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Kinetic characterization for pretreatment of timber varieties and switchgrass using diluted acid hydrolysis

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KINETIC CHARACTERIZATION FOR PRETREATMENT OF TIMBER VARIETIES AND SWITCHGRASS USING DILUTED ACID HYDROLYSIS

By

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Abstract

In recent years, growing attention has been devoted to the use of lignocellulosic biomass as a feedstock to produce renewable carbohydrates as a source of energy products, including liquid alternatives to fossil fuels. The benefits of developing woody biomass to ethanol technology are to increase the long-term national energy security, reduce fossil energy consumption, lower greenhouse gas emissions, use renewable rather than depletable resources, and create local jobs. Currently, research is driven by the need to reduce the cost of biomass-ethanol production. One of the preferred methods is to thermochemically pretreat the biomass material and subsequently, enzymatically hydrolyze the pretreated material to fermentable sugars that can then be converted to ethanol using specialized microorganisms. The goals of pretreatment are to remove the hemicellulose fraction from other biomass components, reduce bioconversion time, enhance enzymatic conversion of the cellulose fraction, and, hopefully, obtain a higher ethanol yield. The primary goal of this research is to obtain kinetic detailed data for dilute acid hydrolysis for several timber species from the Upper Peninsula of Michigan and switchgrass. These results will be used to identify optimum reaction conditions to maximize production of fermentable sugars and minimize production of non-fermentable byproducts.

The structural carbohydrate analysis of the biomass species used in this project was performed using the procedure proposed by National Renewable Energy Laboratory (NREL). Subsequently, dilute acid-catalyzed hydrolysis of biomass, including aspen, basswood, balsam, red maple, and switchgrass, was studied at various temperatures, acid concentrations, and particle sizes in a 1-L well-mixed batch reactor (Parr Instruments,

Model 4571). 25 g of biomass and 500 mL of diluted acid solution were added into a 1-L glass liner, and then put into the reactor. During the experiment, 5 mL samples were taken starting at 100^{o}C at 3 min intervals until reaching the targeted temperature (160, 175, or 190^{o}C), followed by 4 samples after achieving the desired temperature. The collected samples were then cooled in an ice bath immediately to stop the reaction. The cooled samples were filtered using 0.2 μm MILLIPORE membrane filter to remove suspended solids. The filtered samples were then analyzed using High Performance Liquid Chromatography (HPLC) with a Bio-Rad Aminex HPX-87P column, and refractive index detection to measure monomeric and polymeric sugars plus degradation byproducts.

A first order reaction model was assumed and the kinetic parameters such as activation energy and pre-exponential factor from Arrhenius equation were obtained from a match between the model and experimental data.

The reaction temperature increases linearly after 40 minutes during experiments. Xylose and other sugars were formed from hemicellulose hydrolysis over this heat up period until a maximum concentration was reached at the time near when the targeted temperature was reached. However, negligible amount of xylose byproducts and small concentrations of other soluble sugars, such as mannose, arabinose, and galactose were detected during this initial heat up period. Very little cellulose hydrolysis yielding glucose was observed during the initial heat up period. On the other hand, later in the reaction during the constant temperature period xylose was degraded to furfural. Glucose production from cellulose was increased during this constant temperature period at later time points in the reaction.

The kinetic coefficient governing the generation of xylose from hemicellulose and the generation of furfural from xylose presented a coherent dependence on both temperature and acid concentration. However, no effect was observed in the particle size. There were three types of biomass used in this project; hardwood (aspen, basswood, and red maple), softwood (balsam), and a herbaceous crop (switchgrass). The activation energies and the pre-exponential factors of the timber species and switchgrass were in a range of 49 - 180 kJ/mol and from $7.5x10^{4}$ - $2.6x10^{20}$ min^{-1}, respectively, for the xylose formation model. In addition, for xylose degradation, the activation energies and the pre-exponential factors ranged from 130 - 170 kJ/mol and from $6.8x10^{13}$ - $3.7x10^{17}$ min^{-1}, respectively. The results compare favorably with the literature values given by Ranganathan et al, 1985. Overall, up to 92 % of the xylose was able to generate from the dilute acid hydrolysis in this project.

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Chapter 1 Introduction

1.1 Background and Review

The preservation and management of our diverse resources are fundamental political tasks to foster sustainable development in the 21st century. Sustainable economic growth requires safe and sustainable resources for industrial production, a long-term and confident investment and finance system, ecological safety, and sustainable life and work perspectives for the public. Fossil resources are not regarded as sustainable, however, and their availability is more than questionable in the long-term. Because of the increasing price of fossil resources, moreover, the feasibility of their utilization is declining.

Due to these reasons, it is essential to establish solutions which reduce the rapid consumption of fossil resources, which are not renewable (petroleum, natural gas, coal, minerals). A forward looking approach is the stepwise conversion of large parts of the global economy into a sustainable biobased economy with bioenergy, biofuels, and biobased products as its main pillars.

Whereas electricity production can be based on a variety of alternative and renewable raw materials, for example wind, solar, hydro, geothermal, biomass, nuclear fission and fusion, industrial production of liquid transportation fuels is likely to be based on conversion of biomass material in the near term. In the longer term, transportation fuels might be based on hydrogen derived from renewable resources such as solar photovoltaics coupled with electrolysis of water.

A transition from today's production of energy goods and services from fossil to biological raw materials will be essential. The rearrangement of entire production

systems to be based on biomass resources will requires completely new approaches in research and development.

1.1.1 History of Biofuel Development

In the mid 1800s, the feedstocks of corn oil, peanut oil, hemp oil and tallow were used as a strategy for making soap using transesterification. The resulting by-product from the process was alkyl esters, which are now called biodiesel. Prior to the Civil War, ethanol mixed with turpentine, also known as camphene, was widely used as a lamp oil. In 1826, Samuel Morey of Orford, New Hampshire, built the first prototype internal combustion engine using bio-ethanol as the main fuel. Unfortunately, he was not able to attract financial support for his invention.

In the year 1940 the German chemist P. von Walden calculated that in 1940 Germany produced 13 million tons of cellulose leaving 5 to 6 million tons of lignin suitable on as wastage. He then formulated the question: How long can the national economy tolerate this (Walden, 1941)? As early as 1878 A. Mitscherlich, a German chemist, started to improved the sulfite pulp process by fermentation of sugar to ethyl alcohol – it should be mentioned that sugar is a substance in the waste liquor during sulfite pulp production. He also put into practice a procedure to obtain paper glue from the waste liquor. Both processes were implemented in his plant located in Hof, Germany, in the year 1898 (Potsch, 1988).

A historical important step for today's biorefinery developments was the industry-politics-approach of "Chemurgy", founded in 1925 in the US by the Chemist W.J. Hale, son-in-law of H. Dow, the founder of Dow Chemical, and C.H. Herty, a former President of the American Chemical Society. They soon found prominent support from H. Ford and

T.A. Edison. Chemurgy, an abbreviation of “chemistry” and “ergon”, the Greek word for work (Hale, 1934), means by analogy “chemistry from the acre” that is the connection of agriculture with the chemical industry. Chemurgy was soon shown to have a serious industrial political philosophy – the objective of utilizing agricultural resources, nowadays called renewable resources, in industry.

After World War II, numerous inventions and production processes remained, however, and are again highly newsworthy. One was a car, introduced by Henry Ford 1941, whose car interior lining and car body consisted 100% of bio-synthetics; to be specific it had been made from a cellulose meal, soy meal, and formaldehyde resin composite material in the proportions 70%:20%:10%, respectively. The alternative fuel for this car was pyrolysis methanol produced from cannabis. Throughout the 1930s more than 30 industrial products based on soy bean were created by researchers from the Ford Motor Company; this made it necessary to apply complex conversion methods. Hale was a Pioneer of ethyl alcohol and hydrocarbon fuel mixture (Power Alcohol, Gasohol). This fuel mixture, nowadays called E10-Fuel, consisting of 10 percent bioethanol and 90 percent hydrocarbon-based fuel, has been the national standard since the beginning of this millennium in the United States.

During the 1960s wood chemistry had its climax. Projects had been developed, which made it possible to produce nearly all chemical products from wood. Examples are the complex chemical technological approaches of wood processing from Timell 1961, Stamm 1964, James 1969, Brink and Pohlmann 1972, and the wood-based chemical product trees by Oshima 1965. Although these developments did not make their way into

industrial production, they are an outstanding platform for today's lignocellulose conversions and product family trees.

Most of the above mentioned technologies and products, some of which were excellent, could not compete with the fossil-based industry and economy; nowadays, however, they are prevailing again. The basis for this revival started in the 1970s, when the oil crisis and continuously increasing environmental pollution resulted in a broad awareness that plants could be more than food and animal feed. At the same time the disadvantages of intensive agricultural usage, for example over-fertilization, soil erosion, and the enormous amounts of waste, were revealed. From this situation developed complex concepts, which have been published, in which the aim was, and still is, technological and economical cooperation of agriculture, forestry, the food-production industry, and conventional industry, or at least consideration of integrated utilization of renewable resources.

1.2 Energy Overview

Biobased industrial development was pushed by the US President and by the US congress, initially in 2000. In the USA it is intended that by 2020 at least 25% of organic-carbon-based industrial feedstock chemicals and 10% of liquid fuels (compared with levels in 1994) will be produced by biobased industry (RFA, 2005). This would mean that more than 90% of the consumption of organic chemicals in the US and up to 50% of liquid fuel needs would be biobased products.

With only 4.5 percent of the world's population, the United States consumes about 25 percent of global energy and produces roughly 25 percent of the planet's CO_2 emissions. Because of this dubious distinction, the opportunities for positive change in

US energy practices are enormous. The United States presently imports about 60 percent of its oil, and that figure is going to increase in the years ahead (US DOE 2005). This situation also creates balance of trade deficits and energy security concerns.

According to the Energy Information Agency's Country Analysis Brief in November 2005, the United States consumed an average of 20.6 million barrels of petroleum per day during the first nine months of 2005, the same amount year-over-year as in 2004. Average retail regular gasoline prices increased sharply after Hurricanes Katrina and Rita. The average gasoline price for the third quarter of 2005 was $ 2.56 per gallon, up $ 0.67 per gallon from the third quarter of 2004. Thus, conversion of cellulosic biomass to transportation fuels and chemicals presents a powerful opportunity to improve energy security, reduce the trade deficit, reduce green house gas emission, and improve price stability (Wyman, 1999).

1.3 What is Bio-ethanol?

Bio-ethanol is a clear, colorless alcohol fuel made from the sugars found in grains, such as corn, sorghum, and wheat, as well as from potato skins, rice, residues from agriculture and the forest products industry, energy crops, and yard clippings. Ethanol is a renewable fuel because it is made from plants. There are several ways to make ethanol from biomass. The most commonly used processes today use yeast to ferment the sugars derived from starch in corn. Corn is the main feedstock for ethanol in the United States due to its abundance and low price. Most ethanol is produced in the corn-growing states in the Midwest. The starch in the corn is converted into sugar, which is then fermented into alcohol. Other crops such as, barley, wheat, rice, sorghum, sunflower, potatoes, sugar cane and sugar beets can also be used to produce ethanol.

Sugar cane and sugar beets are the most common ingredients for ethanol in other parts of the world. Since alcohol is created by fermenting sugar, sugar crops are the easiest ingredients to convert into alcohol. Brazil, the country with the world's largest ethanol production, makes most of its ethanol this way. Today, many cars in Brazil operate on ethanol made from sugar cane.

A new experimental process which breaks down cellulose in woody fibers can produce what is called "cellulosic ethanol". With this process ethanol can be made from trees, grasses, energy crops, and crop residues. Trees and grasses need less chemical inputs and therefore less energy to grow than grains, which must be replanted every year. Scientists have developed fast-growing trees that grow to harvestable size in ten years. Many grasses can produce two harvests a year for many years (Brigham et al, 1996).

However, existing harvest and collection methods will not be satisfactory to supply the high volume of biomass that will be required for biorefineries in the future. As a result, cost-effective harvesting and collection of biomass is critical to the future feedstock infrastructure (US DOE, 2004). Sustainable harvesting is a key challenge. Researchers will examine various harvesting technologies and methods that will help to meet goals for sustainability and availability of the biomass feedstock. This includes determining what plant components are best suited for feedstock.

1.4 Overview of Biomass Composition and Structure

There are many different types of lignocellulosic biomass, including agricultural residues, herbaceous crops, hardwood and softwood trees, and municipal solid wastes. These biomass types exhibit a wide range of susceptibilities to pretreatment and saccharification because of structural and composition differences. Woody and

herbaceous biomass species are composed mostly of cellulose, hemicellulose, and lignin, but also contain ash and other so-called extraneous materials. Typical compositions of representative lignocellulosic materials are reported in McMillan, 1994. Cellulose is the main component, followed by hemicellulose and lignin; the paper fraction of municipal solid wastes is comprised mostly of cellulose. Hardwoods are composed of about 50% cellulose (dry basis), 23% hemicellulose, and 22% lignin. Herbaceous materials and agricultural residues contain higher proportion of hemicellulose (30-33%) relative to cellulose (38-45%), and have lower levels of lignin (10-17%). The composition and amount of extraneous components vary widely among the different biomass types.

1.4.1 Cellulose

Cellulose consists of a long chain of β-anhydroglucose units linked by β1,4-glucoside bonds. About 50-90% of the cellulose in lignocellulosic materials is bound laterally by hydrogen bonds and forms a crystalline structure. The remaining portion is less ordered, and is often called amorphous cellulose (Beguin, 1994). It is the crystallinity of cellulose that poses the first of the major challenges in effective hydrolysis. Another challenge in cellulose hydrolysis is the physical protection of cellulose provided by hemicellulose and lignin.

1.4.2 Hemicellulose

Hemicellulose consists of branched chains of sugars whose units include mostly aldopentoses, such as xylose and arabinose, and some aldohexoses, such as glucose, mannose, and galactose. In addition to high degrees of polymerization, a hemicellulose polymer typically has substituents on the main chain or its branches. The variety of

linkages, branching, and different monomer units contribute to the complex structure of hemicellulose and thereby its variety of conformations and function. Within biomass, hemicellulose is connected to lignin and cellulose by covalent bonds, but because few hydrogen bonds are involved, it is much more easily broken down than crystalline cellulose. Unlike homogeneous cellulose, the heterogeneity of hemicellulose and the resulting variety of hydrolysis reaction mechanisms involved challenge understanding of the hydrolysis process (Brigham et al, 1996).

1.4.3 Lignin

The third fraction in biomass is lignin, which is a high molecular weight macromolecule based on the phenyl-propyl unit. However, because this portion of biomass remains as a solid after most hydrolysis methods and cannot be fermented to ethanol, it is often burned as boiler fuel to provide process heat and electricity for the ethanol production process (Hsu, 1996).

1.5 Fundamentals of Biorefineries

Biomass, similar to petroleum, has a complex composition. Its primary separation into main groups of substances is appropriate. Subsequent treatment and processing of those substances lead to a whole range of products. Petrochemistry is based on the principle of generating simple to handle and well defined chemically pure products from hydrocarbons in refineries. In efficient product lines, a system based on family trees has been built, in which basic chemicals, intermediate products, and sophisticated products are produced. This principle of petroleum refineries must be transferred to biorefineries. Biomass contains the synthesis performance of the nature and has different C:H:O:N ratio

from petroleum. Biotechnological conversion will become, with chemical conversion, a big player in the future (Figure 1-1).

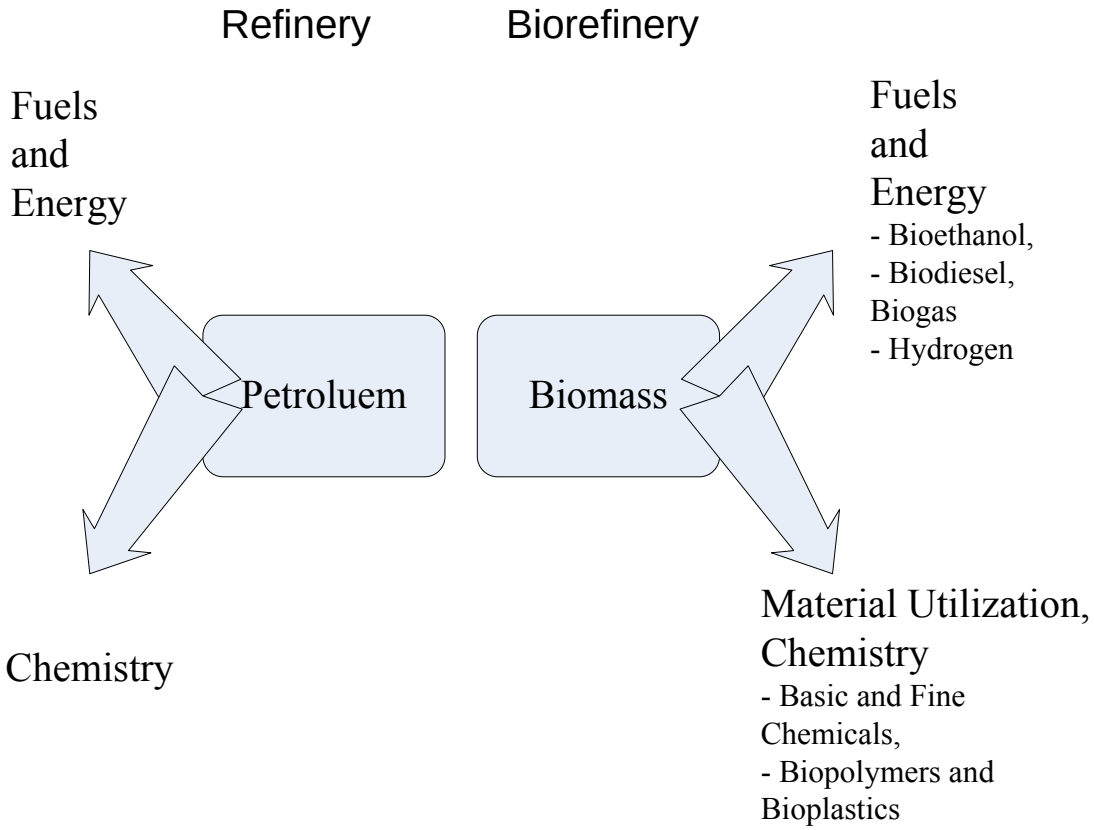

Figure 1-1: Comparison of the basic-principles of the petroleum refinery and the biorefinery (Kamm et al, 2006)

Thus biomass can already be modified within the process of genesis in such a way that it is adapted to the purpose of subsequent processing, and particular target products have already been formed. For those products the term "precursors" is used. Plant biomass always consists of the basic products carbohydrates, lignin, proteins, and fats, and a variety of substances such as vitamins, dyes, flavors, and aromatic essences of very different chemical structure. Biorefineries combine the essential technologies which convert biological raw materials into the industrial intermediates and final products.

A technically feasible separation operation, which would enable separate use or subsequent processing of all these basic compounds, is currently in its initial stages only.

Assuming that of the estimated annual production of biomass by biosynthesis of 170 billion tons 75% is carbohydrates, mainly in the form of cellulose, starch, and saccharose, 20% lignin, and only 5% other natural compounds such as fats (oils), proteins, and other substances, the main attention should first be focused on efficient access to carbohydrates, and their subsequent conversion to chemical bulk products and corresponding final products. Glucose, accessible by microbial or chemical methods from starch, sugar, or cellulose, is, among other things, predestined for a key position as a basic chemical, because a broad range of biotechnological or chemical products are accessible from glucose.

For cellulose this is not yet realized. Cellulose-hydrolyzing enzymes can only act effectively after pretreatment to break up the very stable lignin/cellulose/hemicellulose composites. These treatments are still mostly thermal, thermomechanical, or thermochemical, and require considerable input of energy. The arsenal for microbial conversion of substances from glucose is large, and the reactions are energetically profitable. It is necessary to combine degradation processes via glucose to bulk chemicals with the building processes to their subsequent products and materials.

Among the variety of microbial and chemical products possibly accessible from glucose, lactic acid, ethanol, acetic acid, and levulinic acid, in particular, are favorable intermediates for generation of industrially relevant product family trees. Here, two potential strategies are considered: first, development of new, possibly biologically degradable products or, second, entry as intermediates into conventional product lines of petrochemical refineries (Kamm et al, 2004).

1.6 Bio-ethanol Process

The key components of cellulosic biomass are cellulose, hemicellulose, and lignin. The woody biomass contains ~40-50% cellulose, which yields 6-carbon sugars like glucose using enzymatic hydrolysis; ~25-35% hemicellulose, which provides 5-carbon sugars such as xylose, mannose, galactose, and arabanose through hydrolysis; and ~15-20% lignin, which is a non-fermentable high molecular weight substance based on the phenyl-propene unit; plus lesser amount of minerals, oils, soluble sugars, and other components (Holtzapple, 1993).

The production of ethanol from woody biomass consists of four basic steps: pretreatment, hydrolysis, fermentation, and product purification or separation, as shown in figure 1.

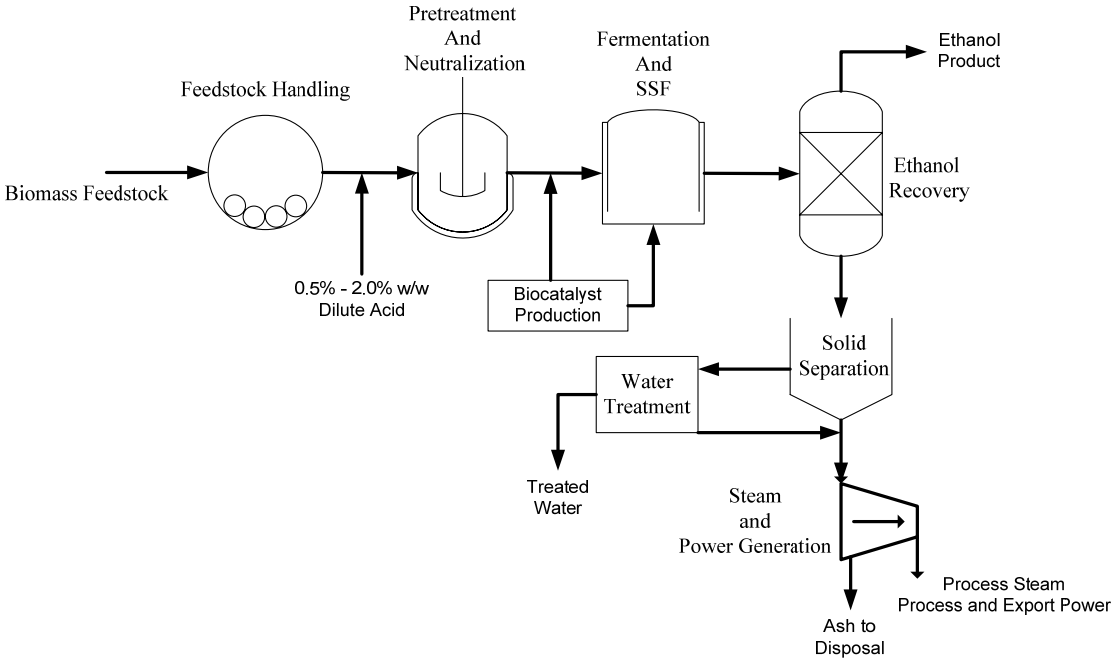

Figure 1-2: Block flow diagram for conversion of biomass to ethanol by the NREL process configuration.

The process configuration on figure 1-2 was proposed by the National Renewable Energy Laboratory of the U.S. Department of Energy. The woody biomass in bulk will

first be processed through the feedstock handling area to alter the biomass macroscopic and microscopic structure so that hydrolysis of the carbohydrate fraction to monomeric sugars can be achieved more rapidly and with greater yield. Then, the reduced size of biomass will be sent to the pretreatment stage. Using diluted sulfuric acid or other pretreatment technologies, the hemicellulose is converted to monosaccharides.

After pretreatment the cellulose fraction is then hydrolyzed to form 6-carbon sugars using an enzymatic treatment in the presence of cellulases. Next, the products of cellulose and hemicellulose, glucose, xylose, and other sugars, are readily fermented to ethanol using genetically engineered microbial strain, for example *Escherichia coli*. Ethanol is recovered from the fermentation by distillation and other separation steps to remove residual water. The byproducts, such as ash, lignin, unreacted cellulose and hemicellulose, will end up at the bottom of the distillation column. These materials can be concentrated, and burned as fuel to supply the power for the process, or convert to other co-products (Wyman, 1999).

1.7 The Advantages of Bio-ethanol

The benefits of developing woody biomass to ethanol technology are to increase the long-term national energy security, reduce fossil energy consumption and greenhouse gas emissions, use renewable rather than depletable resources, cultivate a domestic source of fuels, and create local jobs. These concerns have motivated the research over the last 25 years.

According to the Renewable Fuels Association (2006), the United States imports 64% of its petroleum needs today. By 2025, the Energy Information Administration projects the United States will import 77% of its petroleum. As United States is

increasingly dependent on the imported oil supply to meet our personal, transportation, and industry needs, renewable source of energy can reduce the dependence on foreign oil and enhance US energy security. In fact, in 2004, the use of ethanol reduced the U.S. trade deficit by $5.1 billion by eliminating the need to import 143.3 million barrels of oil (RFA, 2005).

Greenhouse carbon dioxide emissions coupled with a rising demand for fuel are two current strains on the fossil fuel industry. Also, looms the inevitability that current fossil fuel resources will be depleted in the not too distant future. Therefore, there is a growing demand for renewable, alternative fuels that emit less harmful substances to the environment. Ethanol is a promising alternative transportation fuel. It fulfills one requirement in that the emissions of an ethanol-combusting engine consist mainly of carbon dioxide that is not climate active. It is not considered climate active, as fossil based CO_2 is, because the process of growing the biomass sequesters CO_2 from the atmosphere. Upon combustion of fuels derived from biomass CO_2 is simply returned to the atmosphere, closing the cycle and reducing net accumulation of greenhouse gases compared to the case of fossil fuel combustion.

The use of 10% cellulosic ethanol blends reduces greenhouse gas emissions by 6-9% compared with conventional gasoline, according to Argonne National Laboratory (Wang et al. 1999). In 2004, ethanol use in the U.S. reduced CO_2-equivalent greenhouse gas emissions by approximately 7.03 million tons, equal to removing the annual emissions of more than one million cars from the road (Wang, 1999). Also because benzene and tetraethyl lead are not added to the fuel, carbon monoxide and other unhealthy emissions will be drastically reduced (Wyman, 1999). Another benefit of

ethanol is that it can be derived from cellulose and hemicellulose, which are found in almost every type of woody biomass (i.e. trees, corn husks, agricultural waste) and is the most prevalent form of biomass on the Earth. As a result, the source from which ethanol comes is renewable, therefore making it a sustainable fuel in all aspects.

Other benefits of making the ethanol production more feasible are to increase the job opportunities in United States and reduce the dependence on foreign oil supply. In 2004, the ethanol industry provided more than 147,000 jobs in all sectors of the United States economy, boosting United States household income by $4.4 billion (RFA, 2005). In addition, the ethanol production helps to lower the federal farm program cost as the corn demands rise, thus raises the price. Similar economic benefits can be expected for ethanol produced from forest resources, energy crops grown on agricultural and forest lands, and from agricultural/forest residues.

1.8 Research Objectives

In this project, the pretreatment of woody biomass was studied using diluted acid hydrolysis. The goals of the pretreatment are to remove the hemicellulose fraction from other biomass components, reduce bioconversion time for cellulose enzymatic hydrolysis, enhance enzymatic conversion of the cellulose fraction, and, hopefully, obtain a higher ethanol yield. The goals of this research are as follow:

- To measure detailed kinetic data for dilute acid hydrolysis as a pretreatment step for several woody species from the Upper Midwest region of the United States, such as aspen, basswood, balsam fir, red maple, and switchgrass.
- To measure the concentrations of fermentable sugars plus their non-fermentable byproducts.

- To determine the kinetic parameters for xylose formation and degradation from a match between a reaction model and the experimental data.
- To gain a better understanding of optimum reactor conditions for dilute acid pretreatment of these samples in order to maximize production of fermentable sugars and minimize production of non-fermentable byproducts.

1.9 Thesis Organization

This thesis contains seven chapters that discuss the use of biomass as feedstock to produce renewable carbohydrates as a source of energy. Chapter 1 has given a brief background history and overview on the importance of developing biomass-to-ethanol process. By showing the process proposed by NREL, the pretreatment stage plays an important role in overall ethanol production.

Chapter 2 reviews the advantages and disadvantages of current developed pretreatment technologies. The net production cost, the selling price, and the rate of reaction will be discusses in this chapter.

Chapter 3 describes the experimental details of the raw materials, methodologies, and tools used. The characteristics of the raw materials and the specification of the apparatus are then discussed. Chapter 3 discusses the preparation and size reduction procedure for composition analysis; then, the pretreatment setup using the Parr Reactor. Moreover, an introduction is presented of the high performance liquid chromatography (HPLC) analysis to determine pretreatment reaction products, such as glucose, xylose, galactose, arabinose, mannose, and furfural.

Chapter 4 shows the derivation of the kinetic model used to obtain the Arrenius parameters of activation energy (E) and the pre-exponential factor (A) by fitting the model prediction to the experimental data.

Chapter 5 shows the results of all detected sugars and degradation products with different woody species under several pretreatment conditions. The kinetic parameters are calculated using the kinetic model from Chapter 4.

The last 3 chapters, Chapters 6 - 8, provide a discussion and comparison to literature results, summaries and concluding analysis to this dissertation, and gives recommendations for the future work.

Chapter 2 Pretreatment of Biomass

2.1 Introduction

Today's biorefinery technologies are based on the utilization of the whole plant or complex biomass and on integration of traditional and modern processes for utilization of biological raw materials. In the 19th and the beginning of the 20th century large-scale utilization of renewable resources was focused on pulp and paper production from wood, saccharification of wood, nitration of cellulose for guncotton and viscose silk, production of soluble cellulose for fibers, fat curing, and the production of furfural for Nylon. Furthermore, the technology of sugar refining, starch production, and oil milling, the separation of proteins as feed, and the extraction of chlorophyll for industrial use with alfalfa as raw material were of great historical importance. But also processes like wet grinding of crops and biotechnological processes like the production of ethanol, acetic acid, lactic acid, and citric acid used to be fundamental in the 19th and 20th century.

Production of fuels and chemicals from renewable lignocellulosic materials is accomplished by hydrolyzing polysaccharide components to soluble sugars which can be fermented to desired end products. Some type of pretreatment is generally required to render the cellulose fraction susceptible to enzymatic and microbial action because such materials are only partially digestible in their native form (McMillan, 1994).

The specific objectives of pretreatment are dictated by the overall objectives of a biomass conversion process. First, pretreatment must separate the hemicellulose from other biomass components and thereby open up the structure of biomass sufficiently to allow efficient and effective enzyme hydrolysis of the cellulose, which is protected by a

sheath of lignin and hemicellulose. Second, pretreatment must be energetically and chemically efficient for a biomass process to be profitable. Third, pretreatment must promote effective conversion of available carbohydrate to fermentable sugars so that high product yield can be achieved; pretreatment must maximize the formation of sugars or the ability to subsequently form sugars by enzymatic hydrolysis. Hence, degradation or loss of carbohydrate must be avoided. Because it is also desirable to maximize the rate of enzymatic conversion, pretreatment must yield a highly digestible material that is not inhibitory to cell metabolism or extracellular enzyme function. Therefore, it is preferable to avoid the formation of inhibitory products and the need for detoxification or washing; high sugar losses occur if pretreated material is washed prior to enzymatic hydrolysis. Finally, pretreated materials are most efficiently hydrolyzed using low enzyme loadings, so the potential for nonspecific binding of enzymes to lignin and other fractions of pretreated biomass must be minimized.

2.2 Pretreatment Process Economic Analysis

Pretreatment is one of the most costly steps in cellulosic ethanol production, accounting for about 33% of total processing costs in the base-case NREL design (Figure 1-2) (Lynd, 1996). This value likely underestimated the real importance of pretreatment, because pretreatment greatly affects the performance of fermentation and enzyme production. In particularly, it is often producing fermentation inhibitors. Several sources in the literature have reported of inhibitor production for pretreatment with dilute acid pretreatment (McMillan, 1994), steam explosion pretreatment (Forsberg et al, 1988, Mes-Hartree et al, 1983), and acid hydrolysis (Fraser et al, 1991, Tran et al, 1986). Inhibitory compounds originate from 1) hydrolysis of extractive components, organic and sugar

acids esterified to hemicellulose fraction (e.g. acetic, formic, glucuronic, galacturonic), and solubilized phenolic lignin derivatives; 2) degradation products of solubilized sugars (e.g. furfural from xylose, hydroxymethylfurfural from glucose); 3) degradation products of lignin (e.g. cinnamaldehyde, p-hydroxybenzaldehyde, syringaldehyde); and 4) corrosion products (e.g. metal ions). Largely because of inhibition, reports of fermentation of as-received pretreated slurries (including both fiber and liquid hydrolyzate as they emerge from pretreatment) are exceedingly rare (Lynd, 1996).

Rigorous process economic analysis is necessary to determine the best pretreatment process option for a particular feedstock and product opportunity, once the experimental data are available (Aden et al, 2002).

Elander et al (2005) compares the plant level cash costs and minimum ethanol selling price (MESP) for several pretreatment options. The plant level cash cost is also the same as the lowest ethanol price at which the plant will stay operational, even though the plant would be losing money at these market conditions. As such, it defines the competitive position of the proposed facility within the existing ethanol market. In the analysis, cash cost is comprised by six (according to the figure presented in the paper by Elander et al, 2005) components: net stover, other variable costs, and fixed costs without depreciation. Net stover, by analogy with the net corn concept used in corn processing, is defined as the cost of stover feedstock less the value of the electricity co-product. Other variable costs accounts for the cost of enzymes, chemicals, etc. in which the quantities required are tied to the plant production rate. Fixed costs include labor, maintenance, insurance, and other costs not tied to production rate.

2.3 Pretreatment Technologies

It is well known that the high-order molecular packing of cellulose in its crystalline regions limits the heterogeneous chemical reactions to the external surface of crystallites. In addition, the structure of lignocellulosics in the cell wall resembles that of a reinforced concrete pillar with cellulose fiber being the metal rod and lignin the natural cement. Biodegradation of native untreated lignocellulosics is very slow and the extent of degradation is also low, often under 20% (Dunlap et al, 1976). This low rate and extent of conversion inhibits the development of an economically feasible hydrolysis process. To increase the susceptibility of cellulosic material, structural modification by means of various pretreatment schemes is essential.

In general, pretreatment processes produce a solid pretreated biomass residue that is more amenable to enzymatic hydrolysis by cellulases and related enzymes than native biomass. Many pretreatment approaches, such as dilute acid and steam/pressurized hot water based methods, seek to achieve this by hydrolyzing a significant amount of the hemicellulose fraction of biomass and recovering the resulting soluble monomeric and/or oligomeric sugars. Other pretreatment processes, such as alkaline-based methods, are generally more effective at solubilizing a greater fraction of lignin while leaving behind much of the hemicellulose in an insoluble, polymeric form. Most pretreatment approaches do not hydrolyze significant amounts of the cellulose fraction of biomass, but enable more efficient enzymatic hydrolysis of the cellulose by removal of the surrounding hemicellulose and/or lignin along with modification of the cellulose microfibril structure.

Numerous pretreatment approaches have been investigated at many laboratories, universities, and industrial locations over the past 25 years. In the past, it has been difficult to compare the performance and economics of these various approaches due to difference in feedstocks tested, chemical analysis methods, and data reporting methodologies. Recently, a group of pretreatment researchers across North America have begun to collaborate to investigate different pretreatment approaches on a common basis to allow meaningful comparison. These researchers have formed the Biomass Refining Consortium for Applied Fundamentals and Innovation (CAFI) to advance the efficacy and knowledge base of pretreatment technologies. Current participating institutions in the Biomass Refining CAFI are Auburn University (Y.Y. Lee), University of British Columbia (Jack Saddler), Dartmouth College (Charles Wyman; now at UC Riverside), University of Hawaii (Michael Antal), Michigan State University (Bruce Dale), National Renewable Energy Laboratory (NREL) (Rick Elander), Purdue University (Michael Ladisch), University of Sherbrooke (Esteban Chornet), and Texas A&M University (Mark Holtzapple).

Pretreatment methods are either physical or chemical. Some methods incorporate both effects. For the purposes of classification, steam and water are excluded from being considered chemical agents for pretreatment since extraneous chemicals are not added to the biomass. Physical pretreatment methods, such as comminution (mechanical reduction in biomass particulate size), steam explosion, and hydrothermolysis, can be classified into mechanical and non-mechanical pretreatment. Physical forces used in mechanical pretreatments can subdivide lignocellulosic material into fine particles which are substantially susceptible to acid or enzymatic hydrolysis. Non-mechanical physical

pretreatments cause decomposition of lignocellulosics by exposing them to harsh external forces other than mechanical forces.

Chemical pretreatments have been used extensively for removal of lignin surrounding cellulose and for destroying its crystalline structure. Traditionally, the paper industry has utilized pulping processes for delignification of cellulosic materials to produce high strength, long fiber paper products. It has been considered, however, that these processes are quite severe and expensive to be used for pretreatment of lignocellulosics for production of ethanol. Even though chemical pretreatment are usually effective, they have disadvantages which should not be ignored. These include use of specialized corrosion resistant equipment, need for extensive washing, and disposal of chemical wastes. Table 2-1 summarized the advantages and disadvantages of each pretreatment method.

Table 2-1: The Advantages and Disadvantages of Pretreatment Technologies

Pretreatment Technologies	Advantages	Disadvantages
Mechanical Comminution Chemical Used: N/A Methods: Chipping, grinding, milling, and etc	• Increase external surface area • Vibratory ball milling increases the reactivity of cellulose • Reducing the reactor volume as higher slurry concentration	• Require higher energy for smaller particle size • The pretreatment time and processing cost may impractical on a large scale
Alkali Swelling Chemical Used: Diluted NaOH Methods: Soaking	• Hardwood shows increasing efficacy as low lignin content • Improve cellulose digestibility	• Expensive chemical • No effect on softwood as high lignin content • Longer reaction time
Dilute Acid Hydrolysis Chemical Used: 0.5-3.0% H_2SO_4 Methods: Dilute Acid at	• Removes and recovers hemicellulose as dissolved sugars • Glucose yield from cellulose increase with hemicellulose removal	• Corrosion resistant materials needed • Degradation products formed

Moderate Temperature	• Lignin disrupted • High xylan to xylose conversion yield • Shorter reaction time • Lowest Minimum Ethanol Selling Price • Lowest Oligomers	
Steam Explosion Chemical Used: Steam Methods: Explosive Disruption of Biomass	• Ability to separate wood into its three main components • Lignin suitable for conversion to chemical products • Hemicelluloe can be fully utilized and converted to liquid fuels	• Not suitable for softwoods • Washing step required prior enzymatic hydrolysis • Produces relatively low bulk density substrate
pH Controlled Liquid Hot Water Pretreatment Chemical Used: Water Methods: Maintaining pH of water under pressure at high temperature	• Reduces the need of neutralization • Highly digestible cellulose resulted • High yield of sugars from hemicellulose	• Highest Minimum Ethanol Selling Price
Ammonia Explosion, or Ammonia Fiber Explosion (AFEX) Chemical Used: 100% Anhydrous NH_3 Methods: Prewetted Biomass is placed in a Pressure Vessel	• Increase agricultural residue digestibility • High overall hydrolysis yield • No small particle size required • Recyclable • Good for herbaceous biomass	• Need to recycle NH_3 • Causes swelling • Causes partial decrystallization of crystalline cellulose • Not suitable for highly lignified softwoods
Lime Pretreatment Chemical Used: 0.05-0.15 g $Ca(OH)_2$/g Biomass Methods:	• Pressure vessel is not required at low temperature • Sufficient for low-lignin material • Can be recovered and recycled	• Treatment time ranges from weeks to hours

2.3.1 Mechanical Comminution

All pretreatment processes involve an initial mechanical step in which the biomass is comminuted by a combination of chipping, grinding, and milling. Furthermore, the pretreatment processes employ a secondary grinding or milling step to reduce the particle size of chipped biomass. The chipped biomass has a characteristic dimension of 1 cm to 3 cm, in comparison to 0.2 mm to 2.0 mm for milled material. The shearing and compressive forces cause a reduction in crystallinity, a decrease in the mean degree of polymerization, an increase in bulk density, and a decrease in particle size. Ball milled material also allows for a high slurry concentration, thereby reducing the reactor volume and capital cost.

Some researchers have concluded that milling processes, especially vibratory ball milling, increase the reactivity of cellulose, in addition to increasing the external surface area. Ryu et al. (1980) studied the changes in cellulose structure by compression milling. They measured the crystallinity index, accessibility, and moisture regains and reported a considerable decrease in the crystallinity index and a drastic increase in the accessibility of cellulose.

2.3.2 Alkali Swelling

Pretreatment with sodium hydroxide has been used mainly to enhance the digestibility of the lignocellulosic materials rather than pretreatment for hydrolysis (Playne, 1984). Dilute NaOH treatment of lignocellulosic materials causes swelling, leading to an increase in internal surface area, decrease in the degree of polymerization, decrease in crystallinity, separation of structural linkages between lignin and carbohydrates, and disruption of the lignin structure.

The efficacy of alkali pretreatment is dependent upon lignin content. According to Millett et al (1979), for hardwoods soaking in NaOH showed increasing efficacy as lignin content decreases from 24% to 18%. No effect of dilute NaOH pretreatment is, however, observed for softwoods in which the lignin content is 26% - 35%.

Numerous studies of alkaline pretreatment, most of which involve the use of sodium hydroxide alone (Playne, 1984), or sodium hydroxide in combination with other chemicals such as peroxide (Gould et al, 1984), or others (Detroy et al 1981; 1982; Miron et al, 1981), are found in the literature. The effectiveness of alkaline pretreatment appears to vary, depending on such factors as substrate and treatment conditions. Generally, alkaline pretreatment is more effective on agricultural residues and herbaceous crops than on wood materials. In comparison with acid-based pretreatments, the reactor material requirements can be relaxed, but the cost of chemicals may be higher; for example, caustic soda is more than four times as expensive as sulfuric acid according to the Chemical Marketing Reporter on July 8, 2006, and the concentration of alkali used is generally comparable to or higher that that of acid. Process design and economics for ethanol production employing alkaline pretreatment has not been reported. Yet, no alkaline pretreatment techniques appear to have been tested on a pilot scale.

2.3.3 Dilute Acid Hydrolysis

Originally, exposure to concentrated acid and then later to dilute acid was used to directly saccharify lignocellulosic materials (Sherrard et al, 1945). Above moderate temperature, however, direct saccharification suffered from low yields because of sugar decomposition. Thus, the use of dilute acid at high temperature has been developed as a pretreatment prior to enzymatic saccharification to improve overall saccharification rates

and yields, but studies have been initiated only recently. Sulfuric acid has been extensively studied because it is inexpensive and effective. The potential difficulties of the need for corrosion-resistant construction materials for reactors and gypsum generation, however, plague sulfuric acid's prospects as a long-term pretreatment chemical.

NREL currently favors dilute acid hydrolysis as the pretreatment process of choice for a commercial biomass-to-ethanol process (Hinman et al, 1992). In the process, chipped and/or milled biomass particles of nominal 1-mm size are impregnated with approximately 1% (w/w) H_2SO_4 and then incubated at 140°-160°C for a period ranging from several minutes to an hour. High temperature dilute acid treatment causes hemicellulose to hydrolyze. Hemicellulose removal increases porosity and improves enzymatic digestibility of the cellulose.

The major advantage of dilute-acid pretreatment is that significantly higher xylose yields can be obtained. Several studies using a batch dilute-acid pretreatment process showed xylose yields approaching 80% of theoretical (Grohmann et al, 1986; Schell et al, 1992; Torget et al, 1990). However, the main disadvantage of acid pretreatment is that a considerable amount of degradation products were formed, such as furfural and hydroxymethylfurfural.

2.3.4 Steam Explosion

As early as 1929 Mason obtained a patent (Mason, 1929) for his process where saturated steam was brought into contact with wood chips or shavings and the steamed materials were then released rapidly through a valve. Mason's goal was to obtain defibration and particles for board production. The patent clearly stated that saturated

steam leads to condensation on the plant biomass, thereby giving wetted, aqueous lignocellulosics.

Steam treatments of wood are typically carried out using saturated steam at temperatures of 160 ~ 285^{o}C, which corresponds to pressure of 100 – 700 psia (Perry et al, 1984). Residence times are typically tens of seconds to several minutes. Steam explosion can cause extensive hemicellulose degradation and lignin modification. Steam requirements are dominated by the need to heat the moisture content of biomass, and can be reduced by using direct or partially dried wood. Decreasing the chip size to less than 6 mm and/or increasing incubation time and reducing steam temperatures reduces heat transfer heterogeneity. Heat transfer heterogeneity can cause degradation to occur at the outside of chips or undercooking to occur at the center (Brownell et al, 1984).

There are two potential mechanisms of action in steam explosion pretreatment. First, rapid solubilization of hemicellulose opens up the pore structure of biomass, similar to what occurs in dilute acid hydrolysis. This has been demonstrated to occur following high-temperature and acid-catalyzed treatments. Second, explosive decompression exerts a mechanical shear on the biomass, which may increase the specific surface area of the materials by defibrating individual cellulose microfibris or otherwise expanding the lignocellulose matrix. Hemicellulose is thought to be hydrolyzed by the acetic and other acids released during steam explosion pretreatment. Steam explosion involves chemical effects since acetic acid is generated from hydrolysis of acetyl groups associated with the hemicellulose may further catalyze hydrolysis and glucose and xylose degradation. Water also acts as a mild acid at high temperatures (Weil et al, 1998a).

The major advantages and disadvantages of steam explosion have been described previously by Wayman (1980) and are shown in Table 2-1. The major disadvantage is that, although steam explosion greatly enhances the enzymatic hydrolysis of hardwoods and most agricultural residues tested to date, it has not yet been successfully developed for use with softwoods. However, Soderstrom et al (2004) has done the steam explosion on softwood recently in one- and two-step. He claimed that the total yield of fermentable sugars after one-step steam pretreatment was 74% and two-step pretreatment process was 78 ~ 79%.

2.3.5 pH Controlled Liquid Hot Water Pretreatment

The liquid hot water pretreatments use pressure to maintain the water in the liquid state at elevated temperatures (van Walsum et al, 1996). The unique properties need to be exploited to fractionate biomass. This breaking of chemical bonds may be enhanced by the increased disproportionation of water at elevated temperatures. This approach results in very high and often completes solubilization of hemicellulose, significant solubilization of both lignin and overall biomass, and rather low solubilization of cellulose.

The hot compressed liquid water contacts water with biomass for up to 15 min at temperatures of 200-230^{o}C. Between 40% and 60% of the total biomass is dissolved in the process, with 4-22% of the cellulose, 35-60% of the lignin and all of the hemicellulose being removed. Over 90% of the hemicellulose is recovered as monomeric sugars when acid was used to hydrolyze the resulting liquid (Mosier et al, 2005). Therefore, acid is still needed to complete the hydrolysis in this case.

Water pretreatment reduces the need for neutralization and conditioning chemicals since acid is not added. A highly digestible cellulose results when enzyme is added (van Walsum et al, 1996), and high yields of sugars from hemicellulose occur during pretreatment. The pK_a of water is affected by temperature such that the pH of pure water at 200^{o}C is nearly 5.0 (Weil et al, 1998a). The control of pH during pretreatment is to prevent the pH of liquid hot water from falling below 4 limits and/or control the chemical reactions occurring during pretreatment (Weil et al, 1998a).

2.3.6 Ammonia Explosion, or Ammonia Fiber Explosion (AFEX)

In the AFEX process, lignocellulose is soaked with high-pressure (15 atm) liquid ammonia at moderate temperatures (50^{o}C) for about 15 min, causing cellulose to decrystallize. Then, the pressure is instantaneously released, causing the ammonia to flash violently and disrupt the fibrous structure. The combined chemical effects (cellulose decrystallization, hemicellulose prehydrolysis, lignin alterations) and physical effect (increase in accessible surface area) markedly increase the susceptibility of lignocellulose to enzymatic hydrolysis (Dale et al, 1985). All the ammonia, except that which is chemically bound as ammonium ions, will be recovered for reuse. Typically, about 0.5% to 1% ammonia remains in the lignocellulose, which serves as a nitrogen source for the microbes that use the sugars subsequently enzymatically hydrolyzed from the lignocellulose.

AFEX pretreatment has been shown to improve the saccharification rates of numerous herbaceous crops and grasses. Materials pretreated using this process include alfalfa, corn stover, rice stover, wheat straw, barley straw, Bermuda grass, bagasse, and kenaf core (Dale et al, 1985; Holtzapple et al, 1990). Following AFEX pretreatment,

overall hydrolysis yields on these materials are around 90% of theoretical (Teymouri et al, 2004).

AFEX pretreatment has not proven as effective on hardwoods and softwoods, and results of a study on AFEX pretreatment of Bermuda grass, bagasse, and newspaper suggest decreasing AFEX effectiveness with increasing lignin content. AFEX treatment of Bermuda grass (~5% lignin) and bagasse (~20% lignin) resulted in hydrolysis yields of over 90% of theoretical, whereas the hydrolysis yield on AFEX-treated newspaper (~30% lignin) was only about 40% (Holtzapple et al, 1990).

AFEX pretreatment improves somewhat when the process is carried out at higher than ambient temperatures, and remains effective when blowdown pressures are increased from atmospheric to around 44 to 66 psia (Holtzapple et al, 1990). Compression costs represent a major fraction of AFEX process operating costs, so this latter finding may significantly reduce the cost of AFEX treatment, provided NH_3 can be recovered efficiently.

2.3.7 Lime Pretreatment

Pretreatment with lime increase pH and provides a low-cost alternative for lignin removal (Chang et al, 1998). Typically lime loadings are 0.1 g $Ca(OH)_2$/g biomass. A minimum of about 5 g H_2O/g biomass is required. Additional water can be added, but it is neither helpful nor harmful. Lime pretreatment can be performed at a variety of temperatures, ranging from 25 to 130^oC, and the corresponding treatment time ranges from weeks to hours. The advantage of using temperature below 100^oC is that the pressure vessel is not required.

Regardless of the temperature, lime treatment removes approximately 33% lignin and ~100% of acetyl groups. For low-lignin herbaceous materials, such as switchgrass, this level of pretreatment is sufficient to render the biomass digestible (Chang et al, 1997). For high-lignin woody materials, additional lignin removal is required and can be accomplished by adding either oxygen or air to the lime pretreatment system.

Lime pretreatment is effective for improving the enzymatic hydrolysis of switchgrass and corn stover (Chang et al, 1997; Kaar et al, 2000). Washing the pretreated biomass before neutralization reduced the acid required for neutralization by 50%, although the subsequent sugar yield was reduced by 10%. Materials balances show that lime pretreatment is mild (Chang et al, 1998), because the biomass recovery is high. Lime can be easily recovered and recycled, making the pretreatment not only effective, but also economical and environmentally friendly.

2.4 Concluding Remarks for Pretreatment Technologies

From a mechanistic standpoint, there are notable similarities among many pretreatment methods. Except comminution, pretreatments usually employ catalyst because this enables lower temperature operation, which increase yield. In addition, many pretreatments are performed at sufficiently high temperature to hydrolyze hemicellulose. The significance of the resistance of lignocellulosic materials to pretreatment is evidenced by the severe conditions generally required for effective pretreatment.

Overall carbohydrate yield is the most important factor in commercial-scale biomass conversion processes. Research to improve pretreatment processes must therefore focus on minimizing degradation of the carbohydrate fraction of lignocellulosic biomass.

In the near future, the potential to achieve further improvements in existing processes needs to be explored. The economic feasibility of modifying existing processes to enable more rapid enzymatic hydrolysis of pretreated biomass must be evaluated. Sensitivity analyses should be performed to understand the extent to which additional energy and chemicals inputs can be used to improve bioconversion rates and yields or to reduce energy requirements.

In the longer term, improved pretreatments which overcome undesirable features of existing processes and offer the potential of achieving high yields with little or no by-product formation should be pursued. Lower temperature processes are particularly attractive because they eliminate the problem of yield losses caused by high-temperature sugar degradation.

Chapter 3 Materials and Experimental Methods

3.1 Introduction

During recent years, increasing interest has been shown in the utilization of biomass as a renewable resource. Timber species, such as aspen, balsam fir, basswood, and red maple, are available in every county of the Upper Midwest region. This woody biomass may be the primary species used to produce bio-ethanol in the region. As a result, these timber species will be evaluated along with switchgrass in this project.

The material preparation and experimental analysis in the chapter was primarily based on the NREL Laboratory Analytical Procedures (LAPs). The LAPs are similar to procedures from The American Society for Testing and Materials (ASTM) and the Technical Association of the Pulp and Paper Industry (TAPPI). These LAPs provided the basis for the techniques used, including the particle size and the determination of all the biomass components.

The four woody species were kindly supplied by Dr. Christopher Webster, Assistant Professor in the School of Forestry and Environmental Sciences at Michigan Technological University. The detailed preparation of the woody species will be discussed in the following section. The switchgrass was kindly supplied in the form of milled fine particles (10-30 mesh) by Dr. Jim McMillan, who is from Bioprocess Research & Development Group at NREL.

In the following sections, the raw material preparation, experimental setup, and sample analysis of this project will be discussed. The Job Safety Assessments (JSAs) of "Determination of Structural Carbohydrates and Lignin in Biomass", "Kinetic Modeling

of Biomass Pretreatment", and "Determination of Sugars produced in Pretreatment of Dilute Acid Hydrolysis" are attached in Appendix A.

3.2 Experimental Strategy

As the objectives of this project are to establish the kinetic parameters of timber varieties plus switchgrass and to forecast the optimum reactor condition, a selection effect of conditions were investigated, such as acid concentration, operating temperature, and biomass particle size. The test matrix for the experiment is described in Table 3-1.

Table 3-1: Various parameters used in this project

Parameters	Values		
Total Acid Concentrations	0.25 % w H_2SO_4	0.5 % w H_2SO_4	1.0 % w H_2SO_4
Operating Temperature	160^oC	175^oC	190^oC
Particle Size	> 28 mesh	20 – 10 mesh	

The detail sample preparation and sampling methods will be discussed in the following sections.

3.3 Raw Material Preparation

Each tree species was cut into 4 ½ foot log lengths. The 4 ½ foot length of whole wood was down to 15 inches long by using a Chain Saw. Furthermore, the fresh 15 inches wood logs were debarked using a hand axe.

The debarked wood logs were then cut into flakes of approximately 1 mm thickness using a rotary drum blade. Then, the wood flakes were dried in an oven at 105^oC overnight. The total dry solids of the wood flakes averaged 96%, containing about 4% moisture content. The wood flakes were hammer milled into fine particles (10-30 mesh). A hammer mill is a steel drum containing a vertical and horizontal cross-shaped

rotor on which pivoting hammers are mounted. After hammer milling, the wood particles were stored in a closed plastic tub at room temperature. Each species of tree yielded about 8 pounds of dry milled wood biomass.

The switchgrass sample was supplied by Dr. Jim MacMillan of NREL and was used without any modification. No details on the preparation of switchgrass were mentioned by Dr. McMillan.

3.4 Biomass Size Differentiation

The procedure of screening the woody biomass and switchgrass applied in this work was following the LAPs by NREL and with slightly modification (U.S. DOE, 2006). The W.S. TYLER ROTAP (Model RX-29, Serial 9774) was used in this procedure. First, the sieves were stacked in the following order, starting at the bottom: the bottom pan, 28-mesh sieve, 20-mesh sieve, and 10-mesh sieve. Then, the milled fine particles were filled half full in the 10-mesh sieve. The milled sample was prepared in batches. The cover of the sieve was then place on the sieve stack and secured the stack in the sieve shaker.

The stack of sieves was shaken for 15 minutes. The sieved samples were then collected in re-sealable plastic bags. The bags were labeled as follow: +10 mesh, 20-10 mesh, 28-20 mesh, and -28 mesh. The procedure of "Preparation of Samples for Compositional Analysis" can be found on the NREL website (NREL 2006).

3.5 Structural Carbohydrates Analysis

This following structural carbohydrate analysis from an NREL document was used to study the comprehensive biomass analysis. As mentioned in Section 1.4,

carbohydrates and lignin are the major components in the biomass samples. The relative composition of each wood component must be measured. The analysis procedure applied in this work was located in the U.S. Department of Energy (2006) website and was based on the LAP-002 documented by NREL. This procedure used a two-step acid hydrolysis reaction to fractionate the biomass into forms that are more easily quantified. The mass of glucose, xylose, and other sugars in the biomass in this experiment can be directly measured. The sugars were identified and quantified using HPLC Analysis described in Section 3.6.

The concentration of polymeric sugars was calculated from the concentration of the corresponding monomeric sugars obtained by HPLC for each of the hydrolyzed samples using an anhydro correction factor of 0.88 (or 132/150) for C-5 sugars (xylose and arabinose) and a correction of 0.90 (or 162/180) for C-6 sugars (glucose, galactose, and mannose).

The JSA of Structural Carbohydrates Analysis is attached in Appendix A-1. Additionally, the LAP of "Determination of Structural Carbohydrates and Lignin in Biomass" can be found in the NREL website with equations used to calculate the amount of acid addition in the experiment (NREL 2006).

3.5.1 Preparation of Samples for Analysis and Hydrolysis

Triplicate samples of 300 mg of the biomass samples were weighed and put into 100-mL amber glass vials with Teflon screw-tops. Then, 3 mL of 72% sulfuric acid was added to each vial. The samples were thoroughly mixed using a Teflon stir rod to ensure even acid to particle contact. The vials were then placed in a shaker table (LAB-LINE ORBIT Environ-Shaker) at 50 rpm within beakers containing water at 30^{o}C and

incubated for one hour. To ensure even acid to particle contact and uniform hydrolysis, the samples were stirred every ten minutes without removing the sample vials from the beaker. Upon completion of the 60-minute hydrolysis, 84 mL of deionized water was added to each vial to dilute the acid concentration to 4%.

Next, a set of sugar recovery standards (SRS) were prepared. The SRS was used to correct for losses due to the destruction of sugars during a subsequent dilute acid hydrolysis step for each wood sample. A large batch of SRS was prepared and the concentrations were 10 g/L of xylose, 5 g/L of glucose, and 1 g/L each of galactose, arabinose, and mannose. 10 mL of SRS with 348 μL of 72% sulfuric acid was transferred to the amber glass vial and capped tightly.

The triplicate samples and the SRS were autoclaved for 60 min. at 121^{o}C using an Autoclave AC-48 (New Brunswick Scientific). After completion of the autoclave cycle, the contents of the vials were slowly cooled down to room temperature using cool water bath before removing the caps.

3.5.2 Acid Soluble Lignin (ASL) Analysis

The autoclaved samples were vacuum filtered through one of the pre-weighed 25-mL filtering crucibles (Coors Porcelain Gooch Filtering Crucibles, Fisher Catalog No. 08-195D). The filtering crucibles were pre-weighed with 1.6-μm particle retention, 21-mm diameter glass filter paper (Fisherbrand Glass Fiber Circles G6, Catalog No. 09-804-21A). The filtrates were captured in a 250-mL filtering flask (Note: Do not use extra deionized water to wash the remaining solid in this step yet). Then, the absorbance of the filtrate was measured using the Milton Roy Spectronic 21D UV-Visible spectrophotometer at a wavelength of 320 nm. Deionized water was used as the

background solution. The absorbance was used to analyze the acid soluble lignin using the equation provided by NREL LAP # 002. Dilute the sample as necessary to bring the absorbance into the range of 0.7 – 1.0. The Equation (3-1) is also listed as below:

$$\% \ ASL = \frac{UVabs \times Volume_{filtrate} \times Dilution}{\varepsilon \times ODW_{sample}} \times 100 \qquad (3-1)$$

Where: $UVabs$ = average UV-Vis absorbance for the sample at 320 nm

$Volume_{filtrate}$ = volume of filtrate = 87 mL

$$Dilution = \frac{Volume_{sample} + Volume_{diluting\ solvent}}{Volume_{sample}} \qquad (3-2)$$

ε = Absorptivity of biomass at wavelength of 320 nm = 30 L/g-cm

$$ODW_{sample} = \frac{Weight_{air\ dry\ sample} \times \%Total\ solids}{100} \qquad (3-3)$$

3.5.3 Acid Insoluble Lignin (AIL) Analysis

For acid insoluble lignin, deionized water was used to quantitatively transfer all the remaining solids out from the vial into the filtering crucible. The filter cake was dried at 105^{o}C for a minimum of four hours. The oven dried filter cake was then pre-weighed prior to placing the filter cake in a SYBRON Thermolyne 2000 muffle furnace. The crucibles were heated at 575^{o}C for 24 hours. Subsequently, the crucibles were carefully removed from the furnace and placed directly into a desiccator to cool till room temperature. The ash was weighed to a constant weight. From these weight values, the amount of insoluble lignin was determined using the equation provided in NREL LAP # 002. The equation is also shown below:

$$\%AIL = \frac{(Weight_{crucible+residue} - Weight_{crucible}) - (Weight_{crucible+ash} - Weight_{crucible})}{ODW_{sample}} \times 100 \quad (3-4)$$

3.5.4 Structural Carbohydrates Analysis

The remaining filtrates obtained from Section 3.4.2 were used in this step. The filtrates were neutralized with the 6N NaOH to pH 5 – 6 using the Accumet® Model 15 pH Meter. The neutralized samples were collected into HPLC autosampler vials. In this step, a series of calibration standards were also prepared for each sugar. Detail explanation of HPLC analysis setup with samples and calibration standards will be discussed in Section 3.7.

3.6 Dilute Acid Hydrolysis Pretreatment

This experiment was conducted using the High Pressure/High Temperature Parr 4571 Reactor, as shown in Figure 3-1. The aspen, balsam, basswood, red maple, and switchgrass were heated in separate experiments from room temperature to 160^{o}C, 175^{o}C, and 190^{o}C in a diluted acid aqueous solution. The detailed procedures followed are included in the JSA "Dilute Acid Hydrolysis Pretreatment", in Appendix A-2.

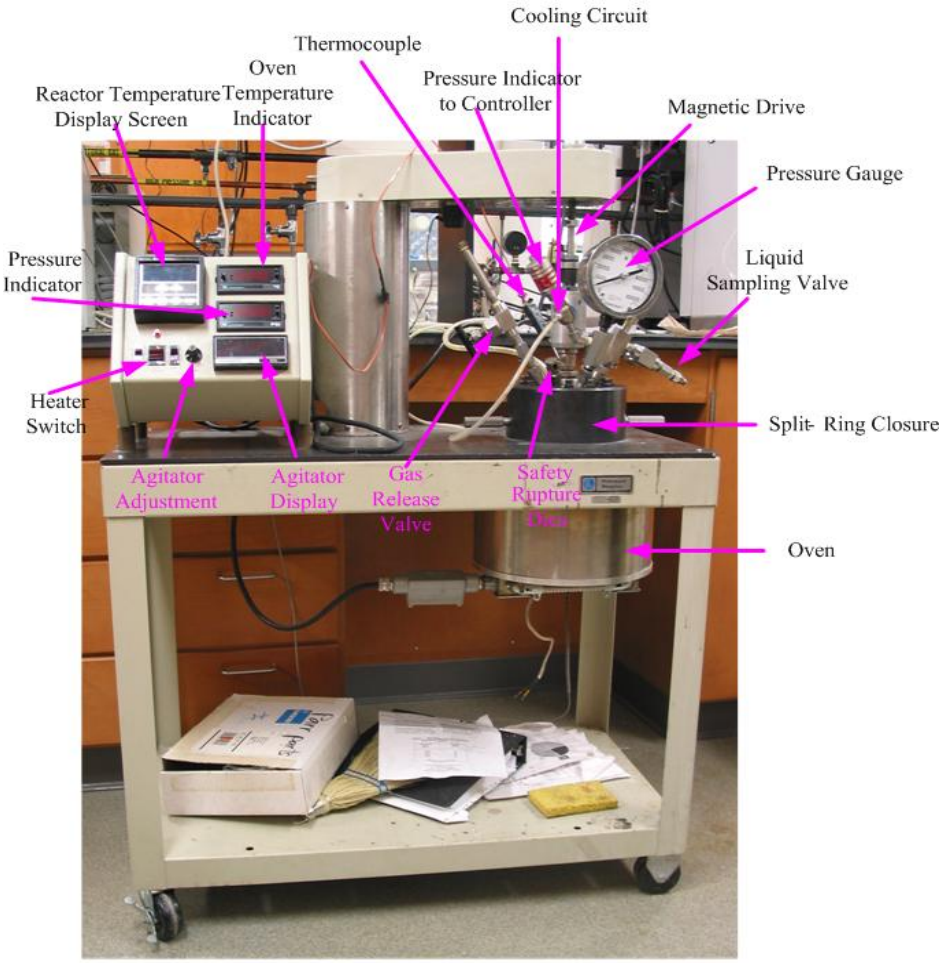

Figure 3-1: High Pressure/High Temperature Parr Stirred Reactor Model 4571, 1000 mL capacity

3.6.1 Start-up Procedure

25 grams of each dry biomass sample was mixed with 500 mL of 0.25-1.0% w/w sulfuric acid in a glass insert for the reactor. The glass liner was then placed into the reactor chamber shown in Figure 3-1. The reactor chamber was put into the oven on the moveable cart. The reactors was tightening using the torque-wrench in the pattern of bypass adjacent screw 180 degree from the starting screw and tighten it to approximately 5 ft/lb. The detail sealing instruction is provided by Parr Instruction Company in Manual No. 274M. The instruction is also attached in Appendix B.

After the reactor was clamped tightly, the cooling circuit was connected to the air valve and the agitator cooling circuit was connected to the cooling water. Moreover, the pressure indicator was adjusted to ambient pressure and the initial temperature setpoint was set to 400^{o}C at the controller. After all the controls were set, the reactor was heated up to the desired temperature by turning on the heater switch on the controller. During the heat up period, the temperature and pressure of the oven and the reactor were recorded every 5 minutes.

3.6.2 Run Time Procedure

5 mL of samples were collected at 100^{o}C, 135^{o}C, and thereafter at 3 minutes intervals until the temperature reached the setpoint (160^{o}C, 175^{o}C, and 190^{o}C). Prior to that, approximately 8 mL of samples were removed from the sampling valve and discarded it to eliminate the sample residue from previous sample. The temperature was maintained at the setpoint for 32 minutes by adjusting the airflow to the cooling coil of the reactor. While the temperature was controlled at the setpoint, four 5 mL of samples were collected at 8 minutes intervals. Approximately 8 mL of samples were removed again to eliminate the unreacted solution in the reactor. A graduated cylinder was used to measure the 8 mL samples and a 20-mL vial was used to collect the 5 mL sample afterward. As a result, an approximately 156 mL of solution sampled from the reactor for 12 vials. The vials were labeled and capped tightly. Thus, there was 344 mL of solution left in the reactor at the end of the experiment.

The collected 5-mL samples were placed in an ice bath to stop the reaction and to cool the samples to room temperature. The cooled samples were filtered through 0.2 μm Millipore membrane filter (25 mm dia, “Isopore”) using the 10-mL syringe. The filtered

samples were collected in the 20-mL vial. In the following procedure, the samples were separated into two analysis; monomeric sugars analysis and total sugars analysis. The monomeric sugars content was analyzed without further hydrolysis. However, the total sugars content was analyzed after the further acid hydrolysis using the autoclave.

For monomeric sugars analysis, 1 mL of sample was drawn from the 20-mL vial using the 100-1000 μL Oxford Benchmate II handheld pipette into the HPLC vial (Agilent Screw Cap Vials 100/pk, Part No. 5182-0716). Then, the samples were neutralized using the 6N NaOH. The samples were slowly neutralized to pH 5 – 6 using the pH 1 – 12 Hydrion Papers. The amount of 6N NaOH added to the HPLC vials for neutralization is listed in Table 3-2 below for different acid conditions.

Table 3-2: Neutralization of monomeric sugars samples in various acid conditions

Sample Concentration	Neutralization using 6N NaOH
0.25 % Dilute Acid Hydrolysis	7 μL
0.5 % Dilute Acid Hydrolysis	15.5 μL
1.0 % Dilute Acid Hydrolysis	26 μL

As the total sugar analysis was simply extra information to identify the amount of oligomers left in the sample after pretreatment, only the even numbered samples were prepared for the second acid hydrolysis. The pH of each sample was measured and recorded in this step using the pH meter. Then, 1 mL of samples, which were sample 2, 4, 6, 8, 10, and 12, were drawn from the 20-mL vial using the pipette into the amber crimp vial (Hewlett Packard 2 mL vial, Part No. 5181-3376). After that, the samples were acidified to 4% acid concentration using the 96% H_2SO_4. For each sample, the volume of 96% sulfuric acid required to bring the acid concentration to a 4% final acid concentration was calculated using the equation provided by NREL LAP # 015 and shown in Equation (3-5) below:

$$V_{96\%} = \frac{[(C_{4\%} \times V_s) - (V_s \times [H^+] \times 98.08 g\, H_2SO_4 / 2\, moles\, H^+)]}{C_{96\%}} \qquad (3-5)$$

Where as: $V_{96\%}$ = volume of 96% acid to be added, mL

V_s = initial volume of sample, mL

$C_{4\%}$ = concentration of 4% w/w H2SO4, 41.0 g/L

$C_{96\%}$ = concentration of 96% w/w H2SO4, 1800 g/L

$[H^+]$ = the concentration of hydrogen ions in the sample = antilog(-pH)

The amount of concentrated sulfuric acid added to the amber crimp vials for acidification is listed in Table 3-3 below for different acid concentration.

Table 3-3: Acidification of total sugar samples for various acid conditions

Sample Concentration	Acidification using 96% H_2SO_4
0.25 % Dilute Acid Hydrolysis	22 μL
0.5 % Dilute Acid Hydrolysis	22 μL
1.0 % Dilute Acid Hydrolysis	20 μL

The acidified samples were crimped tightly and then autoclaved for an hour at 121^{o}C. After completion of the autoclave cycle, allow the hydrolyzates to slowly cool to near room temperature using the cool water before removing the cap. Each sample was subsequently neutralized to pH 5 – 6 using the 6N NaOH. Roughly 133 μL of 6N NaOH was used to neutralize the autoclaved sample.

After neutralizing the monomeric and total sugar samples, the precipitation might be formed in the neutralized sample. The samples were placed into the MARATHON 21K centrifuge (Fisher Scientific) at 8000 rpm for 5 minutes. After completion of centrifuge cycle, the clear liquid was carefully and slowly drawn out from the vials to a

new HPLC vials. The samples were then ready for sugars analysis using the HPLC discussed in the following section.

3.7 HPLC Analysis

High Performance Liquid Chromatography (HPLC) has been commonly used in identification and quantification of sugars and degradation products produced from biomass pretreatment. In this project, an Agilent HPLC Series 1100 instrument was used. The separation and quantitative analysis of neutral sugars and organic acids have been performed by various HPLC methods using different types of columns. The most regularly used HPLC columns for determination of sugars and organic acids are Aminex HPX-87P and Aminex HPX-87H, respectively (Bio-Rad, 2006).

3.7.1 Column Choice for Carbohydrate Analysis

In this project, the Aminex HPX-87P was used as the objective of this project is to optimize a reactor that would be able to produce maximum amount of sugars and minimize the degradation products.

3.7.2 Sample Analysis & Calibration Standards

In this research, a simple HPLC method was developed for quantitative analysis of monomeric sugars, such as glucose, xylose, galactose, arabinose, and mannose, in the reaction samples. The HPLC conditions were as follows:

- Injection volume: 10 μL
- Mobile phase: HPLC grade water

- Guard columns: Filter Guard Column (Agilent Low Dispersion In-line Filter, Catalog No. 01090-68702) and Microguard Deashing Column (BioRad, Catalog No. 125-0118) (Note: these columns was placed outside of the heating unit and kept at room temperature)
- Flow rate: 0.6 mL/min
- Column temperature: 80^{o}C (maximum of the system)
- Refractive Index Detector (RID) temperature: 55^{o}C
- Diode-Array Detector (DAD): Sig=250, 4 Ref=360, 100
- Run Time: 60 min

Signals given out of the RID and DAD were integrated by the computer and were printed out as a short report with chromatogram and details values, such as retention times, peak heights, and peak areas. The RID was used to detect all sugars produced from experiment. The DAD detector was used to monitor for and quantitative furfural. A sample figure and table from RID / DAD report print out is shown in Figure 3-2 and Table 3-4.

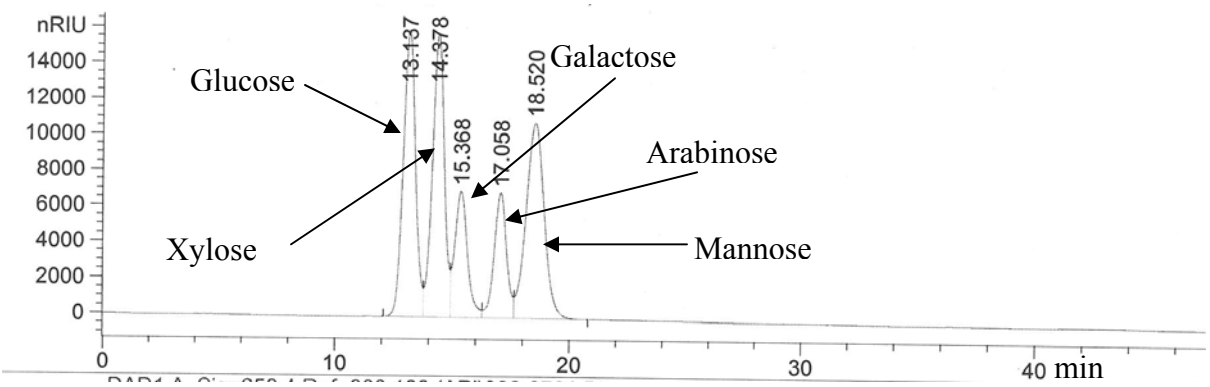

Figure 3-2: Sample HPLC Chromatogram Refractive Index Signals of Sugars Produced from Biomass Pretreatment.

Table 3-4: Sample HPLC Chromatogram Refractive Index Signals Summary of Sugars Produced from Biomass Pretreatment

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Height [nRIU]	Area %
1	13.137	BV	0.5620	5.80303e5	1.59756e4	23.0414
2	14.378	VV	0.5499	5.59088e5	1.58438e4	22.1990
3	15.368	VV	0.5724	2.67390e5	7045.82422	10.6169
4	17.058	VV	0.5693	2.58180e5	6987.20459	10.2513
5	18.520	VB	0.8481	5.91841e5	1.09237e4	23.4995
6	50.778	PB	1.7786	2.61723e5	2327.16479	10.3919
Totals :				2.51853e6	5.91033e4	

Using the Aminex HPX-87P, one of the degradation organics, furfural, was detected by DAD. A sample peak and table from DAD report is shown in Figure 3-3 and Table 3-5.

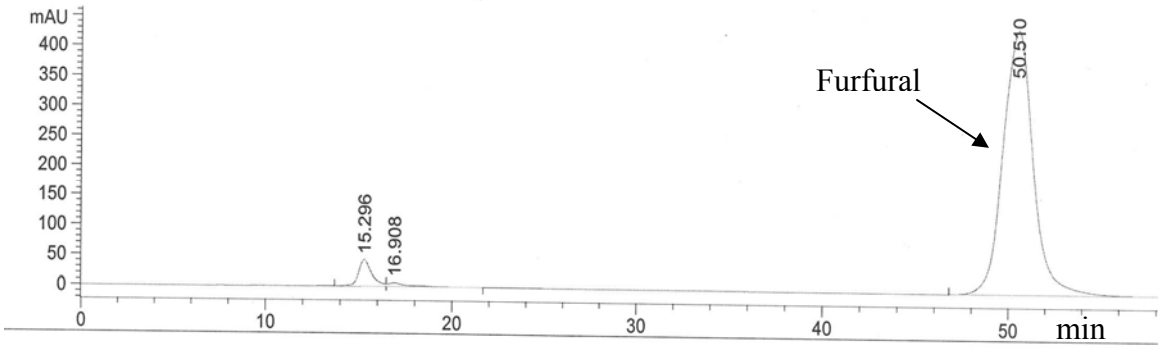

Figure 3-3: Sample HPLC Chromatogram DAD Signal of Furfural Produced from Biomass Pretreatment

Table 3-5: Sample HPLC Chromatogram DAD Summary of Signals Produced

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	15.296	VV	0.7505	2214.21411	43.74676	4.1648
2	16.908	VB	1.1240	546.10559	6.50126	1.0272
3	50.510	BB	1.7952	5.04040e4	441.75473	94.8079
Totals :				5.31643e4	492.00275	

To obtain a quantitative measure of the concentrations, the peak areas obtained from the HPLC analysis was calibrated to obtain an instrument response factor. This

allowed each peak to be representative to an individual reactant and the area of each peak to be related to a concentration (g/L). The calibration curves of all five sugars are shown in Figure 3-4 and 3-5.

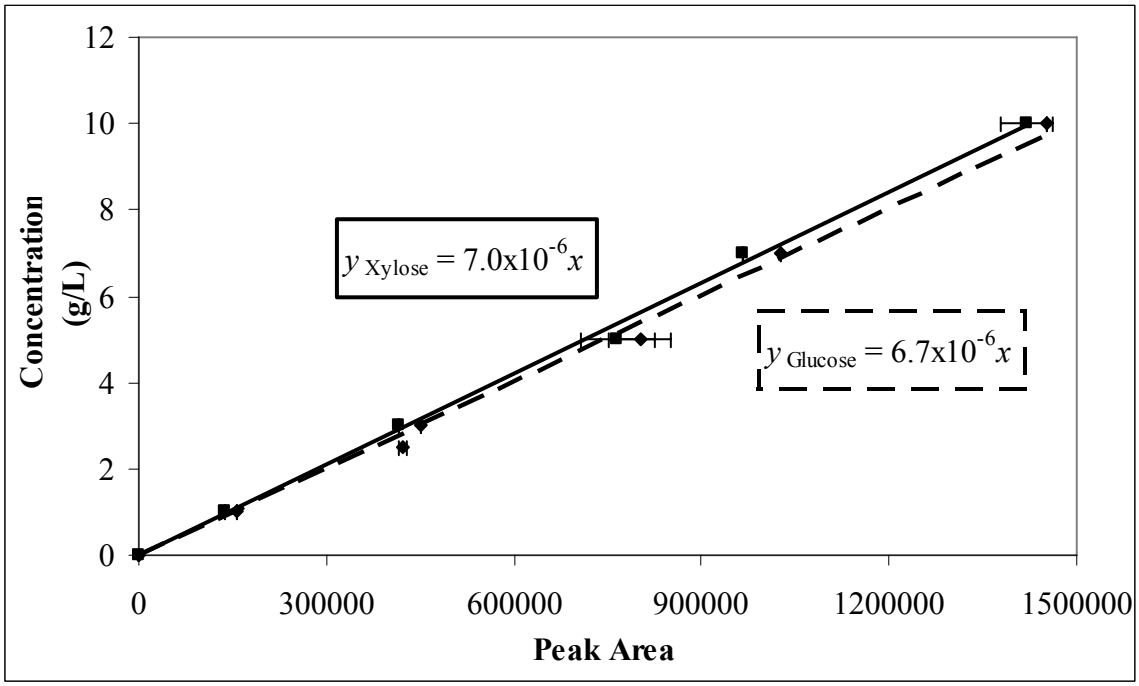

Figure 3-4: HPLC Major Sugars (Xylose and Glucose) Calibration Standards

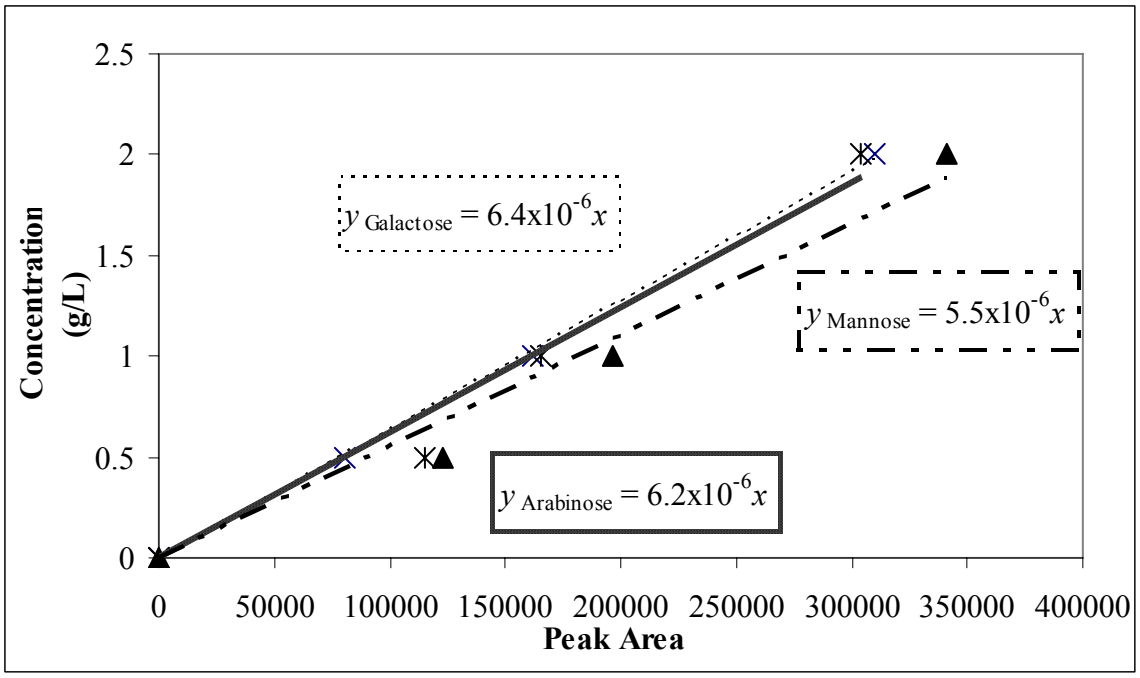

Figure 3-5: HPLC Minor Sugars (Galactose, Arabinose, and Mannose) Calibration Standards

A calibration curve for furfural was performed as shown in Figure 3-6.

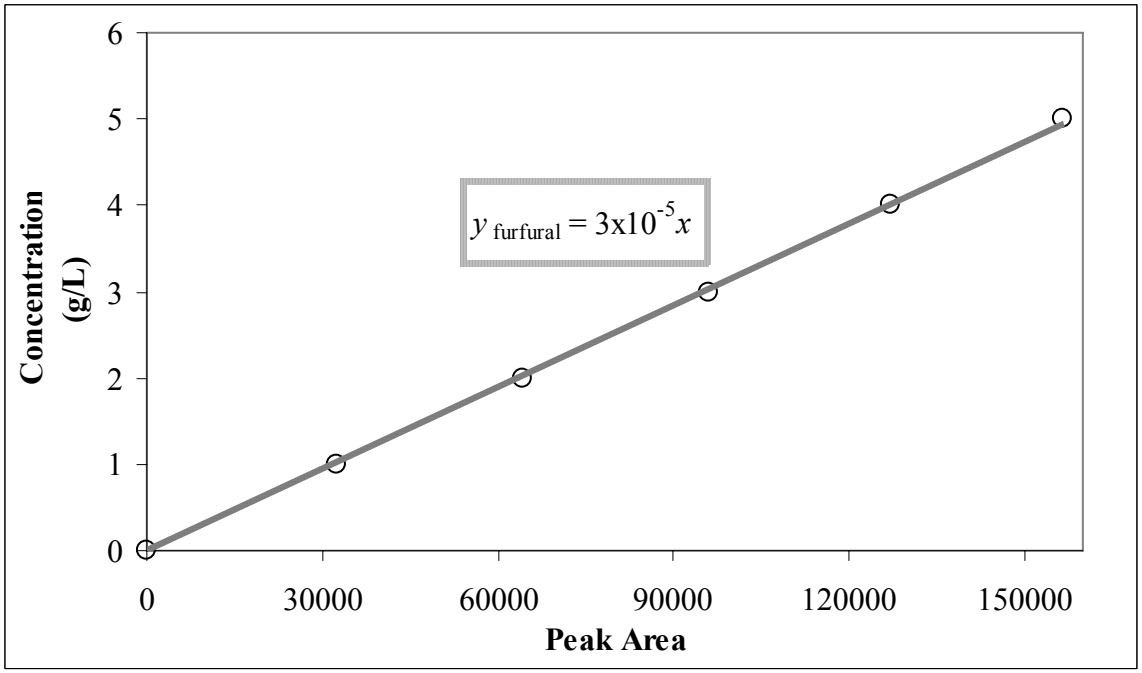

Figure 3-6: HPLC Degradation Product (Furfural) Calibration Standard

Chapter 4 Theoretical Model

4.1 Introduction

With the purposes of obtaining kinetic data and predicting the optimum reactor conditions during the pretreatment, two models were developed and presented in this chapter: xylose formation model and xylose degradation model. The xylose formation model performed the conversion from hemicellulose to xylose through reactor heating period. Yet, the xylose degradation model performed the degradation product formation from xylose during the constant temperature phase.

4.2 Model Development

As mentioned in Section 2.3.3, one of the overall goals of dilute acid pretreatment is to achieve high monomer sugar yields while minimizing the breakdown of sugars into decomposition products. Hemicellulose hydrolysis and sugar degradation reactions can be considered as pseudo first-order processes. High selectivity pretreatment is achieved by maximizing the ratio of the rate constants, k_1/k_2, as shown in Equation (4-1). In order to achieve the pretreatment goal, it is important to design a temperature controlled reactor to avoid the formation of degradation products. A kinetic model for hemicellulose hydrolysis to xylose using the Arrhenius form of the rate constant is required prior to data analysis.

$$\underset{Hemicellulose\,(H)}{} \xrightarrow{k_1} \underset{\substack{Pentoses \\ Hexoses \\ Oligosaccharides}}{} \xrightarrow{k_2} \underset{Degradation\ Product}{} \qquad (4-1)$$

Literature and data on the kinetics of dilute acid pretreatment primarily exist for two types of process conditions, low solids loading (5% - 10% w/w) with high temperature (T > 160^{o}C) and continuous-flow processes (Knappert et al, 1981), and higher solids loading (10% - 40% w/w) with lower temperature (T < 160^{o}C) and batch processes (Grohmann et al, 1986). The kinetics of high-temperature wood saccharification catalyzed by dilute acid was first extensively investigated by Seaman (1945), who demonstrated that cellulose hydrolysis and monomer sugar decomposition follow first-order kinetics, as shown in Equation (4-2).

$$Cellulose \longrightarrow Glucose \longrightarrow Degradation\ Products \qquad (4-2)$$

The kinetics of hemicellulose hydrolysis, Equation (4-3), was first modeled by Mehlberg and Tsao, 1979 in the following reaction:

$$\begin{array}{l} \rightarrow Xylan\ I \rightarrow \\ \uparrow \qquad\quad \downarrow \\ Hemicellulose \qquad Oligomers \rightarrow Xylose \rightarrow Degradation\ Products \\ \downarrow \qquad\quad \uparrow \\ \rightarrow Xylan\ II \rightarrow \end{array} \qquad (4-3)$$

The oligomers obtained during the reaction possess different degrees of polymerization and their reaction rates vary with the degrees of polymerization value. The concentration of the various oligomers of different polymerization degrees cannot be measured as it is difficult to separate them using HPLC; also, separation of xylan I and xylan II is difficult. Grohmann et al. (1986) concluded that at lower temperatures (T < 160^{o}C) hemicellulose

hydrolysis is not homogeneous, with a portion hydrolyzing rapidly while the remainder hydrolyzes more slowly. In the initial stages of hydrolysis, both fast and slow fractions of hemicellulose are hydrolyzed to xylose by parallel first-order reactions. As a result, Equation (4-3) depicts the modified reaction scheme for lower temperature ($T < 160^oC$) hemicellulose hydrolysis.

As the concentrations of xylan I and xylan II and oligomers are very difficult to determine and since the reaction to produce xylan I and xylan II is very rapid at higher temperature ($T > 160^oC$), as mentioned above, the model could be simplified to one that is similar to that for cellulose; that is,

$$Hemicellulose\,(H) \xrightarrow{k_1} Xylose\,(X) \xrightarrow{k_2} Degradation\ \ Products\,(D) \quad (4-4)$$

Two models were developed in this project. The first model includes the first step in conversion of hemicellulose to xylose as discussed in section 4.3. The second model involves the process of xylose degradation. It will be discussed in Section 4.4.

4.3 Modeling of Xylose Formation

Equation (4-4) shows the case in which biomass carbohydrate species H (hemicellulose) hydrolyzes to X (xylose), which then decomposes to D (furfural or other degradation products). Expressions for the net rate of formation of components H, X, and D can be integrated to determine the concentrations of H and X as a function of time and initial conditions. Equations (4-5) and (4-6) are the mass balance equations for H and X in a well-stirred batch reactor under the assumption of constant reactor liquid volume and first order reaction for each species,

$$\frac{dH}{dt} = -k_1 H \qquad (4-5)$$

$$\frac{dX}{dt} = k_1 \frac{H}{0.88} - k_2 X \qquad (4-6)$$

where k_1 and k_2 are first order reaction rate constants for xylose formation and xylose degradation, respectively.

(4-5) was rearranged using the separation of variables for an ordinary differentiation equation,

$$\frac{dH}{H} = -k_1 dt \qquad (4-7)$$

The reaction rate constant, k_1, is assumed to have Arrhenius-type temperature dependence:

$$k_1 = A_1 \exp(-\frac{E_1}{RT_1}) \qquad (4-8)$$

Where as E_1 = Activation energy (kJ/mol)

R = Ideal gas constant = 8.3143 x 10^{-3} (kJ/mol-K)

T_1 = Temperature (K)

A_1 = Pre-exponential factor (min^{-1})

In this project, the pre-exponential factors (A_1) for hemicellulose hydrolysis reactions are assumed to be dependent upon acid concentration

$$A_1 = A_{1o} C^{m_1} \qquad (4-9)$$

where A_{1o} = Pre-exponential factor for hemicellulose hydrolysis (min^{-1})

C = Acid concentration (% w)

m_1 = Acid concentration exponent for the rate constant k_1, dimensionless

Therefore, Equations (4-8) and (4-9) can be combined as follow:

$$k_1 = A_{1o} C^{m_1} \exp(-\frac{E_1}{RT_1}) \qquad (4-10)$$

Equation (4-10) assumes that temperature is constant during the hydrolysis reaction (Esteghlalian et al, 1997). In this project however, the reaction temperature changed overtime. Thus, the reaction rate constant model changed as a function of time as shown in Equation (4-11).

$$k_1 = A_{1o} C^{m_1} \exp(-\frac{E_1}{RT_1(t)}) \qquad (4-11)$$

Consequently, obtaining the analytical solution from Equation (4-7) is absolutely impossible. Therefore, a numerical method was employed. The Equation (4-7) is expressed in finite difference form as follows:

$$\frac{H_{i+1} - H_i}{H_i} = -k_i \Delta t \qquad (4-12)$$

Where i is the time index and Δt is the time step. The numerical solution of Equation (4-12) at each new time step is:

$$H_{i+1} = H_i - k_i H_i \Delta t \qquad (4-13)$$

$$\text{Where } k_i = A_{io} C^{m_1} \exp(-\frac{E_1}{RT_1(t)}) \qquad (4-14)$$

Hence, the xylose formation model is

$$X_{i+1} = \frac{H_{\max} - H_{i+1}}{0.88} \qquad (4-15)$$

Where $H_{\max}$ = the maximum concentration of xylan that can be produced in each species (g xylose oligomer/L) as determined by the total carbohydrate analysis in Section 3.5.4. In equation 4-15 it is assumed that negligible furfural is generated from xylose during the heat up period (period of time for application of the model). The concentration of the

polymeric sugars from the concentration of the corresponding monomeric sugars was calculated using a correction of 0.88 (or 132/150) for C-5 sugar, which is xylose in this case.

Equations 4-13 to 4-15 were integrated using the Trapezoidal Rule of integration with a time step of 0.01, which was determined to be the maximum time step for the convergent of the sum of square error. The determination of time step is shown in Appendix C. The kinetic parameters, A_1 and E_1, were determined using Excel Solver with initial guesses. The sum of squared differences between experimental data and model predictions were minimized by improving the values of the A_1 and E_1 using Equations (4-13), (4-14), and (4-15).

4.4 Modeling of Xylose Degradation

Equation (4-6) was used to model the Xylose degradation products formation. It was relatively easy to solve as the degradation products formed while the desired maximum temperature was constantly maintained. In this section, all the hemicellulose (xylan) was assumed to be reacted to xylose completely ($H = 0$). Therefore, Equation (4-6) becomes

$$\frac{dX}{dt} = -k_2 X \qquad (4-16)$$

To solve the first order ordinary differentiation equation, the separation of variables method was used.

$$\frac{dX}{X} = -k_2 dt \qquad (4-17)$$

The integration is shown in the following step for constant k_2,

$$\int \frac{dX}{X} = -k_2 \int dt \qquad (4-18)$$

$$\ln X = -k_2 t + C \qquad (4-19)$$

where C = an integration constant.

The initial condition was applied in this step. Time zero corresponded with the maximum xylose produced (X_{max}) in the experiment. As a result, Equation (4-19) can be expressed by applying the initial condition.

$$\ln \frac{X}{X_{max}} = -k_2 t \qquad (4-20)$$

By plotting the experimental data according to the form of Equation (4-20) and fit it into linear regression analysis using Microsoft Excel, the reaction rate constant of xylose degradation, k_2, can be found at each maximum temperature for reach biomass species as the slope of the linear regression equation.

To determine the pre-exponential factor (A_2) and activation energy (E_2) of xylose degradation, the Arrhenius form can be represented as follows:

$$k_2 = A_2 \exp(-\frac{E_2}{RT_2}) \qquad (4-21)$$

where T_2 is the maximum temperature at experiment setpoint. The value of k_2 determined at each maximum temperature as per equation 4-20 was used to determine the kinetic parameters in equation 4-21 using linear regression analysis on the transformed Arrhenius equation as follows:

$$\ln k_2 = \ln A_2 - \frac{E_2}{R}\frac{1}{T_2} \qquad (4-22)$$

The slope of the curve (E_2/R) and the constant ($\ln A_2$) could be obtained by plotting the natural log of the calculated value of k_2 at each temperature versus $1/T_2$ using Equation (4 – 22).

As similar to Equation (4-9), the acid concentration exponent (m_2) could also be computed for the Xylose Degradation Model. The Equation (4-9) could then be presented as follow,

$$A_2 = A_{2o} C^{m_2} \qquad (4-23)$$

where A_{2o} is the pre-exponential factor for xylose degradation. Hence, the Equation (4-10) has become,

$$k_2 = A_{2o} C^{m_2} \exp(-\frac{E_2}{RT_2}) \qquad (4-24)$$

By taking the natural log of both the left and right hand sides of Equation (4-24), the equation would become

$$\ln k_2 = m_2 \ln C + \ln[A_{2o} \exp(-\frac{E_2}{RT_2})] \qquad (4-25)$$

Again, the calculated k_2 would be in the y-axis and the solution concentration (C) would be in the x-axis. By plotting the natural log of calculated k_2 from Equation (4-20) at each concentration experiment as a function of natural log of solution concentration, the slope of the linear regression is equal to m_2.

As a result, the pre-exponential factor and activation energy during the xylose degradation were determined from the temperature effect. Conversely, the acid concentration exponent was verified from the acid concentration effect.

Chapter 5 Experimental Results

5.1 Introduction

This chapter presents the pretreatment experimental results of various timber species found in the Upper Peninsula of Michigan plus switchgrass. The typical plots of each species at various reaction conditions are presented in this chapter. All the results in Microsoft Excel format are included in the CD accompanying this thesis (please read the readme.txt for further instructions).

Eight experiments were designed for each woody biomass to investigate the sugars yield and kinetic parameter during pretreatment. The eight experiments are listed in Table 5-1.

Table 5-1: Experimental design of dilute acid pretreatment

Experiment	% w/w Acid Concentration	Setpoint Temperature (^{o}C)	Particle Size (mesh)
1	0.5	175	20-10
2	0.5	175	20-10
3	0.5	160	20-10
4	0.5	190	20-10
5	0.25	175	20-10
6	1.0	175	20-10
7	0.5	175	> 28
8	0.5	175	> 28

In this project, woody biomass acid hydrolysis at 0.5% H_2SO_4, 175^{o}C, and 20-10 mesh particle size was established as the standard and was tested in duplication. The effect of acid concentration was measured with experiments at 1.0% and 0.25% H_2SO_4.

The experiment of the effect of temperature was altered 15 degrees higher and lower. Moreover, duplicate experiments of smaller particle size were analyzed to investigate the effect of size difference.

In addition to woody biomass pretreatment, the carbohydrate composition of each species was investigated in one run using triplicate samples. As a result, a total of nine experiments were run for each tree species and for switchgrass in this project.

5.2 Biomass Composition

The comprehensive structural carbohydrate analysis of woody biomass was determined and described in Chapter 3. Each species was tested in triplicate using a two-step acid hydrolysis to fractionate the biomass into forms that are more easily quantified. Throughout the hydrolysis, the polymeric carbohydrates were hydrolyzed into the monomeric forms, which are soluble in the hydrolysis liquid. The monomeric sugars were then measured by the HPLC. Figure 5-1 shows the biomass composition of all species used in this project; aspen, balsam, basswood, red maple, and switchgrass.

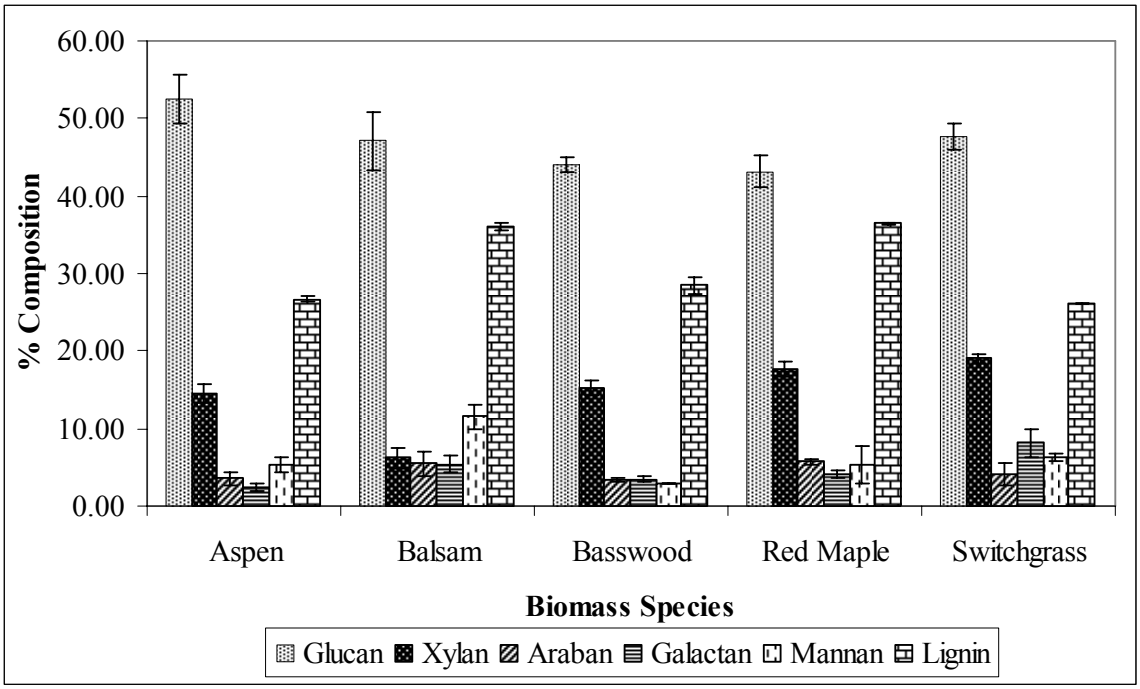

Figure 5-1: Biomass Structural Carbohydrate of 4 Timber Species in North American plus Switchgrass

Table 5-2 includes more details of biomass composition including the ash content of each species. The ash contents were negligible as most showed negative values in the table below. Overall, the compositions of biomass species in Table 5-2 are consistently over 100%. This is due to the propagation errors of measuring devices. As only small amount of biomass sample (300 mg) and acid (3 mL) were measured in the procedure, the calibration of instruments regularly might be necessary. To minimize the errors, a larger amount of sample and acid could be used.

Table 5-2: Biomass Structural Carbohydrate of Timber Species in North America plus Switchgrass

Species	% Biomass Composition							% Gross Composition
	Glucan	Xylan	Araban	Galactan	Mannan	Lignin	Ash	
Aspen	52.43	14.60	3.52	2.41	5.32	26.69	-2.03	103
Balsam	47.09	6.23	5.45	5.41	11.49	36.04	-0.24	111
Basswood	43.99	15.31	3.41	3.49	2.91	28.44	-0.08	97
Red Maple	43.18	17.69	5.71	4.13	5.37	36.49	-0.20	112
Switchgrass	47.72	19.06	4.18	8.11	6.30	26.04	1.19	113

Out of the five biomass species used in this project, aspen exhibits the highest glucan content, about 52%. Switchgrass is second highest in glucan content, 48%, and is highest in xylan content, 19%. Swichgrass also contains the lowest lignin contents.

The balsam was the only softwood species in this project. As expected, the balsam has highest lignin content, 36%, among all species. Although the balsam has the lowest xylan content, 6%, from the table above, it has presented the highest mannan content, 11%. Therefore, the combined hemicellulose content is competitive to other species.

The results of structural carbohydrate analysis are similar to the results published previously (Brooks et al, 1978). The comparison of the results will be discussed in Chapter 6.

5.3 Monosaccharides Formation

The xylan contents from structural carbohydrate of various biomass species used in this project was found to be range from 6% to 19% (dry basis) using the procedure provided by NREL LAP # 002. Thus, the potential xylose was 3 g/L of solution to 9 g/L of solution. The potential of other sugars could easily be calculated based on the results shown in Table 5-2 above.

As discussed above, eight nonisothermal runs were carried out for each of the effects, such as acid concentrations, temperatures, and particle sizes. Figure 5-2 is the typical plot of the time-temperature profile using the Parr 4571 Reactor.

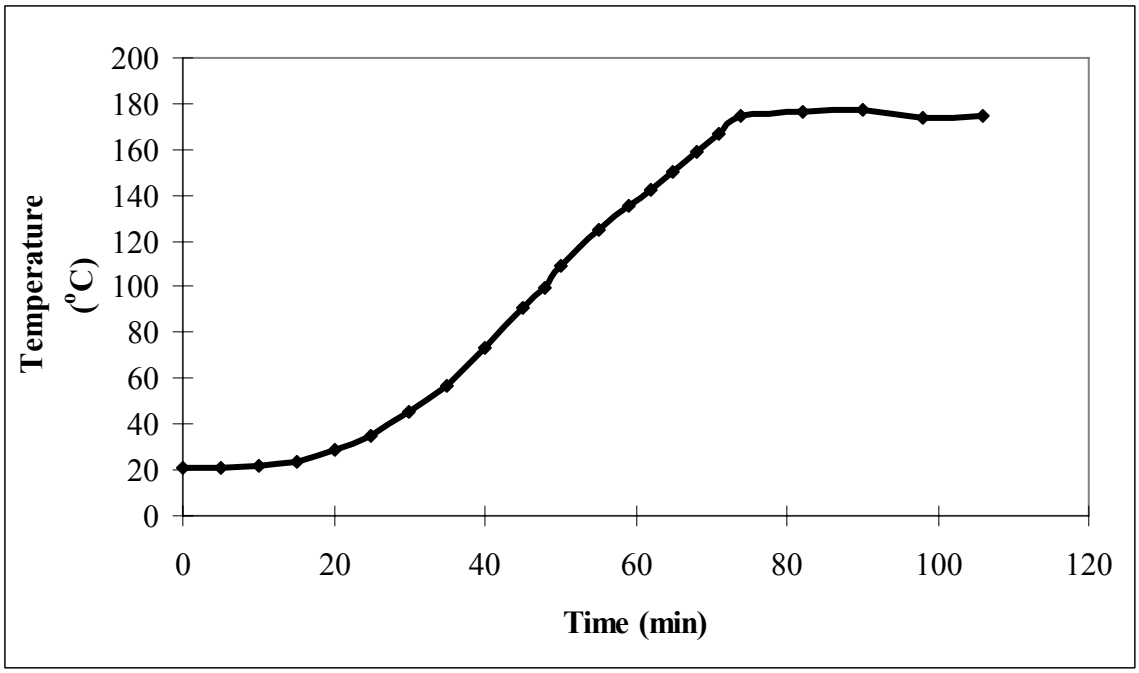

Figure 5-2: Basswood Acid Hydrolysis Time-Temperature History at 0.5% H_2SO_4, 175°C, 20-10 Mesh

The reactor with acid sample solution was heated up from room temperature to the setpoint temperature; 175°C in this case. As shown in Figure 5-2, the reactor temperature increased linearly after 40 minutes from the start of the heating period. From about 55 minutes (for T = 135 ºC) until 70 minutes (T = 175 ºC), temperature increased linearly but at a slightly slower rate compared to earlier times in the experiment. Over this time period most of the hemicellulose was hydrolyzed to sugars, and a linear trendline was fit to the data for subsequent use in kinetic modeling. After reaching the temperature setpoint, compressed air was used to cool the reactor to maintain the temperature constant for another 32 minutes.

Figure 5-3 is a typical plot of the monosaccharide data for biomass hydrolysis at 0.5% sulfuric acid, 175°C, and 20-10 mesh particle size. The figure showed the time-concentration of each sugar plus furfural, one of the main byproducts of dilute acid hydrolysis pretreatment along with insoluble tars.

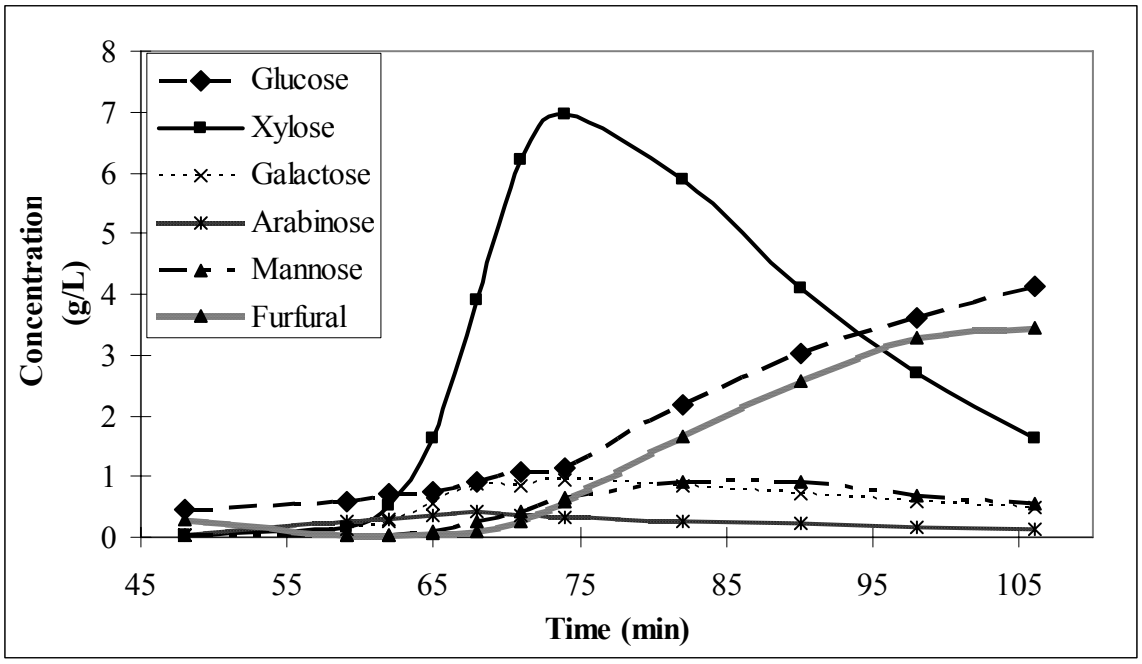

Figure 5-3: Basswood Acid Hydrolysis at 0.5% H_2SO_4, 175°C, 20-10 Mesh (Monomers Content)

The concentration of xylose and other sugars were small prior to achieving 135°C in the reactor. At higher temperatures than 135°C, the xylose concentration increased rapidly until the reactor temperature reached the target. Afterwards, the concentration of xylose decreased while the temperature was maintained at the setpoint. After the setpoint was reached, significant amounts of glucose and furfural were formed. Furfural is the degradation product produced from xylose. Throughout the dilute acid hydrolysis, smaller amounts of minor sugars were formed, such as galactose, arabinose, and mannose.

Balsam was the only softwood in this project. From the structural carbohydrate analysis, balsam has the lowest xylan content but the highest mannan content, which was found to be 11.5% on a dry weight basis. The maximum possible concentration of mannose was therefore 5.6 g/L. Figure 5-4 shows a typical plot of balsam hydrolysis for experiments run at a maximum temperature of 175ºC and 0.5% H_2SO_4. Except for a

relatively small concentration of xylose and large concentration of mannose, the pattern of monomer sugar concentrations generated was same as the other biomass species.

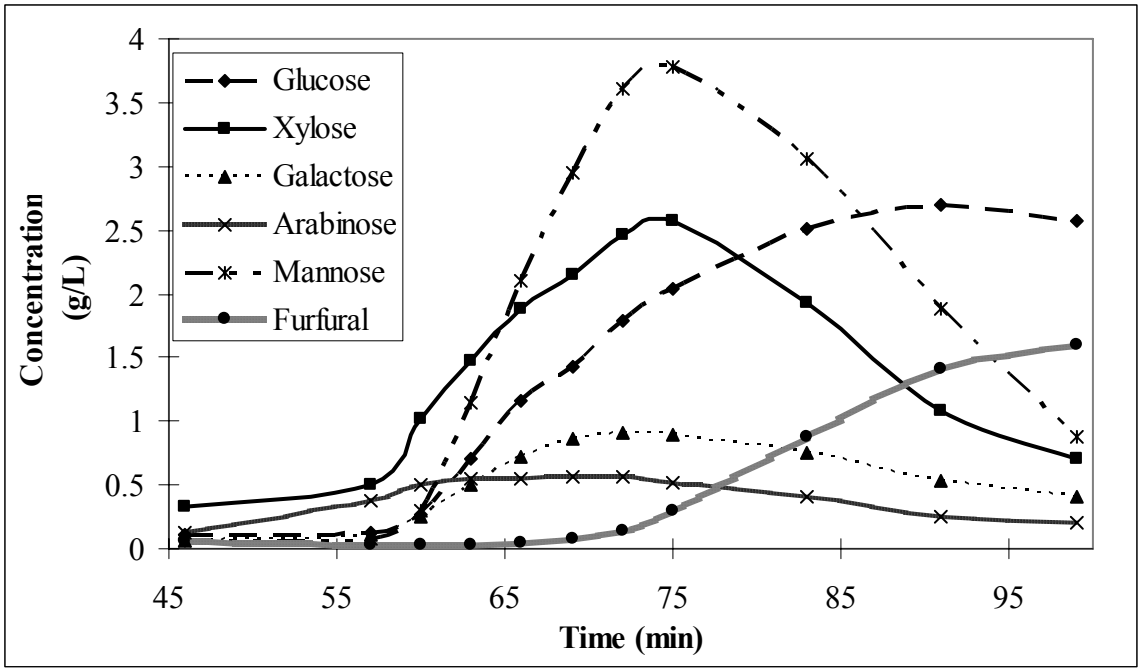

Figure 5-4: Balsam Acid Hydrolysis at 0.5% H_2SO_4, 175°C, 20-10 Mesh (Monomers Content)

The production of furfural in the balsam experiments was smaller as the xylan content is also lower than for other species; however, the mannose was degraded to other products after reaching the setpoint. Therefore, examining the degradation product of mannose would be important in future studies to determine whether the by-product of mannose is inhibitory in the subsequent ethanol fermentation reaction.

5.4 Oligosaccharides Formation

The section above described the monomeric sugar concentrations obtained from biomass hydrolysis during dilute acid pretreatment. However, oligomeric sugars generated from hydrolysis of hemicellulose prior to the forming of the monosaccharides. The oligomeric sugars were evaluated using the method described in Section 3.6.2.

Even numbers of samples were selected for a second acid hydrolysis step for an hour at 121°C using an autoclave. Figure 5-5 is a typical plot for oligomer distribution for all the experiments. The oligomeric sugars were converted in this reaction step into the monomeric form using acid hydrolysis and were quantified by HPLC.

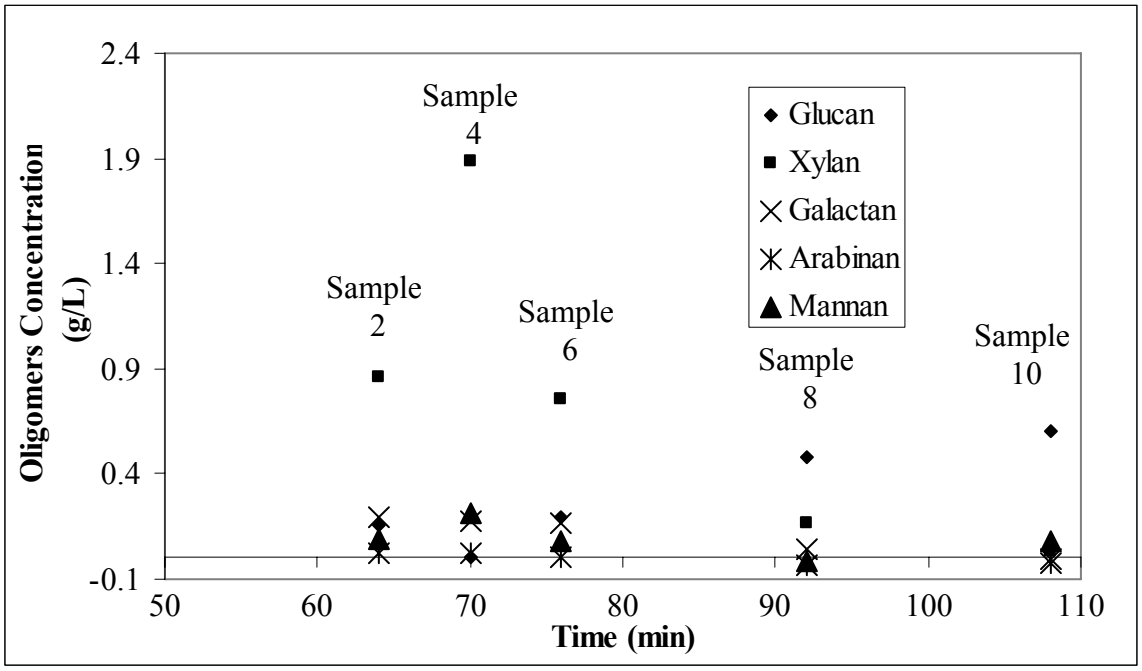

Figure 5-5: Aspen Acid Hydrolysis at 0.5% H_2SO_4, 175°C, 20-10 Mesh (Oligomers Content)

There were five sugars quantified after the secondary hydrolysis as indicated in Figure 5-5. Typically, most of the xylose oligomer appeared early in the pretreatment process as shown in Figure 5-6, which also shows the concentration profile of xylose monomer. The xylose oligomer showed up at the beginning of the reaction, when the temperature of the reactor was around 160°C. Then, the concentration of xylose polymer decreased as it formed monomer; however, the furfural was produced simultaneously.

On the other hand, the glucose oligomer was slowly produced from the cellulose at the later time as the temperature increased. The rest of the minor sugar oligomers were insignificant during the entire pretreatment period.

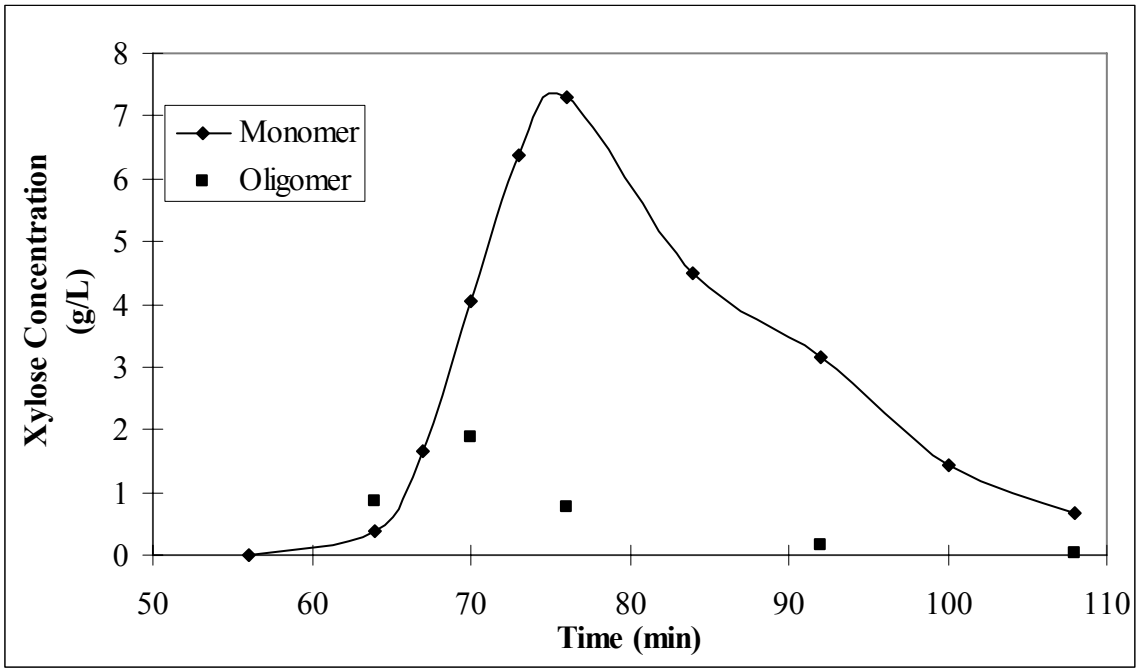

Figure 5-6: Aspen Acid Hydrolysis at 0.5% H_2SO_4, 175°C, 20-10 mesh (Xylose Oligomer & Monomer Profile)

5.5 Temperature Effects

Temperature was one of the three process variables being investigated in this project. The temperature of the reactor was ramped from room temperature to the desired temperatures, which were 160°C, 175°C, and 190°C. Figure 5-7 illustrated the temperature of balsam pretreatment at different runs. Temperature increase was reproduced in the reactor nearly exactly from trial to trial and temperature remained nearly constant after achieving the setpoints.

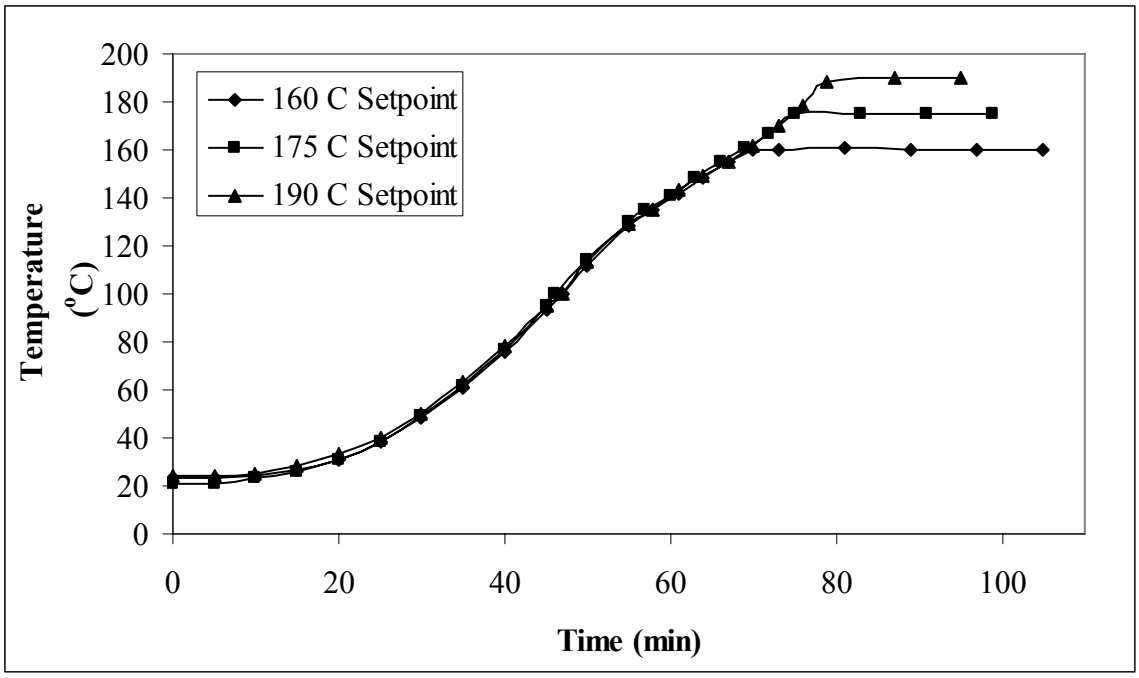

Figure 5-7: Balsam Acid Hydrolysis at 0.5% H_2SO_4, 20-10 Mesh (Temperature Profile)

The representative example of the temperature effect on dilute acid hydrolysis of biomass is shown in Figure 5-8. Because the reactor temperature was nearly exactly reproduced during the heating up period, xylose concentrations for the three runs were observed to increase at the nearly identical rates until the targeted temperature was reached.

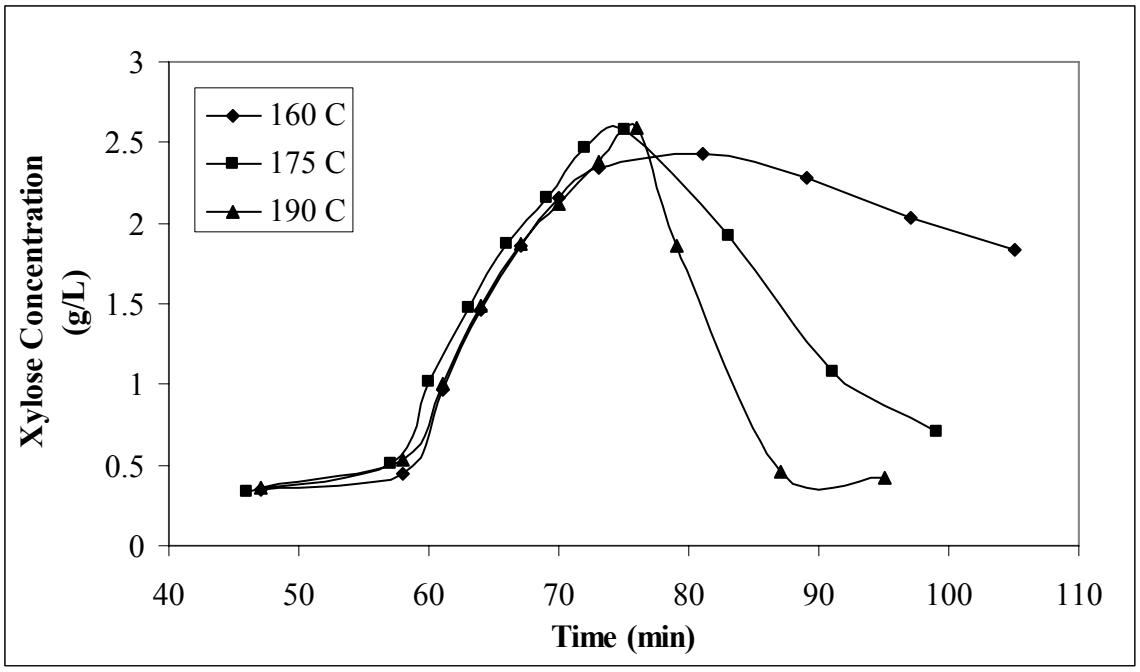

Figure 5-8: Balsam Acid Hydrolysis at 0.5% H_2SO_4, 20-10 Mesh (Xylose Pretreatment Profile with Temperature Dependence)

The xylose concentrations for these three trials showed important differences. For the experiment at a maximum temperature of 160°C, the rate of xylose formation slowed down after achieving maximum temperature and the rate of xylose degradation was small relative to the higher temperature experiments. In the 190°C experiment, xylose degraded rapidly within 10 minutes to near zero concentration.

Figure 5-9 shows glucose concentration versus time for three experiments conducted at maximum temperatures of 160, 175, and 190°C. Similar to the xylose concentration in these experiments during the reactor heat up period, glucose concentration showed a similar trend as the targeted temperature was achieved. After achcieving the maximum temperature, the rate of glucose formation increased significantly at higher maximum temperature. The effect of temperature on mannose, galactose, and arabanose was similar to xylose, characterized by degradation of these sugars with increasing maximum temperature.

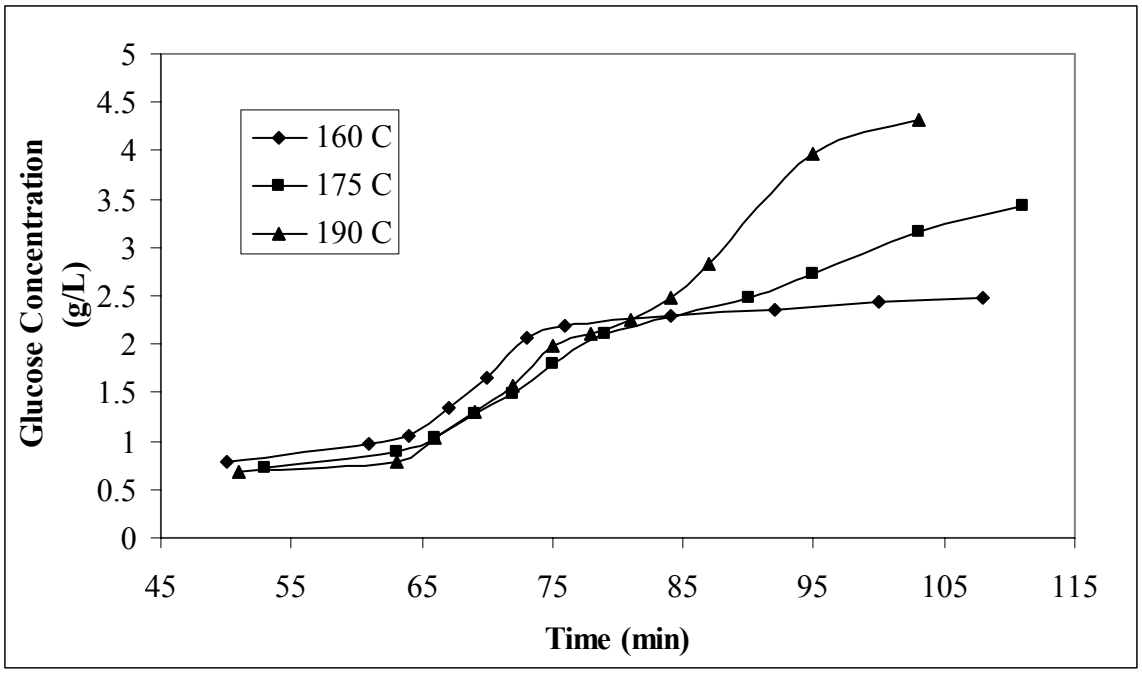

Figure 5-9: Switchgrass Acid Hydrolysis at 0.5% H2SO4, 20-10 Mesh (Glucose Profile with Temperature Dependence)

5.6 Acid Concentration Effects

Another process variable that may affect hemicellulose hydrolysis and xylose formation / degradation during pretreatment is the acid concentration of the reaction medium. Figure 5-10 shows a representative plot of the effect of acid concentration on xylose concentration for all of the biomass species tested. As the concentration of acid increases, the rate of xylose formation and degradation increase. For example, at 0.25% sulfuric acid, the rate of xylose formation was relatively slow and more time was required to achieve maximum xylose concentration. At maximum acid concentration of 1.0%, rate of xylose degradation after achieving maximum temperature of 175^{o}C was the highest for these experiments.

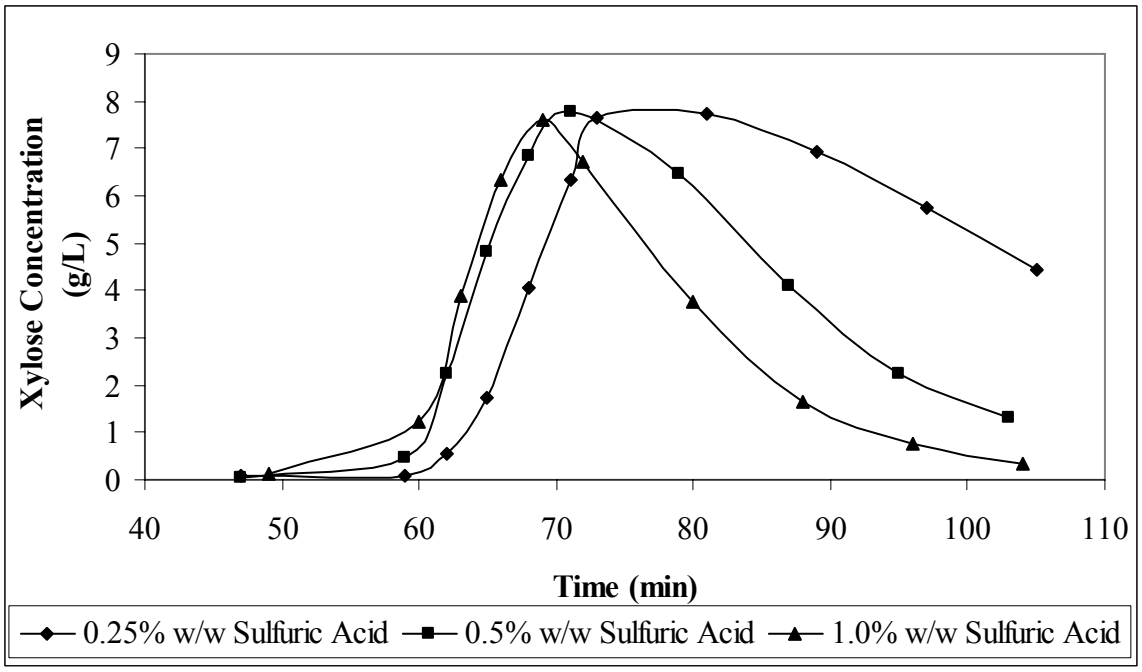

Figure 5-10: Red Maple Acid Hydrolysis at 175°C, 20-10 Mesh (Xylose Pretreatment with Sulfuric Acid Dependence)

Moreover, the xylose oligomer concentration was affected by the acid concentration. At lowest sulfuric acid concentration (0.25 %), for example, the xylose oligomer concentration reached maximum at the sixth sample; however, the xylose oligomer reached maximum at fourth sample at highest sulfuric acid concentration (1.0 %). Roughly twice the amount of xylose oligomer generated at the lowest acid condition compare with at the highest acid concentration.

Similar to the temperature effects shown previously, Figure 5-11 shows an increase of glucose concentration as the concentration of acid in solution increases. Glucose was produced from cellulose more effectively at the most severe acid condition. Although the main objective of pretreatment is to hydrolyze hemicellulose and remove it from other components of the biomass, it is important to note that a significant amount of glucose was generated from cellulose during this first stage of hydrolysis.

The minor sugars of mannose, galactose, and arabanose had seen the similar trends as xylose that is enhanced rates of sugar production and degradation with increasing concentration of acid. However, it is important to keep in mind that except for mannose produced from balsam, the concentrations of these minor sugars was relatively small compared to xylose and glucose.

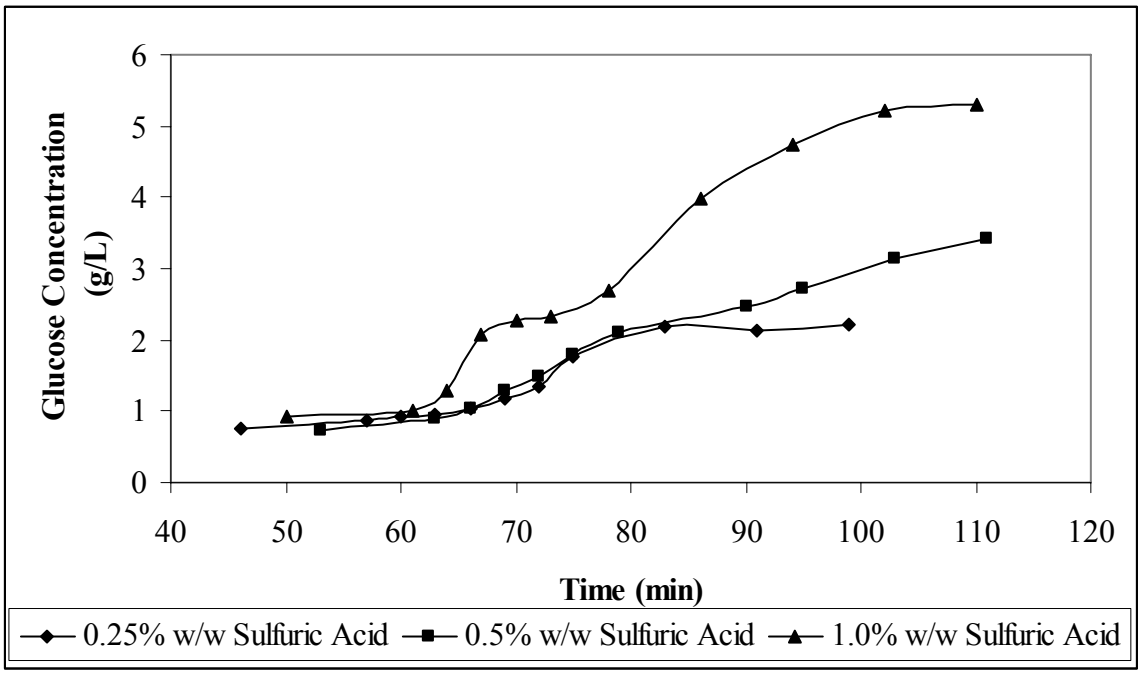

Figure 5-11: Switchgrass Acid Hydrolysis at 175°C, 20-10 Mesh (Glucose Acid Dependence)

5.7 Particle Size Effects

The effect of particle size on conversion of hemicellulose to xylose then subsequently to furfural is shown in Figure 5-12 for basswood at 175°C and 0.5% sulfuric acid. The trends observed for duplicate trials for each particle size seen in the figure for basswood are representative of all species tested. As shown in Figure 5-12, by varying the particle size from the standard, 20-10 mesh, to a smaller one, > 28 mesh, there was no

change in the conversion of xylose formation and degradation. The reaction rates of formation and degradation were almost identical.

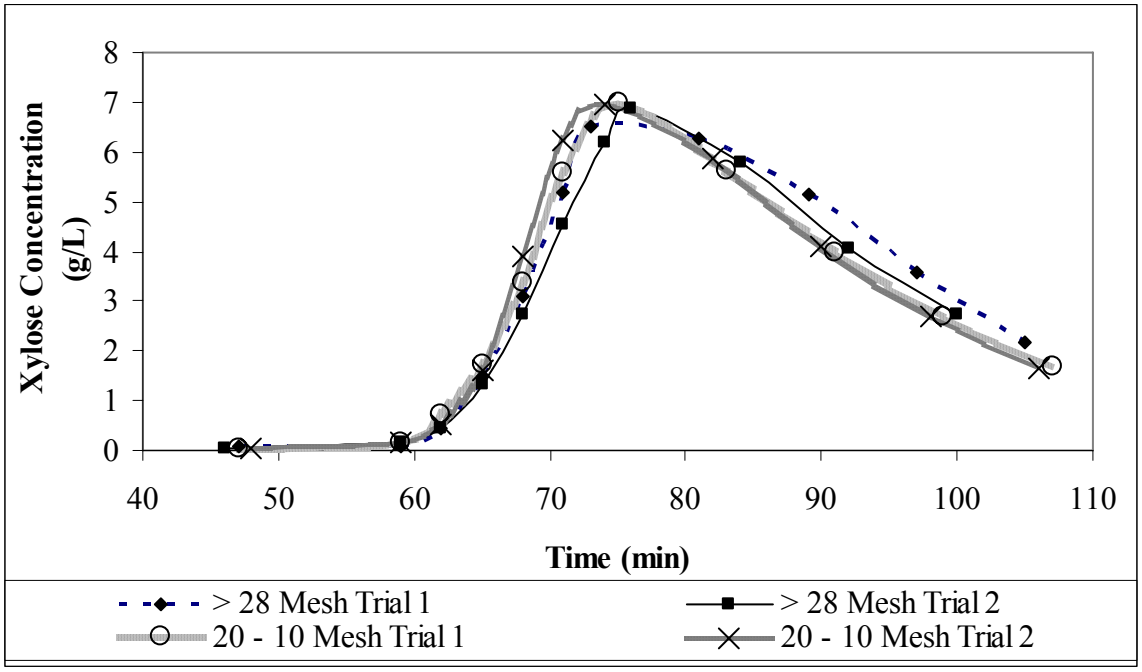

Figure 5-12: Basswood Acid Hydrolysis @ 0.5% H_2SO_4, 175°C (Particle Size Effects)

5.8 Xylose Formation Model

For each of the pretreatment experiments, eleven samples were collected from the reactor and analyzed using HPLC. The peak areas in chromatograms of each sample from the HPLC were then converted to sugar concentration based on calibration experiments discussed in Section 3.7.2. The xylose model was then fitted to the experimental data, as shown in Figure 5-13.

Figure 5-13 is a typical xylose formation modeling plot for all the biomass species and for all process conditions in this project. The xylose formation model was fitted to

the experimental data in order to determine the kinetic parameters of xylose formation. As discussed in Section 4.3, the sum of squared difference between the theoretical model and the experimental data was minimized by varying the pre-exponential factor and the activation energy to get the best fit. The solid line and the solid square were the predicted model and the experimental data, respectively.

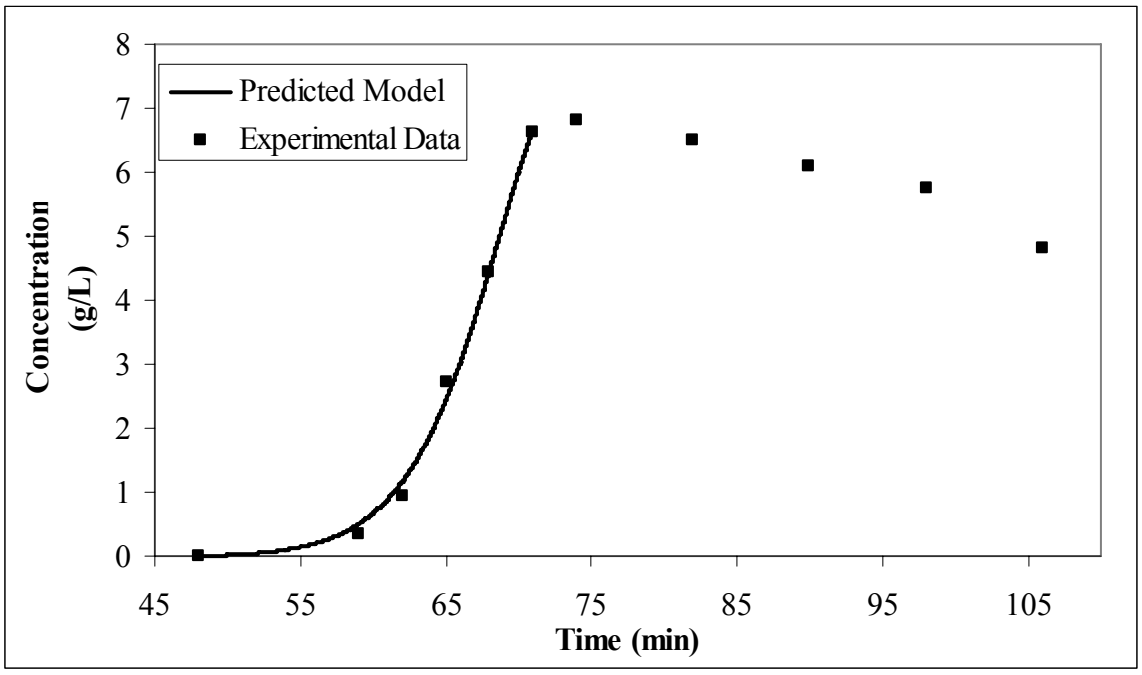

Figure 5-13: Aspen Acid Hydrolysis @ 0.5% H_2SO_4, 160°C, 20-10 Mesh (Comparison of Xylose Formation Model to Xylose Experimental Data)

However, the first data point was excluded in the switchgrass acid hydrolysis because the sum of squared difference was the lowest when the first data point was excluded and made the best fit to the experimental data. As the typical Figure 5-13 shows, the first data point was included in the theoretical model for the other four woody species.

Generally, the predicted model was fitted during the temperature ramp period up to the point when the maximum xylose concentration was achieved. Exceptions to this rule were encountered in this research project. For example, at lower acid concentration (0.25% H_2SO_4) or lower targeted temperature (160^oC), the xylose concentration would only reach the maximum after the temperature is at the steady state maximum. On the other hand, at higher acid concentration (1.0% H_2SO_4) or higher maximum temperature (190^oC), the xylose concentration reached a maximum earlier in the temperature ramp period.

The kinetic parameters of woody biomass pretreatment during xylose formation are summarized in the following Tables 5-3 to 5-7 at different experimental conditions. The pre-exponential factor ranged from $7.53x10^4$ min^{-1} – $2.63x10^{20}$ min^{-1} and the activation energy varied from 49 kJ/mol – 179 kJ/mol. As the acid concentration increased, the kinetic parameter values decreased. Moreover, as can be seen in the tables below, the kinetic parameters decreased as the maximum temperature increased. However, the kinetic parameters remained unchanged at the lower particle size compared with the standard, 20-10 mesh.

Table 5-3: Kinetic Parameters of Aspen during Xylose Formation at various Reactor Conditions

Acid Concentration (% w)	Particle Size	Kinetic Parameters	Temperature (^oC)			
			160	175	175	190
0.25	20-10	E_1 (kJ/mol)		151.85		
		A_1 (min^{-1})		$2.65x10^{17}$		
0.50	20-10	E_1 (kJ/mol)	140.70	134.70	136.10	148.60
		A_1 (min^{-1})	$2.80x10^{16}$	$5.47x10^{15}$	$7.55x10^{15}$	$1.88x10^{17}$
	> 28	E_1 (kJ/mol)		120.23	116.85	
		A_1 (min^{-1})		$4.85x10^{13}$	$1.90x10^{13}$	
1.00	20-10	E_1 (kJ/mol)		97.18		
		A_1 (min^{-1})		$1.53x10^{11}$		

Table 5-4: Kinetic Parameters of Balsam during Xylose Formation at various Reactor Conditions

Acid Concentration (% w)	Particle Size	Kinetic Parameters	Temperature (^oC)			
			160	175	175	190
0.25	20-10	E_1 (kJ/mol)		151.52		
		A_1 (min^{-1})		$2.78x10^{17}$		
0.50	20-10	E_1 (kJ/mol)	89.65	67.83	71.74	67.37
		A_1 (min^{-1})	$8.35x10^{9}$	$1.54x10^{7}$	$4.64x10^{7}$	$1.18x10^{7}$
	> 28	E_1 (kJ/mol)		58.12	74.40	
		A_1 (min^{-1})		$1.00x10^{6}$	$1.25x10^{8}$	
1.00	20-10	E_1 (kJ/mol)		48.72		
		A_1 (min^{-1})		$7.53x10^{4}$		

Table 5-5: Kinetic Parameters of Basswood during Xylose Formation at various Reactor Conditions

Acid Concentration (% w)	Particle Size	Kinetic Parameters	Temperature (^oC)			
			160	175	175	190
0.25	20-10	E_1 (kJ/mol)		179.13		
		A_1 (min^{-1})		$2.63x10^{20}$		
0.50	20-10	E_1 (kJ/mol)	154.36	126.27	134.44	117.36
		A_1 (min^{-1})	$7.58x10^{17}$	$2.42x10^{14}$	$2.58x10^{15}$	$1.94x10^{13}$
	> 28	E_1 (kJ/mol)		139.08	130.94	
		A_1 (min^{-1})		$6.63x10^{15}$	$7.55x10^{14}$	
1.00	20-10	E_1 (kJ/mol)		102.67		
		A_1 (min^{-1})		$4.46x10^{11}$		

Table 5-6: Kinetic Parameters of Red Maple during Xylose Formation at various Reactor Conditions

Acid Concentration (% w)	Particle Size	Kinetic Parameters	Temperature (^oC)			
			160	175	175	190
0.25	20-10	E_1 (kJ/mol)		149.45		
		A_1 (min^{-1})		$1.40x10^{17}$		
0.50	20-10	E_1 (kJ/mol)	145.76	104.07	88.65	88.16
		A_1 (min^{-1})	$1.11x10^{17}$	$6.42x10^{11}$	$5.77x10^{9}$	$6.63x10^{9}$
	> 28	E_1 (kJ/mol)		111.53	96.41	
		A_1 (min^{-1})		$5.56x10^{12}$	$5.86x10^{10}$	
1.00	20-10	E_1 (kJ/mol)		98.31		
		A_1 (min^{-1})		$2.03x10^{11}$		

Table 5-7: Kinetic Parameters of Switchgrass during Xylose Formation at various Reactor Conditions

Acid Concentration (% w)	Particle Size	Kinetic Parameters	Temperature (^{o}C)			
			160	175	175	190
0.25	20-10	E_1 (kJ/mol)		167.48		
		A_1 (min^{-1})		$1.03x10^{19}$		
0.50	20-10	E_1 (kJ/mol)	115.11	70.75	74.36	65.94
		A_1 (min^{-1})	$2.48x10^{13}$	$8.56x10^{7}$	$1.87x10^{8}$	$1.89x10^{7}$
	> 28	E_1 (kJ/mol)		101.29	111.07	
		A_1 (min^{-1})		$3.72x10^{11}$	$6.90x10^{12}$	
1.00	20-10	E_1 (kJ/mol)		78.24		
		A_1 (min^{-1})		$1.21x10^{9}$		

5.9 Xylose Degradation Model

The xylose degradation kinetics was calculated using the equations obtained from Section 4.4. Figure 5-14 illustrates the xylose degradation model with experimental data using Equation (4-20). The trend line was fitted to the degradation data of xylose at constant temperature. The equation on the chart is compared to Equation (4-20); therefore, the k_2 is 0.14 min^{-1} in this case.

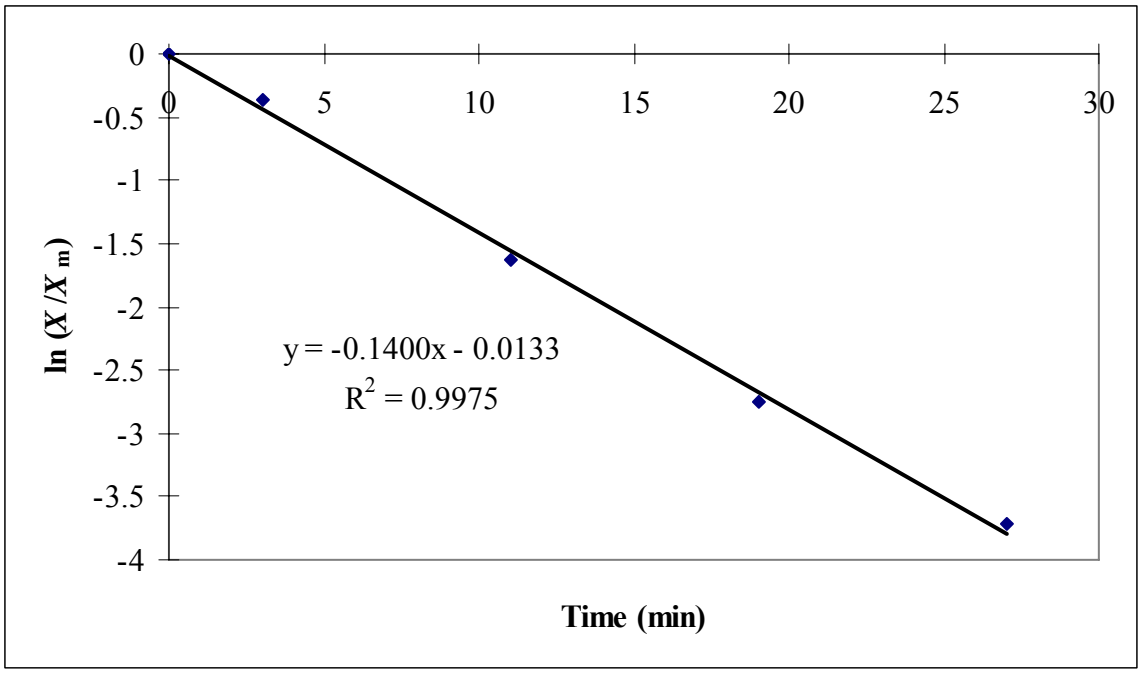

Figure 5-14: Red Maple Acid Hydrolysis @ 0.5% H_2SO_4, 190°C, 20-10 Mesh (Xylose Degradation Model)

By using the k_2 obtained at different temperature, a linear regression trend line could be drawn using Microsoft Excel. The equation shown in Figure 5-15 is then compared to Equation (4-22), where E_2/R = 15593 K or E_2 = 129.6 kJ/mol and ln (A_2) = 31.855 or $A_2 = 6.83 \times 10^{13}$ min^{-1}.

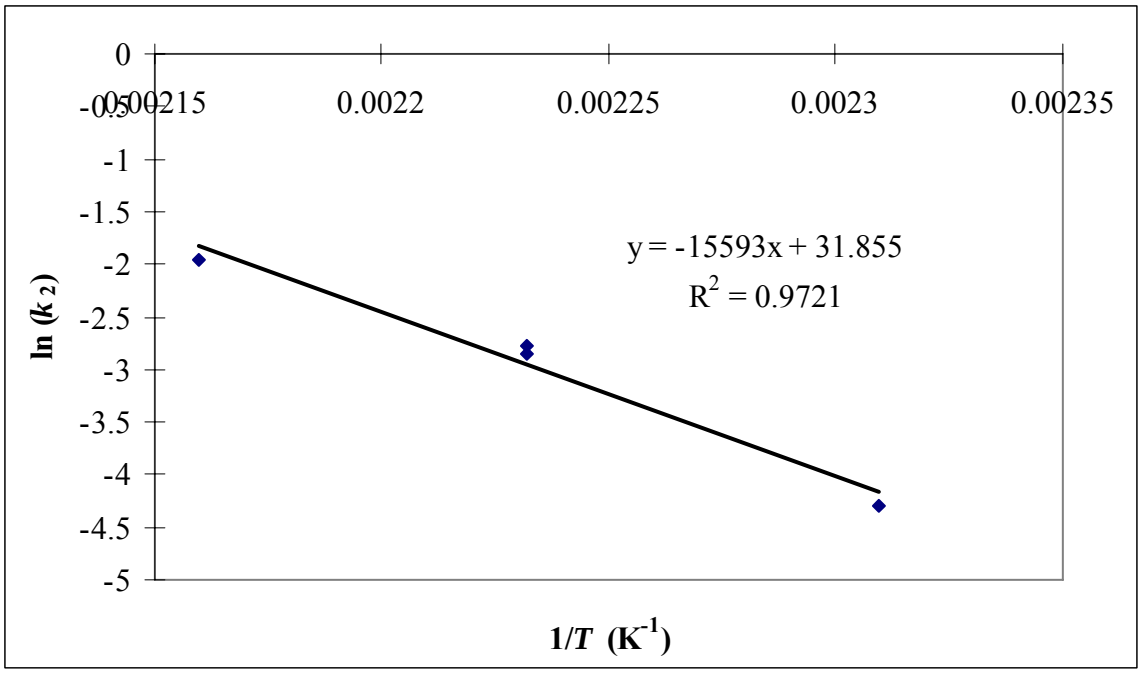

Figure 5-15: Red Maple Acid Hydrolysis Kinetic Parameters Determination of Xylose Degradation using Equation (4-22)

The third parameter of xylose degradation model, which is acid concentration exponents (m_2), could also be computed using the k_2 obtained from Figure 5-14 at different acid concentration conditions. The natural log-log plot of k_2 as a function of C for determining m_2 is shown in Figure 5-16, where the equation on the chart is comparable to Equation (4-25). The slope of the trend line is 1.023, which is m_2 in Equation (4-25), and the y-intercept is -2.228, which is represented as $\ln[A_{2o}\exp(-\frac{E_2}{RT_2})]$ in that equation.

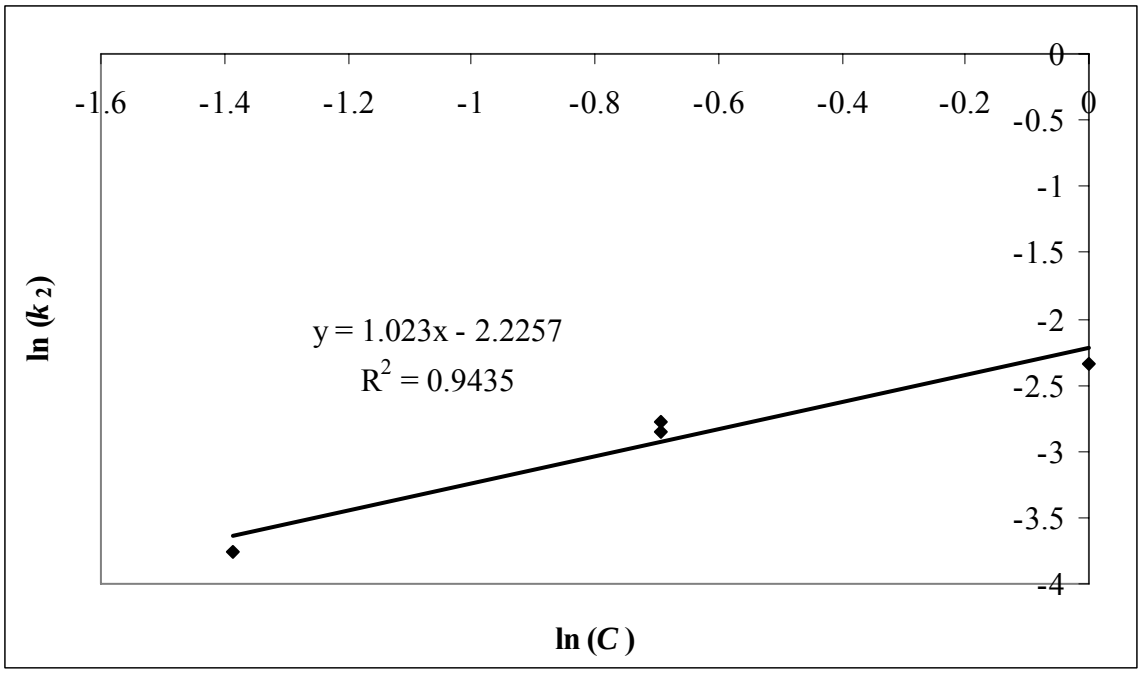

Figure 5-16: Red Maple Acid Hydrolysis Acid Concentration Exponent Determination of Xylose Degradation using Equation (4-25)

The kinetic parameters and the acid concentration exponents are summarized in Table 5-8. The pre-exponential factors and the activation energy were calculated based on the temperature effects at steady temperature and at the maximum concentration as the initial condition. By varying the temperature in this project, the A_2 and E_2 using Equations (4-20) – (4-22) were determined in the table below.

Table 5-8: Kinetic Parameters of Timber Varieties plus Switchgrass during Xylose Degradation

	Pre-exponential Factor, A_2 (min^{-1})	Acid Concentration Exponent, m_2	Activation Energy, E_2 (kJ/mol)
Aspen	$6.51x10^{16}$	1.0	155.36
Balsam	$6.83x10^{13}$	0.9	129.64
Basswood	$2.52x10^{13}$	1.2	126.89
Red Maple	$6.83x10^{13}$	1.0	129.64
Switchgrass	$3.73x10^{17}$	1.4	165.59

The kinetic parameters shown in Table 5-8 are comparable to literature values, as shown in the following chapter.

5.10 Overall Sugars Yield of Various Biomass Species

In this section, the yields of each sugar are reported for the various biomass species. All yields reported were normalized to the total potential sugars in the original untreated feedstock obtained from the total carbohydrate analysis in Table 5-2 to provide a perspective on the relative contribution of each sugar to total sugar recovery. Based on the original substrate composition of glucan, xylan, galactan, araban, and mannan and the appropriate change in mass due to the hydrolysis, the maximum potential recovery of sugars were calculated.

In the calculation of sugar yields, an accounting was made of the 13 mL taken out of the reactor for each sample according to the sampling procedure. The yield was calculated based on the sum of the total mass (monomer and oligomer) of each sugar generated from the drawn samples and of each sugar remaining in the reactor at their maximum concentration. This sum of each sugar was divided by the mass of total potential sugar in the untreated species.

Tables 5-9 to 5-13 show the overall sugars yield of each woody species in the different experimental runs. The overall xylose yield was the major interest in this project. Average xylose yield for each species varied from about 70% for balsam to 92% for switchgrass. No clear trend can be seen for xylose yield under different process conditions. The glucose yield varied from only 10.6% to 12.6% for all species, showing that dilute acid pretreatment is ineffective at hydrolyzing cellulose. Yields of the minor sugars were generally below 50% and often less than 20%. One notable exception was a high yield for mannose from balsam, which has a high percentage of mannose in biomass.

The amount of lignin content in the balsam may affect the overall xylose yield. Table 5-10 shows the high lignin content (balsam) may become the blockage for acid to penetrate cellulose and hemicellulose component. As a result the total potential xylose production might be reduced. However, the low lignin content (switchgrass) has highest gross xylose yield.

Table 5-9: Glucose yield of various biomass species at each reactor condition

% w/w Acid Conc.	Setpoint Temp. (°C)	Particle Size (mesh)	% Overall Glucose Yield				
			Aspen	Balsam	Basswood	Red Maple	Switchgrass
0.5	175	20-10	14.6	10.1	15.2	13.9	11.9
0.5	175	20-10	12.5	8.9	14.5	11.5	12.2
0.5	160	20-10	6.3	7.8	6.4	6.9	9.3
0.5	190	20-10	15.2	14.5	19.0	16.1	17.2
0.25	175	20-10	9.8	7.3	8.7	9.2	8.2
1.0	175	20-10	17.6	13.4	20.6	22.6	18.5
0.5	175	> 28	12.5	11.9	13.7	19.1	12.1
0.5	175	> 28	12.3	10.6	10.8	15.2	10.8
Average			**12.6**	**10.6**	**13.6**	**14.3**	**12.5**
Standard Deviation			**3.45**	**2.58**	**4.86**	**5.14**	**3.59**

Table 5-10: Xylose yield of various biomass species at each reactor condition

% w/w Acid Conc.	Setpoint Temp. (°C)	Particle Size (mesh)	% Overall Xylose Yield				
			Aspen	Balsam	Basswood	Red Maple	Switchgrass
0.5	175	20-10	92.6	64.2	75.7	84.1	87.6
0.5	175	20-10	93.4	76.9	80.2	69.8	85.8
0.5	160	20-10	82.4	77.8	77.5	74.2	95.9
0.5	190	20-10	79.2	74.9	75.1	78.6	98.6
0.25	175	20-10	79.4	61.5	82.9	81.9	89.6
1.0	175	20-10	87.0	63.9	78.7	73.8	97.7
0.5	175	> 28	72.9	67.6	75.8	96.6	90.6
0.5	175	> 28	88.8	73.9	82.0	82.8	93.7
Average			**84.5**	**70.1**	**78.5**	**80.2**	**92.4**
Standard Deviation			**7.20**	**6.51**	**2.98**	**8.29**	**4.75**

Table 5-11: Galactose yield of various biomass species at each reactor condition

% w/w Acid Conc.	Setpoint Temp. (°C)	Particle Size (mesh)	% Overall Galactose Yield				
			Aspen	Balsam	Basswood	Red Maple	Switchgrass
0.5	175	20-10	30.0	36.9	39.8	22.2	27.9
0.5	175	20-10	33.8	35.8	51.2	18.5	24.1
0.5	160	20-10	29.2	42.2	50.4	18.8	35.5
0.5	190	20-10	26.6	38.6	64.5	19.5	45.2
0.25	175	20-10	29.8	32.7	98.0	22.6	50.7
1.0	175	20-10	28.1	33.4	82.8	22.2	26.1
0.5	175	> 28	25.7	31.6	35.6	49.8	29.1
0.5	175	> 28	31.6	29.5	28.9	25.0	46.7
Average			**29.4**	**35.1**	**56.4**	**24.8**	**35.7**
Standard Deviation			**2.62**	**4.12**	**23.97**	**10.33**	**10.47**

Table 5-12: Arabinose yield of various biomass species at each reactor condition

% w/w Acid Conc.	Setpoint Temp. (°C)	Particle Size (mesh)	% Overall Arabinose Yield				
			Aspen	Balsam	Basswood	Red Maple	Switchgrass
0.5	175	20-10	23.9	16.1	18.5	24.2	34.1
0.5	175	20-10	23.1	18.2	17.6	23.0	34.2
0.5	160	20-10	20.1	18.5	19.1	25.0	34.9
0.5	190	20-10	21.7	16.2	19.1	23.5	32.6
0.25	175	20-10	21.9	19.8	20.7	24.4	29.4
1.0	175	20-10	23.4	17.5	17.6	24.1	36.4
0.5	175	> 28	29.2	18.9	9.2	45.0	41.6
0.5	175	> 28	32.0	20.5	10.8	25.7	42.2
Average			**24.4**	**18.2**	**16.6**	**26.9**	**35.7**
Standard Deviation			**4.07**	**1.57**	**4.20**	**7.38**	**4.35**

Table 5-13: Mannose yield of various biomass species at each reactor condition

% w/w Acid Conc.	Setpoint Temp. (°C)	Particle Size (mesh)	% Overall Mannose Yield				
			Aspen	Balsam	Basswood	Red Maple	Switchgrass
0.5	175	20-10	32.3	54.3	34.4	25.4	9.8
0.5	175	20-10	39.9	63.6	52.2	24.5	9.0
0.5	160	20-10	33.8	61.4	39.9	22.9	9.5
0.5	190	20-10	27.8	63.6	41.0	26.8	8.6
0.25	175	20-10	42.0	55.2	42.2	28.4	9.3
1.0	175	20-10	24.6	58.2	45.3	22.9	9.4
0.5	175	> 28	31.7	58.4	28.4	47.8	6.5
0.5	175	> 28	33.0	56.7	26.8	22.4	7.0
Average			**33.1**	**58.9**	**38.8**	**27.6**	**8.6**
Standard Deviation			**5.72**	**3.60**	**8.54**	**8.41**	**1.22**

Chapter 6 Discussion and Comparison to Literature Results

One of the reasons for this section is to compare results from this research to the literature. A second purpose is to discuss assumptions made in the research and the importance of these assumptions.

The main approach of the proposed work was focused on developing the kinetic model of timber species from the Upper Peninsula of Michigan plus switchgrass using dilute acid pretreatment. Four timber species, aspen, balsam, basswood, and red maple, plus switchgrass were investigated. The derived kinetic model was employed to predicting xylose concentration as a function of time throughout the experiment. The model was fitted to the experimental data in order to determine the relevant kinetic parameters. Once kinetic parameters were obtained, it is then possible to use these parameters to predict pretreatment hydrolysis performance for various reactor configurations. Modeling reactor performance will allow for the determination of optimum reactor conditions to maximize production of fermentable sugars and to minimize degradation of sugars to non-fermentable and inhibitory by-products.

The results shown in Table 6-1 are a comparison of biomass composition measured in this study to literature results for the same biomass species. Measured glucan and xylan in this study are generally similar to literature values, with the exception of switchgrass whose glucan value is higher in this study. Lignin content measured in this study for all species is generally higher than in the literature studies reported on here. The minor sugars of galactan, araban, and mannan measured in this study were comparable to literature values.

Table 6-1: Compositional Analysis of Raw Biomass Samples (percent by weight)

Species	% Biomass Composition						References
	Glucan	Xylan	Galactan	Araban	Mannan	Lignin	
Aspen	57.3	16.0	0.8	0.4	2.3	16.3	Brooks et al, 1978
Balsam	46.8	4.8	1.0	0.5	12.4	29.4	
Red Maple	46.6	17.3	0.6	0.5	3.5	24.0	
Switchgrass	32.2	20.3	-	3.7	0.4	19.5	Esteghlalian et al, 1997
Aspen	52.43	14.60	3.52	2.41	5.32	26.69	In this project
Balsam	47.09	6.23	5.45	5.41	11.49	36.04	
Basswood	43.99	15.31	3.41	3.49	2.91	28.44	
Red Maple	43.18	17.69	5.71	4.13	5.37	36.49	
Switchgrass	47.72	19.06	4.18	8.11	6.30	26.04	

After carbohydrate analysis, a series of eight dilute acid pretreatment experiments were conducted under transient temperature conditions for each species. The pretreatment experiments conducted in this research were conducted differently than most studies reported in the literature. In most of the studies reported in the literature, the acid was injected after the sample was heated to the desired temperature (Lloyd et al, 2005; Esteghlalian et al, 1997; Garrote et al, 2001), or the acidified solution was heated quickly from room temperature to the temperature maximum by plunging the reactor into a hot sand bath. Unlike in this research, where small samples were collected as a function of time, studies in the literature collected a sample at the end of each experiment.

In the research reported here the acid was added prior to heating up the reactor, as described in Section 3.6. Notwithstanding these procedural differences, according to the trends reported in Garrote et al, 2001, the trends produced in this project were similar.

Table 6-2 summarizes some of the kinetic parameters of dilute acid pretreatment of other feedstocks from studies found in the literature. These data are comparable with those found in the study reported in this thesis.

The acid concentration exponent for xylose formation is taken in this study to be 1.75, an often quoted literature value, as the methods used in this study were unable to determine its value using either the Microsoft Solver or the Runge-Kutta method described in other studies (Bhandari et al, 1984). The acid concentration exponent of xylose degradation, however, was calculated as described in Chapter 4. The acid concentration exponents in another study were essentially equal to 1 for formation and degradation (McMillan, 1994).

Table 6-2: Kinetic parameters of biomass dilute acid hydrolysis

Materials	Xylose Formation			Xylose Degradation			References
	A_1 (min^{-1})	m_1	E_1 (kJ/mol)	A_2 (min^{-1})	m_2	E_2 (kJ/mol)	
Wheat Straw	$2.25x10^{20}$	1.55	167.0	$1.52x10^{15}$	2.0	141.0	Ranganathan et al, 1985
Switchgrass	$1.9x10^{21}$	0.4	169.0	$3.8x10^{10}$	1.45	99.5	Esteghlalian et al, 1997
Poplar	$3.3x10^{21}$	0.4	176.7	$8.5x10^{10}$	0.55	102.0	
Corn Stover	$6.7x10^{16}$	1.5	129.8	$3.7x10^{10}$	0.5	98.4	
Paper Birch	$2.67x10^{16}$	1.0	126.6	-	-	-	Maloney et al, 1985
Southern Red Oak	$1.04x10^{14}$	1.54	120.1	-	-	-	Kim & Lee, 1987
Aspen	$1.53x10^{11}$ ~ $2.65x10^{17}$	1.75	97.18 ~ 151.85	$6.51x10^{16}$	0.99	155.36	In this project
Balsam	$7.53x10^{4}$ ~ $2.78x10^{17}$	1.75	48.72 ~ 151.52	$6.83x10^{13}$	0.88	129.64	
Basswood	$4.46x10^{11}$ ~ $2.63x10^{20}$	1.75	102.67 ~ 179.13	$2.52x10^{13}$	1.23	126.89	
Red Maple	$5.77x10^{9}$ ~ $1.40x10^{17}$	1.75	88.65 ~ 149.45	$6.83x10^{13}$	1.02	129.64	
Switchgrass	$1.89x10^{7}$ ~ $1.03x10^{19}$	1.75	65.94 ~ 167.48	$3.73x10^{17}$	1.43	165.59	

Most kinetic parameters obtained from other studies, except Ranganathan et al, 1985, were determined using the isothermal method with constant volume, in which the acid was only added while the temperature was constant. In this project, however, a non-isothermal method was used. The acid was added at room temperature prior to starting the pretreatment reaction and the reaction occurred during the heating up period. The samples were collected over time as described in Chapter 3. Although the volume of liquid in the reactor was changing with time due to sampling, a constant volume kinetic model was employed in this project. The volume of each sample (13 mL) drawn out from the reactor was reduced to a minimum that would yield an accurate sampling of reactor contents. About 100 mL of samples was removed from the reactor during the period of time that the kinetic model was fit to the data out of 500 mL of initial volume in the reactor. This is approximately a loss of initial liquid volume equal to 20%. In this research the assumption was made that this relatively small loss of reactor liquid volume would introduce only a small error in the calculated kinetic parameters.

The yield of each sugar for each biomass species was also analyzed as described in Section 5.10. Table 6-3 summarized the xylose monomer yields from hemicellulose hydrolysis by dilute acid pretreatment in some literature studies. Yield data for dilute acid pretreatment was consistent in this project compared with those reported in the literature.

Table 6-3: Results reported in the literature for pretreatment of corn residues

Feedstocks	% w Acid	Temperature (^{o}C)	Time (min)	% Xylose Monomer Yield	References
Corn Stover	0.49	200	32[a]	62.5	Bhandari et al, 1984
Corn Stover	0.92	182	36[a]	78.1	Bhandari et al, 1984
Corn Stover	1.47	155	31[a]	78.7	Bhandari et al, 1984
Corn Stover	0.92	160	5-10	93.0	Torget et al, 1991
Corn Stover	1.2	180	0.5-1	84.0	Esteghlalian et al, 1997
Corn cob/corn Stover	1.22	140	40	90.0	Lee et al, 1994
Corn Stover	1.0	190	1.5	95.0	Tucker et al, 2003
Corn Stover	1.35	191	0.75-1.0	77.0	Schell et al, 2003
Corn Stover	0.22	160	40	27.6	Lloyd et al, 2005 (CAFI Study)
Corn Stover	0.22	180	10	39.5	
Corn Stover	0.22	200	5	45.8	
Corn Stover	0.49	140	80	74.7	
Corn Stover	0.49	160	20	82.1	
Corn Stover	0.49	180	5	78.4	
Corn Stover	0.98	140	40	77.4	
Corn Stover	0.98	160	5	67.4	
Corn Stover	0.98	180	2	77.9	

[a] Target temperature. Non-isothermal heat up from room temperature to target over time period.

Xylose yields reported in the literature for dilute acid pretreatment increased and then dropped with increasing time, while the smaller glucose yields continually increased with pretreatment time. Similar trends were also observed in the research reported in this thesis. Higher temperature and acid concentration accelerated the rate of xylan solubilization relative to xylose degradation, resulting in higher maximum yields (Lloyd et al, 2005). As a result, pretreatment times required for best sugar yields depended on the condition in the reactor.

Overall, the results of this study showed up to 92% of the xylose in switchgrass was recovered during the pretreatment. It is comparable to the results obtained from the literature shown in Table 6-3 for corn stover pretreatment. In general, the yield of fermentable sugars is the most important factor in commercial-scale biomass to ethanol

conversion processes. Research to improve pretreatment processes must therefore focus on minimizing, and preferably eliminating, degradation of the monomer sugars. A significant extent of dilute acid-based processes requiring the use of high temperature has already been developed. However, it might not be attractive in the long term because the high-temperature formation of degradation products reduces yields and increases energy cost (McMillan et al, 1994). As a result, acid catalysis enables processes to be carried out at lower temperatures or using lower residence times, which reduce carbohydrate degradation.

Mixtures of biomass species may be common for commercial production of ethanol from woody biomass. The results in this research on single biomass species can provide insights as to how mixtures of biomass species might respond to dilute acid hydrolysis. Figure 6-1 shows the summary of all species reached maximum xylose concentration under the standard experimental condition.

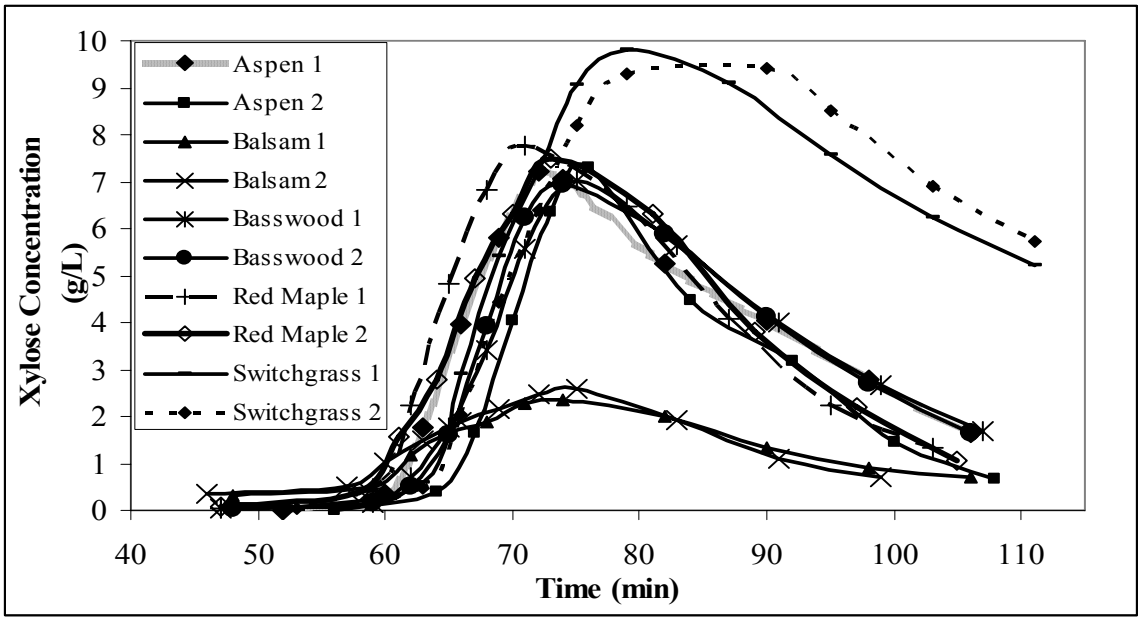

Figure 6-1: Summary of All Biomass Species Acid Hydrolysis at 0.5% H_2SO_4, 175°C, 20-10 mesh (Xylose Concentration)

The switchgrass results achieve xylose concentration maximum at a slightly later time than the wood species samples. The heating rate of the reactor was slower from room temperature to 100^{o}C using the switchgrass as feedstock; however, the amount of time from 100^{o}C to the maximum targeted temperature (175^{o}C) was identical to other species.

Because the reactor residence time for reaching maximum xylose concentration in each reaction was essentially identical, as shown in Figure 6-1, mixtures of two or more biomass species should be feasible from the point of view of maximizing yields. However, as shown in Table 5-10, the high lignin content softwood may reduce the overall xylose yield. Therefore, mixture of softwoods and hardwoods / herbaceous crops may show lower xylose yield as the lignin may become the blockage for the acid.

Figure 6-2 shows the average total mass of sugars produced from the 25g of dry biomass for the various reaction conditions. The mass was calculated based on the sum of the total mass (monomer and oligomer) of each sugar generated from the drawn samples and of each sugar remaining in the reactor at their maximum concentration (occurred at the same time as xylose). As shown in Figure 6-2, the switchgrass produced the highest mass (8 grams) of sugars during the dilute acid pretreatment. However, the softwood (balsam) produced only 5 grams of total sugars during pretreatment.

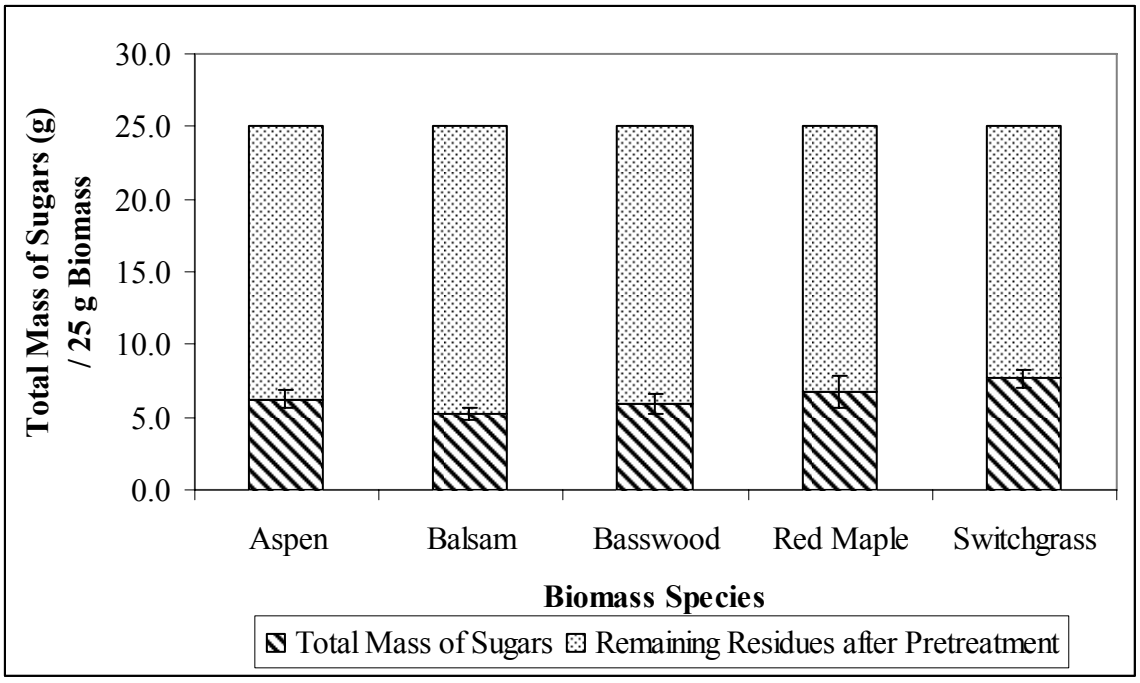

Figure 6-2: The total mass of sugars produced for each biomass species at various reaction conditions

Additionally, the oligomers generated in the reaction might affect the overall performance of a biomass to ethanol process because oligomers are not normally taken up and metabolized by fermenting organisms. It was indicated in Section 5.6 that higher acid concentration could be used to reduce the amount of oligomers produced. However, higher amount of furfural and other high molecular tars are formed in this case. Future studies focused on oligomer are required.

As mentioned in Chapter 2, the main purpose of pretreatment is to separate the hemicellulose component from the biomass, and subsequently increase the efficiency of enzymatic hydrolysis. Therefore, it is critical to the subsequent processes in the production of ethanol from woody biomass. Generally, as the severity of the reaction of pretreatment increases, the yields of xylose and glucose are higher. However, without good control of the reaction residence time, byproducts, such as furfural and high

molecular weight tars, are formed. It is important because the byproducts may act as inhibitors to reduce the rate of the ethanol fermentation. As can be seen in Chapter 5, the effects of temperature and acid concentration were studied. At lower temperature or lower acid concentration, the rate of generation of furfural was slower and easier to control; however, higher temperature or higher acid concentration degraded xylose to nearly zero within 10 minutes. Studies on severity of the reaction may be investigated in the future.

Chapter 7 Conclusions

Among all the pretreatment techniques presented in Chapter 2, the dilute acid catalyzed pretreatment was chosen in this project to characterize pretreatment of woody species from the Upper Peninsula of Michigan plus switchgrass because this technology is a part of the NREL baseline technology and because there is an extensive literature for comparison of results. The main advantage of this process is that significantly higher yield of xylose can be obtained compared to other technologies. The dilute acid catalyzed pretreatment at moderate temperatures effectively hydrolyzed hemicellulose as dissolved sugars.

7.1 Effects of Reaction Conditions

The kinetic coefficient governing the generation of xylose from hemicellulose and the generation of furfural from xylose presented a coherent dependence on both temperature and acid concentration. However, no effect was observed in the particle size. The results indicated that by using a moderate concentration of sulfuric acid (0.5%) at higher temperature (190^{o}C), the rate of generation of xylose was identical to the lower temperatures (160 and 175^{o}C). This is due to the identical temperature versus time behavior of the reactor during heat up. However, the rate of generation of furfural was increased for the higher target temperature. Also, for 160^{o}C trials, the rate of change of xylose concentration during the time of peak concentration was smaller than for higher temperature trials, suggesting that control of reactor to achieve maximum yield would be easier.

The acid concentration results, however, affected both the xylose formation and degradation. At the highest acid concentration (1.0 %), the rate of furfural generation was highest when achieving the maximum reactor temperature (175^{o}C).

7.2 Kinetic Parameters

The activation energy of xylose formation in balsam was found to be the lowest among all other species in this project. This can be attributed to the difference in chemical composition of hemicellulose in woody and herbaceous materials. Table 6-2 summarizes the range of literature kinetic parameters in each condition of feedstock. These data are comparable with those found in this study. A direct comparison, however, cannot be made because of the difference in reaction. The kinetic parameters of xylose degradation are presented in Table 6-2. The rate of xylose conversion is thought to be substrate independent. The results of this study also indicate some similarities to other studies. Activation energies, pre-exponential factors, and the acid concentration exponents are essentially very close.

7.3 Summary

The maximum sugar yields resulting from pretreatment at different reaction conditions are presented in Tables 5-9 through 5-13. The xylose yields from switchgrass were the highest among the species tested for all reaction conditions. This may due to the lowest lignin content in the biomass, affording better access ot the acid catalyst to the hemicellulose. The glucose yields of each species were consistently low at about 10 ~ 15%. In comparison, the minor sugars yields are more variable. The average xylose yield from balsam is lower compared to those resulting from other species using similar

conditions. This might be in part due to the relatively high lignin content in balsam, which might have restricted access to hemicellulose in balsam compared to other species having lower lignin content.

Chapter 8 Recommendations

This research work mainly focused on the hydrolysis of biomass into soluble sugar monomers and oligomers and a solid residue mainly made up of cellulose and lignin. The kinetics of xylose formation and xylose degradation have been satisfactorily modeled on the basis of consecutive, irreversible, first-order reactions leading to xylose and furfural, the main decomposition product. The kinetic coefficients governing the generation of xylose from hemicellulose and the generation of furfural from xylose presented a dependence on both temperature and acid concentration as shown in Chapter 5. This project could be enhanced with future work in the following areas.

1. Recently, most research has focused on combining the pretreatment techniques, such as dilute acid steam explosion and acid-alkali pretreatment. However, only single biomass species have been investigated in pretreatment studies. This study suggests that mixtures of biomass species might be processed together and still achieve maximum yields for each species. However, mixture effects (interactions that might affect species reactivities) must be investigated to confirm this hypothesis. Otherwise, the sugars from one species may form earlier than another and degrade while the sugars from second species are still formed.
2. The kinetic model of dilute acid pretreatment in this project is assumed to be constant volume. However, as the samples were collected over time, the volume of the solution was decreased. Therefore, in order to increase the level of confidence in the predicted kinetic parameters, a larger reactor volume should be used. There are a couple advantages of using larger size of reactor; 1) Minimize the relative amount of samples draw from the reactor, and 2) larger particle size of

biomass species can be investigated. For instance, if a 10-liter reactor is chosen, the 100 mL of samples drawn out from the reactor in this project will be relatively small, which is only 1% of total solution. In addition, particle sizes larger than 10 mesh can be investigated in the larger reactor.

3. As discussed in Chapter 6, the sugar oligomers might reduce the efficiency of the subsequent processes for ethanol production. Therefore, in this case, extra steps are required to convert the oligomers prior enzymatic hydrolysis, such as 1) using higher acid concentration during the pretreatment step; or 2) extra enzymatic hydrolysis using genetically modified enzyme that would consume oligomers. By using higher acid concentration in the reaction medium, the xylose oligomer concentration was reduced roughly 50%, as mentioned in Section 5.6. Further studies are needed on reactor conditions needed to minimize oligomer concentrations.

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Appendix A: Job Safety Assessment Forms

Appendix A-1: JSA of Determination of Structural Carbohydrates and Lignin in Biomass

Equipment Name: Determination of Structural Carbohydrates and Lignin in Biomass
JSA Author: Shu Chiang Yat
Room Number/Building: 205 Chemical Engineering
Faculty Supervisor: Dr. David Shonnard
Revision #: 1
Revision Date: 3/2/2006

Purpose of Experiment / Equipment: Briefly describe what this experiment is designed to achieve and the types of data collected.

The carbohydrates and lignin are the major components in the biomass samples. The contents of biomass must be measured as part of the comprehensive biomass analysis. This procedure uses two-step acid hydrolysis to fractionate the biomass into forms that are more easily quantified. In this experiment, we will be able to exam the amount of glucose, xylose, and other sugars in the biomass samples and compare with the NIST QA standard. The sugars will be measured using HPLC.

Personal Protective Equipment (PPE) – Check all PPE worn during the entire experiment. Do not list these in the procedure section.

☒ Long Pants	☒ Safety Glasses	☐ Hard Hat	☒ Apron
☐ Long Sleeves	☐ Splash Goggles	☒ Insulated Gloves	☐ Ear Protection
☒ Non-porous Shoes	☐ Face Shield	☒ Chemical Gloves	☐ Other:

Hazard Summary – Check all general hazards that are likely to be encountered during this experiment and list the major source of the hazard.

Hazard	Major Source of Hazard
☐ Toxicity	
☐ Fire/Flammability	
☒ Reactivity	72 % w/w Sulfuric Acid
☐ Pressure Hazard	
☒ Electrical Shock	Power Supply
☐ Mechanical Hazard	
☒ Hot Surfaces/ High Temp	Muffle Furnace 575 C

> 150 F	
☐ Biohazard	
☐ Laser Radiation	
☐ Ionizing radiation	
☐ Other: _____	
☐ Other: _____	

Expected Operating Conditions –

Temperature	Pressure
Normal: 25 C	Normal: Ambient pressure
Minimum: Room Temperature	Minimum: Ambient pressure
Maximum: 575 C	Maximum: Ambient pressure

Special Operating Conditions - Check all that apply and consult department Safety Manual.

Unattended Operation: ☐	Drying Oven: ☒
Regulated Chemicals: ☐	Class 3b or 4 Lasers: ☐
Pressures Exceeding 35 atm (515 psia) or Equipment Specifications: ☐	
Temperatures Exceeding 1000^{o}C or Equipment Specifications: ☐	

Available Safety Equipment – Provide the location of each item shown below. Show the location of this equipment on the attached floor plan. If not available, type "NA" in the field.

Item	Location
Fire Extinguisher:	East wall near door (Right hand side when enter the room)
Eyewash:	East wall near door (Right hand side when enter the room)
Safety Shower:	East wall near door (Right hand side when enter the room)
Telephone:	Northeast corner of the room
First Aid Kit:	East wall near door (Right hand side when enter the room)
Other:	
Other:	

Spill Response Supplies - Provide the location of each item shown below. Show the location of this equipment on the attached floor plan. If not available, type "NA" in the field.

Item	Location
Spill Kit:	Northwest of the room, the bottom of classware cabinet
Floor-Dri:	Northwest of the room, the bottom of classware cabinet
Spill Dikes:	Northwest of the room, the bottom of classware cabinet
Sodium Bicarbonate:	Northwest of the room, the bottom of classware cabinet
Drain Plugs:	N/A
Spill Pillows:	N/A
Mercury Spill Kit:	N/A
Other:	

Other:	

Required Attachments:

☐ Diagram of process or equipment Label all valves and identify all equipment for reference in procedure.
☒ Laboratory Floor Plan Identify the location of your experiment and all safety and spill response equipment.
☐ Equipment Specifications Include materials of construction, maximum temperature and pressure, standard operating values, and any other specifications important to the safe operation.
☒ Material Safety Data Sheets (MSDS) Include for all reactants, products and any intermediate or other chemicals which may occur.

Additional Attachments: As necessary.

☒ Evacuation Route	☐
☒ Procedure attached	☐

Chemical Information Page

Fill in as much data below as available. If data are not available, leave the field blank.
List all chemicals, including reactants, products, intermediates, solvents, and any others used.

Chemical Properties and Hazards

Chemical Name	Physical State S, L, G	NFPA Ratings*				Incompatible Chemicals List chemicals present within the laboratory, and any others that may come in contact.	Flash Point Temp.	Flammability Limits	
		H	F	S	Sp.			LFL	UFL
72% w/w Sulfuric Acid	L	3	0	0	WR	Water, potassium chlorate, potassium perchlorate, potassium permanganate, sodium, lithium, bases, organic material, halogens, metal acetylides, oxides and hydrides, metals (yields hydrogen gas), strong oxidizing and reducing agents and many other reactive substances	N/A	N/A	N/A
Calcium Carbanate	S	2	0	0	E	Reactive with oxidizing agents, acids.	N/A	N/A	N/A

*NFPA Ratings: **H** – Health, **F** – Flammability, **S** – Stability, **Sp.** – Special

Chemical Toxicology, Regulation and Disposal: List the same chemicals that appear above, in the same order.

Chemical Name	Toxicology			Hazardous Waste Number[#]	Regulated? See Safety Manual	Personal Protective Equipment Specific to this Chemical
	TWA	PEL	Other			
72% w/w Sulfuric Acid	1 mg/m^3	1 mg/m^3	N/A	N/A	☐	Eyes: Wear protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166 Skin: Wear long sleeve Clothing: Wear apron and long sleeve to prevent skin exposure
Calcium Carbanate	10 mg/m^3	5 mg/m^3	N/A	N/A	☐	Splash goggles. Lab coat. Dust respirator. Be sure to use an approved/certified respirator or equivalent gloves.
					☐	
					☐	
					☐	
					☐	
					☐	
					☐	

[#]**See Chemical Engineering Hazardous Waste Manual.**

Chemical Reactions: Provide details below on any chemical reaction(s) that occur in your process. Please show the species involved, the stoichiometry and the heat of reaction, if available. Also list side reactions and any other reactions that may impact safety. You cannot type subscripts in the form field provided – use the names for the species and the stoichiometric coefficients.

Acid catalyzed hydrolysis of wood yielding soluble sugars, mostly glucose, xylose, and minor amounts of other 5 and 6 carbon sugars.

Job Safety Assessment Form
Safe Operating Procedures Page

Sequence of Steps	Potential Hazards	Procedure to Control Hazard	PPE or Equipment Required
Emergency Shutdown			
1. Turn off main power supply on Parr Reactor	Electric shock/explosion	Use left hand rule	Wear neoprene or rubber gloves, google, full protective clothing, and non-porous shoes
Start-up Procedure			
A) Prepare the sample for analysis and hydrolyze 1. Place an appropriate number of filtering crucibles in the muffle furnace at 575°C for a minimum of four hours. Remove the crucibles from the furnace directly into a desiccator and cool for a specific period of time, one hour is recommended. Weigh the crucibles to the nearest 0.1 mg and record this weight. It is important to keep the crucibles in a specified order, if they are not marked with identifiers. Permanent marking decals are available from Wale Apparatus. Do not mark the bottom of the filtering crucible with a porcelain marker, as this will impede filtration. 2. Place the sample back into the muffle furnace at 575 C and ash to constant weight. Constant weight	Hot surface Hot surface	Handle with care Handle with care	Wear neoprene or rubber gloves, google, full protective clothing, and non-porous shoes

is defined as less than ± 0.3 mg change in the weight upon one hour of re-heating the crucible. 3. Weigh 300.0 mg of the sample or QA standard into a tared pressure tube. Record the weight to the nearest 0.1 mg. Label the pressure tube with a permanent marker. LAP "Determination of Total Solids in Biomass" should be performed at the same time, to accurately measure the percent solids for correction. Each sample should be analyzed in duplicate, at minimum. The recommended batch size is three to six samples and a QA standard, all run in duplicate. 4. Add 3.00 mL (or 4.92 g) of 72% sulfuric acid to each pressure tube. Use a Teflon stir rod to mix for one minute, or until the sample is thoroughly mixed. 5. Place the pressure tube in a water bath set at 30°C and incubate the sample for 60 minutes. Using the stir rod, stir the sample every five to ten minutes without removing the sample from the bath. Stirring is essential to ensure even acid to particle contact and uniform hydrolysis. 6. Upon completion of the 60-minute hydrolysis, remove the tubes from the water bath. Dilute the acid to a 4% concentration by adding 84.00 mL deionized water using an automatic burette. Dilution can also be done by adding 84.00 g of purified water using a balance accurate to 0.01 g. Screw the Teflon caps on securely. 7. Prepare a set of sugar recovery standards (SRS) that will be taken through the remaining hydrolysis	Chemical spill Chemical spill Chemical Spill	Spill kit in the lab Safety shower NE corner of the lab Spill kit in the lab Safety shower NE corner of the lab Spill kit in the lab Safety shower NE corner of the lab	

and used to correct for losses due to destruction of sugars during dilute acid hydrolysis. SRS should include D-(+)glucose, D-(+)xylose, D-(+)galactose, -L(+)arabinose, and D-(+)mannose. SRS sugar concentrations should be chosen to most closely resemble the concentrations of sugars in the test sample. Weigh out the required amounts of each sugar, to the nearest 0.1 mg, and add 10.0 mL deionized water. Add 348 µL of 72% sulfuric acid. Transfer the SRS to a pressure tube and cap tightly. 7.1 A fresh SRS is not required for every analysis. A large batch of sugar recovery standards may be produced, filtered through 0.2 µm filters, dispensed in 10.0 mL aliquots into sealed containers, and labeled. They may be stored in a freezer and removed when needed. Thaw and vortex the frozen SRS prior to use. If frozen SRS are used, the appropriate amount of acid must be added to the thawed sample and vortexed prior to transferring to a pressure tube. 8. Place the tubes in an autoclave safe rack, and place the rack in the autoclave. Autoclave the sealed samples and sugar recovery standards for one hour at 121°C, usually the liquids setting. After completion of the autoclave cycle, allow the hydrolyzates to slowly cool to near room temperature before removing the caps. (If step B is not performed, draw a 10 mL aliquot of the liquor for use in step E.)	Electric shock Hot surface	Handle with care	

Run Time Procedure			
B) Analyze the sample for acid insoluble lignin as follows 1. Vacuum filter the autoclaved hydrolysis solution through one of the previously weighed filtering crucibles. Capture the filtrate in a filtering flask. 2. Transfer an aliquot, approximately 50 mL, into a sample storage bottle. This sample will be used to determine acid soluble lignin as well as carbohydrates. Acid soluble lignin determination must be done within six hours of hydrolysis. If the hydrolysis liquor must be stored, it should be stored in a refrigerator for a maximum of two weeks. It is important to collect the liquor aliquot before proceeding to step B3. 3. Use deionized water to quantatively transfer all remaining solids out of the pressure tube into the filtering crucible. Rinse the solids with a minimum of 50 mL fresh deionized water. Hot deionized water may be used in place of room temperature water to decrease the filtration time. 4. Dry the crucible and acid insoluble residue at 105 °C until a constant weight is achieved, usually a minimum of four hours. 5. Remove the samples from the oven and cool in a desiccator. Record the weight of the cricuble and dry residue to the nearest 0.1 mg. 6. Place the crucibles and residue in the muffle furnace at 575°C for 24 hours. 6.1 A furnace with temperature ramping may	Back presure might occur Sample spill Water spill Hot surface Hot surface Extreme Hot surface	Handle with care Spill kit in the lab Spill kit in the lab Handle with care Handle with care Wear insulated glove handle with care	Wear neoprene or rubber gloves, google, full protective clothing, and non-porous shoes

also be used Furnace Temperature Ramp Program: Ramp from room temperature to 105 °C Hold at 105°C for 12 minutes Ramp to 250 °C at 10°C / minute Hold at 250 °C for 30 minutes Ramp to 575 °C at 20 °C / minute Hold at 575 °C for 180 minutes Allow temperature to drop to 105 °C Hold at 105 °C until samples are removed 7. Carefully remove the crucible from the furnace directly into a desiccator and cool for a specific amount of time, equal to the initial cool time of the crucibles. Weigh the crucibles and ash to the nearest 0.1 mg and record the weight. Place the crucibles back in the furnace and ash to a constant weight. (The amount of acid insoluble ash is not equal to the total amount of ash in the biomass sample. Refer to LAP "Determination of Ash in Biomass" if total ash is to be determined.) C) Analyze the sample for acid soluble lignin as follows 1. On a UV-Visible spectrophotometer, run a background of deionized water or 4% sulfuric acid. 2. Using the hydrolysis liquor aliquot obtained in step B2, measure the absorbance of the sample at an appropriate wavelength on a UV-Visible spectrophotometer. Refer to section 11.3 in attached NREL procedure for suggested	Extreme hot surface Water or chemical spill	Wear insulated glove handle with care Spill kit in the lab	

wavelength values. Dilute the sample as necessary to bring the absorbance into the range of 0.7 – 1.0, recording the dilution. Deionized water or 4% sulfuric acid may be used to dilute the sample, but the same solvent should be used as a blank. Record the absorbance to three decimal places. Reproducibility should be within 0.05 absorbance units. Analyze each sample in duplicate, at minimum. (This step must be done within six hours of hydrolysis.) 3. Calculate the amount of acid soluble lignin present using calculation 11.3 in attached NREL procedure. D) Analyze the sample for structural carbohydrates 1. Prepare a series of calibration standards containing the compounds that are to be quantified, referring to Table 1 for suggested concentration range. Use a four point calibration. If standards are prepared outside of the suggested ranges, the new range for these calibration curves must be validated. 1.1 Table 1- Suggested concentration ranges for D1 calibration standards Component Suggested concentration range (mg/ml) D-cellobiose 0.1-4.0 D(+)glucose 0.1-4.0 D(+)xylose 0.1-4.0	Chemical Spill	Spill kit in the lab	

D(+)galcatose 0.1-4.0 L(+)arabinose 0.1-4.0 D(+)mannose 0.1-4.0 CVS Middle of linear range, concentration not equal to a calibration point (2.5 in attached NREL procedure suggested) 1.2 A fresh set of standards is not required for every analysis. A large batch of standards may be produced, filtered through 0.2 μm filters into autosampler vials, sealed and labeled. The standards and CVS samples may be stored in a freezer and removed when needed. Thaw and vortex frozen standards prior to use. During every use, standards and CVS samples should be observed for unusual concentration behavior. Unusual concentrations may mean that the samples are compromised or volatile components have been lost. Assuming sufficient volume, standards and CVS samples should not have more than 12 injections drawn from a single vial. In a chilled autosampler chamber, the lifetime of standards and CVS samples is approximately three to four days. 2. Prepare an independent calibration verification standard (CVS) for each set of calibration standards. Use reagents from a source or lot other than that used in preparing the calibration standards. Prepare the CVS at a concentration that falls in the middle of the validated range of the calibration curve. The CVS should be analyzed on the HPLC after each calibration set and at regular	Chemical spill Chemical spill	Spill kit in the lab Handle with care Spill kit in the lab Handle with care	

intervals throughout the sequence, bracketing groups of samples. The CVS is used to verify the quality and stability of the calibration curve(s) throughout the run. 3. Using the hydrolysis liquor obtained in step B2, transfer an approximately 20 mL aliquot of each liquor to a 50 mL Erlenmeyer flask. 4. Use calcium carbonate to neutralize each sample to pH 5 – 6. Avoid neutralizing to a pH greater that 6 by monitoring with pH paper. Add the calcium carbonate slowly after reaching a pH of 4. Swirl the sample frequently. After reaching pH 5 – 6, stop calcium carbonate addition, allow the sample to settle, and decant off the supernatant. The pH of the liquid after settling will be approximately 7. (Samples should never be allowed to exceed a pH of 9, as this will result in a loss of sugars.) 5. Prepare the sample for HPLC analysis by passing the decanted liquid through a 0.2 μm filter into an autosampler vial. Seal and label the vial. Prepare each sample in duplicate, reserving one of the duplicates for analysis later if necessary. If necessary, neutralized samples may be stored in the refrigerator for three or four days. After this time, the samples should be considered compromised due to potential microbial growth. After cold storage, check the samples for the presence of a precipitate. Samples containing a precipitate should be refiltered, while still cold, through a 0.2 μm filters.			

6. Analyze the calibration standards, CVS, and samples by HPLC using a Shodex sugar SP0810 or Biorad Aminex HPX-87P column equipped with the appropriate guard column. HPLC conditions: Injection volume: 10 – 50 µL, dependent on concentration and detector limits Mobile phase: HPLC grade water, 0.2 µm filtered and degassed Flow rate: 0.6 mL / minute Column temperature: 80 - 85°C Detector temperature: as close to column temperature as possible Detector: refractive index Run time: 35 minutes Note: The deashing guard column should be placed outside of the heating unit and kept at ambient temperature. This will prevent artifact peaks in the chromatogram.	Chemical Spill Chemical Spill	Spill kit in the lab Handle with care Spill kit in the lab Handle with care	
7. Check test sample chromatograms for presence of cellobiose and oligomeric sugars. Levels of cellobiose greater than 3 mg/mL indicate incomplete hydrolysis. Fresh samples should be hydrolyzed and analyzed.	Chemical spill	Spill kit in the lab Handle with care	
8. Check test sample chromatograms for the presence of peaks eluting before cellobiose (retention time of 4-5 minutes using recommended conditions). These peaks may indicate high levels of sugar degradations products in the previous sample, which is indicative of over hydrolysis. All samples from batches showing evidence of over-			

hydrolysis should have fresh samples hydrolyzed and analyzed. E) Analyze the sample for acetyl content if necessary (not) 1. Prepare 0.01 N sulfuric acid for use as a HPLC mobile phase. In a 2L volumetric flask, add 2.00 mL of standardized 10 N sulfuric acid and bring to volume with HPLC grade water. Filter through a 0.2 µm filter and degas before use. If 10N sulfuric acid is not available, concentrated sulfuric acid may also be used. 278 µl concentrated sulfuric acid brought to volume in a 1L volumetric flask with HPLC grade water will also produce 0.01N sulfuric acid. 2. Prepare a series of calibration standards containing the compounds that are to be quantified. Acetic acid is recommended, formic acid and levulinic acid are optional. A range of 0.02 to 0.5 mg/mL is suggested. An evenly spaced four point calibration is suggested. If standards are prepared outside of the suggested ranges, the new range for these calibration curves must be validated. 3. Prepare an independent calibration verification standard (CVS) for each set of calibration standards, using components obtained from a source other than that used in preparing the calibration standards. The CVS must contain precisely known amounts of each compound contained in the calibration standards, at a			

concentration that falls in the middle of the validated range of the calibration curve. The CVS should be analyzed on the HPLC after each calibration set and at regular intervals throughout the sequence, bracketing groups of samples. The CVS is used to verify the quality and stability of the calibration curve(s) throughout the run. 4. Prepare the sample for HPLC analysis by passing a small aliquot of the liquor through a 0.2 μm filter into an autosampler vial. Seal and label the vial. If it is suspected that the sample concentrations may exceed the calibration range, dilute the samples as needed, recording the dilution. The concentrations should be corrected for dilution after running. 5. Analyze the calibration standards, CVS, and samples by HPLC using a Biorad Aminex HPX-87H column equipped with the appropriate guard column. HPLC conditions: Sample volume: 50 μL Mobile phase: 0.01 N sulfuric acid, 0.2 μm filtered and degassed Flow rate: 0.6 mL / minute Column temperature: 55 -65°C Detector temperature: as close to column temperature as possible Detector: refractive index Run time: 50 minutes			

Shutdown Procedure			
1. Turn off the HPLC in the ChemStation software 2. Turn off the power of the HPLC	Electric shock	Handle with care	Wear neoprene or rubber gloves, google, full protective clothing, and non-porous shoes
Cleanup / Waste Disposal			
1. Disposal the chemical in the sink with plenty of water for at least 15 min. 2. Discard the solid in the waste basket. 3. Rinse all the apparatus with soap	Water spill Chemical spill	Spill kit in the lab	Wear neoprene or rubber gloves, google, full protective clothing, and non-porous shoes

Appendix A-2: JSA of Kinetic Modeling of Hardwood Prehydrolysis

Equipment Name: Kinetic Modeling of Hardwood Prehydrolysis
JSA Author: Shu Chiang Yat
Room Number/Building: 205 Chemical Engineering
Faculty Supervisor: Dr. David Shonnard
Revision #: 7
Revision Date: 4/28/06

Purpose of Experiment / Equipment: Briefly describe what this experiment is designed to achieve and the types of data collected.

The experiment is conducted using the Parr 4571 Reactor HP/HT. Different species of wood chips (~5%wt) is heated to the temperature of 150^{o}C ~ 200^{o}C in a diluted acid aqueous solution. The 0.5-2.0% w/w final concentration of sulfuric acid is added before the reactor is assembled. The reaction will occur when the temperature rises with time. Samples will be collected throughout the experiment for 2 ~ 3 hours.

Personal Protective Equipment (PPE) – Check all PPE worn during the entire experiment. Do not list these in the procedure section.

☒ Long Pants	☒ Safety Glasses	☐ Hard Hat	☐ Apron
☐ Long Sleeves	☐ Splash Goggles	☐ Insulated Gloves	☐ Ear Protection
☒ Non-porous Shoes	☐ Face Shield	☐ Chemical Gloves	☐ Other:

Hazard Summary – Check all general hazards that are likely to be encountered during this experiment and list the major source of the hazard.

Hazard	Major Source of Hazard
☒ Toxicity	Acetic Acid
☒ Fire/Flammability	Acetic Acid (Flash point of 39 C
☒ Reactivity	Sulfuric Acid
☒ Pressure Hazard	300 psi max running in the Parr reactor
☒ Electrical Shock	Power supply
☒ Mechanical Hazard	Parr reactor setup
☒ Hot Surfaces/ High Temp > 150 F	The Parr reactor will be operated from 150 ~ 200^{o}C
☐ Biohazard	
☐ Laser Radiation	
☐ Ionizing radiation	

☐ Other: _____	
☐ Other: _____	

Expected Operating Conditions –

Temperature	Pressure
Normal: 150 ~ 200°C	Normal: 80 ~ 300 psig
Minimum: Room Temperature	Minimum: Ambient pressure
Maximum: 200°C	Maximum: 300 psi

Special Operating Conditions - Check all that apply and consult department Safety Manual.

Unattended Operation: ☐	Drying Oven: ☐
Regulated Chemicals: ☐	Class 3b or 4 Lasers: ☐
Pressures Exceeding 35 atm (515 psia) or Equipment Specifications: ☐	
Temperatures Exceeding 1000°C or Equipment Specifications: ☐	

Available Safety Equipment – Provide the location of each item shown below. Show the location of this equipment on the attached floor plan. If not available, type "NA" in the field.

Item	Location
Fire Extinguisher:	East wall near door (Right hand side when enter the room)
Eyewash:	East wall near door (Right hand side when enter the room)
Safety Shower:	East wall near door (Right hand side when enter the room)
Telephone:	Northeast corner of the room
First Aid Kit:	East wall near door (Right hand side when enter the room)
Other:	
Other:	

Spill Response Supplies - Provide the location of each item shown below. Show the location of this equipment on the attached floor plan. If not available, type "NA" in the field.

Item	Location
Spill Kit:	Northwest of the room, the bottom of classware cabinet
Floor-Dri:	Northwest of the room, the bottom of classware cabinet
Spill Dikes:	Northwest of the room, the bottom of classware cabinet
Sodium Bicarbonate:	Northwest of the room, the bottom of classware cabinet
Drain Plugs:	N/A
Spill Pillows:	N/A
Mercury Spill Kit:	N/A
Other:	
Other:	

Required Attachments:

☒ Diagram of process or equipment Label all valves and identify all equipment for reference in procedure.
☒ Laboratory Floor Plan Identify the location of your experiment and all safety and spill response equipment.
☒ Equipment Specifications Include materials of construction, maximum temperature and pressure, standard operating values, and any other specifications important to the safe operation.
☒ Material Safety Data Sheets (MSDS) Include for all reactants, products and any intermediate or other chemicals which may occur.

Additional Attachments: As necessary.

☒ Evacuation Route	☐
☐	☐

Chemical Information Page

Fill in as much data below as available. If data are not available, leave the field blank.
List all chemicals, including reactants, products, intermediates, solvents, and any others used.

Chemical Properties and Hazards

Chemical Name	Physical State S, L, G	NFPA Ratings*				Incompatible Chemicals List chemicals present within the laboratory, and any others that may come in contact.	Flash Point Temp.	Flammability Limits	
		H	F	S	Sp.			LFL	UFL
Acetic Acid 5 g/L max Reaction product	L	3	2	0		Keep away from caustic soda, lime and strong alkalis, oxidizing agents such as nitric acid, peroxides, amines, sulfuric acid, perchloric acid or chromium trioxide.	39°C	4 %	19.9 %
2-Furaldehyde 4 g/L max Reaction product	L	3	2	1		Materials to avoid: Oxidizing agents, Strong acid	60°C	2.1 %	19.3 %
D-(+)-Glucose ~ 1 g/L Reaction product	S	0	0	0		Materials to avoid: Strong oxidizing agents	N/A	N/A	N/A
Sulfuric Acid 2 ~ 10 M	L	3	0	2		Materials to Avoid: Bases, Halides, Organic materials Incompatible with carbides, chlorates, fulminates, nitrates, picrates, cyanides, alkali halides, zinc iodide, permanganates, hydrogen peroxide, azides, perchlorates, nitromethane, phosphorous, and nitrites. Violent reaction with: cyclopentadiene, cyclopentanone oxime, nitroaryl amines, hexalithium disilicide, and	N/A	N/A	N/A

						phosphorous(III) oxide, Finely powdered metals			
D-(+)-Xylose 10 g/L max Reaction product	S	0	0	0		Materials to avoid: Strong oxidizing agents	N/A	N/A	N/A

*__NFPA Ratings:__ **H** – Health, **F** – Flammability, **S** – Stability, **Sp.** – Special

Chemical Toxicology, Regulation and Disposal: List the same chemicals that appear above, in the same order.

Chemical Name	**Toxicology**			**Hazardous Waste Number#**	**Regulated?** See Safety Manual	**Personal Protective Equipment Specific to this Chemical**
	TWA	**PEL**	**Other**			
Acetic Acid 5 g/L max Reaction product	10 ppm	10 ppm		D001, D002	☐	Skin protection: Wear impervious clothing and gloves to prevent contact. Neoprene is recommended. Eye/face protection: Wear chemical goggles when there is a reasonable chance of eye contact. In addition to goggles, wear a face shield if there is a reasonable chance for splash to the face. Respiratory protection: Use airpurifying respirator with full facepiece and organic vapor cartridge(s) or air-purifying full facepiece respirator with an organic

						vapor canister or a full facepiece powered air-purifying respirator fitted with organic vapor cartridge(s).
2-Furaldehyde 4 g/L max Reaction product	5 ppm	5 ppm			☐	Respiratory: Government approved respirator Hand: Compatible chemical-resistant gloves Eye: Chemical safety goggles Other: Faceshield
D-(+)-Glucose ~ 1 g/L Reaction product	N/A	N/A			☐	Respiratory: Wear dust mask Hand: Protective gloves Eye: Chemical safety goggles
Sulfuric Acid 2 ~ 10 M	1 ppm	1 ppm			☐	Respiratory: Government approved respirator. Hand: Compatible chemical-resistant gloves. Eye: Chemical safety goggles. Other: Faceshield (8-inch minimum).
D-(+)-Xylose 10 g/L max Reaction product	N/A	N/A			☐	Respiratory: Wear dust mask Hand: Protective gloves Eye: Chemical safety goggles
					☐	
					☐	
					☐	

[#]See Chemical Engineering Hazardous Waste Manual.

Chemical Reactions: Provide details below on any chemical reaction(s) that occur in your process. Please show the species involved, the stoichiometry and the heat of reaction, if available. Also list side reactions and any other reactions that may impact safety. You cannot type subscripts in the form field provided – use the names for the species and the stoichiometric coefficients.

It has been observed from other literature that the overall heat of reaction is endothermic

Cellulose Hydrolysis

Cellulose --> Glucose --> formic acid + hydroxymethylfurfural

Hemicellulose Hydrolysis

Hemicellulose --> Xylose --> Acetic Acid + 2-Furaldehyde

Job Safety Assessment Form
Safe Operating Procedures Page

Sequence of Steps	Potential Hazards	Procedure to Control Hazard	PPE or Equipment Required
Emergency Shutdown			
1. Turn off main power supply on Parr Reactor	Electric shock/explosion	Use left hand rule	Wear neoprene or rubber gloves, google, full protective clothing, and non-porous shoes
Start-up Procedure			
Prepare Chemicals Standard Solution: 1. Take the right size tub out from the safety equipment storage cabinet 2. Put the acetic acid and sulfuric acid from the acids storage cabinet to the tub. 3. Transfer the tub to the ventilation hood carefully 4. Take another right size tub out from the safety equipment storage cabinet 5. Put the glucose, xylose, and furfural from the general chemical storage to the tub 6. Transfer the tub to the lab bench top 7. Measure the acids and chemicals to the desired amount. Equipment (Parr Reactor): 1. Fill 500 ml of distilled water and wood chip sample (25g) into the glass liner with 0.5-2.0%	Tripping Chemicals spill	Always make sure that there is nothing lying (tools, rubbish) on the floor. Always make sure the floor is clean and dry Use tub which is just big enough to contain Spill kit in lab Safety shower NE corner of the lab	Wear neoprene or rubber gloves, google, full protective clothing, and non-porous shoes

w/w sulfuric acid 2. Put the glass liner into the reactor chamber 3. A torque-wrench should be used to tighten the cap screws and seal the reactor. 4. Place anti-rotation clamps over the round portion of cylinder handles and over the edge of the cart. 5. Pick a starting screw and tighten it to approximately 5 ft/lb 6. then, bypass the adjacent screw 180 degrees from the start. Torque the second screw and continue in the same pattern until all screws are snug at 5 ft/lb. 7. Repeat this procedure increasing the torque in 10 ft/lb increments until the torque level reaches 25 ft/lb. 8. Turn on the main power of the Parr reactor controller 9. Make sure all valves are close tightly 10. Adjust the pressure indicator to ambient. 11. Adjust the temperature shut off to 450 C 12. Connect the air tube to the cooling circuit 13. Connect the cooling water to the agitator cooling circuit 14. Set the temperature setpoint to 300 (or output 1 to 100 %) 11. Turn on the heater switch 12. Turn on the agitator of the reactor to 50 rpm 13. The reactor will be heating up to the desired temperature (150 - 200 C) at the cut off temperature of 450	Electric shock	Use left hand rule Make sure hands are clean and dry at all time	

14. When the desired temperature (150 ~ 200 C) is reached, open the air valve to control the temperature of the reactor			
Run Time Procedure			
1. 5 mL sample is collected at 100 C and 135 C, then 3 min interval after that until the temperature reaches setpoint (160, 175, and 190 C). 2. 4 samples are collected with 8 min interval at setpoint Steps For Collecting Each Sample: 1. Turn on the sample valve, discard the first 8 ml of the sample using cylinder flask 2. Collect 5 ml of sample after that 3. The hot sample will be cooled using the ice bath. 4. Label the sample 5. The cooled sample is filtered through Millipore membrane (pore diameter, 0.22) 6. The filtered sample will be collected in a 10mL vial 7. Label the sample again 8. Measure and record pH of the samples 9. Transfer the collected samples to duplicate HPLC vials 10. Label the HPLC vial for monomers and total sugar content 11. Repeat step 1 ~ 10. Analyze the sample for monomeric sugars: 1. Prepare a series of sugars calibration standards:	Sample spill Hot sample Sample spill Hot sample	Spill kit in the lab Safety shower NE corner of the lab Handle with care	Wear insulated gloves, google, full protective clothing, and non-porous shoes

D(+)glucose, D(+)xylose, D(+)galactose, L(+)arabinose, D(+)mannose. Use a two point calibration. Suggested concentrations for glucose and xylose are 5 and 10 g/L. Suggested concentrations for galactose, arabinose, and manose 1 and 2 g/L. 2. A large batch of standards may be prepared and stored in the refrigerator. 3. Neutralize the HPLC vial with monomers labelled by using NaOH to pH 5 - 6. 4. Next, go to HPLC Analysis JSA Analyze the sample for total sugar content (monomers and oligomers) 1. Based on the sample pH, calculate the amount of 96% w/w sulfuric acid required to bring the acid concentration of each sample to 4% (refer to section 11.3 of NREL Laboratory Analytical Procedure: Determination of Sugars, Byproducts, and Degradation Products in Liquid Fraction Process Samples) 2. Seal and label the samples 3. Prepare a set of sugar recovery standards (SRS) that will be taken through the analysis and used to correct for losses due to decomposition of sugars during dilute acid hydrolysis. 4. Add the appropriate amount of 96% sulfuric acid to each SRS. 5. Seal and label the SRSs. 6. Autoclave the sealed samples and SRSs for an hour at 121 C.			

7. After completion of autoclave cycle, allow the hydrolyzates to slowly cool to room temperature. 8. Use NaOH to neutralize the sample to pH 5 - 6. 9. Next, go to HPLC Analysis JSA			
Shutdown Procedure			
1. Turn off the sample valve 3. Turn off the agitator 4. Turn off the heater 5. Turn off the main power 6. Turn up the airflow to cool the reactor down	Electric shock	Use left hand rule Make sure hands are clean and dry at all time	Wear neoprene or rubber gloves, google, full protective clothing, and non-porous shoes
Cleanup / Waste Disposal			
1. Open vents and make sure the pressure of the reactor is at ambient before open up the reactor 2. Remove the reactor head when the inside reactor is below 80 C 3. Remove the glass liner from the reactor 4. Filter the residue solids using the coffee filter apparatus 5. Discard the solids to the waste basket 6. The reactor contents and sample vial contents will be discarded into drain with large amount of tap water running to dilute the concentration of the contents 5. Rinse the glass liner, agitator, reactor and reactor head with large amount of soap water	Sample spill Water spill	Spill kit in the lab	Wear neoprene or rubber gloves, google, full protective clothing, and non-porous shoes

Appendix A-3: JSA of Determination of Sugars produced in Pretreatment of Diluted Acid Hydrolysis

Equipment Name: Determination of Sugars produced in Pretreatment of Diluted Acid Hydrolysis
JSA Author: Shu Chiang Yat
Room Number/Building: 205 Chemical Engineering
Faculty Supervisor: Dr. David Shonnard
Revision #: 1
Revision Date: 4/28/06

Purpose of Experiment / Equipment: Briefly describe what this experiment is designed to achieve and the types of data collected.

Carbohydrates make up the major portion of biomass samples. these carbohydrates are polysaccharides constructed primarily of glucose, xylose, arabinose, galactose, and mannose monomeric subunits. During pretreatment of biomass, a portion of these polysaccharides are hydrolyzed and soluble sugars are released into the liquid stream. This method is used to quantify the amount of monomeric sugars release into solution. if the sugars are present in oligomeric form further processing into their monomeric units is required prior to HPLC analysis.

Personal Protective Equipment (PPE) – Check all PPE worn during the entire experiment. Do not list these in the procedure section.

☒ Long Pants	☒ Safety Glasses	☐ Hard Hat	☐ Apron
☐ Long Sleeves	☐ Splash Goggles	☐ Insulated Gloves	☐ Ear Protection
☒ Non-porous Shoes	☐ Face Shield	☐ Chemical Gloves	☐ Other:

Hazard Summary – Check all general hazards that are likely to be encountered during this experiment and list the major source of the hazard.

Hazard	Major Source of Hazard
☐ Toxicity	
☐ Fire/Flammability	
☐ Reactivity	
☐ Pressure Hazard	
☐ Electrical Shock	
☐ Mechanical Hazard	
☐ Hot Surfaces/ High Temp > 150 F	
☐ Biohazard	

☐ Laser Radiation	
☐ Ionizing radiation	
☐ Other: _____	
☐ Other: _____	

Expected Operating Conditions –

Temperature	Pressure
Normal: Room Temperature	Normal: Ambient Pressure
Minimum: Room Temperature	Minimum: Ambient Pressure
Maximum: 80°C	Maximum: 80 bar

Special Operating Conditions - Check all that apply and consult department Safety Manual.

Unattended Operation: ☐	Drying Oven: ☐
Regulated Chemicals: ☐	Class 3b or 4 Lasers: ☐
Pressures Exceeding 35 atm (515 psia) or Equipment Specifications: ☐	
Temperatures Exceeding 1000°C or Equipment Specifications: ☐	

Available Safety Equipment – Provide the location of each item shown below. Show the location of this equipment on the attached floor plan. If not available, type "NA" in the field.

Item	Location
Fire Extinguisher:	
Eyewash:	
Safety Shower:	
Telephone:	
First Aid Kit:	
Other:	
Other:	

Spill Response Supplies - Provide the location of each item shown below. Show the location of this equipment on the attached floor plan. If not available, type "NA" in the field.

Item	Location
Spill Kit:	
Floor-Dri:	
Spill Dikes:	
Sodium Bicarbonate:	
Drain Plugs:	
Spill Pillows:	
Mercury Spill Kit:	
Other:	
Other:	

Required Attachments:

☐ Diagram of process or equipment Label all valves and identify all equipment for reference in procedure.
☐ Laboratory Floor Plan Identify the location of your experiment and all safety and spill response equipment.
☐ Equipment Specifications Include materials of construction, maximum temperature and pressure, standard operating values, and any other specifications important to the safe operation.
☐ Material Safety Data Sheets (MSDS) Include for all reactants, products and any intermediate or other chemicals which may occur.

Additional Attachments: As necessary.

☐	☐
☐	☐

Chemical Information Page

Fill in as much data below as available. If data are not available, leave the field blank.
List all chemicals, including reactants, products, intermediates, solvents, and any others used.

Chemical Properties and Hazards

Chemical Name	**Physical State S, L, G**	**NFPA Ratings***				**Incompatible Chemicals** List chemicals present within the laboratory, and any others that may come in contact.	**Flash Point Temp.**	**Flammability Limits**	
		H	**F**	**S**	**Sp.**			**LFL**	**UFL**

*__NFPA Ratings:__ **H** – Health, **F** – Flammability, **S** – Stability, **Sp.** – Special

Chemical Toxicology, Regulation and Disposal: List the same chemicals that appear above, in the same order.

Chemical Name	**Toxicology**			**Hazardous Waste Number#**	**Regulated?** See Safety Manual	**Personal Protective Equipment Specific to this Chemical**
	TWA	**PEL**	**Other**			
					☐	
					☐	
					☐	
					☐	
					☐	
					☐	

					☐	
					☐	

[#]**See Chemical Engineering Hazardous Waste Manual.**

Chemical Reactions: Provide details below on any chemical reaction(s) that occur in your process. Please show the species involved, the stoichiometry and the heat of reaction, if available. Also list side reactions and any other reactions that may impact safety. You cannot type subscripts in the form field provided – use the names for the species and the stoichiometric coefficients.

Job Safety Assessment Form
Safe Operating Procedures Page

Sequence of Steps	Potential Hazards	Procedure to Control Hazard	PPE or Equipment Required
Emergency Shutdown			
1. Close the Agilent 1100 Online program 2. Click Yes to turn off all Agilent 1100 HPLC intruments. 3. Turn off the main power of the computer and the HPLC	Electric shock/explosion	Use left hand rule	Wear neoprene or rubber gloves, google, full protective clothing, and non-porous shoes
Start-up Procedure			
1. Turn on all the power supply of Agilent 1100 Series HPLC instruments 2. Check and make sure the water, as mobile phase, is in Stream A 3. Go to the Computer Desktop, open the Agilent 1100 Online icon 4. As the window open, there are 2 things need to be setup, Methods Menu and Sequence Menu 5. First, go to the Methods Menu, select Edit entire Method i. The Edit Method window pop up, click OK ii. Then Edit Information, write some comments about the experiment, such as acid concentration, temperaturem and species, then click OK iii. Setup Pump Window, type the flow rate (0.2 mL/min) and stop time (60min) at the Control section, choose stream A for 100% of the Mobile	Electric shock/explosion	Use left hand rule	Wear neoprene or rubber gloves, google, full protective clothing, and non-porous shoes

Phase Solvents, type the Pressure Limit (max=80, min=0 bar), click OK iv. Setup Injector, choose Injection with Needle Wash, the Wash Vial is located at position 100, need to check and make sure the vial is filled with distilled water, click OK v. DAD Signals, choose A (Sig=250,4 Ref=360,100) at the Signals Wavelength, click OK vi. RID Signals, type 55 C at the Optical Unit Temperature, click OK vii. Column Thermostat Method, type 80 C, click OK viii. Signal Details, select RID1 A, Refractive Index Signal and DAD1 A, Sig=250,4 Ref=360,100, click OK ix. then click OK for Edit Integration Events, Specify Report Instrument Curves, and Run Time Checklist. 6. Next, go to Sequence Menu, select Sequence Parameters 7. Type the Operator Name, select Auto in Data File, select Post-Sequence Cmd/Macro and choose PUMPALL OFF in the scroll down window in the Shutdown, click OK 8. Allow the Column Thermostat reaches 80 C, it usually takes about 30 min. 9. Then, increase the flow rate from 0.2 to 0.6 mL/min. 10. Monitor the pressure increases, make sure the pressure doesn't exceed 80 bar 11. When the flow rate, temperature, and pressure			

are stable, purge the reference of RID by clicking on the RID icon, then Control. choose Yes and 30 min under the Purge Reference. 12. After purging, allow the system to run at 0.6 mL/min, 80 C for an hour before start analyzing the samples			
Run Time Procedure			
Analysis the samples 1. Place all the labelled samples on the Autosampler tray. 2. HPLC Conditions: Injection volume: 10 uL Mobile phase: HPLC grade water, 0.2 um filtered and degassed Flowrate: 0.6 mL/min Colume Temperature: 80 C RI Detector Temperature: 55 C Diode-Array Detector: Sig=250,4 Ref=360,100 Run Time: 60 min NOTE: the guard colume should be placed outside of the heating unit and kept at room temperature. 3. Click the sampler tray on the software, locate the sample with name. 4. After getting all the chromatograph, calculate the concentration of the polymeric sugars from the concentration of the corresponding monomeric sugars, using an anhydro correction of 0.88 for C-5 sugars (xylose & arabinose) and a correction of 0.90 for C-6 sugars (glucose, galactose, and mannose)			Wear neoprene or rubber gloves, google, full protective clothing, and non-porous shoes

C(anhydro) = C(corr) x Anhydro correction			
Shutdown Procedure			
1. Turn off the pump flow 2. Turn off the Colume Thermostat. NOTE: Don't turn the rest of the system off as we will need to use it next time	Electric shock/explosion	Use left hand rule	Wear neoprene or rubber gloves, google, full protective clothing, and non-porous shoes
Cleanup / Waste Disposal			
1. Throw the HPLC vials in the glassware disposal box.	Sharp Object	Handle with care, wear glove if needed	Wear neoprene or rubber gloves, google, full protective clothing, and non-porous shoes

Appendix B: Parr 4571 Reactor Sealing Instructions

Manual No. 274M

1317HC SEALING INSTRUCTIONS

A torque-wrench should be used to tighten the cap screws and seal the reactor. The torque required to pre-load the metal gasket is a function of the operating pressure of the bomb. Refer to Table 1 for recommended torque levels.

To insure uniform loading, level the head of the vessel and turn each of the cap screws finger tight. Then use the following procedure when tightening with a torque wrench.

Place anti-rotation clamps over the round portion of cylinder handles and over the edge of the cart. Pick a starting screw and tighten it to approximately 5 ft/lb. Then by-pass the adjacent screw and move around the closure to a screw approximately 180 degrees from the start. Torque the second screw and continue in the same pattern until all screws are snug at 5 ft/lb. Repeat this procedure increasing the torque in 10 ft/lb increments until the torque level shown in Table 1 is reached. Repeated use of the metal gasket may require increased bolt torque up to a maximum of 50 ft/lb. When the gasket does not seal with 50 ft/lb of torque it should be replaced.

TABLE 1. RECOMMENDED BOLT-TORQUE FOR METAL GASKET 1317HC

Pressure (psi)	Torque (ft/lb)
2000	20
3000	30
4000	35
5000	40
Maximum Torque 50 ft/lb	

PARR INSTRUMENT COMPANY
211 Fifty-Third Street
Moline, Illinois 61265 USA
309/762-7716 Fax 309/762-9453

Appendix C: Determination of Maximum Time Step, Δt, for Xylose Formation Model

In Chapter 4, the xylose formation model was difficult to solve analytically because the reactor temperature was not constant. Therefore, an approximation to the solution is required using numerical integration.

Numerical analysis is not only the design of numerical methods, but also the accurate solution of governing equation expressed in finite difference form. One of the central concepts of numerical analysis is convergence, where the numerical method approximates the actual solution. A numerical method is said to be convergent if the numerical solution approaches the exact solution as the step size, which is Δt in this model, approaches zero.

Convergence was tested in the numerical solution by testing the effects of seven time steps between 0.01 and 1 minute. The kinetic equation for hemicellulose hydrolysis and xylose monomer production were integrated in Microsoft Excel running on a PC while keeping an accounting of the sum of squared errors between the model and experimental data. Figure D-1 shows one of the results analysis for an Aspen experiment conducted at 160^{o}C. The sum of squared error converged when Δt approached to 0.01 min. Therefore, a time step of 0.01 is selected as the optimum time step for the solution of the kinetic equations for all species in this research.

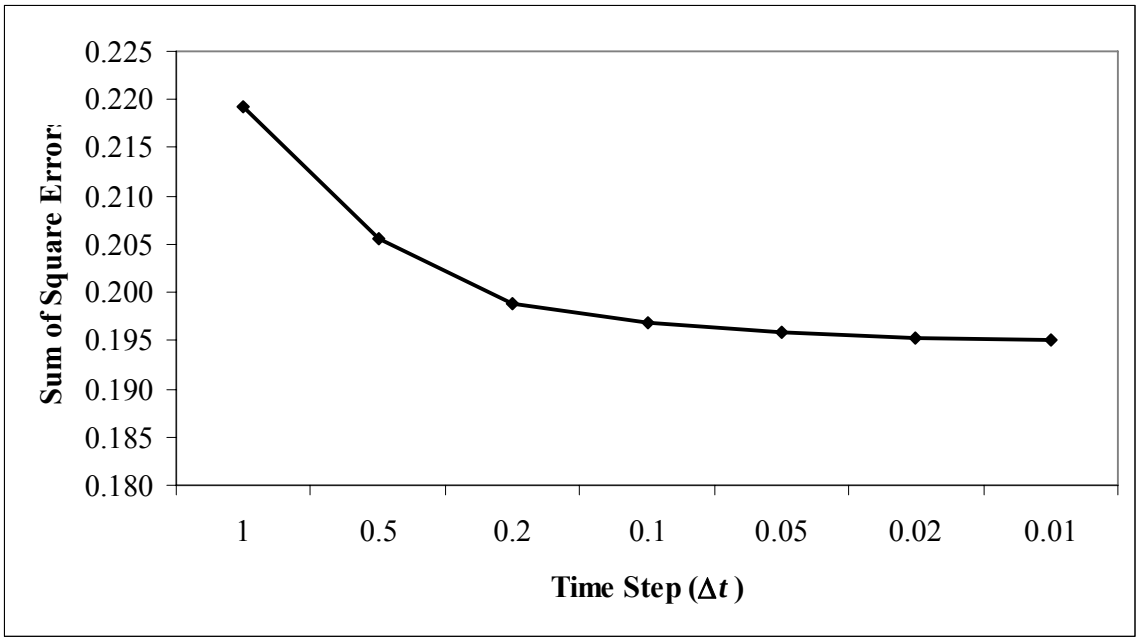

Figure D-1: Aspen Xylose Formation Model Time Step Verification using the Sum of Square Error