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Hydrolysis of concentrated dilute base pretreated biomass slurries.

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HYDROLYSIS OF CONCENTRATED DILUTE BASE PRETREATED BIOMASS SLURRIES

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HYDROLYSIS OF CONCENTRATED DILUTE BASE PRETREATED BIOMASS SLURRIES

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ABSTRACT

Enzymatic hydrolysis is an important, but time limiting step in the process of converting biomass into ethanol. High solids concentrations are desired in order to minimize reactor size and achieve a higher concentration of glucose in the end product stream. However, higher solids concentrations lead to higher viscosities and hence, mixing and mass transfer becomes more difficult.

In this study, a mixer designed to overcome the mass transfer limitations was used to conduct enzymatic hydrolysis of dilute-base pretreated corn stover, wheat, and miscanthus at high solids concentrations. This was done to determine if overcoming the mass transfer limitations would improve glucose release rates and yields, as was the case in a previous study on dilute acid pretreated corn stover. Solids concentrations were tested at 20%, 25%, and 30% for each substrate.

The glucose yields during mixer trials were higher for lower solids concentrations for all three substrates, contradicting the previous results which showed that glucose release rates and yields were maintained as solids content increased in the mixer. The 20% solids corn stover released 70% more glucose than the 30% solids did. Yields for 20% solids were in the 60% range, which is low but close to the expected range. For wheat, 400% more glucose was released for 20% solids than 30% solids. Yields for 20% wheat solids were in the mid 60% range, which is also close to the expected range. For miscanthus, the increase was 36%. Yield for 20% miscanthus solids was below 40%, which was on the order of untreated sawdust in a previous study and indicates an ineffective pretreatment method for this substrate. The contradictory results indicate there

may be some effect other than mass transfer limitations that affects glucose release rates and yields.

The slurries tested had much larger particle sizes and lacked the free water which gives the consistency seen with other pretreatment methods. This is most likely due to poor pretreatment. Due to the consistency, it was difficult to measure viscosity and, hence, determine if mass transfer limitations were overcome in these slurries.

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I. INTRODUCTION

In recent years, the use of scarce energy has become a major topic of concern within various engineering and other fields of study. Fossil fuels constitute the bulk of the sources for current energy usage. The concerning part is that they are in limited supply, since they are not renewable. There are also other concerning factors which lead to more research into renewable energy, including unstable prices for fossil fuel energy and dependence on foreign oil.

Energy consumption is increasing across the globe, so it is getting more difficult for the United States to depend on other countries to supply their oil. Oil consumption rose 3.5% globally in 2007 from the previous year (USA TODAY, 2007). The U.S. Energy Information Administration expects the demand to increase another 22% by 2015. Duncan and Youngquist (2004) project fossil fuels to become extremely limited in the next 40 to 50 years.

The United States has realized this, and has taken steps to help the country move forward into using more renewable energy. The U.S. Environmental Protection Agency (EPA) set standards which require 5.87% of gasoline sold in 2010 and 10% in 2020 to be from renewable fuels (U.S. Environmental Protection Agency, April 2007). The

Department of Energy (DOE) has called for production of ethanol to be 36 billion gallons by 2022.

Bioethanol has been a renewable energy source which has been given much attention due to its promising potential. It is a clean burning fuel which is made by converting the cellulose from biomass to fermentable sugars. The process involves breaking down polymeric cellulose molecules to its monomer glucose molecules. Glucose can then be fermented with the help of yeast or bacteria to produce the end product of ethanol.

Current ethanol production plants use corn as their feedstock, which is useful because corn is produced at a high rate in the United States. It is also easy to degrade the high starch content into fermentable sugar. However, with corn also being a food source, it becomes difficult to maintain it as a fuel source when ethanol demand rises. The cost of corn is rising because of this, as well as other corn products such as feed for cattle and pigs.

Researchers have begun to look at other lower cost sources for the production of bioethanol. Some of the sources readily available in Kentucky which have been researched include corn stover, wheat, and miscanthus. Other sources which have been investigated are shown in Table I, along with the estimated amount available and corresponding ethanol yield (Sun, et al., 2004; Chang et al., 2005).

TABLE I

ESTIMATED AVAILABILITY OF SELECTED FEEDSTOCKS AND POTENTIAL ETHANOL PRODUCTION

Feedstock Type	Estimated Availability (million dry tons/year)	Produced Ethanol (million L/year)
Corn Stover	153	77,777
Wood products	72	36,601
Energy Crops	70	35,584
Other Agricultural Residues	58	29,484
Corn Fiber	4	2034

Corn stover includes the leaves and stalks of corn, so its use does not affect the price of corn to the food industry. In 2010, Kentucky produced over 150,000 bushels of corn, which is 1.2% of the total corn produced in the United States (Kentucky AgriNews, 2011).

Wheat is another commonly used substrate for making biofuels. It is grown in this area as well. Kentucky produced over 16,500 bushels of wheat during 2010, which is 0.7% of the total production for the United States.

Miscanthus is a perennial long grass native to Asia and Africa. It is a high energy crop which grows quickly. Once it is planted, it can be harvested every year for 20 years. Kentucky has recently imported miscanthus and has planted over 800 acres with plans to use it for its high energy content (Associated Press, 2009). A picture of miscanthus is shown in Figure 1.

FIGURE 1: High energy, perennial long grass, miscanthus

The time limiting step in the overall conversion of biomass to bioethanol is the hydrolysis step. Hydrolysis is the process of breaking down cellulose molecules into the monomer glucose molecules. This is done in the presence of water, using hydrogen molecules to separate the monomers. Enzymes help to break apart the polymeric cellulose.

Hydrolysis typically takes in the range of five to seven days for maximum release of glucose, compared to minutes or hours for the other steps of production. Because of this, much research has been done on the topic of speeding up this process, as well as decreasing the volume of reactors for this to take place. The use of enzymes is an answer for decreasing the time it takes for hydrolysis to occur, along with increasing the total release of glucose.

Theoretically, an increase in solids concentration will reduce the reactor volume needed for a set amount of glucose release, and also reduce costly water and heating

expenses. However, higher solids concentrations lead to higher viscosities, which increases power consumption to prohibitive levels at industrial-scale. It has been shown repeatedly that solids concentrations higher than 10% will dramatically reduce the release rate and yield of glucose, which has been hypothesized to be due to insufficient mass transfer (Lübbert and Jørgensen, 2001; Mohagheghi et al., 1992; Spindler et al., 1988).

Previous work by Rezania et al. (2009) showed that glucose yields were maintained as solids content and viscosity increased (up to 30% solids) when the reaction was run in an environment that overcame mass transfer limitations. The main objective of this thesis was to verify this effect using a variety of substrates. This was achieved by utilizing a high intensity mixer to conduct hydrolysis, comparing glucose release rates and yield at different solids concentrations. This mixer is designed specifically to thoroughly mix high viscosity materials and overcome the mass transfer inhibition. The materials to be investigated were dilute-base pretreated substrates: corn stover, wheat, and miscanthus, which are all common in Kentucky. The substrates were tested at solids concentrations ranging from 20% to 30% in the mixer. Shake flask tests were run simultaneously with each mixer test to determine a baseline.

II. LITERATURE REVIEW

Biomass

Biomass is an organic substance which can be converted into energy. The composition of many biomass products includes cellulose, hemicellulose and lignin. In the bulk scale, cellulose and hemicellulose are encased within an outer shell of lignin. Lignin serves as an adhesive to cement the cell walls of a plant together. The structure of this system is shown in Figure 2.

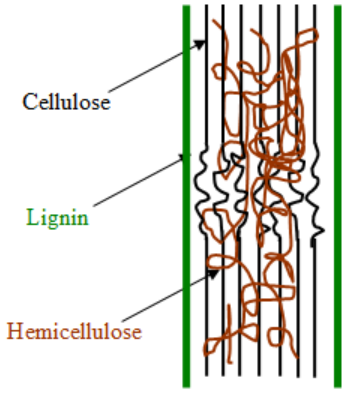

FIGURE 2: Biomass structure

Cellulose is a polymeric substance made up of glucose units, a six-carbon sugar. These units are held together by β-glycosidic linkages. It is a linear form with no branching. The structure is shown below in Figure 3.

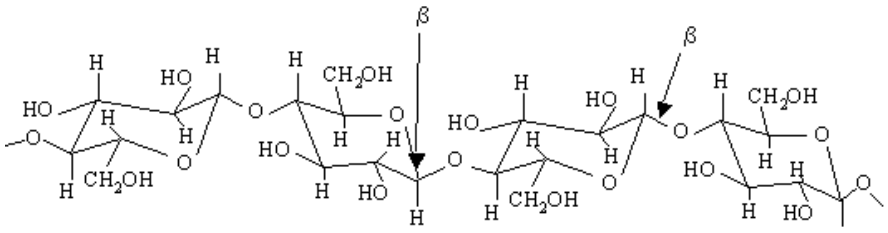

FIGURE 3: Cellulose structure

Cellulose molecules near one another are held together by hydrogen bonds, leading to high stability and resistance to chemical attack. The glucose units within cellulose are high in energy. When cellulose is broken down into its base of glucose molecules, it can lead to a usable energy source.

Hemicellulose is a polymer composed mostly of five-carbon units, but also some six-carbon units. The five-carbon units are mainly xylose and arabinose, while the six-carbon units are mannose, galactose, and glucose. These are also high energy sugars. The structure of hemicellulose is a branched molecule, compared to the linear form of cellulose. This causes it to have less hydrogen bonding between molecules, leading to an amorphous form. Because of its amorphous form, it is easier to break down into its base units.

Lignin is a non-carbohydrate polymer, composed of phenylpropanoic acids. It is complex in nature because of the cross-linking between the units. The monomers are held together by ether and carbon-carbon bonds (Chang et al., 1981; James and David, 1986). Lignin can be used for energy as well, by burning it for heat or converting it to electricity. Lignin and hemicellulose are believed to be bonded together by covalent bonds, enhancing the structural matrix and protecting the valuable cellulose.

Cellulose, hemicellulose, and lignin form a complex composite which strengthens plant cell walls. Table II shows dry mass compositions of various types of raw biomass as shown by Lee in 1997.

TABLE II

COMPOSITION OF VARIOUS TYPES OF BIOMASS

	Corn stover	**Wheat straw**	**Rice straw**	**Rice hulls**	**Bagasse fiber**	**Cotton in trash**	**News print**
Carbohydrate (% of Sugar Equivalent)							
Glucose	39	36.6	41	36.1	38.1	20	64.4
Mannose	0.3	0.8	1.8	3	NA	2.1	16.6
Galactose	0.8	2.4	0.4	0.1	1.1	0.1	NA
Xylose	14.8	19.2	14.8	14	23.3	4.6	4.6
Arabinose	3.2	2.4	4.5	2.6	2.5	2.3	0.5
Non-Carbohydrate (%)							
Lignin	15.1	14.5	9.9	19.4	18.4	17.6	21
Ash	4.3	9.6	12.4	20.1	2.8	14.8	0.4
Protein	4	3	NA	NA	3	3	NA

It is important to consider the areas where a certain type of biomass can grow as well as its ability to produce ethanol. It is not likely to be cost effective to transport biomass great distances to gain a slightly higher glucose release rate. Some examples of biomass which are able to grow well in Kentucky are corn, wheat, and miscanthus.

Using corn or wheat as a source for producing biofuels has the drawback of being major food sources. Much research has involved using corn stover as a source for the biofuels instead of the corn itself. Corn stover includes the leaves and stalks of corn, so its use does not affect the price of corn in the food market. In 2010, Kentucky produced over 150,000 bushels of corn, which is 1.2% of the total corn produced in the United States (Kentucky AgriNews, 2011). Wheat production was 16,500 bushels of wheat during 2010, or 0.7% for the United States.

Miscanthus is a perennial long grass native to Asia and Africa. It is a high energy crop which grows quickly. Once it is planted, it can be harvested every year for 20 years. Kentucky has recently imported miscanthus and has planted over 800 acres with plans to use it for its high energy content (Associated Press, 2009).

Biomass to ethanol process:

The process to convert biomass into ethanol to be used for its energy content is a series of steps. The first of these steps is milling. The feedstock is milled into smaller particles, causing the material to be easier to process. The biomass is then pretreated to ease access to the cellulose. The pretreated feedstock is then hydrolyzed into its base sugars, which are then fermented into ethanol. Upstream processes including pretreatment and hydrolysis are typically 60% of the total cost of manufacture (Nguyen and Saddler, 1991). The overall schematic of the production of ethanol from biomass is shown in Figure 4.

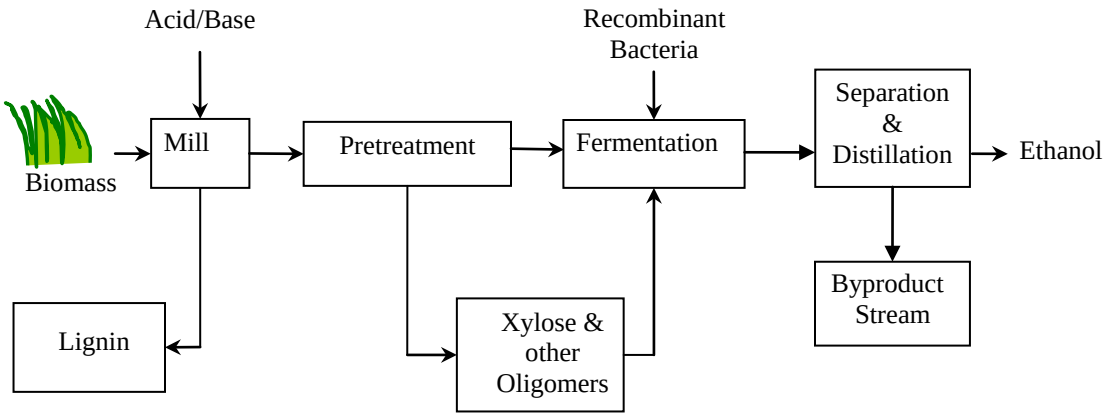

Figure 4: Overall biomass to ethanol process

The pretreatment step breaks apart the lignin crust, enabling the enzyme to reach the cellulose within the biomass product, as shown in Figure 5. The cellulose becomes less crystalline at this point. An enzyme digests amorphous cellulose more easily than when it is crystalline. The surface area of cellulose is increased during this process, increasing the enzyme accessibility to convert the polymers into fermentable sugars. During pretreatment, a small amount of hydrolysis also occurs. This is due to the extreme conditions, such as high temperature and high pressure, during the pretreatment step. The H_2 bonds in the hemicellulose and cellulose are broken down, forming five- and six-carbon sugars, pentoses and hexoses. These sugars can then be fermented into ethanol. The goals of pretreatment are reduction in crystallinity, increase in surface area, and reduction in lignin content.

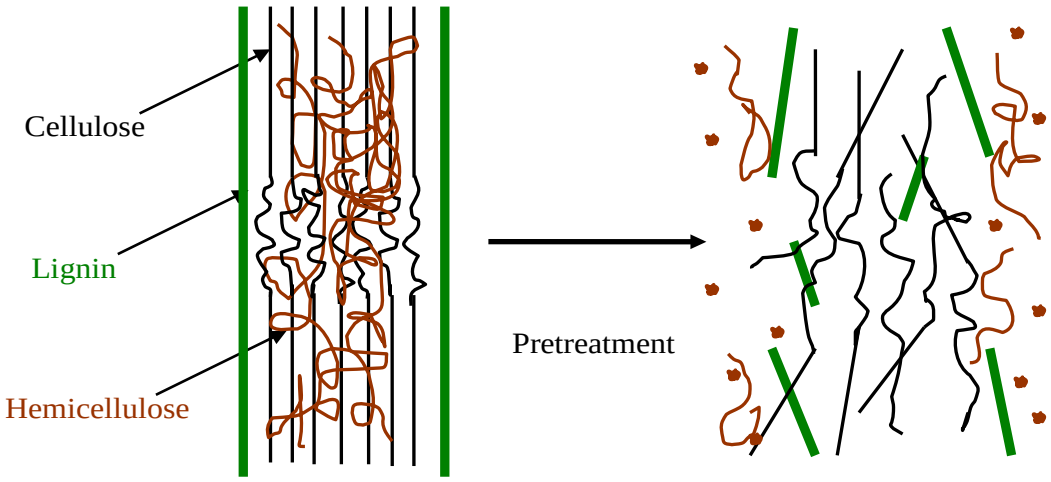

FIGURE5: Biomass being broken down by pretreatment. The lignin breaks up, increasing the accessibility of cellulose and hemicellulose

The two main categories of pretreatment are physical and chemical. Physical pretreatments, such as mechanical and non-mechanical, reduce particle size and crystallinity. Chemical pretreatment methods are used for structurally modifying lignocelluloses. In these methods, lignin is removed, and the pore size is increased (Abraham and Kurup, 1997).

The most common mechanical treatment is the grinding, or milling, process. Reports show grinding and milling improve enzymatic digestibility (Caufield and Moore, 1974; Koullas et al., 1990; Puri, 1984; Matsumura et al., 1977), but it is at high cost and energy intensive. Large scale grinding techniques are not as feasible as chemical pretreatment. Ball milling reduces particle size and crystallinity; however, the added benefits are mostly attributed to the small particle size (Chang and Holtzapple, 2000).

Common methods for chemical pretreatment include dilute-acid pretreatment, treatment with organic solvent or alkali, ammonia fiber explosion, and steam explosion, including acid-catalyzed steam explosion (Walsum et al,. 1996). Acid-catalyzed

pretreatment involves hydrolyzing the hemicellulose layer, but in alkali-catalyzed pretreatment, some of the lignin is removed and the hemicellulose needs to be hydrolyzed by use of hemicellulases (Hagerdal, 2006).

The most researched methods of pretreatment are steam explosion and dilute-acid pretreatment. Dilute-acid pretreatment methods have higher recoveries of hemicellulose sugars and faster reaction rates (Walsum et al., 1996), but they also have more corrosive operating conditions and higher costs. Steam explosion allows partial fractionation the substrate into its components (Schwald et al., 1989) and less corrosive environments, but does not have the high recoveries and reaction rates that dilute-acid pretreatment does. Based on the type of substrate, different pretreatment methods are better than others (Rivers, 1988) because of economic considerations and recovery of acid for recycling (Demirbas, 2006).

The hydrolysis step of the overall conversion to ethanol comes next. Hydrolysis is the process of breaking up cellulose molecules into the monomer glucose molecules. This is done in the presence of water, using hydrogen molecules to separate the monomers. Enzymes can help to break apart the polymeric cellulose.

Glucose is then fermented to form ethanol with the aid of yeast. The yeast ferments the sugar glucose in water to form ethanol and carbon dioxide. Ethanol at the end of the fermentation is still in excess water, so steps are taken to separate the ethanol from water. The solution is distilled until it reaches its azeotrope, then it is sent through molecular sieves to increase the separation in order to maximize the purity of the end ethanol product. Ethanol concentration in the end product is typically around 99%, with

the additional 1% being water. Often, the ethanol is denatured by a percentage of methanol as well.

Enzymatic Hydrolysis

Hydrolysis is the process of breaking a cellulose polymer into its glucose monomers in the presence of water. Glycosidic bonds are broken in this reaction, reducing cellulose to a cellobiose repeat unit. Cellobiose is then broken down further into glucose molecules as in Equation 1.

$$(C_6H_{10}O_5)_n \rightarrow C_{12}H_{22}O_{11} \rightarrow C_6H_{12}O_6 \quad (1)$$

Cellulose Cellobiose Glucose

Enzymatic hydrolysis (saccharification) is this same process helped along by an enzyme, and this is a common process. Enzymes which are able to convert cellulose into its monomer glucose are called cellulases. Cellulases are typically produced from different kinds of fungi. They are used as the biocatalyst for this conversion from cellulose to glucose.

There are four major steps in the mechanism for enzymatic hydrolysis. The enzymes diffuse onto the substrate surface. Glucose is released from the cellulose polymer, still on the enzyme. The glucose is released from the enzyme and into the bulk solution next. Finally, the enzymes are released into the bulk solution.

The surface area and crystallinity are directly related to the initial hydrolysis rate (Ramos et al., 1993 and Walker et al., 1991), so they appear to be major factors in the susceptibility of a substrate to saccharification.

Hydrolysis, even with the help of an enzyme, is a slow process. This is the main factor for time to make bioethanol from raw biomass. The amount of glucose released from this step is directly proportional to the amount of bioethanol which can be produced. Thus, maximum glucose release is crucial to successful enzymatic hydrolysis. Higher solids concentrations can lead to higher concentrations of glucose in the product stream as well as reduced costs in theory. The lower costs are from reduced process water and energy usage, and lower disposal and treatment costs since there is less water used, and reactors do not need to be as large since they have less need to accommodate large volumes of water along with the biomass.

High solids processing will only be feasible if the glucose release rates are similar for the higher solids concentrations as they are at lower solids concentrations. For these higher solids concentrations, the higher viscosity makes the solution more difficult to mix. It has been shown repeatedly that solids concentrations higher than 10% will dramatically reduce the release rate of glucose. This has been hypothesized to be due to insufficient mass transfer (Lübbert and Jørgensen, 2001; Mohagheghi et al., 1992; Spindler et al., 1988).

Mixing

There are multiple ways to provide mixing to a vessel, though an impeller-type mixer is most common among industrial processes. Depending on the type of impeller, radial flow or axial flow can be achieved. Radial flow is in the horizontal direction, with the impeller pushing fluid outward toward the wall in radial flow. Axial flow involves moving liquid parallel to the impeller shaft.

Recently, discoveries have been made in the field of laminar and viscous mixing (Suri, et al., 2002). A liquid can be blended homogenously with chaotic mixing, since the chaotic flow reaches isolated regions within the solution (Yao et al., 1998).

Ultrasound can be generated in a liquid, causing cavitations from the fast compression and expansion. This would cause the flow to be in the same direction as the propagating ultrasound waves (Yao et al., 1998).

Resodyn Acoustic Mixing (ResonantAcoustic® (RAM)) developed a mixer line which operates with low-frequency, high intensity sound energy mixing, called the LAB-RAM Acoustic Mixer (Figures 6 and 7). Designed to operate on a resonant frequency, material is mixed by an electromechanical oscillator. This system allows for rapid mixing, even for viscous materials. The mixing system has great high-viscosity mixing capability, low heat generation, and high rate of filler loading. High intensity mixing can be achieved throughout the entire volume.

The LAB-RAM mixer works effectively for liquid-gas, liquid-liquid, liquid-solid, and powder-powder systems. Colored chalk powder was fully blended with corn syrup in the mixer in only eight seconds. In addition, low bulk density 0.25 micron particle size fumed silica was fully blended with 250 micron particle size sand in only eight seconds.

FIGURE 6: LAB-RAM Mixer

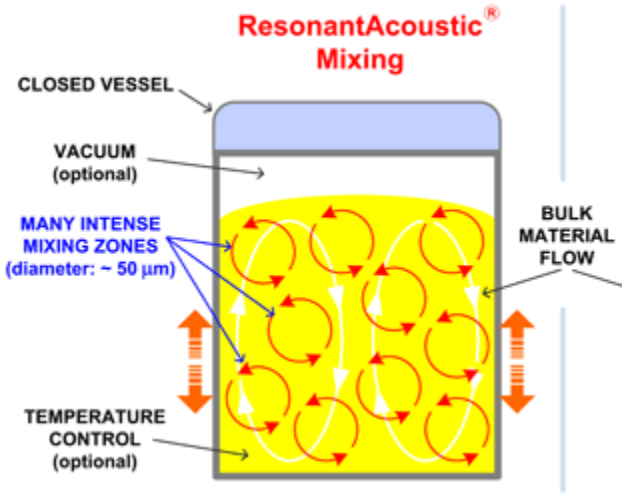

FIGURE 7: Mixing zones in the micro- and macro- scale

Mixing techniques can affect hydrolysis, and must be considered when designing the process. One of the most important factors to saccharification is adsorption of enzymes to the substrate. Due to the heterogeneity of an enzymatic hydrolysis system, adequate mixing is necessary to ensure enough contact between a liquid enzyme and the

solid substrate. Sufficient mixing also promotes increased heat and mass transfer within the system (Mais et al., 2002).

Lübbert and Jørgensen have suggested that substrates with a solids concentration higher that 10% decrease dramatically in effectiveness due to mass transfer limitations. It has been shown that it is possible to decrease these mass transfer limitations in highly viscous slurries by using a high intensity mixer (Rezania, 2009). A comparison of a test done under typical industrial operating conditions and one done with high intensity mixing is shown in Figure 8.

This example is a test with dilute-acid pretreated corn stover. The dotted lines represent the test run in shake flasks in an incubator shaker, while the solid lines represent operation in a high intensity mixer. For each of the different solids concentrations, the high intensity mixing resulted in higher glucose release than mixing in the shake flask. The percentage of glucose release when run in shake flasks decreased significantly at higher solids concentrations. The test at high intensity mixing did not show this behavior. Instead, at the reaction equilibrium, the percentage of glucose released approached the same amount for all the solids concentrations.

Quantification of dispersion coefficients proved that mass transfer limitations were overcome in the high intensity mixer. The dispersion coefficient for a 25,000 cP fluid was 5.81cm^2/s in the axial direction and 1.93cm^2/s in the radial direction. These are comparable to systems with much thinner fluids (Berson et al., 2002), where dispersion was calculated for water as a medium in a roller bottle reactor.

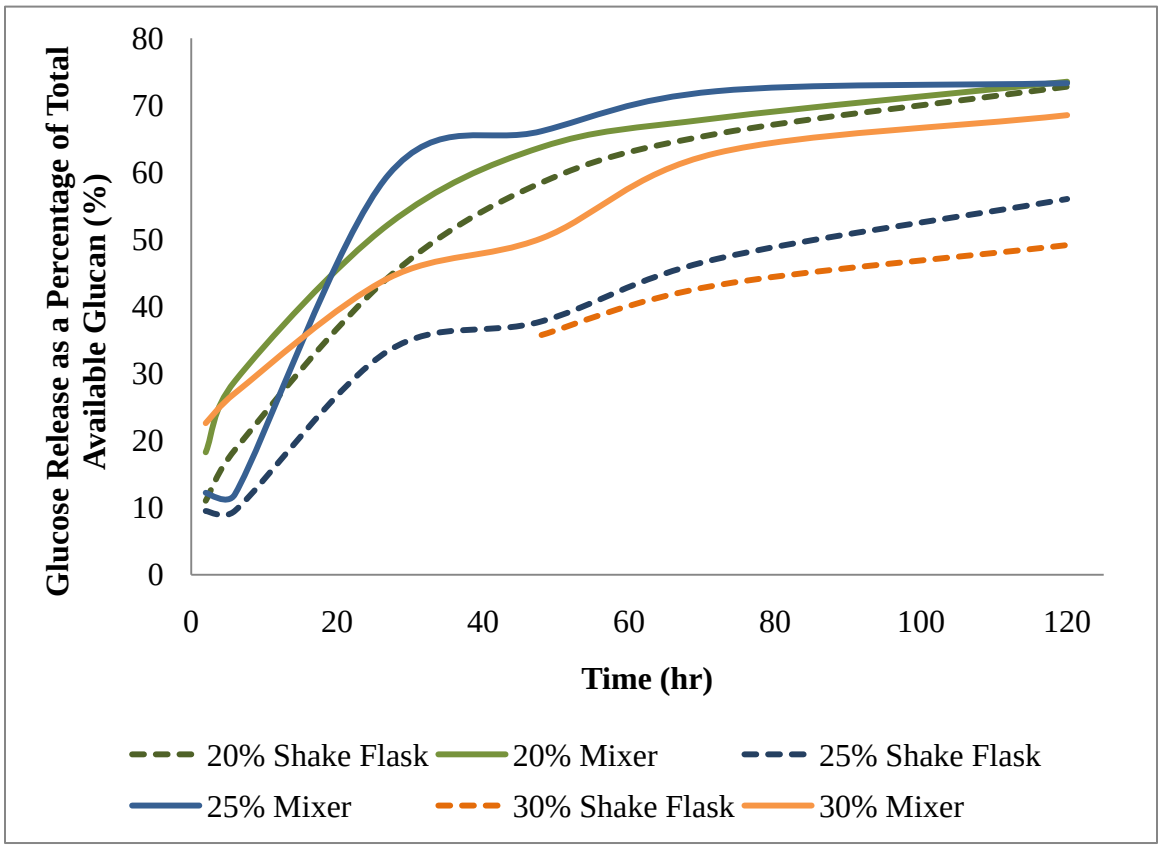

FIGURE 8: Comparison of glucose release in high intensity mixer and shake flasks (Rezania, 2009)

III. MATERIALS AND INSTRUMENTATION

Corn stover, wheat, and miscanthus were the biomass substrates used in this investigation of enzymatic hydrolysis. Each of these was pretreated with 0.4% NaOH for two hours at room temperature to improve glucose release rates. The composition of corn stover was 50.12% cellulose, 24.31% hemicellulose, and 19.60% lignin. The wheat was 44.39% cellulose, 27.29% hemicellulose, and 20.44% lignin. The miscanthus was 55.28% cellulose, 24.54% hemicellulose, and 18.82% lignin.

The samples were washed with deionized water equal to ten times the weight of the solid. Then they were vacuum distilled. This washing and vacuum distilling process was done three times to remove soluble solids and bases, which also decreased the pH of the slurry. Pictures of the substrates after vacuum distillation are shown in Figures 9-11.

FIGURE 9: Dilute-base pretreated corn stover after washing

FIGURE 10: Dilute-base pretreated wheat after washing

FIGURE 11: Dilute-base pretreated miscanthus after washing

The enzyme used to aid the hydrolysis process was Spezyme CP cellulase enzyme. It is a food grade enzyme, made by Genencor (Lot # 301-6295-230), a division of Danisco US, Inc. This enzyme was used for all three substrates.

A buffer solution was made to add to the slurry in order to keep the pH around 4.5. This was made by using 1 mole of citric acid, then filling up to 900mL with deionized water. NaOH pellets were added until the buffer reached a pH of 4.55. Cycloheximide (CAS 66-81-9) was also used in the solution. It was manufactured by Sigma Aldrich Co. (Lot # 030M1524, P-Code: 1000796858, SN C7698-5G).

Shake flask tests were run in an Innova 4230 Refrigerated Incubator Shaker, manufactured by New Brunswick Scientific (SN 101028846, Mfg. # M1233-0001). It is

a heated incubator which also provides is own shaking action and is pictured in Figure 12.

FIGURE 12: Innova 4230 Refrigerated Incubator Shaker

Mixer tests were run with a LABRAM Resonant Acoustic Mixer in a heated insulated box, made by Resodyn Acoustic Mixers (Doc # 100338B). The box was heated by a thermally protected, continuous duty centrifugal heater. It was manufactured by Dayton Electric Manufacturing Co. (Model 4C440, SN 7021 3466). The temperature was controlled by an 8500 Series Microprocessor Based Temperature Control, made by Love Controls (Model 85111-0, SN 949-1275-1). Figures 13 and 14 show the heated box and the temperature controller.

FIGURE 13: LAB-RAM Resonant Acoustic Mixer and centrifugal heater in heating box

FIGURE 14: 8500 Series microprocessor based temperature control

The samples were heated at 95°C for ten minutes to kill the reaction at sampling points. This was done in a VWR Analog Heatblock, manufactured by Henry Troemner LLC (Cat No 12621-104, SN 09021703), shown in Figure 15.

FIGURE 15: VWR Analog Heatblock

The samples were centrifuged to separate the liquid sample from the solid. This was done in a GP Centrifuge, made by Beckman (Model 8H075), shown in Figure 16.

FIGURE 16: GP Centrifuge

Analysis of liquid samples was done in an YSI 2700 Select Biochemistry Analyzer. It was manufactured by Yellow Springs Instrument Co., Inc. (Model 2700-D, SN 95H36904). A calibration standard solution for the YSI 2700 Select was an YSI 2776 Standard (Lot # 10B100606). The concentration of d-glucose (CAS 50997) was 2.50g/L, and l-lactate (CAS 27848802) was 0.50g/L. There is also a buffer solution which goes

with the YSI 2700 Select. A packet of 6.35 ± 0.35 g is mixed with 475 ± 25 mL deionized water to create the buffer. The packet contains disodium phosphate (CAS 7558794), monosodium phosphate (CAS 7558807), sodium benzoate (CAS 532321), dipotassium EDTA (CAS 25102129), sodium chloride (CAS 7647145), and gentamicin sulfate (CAS 1405410). The YSI Biochemistry Analyzer measures glucose content of a solution. The overall system is shown in Figure 17.

FIGURE 17: YSI 2700 Select Biochemistry Analyzer

IV. PROCEDURE

Enzymatic hydrolysis was run on samples of high solids concentration corn stover, wheat, and miscanthus. Each substrate was run at three different solids concentrations: 20%, 25%, and 30%. Each test was performed simultaneously in an incubator shaker and a high intensity mixer. The incubator tests were run at 50°C and 200 rpm in 250 mL shake flasks with a working mass of 75 grams in an Innova 4230 Refrigerated Incubator Shaker (New Brunswick Scientific Co., Inc., NJ). The high intensity mixer tests were run at 40°C to maintain 50°C within the solution and 30% intensity (~30Gs force) in a LAB-RAM Mixer (Resodyn Acoustic Mixing) with a working mass of 150 grams. It has been shown that in the LAB-RAM Mixer the temperature rises significantly in the system. Data shows that with an ambient temperature of 40°C, the solution will be maintained at 50°C (Rezania, 2009).

The pretreated substrates were washed with ten times their weight in deionized water three times, each time running through a vacuum filtration system. After the third wash and vacuum filtering process, the corn stover, wheat, and miscanthus were at 22%, 22%, and 30% solids, respectively. In order to run the tests at higher solids concentrations, all the substrates were dried in an Innova 4230 incubator to a solids concentration of 35%. The solids concentration at this point needed to be higher than the

solids concentration needed for the tests because a buffer solution, enzyme, cycloheximide, and tetracycline needed to be added, which would decrease the solids concentration.

A 1 molar citrate buffer solution was made with NaOH pellets to a pH of 4.8, and used as 5% of the each total slurry. This was to ensure a 0.05 mol/kg molality for each of the tests. The enzyme Spezyme was added at a loading rate of 15 filter paper units (FPU)/g cellulose. Deionized water, cycloheximide, and tetracycline were also in the mixture. Tables III-XI show the composition of solutions for each substrate at each solids concentration.

TABLE III

COMPOSITIONS OF EACH BATCH FOR HYDROLYSIS OF 20% CORN STOVER

	Shake Flask	Mixer
Working Mass (g)	75	150
Substrate (g)	42.86	85.71
Buffer (g)	3.75	7.50
Enzyme (mL)	2.3	4.5
DI Water (mL)	25.57	51.24

TABLE IV

COMPOSITIONS OF EACH BATCH FOR HYDROLYSIS OF 25% CORN STOVER

	Shake Flask	Mixer
Working Mass (g)	75	150
Substrate (g)	53.57	107.14
Buffer (g)	3.75	7.50
Enzyme (mL)	2.8	5.6
DI Water (mL)	14.35	28.71

TABLE V

COMPOSITIONS OF EACH BATCH FOR HYDROLYSIS OF 30% CORN STOVER

	Shake Flask	Mixer
Working Mass (g)	75	150
Substrate (g)	64.29	128.57
Buffer (g)	3.75	7.50
Enzyme (mL)	3.4	6.8
DI Water (mL)	3.04	6.08

TABLE VI

COMPOSITIONS OF EACH BATCH FOR HYDROLYSIS OF 20% WHEAT

	Shake Flask	Mixer
Working Mass (g)	75	150
Substrate (g)	42.86	85.71
Buffer (g)	3.75	7.50
Enzyme (mL)	2.0	4.0
DI Water (mL)	25.87	51.74

TABLE VII

COMPOSITIONS OF EACH BATCH FOR HYDROLYSIS OF 25% WHEAT

	Shake Flask	Mixer
Working Mass (g)	75	150
Substrate (g)	53.57	107.14
Buffer (g)	3.75	7.50
Enzyme (mL)	2.5	5.0
DI Water (mL)	14.65	29.31

TABLE VIII

COMPOSITIONS OF EACH BATCH FOR HYDROLYSIS OF 30% WHEAT

	Shake Flask	Mixer
Working Mass (g)	75	150
Substrate (g)	64.29	128.57
Buffer (g)	3.75	7.50
Enzyme (mL)	3.0	6.0
DI Water (mL)	3.44	6.88

TABLE IX

COMPOSITIONS OF EACH BATCH FOR HYDROLYSIS OF 20% MISCANTHUS

	Shake Flask	Mixer
Working Mass (g)	75	150
Substrate (g)	42.86	85.71
Buffer (g)	3.75	7.50
Enzyme (mL)	2.5	5.0
DI Water (mL)	25.37	50.74

TABLE X

COMPOSITIONS OF EACH BATCH FOR HYDROLYSIS OF 25% MISCANTHUS

	Shake Flask	Mixer
Working Mass (g)	75	150
Substrate (g)	53.57	107.14
Buffer (g)	3.75	7.50
Enzyme (mL)	3.1	6.2
DI Water (mL)	14.05	28.11

TABLE XI

COMPOSITIONS OF EACH BATCH FOR HYDROLYSIS OF 30% MISCANTHUS

	Shake Flask	Mixer
Working Mass (g)	75	150
Substrate (g)	64.29	128.57
Buffer (g)	3.75	7.50
Enzyme (mL)	3.7	7.5
DI Water (mL)	2.74	5.38

A test was run at each of these conditions in a shaker incubator and a high intensity mixer. Three samples were drawn from each test at 4, 8, 24, 48, 72, and 96 hours. The samples were heated at 95°C for ten minutes in order to kill the reaction. The

samples were then centrifuged at 2000 rpm for ten minutes in order to separate the liquid sample from the solids. Glucose released was measured with an YSI Biochemistry Analyzer. Each sample was tested with the YSI two times, for a total of six measurements per sample.

V. RESULTS AND DISCUSSION

Results for dilute-base pretreated substrates are shown in Figures 18 (corn stover), 22 (wheat), and 23 (miscanthus). Each plot has six curves. The dotted lines are those for shake flask results, whereas the solid lines are those for the high intensity mixing. The lines for each solids concentration are the same color for the shake flask and mixer results.

The particle sizes of the corn stover were around ¼", and the solution had no free liquid. A picture of 20% solids corn stover is shown in Figure 18 below. All three substrates had the same general appearance as the corn stover, so this is a representation of all three. At 25% and 30% solids, the solution looked similar, only slightly more solid. This type of solution is not ideal for hydrolysis because of the large stringy particles and lack of flow by the material.

Figure 18: 20% solids corn stover slurry

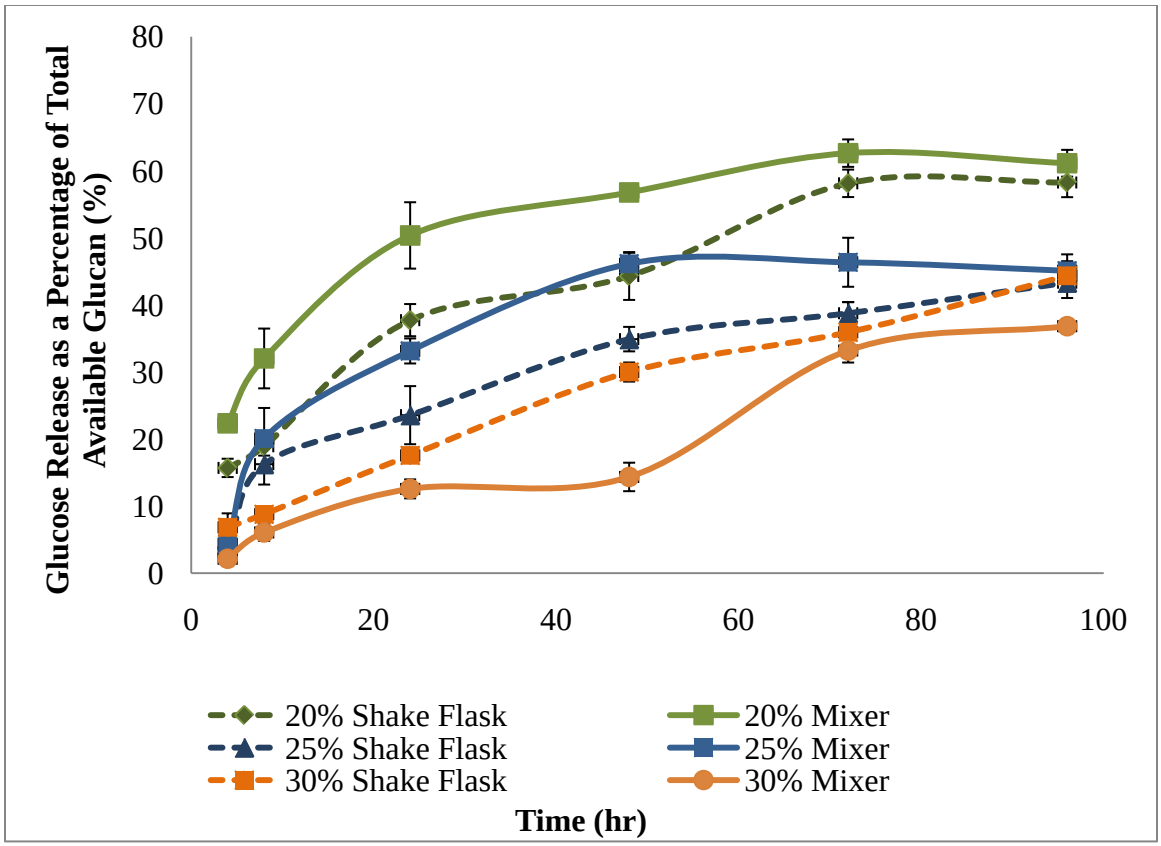

FIGURE 19: Pretreated corn stover glucose release for shake flask and high intensity mixing trials

Most of the glucose released occurred in the first 24-48 hours, and then release seemed to level out over the rest of the time period. This agrees with the typical trend of enzymatic saccharification where there are two main regions. At the onset of the reaction, glucose release rate is high, which is typically observed over the first 48 hours. Then the reaction rate slows over the remainder of the reaction (Dasari, et al., 2009). An example of a typical conversion rate is shown in Figure 19.

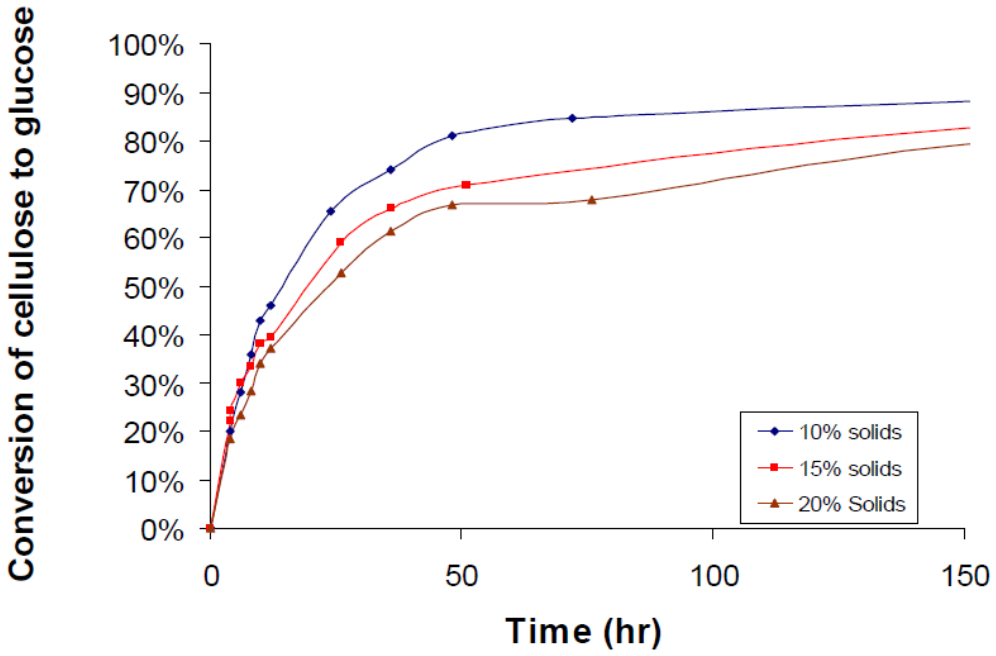

FIGURE 20: General trend of enzymatic saccharification (Rezania, 2009)

The high intensity mixing did not appear to maintain glucose release rates and yields as solids concentrations increased from 20% to 30%. At 30% solids, the glucose release was 36.8% of the available glucan. This is much lower than the 25% solids trial, which achieved 46.1% release. This is in turn significantly lower than at 20%, where the release was 62.6%. The glucose release increased by 70% in the 20% compared to the 30% solids. Preliminary results by Rezania (2009) showed that higher solids concentration corn stover approached the same release rate and yield of glucose as did lower solids concentrations (see Figure 20) due to the mixing environment. At 20% solids, glucose release was 73.5% of the available glucan, and at 30%, the glucose release was 68.5% (a decrease of just 7%). Therefore, the results of the current investigation do not agree with results from Rezania (2009). The glucose release was not maintained at higher solids concentrations.

Analysis of this corn stover solution is difficult due to having no free liquid and high particle sizes and viscosity. Due to the large particle sizes, and the solution appearing to be mostly solid, the viscosity could not be measured with available equipment. Without the viscosity measurement, it cannot necessarily be assumed that the mixer is able to overcome the mass transfer limitations as was the case in the study by Rezania (2009).

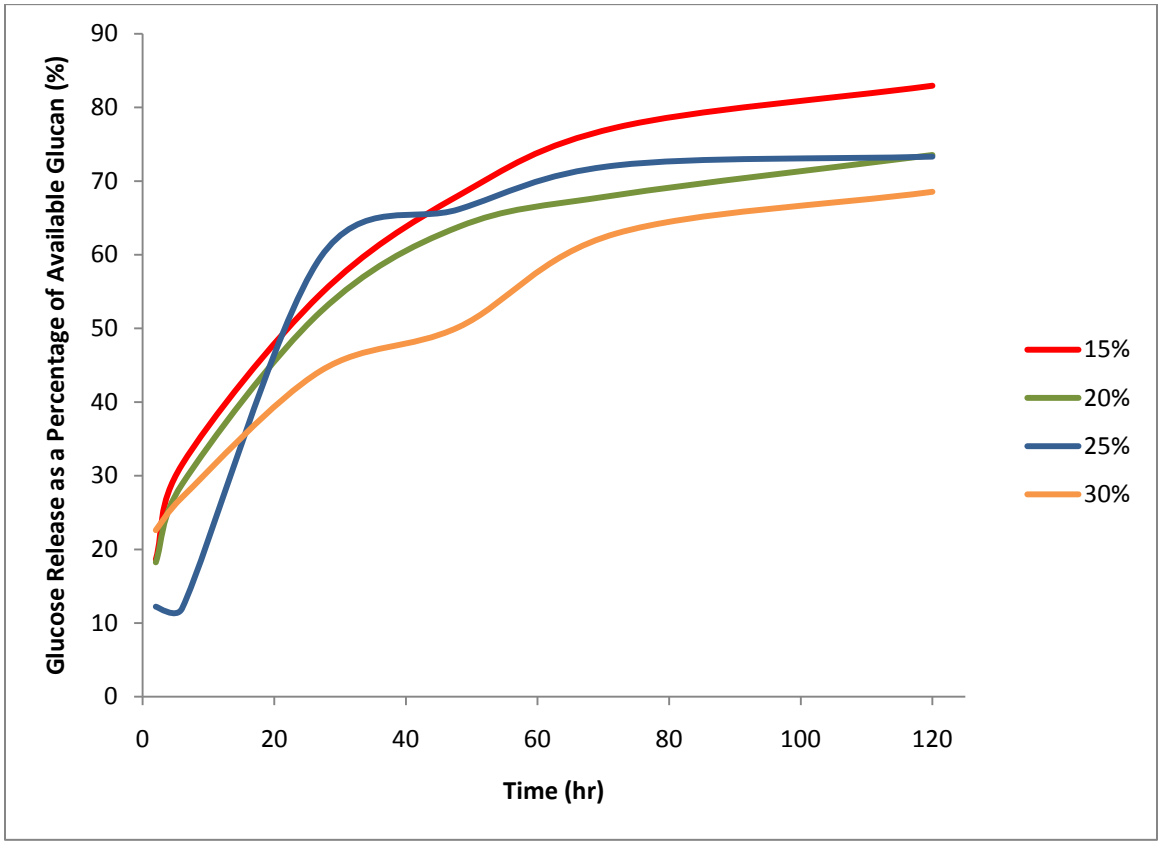

FIGURE 21: Comparison of glucose release between different solids concentrations for dilute-acid pretreated corn stover under high intensity mixing (Rezania, 2009).

High intensity mixing resulted in higher glucose release rates than was achieved in the shake flasks. At 20% solids, the mixer released 2.8% more glucose than the shake

flask. For 30% solids, samples were difficult to obtain because the solution was so viscous (Figure 21). It was difficult to obtain a liquid sample because free liquid was not present and solids dominated the solution. The percent of glucose released from the system at 20% solids is within 10% to 20% of what has been seen previously with corn stover (Dasari, et al., 2009; Dunaway et al., 2010).

FIGURE 22: 30% solids corn stover from the mixer at 96 hours

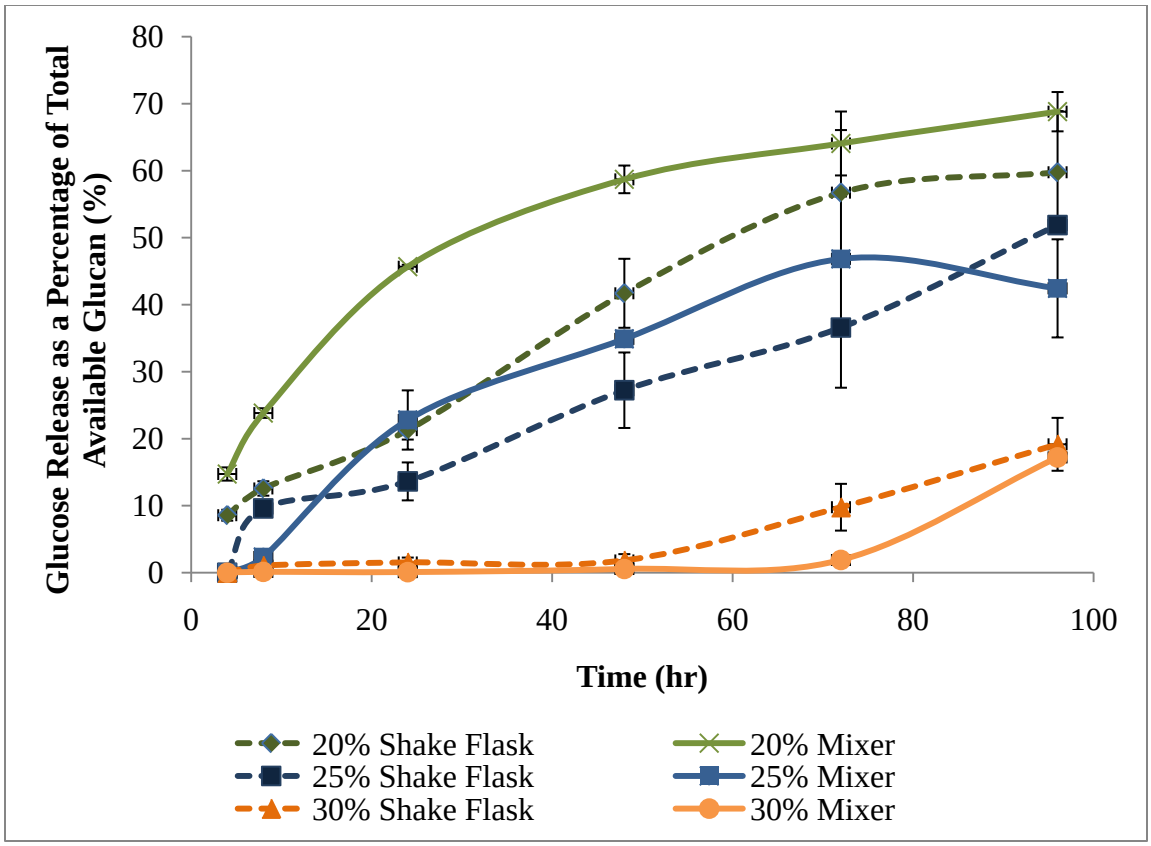

FIGURE 23: Pretreated wheat glucose release for shake flask and high intensity mixing trials

The glucose release trends were similar for wheat. The glucose release at 20% solids was 68.8% of total available glucan. 46.8% of the available glucose was released at 25% solids, and 17.2% glucose at 30% solids. Four times more glucose was released from the 20% solids batch than from the 30% solids batch.

As with corn stover, it was difficult to obtain a liquid sample from the wheat slurry. The 30% solids solution was so thick the slurry was unable to mix well enough to obtain any useful data. Although the data for 30% solids data was not a good representation of what can be expected in a hydrolysis reaction, some evaluation of the 20% and 25% solutions can be done.

With the exception of the 96 hour data point for 25% solids, the trend seemed as if it were nearing the trend of the 20% solution. The solution appeared to have mixed well quickly up to 25% solids, since the glucose release follows the general trend which is expected. The 25% solids still had a lower glucose yield than the 20% solids.

Again, using the mixer results in a higher release rate than the shake flasks. At 20% solids, the mixer released 9.0% more glucose than the shake flask. Once again, it was difficult to obtain liquid samples from the wheat at 30% solids, particularly for the mixer. The total percent of glucose release was on the same order as corn stover.

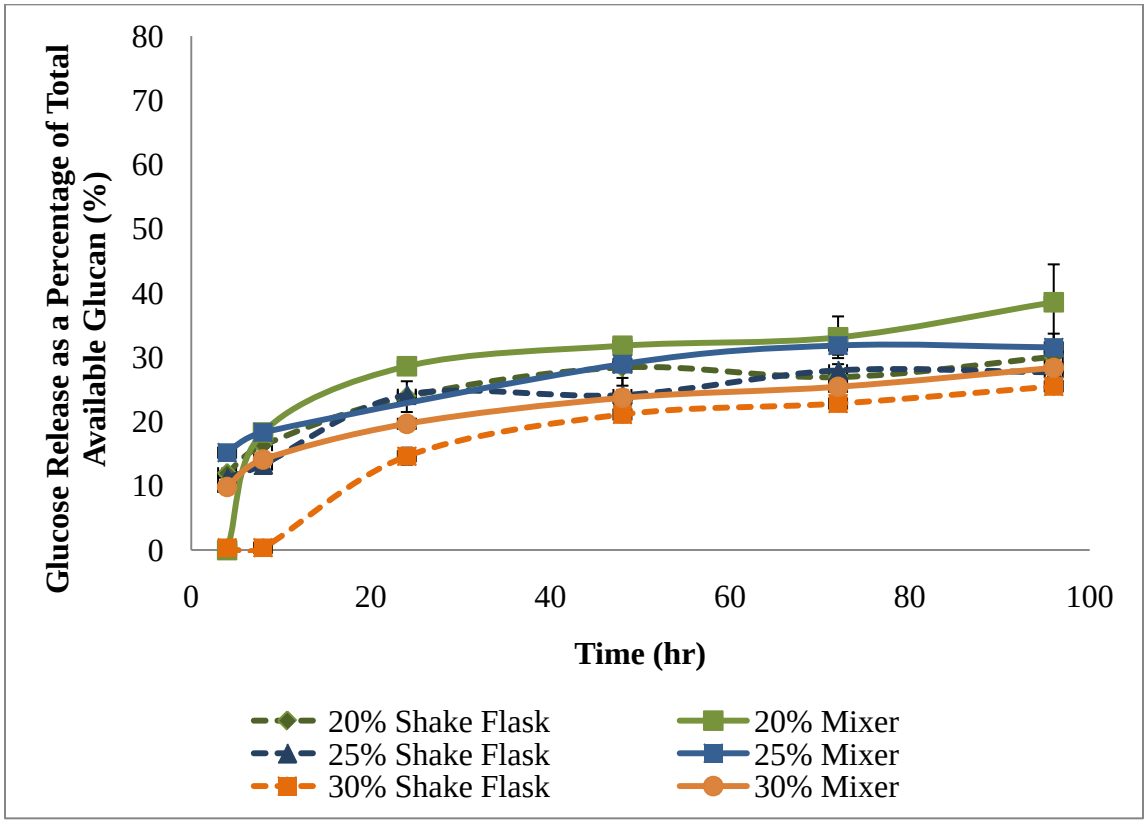

FIGURE 24: Pretreated miscanthus glucose release for shake flask and high intensity mixing trials

Miscanthus also showed that higher solids concentrations yielded less glucose than lower solids concentrations in the mixer. At 20% solids, the glucose released was 38.6% of the total available glucan. At 25% solids, the release was 31.5% and at 30%, the release was 28.4% of the total available glucan. Glucose release was 36% higher for 20% solids than 30% solids.

Miscanthus had higher glucose release from the mixer trials than the shake flask trials at all three solids concentrations. At 20% solids, the mixer released 8.6% more glucose than the shake flask. Sampling was not nearly as difficult in these trials because free liquid was available from both the shake flasks and the mixer. The glucose release rate never reached 40% of total glucan available, which is consistent with hydrolysis of substrates which have not been pretreated. Enzymatic hydrolysis of untreated sawdust resulted in a maximum glucose release rate of 30% (Rezania, 2009).

For miscanthus, it is not reasonable to make conclusions relative to the stated objective. The glucose yields at all solids concentrations were on the order of unpretreated biomass, and therefore, the pretreatment method was not effective for miscanthus.

The glucose release rates from the mixer trials were expected to be similar for 20%, 25%, and 30% solids concentrations for each of the substrates, based on results by Rezania (2009). This expectation did not hold up upon investigation here. The results showed higher glucose yields at lower solids concentrations for all three substrates tested.

For this system with dilute-base pretreated substrates, it was difficult to fully test whether the substrates would maintain glucose yield at high solids concentrations. Due

to the poor slurry characteristics, such as high particle sizes and viscosity, it is not necessarily fair to pull conclusions about minimizing mass transfer inhibition.

Based on results from Rezania with the same system, the mixer trial was expected to release more glucose than the shake flask trials. This expectation held up with results from the current investigation. The glucose released from the mixer trials was higher in most cases than the shake flask trials. The two exceptions were for 30% solids corn stover and wheat. This may be explained by an ineffective pretreatment method. Also, the pretreatment with dilute base gave much larger particle sizes (~1/4") than the pretreatment with dilute acid (~200μm), which leaves less surface area and les open pores for enzyme accessibility.

It can be noted that glucose yield appears to still be increasing after 96 hours in many instances. If this were the case, a test over a longer time period might show glucose release rates for the higher solids concentration trials will approach those of the lower solids.

The substrates in this investigation were pretreated with a dilute base, whereas the corn stover in the Rezania trials was pretreated with a dilute acid. The corn stover pretreated with a dilute base had a noticeably higher particle size than corn stover pretreated with a dilute acid. The dilute-base pretreated corn stover had particle sizes in the range of ¼" whereas the dilute-acid pretreated corn stover had particle sizes in the range of 30 microns. Photos of the two pretreated substrates are shown in Figure 24. The higher particle size causes it to be more difficult to mix well. Also, this may lead to higher crystallinity and lower surface area and pore size which are limiting factors to glucose release due to decreased enzyme accessibility.

FIGURE 25: Dilute-base pretreated corn stover (left) compared to dilute-acid pretreated corn stover (right)

The corn stover responded differently for dilute-base pretreated than dilute-acid pretreated methods. In addition, the miscanthus released glucose at a rate similar to untreated biomass, where corn stover and wheat both released glucose at higher rates. This shows it is necessary to optimize the pretreatment method for individual substrates in order to achieve the highest possible glucose release.

VI. CONCLUSIONS

The main objective of this testing was to determine if dilute-base pretreated corn stover, wheat, and miscanthus would maintain glucose yields at higher solids concentrations when the mass transfer limitations were minimized. For corn stover, there was a 70% glucose release difference between 20% and 30% solids. For wheat, this difference was 400%, and for miscanthus it was 36%, although overall conversion for miscanthus was low due to ineffective pretreatment. The results were unexpected because they contradicted previous results that showed rates and yields were maintained as solids concentrations increased when enzymatic hydrolysis was run in the absence of mass transfer limitations. Results here were inconclusive in regards to mass transfer limitations due to the inability to quantify viscosity for most of the slurries. However, the contradictory results indicate there may be some effect other than mass transfer limitations that affects glucose release rates and yields.

For good hydrolysis conditions, there cannot be particle sizes as large as ¼". Also, it is important to have enough free liquid in a sample to measure glucose content. When the slurry does not allow this, it is difficult to obtain useful results. For the tests in which the slurry appeared to be well mixed and liquid samples could be obtained, there was still a reduction in glucose yield at high solids concentrations. One conclusion that

can be reasonably made from this is that the dilute-base pretreatment method is ineffective for corn stover, wheat, and miscanthus.

For all three substrates, the results show the mixer gave higher glucose release rates than the shake flasks (another indication that mass transfer limits the reaction). At 20% solids, the mixer gave 2.8% more glucose release for corn stover, 9.0% for wheat, and 8.6% for miscanthus.

Based on comparison to previous research, the dilute-base pretreatment method was not effective for all substrates, particularly for miscanthus, as it only achieved glucose release on the order of biomass which had not been pretreated.

VII. RECOMMENDATIONS

Pretreatment should be optimized for each individual substrate. The substrates in this testing had high particle sizes, leading to a higher viscosity than expected, due to the pretreatment by a dilute base. Also, the method did not improve the glucose yield of miscanthus to the same extent it did for corn stover and wheat.

Another recommendation for improved results would be to extend the testing period. Some of the glucose yields appeared to still be increasing at the end of 96 hours. If the testing were done over a longer time period, the final release of glucose would be clear, so these results could be confirmed.

REFERENCES

Brown, Leland E. "Annual Crop Summary 2009-2010 Harvested Acreage, Yield, and Production, Kentucky and United States." *Kentucky Agri-News* 30.1 (2011): 4. Print.

Caufield, D. F., Moore, W. E., 1974.Effect of varying crystallinity of cellulose on enzymatic hydrolysis. Wood Science., 6, 375-379.

Chang M., Chou, T., Tsao, T., 1981.Structure, pretreatment and hydrolysis of cellulose. AdvBiochem Eng., Vol. 20, Pp.15– 42.

Chang, V. S., Holtzapple, M. T., 2000. Fundamental factors affecting biomass enzymatic reactivity. Appl. Biochem. Biotechnol. 84/86, 5-37.

Dasari, Dunaway, and Berson: "A Scraped Surface Bioreactor for Enzymatic Saccharification of Pretreated Corn Stover Slurries", *Energy & Fuels*, **23**(1), 492-497(2009)

Demirbas, A. (2000a). Recent advances in biomass conversion technologies. Energy Edu. Sci. Technol. 6, 19-41.

Demirbas, A. (2006). Biogas potential of manure and straw mixtures. Energy Sources Part A. 28, 71–78.

Dunaway, Dasari, Bennett, and Berson: "Characterization of Changes in Viscosity and Insoluble Solids Content During Enzymatic Saccharification of Pretreated Corn Stover Slurries", *Bioresource Technology*, **101**, 3575-3582 (2010)

Duncan, R. C., Youngquist w., 2004, Encircling the Peak of World Oil Production, Natural Resource Research, Vol. 8, Pp. 219-232.

Hägerdal, B. H., Galbe, M., Grauslund, M. F., Lidén, G., & Zacchi, G. (2006). Bioethanol – the fuel of tomorrow from the residues of today. Trends in Biotechnology. 24 (12), 549-556

Koullas, D. P., Christakopoulos, P. F., Kekos, D., Macris, B. J., Koukios, E. G., 1990. Effect of cellulose crystallinity on the enzymatic hydrolysis of lignocellulosic by *FusariumOxysporum*cellulases. Cellulose Chem., 24, 469-474.

Lübbert, A., and JØrgensen, B. S., 2001. Bioreactor performance: a more scientific approach for practice, J. Biotechnol., 85(2), 187-212.

Matsumura, Y., Sudo, K., Shimizu, K., 1977. Enzymatic hydrolysis of wood II. Effect of grinding and alkali treatment on hydrolysis of woods by *Trichodermaviride cellulase*. Mokuzai Gakkaishi., 23, 562-570.

"Miscanthus Planted in Ky. as Biofuel - U.S. News - Environment - Msnbc.com." *Breaking News, Weather, Business, Health, Entertainment, Sports, Politics, Travel, Science, Technology, Local, US & World News - Msnbc.com*. 21 May 2009. Web. 1 Feb. 2011. <http://www.msnbc.msn.com/id/30808099/ns/us_news-environment/>.

Mohagheghi, A., Tucker, M., Grohman, K, and Wyman, C. E., 1992. High Solid Simultaneous Saccharification and Fermentation of Pretreated Wheat Straw to Ethanol, ppl.Biochem.Biotechnol., 33, 67-81.

Nguyen, Q. A., & Saddler, J. N. (1991).An integrated model for the technical and economic evaluation of an enzymatic biomass conversion, Bioresource Technology.

Puri, V. P., 1984.Effect of crystallinity and degree of polymerization of cellulose on enzymatic saccharification.Biotechnol.Bioeng., 26, 1219-1222.

Ramos, L. P., Breuil, C., & Saddler J. N. (1993).The use of enzyme recycling and the influence of sugar accumulation on cellulose hydrolysis by Trichoderma cellulases.Enzyme and Microbial Technology. 15 (1), 19-25.

Rezania S, Ye Z, and Berson RE: “Enzymatic Saccharification and Viscosity of Sawdust Slurries Following Ultrasonic Particle Size Reduction”, *Appl. Biochem. & Biotech*, **153**, 103-115(2009)

Rivers, D. B., Emert, G. H. (1988). Factors affecting the enzymatic hydrolysis of bagasse and rice straw. Biological Wastes. 26 (2), 85-95.

Saddler, J. N., 1986, Factors Limiting the Efficiency of Cellulase Enzymes, Microbiol. Sci., Vol. 3, Pp. 84-7.

Schwald, W., Breuil, C., Brownell, H. H., Chan, M., & Saddler, J. N. (1989).Assessment of pretreatment conditions to obtain fast complete hydrolysis on high substrate concentrations. Appl. Biochem. Biotechnol.20/21, 2944.

Spindler, D. D., Wyman, C. E., Mohagheghi, A. and Grohmann, K., 1988, Thermotolerant Yeast for Simultaneous Saccharification and Fermentation of cellulose to Ethanol, Appl. Biochem. Biotechnol., Vol. 17, Pp. 279-293.

Sun, X., Sun, R., Sun, X. F, Su, Y., 2004. Fractional and physico-chemical characterization of hemicelluloses from ultrasonic irradiated sugarcane bagasse. Carbohydrate Research, Vol. 339, Pp. 291– 300.

Walker. L. P., & Wilson, D. B. (1991). Enzymatic hydrolysis of cellulose: An overview. Bioresource Technology. 36, 3-14.

Walsum, G. P., Allen, S. G., Spencer, M. J., Laser, M. S., Antal, M. J., & Lynd, L. R. (1996). Conversion of lignocellulosics pretreated with liquid hot water to ethanol. Appl. Biochem. Biotech. 57, 157-170.

Yao, W., Sato, K., Takahashi, K., Koyama, K. 1998. Mixing Performance Experiments in impeller Stirred Tanks Subjected to Unsteady Rotational Speeds. Chem. Eng. Sci., Vol. 53.Pp 3031.

APPENDIX

TABLE XII

CORN STOVER SHAKE FLASK RAW DATA

20% Corn Stover										
Theoretical max		110.264								
	Sample Number							Average	% Theoretical max	
	Glucose (g/L)	1.1	1.2	2.1	2.2	3.1	3.2			Std. Dev.
Time (hr)	4	16.1	15.9	16.7	16.7	19.2	19.2	17.3	15.6896	1.50599
	8	18.06	18.12	15.87	15.39	29.22	28.5	20.86	18.9182	6.29965
	24	44.7	45.3	39.9	40.2	39.6	39.9	41.6	37.7276	2.64726
	48	44.6	45	48.25	48.3	53	54	48.8583	44.3103	3.93172
	72	63	63.6	67.2	66.6	62.4	61.8	64.1	58.1332	2.25832
	96	60.83	61.81	65.31	64.47	66.78	66.22	64.2367	58.2572	2.41258
25% Corn Stover										
Theoretical max		137.83								
	Sample Number							Average	% Theoretical max	
	Glucose (g/L)	1.1	1.2	2.1	2.2	3.1	3.2			Std. Dev.
Time (hr)	4	4.26						4.26	3.09076	#DIV/0!
	8	23.58	23.52	19.98	20.25	23.61	23.4	22.39	16.2446	1.76574
	24	24.99	25.2	34.2	34.2	37.8	38.4	32.465	23.5544	5.97265
	48	46.8	45.2	50	50.4			48.1	34.8981	2.51661
	72	50.5	50.5	55	55	54.5	55	53.4167	38.7555	2.26752
	96	55.8	56.88	63	63.6	59.1	59.82	59.7	43.3142	3.15077
30% Corn Stover										
Theoretical max		165.396								
	Sample Number							Average	% Theoretical max	
	Glucose (g/L)	1.1	1.2	2.1	2.2	3.1	3.2			Std. Dev.
Time (hr)	4	7.06	6.95	12.6	12.1	14.4	14.6	11.285	6.82302	3.45626
	8	15.4	15.3	13.6	13.9	14.5	14.4	14.5167	8.77692	0.72503
	24	28.2	28.4	28.6	28.8	30	30.2	29.0333	17.5538	0.85245
	48	52	52	50	50	47.2	46.4	49.6	29.9886	2.35966
	72	49.86	50.4	66	66.6	61.8	61.8	59.41	35.9199	7.46902
	96	77	72.8	74.2	72.8	76.3	66.88	73.33	44.336	3.6114

TABLE XIII

CORN STOVER MIXER RAW DATA

20% Corn Stover										
Theoretical max		110.264								
	Sample Number							Average	% Theoretical max	
Glucose (g/L)		1.1	1.2	2.1	2.2	3.1	3.2			Std. Dev.
Time (hr)	4	24	24	25	24.8	25	25	24.6333	22.3403	0.49666
	8	33.9	33.6	30.6	30.9	41.7	41.1	35.3	32.0141	4.91732
	24	59.2	59.6	62.4	50	51.2	50.8	55.5333	50.364	5.45772
	48	60.6	61.8	64.2	63	63	63	62.6	56.7728	1.23935
	72	67.48	66.78	72.8	68.39	70.7	68.25	69.0667	62.6375	2.25729
	96	66.33	64.82			69.44	69.02	67.4025	61.1283	2.20509
25% Corn Stover										
Theoretical max		137.83								
	Sample Number							Average	% Theoretical max	
Glucose (g/L)		1.1	1.2	2.1	2.2	3.1	3.2			Std. Dev.
Time (hr)	4	4.84	4.74	7.42	7.36	5.85	6.06	6.045	4.38584	1.16732
	8	27.75	27.6					27.675	20.0791	0.10607
	24	43.2	43.6	43.2	47.6	48.4	48	45.6667	33.1326	2.57268
	48	65	64	65.5	65.5	60.5	61	63.5833	46.1317	2.26752
	72	58.02	57.18	67.8	64.8	67.2	68.4	63.9	46.3615	5.03771
	96	60.62	60.34	63.77	64.19	57.26	66.71	62.1483	45.0906	3.37961
30% Corn Stover										
Theoretical max		165.396								
	Sample Number							Average	% Theoretical max	
Glucose (g/L)		1.1	1.2	2.1	2.2	3.1	3.2			Std. Dev.
Time (hr)	4	3.81	3.74	3.32	3.26			3.5325	2.13578	0.28253
	8	7.59	8.99	11.7	11.8			10.02	6.05819	2.07819
	24	22.8	22.8	18.7	18.8			20.775	12.5608	2.33862
	48	25.05	25.53	19.41	19.11	26.91	26.31	23.72	14.3413	3.51447
	72	54.9	54.9					54.9	33.1931	0
	96	60.6	61.2					60.9	36.8207	0.42426

TABLE XIV

WHEAT SHAKE FLASK RAW DATA

20% Wheat										
Theoretical max		97.658								
	Sample Number							Average	% Theoretical max	
	Glucose (g/L)	1.1	1.2	2.1	2.2	3.1	3.2			Std. Dev.
Time (hr)	4			9.17	8.95	7.64	7.74	8.375	8.57585	0.7971
	8	12.8	13.5			11.4	11.4	12.275	12.5694	1.05
	24	21.4	21.4	21.8	22	19.12	18.94	20.7767	21.2749	1.37395
	48	35.28	34.68	46	46.4	41.2	40.8	40.7267	41.7034	5.02897
	72	55.68	55.62	55.86	55.74	54.36	55.14	55.4	56.7286	0.56625
	96	60.6	59.1	48	47.52	67.8	67.2	58.37	59.7698	8.91806
25% Wheat										
Theoretical max		122.073								
	Sample Number							Average	% Theoretical max	
	Glucose (g/L)	1.1	1.2	2.1	2.2	3.1	3.2			Std. Dev.
Time (hr)	4							#DIV/0!	#DIV/0!	#DIV/0!
	8	11.7						11.7	9.58447	#DIV/0!
	24	13.59	13.56	15.54	15.15	20.79	21.15	16.63	13.6231	3.45777
	48	42	42	27.6	27.57	30	30.3	33.245	27.2338	6.87859
	72	44.8	45.2	45.6	46	43.2	43.2	44.6667	36.5903	1.20444
	96	63	63.5	64.5	63	63.5	62.5	63.3333	51.8817	0.68313
30% Wheat										
Theoretical max		146.487								
	Sample Number							Average	% Theoretical max	
	Glucose (g/L)	1.1	1.2	2.1	2.2	3.1	3.2			Std. Dev.
Time (hr)	4							#DIV/0!	#DIV/0!	#DIV/0!
	8	2.16	2.21	1.33	1.32	1.06	1.15	1.53833	1.05015	0.51152
	24	1.73	1.74	3.63	3.56	1.38	1.37	2.235	1.52573	1.06592
	48	4.39	4.35	2.12	2.11	2.07	1	2.67333	1.82496	1.38172
	72	8.69	8.77	14.1	14	20.2	20.1	14.31	9.76878	5.11141
	96	24.2	24	23.8	25.4	35.6	35.4	28.0667	19.1598	5.78504

TABLE XV

WHEAT MIXER RAW DATA

20% Wheat										
Theoretical max		97.658								
		Sample Number						Average	% Theoretical max	
	Glucose (g/L)	1.1	1.2	2.1	2.2	3.1	3.2			Std. Dev.
Time (hr)	4	14.2	14.3	14.6	14.6	12.8	15.8	14.3833	14.7283	0.96419
	8	23.4	23.6	23.8	24	22.3	22.5	23.2667	23.8246	0.70333
	24					44.8	44.4	44.6	45.6696	0.28284
	48	55.5	55.5	58.5	60.5	56	58	57.3333	58.7083	2.0166
	72			66.6	66.6	58.56	58.5	62.565	64.0654	4.65928
	96	66.6	65.4	70.8	70.8	64.8	64.8	67.2	68.8116	2.86496
25% Wheat										
Theoretical max		122.073								
		Sample Number						Average	% Theoretical max	
	Glucose (g/L)	1.1	1.2	2.1	2.2	3.1	3.2			Std. Dev.
Time (hr)	4							#DIV/0!	#DIV/0!	#DIV/0!
	8	2.85	2.86					2.855	2.33877	0.00707
	24	27.6	27.36	22.95	23.13	37.8	28.08	27.82	22.7897	5.39705
	48			42.8	42.4			42.6	34.8973	0.28284
	72	77	78		37.1	36.6		57.175	46.8369	23.4737
	96	55.74	55.62	35.82	55.56	56.28		51.804	42.4371	8.93986
30% Wheat										
Theoretical max		146.487								
		Sample Number						Average	% Theoretical max	
	Glucose (g/L)	1.1	1.2	2.1	2.2	3.1	3.2			Std. Dev.
Time (hr)	4							#DIV/0!	#DIV/0!	#DIV/0!
	8	0.208	0.199	0.118	0.109	0.186	0.21	0.17167	0.11719	0.04593
	24	0.17	0.144	0.076	0.079			0.11725	0.08004	0.04713
	48	0.655		0.87	0.873			0.79933	0.54567	0.12501
	72	1.39	1.41	3.46	3.43	3.51	3.43	2.77167	1.89209	1.06291
	96	25.2						25.2	17.2029	#DIV/0!

TABLE XVI

MISCANTHUS SHAKE FLASK RAW DATA

20% Miscanthus										
Theoretical max		121.616								
	Sample Number							Average	% Theoretical max	
	Glucose (g/L)	1.1	1.2	2.1	2.2	3.1	3.2			Std. Dev.
Time (hr)	4	14.4	14.2	14.3	14.8	15.1	14.5	14.55	11.9639	0.33912
	8	19.34	19.36	20.2	20.4	19.92	19.88	19.85	16.3219	0.43151
	24	25.8	25.6	32.4	32	29.2	29.2	29.0333	23.873	2.91319
	48	34.76	35.2	30.56	30.48	38.4	38.12	34.5867	28.4392	3.47882
	72	34	33.64	32.68	32.8	31.76	31.68	32.76	26.9372	0.94725
	96	34.68	31.56	37.68	37.72	39	38.96	36.6	30.0947	2.92706
25% Miscanthus										
Theoretical max		152.02								
	Sample Number							Average	% Theoretical max	
	Glucose (g/L)	1.1	1.2	2.1	2.2	3.1	3.2			Std. Dev.
Time (hr)	4	18.3	17.4	20.7	15.8	15.6	15.1	17.15	11.2814	2.11731
	8	19.86	19.95	20.6	20.4			20.2025	13.2894	0.355
	24	36.6	36.9					36.75	24.1745	0.21213
	48	38.92	38.92	31.44	31.48	39.64	39.64	36.6733	24.124	4.05107
	72	41.6	40.8	44.8	42	43.6	42.4	42.5333	27.9788	1.44591
	96	44.4	44.4	41.2	40.8	40.8	40.8	42.0667	27.6718	1.81402
30% Miscanthus										
Theoretical max		182.424								
	Sample Number							Average	% Theoretical max	
	Glucose (g/L)	1.1	1.2	2.1	2.2	3.1	3.2			Std. Dev.
Time (hr)	4	0.621	0.72	0.629	0.623	0.555		0.6296	0.34513	0.05885
	8	0.881	0.872	0.489		0.523	0.529	0.6588	0.36114	0.19934
	24	24.2	24.1	30.2	28.6	26.4	26.4	26.65	14.6088	2.40977
	48	37.2	37.2	35.7	38.7	41.1	41.1	38.5	21.1047	2.22621
	72	38.76	40.8	42.8	42	42.8	42.4	41.5933	22.8004	1.57387
	96	46.4	46	48	48	45.2	45.2	46.4667	25.4718	1.27541

TABLE XVII

MISCANTHUS MIXER RAW DATA

20% Miscanthus										
Theoretical max		121.616								
	Sample Number							Average	% Theoretical max	
Glucose (g/L)		1.1	1.2	2.1	2.2	3.1	3.2			Std. Dev.
Time (hr)	4							#DIV/0!	#DIV/0!	#DIV/0!
	8	21.2	21.6	22.2	22	23.4	23.2	22.2667	18.309	0.87331
	24	36	36	34.8	35.1	33.6	33.3	34.8	28.6147	1.15412
	48	38.08	37.6	39.44	39.64			38.69	31.8132	1.00419
	72	35.4	35.6	44.25	44.15	41	41.25	40.275	33.1165	3.9475
	96	39.7	39.65	55.5	55.5	45.55	45.65	46.925	38.5846	7.15128
25% Miscanthus										
Theoretical max		152.02								
	Sample Number							Average	% Theoretical max	
Glucose (g/L)		1.1	1.2	2.1	2.2	3.1	3.2			Std. Dev.
Time (hr)	4	23	22.4	23.4	23.4			23.05	15.1625	0.47258
	8	27.84	27.87	27.84				27.85	18.32	0.01732
	24							#DIV/0!	#DIV/0!	#DIV/0!
	48	43.6	42.8	47.2	42.4			44	28.9436	2.19089
	72	45.6	45.6	50.8	51.6			48.4	31.8379	3.24962
	96	49.3	49.95	46	46.55			47.95	31.5419	1.96511
30% Miscanthus										
Theoretical max		182.424								
	Sample Number							Average	% Theoretical max	
Glucose (g/L)		1.1	1.2	2.1	2.2	3.1	3.2			Std. Dev.
Time (hr)	4	17.1	17.3	18.6	18.7			17.925	9.82601	0.84212
	8	25.6	25.8					25.7	14.0881	0.14142
	24	35.7	36					35.85	19.652	0.21213
	48	44.8	41.6					43.2	23.6811	2.26274
	72	46.35						46.35	25.4078	#DIV/0!
	96	53.5	53.5	48.65	48.2	53.5	53	51.725	28.3543	2.56744

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