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Enzymatic Enhancement of Water Removal In the Dry Grind Corn to Ethanol Process

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ENZYMATIC ENHANCEMENT OF WATER REMOVAL IN THE DRY GRIND CORN TO ETHANOL PROCESS

by

Ana Beatriz Henriques Thomas

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ABSTRACT OF THE THESIS

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Research Advisors: Professor Muthanna Al-Dahhan, Professor Milorad Dudukovic,

and Dr. David Johnston

The removal of water from coproducts in the fuel ethanol process requires a significant energy input. The drying of the coproducts is responsible for as much as 32% of the total utilities cost of the process. In this study, improvements in the energy and water balances of the corn to ethanol process and a decrease in ethanol production costs were achieved. Significant reductions in water-binding capacity of whole stillage were found for two, commercially available, cell wall degrading enzymes, GC220 and MGC. The addition of a protease, GC106, during fermentation was found to significantly enhance ethanol production rates as well as reduce the water binding capacity of the mash. Improvements in fermentation rates were achieved by the addition of GC106 with either GC220 or MGC. To achieve both enhanced dewatering and increased fermentation rates, it was recommended that a plant dose their fermentors using either a MGC/GC106 volume combination of 0.02/0.02 mL or a GC220/GC106 combination of 0.015/0.01 mL.

A plant trial was conducted to evaluate the scale-up of enhanced water removal from whole stillage by enzyme addition. Enzymes added during this trial proved to be effective, and an increase in the amount of water being removed during centrifugation was observed during the trial. The firing rate of the drier decreased significantly during enzyme addition, resulting in 12% less natural gas required to produce one gallon of ethanol. DDGS composition was not affected by the enzyme addition.

Process simulation results from the enzymatic dewatering model showed a decrease in utility consumption compared to the conventional model. A sensitivity analysis showed a tradeoff between the enzyme cost and the drier's natural gas savings. Because of the non-linear nature of enzyme activity, as the amount of enzyme added was linearly decreased, its resulting effects on the process were non-linear. Even if maximum dewatering effects are not achieved, significant savings in natural gas cost could still be obtained.

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Para a Minha Familia

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Chapter 1 – Introduction and Objectives

1.1 An Overview of Fuel Ethanol

Ethanol as an automobile fuel has been considered as a viable option as far back as 1908, with the design and production of the Ford Model T car by Henry Ford's Motor Company (Jones 2007). The car's engine was capable of running on both gasoline and ethanol (English 2008), and Henry Ford is often quoted as saying:

> We have found that 160-proof alcohol works very well in the ordinary gas engine on our cars and tractors… Using alcohol in an ordinary Ford car, we are able to get 15 per cent more power than with the present gasoline… (Detroit Evening Journal 1916)

However, at that time, with Prohibition and the decreasing cost of gasoline in the United States, the cars that were sold were not capable of operation using ethanol. Despite these obstacles, sometime later events started to take place that eventually led to the ethanol revolution seen in the 21st century.

First, the Arab Oil Embargo of 1973 was the first oil supply disruption to lead to a worldwide energy crisis. Second, there was an increase in oil prices due to a shift in production to less secure parts of the Middle East (EIA 1998). Third, there was the Energy Tax act of 1978, in which car manufacturers began to pay a tax on cars sold that did not meet a combined fuel economy of at least 22.5 mpg. This was also the first time that

Gasohol was officially defined as a blend of gasoline with 10% ethanol by volume, with the requirement that ethanol had to be produced from a renewable resource (EPA 2006). At the same time, countries around the world started to look elsewhere for a more stable, self-sufficient fuel. In 1992, the US Congress passed the Energy Policy Act in an attempt to increase US energy independence and improve air quality by encouraging the use of alternative transportation fuels. This included the requirement that 75% of a new federal fleet must run on an alternative fuel (DOE 2005). All of these measures led to the need for a renewable energy source and the production of an alternative fuel that could directly compete with gasoline in the marketplace.

The United States was not the only country affected by the energy crisis. Brazil quickly realized that it too needed to gain its energy independence. In the early 1980s, large scale plants were built to produce ethanol from sugar cane. After two decades of research, 50% of the Brazilian automotive fleet was running on ethanol and approximately 70% of all cars sold in Brazil were flex fuel (Valdes 2007). More recently, in October of 2002, Neiva, the subsidiary of the Brazilian aviation company, Embraer, presented the first airplane to run on ethanol. The airplane is called the EMB-202 Ipanema, and was designed for the agricultural sector. It uses an American motor that was redesigned to run on ethanol at one of the Embraer's factories. It is estimated that the airplane will save its owner about US$39,000 per year compared to a conventional airplane (Embraer 2002). These savings are attributed to the price difference between jet fuel and ethanol.

1.2 History of Fuel Ethanol from Corn

In the US, fuel ethanol is primarily produced from corn. Production of ethanol from corn was pioneered by corn refiners after WWII. During the postwar time, fuel had a characteristic low price and commercial fuel ethanol production was almost non-existent. At the time corn refiners and farmers who had a need for fuel ethanol to run their machines and tractors began to rustically produce ethanol from corn, a practice that has led to today's corn to ethanol production process. In the 1970s, most of the ethanol produced from corn was used either by the beverage industry or for industrial processes. The Solar Energy Research, Development, and Demonstration Act of 1974 was the first legislative action to promote ethanol as a fuel produced from an organic raw material. In 1975, as the US began phasing out lead in gasoline, ethanol from corn began to be seen as a possible replacement octane booster. Finally in 1979, approximately US $1 billion was handed out in grants for research in biomass for fuel ethanol production, including the production of ethanol from corn (EIA 2003).

According to the latest report by the Renewable Fuels Association (RFA), total yearly ethanol production capacity in the United States was 10.5 billion gallons as of January 2009. By comparison, in 1980, 2000, and 2001, the total production capacities were 175 million, 1.6 billion, and 1.77 billion gallons per year, respectively (RFA, How ethanol is made 2009). There are currently 170 operating corn to ethanol biorefineries in production with 24 more under construction (Figure 1.1) (RFA, Biorefinery locations 2009).

Figure 1.1: Map of corn to ethanol plants in the United States

1.3 Ethanol Production from Corn

Ethanol from corn can be produced by either of two methods: dry grind or wet milling. Approximately 82% of ethanol production facilities use the dry-grind corn to ethanol process while the rest use wet milling (Hardy 2009). The main difference between the two processes is the fractionation of the corn kernel in the wet milling process. This separates the kernel into its major components, starch, gluten, fiber and germ, which are then individually processed into 4 main products, ethanol, gluten meal, gluten feed and oil, respectively.

Furthermore, the starch component can be processed into many other products such as dextrose, thickening agents, adhesives and even as one of the cooling agents for superheated oil drilling bits (CRA 2007).

1.3.1 Wet Milling

The wet milling process for ethanol production has a higher initial capital cost due to the added complexity in the front end of the process reserved for the corn kernel fractionation. A schematic of the wet milling process with its six major products and co-products can be seen in Figure 1.2 (RFA, How ethanol is made 2009).

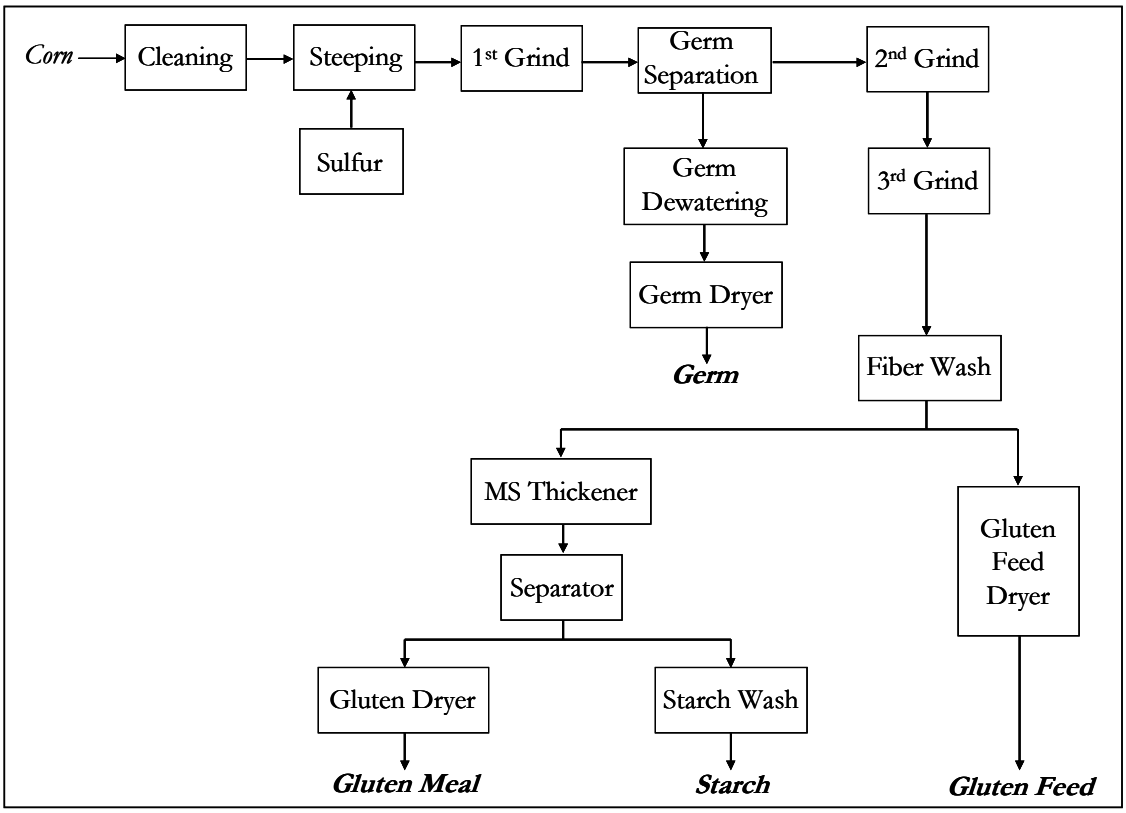

Figure 1.2: Simplified schematic diagram of the wet milling corn to ethanol process

The corn kernels are soaked in a solution of water and sulfurous acid and then separated into the main components: starch, gluten, gluten feed and germ. This preparation of the kernels for fractionation can be done chemically, by pre-treating the kernels with sulfur dioxide in water, or, as recently proposed by Johnston and Singh, by the addition of specific enzymes to break down the protein matrix in the endosperm of the kernels. In both cases, the kernels are prepared for separation in subsequent steps of the process (2005). The latter is called enzymatic milling or E-milling. Each component then goes through a series of processes to be converted to one or more of six primary marketable products: corn oil, wet feed, gluten meal, ethanol, high fructose corn syrup, and corn starch. The conventional wet milling

process uses sulfur dioxide, seen by many as a hazardous chemical, making the e-milling approach more desirable. Although the e-milling process has not been adopted by current wet milling plants, plant trials have been successfully conducted in Malaysia by Johnston and Singh (2005).

1.3.2 Dry Grind

The dry-grind process is less complex and has only three products and co-products: ethanol, distillers dried grains with solubles (DDGS), and CO_2 (Figure 1.3).

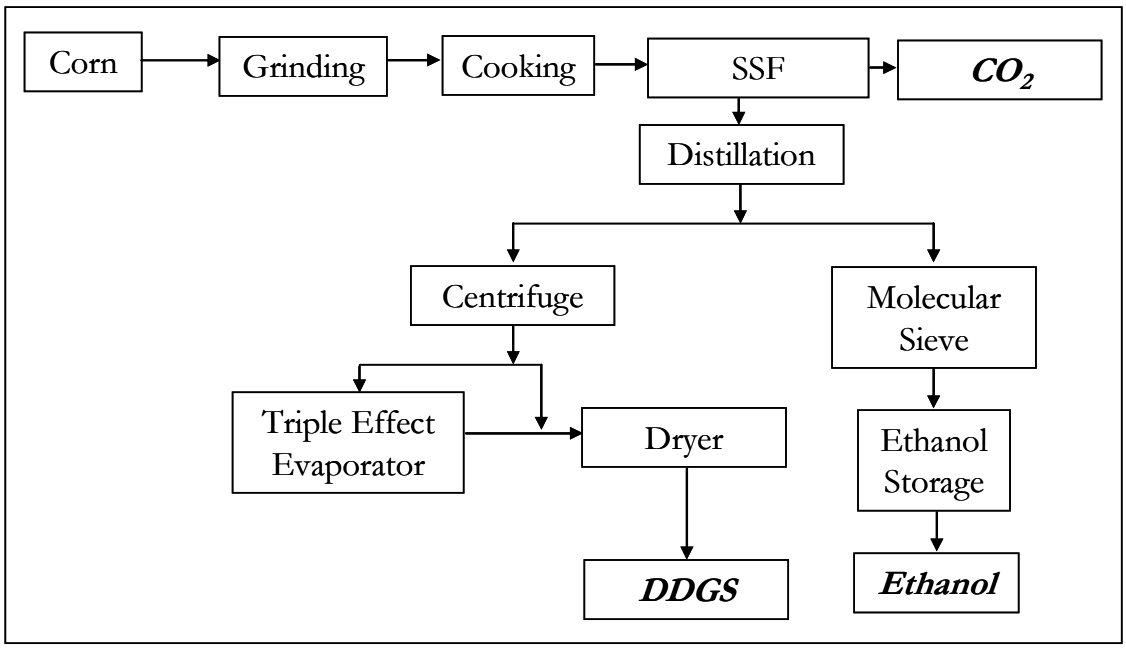

Figure 1.3: Simplified schematic diagram of the dry milling corn to ethanol process

The reason the dry grind process is more prevalent is that it has a few key advantages over the wet milling process. Up front, the dry grind process requires a significantly smaller initial capital cost investment, which makes it easier for companies to get sufficient investment funding to build a plant. The process is also less complex and requires fewer unit operations because the corn kernel is processed as a whole instead of being fractioned into different components as is necessary in the wet milling process. In the dry grind process, the corn is first milled in a hammer mill and screened. The screened corn is then mixed with water and α-amylase and passed through cookers where the starch in the grain is gelatinized and hydrolyzed into dextrins, also known as liquefaction, at 110ºC and a pH of about 5.5 (Wang et al. 2007). With the addition of gluco-amylase the liquefied starch is converted via saccharification into fermentable sugars, glucose, at a pH of about 4.5 (Muller 2000). At the same time, yeast is added to the mash to ferment the glucose into ethanol in a process called simultaneous saccharification and fermentation (SSF). The operating temperature during the SSF process is held between 32 and 35ºC.

In general, SSF takes place in batch fermentors. It is known that some fermentors have a single bottom lift agitator and that others are equipped with multiple impellers on a single shaft. In either case, a recirculation pump continuously circulates the mash at the bottom of the fermentor which increases mixing and therefore mass transfer performance (Shukla et al 2000). In the case of aerobic fermentations, it is important to have large areas of gas-liquid interface to ensure that the gas reaches the surface of the growing yeast cells (Levenspiel 1974). Without adequate mass transfer, glucose to ethanol conversion will not be completed.

The approximate residence time of the fermentation process, in the actual fermentor, in a corn to ethanol plant is about 50 hours. However, fermentation can start to happen before the mash reaches the fermentor, in the yeast propagation tank, and continues to happen after the mash leaves the fermentor and enters the beer well. It is believed that fermentation can occur for as long as 65 hours before the yeast cells become inactive once the mash reaches the distillation part of the process. Furthermore, the amount of CO_2 produced during fermentation enhances mixing by adding a third type of motion in the fermentor which also increases the mass transfer. In this case the CO_2 gas bubbles up through the mash much like having a sparger in the fermentor (Levenspiel 1993).

The yeast used in this step consists of commercially available strains of *Saccharomyces cerevisiae* and most ethanol plants will use one of these during this stage of the process. After SSF, the mash goes through a distillation and dehydration process where the alcohol is removed from the solids and water (SIUE 2001). On a weight basis, about 66% of the processed corn is converted to fuel ethanol and CO_2 via this process (Bothast and Schlicher 2005). The remaining third is processed and becomes what are known as distillers dried grains and are sold to farmers as high-protein feed for livestock, swine, and poultry.

1.4 Motivation

Processing DDGS is an energy intensive part of the dry grind corn to ethanol process. The non-fermentable material left over after SSF is referred to as "whole stillage" and is delivered from the beer well to either a conventional or a solid bowl decanter centrifuge (Figure 1.4).

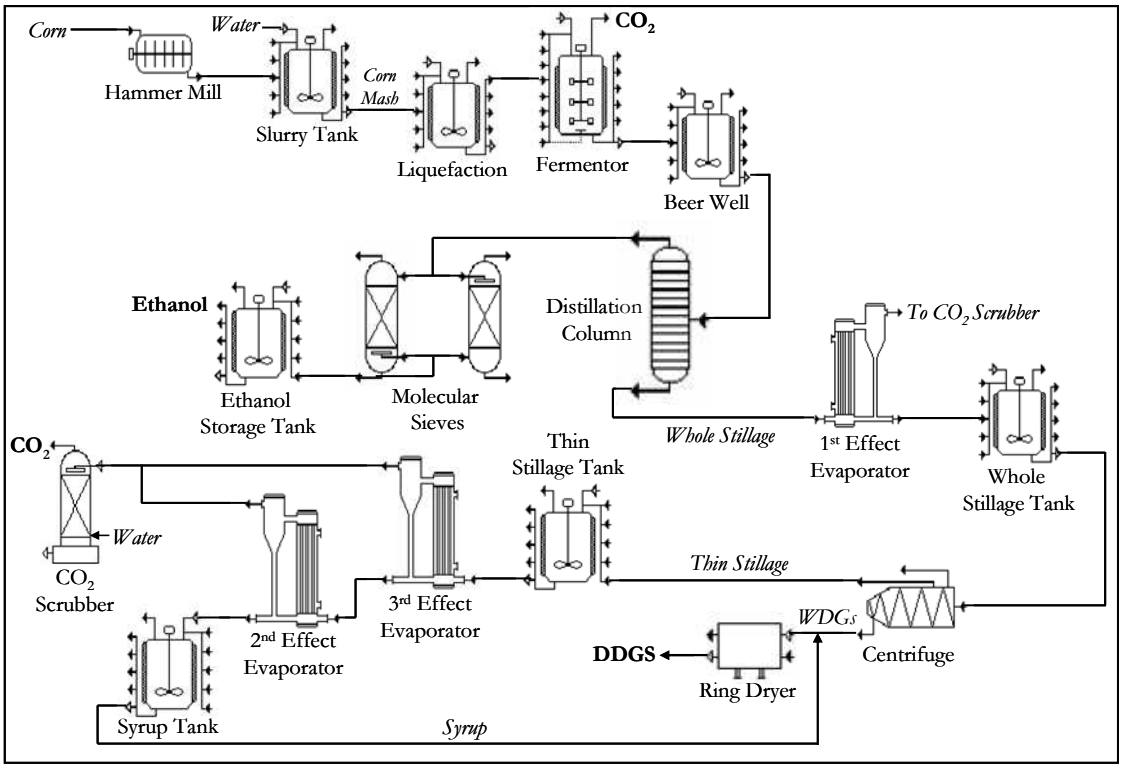

Figure 1.4: Process flow diagram of the dry milling corn to ethanol process

The centrifuge separates the whole stillage into liquid and solid fractions, which are the thin stillage and wet distillers grains (WDGs), respectively. The WDGs are mixed with the concentrated stillage (syrup) coming from the triple effect evaporator, with the resulting

mixture containing approximately 65-70% moisture. As shown in Figure 1.4, the WDGs/stillage mixture is then fed to a ring dryer that reduces the moisture content to about 10% (SIUE 2001). The dried co-product is called Distillers Dried Grains with Solubles or more frequently DDGS.

The DDGS are sold, for the most part, as animal feed for ruminants, but can be also consumed by the swine and poultry industries. Cattle farmers prefer to feed their livestock DDGS that have a color that is in the "golden" range. This can be difficult to achieve since DDGS are easily burned in the drier due to excessive residence times and high operating temperatures. The burned DDGS cause a reduction in the ruminant's digestive abilities (Shurson 2006). Figure 1.5 shows the color variation of the DDGS from facility to facility.

Figure 1.5: DDGS samples from various US ethanol plants, showing that color variation is significant from plant to plant

According to an economic analysis performed by the United States Department of Agriculture (USDA), the drying step is responsible for 32% of the cost of utilities for the entire process (Kwiatkowski et al. 2006). That accounts for roughly US $3.2 million/year for a 40 million gal/year dry grind corn to ethanol plant.

Therefore, by increasing the amount of moisture removed during centrifugation, it will be possible to reach the necessary moisture content in the DDGS using a lower temperature and a shorter residence time in the drier. This will save energy and ultimately reduce the production cost of ethanol. Although wet distillers grains (WDGs), which contain 65-70% moisture, are more economical and perform as well or better than DDGS, the transportation and storage requirements for wet feed present many difficulties such as a short shelf life and flow-ability problems (Ganesan et al. 2006). Furthermore, according to the economic model of fuel ethanol production published by Kwiatkowski et al., decreasing the amount of water removed from the DDGS in the dryer, which would result in a decrease in the retention time in the dryer, presents an important economic benefit that can save up to 6.2% in utility usage in the overall production process (2006). Historically, the sale price of the dry feed has fluctuated between $80 and $140 per ton (Figure 1.6) (USDA 2009).

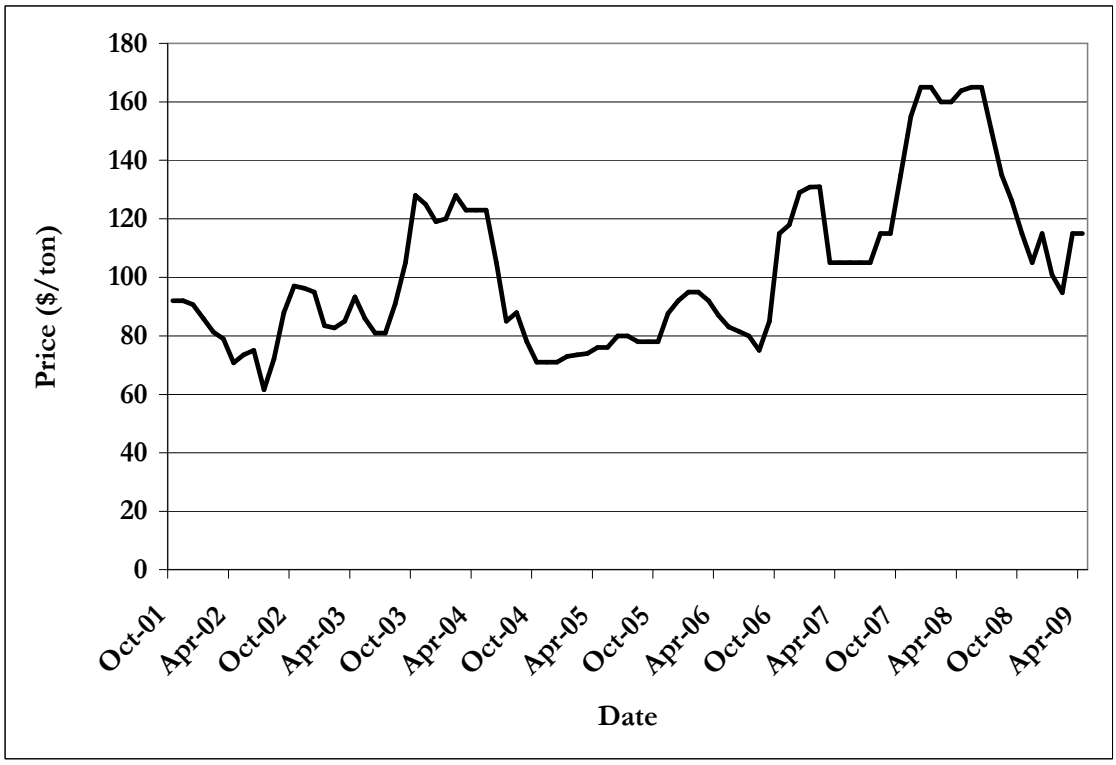

Figure 1.6: Monthly average prices for distillers dried grains

Currently, the average sale price of wet feed (70% moisture) is $46 per ton, while the average sale price of dry feed is $135 per ton (University of Missouri 2009; USDA 2009). Less drying would result in reduced energy requirements for the process, helping to lower the cost of DDGS and make them more attractive in the marketplace (Miller 2000).

DDGS are composed mainly of fiber, protein, and fat, but also include small amounts of water, amino acids, ash, calcium, and phosphorus (Shurson 2004) (Table 1.1).

Table 1.1: High quality DDGS nutrient composition

Component	Content (% dry wt basis)[1]
Fiber	55.4
Crude Protein	30.6
Crude Fat	10.7
Lysine	0.83
Methionine	0.55
Threonine	1.13
Tryptophan	0.24
Calcium	0.06
Phosphorous	0.89

[1] Data from Spiehs et al. 2002

Fiber can be defined as the portions of the cell wall of the DDGS that are made up of cellulose and lignin, both of which are major polysaccharides. The bonds created between water molecules and polysaccharides during hydrolysis can form in a number of different ways and have varying strengths. Maximum water-binding capacity occurs when water molecules share exactly one hydrogen bond with a polysaccharide. In this configuration the greatest hydrophylicity is manifested due to an increase in water concentration in the presence of a polysaccharidic carboxylate group (Chaplin 2003). If this rigid hydrogen bonding structure was disrupted, the centrifugal force of the centrifuge would cause the water molecules to be released from these water-binding components resulting in the retention of less water in the solids phase during centrifugation.

1.5 Objectives

In this research we test the hypothesis that the use of cell wall degrading enzymes, such as cellulase, xylanase, and β-glucanase, to hydrolyze and cleave cellulose, and hemi-cellulose is a way to disrupt the ordered environment of hydrogen bonds found between the polysaccharidic chains and water molecules. We do this via an experimental investigation of the ability of cell wall degrading enzymes to hydrolyze the polysaccharidic chains, which make up the un-fermentable fiber component of the corn kernel, into smaller molecules. We expect that the enzymes will cause these chains to rearrange inter-molecularly and form bonds between chains resulting in the release of water molecules and the weakening of water-to-polysaccharide bonds. There is no information in the literature regarding the use of these enzymes to enhance the dewatering of the DDGS in the dry grind corn to ethanol process and we will conduct such a study.

On the other hand, proteolytic enzymes (proteases), known for their ability to hydrolyze proteins, are already commonly used in the dry grind process to decrease fermentation time, as described later in Section 3.4 (Eckhoff and Tso 1991; Spanheimer et al. 1972; Roushdi et al. 1981; Johnston and Singh 2001; Johnston and Singh 2004; Johnston et al. 2003). Proteases will be studied for their ability to work synergistically with the cell wall degrading enzymes to not only improve dewatering, but also to decrease fermentation time and to increase final ethanol yields.

Cell wall degrading and proteolytic enzymes are also known for their ability to decrease the slurry's viscosity, as described in Sections 2.1 and 3.1 (Harkonen et al. 1996; Bouvier et al. 1992; Karlsson et al. 2001; Ponte et al. 2004; Tahir et al. 2005). It is believed that a decrease in slurry viscosity will have a direct impact on the centrifuge's solids-liquid separation efficiency. Reports in the literature show that there is a relationship between the centrifuge differential speed and performance due to changes in slurry viscosity, as described in Section 6.4.7 (Leung 2001; Beiser et al. 2000; McCabe 2001; Leung 1998). We will attempt from our experimental investigation to establish a relationship between changes in the differential speed of the centrifuge due to enzyme addition and the amount of water removed during centrifugation.

The success in proving the above hypothesis will be useful in the scale-up of the proposed enzyme addition, which will directly affect the economics of the process. Not only would less energy be required during the drying step, an increase in the plant's production capabilities would also be seen. An economic analysis of the dry grind corn to ethanol process can readily establish this and will be done.

1.5.1 Outline of Specific Tasks

The following is an outline of the specific tasks necessary to support the hypothesis stated above.

1. i) Investigate cell wall degrading enzymes for their ability to dewater the

distillers grains after centrifugation.

ii) Study the selected cell wall degrading enzymes for minimum dosage requirement that will cause the desired dewatering of the distillers grains.

2. Investigate proteolytic enzymes for their ability to dewater the distillers grains after centrifugation and increase the final fermentation ethanol yields.

3. i) Assess the combination of the selected cell wall degrading and proteolytic enzymes to maximize dewatering and ethanol yield.

ii) Investigate the minimum dosage requirements of the selected cell wall degrading and proteolytic enzymes that will improve dewatering and ethanol production.

4. Study the effect of enzyme addition on the differential speed and performance of the centrifuge

5. i) Perform large scale testing, with the cooperation of Center Ethanol, Genencor and USDA, by implementing the developed process in a dry-grind corn to ethanol facility in order to investigate production scale results.

ii) Perform economic analysis around co-product processing unit operations to determine viability of the developed process and its industrial implementation.

Chapter 2 – Cell Wall Degrading Enzymes

2.1 Introduction

Corn is mainly composed of 13% water, 8% protein, 68% starch, 8% fiber and 7% fat (Schroeder et al. 1997) (Figure 2.1).

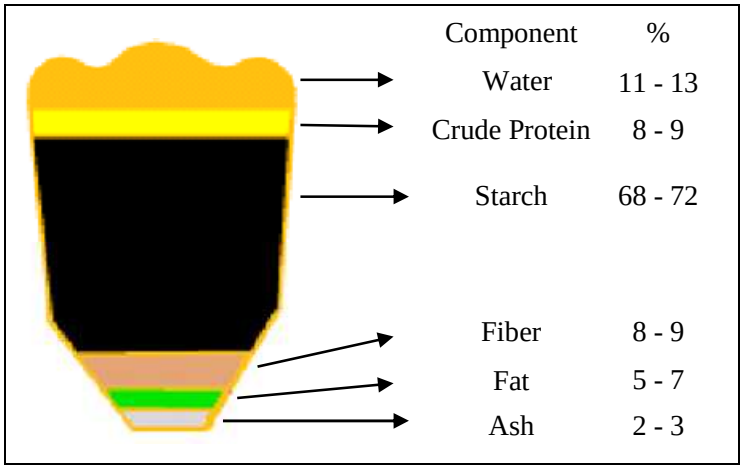

Figure 2.1: Schematic of average of whole mature corn kernel composition on a weight basis

The major components of corn fiber are polysaccharides that consist mainly of cellulose, hemi-cellulose, and lignin (Chaplin 2003; Sugawara et al. 1994). In general, cell wall polysaccharides are responsible for 90% of the cell wall composition and are classified into 3 groups: cellulose, hemi-cellulose, and pectin (McNeill et al. 1984). Cellulose is the most abundant and consists of a linear polymeric chain that gives the cell wall its rigid structure. Hemi-cellulose is also a polymer and can be classified as different types of polymeric chains.

These chains include, but are not limited to, xylan, arabinose, and xyloglucans (de Vries et al. 2001; Gaspar 2007). Pectins are the least abundant and they contain two regions: "smooth" and "hairy" (de Vries et al. 1982; Perez et al. 2000). When combined, cellulose, hemi-cellulose, and pectin create a rigid structure that gives the cell wall its strength.

Polysaccharides can bind water molecules in 3 different ways: intra-molecularly, through a double bond to a water molecule, or through a single bond to a water molecule. The third configuration is known to provide the polysaccharide with "maximum hydration" since it has the largest freedom of movement, thus showing the greatest hydrophilicity. Furthermore, the polysaccharide intra-molecular bonding can form a double hydrogen bonding bridge to a water molecule, thereby also becoming hydrated to a certain extent (Chaplin 2003). These water-to-polysaccharide bonds are the mechanism by which the distillers grains become hydrated. If a way could be found to disrupt these bonds, then the physical nature of the distillers grains could be changed, facilitating the removal of the water molecules.

Cellulases, xylanases, and β-gluconases are some of the enzymes capable of hydrolyzing these polysaccharides. Cellulases will be examined in detail here as they are the most effective at hydrolyzing cellulose, the main component of the corn cell wall. There are four classes of cellulases that are classified according to the method by which they cleave β-1,4-glucosidic linkages, whether they hydrolyze bonds between the main chain and a substitute or whether they cleave bonds at the end of the chain (de Vries et al. 2001; Johnston 2002).

Table 2.1 shows the four classes and their functionality as well as their enzyme commission (EC) number (Whitaker 1994).

Table 2.1: Cellulases divided by class and functionality

Class Name	EC Number	Function
Endoglucanases	3.2.1.4	Split internal bonds
Exo-cellobiohydrolases	32.1.91	Release cellobiose[1] from non-reducing end of cellulose chain
Exo-glucohydrolases	3.2.1.74	Release glucose from non-reducing end of cellulose chain
β-glucosidases	3.2.1.21	Cleave β-1,4,-glucosidic linkages in cellobiose to produce glucose molecules

[1] Cellobiose is a disaccharide that can be hydrolyzed by bacteria to give glucose

All of the functions described above would result in the breaking of bonds between the polysaccharide chains and the molecule bonded to it. By cutting these chains into smaller chains through the cleaving of β-1,4,-glucosidic linkages, it could be expected that the hydrogen bond between the water molecules and the polysaccharide would be weakened or even broken, thus making water more easily extracted by an external force.

Cellulases, as well as xylanases, have long been used in the detergent, textile, pulp and paper, and food processing industries (Johnston et al. 1998). These enzymes play an important role in baking due to their ability to keep dough soft by decreasing the dough's "water-binding capacity" as well as reducing the dough's viscosity (Harkonen et al. 1996). In the juice-

making process, these enzymes are used to reduce the loss of oil during the fiber press and clarification steps by hydrolyzing the cellulase and pectin found in the crude juice (Bouvier et al. 1992; Karlsson et al. 2001). They have been widely used as a supplement to the diet of mono-gastric animals such as poultry for their ability to improve the animal's digestion by hydrolyzing the non-starch polysaccharides (NSP) found in their feed, thereby decreasing the viscosity of the digestive contents (Ponte et al. 2004; Tahir et al. 2005). These enzymes are also used in the textile industry for their ability to abrade denim jeans and other fabrics. Cellulases that are added to the jeans during the washing process help to provide the denim with an array of different shades by hydrolyzing the fibers that contain the fabric's dye, thereby reducing the processing time, minimizing damage to processing equipment, and aiding in the processing of waste (Sariisik 2004). In the agricultural sector, these enzymes have been used to improve starch yields with a reduction in the protein content of the starch in maize and sorghum (Perez-Carrillo and Serna-Saldivar 2006; Serna-Saldivar and Mezo-Villanueva 2003). Experimentally, they have also been used to enzymatically pretreat sorghum bran for ethanol production (Corredor et al. 2007), and to hydrolyze glucose obtained from biomass to produce molecular hydrogen all by means of polysaccharide hydrolysis (Woodward 1997). Unfortunately, no research has been done to examine the ways in which these enzymes could affect the hydration of polysaccharides to aid in the mechanical removal of water from distillers grains.

2.2 Scope

The purpose of these experiments is to study how commercially available, cell wall degrading enzyme preparations can be used in the corn to ethanol process to enhance water removal of the whole stillage by improving centrifugation. The enzymes used were chosen based on their activity under the operating conditions of the SSF portion of the process including a temperature of 30ºC and a pH of 4.5. The enzymes' activities were provided by the enzyme manufacturer. Initially, the enzymes were pre-screened in a preliminary experiment that was conducted with the primary focus of determining the feasibility of the work presented in this thesis without emphasis on statistical significance. The enzymes that showed highest water removal capability were chosen and used in detailed subsequent experiments to investigate reproducibility and repeatability of the results observed, including statistical analyses.

2.3 Experimental Work

2.3.1 Materials and Methods

The enzymes used in this research were donated by Genencor International (a Danisco Company, Palo Alto, CA) and Novozymes (Franklinton, NC). The corn used was a single hybrid variety (33A14) grown at the University of Illinois during the 2004 season. All chemicals used in this study were of analytical grade or better.

2.3.1.1 Mash Preparation

To prepare the mash, a mixture of ground corn and water, 1 kg of corn was removed from the cold room and equilibrated to room temperature. The corn was then ground in a Wiley laboratory mill equipped with a 20-mesh screen. Erlenmeyer flasks (250 mL) were labeled and their tare weights were recorded. Stoppers were also weighed together with 21 gauge 1.5" needles (Figure 2.2).

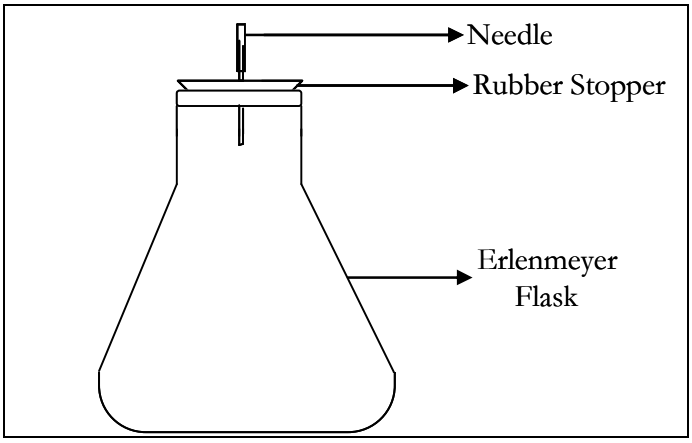

Figure 2.2: Schematic diagram of the fermentation apparatus used in the experiments.

Micro-centrifuge tubes used for collecting samples were labeled with fermentation date, identification number, and sample number. A rubber stopper and a needle were assigned to each fermentation flask. The needle was inserted into the rubber stopper. Each flask closed with a rubber stopper with needle was weighed as an assembly.

Ground corn weighing 227 g (corn weight was adjusted using moisture content to give the desired final solids content of 25% which was the most common concentration found in the literature) was added to 640 mL of water in 1-L beaker and adjusted to pH 5.8 ± 0.1 by adding 1*M* HCl solution. This pH is suggested by the α-amylase enzyme manufacturer to maximize the enzymatic activity. Spezyme Fred α-amylase (1 mL) was added to each flask, which was then placed on a preheated hot plate at 90°C with the stirring rate set to 120 rpm. These were the operating parameters commonly found in the literature, and are similar to current industrial practices (Wang et al. 2006; Singh et al. 2006; Singh 2008). The slurry was liquefied at 90°C for 1 hr. The slurry temperature was brought to 60°C using a water bath and 0.77 g of urea was added to each flask. The urea is added as a nitrogen source for the yeast which improves yeast performance (Narendranath et al. 2000). This amount of urea provides the yeast with 280 to 290 mg N/L of mash (Thomas and Ingledew 1995; Thomas and Ingledew 1990). The slurry was adjusted to pH 4.5 ± 0.1 by adding 1*M* HCl solution and 0.4 mL of Optidex L-400 gluco-amylase was added to the mash which was then saccharified at 60°C for 1 hr. Here, again, the operating parameters chosen were based on the gluco-amylase's manufacturer suggestions to maximize enzymatic activity. Furthermore, this step does not necessarily need to be done separately from the fermentation step. Many corn to ethanol plants combine the saccharification step with fermentation in a process known as Simultaneous Saccharification and Fermentation (SSF). Subsequent experiments, which are presented in the following chapters, will not have a separate saccharification step and will follow experimental parameters of the SSF process.

2.3.1.2 Mash Fermentation

The mash was cooled to 30°C, and 100 g was transferred to the previously weighed Erlenmeyer flasks. This temperature is in accordance to industrial practice and is believed to be optimal for yeast performance (Narendarath et al. 2000). Yeast suspension (Fleischmann's Active Dry Yeast, Fenton, MO) was prepared by mixing 3.3 g of yeast in 30 mL of distilled water and mixing for 10 min at room temperature. The suspension had a viable cell count of $\approx 1.8 \times 10^6$ cells/mL. Each flask was then inoculated with 1 mL of yeast suspension (0.11 g of dry yeast/100 mL of mash). The dewatering enzymes were added to each flask at the indicated amounts. A control flask was also prepared without enzyme addition. All flasks were then sealed with the stopper, the needle was inserted and flasks were moved to a temperature-controlled shaking incubator (30°C at 200 rpm) for 93 hr.

2.3.1.3 Analytical Techniques

Nine samples (1 mL) were taken from each of the flasks throughout the fermentation process. The samples were centrifuged (model 5415 D, Eppendorf, Westbury, NY) for 2 min at 16,110 × *g* and the supernatant (water removed after centrifugation) was filtered using a 0.2-μm syringe filter (model 4455T, Pall, Ann Harbor, MI) into labeled 1-mL micro-centrifuge tubes and frozen until ready for HPLC analysis.

Samples were thawed and injected (5 μL) into a HPLC (model 2350, ISCO, Lincoln, NE) equipped with an Aminex HPX-87H Biorad (Hercules, CA) ion-exclusion column. Compounds were eluted from the column with an aqueous solution of 5 m*M* sulfuric acid, detected with a refractive index detector (model 1047A, Hewlett Packard, Palo Alto, CA) and quantified by HPLC software (Chrom Perfect Spirit v.4.17, Justice Laboratory Software, Fife, UK) using external standard calibrations.

At the end of fermentation, a 40-mL representative sample was taken from each of the 15 flasks and transferred to a 50-mL centrifuge tube (Corning, cat no. 430290, 29.1 mm o.d.). Each tube was then centrifuged in a bench-top centrifuge (model Z320, Hermle, Woodbridge, NJ) for 10 min at 1,400 × *g* to analyze the water-binding capacity of each enzyme treatment. The supernatant (water removed after centrifugation) from each flask was weighed, placed in a 55°C oven for 48 hr, moved to a 135°C oven for 2 hr, and then weighed again. The same was done with the solid pellets (wet grains) obtained after centrifugation.

2.3.1.4 Experimental Design

The procedure described above was used in three experiments. The first experiment had 16 fermenting flasks; one control flask and 15 flasks containing 15 different commercial enzyme preparations. This experiment was used only as a screening to find the enzymes, if any, that would most likely enhance the water removal of mash during centrifugation. The second

experiment used six out of the 15 enzymes used in the screening experiment and a control. The six preparations were chosen based on the highest dewatering capability (largest amount of water removed). Each of the six enzyme preparations was added in volumes of 0.1, 0.5, and 1 mL/100 g of mash. This experiment was done in duplicate to show statistical significance. The last experiment used two out of the six enzymes used in the second experiment. Again, the two preparations chosen had the highest quantity of water removed after centrifugation. The mash volume was increased to 250 mL and the enzyme preparation amounts were scaled accordingly. This experiment was done in triplicate to verify repeatability, reproducibility and statistical significance.

2.3.1.5 Statistical Analyses

A two-way analysis of variance (ANOVA) was used to compare the mean amount of water removed after centrifugation for each enzyme treatment and volume, as well as for the control. A one-way ANOVA (Appendix A) was used to compare the mean amount of water removed after centrifugation for the triplicate experiment. The *t*-test (Appendix B) was used for each pair of enzyme treatments to compare the mean amount of water removed (SPSS for Windows, Chicago, IL). The level selected to show statistical significance was 1% ($P < 0.01$).

2.4 Results and Discussion

Initially, fifteen commercial enzyme preparations were screened for the ability to dewater the whole stillage (Table 2.2). These preparations were chosen based on marketed activities, as well as temperature and pH range.

Table 2.2: Key indicating enzyme preparations used in this study

Enzyme Key	Enzyme Name
A	GC 220
B	AD9990209
C	AD990210
D	AD990208
E	AD990211
F	Multifect Xylanase
G	Multifect GC
H	GC 880
I	GC 440
J	GC 710
K	Protease 899
L	Pulpzyme
M	Multifect B
N	Multifect P3000
O	Viscozyme

Single fermentations at high enzyme dosages (1 mL of enzyme/100 g of mash) were used in this experiment to identify enzyme preparations with the greatest dewatering potential. Significant improvements in dewatering were observed, with some enzyme preparations showing up to 14% more water removed than the control (Figure 2.3).

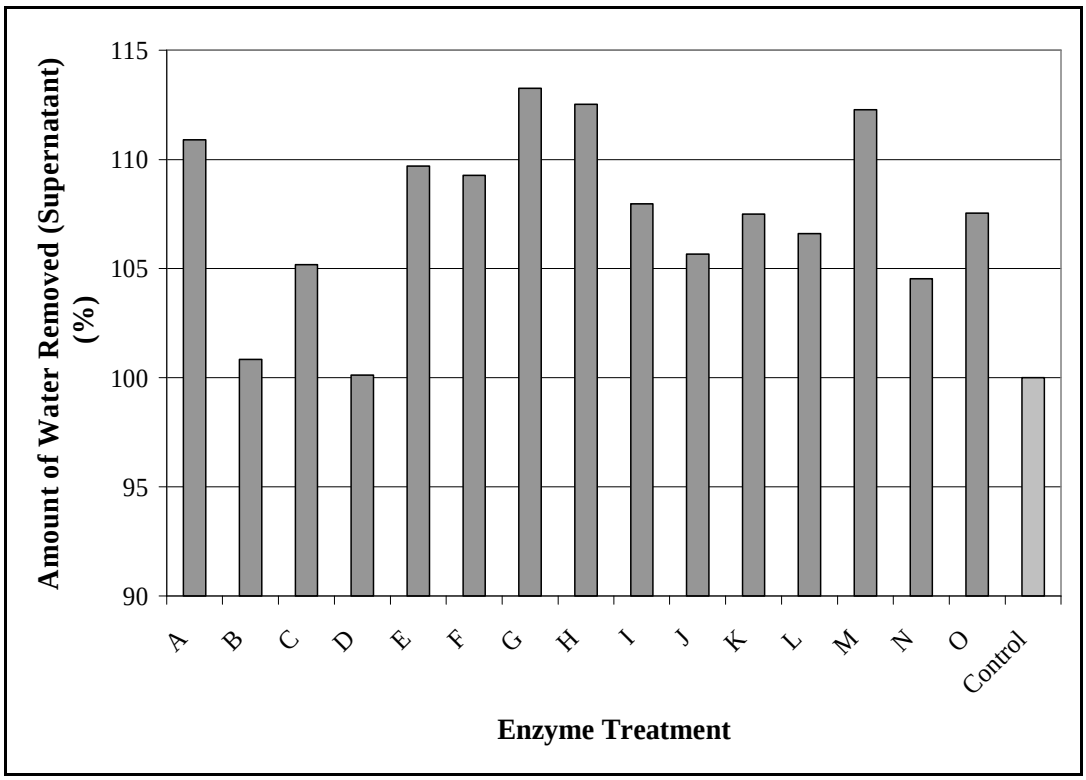

Figure 2.3: Weight of water removed (supernatant) after centrifugation as a percentage of control for each enzyme treated mash for a 40 g subsample of mash; control with the lowest amount of water removed taken as 100%.

The weight of some the dry solid pellets obtained after centrifugation show some reduction in the enzyme treated fractions when compared with the control sample (Figure 2.4). In

Figure 2.5, the dry solid pellet weights are shown as a percentage of the control as the dewatering data was presented above.

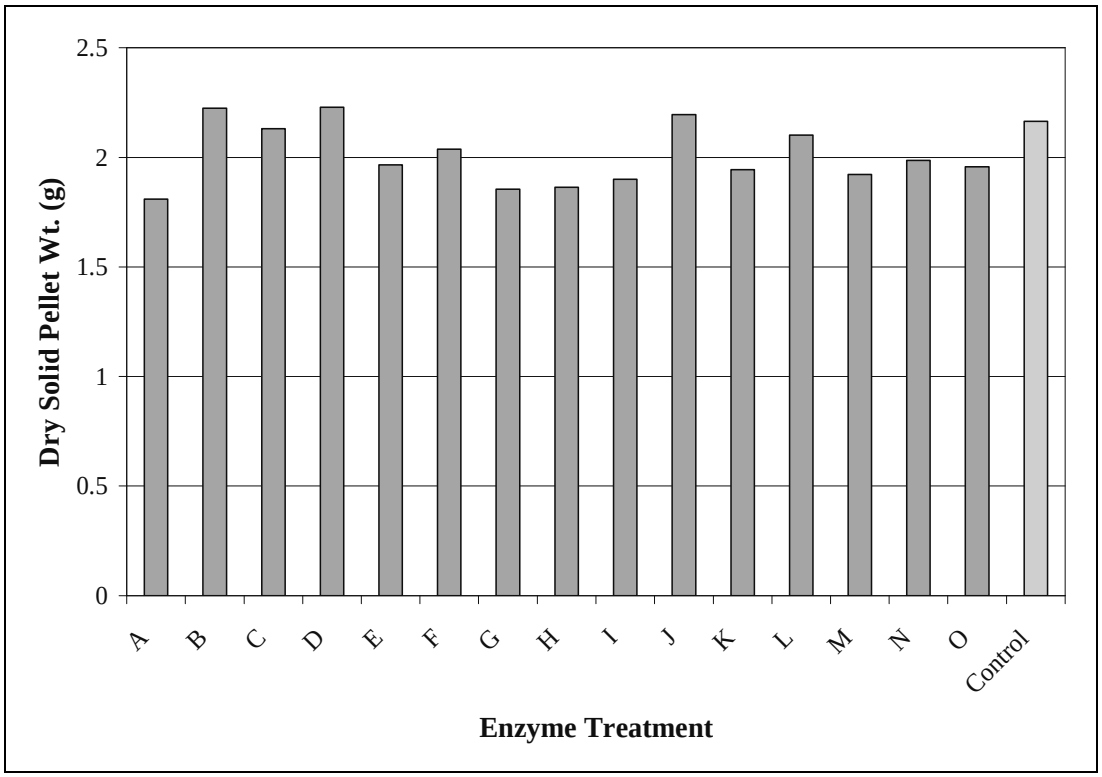

Figure 2.4: Dry solid pellet weights per 40 g of mash shown for each enzyme treated mash and control. Weights were measured from a 40 g sub-sample of the 100 g mash.

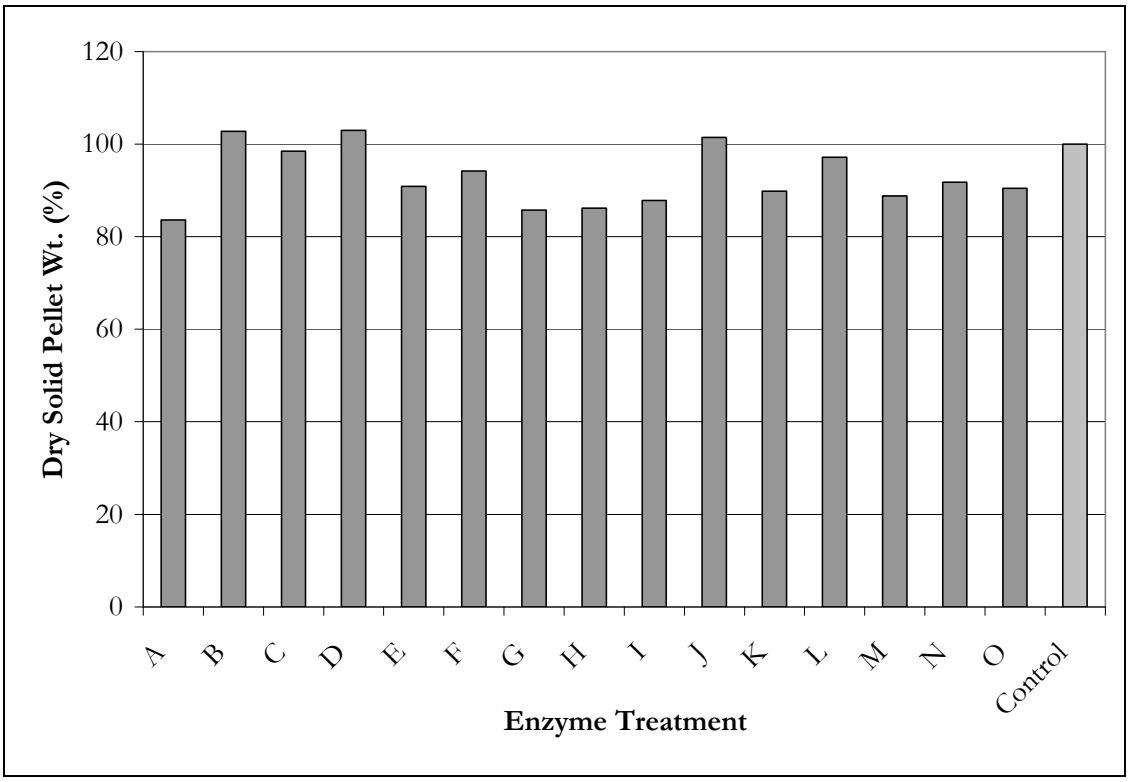

Figure 2.5: Dry solid pellet weights per 40 g of mash shown for each enzyme treated mash and control. Control taken to be 100%, all others are calculated based on the control.

In the cases where the pellet was smaller than the control, a portion of the solid phase was solubilized by the enzyme preparation during fermentation. This increase of solubles in the liquid phase was relatively small, up to 0.5 g with enzyme treatment A, compared with the increase in water removal, which was on average 5 g. In an ethanol facility, the solubilized material will move downstream with the liquid phase and end up in the evaporator. In the evaporator, the water is removed and the soluble solids become concentrated into what is called the syrup. The syrup is then mixed and dried with the distillers grains (insoluble solids) to produce the distillers dried grains with solubles. Six enzyme preparations were chosen to be the most promising in terms of their ability to dewater the whole stillage. Enzyme

preparations A, E, G, H, I, and M had the highest amounts of water removed after centrifugation as well as yielding the lowest wet pellet weights after centrifugation.

The same experiment was repeated for the enzyme preparations that were screened in the first part of this investigation. However, this time, the experiment was done using three different enzyme additions of 0.1, 0.5, and 1 mL for each enzyme treatment. All concentrations of enzyme preparation showed significant improvement over the control (Table 2.3).

Table 2.3: Amount of water removed after centrifugation; final ethanol yield and dry pellet weights given for enzyme treated mashes and control; values followed by the same letter in the same column are not significantly different ($P < 0.01$)

Enzyme	Volume Added (ml)	H_2O Removed[1,2] (%control)	Ethanol Yield[1,2] (%v/v)	Dry Pellet Wt.[1,2] (g)
A	0.1	112 ± 0.188a	13.60 ± 0.11	1.80 ± 0.09c
	0.5	115 ± 0.154a	13.50 ± 0.19	1.96 ± 0.19b
	1	114 ± 0.101a	13.68 ± 0.35	1.77 ± 0.02c
E	0.1	105 ± 0.055b	13.60 ± 0.33	2.17 ± 0.09b
	0.5	112 ± 0.185a	13.34 ± 0.28	1.96 ± 0.09b
	1	114 ± 0.242a	13.53 ± 0.18	1.92 ± 0.16b
G	0.1	114 ± 0.372a	14.25 ± 0.02	2.08 ± 0.06b
	0.5	112 ± 0.299a	14.32 ± 0.07	2.05 ± 0.06b
	1	114 ± 0.120a	14.01 ± 0.28	1.87 ± 0.03c
H	0.1	111 ± 0.27c	14.21 ± 0.31	2.08 ± 0.12b
	0.5	115 ± 0.079a	13.87 ± 0.09	1.98 ± 0.04b
	1	110 ± 0.143c	14.27 ± 0.21	1.84 ± 0.10b
I	0.1	113 ± 0.204a	14.12 ± 0.11	2.38 ± 0.13b
	0.5	112 ± 0.198a	14.10 ± 0.45	2.06 ± 0.10b
	1	114 ± 0.173a	14.06 ± 0.16	1.88 ± 0.12b
M	0.1	115 ± 0.264a	14.25 ± 0.13	2.07 ± 0.12b
	0.5	112 ± 0.071a	14.42 ± 0.15	2.05 ± 0.11b
	1	116 ± 0.308a	14.41 ± 0.16	2.00 ± 0.08b
Control[3]	--	100 ± 0.28d	14.03 ± 0.28	2.67 ± 0.08a

[1] Mean ± standard deviation
[2] Duplicate samples
[3] Control had 26.66 ± 0.27 g of water per 40 g of mash removed

The statistical analysis revealed a significant main interaction, $F(2,12) = 14.38$. Up to 15% more water was removed compared with the control for the mashes treated with A, H, and M. An increase in the amount of water removed during centrifugation was observed for both

H and E with an increase in enzyme preparation addition. As the enzyme preparation E addition was increased from 0.1 to 1 mL, the amount of water removed increased from 4.9 to 14.2% compared with the control. In enzyme preparations G, A, M, and I, the difference observed was not significant when comparing 0.1 to 1 mL. The dry solid pellet weights of the enzyme-treated mashes all showed a significant reduction relative to the untreated control (Figure 2.6).

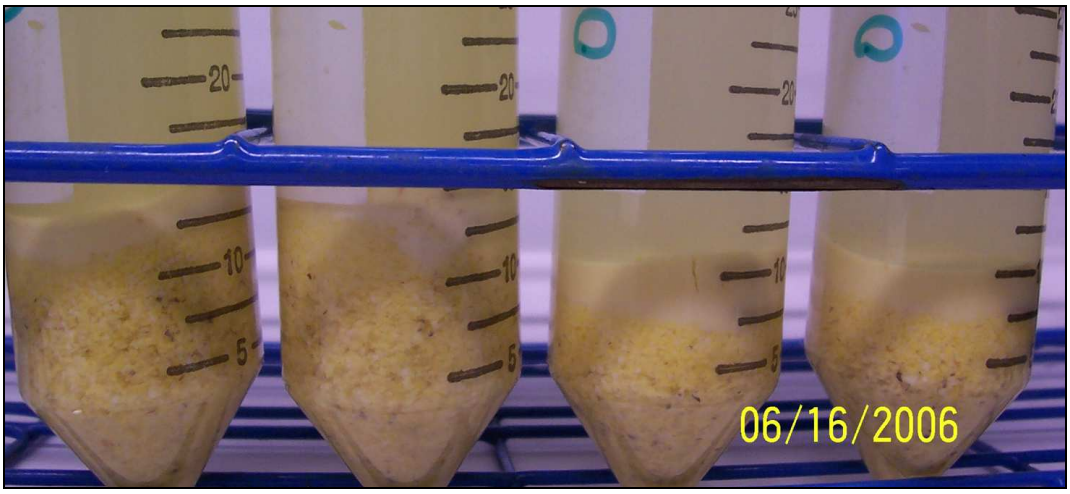

Figure 2.6: Picture of centrifuged samples. The two tubes on the left show solid pellet of control mash with no enzyme treatment. The ones on the right show solid pellet of enzyme treated mash.

At lab scale, this reduction in pellet weight resulted in a reduction in the amount of DDGS co-product produced, but at plant scale this loss in material would not result in a decrease in co-product production. In the corn to ethanol plant the material lost to the liquid phase during centrifugation would be mixed back into the process and dried in conjunction with the distillers grains. It is believed that at the enzyme levels used in this experiment, excessive hydrolysis of the polysaccharides has occurred. Therefore, the loss in solid material was

higher than what is expected to be seen when this process is implemented in a plant at lower enzyme levels.

Final ethanol yields were not found to be significantly different for enzyme-treated mashes when compared with the control (Table 2.3). Also, there were no significant differences between the final ethanol yields for each enzyme-treated mash at the different enzyme levels. Thus, adding additional enzyme preparation to the mash would not aid in increasing the final ethanol production.

The enzyme preparations that showed similar water removal ability at different enzyme additions were deemed favorable due to the need for smaller amounts of enzymes to achieve the best water-removal results. These enzymes removed the water as expected and showed that the dosage could be reduced while maintaining the dewatering results. This would help improve the economics of the process by minimizing additional enzyme costs. Enzyme preparations A and G had this characteristic and were therefore chosen as the most efficient for whole stillage water removal. In enzyme preparations A and G, an enzyme addition of 0.5 mL per 100 g of mash showed the best efficiency in terms of highest water removal and this dosage was chosen to be used in the next experiment.

A third set of fermentations was performed in which the above experiment was repeated in triplicate at a larger scale. The two most efficient enzyme preparations from the previous experiment (A and G) were used to treat the mash of 500 mL with an enzyme addition of

2.5 mL. Both preparations again showed a statistically significant greater amount of water removed relative to the control, $F(2,3) = 46.13$. Enzyme preparation A had an average of 30.4 g of water removed, G had 30.0 g, and the control had only 26.4 g (Table 2.4) from the 40-g subsamples.

Table 2.4: Average weight of H_2O removed; solid pellet and final ethanol yield for mash treated with enzyme A and G; values followed by the same letter in the same column are not significantly different ($P < 0.01$)

Enzyme	H_2O Removed[1,2] Avg. (% control)	Wet Pellet Wt.[1,2] Avg. (% control)	Dry Pellet Wt.[1,2] Avg. (% control)	Ethanol Yield[1,2] (% v/v)
A	115 ± 0.26a	65.39 ± 0.18	79.82 ± 0.04	14.17 ± 0.11a
G	114 ± 0.68a	69.74 ± 0.11	90.30 ± 0.39	13.97 ± 0.29a
Control	100 ± 0.91b[3]	100 ± 0.99[4]	100 ± 0.07[5]	14.19 ± 0.13a

[1] Mean ± standard deviation
[2] Triplicate samples
[3] Control had 26.41 ± 0.91 g of water per 40 g of mash removed
[4] Control had wet pellet weight of 11.47 ± 0.96 g
[5] Control had dry pellet weight of 2.52 ± 0.07 g

For A, this translated into an improvement of 15% more water removed after centrifugation than in the control. The wet solid pellet sizes were also smaller (30–35%) compared with the control. The dry pellet weights were reduced (10–20%) when compared with the control. The standard deviations calculated for each triplicate set showed that this experiment was reproducible and repeatable in terms of the amount of water that was removed after centrifugation and the solid pellet size that was left after the water was removed.

There were no significant differences in the final ethanol yields for the enzyme-treated mashes compared with the control for these runs (Table 2.4). The final ethanol production from the triplicates also proved to be reproducible.

2.5 Conclusions

A significant reduction in water-binding capacity of whole stillage was found for a number of enzymes tested in the initial screening. Average dewatering improvements in whole stillage of 15 and 14% were observed for enzymes A and G, respectively, with 500-mL fermentations done in triplicate. The enzymes were able to disrupt the corn cell wall and release water bound within the grains. The addition of different enzyme amounts to the mash had varying effects, potentially allowing an optimization of enzyme cost with energy savings. In some cases, an enzyme dosage of 0.5 mL/100 g of mash worked as well, if not better, than a 1 mL/100 g of mash dosage. In enzyme A, there was a maximum effect shown with the lowest dose tested, indicating that a significantly smaller amount of this enzyme could be used and still cause a strong dewatering effect. Lower concentrations would be more economical due to a lower enzyme cost.

The addition of these water-removing enzymes during fermentation of the dry grind corn to ethanol process will help in the dewatering of the whole stillage during centrifugation. Furthermore, there will be no capital cost associated with the added enzymes. By removing more water during centrifugation the energy cost of the DDGS drying process could be

significantly reduced, which would translate directly into lower energy consumption, improved energy balance, and reduced ethanol production costs as presented in Chapter 7.

Chapter 3 – Proteolytic Enzymes

3.1 Introduction

Corn contains about 8% protein that is not fermentable by the yeast used for ethanol production. These proteins are mainly divided into albumin (10%), glutelin (40%), globulin (9%) and zein (41%) (Parris et al. 2006; Tsai et al. 1980). Zein and glutelin serve as a nitrogen (N) sink in the corn kernel. They contain as much as 80% of the total N found in the kernel. This is an important function because an increase in plant yield is directly related to an enhancement in the movement of sucrose from the leaves of the corn plant to the kernels. This enhancement in sucrose transport is given by a large assimilation of N in the kernel (Tsai et al. 1980). This matrix of proteins can also interact with the starch chains and prevent them from being converted into sugars and then into ethanol during the SSF step (Vidal et al. 2009; Wang et al. 2009). Proteolytic enzymes, also known as proteases, can hydrolyze proteins into peptides and their amino acid building blocks. They may help release the starch found in corn by weakening the protein matrix that is interacting with the starch chains making them more accessible for saccharification. Proteases are very specific in terms of the peptide bond found in the protein that they hydrolyze. In order for the protein to be hydrolyzed it must be denatured first either by high temperature, low pH treatment or by processing. Proteases are divided into four different groups based on the active site: serine proteases (EC 3.4.21), cysteine proteases (EC 3.4.22), metalloproteases (EC 3.4.24), and aspartic proteases (EC 3.4.23) and each of these groups have a different function and will

hydrolyze proteins at different peptide bond locations (Whitaker 2003). Serine proteases have an optimum pH of 8, while sulfhydryl and metal-containing proteases work most efficiently in a pH range of 6 to 7.5. Aspartic proteases are the only ones that have an optimal pH between 2 and 5, which is in the range at which fermentation operates. Typically commercial enzymes are not pure. They usually contain a mixture of enzymes but are categorized according to the enzyme that is predominant in the preparation. Since SSF is run at a pH of about 4.5, only enzymes that have optimal activity near this pH will be useful. The enzyme preparations chosen to be used in these studies were selected according to their pH activity range. Those that matched best the pH of SSF were deemed most favorable.

Proteases have a variety of applications and are used in many different industries. They have previously been used in the production of glucose for medical applications by decreasing the amount of protein found in the starch of corn grains (Roushdi et al. 1981). In the detergent industry proteases are used as an ingredient in the composition of detergents for their ability to remove soil containing proteins, oils, and grease (Kottwitz et al. 1997). Different studies have looked at the addition of proteases, such as pepsin, papain, bromelain, and trypsin, to whole corn grains, but there was no decrease in the protein content of the starch that was produced. However, a decrease in the amount of protein found in the starch was observed for experiments in which the corn grains were broken (Roushdi et al. 1981). Research involving proteases has also been done to look at modifications of the wet milling pretreatment process (Spanheimer et al. 1972), as well as methods for decreasing drying effects on the starch-gluten separation in the wet milling process (Eckhoff and Tso 1991).

David Johnston and Vijay Singh have improved the wet milling process by using proteases to reduce steep time and sulfur dioxide requirements, and also to improve starch and protein separation by hydrolyzing the proteins attached to the starch particles (2001; 2003; 2004). Furthermore, proteases are known to increase the rate of fermentation by break down the protein into amino acids. These molecules have been observed to increase ethanol yields during fermentation by serving as a nitrogen source to the yeast cells that consume it as a metabolic nutrient (Lantero and Fish 1993).

3.2 Scope

The purpose of this experiment was to identify one or more enzymes that could improve the fermentation rates and potentially enhance the water removal in the corn to ethanol process. All of the enzymes investigated were chosen based on their enzymatic activity at fermentation conditions. The enzymes had to be active and relatively stable at fermentation pH of 4.5 and a temperature of 30ºC. There were 5 commercially available proteolytic enzymes that fit these conditions according to the data sheets provided by the manufacturers. The experiment was designed to quantify the fermentation rates, water removal, and final ethanol yields for each enzyme treatment as well as the amount of enzyme needed to produce these results.

3.3 Experimental Work

3.3.1 Materials and Methods

The corn and enzymes used in this section were the same as described in Section 2.3. All chemicals used were of analytical grade or better.

3.3.1.1 Mash Preparation and Liquefaction

The mash was prepared as previously described in Section 2.3.1.1. In short, corn (30% w/w) was ground and mixed with water. The pH was adjusted to 5.8 ± 0.1 by adding 1*M* HCl solution and the corn mixture was liquefied with the addition of 1.1 mL of Spezyme Fred (1.1 L total mash) for 1 hr at 90ºC.

3.3.1.2 Simultaneous Saccharification and Fermentation

The slurry temperature was cooled to 30°C using a water bath and 1.44 g of urea was added to the slurry. The slurry was adjusted to pH 4.5 ± 0.1 by adding 1 *M* HCl solution, and then 0.75 mL of Optidex L-400 gluco-amylase was added. Using a scale, 100 (± 0.6) g of slurry were transferred to each previously weighed Erlenmeyer flask. In order to accomplish this, each flask was placed on the scale, one at a time, and the scale was zeroed. Then the slurry (that was kept well mixed) was slowly poured into each flask until the weight reading on the

scale reached 100 g. Yeast suspension (Fleischmann's Active Dry Yeast, Fenton, MO) was prepared as previously reported in Section 2.3.1.2. Each flask was then inoculated with 1 mL of yeast suspension (0.11 g dry yeast/100 mL mash). Five proteases at two different amounts were added to each flask according to Table 3.1. A control flask was also prepared without enzyme addition. A duplicate flask was prepared for each condition tested. All flasks were then sealed with the stopper, a 22 gauge needle inserted and moved to a temperature-controlled shaking incubator, (30ºC at 200 rpm) for 72 hours.

Table 3.1: Key indicating enzyme preparations used in this study

Enzyme Key	Enzyme Name
P	Bromelain
Q	GC 106
R	GC 710
S	Protease 899
T	Fungal 500000

3.3.1.3 Analytical Techniques

Throughout the fermentation process, the flasks were periodically taken out of the incubator and their weights were recorded.

At the end of the fermentation process, a 1 mL sample was taken from each of the flasks. The samples were centrifuged (model 5415 D, Eppendorf, Westbury, NY) for 2 min at 16,110 x g and the supernatant was then filtered using a 0.2 μm syringe filter (model 4455T,

Pall, Ann Harbor, MI) into labeled 1 mL microcentrifuge tubes and stored frozen until ready for HPLC analysis. Samples were thawed and analyzed by high-performance liquid chromatography (HPLC) as described in Section 2.3.1.3.

In addition, two 40 mL representative samples were taken from each of the 11 flasks and transferred to a 50 mL centrifuge tube (Corning, cat no. 430290, 29.1 mm OD). Each tube was then centrifuged in a bench top centrifuge (model Z320, Hermle, Woodbridge, NJ) for 5 min at 1,100 x g to analyze the water-binding capacity of each enzyme treatment. The supernatant (water removed after centrifugation) from each flask was weighed, placed in a 55ºC oven for 48 hrs, moved to a 135ºC oven for 2 hrs, and then weighed again. The same was done with the solid pellets (wet grains) obtained after centrifugation.

3.3.1.4 Experimental Design

The procedure described above was used in two separate experiments. The first experiment had 11 fermenting flasks; one control flask and 10 flasks containing 5 different commercial enzyme preparations at two different amounts. Each of the five enzyme preparations were added in volumes of 0.5 and 1 mL/100 g of mash. The second experiment had 10 fermenting flasks; 2 control flasks and 8 flasks containing 4 different volumes of one commercially available enzyme. The volumes were as follows: 0.01, 0.015, 0.02 and 0.03 mL of enzyme/100 g of mash. Two samples were taken from each fermenting flask in each experiment to generate duplicate data for statistical analyses.

3.3.1.5 Statistical Analyses

SPSS software was used to compute a two-way analysis of variance (ANOVA) to compare mean water amount removed after centrifugation for each enzyme treatment and the control as well as for the different enzyme volumes and the control (SPSS for Windows, SPSS Inc., Chicago, IL). A one-way ANOVA was used to compare mean fermentation rates of each enzyme treatment at each enzyme volume added. T-tests were used for each pair of enzyme treatment to compare the means of water amount removed and fermentation rates. The level selected to show statistical significance was 5% ($P < 0.05$).

3.4 Results and Discussion

In the corn to ethanol process, the starch found in the corn kernel must be converted to glucose first before ethanol can be obtained. Starch is converted into glucose via an enzymatic reaction called saccharification. In this reaction, amylases will bind to the starch molecules and produce glucose. Theoretically, it is known that 0.9 g of starch will approximately produce 1 g of glucose, via enzymatic hydrolysis (Equation 3.1), which can be fermented to yield CO_2 and ethanol (Brandam et al. 2002; Ingledew 1993).

$$(C_6H_{10}O_5)_n + n(H_2O) \rightarrow n(C_6H_{12}O_6) \qquad (3.1)$$

During fermentation, ethanol is produced by the following chemical reaction (Equation 3.2):

Yeast, 30°C

$$\underset{\text{glucose}}{C_6H_{12}O_6} \rightarrow \underset{\text{carbon dioxide}}{2CO_2} + \underset{\text{ethanol}}{2C_2H_5OH} \qquad (3.2)$$

In this case, one mole of glucose will give 2 moles of CO_2 and 2 moles of ethanol (Luong 1985).

A relationship between the amount of starch present and the amount of CO_2 produced can now be determined. For example, in 100 g of mash that is 30%/w corn flour there are 30 g of corn flour. From the literature, it is known that the starch content of corn has a small variability with authors reporting values anywhere between 70.6 and 71.8 % w/w (Belyea et al. 2004; Watson 1987; NRC 1982). Assuming that, on average, corn has a starch content of 70%/w then it can be said that there are 21 g of starch present in the mash. Using the relationship between starch and glucose mentioned above, it is calculated that 21 g of starch would give 23.3 g of glucose. Using reaction 3.2, in the presence of yeast, the glucose would be converted into 11.9 g of ethanol and 11.4 g of CO_2.

In this experiment, each mash containing flask had a needle inserted in its stopper. This needle allowed the CO_2 produced during fermentation to escape. When the flasks were weighed during the fermentation process, a decrease in their weight was observed due to the loss of the gas to the atmosphere. Knowing the weight of CO_2 lost allows the amount of ethanol produced to be calculated (disregarding the small amount of water vapor). Since the

weights were taken over a period of time, an estimate of the fermentation rate could be obtained from the observed data. Figure 3.1 shows the weight loss of the flasks throughout fermentation.

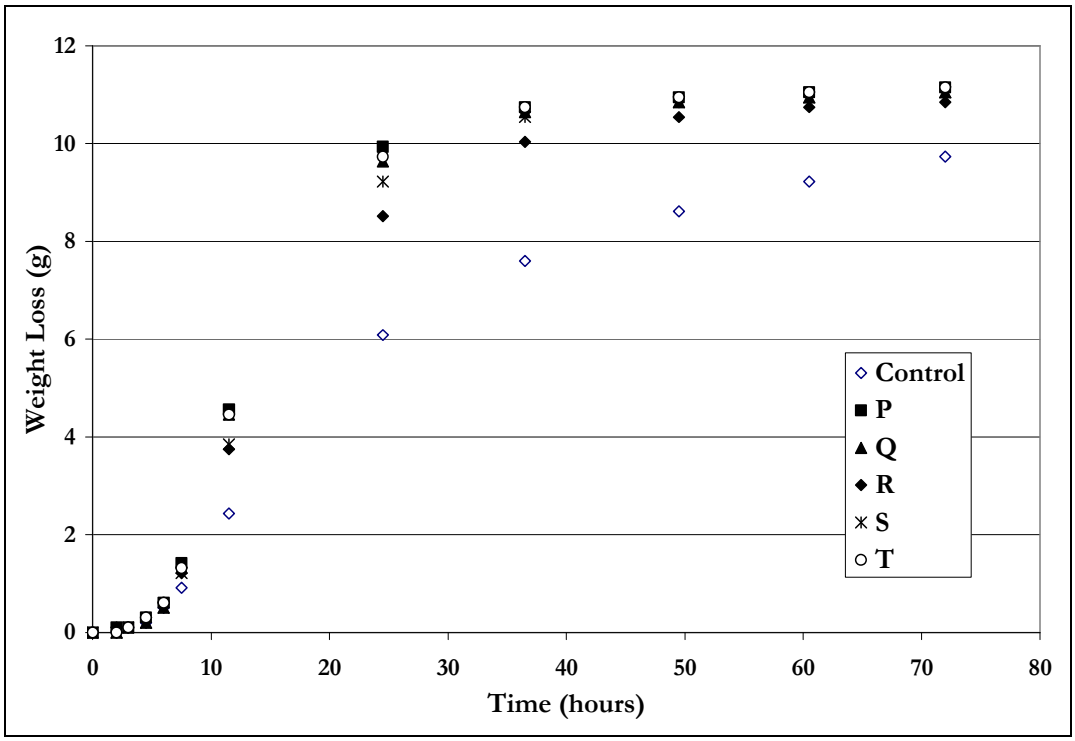

Figure 3.1: Average weight loss for flasks with no enzyme and with 0.5 mL/100 g of mash of 5 different enzymes, P, Q, R, S and T. Weight loss data has been normalized with respect to the control.

Most corn to ethanol plants have a fermentation residence time of 50 hours. This is an approximate time because there is a difficulty in stating the exact start and stop times of fermentation. Furthermore, this time does not include the additional, and somewhat variable, residence time in the beer well or in the seed fermentor. It can be said that, in total, fermentation can occur for as long as 65 to 70 hours when all of the above variable residence times are taken into account. At the end of fermentation, 72 hours, none of the enzymes treatments were statistically different. The control was statistically different from all of the enzyme treatments. According to Figure 3.1 at 50 hours the control was at 88% of its final weight loss whereas the flasks with Bromelain, GC 106, Protease 899 and Fungal 500000

were at 98% completion and 97% for GC 710. Furthermore, at 37 hrs, the control had only reached 76% of its final weight loss whereas the enzyme treated mashes had on average reached 93% of its final weight loss. This means that for all the protease tested the fermentation rate was accelerated and final ethanol yields could be achieved more quickly, which could translate into a higher ethanol throughput in a plant (Wang et al. 2006).

Preliminary experiments showed that protease Q (GC 106) had more promising water removal capability when compared to the other proteases tested. GC 106 was able to remove at least 2% more water than the other proteases and 9% more water than the control. Based on these results an experiment was designed to look at how various amounts of GC 106 would affect water removal and fermentation rates.

This second experiment tested GC 106 at volumes of 0.01, 0.015, 0.02 and 0.03 mL/100 g of mash. Figure 3.2 shows the fermentations rates for GC 106 at the different enzymes volumes.

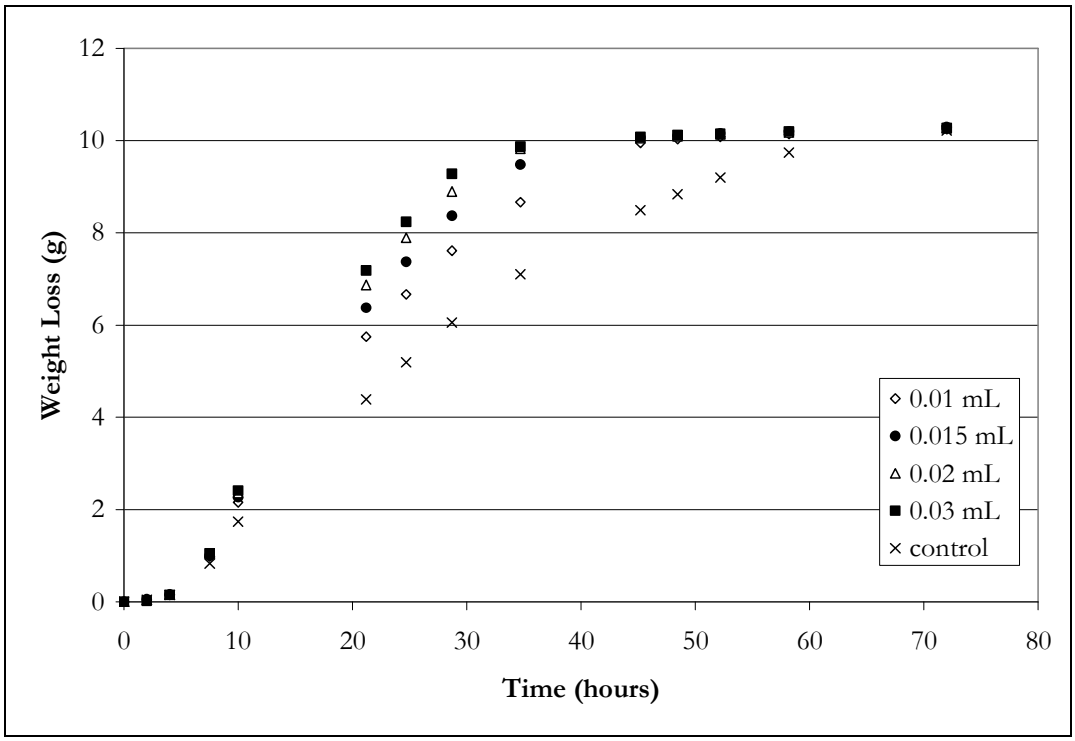

Figure 3.2: Average weight loss for flasks with no enzyme and with GC106 enzyme treated mash. Weight loss data has been normalized with respect to the control.

As can be seen in Figure 3.2, at around 50 hours the flasks containing GC 106 has reached the same weight loss level but the control is on average 2.5 g behind. The enzyme treated flasks reached on average 98% of the final weight loss at 50 hours whereas the control had only reached 86.5%. The average (± standard deviation) final weight loss for each enzyme treatment of 0.01, 0.015, 0.02 and 0.03 mL of enzyme/100 g of mash was 10.245 (± 0.007) g, 10.29 (±0.099) g, 10.265 (±0.035) g and 10.27 (±0.014) g, respectively. The control had an average final weight loss of 10.215 (±0.007) g. From these results it was concluded that an increase in enzyme volume would not necessarily translate into a faster fermentation, but

adding GC 106 to the mash would improve fermentation rate by at least 12% compared to a mash that had not been treated with the enzyme.

An increase in the amount of water removed during centrifugation was observed with an increase in enzyme volume. All volumes of enzyme GC 106 showed a statistically significant improvement relative to the control (Table 3.2).

Table 3.2: Amount of water removed after centrifugation; final ethanol yield and dry pellet weights given for GC106 enzyme treated mashes and control; values followed by the same letter in the same column are not significantly different ($P < 0.05$)

Volume Added (ml)	H_2O Removed[1,2] (%control)	Ethanol Yield[1,2] (%v/v)
0.01	108.2 ± 0.480a	13.58 ± 0.007
0.015	110.4 ± 0.159b	14.63 ± 0.099
0.02	111.2 ± 0.136b	13.60 ± 0.035
0.03	111.4 ± 0.81b	13.61 ± 0.014
control[3]	100 ± 0.352c	13.54 ± 0.007

[1] Mean ± standard deviation

[2] Duplicate samples

[3] Control had 24.50 ± 0.352 g of water/40 g of mash

Up to 11% more water was removed compared with the control for the mash treated with 0.02 and 0.03 mL of GC 106/100 mL of mash. As the enzyme volume was increased from 0.01 to 0.03 mL, the amount of water removed increased from 8.2 to 11.4% compared to the control. When statistically comparing the different enzyme amounts and the amount of water removed, there was only a difference between 0.01 mL and the other amounts. Here

the significance was t(2) = 33.21. There was no statistical difference between 0.015, 0.02 and 0.03 mL of enzyme. Thus, adding more than 0.015 mL/100 g of mash would not significantly improve dewatering and would also increase the cost to the ethanol facilities. From the results above it can be concluded that a GC 106 enzyme dosage of 0.015 mL is the ideal dosage to be used in the corn to ethanol plant process. This would help improve the economics of the process by minimizing additional enzyme costs.

Furthermore, Table 3.2 also shows the final ethanol yields for the enzyme treated mashes and the control. Statistically, there were no differences in the amounts of ethanol produced during fermentation for the enzyme treated mashes and the control. The samples were taken from the flasks at the end of fermentation at 72 hours. Thus, adding GC 106 to the mash in any amount would not aid in increasing the final ethanol production unless fermentation had a residence time of 53 hours of less. In the case of a corn to ethanol plant it can be said that if fermentation time is kept at 50 hours then it would be advantageous to add the enzyme in order to increase ethanol yields. It is important to point out that actual increases in throughput will most likely not be possible due to limits in the downstream processing such as distillation and pressure swing adsorption (PSA). However, more complete fermentations would be expected resulting in more ethanol produced per corn processed.

When comparing enzyme amounts, there was no statistically significant difference in the dry pellet weights as the enzyme volume was increased from 0.01 to 0.03 mL/100 g of mash. The only statistically significant difference was seen when comparing the dry pellet weight of

the control to the enzyme treated mashes, $t(2) = 5.85$. Figure 3.3 shows the average pellet weights for the enzyme treated mashes and the control.

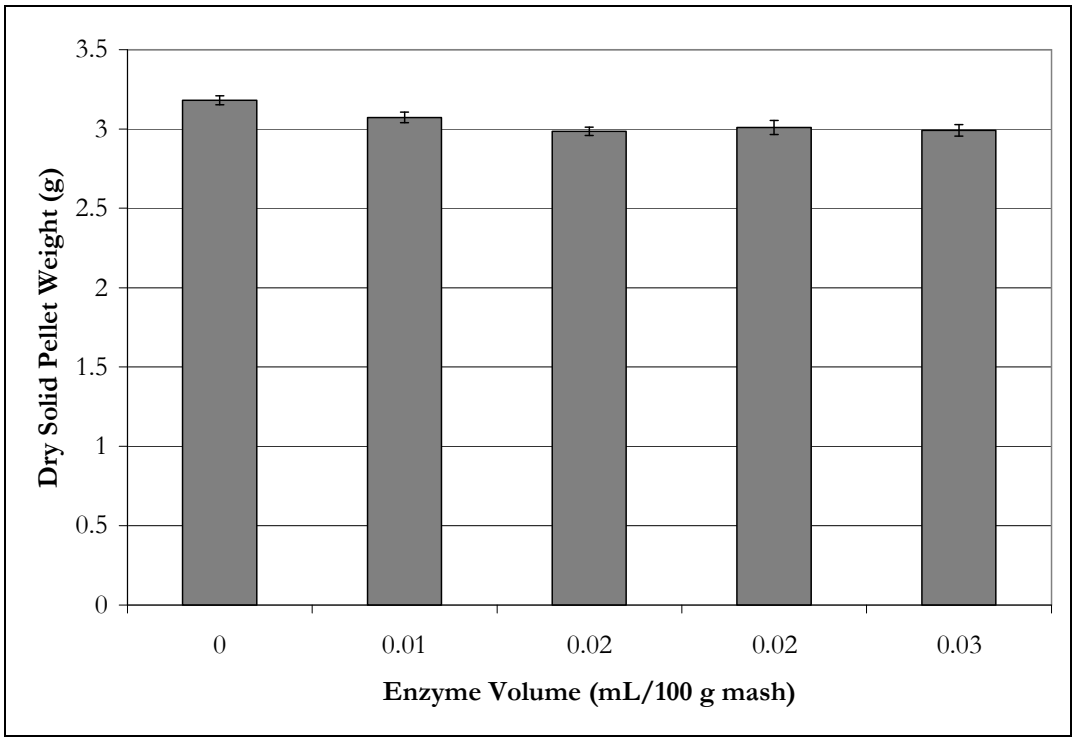

Figure 3.3: Average dry solid pellet weights per 40 g of mash shown for each GC106 enzyme treated mash and control. Weights were measured from a 40 g sub-sample of the 100 g mash.

The average (± standard deviation) dry solid pellet weights were as follows: 3.07 (± 0.05), 2.99 (± 0.04), 3.01 (± 0.06), 2.99 (± 0.05) and 3.18 (± 0.04) g for 0.01, 0.015, 0.02, 0.03 mL of enzyme and control respectively. No trends were observed for the dry pellet weights from the enzyme treated mashes. Thus, adding more of the enzyme to the mash will not affect the amount of solids remaining after fermentation. The small difference between the dry pellet weight of the control and the enzyme treated mashes could be attributed to some of the solids being solubilized into the liquid phase during fermentation. However, since a portion of the liquid stream leaving the centrifuge is recycled back into the process in a corn to

ethanol plant, the amount of solids lost would be negligible and would not translate into a decrease in the amount of DDGS co-product produced by the plant.

3.5 Conclusions

A small but significant reduction in water-binding capacity was found for a number of enzymes tested in the initial screening. Average dewatering improvements in whole stillage of 10% was observed for enzyme GC 106, with 100-mL fermentations done in duplicates. It is thought that the enzyme was able to disrupt the bonding network between protein and water molecules, thus releasing more water during centrifugation. The addition of different enzyme amounts to the mash had a small effect on the dewatering of the mash and the fermentation rate. An enzyme volume of 0.015 ml/100 g of mash proved to be ideal. At this dosage, the fermentation rate reached 98% completion at around 50 hours whereas the untreated mash was only at 86% completion at the same time. At this same enzyme volume, dewatering was increased by 10% compared to the untreated mash. A dosage of 0.015 mL of GC 106 could potentially allow for an optimization of enzyme cost with energy savings.

The addition of this rate-improving enzyme during fermentation of the dry grind corn to ethanol process will not only help in improving ethanol yields, but also in the dewatering of the whole stillage during centrifugation. Corn to ethanol plants can add this enzyme to their process with minimal added capital cost and at the same time reduce their fermentation times and enhance water removal during centrifugation of the whole stillage. By improving

fermentations rates and removing more water during centrifugation, the plant's ethanol production per corn processed could potentially be increased. At the same time the energy input for the DDGS drying process could be reduced by about 14%. This would translate directly into lower energy requirements and higher ethanol production, resulting in a reduction of ethanol production costs that will be looked at in detail in Chapter 7.

Chapter 4 – Dosage Optimization

4.1 Introduction

As discussed earlier, cell wall degrading enzymes have been identified as the most effective for enhancing water removal. Thus, it is necessary to investigate the optimal amount of enzyme needed to achieve the results observed but at the same time ensure that such enzyme 'dosage' is economically viable. Enzyme activity is not linear, thus adding more enzymes to the process will only increase dewatering up to a certain point. After that point is reached, adding more enzymes will not be beneficial. Clearly too high of an enzyme dosage will reflect negatively on the economics of the process due to the high cost of the enzymes. For purposes of this study, an optimal amount of enzyme was defined as the minimal volume of enzyme needed to maximize water removal. The goal of this study is to ensure that the amount of enzyme needed, and therefore the cost to purchase that enzyme, will not be higher than the savings observed in the drier operation by reducing the energy input needed for water removal. Figure 4.1 shows schematically the process used to analyze the data collected and optimize the amount of enzyme used in the plant trial presented in Chapter 6 and the economic analysis described in Chapter 7.

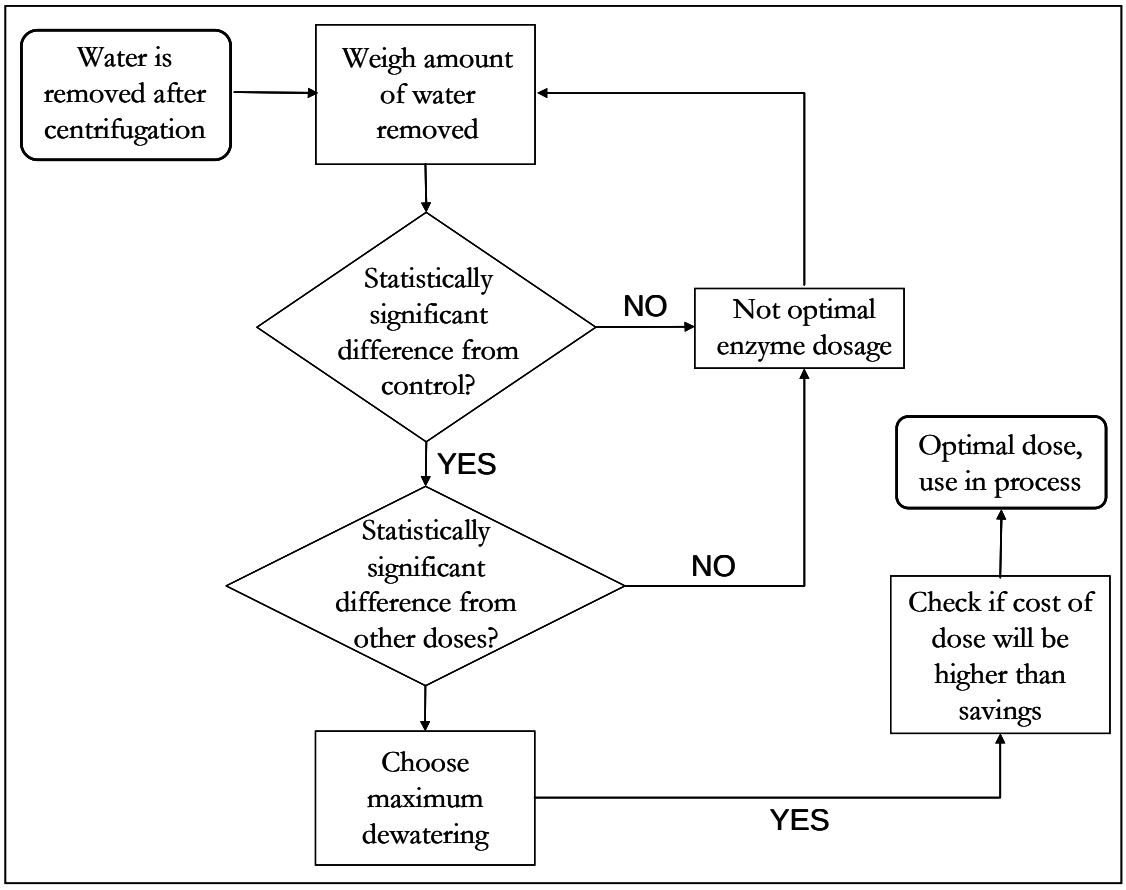

Figure 4.1: Optimization chart used to analyze the data collected during the experiments presented in this chapter.

The optimization process is pretty simple. Each sample collected will be analyzed for dewatering. The sample with the highest amount of water removed will be checked first. If that sample has a dewatering value that is statistically different from the control and the other samples than the enzyme dosage at which that sample was treated will be checked for economic feasibility. The enzyme dosage that proves to be most effective at dewatering and economically viable will be selected as the optimal dosage. An understanding of how the enzyme dosage affects the results observed in the previous chapters will be paramount to the

success of this research. Overdosing the process with enzymes is not beneficial and might not necessarily result in maximum effectiveness of water removal and increased fermentation rates.

4.2 Scope

The purpose of the experiments outlined below is to investigate how different dosages of cell wall degrading enzymes added to the mash affects the amount of water removed during centrifugation. In these experiments two enzymes, which were identified in Chapter 2, will be used. The cell wall degrading enzymes to be used are Multifect GC (MGC) and GC220. The experiments can be divided into two parts. The first experiment uses GC220 in the mash at the amounts of 0.005, 0.01, 0.015, 0.02, and 0.05 mL per 100 g of corn mash. The second experiment uses MGC in the same amounts listed above. The selected enzyme volume ranges were based on the preliminary dosage experiments described in Chapter 2.

4.3 Experimental Work

4.3.1 Materials and Methods

The enzymes and corn used in this section were the same as described in Section 2.2. All enzymes are commercially available preparations. All chemicals used were again of analytical quality.

4.3.1.1 Mash Preparation and Liquefaction

The mash was prepared as previously described in Section 2.3.1.1. In short, corn (30% w/w) was ground and mixed with water. The pH was adjusted to 5.8 and the corn mixture was liquefied with the addition of 1.3 mL of Spezyme Fred (1.3 L total mash) for 1 hr at 90ºC.

4.3.1.2 Simultaneous Saccharification and Fermentation

The slurry temperature was brought to 30°C using a water bath, and 1.25 g of urea (280-290 mg N/L) was added to the slurry for each experiment (Thomas and Ingledew 1995). The slurry was adjusted to pH 4.5 ± 0.1 by adding 1*M* HCl solution, and 0.65 mL of Optidex L-400 gluco-amylase was added. Approximately 100 g of slurry were transferred to each previously weighed Erlenmeyer flask. Yeast suspension (Fleischmann's Active Dry Yeast,

Fenton, MO) was prepared as previously reported in Section 2.3.1.2. Each flask was then inoculated with 1 mL of yeast suspension (≈1.8 × 10^6 cells/mL). The cell wall degrading enzymes, MGC or GC220 were then added to their set of flasks as per Table 4.1. A control flask was also prepared without enzyme addition for each experiment.

Table 4.1: Key indicating experimental design used in this section

Flask	Experiment 1	Experiment 2
	GC220 Volume (mL/100 g mash)	MGC Volume (mL/100 g mash)
1	0.005	0.005
2	0.005	0.005
3	0.01	0.01
4	0.01	0.01
5	0.015	0.015
6	0.015	0.015
7	0.02	0.02
8	0.02	0.02
9	0.05	0.05
10	0.05	0.05
11	None	None
12	None	None

The experiments were done in duplicates. All flasks were then sealed with the stopper, needle inserted, and moved to a temperature-controlled shaking incubator, (30ºC at 200 rpm) for 72 hours.

4.3.1.3 Analytical Techniques

The two experiments followed the analytical techniques described in Section 2.3.1.3 except for the last part. In the case of these experiments, at the end of fermentation, only one 40-mL representative sample was taken from each of the 12 flasks and transferred to a 50-mL centrifuge tube (Corning, cat no. 430290, 29.1 mm o.d.). Each tube was then centrifuged in a bench-top centrifuge (model Z320, Hermle, Woodbridge, NJ) for 5 min at 1,400 × *g* to analyze the water-binding capacity of each enzyme treatment. The supernatant (water removed after centrifugation) from each flask was weighed, placed in a 55°C oven for 48 hr, moved to a 135°C oven for 2 hr, and then weighed again. The same was done with the solid pellets (wet grains) obtained after centrifugation. The samples were placed in a low-temperature oven first to slowly evaporate the water without causing the equipment to smoke. After most of the liquid had evaporated, the samples were placed in the high-temperature oven to completely remove their water content.

4.3.1.4 Statistical Analyses

SPSS was used to compute a one-way analysis of variance (ANOVA) to compare the means of the water amount removed after centrifugation for each enzyme volume and the control (SPSS for Windows, SPSS Inc., Chicago, IL). Tukey's HSD (honestly significant difference) multiple comparison test was used to compute the minimum difference between the two means that is required for the means to differ significantly for each pair of enzyme volume.

The levels tested were the amount of water removed and the weight of the solid pellet. The water removed was separated from the solid pellet after centrifugation and the two parts were then weighed. The level selected to show statistical significance was 5% ($P < 0.05$).

4.4 Results and Discussion

The first experiment investigated the effectiveness of different GC220 enzyme doses on the amount of water removed during centrifugation. The enzyme dosage range chosen for this experiment was based on the results described in Section 2.3. All concentrations of the GC220 enzyme preparation showed significant improvement over the control (Table 4.2).

Table 4.2: Amount of water removed after centrifugation and final ethanol yields given for GC 220 enzyme treated mashes and control; values followed by the same letter in the same column are not significantly different ($P < 0.05$)

Volume Added (ml)	H_2O Removed[1,2] (%control)	Ethanol Yield[1,2] (%v/v)
control[3]	100 ± 0.115d	14.33 ± 0.14a
0.005	110 ± 0.158a	14.05 ± 0.15a
0.01	113.5 ± 0.022b	14.50 ± 0.12a
0.015	115 ± 0.059c	14.32 ± 0.28a
0.02	115.8 ± 0.202c	14.20 ± 0.14a
0.05	115.7 ± 0.321c	14.34 ± 0.31a

[1] Mean ± standard deviation

[2] Duplicate samples

[3] Control had 24.72 ± 0.115 g of water/40 g of mash

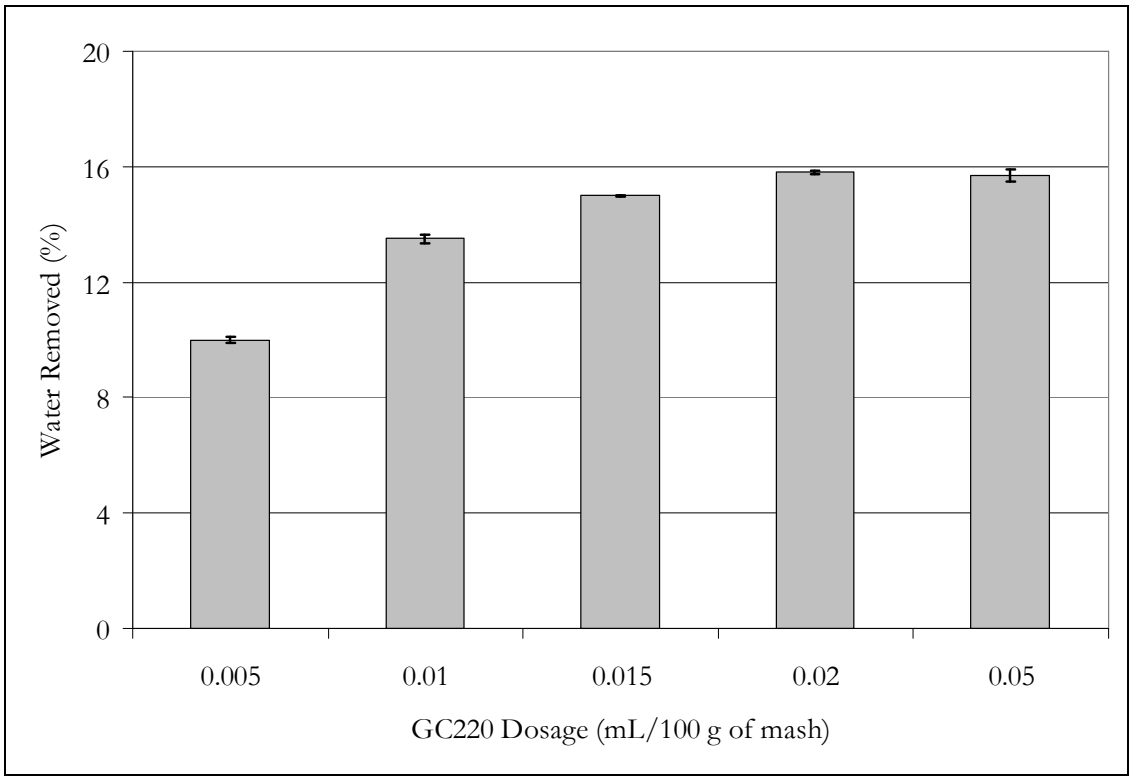

Figure 4.2: Amount of water removed after centrifugation at different GC220 dosages. Values are presented as a percentage of the control where the control is taken to be zero. Error bars represent one standard deviation from the mean.

An increase in the amount of water removed during centrifugation was observed with an increase in enzyme preparation dose. As the enzyme preparation dose was increased from 0.005 to 0.05 mL, the amount of water removed increased from 10 to 15.7% compared with the control. When the enzyme dose was increased from 0.015 to 0.05 mL per 100 mL of mash, the difference observed in the amount of water removed was not statistically significant when compared to one another. Using the optimization chart designed for this experiment, the enzyme dosage that resulted in the maximum dewatering that was statistically different from the control and the lower dosages was 0.015 mL/100 g of mash,

$F(10,11) = 146.48$. Based on these results, it is concluded that the minimum amount of enzyme needed for optimal water removal was an enzyme dose of 0.015 mL/100 g of mash (0.5 kg/MT of corn). In general, enzymatic activity shows nearly linear effects up to a specific enzyme concentration, above which diminishing returns are observed. The data shown in Figure 4.2 is representative of this saturation effect that is typically observed in a system in which enzymes are present.

The dry solid pellet weights of the mashes treated with 0.02 and 0.05 mL of enzyme were the only ones that were found to be significantly reduced in weight relative to the untreated control (Figure 4.2).

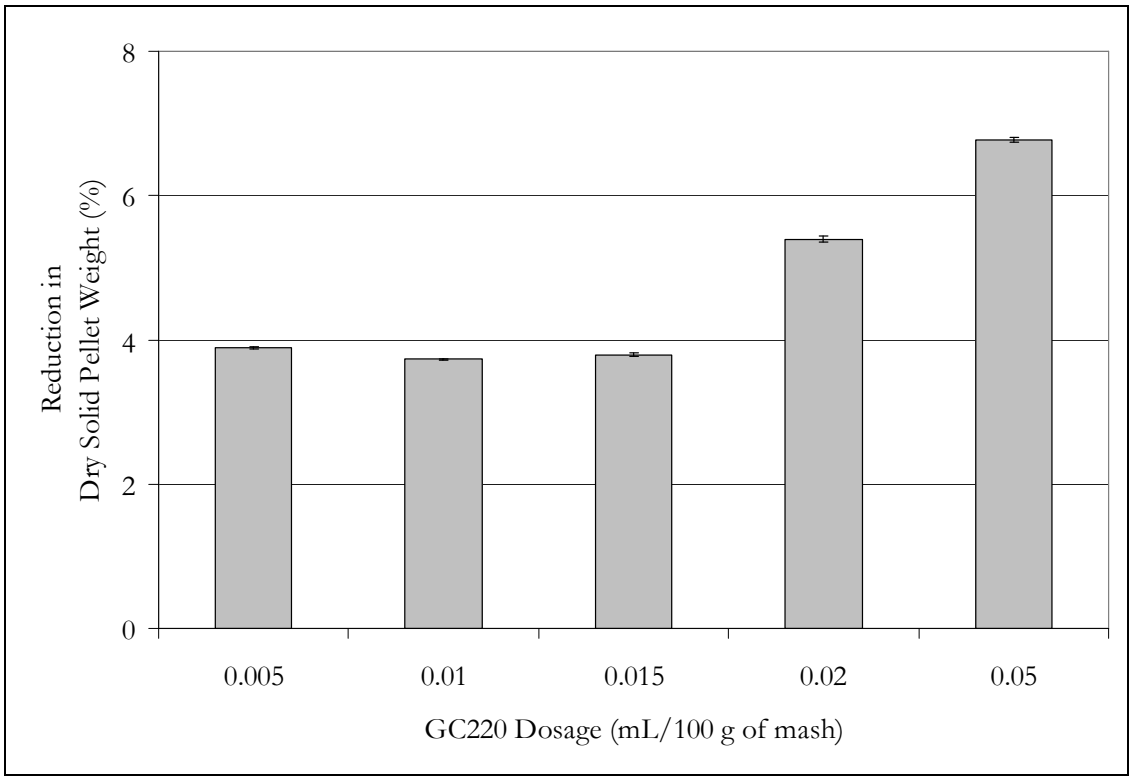

Figure 4.3: Dry Reduction in dry solid pellet weights per 40 g of mash shown for each GC220 enzyme treated mash compared to the control. Weights are shown as a percentage of the control where the control. Error bars represent the standard deviations from the means.

However, the only treated mash that showed a significant difference in the dry solid pellet weight when compared to other treated mashes was the one with an enzyme volume of 0.05 mL, $F(10,11) = 9.12$. When comparing enzyme additions, there was no significant difference in the dry solid pellet weight for the mash treated with enzyme dose between 0.005 and 0.02 mL. This means that an enzyme dose of 0.015 mL/100 g of mash will not affect the amount of solids removed during centrifugation, but it will still maximize the amount of water removed. In a corn to ethanol plant this would translate into an increase in centrifugation efficiency while still maintaining the DDGS production rates. The relatively smaller solid

pellet weights will not cause a loss in final DDGS production rates since the solids that are centrifuged out with the water is recycled back into the process via the process condensate.

Final ethanol yields were not significantly different for enzyme-treated mashes compared with the control (Table 4.3). Also, there were no significant differences between the final ethanol yields for each enzyme-treated mash at the different enzyme levels. Thus, adding more enzyme preparation to the mash would not affect the final ethanol yields of fermentation.

The lowest enzyme dose that showed statistically the same water removal ability as the larger doses was deemed most favorable due to the need of smaller amounts of enzymes to achieve the best water-removal results. This would help improve the economics of the process by minimizing enzyme costs. An enzyme dose of 0.015 mL of GC 220/100 g of mash had this characteristic and was therefore chosen as the most efficient for whole stillage water removal.

The second experiment investigated the effectiveness of different MGC enzyme doses on the amount of water removed during centrifugation. The enzyme dosage range chosen for this experiment was based on the results described in Section 2.3. Just as was observed with GC220, all concentrations of the MGC enzyme preparation showed significant improvement over the control (Table 4.3 and Figure 4.4).

Table 4.3: Amount of water removed after centrifugation; final ethanol yield and dry pellet weights given for MGC enzyme treated mashes and control; values followed by the same letter in the same column are not significantly different ($P < 0.05$)

Volume Added (ml)	H_2O Removed[1,2] (%control)	Ethanol Yield[1,2] (%v/v)
control[3]	100 ± 0.031d	13.88 ± 0.078a
0.005	115.2 ± 0.180a	13.87 ± 0.021a
0.01	116.8 ± 0.197a	13.92 ± 0.011a
0.015	119.8 ± 0.207b	14.01 ± 0.010b
0.02	119.9 ± 0.142b	13.59 ± 0.013c
0.05	123.2 ± 0.038c	14.21 ± 0.056d

[1] Mean ± standard deviation

[2] Duplicate samples

[3] Control had 22.53 ± 0.031 g of water/40 g of mash

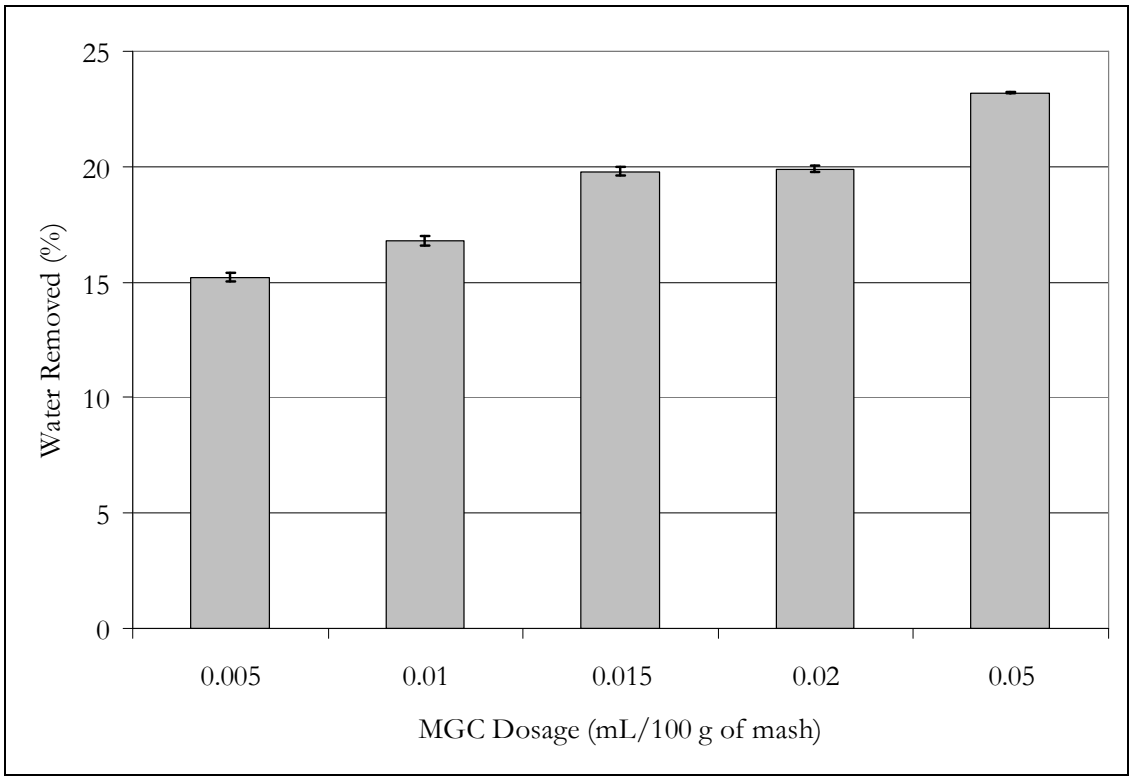

Figure 4.4: Amount of water removed after centrifugation at different MGC dosages. Values are presented as a percentage of the control where the control is taken to be zero. Error bars represent one standard deviation from the mean.

An increase in the amount of water removed during centrifugation was observed with an increase in enzyme preparation dose. As the enzyme preparation dose was increased from 0.005 to 0.05 mL, the amount of water removed increased from 15.2 to 23.2% compared with the control. For dosages of 0.005 and 0.01 mL/100 g of mash, there was no statistically significant difference between the resulting water removal values. Similarly, the same lack of a statistically significant difference was observed for dosages of 0.015 and 0.02 mL/100 g of mash. However, in contrast with the results for GC220, the MGC dosage experiment showed that there was a statistically significant difference in the water removal at the highest

dosage of 0.05 mL/100 g of mash, $F(10,11) = 44.94$. Nevertheless, as is explained later in Chapter 7, using an enzyme dose of 0.05 mL would not be economically viable due to the cost of the enzyme. At this highest dosage, the dewatering effect of 23.2% will not provide enough savings to the processing plant to offset the cost of the enzymes. Furthermore, as it is also shown in Chapter 7, the dosage with the highest dewatering effect may not necessarily be optimal. The savings obtained from using a cell wall degrading enzyme is linear with respect to the amount of enzyme and therefore dewatering. Meanwhile, the dewatering effect observed in non-linear with respect to the enzyme dosage used. Using this rationale, a dosage of 0.05 mL was deemed unfavorable as the optimal amount of MGC to be used in industrial practices. The next dosage considered was 0.02 mL but at this level the amount of water removed was statistically the same as the one obtained with 0.015 mL of MGC. A MGC volume of 0.015 mL was selected as the optimal dosage according to the conditions and parameters defined in this chapter as the most favorable.

As found before with GC220, the dry solid pellet weights of the mashes treated with 0.02 and 0.05 mL of enzyme showed a significant reduction relative to the untreated control (Figure 4.5).

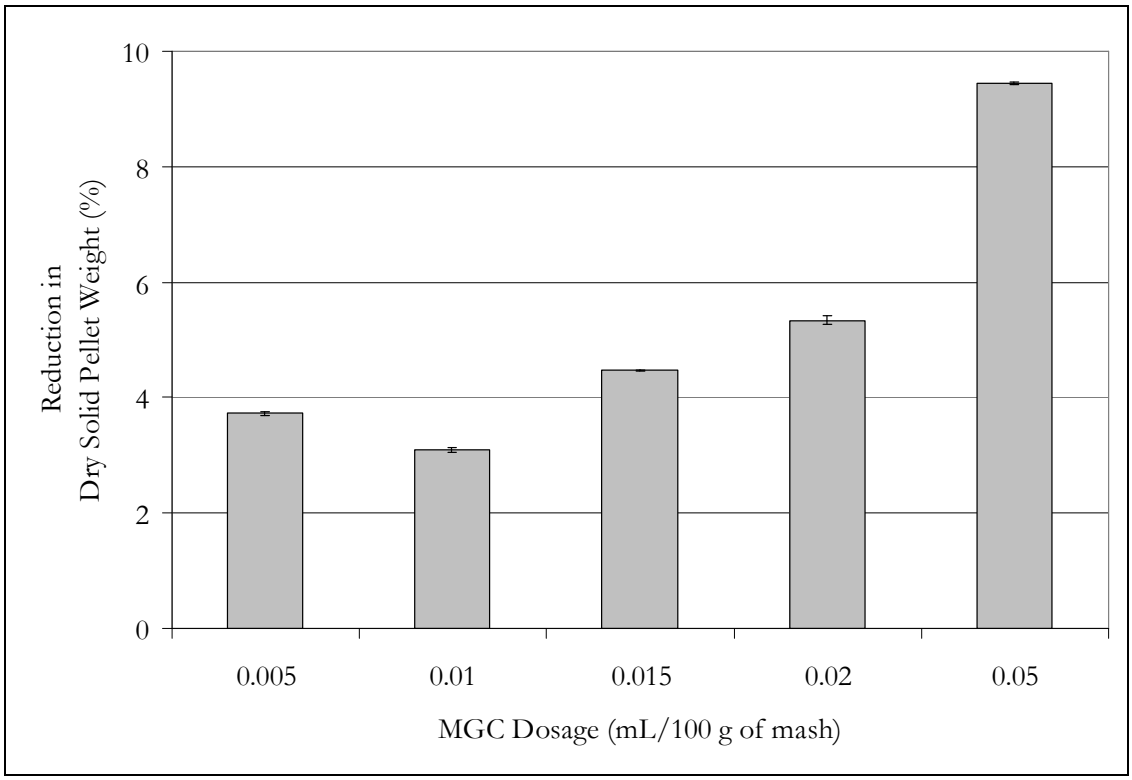

Figure 4.5: Reduction in dry solid pellet weights per 40 g of mash shown for each GC220 enzyme treated mash compared to the control. Weights are shown as a percentage of the control where the control. Error bars represent the standard deviations from the means.

If cell wall degrading enzymes worked perfectly, no loss of material would be observed and only water would be removed during centrifugation. However, it is believed that at the higher enzyme doses of 0.02 and 0.05 mL, the enzyme is solubilizing some of the material into the liquid phase of the mash which would cause a loss of solid material during centrifugation but not to the process as a whole. The only enzyme dosage that showed a statistical difference in the dry solid pellet weight compared to the other dosages was the mash with 0.05 mL of enzyme, $F(10,11) = 20.09$. The mashes treated with an enzyme dosage in the range of 0.005 and 0.02 mL per 100 g of mash were not statistically different. This

means that the optimal dosage presented above will not affect the amount of solids removed during centrifugation in the solid phase and will at the same time maximize the amount of water removed. Even though a decrease in solids removed after centrifugation will not affect the production of DDGS.

When comparing final ethanol yields between the enzyme-treated mashes and the control, there were a few statistically significant differences (Figure 4.5). The final ethanol yield for the control and the mashes treated with 0.005 and 0.01 mL of MGC were not different from one another but were different from the yields from the mashes treated with 0.015, 0.02 and 0.05 mL. Furthermore, the yields for the 3 highest enzyme dosages were also different from one another. A definite trend in the ethanol yields was not observed in this data set. The final amount of ethanol obtained did not increase or decrease with increasing enzyme dosage. These differences in final ethanol yields could be attributed to relatively small differences in the initial amount of starch in the mash and/or the incomplete conversion of starch to glucose during saccharification. It is not believed that once implemented in a processing plant that the enzyme will impact fermentation yields.

4.5 Conclusions

A significant reduction in water-binding capacity was found for both cell wall degrading enzymes (GC220 and MGC) at each enzyme dose tested. Whole stillage dewatering increased from 10 to 15% as GC220 enzyme volume increased from 0.005 to 0.05 mL/100 g of mash, and from 15 to 23% as MGC enzyme volume increased from 0.005 to 0.05 mL/100 g of mash. Since the addition of different enzyme amounts to the mash showed a change in the dewatering effect, it will allow for the optimization of enzyme cost with energy savings in Chapter 7.

In the case of GC220, enzyme dosages above 0.015 mL/100 g of mash gave diminishing returns where dewatering capability was concerned. At this dosage, the curve obtained from plotting enzyme dosage versus water removed reached its leveling off point. Thus, 0.015 mL was determined to be the optimal dosage when using this particular enzyme preparation. In contrast, for MGC, the same trend of diminishing returns was not observed. The maximum dewatering effect was found to be at the highest dosage of 0.05 mL. However, from the way optimization is defined in this work and taking into account economic factors, presented in Chapter 7, a dosage of 0.05 mL/100 g of mash was deemed unfavorable. After analyzing the results for a dosage of 0.02 mL and 0.015 mL, it was determined that there was not statistical difference between the two. Using the optimization chart presented at the beginning of this chapter the optimal enzyme dosage for MGC was determined to be 0.015 mL/100 g of mash.

Final ethanol yields did not significantly differ for the different enzymes amounts and for the control for GC220. For MGC, differences in the final ethanol yield were observed. There were no definite trends for the ethanol yields relative to the enzyme dosage used. The differences were attributed to incomplete starch to glucose conversion or relatively small differences in initial starch content in the mash. It is believed that the glucose to ethanol conversion went to completion in all cases. This means that, with either GC220 or MGC, dry grind corn to ethanol plants could keep the production rates the same and at the same time improve centrifugation efficiency, positively impacting the economics of the process.

Chapter 5 – Enzyme Combination

5.1 Introduction

There are no studies in the open literature that investigate and report the use of both cell wall degrading and proteolytic enzymes in a fermentation process to enhance dewatering and fermentation rates. Some researchers have looked at the addition of multiple enzymes such as cellulases, xylanases, and proteases to increase starch yield or decrease the steeping time during the wet milling corn to ethanol process (Caransa et al. 1988; Hassanean and Abdel-Wahed 1986; Moheno-Perez et al. 1999). Unfortunately, no one has examined how these enzymes can be used in the dry grind corn to ethanol process to reduce the costs of DDGS handling by affecting the amount of water that is removed during centrifugation, and at the same time by decreasing fermentation times of SSF.

5.2 Scope

The purpose of the experiments outlined below was to investigate how cell wall degrading and proteolytic enzymes could be added to the corn to ethanol process in an attempt to enhance both water removal of whole stillage and final ethanol yields of the process. Previous experiments have shown that cell wall degrading enzymes can be added to increase water removal by disrupting the bonding network between the polysaccharides and the water

molecules. The addition of proteases has been proven to increase fermentation rates and decrease fermentation times, allowing for an overall increase in ethanol production rate (Thomas and Ingledew 1990; Thomas and Ingledew 1995; Johnston and Singh 2001; Perez-Carrillo and Serna-Saldivar 2006). Individually, these enzymes showed that they could enhance dewatering and fermentation rates. It was important to see if by adding them together, the results of the separate experiments could be observed in a single experiment.

5.3 Experimental Work

5.3.1 Materials and Methods

The enzymes used in this research were donated by Genencor International (a Danisco Company, Palo Alto, CA). The corn used was donated by the University of Illinois, Urbana-Champagne, and the National Corn to Ethanol Research Center (NCERC) at Southern Illinois University, Edwardsville (SIUE). All chemicals used were of analytical quality.

5.3.1.1 Mash Preparation and Liquefaction

Erlenmeyer flasks (250 mL) were labeled and their tare weights were recorded. Stir bars were also weighed together with stoppers and 21 gauge 1.5" needles. Micro-centrifuge tubes used

for collecting samples were labeled with fermentation date, ID, and sample number. A rubber stopper, a needle, and a stir bar were assigned to each fermentation flask. The needle was inserted into the rubber stopper. Each flask with a stir bar and rubber stopper with a needle was weighed as an assembly.

To prepare the mash for each of the runs performed, 450 g of hammer milled corn (corn weight was adjusted using moisture content to give the desired final solids content of 30%) was added to 910 mL of water in 2-L flask and adjusted to pH 5.8 ± 0.1 by adding 1M HCl solution. Spezyme Fred α-amylase (1.3 mL) was added to the flask, which was then placed on a preheated hot plate at 90°C with the stirring rate set to 120 rpm. The slurry was liquefied at 90°C for 1 hr.

5.3.1.2 Simultaneous Saccharification and Fermentation (SSF)

Simultaneous saccharification and fermentation was carried out as previously reported in Section 4.3.1.2. The cell wall degrading enzymes, MGC and GC220, and the proteolytic enzyme, GC 106, were added to their respective set of flasks Table 5.1. A control flask was also prepared without enzyme addition for each run. The experiment was done in duplicates. All flasks were then sealed with the stopper, needle inserted, and moved to a temperature-controlled shaking incubator (30ºC at 200 rpm), for 72 hours.

5.3.1.3 Analytical Techniques

At the end of the fermentation process, a 1 mL sample was taken from each flask. The samples were centrifuged (model ICE Centra CL2, Thermo Electron Corporation, Waltham, MA) for 2 min at 16,110 x g, and the supernatant was then filtered using a 0.2 μm syringe filter (model 4455T, Pall, Ann Harbor, MI) into labeled 1 mL microcentrifuge tubes and stored frozen until ready for HPLC analysis.

Samples were thawed and analyzed by high-performance liquid chromatography (HPLC) as described in Section 2.3.1.3. Here, the actual HPLC equipment used was from Agilent and model number 1100 (Santa Clara, CA) equipped with an Aminex HPX-87H Biorad (Hercules, CA) ion-exclusion column.

For the final part of the data collection, the experiment followed the procedures described in Section 3.3.1.3 until the final step. In the case of this experiment, at the end of fermentation (72 hours), only one 40-mL representative sample was taken from each of the flasks and transferred to a 50-mL centrifuge tube (Corning, cat no. 430290, 29.1 mm o.d.). Each tube was then centrifuged in the bench-top centrifuge for 5 min at 1,400 × *g* to analyze the water-binding capacity of each enzyme treatment. The supernatant from each flask was weighed, placed in a 55°C oven (model 6555, Thelco Laboratoty Oven, Thermo Electron Corporation, Waltham, MA) for 48 hr, moved to a 135°C oven for 2 hr, and then weighed again. The same was done with the solid pellets (wet grains) obtained after centrifugation.

5.3.2 Experimental Design

The experiment performed in this section of the thesis was set up as a matrix design (Table 5.1). There were 12 runs carried out using this matrix design.

Table 5.1: Experimental setup of enzyme volumes for matrix experiment.

Protease (mL/100 g mash) \ Cell Wall Degrading Enzyme (mL/100 g mash)	0	0.005	0.01	0.015	0.02	0.05
0	0	0	0	0	0	0
0.01	0	0.005	0.01	0.015	0.02	0.05
0.015	0	0.005	0.01	0.015	0.02	0.05
0.02	0	0.005	0.01	0.015	0.02	0.05
0.03	0	0.005	0.01	0.015	0.02	0.05

The shaker-incubator used here could not hold all the flasks needed to perform the matrix experiment, so the experiment had to be divided into different runs. The data for the run with only GC106 was performed separately and is presented in Chapter 3. The runs with only GC220 and only MGC were also performed separately and are described in Chapter 4. The other runs are outlined in Table 5.2. All runs were done in duplicates including a duplicate control for each run.

Table 5.2: Break down of selected runs used in the matrix design experiment. Each pair of enzyme volume was added to two flasks. Each run contained a duplicate control. All enzyme volumes are given for 100 g of mash.

Run 4		Run 5		Run 6		Run 7		Run 8		Run 9		Run 10		Run 11		Run 12	
MGC (mL)	GC106 (mL)	MGC (mL)	GC106 (mL)	MGC (mL)	GC106 (mL)	MGC (mL)	GC106 (mL)	MGC (mL)	GC106 (mL)	GC220 (mL)	GC106 (mL)	GC220 (mL)	GC106 (mL)	GC220 (mL)	GC106 (mL)	GC220 (mL)	GC106 (mL)
0.01	0.015	0.015	0.01	0.05	0.01	0.005	0.01	0.01	0.01	0.005	0.03	0.01	0.03	0.02	0.01	0.05	0.02
0.02	0.01	0.02	0.015	0.015	0.015	0.05	0.015	0.005	0.015	0.015	0.01	0.015	0.015	0.05	0.03	0.005	0.02
0.015	0.02	0.005	0.02	0.05	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.03	0.01	0.01	0.01	0.015
0.05	0.03	0.01	0.03	0.02	0.03	0.005	0.03	0.015	0.03	0.05	0.01	0.005	0.015	0.015	0.02	0.015	0.03
n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	0.005	0.01	0.01	0.02	0.02	0.015	0.05	0.015

n/a - not applicable to this run

5.3.3 Statistical Analysis

SPSS was used to compute an ANOVA to compare the mean amount of water removed after centrifugation, the dry solid pellet weights, and the fermentation rates for each pair of enzyme volumes with the control (SPSS for Windows, SPSS Inc., Chicago, IL). A graphing technique showing the mean of each level tested was used to further investigate whether a statistical relationship was present in the data obtained for the amount of water removed and the dry solid pellet weights. For the fermentation rates, the post-hoc used to analyze the data was the *t*-Test for two samples assuming equal variances. The *t*-Test was performed to compare all pairs of two different enzyme combinations. The level selected to show statistical significance was 5% ($P < 0.05$).

5.4 Results and Discussion

As explained above, due to limitations in the equipment used for the work in this thesis, the experiment presented in this chapter had to be divided into different runs. Therefore, the data within each run is presented as a percentage of the control of that run. The variability of the controls within a run was much less than the variability of the controls between each run. These differences are attributed to unpredictable variations that occurred during each run especially due to the time that elapsed between the runs.

The effect of the different enzyme volumes on the amount of water removed from the 100 g of mash was determined by centrifuging a 40 g sub-sample. Figure 5.1 shows the amount of water removed after centrifugation for each enzyme combination as a percentage of the control.

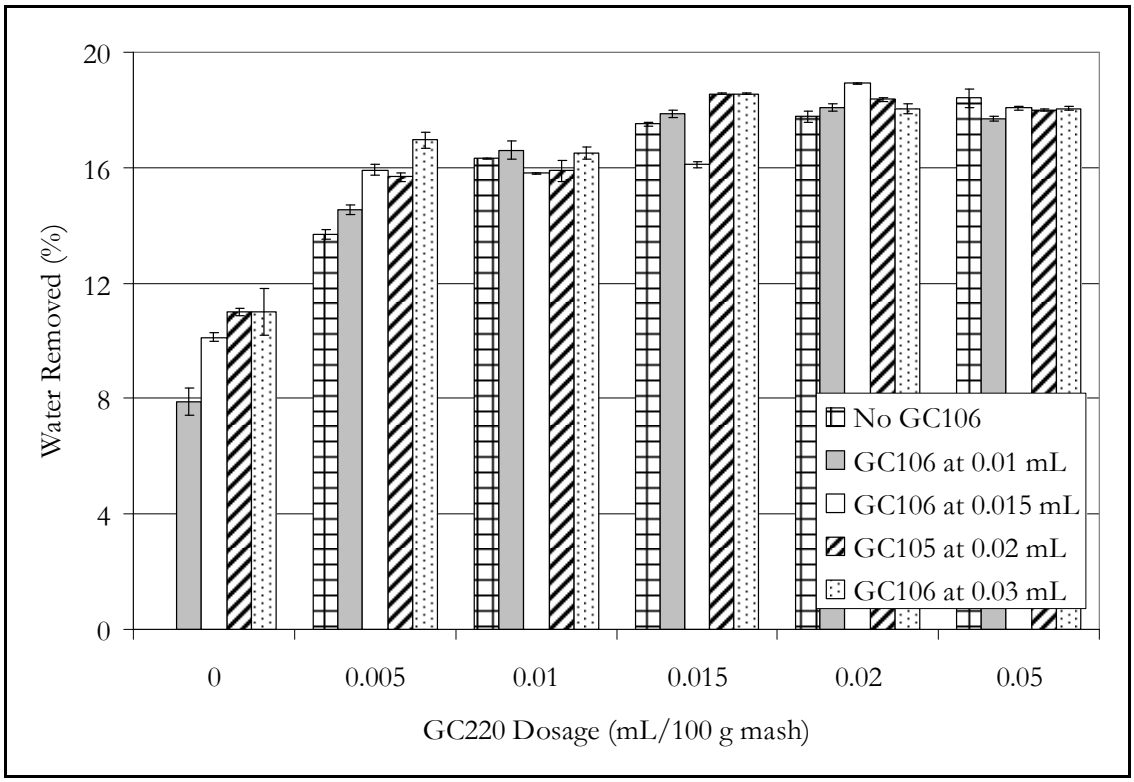

Figure 5.1: Amount of water removed as a percent of the control given for the different volume combinations of GC220 and GC106. The control is taken to be 0%. Error bars represent one standard deviation from the mean.

Each group of bars show the average amount of water removed as a percentage of the control at the different GC106 volumes for a given volume of GC220. In this case the control is taken to be zero percent and any value greater than zero represents an increase in water removed compared to the control. As can be observed from the graph, as the GC220 enzyme volume increased so did the amount of water removed, up to a GC220 volume of 0.015 mL. There was no statistically significant difference between the amount of water removed for the flasks containing 0.015, 0.02 and 0.05 mL of GC220. At a GC220 enzyme volume of 0.015 mL per 100 g of mash, a maximum effect was seen at 0.02 mL of GC106.

The statistical analysis revealed a significant main interaction of GC220 and GC106 volumes, $F(2,12) = 3.34$. At this combination the amount of water removed was on average 18.57 (± 0.351) % of the control. This shows that adding more than 0.015 mL of GC220 may not be beneficial when trying to maximize the effects observed from the enzyme addition.

Furthermore, in terms of the amount of GC106 added to the mash, there is no clear trend for the amount of water removed after centrifugation. No data group showed either an increase or decrease in the amount of water removed during centrifugation as the GC106 amount was increased from 0.01 to 0.03 mL. This indicates that GC106, when added in conjunction with GC220, will not have an adverse effect on the amount of water removed. In the case of a GC220 volume of 0.05 ml, there was no statistical difference in the amount of water removed for all volumes of GC106 added with the greatest amount being on average 18.066 (± 0.033) % of the control at 0.015 mL of GC106. When GC220 was at 0.02 mL level, only the amount of water removed at 0.015 mL of GC106 was statistically different than water removal at other levels of GC106. At this enzyme combination, the amount of water removed was on average 18.914 (± 0.031) % of the control.

When comparing the dewatering at an enzyme combination of 0.015 mL of GC220 and 0.02 mL of GC106 to the dewatering obtained with only 0.015 mL of GC220 (14.97 ± 0.059 %), an increase of 3.6% in the amount of water removed is observed for the mash that contained the enzyme combination. The dewatering obtained at this enzyme combination was higher and significantly different from the dewatering observed at all levels of only GC220. The

same was observed for the amounts of water removed obtained from the mashes with GC106 only. The highest dewatering for this group was 11.39 (± 0.81) % observed at 0.03 mL of GC106. Some dewatering was observed for all of the GC106 volumes added to the mash without GC220 but none of the results was higher than the results observed for the mash containing both GC220 and GC106 as well as with only GC220.

From the results above, it can be said that most favorable results in terms of dewatering were obtained at a GC220 dosage of 0.015 mL in conjunction with a GC106 volume of 0.02 mL per 100 g of mash.

A similar data set as the one presented above can be seen in Figure 5.2 but for enzyme volume combinations of MGC and GC106.

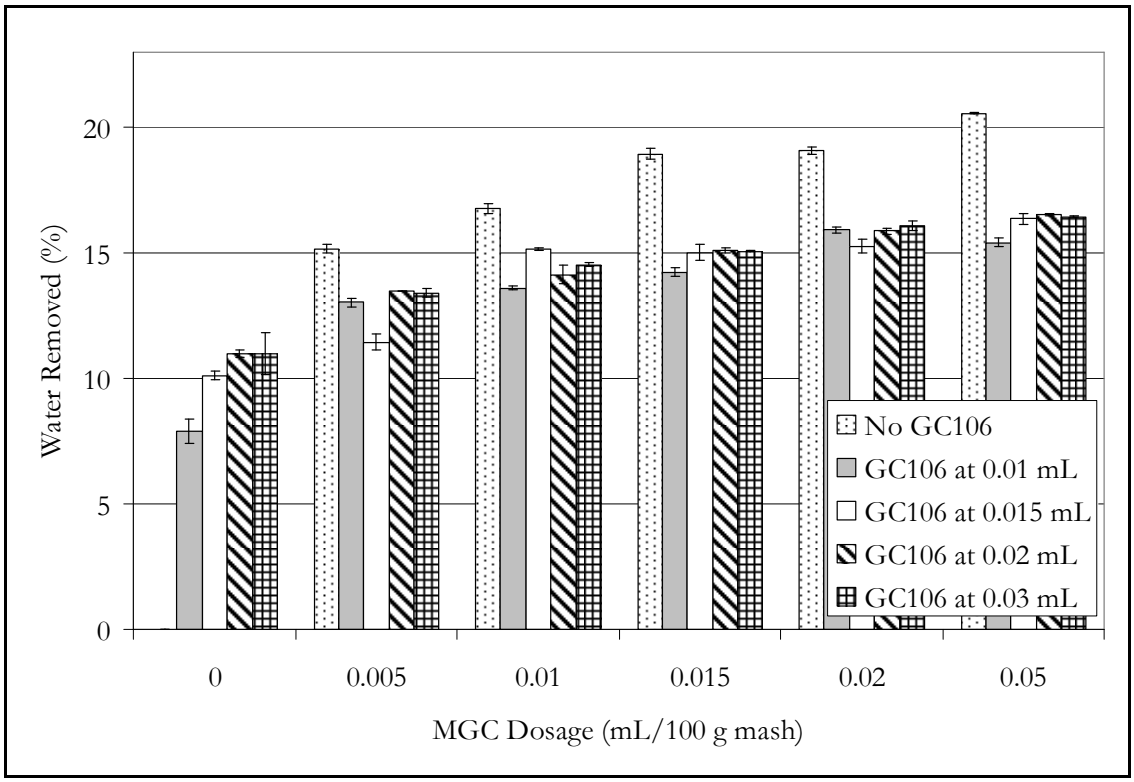

Figure 5.2: Amount of water removed as a percent of the control given for the different volume combinations of MGC and GC106. The control is taken to be 0%. Error bars represent one standard deviation from the mean.

As was the case for the data from the GC220 and GC106 runs, each group of bars show the average amount of water removed as a percentage of the control at the different GC106 volumes for a given volume of MGC. Here again, each control is taken to be zero percent and any value greater than zero represents an increase in water removed compared to the control. The graph shows that as the MGC enzyme volume is increased from 0.005 to 0.05 mL, so is the amount of water removed for the different volumes of GC106. The statistical analysis showed that there was no significant difference in the amount of water removed between MGC enzyme volumes of 0.01 and 0.015 mL, as well as no difference between 0.02

and 0.05 mL. This means that a maximum effect can be observed at 0.02 mL of MGC per 100 g of mash, and that going up to a volume of 0.05 mL would not significantly increase the amount of water removed. The analysis revealed a significant interaction of MGC and GC106 volumes, $F(2,12) = 20.58$. At a MGC enzyme volume of 0.02 mL per 100 g of mash, the highest amount of water removed (29.276 ± 0.215 g) was observed at a GC106 volume of 0.03 mL. However, the statistical results showed that there was no significant difference between the amount of water removed at 0.03, 0.02 and 0.01 mL of GC106. The only statistically significant difference was seen for 0.015 ml of GC106 (27.225 ± 0.281 g) but that weight was smaller when compared to the other weights. This shows that adding more than 0.02 mL of MGC per 100 g of mash and 0.01 mL of GC106 per 100 g of mash may not be beneficial when trying to maximize the effects observed from the enzyme addition.

When examining the amounts of water removed with a given volume of MGC, no trends were observed for the different volumes of GC106. None of the data groups showed either an increase or a decrease in the amount of water removed as the volume of GC106 was increased from 0.01 to 0.03 mL. As was observed in the first experiment, adding GC106 to the mash in conjunction with MGC did not have a detrimental effect on the amount of water removed after centrifugation. Looking at a MGC volume of 0.02 mL, and from the results of the statistical analysis for the different GC106 volumes presented above, it can be seen that adding more than 0.01 mL of GC106 to the mash does not significantly increase the amount of water removed. In this case, even though the maximum effect was not seen at this enzyme volume, adding more than this amount is not effective. At this enzyme

combination of 0.02 mL of MGC and 0.01 mL of GC106, the amount of water removed was on average 15.921 (± 0.139) % of the control.

The data set given for MGC and GC106 differed from the set given for GC220 and GC106 in regard to how much water was removed from the mash with only MGC compared to the mash with only GC220. The results obtained for the mash with only MGC was significantly higher than the water removed with any other enzyme dosage combination of MGC and GC106 as well as with only GC106. In this case the addition of the GC106 to the mash had a detrimental effect in the dewatering of the whole stillage. The effects observed with only GC106 and only MGC were not additive when the enzymes were combined in the same mash. This was not the case for the run with GC106 and GC220 as described above.

Based on the results presented above, the best water removal will be obtained when using a MGC enzyme volume of 0.02 mL in conjunction with a GC106 enzyme volume of 0.01 mL per 100 g of mash. If the only desired effect is dewatering, best results are observed when using only MGC, and optimal results are obtained at 0.015 mL per 100 g of mash as presented in Chapter 4. However, increase in ethanol production rates are also a desired effect, and therefore the results for this parameter must also be examined before a final conclusion can be reached.

As the 40 g sub-samples were centrifuged and water removal data was collected, the weights of the solids present in the mash after centrifugation were also recorded. As was previously

shown in Section 2.3, the unfermentable solids become a pellet that can be removed from the centrifuged tube, dried and weighed. Figure 5.3 and Figure 5.4 show the reduction in dry solid pellet weights for the GC220 and GC106 runs and MGC and GC106 runs, respectively.

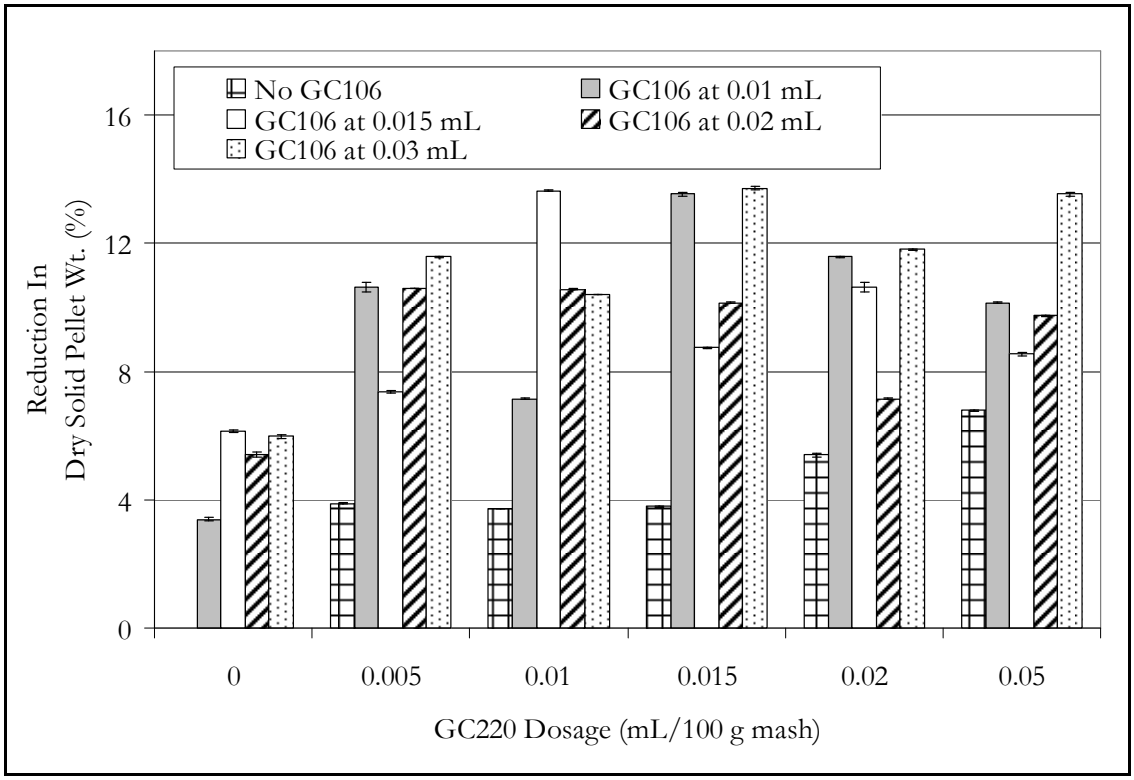

Figure 5.3: Reduction in dry solid pellet weights compared to the control given for the different volume combinations of GC220 and GC106. Control is taken to be 0%. Error bars represent one standard deviation from the mean.

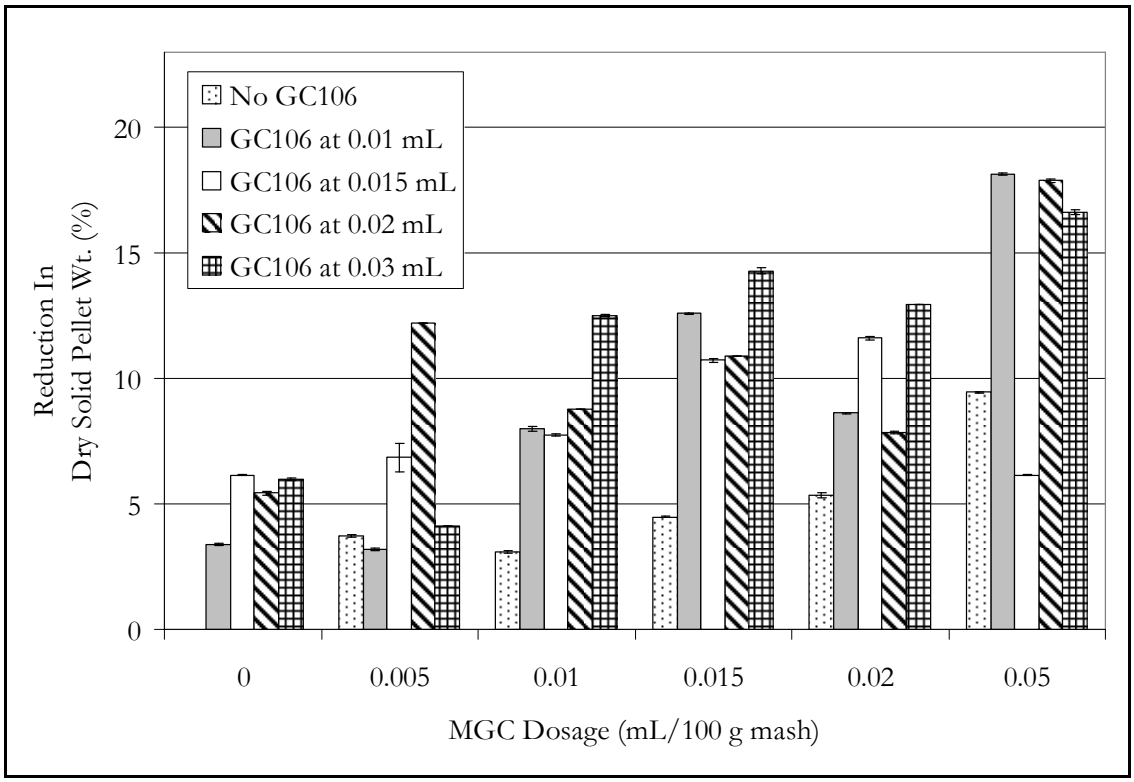

Figure 5.4: Reduction in dry solid pellet weights compared to the control given for the different volume combinations of MGC and GC106. The control is taken to be 0%. Error bars represent one standard deviation from the mean.

The graphs show the reduction in dry solid pellet weights as a percentage of the control. In the case of both graphs the control is given as 0% and since all other measured weights were smaller than the measured weight of the control, all of the percentages shown are positive. As can be seen on the graphs, all dry solid pellet weights from all enzyme volume combinations were smaller than the control. As for the data shown in Figure 5.3 and Figure 5.4, the reduction in the pellet weight increases as the amounts of GC220 and MGC increase. This effect is more evident in the case of MGC and could be due to the different enzymatic activities present in each of the two enzyme preparations. The increased reduction

of pellet weight is attributed to the fact that as the MGC and GC220 volume increases so does the enzymatic activity and more of the solid material is solubilized. Therefore, when the samples are centrifuged more of this soluble material ends up in the liquid phase.

In a dry grind corn to ethanol plant, the solubilized material would be sent to the evaporators where, after the water is driven off, the syrup that is left would contain the solubilized material. This material would then be recycled back into the process by mixing it with the WDGs before going into the drier. In this way, no material (co-product) would be lost during the process. Data collected during the plant trial portion of this work, and presented in Chapter 6, will show that this hypothesis is correct.

When looking at Figure 5.3, for a given volume of GC220, the smallest reduction in pellet weight was observed when there was no GC106 present in the mash. This presence of this protease will result in the break down of protein present in the mash. These smaller protein chains can also end up in the liquid phase after centrifugation. As the GC106 volume was increased no definite trend resulted for the measured pellet weight. In the case of MGC and GC106, a similar result to the GC220 and GC106 run was observed for the reduction of the pellet weight. For a given volume of MGC, there was no trend in the pellet weight reduction as the GC106 enzyme volume increased from 0.01 to 0.03 mL.

Fermentation rates were measured by periodically weighing the flasks during fermentation as explained in Section 3.4. For purposes of these experiments a conversion parameter was

formulated to evaluate whether or not the fermentation rates were affected by the enzyme additions. This parameter is defined as the total weight loss at 31 hr as a percentage of the total weight loss at the end of fermentation (72 hr) for each flask. In this case, 31 hr was selected because at this point most of the mash containing the enzymes had reached an asymptotic point to the leveling off of the weight loss curve. This curve has a characteristic S-shape as presented in Chapter 3, and the leveling off occurs after the curve presents the rapid conversion period, also known as the exponential phase.

Table 5.3 shows the percent increase in weight loss for the MGC/GC106 treated mashes in comparison to the control.

Table 5.3: Data represents increase in weight loss at 31 hrs as a percentage of total weight lost (72 hr) compared to the control. The control is taken to be 0%. Values followed by the same letter in the same row are not significantly different ($P < 0.05$).

		Percent Increase in Weight Loss				
		GC106 (mL)				
		0	**0.01**	**0.015**	**0.02**	**0.03**
MGC (mL)	**0**	0	15.04 ± 1.47a	22.35 ± 0.560b	26.79 ± 0.180c	28.82 ± 1.01d
	0.005	0.67 ± 0.230a	15.86 ± 0.49b	14.24 ± 0.282c	14.59 ± 0.071c	16.88 ± 0.058d
	0.01	-(0.06 ± 0.211)a	13.08 ± 0.398b	14.68 ± 0.142c	14.56 ± 0.032c	14.68 ± 0.142c
	0.015	0.10 ± 0.660a	14.09 ± 0.211b	14.3 ± 0.094b	14.97 ± 0.075c	14.86 ± 0.071c
	0.02	0.40 ± 0.136a	12.56 ± 2.17b	14.68 ± 0.142c	16.51 ± 0.647d	14.6 ± 0.000c
	0.05	1.36 ± 0.796a	15.0 ± 0.131b	16.63 ± 0.372c	14.8 ± 0.278b	15.03 ± 0.109b

As can be observed, the percent conversion values given in the table were all larger than the average value observed for the control. In the case of the mash with only MGC, there was

no statistically significant difference in the percent increase in weight loss as the enzyme volume increased from 0.005 to 0.02 mL compared to the control. There were also no statistically significant differences when those enzyme dosages were compared to one another. The only difference was observed at the highest MGC dose of 0.05 mL. It is believed that this could have happened because MGC may have a small amount of proteolytic activity in its preparation. MGC is a commercially available enzyme preparation and due to its proprietary nature it is not possible to know its exact composition. However, when the MGC enzyme was added at such a high volume, the proteolytic activity was more pronounced and a small difference was seen. When the GC106 enzyme was added by itself there was a significant increase in the percent increase in weight loss as the volume was increased from 0.01 to 0.03 mL. The highest percent increase observed was 28.82% at 0.03 mL. This increase in weight loss was also higher than the increase observed for all of the other enzyme combinations. This could have resulted from a change in the batch of corn used in this experiment. Corn is donated by different sources and once the sample received is exhausted more corn needs to be obtained. During the matrix experiment a batch of corn had been obtained from the University of Illinois but by the time the GC106-only run was performed that batch had been used up and more corn was obtained, this time from Southern Illinois University. As explained earlier in this thesis, corn composition can differ and depending on the hybrid that was harvested, experimental results could be affected.

Statistically, there were differences in the increase in weight loss as the GC106 enzyme volume increase for a given volume of MGC. At MGC volumes of 0.005 and 0.01 mL, the

highest increase in fermentation rates was observed at 0.03 and 0.015 mL, respectively. However, the percent increase at these volumes was only 2.64 and 1.6% higher than the lowest percent increase at 0.015 and 0.01 mL of GC106, respectively. It is believed that at a GC106 enzyme volume of 0.01 mL/100 g of mash, the enzyme activity is already asymptotically reaching its equilibrium phase and the results obtained at this enzyme dosage would be enough to improve the residence time of fermentation in a corn to ethanol processing plant. At the higher volumes of MGC, similar effects are observed. At 0.015 mL of MGC, the difference between the highest and lowest percent increase in weight loss is 0.77%, while at 0.02 and 0.05 mL this difference is 3.95 and 1.83%, respectively. It is believed that in order to obtain more significant results regarding the increase in fermentation rates, another matrix experiment needs to be done to look at GC106 volumes smaller than 0.01 mL. However, at these smaller volumes, the dewatering effect may not be as evident and the results observed above may not be detected.

From the run that combined volumes from both enzymes, which had the flask that showed the highest percent increase in weight loss, it is known that, at 31 hr, that flask had reached 94.78 (± 0.058) % loss in weight compared to the total weight lost at 72 hr. At 31 hr, the control of this run had only reached 77.90 (± 0.007) % in weight loss. This could translate into a decrease in fermentation residence time in a corn to ethanol plant where fermentation is approximately kept at 50 hr or longer under normal plant operating procedures. From the results presented above, it would be possible to achieve the same ethanol production rates at a smaller residence time, between 30 to 36 hr, thus increasing the plant's throughput. This,

of course would depend on the plant's ability to also improve residence times downstream of fermentation.

The calculated percent increase in weight loss for the experiment with GC220 and GC106 can be seen in Table 5.4.

Table 5.4: Data represents percent increase in weight loss at 31 hrs as a percentage of total weight lost (72 hr) compared to the control. The control is taken to be 0%. Values followed by the same letter in the same row are not significantly different ($P < 0.05$).

		Percent Increase in Weight Loss				
		GC106 (mL)				
		0	**0.01**	**0.015**	**0.02**	**0.03**
GC220 (mL)	**0**	0	15.04 ± 1.47a	22.35 ± 0.560b	26.79 ± 0.180c	28.82 ± 1.01d
	0.005	0.74 ± 0.237a	15.05 ± 0.403b	14.92 ± 0.050b	14.75 ± 0.164b	17.15 ± 0.007c
	0.01	-(0.16 ± 0.129)a	13.78 ± 0.971b	13.78 ± 0.97b	14.74 ± 0.175b	15.26 ± 0.072c
	0.015	0.08 ± 0.158a	14.62 ± 0.124b	14.76 ± 0.110b	14.73 ± 0.121b	14.73 ± 0.121b
	0.02	0.15 ± 0.211a	14.59 ± 0.421b	14.68 ± 0.142b	14.72 ± 0.306b	14.83 ± 0.339b
	0.05	0.36 ± 0.438a	15.0 ± 0.131b	14.72 ± 0.306b	14.59 ± 0.421b	14.75 ± 0.164b

The results observed for the mash containing only the addition of GC220 was not statistically different from the control. No improvements in fermentation rates were observed as the GC220 volume was increased from 0.005 to 0.05 mL. At GC220 volumes of 0.005 and 0.01 mL, an increase in fermentation rates was observed at the highest GC106 volume of 0.03 mL. The percent increase at the other GC106 volumes of 0.01, 0.015 and 0.02, were not statistically different from one another. The difference between the highest and the lowest percent increase in weight loss, for the different GC106 volumes, was

calculated to be 2.4 and 1.48% for MGC volumes of 0.005 and 0.01 mL, respectively. For the higher GC220 volumes of 0.015, 0.02 and 0.05 mL, no differences in the percent increase in weight loss was observed as the GC106 volume was increase from 0.01 to 0.03 mL. As was the case for MGC and GC106 data set, using a GC106 volume of 0.01 mL will result in improvements in the fermentation rates. Here again, it is probable that at lower volumes of GC106, less than 0.01 mL/100 g of mash, increase in fermentation rates will still be observed and more definite conclusions could be drawn from the results. However, it is expected that a decrease in the dewatering effect will also be observed.

The enzyme volume combination that showed the highest percent increase in weight loss was 0.03 mL of GC106 and 0.005 mL of GC220. In this run, at 31 hr, the flasks containing this enzyme combination had lost on average 94.02 (± 0.007) % of the total weight lost compared to 76.87 (± 0.330) % observed for the control. These results were not statistically different from the results observed at the same MGC and CG106 volumes as described above. If GC220 is selected as the dewatering enzyme to be used in the plant together with GC106, similar reduction in fermentation residence times, 30 to 36 hr total fermentation times, can be expected as was projected for MGC.

Taking into account both dewatering and percent increase in weigh loss results for this experiment, it is suggested that a plant uses either 0.02 mL of MGC with 0.02 mL of GC106 per 100 g of mash, or 0.015 mL of GC220 with 0.01 mL of GC106 per 100 g of mash.

HPLC data was also collected during this experiment. Table 5.5 and Table 5.6 show the HPLC average results for selected compounds for the duplicate flasks fermented in all of the runs.

Table 5.5: HPLC data given for selected compounds for the MGC and GC106 run. Data presented is the average of the duplicate flasks. Data for the controls is denoted by the absence of enzyme volumes for both MGC and GC106.

HPLC Data					
Enzyme Volume (mL/100 g mash)		Compound			
MGC	GC106	DP4+ (%w/v)	Glucose (%w/v)	Glycerol (%w/v)	Ethanol (%v/v)
0	0	0.368	0.038	0.750	15.038
0	0	0.333	0.050	1.018	15.097
0	0	0.365	0.030	1.050	14.442
0	0	0.400	0.040	1.115	14.840
0	0	0.325	0.010	1.100	15.184
0	0	0.373	0.034	0.958	14.120
0	0	0.134	0.043	1.111	15.714
0	0.01	0.470	0.078	0.968	15.208
0	0.015	0.420	0.049	0.918	15.330
0	0.02	0.343	0.032	0.852	15.837
0	0.03	0.437	0.058	0.876	15.723
0.005	0	0.234	0.017	0.911	15.549
0.01	0	0.288	0.039	0.720	15.224
0.015	0	0.403	0.036	0.781	15.591
0.02	0	0.566	0.043	0.709	15.275
0.05	0	0.542	0.040	0.896	15.442
0.005	0.01	0.500	0.040	0.930	14.867
0.01	0.01	0.480	0.015	0.940	15.501
0.015	0.01	0.595	0.020	0.900	14.195
0.02	0.01	0.555	0.025	0.940	14.867
0.05	0.01	0.655	0.030	0.795	14.994
0.005	0.015	0.460	0.020	0.925	15.558
0.01	0.015	0.530	0.030	0.900	14.816
0.015	0.015	0.585	0.020	0.855	14.861
0.02	0.015	0.555	0.030	0.880	14.785
0.05	0.015	0.625	0.040	0.870	15.323
0.005	0.02	0.518	0.023	0.753	14.851
0.01	0.02	0.475	0.020	0.910	15.684
0.015	0.02	0.550	0.025	0.870	14.582
0.02	0.02	0.595	0.040	0.880	15.380
0.05	0.02	0.635	0.030	0.820	14.829
0.005	0.03	0.530	0.040	0.875	15.368
0.01	0.03	0.515	0.025	0.845	14.823
0.015	0.03	0.515	0.010	0.885	15.469
0.02	0.03	0.595	0.020	0.835	14.797
0.05	0.03	0.630	0.030	0.850	14.620

Table 5.6: HPLC data given for selected compounds for the GC220 and GC106 run. Data presented is the average of the duplicate flasks. Data for the controls is denoted by the absence of enzyme volumes for both GC220 and GC106.

HPLC Data					
Enzyme Volume (mL/100 g mash)		Compound			
GC220	GC106	DP4+ (%w/v)	Glucose (%w/v)	Glycerol (%w/v)	Ethanol (%v/v)
0	0	0.265	0.060	0.830	15.240
0	0	0.240	0.020	0.705	15.233
0	0	0.230	0.040	0.880	15.018
0	0	0.130	0.035	0.643	15.426
0	0	0.373	0.118	0.809	15.676
0	0	0.307	0.035	0.765	15.168
0	0.01	0.470	0.078	0.968	15.208
0	0.015	0.420	0.049	0.918	15.330
0	0.02	0.343	0.032	0.852	15.837
0	0.03	0.437	0.058	0.876	15.723
0.005	0	0.438	0.041	0.805	15.233
0.01	0	0.410	0.070	0.794	15.231
0.015	0	0.368	0.061	0.762	15.005
0.02	0	0.400	0.058	0.828	15.085
0.05	0	0.377	0.051	0.832	15.072
0.005	0.01	0.525	0.060	0.830	15.255
0.01	0.01	0.425	0.030	0.705	15.135
0.015	0.01	0.460	0.025	0.860	15.150
0.02	0.01	0.485	0.060	0.820	15.140
0.05	0.01	0.515	0.050	0.775	14.895
0.005	0.015	0.470	0.035	0.875	15.135
0.01	0.015	0.425	0.030	0.845	15.005
0.015	0.015	0.488	0.040	0.800	14.940
0.02	0.015	0.487	0.026	0.880	15.240
0.05	0.015	0.488	0.029	0.995	15.233
0.005	0.02	0.487	0.029	0.658	15.447
0.01	0.02	0.539	0.038	0.661	14.979
0.015	0.02	0.544	0.039	0.669	14.983
0.02	0.02	0.569	0.040	0.880	15.018
0.05	0.02	0.565	0.042	0.784	14.935
0.005	0.03	0.562	0.041	0.723	15.114
0.01	0.03	0.571	0.039	0.801	14.926
0.015	0.03	0.576	0.052	0.693	15.035
0.02	0.03	0.573	0.048	0.712	15.335
0.05	0.03	0.581	0.290	0.825	14.850

There were no statistically significant differences for the ethanol and glucose contents in the samples obtained at the end of fermentation for the mashes including the controls in all the runs. It is concluded that sugar to ethanol conversion rates were not affected by the addition of GC220, MCG, and GC106, and that glucose depletion remained the same in all runs. Glycerol was measured because it can indicate whether or not the yeast is under stress in the mash. Higher glycerol levels represent a higher level of stress for the yeast. For the most part glycerol levels were not statistically different for the mashes. Five out of seven controls, in Table 5.5, showed an increase in glycerol level compared to the mash that contained the enzymes. In Table 5.6, none of the controls showed this behavior. An increase in glycerol level could be attributed to the lack of certain nutrients that are necessary for proper yeast growth and the presence of compounds that are harmful to the yeast and can influence the metabolic pathways carried out by the cells. An increase in DP4+ levels were observed for the enzyme treated mashes, in both cases, compared to the controls. DP4+ indicates how much there is all components that are soluble and elutes in the first part of the HPLC column. Most of the time, for a corn to ethanol processing plant, this is an important value because it indicates how much of the dextrins present in the mash are not being hydrolyzed into glucose. Usually, the higher the DP4+ level, the lower the final ethanol yield. However, in this case since there were no differences in final ethanol yields and unfermented glucose, it is believed that the increase in DP4+ value was due to the hydrolysis of unfermentable material, mainly soluble fiber such as xylooligosaccharides from xylans and manna-olygosaccharides from yeast cell walls, by the enzymes added to the mash and not due to a decrease in ethanol production (Zhu et al. 2006; Zentek, Marquart and Pietrzak 2002).

5.5 Conclusions

In conclusion, optimal for water removal were obtained at a GC220 dosage of 0.015 mL in conjunction with a GC106 volume of 0.02 mL per 100 g of mash, and a MGC enzyme volume of 0.02 mL in conjunction with a GC106 enzyme volume of 0.01 mL per 100 g of mash. At these enzyme combinations it would be possible to keep a relatively smaller mash-to-enzyme ratio while still maximizing dewatering of whole stillage. The cost-effectiveness of using different dosages of the cell wall degrading enzymes will be examined in the economic analysis presented in Chapter 7.

Furthermore, there was a decrease in the dry solid pellet weights for all of the mashes containing the enzymes. This will not be detrimental to the process since this the presence of a recycle stream after centrifugation will mix in the solubilized solids with the wet grains before this material is sent to the drier. There would be no reduction in the co-product production of the plant.

As for the results from the fermentation rates, it can be concluded that adding GC106 in conjunction with either GC220 or MGC will positively affect the fermentation rates. For the GC220/GC106 run, at 31 hr, the flasks containing 0.005 mL of GC220 and 0.03 mL of GC106 had lost on average 94.02 (± 0.007) % of the total weight lost compared to 76.87 (± 0.330) % observed for the control. In the case of the MGC/GC106 run, the flasks

containing the same enzyme volumes as the previous run, had reached 94.78 (± 0.058) % loss in weight compared to 77.90 (± 0.007) % in weight loss observed for the control.

This means that while a plant that runs without enzymes would be only at approximately 77% completion during fermentation at 31 hrs, the ones that add the enzymes could see an improvement to 94% completion at that same time, potentially decreasing the plant's fermentation residence times. When both enhanced dewatering and increase in fermentation rates are desired, it is recommended that a plant doses their fermentors using either a MGC/GC106 volume combination of 0.02/0.02 mL per 100 g of mash or a GC220/GC106 combination of 0.015/0.01 mL per 100 g of mash.

HPLC results showed that there were no statistically significant differences in the final ethanol yields and glucose levels for all of the flasks fermented in the experiment presented in this section. Furthermore, small differences in the glycerol levels were observed for some of the controls in the MGC/GC106. This increase in the glycerol levels was not enough to affect yeast performance since ethanol yields were not impacted. An increase in DP4+ levels were observed with an increase in cell wall degrading enzyme volume added to the mash. It is believed that this increase was a reflection of the increase in unfermentable material hydrolyzed by the enzymes. There was no evidence that this increase in DP4+ level was due to an increase in dextrins that were not converted into ethanol during fermentation.

Chapter 6 – Plant Trial

6.1 Introduction

Based on the results presented in the previous chapters, the use of cell wall degrading enzymes to enhance water removal during centrifugation is very promising. Laboratory-scale experimental results show that there are statistically significant differences in the process when these enzymes are used. It was shown that water removal was enhanced by 18% when commercially available cell wall degrading enzymes, such as GC220 and Multifect GC, were added to the mash. These enzymes worked to improve water removal at relatively low mash-to-enzyme ratios, about 0.7 kg/MT of corn.

The results obtained prior to the plant trial were from lab-scale experiments done for the most part in 250 mL Erlenmeyer flasks. At this stage it is important to understand how the results obtained will scale up when the process is implemented into a conventional corn to ethanol plant. Moreover, it is critical to understand how the addition of these enzymes affects the energy input of the process, and whether a decrease in the energy usage of the plant will be immediately observed.

Center Ethanol Company, LLC, is a dry grind corn to ethanol plant located in Sauget, IL. The plant currently uses approximately 19.2 million bushels of corn annually to produce

about 54 million gallons of ethanol per year as well as 172,000 tons of DDGS and 1.5 million gallons of corn oil annually. This facility was chosen for conducting a plant scale experiment to determine what scale-up problems, if any, would be seen if the process proposed in this thesis was implemented at full scale.

6.2 Scope

The purpose of the plant trial was to demonstrate, at plant scale, that the separation of the liquid from the whole stillage during centrifugation can be improved by treatment of the corn mash during fermentation with an enzyme preparation that disrupts the water binding capacity of the corn fiber components. Furthermore, the trial was used to study the effects of dewatering enzyme addition on the mass balance surrounding the decanter centrifuge, as well as the drier's energy requirement and the plant's natural gas usage.

6.3 Plant Scale Experimental Design

The plant trial was setup to run in three phases. The first phase consisted of collecting baseline data for about 10 days (Baseline I). The second phase involved the addition of the enzymes to the process and data collection. The final phase was simply a repeat of the first phase after the enzymes were cycled out of the process (Baseline II). It is important to understand that the time at which the enzymes are cycled out of the process is not the same

as the time that the last enzyme-treated fermentor is emptied out. The mash in the fermentor takes about 1.5 hr to enter and exit the beer well and another 0.5 to 1 hr to reach the centrifuge. So for purposes of this plant trial the beginning of the enzyme addition part of the trial is taken to be 2.5 hr after the first enzyme-treated fermentor is emptied. Furthermore, the end of the enzyme addition part of the trial is defined as 2.5 hr after the last enzyme-treated fermentor is emptied. The last phase of the trial, Baseline II, was primarily carried out to show that the data after enzyme removal would be similar to that of the first phase.

Genencor®, a Danisco Division, is the company that produces and commercializes the enzymes that were found in earlier laboratory experiments to be the most effective at removing water and shortening fermentation times. The company agreed to provide the amount of enzyme necessary to carry out the plant trial discussed above.

6.3.1 Baseline Data

Baseline data was collected for five complete fermentations prior to enzyme addition, and for three complete fermentations after enzyme addition was concluded. A complete fermentation is defined as the time at which the fermentor begins to fill to the time at which the fermentor is emptied. Lab data and process data were collected in an identical manner for the baseline and enzyme addition portions of the trial.

During baseline data collection, the Beer Well fill level was reduced to less than 50% (37% is the minimum level for this tank without level alarms being activated). This reduction allowed for a minimization of the volume of enzyme treated beer needed to flush out the downstream processing steps and tanks. Since the downstream tanks are significantly smaller than the beer well, it was not necessary to make changes to the other tank fill levels.

6.3.2 Enzyme Addition Phase

The estimated dose for maximal dewatering effect based on laboratory results was 0.75 kg/MT of corn. For purposes of this plant trial, a slight overdosage was decided upon to ensure that the results observed in the lab could indeed be seen at plant scale. During the enzyme addition phase of the trial, 1.0 kg/MT of corn was used. Genencor® provided 4400 kg of a special preparation that had enzymatic activity identical to the preparation used in the lab. This amount of enzyme was sufficient to treat 5 fermentors with the enzyme at the 1.0 kg/MT of corn dosage level. The enzyme was added to the fermentors through the yeast propagation tank in one dose. This was done during the first half of the fermentor filling to maximize the enzyme reaction time and mixing.

6.3.3 Analytical Tests

The following list shows the analytical tests that were performed in the QC lab of the plant:

1. Spin test on whole stillage
2. Spin test on thin stillage
3. WDGs moisture content
4. HPLC mash composition
5. Total solids of whole stillage
6. Total solids of thin stillage
7. Corn moisture
8. % solids of pre-fermentation mash

All analytical tests, unless otherwise noted, were performed 8 times per day at 3 hour intervals. The standard operating procedures (SOPs) for each test can be found in Appendix C. Daily samples of DDGS were also taken for subsequent compositional analysis (Appendix D).

6.3.4 Process Data Collection

In order to better understand how the enzyme addition affects centrifuge operation and therefore water removal, process data was collected from the centrifuge control panel. Certain centrifuge parameters, such as bowl speed, pinion speed, and differential speed, are

known to affect centrifuge performance and will change when there are process changes (McCabe et al. 2001). Therefore, during the plant trial, the three speeds mentioned above were recorded from the control panel of each of the four centrifuges. The data was recorded as close to every 3 hours as possible.

6.3.4.1 Mass Balance

In order to calculate how much water was coming off the centrifuges in the plant before and during the enzyme addition, a mass balance was performed around those unit operations. Figure 6.1 shows how the centrifugation step of the process was setup at Center Ethanol.

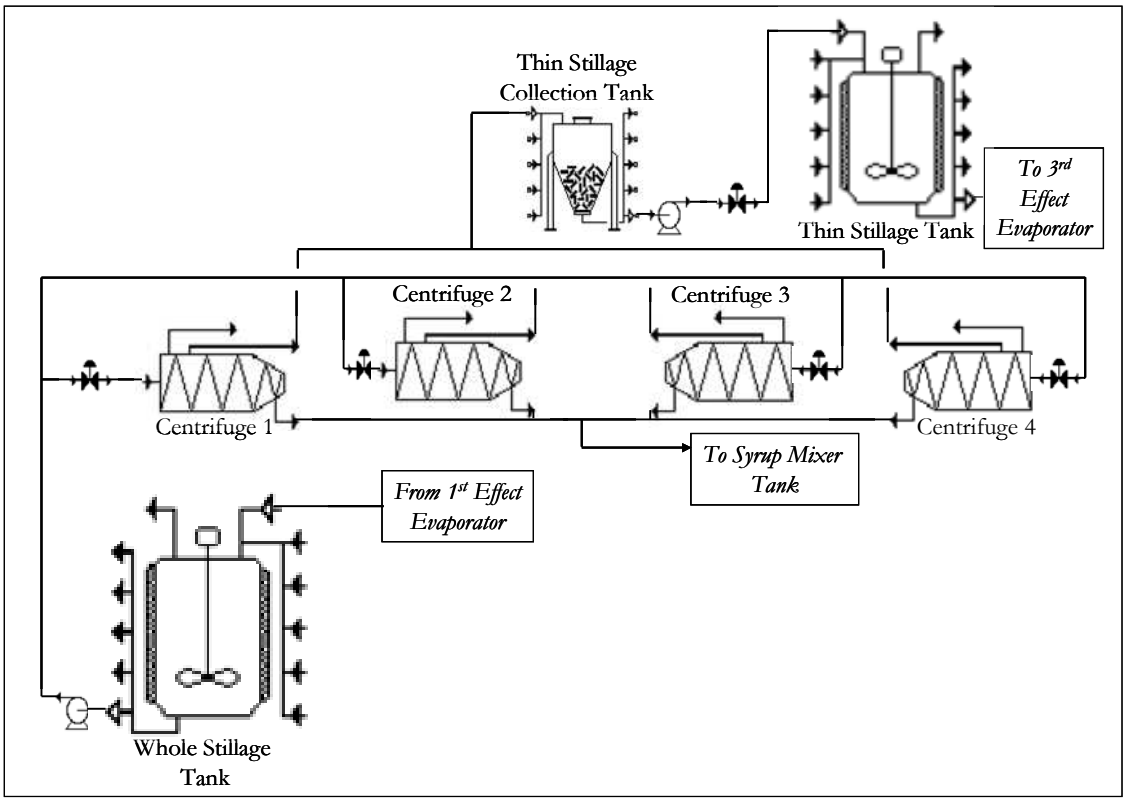

Figure 6.1: Process flow diagram of the centrifugation process at Center Ethanol.

The best way to evaluate the amount of water coming off of the four centrifuges was to perform a mass balance around the centrifuges. There were no mass flow meters present at the outlet of each centrifuge so it was not possible to perform a mass balance at each centrifuge. Since the four streams coming off the centrifuges met before they went into the Thin Stillage Collection Tank, it was easier to calculate the total flow entering the tank. Furthermore, according to the process control scheme of the plant, the Thin Stillage Collection Tank had a level controller that kept the level of the tank at a setpoint of 50%. The process control scheme can be seen in Figure 6.2.

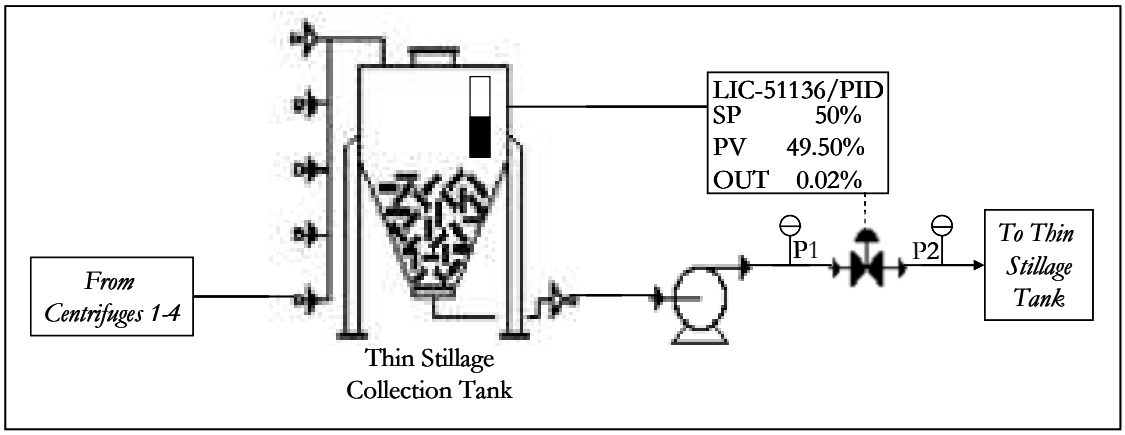

Figure 6.2: Process and instrumentation diagram for the Thin Stillage Collection Tank unit operation.

In order for the level of the tank to stay at the desired 50%, the valve at the outlet of the tank had to open or close to allow for more or less thin stillage to exit the tank. The level controller (LIC-51136) read the tank level and sent a message to the valve, telling it to open or close. For purposes of the plant trial, it was assumed that the amount of thin stillage entering the tank was going to increase due to the enhanced water removal caused by the enzymes. Under this assumption, the level of the tank would increase and the valve would then correct that by increasing its percent open position to allow for a higher outlet flow and to maintain the level at the setpoint. At the same time, to keep the level of the tank constant at the desired setpoint, the inlet flowrate must equal to the outlet flowrate. By calculating the outlet flowrate, then the inlet flowrate would be known. Since there was no mass flow meter at the exit of the tank, the equation for the flow across a valve (Equation 6.1) was used.

$$Q = Cv \times \sqrt{\frac{\Delta P}{G}} \qquad (6.1)$$

Here, Q is the flowrate (gpm), Cv is the valve flow coefficient, ΔP is the pressure drop across the valve (psi), and G is the specific gravity of the liquid going through the valve. The specific gravity is defined as the ratio of the liquid density (lb/in^3) going through the valve to the density of water (lb/in^3), and is therefore dimensionless.

The Cv is dependent on how much the valve is open, or more specifically the percent opening of the valve. In Figure 6.2 the percent opening is given as the OUT variable in the level control loop (LIC-51136) box. The percent opening data was recorded and stored by the Digital Control System (DCS) of the plant. The valve's flow coefficient was obtained from the valve's manufacturer. In the valve's manual, a data table (Table 6.1) is given that relates the Cv to the percent opening of the valve.

Table 6.1: Valve's flow coefficients given for specific percent openings of valve. In this case the valve's size is 6" and the port is 0.4 *Cv* reduced port.

TABLE 7 – FLOW COEFFICIENTS C_V – STANDARD TRIM REVERSE FLOW DIRECTION

Valve Size	Port	Percent of Plug Open										
		Min.	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
1" (DN25)	Full Port	0.1	1.4	2.8	4.2	5.6	7.0	8.4	9.8	11.2	12.6	**14.0**
	0.6 Cv Reduced Port	0.1	1.1	1.8	2.7	3.6	4.6	5.5	6.4	7.3	8.2	**9.1**
	0.4 Cv Reduced Port	0.1	.8	1.1	1.7	2.2	2.8	3.3	3.9	4.4	5.0	**5.5**
	0.2 Cv Reduced Port	0.1	.6	.6	.9	1.2	1.6	1.9	2.2	2.5	2.8	**3.1**
1-1/2" (DN40)	Full Port	0.2	2.5	5.0	7.5	10.0	12.5	15.0	17.5	20.0	22.5	**25.0**
	0.6 Cv Reduced Port	0.2	2.0	3.4	5.0	6.7	8.4	10.1	11.8	13.4	15.1	**16.8**
	0.4 Cv Reduced Port	0.2	1.9	2.5	3.7	5.0	6.2	7.4	8.7	9.9	11.2	**12.4**
	0.2 Cv Reduced Port	0.2	.9	1.0	1.6	2.1	2.6	3.1	3.6	4.2	4.7	**5.2**
2" (DN50)	Full Port	0.6	7.1	14	21	28	36	43	50	57	64	**71**
	0.6 Cv Reduced Port	0.6	5.0	8.4	13	17	21	25	29	34	38	**42**
	0.4 Cv Reduced Port	0.6	4.4	5.8	8.7	12	15	17	20	23	26	**29**
	0.2 Cv Reduced Port	0.6	2.5	2.8	4.2	5.6	7.0	8.4	9.8	11.2	12.6	**14.0**
3" (DN80)	Full Port	1.3	14	30	45	62	77	91	105	121	142	**166**
	0.6 Cv Reduced Port	1.3	10	19	30	39	47	57	67	78	87	**97**
	0.4 Cv Reduced Port	1.3	10	19	27	32	38	43	49	55	60	**66**
	0.2 Cv Reduced Port	1.3	6.9	13	17	20	24	26	28	30	32	**33**
4" (DN100)	Full Port	2.5	29	59	90	113	136	160	184	212	255	**310**
	0.6 Cv Reduced Port	2.6	17	33	53	72	97	112	130	151	167	**195**
	0.4 Cv Reduced Port	2.5	13	27	42	54	65	76	88	104	113	**124**
	0.2 Cv Reduced Port	2.6	11	21	29	36	41	46	51	57	59	**64**
6" & 8" (DN150 & 200)	Full Port	5.2	35	88	146	207	276	330	393	460	569	**648**
	0.6 Cv Reduced Port	5.9	33	67	105	145	183	225	279	341	402	**442**
	0.4 Cv Reduced Port	5.6	28	55	84	114	136	156	189	219	256	**282**
	0.2 Cv Reduced Port	5.3	16	31	47	63	74	88	102	116	127	**132**

NOTE: See Graph No. 2 for corresponding F_L factors.

Using the information given for the 6", 0.4*Cv* Reduced Port, the following graph (Figure 6.3) was created to show the linear relationship between *Cv* and percent opening.

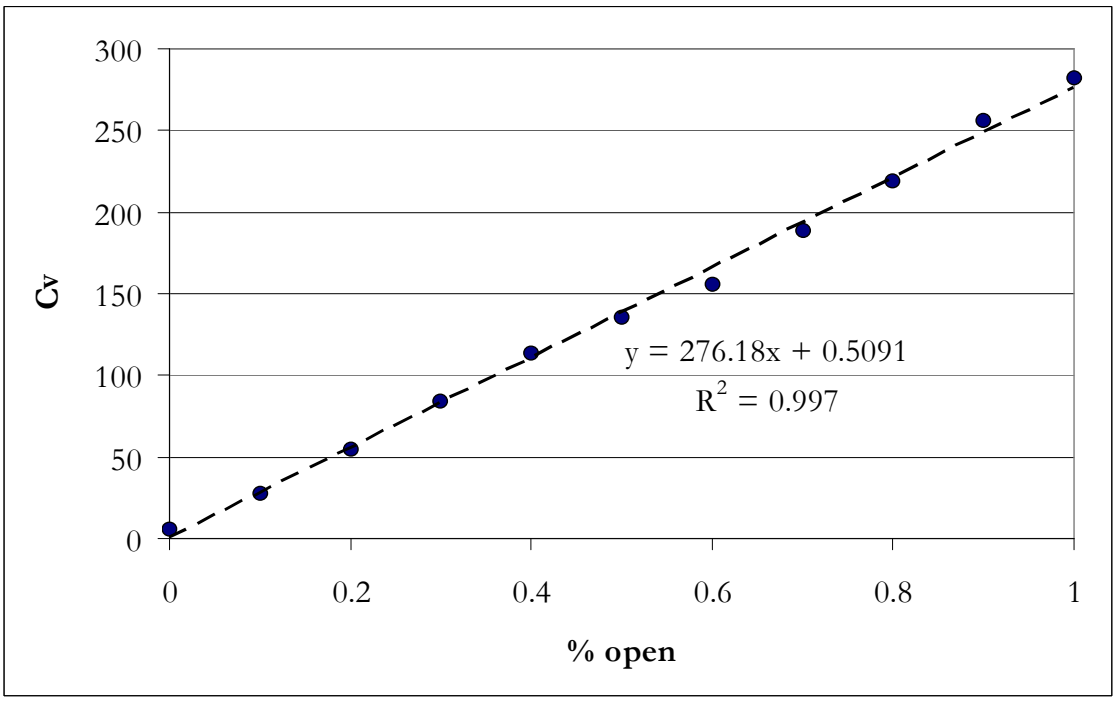

Figure 6.3: Graph of data obtained from valve's manual. *Cv* as a function of the valve's %opening. Linear trend shown by dashed line. Equation for fitted linear trend and R^2 value shown on graph.

On the graph a linear trend is fitted to the data (Equation 6.2) and its R^2 value is obtained. The equation is given as,

$$Cv = 276.18 \times (\%open) + 0.5091 \qquad (6.2)$$

Using this equation, it was possible to calculate the *Cv* given a specific *%open* value.

The pressure drop across the valve was calculated by measuring the pressure at the valve's inlet (P1) and the pressure at the outlet (P2) and subtracting the two numbers. These

pressures were measured by two pressure gauges that were installed at the inlet and outlet of the valve. Both pressures were recorded throughout the trial in three hour intervals.

The specific gravity of the thin stillage was a constant and was estimated to be 0.975 based on the plant's valve sizing documentation. Using the information above and Equation 6.1, the valve's flowrate (Q) was calculated. Since the DCS records the percent opening value every second, a Matlab® program (Appendix E) was developed to automatically take the percent opening data from the DCS and calculate the valve's flowrate creating a graph that shows the flowrate versus time.

6.3.4.2 Process Parameters

Besides the data mentioned above, a set of process parameters were collected from the DCS of the plant to ensure that some of the operating conditions were optimal for enzyme activation as well as to see how they related to the experimental conditions. Some of the parameters were also collected to be used in the economic analysis described in Chapter 7. Table 6.2 shows the parameters that were recorded.

Table 6.2: Parameters that were collected during the plant trial. Parameters have been divided into 4 main areas of the ethanol process. Actual values recorded for the listed parameters can be found in Appendix F.

Area of Ethanol Plant	Parameter
Grain Handling and Milling	1.amount of corn 2.amount of water
Starch to Sugar Conversion	1.amount of ammonia 2.amount of α-amylase 3.amount of gluco-amylase 4.amount of sulfuric acid 5.amount of water
Fermentation	1. fermentor volume 2. fermentor temperature 3. fermentor pH 4. fermentor pressure 5. fermentor residence time 6. fermentor inlet mash flowrate 7. amount of yeast 8. fermentor outlet flowrate 9. ethanol production rate
Co-Product Processing	1. centrifuge inlet flowrates 2. centrifuge residence time 3. centrifuge liquid flowrates 4. centrifuge solid flowrates 5. evaporator inlet flowrates 6. evaporator recycle flowrates 7. dryer inlet flowrate 8. dryer temperature 9. dryer residence time 10. dryer outlet flowrate 11. dryer energy input 12. DDGS production rate

6.3.4.3 Statistical Analysis

For all the data presented in this chapter, a *t*-test, assuming equal variances, was used to compare the means from the two baseline periods of the trial to the enzyme addition data

(SPSS for Windows, Chicago, IL). The level selected to show statistical significance was 5% ($P < 0.05$).

6.4 Results and Discussion

6.4.1 QC Lab Data

Samples were taken from each fermentor just before the fermentors were emptied and the fermented mash was sent to the beerwell. Each sample was analyzed by HPLC according to the SOP in Appendix A. Table 6.3 details the results from the HPLC compositional analysis.

Table 6.3: Average percentage of ethanol and glucose found in the samples collected at the end of each fermentation for the different trial periods, values followed by the same letter on the same row are not significantly different ($P < 0.05$)

Compound (%w/v)	Period of Plant Trial[1]	
	Baseline	Enzyme
Ethanol	13.76 ± 0.41a	14.03 ± 0.16a
Glucose	0.0751 ± 0.017a	0.0774 ± 0.016a
DP4+	0.574 ± 0.025a	0.835 ± 0.014b

[1] Average ± standard deviation

Ethanol, glucose, and DP4+ were the main compounds examined during the plant trial. It was important to see whether the ethanol and glucose concentrations were different during the different phases of the trial. There were no statistically significant differences in the final

ethanol yields and glucose counts between the baseline and enzyme addition parts of the trial. This means that the addition of the enzymes did not affect the ethanol production and glucose to ethanol conversion went unchanged during fermentation. The glucose concentration for the enzyme addition part of the trial was, on average, 0.0774 (± 0.016) % v/v. Glucose concentrations of less than 1% w/v are well within the range accepted by the plant's quality control guidelines. Higher dextrins are usually called DP4+ but DP4+ is more than that. They can be defined as any component that is soluble and dilutes in the first part of the HPLC gel filtration column. In a plant, if the DP4+ value is too high it could mean that the dextrins are not being hydrolyzed into glucose. This would result in a decrease in ethanol yield. In the case of the enzyme addition part of the trial, the DP4+ was higher than the baseline value, $t(13) = 1.77$. However, since the ethanol yields were not significantly different between the two parts of the trial, this increase in DP4+ can be attributed to the hydrolysis of cellulose, hemi-cellulose and xylan by the cell wall degrading enzymes.

Moisture content analysis was also performed on different samples collected throughout the plant trial. The analysis followed the SOP found in Appendix A. Samples from the whole stillage and thin stillage tanks were collected daily, along with WDGs samples from the centrifuges and samples from the syrup mixer tank (Figure 6.1). The data presented in Table 6.4 reflect the average dry matter content for the samples during each part of the trial.

Table 6.4: Average dry content from the whole stillage, thin stillage, wet cake and wet cake plus syrup samples collected during the plant trial; values followed by the same letter on the same row are significantly different ($P < 0.05$)

Dry Content (% /w)	Period of Plant Trial[1]	
	Baseline	Enzyme
Whole Stillage	17.36 ± 0.55a	17.73 ± 0.79b
Thin Stillage	10.67 ± 0.45a	11.76 ± 0.55b
Wet Cake	34.50 ± 0.70a	36.19 ± 0.56b
Wet Cake + Syrup	34.65 ± 0.71a	36.66 ± 0.69b

[1] Average ± standard deviation

All samples, except for the whole stillage, were significantly different when comparing the means from the baseline to the enzyme addition portion of the trial. The differences for thin stillage, wet cake and wet cake plus syrup were $t(115) = -10.14$, $t(92) = -10.68$ and $t(99) = -12.22$, respectively. During the enzyme addition part of the trial, the whole stillage had, on average, 82.27% by weight moisture content compared to 82.64% for the baseline samples. For the results obtained from the wet cake and wet cake plus syrup samples, the average moisture content was smaller for the enzyme addition part of the trial compared to the baseline. On average the samples had 63.81 and 63.34 %/w moisture for the wet cake and wet cake plus syrup, respectively, during the enzyme addition part. The baseline had average moisture content of 65.5 and 65.35 %/w for the wet cake and wet cake plus syrup, respectively. That means the reduction in moisture content was approximately 2% when the plant was running with enzymes. This difference is analogous to the results observed from the centrifuge mass balance presented later in Section 6.4.5.

6.4.2 Drier Firing Rate

The drier firing rate is defined as the percent open of the natural gas supply valve that provides natural gas to the DDGS drier during its operation. Corn to ethanol plants monitor the firing rate, in addition to temperature, in order to determine whether the drier is working properly, and to make sure that there are no fire hazards. Also, it helps to ensure that the distillers grains are drying evenly. The firing rate also gives an instant indication of how much natural gas is being consumed by the drier. The amount of natural gas being consumed depends on how moist the incoming distillers grains are compared to how moist they need to be when they exit the drier. If the grains are too moist it will take a higher temperature, or more natural gas, to drive the water off and bring the moisture content down to the desired 8 to 11%/w.

The plant's DCS automatically keeps track and records the firing rate of the drier. Figure 6.4 shows the data obtained from the records of the DCS of the plant.

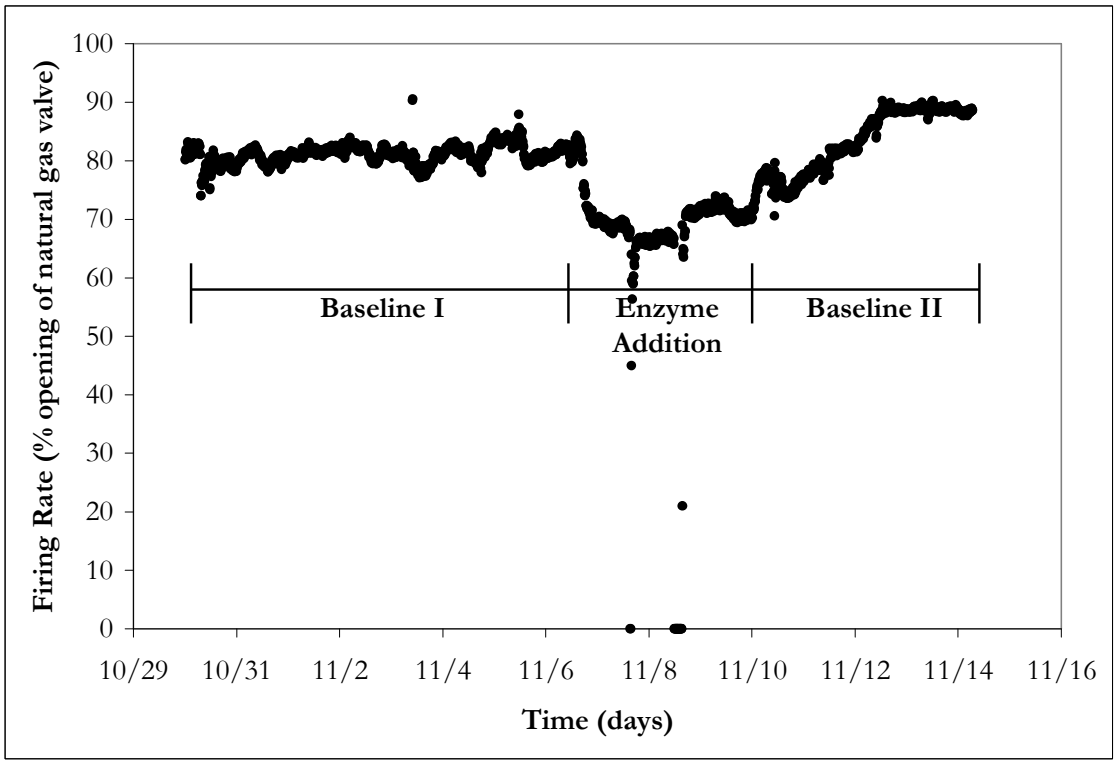

Figure 6.4: Firing rate of the drier given as the percent opening of the natural gas valve to the drier. Data that shows a percent opening of zero corresponds to a plant shutdown due to a mechanical problem on a conveyor belt.

The percent opening of the natural gas valve is directly proportional to the natural gas usage. As the percent opening decreases so does the amount of natural gas being used by the drier. As it can be seen in the figure, during the enzyme addition part of the plant trial the percent opening decreased compared to the two baseline periods. In fact, when a statistical analysis is done on the data, the average percent opening for the enzyme addition period was statistically different from the average percent opening of the baselines, $t(1994) = -22.39$. On average the valve was 70.87 ($\pm$ 3.31) % open during the enzyme addition compared to 82.41 ($\pm$ 3.61) % open during the baselines. That is a 14% reduction in the valve percent opening

and will results in a significant decrease in the amount of natural gas used by the plant as described in Section 6.4.2.

It is important to point out that during the enzyme addition part of the plant trial, on the afternoon of November 7th, the control system of the plant shutdown the drier because the controller that monitors the heat source of the drier, known as fire-eye, stopped functioning. Without this controller, the safety interlocks automatically go into effect to prevent a fire from happening. Furthermore, late afternoon on November 8th, the plant had to be shutdown due to a malfunction of the conveyor belt taking the WDGs from the centrifuges over to the drier. These shutdowns explain why the valve opening suddenly drops to zero twice during the enzyme addition period.

6.4.3 Natural Gas Usage

The next parameter that is closely monitored in a corn to ethanol plant is the amount of natural gas being used by the unit operations. It is important to record this parameter in order to assess the plant's energy balance in an attempt to optimize it. This parameter is also monitored and recorded by the DCS of the plant. In the DCS there is a totalizer that calculates how much natural gas has been used by the plant every 24 hours. This 24 hr period begins at 6 am and ends at 6 am the following day. Figure 6.5 shows the totalizer data for the baselines and enzyme addition periods.

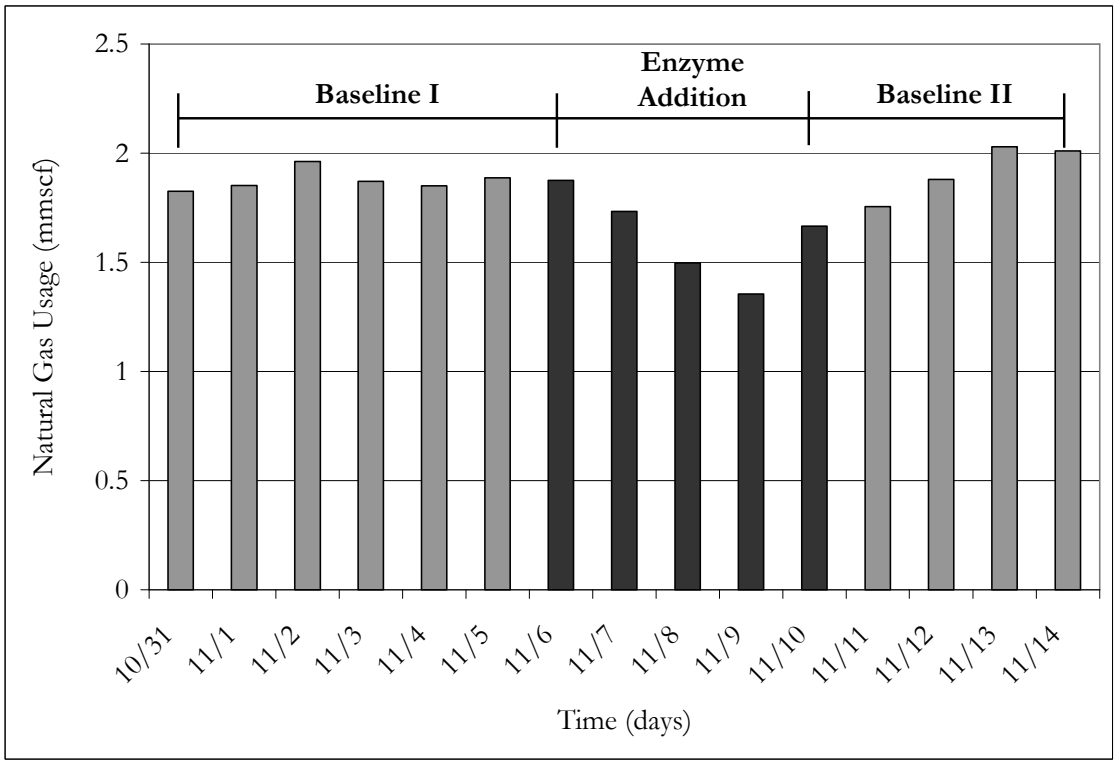

Figure 6.5: Graph shows natural gas used by the plant on a daily basis during the trial; black bars denote days in which plant was running with enzymes

The amount of natural gas is given in millions of standard cubic feet. As can be observed from the graph, during the enzyme addition period there was a decrease in the overall natural gas usage of the plant. When comparing the mean average natural gas usage for each plant trial period there is a statistically significant difference seen in the data. On average, the plant used 1.89 (± 0.11) mmscf of natural gas during the baseline periods compared to 1.62 (± 0.23) mmscf used during the enzyme addition period. That amounts to a 14.11% reduction in the amount of natural gas used by the plant when the plant was running with the enzymes. Since there were two short shutdowns during the enzyme addition part of the trial, it can be argued that some of the reduction seen in natural usage can be attributed to the plant's

shutdown; therefore, in Section 6.4.4, results will be shown for the natural gas usage on a gallon of ethanol produced basis. When the plant shuts down less natural gas is used but at the same time less ethanol is produced.

6.4.4 Production of Ethanol and DDGS

It was important to make sure that during the plant trial the ethanol production remained the same when the enzymes were added to the process, when comparing it to the production during the baseline periods. The volume of ethanol produced on a daily basis can be seen on Figure 6.6.

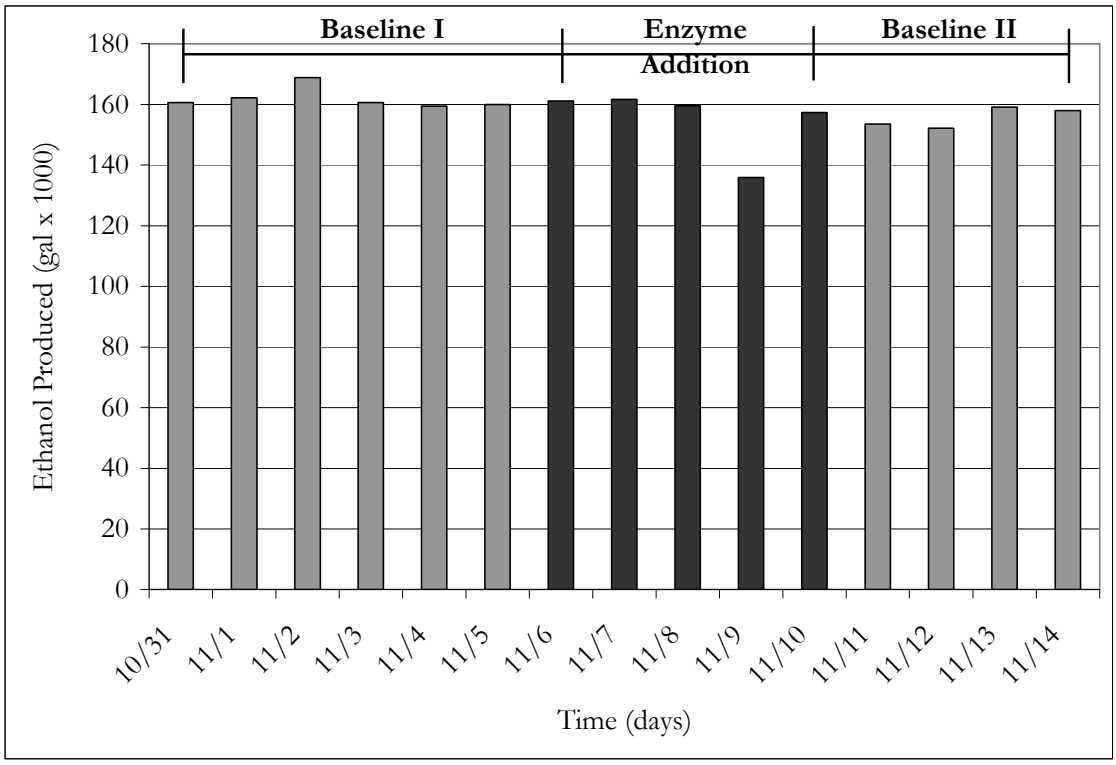

Figure 6.6: Daily ethanol production for baseline and enzyme addition periods of the plant trial; ethanol production given in 1000 x gallons; black bars denote days in which plant was running with enzymes

The data presented above was collected from the DCS of the plant. A totalizer recorded the amount of ethanol produced every 24 hrs so the time chosen to signal the enzyme addition is approximate. This approximation was adjusted to reflect as close as possible the time at which the enzyme treated mash reached the centrifuge. It can be clearly seen in the graph that during the enzyme addition part of the trial there was a slight decrease in production on November 9th. This decrease was due to the plant shutdown that occurred late afternoon on November 8th. When the *t*-test was performed on the data presented on the graph, there was no statistically significant difference between the volumes of ethanol produced during the

enzyme addition period compared to the baseline periods. On average, 155,124 (± 4,582) gal of ethanol were produced during the enzyme addition part of the trial compared to 159,468 (± 10,875) gal of ethanol produced during the baselines. It can be said that there were no changes to the plant's ethanol production when the enzymes were added to the process, which is consistent with lab results obtained and described in Chapter 2.

In order to better compare the volume of ethanol produced and natural gas used, a graph was created to show the ratio of natural gas used to gallon of ethanol produced (Figure 6.7).

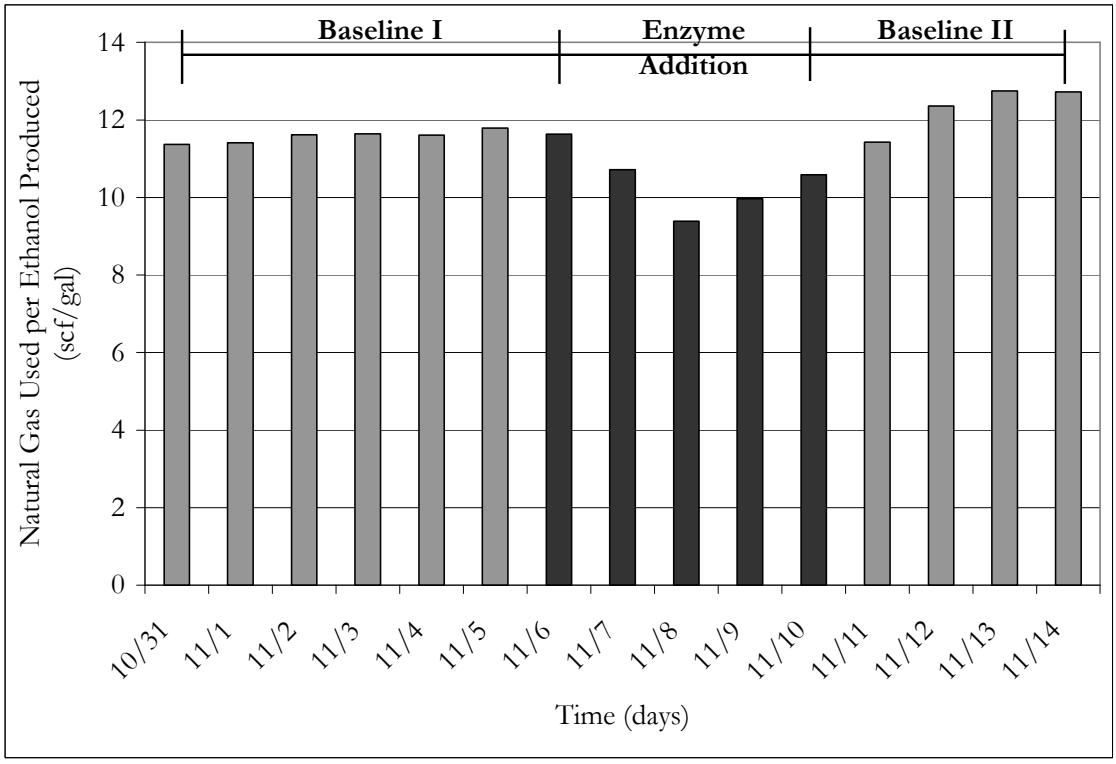

Figure 6.7: Ratio of natural gas used by the plant per gallon of ethanol produced during the plant trial; black bars denote period in which enzyme was added to the process

The graph shows the three phases of the plant trial. The black bars show the volume of natural gas used per gallon of ethanol produced during the enzyme addition part of the trial. Statistically there is a significant difference between the mean natural gas to ethanol ratio for the enzyme addition period compared to the mean of the baselines. On average the volume of natural gas used per gallon of ethanol produced for the enzyme addition period was 10.46 (± 0.85) scf of natural gas/gal of ethanol while for the baselines the mean was 11.87 (± 0.54). This means that when the plant was running with the enzymes less natural gas was required to produce one gallon of ethanol. This will result in energy savings for the plant as explained in Chapter 7.

Center Ethanol does not keep track of the amount of DDGS produced during its operation. A rule of thumb is used by the plant manager that says that 30% of the ethanol production is equivalent to the number in tons of DDGS produced. Since the ethanol production was not affected by the addition of the enzyme, it was assumed that there was also no change to the production of DDGS. Furthermore, the plant's lead engineer did not observe any changes in the amount of distillers grains going through the drier and into the DDGS storage mound.

6.4.5 Mass Balance

A mass balance was performed around the centrifuges and the TS collection tank to better understand how the addition of the enzymes affected the flowrates in that area. The mass

balance was set up so that the four centrifuges were seen as just one unit operation, (1) in Figure 6.8.

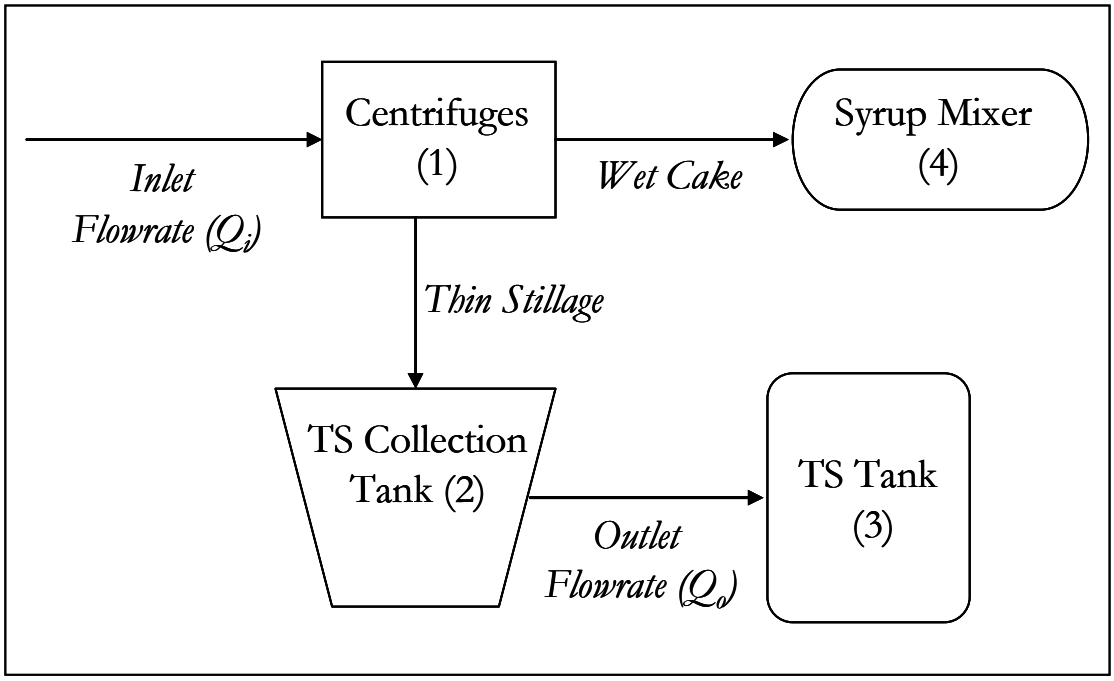

Figure 6.8: Ratio of natural gas used by the plant per gallon of ethanol produced during the plant trial; black bars denote period in which enzyme was added to the process

The flowrate at the inlet of (1), Q_i, is the summation of the flowrates of the four individual centrifuges. This combined flowrate was calculated by a totalizer in the DCS of the plant and recorded in its historian file (Figure 6.9).

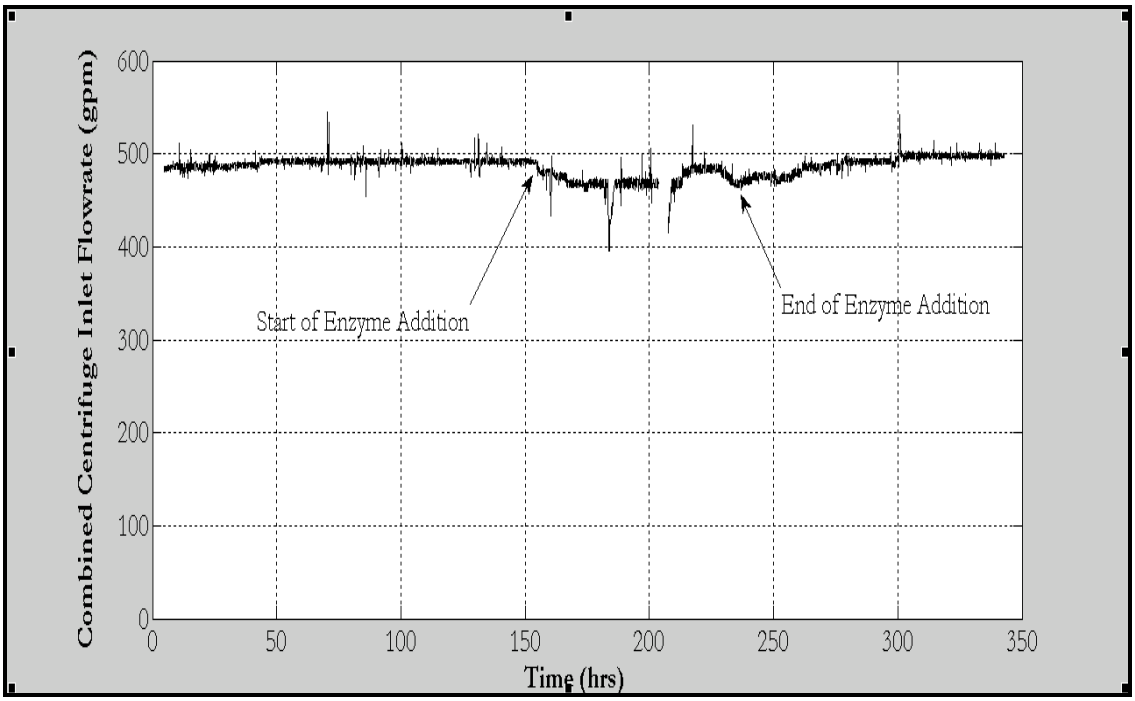

Figure 6.9: Centrifuges' inlet flowrate over time; flowrate given as summation of the inlet of the four individual centrifuges

Approximately two hours after the beerwell containing whole stillage treated with the enzymes started to be emptied, the DCS started to record a 4% decrease in the flowrate to the centrifuges. It is believed that this decrease in flowrate is a result of Center Ethanol's process design. At this plant the whole stillage leaves the beerwell and goes into a first effect evaporator before it enters the centrifuges. If the theory is correct, the enzymes have changed the structural and bonding nature of the polysaccharides present in the slurry, thus allowing for more water to be driven off as it goes through the first effect evaporator. Then, when the slurry gets to the centrifuges, it has already been dewatered by about 4%/v. The amount of solids entering the centrifuges did not change during the different portions of the trial. The solids flowrate remained at about 84 gpm during the baseline and enzyme addition

portions of the trial, which showed that no solid material was lost as the amount of evaporated water increased with the enzyme addition. The small increase in flowrate after it goes to zero during the shutdown was due to the restart of the process and the flushing of the downstream pipelines.

The flowrate at the outlet of (2), Q_o, was calculated as described in Section 6.3.4.1. This flowrate is equivalent to the flowrate called Thin Stillage at the inlet of (2). The wet cake flowrate calculation was performed by subtracting Q_o from Q_i. The valve, located at the outlet of (2), percent open data was also collected from the DCS' historian and is shown in Figure 6.10.

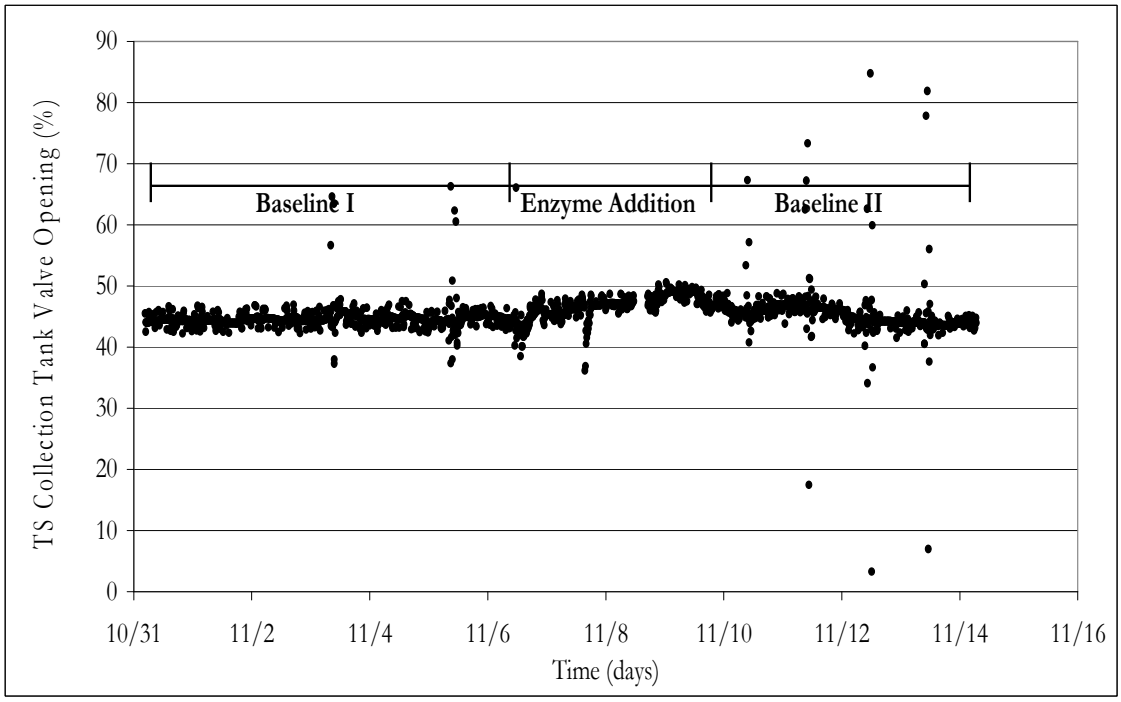

Figure 6.10: TS collection tank outlet valve %open values versus time; data obtained from the DCS' historian

The valve percent open value fluctuates somewhat during periods when the plant is in an unsteady state. However, an increase in the percent open value can be observed during the enzyme addition part of the trial. In fact, when a *t*-test is performed on the data the average percent open value for the baselines, 44.74 (±2.07) %, is statistically different from the average of the enzyme addition part, 47.09 (±1.45) %. This represents a 5.25 % increase in the valve opening. Using this data in Equations 6.1 and 6.2, the average pressure drop across the valve, 35 psig at P1 and 28 psig at P2, and a specific gravity of 0.975, the Matlab® program was used to calculate Q_o over time (Figure 6.11).

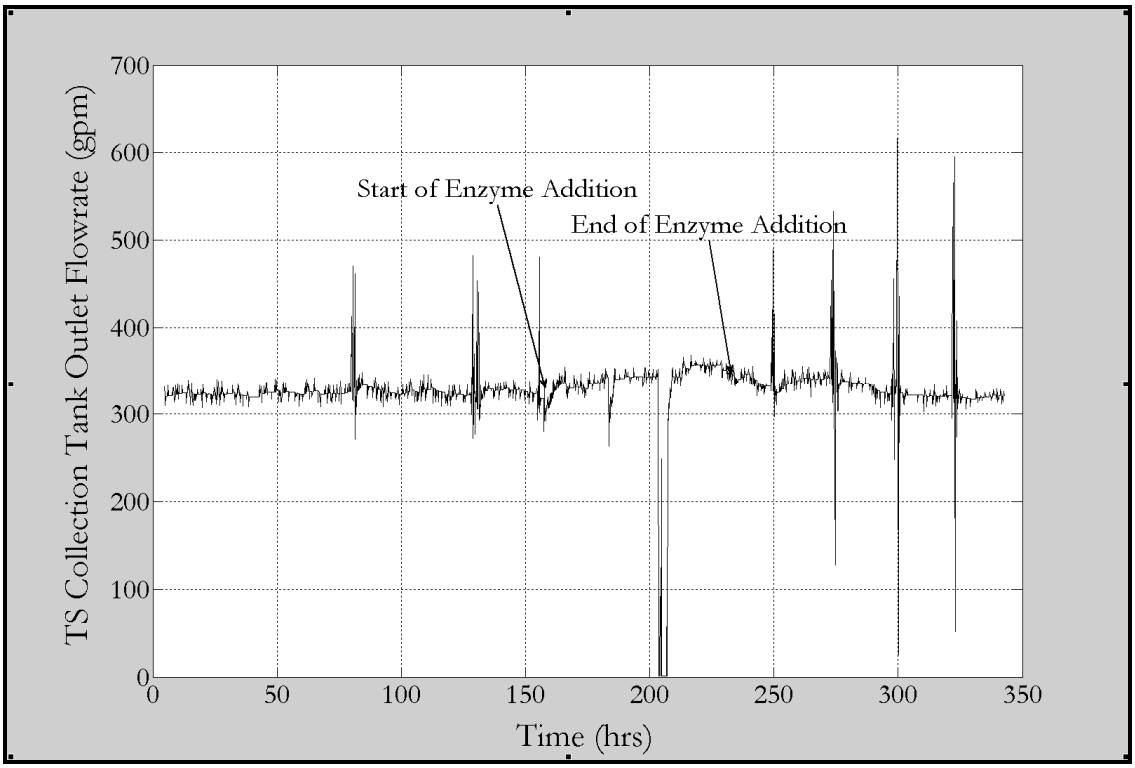

Figure 6.11: Calculated TS collection tank outlet flowrate; data goes to zero when plant is shutdown

The graph shows an increase in the flowrate during the enzyme addition part of the trial which correlates to the increase in the percent open value of the thin stillage valve. The average flowrates for the baselines and enzyme addition parts of the trial, 327.61 gpm and 337.55 gpm, respectively, were shown to be significantly different. This corresponds to a 3% increase in the amount of water removed during centrifugation. Average flowrates were calculated without including the data from the periods in which the plant was shutdown. This number may not be the actual increase for two main reasons: (1) the fluctuations in the data seen as large spikes on the graph due to the periods during which the process was in an unsteady state, and (2) the decrease in centrifuge inlet flowrate described above.

6.4.6 DDGS Composition Profile

Another important aspect of the plant trial was to ensure that the DDGS composition remained the same during the enzyme addition and baseline parts of the trial. It was important to make sure that the enzymes did not alter the composition of the DDGS since its nutritional value is very important to the ruminant's diet and its monetary value as a co-product as explained in Chapter 1. Table 6.5 shows the DDGS composition during the baselines and enzyme addition portions of the trial.

Table 6.5: Data shows the compositional analysis of the DDGS samples collected daily during the baseline and enzyme addition periods; there were no significant differences for the values in the same row ($P < 0.05$)

DDGS Composition[1]	Plant Trial Period	
(% w/w)	Baseline	Enzyme
Moisture	11.00 ± 0.37	10.71 ± 0.63
Ash	4.97 ± 0.17	5.03 ± 0.05
Protein	29.80 ± 0.74	29.99 ± 0.69
Starch	5.63 ± 0.39	5.12 ± 0.58
NDF[2]	31.43 ± 2.89	32.32 ± 1.38
NDICP[3]	12.30 ± 1.09	11.67 ± 2.40
OIL	10.72 ± 0.51	10.97 ± 0.35
NFC[4]	35.38 ± 3.41	33.36 ± 1.68

[1] Average ± standard deviation

[2] Neutral detergent fiber

[3] Neutral detergent insoluble nitrogen expressed as crude protein

[4] Non-fiber carbohydrate

No significant changes were observed in the composition of the DDGS when the enzyme was present in the process. After *t*-tests were performed on the data, there were no statistical differences found between the DDGS components before and during the enzyme addition. NDF is highly digestible and provides a good source of energy for the ruminant, whereas NFC in quantities greater than 40%/w can be harmful to the ruminant and cause ruminal acidosis (Hippen and Garcia 2007; Kaiser 2005). Ruminal acidosis is a condition in which the base component of the ruminant's bodily fluids is in excess when compared to its acid component and it can cause lethargy, diarrhea and anorexia (Owens 1998). The starch content remained the same during the periods of the trial, which means that the starch to glucose conversion in the fermentor was not affected by the addition of the enzymes. All other DDGS components remained in the range previously reported in the literature as well as within the range suggested for ruminant diet (Belyea et al. 2004; Todd et al. 2006; Kleinschmit et al. 2007; Liu 2008; Singh et al. 2005). The use of these enzymes in a dry grind corn to ethanol plant will not affect the nutritional quality of the DDGS, but could potentially increase the digestibility of the feed by limiting the breakdown of the amino acids found in the protein via a reduction in temperature of the heat source of the drier.

6.4.7 Centrifuge Modeling

Decanter centrifuges (Figure 6.12) have been widely used in the agricultural, food, and mineral sectors for the dewatering of slurries (Leung 2002).

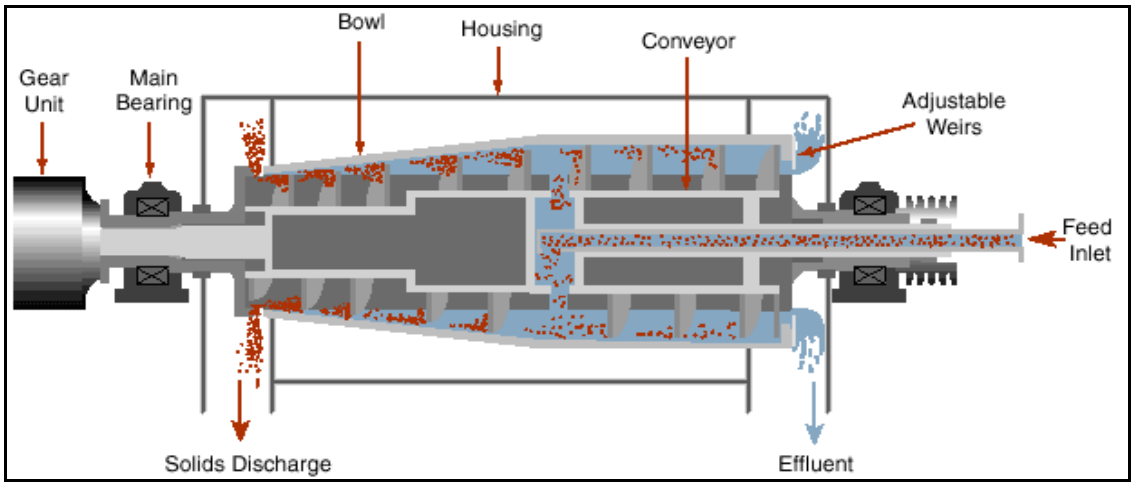

Figure 6.12: Schematic diagram of a decanter centrifuge

Frequently, decanter centrifuges are preferred over other continuous sedimenting centrifuges, mainly disk centrifuges and disk-stack decanter centrifuges, because of their ability to separate a broader range of particle sizes and both dilute and concentrated slurries (Leung 1998). The decanter centrifuge is able to separate small particles, greater than 0.02 mm, while other centrifuges, like the screw press centrifuge, are not capable (Moller et al. 2002). Predicting centrifuge performance based on experimental data is very challenging and frequently requires significant experience in centrifuge operation, since scaling up can not be performed only on a correlation basis (Beiser et al. 2000).

In a decanter centrifuge, the slurry is introduced into the centrifuge through a stationary feed pipe (feed inlet) in the hub of the conveyor where it is accelerated before being fed into the cylindrical section of the bowl. In this section of the bowl, the solids are separated from the liquid as a result of differences in specific gravity coupled with the centrifugal force being

generated by the rotation of the bowl. The heavier solids are forced to the bowl wall while the lighter liquid forms a layer above the solids. The height of this layer is regulated with adjustable weirs at the large end of the centrifuge. The liquid flows toward the large end, allowing the finer particles sufficient time to settle, and is then discharged over these weirs. The solids that have settled against the bowl wall are conveyed in the opposite direction by the scroll conveyor. The conveyor moves the solids toward the conical or tapered section of the bowl, where further dewatering of the settled solids takes place. Solids are conveyed out of the liquid pool onto a beach area where residual liquid is drained away prior to the discharge of the solids out of the bowl. A differential speed between the bowl and conveyor is accomplished by a planetary gear unit or variable speed drive system. The pitch of the conveyor and differential speed between the bowl and conveyor set the retention time of the solids within the bowl (Leung 1998; McCabe 2001).

Usually the plant will set a target wet cake moisture exiting the centrifuge and vary its torque setting to meet that target set point. Wet cake moisture readings are collected on a regular basis and the torque is adjusted accordingly depending on how far off the moisture is compared to the desired value. The plant uses four Alfa Laval decanter centrifuges, type CHNX944B-31G, which are designed with a torque specification of 12 kN-m. However, during the plant trial the control system of the centrifuges was changed, the centrifuges' load, or torque, was set to 25% of the rated value which results in a load of 3 kN-m, or 2212.69 lb-ft, and the cake moisture setting was left variable. This was done so that any changes in the cake moisture as a result of the enzymes being added to the process would be

noticed and quantified in the lab. This modified control system was implemented before data started to be collected during the baseline part of the trial.

In a decanter centrifuge the torque provides the necessary acceleration to maintain the centrifuge at a certain speed regardless of any resistance that it may encounter. The differential speed at which the centrifuge operates at allows for the particulates to be conveyed from one end of the centrifuge to the other. The differential speed is the difference in rpm between the speed at which the bowl rotates compared to the speed of the screw. The torque (*Tsp*) for cake conveyance is defined as

$$Tsp = \frac{ms \times G \times Cf \times L}{\Delta} \qquad (6.3)$$

Where *ms* is the cake solids rate on a dry basis, *Cf* is the coefficient of friction, *L* is the length of the bowl of the centrifuge, and Δ is the differential speed (Leung 1998). As described above, during the plant trial, the value for *Tsp* was set to 3 kN-m. The centrifugal acceleration is set at 3039 ft/sec^2 and the bowl length is 8 ft given by the centrifuge's manufacturer. The coefficient of friction was calculated for this specific plant system in conjunction with the manufacturer and was found to be around 0.013. Using this information, and setting the differential speed value between 0.01 and 0.05 in increments of 0.005 rps, the corresponding wet cake solids rate *(ms)* values were calculated. Figure 6.13 shows the results of this calculation.

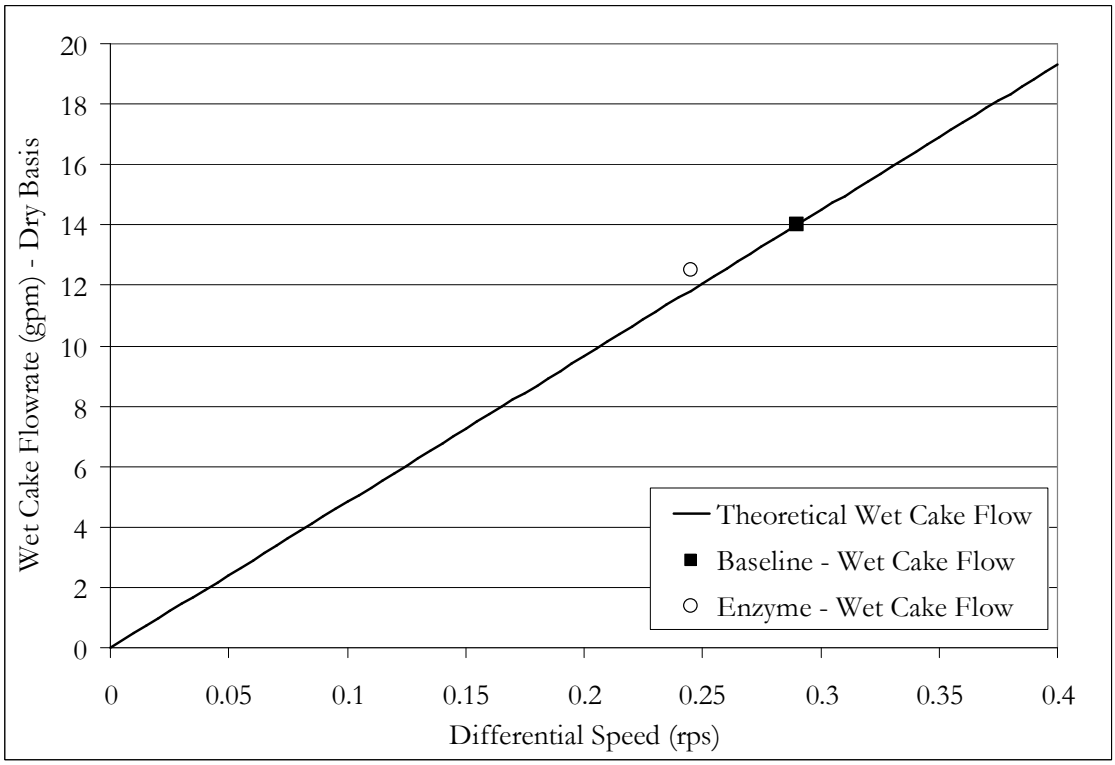

Figure 6.13: Calculated wet cake flowrate (dry basis) as a function of differential speed given by the centrifuge's torque formula; data points show the actual flowrates observed during the plant trial from the baseline and enzyme addition parts of the trial

The differential speed observed during the baseline part of the plant trial was 17.7 rpm, or 0.295 rps. According to the results, at a differential speed of 0.295 rps, the solids flowrate should be 14.24 gpm on a dry basis. Here the solids flowrate is given in gpm because the calculation is done based on the flowrate of the slurry, both the solid and liquid phases. The liquid portion of the slurry is then subtracted from the total flow to give the solids flowrate. The solids flowrate value measured during the baseline portion of the trial was 14.02 gpm which differed by only 1.5% from the calculated value (Table 6.6).

Table 6.6: Comparison of theoretical centrifuge modeling data to experimental data obtained during the plant trial

	Wet Cake Outlet Flowrate[1] - Dry Basis (gpm)	Wet Cake Mositure Content (%)	Thin Stillage Flowrate (centrate) (gpm)	Difference[2] (%)
Theoretical	14.24	65.7[a]	81.75	---
Plant Trial				
Baseline	14.02	65.7	80	2.1
Enzyme Addition	12.5	63.8	87.9	7.5

[1] Values calculated based on a differential speed of 0.295 rps

[2] Given for the Thin Stillage flowrates, plant trial values compared to the theoretical value

[a] Assumed moisture content based on conventional process (plant trial - baseline)

The value obtained during the enzyme addition phase was 12.5 gpm, a 12.22% decrease compared to the calculated value, t(354) = 27.86. However, when the enzyme was added to the process the differential speed decreased from 0.295 rps to 0.248 rps. At this differential speed the calculated solid flow fraction was 12.07 gpm which was only a 2.66% difference from the experimental value of 12.5 gpm. The decrease in the differential speed was given by an increase in the screw conveyor speed while the bowl speed remained constant. This change may be explained by a possible decrease in the viscosity of the medium, which the enzyme used is known to cause, as explained in Chapter 3. Equation 6.4 shows how viscosity is inversely proportional to the terminal velocity (Vsg) and will therefore enhance the rate at which the particles settle in the slurry.

$$Vsg = \frac{(\varrho_s - \varrho) \times g \times d^2}{18 \times \mu} \qquad (6.4)$$

Here, $(\varrho_s - \varrho)$ is the difference in density between the particle and the liquid, g is gravity, d is mean particle size diameter and μ is the viscosity. Assuming that the enzymes added to the

process have indeed decreased the viscosity of the medium, the settling velocity would increase and as a result the centrifuge would not need to rotate as fast to maintain the target setpoint torque (Leung 1998).

Using the solid phase flowrate data obtained above, and by measuring the wet cake moisture content of 65.7% during the baseline part of the trial, the total wet cake flowrate was calculated to be 40.75 gpm. Applying a mass balance around the centrifuge, and given the inlet flowrate was 122.5 gpm, the centrate is given by

$$Q_{in} = Q_s + Q_c \qquad (6.5)$$

where Q_{in} is the inlet flowrate, Q_s is the wet cake flowrate, and Q_c is the centrate flowrate, all in gpm. This gives a thin stillage flowrate, or centrate, of 81.75 gpm, which only differ by 2.1% from the observed value of 80 gpm (Table 6-6). Following the same procedure for the enzyme addition phase of the trial, and given that the cake moisture was observed to be 63.8%, a 1.9% decrease in moisture compared to the baseline, the thin stillage flowrate was calculated to be 81.75 gpm compared to the observed value of 87.9 gpm.

The experimental results collected from the centrifuges during the plant trial were in accordance with the results calculated using some of the centrifuge operation modeling equations. Adding the enzymes to the process caused a direct effect in the performance of the centrifuge by lowering the differential speed, and as a result increasing the amount of centrate exiting the centrifuge.

6.5 Conclusions

In this study, a plant trial was conducted to look at the scale-up of enhanced water removal from whole stillage by enzyme addition. The plant trial was divided into three phases: baseline I, enzyme addition, and baseline II. The trial was conducted at Center Ethanol Company, LLC, which is a 54 mmgpy nameplate plant, located in Sauget, IL.

Final ethanol yield and final glucose concentration obtained from samples collected at the end of each fermentation were found to be statistically equal for the baseline and the enzyme addition portions of the trial. Furthermore, the data obtained for ethanol production showed that on average, 155,124 (± 4,582) gal of ethanol were produced during the enzyme addition part of the trial compared to 159,468 (± 10,875) gal of ethanol produced during the baselines. The difference between the two numbers was not statistically significant. Adding the enzymes to the plant did not affect the final fermentation ethanol yields or the glucose to ethanol conversion expected during this process.

Wet cake samples collected from the outlets of the centrifuges during the three parts of the trial were statistically different. On average, a 2% reduction in moisture was observed for the wet cake samples taken during the enzyme addition phase. The same results were observed from the wet cake plus syrup samples collected from the syrup mixer tank. This resulted in a decrease in the drier's natural gas usage.

The enzymes added during this trial proved to be effective, and a 3% increase in the amount of water being removed during centrifugation was observed during the trial. Additional water was also removed prior to centrifugation, during distillation and evaporation. This caused a decrease in the centrifuge inlet flowrate. The TS collection tank's outlet valve had an increase of 5.25% in its percent open value which directly corresponded to the increase in the tank's outlet flowrate.

The firing rate (percent opening of the natural gas valve) of the drier decreased significantly during the enzyme addition part of the trial. There was a 14% reduction in the valve's percent opening which resulted in a 14.11% reduction in the volume of natural gas used by the plant. On average, the plant used 1.89 (± 0.11) mmscf of natural gas during the baseline periods compared to 1.62 (± 0.23) mmscf used during the enzyme addition period. The ratio of natural usage per gallon of ethanol produced calculated for the three parts of the trial proved to be statistically different. During the enzyme addition period the average ratio was 10.46 (± 0.85) scf of natural gas/gal of ethanol compared to 11.87 (± 0.54) for the baselines. Therefore, 12% less natural gas was required to produce one gallon of ethanol.

The DDGS composition was not affected by the enzymes. There were no significant differences between the composition of the DDGS during the baselines and enzyme addition parts of the trial. All of the nutritional components of the DDGS were well within the range found in the literature and in accordance to the ruminant's dietary guidelines.

Chapter 7 – Process Simulation and Economic Analysis

7.1 Introduction

A lot of work has been done in the area of corn-to-ethanol process modeling and economic feasibility. Many researchers have simulated the process using various modeling software to provide a basis for their economic analysis. Aspen Plus™ has been used as a modeling platform to look at the conversion performance of degermed corn to ethanol, and it was suggested that the process of germ-fiber separation from the corn kernel be improved upon as well as the oil recovery from the germ (Rajagopalan et al. 2004). Aspen Plus™ has also been used to simulate the dry grind corn-to-ethanol process by continuous fermentation and stripping using data collected during laboratory experiments. The simulation was then used to look at the economic viability of the process (Taylor et al. 2000). Neural networks have also been used to model the continuous fermentation process in an attempt to optimize productivity, conversion, and ethanol yield in the fermentor (Rivera et al. 2005). SuperPro Designer®, another simulation software program, was used to model the process and cost of fuel ethanol production using sensitivity analysis. In this study, the increase in ethanol production cost was studied as the price of feedstock increased and the corn's starch content decreased. This simulation allowed for the observation of changes in the production cost of ethanol in a changing market (Kwiatkowski et al. 2006).

The economics of the dry grind corn-to-ethanol process has been looked at with exhaustion, and all of the studies done differ from one another in some ways. Some of the work shows a positive outlook for this process while others deem it not economically beneficial. There are two studies that suggest unfavorable process economics. The major differences between these studies and the ones that show a favorable economic analysis are that the unfavorable analyses do not include the co-products of the process in the energy output calculations, and use outdated data that does not reflect current industrial and agricultural practices (Patzek 2004; Pimentel 2003). The other studies show favorable process economics but differ in the extent of that favorability. Some take into account the production of co-products as a positive energy output while others are able to show favorable economics by simply inputting more current data into their models (FAPRI 2005; Delucchi 2004; De Oliveira et al. 2005). Furthermore, Alexander Farrell et al. developed the Energy and Resources Group Biofuel Analysis Meta-Model (EBAMM) to look at the environmental impact, energy consumption, and greenhouse gases emission previously published in the literature to try to replicate their results and better understand why there is so much discrepancy amongst the studies (2006). Nevertheless, all of the studies mentioned above suggest that there is room for improvement in the process to make it more economically competitive.

7.2 Scope

The purpose of this work was to evaluate economically the process developed in this thesis. The plant trial results presented in Chapter 6 showed promising results, but it was necessary

to look in detail at the economics of the process in order to better understand its cost effectiveness. The main goal was to look at the cost of the enzymes and the energy savings that resulted from their addition. It was important to make sure that the energy savings were greater than the actual cost to buy the enzymes. Two different scenarios were examined in order to make an economical comparison: (1) conventional dry grind corn to ethanol process, and (2) modified process with cell wall degrading enzyme addition and its effects. Sensitivity analyses were done on the cost of the enzyme and the cost of natural gas to understand how the enzymatic dewatering process would behave in the daily changing market.

7.3 Methods

The dry grind corn to ethanol process simulation presented in this chapter was a modification of a simulation done at the United States Department of Agriculture (USDA)'s Agricultural Research Services – Eastern Regional Research Center in Wyndmoor, PA. The main contributors to the models presented in this chapter were Dr. David Johnston, Winnie Yee and Andy McAloon. The process simulation software chosen to perform the simulation was SuperPro Designer®, version 7.5, build 7 (SuperPro Designer®, Intelligen, Inc., Scotch Plains, NJ) mainly because of its ability to model more types of unit operations than any of the other programs available.

7.3.1 Process Model

The existing model was developed to simulate a generic conventional dry grind corn to ethanol processing plant that nominally produces 40 million gallons per year (mmgpy) of ethanol with an input of 46350 kg/hr of corn. Since each corn to ethanol processing plant is unique in its design, the model is a generalization of most plants with universal processing equipment and does not reflect a specific plant's design. This aspect of the model makes it versatile and applicable to different users. The simulation itself contains information such as characteristics and operating conditions of unit operations, streams' composition and flowrates, including mass and energy balances. The model is divided into 5 major areas: grain handling and milling, starch to sugar conversion, fermentation, ethanol processing, and co-product processing. Each of those areas is comprised of different unit operations that are analogous to what is found in a corn to ethanol processing plant (Table 7.1).

Table 7.1: Breakdown of selected unit operations by major areas of the process model

Area	Equipment
Grain Handling & Milling	Silo
	Tanks
	Conveyor Belt
	Tanks
	Hopper
	Pumps
	Hammer Mill
Starch to Sugar Conversion	Heat Exchangers
	Pumps
	Reactors
	Tanks
Fermentation	Fermentors
	Tanks
	CO_2 Scrubber
	Pumps
	Heat Exchangers
Ethanol Processing	Distillation Column
	Tanks
	Molecular Sieve
	Pumps
	Heat Exchangers
CoProduct Processing	Evaporator
	Pumps
	Tanks
	Thermal Oxidizer
	Conveyor Belt
	Drier
	Centrifuge

The information presented in Table 7.1 is only a summary of the main unit operations present in the model (Figure 7.1).

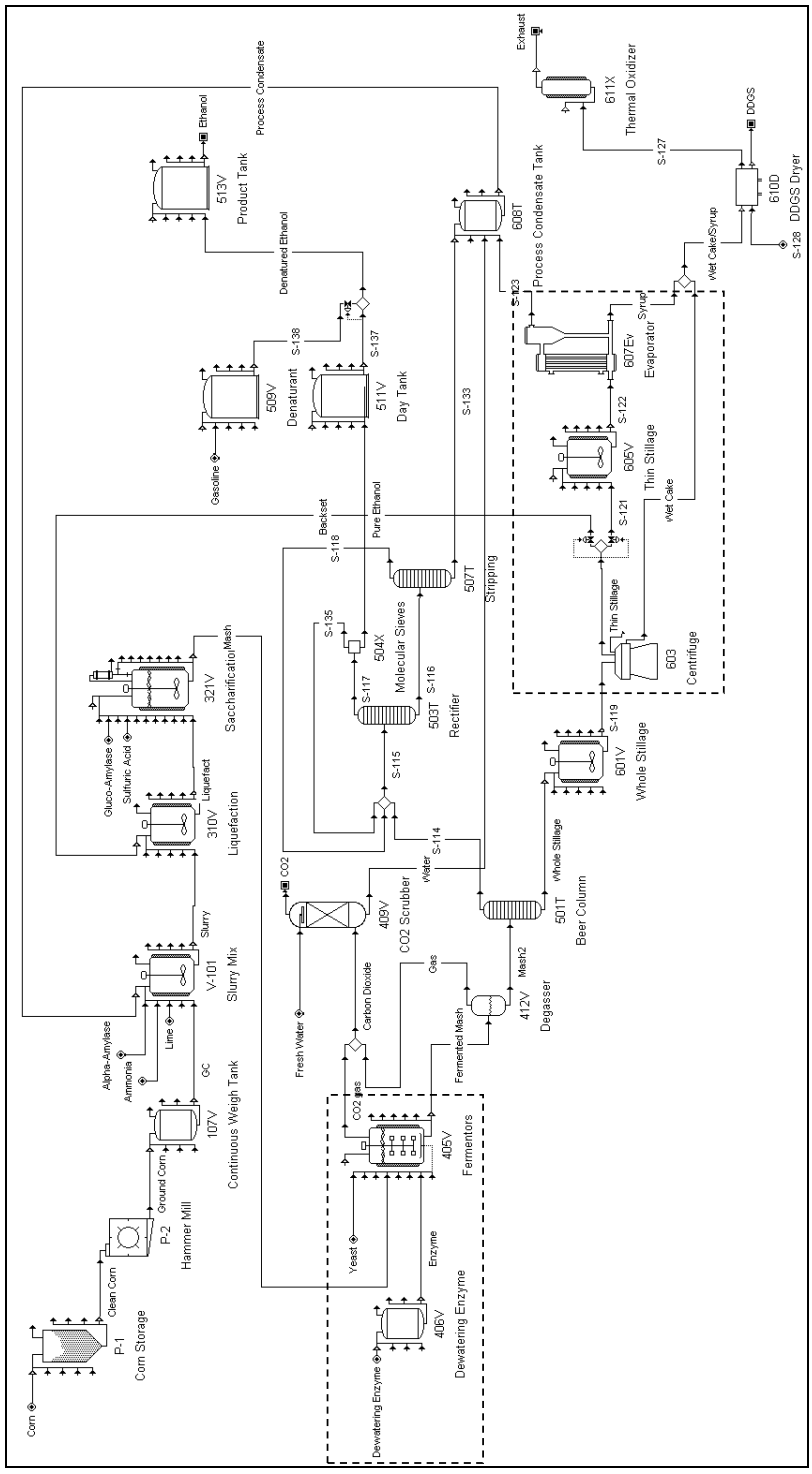

Figure 7.1: Simplified diagram of the enzymatic dewatering model.

For a complete list of all unit operations present in the model please refer to Appendix G. Each unit operation has a tag that is made up of an identification number followed by a letter. The number refers to the area in which the equipment is found and the letter to the type of equipment that it represents. Table 7.2 shows the legend to understand each unit operation's tag.

Table 7.2: Legend to the process' unit operations tags

Number ID	Area of the Process	Letter ID	Equipment
		V	Vessel
100s	Grain Handling and Milling	P	Pump
300s	Starch to Sugar Conversion	E	Heat Exchanger
400s	Fermentation	T	Tank
500s	Ethanol Processing	MH	Conveyor
600s	CoProduct Processing	M	Grinder
		W	Hopper

Using data collected during the plant trial presented in Chapter 6, changes were made to the model to reflect the results obtained when the enzymes were added to the process. Table 7.3 summarizes in detail the specific changes made to each of the model's major areas while Figure 7.1 shows in the highlighted areas where the changes were made.

Table 7.3: List of detailed changes made to the simulation divided by the main areas

Main Areas	Changes Made to the Simulation
Fermentation	1) A tank was added to store the enzyme 2) A pump was added to transfer the enzyme from the tank to the fermentor 3) Enzyme flowrate was set to 32.467 kg/hr
Ethanol Processing	1) Removed corn flowrate design specification
CoProduct Processing	1) Centrifuge's solids concentration was set to 5.47 g/L 2) Evaporator's final solid mass fraction of water was set to 64% 3) Backset recycle ratio was set to 18.5%[1]

[1] The same change was made to the original simulation of the conventional process done at the USDA

In the fermentation area, the tank and the pump were added to provide a way for the enzymes to be stored and transferred to the fermentor. In the plant trial this was not necessary since the enzymes were pumped directly into the fermentors from the totes in which they arrived. The enzyme flowrate was set to 32.5 kg/hr because that corresponded to a dosage of 0.7 kg per metric ton of corn being processed by the plant. This dosage was selected for industrial practice based on the results described in Chapter 5 and Chapter 6. Only one change was made to the Ethanol Processing area, and that was the removal of the corn mass flowrate design specification. This change was optional and done to improve the simulation convergence rate. It was not absolutely necessary to have this specification since the corn input was set to the same amount as the one found in the conventional base case simulation. Furthermore, the presence of this design specification caused problems in the convergence of the model with the specific modifications being made.. Lastly, the changes made to the Co-Product Processing area were necessary to match the results of the

simulation to those obtained during the plant trial. The centrifuge solid's concentration was decreased to 5.47 g/L to achieve the extra water removal after centrifugation observed during the plant trial. The evaporator final mass fraction of water was also changed to match results seen in the plant trial. The only change that had to be made to both the original model and the enzymatic dewatering model was the backset recycle ratio. The model was originally simulated with a backset ratio of 13.5% but in reality the plant recycles 18.5% of the thin stillage and process condensate streams. These changes not only affected the model's simulation results but also the economics of the process.

7.3.2 Economic Model

Once the process models were adjusted and results matched what was observed during the plant trial, an economic analysis was performed. This economic model was created using the economic analysis capabilities of the software itself. However, a significant portion of the information used by the economic analysis package of the software was supplied by the USDA's cost engineer. This information was based on data gathered from vendors, government agencies, and corn to ethanol processing plants, and also found in the literature. The economic model contains information such as equipment cost, utilities usage, raw materials and consumables costs, capital investment as well as labor and operating costs. The economic software package found in SuperPro follows cost engineering methods which are generally accepted in industry (Ramirez et al. 2009; AACE 1990). All of the figures, unless otherwise stated, used for the economic model were based on cost conditions of 2007. The

2007 figures were used because complete economic data for 2008 was not yet available when this work was carried out. Two sensitivity analyses were done on the enzymatic dewatering model to look at how the fluctuating price of natural gas and enzymes would affect the economics of the process. Since the main objective of the enzymatic dewatering process was to decrease the production cost of ethanol, it was necessary to know the point at which the energy savings observed broke even with the purchase cost of the enzymes.

7.4 Results and Discussion

7.4.1 Process Model

Figure 7.1 shows schematically the process model described below. Please refer to this figure for further detail throughout this section.

7.4.1.1 Grain Handling and Milling

Corn, at 15% moisture, comes into the process and is moved to a silo where it is stored. Table 7.4 summarizes the annual consumption of selected bulk materials used by the process including corn.

Table 7.4: List of materials used in the process that remain the same in both models

Bulk Material	Unit Cost ($/kg)	Annual Amount (kg)	Annual Cost ($)
Corn	0.14	367,061,793	50,562,762
Lime	0.09	438,190	39,437
Liq. Ammonia	0.22	733,337	161,334
Alpha-Amylase	2.25	257,139	578,562
Gluco-Amylase	2.25	371,408	835,669
Sulfuric Acid	0.11	733,337	80,667
Caustic	0.01	18,423,742	223,296
Yeast	1.86	96,466	179,426

The silo has a continuous storage capacity of 10.8 days. The corn is then cleaned and transported to the hammer mill where it is ground and then stored is a surge tank. A batch scale weighs the ground corn before it is sent to the Starch to Sugar Conversion area of the process.

7.4.1.2 Starch to Sugar Conversion

In this section of the process, ground corn is mixed in a slurry mix tank with process condensate, lime and ammonia to adjust the pH, and α-amylase to hydrolyze the starch (Table 7.4). The mix tank has a continuous storage capability of 0.25 hr and is kept at a pressure of 1 bar. At this stage the slurry is 67% water. The slurry is then pumped and heated to 88ºC before it enters the liquefaction tank where it is liquefied at a temperature of

90ºC for almost an hour. The liquefied slurry goes through a series of heat exchangers until it reaches a temperature of 60ºC, at which point it enters the saccharification tank. Sulfuric acid, to adjust the pH, and gluco-amylase enzyme are also added to the tank (Table 7.4). The starch in the presence of water reacts with the enzyme to produce glucose at 60ºC for 20 hrs.

7.4.1.3 Fermentation

The saccharified slurry is cooled to 32ºC before it enters the fermentor where it is mixed with yeast suspension that is 95% water. This mixture is known as corn mash. The corn mash is kept at 30% solids via a design specification implemented in the model. This design specification manipulates the water input to the CO_2 scrubber to ensure that the 30% solids target is met during the simulation. At this stage of the process, the first difference between the conventional model and the enzymatic dewatering model appears. Table 7.5 lists the main differences between the two models. These differences reflect actual changes made to the enzymatic dewatering model.

Table 7.5: Description of selected process equipment for modified process model and main differences to conventional process model[1]

Equipment Tag/Type		Enzymatic Dewatering Model	Conventional Model
		Description	Differences
406V	Dewatering Enzyme Transfer Tank	336 hrs residence time 90% working volume 32 kg/hr outlet flowrate	Not Present
409V	CO_2 Scrubber	18017 kg/hr fresh water inlet flowrate 59% removal of water 99.8% removal of ethanol	20507 kg/hr
603	Centrifuge	5.43 g/L solids concentration in solids stream 106654 L/hr volumetric throughput 100% removal of starch, polysaccharides[a] and ins. protein[b]	5.66 g/L 106491 L/hr
607Ev	Evaporator	64% final mass fraction of water at the inlet 4 effect evaporator 95°C steam temperature in 1st effect 79°C solution temperature in 4th effect	65%
610D	Drier	Direct fired ring drier 66 MMBTU of natural used/52980 lb of water evaporated 20 (kg/hr)/m^3 evaporation rate	

[1] Only the differences are noted under the conventional model column, all other parameters remain constant

[a] non-starch polysaccharides

[b] ins. = insoluble

In the enzymatic dewatering model, trial enzyme is added to the fermentor at a rate of 32.467 kg/hr, or 0.7 kg per MT of corn. A transfer tank and a pump were added to the model so that the trial enzyme could be stored and transferred to the fermentor as needed. Fermentation takes place in the reactor at a temperature of 32ºC for 60 hrs. During this time the yeast cells convert glucose into ethanol and CO_2 and reproduce and multiply. The CO_2 is vented off to the CO_2 scrubber while the ethanol, water, and non-fermentable components are transferred to the next area of the process. In the enzymatic dewatering model, the CO_2 scrubber operation is simulated differently from the conventional model. The difference is in the amount of fresh water that is brought into the process. Due to changes made in the

downstream portion of the model, as explained in Section 7.4.1.5, 14.14% less water is required in the enzymatic dewatering model compared to the conventional process (Table 7.5).

7.4.1.4 Ethanol Processing

The mash goes through a series of heat exchangers until it reaches a temperature of 78ºC before it enters the beer column where the mash is distilled to a mixture of 50% ethanol and 50% water at an operating temperature of 104ºC. The bottoms of the beer column, which is composed mainly of water and solids, is transferred to the whole stillage tank; whereas, the ethanol-water mixture is sent to the rectifier, where it is further distilled into a 10% water mixture at a temperature of 95ºC. The water removed in the rectifier is sent to a stripper where any remaining ethanol is removed and the water is recycled back into the process via the process condensate tank. The hot ethanol vapors recovered in the rectifier are used as energy to drive the evaporator, downstream of the process. The 10% water mixture is sent to the molecular sieves where a 99.6% pure ethanol product is removed, mixed with gasoline as a denaturant and stored before it leaves the process. Denatured ethanol as a final product is processed at a rate of 15048.64 kg/hr to meet an annual production of 40 mmgpy. Table 7.6 shows the overall material balance for both the conventional and enzymatic dewatering process models.

Table 7.6: Overall material balance of process models

Component	Enzymatic Dewatering Model		Modified Model	
	In (kg/hr)	Out (kg/hr)	In (kg/hr)	Out (kg/hr)
Corn	39659		39659	
Water	29812	26795	27355	24238
Nitrogen	27063	27063	24415	24415
Carbon Dioxide		14056		14054
Sulfuric Acid	93	93	93	93
Octane	301	301	301	301
Oxygen	8216	8199	7412	7397
Ethanol		14690		14691
Yeast	3	571	3	570
DDGs		13378		13378
TOTAL	105146	105145	99237	99137

The main differences observed in the overall material balance are the amount of water and nitrogen required by each process. In terms of water, as described above, the enzymatic dewatering process requires 14.14% less water than the conventional process due to a decrease in the hot air input of the drier as described in Section 7.4.1.5. This water requirement does not include the cooling water requirement of the process. The simulation estimates about 5.18 billion kg of cooling water per year required for the process in both the conventional and enzymatic dewatering models.

7.4.1.5 Co-Product Processing

Co-product processing is the final stage of the corn to ethanol process. Whole stillage and a mixture of water, oil, and non-fermentable solids is stored in the whole stillage tank at 93ºC. The whole stillage tank has a residence time of 6.12 hrs with an outlet flowrate of 110699 kg/hr. The whole stillage is pumped to the centrifuge where solid-liquid separation takes place. As seen in Table 7.5, the centrifuge operating parameter had to be changed to match liquid-solid separation results observed during the plant trial. In the conventional model, the centrifuge is designed with a solids concentration of 5.66 g/L in the solids stream (wet cake), which results in a solid stream moisture content of 65%. This moisture content differs by 1.2% from the moisture content obtained during the plant trial. Table 7.7 summarizes the main streams found in the co-product processing section including the water content of each stream for both models.

Table 7.7: Water flowrate in selected streams in the Co-Product Processing area

Stream Tag	Description	Conventional Model	Modified Model
		Water (kg/hr)	Water (kg/hr)
S-198	Thin stillage out of centrifuge	77092.50	79643.17
S-181	Wet cake	16627.14	14196.91
S-176	Thin stillage to evaporator	50205.39	52347.75
S-196	Backset	26887.11	27295.42
S-172	Syrup	9887.86	9862.30
S-166	Wet cake plus syrup	26514.99	24059.21
S-127	Process condensate	74140.67	73836.51

In order to achieve a moisture content of 63.8% in the wet cake stream of the enzymatic dewatering process, the solids concentration parameter was changed to 5.43 g/L (Table 7.5). By decreasing the moisture content in the solids stream, the moisture content of the liquid stream (thin stillage) was increased by 5% compared to the conventional model. After centrifugation, the thin stillage stream is divided into two streams; one that goes back into the process as backset and one that goes to the evaporator and eventually becomes part of the process condensate recycle stream. The backset stream flow was set to 18.5% of the combined thin stillage and process condensate flows, which matched the ratio from the plant trial. The wet cake is mixed with the syrup coming off of the evaporator before it enters the drier. The thin stillage, minus the backset flow, enters the evaporator where the water is evaporated, and the solids that remain, also known as syrup, leave the evaporator at 64% water content. In the conventional model the final mass fraction of water to exit the evaporator with the syrup is set to 65%. This 1% difference allows for the wetcake plus syrup stream that enters the drier to match the moisture content of the plant trial, in the case of the enzymatic dewatering model. The final moisture contents for the wetcake plus syrup stream is 66% and 63%, for the conventional and enzymatic dewatering models, respectively. This decrease in water content caused a decrease in the natural gas usage of the drier. In both cases the solids are dried to a 9% moisture setpoint but in the case of the enzymatic dewatering model, less natural gas is needed to achieve this setpoint since less water is present in the system. The dried solids, known as DDGS, are processed at a rate of 15300.7 kg/hr which results in an annual production of 121000 MT.

It is important to point out that in the case of the enzymatic dewatering model, a higher amount of backset is recycled back into the process. This is a result of the enhanced dewatering achieved during centrifugation. When more water is removed during centrifugation, more water is evaporated and condensed to become process condensate. An increase in the flowrate of the process condensate and thin stillage streams will cause an increase in the flowrate of the backset stream to achieve a ratio of 18.5%, as explained above. In the case of the conventional process, the backset stream is set to 29700 kg/hr, compared to 30200 kg/hr in the enzymatic dewatering model. This means that in the case of the enzymatic dewatering model there is more water being recycled back into the process, but since during fermentation the mash is kept at 30% solids, there is a decrease in the fresh water requirement of the process as a whole. The greater the amount of water being recycled the smaller the need to add fresh water to the process. According to ethanol plants, a 40 mmgpy corn to ethanol processing plant is required by federal, state and/or local permits to bring in at least 54 gpm of fresh water into the CO_2 scrubber, otherwise the plant is not allowed to operate. In the enzymatic dewatering model that value is 79 gpm which means that the decrease in the fresh water feed to the scrubber observed in the enzymatic dewatering model still allows for the permit requirements to be met.

7.4.2 Economic Model

For the complete economic evaluation report (EER), please see Appendix H.

7.4.2.1 Annual Operating Costs

The annual operating costs are similar between the conventional and enzymatic dewatering models (Figure 7.2).

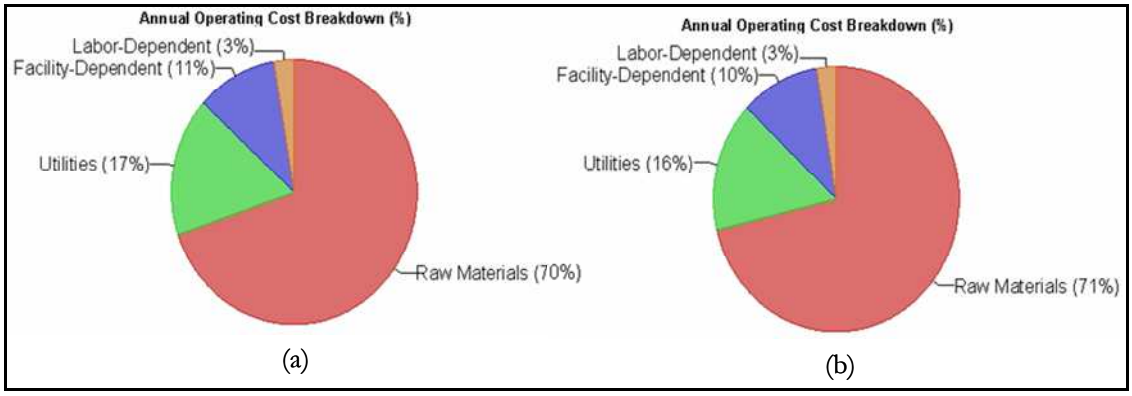

Figure 7.2: Breakdown of annual operating costs for (a) conventional and (b) modified process models

As can be observed, there is a 1% increase in the cost of raw materials in the enzymatic dewatering model, which is attributed to the purchase cost of the trial enzyme. For the purposes of these calculations, the purchase price used for the trial enzyme was $10/kg. Due to the proprietary nature of this commercially available enzyme and Genencor's desire to keep the actual price from being public, this estimate was used based on some potential price range information provided by the company. Based on this information, it is believed that $10/kg is a conservative estimate of how much the enzyme would actually cost. The annual cost to purchase the enzyme for a 40 mmgpy plant that uses the enzyme at a rate of 0.7 kg per metric ton of corn is $2.57 million. Furthermore, even though the cost of raw materials

increase, the utilities consumption by the enzymatic dewatering process decreases by about 1% compared to the conventional model. Labor dependent operating costs remain constant in both models as the facility dependent costs decrease in the enzymatic dewatering model. The decrease in facility dependent costs can be mainly attributed to a decrease in the purchase cost of the ring drier and the thermal oxidizer (TO). The increase in water removal during centrifugation caused a decrease in the sizing requirement of the drier and the TO. This resulted in a smaller moisture content in the inlet of the drier and a decrease in water and ethanol mass flowrate to the TO. The total annual operating cost for the enzymatic dewatering model was calculated to be $79.96 million compared to $77.97 million obtained with the conventional model. These operating costs do not include the cost of capital, corporation administration charges, tax credits, or marketing and distribution charges.

7.4.2.2 Annual Utility Consumption and Costs

Overall, there was a decrease in utility consumption in the enzymatic dewatering model. Table 7.8 shows the annual utility consumption and costs for both models.

Table 7.8: Annual utility consumption and cost for conventional and enzymatic dewatering models

Utility	Conventional Model			Enzymatic Dewatering Model		
	Annual Amount	Annual Cost ($)	%[a]	Annual Amount	Annual Cost ($)	%[a]
Electricity	29872249 (kWh)	1,493,612	11.37	29247875 (kWh)	1,462,394	11.58
Natural Gas	11949842 (kg)	4,202,640	31.98	10781895 (kg)	3,791,885	30.03
Steam	257468235 (kg)	5,496,947	41.83	254167178 (kg)	5,426,469	42.97

[a] Value shown as a percentage of all utilities used by each process

The consumption of electricity by the enzymatic dewatering model was decreased by 2.5% compared to the conventional model. This decrease can be attributed to a decrease in electricity input to the drier even though the evaporator showed an increase in its electricity requirement. The higher the amount of water being centrifuged out, the harder the evaporator has to work to drive off this water. However, the drier's decrease in electricity consumption offsets the increase in the evaporator.

Natural gas accounts for as much as 32.35% of all of the utilities consumed in the conventional model but a reduction to 30% is observed in the enzymatic dewatering model. When directly comparing only the natural gas consumption, the enzymatic dewatering model uses 12% less natural gas than the conventional model. This number is in accordance with the decrease observed during the plant trial presented in Chapter 6 (14%). In the enzymatic dewatering model, natural gas is consumed annually at a rate of 0.09 kg per kg of ethanol produced, whereas in the conventional model the rate is 0.102 kg of natural gas per kg of ethanol produced.

Even though the consumption of steam decreases in the enzymatic dewatering model, it is not significant and therefore does not affect the economics of the process as a whole. A decrease of 1.28% is not enough to significantly affect the economics of the process. Furthermore, in either the conventional or enzymatic dewatering models, the steam is treated as a commodity and is purchased at a certain price. The steam does not come from a boiler and therefore the amount of natural gas used by the models is specific to the drier operation.

Using the information provided by the simulation, it is possible to calculate the amount of BTUs used to produce a gallon of ethanol. To make this calculation, the total energy consumed by the process was converted into BTUs and that number was divided by the total number of gallons of ethanol produced. For the conventional model this ratio was calculated to be 32,641 BTU/gal of ethanol, whereas for the enzymatic dewatering model, this number decreased to 31,039 BTU/gal. That is a 4.91% decrease in the amount of BTUs required to produce a gallon of ethanol.

7.4.2.3 Unit Production Costs

The last important parameter to examine is the unit production cost in each model. The cost to produce a gallon of ethanol is calculated taking the annual total operating cost subtracting

the value added by selling the DDGS and dividing that by the total gallons of ethanol produced by the plant (Equation 7.1).

$$UnitPoductionCost = \frac{(TotalOperatingCost - ValueOfCoproducts)}{TotalGallonsOfEthanol} \quad (7.1)$$

Given that the price of natural gas fluctuates with the market and that there was no definitive enzyme cost, a sensitivity analysis was done to look at how price of natural gas and cost of the enzyme would affect the unit production cost. In this analysis the natural gas price was set between \$3 and \$15 per 1000 ft^3 (1000 ft^3 equals 1 MMBTU). The enzyme price ranged from \$2 to \$20 per kg. Each model was simulated using the price ranges above and the unit production cost was calculated for each case. The unit production cost obtained from the enzymatic dewatering model was then subtracted from the unit production cost of the conventional model in each case (Appendix I). Figure 7.3 shows the results of this sensitivity analysis.

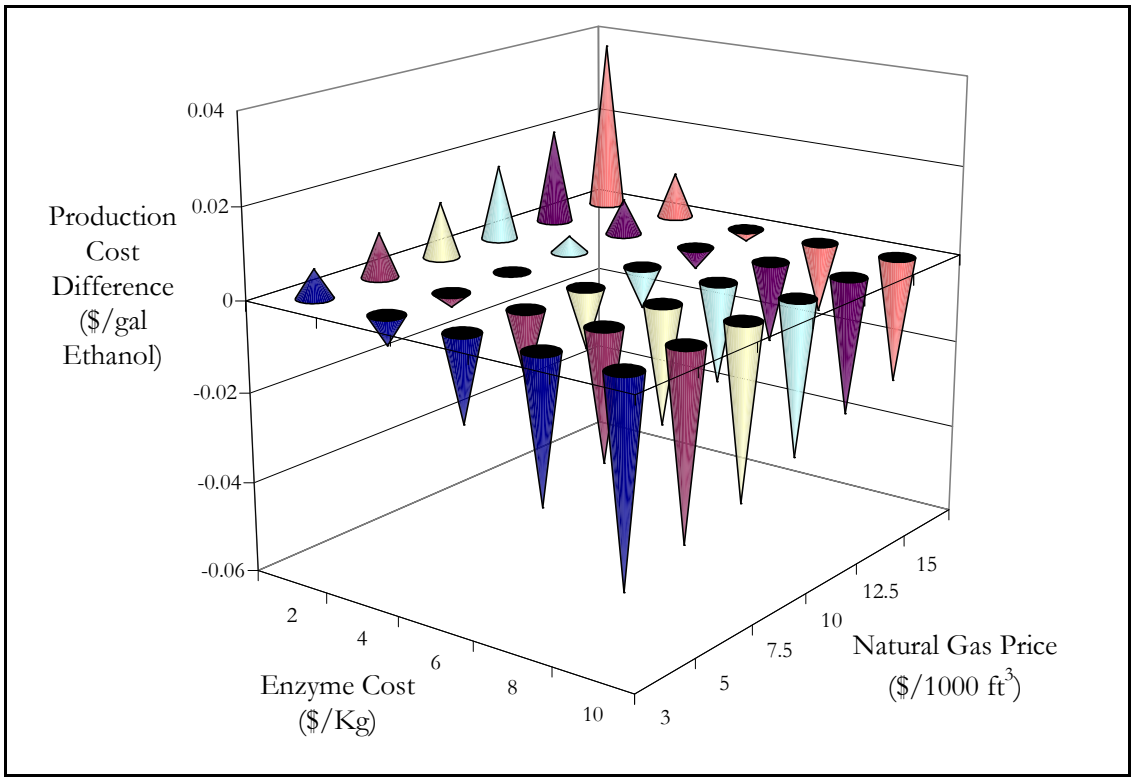

Figure 7.3: Sensitivity analysis of enzyme cost and natural gas prices; a positive difference (cost in conventional model minus cost in enzymatic dewatering model) represents a reduction in the production cost of a gallon of ethanol in the enzymatic dewatering model.

A positive difference in the unit production cost translates into a decrease in the cost of a gallon of ethanol using the enzymatic dewatering model. This means that at these specific combinations of enzyme and natural gas prices, it is more cost-effective to use the enzymatic dewatering process as opposed to the conventional process. However, there is a point at which the natural gas costs too much and the energy savings of the drier are not big enough to offset the purchase price of the enzyme. Given an enzyme cost of $6/kg or higher, the cost to produce a gallon of ethanol is actually higher in the enzymatic dewatering process. At an enzyme price of $4/kg, the natural gas price must be equal to or higher than $7.50 per

1000 ft^3 for the enzymatic dewatering process to be more cost-effective. Nevertheless, at an enzyme cost of $2/kg, any natural gas price within the range used in this study will result in a decrease in the unit production cost for the enzymatic dewatering process. The highest decrease is observed at an enzyme cost of $2/kg and a natural gas price of $15/1000 ft^3. At these prices, the unit production cost in the enzymatic dewatering process is $1.72/gallon of ethanol compared to $1.76/gallon of ethanol in the conventional process.

It is important to understand that unit production cost based on the enzymatic dewatering model can only be finalized when a plant trial involving optimal enzyme dosage is carried out. It is believed that similar dewatering results, and therefore natural gas savings, could be obtained at much smaller enzyme doses. Figure 7.4 shows the projection of possible natural gas savings at lower enzyme addition to the process.

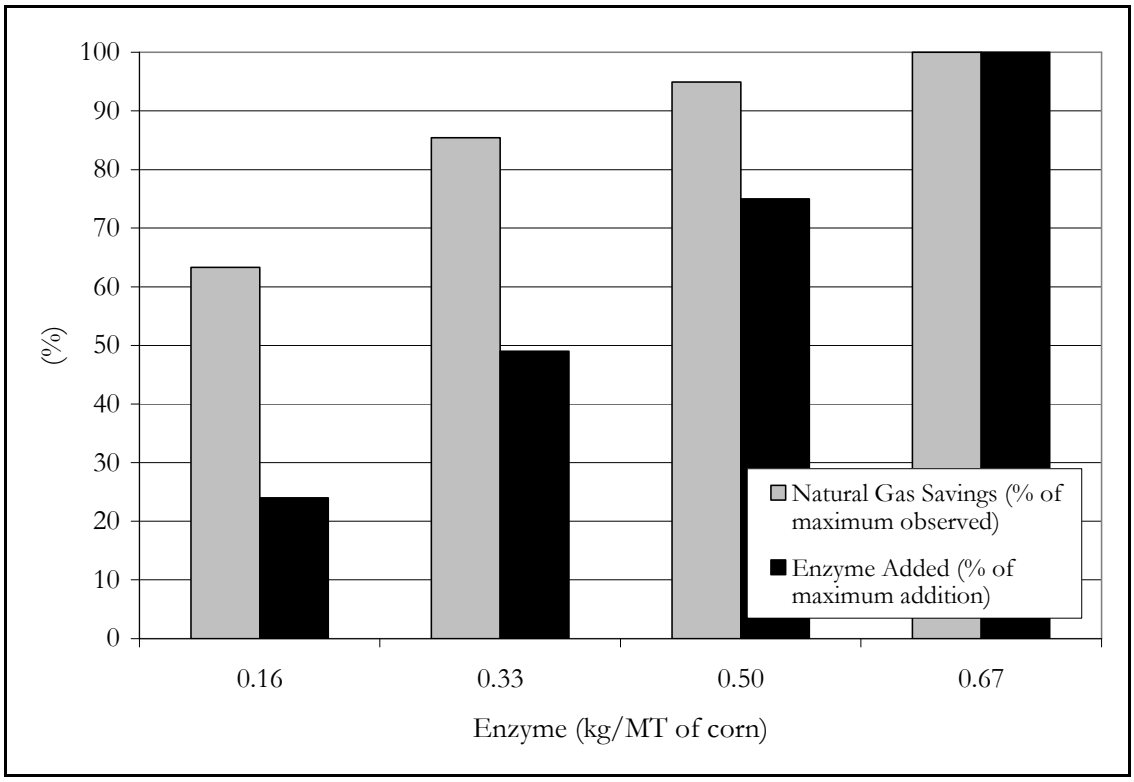

Figure 7.4: Projection of possible natural gas savings at lower enzyme addition based on results from the plant trial and laboratory experiments; 100% represents total gas savings observed in the plant trial and at total enzyme addition of 0.67 kg/MT of corn

Here the total natural gas savings observed in the plant trial is said to be 100% while the amount of enzyme added during the plant trial is also said to be 100%. Based on corresponding laboratory experiments presented in Chapter 4 of this thesis, a ratio between the amounts of enzyme added to each fermentation flask and the resulting dewatering is calculated (Table 7.9).

Table 7.9: Projection of natural gas savings based on lab-scale and plant-scale experimental results, maximum dewatering and natural gas savings shown as total dewatering and total natural gas savings observed in the plant trial

	Lab Enzyme Addition (mL/100 g of mash)/ Enzyme Addition (kg/MT of corn)			
	0.005/0.16	0.01/0.33	0.015/0.50	0.02/0.67
Ratio (Enzyme Addition/Maximum Enzyme Addition)	0.24	0.49	0.75	1.00
Enzyme Added (% of maximum)	24	49	75	100
Lab Dewatering Results (% of water removed)	10.0	13.5	15.0	15.8
Ratio (% Water Removed/Maximum Water Removed)	0.63	0.85	0.95	1.00
Nat. Gas Savings (% of maximum)	63	85	95	100

This same ratio is applied to the maximum natural gas savings which results in a projection of what the natural gas savings would be if the amount of enzyme added was changed. As it can be observed from the graph, when the amount of enzyme decreases from 0.67 to 0.5 kg/MT of corn, a 25% reduction, the natural gas savings decreases by only 5%. When comparing 0.67 to 0.16 kg/MT of corn that translates into a 76% reduction in the amount of

enzyme added but only a corresponding 63% decrease in the natural gas savings. This is possible because of the non-linear nature of enzyme activity. As the amount of enzyme added is linearly decreased, its resulting effects on the process changes nonlinearly.

Furthermore, as explained previously, all of the enzymes used in this thesis are commercially available enzyme preparations that are currently used for other applications. These preparations have specific enzyme activities that are causing the increase in dewatering. The development of a new product that would contain only the necessary activities would not only serve as a means to maximize dewatering but also to hopefully decrease the purchase price. In the end, the companies that make these enzymes will set the price.

7.5 Conclusions

There are a few dry grind corn to ethanol process models in the literature. An enzymatic dewatering model based on the conventional model created at the USDA's Eastern Regional Research Center in Wyndmoor, PA was developed. In the enzymatic dewatering model, a dewatering enzyme was added to the fermentor at a rate of 0.7 kg/MT of corn. Specific changes were made to the centrifuge and evaporator design specifications in order to match plant trial dewatering results. Moisture contents in the wet cake and the wet cake plus syrup streams were adjusted to remain equal to the moistures found during the enzyme addition part of the plant trial. In both the conventional and enzymatic dewatering models the backset recycle ratio was adjusted to 18.5% to correspond to the backset ratio target of the

plant. A decrease in the fresh water input of the enzymatic dewatering process was observed. Since more water was recycled back into the process in the enzymatic dewatering model due to the increase in dewatering during centrifugation, less water addition was required. The conventional model overall used 10% more water than the enzymatic dewatering model.

Simulation results from the enzymatic dewatering model showed a decrease in utility consumption when compared to the results obtained with the conventional model. The consumption of natural gas, electricity, and steam at 50 psi, was decreased by 2.5%, 12%, and 5%, respectively. Annual operating costs also changed from the conventional model to the enzymatic dewatering model. The annual cost from raw materials increased by 1% in the enzymatic dewatering model due to the purchase cost of the enzymes, but at the same time the annual cost from utilities also decreased by 1%, mainly due to the decrease in utility consumption.

A sensitivity analysis showed a tradeoff between the cost to purchase the enzyme and the natural gas savings in the drier. The cost to produce a gallon of ethanol (unit production cost) was calculated using different purchase prices for the enzyme and for the natural gas. A break-even point was observed at an enzyme price of \$4/kg and a natural gas price of \$7.50/1000 ft^3. At this enzyme price, a decrease in the unit production cost was observed for natural gas prices above \$7.50. Furthermore, a decrease in the unit production cost was also observed in the enzymatic dewatering model for an enzyme price of \$2/kg for all natural gas prices used in the analysis.

Because of the non-linear nature of enzyme activity, as the amount of enzyme added is linearly decreased, its resulting effects on the process are non-linear. By relating the results observed in the laboratory experiments to the results of the plant trial, it was calculated that when the amount of enzyme decreases from 0.67 to 0.5 kg/MT of corn, a 25% reduction, the natural gas savings decreases by only 5%. This way, significant savings in natural gas cost can still be obtained even if the maximal dewatering effects are not achieved.

Appendix A – Sample Calculation for One-Way Analysis of Variance (ANOVA)

Table A.1: HPLC data for glucose content of samples collected at the end of fermentation during the plant trial. One sample was collected for each fermentor analyzed during each period of the trial.

HPLC Glucose Data (%w/v)		
Period of the Plant Trial		
Baseline1	Enzyme Addition	Baseline2
0.072	0.079	0.108
0.066	0.07	0.104
0.065	0.065	0.064
0.068	0.104	0.065
0.067	0.069	0.072

Table A.2: Summary of results from the ANOVA analysis performed for the glucose data collected during the plant trial.

Groups	*Count*	*Sum*	*Average*	*Variance*
Baseline1	5	0.338	0.0676	7.3E-06
Enzyme Addition	5	0.387	0.0774	0.000247
Baseline2	5	0.413	0.0826	0.000468

Table A.3: ANOVA results obtained for the glucose data collected during the plant trial.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.0005801	2	0.0002901	1.204596	0.333618	3.885294
Within Groups	0.0028896	12	0.0002408			
Total	0.0034697	14				

In Table A.3, the calculated F value, 1.205, is less than the critical F value, 3.885. Therefore, there is no statistical difference between the glucose content observed at the end of fermentation during the baselines and enzyme addition parts of the trial.

Appendix B – Sample Calculation for t-Test Assuming Equal Variances

Table B.1: Excel generated t-test for the thin stillage solids content data collected during the plant trial.

	Baseline	*EnzymeAddition*
Mean	10.66914	11.7625
Variance	0.2010384	0.30575
Observations	93	24
Pooled Variance	0.2219807	
Hypothesized Mean Difference	0	
df	115	
t Stat	-10.135842	
P(T<=t) one-tail	6.136E-18	
t Critical one-tail	1.6582118	
P(T<=t) two-tail	1.227E-17	
t Critical two-tail	1.9808075	

The t-test presented here was performed for the thin stillage solids content data collected during the plant trial. Here, the t value obtained, -10.136, was smaller than the critical t value, -1.658, for a one-tail test. This means that there is a significantly statistical difference between the data collected during the baseline compared to the enzyme addition part of the trial.

Appendix C – Plant Trial SOPs for QC Lab

Standard operating procedures (SOPs) for each test conducted at the QC lab during the plant trial.

Spin Test On Whole Stillage

1. For each sample weigh and label one 50ml centrifuge tube
2. Take one 40ml sub-sample from each sample and put in the labeled 50ml centrifuge tube
    - Record weight of flask after each sampling
3. Centrifuge all of the tubes in the bench top centrifuge for 5 min at 2200g (approximately 4000 rpm)
    - Record the appearance of oil layer at the top of liquid phase
4. Record the weight and volume of the solid and liquid phases obtained after centrifugation
5. Perform vacuum filtration on the liquid phase using Buchner funnel and filter paper to remove any suspended solids and weight the filtrate
6. Determine the water content of both solid phase and filtrate from above by using double oven method: 55^{o}C until liquid evaporates and then 135^{o}C for 2 hours.

Spin Test On Thin Stillage

1. Fill a 15 mL centrifuge tube to the 10 mL mark and label with sample ID.
2. Place the tube in the centrifuge and make sure it is balanced. Press play button to begin centrifuging. Verify the speed is set to 8000 rpm and centrifuge run time is 10 minutes. Use the "Up" and "Down" arrows to check/change settings for speed and time.
3. After samples are done centrifuging, visually average the amount of solids collected at the bottom of the centrifuge tube using the marking scales located on the side. Record value.
4. This is a visual approximation to see how much solids we are retaining in our stillage samples off our centrifuges in production.
5. Please reset centrifuge run time back to 5 minutes after spins are complete.

Moisture and Solids Content Analyzer

APPARATUS: Moisture analyzer (Mettler HB43-S) and aluminum foil pans.

SAFETY NOTES: Keep combustible materials away from the machine while it is being used. Make sure the top vent is clear and not covered.

PRINCIPLE OF METHOD: Moisture content (as well as total solids) is measured based on the thermo-gravimetric principle, or by the method of "Loss on Drying." At the start of the measurement, the Moisture Analyzer determines the weight of the sample; the sample is quickly heated by the integral halogen dryer and the moisture vaporizes. During the drying operation, the instrument continuously determines the weight of the sample and displays the result. On completion of drying, the result is displayed as % moisture content or as % solids.

The drying methods vary with different materials. A good method should be calibrated by oven method.

PROCEDURE:

1. Switch the instrument on with the "On/Off" key.
2. Open lid to the sample chamber. Place a sample pan flat on the tray, press "O/T" key to tare it, and then close lid to tare.
3. Open lid again, place your sample on a clean sample pan and select the appropriate method on the indicated balance (Table A-1).
    a. Method A (SLURRY) runs the following samples: Slurry, Liquefaction, Fermentation, Beerwell, Whole Stillage, Thin Stillage, Evaporators 1 & 3. A 1.7 to 2.3 g sample size is recommended. Shake the thermos well to ensure the sample is homogeneous before placing the droplets evenly on the pan. Avoid large drops or running streaks when loading samples on pans.
    b. Method B (DDGS) runs the following samples: Wet cake, Wet cake + syrup, syrup, Evaporators 2, DDGS, and corn flour. A 1.7 to 2.3 g sample size is recommended. Place the sample evenly on the sample pan. Avoid large clumps of samples and ensure that solid particles are broken up before distribution on pan.

Table C.1: Method classification for sample type to be used with moisture analyzer

Method	A	B
Sample	Whole Stillage Thin Stillage Slurry	DDGS Corn Flour Wet Cake Wet Cake + Syrup
Profile	RAPID	STD
Steps	1: 202°C 2: 150°C	1: 125°C
Target Weight	2.0 g	2.0 g
Range	1.7 – 2.3 g	1.7 – 2.3 g

4. Record information on data sheet located next to or in binder in Moisture Analyzer drawer.
5. Close lid and analyzer will start automatically.
6. The measurement will be finished when the heating is automatically shut off, the time is no longer running, the temperature is cooling, and the %DC is on the LED is shaded. There will also be an audible "beep" when the analyzer is finished.
7. Results will appear on the screen as % DC (dry content), record information.

HPLC Mash Composition

PRINCIPLE OF METHOD: By running samples using high pressure liquid chromatography, many different components of the sample can be detected and measured. These components include dextrin (DP4+), maltotriose (DP3), maltose (DP2), glucose (DP1), lactic acid, glycerol, acetic acid, and ethanol. A set of standards, which include these eight chemicals in various known concentrations, is prepared every three months. The standards are run with every sequence and are used to establish a calibration line for each of the eight components. By processing the data, the values of these components are automatically reported for each of the samples.

APPARATUS:

Dell Computer with Shimadzu Class VP software

Shimadzu HPLC

LC-20AT (Pump), DGU-20 A3 (Degasser), CBM-20A (Control Tower), SIL-20AC HT (Auto Sampler), RID-10A (Refractive Index), CTO-20A (Oven)

REAGENTS: 0.005 N Sulfuric Acid (Mobile Phase) and Methanol Needle Wash Solution

PROCEDURE:

Pre-Sample Check:

- Check waste bottle, mobile phase, and needle wash solution.

- Make sure that the pump LC-20AT light is green and verify that psi is at about 300.
- Make sure that the oven CTO-20A light is green and verify that it is at 80 degrees actual

- Make sure that green lights are lit under the following equipment:
    - DGU-20 A3 control light
    - LC-20AT remote and pump light
    - CBM-20A connect light
    - SIL-20AC HT remote and cooler
    - RID-10A temp cont and remote
    - CTO-20A oven, remote, and ready
    - Check hoses
    - If RID-10A is not balanced at 0, On software: control tab → direct control → Zero (Dir A)

On Hardware:

- Open door of SIL-20 AC HT → pull out tray → remove lid → place vial in open slot and record the location→ replace lid → replace tray back in appropriate location.

On Software:

- Click on the blue arrow on the toolbar to start a single run.
- Under run information, input the date, sample type, time, and user initials into the sample id box. Example is what is indicated between " ".

  Ex: "04012008 FERM1 0800 AK"

  Date: MMDDYYYY (01012008, 04152008, 12252008, etc.)

  Sample Type: PROP, FERM1, FERM2, FERM3, FERM4, BEERWELL

Time: TTTT (This will be the time of fermentation: 0800, 1600, 2400, 3200, etc.)

User Initials: AK, TCB, PL, etc.

- Make sure that the number of reps is set at 1.
- Make sure that the print method report box is checked.
- Under Autosampler, insert vial number from sample tray.
- Click the start button, on CBM-20A check to make sure that green light under run is lit. Sample will take 25 minutes to run, report will print at conclusion.

Appendix D – Compositional Analysis of DDGS

Procedures for the compositional analysis of the DDGS samples collected during the baseline and enzyme addition phases of the plant trial at Center Ethanol, LLC.

Moisture

Moisture values of DDGS samples were determined by drying 2 g samples at 135°C for 2 hr using American Association of Cereal Chemist's (AACC) method 44-19.

Ash

The ash content was determined as outlined in AACC method 44-19 by heating DDGS samples in a muffle furnace at 550°C until a light gray ash is obtained (16-20 hr).

Oil

Approximately 1 g of sample was extracted with 40 mL of hexane by continuous stirring of the slurry at room temperature for 1 hr. The slurry was filtered through a sintered glass funnel and the filtrate was evaporated under a stream of nitrogen and the oil content determined gravimetrically in (1).

(1) Moreau, R., K. Wayns, R. Flores, and K. Hicks. 2007. Tocopherols and tocotrienols in barley oil prepared from germ and other fractions from scarification and sieving of hulless barley. *Cereal Chemistry*. 84(6):587-592.

Starch

DDGS samples (100 mg) were analyzed using a starch determination kit (Megazyme International Ireland Ltd., Bray Business Park, Bray, Co. Wicklow, Ireland) in accordance to the International Code Council (ICC) Standard Method no. 168, AACC method 32-32 and Association of Official Agricultural Chemists' (AOAC) method 46-30. This method was modified by use of a YSI 2700 Analyzer (YSI Incorporated, Yellow Springs, Ohio) fitted with a YSI 2710 turntable for automated glucose determination of enzymatically hydrolyzed starch containing samples.

Protein

The protein content of DDGS samples was determined in accordance with AOAC method 990.03, and AACC method 46-30 which outline the procedure for use of a combustion instrument and subsequent thermal conductivity detection of nitrogen for the estimation of protein using an appropriate conversion factor. A Flash EA 1112 Elemental Analyzer (CE Elantech Inc., 170 Oberlin Ave., Lakewood, NJ), calibrated with aspartic acid (%N 10.52) was used for the protein determinations. Samples sizes of 50-100 mg were run and the conversion factor used to obtain protein values for barley was 6.25 as outlined in AOAC method 14.067.

β-Glucan

Barley β-glucan was analyzed using a kit (Megazyme International Ireland Ltd., Bray Business Park, Bray, Co. Wicklow, Ireland) in accordance to ICC standard method no. 168 and instructions for the "streamlined method" provided by the manufacturer. This method conforms to AOAC method 995.16 and AACC method 32-23.

Neutral Detergent Fiber

NDF was determined with an Ankom 2000 fiber analyzer (Ankom Technology, 2052 O'Neil Road, Macedon, NY). Fiber was analyzed as per the methods supplied by the manufacturer. About 0.5 g of sample are sealed in a F57 filter bag and extracted with neutral detergent reagent supplied by Ankom. The filter bags are dried and weights are determined. The filter bag technology and extraction process for Crude Fiber is an approved AOAC method 962.09 for the analysis of feeds and grains.

Neutral Detergent Insoluble Crude Protein

NDICP is determined by measuring the protein content of DDGS samples that have been extracted for the determination of NDF. The protein that is insoluble after the neutral detergent extraction process is measured using the same methodology as for total protein. The dried filter bags are simply opened and insoluble material contained within is sampled (50-100 mg) for analysis.

Non Fiber Carbohydrate

NFC is defined as 100% dry matter minus % Crude Protein (CP) minus %Neutral Detergent Fiber (NDF) corrected for neutral detergent fiber insoluble crude protein (NDFICP) less the %Fat and %Ash as outlined in (2).

(2) Hall, M. 2003. Challenges with nonfiber carbohydrate methods. *Journal of Animal Science*. 81:3226-3232. B).

Appendix E – Centrifuge Mass Balance Program

Below is a copy of a Matlab® script used for mass balance calculations. Program was used to calculate outlet flowrate and the ratio of inlet to outlet flowrates, as well as to plot inlet and outlet flowrates and the ratio between the two. A graphical user interface (GUI) was developed to automate both the importing of data collected from the DCS of the plant, and the calculations described in Section 6.3.4.1.

SCRIPT:

```
function varargout = Centrifuge_Mass_Balance_Calculator(varargin)
% TO USE THIS GUI, ENTER THE FOLLOWING ON THE COMMAND LINE:
%   Centrifuge_Mass_Balance_Calculator
%
% Last Modified by GUIDE v2.5 09-Nov-2008 18:49:04

% THIS IS THE INITIALIZATION FUNCTION
% ********************************** DO NOT EDIT ********************************** %
gui_Singleton = 1;
gui_State = struct('gui_Name',       mfilename, ...
                   'gui_Singleton',  gui_Singleton, ...
                   'gui_OpeningFcn', @Centrifuge_Mass_Balance_Calculator_OpeningFcn, ...
                   'gui_OutputFcn',  @Centrifuge_Mass_Balance_Calculator_OutputFcn, ...
                   'gui_LayoutFcn',  [] , ...
                   'gui_Callback',   []);
if nargin && ischar(varargin{1})
    gui_State.gui_Callback = str2func(varargin{1});
end

if nargout
    [varargout{1:nargout}] = gui_mainfcn(gui_State, varargin{:});
else
    gui_mainfcn(gui_State, varargin{:});
end
% ********************************************************************************** %

% THIS FUNCTION ACTIVATES JUST BEFORE WE SEE THE GUI APPEAR ON THE SCREEN.
% ************ ONLY EDIT DEFAULT NUMBERS FOR SPECIFIC GRAVITY AND DELTA P ************ %
function Centrifuge_Mass_Balance_Calculator_OpeningFcn(hObject, eventdata, handles,
varargin)
handles.output = hObject;
% INITIALIZE PERCENT OPEN VARIABLE
handles.percentopen = [];
% INITIALIZE SPECIFIC GRAVITY VARIABLE
handles.specificgravity = 1.015;
```

```
% INITIALIZE VALVE INLET PRESSURE VARIABLE
handles.p1 = 35;
% INITIALIZE VALVE OUTLET PRESSURE VARIABLE
handles.p2 = 28;
% PUT INITIALIZED VARIABLES IN THE GUI
set(handles.edit_specificgravity,'String',num2str(handles.specificgravity));
set(handles.edit_p1,'String',num2str(handles.p1));
set(handles.edit_p2,'String',num2str(handles.p2));
guidata(hObject, handles);
% ********************************************************************************** %

% THIS FUNCTION HANDLES ANY OUTPUTS FROM THE GUI TO THE COMMAND LINE AND IS NOT USED
% ********************************** DO NOT EDIT ********************************** %
function varargout = Centrifuge_Mass_Balance_Calculator_OutputFcn(hObject, eventdata,
handles)
varargout{1} = handles.output;
% ********************************************************************************** %

% THESE FUNCTIONS CREATE SOME OF THE GUI OBJECTS AND NEED TO BE LEFT IN
% ********************************** DO NOT EDIT ********************************** %
function edit_inletinputfile_CreateFcn(hObject, eventdata, handles)
if ispc && isequal(get(hObject,'BackgroundColor'),
get(0,'defaultUicontrolBackgroundColor'))
  set(hObject,'BackgroundColor','white'); end
function edit_specificgravity_CreateFcn(hObject, eventdata, handles)
if ispc && isequal(get(hObject,'BackgroundColor'),
get(0,'defaultUicontrolBackgroundColor'))
  set(hObject,'BackgroundColor','white'); end
function edit_p1_CreateFcn(hObject, eventdata, handles)
if ispc && isequal(get(hObject,'BackgroundColor'),
get(0,'defaultUicontrolBackgroundColor'))
  set(hObject,'BackgroundColor','white'); end
function edit_p2_CreateFcn(hObject, eventdata, handles)
if ispc && isequal(get(hObject,'BackgroundColor'),
get(0,'defaultUicontrolBackgroundColor'))
  set(hObject,'BackgroundColor','white'); end
% ********************************************************************************** %

% THESE FUNCTIONS ARE NOT USED BECAUSE WE NEVER MANUALLY EDIT THESE BOXES
function edit_inletinputfile_Callback(hObject, eventdata, handles)
function edit_outletinputfile_Callback(hObject, eventdata, handles)

% THIS FUNCTION ACTIVATES WHEN THE 'BROWSE...' BUTTON IS PUSHED FOR THE INLET
function pushbutton_browseinletinputfile_Callback(hObject, eventdata, handles)
  % 'UIGETFILE' BRINGS UP THE FILE DIALOG TO ALLOW THE USER TO CHOOSE THE FILE THAT
  % CONTAINS THE TEST DATA. 'FILE' HOLDS THE FILENAME, 'PATH' HOLDS THE PATHNAME, AND
  % FILTER IS ASSIGNED A NUMBER BASED ON WHETHER THE USER CHOOSES 'OPEN' OR 'CANCEL' IN
  % THE DIALOG. IF THE USER CHOOSES 'CANCEL' (FILTER == 0) THEN THE GUI DOES NOTHING.
  % OTHERWISE, THE GUI PUTS THE FULL PATH NAME INTO THE 'INPUT FILE' BOX, AND READS THE
  % EXCEL SPREADSHEET TO RETRIEVE THE DATA.
  %
  % ********** UNCOMMENT THE FOLLOWING LINE TO USE EXCEL INPUT FILE **********
  [inletFile, inletPath, filter] = uigetfile('*.xls', 'Pick Excel File Containing Data');
  % **************************************************************************
  % ********** UNCOMMENT THE FOLLOWING LINE TO USE TEXT INPUT FILE **********
%   [inletFile, inletPath, filter] = uigetfile('*.txt', 'Pick Text File Containing
Data');
  % **************************************************************************
  if filter ~= 0
    % THE FOLLOWING STATEMENT SETS THE STRING PROPERTY OF THE EDIT BOX TO THE FULL PATH
    % NAME OF THE FILE CHOSEN BY THE USER. THE '[inletPath inletFile]' STATEMENT
CONCATENATES THE
    % TWO STRINGS TOGETHER TO FORM ONE LONG STRING.
    set(handles.edit_inletinputfile,'String',[inletPath inletFile]);
    %
    % IF USING EXCEL INPUT FILE:
```

```
    % --> 'Sheet1' CAN BE CHANGED TO REFLECT THE CORRECT SHEET CONTAINING THE DATA
    % --> 'A1:B100' CAN BE CHANGED TO REFLECT THE CORRECT RANGE CONTAINING THE DATA
    % ********** UNCOMMENT THE FOLLOWING THREE LINES TO USE EXCEL INPUT FILE **********
    [num, txt] = xlsread([inletPath inletFile],'cent inlet flow','A1:B65536');
    inletdatetime = txt(:,1);
    handles.inletQ = num(:,1);
    [num, txt] = xlsread([inletPath inletFile],'%open valve','A1:B65536');
    outletdatetime = txt(:,1);
    handles.percentopen = num(:,1)/100;
    % ********************************************************************************
    %
    [inletdate,inlettime] = strtok(inletdatetime);
    [inlettime,inletampm] = strtok(inlettime);
    % PARSE THE DATE CELL ARRAY TO CREATE USABLE DATE DATA
    [MM,inletdate] = strtok(inletdate,'/');
    [DD,inletdate] = strtok(inletdate,'/');
    [YY,inletdate] = strtok(inletdate,'/');
    clear inletdate;
    inletdate = [str2double(YY) str2double(MM) str2double(DD)];
    % PARSE THE TIME CELL ARRAY TO CREATE USABLE TIME DATA
    [H,inlettime] = strtok(inlettime,':');
    [M,inlettime] = strtok(inlettime,':');
    clear inlettime;
    inlettime = [str2double(H)+str2double(M)/60];
    % CREATE THE TIME VECTOR
    inlettimeMultiplier = 0;
    if inletampm{1,1} == ' PM' & str2double(H{1,1}) < 12
      inlettimeVector(1,1) = [inlettimeMultiplier*24+inlettime(1)+12];
    elseif inletampm{1,1} == ' AM' & str2double(H{1,1}) == 12
      inlettimeVector(1,1) = [inlettimeMultiplier*24+inlettime(1)-12];
    else
      inlettimeVector(1,1) = [inlettimeMultiplier*24+inlettime(1)];
    end
    for i = 2:length(inletdate)
      if inletdate(i,3) ~= inletdate(i-1,3)
        inlettimeMultiplier = inlettimeMultiplier + 1;
      end
      if inletampm{i,1} == ' PM' & str2double(H{i,1}) < 12
        inlettimeVector(i,1) = [inlettimeMultiplier*24+inlettime(i)+12];
      elseif inletampm{i,1} == ' AM' & str2double(H{i,1}) == 12
        inlettimeVector(i,1) = [inlettimeMultiplier*24+inlettime(i)-12];
      else
        inlettimeVector(i,1) = [inlettimeMultiplier*24+inlettime(i)];
      end
    end
    % SAVE THE TIME AND PERCENT OPEN VARIABLES TO THE HANDLES STRUCTURE
    handles.inlettime = inlettimeVector;
%     handles.inletQ = flowrate;
    [outletdate,outlettime] = strtok(outletdatetime);
    [outlettime,outletampm] = strtok(outlettime);
    % PARSE THE DATE CELL ARRAY TO CREATE USABLE DATE DATA
    [MM,outletdate] = strtok(outletdate,'/');
    [DD,outletdate] = strtok(outletdate,'/');
    [YY,outletdate] = strtok(outletdate,'/');
    clear outletdate;
    outletdate = [str2double(YY) str2double(MM) str2double(DD)];
    % PARSE THE TIME CELL ARRAY TO CREATE USABLE TIME DATA
    [H,outlettime] = strtok(outlettime,':');
    [M,outlettime] = strtok(outlettime,':');
    clear outlettime;
    outlettime = [str2double(H)+str2double(M)/60];
    % CREATE THE TIME VECTOR
    outlettimeMultiplier = 0;
    if outletampm{1,1} == ' PM' & str2double(H{1,1}) < 12
      outlettimeVector(1,1) = [outlettimeMultiplier*24+outlettime(1)+12];
    elseif outletampm{1,1} == ' AM' & str2double(H{1,1}) == 12
```

```
      outlettimeVector(1,1) = [outlettimeMultiplier*24+outlettime(1)-12];
    else
      outlettimeVector(1,1) = [outlettimeMultiplier*24+outlettime(1)];
    end
    for i = 2:length(outletdate)
      if outletdate(i,3) ~= outletdate(i-1,3)
        outlettimeMultiplier = outlettimeMultiplier + 1;
      end
      if outletampm{i,1} == ' PM' & str2double(H{i,1}) < 12
          outlettimeVector(i,1) = [outlettimeMultiplier*24+outlettime(i)+12];
      elseif outletampm{i,1} == ' AM' & str2double(H{i,1}) == 12
          outlettimeVector(i,1) = [outlettimeMultiplier*24+outlettime(i)-12];
      else
        outlettimeVector(i,1) = [outlettimeMultiplier*24+outlettime(i)];
      end
    end
    % SAVE THE TIME AND PERCENT OPEN VARIABLES TO THE HANDLES STRUCTURE
    handles.outlettime = outlettimeVector;
    %
    % THE FOLLOWING STATEMENT UPDATES THE HANDLES STRUCTURE (WHERE ALL THE DATA IS STORED
    % TO BE ACCESSED LATER) WITH THE NEW VARIABLES WE CREATED HERE --> 'handles.data'
    guidata(hObject, handles);
  end

% THIS FUNCTION ACTIVATES WHEN THE USER ENTERS A NUMBER IN THE 'SPECIFIC GRAVITY' BOX
function edit_specificgravity_Callback(hObject, eventdata, handles)
  % WE ARE STORING THE SPECIFIC GRAVITY VARIABLE INTO THE HANDLES STRUCTURE. THE 'GET'
  % COMMAND GETS THE STRING PROPERTY OF THE SPECIFIC GRAVITY EDIT BOX. THE 'STR2DOUBLE'
  % COMMAND CONVERTS THE STRING TO A NUMBER.
  handles.specificgravity = str2double(get(handles.edit_specificgravity,'String'));
  % THE FOLLOWING STATEMENT UPDATES THE HANDLES STRUCTURE WITH THE NEW VARIABLES WE
  % CREATED HERE --> 'handles.specificgravity'
  guidata(hObject, handles);

% THIS FUNCTION ACTIVATES WHEN THE USER ENTERS A NUMBER IN THE 'VALVE INLET PRESSURE' BOX
function edit_p1_Callback(hObject, eventdata, handles)
  handles.p1 = str2double(get(handles.edit_p1,'String'));
  % THE FOLLOWING STATEMENT UPDATES THE HANDLES STRUCTURE WITH THE NEW VARIABLES WE
  % CREATED HERE --> 'handles.p1'
  guidata(hObject, handles);

% THIS FUNCTION ACTIVATES WHEN THE USER ENTERS A NUMBER IN THE 'VALVE OUTLET PRESSURE'
BOX
function edit_p2_Callback(hObject, eventdata, handles)
  handles.p2 = str2double(get(handles.edit_p2,'String'));
  % THE FOLLOWING STATEMENT UPDATES THE HANDLES STRUCTURE WITH THE NEW VARIABLES WE
  % CREATED HERE --> 'handles.p2'
  guidata(hObject, handles);

% THIS FUNCTION ACTIVATES WHEN THE 'PLOT' BUTTON IS PUSHED
function pushbutton_plot_Callback(hObject, eventdata, handles)
   % CHECK TO MAKE SURE THERE IS DATA IN THE FLOWRATE VARIABLE
   if isempty(handles.flowrate)
     msgbox('There was no inlet flowrate data','ERROR!','error');
     return;
   end
   % CHECK TO MAKE SURE THERE IS DATA IN THE PERCENT OPEN VARIABLE
   if isempty(handles.percentopen)
     msgbox('There was no outlet percent open data','ERROR!','error');
     return;
   end
   % CHECK TO MAKE SURE THERE IS DATA IN THE SPECIFIC GRAVITY VARIABLE
   if isempty(handles.specificgravity)
     msgbox('There was no specific gravity entered','ERROR!','error');
     return;
   end
```

```
 % CHECK TO MAKE SURE THERE IS DATA IN THE INLET PRESSURE VARIABLE
 if isempty(handles.p1)
     msgbox('There was no valve inlet pressure entered','ERROR!','error');
   return;
 end
 % CHECK TO MAKE SURE THERE IS DATA IN THE OUTLET PRESSURE VARIABLE
 if isempty(handles.p2)
     msgbox('There was no valve outlet pressure entered','ERROR!','error');
   return;
 end
% THERE WAS DATA, SO CONTINUE
% ******************
% PLOT INLET FLOWRATE
% ******************
% --> TELL MATLAB WHICH GUI AXIS WE WANT TO USE
axes(handles.axes_inletplot);
% --> TELL MATLAB TO PLOT THE DATA
plot(handles.inlettime,handles.inletQ,'-');
% --> TELL MATLAB THE TITLE TO USE FOR THE PLOT
title('Inlet Flowrate vs Time');
% --> TELL MATLAB THE LABEL FOR THE X AXIS
xlabel('Time (hrs)');
% --> TELL MATLAB THE LABEL FOR THE Y AXIS
ylabel('Flowrate (gpm)');
% --> TURN ON THE GRID
grid on;
% ******************
% PLOT OUTLET FLOWRATE
% ******************
% CALCUATE CV
handles.cv = 276.18 * handles.percentopen + 0.5091;
% CALCULATE DELTA P
handles.deltap = abs(handles.p1 - handles.p2);
% CALCULATE Q
handles.outletQ = handles.cv * sqrt(handles.deltap / handles.specificgravity);
% PLOT Q VERSUS TIME
% --> TELL MATLAB WHICH GUI AXIS WE WANT TO USE
axes(handles.axes_outletplot);
% --> TELL MATLAB TO PLOT THE DATA
plot(handles.outlettime,handles.outletQ,'-');
% --> TELL MATLAB THE TITLE TO USE FOR THE PLOT
title('Outlet Flowrate vs Time');
% --> TELL MATLAB THE LABEL FOR THE X AXIS
xlabel('Time (hrs)');
% --> TELL MATLAB THE LABEL FOR THE Y AXIS
ylabel('Flowrate (gpm)');
% --> TURN ON THE GRID
grid on;
% ********************************************
% PLOT RATIO OF (OUTLET FLOWRATE)/(INLET FLOWRATE)
% ********************************************
% CALCULATE RATIO Q
handles.ratioQ = handles.outletQ./handles.inletQ;
% --> TELL MATLAB WHICH GUI AXIS WE WANT TO USE
axes(handles.axes_ratioplot);
% --> TELL MATLAB TO PLOT THE DATA
plot(handles.inlettime,handles.ratioQ,'-');
% --> TELL MATLAB THE TITLE TO USE FOR THE PLOT
title('Flowrate Ratio (Outlet/Inlet) vs Time');
% --> TELL MATLAB THE LABEL FOR THE X AXIS
xlabel('Time (hrs)');
% --> TELL MATLAB THE LABEL FOR THE Y AXIS
ylabel('Flowrate Ratio (Outlet/Inlet)');
% --> TURN ON THE GRID
grid on;
assignin('base','inlettime',handles.inlettime);
```

```
assignin('base','outlettime',handles.outlettime);
assignin('base','inletQ',handles.inletQ);
assignin('base','outletQ',handles.outletQ);
assignin('base','ratioQ',handles.ratioQ);
guidata(hObject, handles);
```

Appendix F – Plant Trial Process Parameters

Table F.1: Summary of process parameters recorded during the baseline part of the trial

Area of Ethanol Plant	Parameter	Values
Grain Handling and Milling	3. amount of corn	132 klb/hr
	4. amount of water	33.5% Solids
Starch to Sugar Conversion	6. amount of urea	2900 mL/min
	7. amount of α-amylase	140 mL/min
	8. amount of gluco-amylase	400 mL/min
	9. amount of sulfuric acid	2600 mL/min
	10. amount of urea	2600 mL/min
Fermentation	1. fermentor volume	695000 gal
	2. fermentor temperature	33ºC
	3. fermentor pH	5.8
	4. fermentor pressure	N/A
	5. fermentor residence time	Approx. 50 hrs
	6. fermentor inlet mash flowrate	667 gpm
	7. amount of yeast	20 kg/fermentor
	8. fermentor outlet flowrate	N/A
	9. ethanol production rate	60233 gal/day
Co-Product Processing	1. centrifuge inlet flowrates	118.5 gpm
	2. centrifuge residence time	N/A
	3. centrifuge liquid flowrates	N/A
	4. centrifuge solid flowrates	N/A
	5. evaporator inlet flowrates	266 gpm to 3rd effect
	6. evaporator recycle flowrates	2700 gpm
	7. dryer inlet flowrate	N/A
	8. dryer temperature	358ºC
	9. dryer residence time	N/A
	10. dryer outlet flowrate	N/A
	11. dryer energy input	3600 scfh
	12. DDGS production rate	N/A

Appendix G – Process Simulation Equipment Report

List of all equipment used in the simulation. Table obtained from the Equipment Report (EQR) created by SuperPro Designer® for the *40MGY_March08_v75_version_J* simulation.

Table G.1: List of all unit operations used in the simulation of the enzymatic dewatering process.

Name	Type	Units	Standby/ Staggered	Size (Capacity)		Material of Construction	Purchase Cost ($/Unit)
101MH	Belt Conveyor	1	0/0	100.00	m	CS	121,000
102V	Silo/Bin	1	0/0	18,538.87	m^3	CS	979,000
104M	Grinder	1	0/0	46,208.14	kg/h	CS	98,000
105V	Receiver Tank	1	0/0	76.90	m^3	CS	32,000
106W	Hopper	1	0/0	100.92	m^3	CS	51,000
107V	Receiver Tank	1	0/0	76.90	m^3	CS	44,000
307V	Blending Tank	1	0/0	43.54	m^3	SS304	130,000
305V	Hopper	1	0/0	4.02	m^3	CS	9,000
303V	Receiver Tank	1	0/0	8.77	m^3	CS	28,000
301V	Receiver Tank	1	0/0	12.19	m^3	SS304	50,000
302P	Gear Pump	1	0/0	0.20	kW	SS316	4,000
310V	Blending Tank	1	0/0	144.28	m^3	SS304	163,000
321V	Stirred Reactor	1	0/0	53.15	m^3	SS316	104,000
317V	Receiver Tank	1	0/0	17.58	m^3	SS304	84,000
319V	Receiver Tank	1	0/0	18.86	m^3	CS	19,000
401E	Heat Exchanger	1	0/0	202.06	m^2	SS304	288,000
402E	Heat Exchanger	1	0/0	193.66	m^2	SS304	86,000
404P	Gear Pump	1	0/0	0.06	kW	SS316	7,000
405V	Fermentor	1	0/0	10,651.01	m^3	SS316	2,844,000
409V	Absorber	1	0/0	13.41	m^3	SS304	91,000
410P	Centrifugal Pump	1	0/0	2.88	HP-E	CS	7,000
413E	Heat Exchanger	1	0/0	238.27	m^2	SS304	335,000
608T	Receiver Tank	1	0/0	507.49	m^3	CS	99,000
411P	Centrifugal Pump	1	0/0	50.00	HP-E	SS316	16,000

501T	Distillation Column	1	0/0	97.89	m^3	SS304	602,000
MX-101	Mixer	1	0/0	34,560.48	kg/h	CS	0
503T	Distillation Column	1	0/0	113.57	m^3	SS304	254,000
507T	Distillation Column	1	0/0	3.80	m^3	SS304	170,000
511V	Flat Bottom Tank	1	0/0	481.39	m^3	SS304	93,000
509V	Flat Bottom Tank	1	0/0	339.24	m^3	SS304	34,000
513V	Flat Bottom Tank	1	0/0	3,392.22	m^3	SS304	308,000
601V	Blending Tank	1	0/0	755.41	m^3	SS304	197,000
605V	Blending Tank	1	0/0	481.39	m^3	SS304	230,000
604MH	Belt Conveyor	1	0/0	100.00	m	CS	56,000
610D	Rotary Dryer	1	0/0	1,173.80	m^2	SS304	2,200,000
612MH	Belt Conveyor	1	0/0	100.00	m	CS	123,000
FSP-101	Flow Splitter	1	0/0	74,359.68	kg/h	CS	0
MX-103	Mixer	1	0/0	14,701.92	kg/h	CS	0
MX-104	Mixer	1	0/0	138,721.96	kg/h	CS	0
313E	Heat Exchanger	1	0/0	33.66	m^2	SS304	13,000
312E	Heat Exchanger	1	0/0	383.48	m^2	SS304	219,000
304P	Gear Pump	1	0/0	0.25	HP-E	SS316	4,000
318P	Gear Pump	1	0/0	0.25	HP-E	SS316	4,000
320P	Gear Pump	1	0/0	0.02	kW	SS316	4,000
403V	Blending Tank	1	0/0	2.97	m^3	SS304	115,000
611X	Wet Air Oxidizer	1	0/0	12.21	m^3	CS	877,000
514P	Gear Pump	1	0/0	1.79	HP-E	SS316	40,000
510P	Gear Pump	1	0/0	5.00	HP-E	SS316	5,000
314V	Receiver Tank	1	0/0	14.16	m^3	SS304	174,000
412V	Flash Drum	1	0/0	14.62	m^3	SS304	62,000
408E	Condenser	1	0/0	59.49	m^2	SS304	19,000
316E	Heat Exchanger	1	0/0	413.84	m^2	SS304	614,000
308P	Centrifugal Pump	1	0/0	14.25	kW	SS316	25,000
311P	Centrifugal Pump	1	0/0	50.00	kW	SS316	15,000
322P	Centrifugal Pump	1	0/0	50.00	HP-E	SS316	15,000
406P	Centrifugal Pump	1	0/0	25.85	HP-E	SS316	76,000
407P	Centrifugal Pump	1	0/0	13.02	HP-E	SS316	15,000
502P	Centrifugal Pump	1	0/0	50.00	HP-E	SS316	13,000
506P	Centrifugal Pump	1	0/0	20.00	HP-E	SS316	7,000
508P	Centrifugal Pump	1	0/0	10.00	HP-E	SS316	5,000
505P	Centrifugal Pump	1	0/0	0.36	HP-E	SS316	4,000
606P	Centrifugal Pump	1	0/0	20.00	HP-E	SS316	11,000
602P	Centrifugal Pump	1	0/0	50.00	HP-E	SS316	13,000
609P	Centrifugal Pump	1	0/0	20.00	HP-E	SS316	12,000
512P	Centrifugal Pump	1	0/0	10.00	HP-E	SS316	5,000

315E	Heat Exchanger	1	0/0	179.12	m^2	CS	51,000
306P	Gear Pump	1	0/0	5.00	HP-E	SS316	4,000
MX-105	Mixer	1	0/0	15,048.64	kg/h	CS	0
309E	Heat Exchanger	1	0/0	39.30	m^2	CS	23,000
MX-102	Mixer	1	0/0	37,989.83	kg/h	CS	0
607Ev	Evaporator	1	0/0	620.09	m^2	SS304	3,202,000
103MH	Flow Splitter	1	0/0	46,347.19	kg/h	CS	61,000
504X	Component Splitter	1	0/0	19,469.66	kg/h	CS	1,720,000
603	Disk-Stack Centrifuge	1	0/0	1,777.57	L/min	SS316	864,000
split	Component Splitter	1	0/0	110,699.29	kg/h	SS316	0
MX-106	Mixer	1	0/0	22,581.06	kg/h	CS	0
FSP-103	Flow Splitter	1	0/0	88,118.23	kg/h	CS	0
MX-107	Mixer	1	0/0	88,118.23	kg/h	CS	0
406V	Receiver Tank	1	0/0	12.19	m^3	SS304	50,000
405P	Gear Pump	1	0/0	0.20	kW	SS316	4,000

Appendix H – Economic Evaluation Report

Economic evaluation report (EER) created by SuperPro Designer® for the modified dry grind corn to ethanol model.

Table H.1: Executive Summary

Total Capital Investment	57153000	$
Capital Investment Charged to This Project	57153000	$
Main Product Rate	119185254	kg MP/yr
Operating Cost	79963000	$/yr
Unit Production Cost – Gallon of Ethanol	1.69	$/gal
Main Revenue	77470000	$/yr
Other Revenues	12659874	$/yr
Total Revenues	90130000	$/yr

Table H.2: Major equipment specification and FOB cost (2007 prices)

Quantity/ Standby/ Staggered	Name	Description	Unit Cost ($)	Cost ($)
1 / 0 / 0	101MH	Belt Conveyor Belt Length = 100.00 m	121,000	121,000
1 / 0 / 0	102V	Silo/Bin Vessel Volume = 18538.87 m^3	979,000	979,000
1 / 0 / 0	104M	Grinder Size/Capacity = 46208.14 kg/h	98,000	98,000
1 / 0 / 0	105V	Receiver Tank Vessel Volume = 76.90 m^3	32,000	32,000
1 / 0 / 0	106W	Hopper Vessel Volume = 100.92 m^3	51,000	51,000
1 / 0 / 0	107V	Receiver Tank Vessel Volume = 76.90 m^3	44,000	44,000
1 / 0 / 0	307V	Blending Tank Vessel Volume = 43.54 m^3	130,000	130,000
1 / 0 / 0	305V	Hopper Vessel Volume = 4.02 m^3	9,000	9,000
1 / 0 / 0	303V	Receiver Tank Vessel Volume = 8.77 m^3	28,000	28,000
1 / 0 / 0	301V	Receiver Tank Vessel Volume = 12.19 m^3	50,000	50,000
1 / 0 / 0	302P	Gear Pump Power = 0.20 kW	4,000	4,000
1 / 0 / 0	310V	Blending Tank Vessel Volume = 144.28 m^3	163,000	163,000
1 / 0 / 0	321V	Stirred Reactor Vessel Volume = 53.15 m^3	104,000	104,000
1 / 0 / 0	317V	Receiver Tank Vessel Volume = 17.57 m^3	84,000	84,000
1 / 0 / 0	319V	Receiver Tank Vessel Volume = 18.87 m^3	19,000	19,000
1 / 0 / 0	401E	Heat Exchanger	288,000	288,000

		Heat Exchange Area = 202.06 m^2		
1 / 0 / 0	402E	Heat Exchanger Heat Exchange Area = 193.66 m^2	86,000	86,000
1 / 0 / 0	404P	Gear Pump Power = 0.06 kW	7,000	7,000
1 / 0 / 0	405V	Fermentor Vessel Volume = 10651.01 m^3	2,844,000	2,844,000
1 / 0 / 0	409V	Absorber Absorber Volume = 13.41 m^3	91,000	91,000
1 / 0 / 0	410P	Centrifugal Pump Power = 2.88 HP-E	7,000	7,000
1 / 0 / 0	413E	Heat Exchanger Heat Exchange Area = 238.27 m^2	335,000	335,000
1 / 0 / 0	608T	Receiver Tank Vessel Volume = 507.49 m^3	99,000	99,000
1 / 0 / 0	411P	Centrifugal Pump Power = 50.00 HP-E	16,000	16,000
1 / 0 / 0	501T	Distillation Column Column Volume = 97.89 m^3	602,000	602,000
1 / 0 / 0	MX-101	Mixer Size/Capacity = 34560.48 kg/h	0	0
1 / 0 / 0	503T	Distillation Column Column Volume = 113.57 m^3	254,000	254,000
1 / 0 / 0	507T	Distillation Column Column Volume = 3.80 m^3	170,000	170,000
1 / 0 / 0	511V	Flat Bottom Tank Vessel Volume = 481.39 m^3	93,000	93,000
1 / 0 / 0	509V	Flat Bottom Tank Vessel Volume = 339.24 m^3	34,000	34,000
1 / 0 / 0	513V	Flat Bottom Tank Vessel Volume = 3392.22 m^3	308,000	308,000
1 / 0 / 0	601V	Blending Tank Vessel Volume = 755.41 m^3	197,000	197,000
1 / 0 / 0	605V	Blending Tank	230,000	230,000

		Vessel Volume = 481.39 m^3		
1 / 0 / 0	604MH	Belt Conveyor Belt Length = 100.00 m	56,000	56,000
1 / 0 / 0	610D	Rotary Dryer Drying Area = 1173.80 m^2	2,200,000	2,200,000
1 / 0 / 0	612MH	Belt Conveyor Belt Length = 100.00 m	123,000	123,000
1 / 0 / 0	FSP-101	Flow Splitter Size/Capacity = 74359.68 kg/h	0	0
1 / 0 / 0	MX-103	Mixer Size/Capacity = 14701.92 kg/h	0	0
1 / 0 / 0	MX-104	Mixer Size/Capacity = 138721.96 kg/h	0	0
1 / 0 / 0	313E	Heat Exchanger Heat Exchange Area = 33.66 m^2	13,000	13,000
1 / 0 / 0	312E	Heat Exchanger Heat Exchange Area = 383.47 m^2	219,000	219,000
1 / 0 / 0	304P	Gear Pump Power = 0.25 HP-E	4,000	4,000
1 / 0 / 0	318P	Gear Pump Power = 0.25 HP-E	4,000	4,000
1 / 0 / 0	320P	Gear Pump Power = 0.02 kW	4,000	4,000
1 / 0 / 0	403V	Blending Tank Vessel Volume = 2.97 m^3	115,000	115,000
1 / 0 / 0	611X	Wet Air Oxidizer Vessel Volume = 12.21 m^3	877,000	877,000
1 / 0 / 0	514P	Gear Pump Power = 1.79 HP-E	40,000	40,000
1 / 0 / 0	510P	Gear Pump Power = 5.00 HP-E	5,000	5,000
1 / 0 / 0	314V	Receiver Tank Vessel Volume = 14.16 m^3	174,000	174,000
1 / 0 / 0	412V	Flash Drum Vessel Volume = 14.62 m^3	62,000	62,000

1 / 0 / 0	408E	Condenser Condensation Area = 59.49 m^2	19,000	19,000
1 / 0 / 0	316E	Heat Exchanger Heat Exchange Area = 413.83 m^2	614,000	614,000
1 / 0 / 0	308P	Centrifugal Pump Power = 14.25 kW	25,000	25,000
1 / 0 / 0	311P	Centrifugal Pump Power = 50.00 kW	15,000	15,000
1 / 0 / 0	322P	Centrifugal Pump Power = 50.00 HP-E	15,000	15,000
1 / 0 / 0	406P	Centrifugal Pump Power = 25.85 HP-E	76,000	76,000
1 / 0 / 0	407P	Centrifugal Pump Power = 13.02 HP-E	15,000	15,000
1 / 0 / 0	502P	Centrifugal Pump Power = 50.00 HP-E	13,000	13,000
1 / 0 / 0	506P	Centrifugal Pump Power = 20.00 HP-E	7,000	7,000
1 / 0 / 0	508P	Centrifugal Pump Power = 10.00 HP-E	5,000	5,000
1 / 0 / 0	505P	Centrifugal Pump Power = 0.36 HP-E	4,000	4,000
1 / 0 / 0	606P	Centrifugal Pump Power = 20.00 HP-E	11,000	11,000
1 / 0 / 0	602P	Centrifugal Pump Power = 50.00 HP-E	13,000	13,000
1 / 0 / 0	609P	Centrifugal Pump Power = 20.00 HP-E	12,000	12,000
1 / 0 / 0	512P	Centrifugal Pump Power = 10.00 HP-E	5,000	5,000
1 / 0 / 0	315E	Heat Exchanger Heat Exchange Area = 179.12 m^2	51,000	51,000
1 / 0 / 0	306P	Gear Pump Power = 5.00 HP-E	4,000	4,000
1 / 0 / 0	MX-105	Mixer Size/Capacity = 15048.64 kg/h	0	0

1 / 0 / 0	309E	Heat Exchanger Heat Exchange Area = 39.30 m^2	23,000	23,000
1 / 0 / 0	MX-102	Mixer Size/Capacity = 37989.83 kg/h	0	0
1 / 0 / 0	607Ev	Evaporator Evaporation Area = 620.09 m^2	3,202,000	3,202,000
1 / 0 / 0	103MH	Flow Splitter Size/Capacity = 46347.19 kg/h	61,000	61,000
1 / 0 / 0	504X	Component Splitter Size/Capacity = 19469.66 kg/h	1,720,000	1,720,000
1 / 0 / 0	603	Disk-Stack Centrifuge Throughput = 1777.57 L/min	864,000	864,000
1 / 0 / 0	split	Component Splitter Size/Capacity = 110699.29 kg/h	0	0
1 / 0 / 0	MX-106	Mixer Size/Capacity = 22581.06 kg/h	0	0
1 / 0 / 0	FSP-103	Flow Splitter Size/Capacity = 88118.23 kg/h	0	0
1 / 0 / 0	MX-107	Mixer Size/Capacity = 88118.23 kg/h	0	0
1 / 0 / 0	406V	Receiver Tank Vessel Volume = 12.19 m^3	50,000	50,000
1 / 0 / 0	405P	Gear Pump Power = 0.20 kW	4,000	4,000
		Unlisted Equipment		0
			TOTAL	18,300,000

Table H.3: Direct fixed capital cost (DFC) summary (2007 prices in $)

Section Name	DFC ($)
Main Section	216,000
Grain Handling & Milling	4,158,000
Starch to Sugar Conversion	5,268,000
Fermentation	11,880,000
Ethanol Processing	9,779,000
CoProduct Processing	23,652,000
Common Support Systems	2,200,000
Plant DFC	57,153,000

Table H.4: Labor cost – process summary

Labor Type	Unit Cost ($/h)	Annual Amount (h)	Annual Cost ($)	%
Operator	0	0	0	0
Plant Operators	52	39,600	2,059,200	100
TOTAL		39,600	2,059,200	100

Table H.5: Material cost – process summary

Bulk Material	Unit Cost ($/kg)	Annual Amount (kg)	Annual Cost ($)	%
Trial Enzyme	10.00	257,139	2,571,386	4.51
Corn	0.14	367,069,713	50,563,853	88.77
Lime	0.09	438,190	39,437	0.07
Liq. Ammonia	0.22	733,337	161,334	0.28
Alpha-Amylase	2.25	257,139	578,562	1.02
Glucoamylase	2.25	371,408	835,669	1.47
Sulfuric Acid	0.11	733,337	80,667	0.14
Caustic	0.01	18,423,742	223,296	0.39
Yeast	1.86	96,466	179,426	0.31
Water	0.00	143,130,116	6,298	0.01
Octane	0.72	2,383,705	1,722,465	3.02
Air	0.00	252,066,822	0	0.00
TOTAL		785,961,112	56,962,393	100.00

Table H.6: Utilities cost (2007 prices) – process summary

Utility	Annual Amount	Reference Units	Annual Cost ($)	%
Electricity	29247864	kWh	1,462,393	11.58
Steam	4165920	kg	88,942	0.70
Cooling Water	5184932086	kg	518,493	4.11
Chilled Water[1]	0	kg	0	0.00
Natural Gas	10781874	kg	3,791,877	30.03
CT Water	5295747600	kg	370,702	2.94
CT Water NoCost	13179167159	kg	0	0.00
CT Water 35Cout	366029013	kg	25,622	0.20
CT Water 31Cout	3160422808	kg	221,230	1.75
Well Water[1]	0	kg	0	0.00
Steam 50 PSI	75063898	kg	1,602,614	12.69
Steam 6258 BTU	32753326	kg	699,284	5.54
Steam 2205 BTU	179103307	kg	3,823,856	30.28
Rectifier OH[1]	80089532	kg	0	0.00
Steam (High P)	1132400	kg	22,648	0.18
TOTAL			12,627,661	100.00

[1] Limitations in the SuperPro® program restrict the use of heat integration techniques. Utilities with 0 annual costs are computational techniques used to work around the program's limitations.

Table H.7: Annual operating cost (2007 prices) – process summary

Cost Item	Cost ($)	%
Raw Materials	56,962,000	71.24
Labor-Dependent	2,059,000	2.58
Facility-Dependent	8,313,000	10.40
Consumables[1]	0	0.00
Utilities	12,628,000	15.79
Advertising/Selling	0	0.00
Running Royalties	0	0.00
Failed Product Disposal	0	0.00
TOTAL	79,963,000	100.00

[1] Facility related consumables costs are included in these estimates at 0.75% per year of the facility costs and are included under "Facility-Dependent" costs. These include depreciation, maintenance and other facility related charges.

Table H.8: Profitability analysis (2007 prices)

A.	Direct Fixed Capital	57,153,000.00	$
B.	Working Capital	0.00	$
C.	Startup Cost	0.00	$
D.	Up-Front R&D	0.00	$
E.	Up-Front Royalties	0.00	$
F.	Total Investment (A+B+C+D+E)	57,153,000.00	$
G.	Investment Charged to This Project	57,153,000.00	$
H.	Revenue/Credit Stream Flowrates		
	Total flow in DDGS (Other Revenue)	121,181,553.00	kg/yr
	Total flow of stream ETHANOL (Main Revenue)	119,185,254.00	kg/yr
I.	Annual Operating Cost		
	AOC	79,963,000.00	$/yr
K.	Selling / Processing Price		
	Total flow in DDGS	0.10	$/kg
	Total flow of stream ETHANOL	0.65	$/kg
L.	Revenues		
	DDGS (Other Revenue)	12,660,000.00	$/yr
	ETHANOL (Main Revenue)	77,470,000.00	$/yr
	Total Revenues	90,130,000.00	$/yr

Appendix I – Sensitivity Analysis

Figure I.1: Sensitivity analysis results. Unit production cost of a gallon of ethanol ($/gal) at different natural gas prices.

		Conventional Model					
		Natural Gas Price ($/1000 ft^3)					
		4	6	7.5	10	12.5	15
Unit Production Cost		1.596	1.626	1.647	1.684	1.720	1.757

Figure I.2: Sensitivity analysis results. Unit production cost of a gallon of ethanol ($/gal) at different natural gas and enzyme prices.

		Enzymatic Dewatering Model					
		Natural Gas Price ($/1000 ft^3)					
		4	6	7.5	10	12.5	15
Enzyme Price ($/kg)	2	1.592	1.618	1.638	1.671	1.704	1.722
	4	1.605	1.631	1.651	1.684	1.716	1.749
	6	1.617	1.644	1.664	1.696	1.729	1.762
	8	1.630	1.657	1.676	1.709	1.742	1.775
	10	1.643	1.670	1.689	1.722	1.755	1.788

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