

TECHNICAL AND ECONOMICAL ASSESSMENT OF THERMO-MECHANICAL
EXTRUSION PRETREATMENT FOR CELLULOSIC ETHANOL PRODUCTION

by

JUHYUN YOO

B.S., Sungkyunkwan University, 2001

M.S., Kansas State University, 2007

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Grain Science and Industry
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Abstract

The Renewable Fuel Standard (RFS) in the Energy Independence and Security Act of 2007 has set the goal of 36 billion gallons of annual ethanol production in the U.S. by 2022, which is equivalent to 17.5% of the current gasoline consumption in the U.S. However, corn ethanol is expected to plateau at a level of 7.3% of current gasoline consumption on an energy-equivalent basis. Thus, it is essential to utilize a variety of substrates including lignocellulosic biomass from perennial energy crops such as switch grass, crop residues such as corn and sorghum stover, and agri-industrial co-products such as soybean hulls and wheat bran.

Lignocellulosic substrates have a recalcitrant nature and require a pretreatment step that is critical for efficient enzymatic hydrolysis of cellulose and hemicellulose to fermentable sugars. In this study, soybean hulls were used as a model substrate for cellulosic ethanol. A novel thermo-mechanical pretreatment process using extrusion was investigated and compared with two traditional pretreatment methods, dilute acid and alkali hydrolysis, with regard to structural changes in the lignocellulosic substrate, and glucose and ethanol yields. The effect of extrusion parameters, such as barrel temperature, in-barrel moisture and screw speed, on glucose yield from soybean hulls was determined. Optimum processing conditions were screw speed of 350 rpm, maximum barrel temperature of 80°C and 40% in-barrel moisture content, resulting in 95% cellulose conversion to glucose. Compared with untreated soybean hulls, the cellulose to glucose conversion of soybean hulls increased by 69.5, 128.4 and 132.2% for dilute acid, alkali and thermo-mechanical pretreatments, respectively. Glucose and other hexose sugars such as mannose and galactose were effectively fermented by *Saccharomyces cerevisiae*, resulting in ethanol yields of 13.04–15.44 g/L. Fermentation inhibitors glycerol, furfural, 5-(hydroxymethyl)-2-furaldehyde (HMF) and acetic acid were found in the thermo-mechanically pretreated substrate, ranging in concentrations from 0.072–0.431, 0–0.049, 0–0.023 and 0.181–0.278 g/L, respectively, which were lower than those reported from acid hydrolyzed substrates. The economic feasibility of commercial cellulosic ethanol production processes employing dilute acid hydrolysis and thermo-mechanical pretreatment were compared using a system dynamics modeling approach. It was concluded that low feedstock cost and high sugar conversion are important factors that can make cellulosic ethanol production commercially viable. Thermo-

mechanical pretreatment was a more promising technology as compared to dilute acid hydrolysis because of the lower capital and operating costs, and higher sugar conversion.

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Approved by:

Major Professor
Dr. Sajid Alavi

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Dedication

Dedicated to my grandmother

CHAPTER 1 - Introduction

1.1 Biorenewable energy

In 2008, petroleum provided the largest energy source (37%) in U.S. energy consumption (EIA, 2010), and transportation fuels are almost derived from petroleum (>96%) (Wyman, 2009). Whereas high energy price, increasing energy import, concerns about petroleum supplies, and greater recognition of the environmental consequences of fossil fuels have driven interest in transportation biofuels (Hill et al., 2006). To reduce petroleum dependence and ensure energy security, there is a growing interest in renewable energy including solar, hydroelectric, geothermal, biomass and wind energy. Between 2007 and 2008, renewable energy consumption increased about 10 percent and accounted for 7% (7.367 quadrillion Btu) of total energy consumption. Bioethanol derived from corn has been considered as a major alternative for transportation fuel, and the production reached a record high in August 2010 with more than 869,000 barrels per day (bpd). U.S. ethanol production used about 17% of the domestic corn crop in 2006 (Yacobucci and Schnepf, 2007), and about 99% of produced corn ethanol was consumed for E-10 (gasoline blended with 10% ethanol) (EIA, 2009). However corn ethanol solely is not enough to meet the energy registration, it would require more corn than the U.S. currently grows.

Lignocellulosic materials are the world's most widely available low-cost renewable resource to be considered for ethanol production. Such biomass sources include forest residues such as wood, agricultural residues such as sugarcane bagasse, corn cobs, corn stover, sorghum stover, wheat and rice straw, and agricultural by-products such as soybean hulls and wheat bran. Ethanol production from lignocellulosic biomass is different from corn ethanol production. Cost and energy intensive pretreatment process are required to obtain fermentable sugars because the lignocellulosic structure is very complex, highly crystalline, and resistant to enzymatic degradation in its native state.

1.2 Lignocellulosic biomass

Corn stover is the single largest source of agricultural residue in U.S., and there is no doubt that corn stover is a major potential feedstock once the second generation cellulosic biomass conversion technologies are commercialized. However the amount of

ethanol which can be produced from corn stover are only 30 billion liters (Gallagher et al. 2003), and indiscriminate removal of corn stover will increase soil erosion, reduce crop productivity, and deplete soil carbon and nutrient (Graham et al., 2007). Thus various lignocellulosic biomass are required to further reduce petroleum consumption and increase U.S. energy independence on foreign oil.

1.2.1 Soybean hulls

In USA at least 1.8 billion bushels of soybeans are processed for oil, protein, and flour, leaving available 10.8 billion pounds of soybean hulls. Soybean hulls are currently predominantly used as low-value ruminant supplements, but the demand is expected to be reduced by replacing them with distiller's dry grains with solubles (DDGS) (Corredor et al., 2008). Soybean hulls are an attractive source of fermentable sugars for bioethanol production. In addition to their high cellulosic content, soybean hulls are a by-product after grinding and crushing of soybean for oil extraction, so as a result, they do not require as extensive grinding processes as some other cellulosic biomass feedstocks prior to pretreatment. The composition of soybean hull is shown in Table 1.1.

Table 1.1 Chemical composition of soybean hulls^a

Component	% (db)
Carbohydrates	50.7
Starch	1.75 ± 0.08
Cellulose	36.43 ± 0.09
Hemicellulose	12.48 ± 0.32
Total amount lignin	18.20 ± 0.4
Crude fat	3.20 ± 0.4
Crude fiber	32.30 ± 0.32
Crude protein	14.21 ± 0.10
Pectin	6.3 ± 0.2
Ash	4.24 ± 0.03

^a Corredor et al. (2008)

1.3 Pretreatment

There have been many studies of chemical, mechanical, thermo-chemical, and biochemical pretreatments, including acid hydrolysis, alkali hydrolysis, the organosolv process, steam explosion, ammonia fiber explosion (AFEX), pyrolysis, hot water treatment, and microorganism treatment to break down the structural integrity, enhancing enzymatic reactions on cellulose (Sun and Cheng, 2002; Galbe and Zacchi, 2007). There have been some successes in terms of increased ethanol yield. However, no single method has yet been found suitable for commercial application (Saha and Cotta, 2007).

1.3.1 Acid pretreatment

The pretreatment can be done with dilute or concentrated acids. The main reaction that occurs during acid pretreatment is hydrolysis of hemicellulose. Acid pretreatment improves the efficiency of enzymatic hydrolysis by removing the solubilized hemicellulose, opening up the cellulose accessibility and also resulting in high xylose release levels (Sumphanwanich et al., 2008). Concentrated acid hydrolysis is relatively simple and is attractive for its high sugar yield, which approach 100% of theoretical hexose yield. However large volume of acid requirement and complicated acid recovery process are drawback of this process. Dilute acid hydrolysis reduced required amount of acid by increasing the reaction temperature. However oligosaccharides which released at high temperature decomposed to microbial toxins during the process, causing lower sugar yield ranged from 55-60% (Brown, 2003).

1.3.2 Alkaline pretreatment

Some bases can be used for pretreatment of lignocellulosic materials. Alalkine pretreatment is very efficient in delignification of lignocellulosic biomass while retaining the most of hemicelluloses. During alkaline pretreatment the first reactions taking place are salvation and saphonication. This causes a swollen state of the biomass and makes it more susceptible to enzymatic hydrolysis. At strong alkali concentrations dissolution, peeling of end-groups, alkaline hydrolysis and degradation and decomposition of dissolved polysaccharides can take place. Loss of polysaccharides is mainly caused by peeling and hydrolytic reactions (Fengel and Wegener, 1984). One of important aspects of alkaline

pretreatment is the change of the cellulose structure to a form that is denser and thermodynamically more stable than the native cellulose (Pettersen, 1984).

1.3.3 Thermo-mechanical extrusion pretreatment

Extrusion is defined as an operation of shaping a plastic or dough-like material by forcing it through a restriction or die (Riaz, 2000). When the material exits the die, it experiences an expansion, and some of the moisture is flashed into steam as a result of a sudden drop in pressure. In addition, high mechanical energy input will help break down the structure of the raw materials as a result of shear. As a continuous bioreactor, extrusion processing capacity is much higher than for any other existing pretreatment processes. Ning et al. (1991) employed extrusion process as a pretreatment method for corn-based cellulosic material. Dale et al. (1999) adapted ammonia fiber explosion (AFEX) to extrusion processing, increasing the effect of enzymatic hydrolysis up to 3.5 times over untreated biomass. Similarly, extruder was used as a continuous reactor for the woody biomass with ethylene glycol followed by enzymatic hydrolysis, resulting in 62.4% of sugar conversion (Lee et al., 2009). Later the same application using water instead of ethylene glycol showed 54.2% of conversion, which suggested that the effect of mechanical kneading with water on increase in cellulose surface is less than that with ethylene glycol (Lee et al., 2010). Extraction of hemicellulose from cellulosic biomass using extruder was studied (N'Diaye et al., 1996; Prat et al., 1999; N'Diaye and Rigal, 2000; Prat et al., 2002; Prat et al., 2004). Hemicellulose extraction from poplar by using twin screw extruder with addition of sodium hydroxide solution led up to 90% extraction of the initial hemicellulose (N'Diaye et al., 1996). Similarly, wheat straw was extruded with various chemical solutions, such as anthraquinone, anthrahydroquinone, hexamethylenediamine, hexamethylenetetramine, hydrogen peroxide, and ferrous ammonium sulfate by using extrusion-type mixer (Carr and Doane, 1984). Lignin and hemicellulose was effectively removed after washing, which increased enzyme accessibility to cellulose resulting in 92% cellulose to glucose conversion. The extruder has been used as an acid hydrolysis reactor. For example, countercurrent flow of acid solution and co-current flow of alkali solution were employed for hydrolysis and delignification of biomass (Kadam et al., 2009). Three different cellulosic biomass such as corn stover, wheat straw, and sawdust were conditioned to 40% moisture content and

extruded using pilot scale single screw extruder with three acid injection locations, resulting in about 33% sugar conversion. (Noon and Hochstetler, 1982). Extruder-type reactor was used for continuous dilute acid hydrolysis of municipal solid waste, and glucose yield of 50% were achieved (Green et al., 1988). Also domestic organic waste was pretreated by using extrusion process in order to expand the polymer fibers and facilitate their enzymatic hydrolysis (López-Contreras et al., 2000). Twin screw extruder was tested for the concentrated acid hydrolysis of pine softwood sawdust to convert cellulose and hemicellulose into soluble low molecular weight carbohydrate (Miller and Hester, 2007a; Miller and Hester, 2007b). At the most optimum processing condition, 44.4% of cellulose and 73.8% of hemicellulose was converted into soluble carbohydrate. Rouilly et al. (2006) used twin screw extruder to break the sugar beet cell structure, liberating the non cellulosic cell wall polysaccharides. As the severity of extrusion condition increased, micro-structure became thinner, and cells disappeared. Recently single screw extruder was used for corn stover and soybean hull pretreatment followed by enzymatic hydrolysis, resulting in 75% and 62% glucose recovery with no furfural and hydroxymethylfurfural detected (Karunanithy and Muthukumarappan, 2009; Karuppuchamy and Muthukumarappan, 2009). Recently, Lamsal et al. (2010) used extrusion process as a pretreatment to increase the efficiency of enzymatic hydrolysis of wheat bran and obtained 63 to 70% reducing sugar yield.

1.3.4 Formation of fermentation inhibitors

Formation of fermentation inhibitor depends on the type of pretreatment and condition of pretreatments. Harsh conditions such as acidic condition and high temperatures used in pretreatment create a variety of toxic compounds that inhibit the fermentation performance. The possible inhibitors are organic acid such as acetic acid, formic acid and levulinic acid, furan derivatives such as 2-furaldehyde (furfural) and 5-hydroxymethylfurfural (HMF), and phenolic compounds such as *p*-coumaric acid, ferulic acid, 4-hydroxybenzoic acid (4-HBA), vanillic acid, syringaldehyde, and vanillin (Olsson and Hahn-Hägerdal, 1996). If highly toxic hydrolysates are formed, a detoxification stage is necessary prior to fermentation. The main drawback of dilute acid hydrolysis process is degradation of the sugar in hydrolysis reactions and formation of undesirable by-product

which not only lowers the yield of sugars, but also inhibits the formation of ethanol during the fermentation. It has been known that the ammonia fiber explosion (AFEX) does not generate fermentation inhibitors (Dale et al., 1984; Mes-Hartree et al., 1988).

1.4 Economic of cellulosic ethanol

To be a viable substitute for a fossil fuel, cellulosic ethanol should meet several requirements such as environmental benefits, economic competitiveness, sufficient producibility, and positive net energy gain over fossil fuel (Hill et al., 2006). Considering that pretreatment is one of the most costly steps in cellulosic ethanol production accounting for about 33% of the total production cost, there is a window of opportunity to improve biofuel's economic competitiveness to fossil fuel by increasing the conversion efficiency of pretreatment. Many of the conversion technologies currently do not yield products that are cost-competitive with fossil-based products that dominate today's market (Brown, 2003).

Wooley et al. (1999) reported the result of process design study. In their study, predicted production cost of ethanol was \$1.44/gal with current technology, and the production cost could be lowered to \$1.16/gal by adapting an advanced technology model. In 2002, Aden et al. (2002) made change from the design which was used by Wooley et al. (1999). Aden et al. proposed a cellulosic ethanol production design using dilute acid hydrolysis followed by enzymatic hydrolysis to produce fermentable sugar from corn stover. The result presented the minimum ethanol selling price (MESP) of \$1.07/gal which was chosen by the U.S. department of Energy (DOE) as a target price for 2010. Several The model used by Aden et al. (2002) was adapted in several studies as a template and used for the economic analysis of different pretreatments technologies and various biomass (Eggeman and Elander, 2005; Foust et al., 2009; Huang et al., 2009; Kazi et al., 2010).

Since none of cellulosic biofuel technologies have been commercialized yet, uncertainties and limits are unavoidable in the economic analysis and comparison of conversion technologies. Therefore, identifying the process economic impact of different pretreatments related to productivity, capital cost, and operating cost as well as well defined assumptions are important as conducting the economic analysis of biofuel to obtain reliable and creditable cost prediction.

1.5 Scope of this study

Chapter 2 discusses the developing process of thermo-mechanical pretreatment using extrusion. In this chapter, soybean hulls were used as model system for lignocellulosic material. Several approaches including optimization of enzyme cocktail, various temperature and screw speeds, processing aids, and different in-barrel moisture contents were applied to optimize the extrusion pretreatment. Two pretreatments, dilute acid hydrolysis and alkali hydrolysis which are most studied pretreatment technologies were compared with a focus on glucose yield.

Chapter 3 focuses on systematic investigation of extrusion parameters such as in-barrel moisture content and screw speed and their effect on the efficiency of enzymatic hydrolysis and fermentation of extruded soybean hulls. The amount of fermentation inhibitors and their impact on ethanol yield are discussed as well.

Chapter 4 estimates the production cost of cellulosic ethanol employing thermo-mechanical extrusion pretreatment or dilute acid hydrolysis pretreatment. With experimental and publicly available data, plant scale cellulosic ethanol production was simulated by using system dynamic model, showing economic competitiveness of thermo-mechanical extrusion pretreatment on cellulosic ethanol production over dilute acid pretreatment.

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**CHAPTER 2 - Thermo-mechanical Extrusion Pretreatment for
Conversion of Soybean Hulls to Fermentable Sugars**

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2.1 Abstract

Thermo-mechanical pretreatment for lignocellulosic biomass was investigated using soybean hulls as the substrate. The enzyme cocktail used to hydrolyzed pretreated soybean hulls to fermentable sugar was optimized using response surface methodology (RSM). Structural changes in substrate and sugar yields from thermo-mechanical processing were compared with two traditional pretreatment methods that utilized dilute acid (1% sulfuric acid) and alkali (1% sodium hydroxide). Extrusion processing parameters (barrel temperature, in-barrel moisture, screw speed) and processing aids (starch, ethylene glycol) were studied with respect to reducing sugar and glucose yields. The conditions resulting in highest cellulose to glucose conversion (95%) were screw speed 350 rpm, maximum barrel temperature 80°C and in-barrel moisture content 40% wb. Compared with untreated soybean hulls, glucose yield from enzymatic hydrolysis of soybean hulls increased by 69.6, 128.7 and 132.2%, respectively, when pretreated with dilute acid, alkali and extrusion.

Keywords: soybean hulls; lignocellulosic; ethanol; pretreatment, extrusion

2.2 Introduction

The National Biofuels Action Plan released on October 2008 stated that expanding annual biofuel production to 36 billion gallons by 2022 would be a key component in America's movement towards clean, affordable, and secure energy solution. Corn ethanol, which is categorized as a first generation biofuels, has grown steadily over the last few years to 12.1 billion gallons in 2010 (Service, 2010). However, corn ethanol alone cannot meet the increasing demand for biofuels because of sustainability issues (Perlack et al., 2005). The Renewable Fuel Standard 2 (RFS2), which was published in March 2010, introduced a 15 billion gallon cap for corn ethanol by 2015 and set the goal of 21 billion gallons for advanced biofuels by 2022. The latter include cellulosic ethanol, biodiesel and other nonconventional biofuels.

Lignocellulosic biomass is abundantly available, and has potential for better energy balance than corn ethanol. Additionally, cellulosic ethanol provides the benefit of reducing greenhouse gas emissions by up to 86% (Wang et al., 2007). Potential feedstock includes perennial energy crops such as switch grass, forest residues such as wood, crop residues such as sugarcane bagasse, corn cobs, corn stover and wheat straw, and agricultural co-products such as soybean hulls and wheat bran. In the USA 10.8 billion pounds of soybean hulls are produced every year, as a result of processing 1.8 billion bushels of soybeans for oil, protein and flour (Corredor et al., 2008). This co-product is used as a low-value protein/fiber supplement in ruminant animal feed, but its demand is expected to reduce due to availability of distiller's dry grains with soluble (DDGS) as a substitute. Soybean hulls contain 46-51% cellulose, 16-18% hemicelluloses and 1.4-2% lignin (Corredor et al., 2008; Blasi et al., 2000), which makes them an attractive source of fermentable sugars for ethanol production. In addition to their high cellulosic content, soybean hulls do not require as extensive a grinding process prior to pretreatment as some other feedstocks.

Cellulosic ethanol production is different from corn ethanol, due to the 'recalcitrance' of the substrate. An additional pretreatment step is required because the lignocellulosic structure is very complex, highly crystalline, and resistant to enzymatic degradation in its native state. Despite the ongoing research on pretreatment technologies and other aspects of cellulosic ethanol production the current production costs are still too

high to compete in the market place (Galbe et al., 2007). In 2010, production of cellulosic ethanol in the USA, all by demonstration facilities, was expected to total 25.5 million gallons (Service, 2010). This is far less than original expectations, due to the economic slump, uncertainty among policymakers, an oversupply of first generation biofuels, transportation logistics for raw materials, and technological gaps that make a cellulosic ethanol plant unviable. To overcome at least part of these obstacles, transformational scientific breakthroughs are urgently needed in pretreatment procedures, enzyme systems for conversion of pretreated substrate to fermentable sugars and efficient microorganisms to ferment mixed sugars to ethanol (Saha and Bothast, 1999).

In order to break down the structural integrity of lignocellulosic biomass and enhance enzymatic action on cellulose, various chemical, mechanical, thermo-chemical and biochemical pretreatment have been investigated, including acid hydrolysis, alkali hydrolysis, the organosolv process, steam explosion, ammonia fiber explosion (AFEX), pyrolysis, hot water treatment and microorganism treatment (Corredor et al., 2008; Galbe et al., 2007; Galbe and Zacchi, 2007; Sun and Cheng, 2002). There have been some successes in terms of increased ethanol yield, however no single method has yet been found suitable for commercial application (Saha and Cotta, 2007). The challenge lies in increasing the efficiency of cellulose conversion to the extent that the overall ethanol production process becomes cost-effective. This article primarily focuses on pretreatment methods, and to a certain degree on optimum enzyme systems. Most of the above mentioned pretreatment technologies have inefficiencies related to batch processing, small capacity and low solids loading. A recent publication by our laboratory (Lamsal et al., 2010) described the application of a promising technology, extrusion processing, which can provide a unique and continuously stirred thermo-chemical reactor environment in combination with mechanical energy or shear. The shear forces applied by the extrusion screw serve to continuously remove the softened surface regions of the substrate and expose the interior to chemical and/or thermal action, thus improving the overall rate of cellulose conversion. The high throughputs associated with extrusion further increase its attraction as an effective pretreatment technology. In this study soybean hulls were used as the model lignocellulosic substrate, with the objectives of development of a protocol for thermo-mechanical extrusion pretreatment, optimization of the enzyme cocktail for downstream hydrolysis to sugars, and

comparison of conversion efficiency of extrusion with two traditional pretreatments, acid hydrolysis and alkali hydrolysis.

2.3 Materials and Methods

2.3.1 Lignocellulosic biomass

Soybean hulls (Archer Daniels Midland Company, Salina, KS) were ground to a particle size less than 1041 μm by using the experimental laboratory Ross Roller Mill (Ross Machine & Mill Supply Inc., Oklahoma City, OK). All experiments described in this study utilized ground soybean hulls as the substrate. The lignocellulosic composition of soybean hulls was determined using the ANKOM Fiber Analyzer (ANKOM Technology, NY), and starch content was determined using the standard glucoamylase method (AOAC 920.40; AOAC 2010).

2.3.2 Dilute acid hydrolysis and alkali hydrolysis

Ground soybean hulls were subjected to two traditional pretreatment methods, dilute acid hydrolysis and alkali hydrolysis, which were adapted from previous studies (Corredor et al., 2008; Saha and Bothast, 1999). The substrate (10%, w/w) was added to 1% (w/w) sulfuric acid or 1% (w/w) sodium hydroxide solutions (both chemicals were obtained from Sigma Aldrich, St. Louis, MO), and autoclaved (SS-325E; Tomy Tech USA, Inc., Fremont, CA) at a temperature of 121°C for 30 min. The remaining solids were neutralized and washed three times with 500 ml of deionized water at 85°C. This was followed by 10 min of centrifugation (10,000 \times g) and subsequent drying in an air oven at 45°C for 24 h.

2.3.3 Enzyme cocktail optimization

A combination of cellulase (Celluclast 1.5L), β -glucosidase (Novozyme 188) and a cell-wall degrading enzyme complex (Viscozyme® L) were used for enzymatic saccharification of pretreated soybean hull. All enzymes were obtained from Novozyme, Franklinton, NC. Cellulase was used to break down cellulose into glucose, cellobiose and higher glucose polymers. The enzyme activity of Celluclast 1.5L was 80 FPU (filter paper unit)/mL with optimal pH range 4.5 – 6.0 and the optimal temperature range of 50 – 60°C. β -glucosidase with enzyme activity of 322 CBU (cellobiase unit)/mL was used to hydrolyze

the cellobiose, which inhibits the initial stage of cellulose hydrolysis. Viscozyme® L contained a wide range carbohydrases, including arabinase, cellulase, β -glucanase, hemicellulase, and xylanase, which act on branched pectin-like substances found in plant cell walls. Its activity was 134 FBG (fungal beta-glucanase unit)/mL. Response surface methodology was used to optimize the amount of each enzyme for maximum reducing sugar yield, using soybean hulls pretreated with acid hydrolysis as the substrate. The experimental design (Table 2.1) had fifteen combinations with 3 levels (-1, 0, 1) of each enzyme and 3 replicates at the center point (Box and Draper, 1987). Results were analyzed using SAS software (v.9.2, SAS Institute, Cary, NC) at $p < 0.05$. Three dimensional plots of reducing sugar yield for various enzyme dosages were generated, model coefficients were obtained using the response surface regression (RSREG) procedure, and optimum enzyme dosage determined.

2.3.4 Extrusion pretreatment – high starch

Thermo-mechanical or extrusion pretreatment of soybean hulls, the primary focus of this study, was conducted using a laboratory-scale twin-screw extruder (Micro-18, American Leistritz, Somerville, NJ) with a six-head configuration, screw diameter of 18 mm, L/D ratio of 30:1 and 2.4 mm circular die opening. Prior to extrusion, soybean hulls were mixed with 10 and 20% corn starch (Archer Daniels Midland Company, Salina, KS) as a processing aid to facilitate the flow of soybean hulls. The soybean hull and corn starch blends were hydrated using a Hobart mixer (The Hobart Mfg. Co., Troy, OH) and equilibrated for 24 h at room temperature before extrusion in order to obtain 20 and 25% (wet basis or wb) moisture. This is also referred to as the process moisture or in-barrel moisture. The extruder screw configuration and barrel temperature profile are shown in Fig. 2.1. Three temperature profiles with maximum barrel temperatures of 80, 110 and 140°C (at the discharge end) were used. Extruder screw speed was fixed at 420 rpm. The feeder screw speed rate was set at 275 rpm, which resulted in a feed rate ranging from 1.4 to 2.0 kg/h depending on the moisture and starch content of the feed. Extruded soybean hull pellets were dried in a convection oven at 45°C for 24 h. The dried pellets, having a moisture content of 9-10% (wb), were stored at room temperature. Unlike acid or alkali hydrolyzed soybean hulls, the extruded soybean hulls did not require neutralization or

washing steps prior to enzymatic hydrolysis.

Specific mechanical energy (SME) input during the extrusion process was computed as follows:

$$SME(kJ kg^{-1}) = \frac{(\tau - \tau_0)/100 \times (N/N_r) \times P_r}{\dot{m}} \quad (1)$$

where, τ is the % torque; τ_0 is the no load % torque; N is the screw speed; N_r is the rated screw speed (500 rpm); P_r is the rated motor power (2.2 kw); and \dot{m} is the mass flow rate or feed rate (kg/s).

2.3.5 Extrusion pretreatment – low starch

Another set of thermo-mechanical pretreatments was carried out with low levels of starch addition (0 and 5%). Results from the above experiment and also a previous study (Lamsal et al., 2010) indicated that in the case of soybean hulls reduced amount or absence of the processing aid led to surging, burning of the substrate and even blocking of the die during extrusion. To avoid these problems, higher levels of in-barrel moisture were required. Soybean hulls with 5% starch were hydrated to moisture levels of 20, 25, 30, 35 and 40% (wb), whereas the moisture levels for soybean hull without starch were adjusted to 40, 45 and 50% (wb). All other process parameters were same as in the previous experiment. At the same feeder screw speed of 275 rpm, the resultant feed rate however was much lower, ranging from 0.48 to 0.84 kg/h, because of the higher moisture and lower starch level. Only one barrel temperature profile was evaluated (maximum 80°C). In an additional experiment, thermo-mechanical pretreatment of soybean hulls without starch was evaluated at 40% (wb) in-barrel moisture content and three different extruder screw speeds (280, 350 and 420 rpm). All other process conditions were same as above.

2.3.6 Extrusion pretreatment – ethylene glycol

Ethylene glycol has been used for fibrillation of lignocellulosic materials such as wood (Lee et al., 2009). One set of experiments was conducted to study its effectiveness with soybean hulls in combination with extrusion pretreatment. Solutions were prepared at ratios of ethylene glycol (Sigma-Aldrich, St. Louis, MO) to water of 1:1, 1:3 and 1:9. Pure water and ethylene glycol were used as controls. Soybean hulls were blended with solvent

at a ratio of 2:1. The same extrusion processing conditions were used as described above (Section 2.5).

2.3.7 Enzymatic hydrolysis and sugar analysis

Pretreated soybean hull (10%, w/w) were hydrolyzed by using the optimized enzyme cocktail dosage in 0.05M sodium acetate buffer at pH 5, and incubated at 50°C for 72 h. Soybean hulls without any pretreatment were also subjected to enzymatic saccharification as a control. Total reducing sugar yield was determined by using the 3,5-dinitrosalicylic acid (DNS) reagent method (Miller, 1959), while glucose concentration was analyzed by high performance liquid chromatography (HPLC) (Agilent 1100; Agilent Technologies; Santa Clara, CA) using a Phenomenex Rezex ROA organic acid column (130x7.8 mm, H+ (8%); Phenomenex, Torrance, CA). The mobile phase was 0.005N sulfuric acid at 60°C with flow rate 0.6 ml/min.

Reducing sugar and glucose yield was expressed in terms of g/g soybean hull or g/g extruded pellet. The degree of conversion to the reducing sugar ($C_{RS}\%$) was calculated based on total cellulose and hemicellulose content in soybean hulls and added starch as follows.

$$C_{RS}\% = \frac{Y_{RS}}{(C + H) \times 1.1} \times 100 \quad (2)$$

where, C = amount of cellulose in 1 g of pellet (g/g), H = hemicellulose g in 1 g of pellet (g/g), 1.1 = convert factor for polymer to monomer sugar, Y_{RS} = reducing sugar yield (g/g). The degree of conversion from cellulose to glucose conversion ($C_{CG}\%$) was determined as the ratio of the glucose obtained to the theoretical yield based on the amount of cellulose in soybean hulls.

$$C_{CG}\% = \frac{Y_G}{C \times 1.1} \times 100 \quad (3)$$

where, Y_G = glucose yield (g/g) and C = amount of cellulose in 1 g of pellet (g/g). Both reducing sugar and glucose yield were corrected for the contribution of starch, assuming 100% conversion of the latter.

2.3.8 Structural characteristic by using X-ray diffraction (XRD)

X-ray diffraction (XRD) patterns of the lignocellulosic substrate before and after pretreatment were analyzed using an X-ray diffractor (Advance D-8, Bruker AXS, Inc., Karlsruhe, Germany) set at 40 KV and 40 mA. Samples were scanned in the range of diffraction angle $2\theta = 10\text{-}40^\circ$ with a step size of 0.05° and a scan speed of $4^\circ/\text{min}$. The crystallinity index (CrI) was the percentage of crystalline material in the biomass and was used to compare the effects of various treatments on the structural modification of lignocellulosic biomass. It was defined as follows.

$$CrI = \frac{(I_{002} - I_{am})}{I_{002}} \times 100 \quad (4)$$

where I_{002} is the diffraction intensity at $2\theta = 22.6^\circ$, which represents both the crystalline and amorphous regions, and I_{am} is the intensity of diffraction at $2\theta = 18.7^\circ$, representing the amorphous material (Yoshida et al., 2008; Zhao et al., 2008)

2.4. Results and Discussion

2.4.1 Lignocellulosic structure of soybean hulls

Soybean hulls contain 35.35, 17.21 and 2.33 (dry basis or db) of cellulose, hemicellulose and lignin, respectively (Table 2.2), and 0.83% (db) starch. The remaining portion is reported to comprise of protein, fat, pectin, sugars and ash (Corredor et al., 2008; Schirmer-Michel et al., 2008). Cellulose is mainly comprised of glucose monomers, while hemicellulose is composed of mannose and xylose (Huisman et al., 1998; Stombaugh et al., 2000). Typically, cellulose exists as microfibrils, which are sheathed with hemicellulose and aligned in the direction of cell walls. Lignin forms the glue that holds the fibers together, and has a complex, cross linked structure whose building units include monomers such as guaiacyl and syringyl. Pectin performs a similar function and mainly consists of arabinose, galactose, rhamnose, fructose and uronic acid. As cellulose is the only source of glucose, the theoretical yield of latter was calculated as 0.39 g/ g soybean hull based on cellulose composition, and by multiplying by 1.1 to account for the water of hydrolysis.

2.4.2 Enzyme cocktail optimization

Response surface analysis of enzymatic saccharification data resulted in a statistical model for describing reducing sugar yield from dilute acid pretreated soybean hulls. The model ($R^2 = 0.96$) equation is described below.

$$Y_{RS} = 5.18 + 699.51X + 75.72Y + 52.02Z - 3623.71X^2 - 365.15Y^2 - 375.27Z^2 - 824.76XY - 1.69XZ + 463.82YZ \quad (5)$$

where, Y_{RS} = reducing sugar yield (g/g), X = cellulase (mL/g cellulose), Y = β -glucosidase (mL/g cellulose), and Z = cell-wall degrading enzyme complex (mL/g cellulose).

Based on the above, maximum reducing sugar release was predicted at 0.0873, 0.0806, and 0.1189 ml/g cellulose for cellulase (Celluclast 1.5L), β -glucosidase (Novozyme 188) and cell-wall degrading enzyme (Viscozyme), respectively. This combination of enzymes was used for all subsequent enzymatic saccharification experiments in this study. Cellulose concentration and its second order term, i.e. X and X^2 , had the greatest contribution to the statistical model with $p=0.0002$ and 0.0037 , respectively. Cellulase acts directly on cellulose and degrades it to lower molecular weight glucans and glucose. Previous studies on enzymatic saccharification of lignocellulosic substrates have used cellulase loadings in the wide range of 7 to 33 FPU/g substrate (Sun and Cheng, 2002). In this study, the optimum amount of 0.0873 ml/g cellulose corresponded to 6.74 FPU/g cellulose, which is a lower cellulose dosage than that used in most previous studies. The addition of β -glucosidase achieved better saccharification by hydrolyzing cellobiose, which as mentioned before was an intermediate product of cellulose hydrolysis and inhibited cellulase activity. Cellulase has been supplemented with β -glucosidase in many other studies, and the application of Novozyme 188 was recommended by the manufacturer at the ratio of 1:0.2 for Celluclast 1.5L to Novozyme 188 for initial industrial trials (Note, 1990). Substantial amount of hemicellulose was presumed to be dissolved and removed by the dilute acid pretreatment. However, the presence of cell wall degrading enzyme complex did contribute to the efficiency of enzymatic saccharification. The primary mechanism was hydrolysis of the residual hemicellulose and increase in cellulase accessibility to the cellulose (Fan et al., 1987). Cell wall degrading enzymes have been reported to act on even untreated soybean hulls, resulting in more than 97% increase in sugar yield during enzymatic hydrolysis with cellulase (Corredor et al., 2009).

It was clear from optimization results that enzyme concentration beyond a certain level led to a decrease in the amount of reducing sugar liberated. Faster sugar production with an enzyme overdose might lead to inhibition of the hydrolysis process (Drissen et al., 2009). Enzyme dosage also significantly affects the cost of the overall ethanol production process, which is another reason for optimizing the dosage.

2.4.3 Crystallinity of pretreated soybean hull

The crystallinity index (CrI) of soybean hulls prior and subsequent to various pretreatments is shown in Fig. 2.2. Increase in cellulose crystallinity after pretreatment of lignocellulosic substrates using sulfuric acid, sodium chlorite, peracetic acid or other chemicals has been observed previously (Weimer et al., 1995; Corredor et al., 2008; Yoshida et al., 2008; Zhao et al., 2008). In the current study also the average crystallinity of the substrate increased due to chemical pretreatment with acid (AH) or alkali (ALH). The two processes led to removal of hemicellulose and delignification, respectively, and thus increased the concentration of cellulose and CrI. This was confirmed by the lignocellulosic composition data (Table 2.2). After sulfuric acid and sodium hydroxide pretreatments, the concentration of cellulose increased by 75 and 89%, respectively, due to removal of other components such as hemicellulose from the substrate. This would lead to greater exposure of cellulose to enzymatic action and more efficient saccharification. It was clear that the mechanism by which acid and alkali pretreatments render the substrate more susceptible to enzymatic hydrolysis does not rely on decreased crystallinity. Lee et al. (2009) suggested that opening of the cell wall structure at the microscopic scale due to some pretreatment would be sufficient for enzymatic saccharification regardless of cellulose crystallinity. Yoshida (2008) also found that delignification increased the rate of enzymatic hydrolysis of cellulose and hemicellulose, although delignified biomass showed higher crystallinity. They concluded that lignin is the most significant factor interfering with the enzymatic hydrolysis of lignocellulosic biomass. However, lowering of cellulose crystallinity and increase in substrate surface area due to mechanical pretreatment processes such as ball milling (Ouajai and Shanks, 2006; Yoshida et al., 2008) can also lead to improved enzymatic saccharification.

In comparison, thermo-mechanical extrusion pretreatment appeared to have a

unique impact on substrate morphology. Cellulose crystallinity increased by 82% (Fig. 2.2), even though there was no significant change in lignocellulosic composition (Table 2.2). This indicated crystallization of the amorphous phase of cellulose during thermo-mechanical processing. Other researchers have observed recrystallization of cellulose in the presence of moisture and heat, during or subsequent to pretreatment processes such as steam explosion and ball milling (Tanahashi et al., 1983; Bertran and Dale, 1985; Ouajai and Shanks, 2006). Different degrees of recrystallization of ball milled hemp cellulose, resulting from various water amounts, drying rates and agitation speed, were observed by Ouajai and Shanks (2006). Bertran and Dale (1985) also reported that amorphous cellulose from cotton linter and wood was recrystallized in aqueous media subsequent to ball milling, and concluded that water promoted recrystallization. Tanahashi et al. (1983) found that the crystallinity of cellulose in wood increased after high pressure steam explosion due to the transformation of cellulose from amorphous to crystalline phase. Similarly, extrusion is a high pressure and temperature process, and it is reasonable to expect inducement of cellulose crystallization in the presence of moisture as supported by data.

2.4.4 Effect of extrusion pretreatment on saccharification

Extrusion is widely used in in the food and plastics industry and the corresponding process dynamics have been extensively studied and understood. In contrast, extrusion of lignocellulosic material is far more complex and challenging, primarily because the material does not melt even at high temperatures and consequently shows poor flow properties inside the extruder barrel and die (Prat et al., 1999). This was observed in the current study also as described below.

2.4.4.1 Barrel temperature

Extrusion experiments were conducted at maximum barrel temperatures of 80, 110 and 140°C. At higher barrel temperature (110 and 140°C), processing conditions were unstable. For example, large fluctuations in motor torque and die pressure were observed, the flow occurred in surges, and burning of soybean hulls inside the barrel and die led to frequent blockage and cessation of screw rotation. The little amount of soybean hulls that could be extruded at 110 and 140°C appeared to be darker than those extruded at 80°C, which again pointed towards burning. Reducing sugar yields are summarized in Fig. 2.3.

The contribution of starch, assuming complete hydrolysis, was expressed in darker shade for each treatment. Y_{RS} ranged from 0.46-0.61 g/g pellet, which corresponded to conversion percentage ($C_{RS}\%$) of 79-104% after subtracting the contribution of starch. Greater than 100% conversion was probably because of naturally present sugars in untreated soybean hull. Significant trends in Y_{RS} were however not observed with respect to the barrel temperature. As soybean hulls could be processed with relative ease at 80°C without any impact on sugar yield, that barrel temperature was considered to be optimum and utilized in all subsequent experiments and analyses.

2.4.4.2 Starch and in-barrel moisture

As mentioned earlier, starch was added to soybean hulls at levels of 5, 10 and 20% as a processing aid that facilitated flow during extrusion. Lignocellulosic materials such as soybean hulls are otherwise tough to extrude especially at low in-barrel moisture (<35% wb), as determined previously by our research group (Lamsal et al., 2010). Materials like wheat bran are an exception, as they naturally contain as much as 19% starch, which allows extrusion at in-barrel moistures of 20-25% wb (Lamsal et al., 2008; Lamsal et al., 2010). The starch is gelatinized 350 during extrusion and forms a viscous melt, which assists in pushing the lignocellulosic material through the extruder. Glucose yields (Y_G) from experiments at barrel temperature of 80°C and constant screw speed of 420 rpm are summarized in Fig. 2.4. As before the contribution of starch, assuming complete hydrolysis, was expressed in darker shade. Without taking the starch into account, the conversion percentage was as high as 130%. Thus it was obvious that glucose and higher molecular weight dextrans were produced from hydrolysis of starch during thermo-mechanical treatment and subsequent enzymatic saccharification. The latter effect was probably due to side activities of Viscozyme, Novozyme 188 and Celluclast 1.5L, which is typical of enzymes produced from natural strains of fungi. Overall glucose yields (Y_G) ranged from 0.32-0.38 g/g pellet, and did not exhibit any trends with respect to starch level. It should be noted that soybean hulls without any starch could also be extruded, provided in-barrel moisture was high enough (40-50% wb), resulting in Y_G comparable to treatments with 5-20% starch addition. After subtracting the contribution of starch, glucose yields ranged from 0.15-0.35 g/g and the corresponding cellulose to glucose conversion percentage

($C_{CG}\%$) ranged from 51-90%. $C_{CG}\%$ had a clear decreasing trend with respect to starch level. For example, at 20% in barrel moisture $C_{CG}\%$ decreased from 78% to 51% as starch increased from 10 to 20%. The corresponding decline was from 63% to 51% at 25% in-barrel moisture. Such a trend was also observed for reducing sugar conversion percentage ($C_{RS}\%$) (Fig. 2.3). Specific mechanical energy (SME) ranged from 738-1905 kJ/kg, and increased starch level resulted in higher SME due to greater viscosity development. However, it was clear that both Y_G and $C_{CG}\%$ were not proportional to SME. One reason could be that mechanical energy was distributed unevenly between soybean hulls and starch, in favor of the latter.

The above reasoning also appeared to be valid for the relationship between in barrel moisture, SME and glucose yield at high starch levels (10-20%). Higher motor torque was obtained as in-barrel moisture increased from 20 to 25% due to greater viscosity development, which in turn led to increase in SME. However, Y_G and $C_{CG}\%$ either decreased or remained the same. On the other hand at lower starch levels (0-5%), torque exhibited the near universal trend of increasing with lower in-barrel moisture. In the absence of adequate starch, mechanical energy was directed towards soybean hulls and exposed the interior of the substrate to thermal energy and subsequent enzymatic saccharification, thus leading to greater Y_G and $C_{CG}\%$. This demonstrated the primary mechanism involved in thermo-mechanical pretreatment of lignocellulosic substrates, with higher torque breaking down the cell wall structure more efficiently and liberating the cellulose microfibrils (Lee et al., 2009; Lamsal et al., 2010). The drop in glucose yield observed as in-barrel moisture decreased from 45 to 40% was contrary to the above mentioned trend and might be due to degradation of sugars. Factors other than those discussed above might also be at work, including interactions between starch and lignocellulosic components, competition between them for water, and also residence time of material inside the extruder barrel.

2.4.4.3 Screw speed

Fig. 2.5 summarizes glucose yields (Y_G) from thermo-mechanical pretreatment experiments at screw speeds ranging from 280 to 420 rpm, while barrel temperature and in-barrel moisture were constant at 80°C and 40% wb, respectively. Specific mechanical

energy (1478-1528 kJ/kg) increased with screw speed, which is again a universal trend observed during extrusion. Y_G ranged from 0.22 g/g pellet to 0.37g/g pellet, with the maximum value obtained at the intermediate screw speed of 350 rpm. As discussed in section 2.4.4.2, in the absence of starch mechanical energy was directed at soybean hulls, thus improving glucose yield as screw speed increased from 280 to 350 rpm. The excessive mechanical energy input at the highest screw speed of 420 rpm possibly resulted in degradation of sugars and reduction in glucose yield. The low residence time associated with high screw speeds might also be a factor, and could have led to reduced thermal exposure of the substrate during extrusion and subsequently lower saccharification.

2.4.4.4 Ethylene glycol

The use of ethylene glycol (EG) as a processing aid was based on a previous study involving thermo-mechanical pretreatment of wood (Lee et al., 2009). EG was used because of its cellulose affinity. Its addition was shown to accelerate the fibrillation process during extrusion of lignocellulosic materials because it intercalates between cellulose microfibrils, thus preventing reaggregation of liberated microfibrils due to strong hydrogen bonds. While Lee et al. (2009) observed 62.4% cellulose to glucose conversion by extruding Douglas fir with 200% EG, this approach was not successful in the current study. Soybean hulls could not be processed with either 100% EG or 1:1 solution of EG: water. Decreasing the EG: water ratio to 1:3, 1:9 and 100% water allowed extrusion of the soybean hulls and led to successively higher glucose yield at 0.19, 0.24 and 0.27 g/g pellets, respectively, which corresponded to 48-70% cellulose to glucose conversion percentage. The low amount of solvent used (50%) and differences in the extrusion process (counter rotating extrusion was used by Lee et al., 2009) might be some reasons for the poor performance of EG.

2.4.5 Comparison of thermo-mechanical pretreatment with other methods

Fig. 6 shows data for cellulose to glucose conversion percentage ($C_{CG}\%$) after enzymatic saccharification of soybean hulls subjected to different pretreatments, including acid (AH), alkali (ALH) and thermo-mechanical extrusion (EX). Enzymatic hydrolysis of untreated soybean hulls (SH) was the control, which resulted in glucose yield and conversion percentage of 0.16 g/g and 40.8%, respectively. The grinding process for

soybean hulls exposed enough of the cellulosic structure to allow substantial saccharification even without any pretreatment. All three pretreatments led to improvement in glucose yield as compared to the control (69.6, 128.7 and 132.2% increase for AH, ALH and EX, respectively) although the mechanisms by which it was brought about differed greatly.

After enzymatic hydrolysis of AH and ALH pretreated soybean hulls, glucose yield was 0.27 and 0.36 g/g, respectively, which corresponded to 69.2 and 93.3% cellulose conversion. Dilute sulfuric acid pretreatment improved the efficiency of enzymatic hydrolysis by solubilization and removal of hemicellulose and increasing cellulose accessibility (Torget et al., 1990). Alkaline pretreatment promotes cellulose conversion by delignification due to the degradation of ester bonds and cleavage of glycosidic linkages in the cell wall matrix, which lead to the alteration of the structure of lignin and reduction of the lignin-hemicellulose complex (Cheng et al., 2010). This might also be accompanied by swelling of cellulose and its partial decrystallization. The cellulose conversion from AH and ALH treatments in this study were comparable with results reported previously. Corredor et al. (2009) observed a maximum cellulose to glucose conversion of 73% for soybean hulls by applying the combination of 2% H₂SO₄ at 140°C, followed by steam explosion and enzymatic hydrolysis for 36 h. Martin et al. (2007) pretreated rice hulls with 2% of H₂SO₄ solution at a solid to liquid ratio of 1:10 at 121°C, resulting in 61.4% cellulose conversion after saccharification. Saha et al. (2005) showed that fermentable sugar yields from rice hulls varied, depending on the acid solution concentration, reaction time and temperature. Less fermentation inhibitors were observed at lower reaction temperature, and the maximum monomeric sugar yield of 60% was obtained by treatment with 1% H₂SO₄ at 121°C. Glucose yield from coastal Bermuda grass after alkali hydrolysis using 0.75% NaOH at 121°C reached up to 90% with 86% of lignin removal (Wang et al., 2010). Thermo-mechanical pretreatment (EX) was comparable or better than the more established chemical methods (AH and ALH), with regard to saccharification efficiency. Enzymatic hydrolysis of EX pretreated soybean hulls (no starch, 40% moisture, 350 rpm) led to glucose yield of 0.37 g/g and conversion of 94.8%. The primary mechanism was disruption of cell wall structure due to a combination of mechanical and thermal energy. The conversion percentage for EX was even better than that reported by some recent studies

involving extrusion. Our lab group previously reported reducing sugar yields of 60-73% and 25-36% for wheat bran and soybean hulls, respectively, after pretreatment with twin screw extrusion followed by saccharification (Lamsal et al., 2010). Karuppuchamy and Muthukumarappan (2009) and Karunanithy and Muthukumarappan (2010a; 2010b) used a single screw extruder for pretreatment of soybean hulls, corn stover, switchgrass and prairie cord grass followed by enzymatic hydrolysis, resulting in 62, 61, 45 and 66% sugar recovery, respectively. These authors also reported absence of fermentation inhibitors such as hydroxymethylfurfural, which are often produced during chemical pretreatment (Torget et al., 1990; Saha et al., 2005).

2.5 Conclusions

Thermo-mechanical extrusion was shown to be a feasible pretreatment method for lignocellulosic ethanol production. The challenge of processing lignocellulosic substrates with poor flow properties can be overcome by utilizing high in-barrel moistures and/ or processing aids such as starch. Cellulose conversion from extrusion pretreatment of soybean hulls was comparable or better than that obtained from traditional chemical pretreatments utilizing acid and alkali. The enzyme loading used in this study was much lower than in most previous studies, yet higher glucose yields were obtained. This continuous pretreatment technology shows great promise, especially since it can be scaled up easily to obtain high throughputs. Absence of fermentation inhibitors is another advantage over traditional pretreatment methods.

2.6 Acknowledgements

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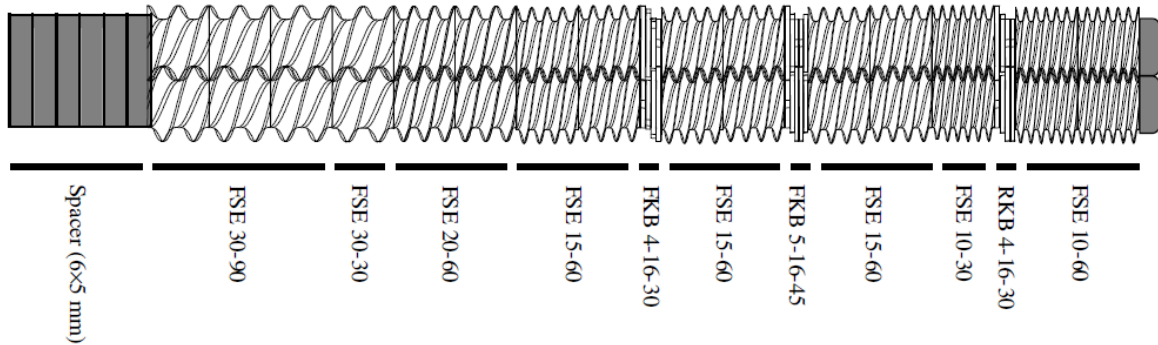
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Head No.	1	2	3	4	5	6
Barrel temperature						
Temp.(°C)	40	50	60	70	75	80
	40	50	65	80	90	110
	40	60	80	100	120	140



FSE: forward conveying screw element (all double flight, intermeshing)
 FKB: forward kneading block; RKB: reverse kneading block
 Numbers on screw elements: pitch (mm)-element length (mm)
 Numbers on kneading blocks: number of disks-total block length (mm)-staggering angle of disks

Figure 2.1 Schematic of lab-scale twin extruder screw profile and barrel temperature settings.

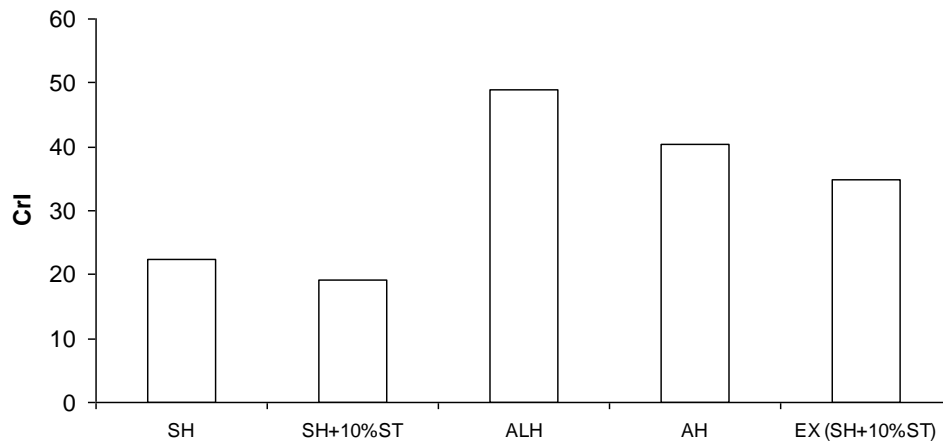


Figure 2.2 Crystallinity of soybean hulls before and after pretreatment. SH: raw soybean hull; SH+10%ST: raw soybean hull + 10% starch; ALH: alkali pretreated soybean hull; AH: dilute acid pretreated soybean hull; EX: extruded pellet from soybean hull + 10% starch.

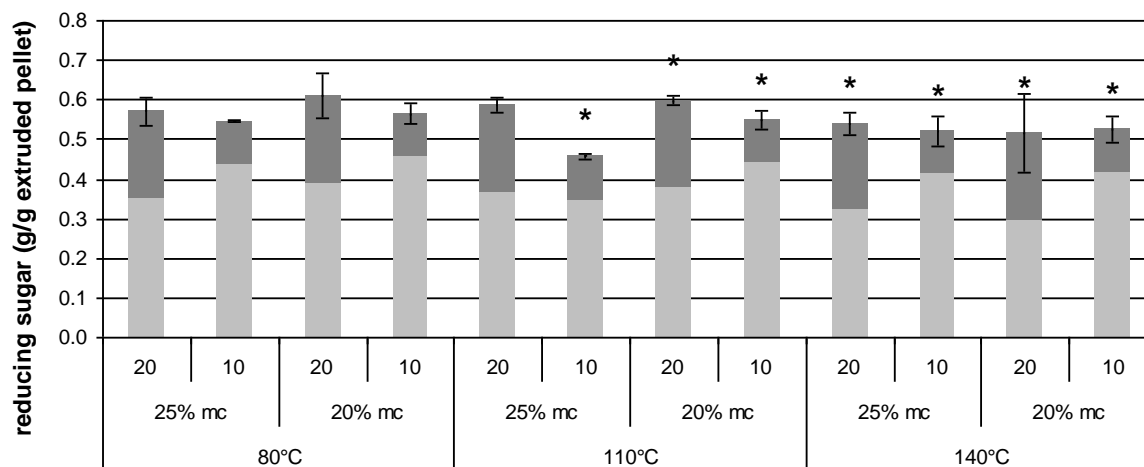


Figure 2.3 Reducing sugar amount (g/g pellet) as determined by DNS assay.

Dark shaded portion of each bar indicates starch contribution assuming 100% hydrolysis.

Error bars represent standard deviation; x-axis from top to bottom: starch addition %, in-

barrel moisture content %, maximum barrel temperature; * Surging and burning problem.

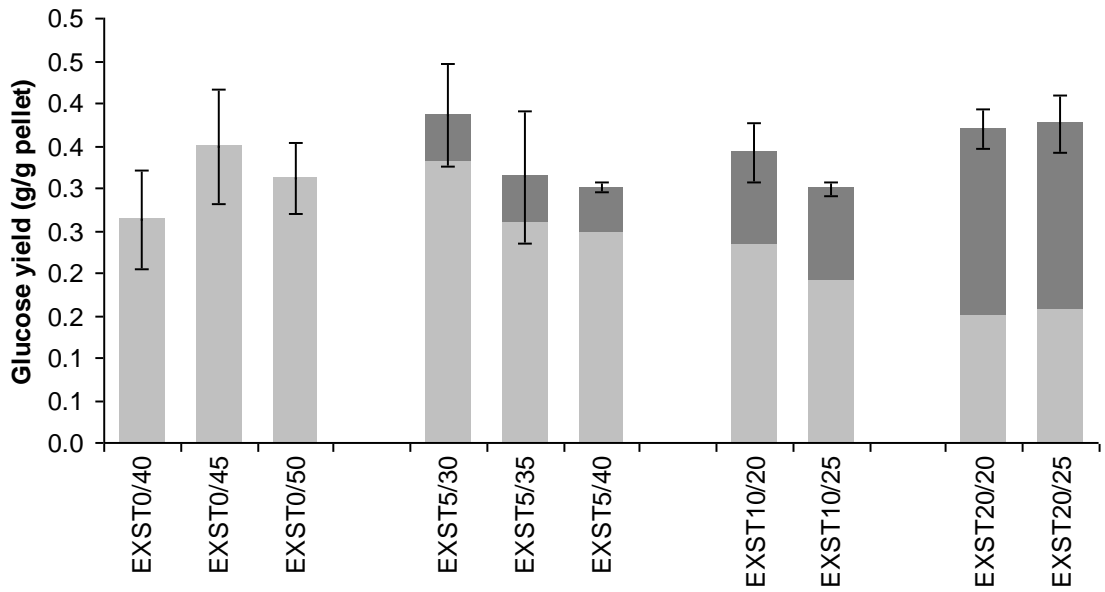


Figure 2.4 Effect of in-barrel moisture content and starch addition on glucose concentration (g/g pellet). Glucose yield was analyzed by using HPLC. Screw speed 420 rpm and barrel temperature 80°C. Dark shaded portion of each bar indicates starch contribution assuming 100% hydrolysis. Error bar represent standard deviation; EXST0/40: extrusion with 0% starch addition and 40% in-barrel moisture content).

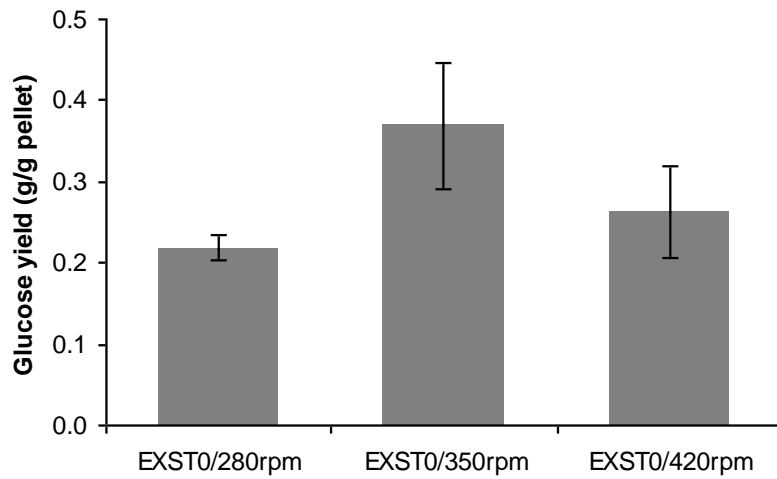


Figure 2.5 The effect of screw speed on glucose concentration (g/g pellet). Glucose yield was analyzed by using HPLC. No starch, barrel temperature 80°C and in-barrel moisture 40% wb. (X-axis:EXST0/screw speed).

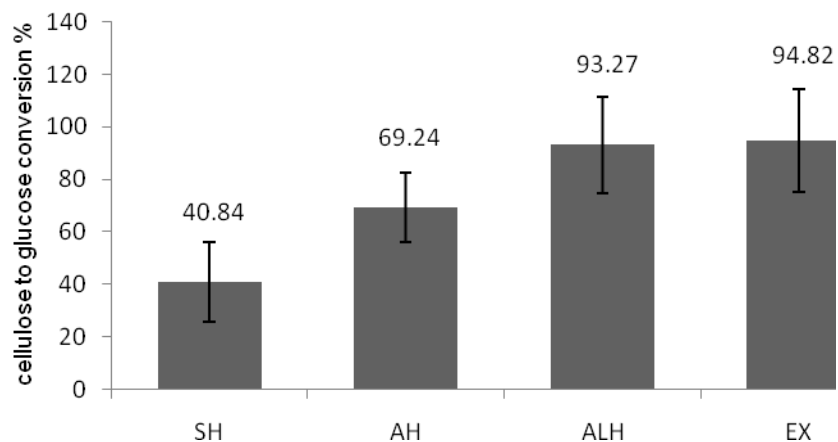


Figure 2.6 Cellulose to glucose conversion (%) after saccharification of soybean hulls subjected to different pretreatments. SH: raw soybean hull; AH: dilute acid pretreated soybean hull; ALH: alkali pretreated soybean hull; EX: extruded soybean hull (no starch, screw speed 350 rpm and in-barrel moisture content 40% wb). Error bars represent standard deviation.

Table 2.1 Experimental design for enzyme cocktail optimization (response surface methodology)

		mL/g cellulose		
		-1	0	1
X	cellulose (Celluclast 1.5L)	0.0254	0.0609	0.1016
Y	β -glucosidase (Novozyme 188)	0.0254	0.0609	0.1016
Z	cell-wall degrading enzyme (Viscozyme® L)	0.0254	0.0609	0.1016

Test no.	X	Y	Z
1	-1	-1	0
2	1	-1	0
3	-1	1	0
4	1	1	0
5	-1	0	-1
6	1	0	-1
7	-1	0	1
8	1	0	1
9	0	-1	-1
10	0	1	-1
11	0	-1	1
12	0	1	1
13	0	0	0
14	0	0	0
15	0	0	0

Table 2.2 Lignocellulosic composition (% d.b) of soybean hull (SH) before and after pretreatment^a

	Lignin	Hemicellulose	Cellulose
Raw SH	2.33±0.05	17.21±0.06	35.35±0.20
Acid hydrolyzed SH	5.22±0.11	7.80±0.14	61.79±0.43
Akali hydrolyzed SH	11.66±0.21	14.59±0.12	66.72±0.48
(SH + 0% starch) extrudate	1.52±0.01	20.42±0.22	36.88±0.05

^a ANKOM Fiber Analyzer (ANKOM Technology, NY)

**CHAPTER 3 - A Technical Assessment of Thermo-mechanical
Extrusion Processing Conditions for Ethanol Production from
Soybean Hulls**

Submitted, Applied Biochemistry and Biotechnology, December, 2010.

3.1 Abstract

Thermo-mechanical extrusion pretreatment was studied to produce cellulosic ethanol from soybean hulls with various in-barrel moisture contents and screw speeds. Extrusion pretreatment enhanced the accessibility of enzymes to cellulose and hemicellulose by breaking down the lignocellulosic structure, increasing the efficiency of enzymatic hydrolysis of soybean hull by up to 155% compared to control soybean hull. At maximum, 74% of cellulose was hydrolyzed and converted into glucose at 50% in-barrel moisture content with 350 rpm screw speed. Adding the cell wall degrading enzyme in addition to cellulase and β -glucosidase helped to improve the cellulose conversion up to 87%. Glucose and other hexose sugars such as mannose and galactose were effectively fermented by *Saccharomyce. cerevisiae* and produced ethanol from 13.04–15.44 g/L. Fermentation inhibitors, glycerol, furfural, 5-(hydroxymethyl)-2-furaldehyde (HMF), and acetic acid were found in the extruded pellet, ranging from 0.072–0.431, 0–0.049, 0–0.023 and 0.181–0.278 g/L, respectively. After enzymatic hydrolysis and fermentation, furfural and HMF were not detectable, and the effects of other fermentation inhibitors on ethanol fermentation were not detrimental.

Index entries: soybean hulls, lignocellulosic, pretreatment, extrusion, ethanol

3.2 Introduction

According to the Energy Information Administration (EIA), U.S. ethanol production reached a record high in August 2010 with more than 869,000 barrels per day (bpd). Also, ethanol demand rose to an all-time high at 911,000 bpd, which is an increase of 177,000 bpd from a year ago. Energy legislation from 2007 mandated an increasing share of advanced biofuels, including cellulosic ethanol and biodiesel, that should reach up to 21 billions of gallons by 2022. There has been steady progress in increasing the efficiency of cellulosic ethanol production. However, over the last years, the optimistic promise for cellulosic ethanol has faded, a reflection upon the economic slump, uncertainty among policymakers and an oversupply of first generation biofuels (Service, 2010). Therefore, breakthroughs in conversion science and technology will be urgently needed to make cellulosic ethanol plants economically and technically viable.

Extrusion processing has been used in the polymer industry for a long time and has become very popular in the foods and feeds industries as well. As material passes through an extruder barrel, high pressure and temperature are developed as a result of the intense mechanical shear exerted by the screw. When the material exits the die, it experiences an expansion, and some of the moisture is flashed into steam as a result of a sudden drop in pressure. In addition, high mechanical energy input will help break down the lignocellulosic structure of the raw materials. Chemical reactions and material processing normally take place in one apparatus, which results in saving equipment and energy costs (Rozeń et al., 2001). The application of extrusion technology has been very versatile, and is expanding to the treatment of biomass as a continuous reactor, but very little basic information is available (Carr and Doane, 1984). The extruder has been used as an acid hydrolysis reactor. For example, wheat straw and corn stover were extruded using a pilot scale single screw extruder with three acid injection locations, achieving about 33% cellulose to glucose conversion (Noon and Hochstetler, 1982). In another study, wheat straw was extruded with various chemical solutions, such as anthraquinone, anthrahydroquinone, hexamethylenediamine, hexamethylenetetramine, hydrogen peroxide, and ferrous ammonium sulfate by using an extrusion-type mixer (Carr and Doane, 1984). This process removed lignin and hemicellulose effectively and increased enzyme accessibility to cellulose, resulting in a maximum of 92% cellulose to glucose conversion. Kadam et al. (2009)

introduced a continuous biomass fractionation process using a twin screw extruder. The resulting cellulose rich solids were subjected to enzymatic hydrolysis, and the cellulose conversion rate reached 80%. In a recent study, a single screw extruder was used for corn stover and soybean hull pretreatment, followed by enzymatic hydrolysis, resulting in 75% and 62% glucose recovery (Karunanithy and Muthukumarappan, 2010; Karuppuchamy and Muthukumarappan, 2009). Lamsal et al. (2010) used extrusion process and particle size reduction as a pretreatment, and compared increase in efficiency of enzymatic hydrolysis of wheat bran and soybean hulls. Extrusion processing in combination of chemicals obtained the maximum of 73 and 36% reducing sugar yield from wheat bran and soybean hulls, respectively, while the particle size reduction obtained 30% reducing sugar yield from wheat bran. Further work in our lab was conducted to compare extrusion pretreatment with traditional pretreatments, such as dilute acid hydrolysis and alkaline pretreatment, and observed that cellulose conversion of soybean hulls was increased up to 95, 93 and 69% by extrusion, alkaline and dilute acid pretreatment, respectively (Yoo et al., 2010).

This article discusses the process development effort for thermo-mechanical pretreatment of lignocellulosic biomass. The study is focused on systematic investigation of extrusion parameters such as in-barrel moisture content and screw speed and their effect on the efficiency of enzymatic hydrolysis and fermentation of extruded soybean hulls. The amount of fermentation inhibitors and their impact on ethanol yield were studied as well.

3.3 Materials and Method

3.3.1 Lignocellulosic biomass

Soybean hulls (ADM, Salina, KS) were used as a model system in this study because of the benefit of soybean hulls as an agricultural co-product which is produced consistently and collected centrally. Soybean hulls were ground and screened into a particle size less than 1041 μm by using the laboratory Ross Roller Mill (Ross Machine & Mill Supply Inc., Oklahoma City, OK). The lignocellulosic composition of soybean hulls was determined using an ANKOM Fiber Analyzer (ANKOM Technology, NY), and starch content was determined following the AOAC 979.10 standard method. The soybean hulls used in this study contained about 36.2, 17.7, 2.0 and 0.83% (db) of cellulose, hemicellulose, lignin, and starch, respectively. The rest of soybean hull is comprised of crude protein, crude fat, pectin, and ash, and their contents are 14.2, 3.2, 6.3 and

4.2%, respectively. (Corredor et al., 2008; Blasi et al., 2000; Schirmer et al., 2008). The cell wall polysaccharide of soybean hull consists of 30% pectin, 50% hemicellulose, and 20% cellulose. Pectin is mainly arabinose, galactose, rhamnose, fucose, and uronic acid. Hemicellulose is composed mannose and xylose, and cellulose is mainly glucose (Huismann et al., 1998; Snyder and Kwon, 1987; Stombaugh et al., 2000). The theoretical yield of glucose from soybean hulls was calculated as 0.40 g glucose/g soybean hull, based on cellulose composition and by multiplying by 1.1 to account for the water of hydrolysis.

3.3.2 Extrusion pre-treatment with various processing conditions

A laboratory-scale twin-screw extruder (Micro-18, American Leistritz, Somerville, NJ) with a six-head configuration, screw diameter of 18 mm, L/D ratio of 30:1, and tapered die with 2.4 mm opening was used for thermo-mechanical pretreatment of soybean hulls. Soybean hulls were hydrated with water to 40, 45, or 50% moisture content and equilibrated for 24 h at room temperature before extrusion. The screw configuration and barrel temperature profile are shown in Fig. 3.1. Barrel temperature was set to increase gradually from feed inlet toward the die. A thermocouple measured the temperature at the die. In feeding, hydrated soybean hulls at 50% moisture content stacked up and blocked the feed inlet. Thus, for higher moisture content feed screw speed was lowered. Feed screw speed varied from 250 to 300 rpm to maintain a constant feed rate of 0.6 kg/h, depending on the moisture content of the hydrated soybean hulls. In addition to moisture content, three extruder screw speeds, 280, 350 and 420 rpm were compared. Therefore, 9 treatments were tested following a factorial design. Soybean hull pellets extruded under 9 different conditions were designated by in-barrel moisture content/screw speed (rpm) (e.g. 35/420: 35% in-barrel moisture content with screw speed of 420 rpm) in this paper.

Specific mechanical energy (SME) was computed for the extrusion process as follows:

$$SME(kJ kg^{-1}) = \frac{\left(\frac{\tau - \tau_0}{100}\right) \times \frac{N}{N_{rated}} \times P_{rated}}{\dot{m}} \quad (1)$$

where, τ is the % torque; τ_0 is the no load torque; N is the screw speed; N_{rated} is the rated screw speed, 500 rpm; P_{rated} is the rated motor power, 2.2 kJ s⁻¹; \dot{m} is the mass flow rate, kg/s.

3.3.3 Enzymatic hydrolysis

Extruded soybean hull (10%, w/w) and two enzymes, cellulase (Celluclast 1.5L; Novozyme, Franklinton, NC) and β -glucosidase (Novozyme 188; Novozyme, Franklinton, NC) were added into pH 4.8, 0.05M citrate buffer solution, and incubated at 50°C for 72 h in 150 rpm shaker (Innova 44; New Brunswick, Edison, NJ). The cellulase dose was 25FPU/g cellulose, and β -glucosidase was used at a ratio of 1:0.2 of Celluclast 1.5L to Novozyme 188 (Beck et al., 1990).

A set of experiments was also carried out to see the effect of addition of cell wall degrading enzyme on improving the efficiency of enzymatic hydrolysis. Three enzymes consisting of cellulase, β -glucosidase and cell wall degrading enzyme complex (Viscozyme (complex of arabanase, cellulase, β -glucanase, hemicellulase, and xylanase); Novozyme, Franklinton, NC) were added to soybean hulls pretreated in the extruder with 40, 45, and 50% moisture content at 350 rpm. The cell wall degrading enzyme complex was used at a 1:1 ratio with cellulase. Untreated soybean hulls were also subjected to the enzymatic hydrolysis, and the resulting glucose yield value was used as a control. The hydrolysate obtained was centrifuged at 10,000 \times g for 10 min. Supernatant was collected for further analysis and fermentation. Glucose concentration was determined by using high performance liquid chromatography (HPLC; Agilent 1100; Agilent Technology, Santa Clara, CA) equipped with a refractive index detector (Agilent 1200; Agilent Technology, Santa Clara, CA) and Rezex ROA organic acid column (130 \times 7.8 mm; Phenomenex Inc., Torrance, CA). An aqueous mobile phase (0.005N sulfuric acid in water) was used, with a flow rate of 0.6 ml/min at 60°C. Cellulose to glucose conversion % was determined as ratio of the glucose obtained by HPLC analysis to the theoretical yield based on the amount of cellulose in the soybean hulls.

$$\begin{aligned} & \text{Glucose conversion efficiency (\%)} \\ & = \frac{\text{Released glucose (g) from given amount of treated biomass (g)}}{\text{Theoretical yield (g) in same amount of untreated biomass (g)}} \times 100 \quad (2) \end{aligned}$$

3.3.4 Fermentation inhibitors

The amount of fermentation inhibitors present in extruded soybean hull pellets, hydrolysate and fermented broth were monitored by using HPLC as described above. The inhibitors studied were carbohydrate degradation products such as furfural and 5-

(hydroxymethyl)-2-furaldehyde (HMF), as well as other organic compounds such as acetic acid, lactic acid and glycerol. To measure the amount of inhibitors in the extruded pellets, ground extrudates were suspended at 10% w/w in distilled water and stirred for 20 min. Samples were centrifuged for 10 min at 10,000 ×g, and supernatants were collected. Hydrolysate and fermented broth were analyzed as they were obtained from the centrifugation.

3.3.5 Ethanol fermentation

Fermentation was carried out subsequent to the hydrolysis. One g of active dry yeast (*Saccharomyces Cerevisiae*; Red Star Ethanol Red®, Lesaffre Group, Milwaukee, WI), was dispersed in 20 mL of the nutrient media containing glucose (10 g/L), peptone (5 g/L), and yeast extract (5 g/L) and inoculated for 24h at 32°C in a shaking incubator at shaking speed of 100 rpm. Ten mL yeast in the nutrient medium were added to the hydrolysate. Ethanol fermentation was carried out at 32°C in a shaking incubator at a shaking speed of 100 rpm for 48 h. Fermented broth obtained was centrifuged at 10,000 ×g for 10 min. Supernatant was collected to determine ethanol concentration and yield.

Ethanol concentration in the fermentation broth was used to define the ethanol yield. Ethanol concentration was determined by using HPLC (Agilent 1100; Agilent Technology, Santa Clara, CA) equipped with a refractive index detector (Agilent 1200; Agilent Technology, Santa Clara, CA) and Rezex ROA organic acid column (130x7.8 mm; Phenomenex Inc., Torrance, CA). An aqueous mobile phase (0.005N sulfuric acid in water) was used with a flow rate of 0.6 ml/min. Column temperature was set 60°C. Specific gravity of ethanol, 1.25 g/L was used for unit conversion of ethanol concentration. The ethanol yield ($Y_{P/S}$) is presented as following

$$Y_{P/S} = \frac{\text{Amount of ethanol formed (g)}}{\text{Amount of sugar consumed (g)}} \quad (3)$$

where, P/S stands for product/substrate.

Fermentability of sugars including glucose, galactose, mannose, xylose, arabinose, and cellobiose by the Red Star Ethanol Red® yeast preparation was investigated by using pure sugar under the same fermentation conditions described above. All results were analyzed by PROC GLM using SAS software (v.9.2, SAS Institute, Cary, NC) at $P < 0.05$.

3.4 Results and Discussion

3.4.1 Efficiency of enzymatic hydrolysis

The specific mechanical energy (SME) for the three levels of in-barrel moisture content and screw speed ranged from 566 to 2615 kJ/kg (Fig. 3.2). Comparing the treatments of 40% and 45% in-barrel moisture content, the SME for the 45% in-barrel moisture contents were lower than those for the 40% in-barrel moisture contents for each screw speed, and SME increased as screw speed increase. However, as mentioned in the materials and methods section, high moisture content in the feed, i.e. 50%, caused problems at the feed inlet. So as a result, the feed rate was not constant throughout the process. This problem resulted in a low mass flow rate and consequently a higher SME. In-barrel moisture content also affects motor torque. Motor torque was slightly changed. Higher torque was observed at lower in-barrel moisture contents, and the overall range was from 0.18 to 0.25. Lee et al. (2009) emphasized the importance of motor torque for the higher cellulose to glucose conversion by extrusion processing when followed by enzymatic hydrolysis. However, it appeared that although in-barrel moisture content is a significant factor affecting motor torque, higher motor torque was not a factor necessary to produce higher glucose yield. Previous studies (Lamsal et al., 2010) from our laboratory observed that SME input during extrusion pretreatment of wheat bran ranged from 800 to 2300 kJ/kg. In that study, the highest reducing sugar yield was obtained in the range of 1500 kJ/kg with a combination of mild temperatures at 110°C, with a longer residence time. It was clear that the relationship between sugar yield and SME or other processing conditions was not linear, and the interaction of such conditions was more important. This observation was confirmed again through this study.

Glucose yield ranged from 0.25 to 0.30 g/g pellet, which corresponded to 63 to 74% conversion, and the highest glucose yield was obtained from the soybean hull pellets extruded at 50/350 (Fig. 3.3). Extrusion pretreatment increased the efficiency of enzymatic hydrolysis of soybean hull by up to 155%, compared to the control non-extruded soybean hulls. Our previous study reported that dilute acid and alkaline pretreated soybean hull increased glucose yield by 69 and 125% as compared to untreated soybean hulls, respectively (Yoo et al., 2010).

In-barrel moisture content, screw speed, and their interactions were the significant ($p < 0.05$) factors affecting the glucose yield. Higher glucose yield resulted from lower screw speeds (280 and 350 rpm) as compared with the higher screw speed at 420 rpm. Lower screw speeds increased the residence time of biomass inside the extruder during pretreatment.

Increasing residence time involves the higher possibility of more time for physical and chemical reactions, i.e., grinding action, reducing the particle size, opening up the cellulosic structure, increasing the exposed surface area and releasing the shorter chains of cellulose and hemicellulose (Lue et al., 1991), and which lead to more efficient enzymatic hydrolysis and higher glucose yields.

Also, higher in-barrel moisture treatments of 45 and 50% resulted in higher glucose yields as compared with the 40% in-barrel moisture treatment. Water has been used elsewhere to soften lignocellulosic material prior to or during pretreatments. For example, during the wet ball milling process for corn stover, the solid/liquid ratio was a factor key to the process efficiency because water controlled the viscosity of material, and also was required to soften the cellulose so that microfibril bonds could easily be broken by shear force (Lin et al., 2010). Lin et al. (2010) obtained a maximum glucose yield with a solid/liquid ratio at 1:10 from corn stover ball milling, and described that excess liquid formed a thin slurry, reducing the effectiveness of milling. On the other hand, not enough liquid lead to a highly viscous slurry, restricting the ball's movement. The importance of water in the treatment of cellulosic material can also be found in the thermo-mechanical pulping (Brucato, 1981). In the thermo-mechanical pulping process wood chips are preheated with steam at an elevated temperature and pressure, and are then defibered in a disc refiner. During preheating, lignin is softened to a greater extent and cellulosic fibers are pulled apart and separated more easily than in the conventional mechanical pulping process.

3.4.2 Effect of two vs. three enzymes on enzymatic hydrolysis

The yields of glucose obtained after enzymatic hydrolysis using two enzymes consisting of cellulolase and β -glucosidase and three enzymes consisting of cellulase, β -glucosidase and cell wall degrading enzyme complex were compared (Fig. 3.4). Glucose yield was 0.26, 0.28 and 0.29 g/g pellet for the treatments of 40/350, 45/350 and 50/350, respectively, when only two enzymes, cellulase and β -glucosidase, were applied. By adding the cell wall degrading enzyme in addition to cellulase and β -glucosidase, glucose yield was increased by 16, 24 and 12%, respectively for the treatments of 40/350, 45/350 and 50/350 samples, and the maximum glucose conversion reached 87% at 45/350.

Cell wall degrading enzyme has been used to reduce the viscosity by breaking down branched pectin-like substances found in plant cell walls, increasing the extraction yield of

intercellular constituents (Guan and Yao, 2008). Unlike other chemical or physical pretreatments, such as alkali and dilute acid pretreatment which partially remove hemicellulose, extrusion pretreatment does not remove hemicellulose components from the biomass. Carr and Doane (1984) used an extrusion type mixer to extrude wheat straw and measured the change in composition of cellulose, pentosan, and lignin. In that study, 4% of the lignin was removed, but the amount of pentosan remained the same after 5 washing times. On the other hand, over 60% of the lignin and 40% of the pentosan were removed when an alkaline solution was fed into the extruder instead of water. Although extrusion processing opened and disrupted the cellulosic structure, the hemicellulose remaining in the matrix interfered with cellulase and cellobioase access to the substrate. Soybean hulls used in this study contained 17.7% of hemicellulose. Therefore, the cell wall degrading enzyme complex consisting of arabanase, cellulase, β -glucanase, hemicellulase, and xylanase hydrolyzed cellulose as well as hemicellulose, and helped the other two enzymes gain access to the substrates, leading to higher efficiency of overall enzymatic hydrolysis.

3.4.3 Efficiency of fermentation and ethanol yield

The fermentability tests on pure glucose, mannose and galactose showed that all of these sugars were used up by the Red Star Ethanol Red® yeast strain during fermentation to produce ethanol and other fermentation by-products such as glycerol, lactic acid and acetic acid, and there was no residual sugar peak found by HPLC after fermentation. Ethanol yields ($Y_{P/S}$) were 0.46, 0.43 and 0.41 for glucose, mannose, and galactose, respectively. The amounts of arabinose, xylose and cellobiose were slightly reduced after fermentation but did not produce ethanol, which means that they were not fermented to alcohol but utilized to produce other by-products or energy.

The chromatograms obtained by HPLC analysis of the hydrolysates and the fermented broths are shown in Fig. 3.5. A glucose peak appeared at 9.790 min from the hydrolysate (Fig. 3.5; top), but it was not found in the fermented broth (Fig. 3.5; bottom). An ethanol peak appeared at 21.991 min in the fermented broth. Standard peaks for xylose, galactose, and mannose appeared at 10.440, 10.395 and 10.354 min, respectively. Although these three sugars were present in hydrolysates of extruded soybean hull pellets, their retention times were too close together to be resolved into individual sugar peaks. The third highest peak appeared at

10.388 min on the hydrolysate chromatogram for hydrolysate, and this peak was a combination of three sugars, xylose, galactose and mannose. After fermentation, mannose and galactose were utilized to produce ethanol, and only the xylose remained. Its peak showed up at 10.404 min, which corresponded exactly to the standard peak for xylose. The same results were observed for all soybean hull extruded pellets.

Fermentation of galactose and mannose by yeast was evident from the ethanol yield ($Y_{P/S}$) data as shown in Fig. 3.6. Ethanol yields shown in Fig. 3.6 were calculated, based on the glucose amount in the hydrolysate and did not count the amount of galactose and mannose that may have been converted. Thus, except for the 50/420 sample, ethanol yields for all treatments were over the theoretical maximum yield, i.e. 0.51. They ranged from 0.54 to 0.63. Treatments which showed higher glucose yields from enzymatic hydrolysis led to more ethanol production (Fig. 3.7). Generally, more ethanol was produced from extruded pellets treated with the lower screw speeds of 280 and 350 rpm and higher in-barrel moisture contents (45 and 50%). Ethanol produced for all 9 treatments ranged from 13.04 to 15.44 g/L, and the maximum ethanol production resulted when soybean hulls was extruded at 280 rpm with 45% in-barrel moisture content.

3.4.4 Fermentation inhibitors

The inhibition mechanisms of inhibitors to ethanol fermentation by *S. cerevisiae* are classified into three categories, including chemical interference with cell maintenance functions, direct inhibition of ethanol pathway and osmotic pressure effect of cells (Luo et al., 2002). The inhibition mechanisms of acetic acid, glycerol and lactic acid are belonged to the chemical interference (Maiorella et al., 1983). Furfural and 5-(hydroxymethyl)-2-furaldehyde (HMF) inhibit the glycolysis pathways of many organisms as well as their protein and RNA synthesis (Luo et al., 2002). The amounts of acetic acid, glycerol, lactic acid, furfural, and HMF present in the pretreated soybean pellet, hydrolysate, and fermented broths are shown in Table 3.1. Extrusion pretreatment has many similarities to steam explosion pretreatment, as both processes involve heat (thermo), shear force (mechano) and hydrolysis of glycosidic bonds (chemical) (Chornet and Overend, 1988). Martin et al. (2008) compared inhibitor formation of wet oxidation and steam explosion samples and observed lower amounts of inhibitors such as formic acid, phenolic compounds and acetic acid, and higher amounts of HMF and furfural in the liquid

fraction from the steam explosion process, as compared with that from the wet oxidation process. HMF and furfural are formed under thermal and acidic conditions as a result of dehydration of hexoses and pentoses. High temperature and water acting as an acid during steam explosion (Kumar et al., 2009), could be the reason for the higher formation of HMF and furfural. Steam explosion shows a limited extent of lignin removal (Kumar et al., 2009), resulting in lower amounts of phenolic compounds such as ferulic acid, vanillic acid, syringaldehyde, and vanillin in the liquid fraction.

The extruder barrel temperature used in the extrusion pretreatment was designed to gradually increase from feed inlet toward the discharging, ranging from 40 to 80°C, and the temperature read at the die ranged from 89 to 100°C. Temperatures used in extrusion processing are generally lower than those used in steam explosion pre-treatment, which typically range from 120 to 250°C. Also, no acidic solvents were involved that would disrupt the microstructure of the lignocellulosic materials used in this study. Therefore, lower amounts of HMF and furfural were formed, which are known for their significant inhibitory effect on fermentation, were found (Table 3.1). Furfural was found from most of the pretreated soybean hull pellets except for the 50/350 sample, ranging from 0.001 to 0.049 g/L. HMF was found only from three pretreatment conditions, 45/420, 50/280, and 50/350, and it ranged from 0.001 to 0.023 g/L. Banerjee et al. (Banerjee et al., 1981) found that furfural inhibited ethanol production by *S. cerevisiae* at as low as 0.5 g/L and complete inhibition occurred at 4 g/L. HMF inhibits the organisms in the same manner, but the threshold concentration is slightly higher. In our study, the amount of furfural and HMF was much less than the critical inhibitory concentration, and they were not even detectable in the hydrolysate or the fermented broth.

Karunanithy and Muthukumarappan (2010) and Karuppuchamy and Muthukumarappan (2009) used extrusion pre-treatment on corn stover and soybean hull at 25 to 125°C, with screw speeds varying from 25 to 125 rpm, and found acetic acid resulted from most of the treatments. However furfural and HMF were not found from any of the pretreatment condition. According to Shirmer-Michel et al. (2008), 0.07 g/L of furfural and 1.10 g/L of acetic acid were present in the hydrolysate of soybean hulls pretreated by dilute acid hydrolysis, and their inhibitory effect were neglect able.

Different cellulosic biomasses and pretreatment processes generate different combinations of toxic compounds. Different yeast strains show different levels of tolerance to

toxic compounds, and fermentation conditions can change their level of resistance. Acetic acid is formed by the hydrolysis of acetyl groups in hemicellulose, and usually found at the highest concentration in the hydrolysate (Martin et al., 2007). Its inhibitory effect is pH dependent (Olsson and Hanh-Hägerdal, 1996). It has been reported that acetic acid concentration increased during enzymatic hydrolysis, and a further increase is observed during fermentation (Olsson and Hanh-Hägerdal, 1996; Thomsen et al., 2009). Increases in acetic acid concentrations during processing were observed during hydrolysis and fermentation. The concentrations were 0.181 – 0.278 g/L in the extruded pellet, 1.119 – 1.417 g/L in the hydrolysates, and 2.683 – 3.332 g/L in the fermented broths. Phowchinda et al. (Phowchinda et al., 1995) reported a 74% inhibitory effect on the fermentation by *S. cerevisiae* by 6.0 g/L acetic acid. Pampulha and Loureiro (1989) showed 50% inhibition by 1.4 and 4.3 g/L of acetic acid at pH 4.5 and pH 5.5, respectively. Considering the high ethanol yield in this study, the acetic acid generated did not seem to significantly influence the ethanol fermentation by *S. cerevisiae*. In comparison with acetic acid, high concentrations of glycerol and lactic acid were found in the fermented broths. However, similar to acetic acid, the inhibitory effect of these two compounds on fermentation was not significant, did not prevent high ethanol yields.

In cellulosic ethanol production, various pretreatment processes, in addition to generating suitable substrates for conversion to biofuel, typically produce a range of compounds that inhibit the organisms used for fermentation (Pienkos and Zhang, 2009). Therefore, detoxification processes have been employed prior to fermentation to improve fermentability, which increases the cost of the overall process. In our previous study (Lamsal et al., 2010), when wheat bran and soybean hull were extruded at 110°C, the washing of pretreated samples prior to enzymatic hydrolysis led to substantial improvement in the reducing sugar yield. However, as shown in this study, extrusion pretreatment conducted at 80°C resulted in relatively small amounts of furfural, HMF, acetic acid, and glycerol during pretreatment. Although fermentation inhibitors increased during enzymatic hydrolysis and fermentation, the inhibitory effect was not sufficient to prevent high yields of ethanol. Therefore, detoxification process was not a necessary step for the extrusion pretreatment of soybean hulls. This will contribute to lowering the production costs.

3.5 Conclusions

To ensure a sustainable biofuel supply for cellulosic ethanol production, it is necessary to develop an efficient pretreatment technology which produces high yields of fermentable sugars and at the same time avoids producing fermentation inhibitors. Thermo-mechanical extrusion pretreatments effectively disrupt the cellulosic structure of soybean hulls, enhancing the enzymatic hydrolysis. Conversion of cellulose to glucose was obtained to 87% at 45% in-barrel moisture content with 350 rpm screw speed. Fermentation inhibitors present in the extruded pellets, hydrolysates, and fermented broths were under the detrimental concentrations for efficient fermentation, and the maximum ethanol yield reached up to 15.4 g/L. Unlike other chemical pretreatments, extrusion pretreatment does not required detoxification and neutralization steps prior to the fermentation, which makes thermo-mechanical extrusion pretreatment environmentally friendly and more cost and time effective.

Although extrusion processing is technically feasible and shows high yields of cellulosic ethanol from soybean hulls, certain technical and economic aspects still require refinement. This study also needs to be extended to other varieties of biomass. For economic analysis, a systematic dynamic approach needs to be employed to solve the uncertainties in economic aspects of extrusion pretreatment.

3.6 Acknowledgements

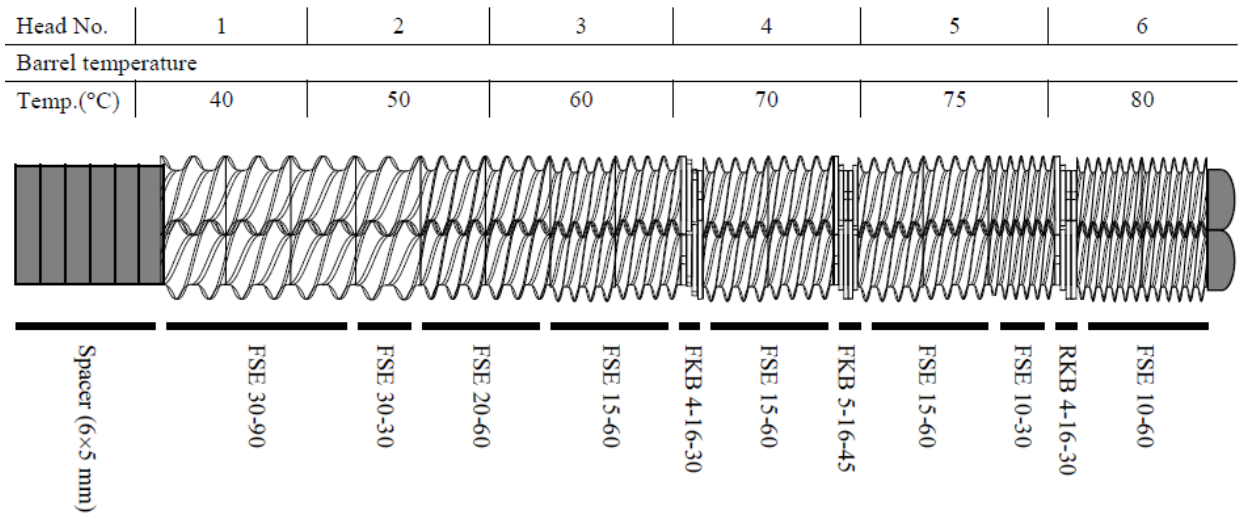
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FSE: forward conveying screw element (all double flight, intermeshing)

FKB: forward kneading block; RKB: reverse kneading block

Numbers on screw elements: pitch (mm)-element length (mm)

Numbers on kneading blocks: number of disks-total block length (mm)-staggering angle of disks

Figure 3.1 Schematic of pilot-scale twin screw extruder profile and barrel set temperatures.

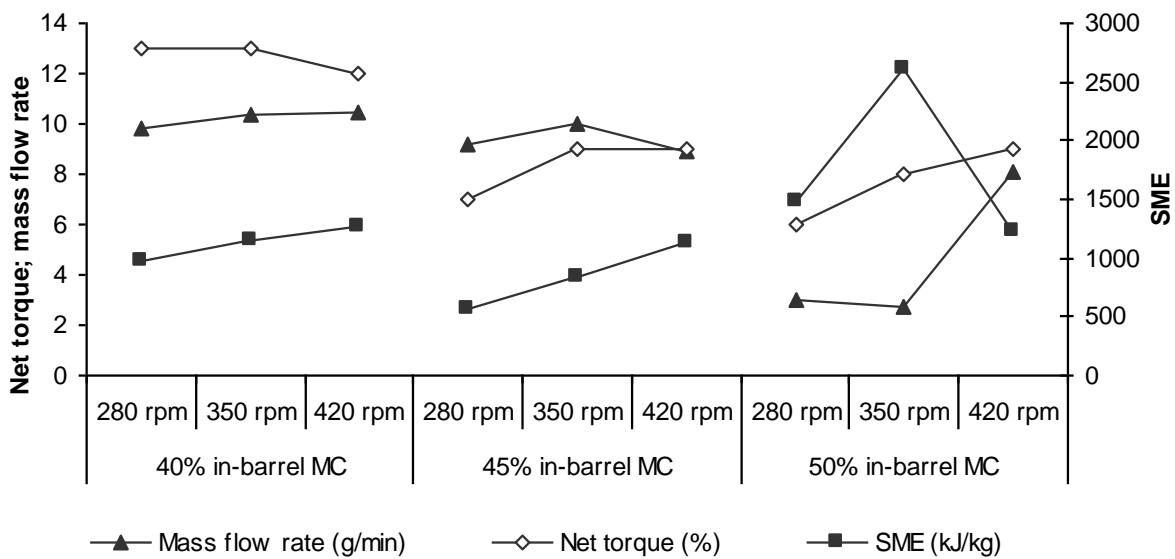


Figure 3.2 Specific mechanical energy for three levels of in-barrel moisture contents and screw speeds.

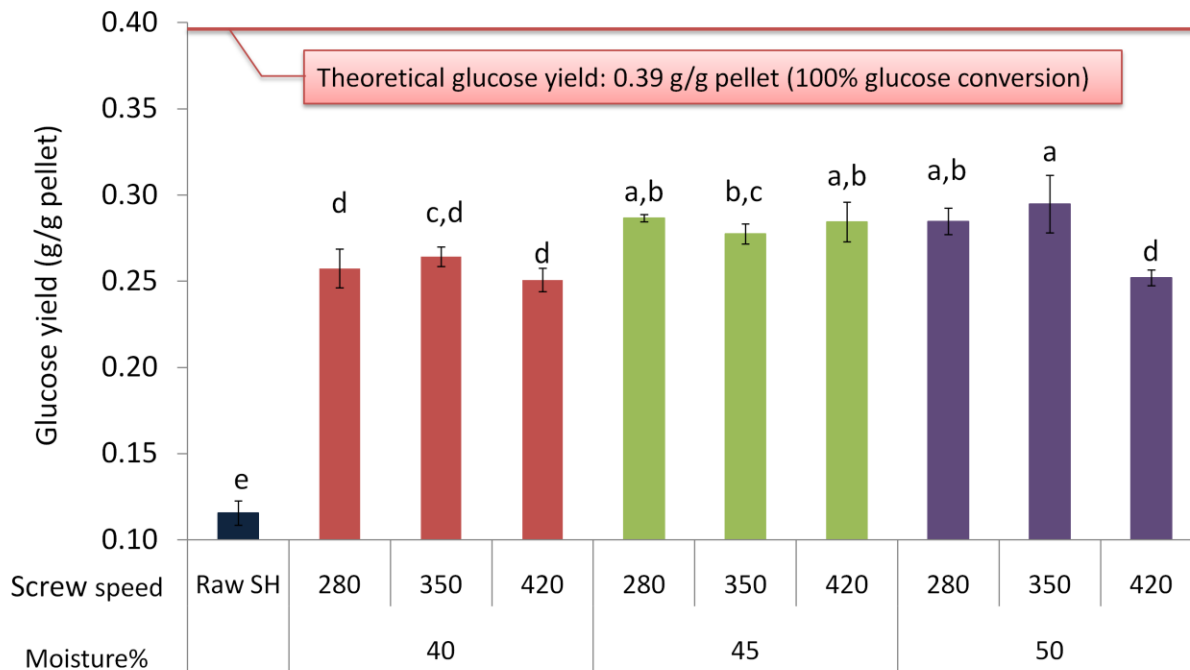


Figure 3.3 Glucose yield (g/g pellet) after 72 h enzymatic hydrolysis for control (Raw SH) and nine treatments from 3×3 factorial design. Glucose yields were determined by HPLC. Results sharing the same letters are not significantly different (p<0.05). Raw SH: soybean hull without pretreatment (control).

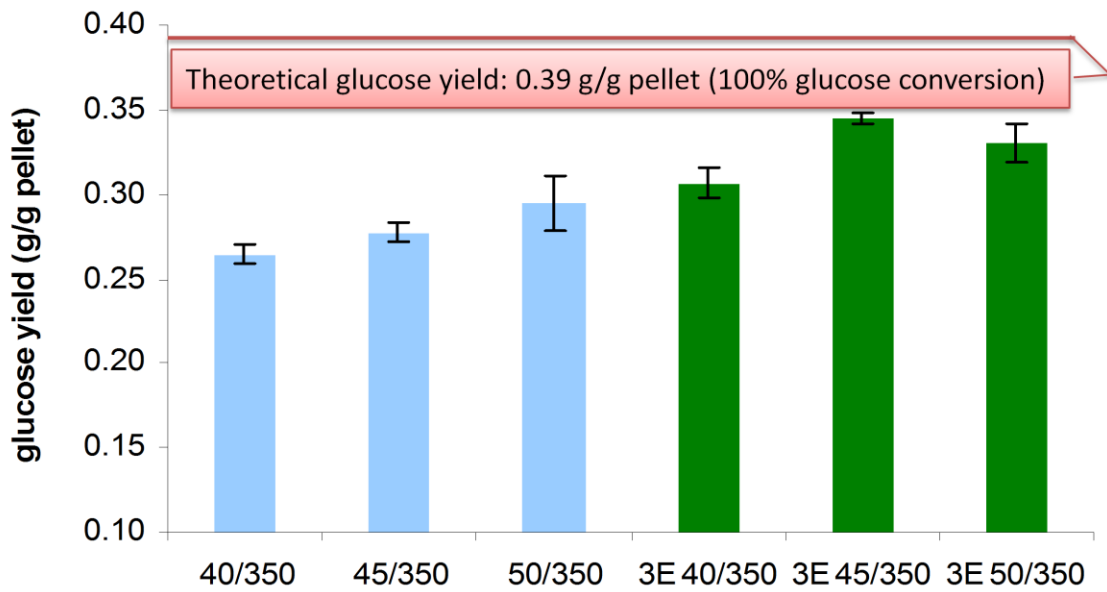


Figure 3.4 Glucose yield (g/g pellet) after 72 h enzymatic hydrolysis with two enzymes vs. three enzymes. Glucose yield was determined by HPLC. On x-axis: a/b = in-barrel moisture content/screw speed. 2E = two enzymes; 3E = three enzymes.

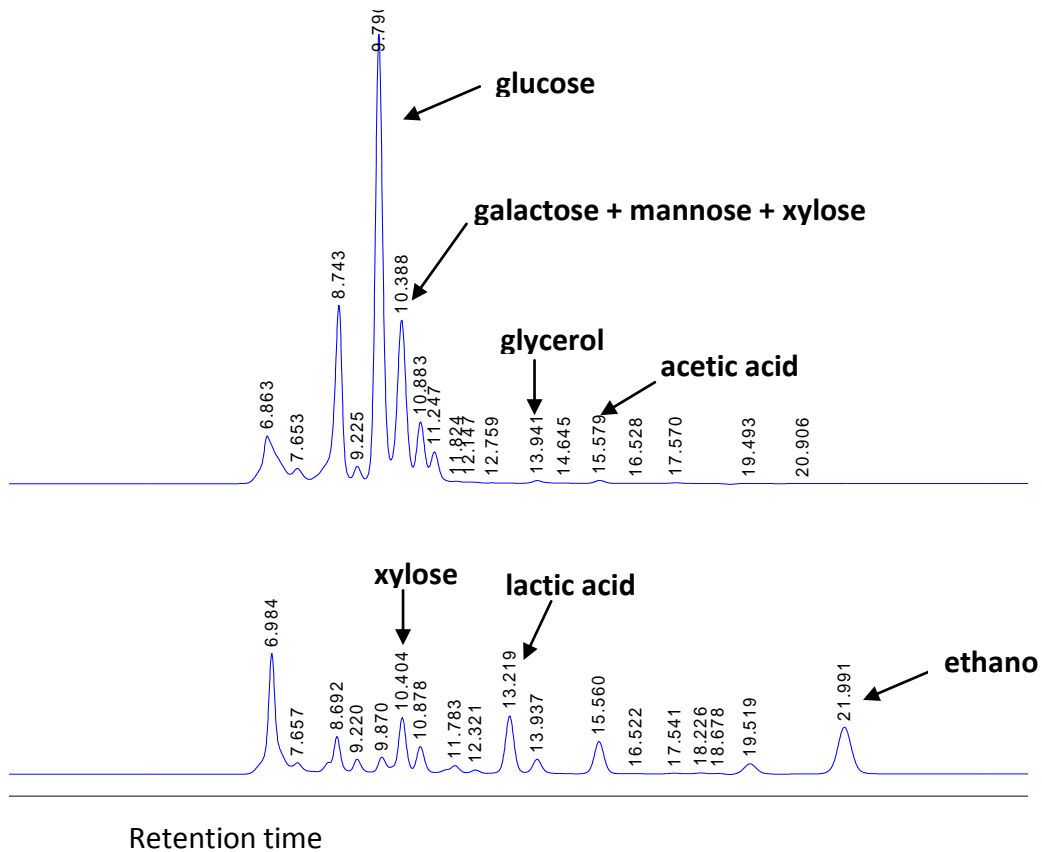


Figure 3.5 Chromatogram of hydrolysate (top) and fermented broth (bottom) for treatment with screw speed 280 rpm at 50% in-barrel moisture content.

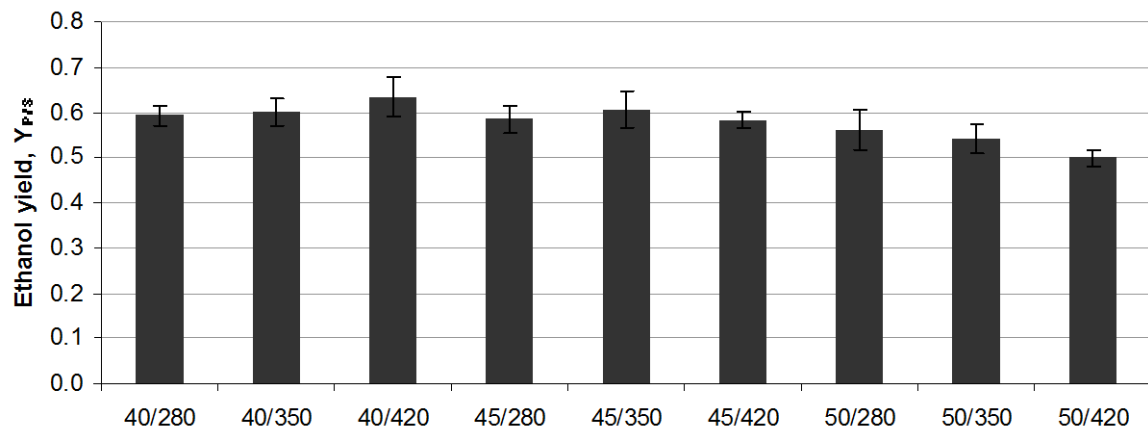


Figure 3.6 Ethanol yield, $Y_{P/S}$. $Y_{P/S}$ is based on glucose consumption. On x-axis: a/b = in-barrel moisture content/screw speed. P/S = product/substrate.

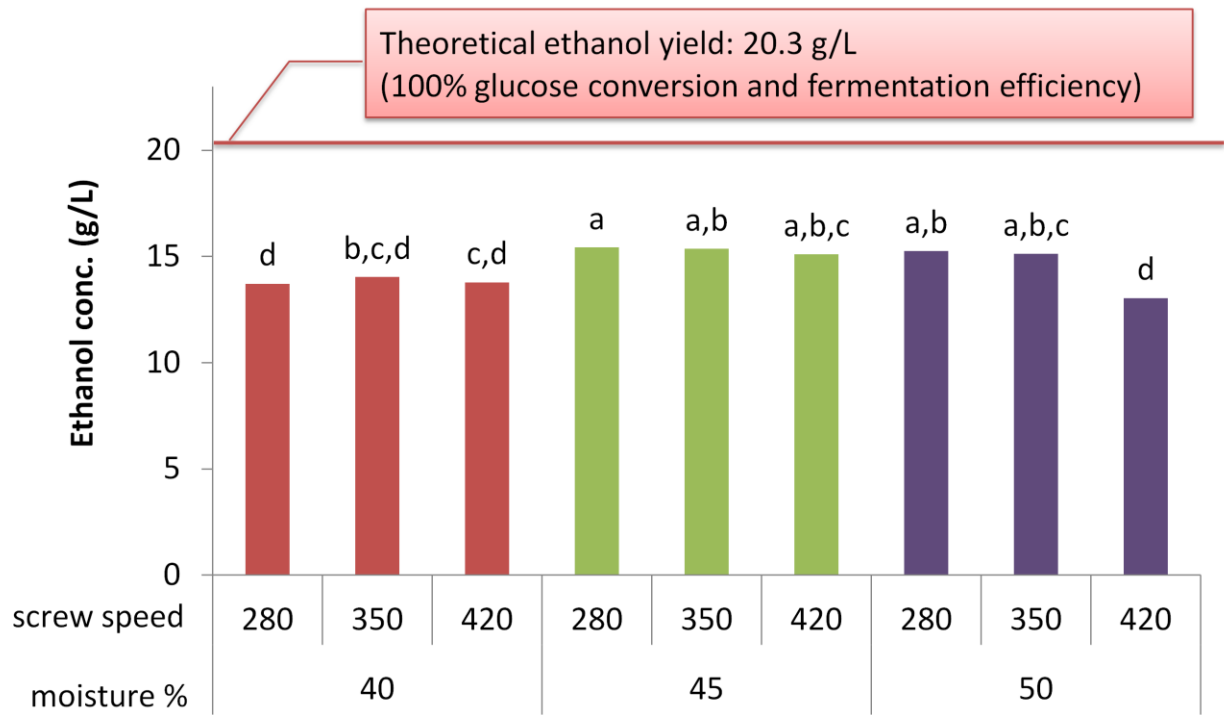


Figure 3.7 Ethanol concentration (g/L) in fermented broth after 48 h fermentation.

Table 3.1 Fermentation inhibitors (g/L) present in extruded pellet, hydrolysate and fermented broth

		40/280	40/350	40/420	45/280	45/350	45/420	50/280	50/350	50/420
Lactic acid	Pellet	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
	Hydrolysate	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
	FB	8.422	9.035	9.166	8.276	9.261	8.389	7.762	7.477	7.275
Glycerol	Pellet	0.349	0.363	0.362	0.371	0.360	0.369	0.422	0.431	0.072
	Hydrolysate	0.518	0.526	0.474	0.539	0.526	0.536	0.675	0.686	0.239
	FB	4.189	4.287	4.238	4.485	4.467	4.433	4.580	4.536	4.106
Acetic acid	Pellet	0.197	0.190	0.192	0.209	0.212	0.214	0.278	0.211	0.181
	Hydrolysate	1.398	1.401	1.239	1.388	1.393	1.417	1.325	1.368	1.119
	FB	2.899	2.894	2.683	3.018	2.875	2.783	3.215	3.321	2.954
Furfural	Pellet	0.042	0.047	0.044	0.044	0.046	0.049	0.029	n/d	0.001
	Hydrolysate	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
	FB	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
HMF	Pellet	n/d	n/d	n/d	n/d	n/d	0.002	0.023	0.001	n/d
	Hydrolysate	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
	FB	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d

HMF: 5-(hydroxymethyl)-2-furaldehyde; FB: fermented broth; n/d: not detected.

CHAPTER 4 - Bioenergy from soybean hulls: Efficiency and economics comparison of dilute acid hydrolysis and thermo-mechanical extrusion pretreatment

Juhyun Yoo¹, Vincent Amanor-Boadu² and Sajid Alavi^{1,3}

¹ Department of Grain Science and Industry, Kansas State University, Manhattan, KS 66506

² Department of Agricultural Economics, Kansas State University, Manhattan, KS 66506

³ Corresponding author. Phone: (785) 532-2403, E-mail: salavi@ksu.edu

4.1 Abstract

Technical and economical feasibility of thermo-mechanical extrusion pretreatment for cellulosic ethanol production was compared with dilute acid hydrolysis. Two pretreatments were conducted in lab scale, and resulting sugar conversion efficiency along with two publicly available experimental data were utilized for the economic analysis of each pretreatment in plant scale. Plant scale models were developed, and cellulosic ethanol production costs were estimated. The system dynamics modeling approach was employed by using Monte Carlo simulator with built in technological enhancements with respect to the production and sugar yield over a production year.

Net present value for 20 year simulation period indicated negative value for both pretreatment because of the high price (\$106/ton) of soybean hulls used in this study. As reduced the feedstock cost, net present value of cellulosic ethanol production using extrusion pretreatment and dilute acid hydrolysis turned into positive value at soybean hull price of \$54.9/ton and \$13.5/ton, respectively. From the simulation of two different scenarios, it was concluded that low feedstock cost and high sugar conversion are important factors to make cellulosic ethanol production commercially viable. Extrusion pretreatment was a promising pretreatment technology over dilute acid hydrolysis because of the lower capital and operating costs, and higher sugar conversion efficiency.

4.2 Introduction

According to the National Biofuels Action Plan released on October 2008, expanding annual biofuel production to 36 billion gallons per year over 15 years on a sustainable basis will be a key component in America's movement toward a clean, affordable, and secure energy solution. Second generation biofuels derived from lignocellulosic raw materials such as agricultural and forestry residues and herbaceous and woody energy crops, will play an important role in approaching to the goal. Although production cost is still high to compete in the market place, there have been significant reductions in ethanol production costs (Galbe et al., 2007) with advanced technology in pretreatment, fermentation, enzyme production, and enzymatic hydrolysis (Yang and Wyman, 2008).

The lignocellulosic structure is very complex, highly crystalline, and resistant to enzymatic degradation in its native state. Therefore, cellulosic ethanol production is different from first generation biofuel, corn ethanol production, that is, an additional pretreatment is required to break down the 'recalcitrant' structure and improve efficiency of enzymatic hydrolysis of cellulosic material. Previous economic analysis has shown that about 40% of production cost is related to pretreatment, enzyme production, and enzymatic hydrolysis, with pretreatment responsible for almost half of this total (Aden et al., 2002; Wooley et al., 1999a; Wooley et al., 1999b). In other word, the effectiveness of the pretreatment conditions determines the yield and economics of the overall process.

There have been many studies on pretreatment technologies of cellulosic material which can be classified as biological, chemical, physical, or thermal processes. These include acid hydrolysis, alkali hydrolysis, organosolv process, steam explosion, ammonia fiber explosion (AFEX), pyrolysis, hot water treatment, and microorganism treatment (Galbe and Zacchi, 2007; Sun and Cheng, 2002; Yang and Wyman, 2008). There have been some successes in terms of increased ethanol yield. However, the challenge lies in increasing the efficiency of these methods to make the overall cellulosic ethanol production process cost effective, and no single method has yet been found suitable for commercial application (Saha and Cotta, 2007).

Extrusion processing can be employed as a pretreatment with much higher capacity than any other existing pretreatment processes. When material passes through the extruder barrel, high pressure and temperature are developed as a result of the intense mechanical shear exerted

by the screw. When the extruded material comes out of the die, some of moisture present in material is flashed into steam because of sudden drop in pressure, and the material is expanded. In addition, high mechanical energy input can help break down the lignocellulosic structure of the raw materials as a result of shear. Another aspect of extrusion processing is that chemical reactions and material processing can be carried out in one apparatus, which results in saving equipment and energy (Rozeń et al., 2001). Lamsal et al. (2010) described several examples of utilization of extrusion processing as a bioreactor for processing lignocellulosic biomass.

There is a large amount of information regarding conversion technologies for lignocellulosic material to ethanol based on studies mostly focused on technical feasibility and basic science. However, this information is very difficult to use on comparison basis due to different underlying assumptions (Foust et al., 2009). In 2000, the Consortium for Applied Fundamental and Innovation (CAFI) was formed to develop the information on cellulosic biomass pretreatment by leading technologies. Chemical pretreatments such as dilute acid pretreatment and alkali treatment appeared to be the most effective pretreatments with significant removal of hemicellulose and lignin. Aden et al. (2002) described the base case of a cellulosic ethanol process with corn stover as raw material by using dilute acid pretreatment and provided the minimum ethanol selling price at \$1.07/gal.

Thermo-mechanical extrusion pretreatment has been used in our laboratory to produce ethanol using soybean hulls as a model lignocellulosic substrate, resulting in high efficiency of enzymatic hydrolysis and ethanol yield. The objectives of this study were to estimate the production cost of cellulosic ethanol using thermo-mechanical extrusion pretreatment over a 20 year time horizon, to determine the effect of identified scenarios on the cost estimation, and to analyze the economic competitiveness of thermo-mechanical extrusion pretreatment with conventional dilute acid pretreatment. In assessing the economic feasibility of the thermo-mechanical pretreatment method, technical analysis from the laboratory was scaled up to a plant of commercial size, making specific assumptions about the scale effects.

4.3 Materials and Method

4.3.1 Assessment of technical feasibility

For the simulation of plant scale cellulosic ethanol production from feedstock handling to downstream process, the cellulosic ethanol production models employing dilute acid hydrolysis

and extrusion pretreatments were developed and depicted in Fig 4.1 and Fig 4.2. As shown in Fig. 4.1, ‘process area A’ including biomass storage, transportation and handling (A1) and ‘process area C’ including enzymatic saccharification (C1), fermentation (C2), distillation (C3), solid/liquid separation (C4), waste management (C5), combustor/boiler (C6) and turbin/generator (C7) were assumed to be the same for both pretreatments. Dilute acid hydrolysis model used in this study heavily adapted the work by Aden et al. (2002) with some modification.

In the plant scale process, dilute acid hydrolysis (B-D1) will be carried out in three reactor trains which consist of presteamer, blow tank, and reactor. The biomass is moved by screw conveyor from presteamer to blow tank, and blow tank to reactor. The exiting materials from reactor are cooled down and filtered to separate solid from liquid by using pressure filter (B-D2) (Aden et al. 2002). Separated product liquor is conditioned with sufficient amount of lime to raise the pH to 10.0 (B-D3), resulting in a gypsum precipitate (B-D4). Conditioned liquor fraction is reacidified to proper pH for enzyme system (B-D3) and combined again with solid materials in slurry tank, and then resulting slurry is sent to enzymatic saccharification (C1).

Extrusion process in plant scale is simpler and requires less equipments (Table B.1) compared with dilute acid hydrolysis (Table B.2) because it does not require solid/liquid separation and conditioning/detoxification process (Yoo et al., 2011a). The extrusion system consists of a preconditioner, extruder, and peripheral devices (B-E1). In the preconditioner, steam and water are injected to hydrate the biomass to the target in-barrel moisture and soften it in order to facilitate the extrusion process. Wet extruded pellets are mixed with additional water in slurry tank and send to enzymatic saccharification (C1).

Hydrolysate is fermented by *Zymomonas Mobilis* (C2), and fermented broth is then distilled in a column system to produce ethanol (C3). In solid/liquid separation (C4), lignin rich residue are recovered and sent to biomass combustion system (C6) to generate the electricity (C7) which is sufficient to support the biorefinery.

To assess the technical feasibility of extrusion process as a pretreatment and compare with dilute acid hydrolysis pretreatment which has been demonstrated its technical and economical feasibility, two lab-scale pretreatment, extrusion and dilute acid hydrolysis were conducted in our laboratory (Yoo et al., 2011b). Soybean hulls consisting of 35.35, 17.21, 2.33 and 0.83% (db) of cellulose, hemicellulose, lignin, and starch, respectively, were used as a feedstock. Soybean hulls were ground into particle size less than 1041 μ m. Unlike acid

hydrolyzed soybean hull, extruded soybean pellets did not required conditioning and neutralizing step prior to enzymatic hydrolysis. Resulting hydrolysate from each pretreatment was subject to enzymatic hydrolysis using cellulase and β -glucosidase. Glucose concentration in hydrolysate was determined by using high performance liquid chromatography (HPLC; Agilent 1100). Cellulose to glucose conversion % was determined as ratio of the glucose obtained with HPLC to the theoretical yield based on the amount of cellulose in soybean hulls.

In addition to the results from our laboratory, for the comparison of two pretreatments, other publicly available experimental results were utilized, including Corredor et al. (2008) and Karuppuchamy and Muthukumarappan (2009). The processing conditions and the yield for each pretreatment are summarized in Tables 4.1.

In the simulation of the fermentation stage, fermentation by *Zymomonas mobilis* was adapted. *Z. mobilis* ferments both hexose and pentose sugar to ethanol. Fermentation efficiency by *Z. mobilis* was assumed to be same for all scenarios at 95% for hexose sugar and 85% for pentose sugar. In contrast, in our lab scale fermentation experiment (data is not shown), *Saccharomyces cerevisiae* (Red Star Ethanol Red® , Lesaffre Group, Milwaukee, WI) was employed resulted in 100% conversion efficiency of glucose to ethanol, while less than 1% conversion efficiency for xylose was reported (Yoo et al., 2011a). Joachimsthal et al. (1999) reported that fermentation efficiency of *Z. mobilis* for mixed sugar of glucose and xylose is comparable (if not better) to the efficiency for glucose by *S. cerevisiae*. Nguyen and Glassner (2001) reported that fermentation time by *Z. mobilis* is shorter than that by yeast as well as ethanol yield is higher at 92-94% compared with 88-90% for yeast.

4.3.2 Comparing the frameworks for economic analysis: The Assumptions

Fig. 4.1 shows the process flow for cellulosic ethanol production utilizing dilute acid pretreatment and thermo-mechanical extrusion pretreatment with a focus on their points of differentiation. The production cost of each pretreatment was estimated, and they were compared to the value of product produced. The economic feasibility study was conducted at the plant scale under some well-defined assumptions after modification of the analysis by Aden et al. (2002). The principal assumptions influencing the analysis are given below.

4.3.3 Operational Assumptions

Access to raw material is assumed to be available throughout the year because soybean is processed by soybean processing plants throughout the year. Thus, unlike corn stover and other cellulosic materials that need to be collected and processed within a very short period, soybean hulls are procured on a flow basis from processing facilities year round. The foregoing eliminates significant amount of storage costs for soybean hulls by the processing plants since they can base their operations on production rates of the soy processing facilities from where they procure soybean hulls.

Plant processing capacity of 2,000 dry metric tons of biomass per day is assumed for both pretreatment approaches, which means the ethanol plant should have access to several soybean processing plants nearby with approximately total 20,000 tons capacity daily, and raw material utilization rates are about 83.3 metric tons of dry biomass per hour. The thermo-mechanical pretreatment is a continuous process with the extruder capacity assumed to be an average of 6 metric tons per hour. Thus, 14 extruders with this mean flow capacity are installed to ensure the average daily biomass processing capacity. Based on the quantities of biomass processed, the operating time, and the results from the experiments conducted in the previous study (Yoo et al., 2011b), the process uses about 55,555 liters of water (66.7 % of soybean hull weight) per hour to achieve 40% in-barrel moisture content (Table 4.1). In dilute acid hydrolysis (B-D1), water is required 9 times biomass weight. After solid/liquid separation (B-D2), liquid was recaptured and recycled. Extrusion pretreatment (B-E1) does not require solid/liquid separation step. Extruded pellets are directly sent to enzymatic saccharification (C1).

The dilute acid process is a batch process with a mean residence time of 30 minutes per batch. The capital outlay for the plant is based on this mean residence time and is drawn from NREL research (Aden et al., 2002). The variability in productivity is caused by both technical and human factors. Total plant operation time is 50 weeks per year, allowing for two weeks (336 h) of downtime for maintenance and cleaning. Assuming a 24 hour, 3 shift operation, this is equivalent to 8,400 h/year in operation for both plants. All scheduled and unscheduled maintenance work is assumed to be included in the 336 h of downtime for operations.

Sugar yield from the enzymatic saccharification process were based on the experiments conducted in our laboratory. We also considered alternative sugar release levels presented in the

literature by Corredor et al. (2008) and Karuppuchamy and Muthukumarappan (2009) for dilute acid hydrolysis and extrusion pretreatment, respectively (Table 4.1).

4.3.4 Capital Cost Assumptions

Costs were procured and extrapolated from the Chemical Engineering Plant Cost Index (CEPCI, 2009) and from Aden et al. (2002). Total equipment capital costs for acid hydrolysis and conditioning was estimated at \$39.2 MM that was 23.6% of total equipment cost for overall ethanol production. The largest single item in pretreatment is the pre-hydrolysis, screw feeder and reactor, estimated to account for 64 % of total capital cost of pretreatment/conditioning (Table B.2). The next most expensive item is pneumatic press filter, which was estimated to account for about 18 % of the total capital cost of pretreatment/conditioning. The remainder of the near 32 capital equipment items account for 18 %, and these ranged from 0.01 to 3.52 % of total estimated pretreatment/conditioning capital costs. Kazi et al. (2010) reported that capital cost for pretreatment using dilute acid hydrolysis accounts for 22.1 % of total capital cost and is the second highest after combustor, boiler, and turbogenerator. The equipments for acid hydrolysis, screw feeder and reactor were “straight line” depreciated over 20 years straight line, with zero salvage value.

The extrusion equipment was estimated at about \$25 MM. This includes 14 high capacity extruders, which together account for 78.6 % of total capital equipment cost in pretreatment (Table B.1). Equipment cost for extrusion pretreatment was 16.4% of total capital cost of \$152 MM for whole ethanol production line. Extruders are “straight line” depreciated over 20 years, with zero salvage value.

4.3.5 Operating Cost Assumptions

Operating costs were considered focusing on four main components (Tables 4.3 and 4.4):

1. Feedstock: Feedstock is the single most expensive component in bioethanol production (Foust et al., 2009). In the economic analysis by Foust et al. (2009), biomass price was approximately 38% (\$0.51/gal) of overall cost, i.e. minimum ethanol selling price (MSEP) of \$1.33/gal. Soybean hull price at \$160 in 2011 (By-product feed price listing, 2011) was adjusted to 2008\$ (Bureau of Labor Statistics, 2011). Both methods are assumed to process the same quantity of feedstock. Thus, biomass procurement costs—product and logistics—are assumed to be the same. However the soybean hulls used in the study by Yoo et al. (2011b), Corredor et al.

(2008) and Karuppuchamy and Muthukumarappan (2009) indicated the different cellulose and hemicelluloses contents. This was taken into account for the product yield calculation.

2. Energy: Studies in economic analysis of cellulosic bioethanol production have indicated that ethanol plant will generate the energy from combustion of by-product such as lignin-rich residue to power the whole production process, and the energy generated in excess of plant needs will be sold. For example, Aden et al. (2002) showed electricity credit of 9.29 cents/gal. Kazi et al. (2010) concluded that electricity credit was 7.1% of total operating cost for dilute acid pretreatment. The co-product revenue in the form of electricity credit are important for the low ethanol yield production, and become predictably less important as the yield improves (Wyman, 1995). In this study, we assumed that all the electricity needed are generated in the plant and did not count the electricity credit for the revenue.

3. Variable operating cost: Extrusion pretreatment does not employ any chemical during the pretreatment. Also the water amount required is significantly lower than that for the dilute acid hydrolysis, as solid loading during the acid hydrolysis is about 10% while it is 60% in extrusion process. The extrusion pretreatment does not generate any liquid waste prior to saccharification because the added water to the biomass is absorbed in the extrusion process. On the contrary, the dilute acid hydrolysis generates waste water from most of the added water, and the former must be treated in order to reuse it through a recycling process. As a result, the chemical process has water recycling (processing) cost included in the operating cost. The variable processing costs were modified based on Aden et al. (2002).

4. Fixed operating cost (office, labor and management): The same number of people work in the front and back offices of the two evaluated pretreatment methods. Also, the same number of yard workers work in both methods. The difference comes in the number of people directly involved with the two operations. Because the extrusion system is a continuous process, the only labor required is to ensure that screw speed and other parameters are constantly operating optimally with any changes in incoming biomass quality, ambient temperature, humidity and flow characteristics. It is assumed further that each operation runs three shifts and each operations worker has a 40 h work per week. Acid hydrolysis requires total 77 people including 12 shift operators. Extrusion pretreatment requires total 66 people including 9 shift operators.

4.3.6 The System dynamic models for the two approaches

In addition to the foregoing, the model was built as a system dynamic simulation model that allows from improvements in technology, which contribute to better yield of glucose from cellulosic materials, such as soybean hulls. The model was used to simulate costs over 20 years.

System dynamics modeling approach (Fig. 4.2) is grounded in nonlinear dynamics' theory (Lane, 2001) and uses well-defined assumptions to describe and analyze complex and dynamic feedback problems (Forrester, 1968; Sterman, 2000). Many people have used this approach to evaluate systemic and dynamic problems which are complex in their interrelationships, for example, Philbin (2008) in his application to the management of complex technology projects; Pawlak and Malyszek (2008) in their work on collaboration in supply chains; and Ndiyo (2007) in the relationship between education and economic growth. It is useful in this problem because of its ability to handle the complex feedback relationships among technological improvements, market and policy changes and consumer ethical responses to changing environmental conditions. To operationalize the model, we employed a Monte Carlo simulator with built in technological enhancements with respect to the production and sugar yield over a production year and then aggregated them to annual to generate 20 years outcomes.

Projected annual ethanol yields and percentage yield was obtained with all experimental data and assumption as following

$$Y = f \times C_e \times C_f \times 0.51 \times D \times C \quad (1)$$

where, Y=annual ethanol yield; f=fraction of cellulose (or hemicellulose); C_s =conversion efficiency of enzymatic hydrolysis; C_f =conversion efficiency of fermentation; D=plant operating days per year; C=plant capacity; 0.51=fermentation factor from sugar to ethanol. Ethanol density of 0.789g/ml was used for unit conversion of ethanol concentration.

$$Y\% = \frac{Y}{Y_t} \times 100 \quad (2)$$

where, Y%=percentage yield; Y_t =theoretical yield when C_s and C_f are 100%; Y=annual ethanol yield.

Results described are for subsequent simulation. The terms extrusion pretreatment and thermo-mechanical pretreatment are used interchangeably.

4.4 Results and Discussion

4.4.1 Comparing the efficiency of pretreatments on enzymatic hydrolysis

Glucose yield after enzymatic hydrolysis of dilute acid hydrolyzed soybean hull and extruded soybean hull were compared (Table 4.1). Yoo et al. (2011b) reported that glucose yields after enzymatic hydrolysis were 0.27 and 0.37 g/g soybean hull utilizing dilute acid hydrolysis and extrusion pretreatment, respectively. These yields were corresponding to 69.2 and 94.8% conversion based on 35.35% cellulose (0.39 g theoretical sugar) in 1 g soybean hulls. Karuppuchamy and Muthukumarappan (2009) reported 62.5% cellulose to glucose conversion by extrusion pretreatment followed by enzymatic hydrolysis of soybean hull consisting of 46% cellulose. Corredor et al. (2008) reported 79 and 96% conversion for cellulose and hemicelluloses, respectively. Projected annual ethanol yields based on a plant capacity of 2000 tons/day, from both hexose and pentose sugars in each scenario are shown in Table 4.1. Extrusion pretreatment by Yoo et al. (2011b) resulted in the highest annual ethanol yield of 52.2 MM gal/year with 83.1% conversion. The percentage yield of the thermo-mechanical pretreatment employed by Karuppuchamy and Muthukumarappan (2009) was the lowest at 62.3%, even though their corresponding projected annual ethanol yield of 47.6 MM gal was second highest, because the soybean hulls used contained higher cellulose and hemicellulose. It was concluded that efficient pretreatment along with high cellulose and hemicelluloses content feedstock are important to maximize the efficiency of overall cellulosic ethanol production.

4.4.2 Base scenario results

The base scenario simulation was conducted by computing the results of lab scale experiment from our laboratory. Cellulose to glucose conversion used for, extrusion pretreatment and dilute acid hydrolysis were 94.8 and 69.2%, respectively (Table 4.1). As described earlier, hemicelluloses conversion was assumed to be 90%. Ethanol price used in the simulation was \$1.90/gal (Ethanol Market, 2011).

The extrusion process produced, on average, 53.7 MM gal/year of ethanol, which was about 23.4% more ethanol than that produced from acid hydrolysis pretreatment, 43.5 MM gal/year. It was because of the higher efficiency in glucose conversion of extrusion pretreatment. In the period of simulation, total fixed operating cost including salary and fringe and benefit increased by the inflation over time from \$3.1 to 5.9 MM/year and from \$3.6 to 6.8MM/year for ethanol production with extrusion or dilute acid hydrolysis process, respectively. Over 20 years

of operations, the annual variable operating cost under the extrusion process also increased from \$91.4 to 172.6MM/year with 5% inflation rate which was lower as compared with \$105.5 to 199.2MM/year for ethanol production employing dilute acid hydrolysis.

In this study, soybean hull feedstock cost, \$105/ton accounted for more than 70% of total variable operating cost. Therefore, sufficient supply of cheap feedstock is important to reduce the operating cost, and which would be the key for the economically feasible cellulosic ethanol production. For example, in the economic analysis of cellulosic ethanol production by Aden et al. (2002), corn stover was used as the feedstock, and estimated feedstock price was \$25/ton. As a result of this low feedstock cost, the total operating variable cost \$31.6MM/year. Kaylen et al. (2000) modeled cellulosic ethanol plant with 6 cellulosic biomass such as corn stalks, grain sorghum stalks, wheat straw, energy crops, primary wood processing, logging residues. The price of these feedstocks ranged from \$25 to 44/ton, and their cellulosic composition was between 52% and 71%. Again, the importance of feedstock with lower price and higher cellulosic composition was emphasized for the feasible cellulosic ethanol production.

In Fig. 4.3, net present value (NPV) of the total cash flow for various feedstock prices is shown. With the market soybean hull price of \$105.5/ton (2008\$), NPV for both pretreatment were minus, indicating that it is not technically feasible. By reducing the feedstock price, NPV for extrusion pretreatment turned into positive value at \$54.9/ton and \$13.5/ton for extrusion pretreatment and dilute acid hydrolysis, respectively.

When the efficiency of glucose conversion for the extrusion pretreatment was assumed to be the same as dilute acid hydrolysis at 69.2% cellulose to glucose conversion (Fig. 4.4), NPV for the extrusion process was \$34.6MM while NPV for dilute acid pretreatment was -\$97.5MM with feedstock cost at \$30/ton. As increase the conversion rate of dilute acid hydrolysis, resulting NPV was increased and became equal to NPV of extrusion process at 98.8%. From the results, it was proven that extrusion process is a promising pretreatment method for cellulosic ethanol production.

4.4.3 Randomized scenario analysis

Model simulation was randomized with two different yields and feedstock cellulosic fraction for each pretreatment. For extrusion pretreatment, conversion % was randomized between 62.5% (Karuppachamy and Muthukumarappan, 2009) and 94.8% (Yoo et al., 2011b).

Dilute acid hydrolysis yield was also randomized between 69.2% (Yoo et al., 2011b) and 79% (Corredor et al., 2008). Range of cellulose and hemicellulose fraction was from 35 to 46% and 12 to 18%.

Cellulosic ethanol production for 20 years at soybean hull price of \$105.5/ton resulted in shortfall of cash flow for both of pretreatment irrespective to the randomization (Fig.4.5). Soybean hull price of \$23/ton and \$48/ton turned ethanol production into excess of cash flows for dilute acid hydrolysis and extrusion pretreatment, respectively.

The simulation for randomized model was conducted at soybean hull price of \$10/ton and the results are shown in Table 4.4. Revenues from ethanol sales averaged \$90.83MM and \$94.48MM for the dilute acid hydrolysis and extrusion processes respectively, the difference being equal to the 4.02% higher production observed because the same ethanol price was applied to both processes. Total cost for the extrusion process was 38.8% lower while net income was 58.78% higher. The time-trends in the costs and revenues are presented in Table D.1.

The net present value (NPV) of the total cash flow over the simulation period under the randomized scenario for the extrusion process was \$234MM at a discount rate of 7.5 %. The internal rate of return (IRR) was estimated at 23 %. For the dilute acid hydrolysis, the NPV was \$76 MM at the same discount rate and the IRR was 13%. Thus, when the average extruder capacity is 6 metric tons per hour, and given all the other assumptions, the extrusion process yields a superior financial outcome than the dilute acid hydrolysis.

4.5 Conclusions

Specifically, the purpose of this study was to determine the economic feasibility of extrusion pretreatment method having demonstrated its technical feasibility in the previous study by Yoo et al. (2011b). By comparing the economic feasibility of two pretreatment methods, such as dilute acid hydrolysis and extrusion pretreatment, it was ascertained that extrusion pretreatment would be the promising pretreatment method for cellulosic ethanol production over dilute acid hydrolysis which has been studied most but has not been proven to be feasible for commercialization.

A system dynamic modeling approach was employed because of the differences in capital outlay between the two methods and the dynamic effects of marketplace and policy risks on their application. For example, fresh water availability is becoming increasingly important in

environment policy discussion, and solutions to current energy problems are being evaluated within the context of their systemic effects on other environmental resources. The effect of various feedstock cost and cellulosic composition on NPV showed the selection of feedstock and reduction in price by developing the efficient logistic would be very important. Development of technologies to reduce the variable cost including enzyme and yeast cost and to increase the efficiency of pretreatment will lead to earlier commercialization of cellulosic ethanol.

4.6 Acknowledgement

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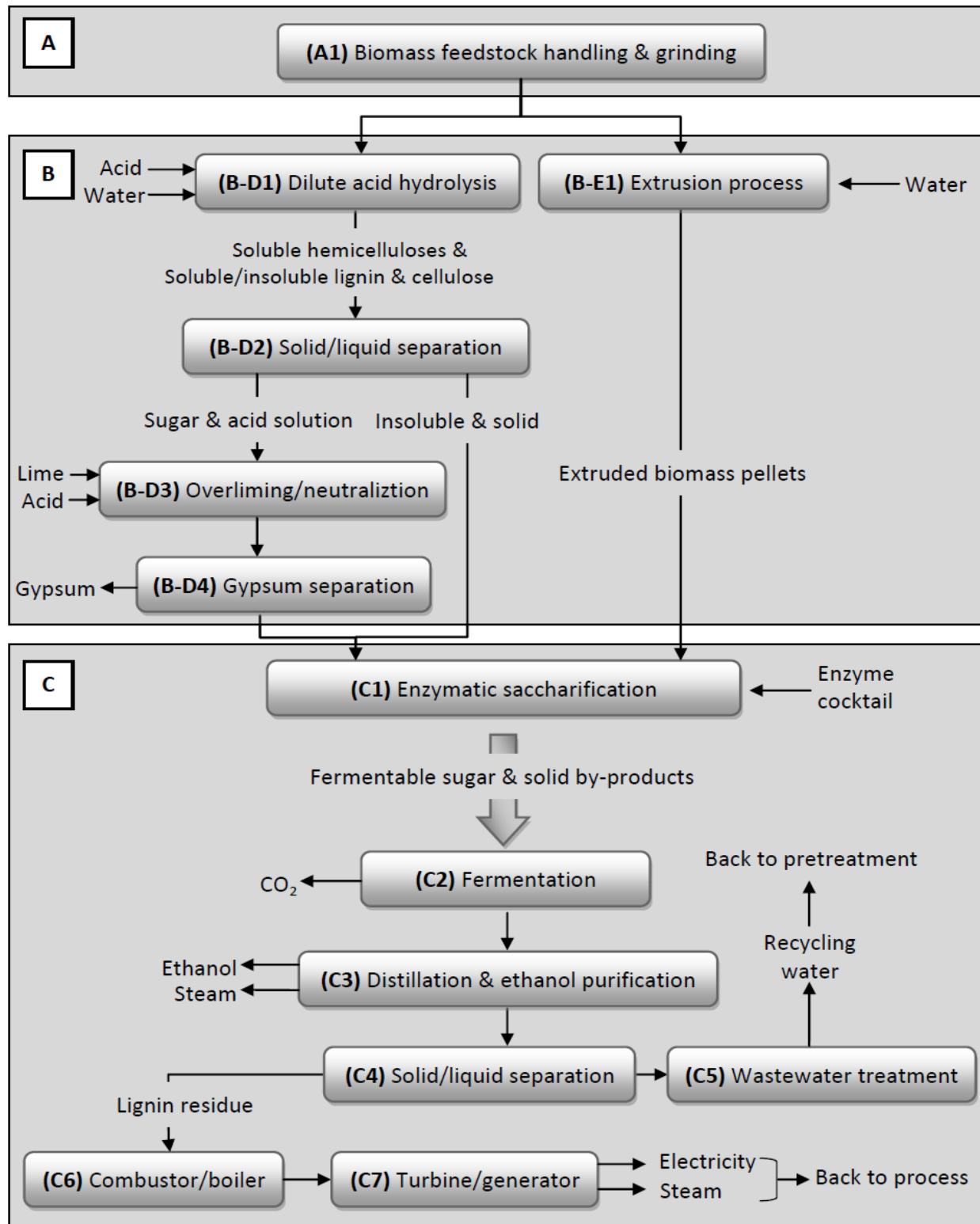


Figure 4.1 Comparative frameworks of dilute acid hydrolysis and thermo-mechanical extrusion pretreatment processes. Area A and C: assumed to be the common process for both pretreatments; Area B: differentiated process.

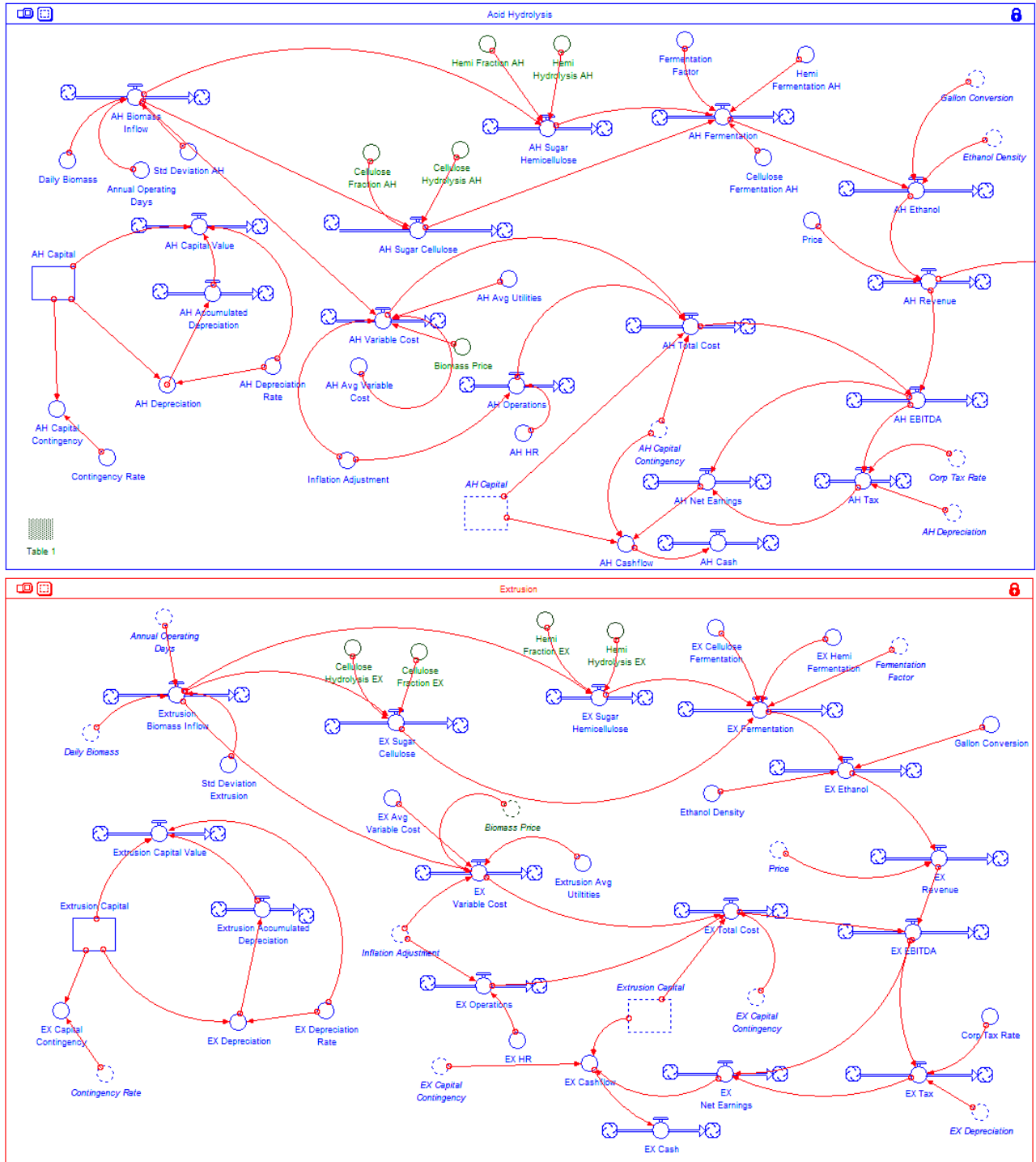


Figure 4.2 Model schematic for the economic analysis of cellulosic ethanol production by using acid hydrolysis (top) and extrusion pretreatment (bottom).

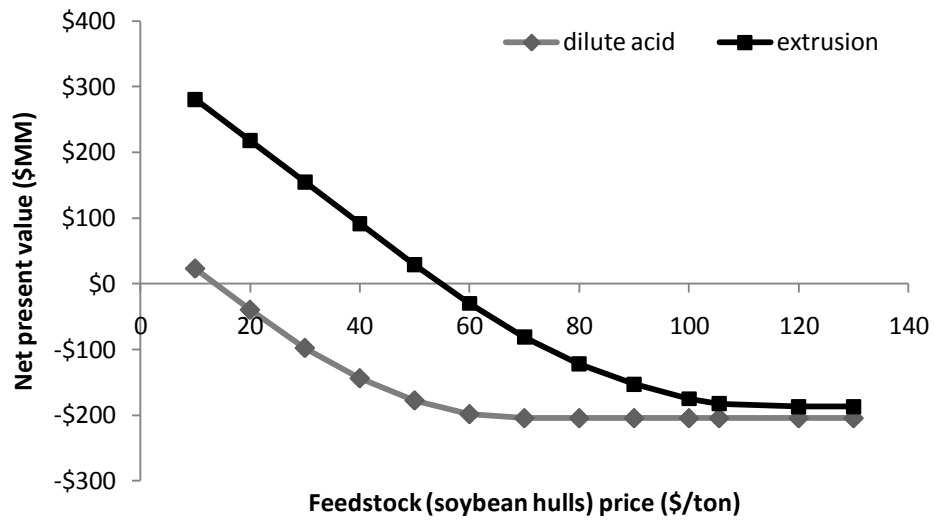


Figure 4.3 Net present value of cellulosic ethanol production by using dilute acid hydrolysis and extrusion pretreatment with various feedstock cost (non randomized pretreatment yield from Yoo et al. (2011b)).

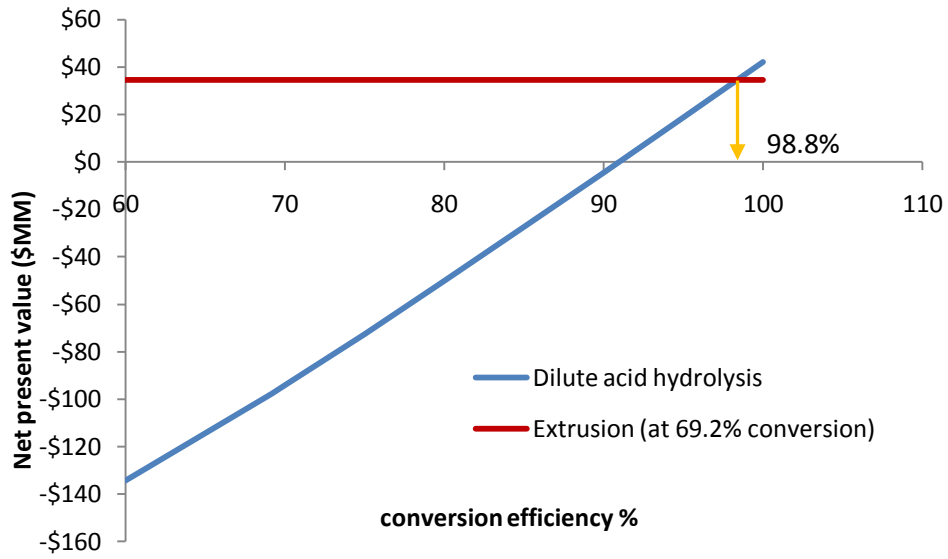


Figure 4.4 Comparison of net present value for dilute acid hydrolysis with various conversion yield and extrusion pretreatment with fixed conversion yield (Feedstock price: \$30/ton).

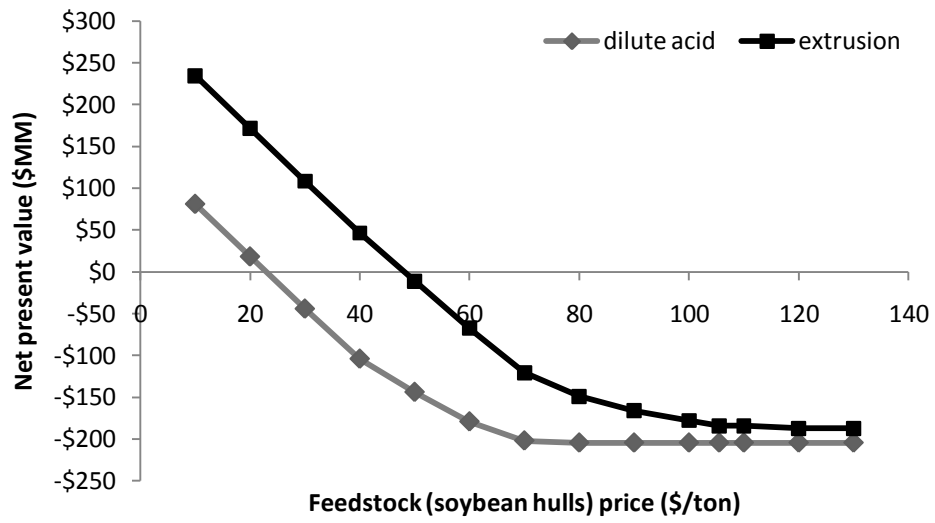


Figure 4.5 Net present value of cellulosic ethanol production by using dilute acid hydrolysis and extrusion pretreatment with various feedstock cost (randomized pretreatment yield).

Table 4.1 Process parameters for four different scenarios (based on capacity of 2000 tons/day)

Process area	Process Parameter	Model value		Process Parameter	Model value	
Pretreatment		EX ^a	EX ^b		DAH ^c	DAH ^d
	Feed rate (kg/h)	1.61	- ^e	Sulfuric acid concentration (%)	1.0	2.0
	Screw speed (rpm)	350	60	Reaction Time (min)	30	30
	Maximum temperature (°C)	80	130	Temperature (°C)	121	140
	In-barrel moisture (%)	40	12.5	Solids in the reactor (%)	10	10
Feedstock	Cellulose fraction %	35.4	46.0	Cellulose fraction %	35.4	36.4
	Hemicelluloses fraction %	17.2	18.0	Hemicelluloses fraction %	17.2	12.5
Saccharification	Cellulose conversion %	94.8	62.5	Cellulose conversion %	69.2	79.0
	Hemicellulose conversion %	(90.0) ^f	(90.0)	Hemicellulose conversion %	(90.0)	96.0
Fermentation	Glucose to ethanol yield %	(95.0)	(95.0)	Glucose to ethanol yield %	(95.0)	(95.0)
	Pentose to ethanol yield %	(85.0)	(85.0)	Pentose to ethanol yield %	(85.0)	(85.0)
Ethanol yield	MM gal/year	52.2	47.6	MM gal/year	42.2	43.5
	%	83.1	62.3	%	67.2	74.4

EX: extrusion pretreatment; DAH: dilute acid hydrolysis

^a Yoo et al. (2011b)

^b Karuppuchamy and Muthukumarappan (2009)

^c Yoo et al. (2011b)

^d Corredor et al. (2008)

^e Manual feeding

^f Numbers in parenthesis indicate that they were assumed for simulation according to Aden et al. (2002).

Table 4.2 Total ethanol production cost analysis for dilute acid hydrolysis

1. Capital cost				\$M
Feed handling (A1) ^a				10.95
Pretreatment, etc. (B-D1~B-D2) ^b				27.74
Neutralization/conditioning (B-D3~B-D4) ^b				11.39
Saccharification & fermentation (C1 & C2)				13.72
Distillation & solid recovery (C3)				31.83
Wastewater treatment (C5)				4.82
Storage				2.92
Boiler/turbogenerator (C6 & C7)				55.92
Utility				6.86
<u>Total installed equipment cost</u>				166.15
Site development (15% of total installed equip.)				24.92
<u>Total project investment</u>				191.07
2. Operating variable cost				\$MM/year
Feedstock (A1)				73.85
Cellulase (C1)				7.00
Corn steep liquor (C2)				4.40
Other raw material cost				8.81
Waste disposal				4.63
<u>Total variable cost</u>				98.69
3. Operating fixed cost				
	Salary (\$/year)	Personnel		\$MM/year
Office Support	24718	5		0.12
General Manager	123592	1		0.12
Lab Manager	61796	1		0.06
Lab Technician	30898	2		0.06
Maintenance Supervisor	74155	1		0.07
Maintenance Technician	34606	8		0.28
Plant Engineer	80335	1		0.08
Plant Manager	98873	1		0.10
Shift Operators	30898	20		0.62
Shift Supervisor	45729	5		0.23
Yard Employees	24718	32		0.79
<u>Total Salaries</u>				2.54
Fringe and benefit (32% of labor)				0.81
<u>Total fixed operating cost</u>				3.35

^aRefer to Fig. 4.1 for codes shown in capital cost and variable operating cost. Codes represent major unit operation.

^bRefer to Appendix B.2 for detailed capital cost for pretreatment, neutralization, conditioning, etc.

Table 4.3 Total ethanol production cost analysis for extrusion process

1. Capital cost				\$MM
Feed handling (A1) ^a				10.95
Pretreatment (B-E1)				25.00
Saccharification & fermentation (C1 & C2)				13.72
Distillation & solid recovery (C3)				31.83
Wastewater treatment (C5)				4.82
Storage				2.92
Boiler/turbogenerator (C6 & C7)				55.92
Utility				6.86
<u>Total installed equipment cost</u>				152.02
Site development (15% of total installed equip.)				22.80
<u>Total project investment</u>				174.82
2. Operating variable cost				\$MM/year
Feedstock (A1)				73.85
Cellulase (C1)				7.00
Corn steep liquor (C2)				4.40
Waste disposal				4.63
<u>Total variable cost</u>				89.88
3. Operating fixed cost				\$MM/year
	Salary (\$/year)	Personnel		
Office Support	24718	5		0.12
General Manager	123592	1		0.12
Lab Manager	61796	1		0.06
Lab Technician	30898	2		0.06
Maintenance Supr	74155	1		0.07
Maintenance Tech	34606	8		0.28
Plant Engineer	80335	1		0.08
Plant Manager	98873	1		0.10
Shift Operators	30898	9		0.28
Shift Supervisor	45729	5		0.23
Yard Employees	24718	32		0.79
<u>Total Salaries</u>				2.20
Fringe and benefit (32% of labor)				0.70
<u>Total fixed operating costs</u>				2.90

^aRefer to Fig. 4.1 for codes shown in capital cost and variable operating cost. Codes represent major unit operation.

Table 4.4: Summary of randomized simulation results^a (\$MM)

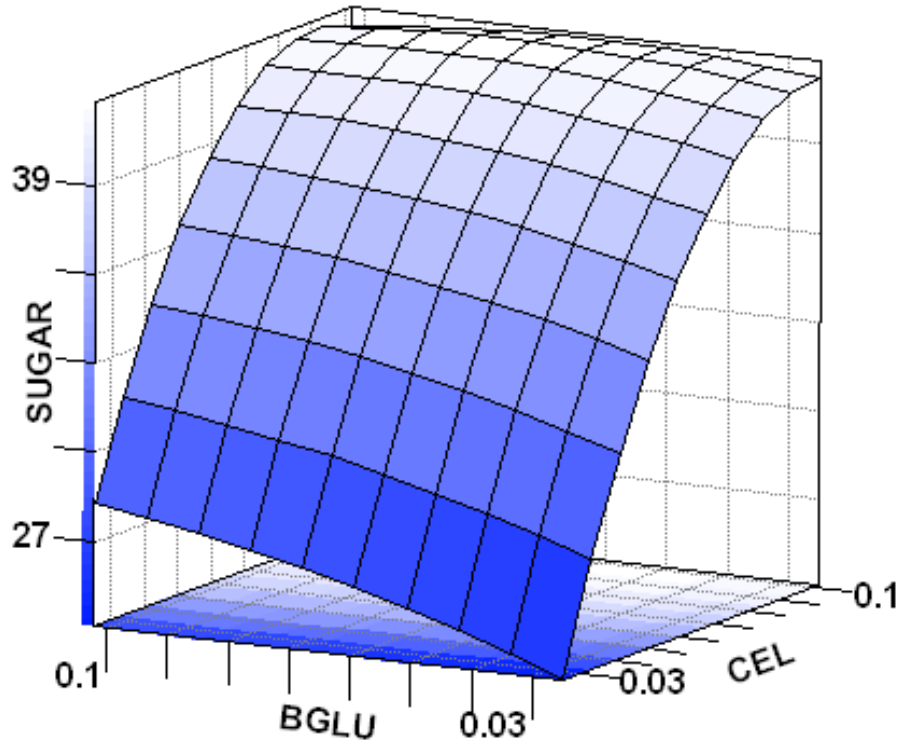
Variables	Years	Minimum	Maximum	Mean	SD
DAH Revenue	20	83.71	98.17	90.83	3.84
Extrusion Revenue	20	84.53	106.76	94.48	6.19
DAH Total Cost	20	37.61	71.05	54.33	10.41
Extrusion Total Cost	20	23.02	43.48	33.25	6.37
DAH Variable Cost	20	34.03	64.28	49.16	9.42
Extrusion Variable Cost	20	19.92	37.62	28.77	5.51
DAH Operations Cost	20	3.58	6.76	5.17	0.99
Extrusion Operations Cost	20	3.10	5.86	4.48	0.86
DAH EBITDA	20	20.64	48.14	36.50	9.14
Extrusion EBITDA	20	43.90	74.92	61.24	7.86
DAH Tax Bill	20	6.20	12.39	9.60	1.82
Extrusion Tax Bill	20	14.19	23.01	18.53	2.27
DAH Net Income	20	13.41	35.75	26.90	7.74
Extrusion Net Income	20	28.54	52.78	42.71	6.35

DAH: dilute acid hydrolysis pretreatment

EBITDA: Earnings Before Interest, Taxes, Depreciation and Amortization

^a soybean hull price at \$10/ton

Appendix A - Predicted response surface plot



Fixed levels: cell wall degrading (g/g cellulose) = 0.102

Figure A.1 Predicted response surface for reducing sugar released from soybean hull pretreated with acid hydrolysis, CEL=cellulase (ml/g cellulose), BGLU= β -glucosidase (ml/g cellulose). Concentration of cell-wall degrading enzyme complex fixed at 0.102 ml/g cellulose; SUGAR=reducing sugar release g/g soybean hull.

Appendix B - Equipment and Cost Estimates for Pretreatment (in 2008 \$MM)

Table B.1 Installed equipment costs for extrusion pretreatment

Number	Equipment Name	Equipment Cost	Share
14	Preconditioner/Screw Feeder/Extruder	19.66	78.6%
14	Saccharification Feed Pump	3.63	14.5%
14	Conveyor Feeders	1.62	6.5%
1	Slurrying tank	0.09	0.4%
	Total installed equipment cost	25.00	100%

^a (B-E1) shown in Fig. 4.1

Table B.2 Installed equipment costs for dilute acid hydrolysis^a

Number	Equipment Name	Equipment Cost	Share
3	Prehydrolysis/Screw Feeder/Reactor	25.20	63.94%
3	Pneumapress Filter	7.04	17.97%
2	Beer Column Feed Economizer	1.38	3.52%
2	Saccharification Feed Pump	0.86	2.20%
2	Waste Vapor Condensor	0.48	1.21%
1	Wash Filtrate Pump	0.39	1.00%
1	Hydroclone & Rotary Drum Filter	0.39	0.99%
1	Primary Filtrate Pump	0.34	0.87%
1	Reacidification Tank	0.34	0.86%
1	LimeDust Vent Baghouse	0.31	0.80%
1	Lime Storage Bin	0.26	0.67%
2	Pneumapress Feed Pump	0.22	0.55%
1	Overliming Tank	0.21	0.53%
1	Lime Unloading Blower	0.21	0.53%
1	Hydrolysate Washed Solids Belt Conveyor	0.16	0.42%
1	Reacidification Tank Agitator	0.15	0.38%
1	Filtered Hydrolyzate Pump	0.13	0.34%
1	Reacidified Liquor Pump	0.13	0.34%
1	Overlimed Hydrolyzate Pump	0.13	0.33%
1	Primary Filtrate Tank	0.13	0.33%
1	Blowdown Tank	0.12	0.30%
1	Hydrolyzate Screw Conveyor	0.12	0.29%
1	Wash Filtrate Tank	0.10	0.24%
1	Slurrying Tank	0.09	0.23%
1	Hydrolyzate Cooler	0.07	0.19%
1	Reslurrying Tank Agitator	0.07	0.18%
1	Sulfuric Acid Pump	0.07	0.18%
1	Hydrolyzate Mixing Tank	0.06	0.15%
1	Hydrolyzate Mix Tank Agitator	0.05	0.13%
1	Overliming Tank Agitator	0.05	0.13%
1	Pneumapress Vent Condensor	0.04	0.11%
1	Sulfuric Acid Tank	0.02	0.05%
1	Lime Solids Feeder	0.08	0.02%
1	In-line Sulfuric Acid Mixer	0.04	0.01%
	Total installed equipment cost	39.19	100%

^a (B-D1) through (B-D4) shown in Fig. 4.1

Appendix C - Model equation for base scenario

AH_Capital(t) = AH_Capital(t - dt)

INIT AH_Capital = 191074849.023091

Extrusion_Capital(t) = Extrusion_Capital(t - dt)

INIT Extrusion_Capital = 174821733.57016

UNATTACHED:

AH_Accumulated__Depreciation = AH_Depreciation*TIME

UNATTACHED:

AH_Biomass__Inflow = IF(TIME<1) THEN(0) ELSE(RANDOM((Daily_Biomass*Annual_Operating_Days - 2*Std_Deviation_AH),(Daily_Biomass*Annual_Operating_Days+2*Std_Deviation_AH),3))

UNATTACHED:

AH_Capital_Value = IF(TIME<=AH_Depreciation_Rate) THEN(AH_Capital-AH_Accumulated__Depreciation) ELSE(0)

UNATTACHED:

AH_Cash = AH_Cashflow

UNATTACHED:

AH_EBITDA = AH_Revenue-AH_Total_Cost

UNATTACHED:

AH_Ethanol = AH_Fermentation*Gallon_Conversion*1000/Ethanol_Density

UNATTACHED:

AH_Fermentation = Fermentation_Factor*(AH_Sugar_Cellulose*Cellulose_Fermentation_AH+AH_Sugar__Hemicellulose*Hemi_Fermentation_AH)

UNATTACHED:

AH_Net_Earnings = (AH_EBITDA-AH_Tax)

UNATTACHED:

AH_Operations = IF(TIME<1) THEN(0) ELSE(AH_HR*(1+Inflation_Adjustment*TIME))

UNATTACHED:

AH_Revenue = AH_Ethanol*Price

UNATTACHED:

AH_Sugar_Cellulose = AH_Biomass__Inflow*((Cellulose_Fraction_AH*Cellulose__Hydrolysis_AH))

UNATTACHED:

AH_Sugar__Hemicellulose = AH_Biomass__Inflow*((Hemi__Hydrolysis_AH*Hemi_Fraction_AH))

UNATTACHED:

AH_Tax = IF(AH_EBITDA>0)THEN((AH_EBITDA-AH_Depreciation)*Corp_Tax_Rate)ELSE(0)

UNATTACHED:

AH_Total_Cost = IF(TIME<1)THEN(AH_Capital+AH_Capital_Contingency)ELSE(AH_Operations+AH_Variable_Cost)

UNATTACHED:

AH_Variable_Cost = IF(TIME<1) THEN(0) ELSE((AH_Biomass__Inflow*(Biomass_Price+AH_Avg_Utilities+AH_Avg_Variable__Cost))*(1+Inflation_Adjustment*time))

UNATTACHED:

Extrusion_Accumulated_Depreciation = EX_Depreciation*TIME

UNATTACHED:

Extrusion_Biomass_Inflow = IF(TIME<1) THEN(0) ELSE(RANDOM((Daily_Biomass*Annual_Operating_Days - 2*Std_Deviation_Extrusion),(Daily_Biomass*Annual_Operating_Days+2*Std_Deviation_Extrusion),7))

UNATTACHED:

Extrusion_Capital_Value = IF(TIME<=EX_Depreciation_Rate) THEN(Extrusion_Capital-Extrusion_Accumulated_Depreciation) ELSE(0)

UNATTACHED:

Extrusion_Tax_Bill = IF(Extrusion_EBITDA1>0) THEN((Extrusion_EBITDA1-EX_Depreciation)*Corp_Tax_Rate)ELSE(0)

UNATTACHED:

EX_Cash = EX_Cashflow

UNATTACHED:

EX_EBITDA = EX__Revenue - EX_Total_Cost

UNATTACHED:

EX_Ethanol = EX_Fermentation*1000*Gallon_Conversion/Ethanol_Density

UNATTACHED:

EX_Fermentation = (EX_Sugar_Cellulose*EX_Cellulose_Fermentation+EX_Sugar_Hemicellulose*EX_Hemi__Fermentation)*Fermentation_Factor

UNATTACHED:

EX_Net_Earnings = (EX_EBITDA-EX_Tax)

UNATTACHED:

EX_Operations = IF(TIME<1) THEN(0) ELSE(EX_HR*(1+Inflation_Adjustment*TIME))

UNATTACHED:

EX_Sugar_Cellulose = Extrusion_Biomass_Inflow*Cellulose_Fraction_EX*Cellulose_Hydrolysis_EX

UNATTACHED:

EX_Sugar_Hemicellulose = Extrusion_Biomass_Inflow*Hemi__Fraction_EX*Hemi_Hydrolysis_EX

UNATTACHED:

EX_Tax = IF(EX_EBITDA>0) THEN((EX_EBITDA-EX_Depreciation)*Corp_Tax_Rate) ELSE(0)

UNATTACHED:

EX_Total_Cost = IF(TIME<1)THEN(Extrusion_Capital+EX_Capital_Contingency) ELSE(EX_Operations+EX_Variable_Cost)

UNATTACHED:

EX_Variable_Cost = IF(TIME<1) THEN(0) ELSE((Extrusion_Biomass_Inflow*(Biomass_Price+EX_Avg_Variable_Cost+Extrusion_Avg_Utilities))*(1+Inflation_Adjustment*time))

UNATTACHED:

EX__Revenue = EX_Ethanol*Price

AH_Avg_Variable__Cost = 35.4898266767144

AH_Capital_Contingency = AH_Capital*Contingency_Rate

AH_Cashflow = IF(TIME=1) THEN(-1*(AH_Capital_Contingency+AH_Capital)) ELSE(AH_Net_Earnings)

AH_Depreciation = IF(TIME<= AH_Depreciation_Rate) THEN(AH_Capital/AH_Depreciation_Rate) ELSE(0)

AH_Depreciation_Rate = 15

AH_EBITDA1 = AH_Revenue-AH_Total_Cost

AH_HR = 3350918.28220859

AH_Net_Income = AH_EBITDA1-AH_Tax_Bill

AH_tax_Bill = IF(AH_EBITDA1>0) THEN((AH_EBITDA1-AH_Depreciation)*Corp_Tax_Rate)ELSE(0)

Annual_Operating_Days = 350

Biomass_Price = 105.5

Cellulose_Fermentation_AH = .95

Cellulose_Fraction_AH = .3535

Cellulose_Fraction_EX = 0.3535

Cellulose_Hydrolysis_EX = .948

Cellulose__Hydrolysis_AH = 0.692

Contingency_Rate = 0.15
Corp_Tax_Rate = 0.35
Daily_Biomass = 2000
Ethanol_Density = 0.79
Extrusion_Avg_Utilities = 0
Extrusion_EBITDA1 = EX__Revenue-EX_Total_Cost
Extrusion_Net_Income = Extrusion_EBITDA1-Extrusion_Tax_Bill
EX_Avg_Variable_Cost = 16.620734201744
EX_Capital_Contingency = Extrusion_Capital*Contingency_Rate
EX_Cashflow = IF(TIME=1) THEN(-1*(EX_Capital_Contingency+Extrusion_Capital)) ELSE(EX_Net_Earnings)
EX_Cellulose_Fermentation = .95
EX_Depreciation = IF(TIME<= EX_Depreciation_Rate) THEN(Extrusion_Capital/EX_Depreciation_Rate) ELSE(0)
EX_Depreciation_Rate = 15
EX_Hemi__Fermentation = .85
EX_HR = 2902280.24539877
Fermentation_Factor = .51
Gallon_Conversion = 0.26417
Hemi_Fermentation_AH = .85
Hemi_Fraction_AH = .1721
Hemi_Hydrolysis_EX = .9
Hemi__Fraction_EX = 0.1721
Hemi__Hydrolysis_AH = .9
Inflation_Adjustment = 0.05
Price = 1.90
Std_Deviation_AH = 0
Std_Deviation_Extrusion = 0

Appendix D - Production cost, revenues, and net financial situation for dilute acidic hydrolysis and extrusion pretreatment

Table D.1 Randomized model simulation at \$10/ton soybean hull cost

Year	Fixed cost (\$MM/year)		Variable cost (\$MM/year)		Total cost (\$MM/year)		Ethanol production (gal/year)		Revenue (\$MM/year)		EBITDA (\$MM/year)		Tax bill (\$MM/year)		Net income (\$MM/year)		
	DHA	EX	DHA	EX	DHA	EX	DHA	EX	DHA	EX	DHA	EX	DHA	EX	DHA	EX	
Initial																-219.74	-201.04
1	3.58	3.10	34.03	19.92	37.61	23.02	44.65	45.48	84.84	86.41	47.23	63.40	12.07	18.11	35.16	45.29	
2	3.75	3.25	35.62	20.85	39.37	24.09	46.06	50.16	87.52	95.30	48.14	71.20	12.39	20.84	35.75	50.36	
3	3.92	3.39	37.22	21.78	41.13	25.17	46.69	46.83	88.70	88.97	47.57	63.80	12.19	18.25	35.38	45.55	
4	4.08	3.54	38.81	22.71	42.89	26.25	45.26	53.25	86.00	101.17	43.10	74.92	10.63	22.14	32.48	52.78	
5	4.25	3.68	40.40	23.64	44.65	27.32	44.06	44.49	83.71	84.53	39.06	57.21	9.21	15.94	29.85	41.26	
6	4.42	3.83	41.99	24.57	46.41	28.40	48.08	46.28	91.36	87.94	44.95	59.53	11.27	16.76	33.67	42.78	
7	4.59	3.97	43.58	25.51	48.17	29.48	47.72	49.51	90.66	94.07	42.49	64.59	10.41	18.53	32.08	46.06	
8	4.75	4.12	45.18	26.44	49.93	30.56	48.74	50.67	92.61	96.28	42.68	65.72	10.48	18.92	32.20	46.80	
9	4.92	4.26	46.77	27.37	51.69	31.63	48.11	53.03	91.42	100.75	39.73	69.12	9.45	20.11	30.28	49.00	
10	5.09	4.41	48.36	28.30	53.45	32.71	50.44	50.18	95.83	95.33	42.38	62.62	10.37	17.84	32.00	44.79	
11	5.26	4.55	49.95	29.23	55.21	33.79	50.55	53.70	96.04	102.04	40.83	68.25	9.83	19.81	31.00	48.44	
12	5.42	4.70	51.55	30.16	56.97	34.86	47.52	50.48	90.30	95.91	33.33	61.05	7.21	17.29	26.12	43.76	
13	5.59	4.84	53.14	31.10	58.73	35.94	50.79	46.38	96.50	88.13	37.77	52.19	8.76	14.19	29.01	38.00	
14	5.76	4.99	54.73	32.03	60.49	37.02	47.86	51.46	90.93	97.77	30.44	60.76	6.20	17.19	24.25	43.57	
15	5.93	5.13	56.32	32.96	62.25	38.09	48.42	56.19	91.99	106.76	29.74	68.66	9.30	23.01	20.45	45.65	
16	6.09	5.28	57.91	33.89	64.01	39.17	46.30	49.74	87.96	94.50	23.96	55.33	8.38	19.37	15.57	35.97	
17	6.26	5.42	59.51	34.82	65.77	40.25	51.67	51.91	98.17	98.62	32.40	58.37	11.34	20.43	21.06	37.94	
18	6.43	5.57	61.10	35.75	67.53	41.32	47.11	46.23	89.52	87.83	21.99	46.51	7.70	16.28	14.29	30.23	
19	6.60	5.71	62.69	36.69	69.29	42.40	47.78	52.62	90.78	99.98	21.50	57.58	7.52	20.15	13.97	37.43	
20	6.76	5.86	64.28	37.62	71.05	43.48	48.25	45.99	91.68	87.38	20.64	43.90	7.22	15.37	13.41	28.54	
Avg.	5.17	4.48	49.16	28.77	54.33	33.25	47.80	49.73	90.83	94.48	36.50	61.24	9.60	18.53	26.90	42.71	
Std.	0.97	0.84	9.18	5.37	10.15	6.21	1.97	3.18	3.74	6.04	8.91	7.66	1.77	2.21	7.54	6.19	
NPV																76	234

IRR

13%

23%

DAH: dilute acid hydrolysis pretreatment; EX: extrusion pretreatment.