein (%)	Oil (%)	Fiber (%)
No.6	3.360	7.9	28.2	15.3	7.4
		(0.28)	(0.00)	(0.07)	(0.00)
No.8	2.580	8.2	29.1	15.5	7.3
		(0.14)	(0.14)	(0.14)	(0.07)
No.10	2.000	9.1	29.6	15.1	7.2
		(0.00)	(0.07)	(0.14)	(0.00)
No.14	1.400	9.6	28.7	13.8	7.3
		(0.07)	(0.07)	(0.00)	(0.00)
No.16	1.190	10.1	28.0	12.8	7.4
		(0.07)	(0.07)	(0.00)	(0.07)
No.20	0.841	10.5	28.3	12.5	7.3
		(0.07)	(0.07)	(0.00)	(0.07)
No.30	0.585	11.1	29.0	11.5	7.2
		(0.07)	(0.14)	(0.07)	(0.00)
No.40	0.420	11.3	30.0	11.0	7.2
		(0.07)	(0.00)	(0.07)	(0.00)
No.50	0.297	11.4	30.8	10.5	7.3
		(0.28)	(0.07)	(0.21)	(0.07)
No.70&Pan	0.210	11.0	31.0	10.1	7.5
		(0.14)	(0.35)	(0.07)	(0.14)

[a] Values are reported as means of two batches and two replicates from each batch, and values in parentheses are standard deviation.

Table 4.6 Statistical analysis of properties of fractionated distillers dried grains with solubles (DDGS) by ANOVA test (Independent Variable: Particle Size).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	9.90357724	2.47589431	68.94	0.0001
Error	5	0.17956886	0.03591377		
Corrected Total	9	10.08314610			

R-Square	Coeff Var	Root MSE	Sieve Mean
0.982191	14.71003	0.189509	1.288300

Source	DF	Type I SS	Mean Square	F Value	Pr > F
mc	1	9.71159198	9.71159198	270.41	<.0001
protein	1	0.01579678	0.01579678	0.44	0.5365
oil	1	0.00238079	0.00238079	0.07	0.8071
fiber	1	0.17380770	0.17380770	4.84	0.0791

Source	DF	Type III SS	Mean Square	F Value	Pr > F
mc	1	0.69313432	0.69313432	19.30	0.0071
protein	1	0.09060627	0.09060627	2.52	0.1731
oil	1	0.14582440	0.14582440	4.06	0.1000
fiber	1	0.17380770	0.17380770	4.84	0.0791

Table 4.6 Statistical analysis of properties of fractionated distillers dried grains with solubles (DDGS) by ANOVA test (Independent Variable: Particle Size) (continued).

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	45.66950392	16.09770378	2.84	0.0364
mc	-1.46061392	0.33247366	-4.39	0.0071
protein	-0.13588536	0.08555079	-1.59	0.1731
oil	-0.44657052	0.22161822	-2.02	0.1000
fiber	-2.74253347	1.24665977	-2.20	0.0791

Table 4.7 Correlation coefficient (r) values between properties of DDGS fractions. [a]

	Particle Size Diameter(mm)	Moisture	Protein	Oil	Fiber
Particle Size Diameter (mm)	1				
Moisture	-0.98	1			
Protein	-0.54	0.53	1		
Oil	0.93	-0.96	-0.61	1	
Fiber	0.08	-0.13	0.08	-0.12	1

[a] Denotes that significant differences in a given property between fractions are present ($p < 0.05$)

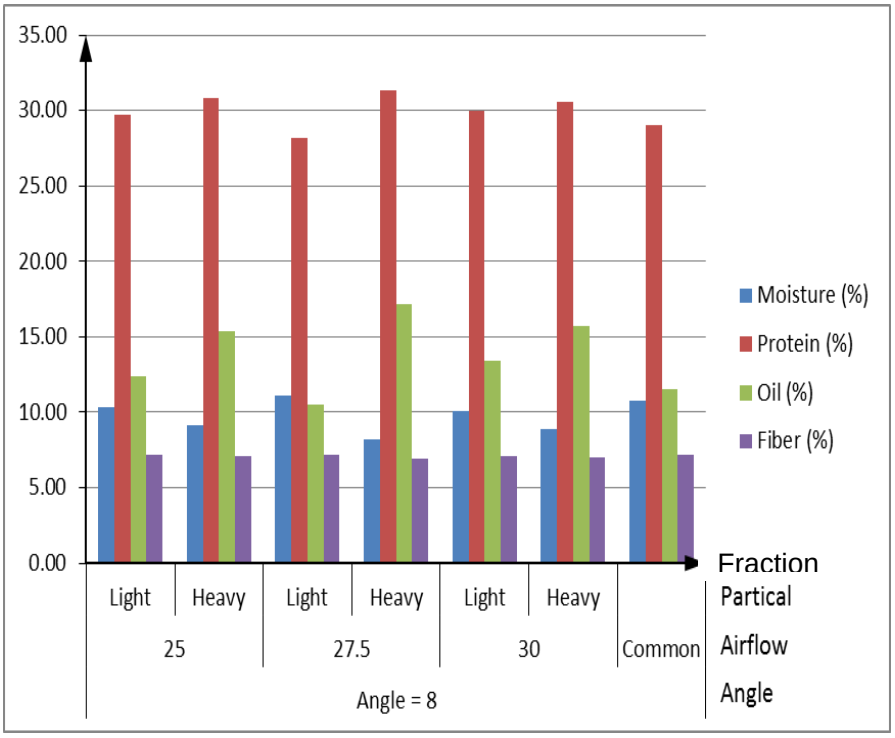

Figure 4.1 Composition of DDGS fractions separated by a destoner at Angle = 8°.

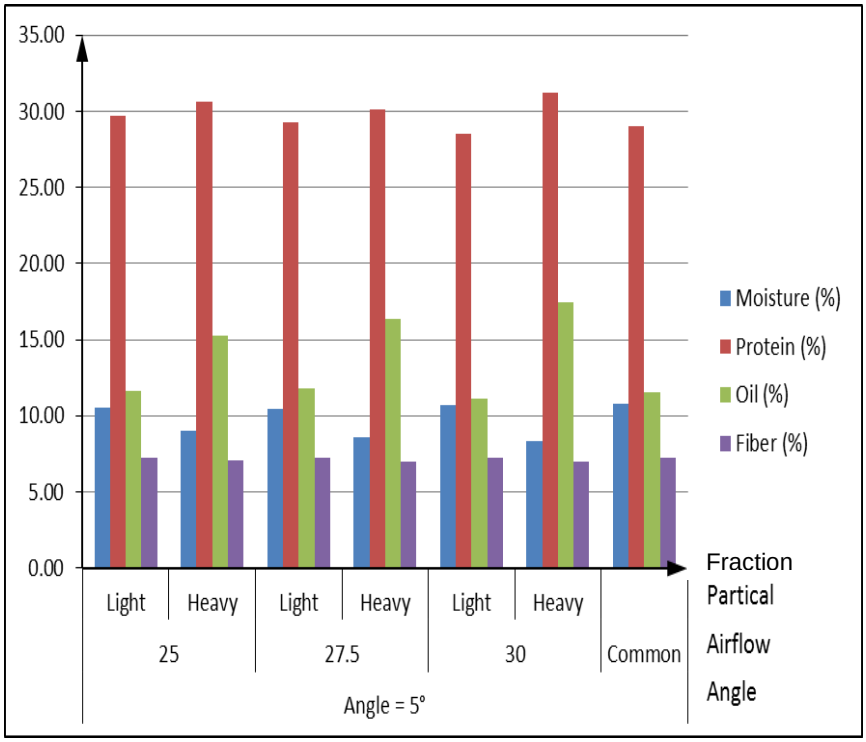

Figure 4.2 Composition of DDGS fractions separated by a destoner at Angle = 5°.

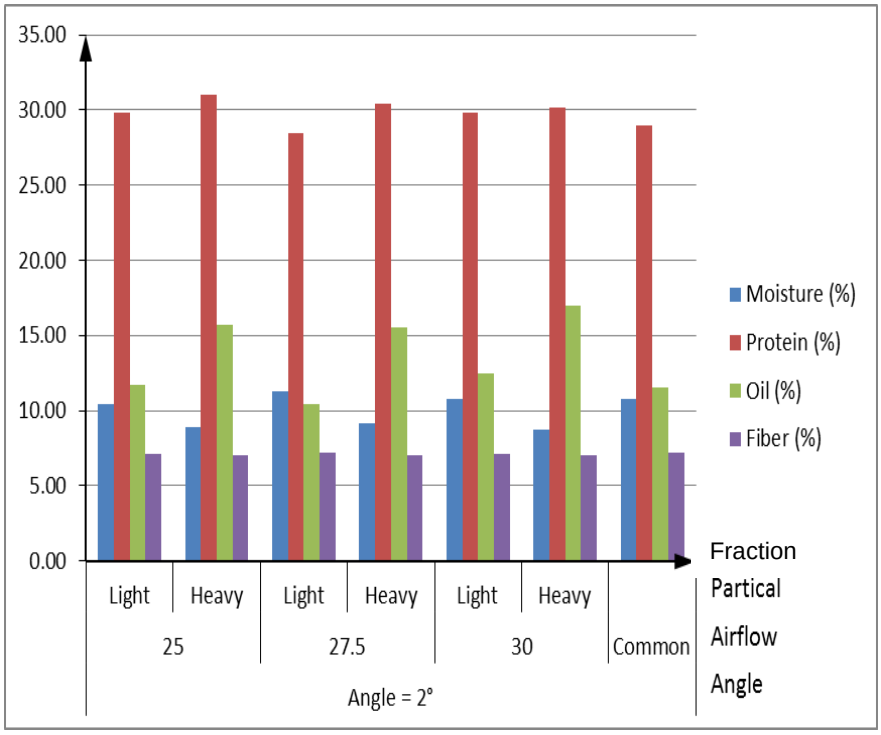

Figure 4.3 Composition of DDGS fractions separated by a destoner at Angle =2°.

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CHAPTER 5: Pretreatment of distillers dried grains with solubles (DDGS) using low-moisture anhydrous ammonia (LMAA) process

5.1. Introduction

With pressure from shortage of fossil fuels, bioethanol as a fuel additive continues its rapid growth in United States, and the ethanol industry also has grown rapidly in recent years (Schnepf and Yacobucci, 2013). Approximately 14.8 U.S. liquid billion gallons (52.6 billion liters) of ethanol was produced in 211 plants operating in 29 states, which is mostly from corn grains and the top producer in the world (RFA, 2013). In the US, the dominant process for producing bioethanol is dry grind process, which contributes more than 80% of current ethanol production (RFA, 2009).

In the corn-based fuel manufacturing, distiller dry grain and solubles (DDGS) is created as a co-product, and 42.5 million metric tons of this material was produced in 2012 (RFA, 2013). Among all products from bioethanol industry, marketing of DDGS as an important ingredient is directly related to sustainability of the dry grind plant, which is sold at a varying market price (US$85–140/ton) (Liu, 2008).

However, increasing production of DDGS caused the price is expected to decrease in relation to other feeds such as soybean meal. Thus, it is necessary to increase the value of DDGS to keep cost competitive and enzymatic hydrolysis of DDGS is a possible method to increase its value (Bals et al., 2006). However, lignin and

hemicellulose are tightly associated with each other, which protect polysaccharides and cellulose from enzymatic hydrolysis (Fengel and Wegener, 1984; Hendriks and Zeeman, 2009). Therefore, pretreatment is necessary to break down the structure of lignin-hemicellulose association, and then the resulting cellulose can be hydrolysis into glucose (Mosier et al., 2005).Thus, Tucker et al (2004) attempted dilute–sulfuric acid to pretreat distiller grains and obtained soluble sugar yields of 73% of the theoretical value. Bals et al (2006) pretreated DDGS with ammonia fiber expansion (AFEX) and obtained a conversion yield of 190g glucose/kg dry biomass. Then Lau et al., (2008) used simultaneous saccharification and fermentation (SSF) on pretreated DDGS, and found an ethanol productivity of 1.2 g/h/L.

Through some research has been done to study the effectiveness to pretreatment of DDGS, these methods have only met with limited, varying degrees of success. For example, inhibition of acid to sugar fermentation and high cost are the disadvantages of acid pretreatment method (Li et al., 2010; Kootstra et al., 2009); economics, water and chemical consumption, and environmental concerns are problematic to AFEX (Chundawat et al, 2007; Eggeman and Elander, 2005).

In order to avoid the washing step and reduce capital costs in the pretreatment process, low-moisture anhydrous ammonia (LMAA) process has been developed by Yoo et al. (2011). LMAA pretreat biomass with low moisture using gaseous ammonia, which leads to short exposure time and can be carried out under ambient conditions. With the condition of 80°C for 84 h and 0.1g NH_3/g biomass loading rate, Yoo et al. (2011) obtained 89% of theoretical ethanol yield from corn stover, which is a higher conversion

yield than other pretreatment methods. However, Yoo et al. (2011) utilized a very small sealed batch reactors (8.1 cm *8.1 cm *18.5 cm, 690 mL internal volume) so that it may lead to inappropriate conditions for optimal ethanol production at larger scales.

Thus, the main objective of this research was to explore whether using LMAA is an efficient and useful method to pretreat DDGS and enzymatic hydrolysis with a higher efficiency. In addition, optimal conditions with pretreatment temperature, pretreatment time, and moisture content of DDGS, ammonia loading rate for highest enzymatic hydrolysis yield were obtained in a larger-scale reactor.

5.2. Materials and Methods

5.2.1. Materials

5.2.1.1. DDGS

DDGS samples were supplied by one dry grind corn ethanol facility located in Iowa, collected during the fall of 2011, and were stored at room temperature (24 ± 1^{o}C) in sealed plastic storage bags. All composition contents were measured at room temperature and studied with a completely randomized design.

5.2.1.2. Enzymes

Cellulase GC 220 (Lot#301-042320162) and Multifect-xylanase (Lot #301-04021-015) were provided from Genencor International, Inc. (Rochester, NY, USA). The average activity of the enzyme was expressed with 45 filter paper units (FPU)/ml

and 8000 Genencor xylanase units (GXU)/ ml. The β-glucosidase enzyme (Novozyme 188) was provided from Sigma-Aldrich, Inc. (St. Louis, Missouri, USA). The average activity of Novozyme 188 was 750 cellobiase units (CBU) / mL.

5.2.2. Equipment

The large scale reactor (Figure 5.1) for pretreatment process was provided from Pall Corporation, Ann Arbor, Michigan, USA. Comparing with previous study, the new sealed reactor was about 16 times larger than Yoo et al (2011), which had a volume of 0.7 L. Sugars content was measured by HPLC with a Bio-Rad Aminex HPX-87P column (Aminex HPX-87P, Bio-Rad Laboratories, Hercules, CA, USA) and a refractive index detector (Varian 356-LC, Varian, Inc., CA, USA). Acid soluble lignin (ASL) content was determined by UV-Visible spectrophotometer (UV-2100 Spectrophotometer, Unico, United Products & Instruments, Inc., Dayton, NY, USA).

5.2.3. Experimental Design

In this study, four independent variables were investigated, including DDGS moisture, ammonia loading rate, pretreatment time and pretreatment temperature, which may influenced the reaction severity. Moisture contents of DDGS were set as 20 %, 40 % and 80 % (w.b. - wet basis); ammonia loading rate was set to 0.1 g, 0.3 and 0.5g NH_3/g-DDGS; pretreatment times were targeted as 24 h, 96 h, 168 h; the pretreatment temperatures were set as 20°C, 50 °C, 80 °C, with higher temperature (>80 °C) could burn DDGS to char. By controlling these independent variables, there were 17

treatments in this study (i.e. 2 × 2 × 2 × 2 + 1 center point). Glucan, xylan, galactan, arabinan, mannan, lignin and ash content were measured as dependent variables during the experiment. The experimental design for this study is shown in Table 5.1.

5.2.4. Experimental Operation

5.2.4.1. Moisturization

Moisture content was determined following the standard Forage Analysis Procedure (NFTA, 2002), using a forced-convection laboratory oven (Thermo OGH & OMH180, Scientific Heratherm, Langenselbold, Germany) at 105 ºC for 3 h. The average moisture content of original DDGS was 7.72% (w.b. – wet basis). In order to adjust to the various conditions, additional water was added to 20, 40, and 60 % (w.b. – wet basis) and steeped for 24h. Each sample was ammoniated, pretreated, and dried under same conditions.

5.2.4.2. Pretreatment

Moisturized DDGS was placed in the sealed reactor, which was connected to an ammonia gas cylinder with single stage gas regulator. A pipe was connected between the top of the reactor and the fume hood to ventilate surplus ammonia. Gauges were equipped on the reactor to monitor the pressure and temperature during the ammoniation process. After air valve was open, anhydrous ammonia was added up to the various targeted pressure to achieve 0.1 g, 0.3 and 05 NH_3/ g biomass. After ammonia loading, the connection to the valve was closed, and system pressure was maintained below 25

psi, which lasted up to 30 min in order to have a complete ammonization reaction. Temperature changes could be observed with 30 ºC increasing to about 60 ºC, but it was not controlled during this study. After the ammoniation process was finished, DDGS was transferred to glass bottles (250 mL) with a screw cap and covered with parafilm and aluminum foil tightly. The bottles packed with ammoniated DDGS were placed in heating ovens at various pretreatment temperatures (20°C, 50°C, and 80°C) for 24 h, 96 h, and 168 h. After the pretreatment process was finished, the pretreated samples were dried in fume hood and surplus ammonia was evaporated for 24 h.

5.2.5. Analytical Methods

5.2.5.1. Compositional analysis

Carbohydrates and lignin were determined by NREL LAP (NREL, 2008), which each samples was analyzed in duplicate. According to the NREL standards, the content of glucan and xylan in the DDGS could be analyzed by HPLC, and avicel (PH-101, particle size: ~ 50 μm, Sigma-Aldrich) was used as a sugar conversion standard. Acid soluble lignin was measured by UV-Visible Spectrophotometer, and moisture content was determined by an oven drying method (NREL, 2008).

5.2.5.2 Enzymatic digestibility test

The enzymatic digestibility of LMAA-treated DDGS was carried out based upon NREL LAP (NREL, 2008). The test was conducted in duplicate under conditions of pH = 4.8 (0.1M sodium citrate buffer) with 40 mg/L tetracycline and 30 mg/L

cyclohexamide in 250 mL Erlenmeyer flasks. The reaction was conducted at 50°C ± 1°C and 150 rpm in an incubator shaker for 96h (Excella E24 Incubator Shaker Series, New Brunswick Scientific, Edison, NJ, USA).

The initial glucan concentration was 1% (w/v), which was considered as an optimal biomass loading rate (Li and Kim, 2011; Yoo et al, 2011). Cellulose (GC 220) was loading at 15 FPU/g of glucan, ß-glucosidase (Novozyme 188) loading was at 30 CBU/g of glucan, and xylanase enzyme loading was equal to 2000 GXU/g of xylan. Total glucose and xylose detected from HPLC was used to calculate the glucan and xylan digestibility following equation 1and 2 below. The conversion factor for glucose to equivalent glucan was 0.9 and conversion factor for xylose to equivalent xylan was 0.88, which depends on molecular weight.

$$\text{Glucan digestibility } [\%] = \frac{\text{Total released glucose} \times 0.9}{\text{Initial glucan loading}} \times 100\%$$

Equation 1: The Equation for Glucan Digestibility from released glucose

$$\text{Xylan digestibility } [\%] = \frac{\text{Total released xylose} \times 0.88}{\text{Initial xylan loading}} \times 100\%$$

Equation 2: The Equation for Xylan Digestibility from released xylose

5.3. Data Analysis

All collected data were analyzed with Microsoft Excel v. 2010 (Microsoft Corp, Redmond, WA), and SAS (SAS Institute, Cary, NC) software. Summary statistics, and ANOVA were used to test each property to determine whether significant differences

existed with using a Type I (α) error rate of 0.05; if so, post-hoc LSD tests were conducted using a 95% confidence level to determine where those differences occurred (Meier, 2006).

5.4. Results and Discussion

5.4.1. Effects of LMAA pretreatment on DDGS composition

The use of low-moisture anhydrous ammonia (LMAA) pretreatment didn't result in many significant changes in glucan, xylan and ash contents, as shown in Table 5.2 (main effects) and Table 5.4 (treatment effects). In addition, as Table 5.3 shows, the majority of the p-values for interactions among these independent variables were higher than 0.05, which indicates little evidence of significant interactions among independent variables was obtained in this study. The reason for insignificant compositional analyses result was because the ammonia used in the pretreatment process was meant to break down lignocellulosic structure, not directly to hydrolyze cellulose and hemicellulose structure.

However, there were some significant changes in lignin, galactan, arabinose and mannan in the pretreatment of LMAA, as shown in Table 5.2 (main effects) and Table 5.4 (treatment effects), especially to the samples treated with a higher temperature and longer reaction time. What's more, interaction effect test (Table 5.3) indicates that some of the p-values of interactions among temperature and reaction time were lower than 0.05, which meant strong evidence of significant interactions among independent variables was obtained in this study. The reason for the change of compositional

analyses result may was that ammonia had the ability of some degrees to break down lignin content and related structure, which was similar with other researcher's results (Lau et al, 2008; Lau et al, 2009; Li and Kim, 2011). In addition, broken lignin strucutre could release and convert some sugar such as galactan, arabinose and mannan, which may lead to the change of these contents. Comparing with other pretreatment methods, LMAA didn't contain a washing step so that separated lignin and released sugar content would stay with samples so that results was higher than untreated one, which was different from other methods such as AFEX and dilute acid pretreatment (Lau et al, 2009; Li and Kim, 2011; Yoo et al, 2011). Besides these, DDGS had a more complicated structure with higher protein and oil content, which may lead to some change and hydrolysis by ammonia and need to be investigated in future work.

5.4.2. Effects of LMAA pretreatment on enzymatic digestibility

Figure 5.2 shows the enzymatic digestibility results for the 17 treatments listed in Table 5.1, while Figure 3 compares digestibility results for avicel (used as a reaction blank for the substrate), untreated corn stover, and the best digestibility. From Figure 5.2, different combinations of the four factors resulted in various digestibility. The enzymatic digestibility of glucan to all treated DDGS measured at 96 h was 36.67- 63.03%. As shown in Figure 5.3, the highest glucan digestibility (63.03%) of LMAA pretreated DDGS with a pretreatment condition of 80˚C pretreatment temperature, 96 h pretreatment time, 60% (w.b.- wet basis) moisture content of DDGS, and 0.1 NH_3/ g biomass ammonia loading rate, which was 1.74 times compared to untreated DDGS

(36.21%). Among the 17 treatments, the median treatment, which was 50˚C pretreatment temperature, 72 h pretreatment time, 50% (w.b.- wet basis) moisture content of DDGS, and 0.1 NH_3/ g biomass ammonia loading rate, achieved a glucan digestibility with 50.04%.

Four pretreatment factors were tested in this study: pretreatment temperature, pretreatment time, moisture content of DDGS, and ammonia loading rate. Among these factors, due to the highest p-value (0.0034), pretreatment temperature was selected as the most important variable factor. With other factors keeping constant (i.e., main effects), the differences of average glucan digestibility between high pretreatment temperatures and low temperatures were shown in Table 5.5. It is clearly shown that the higher temperature resulted in increasing digestibility, which is similar to the results of Bals et al (2006). Considering the effect of furnace, 80 °C could be optimal temperature to LMAA for DDGS pretreatment, which higher temperature (>80 °C) could start to burn DDGS to char and substantially decrease the cellulose content.

Similar to pretreatment temperature, the difference of pretreatment time between longer time and shorter time was also significant (Table 5.5). The data indicated that for pretreatment time, glucan digestibility increased as the pretreatment time increased. The average glucan digestibility at 168 h pretreatment time was higher than the average for 24 h pretreatment time. It is a trend that longer pretreatment time could increase the glucan digestibility, which was proved by most researchers' groups (Bals et al, 2006; Lau et al, 2008; Lau et al, 2009; Li and Kim, 2011; Yoo et al, 2011). The reason for this

trend is considered that enzyme needs enough time to hydrolyze cellulose from the collapsed structure of DDGS, which is certified by the reaction curve of enzyme.

As for moisture content, it was observed that DDGS resulted in higher glucose digestibility with higher moisture content of DDGS. The average glucan digestibility at 60% (w.b. - wet basis) was higher than the average for 20% (w.b. - wet basis) moisture content of DDGS. Considering the ability of DDGS to absorb water, moisture content of 60% is a better degree to DDGS. The reason for this may result from the effect of water molecule bind ammonium ion during the ammoniation. Due to the presence of bound water in biomass, ammonium ion (NH_4^+) and hydroxyl ion (OH^-) can formed from ammonia molecule and related NH_3–H_2O, which is responsible for the reaction with lignin. In addition, hydrogen bonds with cellulose are formed from bound water, which causes swelling of crystalline cellulose structure and increases the accessibility to enzymes (Yoo et al, 2011).

Different from the three other factors, ammonia loading rate results in weak significant difference between lowest and highest loading rate. The ammonia loading rate of 0.1g NH_3/g-DDGS average obtained 50.34% of glucan digestibility, which is just a little higher than the samples of 0.5g NH3/g-DDGS average and 0.3g NH3/g-DDGS average. It indicates that LMAA is a useful and efficient method to pretreat DDGS with a lower amount of ammonia, which is important in industry production.

For xylan digestibility, the xylose yields were negligible to all four effect factors. Multifect Xylanase was added 2000 GXU/g-xylan to pretreated DDGS. However, the data indicated that less than 15% of the xylan was hydrolyzed in all samples and no

relationship was founded between four effect factors. The reason may be resulted that hemicellulose in DDGS has a complex arabinoxylan structure, which is consisted of a xylan backbone with several branching and cross-linked chains (Leathers 2003; Koukiekolo et al, 2005). For effectively hydrolyzing this structure, it requires breaking several different bonds and more enzymes than simply xylanase (Bals et al, 2006). In addition, the higher protein and oil content may affect the efficiency of xylanase, which need to be explored in the future study.

5.5. Conclusions

LMAA pretreatment is considered as an effective potential method to pretreat DDGS and other biomass, which has the advantages of using low amount ammonia, no washing step and low energy consumption. In this study, it explored the effect of LMAA pretreatment to DDGS and the efficiency of enzymatic hydrolysis under various conditions. According to experimental result, DDGS achieved a higher glucose yield after pretreatment, which reflected LMAA method had a potential to pretreat DDGS in industry producution. When DDGS (60 wb% moisture content) was pretreated with 0.1g NH3/g-DDGS ammonia loading rate at 80˚C and 168 h, it was obtained with the maximum glucan digestibility of 63.03%. Higher pretreatment temperature, longer pretreatment time, higher moisture content of DDGS and lower ammonia loading rate have a trend to pretreat DDGS and obtain a higher enzymatic hydrolysis.

Table 5.1 Experimental design for pretreatment of distillers dried grains with solubles (DDGS) using low-moisture anhydrous ammonia (LMAA) process.

Treatment	Moisture Content (wb %)	Time (h)	Temperature (°C)	Ammonia Loading Rate (g NH_3/g-DDGS)
1	20	24	20	0.5
2	20	24	20	0.1
3	20	24	80	0.5
4	20	24	80	0.1
5	20	168	20	0.5
6	20	168	20	0.1
7	20	168	80	0.5
8	20	168	80	0.1
9	60	24	20	0.5
10	60	24	20	0.1
11	60	24	80	0.5
12	60	24	80	0.1
13	60	168	20	0.5
14	60	168	20	0.1
15	60	168	80	0.5
16	60	168	80	0.1
CP[a]	40	96	50	0.3

[a]: CP denotes center point of the design.

Table 5.2 Main effects on resulting compositional analysis to treated DDGS [a]

Factor	Levels	Lignin (%)	AIL (%)	ASL (%)	Glucan (%)	Xylan (%)	Galactan (%)	Arabinose (%)	Mannan (%)	Ash (%)
Temperature (°C)	20	13.23a (1.23)	8.92a (1.96)	4.31a (1.24)	23.24a (2.58)	7.39a (1.63)	5.70a (0.16)	4.81a (0.65)	1.96a (0.11)	3.90a (0.53)
	50	14.55b (0.25)	8.31a (0.48)	6.24b (0.74)	23.26a (2.75)	8.42a (0.24)	6.07b (0.01)	5.85b (0.07)	1.87b (0.08)	3.55a (0.30)
	80	15.62c (2.51)	10.62b (2.24)	4.99c (1.06)	24.51a (1.74)	7.83a (1.13)	5.84c (0.27)	5.30b (0.70)	2.01c (0.11)	3.85a (0.41)
Time (h)	24	13.43a (1.79)	9.20a (2.36)	4.23a (1.15)	24.38a (2.33)	7.25a (1.51)	5.73a (0.21)	4.99a (0.63)	1.90a (0.05)	3.94a (0.41)
	96	14.55b (0.25)	8.31a (0.48)	6.24b (0.74)	23.26a (2.75)	8.42b (0.24)	6.07b (0.01)	5.85b (0.07)	1.87a (0.08)	3.55a (0.30)
	168	15.41b (2.36)	10.34b (2.04)	5.07c (1.10)	23.37a (2.14)	7.98b (1.22)	5.80c (0.24)	5.12a (0.80)	2.07b (0.10)	3.81a (0.52)
Moisture Content	20	13.88a (2.20)	9.12a (1.52)	4.76a (1.36)	24.20a (2.35)	6.94a (1.08)	5.75a (0.23)	5.48a (0.57)	1.95a (0.10)	3.92a (0.54)
(wb%)	40	14.55ab (0.25)	8.31a (0.48)	6.24b (0.74)	23.26a (2.75)	8.42b (0.24)	6.07b (0.01)	5.85a (0.07)	1.87b (0.08)	3.55a (0.30)
	60	14.97b (2.32)	10.42b (2.68)	4.55a (1.02)	23.55a (2.18)	8.29b (1.38)	5.79a (024)	4.63b (0.57)	2.02c (0.12)	3.82a (0.39)
Ammonia Loading Rate	0.1	14.41a (2.79)	9.03a (2.46)	5.38a (0.93)	24.06a (2.03)	8.15a (1.17)	5.85a (0.15)	5.15a (0.69)	2.01a (0.11)	3.89a (0.56)
(g NH3/g-DDGS)	0.3	14.55a (0.25)	8.31a (0.48)	6.24a (0.74)	23.26a (2.75)	8.42a (0.24)	6.07b (0.01)	5.85b (0.07)	1.87b (0.08)	3.55a (0.30)
	0.5	14.43a (1.75)	10.51b (1.79)	3.93b (0.96)	23.69a (2.52)	7.08b (1.44)	5.69c (0.27)	4.96a (0.74)	1.96c (0.11)	3.85a(0.37)

[a]: Similar letters after means in each level of the main factor indicates insignificant difference at α=0.05, LSD, for that dependent variable. Values in parentheses are standard deviation. AIL = Acid Insoluble Lignin, ASL = Acid Soluble Lignin.

Table 5.3 Interaction effects on resulting compositional analysis (p-values) [a].

INTERACTION EFFECT									
Factor	Lignin (%)	AIL (%)	ASL (%)	Glucan (%)	Xylan (%)	Galactan (%)	Arabinose (%)	Mannan (%)	Ash (%)
Temp	<.0001	<.0001	0.0028	0.0608	0.0878	<.0001	0.0013	0.0008	0.6677
Time	<.0001	0.0011	0.0005	0.1318	0.0078	0.0061	0.3298	<.0001	0.3003
MC	0.0006	0.0318	0.3466	0.4142	0.0862	<.0001	0.6636	0.0126	0.3862
Loading	<.0001	0.0003	0.2965	0.3154	<.0001	0.1362	<.0001	<.0001	0.3973
Temp*Time	0.0027	0.0002	0.0053	0.1392	0.618	<.0001	0.2018	0.005	0.0007
Temp*MC	0.0486	0.8686	0.0625	0.0138	0.4007	<.0001	0.3935	0.0004	0.1547
Temp*Loading	<.0001	0.0022	0.6684	0.4833	0.0164	0.0009	0.4422	0.1041	0.1906
Time*MC	0.9244	<.0001	<.0001	0.5732	0.0004	<.0001	0.161	0.0008	0.7678
Time*Loading	0.0006	<.0001	0.0043	0.0766	0.092	0.5117	0.5758	0.01	0.0489
MC*Laoding	<.0001	0.0002	0.8153	0.7151	0.4035	0.003	0.5004	0.0466	0.178
Temp*Time*MC	<.0001	0.0297	0.007	0.9721	0.1748	<.0001	0.0378	0.0573	0.8479
Temp*Time*Loading	<.0001	0.0008	0.7287	0.8948	0.828	0.0314	0.0141	0.0003	0.0662
Temp*MC*Loading	0.3179	0.1872	0.3497	0.0413	0.0777	<.0001	0.0007	0.254	0.0531
Time*MC*Loading	0.1879	0.8619	0.1039	0.5996	0.0036	0.547	0.6776	0.0702	0.0689
Temp*Time*MC*Loading	0.0362	0.0277	0.267	0.0195	0.0009	0.0048	0.027	0.0857	0.3191

[a]: Temp = Temperature, MC = Moisture Content, Loading = Ammonia Loading Rate, AIL = Acid Insoluble Lignin, ASL = Acid Soluble Lignin.

Table 5.4 Treatment effects on resulting compositional analysis to treated DDGS [a]

Treatment	Lignin (%)	AIL (%)	ASL (%)	Glucan (%)	Xylan (%)	Galactan (%)	Arabinose (%)	Mannan (%)	Ash (%)
1	13.07 fg	10.58 bc	2.49 h	24.84 ab	6.75 cd	5.66 e	5.45 bc	1.83 f	4.02 a-d
2	12.61 gh	7.84 e	4.77 c-f	25.95 ab	6.08 de	5.74 de	5.01 cd	1.93 de	4.62 a
3	13.31 e-g	10.47 bc	2.84 gh	20.35 d	5.22 e	5.80 cd	4.25 de	1.93 de	3.54 de
4	11.71 h	5.86 f	5.85 bc	22.78 a-d	9.18 a	5.79 cd	4.17 e	1.90 d-f	3.89 b-d
5	12.60 gh	8.71 de	3.89 fg	20.41 d	5.82 de	5.34 f	4.91 c-e	1.84 f	3.61 de
6	13.93 d-f	8.64 de	5.29 b-d	25.04 ab	8.48 a	5.73 de	5.94 ab	2.04 c	4.54 ab
7	15.74 c	11.89 ab	3.85 fg	24.25 a-c	8.78 a	5.73 de	4.41 de	2.08 a-c	3.86 b-d
8	12.90 f-h	7.36 ef	5.54 b-d	22.31 b-d	8.86 a	5.81 cd	4.36 de	2.15 a	3.13 e
9	12.89 f-h	8.09 de	4.80 c-f	26.14 a	6.20 de	5.74 de	5.59 a-c	1.87 ef	3.93 a-d
10	12.12 gh	7.59 ef	4.53 ef	25.38 ab	6.85 cd	6.04 ab	5.42 bc	1.96 d	3.74 c-e
11	17.37 b	13.37 a	4.00 f-g	24.99 ab	9.10 a	5.28 f	4.49 de	1.95 d	3.74 c-e
12	14.42 de	9.81 cd	4.61 ef	24.61 a-c	8.62 a	5.81 cd	5.57 a-c	1.88 ef	3.91 b-d
13	14.78 cd	9.69 cd	5.10 b-e	24.87 ab	6.96 b-d	6.07 a	6.32 a	2.07 bc	3.60 de
14	19.06 a	11.82 ab	7.23 a	21.01 cd	8.39 ab	5.70 de	5.25 bc	2.08 a-c	3.34 de
15	15.74 c	11.28 bc	4.47 d-f	23.71 a-d	7.82 a-c	5.93 bc	4.29 de	2.13 ab	4.43 a-c
16	18.59 ab	13.34 a	5.24 b-d	25.40 ab	8.76 a	6.13 a	5.48 bc	2.14 a	3.97 a-d
CP	14.55 cd	8.31 de	6.24 ab	23.25 a-d	8.42 a	6.07 a	5.85 ab	1.87 ef	3.55 de

[a]: Similar letter after means in each treatment indicates insignificant difference at $\alpha = 0.05$, LSD, for the dependent variable. CP denotes center point in this study. AIL = Acid Insoluble Lignin, ASL = Acid Soluble Lignin.

Table 5.5 Main effects on enzymatic digestibility results to treated DDGS (at t=96 h).

Factor	Level	Enzymatic Digestibility to Glucose Yield (%)
Pretreatment Temperature (°C)	20	45.03 (6.73)
	50	50.04 (-)
	80	52.76 (5.62)
Pretreatment Time (h)	24	47.04 (7.31)
	96	50.04 (-)
	168	51.42 (5.93)
Moisture Content of DDGS	20	46.64 (6.69)
(wb%)	40	50.04 (-)
	60	51.82 (6.28)
Ammonia Loading Rate	0.1	50.34 (7.24)
(g NH3/g-DDGS)	0.3	50.04 (-)
	0.5	48.11 (6.66)

Figure 5.1 11 L ammoniation reactor

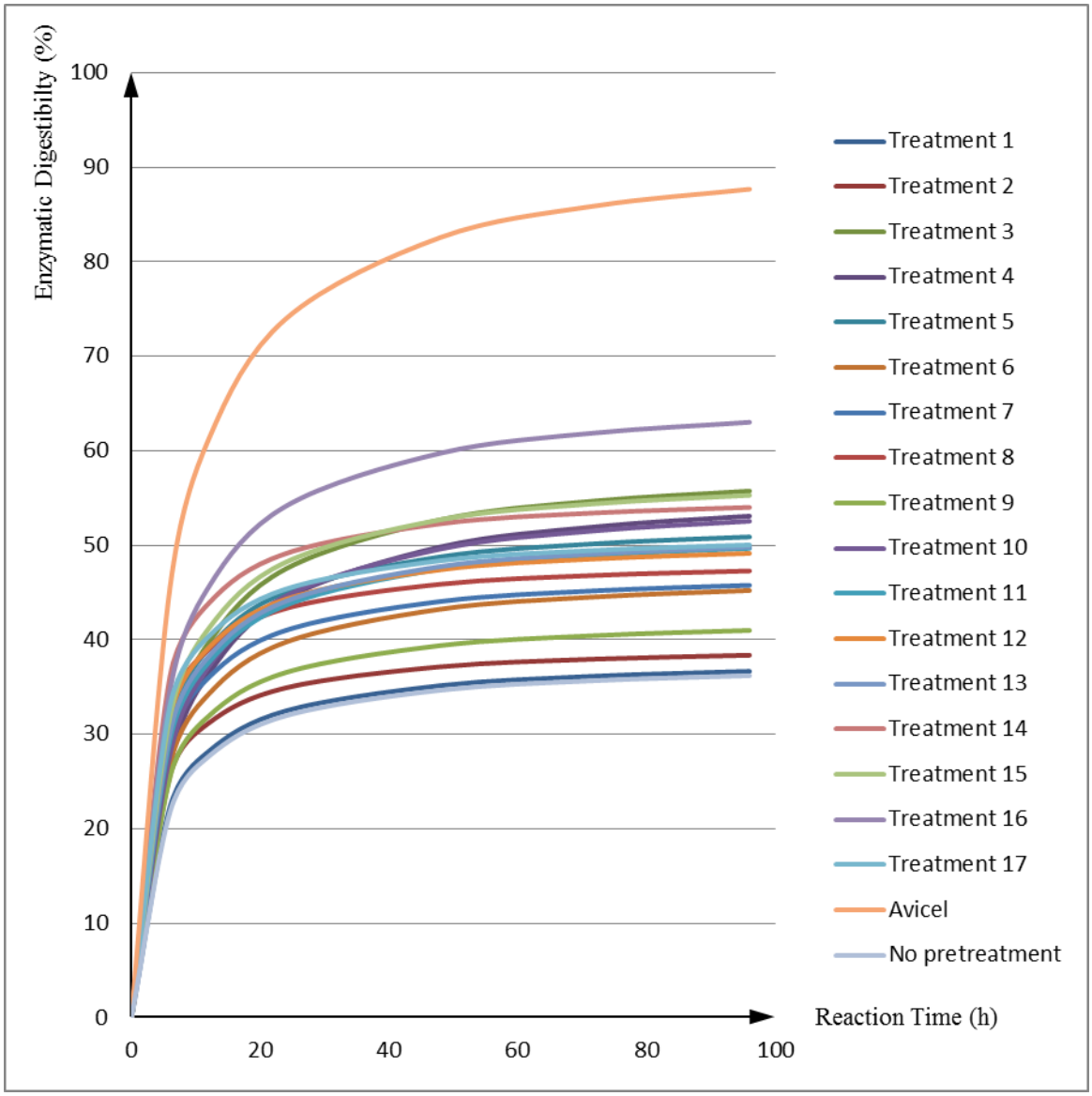

Figure 5.2 Enzymatic digestibility results for all treatments. Treatment 17 denotes center point.

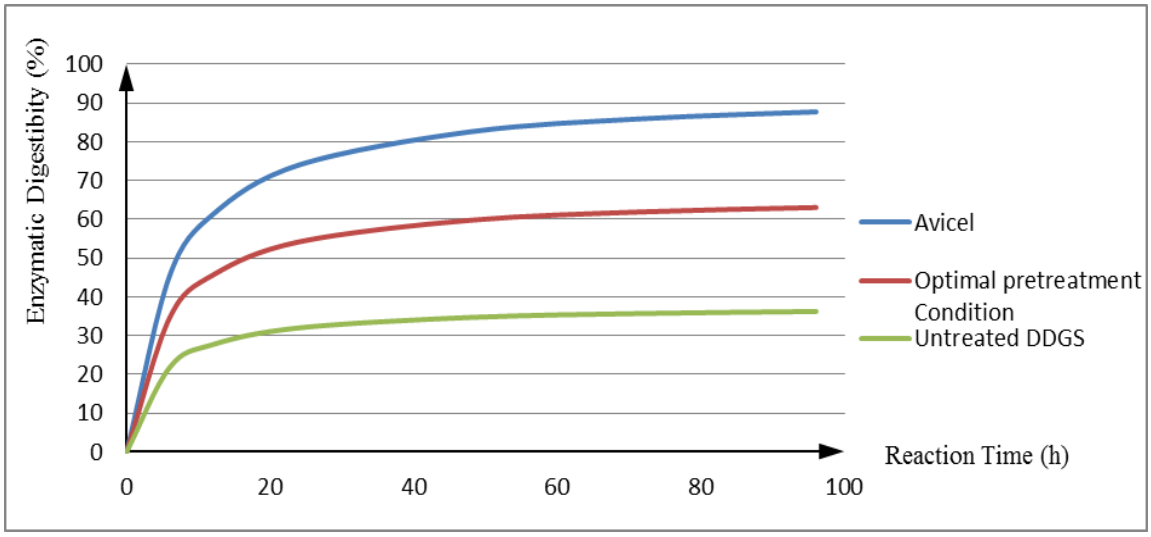

Figure 5.3 Enzymatic digestibility results for avicel, untreated DDGS, and maximum LMAA-treated DDGS (60 wb% moisture content, pretreated with 0.1g NH3/g-DDGS ammonia loading rate at 80˚C and 168 h).

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Charter 6: General Conclusions

6.1. Overall Conclusions

This thesis represents a summary of the research project "Analysis of properties to Distillers Dried Grainswith Solubles (DDGS) and using destoner and low moisture anhydrous ammonia (LMAA) to utilize DDGS". With pressure from shortage of fossil fuels, bioethanol as a fuel additive is gradually utilized to reach the demand for fuel (Schnepf and Yacobucci, 2013). Conversion corn to ethanol is an efficient method in the US ethanol industry, and has grown rapidly in recent years. According to Rosentrater and Muthukumarappan (2006), more than 95% US fuel ethanol plants are used corn as a major raw material to produce ethanol. In the corn-based fuel manufacturing, bioethanol, distillers dried grains with solubles (DDGS), and carbon dioxide are three main products. Among all products from bioethanol industry, DDGS is an important ingredient, which is wet distillers grains (WDG) that has been dried with the concentrated thin stillage to 10~12 percent moisture. With the rapid development of the ethanol industry, various research on distillers dried grains with solubles (DDGS) as a main co-product from the ethanol industry has been done in recent years.

Chapter two, it is a literature review that discussed related background information about three major topics, including: properties to DDGS, using destoner to separate DDGS and using the method of LMAA to pretreat DDGS for higher efficiency to enzymatic conversion. To first topic, this thesis chose seven common physical properties, including moisture content, water activity, angle of repose, particle size, bulk

density, color and shear strength, to discuss the results of previous study. In the second part, sieving and aspiration used as the methods of separation to DDGS has been discussed and destoner is also simply introduced in this part. Finally, this literature review discussed published papers of pretreatment methods on different lignocellulosic biomass, and investigated how these methods have been utilized.

Chapter three attempt to provide baseline property data for typical DDGS from Midwest from USA in 2011 and 2012. After experimental test, this study got the data of DDGS properties and compared with other researcher's results, which included moisture content, water activity, angle of repose, geometric mean diameter (dgw), geometric standard deviation (Sgw), loose bulk density, packed bulk density, color content, shear strength. This research supplies up to date engineering data which is key to storing and handling DDGS, designing and utilizing equipment, and producing co-products from DDGS. Future work will focus on examining correlations between physical and chemical properties and explore the reasons why the differences occur in different samples.

To explore a reliable and useful method to separate DDGS into various compositions, chapter four focused on whether destoner fractionation was effecting in separating DDGS into components, and to examine the relationships between particle size and chemical content. The final results showed that destoner fractionation was somewhat efficient to separate oil fractions of DDGS, and 8° angle and 27.5% air flow had the highest value. Also, compared with other methods, destoner fractionation has advantages of relatively high efficiency and low cost, after considering the whole

procedure. Particle size distribution had a positive correlation to oil, and a negative correlation to water. Fiber had no relationship with particle size, while protein had a weak correlation with particle size. Further fractionation should be explored reasons in future research.

In Chapter five, low-moisture anhydrous ammonia (LMAA) pretreatment, which is considered as a potential method with advantages of using low amount ammonia, no washing step and low energy consumption, has been attempted to pretreat DDGS for more efficient enzymatic hydrolysis. In this chapter, it explored the effect of LMAA pretreatment to DDGS and the efficiency of enzymatic hydrolysis under various conditions. As expected, LMAA pretreatment is a potential method to pretreat DDGS and achieve a higher glucose yield. When DDGS (60 wb% moisture content) was pretreated with 0.1g NH3/g-DDGS ammonia loading rate at 80˚C and 168 h, it was obtained with the maximum glucan digestibility of 63.03%. Higher pretreatment temperature, longer pretreatment time, higher moisture content of DDGS and lower ammonia loading rate have a trend to pretreat DDGS and obtain a higher enzymatic hydrolysis.

Overall, various research have been done on distillers dried grains with solubles (DDGS), which is related to basic physical properties, separation of destoner and the possibility to enzymatic hydrolysis with a pretreatment of LMAA.

6.2. Future Work

In this thesis, chapter three has investigated some physical properties of DDGS and explores the relation between them. But to DDGS, chemical and nutritional qualities are another two important facets, which needs to be explored and studied. So it focuses on examining correlations between physical and chemical properties and explore the reasons why the differences occur in different samples.

Chapter four has explored that destoner fractionation was effecting in separating DDGS into components, and to examine the relationships between particle size and chemical content. The final result showed that particle size distribution had a positive correlation to oil, and a negative correlation to water. Fiber had no relationship with particle size, while protein had a weak correlation with particle size. But the reasons to these relation are not discussed, which could be explored in future work. What's more, various types and sizes of DDGS should be attempted to explore the optimal condition to most destoner to the fractionation of DDGS.

In chapter five, LMAA has been utilized to pretreat DDGS and increase enzymatic hydrolysis with a higher efficiency. Ammonia has been considered as an efficiency reagent and studied how to pretreat biomass (Bariska, 1975; Streeter and Horn, 1982; Dale, 1986; Holtzapple et al., 1992; Foster et al., 2001; Kim and Lee, 2005; Mosier et al., 2005, Kim and Lee, 2007), but the principle of LMAA to pretreat DDGS is still not clear at the molecule level, which could be explored in the future work. What's more, DDGS had a more complicated structure with higher protein and oil content, which may lead to some change and hydrolysis by ammonia and need to be investigated

in future work. In addition, xylan digestibility from treated DDGS are very low in this study and reason should be explored in the future, which the higher protein and oil content may affect the efficiency of xylanase.

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