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Evaluation of low-moisture anhydrous ammonia (LMAA) pretreatment method of lignocellulosic biomass for bioethanol production

by

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Kurt A. Rosentrater, Major Professor
Carl J. Bern
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DEDICATION

This thesis is dedicated to my parents for their endless love, support and patience. Thank you for being my role models and cheerleading squad whenever I needed!

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ABSTRACT

The major objective of this project was to evaluate the low-moisture anhydrous ammonia (LMAA) pretreatment process of corn stover for bioethanol production. In order to fully evaluate this pretreatment method, three sub-objectives were developed: the optimization of the conditions for the LMAA pretreatment process; the influence of the LMAA pretreatment process during long-term storage of corn stover; and the techno-economic analysis (TEA) of this LMAA pretreatment process in bioethanol production.

In optimization experiments, several influencing factors were being investigated. As a result, the LMAA process increased the glucose yield by nearly 3 times compared with un-pretreated corn stover. And the optimal conditions determined by the experiments were 50 wt % moisture content, 72h of pretreatment time, 75°C of pretreatment temperature and 0.1 g NH_3/g DM biomass.

In storage experiments, two sealing conditions were being explored equipped with two independent variables for each scenario. The results showed that no significant reduction in carbohydrates was found in either sealed or open treatments, but lignin reduction was observed in sealed treatments. In terms of mass loss, it increased with time in sealed treatments, but in open treatments, the mass loss was affected by the environment.

As for the techno-economic analysis of the LMAA pretreatment process, the detailed cost analysis was conducted and the unit cost of the bio-ethanol was compared among three various scales. The results indicated that the minimum ethanol selling price corresponding to ethanol production of 50 MM gal/y was $3.86/ gal. However, this was still high compared with the current gasoline price.

CHAPTER 1. INTRODUCTION

General Introduction

At present, fossil energy, such as coal and natural gas, has become the largest source of the world’s economy (Uihlein and Schebek, 2009). However, due to the unsustainable nature of fossil energy in the long run and concerns over the global environmental change, there is an increasing interest in developing the alternatives to fossil fuels (Himmel et al., 2007). As a result, renewable fuels have attracted much attention since they are sustainable. There are a variety of alternative renewable sources in the energy industry, for instance, wind power, hydropower, solar energy and biofuel (Lynd and Wang, 2003). The utilization of biofuel is considered to be environmental-friendly since it makes no net contribution to global warming. This is mainly because the carbon cycle balances in nature: carbon dioxide released in the burning would be absorbed during photosynthesis in plant growth (Naik et al., 2010).

Biofuels, such as bioethanol and biodiesel, are primarily derived from biomass. Bio-ethanol produced from edible sources, such as sugar cane and corn, is generally considered as the *first generation bio-ethanol*. Bio-ethanol produced from sugar- or starch-based materials is efficient but challenged due to the problem of land use, competition with food crops, and the debate on the amount of reduction of greenhouse gas emission (Sims et al., 2010). The *second generation biofuel* is mainly produced from lignocellulosic biomass, which refers to agricultural residues, energy crops, forestry and wood residues (Cheng and Timilsina, 2011).

Bio-ethanol production from lignocellulosic biomass has many advantages not only from the environmental aspect, but also from long-term economic development and national security (Mosier et al., 2005b). In June 2007*, the Energy Independence and Security Act of 2007* was passed. And in this act, 36 billion US gallons of renewable fuel use by 2022 has been required, and the target for second generation biofuel was 16 billion US gallons by 2022 (EPA, 2007), which accounts for nearly half of the renewable fuel.

Currently in the United States, as shown in Fig. 1.1, over 1.3 billion dry tons per year of biomass potential from the forestland and agricultural land was found; on forestlands, nearly 368 million dry tons of biomass could be produced, and about 998 million dry tons from agricultural lands (Perlack et al., 2005). As established by the Biomass Research and Development, 7% of the biomass will be used to supply the nation’s power by 2020, 20% for its bio-based transportation fuels by 2030, and 55.3 billion lbs of its bio-based chemicals and materials production by 2030 (BRD Committee, 2006).

In terms of lignocellulosic biomass, it is a promising material for biofuel production due to its low cost and large quantities (Lee et al., 2013). However, in order to resist the assault from the microbial and animal kingdoms, the lignocellulosic biomass has evolved the complex structure of its cell wall (Himmel et al., 2007). The major components of cellulosic biomass contain three parts: cellulose, hemicellulose and lignin.

Cellulose, with the formula $(C_6H_{10}O_5)_n$, is the main constituent of lignocellulosic biomass. As estimated, the cellulose content of wood is about 40-50% (Sjöström, 1993). Cellulose is a polysaccharide composed of linear chains of D-glucose linked by β-1, 4-glycosidic bonds, as shown in Fig.1.2, with the degree of polymerization ranging from 300 in wood pulp to 10,000 in cotton and other plant fibers (Klemm et al., 2005). Many properties of cellulose depend on degree of polymerization; Short chain molecules, in other words low degree of polymerization, are relatively soluble in water and other organic solvent compared with long chain (Klemm et al., 2005).

Due to the hydroxyl groups at C2, C3 and C6 positions, cellulose has a strong tendency to form intra- and inter-molecular hydrogen bonds on the linear chains. The extensive hydrogen bond networking results in the aggregation of crystalline fiber structure and morphologies (Klemm et al., 2005). And the crystalline cellulose is quite stiff and has little access for chemicals and water (Nishiyama et al., 2003).

Unlike cellulose, hemicellulose is a polysaccharide containing various sugar monomers, including pentose (β-D-xylose, α-L-arabinose) and hexose (β-D-mannose, β-D-glucose, and α-D-galactose) (Gírio et al., 2010). Moreover, the average degree of polymerization in hemicellulose ranges from 40-600, which are relatively small chain compared with cellulose (Gírio et al., 2010). Xylans are the most abundant hemicellulose components of the cell wall and constitute, approximately about 20-30% of the biomass (Gullón et al., 2011). Due to the amorphous and branched structure, hemicelluloses are easy to be hydrolyzed into their monomers compared with cellulose (Li et al., 2010).

Lignin, a complex aromatic polymer, is the second most abundant component of the cell wall. The composition of lignin varies from one species to another. As shown in Fig. 1.3, lignin is a highly disordered polymer that fills the spaces in the cell wall between cellulose and hemicellulose (Chabannes et al., 2001). It is commonly used as the protection and support of the cells. However, because of the complex and irregular properties of lignin, its chemical structure is not completely known yet (Borejan et al., 2003). The lignin-carbohydrate complex (LCC) linkages in lignocellulosic biomass are believed to be the largest obstacles in bioethanol production (Yuan et al., 2011).

At present, the bioethanol production from lignocellulosic biomass comprises four major procedures: biomass pretreatment, enzymatic hydrolysis, sugar fermentation and ethanol recovery. Pretreatment step is the most essential and critical step because it is typically used to reduce the recalcitrance of the LCC linkage by removing lignin and solubilizing hemicellulose and decreasing the physical barriers for enzymes (Himmel et al., 2007). The objective of pretreatment is to break down the lignin structure and disrupt the crystalline structure of cellulose for enhancing enzyme accessibility to the cellulose during the following step. Enzymatic hydrolysis is primarily conducted to convert cellulose into glucose and hemicellulose into pentose and hexose (Cheng and Timilsina, 2011). The enzymes used are highly specific, and this process is usually carried out under mild conditions (Beguin and Aubert, 1994). In terms of the fermentation process, it is usually used to ferment monosaccharide into ethanol, with the help of microorganisms such as *S. cerevisiae*, *E.coli*, and *Z. mobilis* (Sarkar et al., 2012). After fermentation, ethanol will be purified and distilled in order to meet the market requirements. Therefore, an appropriate pretreatment method plays a crucial role in the whole ethanol production process.

Literature Review

Various pretreatment methods, such as supercritical carbon dioxide, lime, liquid hot water, dilute sulfuric acid, ammonia fiber explosion, aqueous ammonia, and ammonia recycle percolation, have been invented and investigated in recent decades. Although a few methods are effective in some certain lignocellulose biomass, they may have little effect in other biomass.

In this review, the author lists several major pretreatment methods for lignocellulosic biomass used in the last 30 years based on different reagents: carbon dioxide, lime, water, acid, and ammonia. Those pretreatment methods have been reviewed from published studies on various lignocellulose materials for ethanol production, and how these methods have been utilized. The main objectives were to compare pretreatment conditions, reducing sugar yield, enzymatic digestibility, and lignin removal under different pretreatment methods.

Supercritical Carbon Dioxide (SC-CO_2)

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). Papers about supercritical carbon dioxide (SC-CO_2) pretreatment have shown that the SC-CO_2 method was effective on some specific 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, 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 content 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 close to the result of Santos et al (2011) with 72.0% of glucose theoretical yield; and at a 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, which was higher 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) achieved glucose yield of only 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 appeared.

For the SC-CO_2 pretreatment method, the reason for wide usage is due to the advantages of its economic cost 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 a barrier 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 pretreatment may be a future research direction at industrial plants (Benazzi et al., 2013).

Lime

Lime pretreatment, which is a mild alkaline pretreatment method, has been studied as well. Lime pretreatment has been used in various biomasses, such as corn stover, switchgrass, rice straw, and 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 and Cheng, 2011). 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 has the potential to result in better enzymatic saccharification results (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 pre-hydrolysate analysis after lime pretreatment and the evaluation of fermentation potential from other biomass.

Liquid Hot Water (LHW)

Liquid hot water (LHW), which is the pretreatment method using hot compressed water, has been proved to be efficient in separating hemicelluloses, cellulose and lignin (Wang et al., 2012).

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., 2005a). 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 that 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 SO_2 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., 2012). 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., 2010), which has also been proved effective in hydrolyzing hemicellulose.

The advantages of the liquid hot water method include 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 (Perez 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.

Dilute 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.

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 H_2SO_4 (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 (Redding et al., 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., 2013).

Ammonia Fiber Explosion (AFEX)

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 biomasses, 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 et al., 2004). With further study using simultaneous saccharification and fermentation (SSF), the maximum ethanol was 96% of theoretical yield from pretreated corn stover (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 et al., 2007) and higher ethanol yield with 0.2g/g biomass (Alinia et al., 2010). In addition, AFEX was used to pretreat reed canary grass (Bradshaw et al., 2007) and coastal Bermuda grass (Lee et al., 2010), and had very similar results to the data of corn stover.

By modifying the ammonia fiber explosion process, ammonia fiber expansion, also termed as AFEX, was developed in 2006, and used to pretreat more than 10 types of biomass in the following 5 years. Bals et al. (2006) pretreated DDGS and obtained a glucose yield of 190g glucose/kg dry biomass, using the pretreatment condition of 70°C with a loading rate of 0.8 anhydrous NH_3/ kg dry biomass in 5 min. Lau et al. (2008) used simultaneous saccharification and fermentation (SSF) to 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 et al., 2010; Gao et al., 2010) with an ethanol yield variation 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, respectively. Besides these results, 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.

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.

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, an 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 to 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.

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.

Future work could 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 enzymes and using microorganisms capable of utilizing xylose to increase ethanol production yield (Teymouri et al., 2004).

Ammonia Recycle Percolation (ARP) and 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 gives 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.

Conclusions

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., 2005b). Dilute acid pretreatment offers good performance in terms of recovering hemicellulose, cellulose digestibility, and sugars, but suffers from its use of H_2SO_4. Ammonia is a better reagent than lime because it makes biomass delignified, and also swells and preserves cellulose for a relatively 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. Further research could be focused on decreasing the effect on environment, equipment, financial cost and producing higher glucose yield.

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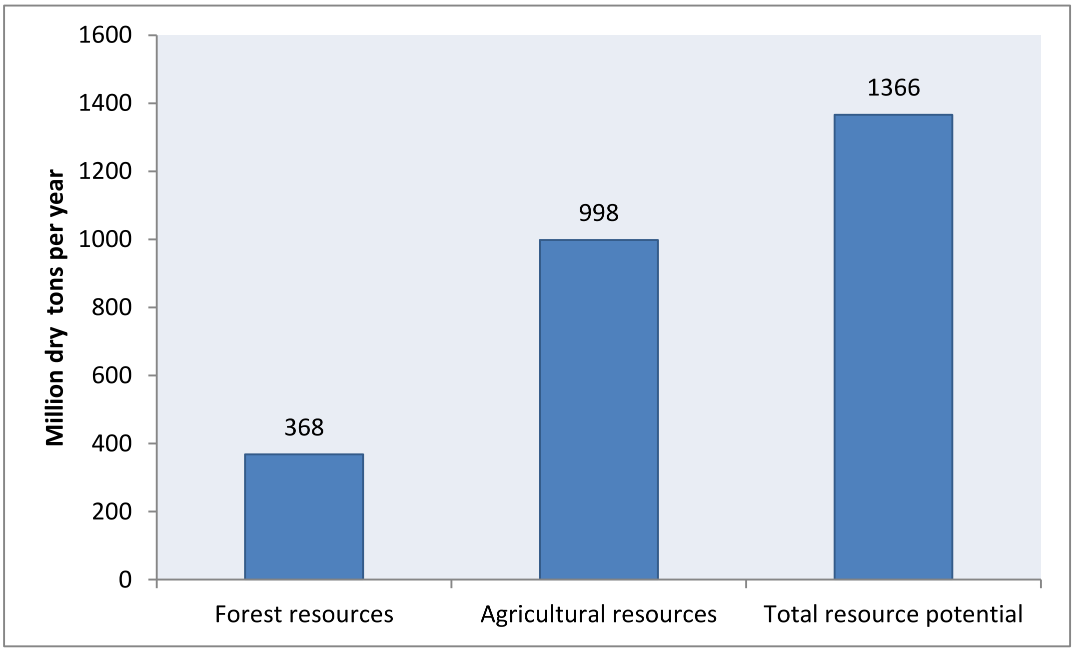

Figure 1.1. Annual biomass resources (Perlack et al., 2005).

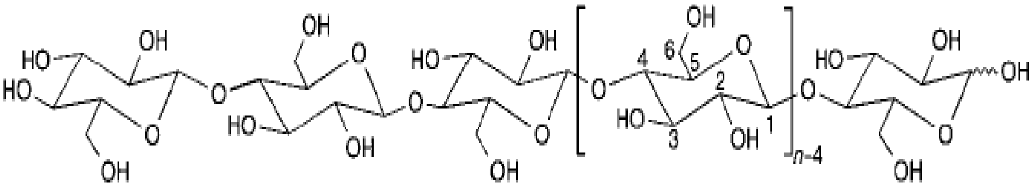

Figure 1.2. Cellulose structure (Klemm et al., 2005).

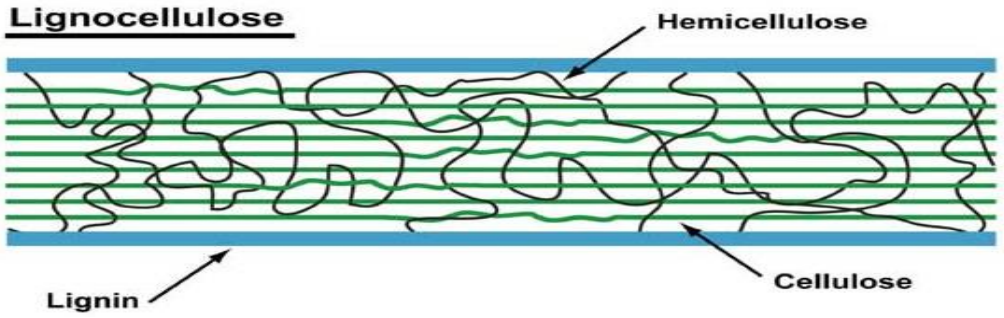

Figure 1.3. Structure of lignocellulosic biomass (USDA Agricultural Research Service, 2014).

CHAPTER 2. OBJECTIVES

The overall objective of this study was to evaluate the effectiveness of the Low-Moisture Anhydrous Ammonia (LMAA) pretreatment method for bioethanol production.

More specifically, the sub-objectives of this study with the specified hypotheses were:

1. To optimize of LMAA pretreatment process with the factors of moisture content, particle size, pretreatment temperature and time;

   (H_o: the LMAA pretreatment process has the potential to increase the glucose yield compared with un-pretreated corn stover);

2. To determine the effects of LMAA pretreatment method in long-term storage;

   (H_o: the LMAA pretreatment process could help to maintain carbohydrates in the corn stover during long-term storage);

3. To investigate the unit cost of bioethanol by conducting the techno-economic analysis (TEA) of LMAA pretreatment process;

   (H_o: the bioethanol produced by the LMAA pretreatment process could be competitive as the current gasoline market).

CHAPTER 3. LOW-MOISTURE ANHYDROUS AMMONIA (LMAA) PRETREATMENT METHOD OF CORN STOVER

Modified from a paper to be submitted to *Industrial Crops and Products.*

Abstract

In recent years, much attention has been devoted to ethanol production from lignocellulosic biomass. In previous research, low-moisture anhydrous ammonia (LMAA) pretreatment was investigated due to its relatively high efficiency and less washing steps compared with other pretreatment methods. The purpose of this study was to evaluate the effect of the LMAA pretreatment process in bioethanol production. In this experiment corn stover was used with different moisture contents (20, 50, and 80 wt %) and particle sizes (<0.09, 0.09-2, >2 mm). Corn stover was ammoniated with a loading rate of 0.1g NH_3 /g DM biomass. Ammoniated corn stover then was subjected to different pretreatment times (24, 72, 144 h). After that, compositional analysis, and enzymatic digestibility were used to determine the glucose yield. The maximum glucose yield obtained in this study was 57.2%. Compared with the untreated corn stover (29.02%), the LMAA showed its potential in increasing glucose yield.

Keywords. Ammonia, biomass, cellulosic ethanol, LMAA, pretreatment.

Introduction

In recent years, there has been increasing interest in renewable, domestic sources of fuels to replace fossil fuel due to the concerns about environment, long-term economics, and national security (Mosier et al., 2005b). Bioethanol, which is renewable and environmental-friendly, can be used as an alternative to gasoline. Currently, bioethanol is mainly produced from sugar- or starch-based materials, such as corn, which is efficient but problematic due to land use and competition with food crops, also known as the food versus fuel debate (Sim et al., 2010). Bioethanol can also be produced from lignocellulosic biomass. Generally, there are four major processes in converting lignocellulosic biomass to ethanol: pretreatment, hydrolysis, fermentation, and ethanol recovery (Naik et al., 2010). Among these steps, pretreatment is critical because it could increase the enzyme accessibility of cellulose, which is protected under a shield of hemicellulose and lignin (Mosier et al., 2005a).

Over the past few decades, numerous research had been focused on various pretreatment methods to improve the efficiency of second generation biofuel production (Alinia et al., 2010; Alizadeh et al., 2005; Bals et al., 2006; Gao et al., 2011; Garlock et al., 2009; Gupta and Lee, 2009; Hanchar et al., 2007; Kim and Hong, 2001; Kim and Lee, 2005; Lau et al., 2009; Lee et al., 2010; Narayanaswamy et al., 2011; Screenath et al., 1999; Srinivasan and Ju, 2010; Teymouri et al., 2004, 2005; Wan and Li, 2011; Wang et al., 2012; Yoo et al., 2011; Yourchisin and Walsum

2004; Zhang et al., 2011). Additionally, various pretreatment reagents have been studied, each with its own advantages and disadvantages.

For instance, carbon dioxide (CO_2) has been used because of its many advantages: environmentally friendly, inexpensive, and easy to recover after use. The supercritical carbon dioxide (SC-CO_2) pretreatment method has been applied to a few lignocellulosic biomasses, such as aspen and southern yellow pine (Kim and Hong, 2001), wheat straw (Alinia et al., 2010), guayule (Srinivasan and Ju, 2010), switchgrass, and corn stover (Narayanaswamy et al., 2011). The maximum glucose yield (30g/100g dry biomass) for corn stover was under 3500 psi at 150°C (Narayanaswamy et al., 2011). However, low-efficiency and high capital cost for high-pressure equipment could be barriers to large-scale production using SC-CO_2 pretreatment method (Kim and Hong, 2001).

Hot water has also been used as the reagent in pretreatment studies. Hot water has been used in aspen (Yourchisin and Walsum, 2004), soybean straw (Wan and Li, 2011), corn stover (Wan and Li, 2011; Yourchisin and Walsum, 2004), alfalfa (Screenath et al., 1999), and cattails (Zhang et al., 2011). Liquid hot water as a pretreatment method may be effective for soybean straw when combined with fungal degradation pretreatment, but it is not efficient for corn stover compared with fungal degradation pretreatment alone (Wan and Li, 2011).

Ammonia is another reagent widely used in pretreatment methods. Various studies around ammonia have been investigated, such as ammonia fiber explosion

(Alizadeh et al., 2004, 2005; Hanchar et al., 2007; Lee et al., 2010; Teymouri et al., 2005), ammonia fiber expansion (Bals et al., 2006; Garlock et al., 2009; Gao et al., 2011; Lau et al., 2010), and aqueous ammonia soaking (Gupta and Lee, 2009; Kim and Lee, 2007). Previous studies have shown the efficiency in delignification and in glucose yield using ammonia. However, economics, water and chemical consumption, and environmental concerns are problematic for ammonia based methods.

In order to eliminate the washing step and reduce capital costs of the pretreatment process, Yoo et al. (2011) developed a low moisture anhydrous ammonia (LMAA) pretreatment method. In his research, corn stover treated at 80°C for 84 h with 3% glucan loading resulted in the highest yield (89% of theoretical ethanol yield). However, the batch reactor (2.9 inch (8.1 cm) internal diameter × 6.5 inch (18.5 cm) length, 690 mL internal volume) in Yoo’s (2011) research was in relatively small scale, which may lead to inappropriate conditions for optimal ethanol production at larger scales.

In this study, a deeper investigation of the LMAA pretreatment process with a larger-scale reactor, under a range of pretreatment conditions (moisture content, particle size, pretreatment temperature, and pretreatment time) was investigated to determine the effect of the LMAA process.

Materials and Methods

Materials

In this study, freshly-harvested, air-dried corn stover was supplied from central Iowa in 2012 and stored at ambient temperature. The biomass was then ground and sieved into three size fractions prior to pretreatment (<0.09 mm, 0.09-2.0mm, >2.0 mm). The sieved corn stover was stored at room temperature (~21°C) until use. Avicel PH-101, which was purified microcrystalline cellulose with the molecular formula $(C_{10}H_{18}O_6)_n$, was used in the enzymatic digestibility test. It was a white and odorless powder, and the particle size of Avicel PH-101 used in this study was around 50 µm (Sigma-Aldrich).

Equipment

The reactor (Fig. 3.1) used for the ammoniation process was purchased from Pall Corporation, Ann Arbor, Michigan, USA. The capacity of the sealed reactor was 3 L, which was about 4.35 times larger compared to Yoo's (2011) previous study. The use of a larger reactor may reduce the potential bias that may be caused by different ammonia loadings and reaction times. In order to measure sugar content, 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) were used. Acid soluble lignin (ASL) content was determined by UV-Visible spectrophotometer (UV-2100 Spectrophotometer, Unico, United Products & Instruments, Inc., Dayton, NY, USA).

Enzymes

In this study, GC-220 and Novozyme 188 were used in the enzymatic digestibility test. GC-220 (cellulose enzyme) was purchased from Genencor International, Inc. (Rochester, NY, USA). The cellulose activity was expressed in filter paper units (FPU); the average activity of GC-220 was determined to be 45 FPU / mL. Novozyme 188 (β-glucosidase enzyme) was provided from Sigma-Aldrich, Inc. (St. Louis, Missouri, USA); the activity of Novozyme 188 was 750 cellobiase units (CBU) / mL.

LMAA pretreatment process

Before ammoniation, different amounts of water were added to the ground corn stover in order to meet the required moisture content (20, 50, 80 wt %); samples were equilibrated for over 24 h.

Moisturized corn stover was placed in the sealed reactor, and ammonia was introduced. A pipe was connected between the top of the reactor and the ammonia pump. A pressure gauge was equipped on the reactor to monitor the pressure change during the ammoniation process. Anhydrous ammonia was added up to the targeted pressure to achieve 0.1 g NH_3/ g DM biomass. The whole ammoniation process lasted up to 30 min in order to obtain a complete reaction. Temperature change during ammoniation process was not controlled in this study. After the ammoniation process, the reactor was cooled down for 5 min, the lid was removed in the fume hood, and then the ammoniated corn stover was transferred into several glass bottles (250 mL) with screw caps.

The bottles packed with ammoniated corn stover were placed in heating ovens at various pretreatment temperatures (20°C, 75°C, and 120°C) for 24 h, 72 h, and 144 h. As soon as the pretreatment process was complete, the lids of the glass bottles were removed in fume hood and surplus ammonia was evaporated for 12 h.

Experimental design

In this study, four independent variables that influenced the reaction severity were investigated. Raw material moisture contents were 20 wt %, 50 wt % and 80 wt %; the pretreatment times were 24 h, 72 h, 144 h; the pretreatment temperatures were 20°C, 75°C, 120°C, and the particle sizes were <0.9mm, 0.9-2.0mm and >2.0mm, respectively. By controlling these independent variables, 17 combinations were used in this study (i.e. $2 \times 2 \times 2 \times 2 + 1$ center point). Lignin, glucan, xylan, galactan, arabinan, mannan and ash content were measured as dependent variables during the experiment. The experimental design for this study is shown in Table 3.1.

Compositional analysis

Carbohydrates and lignin content were determined by NREL LAP (NREL, 2008)**.** Each sample was analyzed in duplicate. The content of glucan and xylan in the corn stover were analyzed by HPLC, following the NREL standards. Acid soluble lignin was measured by UV-Visible Spectrophotometer. In terms of the moisture content, it was determined by the oven drying method (NREL, 2008).

Enzymatic digestibility

Enzymatic digestibility was determined following NREL LAP-009 (NREL, 2008). The test was done in duplicate under the 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 initial glucan concentration was 1% (w/v). Cellulase enzyme (GC-220) loading was equal to 15 FPU/g of glucan, and ß-glucosidase enzyme (Novozyme 188) loading was equal to 30 CBU/g of glucan. Flasks were incubated at 50°C ± 1°C and 150 rpm in an incubator shaker (Excella E24 Incubator Shaker Series, New Brunswick Scientific, Edison, NJ, USA). Enzymatic digestibility time ranged from 0 h to 144 h for sugar analysis.

Total glucose detected from HPLC was used to calculate the glucan digestibility following equation 3.1 below. The conversion factor for glucose to equivalent glucan was 0.9.

$$\text{Glucan digestibility } [\%] = \frac{\text{Total released glucose} \times 0.9}{\text{Initial glucan loading}} \times 100\% \qquad (3.1)$$

Results and Discussion

Effects of LMAA pretreatment on biomass composition

In this study, the use of low-moisture anhydrous ammonia (LMAA) pretreatment didn’t result in many significant changes in lignin, glucan, xylan, arabinose, mannan or ash contents, as shown in Table 3.2 (main effects) and Table 3.4

(treatment effects). What’s more, as Table 3.3 shows, the majority of the p-values of interactions among these independent variables were higher than 5%, which indicates little evidence of significant interactions among independent variables was obtained in this study. The reason for the insignificant composition analyses result was because the anhydrous ammonia used in the pretreatment process was meant to separate lignin from cellulose and break down cellulose for enzymatic saccharification, not to change composition *per se*.

Effects of LMAA pretreatment on enzymatic digestibility

Fig. 3.2 shows the enzymatic digestibility results for the 17 treatments listed in Table 3.1, while Fig. 3.3 compares digestibility results for avicel (used as a reaction blank for the substrate), untreated corn stover, and the best digestibility trial. From Fig 3.2, different combinations of the four factors resulted in varying digestibility. As shown in Fig. 3.3, the highest glucose digestibility (57.23%) of LMAA pretreated corn stover was 1.97 times compared to untreated (29.02%). Among the 17 treatments, the median treatment, which was 50% moisture content (wet base), 0.9-2.0 mm particle size, 72 h pretreatment time and 75˚C pretreatment temperature, achieved the highest enzymatic digestibility result. In Yoo’s research (2011), the optimal pretreatment condition was 80˚C for 84 h pretreatment time, which was also the median treatment in his study. Thus our results compare favorably with prior research on the smaller scale.

In this study, four pretreatment factors were tested: pretreatment temperature, pretreatment time, moisture content, and particle size. Among these factors, pretreatment temperature was selected as the most important variable, because it had the highest p-value (0.0013). Table 3.5 shows the differences of average glucose digestibility between high pretreatment temperatures and low temperatures, while other factors were kept constant (i.e., main effects). As can be seen from the table, the highest temperature resulted in decreased digestibility in this study.

In terms of pretreatment time, the difference between longer time and shorter time was also significant (Table 3.5). The average glucose digestibility at 168 h was considerably lower than the average for 24 h pretreatment time. Based on these results, there was an average of 92.7% increase from 6 h to 18 h, which was the maximum rate of increase during the entire enzymatic digestibility. The reason for this may be longer pretreatment times cause the collapse of the structure of corn stover.

As for moisture content, it was observed that with higher moisture content, corn stover resulted in lower glucose digestibility. The reason for this may be the reduction of retaining ammonia with higher moisture, which may result in lower delignification. As for the effect of particle size, as shown in Table 3.5, there were some differences between small size and large size corn stover, with larger stover pieces being somewhat more digestible.

Conclusions

In this study, the effect of LMAA pretreatment under various conditions was explored. As expected, LMAA pretreatment has the potential to achieve higher glucose yield than untreated corn stover. When corn stover (50 wt % moisture content) was pretreated at 75˚C and 96 h, the maximum glucose yield (57.23%) was obtained. What's more, LMAA pretreatment may eliminate water consumption because there was no washing step during this study.

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Figure 3.1. Ammoniation reactor (3L).

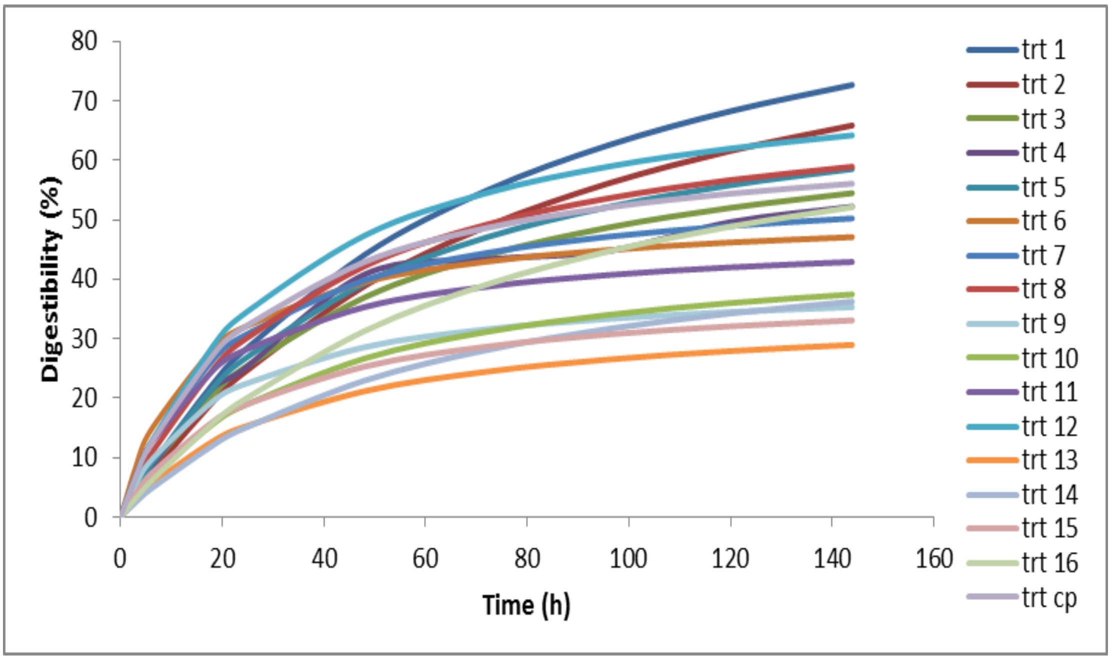

Figure 3.2. Enzymatic digestibility results for all treatments . (Trt denotes treatment; CP denotes center point.)

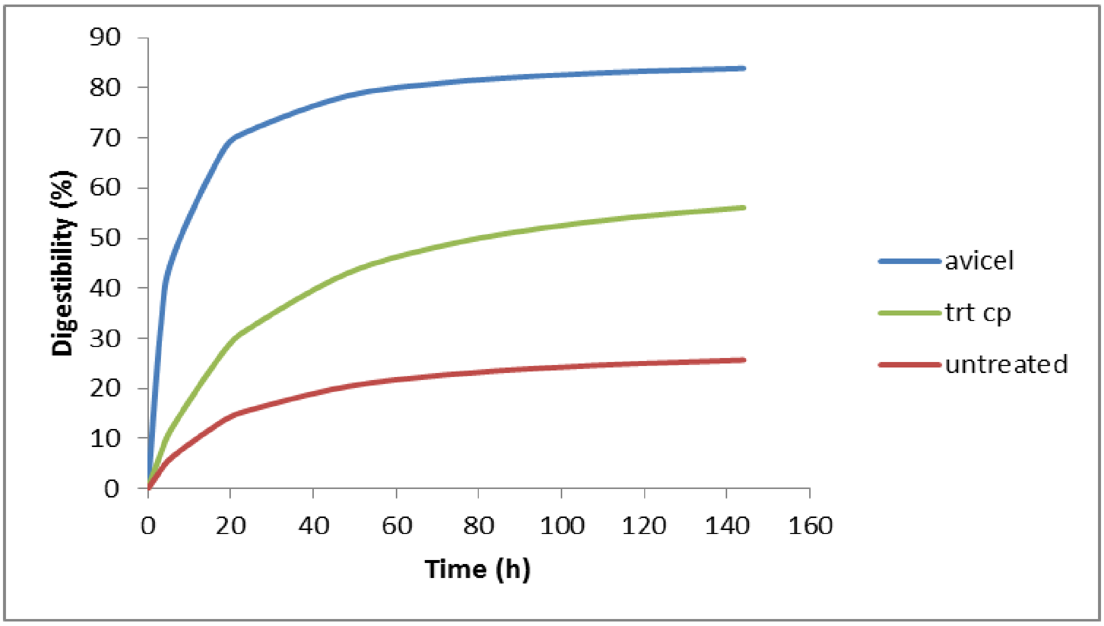

Figure 3.3. Enzymatic digestibility results for avicel, untreated corn stover, and maximum LMAA-treated corn stover (treatment cp). (Trt denotes treatment; CP denotes center point.)

Table 3.1. Experimental design.*

Treatment	Moisture Content (wt %)	Time (h)	Temperature (°C)	Particle size (mm)
1	20	24	20	<0.9
2	20	24	20	>2.0
3	20	24	130	<0.9
4	20	24	130	>2.0
5	20	144	20	<0.9
6	20	144	20	>2.0
7	20	144	130	<0.9
8	20	144	130	>2.0
9	80	24	20	<0.9
10	80	24	20	>2.0
11	80	24	130	<0.9
12	80	24	130	>2.0
13	80	144	20	<0.9
14	80	144	20	>2.0
15	80	144	130	<0.9
16	80	144	130	>2.0
CP	50	72	75	0.9-2.0

*CP denotes center point of the design

Table 3.2. Main effects on resulting compositional analysis. *

Factor	Levels	Lignin (%)	AIL (%)	ASL (%)	Glucan (%)	Xylan (%)	Galactan (%)	Arabinose (%)	Mannan (%)	Ash (%)
Temperature (°C)	20	20.86a (0.73)	16.86a (0.74)	3.99a (0.44)	35.73a (2.97)	21.35a (2.96)	0.67a (0.34)	3.7a (0.47)	0.05a (0.05)	1.67a (0.69)
	75	21.2ab (0.26)	16.23a (0.48)	4.97b (0.74)	38.89a (2.75)	25.59b (3.07)	0.55a (0.06)	4.31a (0.64)	0.02b (0.01)	1.96ab (0.3)
	130	21.36b (0.8)	17.83b (0.87)	3.54c (0.57)	37.08a (2.86)	22.47ab (1.77)	0.83a (0.4)	3.88a (0.53)	0.04b (0.02)	2.2b (0.55)
Time (h)	24	20.89a (0.95)	17.27a (1.28)	3.62a (0.58)	35.38a (3.25)	21.89a (2.93)	0.75a (0.41)	3.75a (0.64)	0.05a (0.05)	1.94a (0.69)
	96	21.2ab (0.26)	16.23a (0.48)	4.97b (0.74)	38.89ab (2.75)	25.59b (3.07)	0.55a (0.06)	4.31a (0.64)	0.02b (0.01)	1.96a (0.3)
	168	21.33b (0.55)	17.42a (0.72)	3.91a (0.5)	37.43b (2.26)	21.92ab (2)	0.75a (0.34)	3.83a (0.32)	0.04b (0.02)	1.93a (0.66)
Moisture Content (wb%)	20	21.12a (0.95)	17.32a (1.08)	3.8a (0.45)	35.54a (2.76)	22.02ab (2.66)	0.82a (0.45)	3.88a (0.62)	0.06a (0.04)	1.79a (0.65)
	50	21.2a (0.26)	16.23a (0.48)	4.97b (0.74)	38.89a (2.75)	25.59a (3.07)	0.55a (0.06)	4.31a (0.64)	0.02b (0.01)	1.96a (0.3)
	80	21.1a (0.64)	17.36a (0.81)	3.73a (0.65)	37.27a (2.95)	21.79b (2.33)	0.69a (0.27)	3.7a (0.35)	0.03b (0.02)	2.08a (0.68)
Size	S	21.31a (0.92)	17.56a (1.12)	3.75a (0.6)	35.6a (2.77)	20.67a (2.38)	0.79a (0.36)	3.65a (0.46)	0.04a (0.04)	2.32a (0.59)
	M	21.2a (0.26)	16.23b (0.48)	4.97b (0.74)	38.89a (2.75)	25.59b (3.07)	0.55a (0.06)	4.31a (0.64)	0.02b (0.01)	1.96ab (0.3)
	L	20.91a (0.62)	17.12ab (0.67)	3.78a (0.51)	37.21a (2.98)	23.14b (1.91)	0.71a (0.39)	3.94a (0.52)	0.04a (0.03)	1.56b (0.51)

* 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. S denotes size less than 0.9 mm, M denotes size between 0.9-2.0 mm, while L denotes size larger than 2.0 mm. AIL = Acid Insoluble Lignin, ASL = Acid Soluble Lignin.

Table 3.3. Interaction effects on resulting compositional analysis (p-values). *

Factor	Lignin (%)	AIL (%)	ASL (%)	Glucan (%)	Xylan (%)	Galactan (%)	Arabinose (%)	Mannan (%)	Ash (%)
Temp	0.004	0.004	0.029	0.160	0.188	0.120	0.245	0.014	0.004
Time	0.978	0.612	0.150	0.038	0.967	0.955	0.580	0.003	0.978
MC	0.089	0.885	0.738	0.075	0.788	0.193	0.239	<.0001	0.089
Size	<.0001	0.150	0.865	0.097	0.008	0.418	0.072	0.807	<.0001
Temp*Time	0.437	0.793	0.772	0.546	0.283	0.618	0.010	<.0001	0.437
Temp*MC	0.285	0.110	0.065	0.426	0.466	0.672	0.036	<.0001	0.285
Temp*Size	0.922	0.282	0.678	0.205	0.190	0.056	0.927	1.000	0.922
Time*MC	0.083	0.244	0.240	0.178	0.308	0.426	0.765	0.000	0.083
Time*Size	0.377	0.410	0.753	0.722	0.507	0.003	0.053	0.807	0.377
MC*Size	0.507	0.946	0.423	0.714	0.308	0.236	0.233	0.807	0.507
Temp*Time*MC	0.097	0.219	0.975	0.073	0.344	0.077	0.188	0.005	0.097
Temp*Time*Size	0.272	0.939	0.865	0.407	0.457	0.358	0.552	0.155	0.272
Temp*MC*Size	0.070	0.361	0.738	0.836	0.650	0.015	0.765	0.335	0.070
Time*MC*Size	0.512	0.852	0.701	0.315	0.635	0.654	0.510	0.100	0.512
Temp*Time*MC*Size	0.806	0.340	0.356	0.956	0.502	0.100	0.685	0.064	0.806

* Temp = Temperature, MC = Moisture Content, AIL = Acid Insoluble Lignin, ASL = Acid Soluble Lignin.

Table 3.4. Treatment effects on resulting compositional analysis. *

Treatment	Lignin (%)	AIL (%)	ASL (%)	Glucan (%)	Xylan (%)	Galactan (%)	Arabinose (%)	Mannan (%)	Ash (%)
1	20.01de	16.01d	4.00a-c	30.035c	16.365c	0.47c	2.895c	0.13a	2.225a-d
2	19.78e	16.22cd	3.56bc	34.435bc	23.34ab	1.195ab	3.685bc	0.12a	0.57e
3	21.46a-c	17.86a-c	3.6bc	36.685ab	20.61bc	0.55c	3.51bc	0.03cd	2.345a-c
4	21.02b-e	16.88b-d	4.135a-c	38.795ab	23.19ab	0.56c	3.7bc	0.025cd	1.805cd
5	20.99b-e	17.03b-d	3.955a-c	36ab	21.11a-c	0.785bc	3.945b	0.03cd	2.195a-d
6	20.94c-e	17.09a-d	3.845a-c	37.51ab	23.8ab	0.585c	3.975b	0.035b-d	1.475c-e
7	21.31a-c	16.76b-d	4.54ab	34.6a-c	20.12bc	0.645bc	3.92b	0.01d	1.47c-e
8	21.36a-c	16.99b-d	4.36ab	37.81ab	22.25ab	0.585c	3.995b	0.025cd	1.285de
9	22.465a	18.775a	3.685bc	35.145a-c	22.49ab	0.895bc	3.955b	0.035b-d	2.19a-d
10	21.12b-d	17.27a-d	3.845a-c	35.865ab	24.09ab	0.54c	4.92a	0.03cd	1.615cd
11	20.79c-e	17.78a-c	3.005c	37.1ab	22.04ab	0.56c	3.46	0.015d	2.795ab
12	20.47c-e	17.33a-d	3.135c	34.97a-c	23.01ab	1.22ab	3.885b	0.035b-d	1.955b-d
13	22.19ab	18.375ab	3.815a-c	37.09ab	21.45ab	1.52a	3.8b	0.03cd	2.295a-c
14	21.46a-c	17.78a-c	3.7bc	38.235ab	23.52ab	0.55c	3.89b	0.05bc	1.805cd
15	21.29a-c	17.89a-c	3.405bc	38.175ab	21.19a-c	0.91bc	3.72bc	0.065b	3.005a
16	21.11b-d	17.41a-d	3.695bc	40.045a	21.97ab	0.455c	3.43bc	0.035b-d	1.96b-d
CP	21.2b-d	16.23cd	4.97a	38.895ab	25.59a	0.55c	4.31ab	0.015d	1.955b-d

*Similar letter after means in each treatment indicates insignificant difference at α = 0.05, LSD, for the dependent variable. CP denotes center point in this study. AIL = Acid Insoluble Lignin, ASL = Acid Soluble Lignin.

Table 3.5. Main effects on enzymatic digestibility results (at t=144 h).*

Factor	Levels	Digestibility (%)
Temperature (°C)	20	47.76 (16.11)
	75	56.07 (-)
	130	51.02 (9.56)
Time (h)	24	53.14 (13.83)
	96	56.07 (-)
	168	45.65 (11.55)
Moisture Content (%)	20	57.51 (8.47)
	50	56.07 (-)
	80	41.28 (11.60)
Size	S	47.02 (14.80)
	M	56.07 (-)
	L	51.77 (11.17)

* Values in parentheses are standard deviation.

CHAPTER 4. OPTIMIZATION OF LOW-MOISTURE ANHYDROUS AMMONIA (LMAA) PRETREATMENT OF CORN STOVER

Modified from a paper to be submitted to *Industrial Crops and Products.*

Abstract

With many environmental benefits, corn-based ethanol has been widely used in recent years. Cellulosic ethanol, however, will require pretreatment to break down lingo-cellulosic structures prior to fermentation. Among all the pretreatment reagents which can be used, ammonia has been shown to be one of the most effective, because it can readily delignify, swell, and preserve the cellulose. Previous work evaluated the effectiveness of the LMAA pretreatment method. The purpose of this study was to determine optimal conditions (i.e. highest glucose yield) using the LMAA pretreatment process. In this experiment, corn stover was prepared with different moisture contents (20%, 50%, 80 wt.%) and particle sizes (9-30 mesh, 30-144 mesh). Corn stover was ammoniated at 20 psi for 30 minutes. Ammoniated corn stover then was subjected to different incubation times (24h, 75h, and 144h) under different temperatures (20°C, 70°C, 120°C). After that, compositional analyses, including ash content, solids content, structural carbohydrates, and lignin content, were conducted. Enzymatic digestibility tests were also conducted. Results showed that under the conditions of 50 wt. % moisture content, 72h, 75°C, and 0.1g NH_3/g DM biomass, the highest glucose yield was obtained (71.6%).

Keywords Ammonia, biomass, cellulosic ethanol, LMAA, pretreatment.

Introduction

Over the past few years, more and more research has focused on the alternative of fossil fuel due to the concerns about environmental, economic and security issues (Mosier et al., 2005). Bioethanol, which is renewable and environmental-friendly, can be used as an alternative to gasoline. Currently, bioethanol is mainly produced from sugar- or starch-based materials, which is called first generation biofuel. Corn ethanol produced in the US and sugarcane ethanol produced in Brazil has been reported to be the world's predominant biofuel nowadays. However, concerns exist about the sources of the first generation biofuel, known as the food versus fuel debate (Sims et al., 2010). Studies showed that the production of starch- or sugar- based biofuel may be one of the reasons of increasing food prices, and the ability of replacing fossil energy has been questioned also (Searchinger et al., 2008).

Bioethanol produced from lignocellulosic biomass, which referred to agricultural residues, forestry residues and energy crops, is regarded to be another available material because of its low cost and less competition with food (Cheng and Timilsina, 2011). Lignocellulosic biomass is the most abundant raw materials on the earth. The main components in lignocellulosic biomass are cellulose, hemicellulose and lignin. Typically, four major steps are needed in bioethanol production: pretreatment, enzymatic hydrolysis, fermentation and ethanol recovery (Naik et al., 2010). These carbohydrate polymers contain different sugars, such as pentose and hexose. Moreover, they are tightly bound with each other. Because of such characteristics of lignocellulosic biomass, pretreatment is required for efficient enzymatic hydrolysis (Mosier et al., 2005).

Numerous biomasses have been studied for biofuel production, such as wheat straw (Alinia et al., 2010), aspen (Yourchisin and Walsum, 2004), soybean straw (Wan and Li, 2011), and corn stover (Narayanaswamy et al., 2011). One of the primary lignocellulosic biomass suitable is corn stover, which is regarded as one of the most important sources of bioenergy, bioethanol and a few commodity chemicals (Sassner et al., 2008). What’s more, various studies focused on pretreatment method have been conducted to enhance enzymatic digestibility and improve ethanol yield based on corn stover. Among all chemical reagents used for pretreatment, ammonia stands out because of its delignification, high preservation of glucose and swelling effect (Yoo et al., 2011).

In 2011, Yoo et al. developed a new pretreatment method named low moisture anhydrous ammonia (LMAA). In their study, a small sealed batch reactor (690 mL internal volume) was used and achieved 89% of theoretical ethanol yield. However, the optimal conditions of small size reactor may not be accurate when scaled up.

In this study, a deeper investigation of the Low-Moisture Anhydrous Ammonia (LMAA) pretreatment method process with a larger-scale reactor (3L), under a range of pretreatment conditions (moisture content, particle size, pretreatment temperature, and pretreatment time) was studied. Optimal conditions for glucose yield were explored.

Materials and Methods

Biomass

In this study, corn stover which had been air-dried was supplied from central Iowa in 2012 and stored at ambient temperature. The biomass was then ground and sieved into three various sizes prior to pretreatment (<0.09 mm, 0.09-2.0mm, >2.0 mm). The sieved corn stover was kept at room temperature (~21°C) until use.

Equipment

The reactor (Fig. 3.1.), which was about 4.35 times larger compared to Yoo's (2011) previous study, used for the ammoniation process was purchased from Pall Corporation, Ann Arbor, Michigan, USA. The use of a larger reactor may reduce the potential errors that may be caused by different ammonia loadings and reaction times. In order to measure mono-saccharides, an HPLC installed 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) were used. Acid soluble lignin (ASL) content was determined by UV-Visible spectrophotometer (UV-2100 Spectrophotometer, Unico, United Products & Instruments, Inc., Dayton, NY, USA). And acid insoluble lignin (AIL) content was determined by oven and furnace.

Enzymes

GC 220 cellulase was purchased from Genencor International, Inc. (Rochester, NY, USA). The cellulose activity was expressed in filter paper units (FPU). In this study, the average activity of GC 220 was determined to be 45 FPU / mL. The β-glucosidase enzyme (Novozyme 188) was provided from Sigma-Aldrich, Inc. (St. Louis, Missouri, USA). The activity of Novozyme 188 was 750 cellobiase units (CBU) / mL.

LMAA pretreatment process

Before ammoniation, different amounts of water were added to the corn stover in order to meet the required moisture content (20, 50, 80 wt. %); samples were equilibrated for over 24 h.

Moisturized corn stover was placed in the sealed reactor, and ammonia was introduced. A pipe was connected between the top of the reactor and the fume hood to ventilate surplus ammonia. A pressure gauge was installed on the reactor to monitor the pressure change during the ammoniation process. Anhydrous ammonia was added up to the targeted pressure to achieve 0.1 g NH_3/ g DM biomass. The whole ammoniation process lasted up to 30 minutes in order to achieve a complete reaction. Temperature changes could be observed from the temperature gauge which was also installed on the top of the reactor, but it was not controlled during this study. After the ammoniation process was finished, the reactor was cooled down for 5 minutes, the lid was removed in the fume hood, and then the ammoniated corn stover was transferred into several glass bottles (250 mL) with screw caps.

The bottles packed with ammoniated corn stover were placed in heating ovens at various pretreatment temperatures (20°C, 75°C, and 120°C) for 24 h, 72 h, and 144 h. As soon as the pretreatment process was complete, the lids of the glass bottles were removed in the fume hood and surplus ammonia was evaporated for 12 h.

Experimental design

In this study, four independent variables that influenced the reaction severity were investigated. Biomass moisture contents were 20 wt. %, 50 wt. % and 80 wt. %; the pretreatment times were 24 h, 72 h, 144 h; the pretreatment temperatures were 20°C, 75°C, 120°C, and the particle sizes were <0.9mm, 0.9-2.0mm and >2.0mm, respectively; with five 0h pretreated samples and five un-pretreated samples. There were 27 treatments in this study. Moisture content, lignin, glucan, xylan, galactan, arabinan, and mannan were measured as dependent variables during the experiment. The experimental design for this study is shown in Table 4.1.

Compositional analysis

Carbohydrates and lignin were determined followed by NREL LAP (NREL, 2011). Each sample was analyzed in duplicate. The content of glucan and xylan in the corn stover were analyzed by HPLC, following the NREL standards. Acid soluble lignin (ASL) was measured by UV-Visible Spectrophotometer. And moisture content was determined by the oven drying method (NREL, 2011).

Enzymatic digestibility

Enzymatic digestibility was determined following NREL LAP (NREL, Enzymatic saccharification of lignocellulosic biomass. Laboratory Analytical Procedure (LAP). 2008). The test was done 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 initial glucan concentration was 1% (w/v). Cellulase enzyme (GC 220) loading was 15 FPU/g of glucan, and ß-glucosidase enzyme (Novozyme 188) loading was equal to 30 CBU/g of glucan. Flasks were incubated at 50°C ± 1°C and 150 rpm in an incubator shaker (Excella E24 Incubator Shaker Series, New Brunswick Scientific, Edison, NJ, USA). Enzymatic digestibility time ranged from 0 h to 120 h for sugar analysis.

Total glucose detected from HPLC was used to calculate the glucan digestibility following equation 4.1 below. The conversion factor for glucose to equivalent glucan was 0.9.

$$\% \text{ digestion} = \frac{\text{grams cellulose digestied}}{\text{grams cellulose added}} \times 100 \times 0.9 \qquad (4.1)$$

Results and Discussion

Effects of LMAA pretreatment on biomass composition

As shown in Table 4.2 (main effects on composition analysis), the use of anhydrous ammonia didn’t result in large differences in terms of lignin, glucan, xylan, galactan, arabinan, and mannan content, as can be indicated from the similar letters after each mean number. What’s more, a majority of the p-values of the interaction effects (Table 4.3) between factors were higher than 0.05, which indicated little evidence of significant interaction among the four factors. As to the treatment effects on composition analysis, as shown in table 4.4, same result as the main effects can be applied. Many studies using ammonia as the base reagent have reported the same result (Alizadeh et al., 2005). It is mainly because the ammonia used was meant to break down cellulose and separate lignin from cellulose and hemicellulose. In other words, ammonia didn’t have the effect of changing composition.

Effects of LMAA pretreatment on enzymatic digestibility

All enzymatic digestibility results are presented in Fig. 4.1. As can be observed from Fig. 4.2, the highest digestibility from enzymatic digestibility of pretreated corn stover (71.6%) was much higher than unpretreated sample (26.6%), which validated the effect of anhydrous ammonia in breaking down cellulose into monosaccharide. Among the 27 treatments, 72h, 75°C, and 50 wt % moisture content with 0.1 g NH_3/g DM corn stover had the highest digestibility (71.6%). This result is similar to Yoo's work (84h, 85°C, 50 wt % moisture content with 0.1g NH_3/g DM corn stover). As to the time effect on enzymatic digestibility, longer time may improve digestibility, but not very significantly (Fig. 4.3). However, Fig. 4.4 confirms that lower incubation temperature may improve digestibility compared with the higher one.

Conclusions

In this paper, the effect of LMAA pretreatment method in larger scale reactor was studied. The results were similar compared with previous research, which indicated that LMAA pretreatment has the potential to achieve higher glucose yield (71.6%) under the conditions of 50 wt % moisture content, 72h, 75°C, and 0.1g NH_3/g DM biomass.

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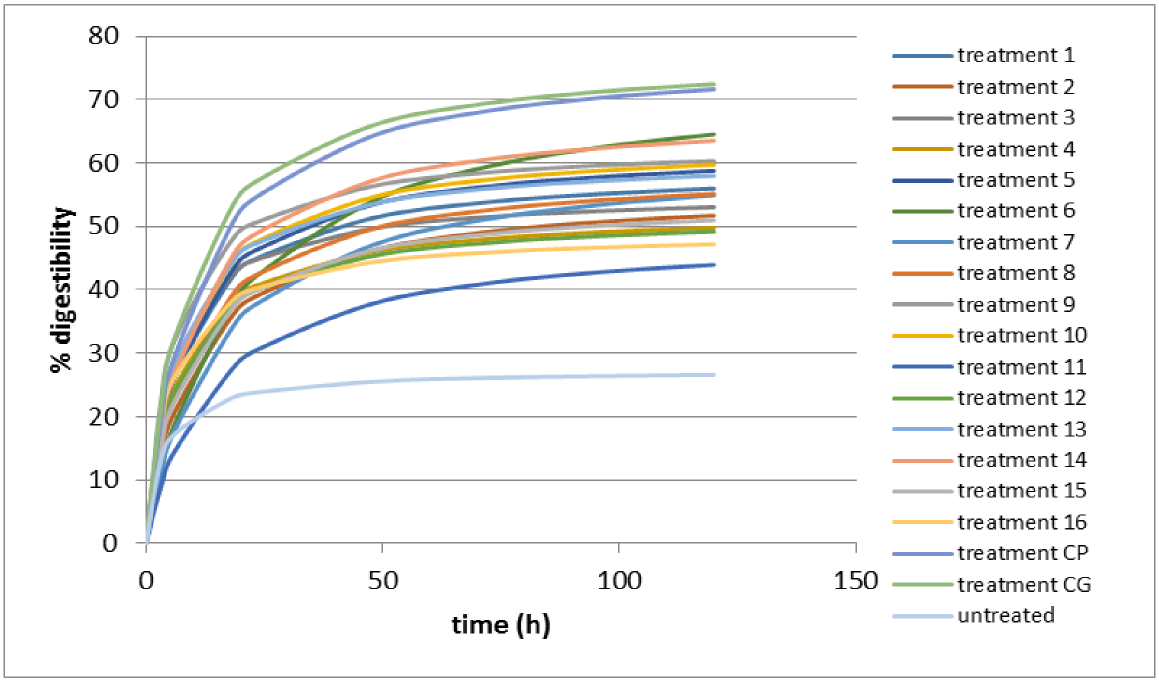

* CP denotes center point of the design; CG denotes previous research.

Figure 4.1. Enzymatic digestibility of all treatments.*

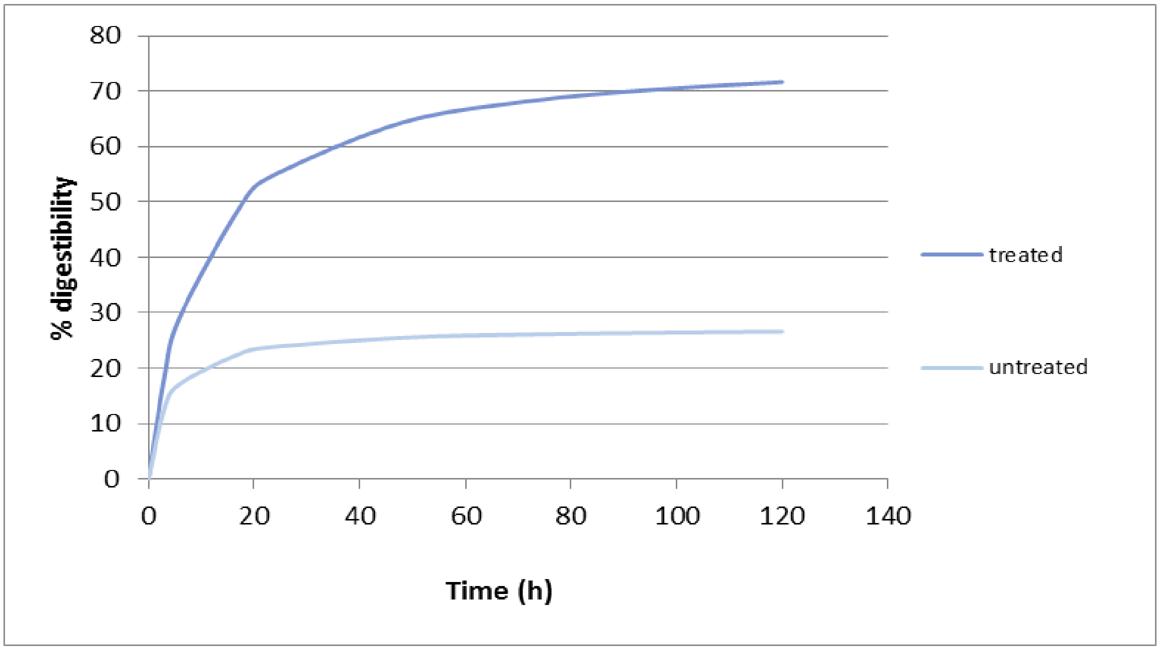

Figure 4.2. Enzymatic digestibility of treated and untreated corn stover.

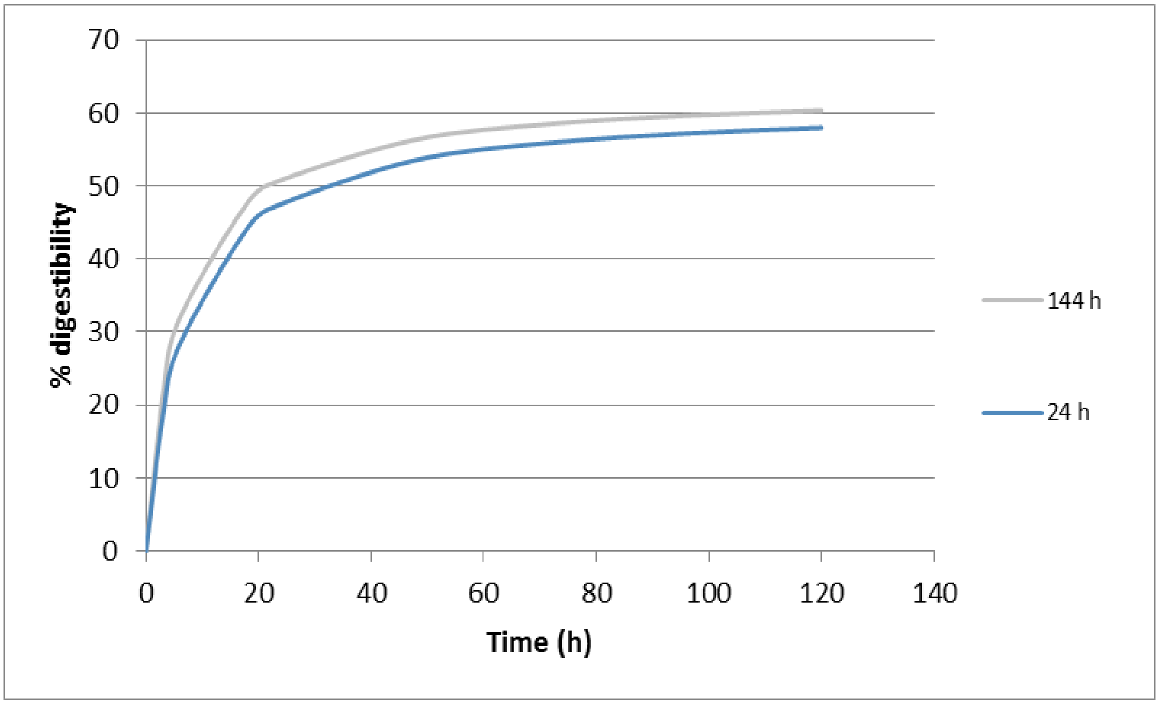

Figure.4.3. Time effects on enzymatic digestibility.

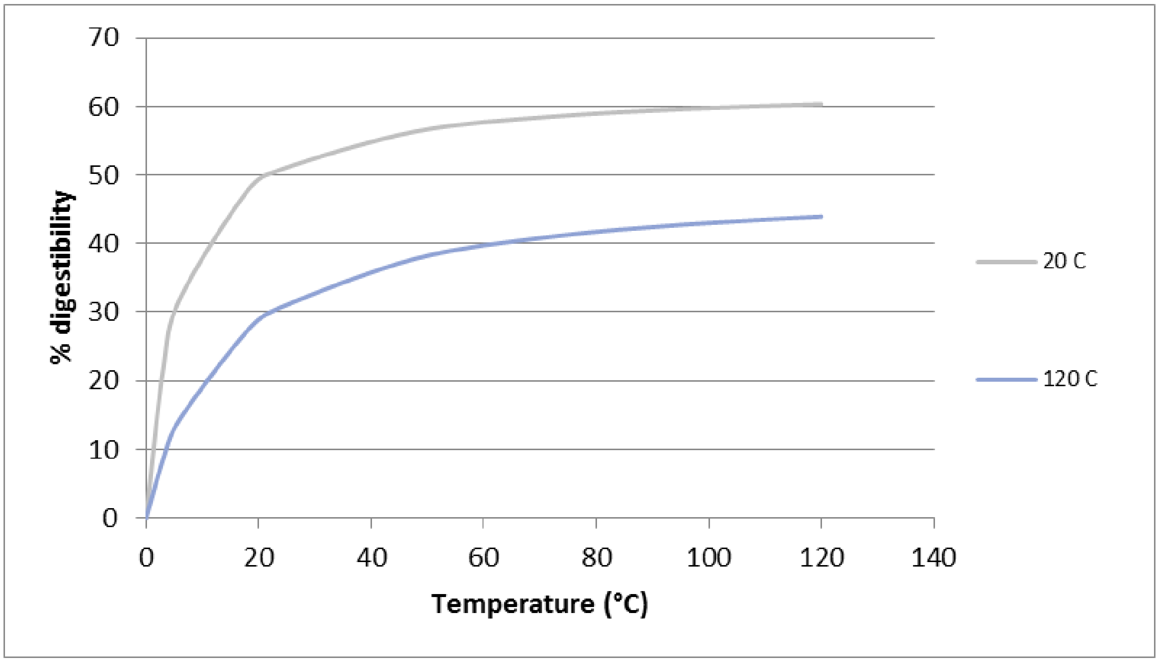

Figure 4.4. Temperature effects on enzymatic digestibility.

Table 4.1. Experimental design *

Treatment	Moisture content (wb %)	Time (h)	Temperature (˚C)	Particle size
1	20	24	20	S
2	20	24	20	L
3	20	24	120	S
4	20	24	120	L
5	20	144	20	S
6	20	144	20	L
7	20	144	120	S
8	20	144	120	S
9	80	24	20	L
10	80	24	20	S
11	80	24	120	L
12	80	24	120	S
13	80	144	20	L
14	80	144	20	S
15	80	144	120	S
16	80	144	120	L
CP	50	72	75	M
18	20	0		S
19	20	0		L
20	80	0		S
21	80	0		L
22	50	0		M
23	20			S
24	20			L
25	80			S
26	80			L
27	50			M

* CP denotes center point of the design; Treatment 18- 22 denote 0h pretreated corn stover; Treatment 23-27 denotes un-pretreated corn stover.

Table 4.2. Main effects on resulting compositional analysis. *

Factor	Levels	Lignin (%)	Glucan (%)	Xylan (%)	Galactan (%)	Arabinan (%)	Mannan (%)
Time (h)	24	16.91a(1.29)	43.90a(4.16)	23.50a(5.04)	0.24a(0.68)	4.46a(1.01)	0.22a(0.23)
	72	16.08a(0.15)	42.80a(4.00)	28.16a(11.26)	1.69a(2.39)	5.19a(1.49)	0.69b(0.39)
	144	17.33a(0.73)	43.44a(4.01)	21.33a(0.88)	0.27a(0.58)	3.54b(0.69)	0.06a(0.17)
Temperature (°C)	20	16.70a(1.15)	43.68a(4.47)	22.53a(3.77)	0.28a(0.59)	4.18a(0.82)	0.12a(0.21)
	75	16.08a(0.15)	42.80a(4.00)	28.16a(11.26)	1.69a(2.39)	5.19a(1.49)	0.69b(0.39)
	120	17.54b(0.78)	43.67a(3.67)	21.97a(4.59)	0.23a(0.67)	3.81a(1.11)	0.17a(0.22)
Moisture Content (%)	20	17.16a(0.91)	41.00a(3.69)	21.78a(3.72)	0.25a(0.55)	3.72a(0.91)	0.21a(0.24)
	50	16.66a(0.74)	40.46ab(10.72)	21.39a(6.51)	0.90b(1.59)	4.00a(1.00)	0.24b(0.36)
	80	17.13a(1.01)	44.21b(5.01)	20.88a(4.78)	0.31a(0.73)	3.71a(0.99)	0.10a(0.21)
Size	L	17.21a(0.94)	42.78a(4.95)	21.62a(4.77)	0.21a(0.54)	3.57a(0.89)	0.18a(0.25)
	M	16.66a(0.74)	40.46a(10.72)	21.93a(6.51)	0.90b(1.59)	4.00a(1.00)	0.24b(0.36)
	S	17.08a(0.98)	42.43a(4.40)	21.04a(3.76)	0.34a(0.73)	3.86a(0.99)	0.13a(0.21)

* Similar letters after means in each level of the main factor indicates insignificant differences at α=0.05, LSD, for that dependent variable. Values in parentheses are standard deviation. S denotes size less than 0.9 mm, M denotes size between 0.9-2.0 mm, while L denotes size larger than 2.0 mm.

Table 4.3. Interaction effects on resulting compositional analysis (p-values). *

Factor	Lignin (%)	Glucan (%)	Xylan (%)	Galactan (%)	Arabinose (%)	Mannan (%)
Temp	0.0456	0.9980	0.7748	0.8714	0.2615	0.5609
Time	0.2887	0.7945	0.2105	0.9127	0.0101	0.0648
MC	0.8146	0.0294	0.6197	0.7741	0.9570	0.1228
Size	0.9091	0.7585	0.8642	0.5096	0.2154	0.9938
Temp*Time	0.2050	0.6462	0.8522	0.3058	0.0947	0.5404
Temp*MC	0.8122	0.3637	0.4369	0.5140	0.3652	0.9689
Temp*Size	0.8368	0.9004	0.8110	0.6654	0.2376	0.9814
Time*MC	0.2435	0.5949	0.7196	0.6629	0.4889	0.9938
Time*Size	0.5141	0.6482	0.7362	0.5207	0.3024	0.9814
MC*Size	0.8616	0.9463	0.7059	0.8659	0.6728	0.5203
Temp*Time*MC	0.4923	0.631	0.9525	0.6185	0.6589	0.9938
Temp*Time*Size	0.8541	0.535	0.7797	0.6579	0.4819	0.1194
Temp*MC*Size	0.7041	0.8605	0.4447	0.2131	0.1887	0.5007
Time*MC*Size	0.6354	0.2396	0.8293	0.377	0.7497	0.5007
Temp*Time*MC*Size	0.8866	0.9241	0.7525	0.9071	0.9662	0.0337

* Temp = Temperature, MC = Moisture Content.

Table 4.4. Treatment effects on resulting compositional analysis. *

Treatment	Lignin (%)	Glucan (%)	Xylan (%)	Galactan (%)	Arabinose (%)	Mannan (%)
1	16.67a-c	41.42a-c	24.93a	0.31a	5.21a	0.48ab
2	16.19bc	38.10bc	21.13a	0.00a	3.15d-i	0.00b
3	18.28a	43.84ab	23.00a	0.11a	4.49a-e	0.20ab
4	17.63a-c	42.20ab	25.51a	0.00a	4.57a-d	0.46ab
5	16.98a-c	39.72a-c	21.96a	0.99a	4.25a-g	0.00b
6	17.36a-c	44.06ab	20.98a	0.10a	3.56b-i	0.26ab
7	16.99a-c	40.69a-c	21.88a	0.00a	3.17c-i	0.25ab
8	17.37a-c	43.00ab	22.52a	0.12a	3.65a-i	0.00b
9	16.12bc	47.16ab	23.63a	0.00a	4.88ab	0.00b
10	15.97c	47.33ab	24.73a	0.00a	4.20a-h	0.21ab
11	17.19a-c	43.72ab	23.12a	1.46a	4.75a-c	0.21ab
12	17.24a-c	47.46ab	22.00a	0.00a	4.41a-f	0.21ab
13	17.31a-c	45.77ab	19.88a	0.00a	3.99a-i	0.00b
14	17.05a-c	45.86ab	23.01a	0.87a	4.22a-g	0.00b
15	17.64a-c	44.92ab	18.27a	0.12a	2.88f-i	0.00b
16	18.10ab	43.55ab	19.46a	0.00a	2.58i	0.00b
CP	16.08bc	43.80ab	28.16a	1.69a	5.19a	0.69a
CG	16.10bc	50.92a	21.33a	1.79a	3.67a-i	0.27ab
20	17.36a-c	37.88bc	19.36a	0.00a	2.96e-i	0.27ab
21	17.08a-c	41.98ab	24.28a	0.38a	3.82a-i	0.26ab
22	17.05a-c	45.34ab	19.76a	0.12a	3.59b-i	0.00b
23	17.45a-c	40.30a-c	18.98a	0.13a	2.61hi	0.38ab
24	17.64a-c	29.61c	18.10a	0.00a	3.79a-i	0.00b
25	16.33a-c	39.24a-c	17.64a	1.02a	2.76g-i	0.19b
26	17.87a-c	39.88a-c	18.18a	0.00a	3.03d-i	0.19ab
27	17.11a-c	39.44a-c	19.03a	0.00a	3.37b-i	0.00b
28	17.33a-c	39.72a-c	18.69a	1.01a	3.02d-i	0.18b
29	16.82a-c	38.53bc	18.00a	0.13a	3.34b-i	0.00b

* Similar letter after means in each treatment indicates insignificant difference at α=0.05, LSD, for the dependent variable. CP denotes center points of the study; CG denotes previous optimal conditions.

CHAPTER 5. LONG TERM STORAGE OF CORN STOVER USING LOW-MOISTURE ANHYDROUS AMMONIA PRETREATMENT METHOD

Modified from a paper to be submitted to *Industrial Crops and Products.*

Abstract

As a promising material for bioethanol production, corn stover has been studied under various pretreatment methods prior to production of bioethanol. However, the storage of pretreated corn stover is still challenged by both weather conditions and the physical properties of its own. The objective of this experiment is to evaluate the effect of low-moisture anhydrous ammonia (LMAA) pretreatment method on biomass quality during long periods of storage. In this study, corn stover was contacted with various ammonia loadings (0, 0.1, and 0.2 g/g DM biomass) and moisture content (20 wt %, 40 wt %, and 60 wt %) from 1 day to 90 days both in sealed and open containers. As a result, the mass loss in sealed container increased with time; however, the mass loss in open container was affected by the conditions of the environment. In terms of the carbohydrate, no significant reduction was observed in either sealed or open containers.

Keywords LMAA, corn stover, bioethanol, storage, dry matter loss, mold growth

Introduction

Bioethanol, a promising replacement of fossil fuel, has been studies in various ways. Typically, bioethanol can be produced by food crops, such as corn and sugarcane, or lignocellulosic biomass, which is non edible plant and energy crops (Nagarajan et al., 2013). With the aim of producing 36 billion gallons of ethanol per year by 2022, 16 billion gallons was supposed to come from cellulosic biomass (Schnoor, 2011).

Corn stover, mainly comprised of the stalks and leaves, has the great potential to serve as the biofuel feedstock. According to the estimation of Kadam (2003), 80-100 million dry tonnes/year of corn stover could be collected, among which 80% is available for ethanol production (Kadam and McMillan, 2003). Currently, the potential of the conversion of corn stover to biofuel is targeted to be 90 gal/ton in the near future (DOE-EERE 2009). However, the sturdy structure of lignocellulosic biomass increases the difficulty in bioethanol production.

Generally speaking, lignocellulosic biomass is composed of three parts: cellulose, hemicellulose and lignin. As is known, cellulose is a linear polymer of glucose; hemicellulose is a branched polymer containing xylose, arabinose, mannose, and some other polysaccharides. In terms of lignin, it is a highly disordered polymer which serves as the protection since cellulose is embedded in the matrix of lignin and hemicellulose (Menon and Rao, 2012). In order to open the structure and expose cellulose within lignocellulosic biomass, a pretreatment process before hydrolysis and fermentation is critical.

Pretreatment processes have been developed by numerous studies. One of the base reagents adopted by researchers is ammonia. Ammonia fiber expansion (AFEX) uses concentrated ammonia to break down the inner structure of lignocellulosic biomass for the enzymatic hydrolysis and fermentation process (Lau et al., 2010); Soaking in aqueous ammonia (SAA) for pretreatment is proved to have the ability of retaining the hemicellulose at low temperature and increasing the fermentation yield (Kim and Lee, 2005); And the low-moisture anhydrous ammonia (LMAA) process is developed to minimize the water and ammonia input for bioethanol production (Yoo et al., 2011).

In terms of corn stover storage, two common approaches are applied: dry storage and wet storage (Cui et al., 2012). Dry product, which refers to 20 - 25% moisture content in raw corn stover, is typically harvested and packaged in round bales (Shinners et al., 2007), but the high drying cost and high dry matter losses during storage are the remaining problems (Richard, 2010). On the other side, wet storage, also named ensilage, is a method of preserving biomass at high moisture content (> 45%) (Cui et al., 2012). It could minimize the loss of nutrients and reduce the drying cost (Weinberg and Ashbell, 2003), but it still has the problem of mold growth, which may be hazardous to downstream operations (Essien et al., 2005). In order to produce bioethanol, higher effective preservation of carbohydrates during storage is required.

In this study, a low moisture anhydrous ammonia (LMAA) pretreatment process is applied before corn stover storage since ammonia could result in higher efficiency in ethanol production (Ko et al., 2009) as well as impeding mold growth. The objective of this research is to evaluate the effects of the LMAA pretreatment process on biomass quality (changes in carbohydrates, ash, and mass losses) during long periods from 1 day to 3 months. In addition, growth of fungi or other microorganisms will be monitored in these days.

Materials and Methods

Biomass

Corn stover, freshly harvested and delivered in bales, was obtained from central Iowa, USA, 2013. It was air-dried before baling and receiving by the lab. Then, the corn stover was ground through a 2-mm screen using a grinder (Wiley Model 4), and stored at room temperature. After that, deionized water was mixed with corn stover to achieve the target moisture contents (20 wt %, 40 wt %, and 60 wt %). Moisturized corn stover was placed overnight at ambient temperature to reach equilibrium.

Low-moisture anhydrous ammonia (LMAA) pretreatment process

Before contacting with anhydrous ammonia, moisture content of treated corn stover was determined and recorded using the moisture tester. Then the corn stover was placed into the ammoniation reactor, as shown in Fig. 3.1, to contact with various loadings of anhydrous ammonia (0.1 g/g DM biomass and 0.2 g/g DM biomass), tightly closed the valve of the reactor for 30 minutes after reaching the target pressure. After that, the ammoniated corn stover was transferred into several heavy-duty Ziploc plastic bags and open containers, thoroughly mixed and weighed. Sealed containers (Fig. 5.1) and open containers (Fig. 5.2) were placed at ambient temperature for 0h, 6h, 1d, 5d, 12d, 30d, 60d, and 90d.

Compositional analysis

Once the duration time was achieved, pretreated samples were weighed, and surplus ammonia was evaporated in the fume hood. Then the compositional analysis was followed by the NREL LAP procedure (Sluiter et al., 2011). The monosaccharides were analyzed by high performance liquid chromatography (HPLC) installed 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). The content of acid soluble lignin (ASL) was determined by UV-Visible spectrophotometer (UV-2100 Spectrophotometer, Unico, United Product & Instruments, Inc., Dayton, NY, USA). All samples were analyzed in duplicate.

Mold growth observation

The observation experiment was conducted both in sealed container samples and open containers. Mold growth was monitored everyday during the whole experimental period by obseving the changes in color and shape.

Experimental design

In this study, two independent variables were designed to investigate the storage effect: ammonia loading, and moisture content. Each has three levels. Moreover, full factorial design was used as shown in Table 5.1.

Results and Discussion

Mold growth

Mold growth in both sealed containers and open containers was observed during 90 days storage of corn stover. The first appearance of mold was found in treatment 3 after 16 days (Fig. 5.3). After one day, the mold growth was observed in treatment 2. However, there was no other mold appearance in other treatments until 90 days.

The results indicate that high moisture content was the main reason of the mold growth since the moisture content in treatment 2 and 3 were 40 wt % and 60 wt %, respectively. Moreover, under the protection of ammonia, pretreated corn stover could be well preserved without mold growth. This was due to the anti-microbial characteristic of ammonia (Rideal, 1895). Even though ammonia is not currently listed as the disinfectant by the Environmental Protection Agency (EPA), its effect in killing microbes and molds has been proved (Tajkarimi et al., 2008).

Storage mass losses

Mass loss during storage was measured in the changes of entire treatment. Results for sealed container (Table 5.3) and open container (Table 5.4) were quite different. And Table 5.5 showed the moisture content (% dry basis) and dry matter at t=0h and t=90 d.

For sealed container treatments, as time increased, mass loss also increased. Their relationship could be seen from Fig. 5.4. Moreover, under the same moisture content, treatments with 0.2 g/g DM biomass ammonia loadings tend to have higher mass losses.

However, as for open container treatments, the mass changes during 90 days were highly dependent on the ambient temperature and humidity; the relationship between time and mass losses was not as straightforward as sealed container treatments. What’s more, under the same ammonia loading, treatments with 60 wt % moisture content lost more mass than the other two levels; but there was no obvious difference in mass losses among three levels of ammonia loading under the same moisture content.

Ash content

Ash content was measured following the NREL standard lab procedure (Sluiter et al., 2008). Distribution of ash content in the sealed container treatments was shown in Fig. 5.5. As can be seen from the graph, higher moisture content under the same ammonia loading tended to have higher ash content; and treatment 3 (60 wt % moisture content with no ammonia loading) has the highest ash percentage, a similar trend was also found in open container treatments. The results indicated that anhydrous ammonia may not have significant effect in retaining the ash.

Ash content was also analyzed in the difference between sealed containers and open containers (Table 5.6). As time increased, the ash content decreased; and the corresponding p-value for time was larger than 0.0001, which indicated that there was little evidence of difference between ash content in either sealed containers or open containers.

Lignin content

As can be seen in Fig. 5.6, lignin content in sealed containers was higher in the first three treatments (without ammonia), and there was no significant evidence of difference between the two levels of ammonia loading in lignin content (p-value=0.0816).

The lignin content in open containers didn't resulted in significant difference among nine treatments (p-value=0.4647); however, under the same moisture content, the lignin content decreased rapidly with higher ammonia loading, which could be seen from Fig. 5.7.

Those results were the evidence that anhydrous ammonia has the potential to remove lignin, which could help to increase the accessibility of enzyme in hydrolyzing. Similar reduction in lignin content was also reported by other researchers (Lau et al., 2010).

Sugar content

Sugar content analysis focused on glucan content and xylan content in this study.

As for glucan content (Table 5.7), the overall trend in both sealed containers and open containers was decreasing over time. In sealed containers, there was no significant decline in glucan content among the nine treatments since p-value =0.6714; in open containers, the reduction trend was not obvious either (p-value=0.4468). The reason for this insignificant change was because the effect of the low-moisture anhydrous ammonia pretreatment was to break down the lignin-carbohydrate-compounds (LCC) for higher enzymatic hydrolysis rate; it didn’t affect the glucan content in biomass.

In terms of xylan content (Table 5.8), the reduction in sealed containers under the same ammonia loading was observed, as shown in Fig. 5.8, but with the same moisture content, higher ammonia loading tended to retain more xylan. In open containers, no obvious reduction was observed. By analyzing the difference between two sealing conditions, even though xylan content in open containers was higher than in sealed containers, the difference was not obvious (p-value=0.4978). This could also be explained by the pretreatment effect; LMAA didn’t affect the xylan content significantly in biomass.

Conclusions

LMAA pretreated corn stover could be well preserved up to 90 days without mold growth and reduction in carbohydrates. Compared between two sealing conditions (sealed containers and open containers), the effect of lignin removal was more obvious in sealed containers, but the sugar contents in both conditions were nearly the same. As for ammonia loading, mass losses in 0.2- was higher than 0.1 g/g DM biomass, however, no other significant differences were found in terms of ash content, lignin content and sugar contents. For future work, more attention could be focused on the interaction effect of time and temperature during long-term storage.

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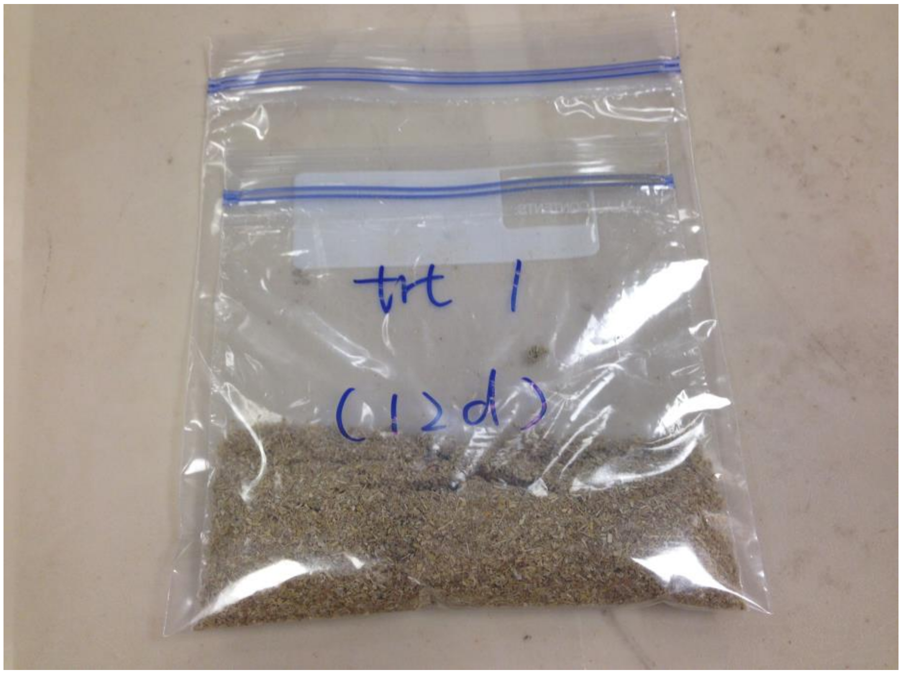


Figure 5.1. Sealed container.

Figure 5.2. Open container.

Figure 5.3. Visible mold grown in sealed containers after 16 days of storage.

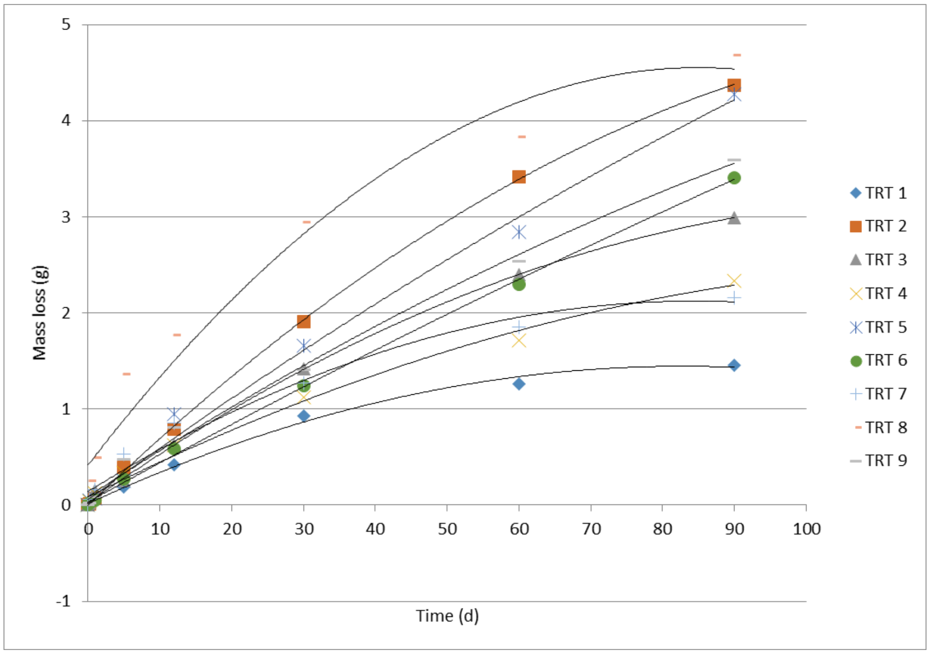
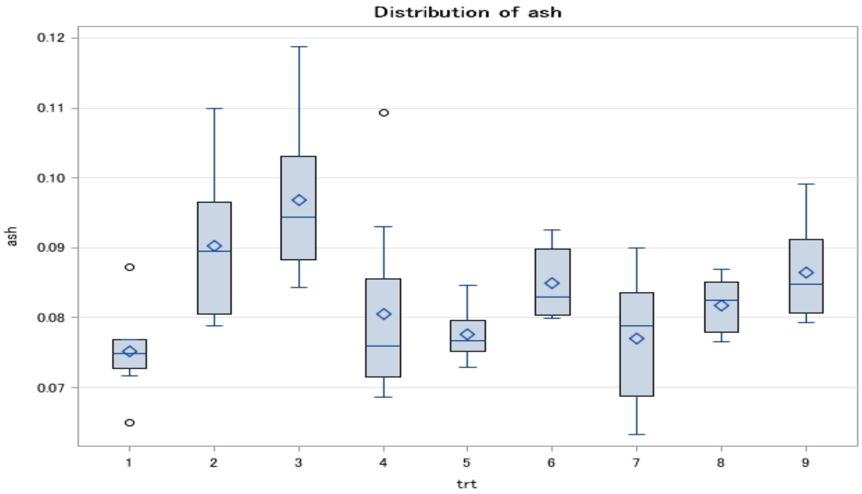
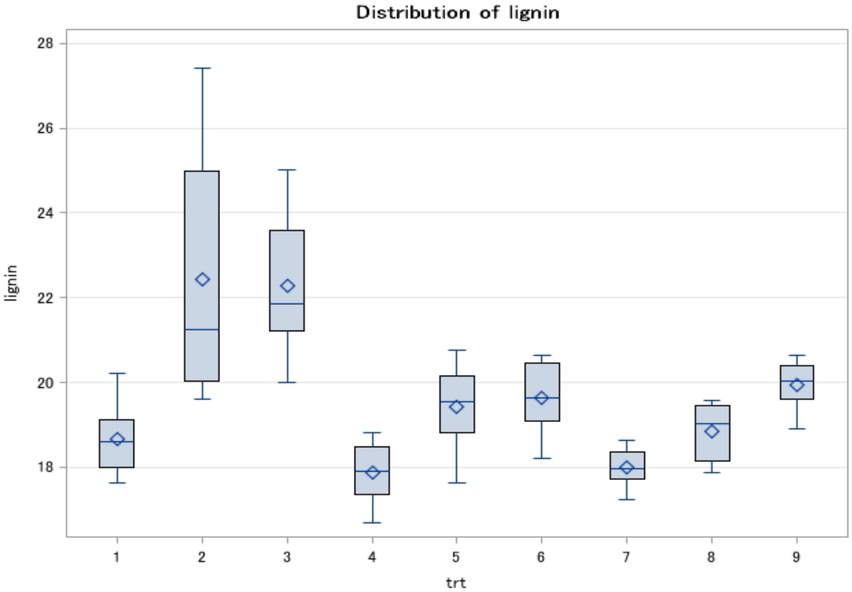


Figure 5.4. Relationship between mass changes (wet basis) and time in sealed containers.



S.D.: trt1=0.006; trt2=0.011; trt3=0.011; trt4=0.014; trt5=0.004; trt6=0.005; trt7=0.009; trt8=0.004; trt9=0.007.

Figure 5.5. Distribution of ash content in sealed containers.



S.D.: trt1=0.860; trt2=3.149; trt3=1.802; trt4=0.737; trt5=1.016; trt6=0.855; trt7=0.472; trt8=0.695; trt9=0.580.

Figure 5.6. Distribution of lignin content in sealed containers.

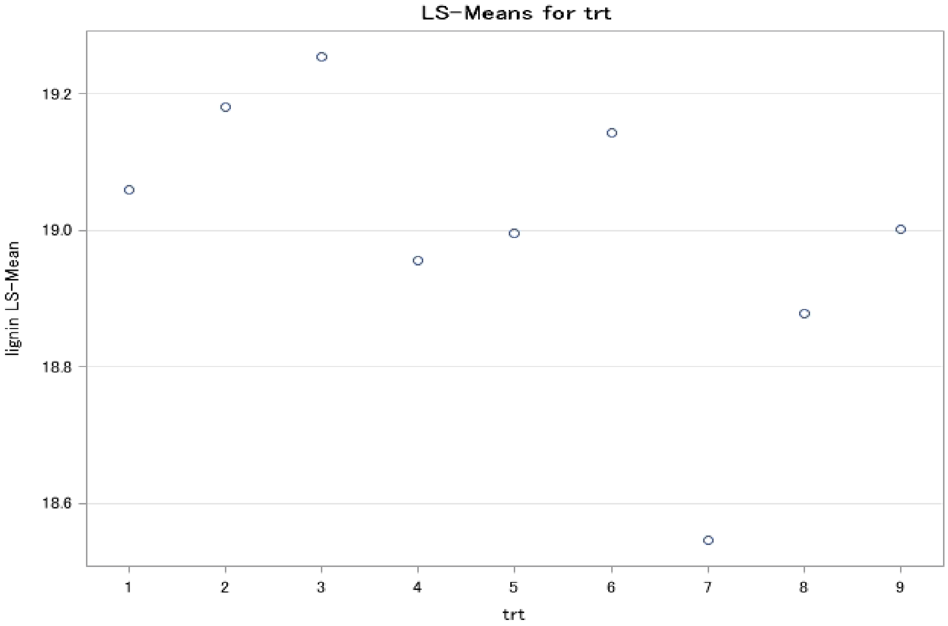


Figure 5.7. Least square means of lignin content in open containers.

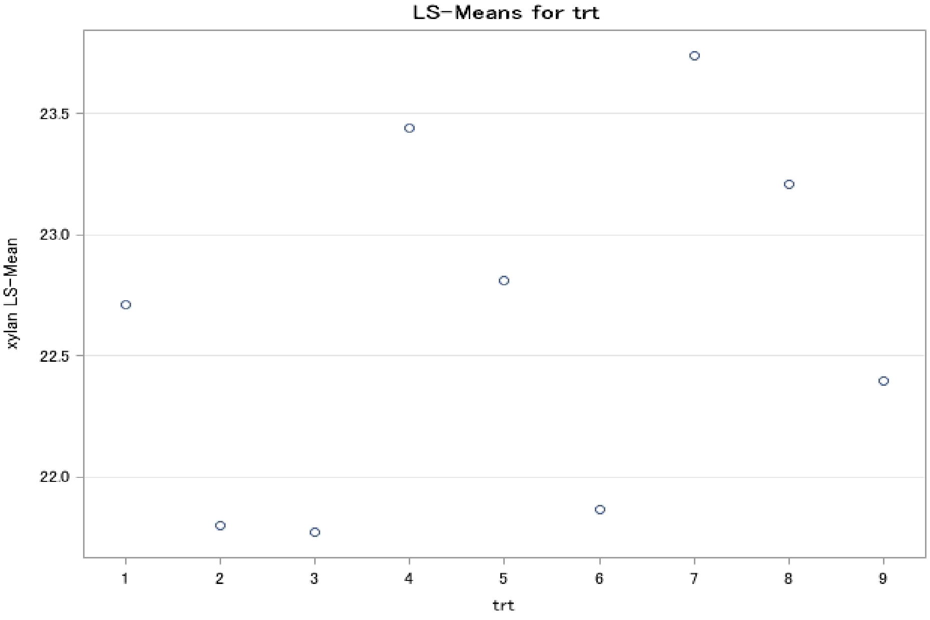


Figure 5.8. Least square means of xylan content in sealed containers.

Table 5.1. Experimental design.

Treatment	Ammonia Loading	M.C.
1	0	20%
2	0	45%
3	0	60%
4	0.1	20%
5	0.1	45%
6	0.1	60%
7	0.2	20%
8	0.2	45%
9	0.2	60%

Table 5.2. Regression analysis of the mass loss of the seal containers.

	Regression Analysis	R^2
TRT 1	$Y=-0.0002X^2+0.0345X+0.0162$	0.9952
TRT 2	$Y=-0.0003X^2+0.0723X-0.0029$	0.9998
TRT 3	$Y=-0.0002X^2+0.0529X+0.0239$	0.9992
TRT 4	$Y=-0.0001X^2+0.0376X+0.0861$	0.9922
TRT 5	$Y=-9*10^{-5}X^2+0.0545X+0.0584$	0.9939
TRT 6	$Y=-4*10^{-5}X^2+0.0413X+0.0325$	0.999
TRT 7	$Y=-0.0003X^2+0.0469X+0.1411$	0.9784
TRT 8	$Y=-0.0006X^2+0.0973X+0.4176$	0.9683
TRT 9	$Y=-0.0001X^2+0.0488X+0.09$	0.9943

Table 5.3. Storage mass loss results for sealed containers.

	0h	6h	1d	5d	12d	30d	60d	90d
TRT 1	0	0.01	0.04	0.19	0.42	0.93	1.26	1.46
TRT 2	0	0.01	0.07	0.39	0.79	1.91	3.42	4.37
TRT 3	0	*0.02*	0.15	0.25	0.62	1.42	2.4	2.99
TRT 4	0	0.04	0.12	0.34	0.62	1.12	1.71	2.33
TRT 5	0	0.05	0.07	0.25	0.95	1.66	2.84	4.28
TRT 6	0	0.02	0.05	0.27	0.59	1.24	2.30	3.41
TRT 7	0	0.06	0.16	0.53	0.85	1.27	1.85	2.16
TRT 8	0	0.25	0.49	1.36	1.77	2.94	3.83	4.68
TRT 9	0	0.02	0.09	0.48	0.81	1.41	2.54	3.59

Table 5.4. Storage mass loss results for open containers.

	0h	6h	1d	5d	12d	30d	60d	90d
TRT 1	0	1.37	2.14	0.33	4.02	-0.15	2.99	-0.37
TRT 2	0	3.44	5.6	1.4	11.13	-0.08	10.4	-0.07
TRT 3	0	5.31	7.7	3.32	17.72	-0.13	17.19	-0.08
TRT 4	0	2.14	1.44	0.35	3.93	-0.25	3.08	-0.07
TRT 5	0	5.09	4.96	0.77	9.76	-0.18	8.74	-0.07
TRT 6	0	4.5	8.4	2.63	17.98	-0.09	16.59	-0.04
TRT 7	0	2.83	1.6	0.32	4.43	-0.13	4.23	-0.24
TRT 8	0	5.77	5.36	1.03	10.63	-0.1	10.63	-0.04
TRT 9	0	6.8	7.64	2.45	17.8	-0.11	16.8	-0.05

Table 5.5. Moisture content and dry matter at t=0h and t=90d.

SEALED CONTAINERS				
	Moisture content (dry basis)		*Dry matter (g)*	
	t=0h	*t=90d*	*t=0h*	*t=90d*
TRT 1	19.20%	12.16%	11.48	11.20
TRT 2	43.29%	36.21%	11.39	10.02
TRT 3	58.38%	41.87%	7.21	8.34
TRT 4	19.20%	11.49%	15.91	15.37
TRT 5	43.29%	32.59%	12.66	12.17
TRT 6	58.38%	48.50%	7.98	8.12
TRT 7	19.20%	9.41%	14.80	14.64
TRT 8	43.29%	28.00%	11.14	10.78
TRT 9	58.38%	45.83%	7.50	7.81
OPEN CONTAINERS				
	Moisture content (dry basis)		*Dry matter (g)*	
	t=0h	*t=90d*	*t=0h*	*t=90d*
TRT 1	19.20%	10.13%	12.14	12.15
TRT 2	43.29%	15.95%	17.05	16.53
TRT 3	58.38%	29.89%	12.49	9.00
TRT 4	19.20%	8.98%	12.38	12.55
TRT 5	43.29%	18.96%	17.04	17.27
TRT 6	58.38%	29.75%	12.65	9.69
TRT 7	19.20%	9.12%	24.31	23.50
TRT 8	43.29%	18.19%	17.48	16.53
TRT 9	58.38%	27.56%	12.58	9.72

Table **5.6.** Differences (db %) in ash between sealed and open containers.*

	0h	**6h**	**1d**	**5d**	**12d**	**30d**	**60d**	**90d**
TRT 1	-0.90(0.41)	1.35(0.91)	0.30(0.04)	0.74(0.27)	-1.77(1.57)	-1.95(1.90)	2.81(3.94)	-3.90(7.61)
TRT 2	0.44(0.09)	1.16(0.07)	2.33(2.70)	0.61(0.19)	1.57(1.23)	2.73(3.72)	2.42(2.92)	0.10(0.01)
TRT 3	0.31(0.05)	0.34(0.06)	1.92(1.84)	-0.26(0.03)	0.40(0.08)	1.97(1.95)	1.27(0.80)	-0.07(0.01)
TRT 4	0.82(0.33)	1.64(1.33)	2.56(3.27)	-0.65(0.21)	0.76(0.28)	1.07(0.57)	3.35(5.61)	-4.03(8.12)
TRT 5	0.91(0.42)	0.63(0.20)	1.92(1.84)	0.08(0.01)	0.47(0.11)	0.26(0.03)	0.71(0.25)	-2.79(3.88)
TRT 6	0.78(0.30)	0.57(0.16)	0.85(0.36)	0.75(0.28)	-0.53(0.14)	-0.39(0.07)	-0.58(0.16)	-3.88(7.52)
TRT 7	0.69(0.24)	1.18(0.69)	1.22(0.75)	1.23(0.58)	1.08(0.99)	1.41(0.52)	-1.03(5.99)	-3.46(0.40)
TRT 8	0.90(0.40)	0.23(0.03)	1.04(0.54)	0.21(0.02)	-0.06(0.01)	1.02(0.51)	-0.01(0.01)	-4.58(1.05)
TRT 9	0.60(0.17)	-0.13(0.01)	2.52(3.18)	1.11(0.62)	-0.66(0.21)	-0.06(0.01)	-0.66(0.21)	-3.52(0.62)

* Difference = Ash (sealed container) - Ash (open container). Values in parentheses are standard deviation.

Table 5.7. Differences in glucan content (db %) in sealed and open containers.*

	0h	**6h**	**1d**	**5d**	**12d**	**30d**	**60d**	**90d**
TRT 1	-7.73(2.98)	-0.74(0.28)	-1.92(1.85)	-3.81(7.28)	2.17(2.34)	2.04(2.08)	4.88(1.19)	-2.23(2.49)
TRT 2	-2.72(3.71)	0.40(0.08)	-3.07(4.73)	-2.46(3.03)	-1.83(1.67)	-5.30(1.41)	-3.15(4.97)	-1.09(0.59)
TRT 3	-2.33(2.73)	0.99(0.48)	-1.79(1.61)	-3.91(7.62)	-1.71(1.46)	-1.55(1.19)	-3.09(4.76)	-1.65(1.36)
TRT 4	-3.33(5.53)	-3.59(3.46)	-1.17(0.68)	-2.74(3.75)	0.58(0.17)	-1.06(0.56)	4.76(1.13)	-2.87(4.11)
TRT 5	-3.97(7.89)	-2.46(3.01)	-1.50(1.12)	-5.87(1.72)	0.44(0.09)	0.71(0.25)	-0.17(0.01)	-3.05(4.64)
TRT 6	-6.39(2.04)	-7.86(3.08)	-1.10(0.61)	-5.19(1.35)	0.64(0.20)	-0.72(0.26)	6.41(2.05)	-3.54(6.27)
TRT 7	-4.92(1.21)	1.14(0.65)	-0.48(0.12)	-1.45 (1.06)	-0.20(0.02)	-4.70(1.11)	5.95(1.77)	-2.85(4.07)
TRT 8	-5.58(1.56)	1.25(0.78)	1.89(1.78)	-4.00(0.81)	1.15(0.65)	1.96(1.92)	1.10 (0.61)	-1.17(0.68)
TRT 9	-6.84(2.34)	-0.33(0.05)	-1.22(0.75)	-4.34(9.43)	-0.93(0.44)	-1.12(0.63)	0.21(0.02)	-3.47(6.00)

* Difference = Glucan (sealed container) - Glucan (open container). Values in parentheses are standard deviation.

Table 5.8. Differences in xylan content (db %) in sealed and open containers.*

	0h	**6h**	**1d**	**5d**	**12d**	**30d**	**60d**	**90d**
TRT 1	2.24(2.52)	0.24(0.03)	1.33(0.88)	2.79(3.89)	-3.08(4.75)	1.62(1.31)	-1.72(1.48)	-1.10(0.61)
TRT 2	-1.23(0.76)	1.96(1.92)	5.28(1.39)	2.57(3.30)	-0.57(0.17)	4.60(1.06)	-1.64(1.35)	-7.03(2.47)
TRT 3	4.80(1.15)	-1.02(0.52)	0.89(0.39)	1.49(1.11)	-0.72(0.26)	2.69(3.61)	-1.86(1.73)	3.95(7.82)
TRT 4	1.54(1.18)	1.25(0.78)	0.54(0.15)	1.65(1.36)	-1.63(1.33)	3.87(7.48)	0.36(0.06)	-4.45(2.99)
TRT 5	4.24(8.98)	0.60(0.19)	1.49(1.12)	1.33(0.88)	-0.66(0.22)	4.04(8.15)	-2.10(2.19)	-5.38(1.45)
TRT 6	2.65(3.52)	2.96(4.38)	-0.17(0.01)	1.20(0.73)	-0.79(0.32)	4.28(9.14)	1.09(0.60)	-6.05(1.83)
TRT 7	1.25(0.78)	-4.04(8.18)	-0.07(0.02)	-0.42(0.08)	-0.34(0.06)	2.02(2.04)	-1.75(1.53)	-5.71(1.63)
TRT 8	2.19(2.41)	-1.27(0.81)	-1.75(1.54)	3.16(5.01)	0.06(0.01)	2.77(3.85)	-1.45(1.05)	-5.62(1.58)
TRT 9	2.21(2.44)	-1.74(1.52)	-0.08(0.03)	3.41(5.81)	2.88(4.16)	4.21(8.87)	-2.93(4.31)	-4.85(1.17)

* Difference = Xylan (sealed container) - Xylan (open container). Values in parentheses are standard deviation.

CHAPTER 6. TECHNO-ECONOMIC ANALYSIS (TEA) OF LOW-MOISTURE ANHYDROUS AMMONIA (LMAA) PRETREATMENT METHOD FOR CORN STOVER

Modified from a paper to be submitted to *Industrial Crops and Products.*

Abstract

Techno-Economic Analysis (TEA) plays an important role in assessing economic performance and potential market acceptance for new technologies. Previous work has shown that the construction and operation of a cellulosic bioethanol plant can be very expensive. One of the largest cost categories is pretreatment processing. The purpose of this study was to conduct a detailed cost analysis to assess low moisture anhydrous ammonia (LMAA) pretreatment process at the commercial-scale, and to estimate the breakeven point in large-scale production. In this study, capital expenses, including annualized purchase and installation fees, and annual operating costs associated with each unit operation were determined. The lowest unit cost obtained from this study was $3.86 / gal, but it was still high compared with current gasoline price.

Keywords. Ammonia, biomass, cellulosic ethanol, LMAA, pretreatment, techno-economic analysis.

Introduction

With an increasing demand for energy, more and more research have been focused on bioethanol production. Bioethanol, a promising replacement of fossil fuel, can be obtained from lignocellulosic biomass, such as energy crops and residues from arable land (Singh et al., 2010). Typically, ethanol production from lignocellulosic biomass follows several steps: pretreatment, enzymatic hydrolysis, fermentation of sugar, and ethanol recovery (Alvira et al., 2010). Among the ethanol production processes, pretreatment is regarded as the critical step because it is required for efficient hydrolysis. Various pretreatment methods have been developed, such as dilute acid, hot water extraction, and ammonia fiber expansion (AFEX). Each method has its own advantages and disadvantages. Ammonia has been chosen because of its delignification effect (Kim and Lee, 2007) and swelling effect (Mosier et al., 2005). In 2011, a new method named low moisture anhydrous ammonia (LMAA) has been performed in lab scale (Yoo et al., 2011). In their study, LMAA process resulted in 89% of the maximum theoretical ethanol yield and showed the potential to decrease ammonia and water inputs compared with other pretreatment methods.

The technician report published by National Renewable Energy Laboratory (NREL) in 2010 entitled Techno-economic analysis of biochemical scenarios for production of cellulosic ethanol compared four different models of pretreatment processing (dilute acid, two-stage dilute acid, hot water, and ammonia fiber expansion (AFEX)) (Kabir Kazi et al., 2010). It was concluded that without any downstream process variation, the dilute acid process had the lowest product value (PV) of $ 3.40/gal of ethanol in 2007, which was equivalent to $5.15 / gal of gasoline. One year later, in

2011, the NREL published another technical report entitled Process design and economics for biochemical conversion of lignocellulosic biomass to ethanol (Humbird et al., 2011) focused on the dilute acid pretreatment process. In that report, detailed bioethanol conversion design was built on eight specific areas. The minimum ethanol selling price reported from NREL was \$2.15 / gal, which was equivalent to \$3.27 /gal gasoline. When broken down into process sections, \$0.74 / gal was contributed from the feedstock, enzyme and wastewater treatment each contributed \$0.34 / gal, and the rest (\$0.73 / gal) was contributed from the remaining conversion process areas. Even though the selling price was still higher than market price, the latter one was \$0.13 / gal lower than previous.

With the recent development of pretreatment technology and updated cost estimation, an updated techno-economic analysis of biofuel production was required. As far as the authors know, cost analysis based on low-moisture anhydrous ammonia (LMAA) pretreatment has not been published in any journals yet. This research is focused on estimating unit costs of bioethanol production based on LMAA pretreatment process, and comparing it among three different production scales.

Materials and Methods

This study began by developing process flow diagrams (Fig. 6.1), and all the economic and environmental analyses were then calculated in an Excel-based spreadsheet with an accuracy of ± 30% (Coker, 2010). The whole process was divided into six sections: feedstock handling, ammoniation process, incubation process, simultaneous saccharification and co-fermentation (SSCF) process, evaporation process, and combustor or burner. Waste water treatment, biomass and ethanol storage were not considered in this research.

This study was a derivative estimation from the NREL's report (Humbird et al., 2011). What's more, the calculation was based on a plant size of 2,000 metric tonne (MT) of corn stover per day; the other two scales were 100 MT/d and 800 MT/d, respectively. The following exponential expression (6.1) was used for scaling, in which the exponent was assumed to be 0.6 (Aden et al., 2002).

$$New\ cost = Original\ cost\ \left(\frac{New\ size}{Original\ size}\right)^{exp} \tag{6.1}$$

This bioethanol plant was assumed to work 24 hours per day, 7 days per week, and 45 weeks annually, which was 315 online processing days per year. The main product of the plant was ethanol, and electricity was generated as a by-product. Other major assumptions were listed in Table 6.1.

Techno-Economic Analysis

TEA of the whole process will be analyzed on the six sections mentioned in previous part. Equipment costs are obtained from factory quotation and previous study.

Feed handling

The feedstock used in this study is corn stover. Table 6.2 shows the average composition (% dry basis) of corn stover based on NREL studies (Aden et al., 2002). The composition of biomass has a strong influence on ethanol yield. Corn stover is delivered in bales and the cost is $36.25 /dry tonne (Pennington, 2013).

As Fig. 6.2 shows, corn stover bales were received by belt conveyors, including transport conveyors and unwrapping conveyors. Then the unwrapped feedstock is transported to a hammer mill where the size of the material was reduced and became more homogeneous.

Since the plant size is 2,000 MT/d, two lines of the transport conveyor and unwrapped conveyor with the capacity of 45 tonne/h are used to receive corn stover. Then the stover is introduced into the hammer mill with a capacity of 75 tonne/h. Water is sprayed on the biomass during transporting process to wash off dirt, yet the amount is not considered here.

LMAA Pretreatment and incubation

Before ground corn stover was contacted with anhydrous ammonia, a hydrolysate process was conducted in order to remove acetic acid and part of furfural, which may be toxic to downstream fermentation microorganisms (Aden et al., 2002). Ammoniation was designed for 20 minutes of residence time, and ammonia loading was 0.1g ammonia / g DM biomass. Ammoniated stover was then transferred into incubation tank for 3.5 days. After incubation, solids were used for the saccharification and co-fermentation processes. Other assumptions for pretreatment conditions are listed in Table 6.3. And Fig. 6.3 and Fig. 6.4 are the flowcharts representing the ammoniation and incubation process. Surplus NH_3 from incubation tank was recycled to ammoniation tank.

The washed and ground corn stover was fed to two screw conveyors with four water pumps for hydrolysis in this process. Then two hold tanks with a capacity of 15,000 gal each were used because of ammoniation resistance time. Every half an hour, stover was fed to the ammoniation tank. Anhydrous ammonia was inlet into the tank by two pumps. After this, ammoniated corn stover was transferred into 10 incubation tanks with a capacity of 100,000 gal each. Hydrolysate from incubation was delivered to the saccharification process by four belt conveyors. Waste water was collected and treated using both anaerobic and aerobic digesters, which will not be discussed in this study.

Saccharification and Co-Fermentation process

Hydrolysate from pretreatment is fed to the saccharification tank along with enzymes. After saccharification, the microorganism *Z. mobilis*, grown in seed tank (Fig. 6.5), is used as the biocatalyst in fermentation process. Then, the seed inoculum, nutrients, and saccharified slurry are added to ethanol fermenter (Fig. 6.6).

In the saccharification process, five 1,000,000-gallon tanks are used. The enzyme loading is calculated based on the cellulose content and target hydrolysis conversion level. A cooler is used for saccharified slurry. Other assumptions are listed in Table 6.4.

In terms of seed production process, 10% of the saccharified slurry was sent for seed production (Aden et al., 2002). Two trains were used for turn-around time for each seed fermenter for 12 hours; five fermenters were needed in each train. Other assumptions are listed in Table 6.5.

The fermentation process was conducted in five 1,000,000-gal ethanol fermenters. The total residence time was assumed to be 36 hours. The fermenters were cooled before the distillation and evaporation processes. Table 6.6 lists the assumptions used in the fermentation process.

Evaporation process

During this process, molecular sieves and distillation are used for ethanol recovery. Fig. 6.7 represents the process.

Five beer columns with the capacity of 1,000L are used in the distillation process to remove the dissolved CO_2 and most of the water. The ethanol is collected as vapor from the beer column and fed to the rectification column. After rectification, overhead vapor of ethanol is given to molecular sieve adsorption unit. Nice pieces are contained in this unit, such as product cooler, condenser, and mole sieve columns. The mixture is condensed and returned to the rectification column.

Liquids from the beer column is sent to the 1st evaporator, about 24% of the water entering could be evaporated (Aden et al., 2002). Then the slurry is fed to the 2nd evaporator, about 44% of the water could be evaporated. The 3rd evaporator can evaporate nearly 76% of the water. The final vapor is condensed, and solids are sent to burner.

Combustor and Generator process

The purpose of this process is to burn solids or by-products downstream for electricity generation. All the remaining lignin and hemicellulose from the feedstock are burnt in the fluidized bed combustor. A generator is used to generate electricity. The flow diagram of this process is shown in Fig. 6.8.

Results and Discussion

The techno-economic analysis (TEA) of the cellulosic ethanol plant was conducted on three different scales based on corn stover capacity: 100 MT/d, 800 MT/d and 2,000 MT/d. Results showed that the larger the plant scale, the lower the product cost, which is illustrated in Fig. 6.9. The approximate ethanol yield per year in compatible with the corn stover capacity was 2.5 MM gal/y, 20 MM gal/y, and 50 MM gal/y, respectively. The lowest cost of ethanol was $3.86/gal for a commercial plant of 50 MM gal ethanol yield per year, which was still higher compared with market gasoline price ($3.704) (U.S. Energy Information Administration, 2014). However, this cost would be much higher in real commercial scale since the waste water treatment, storage cost, and utility cost were not considered in this study. In terms of the small scale ethanol plant (2.5 MM gal/y), the ethanol cost would be $5.08/gal; and $4.28/gal for medium scale ethanol plant (20 MM gal/y).

In terms of cost per ton of feedstock, as shown in Fig. 6.10, the larger the plant size, the lower the unit cost. For small scale, the cost per unit feedstock is $451.27; for medium scale, it decreases to $380.85; and $342.79/ton of corn stover for large scale ethanol production.

Final Reports

The final report based on 2000 MT corn stover/d with the ethanol production of 50 MM gal/y is shown in Table 6.7.

The final report based on 1200 MT corn stover/d with the ethanol production of 20 MM gal/y is shown in Table 6.8.

The final report based on 100 MT corn stover/d with the ethanol production of 2.5 MM gal/y is shown in Table 6.9.

Conclusions

In this study, a cellulosic bioethanol plant based on six major processing sections was built in three different scales: 100 MT corn stover/d, 800 MT corn stover/d, and 2,000 MT corn stover/d. After the techno-economic analysis, the result showed that the larger the ethanol plant, the lower the unit cost both in $/gal of ethanol and $/ton of feedstock. However, the minimum ethanol selling cost obtained from this study ($3.86 / gal) was still high compared with the current gasoline price. In order to further reduce the unit cost to make bioethanol more competitive, improvements in process design and ethanol conversion rate need to be made. As the development of bio-renewable energy industry and the techno-economic analysis, lower price in bioethanol could be achieved in the near future.

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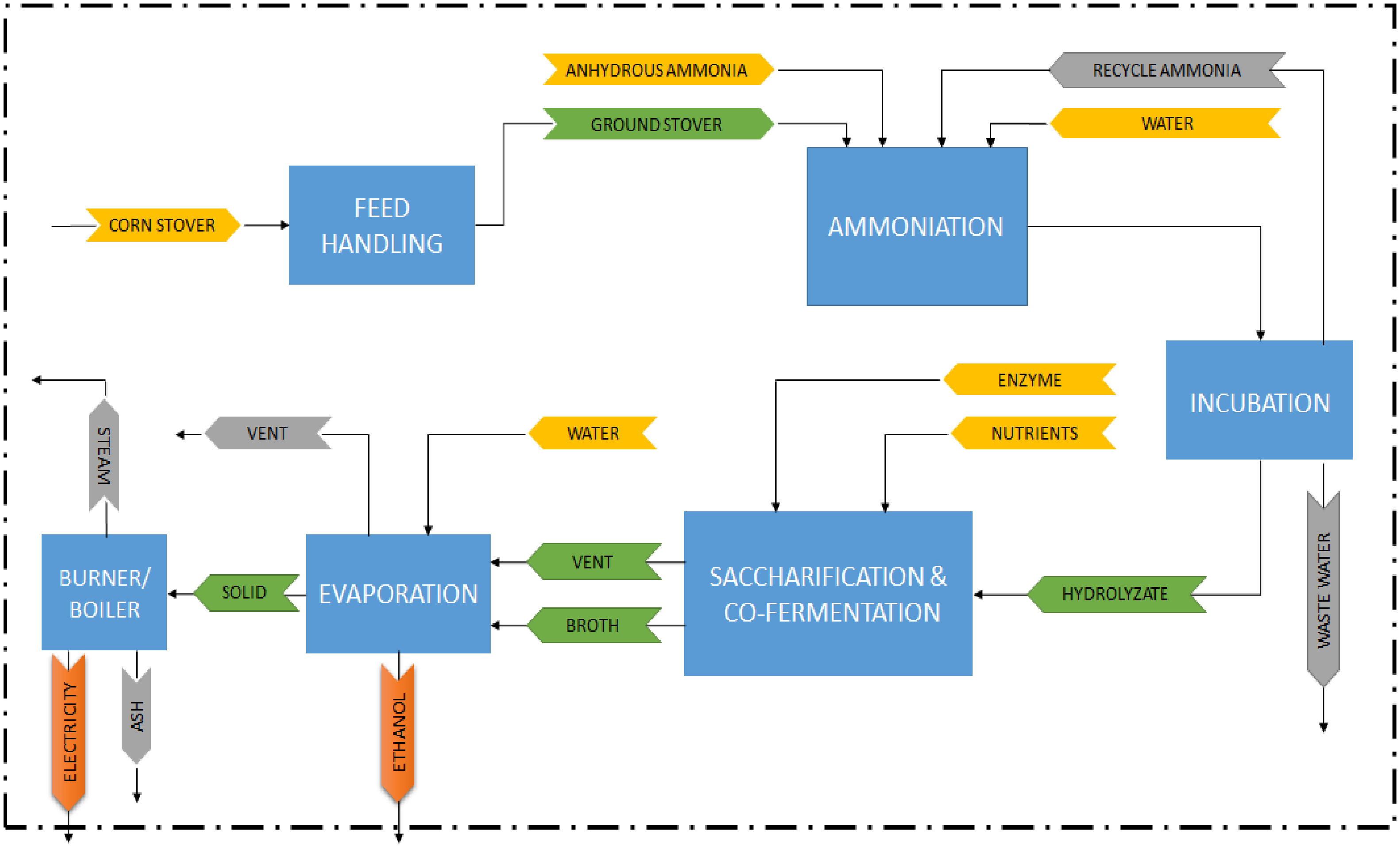


Figure 6.1. Overall process of LMAA-based bioethanol production.

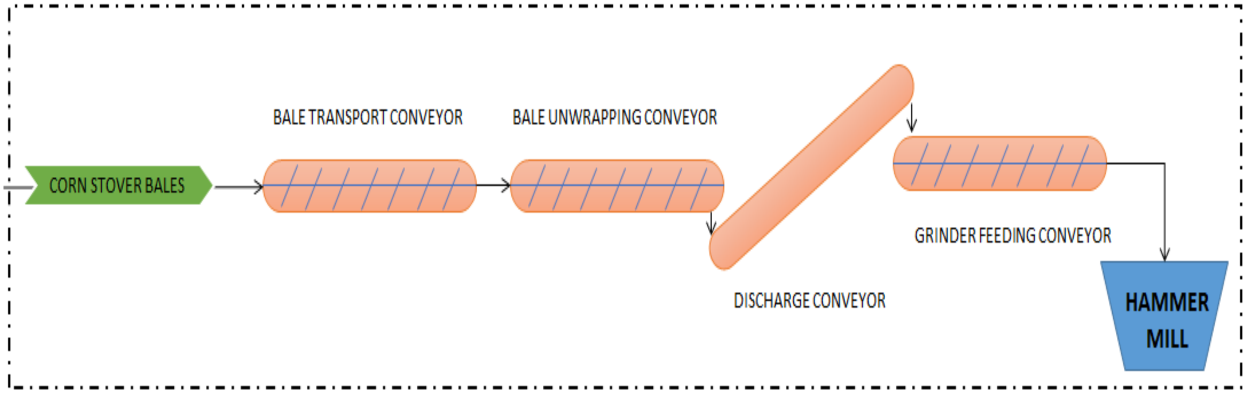


Figure 6.2. Feed handling process.

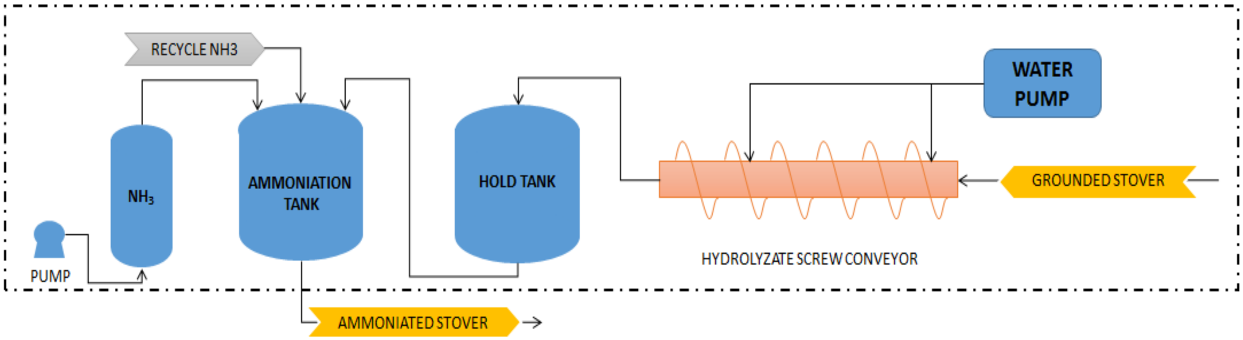


Figure 6.3. Ammoniation process.

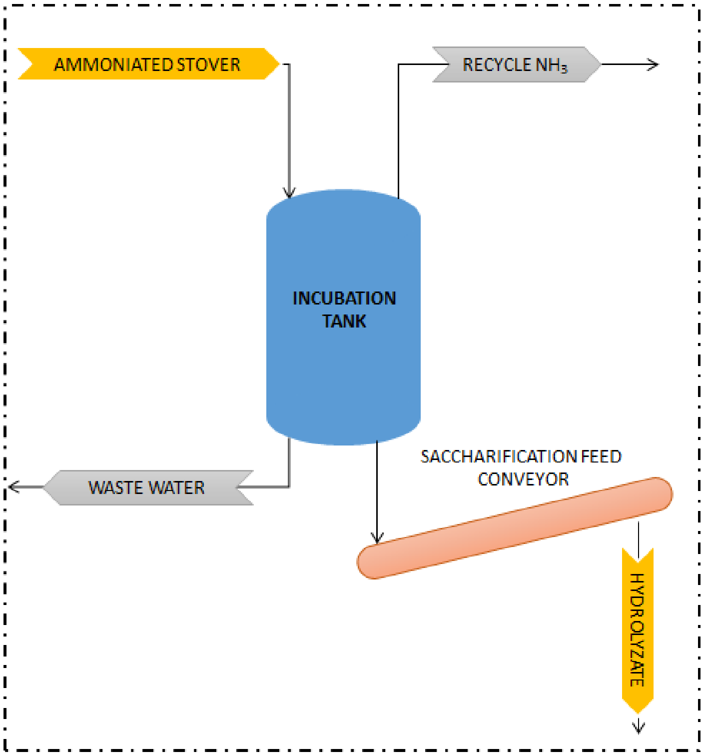


Figure 6.4. Incubation process.

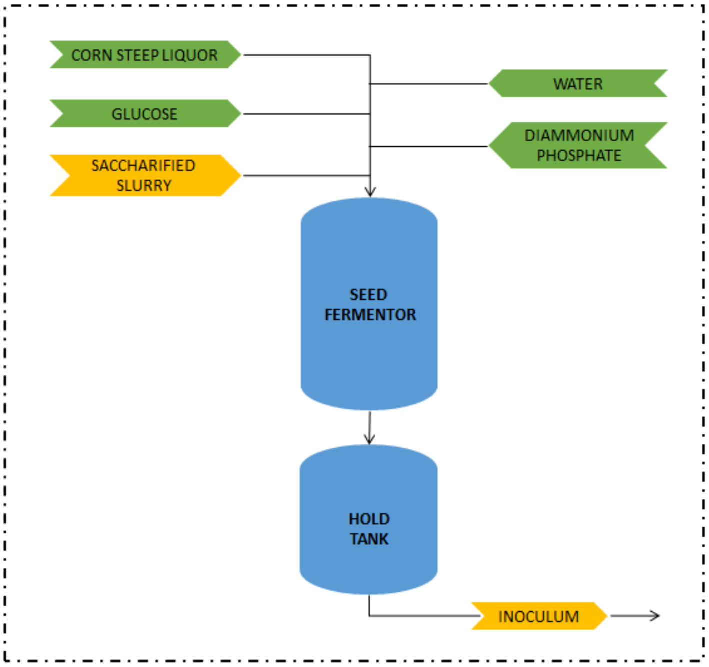


Figure 6.5. Seed production process.

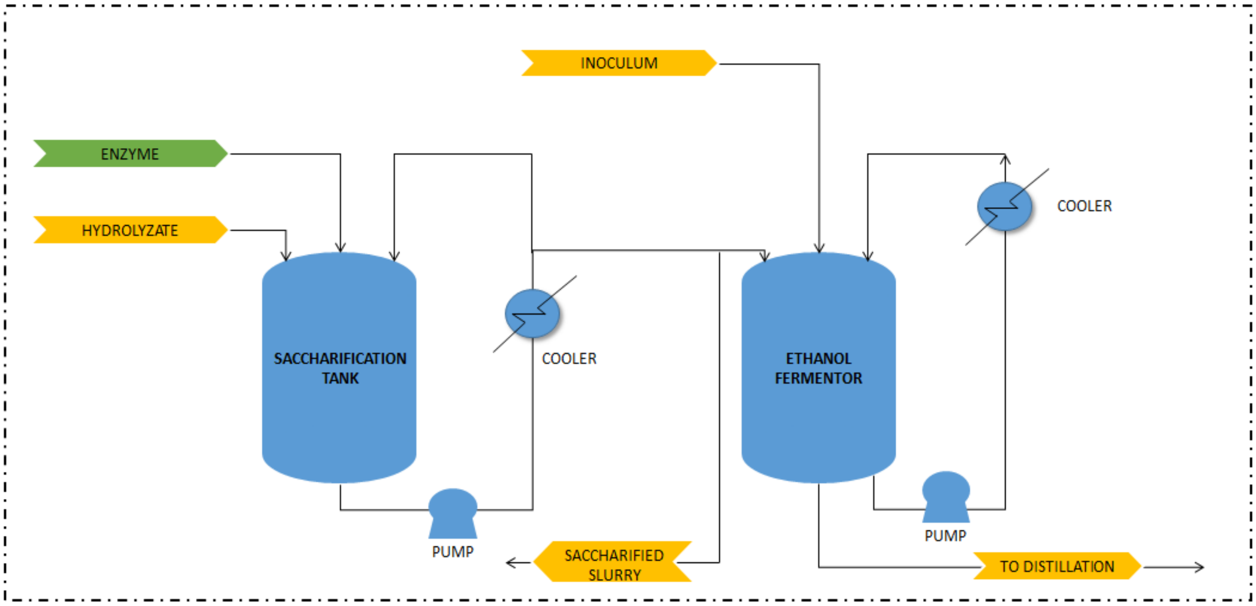


Figure 6.6. Saccharification and co-fermentation process.

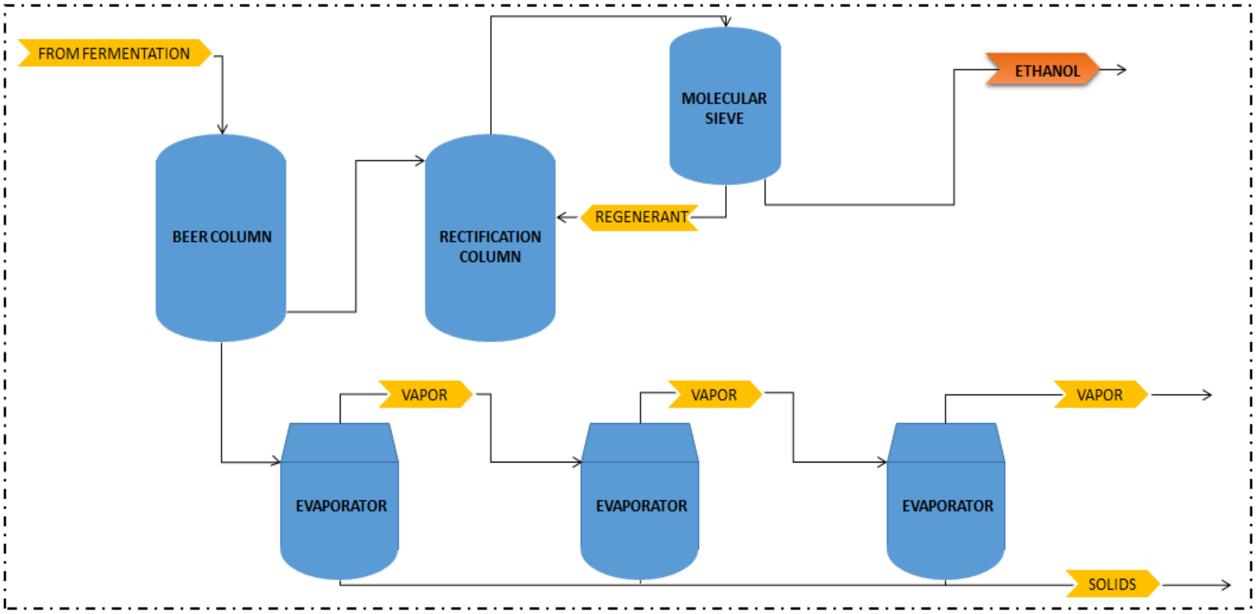


Figure 6.7. Distillation and evaporation process.

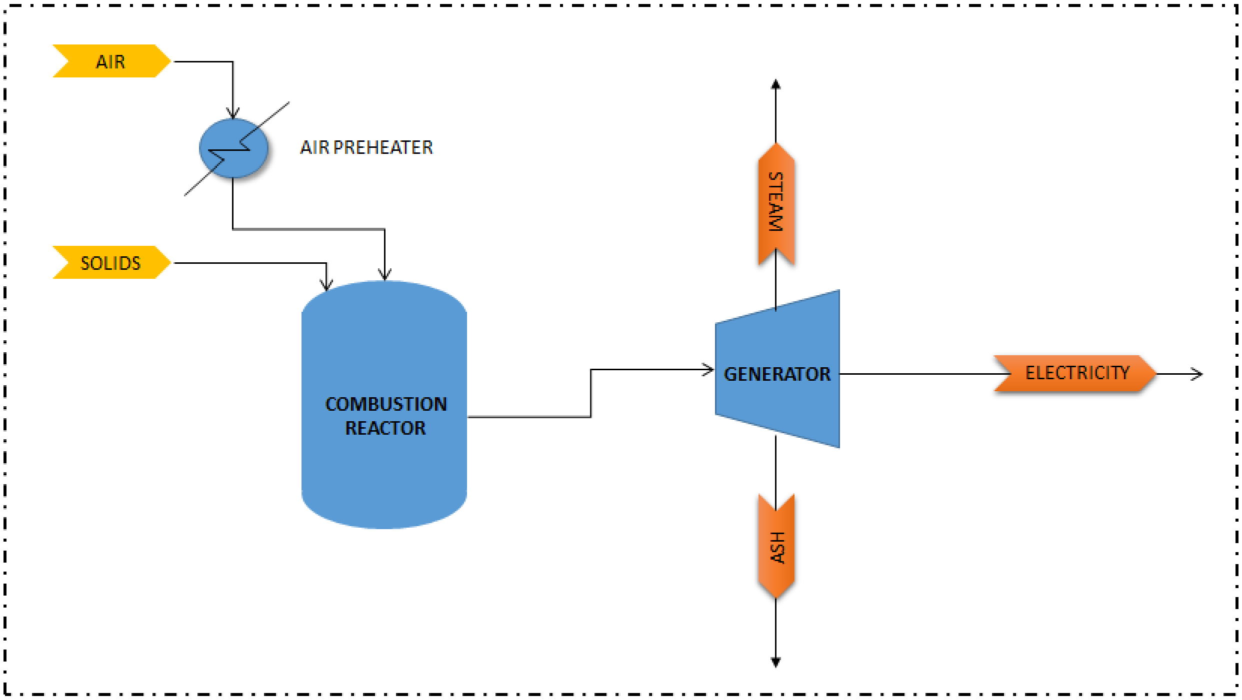


Figure 6.8. Burning process.

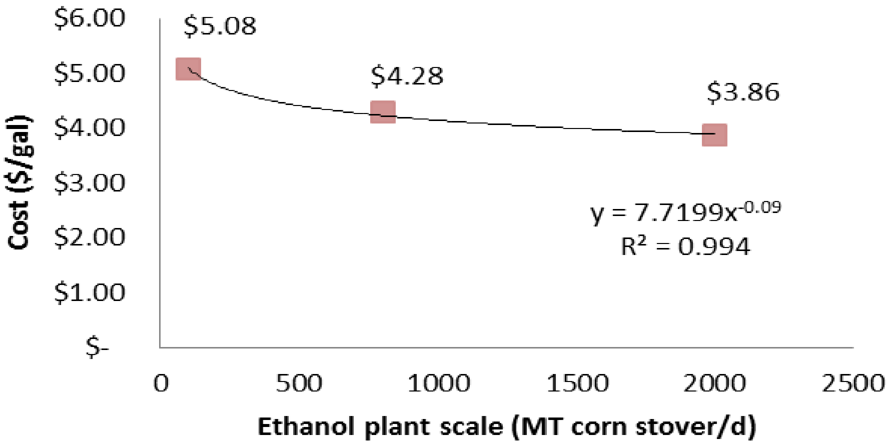


Figure 6.9. Cost per unit ethanol in different scales.

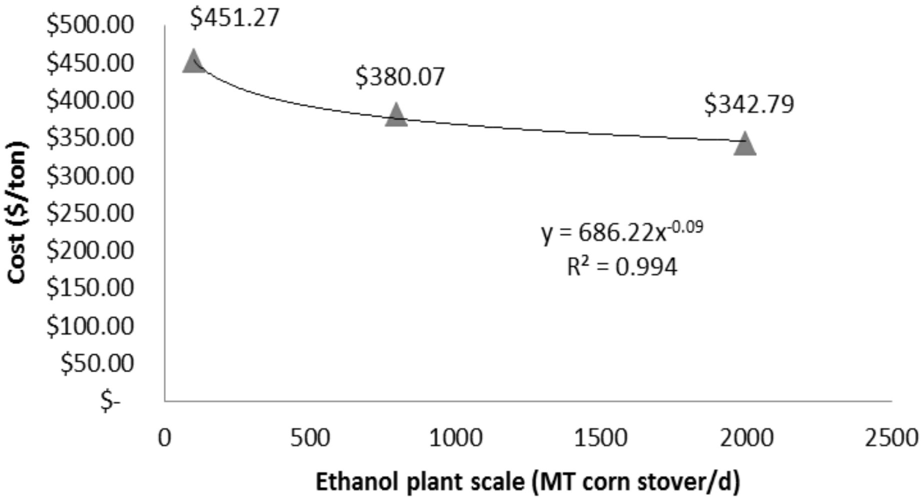


Figure 6.10. Cost per unit feedstock in different scales.

Table 6.1. General assumptions for bioethanol plant (Aden et al., 2002).

Feedstock cost is $36.25 / dry tonne.[a]
The plant is located in the center of corn farmland in IOWA.
Electricity price is $0.0062/kWh.[b]
Water price is \$0.027/ft^3.[c]
Power efficiency for equipment is assumed to be 85%.
Heat loss is not accounted for in the energy balance calculations.
Building cost is not considered in this report.
Construction time and start-up period are not considered.
Labor fee is not considered in this report.
No leakage happens during the whole process.
Insurance and tax are estimated to be 1.5% of the installed price.
Annual interest is 6.0% in US Bank.[d]
Equipment life expectancy is 10 years.
Plant life is 20 years.
Electrical wiring and controls fee is assumed to be 4% of the purchase price.
Equipment freight is assumed to be 1% of the purchase price.
Overhead fee is $0.16/ton.
Maintenance and repair cost are assumed to be 2% of the installation fee.
Enzyme price is $2 /kg.[e]

a. Available at: http://msue.anr.msu.edu/news/corn_stover_what_is_its_worth
b. Available at: http://www.cityofames.org/index.aspx?page=113
c. Available at: http://www.cityofames.org/index.aspx?page=355
d. Available at: https://www.usbank.com/calculators/jsp/MortgageCompare.jsp#3
e. Available at: http://www.alibaba.com/showroom/cellulase-enzyme.html

Table 6.2. Composition of corn stover (Aden et al., 2002).

Components	***Composition (%)***
Extractives	4.70
Glucan	37.40
Xylan	21.10
Galactan	2.00
Arabinan	2.90
Mannan	1.60
Lignin	18.00
Ash	5.20
Acetate	2.90
Protein	3.10
Soluble solids	1.10
Moisture	18.00

Table 6.3. Pretreatment conditions (Yoo et al., 2011).

Ammonia loading	0.1g NH_3/ g DM biomass
Water loading	1g /g DM biomass
Residence time	20 minutes
Solids in the ammoniation	70%
Incubation temperature	80°C
Incubation time	3.5 days (84 hrs)

Table 6.4. Saccharification conditions.

Temperature	65°C
Residence time	2 days
Cellulose loading	12 FPU/g cellulose
Number of continuous trains	1

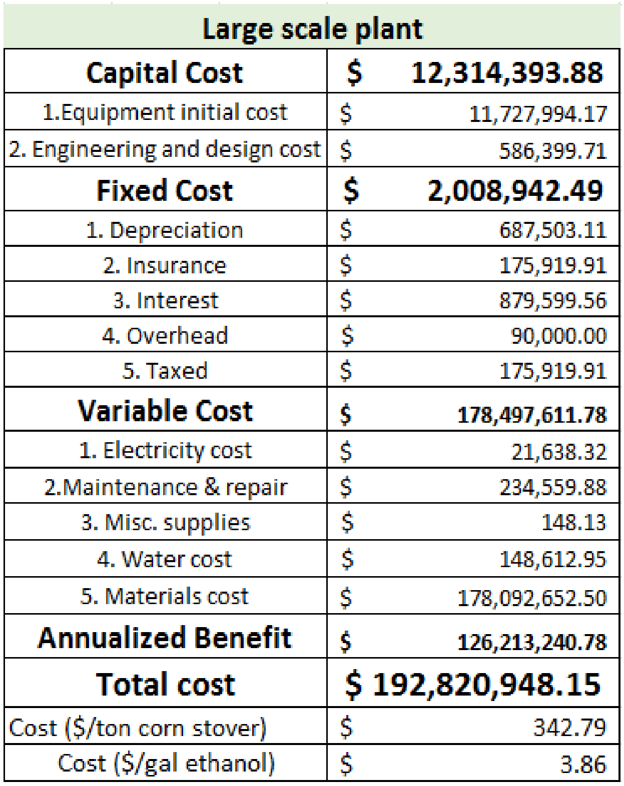
Table 6.5. Seed production conditions.

Number of trains	2
Number of fermenter	5 /train
Max fermenter volume	10,000L
Min fermenter volume	100L
Corn steep liquor level	0.5%
Diammonium phosphate level	0.67 g/L broth

Table 6.6. Fermentation conditions.

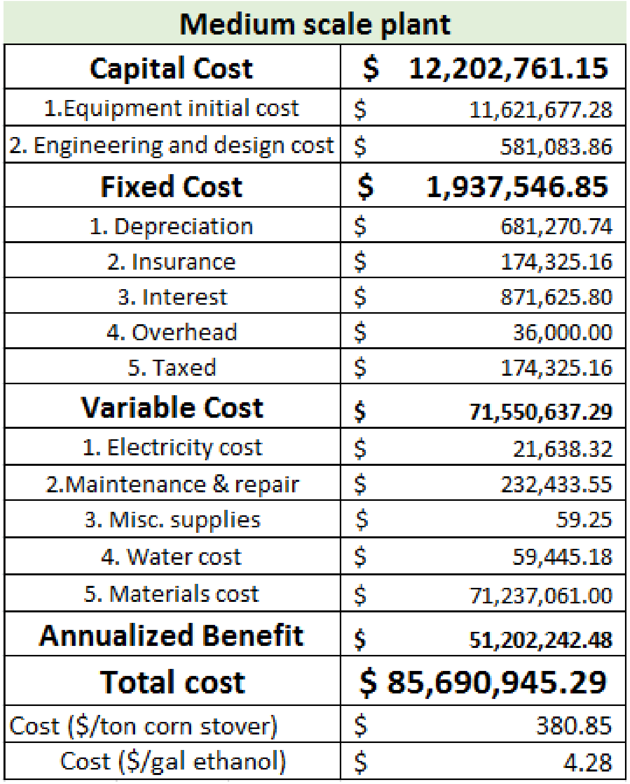
Microorganism	*Z. mobilis*
Residence time	36 hrs
Number of fermenter	5
Temperature	41°C
Corn steep liquor level	0.25%
Diammonium phosphate level	0.33 g/L broth

Table 6.7.TEA report of 50 MMgal/y ethanol production plant.



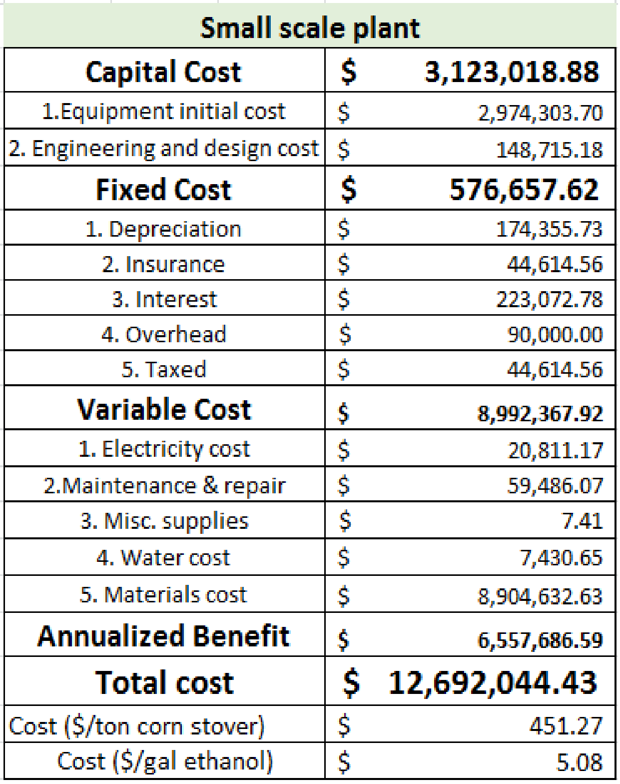
Large scale plant		
Capital Cost	**$**	**12,314,393.88**
1.Equipment initial cost	$	11,727,994.17
2. Engineering and design cost	$	586,399.71
Fixed Cost	**$**	**2,008,942.49**
1. Depreciation	$	687,503.11
2. Insurance	$	175,919.91
3. Interest	$	879,599.56
4. Overhead	$	90,000.00
5. Taxed	$	175,919.91
Variable Cost	**$**	**178,497,611.78**
1. Electricity cost	$	21,638.32
2.Maintenance & repair	$	234,559.88
3. Misc. supplies	$	148.13
4. Water cost	$	148,612.95
5. Materials cost	$	178,092,652.50
Annualized Benefit	**$**	**126,213,240.78**
Total cost	**$**	**192,820,948.15**
Cost ($/ton corn stover)	$	342.79
Cost ($/gal ethanol)	$	3.86

Table 6.8.TEA report of 20 MMGal/y ethanol production plant.



Medium scale plant		
Capital Cost	**$**	**12,202,761.15**
1.Equipment initial cost	$	11,621,677.28
2. Engineering and design cost	$	581,083.86
Fixed Cost	**$**	**1,937,546.85**
1. Depreciation	$	681,270.74
2. Insurance	$	174,325.16
3. Interest	$	871,625.80
4. Overhead	$	36,000.00
5. Taxed	$	174,325.16
Variable Cost	**$**	**71,550,637.29**
1. Electricity cost	$	21,638.32
2.Maintenance & repair	$	232,433.55
3. Misc. supplies	$	59.25
4. Water cost	$	59,445.18
5. Materials cost	$	71,237,061.00
Annualized Benefit	**$**	**51,202,242.48**
Total cost	**$**	**85,690,945.29**
Cost ($/ton corn stover)	$	380.85
Cost ($/gal ethanol)	$	4.28

Table 6.9.TEA report of 2.5 MMgal/y ethanol production plant.



Small scale plant		
Capital Cost	**$**	**3,123,018.88**
1.Equipment initial cost	$	2,974,303.70
2. Engineering and design cost	$	148,715.18
Fixed Cost	**$**	**576,657.62**
1. Depreciation	$	174,355.73
2. Insurance	$	44,614.56
3. Interest	$	223,072.78
4. Overhead	$	90,000.00
5. Taxed	$	44,614.56
Variable Cost	**$**	**8,992,367.92**
1. Electricity cost	$	20,811.17
2.Maintenance & repair	$	59,486.07
3. Misc. supplies	$	7.41
4. Water cost	$	7,430.65
5. Materials cost	$	8,904,632.63
Annualized Benefit	**$**	**6,557,686.59**
Total cost	**$**	**12,692,044.43**
Cost ($/ton corn stover)	$	451.27
Cost ($/gal ethanol)	$	5.08

CHAPTER 7. CONCLUSIONS

In this project, the low-moisture anhydrous ammonia (LMAA) pretreatment method has been explored thoroughly.

1. When LMAA pretreatment method was contacted with corn stover in a 3L scale reactor, the glucose yield were much higher compared with unpretreated corn stover; and the optimal conditions to achieve higher glucose yield were 50 wt % moisture content, 72h, 75°C, and 0.1g NH_3/g DM biomass.
2. When corn stover was pretreated with LMAA method, it could be well preserved up to 90 days without mold growth and reduction in carbohydrates. And the effect of lignin removal was more significant in sealed containers, but the sugar contents in both sealed and open conditions were nearly the same. As for ammonia loading, mass losses in 0.2- was higher than 0.1 g/g DM biomass, however, no other significant difference were found in terms of ash content, lignin content and sugar contents.
3. The TEA of cellulosic bioethanol plant based on LMAA pretreatment indicated that the larger the ethanol plant, the lower the unit cost both in $/gal of ethanol and $/ton of feedstock. However, the minimum ethanol selling cost obtained ($3.86 / gal) was still high compared with the current gasoline price.

For future work, the influence of ammonia loading in glucose yield could be investigated; and the improvement in bioethanol plant process would help to reduce the unit cost of bioethanol in order to make it more competitive to gasoline.