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PROCESS DEVELOPMENT FOR THE FRACTIONATION AND ISOLATION OF CORN FIBER HEMICELLULOSE

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PROCESS DEVELOPMENT FOR THE FRACTIONATION AND ISOLATION OF CORN FIBER HEMICELLULOSE

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ABSTRACT

Corn fiber is a co-product of the corn wet-milling process that holds potential to become a value-added product. A process was developed to fractionate and isolate the hemicellulose B component of corn fiber generated by corn wet milling. The process consisted of pretreatment by soaking in aqueous ammonia (SAA) of starch-free corn fiber followed by enzymatic cellulose hydrolysis, during which the hemicellulose B was solubilized by cleavage into xylo-oligosaccharides. The hemicellulose A and B fractions were separated by adjustment of pH, and the hemicellulose B recovered by precipitation with ethanol.

The pretreatment step resulted in a high retention of major sugars, with 94% of initial glucan, 76% of initial xylan, and 78% of initial arabinan remaining in the pretreated material. Additionally, the pretreated material demonstrated a high glucan digestibility, with 85% of available glucan released as glucose after 72 hours of hydrolysis with cellulase. Xylan and arabinan digestibilities were low and very little xylan and arabinan remained in the solid phase after hydrolysis, indicating their cleavage to soluble xylo-oligosaccharides. A mass balance conducted around the process accounted for 87% of the initially present glucan, 91% of the initially present xylan, and 90% of the initially present arabinan.

The hemicellulose B was then hydrolyzed by a cocktail of enzymes that consisted of β-glucosidase, pectinase, xylanase, and ferulic acid esterase. Used by itself, the xylanase was very ineffective, demonstrating yields of less than 2% of xylose and arabinose. The greatest xylose and arabinose yields, 44% and 53%, respectively, were

obtained by the combination of pectinase, used at 100 x manufacturer recommended dosage, and ferulic acid esterase, loaded at 10 x manufacturer recommended dosage. Addition of xylanase to this mixture had very little effect, increasing xylose yield by 0.03% while decreasing arabinose yield by 0.44%.

The glucose solutions resulting from the hydrolysis of the cellulose and starch fractions were then utilized in ethanol fermentation. They were combined in equal volumes and used instead of water to produce a corn mash, which was fermented for 70 hours. Compared to the fermentation of corn mashed with water, the use of the glucose solutions resulted in an increase of ethanol concentration in the beer of 2% (v/v). The overall fermentation efficiency was increased by 7% when the hydrolysis solutions were used in mashing.

DEDICATION

This work is dedicated to my parents, Peter and Deborah Montanti, for their love, support, and encouragement; and to Sarah Lovesky for her unfailing companionship and support. I would also like to thank Rebecca Crane, Lauren Staples, and Keelin Cassidy, whose friendship has been of great importance to me.

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CHAPTER ONE

INTRODUCTION

"The fuel of the future is going to come from fruit like that sumac out by the road, or from apples, weeds, sawdust -- almost anything. There is fuel in every bit of vegetable matter that can be fermented. There's enough alcohol in an acre of potatoes to drive the machinery necessary to cultivate the field for a hundred years."

-- Henry Ford, New York Times, 1925.

BACKGROUND INFORMATION

A Historic and Market View of Ethanol Production in the United States

Ethanol has been produced as a commodity in the US for over a century, predominantly from corn. The early Ford Model T was designed with the capability of utilizing both ethanol and gasoline as a fuel. Ethanol was used as a fuel until the emergence of cheap and abundant petroleum-based fuels following World War II. Ethanol did not come into favor again until the oil embargo in the 1970s spawned a renewed interest in alternatives to petroleum-based fuels. The US ethanol production in 1979 was approximately 10 million gallons (Bothast and Schlicher, 2005). Since that time, the industry has grown steadily, with production increasing rapidly during the last ten years. According to the Renewable Fuels Association (RFA), US ethanol production reached a record 10.6 billion gallons in 2009 (Figure 1.1) (RFA, 2010).

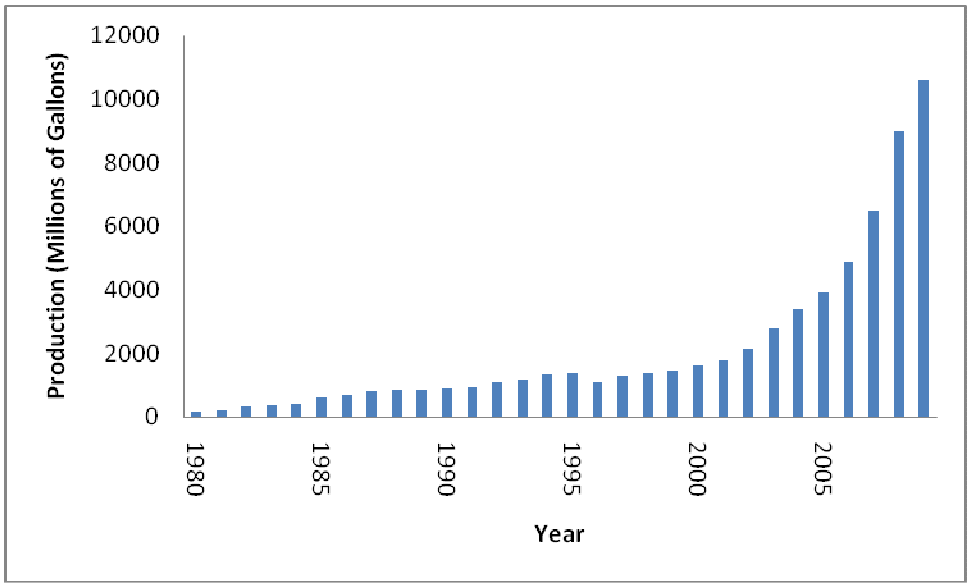

Figure 1.1. Historic ethanol production in the United States from 1980 to 2009 (RFA, 2010).

The tremendous growth in the fuel ethanol industry over the past several decades is due to a variety of factors. In 1990, congress amended the Clean Air Act (CAA), mandating the use of oxygenated fuels. Methyl tertiary-butyl ether (MTBE) was the primary oxygenating agent blended with gasoline. However, due to its detrimental health and environmental effects, MTBE has largely been replaced with ethanol. Ethanol is attractive as a fuel and a fuel oxygen source for several reasons. As a fuel additive, ethanol improves the octane level of gasoline and replaces MTBE as an oxygen source. From an environmental perspective, ethanol burns more cleanly than gasoline, and can be produced from renewable feedstocks (Bothast and Schlicher, 2005). The net energy value (NEV) of a compound is a measure of the amount of energy gained or lost in the production of that compound, i.e. the difference between the energy contained in the final product and the energy consumed in its production. A positive NEV represents a net energy gain, while a negative NEV represents a net loss of energy. The NEV of ethanol is positive, whereas gasoline exhibits a negative NEV. Although NEV data for ethanol varies widely due to model

assumptions and technological advances, the United States Department of Agriculture (USDA) reported corn based ethanol's NEV to be 1.34, i.e. a 34% energy gain. Conversely, the NEV of gasoline was estimated in the same study to be 0.805, a 19.5% energy loss (Shapouri et al, 2002).

The trend of increasing ethanol production is expected to continue in the near future. In a 2005 amendment to the CAA, the Renewable Fuels Standard (RFS1) was created. RFS1 mandated the blending of fuels from renewable sources into the US transportation fuel market. In May 2009, the United States Environmental Protection Agency (EPA) proposed a revised renewable fuels standard (RFS2), which was finalized in February 2010. Under RFS2, the use of 11.1 billion gallons of renewable fuels was mandated for 2009, increasing to 12.95 billion gallons in 2010. RFS2 extends through 2022, when the use of 36 billion gallons of renewable fuels is mandated (EPA, 2009). These requirements are *in addition to* ethanol blended with gasoline as an oxygen source (Figure 1.2). The EPA considers a fuel to be renewable if it reduced green house gas (GHG) emissions (determined by lifecycle analysis) by at least 20% as compared to gasoline. Advanced biofuels are those that reduce lifecycle GHG emissions by 50%, and cellulosic biofuels are those that reduce lifecycle GHG emissions by 60% (EPA, 2010).

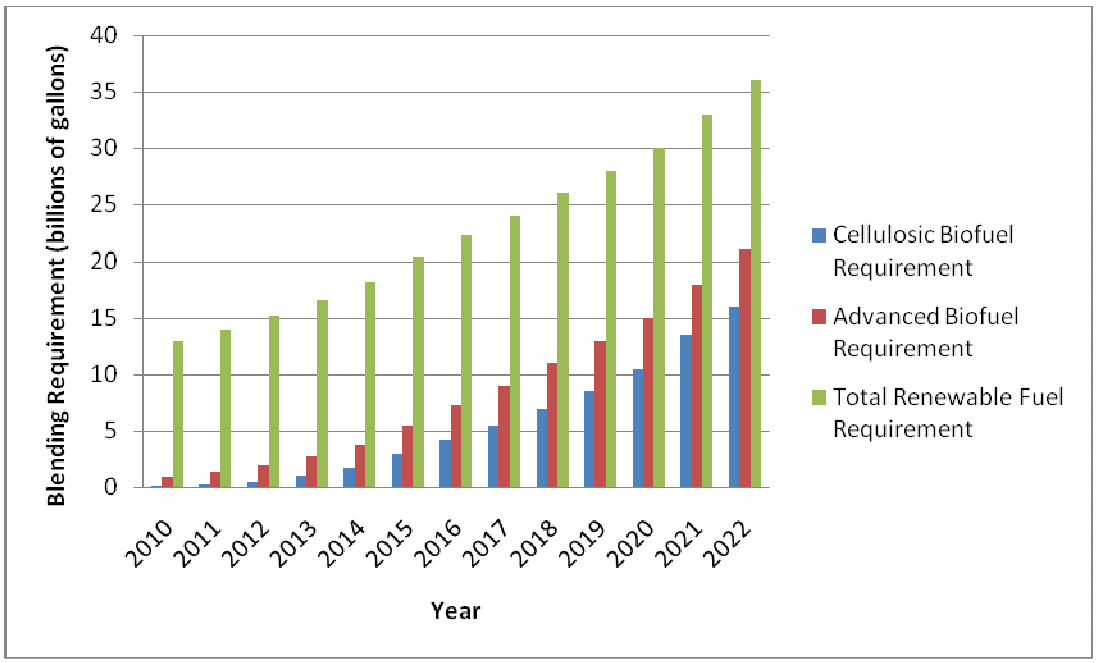

Figure 1.2. Renewable Fuels Standard 2 blending requirements (EPA, 2010).

Federal regulation such as RFS1 and 2, as well as legislative efforts to increase the maximum allowable ethanol blend (10% v/v, currently); contribute to the expectation that a market for ethanol will exist in the near future (RFA, 2009, RFA, 2010).

Ethanol Feedstocks and Biomass Conversion

Although potential feedstocks for ethanol production are many, they consist primarily of sugars derived from plant biomass. These sugars are typically in the form of cellulose or starch. In the United States, the large majority of fuel ethanol production currently is from starch derived from corn. The production of ethanol from cellulose is an industry in its infancy. Much work has been done, and many processes developed, on the laboratory and pilot scale. Commercialization of these processes, however, is only just beginning. As of February 2010, at least 28 cellulosic ethanol facilities were under construction in the United States, utilizing a wide

variety of cellulosic feedstocks (RFA, 2010). Biofuels Digest, an industry publication, surveyed biofuels producers on biomass ethanol plant capacity. Cellulose to ethanol capacity is predicted to grow rapidly, similar to the growth in corn ethanol production over the past ten years (Figure 1.3) (Biofuels Digest, 2010).

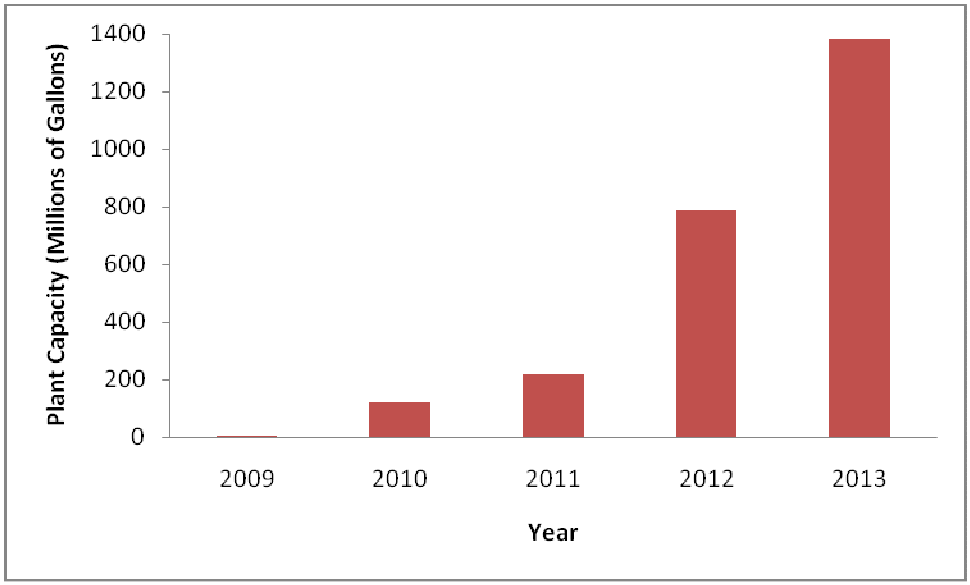

Figure 1.3. Predicted near-term cellulosic ethanol capacity based on existing and planned plant construction projects (Biofuels Digest, 2010).

Lignocellulosic Biomass

Cellulosic biomass used for ethanol production may be derived from many sources, including dedicated energy crops such as switchgrass or hybrid poplar, agricultural residues such as corn stover or corn fiber, forest residues such as woodchips, or waste streams from paper processing or municipal solid waste. Producing enough ethanol to meet any significant portion of current transportation fuel demand will require the development of ethanol production processes utilizing feedstocks that are available sustainably in large quantities and capable of generating

fermentable sugars at low cost. Lignocellulosic feedstocks are the only ones that are available in sufficient quantities to meet fuel demands (Bothast and Schlicher, 2005). Lignocellulosic biomass, regardless of source, consists primarily of three major components; cellulose, hemicellulose, and lignin. Cellulose and hemicellulose are carbohydrate polymers, and lignin is a complex phenolic polymer. Each of these components will be considered below.

Cellulose

According to the US Department of Energy (DOE), cellulose is the most abundant biological material on earth, and thus represents enormous potential for ethanol production (US DOE, 2006). Cellulose is a chemically homogenous linear polymer of β-1,4 linked D-glucose monomers (Figure 1.4).

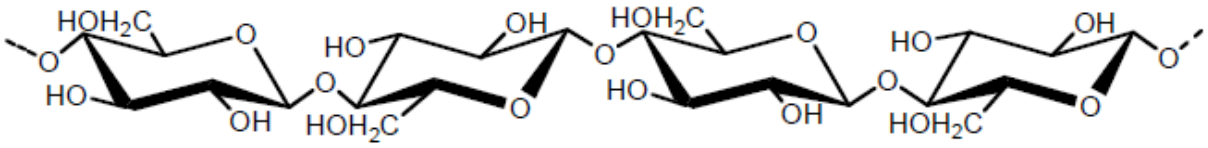

Figure 1.4. Cellulose structure (NSF, 2008).

The repeat unit of the polymer is cellobiose, which consists of two glucose monomers. Linear cellulose chains in biomass are typically arranged parallel to one another, resulting in a highly ordered and crystalline structure. Tight packing of cellulose molecules within this crystalline structure prohibits penetration by large molecules such as cellulose degrading enzymes, contributing to the recalcitrance of native biomass. Although the composition of cellulosic biomass varies widely, cellulose content typically represents one third to half of the dry weight of biomass (Lynd et al, 2002). Because it consists solely of glucose, cellulose, once broken down

to its monomeric component, is fermentable by *Saccharomyces cerevisiae*, the yeast used universally for ethanol production from corn starch and cane sugar. Thus, the challenges to utilizing cellulose in ethanol production arise primarily from its recalcitrance to hydrolysis. Overcoming this recalcitrance will be considered in more detail later.

Hemicellulose

Hemicellulose is the second most abundant polysaccharide in nature. It typically represents about 20 – 35% of the dry weight of biomass (Saha, 2003). Hemicellulose is a heterogeneous polymer consisting primarily of pentose and hexose sugars, plus associated sugar acids. The composition of hemicellulose varies widely across biomass sources. Xylans, hemicelluloses based on a xylan backbone, are considered the most abundant type of hemicellulose in nature (Saha, 2003). An example of xylan, derived from corn fiber, is shown in Figure 1.5.

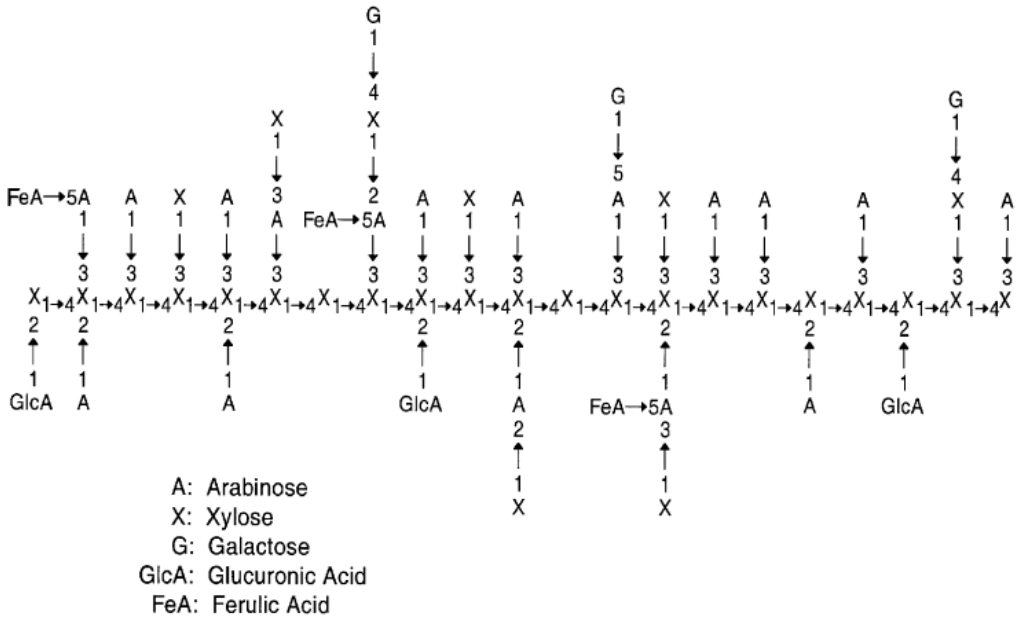

Figure 1.5. Structure of corn fiber hemicellulose. From (Saha, 2003).

Because pentose sugars, common in hemicellulose, are not fermentable by *S. cerevisiae*, alternative fermentation strategies for ethanol must be developed. It is clear, however, that due to its abundance in biomass, the utilization of hemicellulose is critical to the efficient utilization of cellulosic biomass for commercial ethanol production.

The amount, structure, and composition of hemicellulose vary widely across different biomass sources. Hemicellulose occurs in association with cellulose in the cell wall, and there is evidence suggesting that it plays a role in the regulation of cell wall biosynthesis (Lynd et al, 2002). Unlike cellulose, hemicellulose occurs most frequently with a branched, non-crystalline structure. Carbohydrates commonly found in hemicellulose include glucose, galactose, mannose, xylose, and arabinose. Xylose is the most common component of hemicellulose in many plants (Lynd, 1996). While usage of the term hemicellulose is considered correct, to account for the wide variation in their chemical composition, hemicelluloses are more specifically named for their major carbohydrate components. Arabinoxylan, the hemicellulose found in agricultural

products such as wheat or corn fiber, consists primarily of xylose and arabinose (Saha, 2003). Galactoglucomannan, the hemicellulose found in softwood such as spruce, consists primarily of galactose, glucose, and mannose (Lundqvist et al, 2002).

Lignin

Lignin is a complex phenolic polymer that may account for up to 30% of the dry weight of biomass (Lynd, 1996). It is composed primarily of three building blocks, syringyl alcohol, coniferyl alcohol, and ρ-coumaryl alcohol. The exact structure of lignin is variable and not fully characterized, though an example is shown in Figure 1.6.

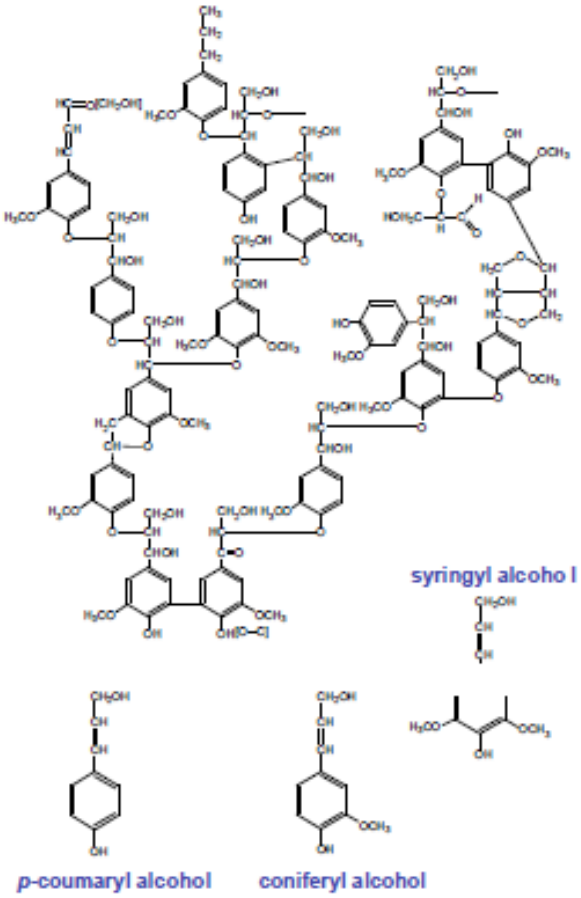

Figure 1.6. Partial structure of lignin and lignin components (NSF, 2008).

Lignin is not a source of biomass carbohydrate, limiting its ability to be converted to ethanol by biochemical means. Cellulose and hemicellulose may be converted to ethanol by hydrolysis and subsequent fermentation of the sugars. Lignin, however, must be gasified to synthesis gas prior to conversion to ethanol. The gasification process has received significantly less attention than the hydrolysis / fermentation process (Drapcho et al, 2008). However, with recent process improvements, at least two cellulosic ethanol plants are under construction utilizing this platform (Biofuels Digest, 2010).

Lignocelluloses from Agricultural Residues

Agricultural residues are materials left on the field after harvest, or byproducts from processing of agricultural products. Examples include corn stover (corn stalks and leaves), wheat straw, barley straw, sugarcane bagasse, rice hull, barley hull, and corn fiber. An estimated 113 million MT / yr of dry agricultural residues are available in the US, with corn stover representing the single largest source (Drapcho et al, 2008). As ethanol production from corn is a mature industry, co-products generated in corn ethanol plants are of particular interest. Corn ethanol is produced typically by either the dry grind or the wet milling process (Rausch and Belyea, 2006). The marketing of co-products is considered critical to the economic viability of ethanol (Bothast and Schlicher, 2005). Typically, co-products from both wet milling and dry grind ethanol plants are marketed as animal feed. However, they are of relatively low monetary value. Because these co-products contain cellulosic material, they hold the potential for conversion into additional ethanol or high value-added products. Utilization of these co-products in such a way not only adds a revenue stream to the plant, but increases the value in other aspects of the process as well. For example, in a dry grind ethanol plant, the only co-product generated is distillers' dried grains

with solubles (DDGS), which is primarily sold as an animal feed. Because the dry grind process does not involve the fractionation of the corn kernel, the fiber fraction ends up in the DDGS. This material is not readily digested by non-ruminant animals, limiting DDGS to ruminant diets only. Fractionation of the fiber component, however, would generate an additional co-product, corn fiber, and increase DDGS value by reducing its fiber content, thereby expanding its market to non-ruminant diets (Rausch and Belyea, 2006).

Biochemical Conversion of Lignocellulosic Biomass to Ethanol

The conversion of biomass to ethanol may be accomplished by processes falling into two categories, biochemical and thermochemical. The thermochemical platform involves gasifying the entirety of the biomass to a synthesis gas, which is commonly referred to as syngas. The syngas contains primarily hydrogen and carbon monoxide, which can be converted to ethanol via fermentation or chemical catalysis. The biochemical platform has received significantly more attention, and will be the focus of this section. In this process, the carbohydrate components of the biomass are hydrolyzed to monosaccharides, which are then fermented to ethanol. This typically involves three steps; pretreatment, hydrolysis, and fermentation (Figure 1.7).

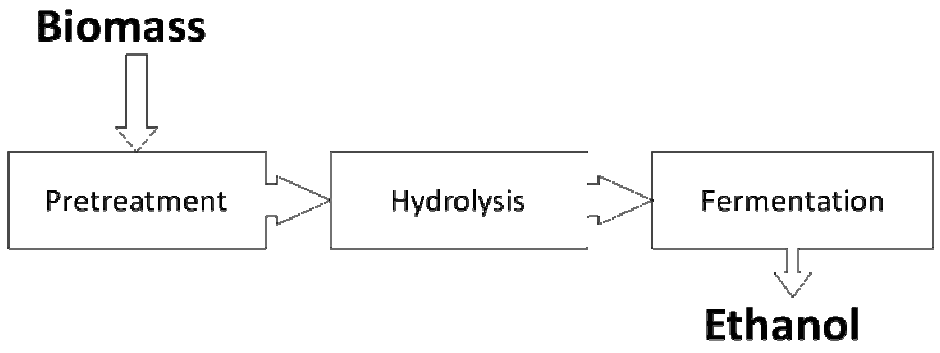

Figure 1.7. Schematic representation of biochemical cellulosic ethanol processing scheme.

While additional product recovery steps such as distillation are required, they will not be covered here as they are standard practice within the corn ethanol industry. Each of the three primary steps will be considered individually, although in practice options exist for their various combinations. The combined processing options will be discussed later.

Pretreatment

Typically the first step in the biochemical conversion of lignocellulose to ethanol is pretreatment. Because of the structure of cellulosic biomass and the recalcitrance that results, biomass in its native form is highly resistant to enzymatic attack. Pretreatment processes are designed to overcome this problem. The primary goal of pretreatment is to alter the structure of the biomass to increase the rate and yield of hydrolysis (Drapcho et al, 2008). Fermentable sugar yields in enzymatic hydrolysis of biomass without pretreatment (i.e. the hydrolysis of raw native biomass) are typically less than 20% of theoretical. After pretreatment, however, this value may increase to greater than 90% (Lynd, 1996). An ideal pretreatment will maximize yields while minimizing cost. More specifically, in addition to improving upon rates and yields in hydrolysis, a pretreatment process should involve the use of inexpensive materials and mild conditions to minimize capital costs, losses of sugars, and formation of fermentation inhibitors, produce minimal waste, and allow recycling of materials for reduced operating costs (Drapcho et al, 2008). Though considered ideal, no pretreatment technologies currently exist that meet all of the above criteria. The selection of a pretreatment process must be based on which of the criteria are most important given the feedstock or process the pretreatment is to be associated with. Several pretreatment processes are discussed below.

Dilute Acid (DA) Pretreatment

Dilute acid pretreatment has been considered a promising technology, and is favored by the National Renewable Energy Laboratory (NREL) (Lynd, 1996). It is capable of achieving high sugar recoveries. In DA pretreatment, biomass is exposed to a dilute acid. Most commonly, sulfuric acid is used. Much work has been done on the optimization of DA pretreatment conditions, typically by varying acid concentration, treatment temperature, and treatment time. In two typical studies utilizing corn fiber, acid concentrations ranging from 0.25% (v/v) to 1% (v/v) H_2SO_4 were attempted over a period of time ranging from 15 to 90 min. The treatment temperature was 121°C (Saha and Bothast, 1999, Buhner and Agblevor, 2004). In both cases, longer treatment time at higher acid concentrations resulted in increased sugar yields. However, Buhner and Agblevor (2004) noted the highest concentration of inhibitory compounds under these conditions.

Under acidic conditions and especially at elevated temperatures, a number of inhibitory compounds are formed. Pentoses may be degraded to furfural, and hexoses to 5-hydroxy-methyl furfural (5-HMF), which then may further degrade to levulinic acid and formic acid. Lignin may partially degrade to low molecular weight phenolic compounds (Larsson et al, 1999). In addition to the inhibitory effect these compounds have on fermentation, their formation from sugars means that some sugars were lost, resulting in a lower potential yield, even if the inhibition effects could be reduced or eliminated. In order to relieve fermentation inhibition, several methods aimed at the removal of these compounds have been developed. These include over liming, partial neutralization, activated charcoal treatment, anion exchange, and simple dilution.

Over liming is a process that involves increasing pH to values as high as 10 by the addition of calcium hydroxide. This degrades furfural and 5-HMF. However, readjustment of pH

to a much lower value (about 5) is necessary prior to hydrolysis and subsequent fermentation, contributing to significant increases in operating cost. A large amount of gypsum is also formed in the process, which must be removed and disposed of, further increasing operating cost (Buhner and Agblevor, 2004, Drapcho et al, 2008).

Partial neutralization involves the addition of calcium hydroxide to increase pH to 4.0. While this incurs less raw material and disposal costs than over liming, it has not been found to remove a significant portion of inhibitory compounds, thus necessitating additional treatment prior to fermentation. Treatment with activated charcoal has been combined with partial neutralization to some success, although the addition of an activated charcoal treatment again contributes to increased cost (Buhner and Agblevor, 2004). Anion exchange has also been utilized to effectively remove inhibitory compounds, but the process is expensive (Drapcho et al, 2008).

Dilution of the hydrolysate obtained by enzymatic hydrolysis of the pretreated material, to reduce the inhibitory compound concentrations to below inhibitory levels, can be effective in the relief of inhibition. In one study, non-diluted hydrolysate (derived from corn fiber) was found to inhibit fermentation, and no microbial growth occurred. Dilution with 2 volumes of water allowed microbial growth (Nghiem et al, 2009). However, such dilution lowers sugar concentrations and thus reduces product yields, and necessitates a significant increase in fermenter capacity or a reduction in plant flow through, resulting in increased cost or reduced revenue.

Liquid Hot Water (LHW) Pretreatment

Pretreatment with liquid hot water (LHW) effectively solubilizes a significant fraction of hemicellulose and lignin, resulting in a solid material containing primarily cellulose with an increased susceptibility to enzymatic hydrolysis. Unlike DA pretreatment, little or no inhibitory compounds are produced. LHW pretreatment has been reported to result in a cellulose rich fraction which is completely hydrolysable and less than 1% of carbohydrates lost to inhibitory compounds, keeping their concentration well below inhibitory levels. These results, determined on the lab scale, were duplicated on the industrial scale by the same investigators, demonstrating LHW pretreatment's ability to function at the industrial scale. The parameters used in the study were a reaction temperature of 160°C for 20 min at a pH above 4.0. This pH was chosen because above pH 4.0, there is minimal production of monosaccharides, which is the likely reason for the very low production of inhibitory compounds (Mosier et al, 2005, Kim et al, 2009). Kim et al reported similar results for these conditions (2008).

In addition to its low inhibitor production, LHW pretreatment requires very little pH adjustment or disposal of waste products, and is carried out under less corrosive conditions, all contributing to a reduced cost when compared to DA pretreatment (Taherzadeh and Karimi, 2008). However, because LHW solubilizes the hemicellulose fraction of the biomass, those sugars must either be recovered and hydrolyzed for fermentation or lost. In either case, the economic impacts upon the process must be considered, possibly offsetting any savings from the use of LHW (Drapcho et al, 2008).

Ammonia Fiber Explosion (AFEX) Pretreatment

The Ammonia Fiber Explosion (AFEX) process involves reacting biomass with anhydrous ammonia at elevated temperatures for a specified period of time. During the process, high pressures are achieved within the reaction chamber. At the end of the reaction time, the pressure is released rapidly, exploding the biomass into a collection vessel. Unlike the other processes discussed, this process does not result in a slurry requiring solid / liquid separation, simplifying its implementation. In fact, the only stream exiting the process (aside from the pretreated biomass) is a mixture of gaseous ammonia and water vapor (Drapcho et al, 2008). The high volatility of the ammonia provides two key benefits. First, the evaporation of ammonia into the gaseous phase effectively removes it from the biomass, minimizing the need for pH adjustment. Second, it facilitates simple recovery of the ammonia for reuse, leading to a significant savings in cost.

AFEX pretreatment is typically carried out at an anhydrous ammonia:dry biomass loading of 1:1 by mass under conditions ranging from ambient temperature to temperatures up to 120°C for just a few minutes (Hendriks and Zeeman, 2009). DDGS has been pretreated using the AFEX process by loading DDGS and anhydrous ammonia into a pressure vessel at a loading of 0.8 g/g DDGS (dry basis). The mixture was then heated to 70°C (the treatment temperature) over a period of 14 to 18 min. Once the treatment temperature was achieved, it was maintained for 5 min before pressure was rapidly released and the reactor allowed to cool (without any external heat transfer methods such as a water bath or refrigeration) to room temperature. Prior to explosive release, pressure in the vessel reached 430 psi. These conditions resulted in about 90% glucan digestibility after 24 hours of hydrolysis, reaching complete digestion by 70 hours.

Conversely, the untreated material achieved about 60% digestion within 24 hours, increasing to 70% after 70 hours (Kim et al, 2008).

Phosphoric Acid and Acetone Pretreatment

Phosphoric acid and acetone have been used successfully as cellulose solvents. Phosphoric acid was chosen as a cellulose solvent for its ability to dissolve cellulose at low temperatures and in the presence of water, the amorphous form of the regenerated cellulose, and the fact that phosphoric acid does not inhibit hydrolysis or fermentation at residual levels (Zhang et al, 2007). Concentrated phosphoric acid (83%, w/w) was combined with biomass at a loading of 8 ml / g dry biomass. Douglass fir, corn stover, switchgrass, and hybrid poplar were utilized in the study. The acid and biomass were reacted for 30 – 60 min at 50°C, dissolving the cellulose and hemicellulose and hydrolyzing them to oligosaccharides. Acetyl groups were cleaved from the hemicellulose, generating acetic acid. Acetone was then used to precipitate the cellulose and hemicellulose. Acetone was removed with the addition of water, and phosphoric acid and acetone were recovered based on their differing volatilities. The pretreated biomass was then hydrolyzed with cellulase. Glucan digestibilities were high, reaching 96 – 97% after 24 hours for corn stover, switchgrass, and hybrid poplar. Douglas fir glucan digestibility was 75% after 24 hours (Zhang et al, 2007).

Organosolv Pretreatment

The organosolv process utilizes an organic solvent such as ethanol or methanol at high temperatures and acidic pH to dissolve lignin and hemicellulose, while leaving cellulose intact. While a variety of alcohols or other organic solvents may be used, ethanol and methanol are

considered to be the most beneficial due to their low cost, miscibility with water, and ease of recovery (Zhao et al, 2009). In the organosolv process utilizing ethanol or methanol, the biomass is reacted with the alcohol at high temperature (185 - 210°C). After the treatment, the solid fraction (mainly cellulose) is recovered, washed, and carried on to hydrolysis. The liquid fraction, containing the hemicellulose, lignin, and solvent, is condensed by evaporation. This recovers the solvent for reuse. The condensed liquid, referred to as black liquor, is diluted with water, causing the precipitation of lignin. The lignin is recovered by filtration and dried for use in the production of a high value product. The filtrate is an aqueous solution of sugars derived from the hemicellulose (Zhao et al, 2009). The cellulose derived from this may be hydrolyzed to glucose at high rate and yield, as in the Lignol process, described below (Drapcho et al, 2008).

The Lignol Process

The Lignol process is a process utilized for the biorefining of woody biomass, which includes ethanol organosolv pretreatment. In the Lignol process, the biomass is pretreated using ethanol by the organosolv process, followed by hydrolysis and fermentation of the cellulose to produce ethanol (Zhao et al, 2009). In the organosolv section of the Lignol process, woody biomass is treated with ethanol for 30 – 90 min at temperatures of 180 - 195°C and low pH (2.0 – 3.8). The treatment conditions vary depending upon the specific feedstock (Arato et al, 2005).

During organosolv pretreatment, the cellulose is partially hydrolyzed to smaller fragments, though it remains in the solid phase. The hemicellulose is hydrolyzed to soluble oligosaccharides. Acetic acid is generated by the removal of acetyl groups from the hemicellulose, and serves as an acid catalyst in the hydrolysis of other components, including the degradation of xylose to furfural. The amount of xylose degraded varies depending upon

treatment conditions, with milder conditions reducing the xylose loss. The lignin is broken down into soluble low molecular weight fragments. The resulting liquid and its soluble contents are referred to as black liquor. The black liquor may be processed to recover the lignin, recover and recycle the ethanol, recover xylose and acetic acid, and produce monomeric sugars from the oligosaccharides via dilute acid hydrolysis (Arato et al, 2005).

The solid material resulting from the organosolv treatment, primarily cellulose, may be hydrolyzed by enzymes and the resulting glucose subsequently fermented to ethanol. In one study, hybrid poplar was treated by the Lignol process with 50% ethanol for one hour at 180°C. 1.25% (w/w) sulfuric acid was added as a catalyst. The resulting solids contained 27% (w/w) of the original lignin, 88% (w/w) of the original glucose, and 19% (w/w) of the original xylose. The liquid phase contained 1% (w/w) of the original glucose, 52% (w/w) of the original xylose, and 85% (w/w) of the original arabinose. The total sugar degradation products (furfural and HMF) concentration in the liquid phase was 3% (w/w). The glucose digestibility of the solids after 48 hours of enzyme hydrolysis was 97% (w/w), corresponding to an 85% (w/w) glucose yield as compared to the glucose content of the untreated biomass (Pan et al, 2006).

Soaking in Aqueous Ammonia (SAA) Pretreatment

The Soaking in Aqueous Ammonia (SAA) process is a simple batch process where biomass is mixed with aqueous ammonia (typically 15 – 30% w/w) and allowed to soak at mild temperatures (typically less than 90°C) for the desired period of time. Temperature is often elevated above room temperature because such low temperatures necessitate treatment times on the order of days, whereas at elevated temperature this may be reduced to a matter of hours (Zhu et al, 2006, Kim and Lee, 2007, Kim et al, 2008b). The SAA process has the advantage of being

a very simple batch process. Thus, it is much more easily implemented in a processing plant, and requires less instrumentation and control systems. Like AFEX, SAA is an ammonia-based process and does not generate inhibitory compounds. It also demonstrates a high retention of major sugars, a significant degree of lignin removal, and results in a pretreated material that is highly digestible (Drapcho et al, 2008). In a study utilizing corn fiber, under the optimum study condition, glucan, xylan, and arabinan retentions were 97.3%, 81.3%, and 90.7% (w/w, db), respectively (as compared to masses initially present prior to treatment). The treatment retained 71.7% of the total solid material, and removed 20% of the total lignin. The resulting pretreated material demonstrated a glucan digestibility of 85% (unpublished data, Kim, 2008).

Soaking in Ethanol and Aqueous Ammonia (SEAA) Pretreatment

The Soaking in Ethanol and Aqueous Ammonia (SEAA) process is similar to the SAA process; with the modification that ethanol is added to the ammonia solution. While the SAA process is effective at retaining nearly all available glucan, roughly 20% of available xylan is lost. In theory, because ethanol is effective in precipitating soluble hemicellulose, its presence in the pretreatment step would cause the precipitation of xylan solubilized by the pretreatment, thus allowing it to be retained in the solid form (Kim et al, 2009b). To test this, corn stover was pretreated with ethanol and ammonia. The ammonia solution (15% w/w) was loaded at a solid: liquid ratio of 1:9, and supplemented with ethanol at 0, 1, 5, 20, or 49% (w/w) ethanol (% of the total liquid). The treatment conditions were 60°C for 24 hours. With no added ethanol, 17.2% of available xylan was lost, which is consistent with the results seen in the SAA process. The xylan retention increased with the addition of ethanol, with only 6.3% lost at the highest ethanol loading. Ethanol addition did not significantly affect glucan retention. The addition of ethanol at

20% (w/w) or lower did not significantly affect the glucan or xylan digestibility, as compared to the glucan and xylan digestibilities of the material treated with ammonia only. However, ethanol loaded at 49% caused a decrease in digestibility. Thus, it was determined that the optimum ethanol concentration for the SEAA process was 20% (w/w) (Kim et al, 2009b).

Enzymatic Hydrolysis

After pretreatment, the biomass must be hydrolyzed to fermentable sugars prior to conversion to ethanol or other products, typically through the action of enzymes. The hydrolysis of cellulose to glucose is carried out by a system of enzymes, referred to collectively as cellulase. The cellulase system contains three enzymes, endoglucanase, exoglucanase, and β-glucosidase. Each of these enzymes operates by cleaving the β-1,4 linkages at various points along the cellulose chain. Exoglucanase acts on the ends of the chain, liberating cellobiose. Endoglucanase acts within the chain, cleaving it into oligosaccharides of various lengths. Additionally, this produces new chain ends for exoglucanase to act upon. β-glucosidase then cleaves the liberated cellobiose to glucose (Drapcho et al, 2008, Lynd et al, 2002). This process is shown schematically in Figure 1.8.

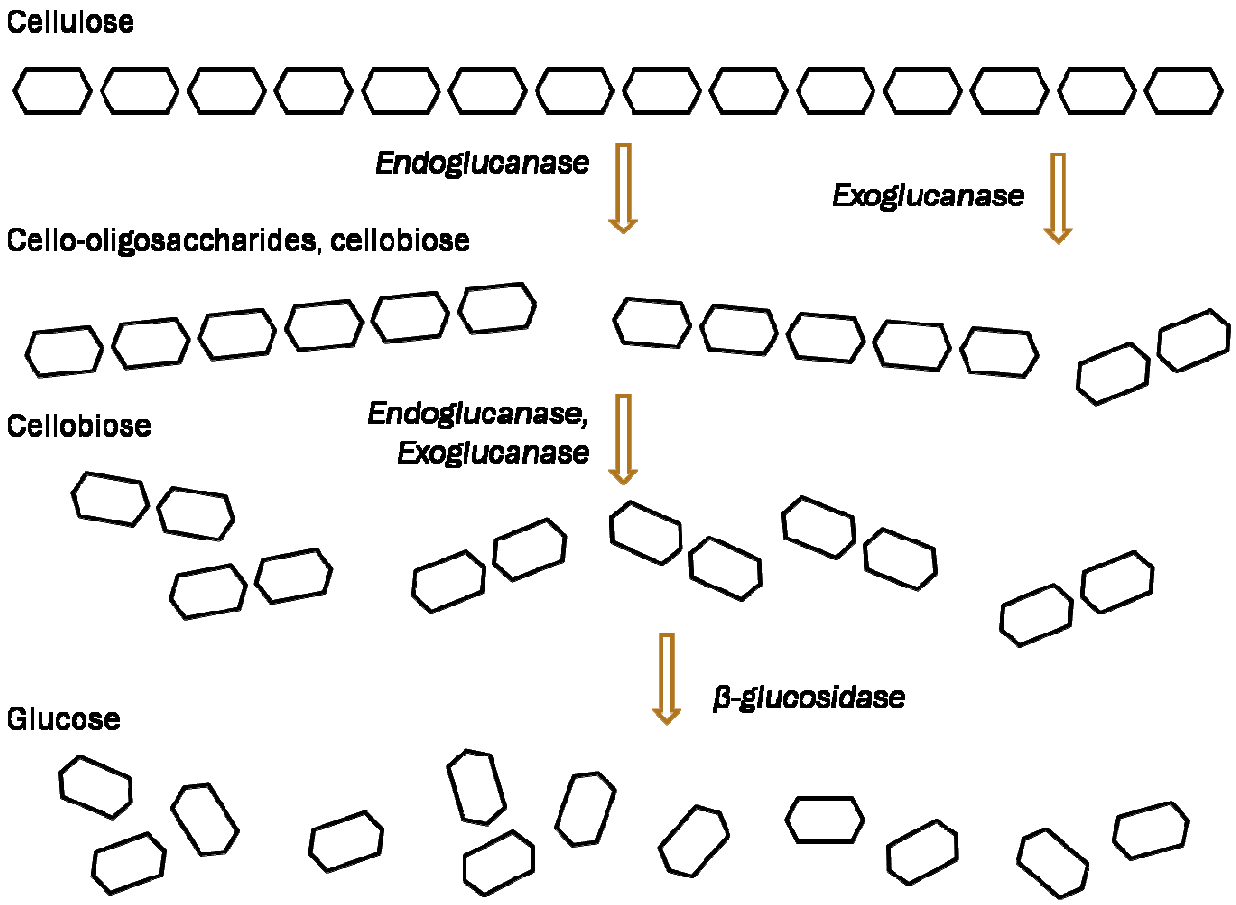

Figure 1.8. Schematic representation of cellulose hydrolysis by the cellulase system.

The cellulase system described is well understood, and can be highly effective in practice, as indicated by the high glucan digestibilities described previously. For a more comprehensive review of the biochemical and microbiological factors involved with this enzyme system, the reader is encouraged to refer to Lynd et al (2002); as such a review is beyond the scope of this work.

Due to the heterogeneous nature of hemicellulose, its hydrolysis requires a more complex enzyme system than cellulose. Hydrolysis of xylan involves primarily a xylanase system which is analogous to the cellulase system. It is composed of endoxylanase, exoxylanase, and β-xylosidase. Endoxylanase cleaves the β-1,4 linkages between xylose within the chain, creating

xylo-oligosaccharides. Exoxylanase acts on the ends of the chain, releasing xylobiose, a xylose dimer. The xylobiose is cleaved to release xylose by β-xylosidase, which may also release some xylose from shorter xylo-oligosaccharides (Saha, 2003, Drapcho et al, 2008).

In addition to the xylanase system described, several accessory enzymes may be required. Because of the branched structure of the hemicellulose, the xylanolytic enzymes may not have sufficient access to the xylose backbone to achieve complete degradation. When that is the case, debranching via accessory enzymes must occur to allow xylan digestion. These enzymes include α-arabinofuranosidase, α-glucoronidase, acetylxylan esterase, ferulic acid esterase, and ρ-coumaric acid esterase. A description of the methods of action of these enzymes is provided in Table 1.1.

Table 1.1. Summary of xylanolytic enzymes and their mechanisms (Saha, 2003).

Enzyme	Method of Action
Endoxylanase	Hydrolyzes β-1,4 xylose linkages within the xylan chain, creating xylo-oligosaccharides
Exoxylanase	Hydrolyzes β-1,4 xylose linkages at the end of the chain, liberating xylobiose
β-xylosidase	Liberates xylose from xylobiose and short xylo-oligosaccharides
α-arabinofuranosidase	Removes terminal α-arabinofuranose from arabinoxylan
α-glucoronidase	Removes glucuronic acid from glucuronoxylans
Acetylxylan esterase	Hydrolyzes acetyl ester bonds in acetylated xylan
Ferulic acid esterase	Hydrolyzes feruloylester bonds in xylan
ρ-Coumaric acid esterase	Hydrolyzes ρ-Coumaryl ester bonds in xylan

Each of these accessory enzymes acts against a specific side group or chain which, depending upon the source and structure of the hemicellulose, may or may not be present. For example, α-arabinofuranosidase is active against arabinoxylan, while α-glucoronidase is active against glucuronoxylan.

Fermentation

Complete degradation of biomass will yield hexoses (glucose, galactose, and mannose) and pentoses (xylose and arabinose). The fermentation of glucose to ethanol is represented by the following chemical equation,

$$\text{glucose} \rightarrow 2\ \text{ethanol} + 2\ \text{carbon dioxide}$$

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

Assuming 100% of the glucose is utilized for ethanol production, the theoretical yield is 0.511 g ethanol per g glucose. In practice, however, this cannot be achieved because some of the glucose is utilized by the cells for biomass production and cell maintenance. Typically, 90 – 95% of the theoretical yield may be achieved (Drapcho et al, 2008). The yeast *Saccharomyces cerevisiae*, used industrially for nearly all ethanol production, is capable only of utilizing the hexose sugars for growth and thus ethanol production. For complete utilization of the biomass sugars, alternative fermentation strategies must be developed. This has been the subject of much work, and the strategies developed often involve one of three main premises:

- Development of an organism capable of metabolizing all of the sugars present to produce ethanol,
- Development of a co-fermentation strategy using two different organisms each capable of metabolizing different sugars, and
- Separation of pentose sugars for separate fermentation to ethanol or high-value products.

Each of these strategies will be discussed briefly.

Organism Development

The development of organisms capable of utilizing hexose and pentose sugars for ethanol production has received a significant amount of attention. Typically, organisms naturally possess the ability to utilize both pentose and hexose sugars, or the ability to produce ethanol in industrially significant amounts, but not both. This strategy has focused on the development of an organism with both of these traits (Drapcho et al, 2008). *Zymomonas mobilis* is a facultative anaerobic bacterium that has received a significant amount of attention due to its rates of ethanol production and volumetric ethanol productivity both being significantly higher than observed in *S. cerevisiae* (Panesar et al, 2006). Conversely, *Escheria coli* has the ability to utilize the major sugars found in biomass, but does not produce ethanol at significant levels. Thus, metabolic engineering strategies have focused on combining these traits.

A recombinant *Z. mobilis* strain capable of xylose utilization was created by the addition of *E. coli* genes associated with xylose metabolism. The recombinant *Z. mobilis* was able to produce ethanol from both glucose and xylose at 95% of theoretical yield after 30 hours of fermentation. Glucose was utilized preferentially by the organism (Zhang et al, 1995).

The opposite strategy, the production of recombinant *E. coli* from *Z. mobilis* genes, has also been attempted. Genes associated with ethanol production in *Z. mobilis* were successfully transformed into *E. coli*, creating a recombinant strain referred to as *E. coli* KO11. This strain was capable of utilizing all of the major sugars found in biomass to produce ethanol at greater than 95% efficiency (Ingram et al, 1998).

Co-fermentation

In order to avoid the problems associated with metabolic engineering, co-fermentation has been investigated. Rather than producing an organism with both the ability to utilize a wide range of sugars and the ability to produce ethanol, the co-fermentation strategy involves fermenting the biomass hydrolysate (containing pentose and hexose sugars) with two different organisms simultaneously. This is a strategy that is met with many difficulties, primarily involved with an incompatibility of growth conditions, typically oxygen requirement. For example, many yeast strains exist which have the ability to metabolize pentose sugars. However, under anaerobic conditions, their growth is severely limited. Under aerobic conditions growth occurs, but no ethanol is produced (Lynd 1996).

Separate Fermentation

To overcome incompatible growth conditions, separation of the fermentation of pentose and hexose sugars has been proposed. This may be accomplished in many ways. In one study, pentose sugars derived from hydrolysis of hemicellulose were utilized by *E. coli* to produce a modest amount of ethanol. After pentose fermentation, the beer was transferred to a second fermenter where glucose was added and fermented with *S. cerevisiae.* The ethanol produced in the first fermentation was not enough to cause inhibition in the second, and the overall result was efficient ethanol fermentation (Lindsay et al, 1995). Another alternative for the separate fermentation of biomass sugars is to use the glucose for ethanol fermentation and the xylose and arabinose for fermentation of a high value product(s). This has the benefit of providing simplicity in hexose utilization (simply add the stream to existing fermenters), flexibility to respond to changing market conditions, and the potential for significant revenue generation.

There are many compounds that can be produced from hemicellulose hydrolysates. Several examples are xylitol, 2,3-butanediol, succinic acid, lactic acid, ferulic acid, and astaxanthin.

Xylitol is a sugar alcohol of interest to the food industry as a sweetener, and to the pharmaceutical industry for its anti-inflammatory properties. Xylitol is produced naturally by strains of the yeast *Candida*, and pentose sugars derived from prairie grass hemicellulose have been proposed as a potential feedstock for xylitol production (West, 2009).

2,3-Butanediol (2,3-BD) is a bulk chemical that is useful as a chemical intermediate (Saha, 2003). As such, its monetary value is intrinsically low, requiring that a low cost feedstock be used for its production to be economically feasible. To this end, hemicellulose from agricultural or forest residues has been proposed. One of the best known 2,3-BD producing organisms, *Klebsiella oxytoca*, has the ability to utilize all of the major sugars present in biomass for efficient 2,3-BD production (Celinska and Grajek, 2009).

Succinic acid is another bulk chemical useful as a feedstock for the production of various high-value products. It may be produced synthetically in a petroleum-based process, but its microbial production from renewable biomass sources is gaining increasing attention. Much work has been done on the production of succinic acid by *Actinobacillus succinogenes* and recombinant *E. coli*. Both organisms are capable of utilizing pentose sugars in the formation of succinic acid (Li et al, 2010, Andersson et al, 2007).

Lactic acid is another bulk chemical widely used in many industries, including use as a component in bioplastics (Saha, 2003). It has been produced previously from glucose (derived from starch) or sucrose, but lignocellulosic materials have been pursued as a lower cost alternative. Much work has been done to develop organisms capable of utilizing pentose sugars for lactic acid formation. One of the more promising organisms is *Lactobacillus pentosus*. The

organism was able to utilize hydrolysate derived from SAA pretreated corn stover to efficiently produce lactic acid from all sugars present at greater than 90% theoretical yield (Zhu et al, 2007). Recombinant *E. coli* has also been used successfully in lactic acid production from glucose and xylose (Saha, 2003).

Ferulic acid is present in plant cell walls, where it acts as a crosslinking agent (Shin et al, 2006). Once recovered from the cell wall, ferulic acid may be used as a substrate for the microbial production of vanillin, a high value flavoring compound (Shin et al, 2006). In corn fiber, ferulic acid crosslinks the xylan backbones of the hemicellulose, which is believed to contribute to its recalcitrance. The release of ferulic acid from compounds such as corn fiber hemicellulose will aid in their degradation, and data indicates that enzyme cocktails containing ferulic acid esterase (FAE) are more effective at releasing xylose and arabinose from corn fiber hemicellulose than those lacking FAE (Dien at al, 2008, Akin and Rigsby, 2008, this paper). Thus, the recovery of ferulic acid from materials such as corn fiber presents both the opportunity for a high-value product, and an improvement in xylose and arabinose yields, allowing for further high-value product formation.

Astaxanthin is the carotenoid that provides salmon and crustaceans with their characteristic coloration. As these organisms lack the ability for the *de novo* synthesis of astaxanthin, they must obtain it through their diet. In the marine environment, algae and plankton which naturally produce astaxanthin are consumed. In an aquaculture setting this is not the case, necessitating the addition of astaxanthin or astaxanthin containing materials to the feed. Additionally, astaxanthin has been found to be a powerful antioxidant, attracting the attention of the nutraceutical industry. The red yeast *Phaffia rhodozyma* is a natural producer of astaxanthin, and has the ability to utilize hexose and pentose sugars (Nghiem et al, 2009).

Lignocellulosic Biomass Processing Schemes

The three main steps involved in the production of ethanol from lignocellulosic biomass, pretreatment, hydrolysis, and fermentation, may be conducted separately or in combination(s). This is illustrated in Figure 1.9.

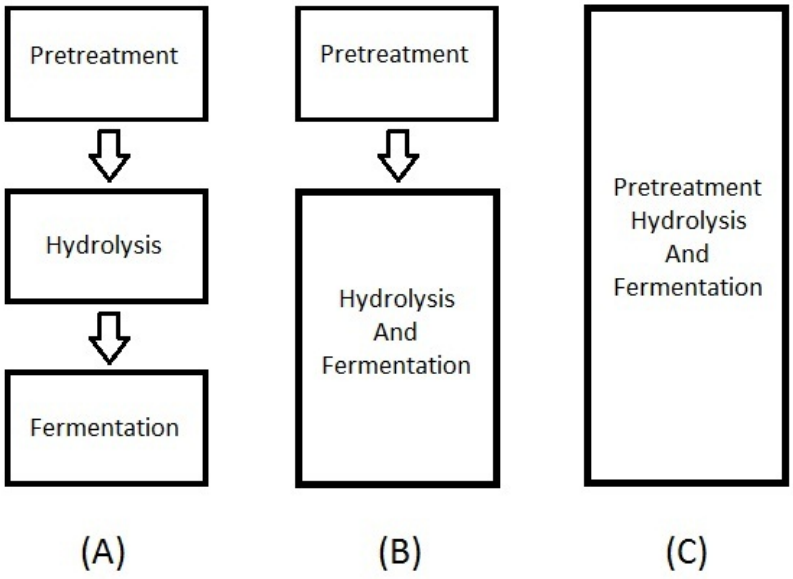

Figure 1.9. Cellulosic ethanol process schemes. (A) Separate Hydrolysis and Fermentation (SHF). (B) Simultaneous Saccharification and Fermentation (SSF). (C) Consolidated Bioprocessing (CBP). Adapted from (Lynd et al, 2002)

In the figure, each block represents a separate reactor. The separation of boxes (i.e. reactors) represents spatiotemporal separation in processing. In Separate Hydrolysis and Fermentation (SHF), each step is carried out in separate reactor vessels, allowing the conditions in each to be optimized for each process. However, this process necessitates increased capital cost, as more processing equipment is involved.

In Simultaneous Saccharification and Fermentation (SSF), the pretreatment step is carried out first, followed by the simultaneous hydrolysis and fermentation of the pretreated biomass. This has the benefit of reducing inhibition of enzymatic cellulose hydrolysis caused by the accumulation of glucose, as the glucose is utilized in ethanol fermentation as it is produced. This processing scheme introduces the challenge of matching the conditions for optimum cellulase production or hydrolysis to those of optimum ethanol fermentation. Consolidated Bioprocessing (CBP) is a term coined by Lynd in 1996 to describe the pretreatment, hydrolysis, and fermentation of cellulosic biomass in a single step (Lynd et al, 2002). The microorganism utilized for CBP ideally must be able to synthesize a highly active system of enzymes capable of reducing the biomass to its component fermentable sugars, metabolize those sugars, and produce and withstand significant ethanol volumes, all doing so in a medium that does not require expensive micronutrients, antibiotics, etc. No naturally occurring organism is known to meet these criteria (Lynd et al, 2002). In 2009, Mascoma Corporation reported breakthroughs in CBP organism production, providing proof of concept for CBP. In February 2009, Mascoma reported that its pilot scale CBP facility had begun ethanol production (Green Car Congress, 2009).

Ethanol from Corn

Corn is the predominant feedstock used in the production of ethanol in the United States. The RFA estimates that in 2009, 3.8 billion bushels of corn (90.4 million metric tons) were converted to 10.6 billion gallons of ethanol and 30.5 million metric tons of co-products (RFA, 2010). Corn based ethanol is produced from the starch component of the corn kernel, which comprises 70 – 72% of the kernel on a dry weight basis (Bothast and Schlicher, 2005). Two different processes, wet milling and dry grind, are used in the conversion of corn to ethanol.

Wet milling plants fractionate the corn into its starch, germ, fiber, and protein components prior to fermentation. Only the purified starch component is utilized in fermentation to produce ethanol, while the other components are further processed to a variety of co-products. Because of this, wet milling plants are much more expensive to build, and thus must be larger than dry grind plants in order to be economically favorable (Drapcho et al, 2008). Because of the increased size and expense, wet milling plants are typically corporate owned. Dry grind plants, on the other hand, do not fractionate the corn kernel prior to fermentation. This results in a greatly reduced capital cost, but also fewer co-products. Dry grind plants are typically producer owned, and thus are more beneficial to their local economies. Due to their decreased capital cost and slightly higher ethanol yields (2.8 vs. 2.5 gal / bu), dry grind plants account for roughly two thirds of corn ethanol production in the United States (Bothast and Schlicher, 2005).

Dry Grind Process

Corn is first milled to reduce its size and increase water infiltration. The milled corn is then mixed with water to form a slurry commonly referred to as mash, and its pH is adjusted to 6.0. A thermostable α-amylase preparation is added, and the mash is cooked at temperatures as high as 100°C to facilitate the enzymatic hydrolysis of the α-1,4 linkages within the starch molecules, yielding dextrins. Temperature is maintained for several minutes, after which time the mash temperature is reduced to 80 - 90°C. Additional α-amylase is added and temperature maintained for at least 30 min. This step is referred to as liquefaction. After liquefaction, the temperature of the mash is reduced, and pH adjusted to 4.5. Glucoamylase is added to hydrolyze the dextrins to glucose, and the mixture is transferred to the fermenter. Yeast is added and the mash is supplemented with a nitrogen source, commonly urea, to facilitate growth and ethanol

production. Hydrolysis of starch to glucose occurs simultaneously with fermentation. After 72 h of fermentation, ethanol concentration in the broth, now referred to as beer, typically reaches 14-15% by volume. CO_2 emitted during fermentation typically is collected and sold as a co-product, commonly to the beverage industry to be used as a carbonating agent. After fermentation, the beer is transferred to a distillation column where the ethanol is removed, resulting in a 95% ethanol solution. The remaining 5% water is removed by molecular sieve, resulting in anhydrous ethanol.

The material left over after distillation contains the non-fermentable material initially present in the corn kernel, including the protein, oil, fiber, and any non-fermented starch. This material is referred to as whole stillage. It is concentrated by centrifugation and evaporation, to yield a liquid stream (thin stillage) and a viscous slurry containing residual liquid and the solids, referred to as wet distillers' grains with solubles (WDGS). The WDGS is typically dried to low moisture content, producing DDGS. DDGS is a valuable livestock feed, and represents a significant source of revenue for the dry grind plant (Bothast and Schlicher, 2005). However, because it contains corn fiber, its use is limited to ruminant diets. Fractionation of the fiber component in dry grind plants is attractive not only to widen the market for DDGS (which is quickly becoming saturated due to the rapid growth of ethanol production by the dry grind process), but also to provide a valuable feedstock for the production of high-value compounds, as described previously. Additionally, the removal of the fiber fraction prior to fermentation reduces the amount of material in the fermenter, thus increasing fermenter capacity, or reducing the size requirement, and thus the capital cost.

To this end, modifications to the dry grind process have been proposed that allow the recovery of the corn fiber prior to fermentation. In a process developed at the University of

Illinois, the germ and fiber fractions are recovered in a hydroclone immediately after milling. A wash step then recovers some additional starch from the germ and fiber, and the fiber is separated from the germ via aspiration. Corn fiber isolated in this way is referred to as "quick fiber". The composition of quick fiber is shown below, and compared to the composition of traditional corn fiber.

Table 1.2. Comparison of quick fiber and corn fiber compositions.

Component	Corn Fiber[1] (% w/w, db)	Quick Fiber[2] (% w/w, db)
Starch	10.61 - 25.6	15
Glucan[3]	10.3 - 22.1	17
Xylan	17.2 - 28.5	22
Arabinan	10.7 - 18.3	11
Protein	8.3 - 9.7	11

[1]Data represents the range of data appearing below in Table 1.5.
[2] Data from Dien et al, 2004.
[3] Glucan is in the form of cellulose.

The modified process results in quick fiber with a carbohydrate composition within the range of traditional corn fiber. Quick fiber yields in the process were 3.04 lb / bushel (dry basis), or 5.4% by weight. This is significantly lower than the corn fiber recovery in traditional wet mills (see Table 1.4, below) (Dien et al, 2004). The reason for this was not clear, nor was any speculation offered by the authors.

Wet Milling Process

Unlike the dry grind process, wet milling seeks a purified starch stream that may be used for ethanol production (most commonly), or the production of products such as high fructose corn syrup. First, the whole kernel is steeped in a very weak solution of sulfurous acid. This

softens the kernel, increasing the ease of downstream processing. Soluble compounds are removed in this stage. The steeping solution, referred to as light steep water, which contains less than 10% solids, is concentrated to heavy steep water, which contains up to 50% solids, by evaporation. The solids in the heavy steep water may have a protein content of up to 50%. After steeping, the corn kernels are gently milled and the germ removed in a hydroclone. Residual starch and gluten are removed from the germ, and then are dried. The germ contains corn oil, which may either be extracted onsite or by a third party. The fiber is recovered by screening and then washed. Traditionally, the fiber is then combined with the heavy steep water and dried to create corn gluten feed (CGF). Alternatively, the fiber may be reserved for use as a feedstock in the production of additional ethanol or a high-value product.

The solids remaining after germ and fiber separation consist primarily of starch and protein, which are separated from one another via centrifugation. The starch fraction is then further purified to >99.5% purity in hydroclones. The protein (gluten) is concentrated and dewatered by centrifugation and filtration, then dried to produce corn gluten meal (CGM). The starch fraction is then fermented to ethanol (Rausch and Belyea, 2006, Drapcho et al, 2008). A comparison of the co-products from the dry grind and wet mill processes is shown in Table 1.3.

Table 1.3. Co-products from dry grind and wet mill ethanol production.

Co-Product	Process	Use
DDGS	Dry Grind	Animal Feed (ruminant diet)
CGF	Wet Mill	Animal Feed (ruminant diet)
CGM	Wet Mill	Animal Feed (non-ruminant diet)
Corn Oil	Wet Mill	Valuable food ingredient
Corn Fiber	Wet Mill	Ethanol, high value products
CO_2	Dry Grind, Wet Mill	Beverage carbonation, other industrial processes

Co-products resulting from process modifications to dry grinding, as mentioned above, were not considered in the table. Corn fiber is a potentially very valuable co-product, and is discussed below.

Corn Fiber

Corn fiber is a co-product of the corn wet milling process and is composed of the cellulosic components of the corn kernel, namely the pericarp and endosperm fiber. Recently, modifications to the dry grind process have been developed to allow pericarp fiber production there as well (Dien et al, 2004). While industrial yields of corn fiber vary, corn fiber production averages 11.5% of the mass of corn processed (dry basis) in corn wet milling facilities (Table 1.4).

Table 1.4. Industrial corn fiber yields in corn wet milling facilities.

Corn Fiber Yield (% w/w, db)	Reference
10	Rausch and Belyea, 2006
11	Saha and Bothast, 1999
11.5	Gulati et al, 1996
13	Gulati et al, 1996
12.8	Gulati et al, 1996
10.7	Doner and Hicks, 1997
11.5	Average

Wet milling corn fiber is of particular interest because of its high carbohydrate content and its (currently) low value (Hanchar et al, 2007). In addition, unlike many other agricultural residues, corn fiber is already available onsite in ethanol facilities. Thus, its utilization as a feedstock for other processes within the facility avoids the costs of feedstock gathering and transportation associated with other agricultural residues such as corn stover. The fractionation

of corn fiber prior to fermentation also provides the benefits of increased fermenter capacity, and increased value of the DDGS due to reduced fiber content, as discussed previously. Although the composition of corn fiber varies by source, cellulose and hemicellulose typically account for about 50% of the dry weight, with adherent starch comprising another 10 – 20%. Thus, corn fiber typically consists of about 70% recoverable sugars. A more detailed compositional analysis, compiled from multiple sources to illustrate compositional variability, is provided in Table 1.5.

Table 1.5. Corn fiber composition.

Component (% w/w, db)									
Glucan[1]	Xylan	Arabinan	Galactan	Mannan	Starch	Lignin	Protein	Ash	Reference
18.8	21.3	11.7	NR[2]	NR	11.5	NR	NR	NR	Gaspar et al, 2007
19.71	28.5	13.7	3.8	0.39	10.61	12.41	NR	NR	Hanchar et al, 2007
22.1	25.7	16.1	4.5	1.9	NR	8.8	NR	NR	Kim, 2008
21.2	17.2	12.9	4.1	NR	17.5	NR	NR	NR	Nghiem et al, 2009
20.1	25.18	18.3	3.78	NR	12.7	NR	NR	NR	Gulati et al, 1996[3]
14.4	20.8	13.6	3.5	NR	15.7	9.9	8.3	1	Schell et al, 2004[4]
12.6	18.6	11.2	6.9	NR	24.9	15.6	8.7	0.9	Schell et al, 2004
10.3	18.8	10.7	6.8	NR	25.6	16.6	9.7	0.9	Schell et al, 2004

[1]Glucan here refers to cellulose only. Although also a source of glucan, starch is listed separately.
[2]NR: Not Reported.
[3]Values were originally reported as monomers. They have been converted to polymers here for consistency.
[4]Reference appears multiple times because the composition of multiple batches was reported.

The carbohydrates in corn fiber exist in three main forms, cellulose, hemicellulose, and starch. The hemicellulose component accounts for the xylan, arabinan, galactan, and mannan. While corn fiber hemicellulose is classified as an arabinoxylan (containing primarily arabinose and xylose), a small amount of glucose, mannose, and galactose are also present. The structure of corn fiber hemicellulose will be considered below.

Corn Fiber Hemicellulose

The hemicellulose fraction of corn fiber is typically referred to as corn fiber gum (CFG). Industrially, it has several uses. In its native form, CFG may be extracted and used as an emulsifier in the beverage industry (Yadav et al, 2007). If hydrolyzed, CFG could serve as a source of xylose and arabinose for use in a number of high-value product fermentations. Corn fiber hemicellulose is composed of two fractions, hemicellulose A and hemicellulose B. Hemicellulose A typically accounts for less than 10% of the total hemicellulose, and is discarded in most processes that extract corn fiber hemicellulose (Doner and Hicks, 1997). Hemicellulose A is insoluble in water at acidic pH, while hemicellulose B remains soluble under these conditions. Thus, the fractionation of the hemicellulose A component from the total hemicellulose is simple, and may be carried out relatively easily.

Corn fiber gum is composed of β-1,4 linked xylose, forming the xylan backbone. This constitutes about half of the CFG, by mass (db). Typically about 80% of the xylose residues composing the xylan backbone are substituted with various side chains that could include arabinose or glucuronic acid, or various oligomeric compounds (Saha, 2003). The composition of CFG is shown in Table 1.6.

Table 1.6. Corn fiber gum composition

Component (% w/w, db)[1]					
Xylan	Arabinan	Glucan	Galactan	Mannan	Reference
38.57	18.13	3.06	6.78	0	Hanchar et al, 2007
42.24 - 47.52	29.04 - 30.8	NR[2]	4.5 - 9.9	NR	Doner and Hicks, 1997
39.6	26.4	NR	6.66	NR	Dien et al, 2008
47.92 - 49.32	30.32 - 30.67	1.71 - 2.30	5.27 - 5.94	NR	Singh et al, 2000
42.33 - 43.82	33.18 - 35.10	0.72 - 0.90	4.86 - 7.56	NR	Doner et al, 1998
36.96	23.76	0.003	0.1	NR	Li et al, 2005
42.24	30.8	NR	6.3	NR	Benko et al, 2007

[1]Values were originally reported as monomers. They have been converted to polymers here for consistency.
[2]NR: Not Reported

The xylan backbone of CFG is substituted primarily with arabinose, and the xylan chains are crosslinked to one another by diferulic bridges. To form the diferulic bridge, two ferulic acid residues (each substituted to a separate xylan chain) form an ester linkage with one another. The cellulose fibers, along with structural proteins, embed within the lattice formed by the crosslinked xylan, thus forming the corn fiber cell wall (Figure 1.10).

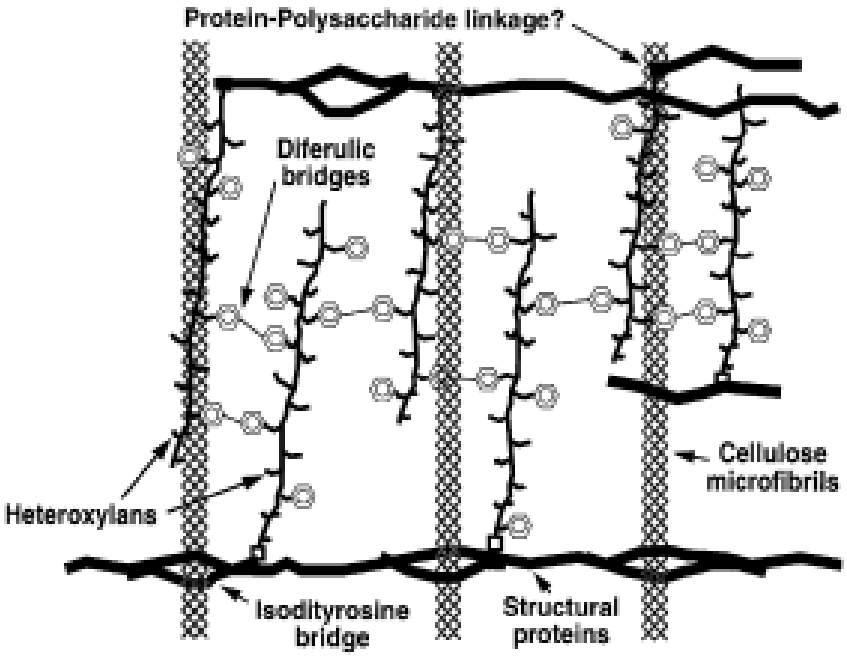

Figure 1.10. Schematic of the corn fiber cell wall. From (Saha, 2003).

Proteins are associated with the hemicellulose, as even purified CFG may contain up to 5% protein (Yadav et al, 2007b). It is the highly crosslinked, complex, and substituted nature of corn fiber hemicellulose that is thought to impart its recalcitrance. The enzymatic hydrolysis of corn fiber hemicellulose will be discussed in more detail later.

Extraction of CFG

The extraction of hemicellulose from corn fiber is desirable for several reasons. First, the fractionation of hemicellulose allows it to be hydrolyzed separately from the cellulose, yielding separate hexose and pentose streams. This allows great flexibility, as the pentose sugars can be utilized for any of a number of high-value product fermentations, while the glucose may be added back into ethanol fermentations without the need for modifications to the existing ethanol

process. Additionally, if hydrolysis of CFG is not to be performed, the CFG may be sold as a valuable product, for use as a beverage flavor emulsifier, for example.

Several processes have been proposed to extract CFG from corn fiber. They typically involve an alkaline hydrogen peroxide (AHP) extraction (Doner and Hicks, 1997). In the AHP process, corn fiber was added to an aqueous solution of 1% H_2O_2 to achieve an H_2O_2 to dry fiber ratio of 0.25. The pH was raised to 11.5 with NaOH, and the mixture stirred rapidly. During the extraction, pH was maintained at 11.5 by the addition of NaOH as needed. After extraction, the mixture was centrifuged to remove the non-hemicellulosic solids. The supernatant (containing the hemicellulose) was adjusted to pH 4, at which hemicellulose A is no longer soluble, causing it to precipitate. The hemicellulose A was then separated via centrifugation. The hemicellulose B, located in the supernatant, was then precipitated by adding the supernatant to two volumes of isopropanol. The hemicellulose B precipitate was allowed to settle out, and the liquid removed by decanting. Because the presence of water (from the hemicellulose B containing supernatant) causes the hemicellulose B to be sticky and difficult to work with, it was washed with fresh isopropanol, then collected via filtration or centrifugation, and finally dried. The process produces an off-white CFG powder in yields as high as 42% (w/w, db) of the initially available hemicellulose B. The off-white color observed is thought to be due to the presence of contaminating lignin or protein, and it is speculated that the coloration may limit commercial applications of the extracted CFG (Doner and Hicks, 1997). For example, in beverage applications where color is an important factor in product quality, CFG with no coloration would be necessary.

In a modification to the process designed to improve the color of the product and minimize H_2O_2 usage, alkaline and H_2O_2 treatment are separated and performed sequentially.

Corn fiber was added to an NaOH solution, and boiled for 1 hr. The non-hemicellulosic residue was removed by centrifugation, and the hemicellulose containing supernatant decanted. Hemicellulose lost in the residue was recovered by boiling the residue in water, centrifuging, and combining the supernatant with the original supernatant. H_2O_2 was added to the pooled supernatants and the pH adjusted to 11.5 as described previously. The mixture was stirred for 2 hr at room temperature, and the pH was lowered to 4 to precipitate the hemicellulose A, which was removed by filtration or centrifugation. Two volumes of ethanol were then added to the filtrate to precipitate the hemicellulose B. After the precipitate was allowed to settle, the liquid was decanted and the hemicellulose B washed with fresh ethanol. The hemicellulose B was then recovered and dried. The CFG yield was 40% (w/w, db), which is comparable to the previous process. In the modified process, the color of the CFG was improved, and a small reduction in the quantity of raw materials utilized was realized (Doner et al, 1998).

Enzymatic Hydrolysis of CFG

As described previously, the complex nature of corn fiber hemicellulose imparts recalcitrance to enzymatic hydrolysis. The enzymes needed to hydrolyze hemicellulose were listed previously in Table 1.1. Due to the complexity of CFG hydrolysis, reported yields of xylose and arabinose are generally much lower than those typical of glucose in cellulose hydrolysis. It has been noted that currently no commercially available xylanase preparation is able to efficiently hydrolyze CFG (Hanchar et al, 2007). However, it is theorized that a combination of commercially available enzymes may contain the appropriate activities.

Mixtures of commercial enzyme preparations have been utilized in an attempt to yield xylose and arabinose from WDGS and DDGS. Xylose and arabinose present in WDGS and

DDGS are in the form of CFG. The hydrolysis of AFEX pretreated WDGS utilizing cellulase, β-glucosidase, xylanase, and FAE was able to release 45% of the available xylose, an increase from only a 12% yield in hydrolysis with cellulase and β-glucosidase alone. Arabinose data was not reported, nor was any attempt made to separate the effect of xylanase from that of FAE (Kim et al, 2008). DDGS was pretreated either through the LHW or AFEX processes, and hydrolysis was carried out with cellulase and β-glucosidase. This was effective at releasing glucose (93%), but ineffective at releasing xylose (14%) and arabinose (20%). The cellulase system was supplemented with commercially available xylanase, which succeeded only in achieving a marginal increase in xylose yield (22%). Supplementation with pectinase instead of xylanase (chosen for possible hemicellulose side activities) achieved a significant increase in xylose (66%) and arabinose (79%) yields, while the supplementation with both xylanase and pectinase slightly lowered xylose and arabinose yields to 61% and 75%, respectively. The addition of pectinase and ferulic acid esterase (FAE), without xylanase, further improved xylose and arabinose yields to 81% and 98%, respectively (Dien et al, 2008).

It is unsurprising that the addition of FAE improved yields. The diferulic bridges crosslinking the xylan chains hinder the action of xylanase unless FAE activity is present (Dien et al, 2006). Further evidence demonstrating the importance of FAE is provided by the microbial production of FAE by several organisms when grown on corn fiber or corn fiber fractions. *Aspergillus niger* NRRL 2001 and *Trichoderma reesei* Rut C30, both known for their production of biomass degrading enzymes, were grown on corn fiber. An activity assay of the enzymes produced showed the production of ferulic acid esterase by *A. niger* when cultured on LHW pretreated corn fiber (Dien et al, 2006). Similar results were obtained by the growth of *Fusarium*

proliferatum NRRL 26517 and of *Neosartorya spinosa* NRRL 185 (Shin and Chen, 2006, Shin et al, 2006).

SCOPE OF COMPLETED RESEARCH

Project Objectives

The goal of the project was to develop a process to fractionate corn fiber hemicellulose and obtain xylose and arabinose from it. The objectives of the research were:

1. to develop an enzyme-based process to fractionate corn fiber cellulose and hemicellulose and isolate the hemicellulose fraction
2. to investigate enzymatic systems for hydrolysis of the isolated hemicellulose fraction to produce xylose and arabinose
3. to develop a mass balance to quantify process yields

Document Summary

This document is organized into three chapters, as follows:

1. *Introduction*: A review of literature pertaining to topics relevant to the ethanol industry, the production of ethanol and other compounds from corn and cellulosic biomass, and corn fiber is presented. The objectives of the completed research are presented.
2. *Process Development for the Fractionation and Isolation of Corn Fiber Hemicellulose*: The methods and data relevant to the completion of the stated objectives are presented. This chapter is formatted for individual publication.
3. *Concluding Remarks*: Concluding remarks on the project are made, and directions for future research, including comments on the scaling of the developed process, are presented.

NOMENCLATURE

Table 1.7. Definition of acronyms appearing in Ch. 1.

Acronym	Meaning
2,3-BD	2,3-butanediol
5-HMF	5-hydroxymethyl furfural
AFEX	ammonia fiber explosion
AHP	alkaline hydrogen peroxide
CAA	Clean Air Act
CBP	consolidated bioprocessing
CFG	corn fiber gum
CGF	corn gluten feed
CGM	corn gluten meal
DA	dilute acid
DDGS	distillers' dried grains with solubles
DOE	Department of Energy
EPA	Environmental Protection Agency
FAE	ferulic acid esterase
GHG	green house gas
LHW	liquid hot water
MTBE	methyl tertiary-butyl ether
NEV	net energy value
NR	not reported
NREL	National Renewable Energy Lab
RFA	Renewable Fuels Association
RFS1	Renewable Fuels Standard 1
RFS2	Renewable Fuels Standard 2
SAA	soaking in aqueous ammonia
SEAA	soaking in ethanol and aqueous ammonia
SHF	separate hydrolysis and fermentation
SSF	simultaneous saccharification and fermentation
USDA	United States Department of Agriculture
WDGS	wet distillers' grains with solubles

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CHAPTER TWO

PROCESS DEVELOPMENT FOR THE FRACTIONATION AND ISOLATION OF CORN FIBER HEMICELLULOSE

INTRODUCTION

Corn is the predominant feedstock used in the production of ethanol in the United States. The Renewable Fuels Association (RFA) estimates that in 2009, 3.8 billion bushels of corn (90.4 million metric tons) were converted to 10.6 billion gallons of ethanol and 30.5 million metric tons of co-products (RFA, 2010). Corn based ethanol is produced from the starch component of the corn kernel, which comprises 70 – 72% of the kernel on a dry weight basis (Bothast and Schlicher, 2005). Two different processes, wet milling and dry grind, are used in the conversion of corn to ethanol.

Wet milling plants fractionate the corn into its starch, germ, fiber, and protein components. The purified starch component is utilized in fermentation to produce ethanol, while the other components are further processed to a variety of co-products. Dry grind plants, on the other hand, do not fractionate the corn kernel prior to fermentation. This results in a greatly reduced capital cost, but also fewer co-products. The marketing of co-products is considered critical to the economic viability of ethanol (Bothast and Schlicher, 2005). Typically, co-products from both wet milling and dry grind ethanol plants are marketed as animal feed. However, they are of relatively low monetary value. Because these co-products contain cellulosic material, they hold the potential for conversion into additional ethanol or high value-added products. Utilization of these co-products in such a way adds a revenue stream to the

plant, potentially improving the overall cost effectiveness of ethanol production. Corn fiber is a co-product of the corn wet milling process and is composed of the cellulosic components of the corn kernel, namely the pericarp and endosperm fiber. Recently, modifications to the dry grind process have been developed to allow pericarp fiber production there as well (Dien et al, 2004). While industrial yields of corn fiber vary, corn fiber production averages 11.5% of the mass of corn processed (dry basis) in corn wet milling facilities.

Wet milling corn fiber is of particular interest because of its high carbohydrate content and its (currently) low value (Hanchar et al, 2007). In addition, unlike many other agricultural residues, corn fiber is already available onsite in ethanol facilities. Thus, its utilization as a feedstock for other processes within the facility avoids the costs of gathering and transportation associated with other agricultural residues such as corn stover. Corn fiber is a lignocellulosic biomass, composed primarily of cellulose, hemicellulose, and lignin. Although the composition of corn fiber varies by source, cellulose and hemicellulose typically account for about 50% of the dry weight, with adherent starch comprising another 10 – 20%. Thus, corn fiber typically consists of about 70% recoverable sugars.

Because of the complexity and recalcitrance of cellulosic biomass, effective utilization of the non-starch polysaccharides requires pretreatment prior to enzymatic hydrolysis to release fermentable sugars. While a number of pretreatment methods exist, the soaking in aqueous ammonia (SAA) process offers several benefits. SAA is carried out at moderate temperature (typically less than 90°C), allowing for reduced reactor cost. The SAA process has the advantage of being a very simple batch process. Thus, it is much more easily implemented in a plant, and requires less instrumentation and control systems. It does not generate inhibitory compounds that affect the growth of yeast, demonstrates a high retention of major sugars, a significant degree of

lignin removal, and results in a pretreated material that is highly enzymatically digestible (Drapcho et al, 2008).

After pretreatment and enzymatic hydrolysis, the resulting sugar stream will contain hexose and pentose sugars, derived from the cellulose and hemicellulose components, respectively. Because *Saccharomyces cerevisiae*, the organism used in industrial fuel ethanol fermentation, cannot utilize pentose sugars, it may be advantageous to fractionate the cellulose and hemicellulose components from one another to allow separation of the hexose and pentose sugars.

The hemicellulose fraction of corn fiber is an arabinoxylan, typically referred to as corn fiber gum (CFG). It is considered to be one of the most complex and recalcitrant hemicelluloses. It is composed typically of 42.24 – 47.52% xylan and 29.04 – 30.8% arabinan (Doner and Hicks, 1997) Industrially, it has several uses. In its native form, CFG may be extracted and used as an emulsifier in the beverage industry (Yadav et al, 2007). If CFG were hydrolyzed, it could serve as a source of xylose and arabinose for use in a number of high-value product fermentations, including xylitol, astaxanthin, and many others. Corn fiber hemicellulose is composed of two fractions, hemicellulose A and hemicellulose B. Hemicellulose A typically accounts for less than 10% (w/w) of the total hemicellulose, and is discarded in most processes that extract corn fiber hemicellulose (Doner and Hicks, 1997). Hemicellulose A is insoluble in water at acidic pH, while hemicellulose B remains soluble under these conditions. Thus, the fractionation of the hemicellulose A component from the total hemicellulose is simple, and may be carried out relatively easily.

Corn fiber gum is composed of β-1,4 linked xylose, forming the xylan backbone. This constitutes about half of the CFG mass (db). Typically about 80% of the xylan backbone of CFG

is substituted primarily with arabinose. The xylan chains are crosslinked to one another by diferulic bridges (Saha, 2003). To form the diferulic bridge, two ferulic acid residues (each substituted to a separate xylan chain) form an ester linkage with one another. The cellulose fibers, along with structural proteins, embed within the lattice formed by the crosslinked xylan, thus forming the corn fiber cell wall. Proteins are associated with the hemicellulose, as even purified CFG may contain up to 5% protein (Yadav et al, 2007b).

The extraction of hemicellulose from corn fiber is desirable for several reasons. First, the fractionation of hemicellulose allows it to be hydrolyzed separately from the cellulose, yielding separate hexose and pentose streams. This allows great flexibility, as the pentose sugars can be utilized for any of a number of high-value product fermentations, while the glucose may be added back into ethanol fermentations without the need for modifications to the existing ethanol process. Additionally, if hydrolysis of CFG is not to be performed, the CFG may be sold as a valuable product, for use as a beverage flavor emulsifier, for example.

Due to the complexity of CFG, reported yields of xylose and arabinose are generally much lower than those typical of glucose in cellulose hydrolysis. It has been noted that currently no commercially available xylanase preparation is able to efficiently hydrolyze CFG (Hanchar et al, 2007). However, it is theorized that a combination of commercially available enzymes may contain the appropriate activities. The objectives of the present work were to develop an enzyme-based process to fractionate corn fiber cellulose and hemicellulose and isolate the hemicellulose fraction, and to investigate enzymatic systems for hydrolysis of the isolated hemicellulose fraction to produce xylose and arabinose.

MATERIALS AND METHODS

Chemicals and Corn Fiber

Corn fiber was provided by Archer Daniels Midland (Decatur, IL, USA). All chemicals were reagent grade and purchased from Sigma. Anhydrous ethanol was used for hemicellulose extraction, and was stored in the freezer prior to use. Novo 188 (β-glucosidase) was purchased from Sigma-Aldrich (St. Louis, MO, USA). GC-220 (cellulase), Spezyme Fred (α-amylase), Spezyme Xtra (α-amylase), PEKTOZYME Essential (pectinase), and Optidex L-300 (glucoamylase) were provided by Genencor, a Danisco Division (Rochester, NY, USA). Novozymes NS50030 (xylanase), Novozymes NS50012 (multi-enzyme complex), and Novozymes NS50014 (xylanase) were provided by Novozymes (Franklinton, NC, USA). Depol 692 L (ferulic acid esterase) was provided by Biocatalysts Inc. (Wales, UK). Active Dry Ethanol Red yeast was provided by LeSaffre Yeast Corporation (Milwaukee, WI, USA), and stored at 4°C until use.

Corn Fiber Starch Removal

To separate the glucose contributions from starch and from cellulose, starch was removed from the corn fiber prior to any other experiments. Corn fiber was mixed with water at 10% dry solids loading, and the pH adjusted to 6.0. Spezyme Fred (α-amylase) was added at a loading of 42μL / g dry corn fiber. The mixture was then heated to 80°C for 1 hour with mechanical stirring, and allowed to cool to 55°C with the aid of a chilled water bath. The mixture was weighed to determine the amount of water lost due to evaporation during heating, and that amount was added back using deionized water. The pH was adjusted to 4.5, and Optidex L-300

(glucoamylase) was added at a loading of 50µL / g dry corn fiber. The mixture was then placed in an orbital shaker at 55°C with 250 RPM orbital shaking overnight. The solids (destarched corn fiber, DCF) were recovered by filtering and pressing with cheese cloth. The glucose-rich liquid resulting from starch hydrolysis (destarching water, DSW) was frozen until use. The DCF was then spread in a thin layer in a large dish and dried at 40°C with occasional stirring to allow even drying. Once dried, the recovered DCF was stored in a sealed container at 4°C until use. The destarching procedure was carried out one time initially, and this batch of DCF was used as the starting point for all subsequent experiments.

Soaking in Aqueous Ammonia (SAA) Pretreatment

SAA pretreatment was carried out by combining DCF and 15% (w/w) NH_4OH (7.75 *M*) at a solid to liquid ratio of 1:11 based on dry DCF in a sealed media jar. The well mixed slurry was then held at 65°C for 8 hours. At the end of this time, the cap was removed and the open jar left at room temperature (about 25°C) in a fume hood for three hours to allow ammonia evaporation. The pretreated biomass was then washed with de-ionized water to remove soluble lignin and residual ammonia. The pretreated solids were separated from the wash water by centrifugation at 8,000 RPM for 30 minutes. The supernatant was then decanted and the pretreated solids were recovered. This washing process was carried out a total of three times. The recovered solids (pretreated destarched corn fiber, PTDCF) were sampled for moisture determination and compositional analysis, and stored in a sealed container in the refrigerator until use.

Cellulose Hydrolysis

The PTDCF was combined with water at a solids loading of 5% (db). The pH of the slurry was adjusted to 5 with H_2SO_4 (either 72% w/w [13.2 *M*] or 5% w/w [0.92 *M*] depending on distance from target pH). 1N NaOH was used to correct in the case of overshoot. Novo-188 (β-glucosidase) was loaded at 0.04 ml / g glucan (30 CBU / g glucan) and GC-220 (cellulase) was loaded at 0.35 ml / g glucan (15.75 FPU / g glucan). Hydrolysis was then carried out for 72 hours at 55°C with 250 RPM orbital shaking.

Hemicellulose A Removal

Following hydrolysis, the pH was about 4.5, sufficient to cause the hemicellulose A fraction to precipitate. 1N NaOH was added to the hydrolysate to increase the pH to 8 – 9. The hydrolysate was then mixed to dissolve the hemicellulose A. The hydrolysate was then centrifuged and the residual solids (RS) were set aside. The supernatant (hydrolysate supernatant, HSN) contained the liberated glucose from hydrolysis as well as the hemicellulose A and B. The pH of the HSN was then lowered to 3 with 72% (w/w) (13.2 *M*) H_2SO_4. The HSN was mixed to allow the hemicellulose A to precipitate out, and passed through a Whatman #1 filter paper to remove the precipitated hemicellulose A, which was discarded.

Hemicellulose B Precipitation

To precipitate the hemicellulose B, seven volumes (i.e. seven times the volume of the HSN used) of cold ethanol (stored in the freezer prior to use) was slowly added one volume (i.e. the same volume as that of the HSN) at a time to the HSN (the filtrate from the above step) with stirring. Mixing was continued for about 15 min after addition of the final volume. The mixture

was allowed to settle for about 1 hr. After settling, the liquid phase (which was still somewhat cloudy) was carefully decanted. The decanted liquid was passed through a pre-weighed Whatman #1 filter paper. After filtration of the ethanol, the filter was dried at 105°C overnight, and then weighed to quantify the mass of hemicellulose lost during ethanol decanting. A small amount (~100 ml) of cold ethanol was added to the precipitate and mixing was resumed for about 15 min. This second ethanol addition removed residual water from the precipitate, reducing its stickiness. The mixture was then passed through a fresh pre-weighed Whatman #1 filter paper. The cake was washed with a small amount of cold ethanol (~100 ml), and dried at 105°C overnight. The resulting powder was the dried hemicellulose B, which was stored in a sealed vial until use. The efficiency of the hemicellulose recovery was determined by dividing the xylan and arabinan content of the recovered CFG by the xylan and arabinan initially present in the pretreated material that was subjected to cellulose hydrolysis.

Corn Fiber Gum Enzyme Hydrolysis

The CFG was dissolved in water to a concentration of about 35 g dry CFG / L. The pH was adjusted to 5 with 1*N* NaOH, and the solution was then added to a series of microcentrifuge tubes, at 1 ml solution each. Each tube was dosed with the enzyme(s) called for in the particular experiment at the dosage indicated. The enzymes used in the experiments are listed in the Table 2.1.

Table 2.1. Enzymes used in corn fiber gum hydrolysis experiments.

Symbol[1]	Name	Advertised Activity[2]	MRD [3]
E	PEKTOZYME Essential	Pectinase	150 mg / L
D	Biocatalysts Depol 692 L	Ferulic Acid Esterase	5 % (w/w)
B	Novozymes Novo188	β-glucosidase	4 % (w/w)[4]
N30	Novozymes NS50030	Purified Endoxylanase	0.5 % (w/w)
N12	Novozymes NS50012	Complex	0.4 % (w/w)
N14	Novozymes NS50014	Endoxylanase	0.4 % (w/w)

[1]The symbols used to refer to the corresponding enzymes for the remainder of this document.
[2]The term "Advertised Activity" refers to the manufacturer's description of the enzyme's activity. In reality, the enzymes are likely to have many other activities as well.
[3]MRD: Manufacturer Recommended Dosage. In cases where the manufacturer recommended a dosage range, the high value is reported and used.
[4]Based on (Kim et al, 2008b)

Hydrolysis was carried out at 50°C for 72 hours. Each experiment was conducted in duplicate, with the averages reported. Initial and final samples were taken and analyzed for xylose and arabinose content via HPLC (described below). The theoretical yield (100% conversion) was determined by dilute acid hydrolysis of the CFG, as described below.

Ethanol Fermentation

To determine the effects of the glucose liberated by the process on corn ethanol fermentation, samples of the glucose solutions from destarching (destarching water, DSW) and from cellulose hydrolysis (hydrolysate supernatant, HSN) were utilized in corn mashing, and the mash subsequently fermented to ethanol. The corn was mashed at 25% dry solids by combining ground corn with water (as a control) or with a solution composed of equal parts DSW and HSN. (A note on nomenclature: experiments carried out on the corn mashed with water are herein referred to as "water", and those carried out on the mash produced with DSW and HSN are referred to as "DSW+HSN"). The pH was adjusted to 5.2 and Spezyme Xtra (α-amylase) was

added at a loading of 0.38 µL / g dry solids. The mixture was heated to 85°C for two hours with mechanical mixing, and then cooled to 32°C with the aid of a chilled water bath. Water lost during heating was added back to the mash. The pH was adjusted to 4 and Fermenzyme L-400 (glucoamylase) was added at a loading of 0.75 µL / g dry solids. Urea (0.2 g) was added to provide a nitrogen source. The mash was then split into five 250 ml Erlenmeyer flasks, each flask receiving 100 g. Yeast inoculum was prepared by dissolving dry dehydrated yeast in water at 5% (w/w) loading. Each flask was inoculated with 0.75 ml and the flasks were sealed with rubber stoppers pierced by a needle to allow CO_2 evolved during fermentation to escape. Flasks were weighed initially and incubated at 32°C with 150 RPM orbital shaking for 71 hours. Ethanol production was tracked by determining the decrease in mass of the flasks (mass loss due to CO_2 evolution) daily, and the final ethanol concentration was determined via HPLC (described below). Five flasks of each mash (water or DSW+HSN) were fermented, and the average is reported.

Analytical Procedures

The glucan, xylan, and arabinan content of the DCF, PTDCF, and RS were determined according to NREL LAP Determination of Structural Carbohydrates and Lignin in Biomass (Sluiter et al, 2008). The composition of the CFG was determined by the method of Hanchar et al (2007), with the modification that a sample of synthetic sugars mimicking the expected composition of the CFG was also hydrolyzed under the same conditions to quantify sugar destruction, and the data adjusted accordingly, as recommended in the NREL procedure. In both procedures, monomeric sugars were determined by HPLC. The HPLC was a Shimadzu system utilizing either the Bio-Rad Aminex HPX-87P or Bio-Rad Aminex HPX-87H columns.

Standards containing glucose, xylose, and arabinose were analyzed with each set of samples to correct for any bias caused by the differing columns, though none was observed. The flow rate and mobile phase were 0.6 ml / min of nanopure 18 MΩ deionized water (HPX-87P column) or 0.6 ml / min of 5 m*M* H_2SO_4 (HPX-87H column), and the system was equipped with an RID detector. All analytical procedures were carried out in duplicate, and the averaged results reported.

Mass Balance

To determine yields throughout the process, mass balances with respect to glucan, xylan, and arabinan were constructed. In cases where data was in the form of glucose, xylose, or arabinose, it was converted to glucan, xylan, or arabinan by multiplying by 0.9 (glucose) or 0.88 (xylose and arabinose) as described by Gulati et al (1996). It should be noted that the term arabinan is used here to describe arabinose, because it is considered standard to report sugars on a polymeric basis. However, arabinan is not actually present in corn fiber. Rather, arabinose monomers are present, substituted onto the xylan backbone of the hemicellulose. The composition of the destarched corn fiber (DCF), pretreated destarched corn fiber (PTDCF), and corn fiber gum (CFG) were determined as mentioned above. The composition of the DCF was used to determine the initial mass of glucan, xylan, and arabinan present prior to pretreatment. All percentages reported in the mass balance were calculated as the percentage of the initial mass of the component present in the DCF. After pretreatment, the composition of the pretreated solids was used to determine the amount of glucan, xylan, and arabinan recovered. These were subtracted from the amounts initially present to determine the pretreatment loss, referred to as "PT Loss". Samples of the PTDCF were taken for moisture determination and compositional

analysis. The amounts of glucan, xylan, and arabinan in the samples were accounted for in the mass balance as “Sample Loss”. Also included in this category were losses due to material left in containers after collection, for example the amount of pretreated material stuck to the side of the centrifuge bottle after washing recovery. This amount was small, but included for completeness. Such material losses were calculated by weighing containers prior to use, and re-weighing after use to obtain the mass of solids not recovered. Next, the glucan, xylan, and arabinan content of the recovered CFG was subtracted from that of the PTDCF subjected to cellulose hydrolysis, representing the masses of sugars present that were not recovered as CFG. These are accounted for in the category labeled “Pass Through”. The glucan, xylan and arabinan content of the recovered CFG was then accounted for, as was the glucan, xylan, and arabinan content of the CFG lost during the initial ethanol decanting. These values were summed and included as one value, “CFG”. The masses of each component in each category were then summed to determine the total percentage of the component accounted for in the mass balance, labeled “Sum”.

Statistical Analysis

Presented results represent the average of two trials except when noted otherwise. Standard deviations are also presented where applicable.

RESULTS AND DISCUSSION

The process developed to fractionate corn fiber gum is shown in Figure 2.1. Corn fiber (CF) was first destarched, and the resulting glucose solution (DSW) utilized in corn mashing for ethanol fermentation. The composition of the destarched corn fiber (DCF) is shown in Table 2.2.

Table 2.2. Destarched corn fiber major sugars composition

Component (% of DCF, w/w, db)		
Glucan	Xylan	Arabinan
38.08%	24.73%	18.00%

The DCF was pretreated by the SAA process, and then washed to remove solubilized lignin. The recovered solids (pretreated destarched corn fiber, PTDCF) were hydrolyzed with cellulase, and β-glucosidase was supplemented to relieve glucose inhibition. During hydrolysis, cellulose was converted to glucose, and hemicellulose was broken down to xylo-oligosaccharides, which were soluble in the aqueous phase. After hydrolysis, the hydrolysate was centrifuged to separate the residual solids (RS) which were primarily lignin. The liquid phase (hydrolysate supernatant, HSN) was recovered by decanting, and the pH was raised to about 9 to ensure any hemicellulose A remaining was in solution. The HSN was filtered to remove the small amount of residual solids remaining, and the pH of the filtrate reduced to 3 to precipitate the hemicellulose A. This was removed via filtration. Seven volumes of cold ethanol were added to the filtrate to precipitate the hemicellulose B, which was then washed with additional ethanol, collected via filtration, and dried. The resulting product was a white or off-white powder, which was used in subsequent enzyme hydrolysis experiments.

Batches (defined as an iteration of the process beginning with pretreatment through obtaining the precipitated CFG) were conducted with the pretreatment of 7 g dry DCF for the construction of the mass balance, in order to conserve material and allow for all steps to be carried out analytically. Additional batches were conducted beginning with 70g dry DCF for the purpose of obtaining enough dry CFG to carry out subsequent CFG enzymatic hydrolysis experiments.

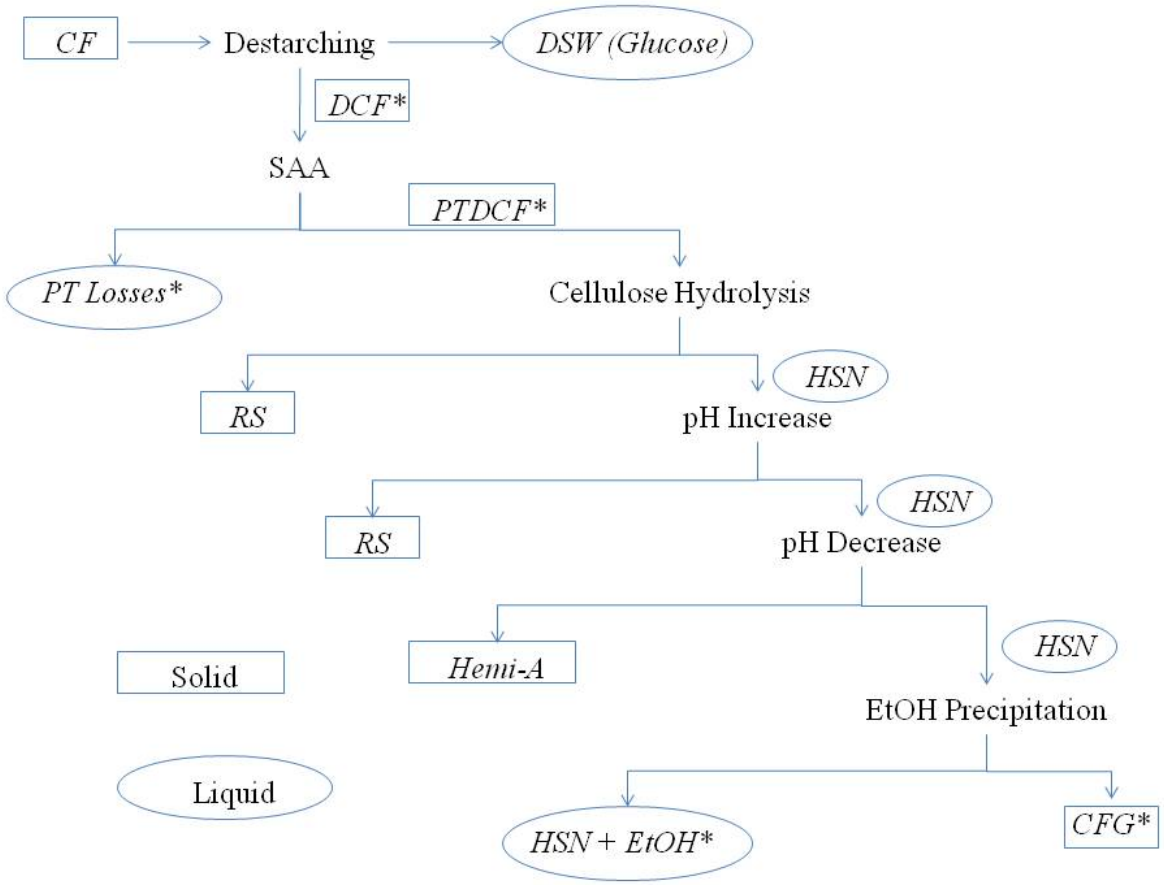

Figure 2.1. Process for the extraction and isolation of corn fiber hemicellulose. Solid materials are indicated by a box, liquids are indicated by an oval. All material streams are represented in italic font. Unit operations are in plain text with no symbol (box or oval). Streams marked with * are those utilized in the mass balance.

SAA Pretreatment and Cellulase Hydrolysis

DCF was pretreated using the SAA process. The glucan, xylan, and arabinan content of the resulting material were then determined (Table 2.3).

Table 2.3 Compositional analysis of pretreated destarched corn fiber.

	Component (% of PTDCF w/w, db)		
Batch	Glucan	Xylan	Arabinan
1	50.47	26.66	20.19
2	47.53	26.87	20.18
3	51.07	24.37	17.47
Average	49.69	25.97	19.28
St. Dev.	±1.89	±1.39	±1.57

The recovery percentages of these components in the washed PTDCF, as compared to the untreated DCF, (i.e. the fraction of each component retained) are shown in Table 2.4

Table 2.4 Soaking in aqueous ammonia pretreatment recoveries.

	Recovery (% w/w, db)			
Batch	Solids	Glucan	Xylan	Arabinan
1	69.40	91.99	74.82	77.85
2	75.85	94.68	82.42	85.04
3	71.75	96.23	70.71	69.64
Average	72.34	94.30	75.98	77.51
St. Dev.	±3.26	±2.15	±5.94	±7.71

These data indicate good recoveries of the major sugars, specifically glucan. The recoveries are comparable to data previously obtained by others (Kim, 2008, unpublished data).The pretreated material was hydrolyzed with cellulase enzymes, releasing, on average, 85.41% of available glucan as glucose. Hydrolysis efficiencies for xylan and arabinan were, as expected, much lower, 8.15% and 16.95%, respectively. This, combined with the observation that only a small amount of residual solids were recovered after hydrolysis, containing very little xylan or arabinan, confirms that the xylan and arabinan were largely degraded into soluble oligomers during the hydrolysis.

Hemicellulose Recovery

The efficiency of the hemicellulose recovery was low, 45% for xylan and only 28% for arabinan (based on total hemicellulose content of the pretreated material subjected to cellulose hydrolysis), on average. This is likely due to the fact that the solubilities of the xylo-oligosaccharides in ethanol are a function of chain length, i.e. longer chain xylo-oligosaccharides are precipitated more easily, at lower ethanol concentration. The low efficiencies, thus, are likely a result of lower molecular weight xylo-oligosaccharides that remain soluble upon the addition of 7 volumes of ethanol. The observation that a precipitate began to form (presumably from the largest xylo-oligosaccharides present) upon addition of the second volume of ethanol supports this theory. It was expected that the arabinan recovery would be lower than that observed for xylan, due to the mechanism of hydrolysis of corn fiber hemicellulose. In order for the xylan backbone to be accessible by enzymes, the substituted species (i.e. arabinose) must first be removed. If the hemicellulose is solubilized by cleavage into xylo-oligosaccharides, it follows that some arabinose must be liberated from the hemicellulose, and thus not available for precipitation. This was supported by the observation of a greater hydrolysis yield for arabinose, as mentioned above. The composition of the recovered hemicellulose (corn fiber gum, CFG) is shown in Table 2.5.

Table 2.5 Corn fiber gum composition

	Component (% of CFG w/w, db)			
Batch	Glucan	Xylan	Arabinan	X:A[1]
1	0.31	30.67	13.93	2.20
2	0.27	28.63	13.01	2.20
3	0.22	27.17	12.50	2.17
Average	0.27	28.82	13.15	2.19
St. Dev.	±0.05	±1.76	±0.72	±0.02

[1]X:A: Xylan:Arabinan Ratio, calculated by dividing xylan content by arabinan content.

The xylan and arabinan content of the recovered CFG was lower than expected. This is likely due to a mass dilution effect. After pretreatment, the solids were washed minimally to minimize the loss of pretreated material in the wash water. Thus, some of the lignin solubilized during pretreatment was not washed away, and ended up in the CFG, causing a reduction in the mass percent of the xylan and arabinan. In the larger batches conducted with the goal of producing CFG for hydrolysis experiments, the pretreated solids were washed more thoroughly, as analytical solids recovery for the mass balance was not a concern. The CFG recovered from these batches had the expected composition, shown in Table 2.6.

Table 2.6 Additional corn fiber gum composition

	Component (% of CFG w/w, db)			
Batch	Glucan	Xylan	Arabinan	X:A
A	0.52	53.48	23.24	2.30
B	0.39	47.62	21.16	2.25
C	0.62	56.80	25.99	2.19
D	0.49	55.47	25.83	2.15
Average	0.51	53.34	24.06	2.22
St. Dev.	±0.10	±4.05	±2.31	±0.07

It should be noted that, while the mass percentages in Table 2.5 are lower than those in Table 2.6, the xylan:arabinan ratios are comparable, indicating the mass dilution effect. Further, it was observed that less CFG was recovered per volume of HSN used in extraction in batches A – D. The compositions shown in Tables 2.5 and 2.6 were used to determine the mass of xylan and arabinan recovered per HSN volume, shown in Table 2.7.

Table 2.7 Xylan and arabinan content of recovered corn fiber gum

	Component per ml HSN (mg, db)		
Batch	CFG	Xylan	Arabinan
A	6.40	3.42	1.49
B	8.14	3.88	1.72
C	7.08	4.02	1.84
D	6.79	3.76	1.75
Average	7.10	3.77	1.70
St. Dev.	±0.75	±0.25	±0.15
1	11.05	4.10	1.92
2	14.66	5.12	2.50
3	15.52	5.12	2.45
Average	13.74	4.78	2.29
St. Dev.	±2.37	±0.59	±0.32

While a greater mass of CFG was recovered per ml HSN in batches 1 – 3, the mass of xylan and arabinan therein was comparable. This provides further support for the mass dilution effect previously mentioned.

Hemicellulose Hydrolysis

Commercially available enzymes were used individually and in combinations in an attempt to hydrolyze CFG. Xylanases (N30, N12, N14) were chosen for their expected activity

against the xylan backbone. Ferulic acid esterase (D) was chosen to cleave the diferulic bridges cross linking the arabinoxylans, which was expected to improve their digestibility. Pectinase (E) and β-glucosidase (B) were chosen for their possible side activities, which have shown some success in hydrolyzing CFG in previous work (Dien et al, 2008) The enzymes were first tested individually at one and ten times the manufacturers recommended dosage (MRD) (see Table 2.1). The yields, based on total xylan and arabinan available in the hydrolysis, are shown in Table 2.8.

Table 2.8. Xylose and arabinose yields in individual enzyme hydrolysis

	Component Yield (% w/w, db)							
Dosage:	1 x MRD				10 x MRD			
Enzyme	Xylose	St. Dev X[1]	Arabinose	St. Dev. A[2]	Xylose	St. Dev. X	Arabinose	St. Dev. A
E	4.99	±0.08	16.43	±0.03	8.44	±0.93	24.53	±1.16
N30	0.51	±0.01	1.97	±0.06	1.38	±0.01	1.90	±0.03
D	ND[3]	NA[4]	5.34	±0.14	7.39	±0.14	32.63	±0.64
B	3.35	±0.04	12.41	±0.12	2.08	±0.01	11.41	±2.63
N12	ND	NA	ND	NA	1.62	±1.32	7.49	±0.43
N14	ND	NA	ND	NA	ND	NA	ND	NA

[1]St. Dev. X: Standard deviation of the xylose yield data in the column to the left
[2]St. Dev. A: Standard deviation of the arabinose yield data in the column to the left
[3]ND: Not Detected
[4]NA: Not Applicable

At the 1x dosage level, the highest yields were produced by pectinase, which is unsurprising because it was chosen for its wide range of side activities. At the 10x level, the yields achieved with D were slightly higher than with E. The low yields demonstrated by xylanases N30 and N14 were not surprising, as these enzymes were advertised as being relatively pure in their activity (see Table 2.1). The low yield demonstrated by N12 was more surprising, as this enzyme was advertised as a complex with multiple activities.

To investigate potential synergism between these enzymes, combinations of the enzymes were tested for their ability to hydrolyze CFG. The first set of experiments utilized N30 as the xylanase. The results are shown in Table 2.9.

Table 2.9 Xylose and arabinose yields in mixed enzyme hydrolysis with xylanase N30

	Component Yield (% w/w, db)							
Dosage:	1 x MRD				10 x MRD			
Enzymes	Xylose	St. Dev. X	Arabinose	St. Dev. A	Xylose	St. Dev. X	Arabinose	St. Dev. A
E+D	5.36	±0.05	18.27	±0.40	14.45	±0.20	32.47	±0.46
E+D+B	5.99	±0.02	20.36	±0.36	9.11	±0.10	26.81	±0.21
E+D+N30	5.61	±0.11	18.43	±0.34	17.38	±0.34	34.85	±0.58
E+D+N30+B	6.40	±0.08	20.34	±0.22	10.82	±1.64	27.73	±1.90
E+B	5.59	±0.01	18.36	±0.12	7.36	±0.03	28.27	±0.14
D+B	4.45	±0.01	17.15	±0.12	3.79	±0.25	18.70	±0.53
D+B+N30	4.75	±0.05	17.35	±0.17	7.63	±1.23	23.92	±1.15
N30+B	3.92	±0.18	12.35	±0.25	2.19	±0.10	9.91	±2.88

At the 1x dosage level, the highest yields were obtained by the combination of all four enzymes. However, the yields in the E+D+B trial were only slightly lower, suggesting that N30 was very ineffective. This is consistent with the results found in the individual trials. This was also seen when N30 was added to E+D and to D+B. It was also observed that B was not very effective at the 1x dosage level, causing very modest increases when added to E+D. However, the combination of D and B exhibited a much higher yield that D alone, suggesting a synergistic effect between the two enzymes.

At the 10x dosage level, the greatest yields were exhibited by E+D+N30, although the increase over E+D was very modest, consistent with the 1x dosage results and those of the individual trial. Interestingly, the addition of B to E+D and to E+D+N30 both caused a decrease in yield of about 5 – 7%. This effect was not seen in the 1x dosage trials. In the case of N30+B, yields were actually decreased by the increase in dose. These observations suggest that B is problematic when used in high concentration.

The yields exhibited by N30 were poor, as expected. Thus, xylanases N12 and N14 were investigated as alternatives. The results are shown in Table 2.10

Table 2.10 Xylose and arabinose yields in mixed enzyme hydrolysis with xylanases N12 and N14

	Component Yield (% w/w, db)							
Dosage:	1 x MRD				10 x MRD			
Enzymes	Xylose	St. Dev. X	Arabinose	St. Dev. A	Xylose	St. Dev. X	Arabinose	St. Dev. A
N14+D	ND	NA	5.52	±0.02	6.46	±0.32	28.68	±0.79
N14+E+D	1.56	±0.04	8.28	±0.26	13.70	±0.05	38.51	±0.27
N12+N14+E+D	1.49	±0.05	9.31	±0.08	13.72	±0.57	38.28	±0.95
N12+D	0.49	±0.01	6.25	±0.24	6.66	±0.14	28.51	±0.20
N12+E+D	1.61	±0.01	9.22	±0.06	14.07	±0.39	38.81	±0.52
N12+N14	ND	NA	2.06	±0.11	ND	NA	10.14	±0.43
N12+N14+D	ND	NA	5.89	±0.19	6.25	±0.38	27.53	±0.71

At the 1x dosage level, the yields obtained by N14+E+D, N12+E+D, and N14+N12+E+D were very similar, suggesting little difference in the effectiveness of N12 and N14. The same trend was observed at the 10x dosage level. At both dosage levels, the combination of N12+N14 was ineffective, which is consistent with the individual trials.

The highest yields in the multiple enzyme trials were obtained by the combinations of E+D and E+D+xylanase (little difference was seen between xylanases). In the single enzyme trials, the highest yield was obtained by D, though it was a small increase over E at a significantly greater loading (see Table 2.1). Thus, E was loaded at 100x dosage and utilized alone and in combinations of E+D and E+D+N30. D and N30 were loaded at the 10x dosage level. The results are shown in Table 2.11.

Table 2.11 Xylose and arabinose yields in enzyme hydrolysis with 100x pectinase loading.

	Component Yield (% w/w, db)			
Enzyme	Xylose	St. Dev. X	Arabinose	St. Dev. A
E	34.49	±2.45	47.20	±2.77
E+D	44.45	±0.77	52.89	±0.05
E+D+N30	44.48	±6.92	52.45	±2.81

When E is loaded at the 100x dosage level, the highest yields are observed. The addition of D increased yields over E alone, while the addition of N30 to the mixture was ineffective. Because of the ineffectiveness of the addition of N30, the highest performing combination was considered to be E+D.

Process Mass Balance

A mass balance was conducted around the process for glucan, xylan, and arabinan. The overall mass balance was good, with 86.89% glucan, 90.59% xylan, 89.90% arabinan accounted

for on average. The major sugars compositions of each stream utilized in the mass balance calculation were provided previously in Table 2.2 (DCF), Table 2.3 (PTDCF), and Table 2.5 (CFG). A detailed mass balance is provided in Table 2.12.

Table 2.12 Process mass balance

	Stream:	DCF		PT Loss		Sample Loss		Pass Through		CFG		Sum	
Batch	Component	Mass (g)	Percent Initial	Mass (g)	Percent Initial	Mass (g)	Percent Initial	Mass (g)	Percent Initial	Mass (g)	Percent Initial	Mass (g)	Percent Initial
1	Glucan	2.662	100	0.213	8.01	1.321	49.63	0.864	32.45	0.002	0.08	2.400	90.16
	Xylan	1.729	100	0.435	25.18	0.698	40.37	0.288	16.64	0.199	11.51	1.620	93.70
	Arabinan	1.258	100	0.279	22.15	0.528	42.00	0.269	21.40	0.090	7.18	1.167	92.73
2	Glucan	2.666	100	0.142	5.32	1.188	44.55	0.880	33.03	0.002	0.08	2.212	82.99
	Xylan	1.731	100	0.304	17.58	0.671	38.78	0.301	17.42	0.220	12.73	1.498	86.52
	Arabinan	1.260	100	0.189	14.96	0.504	40.02	0.285	22.62	0.100	7.95	1.078	85.54
3	Glucan	2.666	100	0.101	3.77	1.307	49.03	0.924	34.65	0.002	0.07	2.333	87.52
	Xylan	1.731	100	0.507	29.29	0.624	36.03	0.239	13.81	0.215	12.41	1.585	91.54
	Arabinan	1.260	100	0.383	30.36	0.447	35.49	0.223	17.73	0.099	7.84	1.152	91.42
Avg	Glucan	2.664	100	0.152	5.70	1.272	47.74	0.889	33.37	0.002	0.07	2.315	86.89
	Xylan	1.730	100	0.416	24.02	0.664	38.39	0.276	15.96	0.211	12.22	1.567	90.59
	Arabinan	1.259	100	0.283	22.49	0.493	39.17	0.259	20.58	0.096	7.66	1.132	89.90
St. Dev.	Glucan	0.002	0.00	0.057	2.15	0.073	2.77%	0.031	1.14	0.000	0.01	0.095	3.63
	Xylan	0.001	0.00	0.103	5.94	0.038	2.19%	0.033	1.90	0.011	0.63	0.063	3.69
	Arabinan	0.001	0.00	0.097	7.71	0.042	3.34%	0.032	2.54	0.005	0.41	0.048	3.83

Ethanol Fermentation of DSW and HSN

Corn was mashed with either water or a mixture of DSW and HSN from the above process, and fermented into ethanol. At the end of the fermentation period, all flasks tested negative for starch (iodine test) and glucose (HPLC). The mass loss from the flasks is shown in Figure 2.2. In the figure, the mashing and fermentation of corn utilizing water as the liquid is referred to as "water". When the mixture of sugar solutions was used as the liquid in mashing, it is referred to as "DSW+HSN".

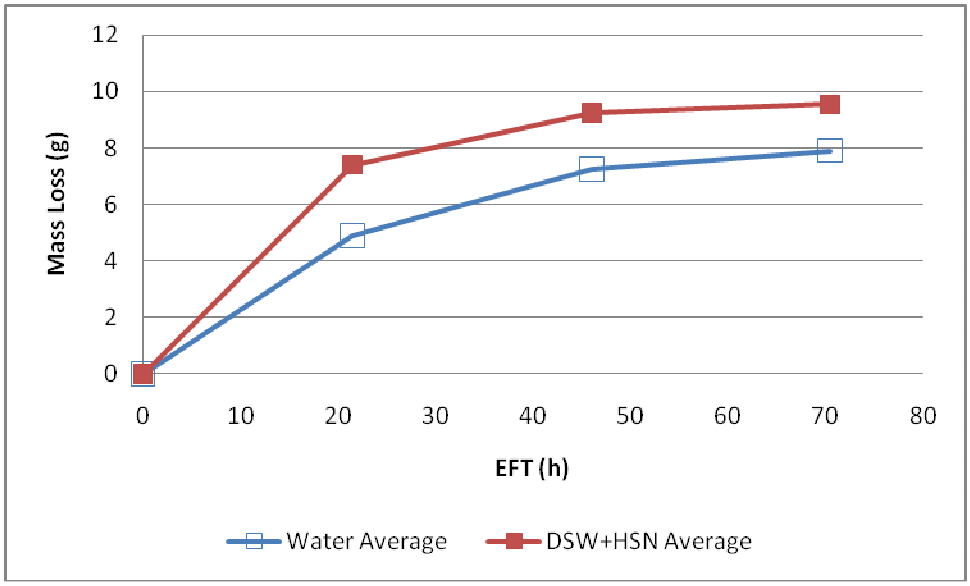

Figure 2.2. Mass lost from flasks during ethanol fermentation.

It can be seen that additional mass was lost (and thus additional ethanol produced) from the flasks mashed with a mixture of DSW and HSN, due to the glucose added by using these materials rather than water. The final ethanol concentrations were 11.52% (v/v) in

the water trials and 13.55% (v/v) in the DSW+HSN trials. The efficiency of the mashing and fermentation was calculated by comparing the ethanol production to the theoretical production based on total glucan (and glucose for DSW+HSN trial) initially present in the mash. The efficiencies for the water and DSW+HSN trials were 80% and 87%, respectively. While it was expected that additional ethanol would be produced (due to additional glucose) in the DSW+HSN trial, the increase in overall efficiency was unexpected. It is likely due to the presence of additional nutrients and enzymes in the DSW and HSN. No attempt was made to denature the enzymes remaining in these materials prior to their use. The enzymes present in the HSN would be unlikely to retain activity after the heating during the mashing process. Those present in the DSW, however, were thermostable and would likely have remained active. This was supported by the observation that the mash utilizing the DSW+HSN had a lower viscosity than the mash produced with water.

CONCLUSION

A process was developed to fractionate the hemicellulose component of corn fiber. Destarched corn fiber was pretreated by soaking in aqueous ammonia, which retained 94% of the available glucan, 76% of the available xylan, and 78% of the available arabinan. The pretreated material demonstrated a glucan digestibility of 85% when hydrolyzed with cellulase enzymes. Xylan and arabinan digestibilities were low but very little xylan and arabinan remained solid after hydrolysis, suggesting that the

majority of the arabinoxylan was solubilized in the form of xylo-oligosaccharides. These were precipitated by the addition of ethanol. The precipitated solids were the corn fiber gum. A mass balance was constructed around the process, which accounted for 87% of glucan, 91% of xylan, and 90% of arabinan. A variety of enzymes were utilized in an attempt to release xylose and arabinose from the recovered corn fiber gum. The greatest xylose and arabinose yields (44.45% and 52.89%, respectively) were achieved when PEKTOZYME Essential, a pectinase, was loaded at 100x manufacturer recommended dosage along with Biocatalysts Depol 692 L, a ferulic acid esterase loaded at 10x MRD. The glucose liberated from the destarching of the corn fiber was utilized in ethanol fermentation along with the hydrolysate resulting from cellulose hydrolysis to increase ethanol concentration in the beer by 2% (v/v). The efficiency of the fermentation was increased by 7%.

While it has been demonstrated that the developed process is technically feasible, much work remains in its optimization. A significant amount of the hemicellulose was not recovered by ethanol precipitation, presumably due to low chain length. Thus, a cellulose hydrolysis method should be developed that retains the high glucan digestibility demonstrated above, but solubilizes the arabinoxylan by producing longer chain xylo-oligosaccharides. This would allow a greater recovery of xylan and arabinan from a smaller volume of ethanol. Additionally, the volume of the hydrolysate could be reduced by evaporation or ultrafiltration, again reducing the needed amount of ethanol. Finally, the enzymatic hydrolysis of corn fiber gum remains a challenge, and additional work is needed to generate improvements in this step.

NOMENCLATURE

Table 2.13. Definition of acronyms appearing in Ch. 2.

Acronym	Meaning
CFG	corn fiber gum
DCF	destarched corn fiber
DSW	destarching water (glucose solution)
EFT	elapsed fermentation time
HSN	hydrolysate supernatant
LAP	laboratory analytical procedure
MRD	manufacturer's recommended dose
ND	not detected
NREL	National Renewable Energy Lab
PTDCF	pretreated destarched corn fiber
RFA	Renewable Fuels Association
RS	residual solids
SAA	soaking in aqueous ammonia

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CHAPTER THREE

CONCLUDING REMARKS

Though the ability of the developed process to fractionate hemicellulose from corn fiber was demonstrated, additional research is recommended. Several areas for improvement are suggested.

1. *Pretreatment*. Although the glucan retention was high (94%), the xylan and arabinan retentions were lower, 76% and 78%, respectively. A pretreatment process that demonstrated higher retentions of xylan and arabinan would improve the overall efficiency of the process. This could be achieved by further optimizing the pretreatment conditions. As an alternative, pretreatment by soaking in ethanol and aqueous ammonia (SEAA) has been shown to significantly increase xylan and arabinan retention by causing xylo-oligosaccharides solubilized by ammonia to precipitate, allowing their recovery with the pretreated solids. Addition of ethanol to the pretreatment stage may prove beneficial to the overall process.
2. *Cellulose Hydrolysis and Precipitation Efficiency*. During this step of the process, cellulose is hydrolyzed to glucose and the arabinoxylan is solubilized by cleavage into xylo-oligosaccharides. These are recovered by the addition of ethanol, with larger chains precipitating at lower ethanol concentrations. The shortest chains may not be able to precipitate at all. Development of a hydrolysis procedure which still solubilized the hemicellulose while maximizing the chain length of the xylo-oligosaccharides would allow a greater recovery of CFG.

3. *Ethanol Volume.* Currently, seven volumes of ethanol are used in the precipitation. However, the formation of precipitate was observed after the addition of only two volumes. Maximizing xylo-oligosaccharide chain length, discussed above, would allow fewer volumes of ethanol to be used. Additionally, volume reduction of the hydrolysate, possibly through evaporation or ultrafiltration, could be carried out prior to precipitation. This would further reduce the volume of ethanol needed to recover the CFG.
4. *Hemicellulose Hydrolysis.* This step remains a significant challenge to the utilization of corn fiber. For the greatest efficiency to be realized, improvements to the hydrolysis must occur. To achieve this, a wider range of commercially available enzymes should be screened, and work conducted to optimize the loadings of the enzymes.

The process as described herein was developed and carried out at the laboratory scale. Thus, modifications to the process are necessary prior to its scaling. Several options for process improvements, with scale up in mind, are suggested.

1. *Chemical Recycling*. The recovery and re-use of ammonia from the pretreatment is a simple operation that would be necessitated upon scale up of the process. Similarly, the recovery and re-use of ethanol used for precipitation would improve the process economics. Both of these operations are commonplace in processes that utilize volatile solvents such as ethanol and ammonia. Thus, they are well known and their implementation would not be complex.

2. *Process Optimization*. The process as described was not optimized. Thus, significant increases in yield could be achieved through optimization of each stage of the process. As an example, the process as described utilized seven volumes of ethanol to precipitate CFG. The amount of CFG precipitated by each volume could be characterized, in order to determine the most cost effective ethanol concentration.
3. *Additional Product Recovery*. In the process as developed, lignin removed by pretreatment is discarded. However, in an industrial setting it could be burned to provide process heat or energy or converted to synthesis gas and then to ethanol. Additionally, corn fiber contains valuable oil, which was not separated by the described process. Fractionation of the corn fiber oil could provide an additional high value revenue stream for the process.

APPENDICES

APPENDIX A

Equations and Sample Calculations

In this section, several of the equations used to calculate various parameters found in Chapter 2 are described. Each equation is accompanied by an example of its use based on the data presented in Chapter 2. Often, the calculations were carried out individually for glucan, xylan, and arabinan. In those cases, the example is shown for only one of the sugars, as the calculation is carried out in exactly the same manner for each sugar.

Sugar Mass Present

The mass of major sugars present in each material were calculated based on the material's composition, the determination of which was described in Chapter 2. Once the material's composition is known, the mass of the components, on a dry basis, may be determined with the following equation:

$$[M_w(1-m)] \times c = M_c \qquad \text{(A1)}$$

Where,

M_w = mass of the material, wet basis (g)

m = moisture content of the material (%)

c = component content (from compositional analysis) (%)

M_c = mass of the component (g)

Example:

7.23 g (wet basis) of destarched corn fiber (DCF) was pretreated. The DCF was determined to have a composition of 38.08% glucan, 24.73% xylan, and 18.00% arabinan. It had a moisture content of 8.19%. The masses of glucan, xylan, and arabinan contained in the DCF prior to pretreatment were determined.

$$[M_w(1-m)] \times c = M_c \quad \text{(A1)}$$

$$[M_{w,DCF}(1-m)] \times c_{glucan} = M_{glucan}$$

$$[7.23\ g\ (1-0.0819)] \times 0.3808 = 2.53\ g\ glucan$$

By the same method, the mass of xylan and arabinan in the DCF were determined. This method is applicable to any material with known composition.

Pretreatment Recovery

The percentage of major sugars that were retained during pretreatment was calculated based on the compositions of the DCF and the pretreated DCF. Equation A1 was first used to calculate the masses of the major sugars present in both the raw and pretreated materials. They were then compared as follows:

$$\frac{M_{c,PT,DCF}}{M_{c,DCF}} = R_c \quad \text{(A2)}$$

Where,

$M_{c,PTDCF}$ = mass of the component in the PTDCF (g)

$M_{c,DCF}$ = mass of the component in the DCF (g)

R_c = recovery of the component (%)

Example:

7.0 g (dry basis) of DCF were pretreated. Prior to pretreatment, the DCF contained 2.66 g glucan, 1.73 g xylan, and 1.26 g arabinan. After pretreatment, 4.28 g (dry basis) of solids were recovered, containing 2.45 g glucan, 1.29 g xylan, and 0.98 g arabinan. The recoveries were determined.

$$\frac{M_{c,PTDCF}}{M_{c,DCF}} = R_c \qquad \text{(A2)}$$

$$\frac{M_{glucan,PTDCF}}{M_{glucan,DCF}} = R_{glucan}$$

$$\frac{2.45\ g}{2.66\ g} = 0.9211\ or\ 92.11\%$$

The same method was used to calculate the recoveries of xylan and arabinan. To determine the total solids recovery, the *c* term in equation A1 was set to 1.0 (100%), thus calculating the mass of dry solids.

Hemicellulose Recovery Efficiency

The hemicellulose recovery efficiency is a measure of the amount of hemicellulose that was recovered through precipitation as compared to the total amount that was subjected to hydrolysis with cellulase. The recovery efficiency was calculated separately for xylan and arabinan, as follows:

$$\frac{M_{c,CFG}}{M_{c,PTDCF}} = E_c \qquad \text{(A3)}$$

Where,

$M_{c,CFG}$ = mass of the component in the CFG (g)

$M_{c,PTDCF}$ = mass of the component in the PTDCF (g)

E_c = efficiency with respect to the component (%)

Example:

The pretreated destarched corn fiber (PTDCF) that was subjected to hydrolysis with cellulase was found to contain 0.46 g xylan (by equation A1). After hydrolysis, the corn fiber gum (CFG) was precipitated with ethanol and dried. The CFG was determined to contain 0.2 g xylan (by equation A1). The efficiency of the xylan recovery was then determined.

$$\frac{M_{c,CFG}}{M_{c,PTDCF}} = E_c \quad \text{(A3)}$$

$$\frac{M_{xylan,CFG}}{M_{xylan,PTDCF}} = E_{xylan}$$

$$\frac{0.2\ g}{0.46\ g} = 0.4348, or\ 43.48\%$$

The efficiency of the recovery with respect to arabinan was determined by the same method.

Determination of "Pass Through" Stream

To determine the amount of material that "passed through" the process, i.e. the material that remained in the liquid after precipitation and recovery of the hemicellulose, the masses of recovered material (in the CFG) were subtracted from the total mass (in the PTDCF subjected to hydrolysis) as follows:

$$M_{c,PTDCF} - M_{c,CFG} = M_{c,PT} \quad \text{(A4)}$$

Where,

$M_{c,PT}$ = mass of the component in the pass through stream (g)

Example:

1.7 g (dry basis) PTDCF was subjected to cellulase hydrolysis. Prior to hydrolysis the PTDCF contained 0.87 g glucan, 0.46 g xylan, and 0.35 g arabinan. After hydrolysis,

hemicellulose was precipitated. 0.0017 g glucan, 0.17 g xylan, and 0.08 g arabinan were recovered in the hemicellulose stream. The material not recovered, i.e. the pass through stream, was then calculated.

$$M_{c,PTDCF} - M_{c,CFG} = M_{c,PT} \quad \text{(A4)}$$

$$M_{xylan,PTDCF} - M_{xylan,CFG} = M_{xylan,PT}$$

$$0.46\ g - 0.17\ g = 0.29\ g$$

The masses of glucan and arabinan found in the pass through stream were calculated in the same way.

Theoretical Yield for Hemicellulose Hydrolysis

To determine the hydrolysis efficiency observed in the CFG hydrolysis experiments, the theoretical yields were first determined. The theoretical yield of xylose or arabinose (100% conversion of xylan to xylose and arabinan to arabinose) was calculated for each experiment as follows:

$$\frac{\left(\frac{M_{CFG}}{V_W + V_{pH}}\right) \times V_{exp} \times c \times a_5}{V_{exp} + V_{enz}} = Y_{c,T} \quad \text{(A5)}$$

Where,

M_{CFG} = mass of corn fiber gum dissolved (g)

V_W = volume of water CFG dissolved in (ml)

V_{pH} = volume used to adjust pH (ml)

V_{exp} = volume of CFG solution used in the experiment (ml)

c = component content (from compositional analysis) (%)

a_5 = 0.88 (anhydrous correction factor for pentose sugars, see Gulati et al, 1996)

V_{enz} = volume of enzyme added to the experiment (ml)

$Y_{c,T}$ = theoretical yield of the component (g / L)

Example:

To prepare the CFG solution for use in a hydrolysis experiment, 2.3097 g of CFG was dissolved in 59.60 ml of water. The pH was adjusted to 5 by the addition of 500 µL 1N NaOH. The CFG was composed of 53.48% xylan. The hydrolysis experiment was carried out by distributing 1 ml CFG solution into a centrifuge tube, and adding 19 µL of enzyme. The theoretical yield of xylose was determined.

$$\frac{\left(\frac{M_{CFG}}{V_W + V_{pH}}\right) \times V_{exp} \times c \times a_S}{V_{exp} + V_{enz}} = Y_{c,T} \quad \text{(A5)}$$

$$\frac{\left(\frac{M_{CFG}}{V_W + V_{pH}}\right) \times V_{exp} \times C_{xylan} \times a_S}{V_{exp} + V_{enz}} = Y_{xylan,T}$$

$$\frac{\left(\frac{2.3097\ g}{59.60\ ml + 0.5\ ml}\right) \times 1\ ml \times 0.5348 \times 0.88}{1\ ml + 0.019\ ml} = 0.01775 g/ml, or\ 17.75\ g/L$$

The theoretical yield of arabinose was determined by the same method.

Actual Yield for Hemicellulose Hydrolysis

After hydrolysis of the CFG solution described in the previous example, a sample of the CFG hydrolysate was analyzed for xylose and arabinose content via HPLC. The resulting concentration data was used to determine the actual yield observed in the hydrolysis experiment as follows:

$$\frac{Y_{c,Obs}}{Y_{c,T}} = Y_{c,A} \quad \text{(A6)}$$

Where,

$Y_{c,Obs}$ = observed yield of the component (g / L)

$Y_{c,T}$ = theoretical yield of the component (g / L)

$Y_{c,A}$ = actual yield of the component (%)

Example:

The HPLC results indicated that the hydrolysate contained 2.709 g / L xylose. The actual yield of the experiment was calculated.

$$\frac{Y_{c,Obs}}{Y_{c,T}} = Y_{c,A} \qquad \text{(A6)}$$

$$\frac{Y_{xylose,Obs}}{Y_{xylose,T}} = Y_{xylose,A}$$

$$\frac{2.709\ g/L}{17.75\ g/L} = 0.1526, or\ 15.26\%$$

The actual yield of arabinose was determined by the same method.