Project Number DDB-2012

Enzymatic Hydrolysis of Cellulosic Biomass for the Production of Second Generation Biofuels

A Major Qualifying Project Report
Submitted to the faculty of

Worcester Polytechnic Institute

In partial fulfillment of the requirements for the
Degree of Bachelor of Science

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Abstract:

The Apple pomace left over from the apple pressing process has shown to be a good substrate in the conversion process from cellulosic biomass into fermentable sugars for the production of second generation biofuels such as cellulosic ethanol. This fuel can then be used to replace fossil fuels in the transportation sector. This reaction is analyzed in a bench scale bioreactor, using commercial cellulase enzymes to break down the available cellulose in the apple pomace, wood shavings and swithchgrass feedstocks.

Introduction

Energy availability has been an issue since the Vietnam War. With the potential of fossil fuels, the most common energy source in use today, becoming depleted, new sources of energy are being developed. These new forms of energy include solar, wind, geothermal, nuclear, and other forms of renewable energy. Included within the renewable energy field lies that of using ethanol as either a fuel additive or as a fuel itself. There are several ways to produce this ethanol. Cellulose based materials can be broken down into individual sugars. These sugars can be futher broken down and fermented into ethanol. Breaking down the cellulose into sugars, predominantly glucose, can pose a challenge. One way to do so is by enzymatic hydrolysis. This uses enzymes, biological catalysts, to break down the cellulose polymers into monomeric sugars.

Enzymatic hydrolysis of cellulosic biomass is a key obstacle that must be overcome in the advancement of second generation biofuels technology. There are several steps involved in obtaining that usable fuel. First, the cellulose is obtained from plants and separated from the lignin present in the mixture. The cellulose is then broken down into 5 or 6-carbon sugars for conversion into the fuel source. Hydrolysis, or saccharification are processes utilized to break down long chain cellulose, starch, protein and fat molecules into smaller, fermentable molecules such as amino acids, fatty acids and simple sugars. This MQP will focus on the hydrolysis and pretreatment steps, and from there, design a process capable of saccharifying the cellulose into the fermentable sugars.

The hydrolysis step is the part of the process the project will focus most specifically on. The enzymatic hydrolysis reaction is carried out by means of enzymes that act as catalysts to break the glycosidic bonds. There are advantages to both types of reaction. The chemical catalytic hydrolysis is a faster acting reaction that requires much less residence time in the reactor. Enzymatic hydrolysis can be run at much lower temperatures which bring utility costs of a process down.

A notable biofuels production process which employs enzymatic hydrolysis has been optimized by the National Renewable Energy Laboratory (NREL). In their process, pre-treatment is necessary to break down the crystalline structure of the lignocellulosic material, isolating the cellulose away from the lignin in the cell walls for hydrolysis. The cellulose is then hydrolyzed with three types of cellulase enzymes: exoglucanase, endogluconase and betaglucosidase. These three enzymes have been utilized to streamline the process, reduce expenses and increase yields. (Zacchi, 2002)

The next step in each these biochemical conversion options, microbial fermentation, also known as Acetogenesis, is the process by which microorganisms most commonly *Saccharomyces cerevisiae,* also known as Baker or Brewer's yeast, use 6-carbon carbohydrates such as glucose for food. The sugars to feed the yeast can be derived from a variety of sources such as cellulose in the cell walls of plants obtained either from agricultural waste or dedicated energy crops such as switchgrass. Ethanol is produced in the metabolic process along with other by-products. The chemical reaction for the fermentation of glucose by yeast to form ethanol is given by:

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2$$

(Thomson, 2006). Considering fermentation is already a highly researched and tested technology, existing infrastructure will ease the transportation sector's transition into biochemical conversion of cellulosic biomass.

Bioethanol burns cleaner than unleaded gasoline, producing lower emissions of particulate matter and toxics. Production of cellulosic ethanol from agricultural or forestry residue can and conserve agricultural lands and reduce the pressure on landfills.

Background

What are Biofuels?

Biofuels are a source of energy derived from biomasses, such as corn, grains, and other plant life. There is currently a large push in research and development of these energy sources, as they are renewable as opposed to the currently used fossil fuels.

Cellulosic vs. Fossil Fuels

Currently, the majority of energy in the United States is produced by the fossil fuels coal, oil, and natural gas. Unfortunately, there have been problems with the fact that these resources are not renewable. There has been a recent push to develop a renewable energy souce with which to fuel energy needs, such as for vehicles and other personal use. Ethanol produced from corn is one such step. However, other forms of plants are able to produce much more energy from ethanol they produce. Figure 1 shows the ratios of energy input by fossil fuels against how much energy they are able to produce. Cellulosic ethanol sources produce more than ten times as much energy as is required to make them. This compares favorably to

corn ethanol, at 1.36 times. Coal and gasoline provide slightly less than the energy put into them, while electricity only produces half of the energy put in.

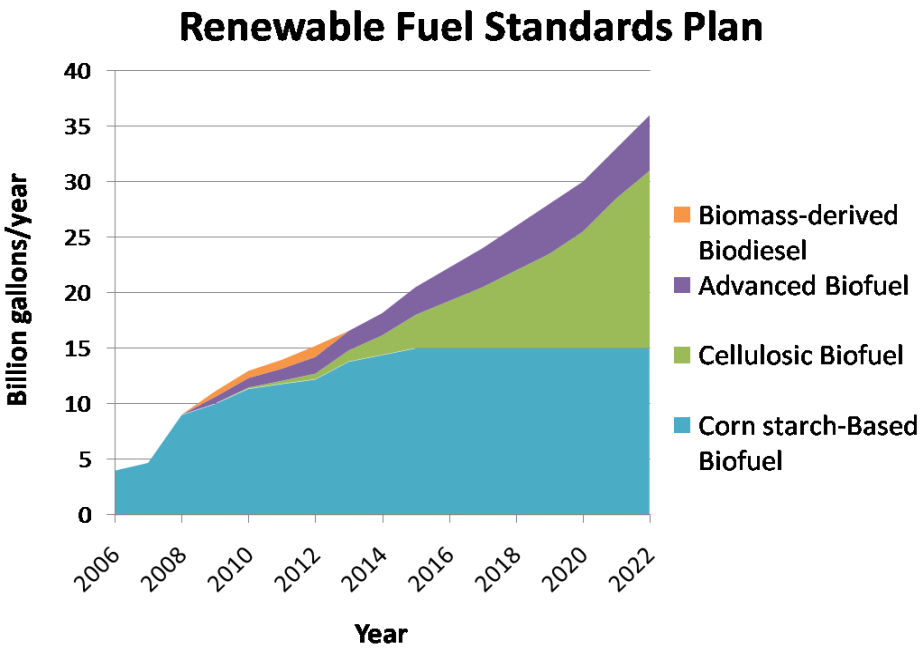

Figure 1: (EISA: 2007)

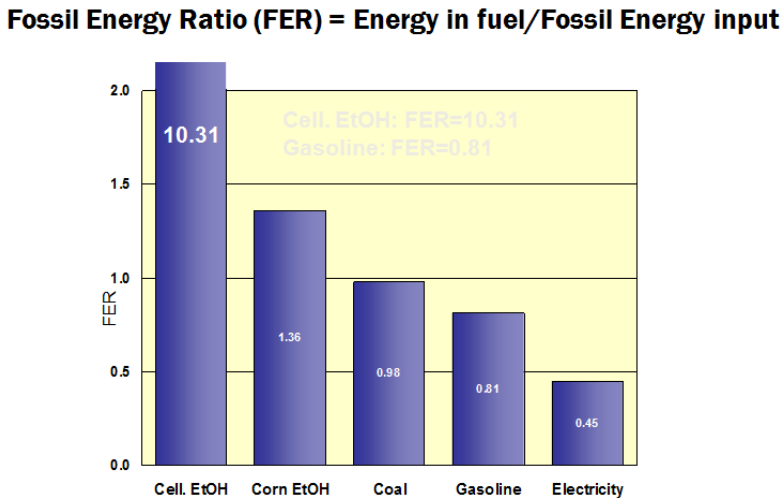

Figure 2: Ratio of the energy in Fuels to the Fossil energy input

(Energy and GHG, 2005)

What is Cellulose?

Cellulose and hemicellulose are the major materials that will be broken down through the trial runs. Cellulose is a long chained glucose polymer, connected by β(1→4)-glycosidic linkages with the chemical formula $(C_6H_{10}O_5)_n$, where "n" is the degree of polymerization of the polymer. This varies from starch and glycogen found in animals because those contain α(1→4) linkages between the individual units. The two types are differentiated by α-linkages occurring below the plane of the sugar, while the β-linkages occur above the plane. As the degree of polymerization increases, various properties of the cellulose also change. Typical ranges of cellulose chains in plants are anywhere from 300 to 10,000 units, varying from plant species to plant species.

Hemicellulose, the other major component of plant structure, is similar to cellulose in that it is a polymer of sugars. However, in addition to glucose, hemicelluloses are made up of additional sugars, including arabinose, xylose, mannose, and galactose. Hemicelluloses are also shorter than its cellulose counterpart, with a degree of polymerization around 200. This material is also branched in its polymerization, as opposed to the linear cellulose.

Sources of Cellulose

The goal of this project was to develop a process capable of converting a feedstock, in this case predominantly apple pomace, into fermentable sugars. The apple pomace used was donated by the Carlson Orchards located in Harvard, MA. This facility presses apples for the eventual production of juices and ciders. There is a good deal of biomass remaining after the pressing process that gets used either as compost or animal feed. This waste biomass has the

potential to be used as a sustainable energy source if converted to ethanol or another biofuel. The company, in looking for new ways to use this material, is looking into the potential for using this material as an energy source.

With other biomasses there must be a pretreatment step to separate the cellulose from lignin however apple pomace is shown to have a low concentration of lignin (Gullon, et al. 2007), therefore no separation pretreatment step will be used. The enzymatic hydrolysis step is the part of the process this project specifically focuses on. The enzymatic hydrolysis reaction is carried out by means of enzymes that act as catalysts to break the glycosidic bonds. There are advantages to both types of reaction. The chemical catalytic hydrolysis is a faster acting reaction that requires much less residence time in the reactor. Enzymatic hydrolysis can be run at much lower temperatures which bring utility costs of a process down. Enzymatic hydrolysis also allows for a very high rate of conversion. Acid hydrolysis, the other major method for saccharification of cellulose, is more equilibrium driven which allows for less conversion. Because of the combination of higher conversion and cheaper utility costs, enzymatic hydrolysis is the method of choice for this project. The eventual goal of this project would result in a bioreactor unit usable by local farms, operated by workers with limited knowledge of chemical and reactor engineering principles.

Why is Enzymatic Hydrolysis Important?

This process is very important because it reduces the strain put on the food industry from other forms of ethanol production. Instead of utilizing food crops to produce fuels, other forms of organic matter containing cellulose can be used. There are a variety of uses for the

sugars produces from this reaction; ethanol, acetic acid, amino acids, antibiotics, and other chemicals are all potential products of these sugars.

Alternative to petroleum products

The Energy Policy Act of 2005 requires that 250 million gallons of the renewable fuel consumed from 2013 and beyond be cellulosic ethanol. The act considers any fuel that "is derived from any lingocellulosic or hemicellulosic matter that is available on a renewable or recurring basis including dedicated energy crops and trees, wood and wood residues, plants, grasses, agricultural residues, fibers, animal wastes, and other waste materials and municipal solid waste." (Regulatory Impact, 2007) This act as well as other follow up legislation, provides a platform for cellulosic ethanol to incrementally replace fossil fuels in the transportation sector in the coming years.

Highly renewable resource

Cellulosic ethanol is also a better choice than corn ethanol because of the comparative accessibility and abundance of forest, agricultural, and other cellulosic resources which do not compete with existing food reserves. The traditional method of producing ethanol from grains such as corn and wheat sorghum is known as fermentation. This fermentation process uses some type of fossil fuel to heat the boilers in the distillation columns and power the process. New lignocellulosic biomass conversion processes can be mostly run on the otherwise wasted lignin byproduct, saving money, energy and the environment. (Bergman, 2008)

Significant breakthroughs/developments

A notable method employed to produce ethanol from lignocellulosic biomass is known as simultaneous saccharification and fermentation (SSF). The glucose and other fermentable

sugars are first produced in the saccharification step and then fermented in the next step. This is very promising for producing ethanol from lignocellulose due to its ability to improve hydrolysis rates, yields, and product concentrations compared to separate hydrolysis and fermentation (SHF) systems. Experiments using Genencor 150L cellulase and mixed yeast cultures have produced yields and concentrations of ethanol from cellulose of 80% and 4.5% respectively.

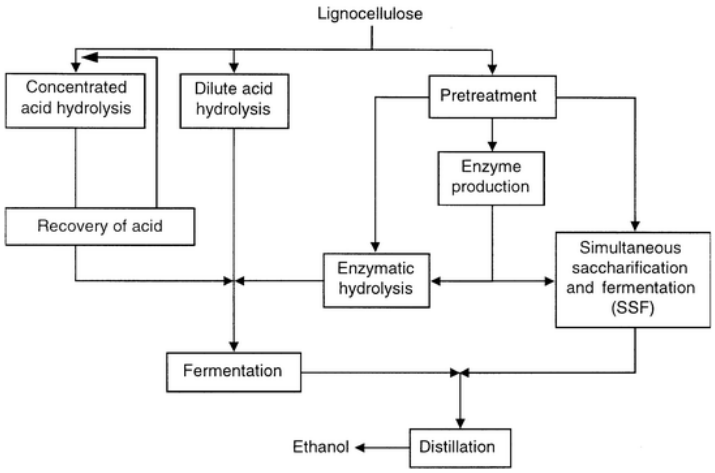

Figure 3: Cellulosic Ethanol Production Process

(Zacchi, 2002)

Reaction Pathway

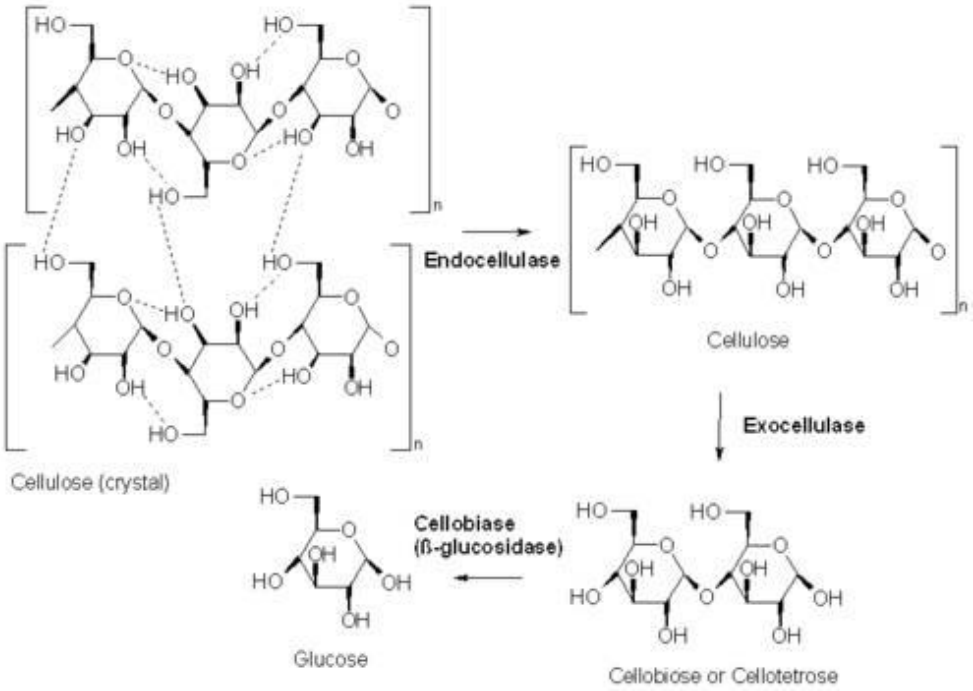

Figure 4: Reation pathway from cellulose to glucose (Enzymeindia, 2008)

The above reaction pathway shows the progression from the cellulose substrate to the glucose product through the activity of three enzymes: endocellulase, exocellulase and β-glucosidase. The endocellulase and exocellulase enzymes incrementally cleave cellobiose molecules off of the long chain polysaccharide molecules. This cellobiose intermediate then becomes the substrate in the hydrolysis reaction with β-glucosidase, producing the final glucose product.

Methodology

Experimentation consisted of the hydrolysis of substrates via several different enzymes. Glucose concentration was measured over time, while conditions such as temperature and pH were kept constant.

Materials used

There were various materials used throughout the experimental runs. Some of the feeds used during the experiments were: Apple pomace, (50 mL each): wood (poplar) with bark, and switch grass. The apple pomace used during the experiments was obtained at The Carlson Orchards[1] in Harvard, Ma. The apple pomace was used at several different amounts for the feed, varying from 24g to 80g. It was also used at different conditions such as rinsed and dried, and also apple pomace that had not been pretreated. With a 100g sample of apples, the majority of the composition is water, which is about 85 grams. Contents of more importance include the carbohydrates which consist of: 2210mg of glucose, 6040mg of fructose, 2470mg of sucrose, 600mg of starch, and 500mg of sorbit (Matthias Besler, 1999). Many background sugars are present in apple pomace that may affect the results of the experiments, which will be discussed later on in the results section. The figure below shows rinsed and dried apple pomace:

[1] Carlson Orchards, 115 Oak Hill Road, Harvard, MA 01451

Figure 5: Rinsed and dried apple pomace

The wood shavings were purchased at the pet store Pet Barn[2], and were already dried. Further pre-treatment, blending, was done to the wood shavings to increase the surface area for the experiement. The figure below is a mixed blend of wood shavings:

Figure 6: Mixed wood shavings

Switchgrass, provided from the Gateway Bioprocessing Center[3], was already pre-treated by blending, for the most productive conditions. No further pre-treatment was needed. Switchgrass has an extremely high initial amount of background sugars. The figure below shows blended switchgrass:

[2] Pet Barn, 310 Park Avenue, Worcester, MA 01609.
[3] WPI Life Sciences &Bioengineering Center, 60 Prescott Street, Worcester, MA 01609

Figure 7: Blended switchgrass

The enzymes used during the experiments were the Novozym 50010 β -glucosidase, Novozym 50013 cellulase, GC 230 cellulase. Both the Novozym and Genencor cellulases were used to convert the cellulose to cellobiose. Once there was cellobiose, the Novozym β-glucosidase would further convert it into glucose. This reaction pathway is also illustrated in Figure 2 (page 10).

Equipment

The equipment used for these experiments were located in the Unit Operations lab on the second floor of Goddard Hall. There are several different pieces of equipment that were used to create the experimental set-up, such as, the MultiGen agitator, pH controller, and temperature controller. Other equipment was used for pre-treatment steps and those included, the MicroFermentor, a larger scale bio reactor than the MultiGen, used to rinse the apple pomace. Another piece of equipment used for the pre-treatment in the Unit Operations lab was the oven, which was used to dry the apple pomace. A One-Touch blood glucose meter was used to measure the amount of glucose produced during the runs at various time points.

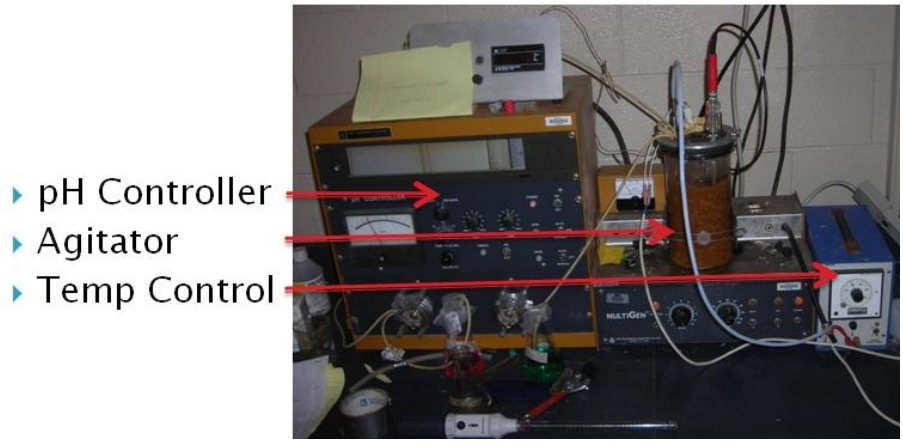

Figure 8: Bench scale bioreactor set-up

The MultiGen agitator came with both 1 liter and 2 liter beakers. The 1 liter beaker was used for some of the initial runs, then up-scaled to the 2 liter beaker. The pH controller would maintain the desired pH for each experiment, by adding a 4 or 9 pH buffer depending on feedback from the pH probe . The MultiGen agitator did come with its own built in water temperature control, but unfortunately it did not work. For a replacement temperature control, an electrical temperature controller was used. The controller had a temperature probe and a heating element that were submerged into the slurry; the probe would give a temperature reading, while the heating element provides the heat. The temperature controller would be set at a desired temperature and once the temperature started to stray away, the heating element would turn on and heat up the slurry until it reached the desired temperature once again.

The MicroFermentor was used for pre-treatment of the apple pomace, by way of rinsing. The apple pomace would need to be left rinsing for a few hours; afterwards it can be

filtered out. The oven was used for several hours to dry the apple pomace that was both rinsed and non-rinsed.

Experimental Analysis

The One-Touch blood glucose meter was used to measure the amount of glucose produced at the various times throughout the run. The way the meter is able to obtain a reading is through a process of chemical reactions. First, the glucose in the blood or in the case of our experiment, the slurry, reacts with the glucose oxidase to form gluconic acid. The gulconic acid will then react with ferricyanide to form ferrocyanide. The electrode in the meter then oxidizes the ferrocyanide which will create a current that is directly proportional to the amount of glucose in the slurry at that specific time point in the experiment. (Sherman, 2006) Below is a figure of the chemical reaction process. The instrument came with a test solution of a known glucose concentration to ensure accurate readings from the device. The glucose meter has a range of 20-600 mg/dL of glucose. If samples gave a reading which was to high, samples were diluted to provide a reading within the acceptable range. If a sample was to read to low, the value was recorded as “Lo”.

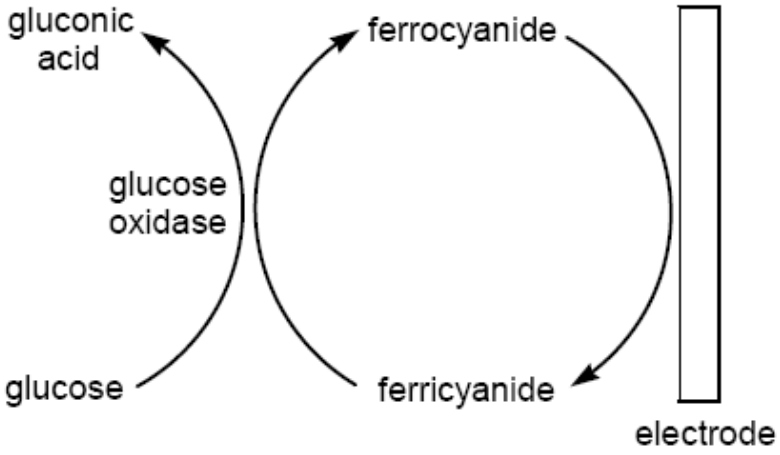

Figure 9: Glucose meter chemical reaction (Sherman, 2006)

Apple pomace pretreatment

The pretreatment processes used were rinsing and drying. Apple pomace weighing 200 g was loaded into the Microferm Fermenter vessel containing 2 liters of DI water. The resulting slurry was mixed using the agitator drive for approximately four hours. Washed contents were filtered through a standard sized four inch diameter filter. Water runoff from this procedure was set aside and the separated apple pomace substrate was placed in a pyrex container to be dried. Washed and filtered apple pomace was placed in an oven operating at 50 °C until dried.

Hydrolysis Step

Apple pomace, whether pretreated or not, was placed in the bioreactor vessel at varying weights. The initial weight of the substrate was varied to discover the appropriate amount of substrate required for high enzyme activity throughout the process. These weight variations were accounted for when analyzing the results by standardizing the different trials by the initial apple pomace weight. A pH probe and pH controller system along with a temperature control system was used to monitor and control their respective parameters. Temperature and pH

were kept constant at 40 °C and 5, respectively, as recommended by Professor Alex DiIorio. These parameters were maintained throughout all experiments unless otherwise noted. Bioreactor contents were allowed to reach the desired operating conditions before the initial sample at time zero was measured. An enzyme cocktail was added to hydrolyze the glycosidic bonds of the cellulose, producing glucose. The hydrolysis reaction works through the addition a water molecule to the anomeric (1) carbon of a glucose unit in the cellulose, causing the bridge oxygen to go off with the other (4) carbons, severing the chain (refer to figure 9). This process occurs towards the end of the chains, separating one or two glucose molecules at a time; if two glucose molecules are freed this way, then another enzyme will cleave the dimer into two monomers. After the initial glucose was determined the enzyme was added in at the appropriate concentration, 0.004 ml enzyme/ ml DI water. Samples were taken and glucose levels measured using a One-Touch Blood Glucose Meter at two, five, eight, eleven, thirteen, and fifteen hours after the start time.

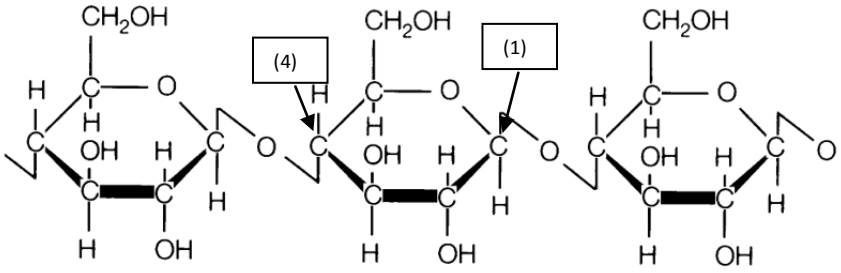

Figure 10: Cellulose Chain (http://www.greenspirit.org.uk/resources/cellulose.gif)

Results:

In order to standardize the readings for all the different experiments, the results are shown in the units of:

$$\frac{mg\ Glucose\ Produced}{g\ Feedstock}$$

referred to as mg/g. The glucose obtained from rinsing the apple pomace during pretreatment is not included in the calculations here of glucose production. The enzyme was added in same volumetric ratio to the solvent, so the volume of the containers was not taken into account for this analysis. All of the runs referred to in this section have all raw data, including operating conditions and enzymes used, available in Appendix A. The various runs showed a multitude of trends. In Figure 1, taken from the data collected from "Run 3" found in Appendix A, it can be seen that the amount of glucose produced increased with time. This is the expected, normal result of the data, as the enzymes are able to produce more glucose from the cellulose with a larger amount of time passing. This is a characteristic seen within all of the data collecting trials.

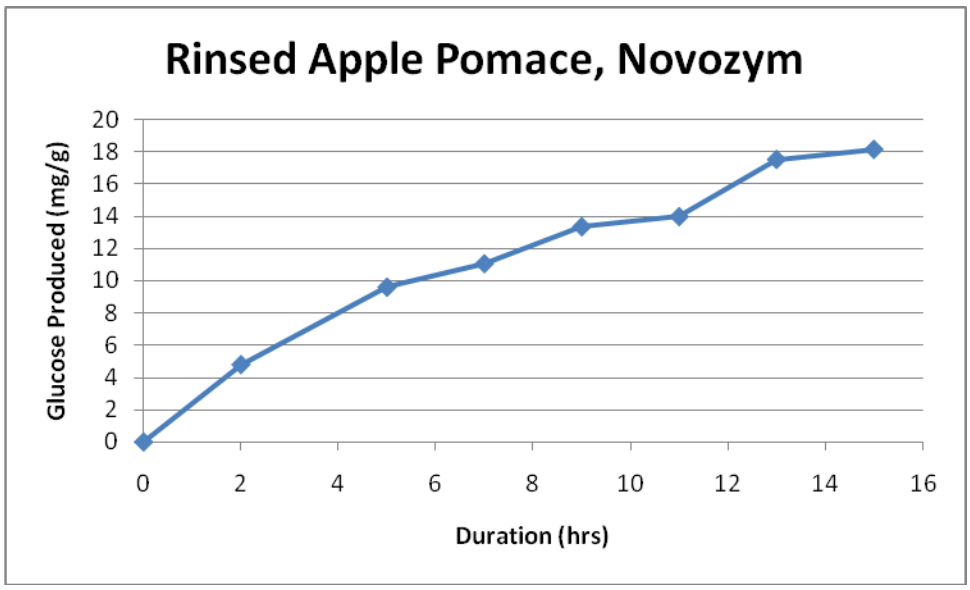

Figure 11: Rinsed Apple Pomace Data shows an increasing amount of glucose produced with time.

There are some trials, such as "Run B2" using apple pomace with the Novozym Cellulase, where a maximum amount of glucose produced is reached, and then the readings begin to fluctuate, as shown in Figure 2. This could be the result the substrate becoming depleted in the reactor, causing the values to begin to level off. Furthermore, the resulting drop in the reading could be the result of the glucose and cellobiose being a non-competitive inhibitor of the cellulase (Holtzapple, Cognata, Yuancai, & Hendrickson, 1990). This causes the glucose and cellobiose to bind to sites on the enzyme different than the active site, changing the morphology of the enzyme so that the active site is no longer able to accept the desired substrate for future conversion, lowering reaction rates. This product-enzyme complex would not be detected by the glucose meter. The combination of all these readings could cause the readings to be lower. Cellobiose would not act as an inhibitor for the β-glucosidase, as that is the substrate to be converted into glucose, which acts as a feedback inhibitor to this enzyme. Because of these fluctuations, some runs were ended before the 15 hour time trial ended.

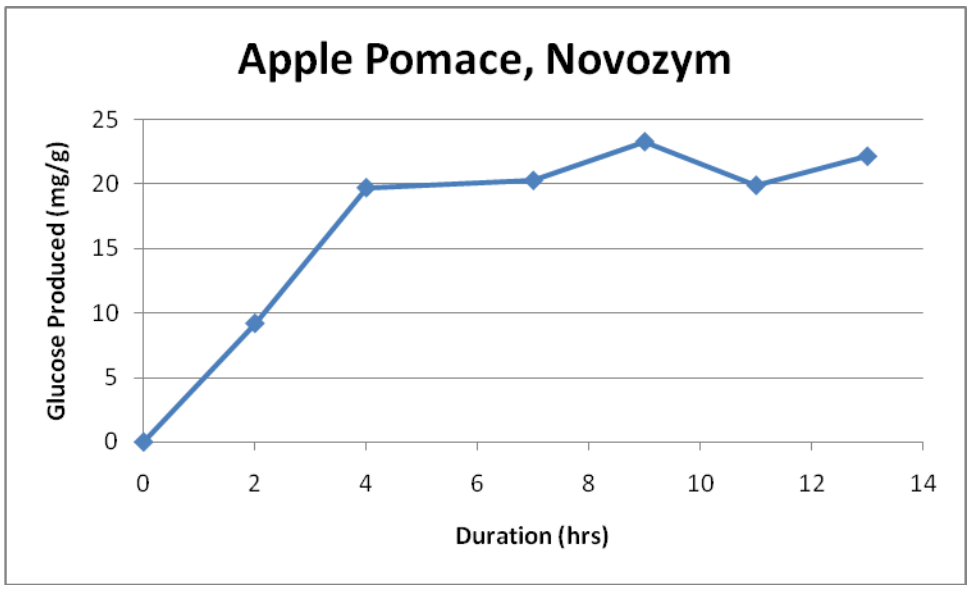

Figure 12: The glucose produced begins to vary because of the products inhibiting the productivity of the enzyme

The different conditions for the apple pomace produced varied results. The most notable difference, as illustrated in figure 3, is in the different enzyme.

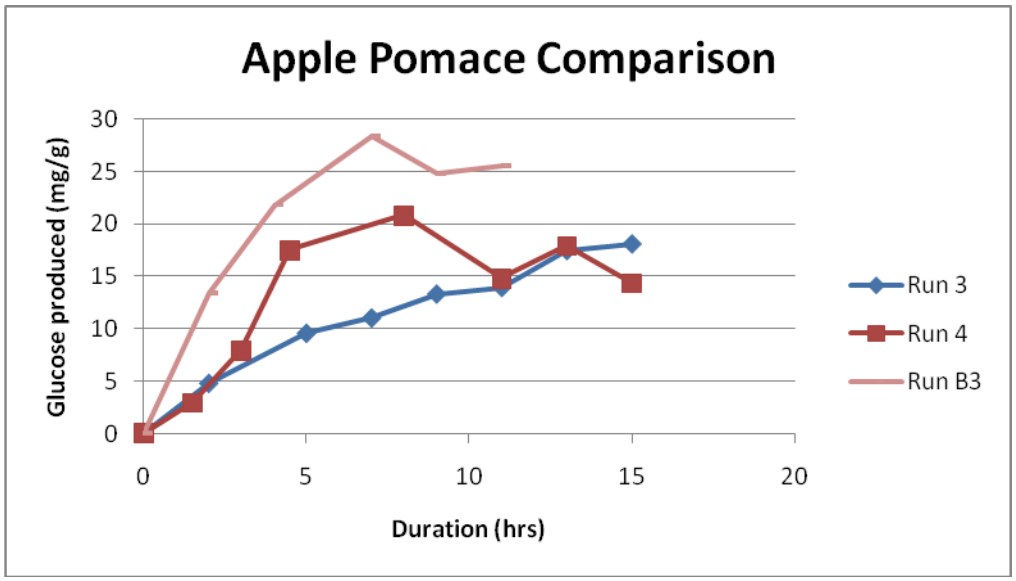

Figure 13: Different conditions of apple pomace runs; Run 3 was rinsed and dried with Novozym, Run 4 was un-risned with Novozym, and Run B3 with un-rinsed and Genencor.

The run B3, used with the Genencor enzymes, produced a much higher level of glucose than the other runs which utilized the Novozym enzymes. This could be an effect of the Genencor enzymes having a higher affinity for the substrate than the Novozym enzymes, leading to a higher efficiency. Even after the fluctuations in the readings began, the levels of the glucose produced were still higher. Runs 3 and 4 also gave some differing results; Run 3 was using rinsed apple pomace, and Run 4 was not rinsed. The end results over time were that Run 4 peaked earlier at 20.8 mg/g, then dropped to 14.375 mg/g several hours later. The rinsed apple Pomace from Run 3 gave more steady results, going up to its maximum value of 18.125 mg/g over the duration of the run without fluctuating down. From these results, it can be drawn that rinsing is a good method of material preparation. The glucose rinsed out from the apple pomace cannot inhibit the activity of the enzymes. The peak in the unrinsed run could be the result of glucose already present in the apple pomace, with the decline being that of the enzyme-inhibitor complex, which would not be detected by the methods used for glucose measurements. The same thing could have occurred in the Genencor run, which was with unrinsed apple pomace. The quick spike at 28.3 mg/g is attributed to the same reason, while the prolonged point of 25.5 mg/g is still higher due to the better efficiency of the Genencor enzyme.

When trials were executed using wood shavings, results were not useful for this experiment, labeled “Run 9” in Appendix A. As seen in Table 1, the amount of glucose produced were below the minimum of 20 mg/dl of glucose required for the glucose reader to detect. Readings from the “Lo” readings could be obtained via adding a small, measured amount of glucose added to a known volume of sample to get the reading within the measuring range. The

concentration of the initial sample could then be calculated. However, due to the low amount of glucose present and the inefficiency of the wood shavings to produce glucose, this calculation was not done. It is not feasible to use this type of wood shaving for glucose production.

Table 1: Wood Shavings Results				
Temp: 40 °C	pH: 5	Feed mass: 44g	Volume: 1.5 L	Enzyme: GC
Duration (hrs)		Glucose Concentration (mg/dl)		
0		"Lo" (<20)		
2		"Lo" (<20)		
5		"Lo" (<20)		
8		23		
11		26		
13		"Lo" (<20)		
15		"Er 4"		

One reason for the much lower glucose production is the higher lignin content in the wood compared to apples. The lignin is not able to be broken down in the same way, and acts as a protective layer around the cellulose, slowing down the enzyme activity. A pretreatment step to eliminate the lignin could be used to break down that barrier and increase the glucose production.

An overall scheme of the glucose production is shown in Figure 4. The run designations correspond to the run labels in Appendix B. Table 2 also shows the time points at which the different runs reached the maximum glucose production for that trial. The data shown in Figures 11 and 12 are duplicated here. The variances in the times where the apple pomace reached the maximum value could be the result of glucose pockets within the material

becoming exposed to the solvent and entering solution, with the subsequent drops in concentration being formed by the non-competitive inhibition described earlier.

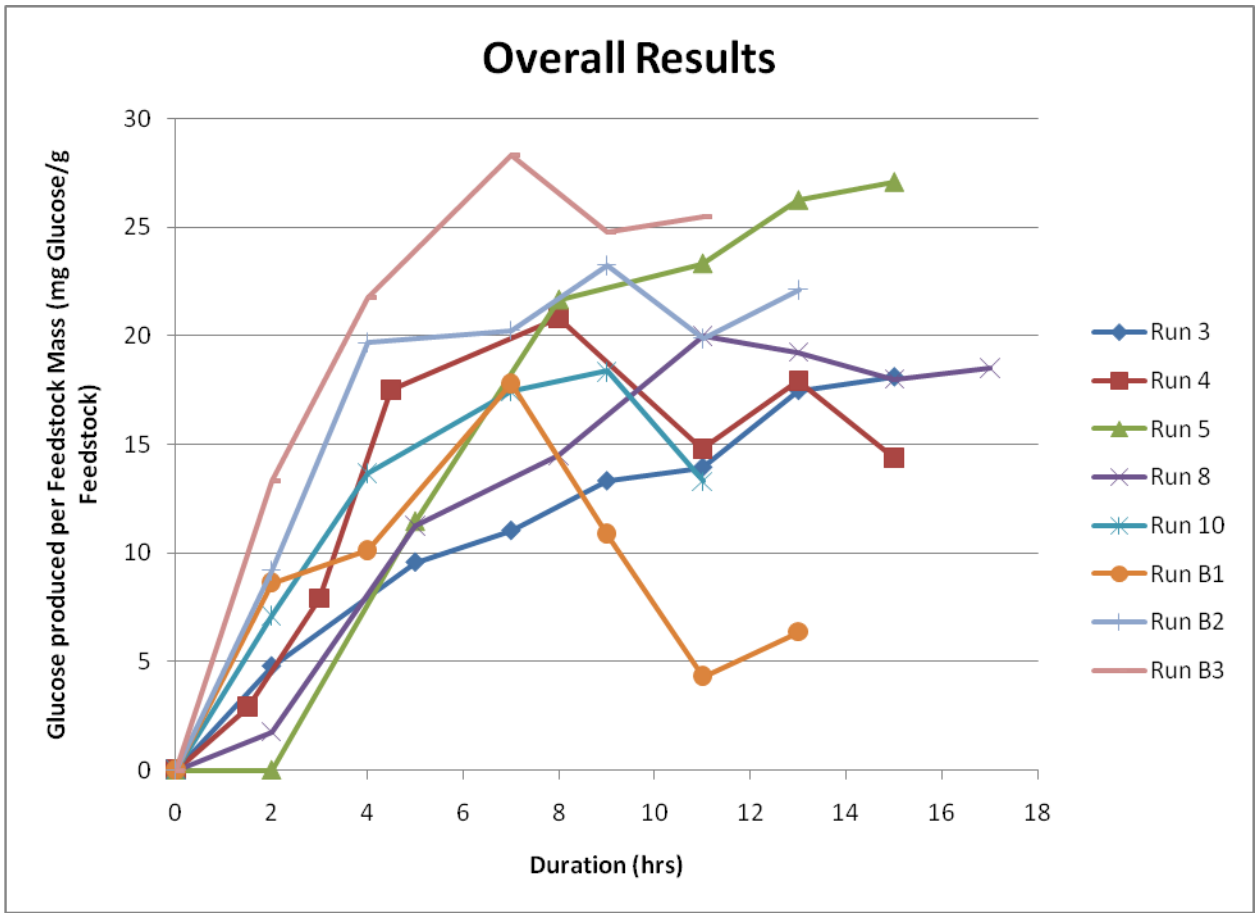

Figure 14: Overall results of the different trial runs

Table 2: Maximum Glucose Production		
Run Designation	Time Duration (hrs)	Glucose Produded (mg/g)
Run 3	15	18.125
Run 4	8	20.83
Run 5	15	27.1
Run 8	11	20
Run 10	9	18.375
Run B1	7	17.81
Run B2	9	23.25
Run B3	7	28.31

Discussion

Apple pomace has the capability to produce a usable amount of glucose for future fermentation into ethanol. For every gram of apple pomace put into the bioreactor, approximately 0.105 g of glucose can be produced at optimum levels. This output appears promising, especially since the pomace is regarded as waste by apple companies. At their maximum, during the fall, Carlson Orchards produces 72 tons of apple pomace per week. If they were to allot half of that amount to biofuel production, assuming a 60% conversion of glucose to ethanol, there is the potential of more than 300 gallons of ethanol being produced per week. Further economic analysis may help to show exactly how beneficial these results can be.

Further analysis of the rinsing process for the apple pomace has some surprising results. Samples of which to measure the runoff were not available for analysis, so this data must be inferred from other collected data. By looking at different runs, those with rinsed apple pomace (3 and B3) had initial glucose concentrations in the range of 50-65 mg/dL, while the initial glucose concentration of unrinsed apple pomace was much higher, in the range of 250-300 mg/dL. This difference of approximately 200 mg/dL is comparable to the amount of glucose actually produced by the enzymatic hydrolysis. In an industrial or commercial setting, a vigorous rinsing process would be desired to have before the actual reactor, so that glucose recovery and production can be optimized.

The DNS assay could be very useful in producing more accurate results. However, due to the inability to have it work, this method of glucose detection could not be used. The resulting mixtures of the reagent and the samples were too dark to be able to generate accurate

readings from the spectrometer. If these readings could be obtained, the overall results of the experiments could be better.

Conclusion:

Based on experimental results, apple pomace has shown to be the best feedstock among our results for a second generation biofuels production process. This is based on its high initial level of background sugars which can be removed before hydrolysis and used as additional feedstock for the fermentation process. This is then added to the other sugar products of the enzymatic hydrolysis reaction and fermented into cellulosic ethanol.

Experimentation with cellulase enzymes has resulted in an increase in glucose concentration over time, but readings begin to fluctuate after several hours of operation. Fluctuations and decreases in glucose concentration are thought to be the results of non-competitive inhibition of glucose and cellobiose on the cellulase enzymes. The enzymes produced by Genencor have shown a higher conversion for our substrate than that of the Novozym cellulases. Further experimentation could help develop an understanding as to why this occurs. The addition of the enzyme β-glucosidase also drastically increases the production of glucose, as it hydrolyzes the cellobiose into the two respective monosaccharides desired by this process.

The rinsing of apple pomace is a crucial step in the production of glucose. It eliminates the background sugars which can be recovered, allowing for more efficient enzyme activity. This rinsing has a dual purpose; it removes the enzymatic inhibitors for better production and reaction rates, and the excess sugars rinsed off can be utilized for fermentation.

Recommendations

The yield of the glucose product from the enzyme catalyzed hydrolysis reaction could be improved by altering the process in several ways. Conversion improvements could be made through the addition of further pre-treatment of the feed stocks, experimentation with various enzyme cocktails, and also using more up-to-date and efficient equipment.

It was found that the implementation of a feed stock pretreatment step would provide better experimental data than it would if the feed were not pre-treated. Also, when running the experiments, it was found that certain enzymes would provide better results with specific feed stocks. So, finding the most productive enzyme cocktails for specific feed stocks would be best. The equipment used in the lab provided some problems during the experiments. It was extremely hard to obtain replacement parts for some equipment, such as the MultiGen agitator. Even though the equipment set-up was complete, MultiGen's temperature controls did not work, and a lot of time and effort was put into the equipment setup instead of experimentation. Also, if there was a more efficient filtering process to use when removing the rinse-off water after pre-treating the apple pomace feed, the process would have been much improved. This would allow for higher retention of substrate; it seemed some of the substrate had still passed through the filter and may have affected readings. Following through with these recommendations would make these experiments more effective for future students and much more efficient of a conversion process.

Works Cited

Bergman, Richard, and John Zerbe. "Primer on Wood Biomass for Energy." USDA Forest Service. Jan. 2008. USDA. <http://www.fpl.fs.fed.us/tmu/resources/documents/primer_on_wood_biomass_for_energy.pdf>.

Besler, Matthias Composition of Apple, 1999

<http://www.food-allergens.de/symposium-vol1(3)/data/apple/apple-composition.htm>

“Cellulase.” Worthington Enzyme Manual. Worthington Biochemical Corporation, 1993

"Energy and Greenhouse Gas Emissions Impacts of Fuel Ethanol." Chart. Argonne National Laboratory. Center for Transportation Research: Michael Wang. August 23, 2005

Holtzapple, Mark, Cognata, Mona, Shu, Yuancai, and Hendrickson, Christie. “Inhibition of *Trichoderma ressei* cellulase by sugars and solvents.” Biotechnology and Bioengineering, Volume 36, Issue 3 pg. 275-287. John Wiley & Sons, 1990

Regulatory Impact Analysis: Renewable Fuel Standard Program." EPA. 20 Apr. 2007. EPA. 8 Mar.-Apr. 2008 <http://www.epa.gov/otaq/renewablefuels/420r07004.pdf>.

Sherman, Marie “How do blood glucose meters work?” Chem 13 News, Issue 343 p. 5-6, Dec. 2006

Thomson, Alexander. "An Investigation Into the Implications of Using Very Wet Biomass as a Fuel" Sept. 2006. University of Strathclyde Mech. Eng. Dept. <http://www.esru.strath.ac.uk/Documents/MSc_2006/thomson.pdf>.

Zacchi, G., and M. Galbe. "A Review of the Production of Ethanol From Softwood." Springer- Verlag. 17 July 2002. Dept. of CHE Lund University, Sweden. 04 Mar.-Apr. 2008 <http://www.springerlink.com/content/94bkt3ntvflxgyyq/fulltext.html>.

Appendix A

Run Code:	Run 3	Duration:	Time:	Temp reading: ° C	pH reading:	Glucose Reading (mg/dL):	Glucose Total(mg)	Glucose Produced (mg)	Glucose produced /Initial Feed Mass (mg/g)
Date:	3/27/2008	0:00:00	8:30:00	41	5				
Feedstock Name:	Rinsed Apple Pommace	0:40:00	9:10:00	41	5	62	310	0	0
Feedstock Mass &or Volume:	24 G	2:30:00	11:00:00	41	5	85	425	115	4.79
Enzyme Name:	Novozyme Cellulase	5:30:00	14:00:00	41	5	108	540	230	9.58
Enzyme Volume:	5 mL	7:30:00	16:00:00	41	5	115	575	265	11.04
DI H2O Volume:	500 mL H2O	9:30:00	18:00:00	41	5	126	630	320	13.33
Buffer Name & pH:	pH 10	11:30:00	20:00:00	41	5	129	645	335	13.96
Buffer Name & pH:	ph 4	13:30:00	22:00:00	41	5	146	730	420	17.5
Initial Slurry pH:		15:30:00	0:00:00	41	5	149	745	435	18.13
Initial Slurry Temp (° C):									

Run Code:	Run 4	Duration:	Time:	Temp reading: ° C	pH reading:	Glucose Reading (mg/dL):	Glucose Total(mg)	Glucose Produced (mg)	Glucose produced /Initial Feed Mass (mg/g)
Date:	3/29/2009	0:00:00	0:00:00	40	5	301	1505	0	0
Feedstock Name:	Apple Pommace	1:30:00	1:30:00	41	5	315	1575	70	2.92
Feedstock Mass &or Volume:	24 G	3:00:00	3:00:00	41	5	339	1695	190	7.92
Enzyme Name:	Novozyme Cellulase	4:30:00	4:30:00	41	5	385	1925	420	17.5
Enzyme Volume:	5 mL	8:00:00	8:00:00	41	5	401	2005	500	20.83
DI H2O Volume:	500 mL H2O	11:00:00	11:00:00	41	5	372	1860	355	14.79
Initial Slurry pH:		13:00:00	13:00:00	41	5	387	1935	430	17.92
Initial Slurry Temp (° C):		15:00:00	15:00:00	41	5	370	1850	345	14.38

Run Code:	Run 5	Duration:	Time:	Temp reading: ° C	pH reading:	Glucose Reading (mg/dL):	Glucose Total(mg)	Glucose Produced (mg)	Glucose produced /Initial Feed Mass (mg/g)

Appendix A

Date:	4/2/2009	0:30:00	6:00:00	40	4.2	377	1885	0	0
Feedstock Name:	SWITCHGF	2:00:00	8:00:00	38	5	362	1810	0	0
Feedstock Mass &or Volume:	24 G	5:00:00	11:00:00	41	5	432	2160	275	11.46
Enzyme Name:	Genencor 220 Cellulase	8:00:00	14:00:00	41	5	481	2405	520	21.67
Enzyme Volume:	2 mL	11:00:00	17:00:00	41	5	489	2445	560	23.33
DI H2O Volume:	500 mL H2O	13:00:00	19:00:00	41	5	503	2515	630	26.25
Initial Slurry pH:	RPM: 400	15:00:00	21:00:00	41	5	507	2535	650	27.08
Initial Slurry Temp (° C):	ph 4								
Initial Glucose Reading:	324								
Run Code:	Run 8	Duration:	Time:	Temp reading: ° C	pH reading:	Glucose Reading (mg/dL):	Glucose Total(mg)	Glucose Produced (mg)	Glucose produced /Initial Feed Mass (mg/g)
Date:	4/11/2008	0:00:00	10:30:00	38	5	217	3255	0	0
Feedstock Name:	Apple Pommace	2:00:00	12:30:00	41	5	224	3360	105	1.75
Feedstock Mass &or Volume:	60 G	5:00:00	15:30:00	41	5	262	3930	675	11.25
Enzyme Name:	Novozyme Cellulase	8:00:00	18:30:00	41	5	275	4125	870	14.5
Enzyme Volume:	6 mL	11:00:00	21:30:00	40	5	297	4455	1200	20
DI H2O Volume:	1500 mL H2O	13:00:00	23:30:00	40	5	294	4410	1155	19.25
Buffer Name & pH:	pH 10	15:00:00	1:30:00	41	5	289	4335	1080	18
Buffer Name & pH:	ph 4	17:00:00	3:30:00	41	5	291	4365	1110	18.5
Initial Slurry pH:									
Initial Slurry Temp (° C):									
Run Code:	Run 10	Duration:	Time:	Temp reading: ° C	pH reading:	Glucose Reading (mg/dL):	Glucose Total(mg)	Glucose Produced (mg)	Glucose produced /Initial Feed Mass (mg/g)
Date:	4/13/2008	0:00:00	14:00:00	40	4	109	1635	0	0

Appendix A									
Feedstock Name:	1.) Apple Pomace 2.)Blended Wood Shavings	2:00:00	16:00:00	40	5	147	2205	570	7.13
Feedstock Mass &or Volume:	1.) 40 g 2.) 40 g	4:00:00	18:00:00	40	5	182	2730	1095	13.69
Enzyme Name:	Novozyme Cellulase	7:00:00	21:00:00	40	5	202	3030	1395	17.44
Enzyme Volume:	5 mL	9:00:00	23:00:00	40	5	207	3105	1470	18.38
DI H2O Volume:	1500 mL H2O	11:00:00	1:00:00	40	5	180	2700	1065	13.31
Buffer Name & pH:	pH 10								
Buffer Name & pH:	ph 4								
Initial pH:									
Initial Slurry pH:									
Initial Slurry Temp (° C):									
Initial pH:									
Initial Temp:									
Run Code:	B1	Duration:	Time:	Temp reading: ° C	pH reading:	Glucose Reading (mg/dL):	Glucose Total(mg)	Glucose Produced (mg)	Glucose produced /Initial Feed Mass (mg/g)
Date:	4/14/2008	0:00:00	9:00:00	40	5	191	2865	0	0
Feedstock Name:	1.) Apple Pomace 2.)Blended Wood Shavings	2:00:00	11:00:00	40	5	237	3555	690	8.63
Feedstock Mass &or Volume:	1.) 40 g 2.) 40 g	4:00:00	13:00:00	40	5	245	3675	810	10.13
Enzyme Name:	Novozyme Cellulase	7:00:00	16:00:00	40	5	286	4290	1425	17.81
Enzyme Volume:	5 mL	9:00:00	18:00:00	40	5	249	3735	870	10.88
DI H2O Volume:	1500 mL H2O	11:00:00	20:00:00	40	5	214	3210	345	4.31
Initial Slurry pH:		13:00:00	22:00:00	40	5	225	3375	510	6.38
Initial Slurry Temp (° C):									

Appendix A

Run Code:	B2	Duration:	Time:	Temp reading: ° C	pH reading:	Glucose Reading (mg/dL):	Glucose Total(mg)	Glucose Produced (mg)	Glucose produced /Initial Feed Mass (mg/g)
Date:	4/16/2008	0:00:00	9:00:00	40	4.5	224	3360	0	0
Feedstock Name:	Apple Pomace	2:00:00	11:00:00	40	4.5	273	4095	735	9.19
Feedstock Mass &or Volume:	80 g	4:00:00	13:00:00	40	4.5	329	4935	1575	19.69
Enzyme Name:	Novozyme Cellulase	7:00:00	16:00:00	40	4.5	332	4980	1620	20.25
Enzyme Volume:	5.5 mL	9:00:00	18:00:00	40	4.5	348	5220	1860	23.25
DI H2O Volume:	1500 mL H2O	11:00:00	20:00:00	40	4.5	330	4950	1590	19.88
Initial Slurry pH:	4.5	13:00:00	22:00:00	40	4.5	342	5130	1770	22.13
Initial Slurry Temp (° C):	40								

Run Code:	B3	Duration:	Time:	Temp reading: ° C	pH reading:	Glucose Reading (mg/dL):	Glucose Total(mg)	Glucose Produced (mg)	Glucose produced /Initial Feed Mass (mg/g)
Date:	4/17/2008	0:00:00	9:00:00	40	5	54	810	0	0
Feedstock Name:	Apple Pomace	2:00:00	11:00:00	40	5	125	1875	1065	13.31
Feedstock Mass &or Volume:	80 g	4:00:00	13:00:00	40	5	170	2550	1740	21.75
Enzyme Name:	Genencor 220 Cellulase	7:00:00	16:00:00	40	5	205	3075	2265	28.31
Enzyme Volume:	5 mL	9:00:00	18:00:00	40	5	186	2790	1980	24.75
DI H2O Volume:	1500 mL H2O	11:00:00	20:00:00	40	5	190	2850	2040	25.5
Initial Slurry pH:	5								
Initial Slurry Temp (° C):	40								

Appendix B						
Enzymatic Hydrolysis of Cellulosic Biomass						
Run Code:	A 1		Time:	Temp reading: ° C	pH reading:	Glucose Reading (mg/dL):
Date:	3/23/2008		13:35:00	19	4.5	
Feedstock Name:	Apple Pomace		13:46:00	19	4	
Feedstock Mass &or Volume:	24 G		14:00:00	19	4.25	
Enzyme Name:	Novozyme Cellulase		14:22:00	21	4.8	
Enzyme Volume:	5 mL		14:37:00	37	5.1	
DI H2O Volume:	500 mL H2O		14:56:00	40	5.4	
Buffer Name & pH:	pH 10		15:06:00	40	5.4	
Buffer Name & pH:	ph 4					
Initial pH:						
Initial Slurry pH:						
Initial Slurry Temp (° C):						
Initial pH:	4.5					
Initial Temp:	19° C					
Run Code:	A2		Time:	Temp reading: ° C	pH reading:	Glucose Reading (mg/dL):
Date:	3/24/2008					
Feedstock Name:	Distillers grain					
Feedstock Mass &or Volume:	20 mg, 50 mL					
Enzyme Name:	Novozyme Cellulase					
Enzyme Volume:	5 mL					
DI H2O Volume:	500 mL					
Buffer Name & pH:	7					
Buffer Name & pH:	4					
Initial Slurry pH:						
Initial Slurry Temp (° C):						
Assay Type:						
Assay Readings/ Color Change:						
Initial pH:						
Initial Temp:						
Run Code:	A3		Time:	Temp reading: ° C	pH reading:	Glucose Reading (mg/dL):
Date:	3/25/2008		9:30:00	40	5	392
Feedstock Name:	Apple Pomace		11:30:00			

Appendix B						
Feedstock Mass &or Volume:	24 G		13:30:00	41	5	275
Enzyme Name:	Novozyme Cellulase		14:30:00			
Enzyme Volume:	5 mL		17:30:00			
DI H2O Volume:	500 mL H2O		19:30:00			
Buffer Name & pH:	pH 10		21:30:00			
Buffer Name & pH:	ph 4		23:30:00			
Initial pH:						
Initial Slurry pH:						
Initial Slurry Temp (° C):						
Run Code:	Run 2		Time:	Temp reading: ° C	pH reading:	Glucose Reading (mg/dL):
Date:	3/26/2008		11:30:00	20	3.9	
Feedstock Name:	Apple Pomace		15:45:00	41		
Feedstock Mass &or Volume:	24 G		18:32:00	273		
Enzyme Name:	Novozyme Cellulase					
Enzyme Volume:	2.5 mL					
DI H2O Volume:	500 mL H2O					
Run Code:	Run 3	Duration:	Time:	Temp reading: ° C	pH reading:	Glucose Reading (mg/dL):
Date:	3/27/2008	0:00:00	8:30:00	41	5	
Feedstock Name:	Rinsed Apple Pommace	0:40:00	9:10:00	41	5	62
Feedstock Mass &or Volume:	24 G	2:30:00	11:00:00	41	5	85
Enzyme Name:	Novozyme Cellulase	5:30:00	14:00:00	41	5	108
Enzyme Volume:	5 mL	7:30:00	16:00:00	41	5	115
DI H2O Volume:	500 mL H2O	9:30:00	18:00:00	41	5	126
Buffer Name & pH:	pH 10	11:30:00	20:00:00	41	5	129
Buffer Name & pH:	ph 4	13:30:00	22:00:00	41	5	145 & 146
Initial pH:		15:30:00	0:00:00	41	5	149
Initial Slurry pH:						
Initial Slurry Temp (° C):						
Initial pH:						
Initial Temp:						

Appendix B

		11:30:00				
Run Code:	Run 4	Duration:	Time:	Temp reading: ° C	pH reading:	Glucose Reading (mg/dL):
Date:	3/29/2009	0:00:00	11:30:00	40	5	301
Feedstock Name:	Apple Pommace	1:30:00	13:00:00	41	5	
Feedstock Mass &or Volume:	24 G	3:00:00	14:30:00	41	5	339
Enzyme Name:	Novozyme Cellulase	4:30:00	16:00:00	41	5	385
Enzyme Volume:	5 mL	8:00:00	19:30:00	41	5	401
DI H2O Volume:	500 mL H2O	11:00:00	22:30:00	41	5	372
Buffer Name & pH:	pH 10	13:00:00	0:30:00	41	5	387
Buffer Name & pH:	ph 4	15:00:00	2:30:00	41	5	370
Run Code:	Run #5	Duration:	Time:	Temp reading: ° C	pH reading:	Glucose Reading (mg/dL):
Date:	4/2/2009	0:30:00	6:00:00	40	4.2	377
Feedstock Name:	SWITCHGR/	2:00:00	8:00:00	38	5	362
Feedstock Mass &or Volume:	24 G	5:00:00	11:00:00	41	5	432
Enzyme Name:	Genencor 220 Cellulase	8:00:00	14:00:00	41	5	481
Enzyme Volume:	2 mL	11:00:00	17:00:00	41	5	489
DI H2O Volume:	500 mL H2O	13:00:00	19:00:00	41	5	503
Initial Slurry pH:	RPM: 400	15:00:00	21:00:00	41	5	507
Initial Slurry Temp (° C):	ph 4					
Initial Glucose Reading:	324					
Run Code:	Run #6	Duration:	Time:	Temp reading: ° C	pH reading:	Glucose Reading (mg/dL):
Date:	4/3/2008	0:00:00	5:00:00	40	5	LO
Feedstock Name:	1.) Switchgrass 2.)Wood w/ bark	0:45:00	5:45:00	40	5	28
Feedstock Mass &or Volume:	1.)8.18 g 2.)1.71g	2:30:00	7:30:00	40	5	29
Enzyme Name:	Novozyme Beta-Glucosidase					
Enzyme Volume:	2 mL					
DI H2O Volume:	500 mL H2O					
Initial Slurry pH:	5.4					

Appendix B						
Initial Slurry Temp (° C):	40					
Run Code:	Run #7	Duration:	Time:	Temp reading: ° C	pH reading:	Glucose Reading (mg/dL):
Date:	Tuesday 4/7/2008	0:00:00	10:45:00	40	5.9	72
Feedstock Name:	Rinsed and Dried Apple Pomace	2:00:00	13:00:00	40	5.9	107
Feedstock Mass &or Volume:	25 G					
Enzyme Name:	Novozyme Cellulase					
Enzyme Volume:	5 mL					
DI H2O Volume:	500 mL H2O					
Buffer Name & pH:	pH 10					
Buffer Name & pH:	ph 4					
Initial Slurry pH:						
Initial Slurry Temp (° C):						
Run Code:	Run#8	Duration:	Time:	Temp reading: ° C	pH reading:	Glucose Reading (mg/dL):
Date:	4/11/2008	0:00:00	10:30:00	38	5	217
Feedstock Name:	Apple Pommace	2:00:00	12:30:00	41	5	224
Feedstock Mass &or Volume:	60 G	5:00:00	15:30:00	41	5	262
Enzyme Name:	Novozyme Cellulase	8:00:00	18:30:00	41	5	275
Enzyme Volume:	6 mL	11:00:00	21:30:00	40	5	297
DI H2O Volume:	1500 mL H2O	13:00:00	23:30:00	40	5	294
Buffer Name & pH:	pH 10	15:00:00	1:30:00	41	5	289
Buffer Name & pH:	ph 4	17:00:00	3:30:00	41	5	291
Initial Slurry pH:						
Initial Slurry Temp (° C):						
Run Code:	Run #9	Duration:	Time:	Temp reading: ° C	pH reading:	Glucose Reading (mg/dL):
Date:	4/13/2008	0:00:00	10:30:00	40	5	Lo
Feedstock Name:	Wood Shavings	2:00:00	12:30:00	40	5	Lo

Appendix B						
Feedstock Mass &or Volume:	44.6 G	5:00:00	15:30:00	40	5	Lo
Enzyme Name:	Genencor 220 Cellulase	8:00:00	18:30:00	40	5	23
Enzyme Volume:	5 mL	11:00:00	21:30:00	40	5	26
DI H2O Volume:	1500 mL H2O	13:00:00	23:30:00	40	5	Lo
Buffer Name & pH:	pH 10	15:00:00	1:30:00	40	5	"Er 4"
Buffer Name & pH:	ph 4					
Initial pH:						
Initial Slurry pH:						
Initial Slurry Temp (° C):						
Initial pH:						
Initial Temp:						
Run Code:	Run #10	Duration:	Time:	Temp reading: ° C	pH reading:	Glucose Reading (mg/dL):
Date:	4/13/2008	0:00:00	14:00:00	40	4	109
Feedstock Name:	1.) Apple Pomace 2.) Blended Wood Shavings	2:00:00	16:00:00	40	5	147
Feedstock Mass &or Volume:	1.) 40 g 2.) 40 g	4:00:00	18:00:00	40	5	182
Enzyme Name:	Novozyme Cellulase	7:00:00	21:00:00	40	5	202
Enzyme Volume:	5 mL	9:00:00	23:00:00	40	5	207
DI H2O Volume:	1500 mL H2O	11:00:00	1:00:00	40	5	180
Buffer Name & pH:	pH 10					
Buffer Name & pH:	ph 4					
Initial pH:						
Initial Slurry pH:						
Initial Slurry Temp (° C):						
Initial pH:						
Initial Temp:						
Run Code:	B1	Duration:	Time:	Temp reading: ° C	pH reading:	Glucose Reading (mg/dL):
Date:	4/14/2008	0:00:00	9:00:00	40	5	191

Appendix B						
Feedstock Name:	1.) Apple Pomace 2.) Blended Wood Shavings	2:00:00	11:00:00	40	5	237
Feedstock Mass &or Volume:	1.) 40 g 2.) 40 g	4:00:00	13:00:00	40	5	245
Enzyme Name:	Novozyme Cellulase	7:00:00	16:00:00	40	5	286
Enzyme Volume:	5 mL	9:00:00	18:00:00	40	5	249
DI H2O Volume:	1500 mL H2O	11:00:00	20:00:00	40	5	214
Initial Slurry pH:		13:00:00	22:00:00	40	5	225
Initial Slurry Temp (° C):						
Run Code:	B2	Duration:	Time:	Temp reading: ° C	pH reading:	Glucose Reading (mg/dL):
Date:	4/16/2008	0:00:00	9:00:00	40	4.5	224
Feedstock Name:	Apple Pomace	2:00:00	11:00:00	40	4.5	273
Feedstock Mass &or Volume:	80 g	4:00:00	13:00:00	40	4.5	329
Enzyme Name:	Novozyme Cellulase	7:00:00	16:00:00	40	4.5	332
Enzyme Volume:	5.5 mL	9:00:00	18:00:00	40	4.5	348
DI H2O Volume:	1500 mL H2O	11:00:00	20:00:00	40	4.5	330
Initial Slurry pH:	4.5	13:00:00	22:00:00	40	4.5	342
Initial Slurry Temp (° C):	40					
Run Code:	B3	Duration:	Time:	Temp reading: ° C	pH reading:	Glucose Reading (mg/dL):
Date:	4/17/2008	0:00:00	9:00:00	40	5	54
Feedstock Name:	Apple Pomace	2:00:00	11:00:00	40	5	125
Feedstock Mass &or Volume:	80 g	4:00:00	13:00:00	40	5	170
Enzyme Name:	Genencor 220 Cellulase	7:00:00	16:00:00	40	5	205
Enzyme Volume:	5 mL	9:00:00	18:00:00	40	5	184 / 186
DI H2O Volume:	1500 mL H2O	11:00:00	20:00:00	40	5	190
Initial Slurry pH:	5					
Initial Slurry Temp (° C):	40					

Appendix B						
Run Code:	B4	Duration:	Time:	Temp reading: ° C	pH reading:	Glucose Reading (mg/dL):
Date:	4/21/2008	0:00:00		40	5	34
Feedstock Name:	Apple Pomace	2:00:00		40	5	110
Feedstock Mass &or Volume:	80 g	4:00:00		40	5	153
Enzyme Name:	Genencor 220 Cellulase	7:00:00		40	5	
Enzyme Volume:	5 mL	9:00:00		40	5	
DI H2O Volume:	1500 mL H2O	11:00:00		40	5	
Initial Slurry pH:		13:00:00		40	5	
Initial Slurry Temp (° C):						
Run Code:	B5	Duration:	Time:	Temp reading: ° C	pH reading:	Glucose Reading (mg/dL):
Date:	4/25/2008	0:00:00		40	5	
Feedstock Name:	Blended Wood Shavings	2:00:00		40	5	
Feedstock Mass &or Volume:	80 g	4:00:00		40	5	
Enzyme Name:	Genencor 220 Cellulase	7:00:00		40	5	
Enzyme Volume:	5 mL	9:00:00		40	5	
DI H2O Volume:	1500 mL H2O	11:00:00		40	5	
Initial Slurry pH:		13:00:00		40	5	
Initial Slurry Temp (° C):						