AN ABSTRACT OF THE DISSERTATION OF

Alireza Esteghlalian for the degree of Doctor of Philosophy in Bioresource Engineering presented on September 25, 1996. Title: Modeling and Optimization of the Dilute-Sulfuric Acid Pretreatment of Lignocellulosic Feedstocks.

Abstract approved: _*Redacted for Privacy*_____

Andrew G. Hashimoto

Environmental concerns about urban air quality, global climate change, energy security and economic considerations motivate a growing interest in alternative fuels for the transportation sector. Ethanol, a fermentation-derived fuel, can be produced by bioconversion of renewable materials, such as wood, grass, and waste. Combustion of ethanol fuel, in both neat and blended form, can improve the engine efficiency, and lower the emission of CO, NO_X, and volatile organic compounds (VOC), hence reducing the urban ozone level. Moreover, enhanced agricultural activities for production and collection of lignocellulosic feedstocks and industrial developments for production of ethanol will help the economic growth by creating new jobs and new income sources. Bioconversion of lignocellulosic feedstocks into ethanol requires a pretreatment process to increase the digestibility of cellulose by cellulolytic enzymes. The dilute-sulfuric acid pretreatment can hydrolyze hemicelluloses (xylan), disrupt lignin structure, and increase the yield of ethanol production from fermentation of monomeric units of cellulose (glucose). In this study, herbaceous (corn stover and switchgrass) and woody (poplar

chips) feedstocks were pretreated with dilute sulfuric acid (0.6, 0.9, and 1.2% w/w) in a batch reactor at relatively high temperatures (140, 160 and 180 °C). A unifying kinetic model including reaction time, temperature and acid concentration was developed, and pertinent kinetic parameters were determined. This model can predict the percentages of xylan remaining in the pretreated solids, net xylose yield in the liquid prehydrolysate, and xylose loss after pretreatment of a feedstock at a certain set of reaction conditions. Using this model, four optimum reaction conditions for obtaining maximum net xylose yield in the liquid prehydrolysate were identified. The yield and rate of ethanol production from the optimum prehydrolysates by the pentose fermenting yeast, *Pichia stipitis*, were determined. It was found that pretreating the selected feedstocks at 170-180 °C with 1.0-1.2% sulfuric acid for 1-3 min resulted in the recovery of 80-85% of the original xylan in the liquid prehyrolysate. It was also found that feedstocks with higher neutralizing capacity (e.g., corn stover) produced lower sugar yields as a result of acid neutralization. Pretreatment of feedstocks at conditions beyond the optimum reaction conditions would increase the extent of xylose degradation, and lower the yield and rate of ethanol production due to loss of fermentable sugars and formation of toxic byproducts. The optimum prehydrolysates of corn stover produced the highest yields of ethanol (0.39-0.47 g ethanol/g xylose) followed by switchgrass (0.36-0.45) and poplar (0.26-0.44). The inhibitory effects of byproducts (e.g., acetate) was more pronounced in poplar prehydrolysates.

Modeling and Optimization of the Dilute-Sulfuric Acid Pretreatment of Lignocellulosic

Feedstocks

by

Alireza Esteghlalian

A DISSERTATION

Submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Presented September 25, 1996
Commencement June 1997

Doctor of Philosophy dissertation of <u>Alireza Esteghlalian</u> presented on <u>September 25, 1996</u>

APPROVED:

*Redacted for Privacy*_____

Major Professor, representing Bioresource Engineering

_*Redacted for Privacy*_____

Chair of Department of Bioresource Engineering

*Redacted for Privacy*_____

Dean of Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

*Redacted for Privacy*_____

Alireza Esteghlalian, Author

ACKNOWLEDGMENT

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF TABLES

LIST OF APPENDIX FIGURES

Modeling and Optimization of the Dilute-Sulfuric Acid Pretreatment of Lignocellulosic Feedstocks

1. INTRODUCTION

Lignocellulosic materials, such as municipal solid waste, different types of hardwoods and softwoods, herbaceous plants, and agricultural and forestry residues are currently "overproduced" and "underutilized". These materials, which are mainly composed of cellulose, hemicellulose and lignin, can be a good source for production of chemicals, biochemicals, and alternative transportation fuels, such as ethanol.[23,33-36,49,59,72,73] Today, environmental concerns about global climate change, air quality, ozone layer depletion, as well as the need for an alternative to finite sources of fossil fuels, motivate a growing interest in utilization of lignocellulosic materials for production of ethanol fuel. In the United States, in particular, energy security and trade deficit are the two additional important factors that necessitate the development of biomass utilization technology.

Ethanol can be used as a primary fuel either in pure form or in combination with gasoline. Ethanol-gasoline mixtures, even low-level blends which consist mainly of gasoline, increase octane number and fuel oxygen content, providing more complete combustion in older cars. Three billion gallons of fuel ethanol are produced annually in Brazil, and used primarily as unblended fuel.[17,56] Combustion of ethanol in internal combustion engines designed for alcohols will give higher thermal efficiency and more power than combustion of gasoline in conventional engines. Emission reduction, urban

ozone level improvement, and decrease in atmospheric contaminants are among other benefits of ethanol fuel according to the Environmental Protection Agency.[36]

The abundance and renewability of lignocellulosic biomass have made these materials a promising source for biological production of ethanol. Although, during the past few decades, the concern about the economic feasibility of such technology has faced researchers in different fields including agriculture, economy, science and engineering with the challenge of finding the most appropriate feedstocks, developing an efficient bioconversion process with minimal adverse impacts on the society and environment, and determining when and where to use these materials for energy production. Intensive research in the above mentioned areas has enabled researchers to identify several model energy crops with sufficient yield and environmental benefits, examine numerous pretreatment methods and fermentative microorganisms, and evaluate the digestibility of different feedstocks. The short- and long-term impacts of biofuel production on environment and society have also been studied, and several viable processes have been developed. There is still much to be done in designing an efficient and economic process for producing ethanol. Use of land and natural resources, currently used by food- and feed-oriented agriculture, for production of energy crops also needs to be well justified to gain the public's support.

Pretreatment of biomass prior to the bioconversion process proved to be necessary for increasing the ethanol yield and lowering the price of final product. Chemical pretreatment can affect the structure of lignocellulosic feedstocks by solublizing or otherwise altering hemicelluloses, altering lignin structure, reducing cellulose crystallinity,

and increasing the available surface area and pore volume of the substrate, hence increasing the digestibility of the pretreated solids.[10,22,23,30,32,35,3947,49,69,74]

The objective of this study was to develop a unifying kinetic model including temperature, acid concentration and time for predicting the extent of hemicellulose (xylan) hydrolysis and net xylose yield in the resulting liquid stream after pretreatment of selected feedstocks by dilute sulfuric acid. The other objective was to determine the optimum reaction conditions for maximum xylose recovery in the liquid prehydrolysate to increase the ethanol production potential.

The three feedstocks used in this study, namely poplar chips, switchgrass and corn stover (stalks and leaves), were provided by the National Renewable Energy Laboratory. Poplar and switchgrass are two of the three plant species identified as the model energy crops in the United States, i.e., switchgrass (*Pancium virgatum*), hybrid poplar (*populus spp.*) and hybrid willow. Corn stover is the most abundant agricultural residue in the United States, and is estimated to furnish 10% of the U.S. liquid fuel requirements, if efficiently bioconverted to ethanol.[9]

This introduction is followed by four chapters intending to provide answers to the following questions:

1. Why is there a need for an alternative transportation fuel? What are the possible resources for production of such fuel? What are the environmental, economic and societal impacts of agricultural and industrial developments for production of biofuels? (Section 2.1)

2. What are the lignocellulosic feedstocks that can be converted to ethanol? What are the chemical and structural characteristics of these materials? (Section 2.2)

3. Why should the feedstocks be pretreated before digestion? and what are the pretreatment methods? (Section 2.3)

4. How were the pretreatment experiments and analytical measurements done in this particular work? (Chapter 3)

5. How was the kinetic model developed? (Chapter 4)

6. What are the results and implications of this study? (Chapter 5)

2. LITERATURE REVIEW

The purpose of this section is to explain the benefits of using ethanol as alternative to gasoline, elucidate the consequences of biomass production and utilization on the environment, economy and society, and provide the reader with sufficient information regarding the chemical make-up and physical characteristics of lignocellulosic materials.

2.1 Production of Ethanol-Fuel from Lignocellulosic Biomass

In the United States, there are two major factors motivating interest in alternative fuels: energy security and air quality. Among the four major energy sources in the United States (i.e., petroleum, coal, natural gas and nuclear power), petroleum supplies the largest share (40%) of total energy used ($8.6X10^{10}$ gigajoules). Energy use by the transportation sector was $22x10^{15}$ Btu in 1995, 97% of which was supplied by petroleum.[35,36,80] While imported oil is the largest contributor to the United States trade deficit (40% of the net deficit), more than half of the petroleum used in the country is imported.[36,79] Moreover, there is virtually no other fuel to which transportation sector can switch in case of supply shortage or disruption.[36-37]

Transportation fuels account for 27% of the 3.3 billion metric tons of CO_2 released annually in the United States, and are responsible for about two-thirds of the carbon monoxide pollution and about 40% of urban smog pollution. Vehicles account for 4.7% of total worldwide CO_2 emissions, with the U.S. vehicles being responsible for 2.5% of total emissions.[35,36,78]

In order to reduce the release of greenhouse gases (CO_2, CH_4, N_2O) produced by combustion of fossil fuels in vehicles, it may be suggested to reduce the number of vehicle miles traveled, or improve the efficiency of internal combustion engines to reduce the fuel consumption rate. These solutions, however, are not easily achievable. Limiting the vehicle miles traveled will limit the economic activities of automobile manufacturing companies and other businesses that depend on transportation system for survival and profitability. Under today's economic circumstances imposing such limitations is not feasible. Although improving the efficiency of automobile engines could lower the fuel consumption, and reduce the CO emission, it has the disadvantage of increasing the production and maintenance cost of vehicles which will lower their marketability and sales.[80]

The other possible alternative is to use a "cleaner" fuel with less toxic emissions. One such alternative fuel is ethanol which can also be produced from renewable lignocellulosic resources. Lignocellulosics are the most abundant products of photosynthesis which consumes CO_2 from the atmosphere. As a result, the photosynthesis-ethanol production-ethanol combustion cycle will result in very little, if any, net release of carbon dioxide. The energy balance for ethanol production from lignocellulosic feedstocks is also favorable.[35-36,75,80]

Ethanol can be used in low-ethanol gasoline blends, in neat (unblended) form, or as a primary fuel with small amounts of gasoline. All forms of ethanol-gasoline blends have higher octane number and oxygen content and give higher thermal efficiency and power than does the combustion of gasoline in conventional engines.[35,36] This is because ethanol has a lower tendency for pre-ignition owing to its higher octane number, and can

therefore be used in engines with higher compression ratios. Such engines require leaner fuel mixtures and provide better power and fuel economy. The approximate volumetric mileage for ethanol blended 10% with gasoline vary from 96% to 107% relative to gasoline. The average value would be equivalent of gasoline volumetric mileage. In case of neat ethanol fuel, however, approximately 1.25 gallons of ethanol are needed to travel the same distance as that obtained from one gallon of gasoline in optimized engines.

Use of ethanol as a neat fuel requires carburetor modification, because of its lower specific gravity and higher viscosity than gasoline. Gasoline contains many light hydrocarbon contents that are easily volatalized, whereas ethanol has a single boiling point (78 °C) and requires provision for cold starts since it lacks the light ends present in gasoline. In order to overcome the cold starting problem in neat ethanol fuel engines, it has been suggested to use an auxiliary starting system that can use gasoline, add more volatile compounds to ethanol, or use a small electric heater to warm up a small amount of fuel for initial ignition.[78-80] Moreover, hydrous ethanol fuels (95% ethanol-5% water) are known to have some corrosion effects on the engine fuel system which would require the use of special materials in certain areas of the engine.[16,78]

Ethanol can be produced from different forms of lignocellulosic materials including:

1. Wastes: agricultural, forestry, and municipal solid waste (MSW),

2. Hardwoods and softwoods, and

3. Agricultural products: grasses and starch-rich crops.

Wyman[79] has shown that ethanol derived from these resources is more than sufficient to replace all gasoline now used in the United States. Relative contribution of these different

resources are: 42% for wastes, 39% for energy-devoted forestry, and 19% for energy-devoted agriculture.[35]

Among agricultural products, sugar and starch-rich crops are generally considered to have less favorable energy balances compared to lignocellulosic waste. When by-products from ethanol production are used for animal feed, corn is the most attractive of the grain and sugar crops for ethanol production.[35-36] Currently, 1426.5 million gallons of ethanol are produced annually in the United States, with 86.5 % of this (1235 million gallons) produced from corn.[14] Although there is a 24% energy gain for every BTU spent to produce and convert corn to ethanol, the price of corn-derived ethanol is still too high to compete with gasoline.[60]

Herbaceous materials, such as prairie and forage grasses, have a more favorable energy balance and lower potential for adverse environmental impacts as compared with those of corn. The energy consumed to grow, harvest and convert the energy crops into ethanol is much lower than that required for corn. Furthermore, economic utilization of grass does not depend on byproduct utilization. The energy out/energy in ratio for herbaceous energy crops is estimated to be greater than 5:1. As an example, an acre of switchgrass, one of the designated energy crops, can produce 20.6 times the energy required to produce it.[14] Previous studies predicted a larger role for grasses among agricultural products; however, there is still considerable uncertainty regarding the availability of agricultural land for energy crops in the year 2000.[35]

Use of municipal solid waste (MSW) for energy production will reduce the problem of waste disposal. In 1992, over 178 million metric tons of MSW were produced. The carbohydrate fraction of MSW is a very competitive substrate for ethanol production

compared to, for example, hardwood chips, even after considering the cost of separation of the degradable fraction. In 1992, MSW consisted of 38% paper and paperboard and 6% solid wood waste, both suitable feedstocks for biofuel production.[25] From energetic and product value considerations, ethanol production is an attractive alternative to other new methods of municipal solid waste management, including waste separation, recycling, and electricity generation by incineration.[35]

Utilization of agricultural and forest residues for ethanol production captures the potential energy of these materials which will otherwise be burned or returned to the soil. Chaff, the residual material from cereal grain crops, is produced in large quantities in cereal growing areas of the United States, and may be used as a suitable feedstock for production of ethanol. Collection of chaff removes the weed and cereal grain seeds from the agricultural lands, hence lowering the herbicide and tillage requirements; and also preventing the chaff from accumulating in windrows which may cause poor seed placement and limit the nitrogen availability for the new crop. The low bulk density of chaff, however, makes the transportation and use of this feedstock uneconomical. Therefore, additional operations, such as compaction, particle size reduction and particle orientation are required to increase the chaff density and reduce its transportation cost.[55]

In addition to agricultural products and waste, forest-derived biomass is another important source of raw materials for ethanol production. The recent changes in forest management philosophies, however, have had a significant impact on cost and supply of forest biomass for production of liquid fuels. The emphasis on partial harvest and thinning rather than clear-cut harvest, and leaving slash and debris on site instead of collecting them as potential biomass products will increase the cost of logging operation and decrease the

amount of biomass recovered per acre. This in turn increases the price of forest biomass products.[41] Apart from its higher cost, using the new methods of silviculture management and enviornmentally-friendly techniques of forestry, forest derived biomass can be supplied and utilized. In addition, hardwood trees such as poplar and willow are also among the main energy crops. It is interesting that cultivation and production of such trees, known as short-rotation woody crops, is not considered forestry, but "intensive culture" or simply agriculture. This is because of the difference between the yield of these crops and usual forest species. Yield is the major criterion in growing the energy crops and is defined as the average amount of harvestable dry plant matter (biomass) per unit of land area per year. The desirable yields of poplar and willow trees are in the range of 5-20 dry tons/acre/year (dt/a/y) which is much larger than the yields of forests (below 1 dt/a/y).[28]

According to Lynd[35], all things considered, ethanol production potential in the United States is significant relative to the current demand for liquid transportation fuel, and that this end use for ethanol cannot be dismissed based on substrate supply limitations.

2.1.1 Environmental Impacts

The environmental impacts of ethanol production and consumption are often studied from three perspectives:

1. The problems associated with the waste streams of the ethanol production process

2. The effects of substrate production on land resources, such as soil and wildlife habitat

3. The environmental impacts of using ethanol as a transportation fuel

1. <u>The problems associated with the waste streams of the ethanol production process</u>

The main waste streams of the ethanol production process include: low-pH liquid effluents with high concentrations of BOD and organic matter (both dissolved and suspended solids); solids in the form of ash and insoluble salts resulting from neutralization of acid; and airborne emissions. The water quality problems associated with fuel-ethanol plants are claimed to be solvable by using the conventional wastewater treatment technologies. Infiltration and runoff of waste water can be controlled by installation of impermeable pads and using treatment ponds.[35,73] In several process designs, the lignin residual and the sludge from wastewater treatment are burned to eliminate the organic solid waste, and provide the energy required by the process.[21,35,73] Stillage has a high pollution potential if discharged, and therefore, must be processed within the ethanol production plant to produce animal feed.[35,73] The major atmospheric emissions from an ethanol production plant, including the lignin incineration facilities, are hydrocarbons, particulates of dust, SO_2, CO_2, and NO_X. Particulate matter and carbon dioxide are generated in the largest quantities. Even though there is some potential for air quality degradation by these contaminants, application of required air pollution abatement systems are able to minimize the potential pollution.[73]

2. <u>The effects of substrate production on land resources, such as soil and wildlife habitat</u>

The production and harvest of agricultural and forestry biomass have some destructive effects on the soil quality. Complete removal of plant residues exposes the soil to water and wind. The resulting increase in runoff destroys surface soil structure, causing puddling, decreased water infiltration, soil compaction, reduced porosity, and a loss of organic matter. Grain and sugar crops have the highest potential for causing such adverse impacts on soil properties. Herbaceous energy crops, in contrast, have significant positive impacts and definite advantages over annual row crops because of their perennial growth habit. These crops can improve soil structure, increase water-holding capacity and infiltration through structural and porosity changes, improve nutrient conservation and availability, and decrease soil erosion. Herbaceous energy crops, in general, have less nutrient and fertilizer requirements, and prevent fertilizer runoff as a result of their compact root system.[8] Both herbaceous and short-rotation woody crops can increase biodiversity in agricultural landscapes and provide habitat for a variety of animals and birds.[68] Wood and food-processing wastes, and collected logging residues have no significant potential for environmental deterioration.[35,72] Generally, the impacts of ethanol production on aquatic and terrestrial ecology are highly site-specific, and can be avoided by siting plants in locations with minimal adverse effect on ecological resources.[73]

   3. <u>The environmental impacts of utilization of ethanol as a transportation fuel</u>

Transportation fuels derived from renewable biomass do not add greenhouse gases (CO_2, NO_2, CH_4) to the atmosphere during their life cycle as do fossil fuels. The CO_2 produced during the fermentation process and combustion of ethanol is consumed during the growth of biomass. The other benefit of transformation of biomass to ethanol is the omission of methane gas which would be produced if biomass decayed naturally.

According to most air-quality calculations, some improvement in urban ozone levels and a decrease in air toxins are expected by using ethanol as the transportation fuel.[35] This is primarily because of higher oxygen content of ethanol as compared to gasoline, and improved efficiency and fuel economy of the engines designed for ethanol fuels. Use of anhydrous ethanol-gasoline blends has a leaning effect on the fuel combustion in the engines which reduces the emission of CO gas. Such leaning effects, however, could result in less power and poor fuel economy.[78]

The formation of tropospheric ozone, the major component of smog, is enhanced by presence of nitrogen oxides, unburned fuel, and volatile organic compounds (VOC). The latter are emitted form the tailpipe, through evaporative loss in the fuel system and as evaporative loss during refueling. In the presence of light, VOC will photochemically react with nitric oxide and produce atomic oxygen, and subsequently ozone (O_3) molecules. Sine the emission of VOC is primarily due to high volatility of a fuel, ethanol blends with lower vapor pressures are expected to result in lower concentrations of volatile organic compounds. On the other hand, the photochemical reactivity of ethanol is much less than for hydrocarbons, and concentration of highly reactive components produced by combustion products are very low. The emission of NO_X is decreased by combustion of neat ethanol fuel, because ethanol burns at lower temperatures and NO_X formation decreases as temperature decreases.[78]

Ethanol produced from lignocellulosic feedstocks can also be used for production of ethyl tertiary butyl ether (ETBE) which is a high octane, oxygenated fuel additive. ETBE is produced by reacting ethanol with isobutylene, and is expected to perform as

well as the widely used additive, methyl tertiary butyl ether (MTBE), and have the same effect in reducing the level of CO production.[78,79]

2.1.2 Economic and Societal Impacts

Various studies on the economic impacts of utilization of renewable resources for energy production indicate that investment in biomass energy technology in addition to creating new jobs and income can provide economic development for rural areas of the United States. The total economic impacts of production of bioenergy are usually divided into three different categories:

1.   Direct impacts: resulting from expenditures of the agricultural and industrial enterprises on goods and services. Development of both agricultural and industrial activities create new employment opportunities and more income sources.

2.   Indirect impacts: resulting from the creation of income and new jobs in businesses that provide service and support for the renewable energy production activities. The supporting businesses include machine and parts manufacturers, office suppliers, maintenance providers, and all other services necessary for establishment and operation of agricultural and industrial enterprises involved in production of biofuels.

3.   Induced impacts: resulting from personal expenditures of employees (and their families) supported by the bioenergy industry in food, clothing, health, education, real estate and recreation.[57]

The results of a study by the U.S. Department of Agriculture, Economic Research Service, indicated that increasing the annual ethanol production to 5 billion gallons per year would create almost 108,000 new jobs nationwide, including 34,000 direct and indirect ethanol jobs from ethanol processing, 14,000 temporary construction jobs, and 60,000 jobs from additional crop production. If all this ethanol is produced from 10 major crops (corn, sorghum, barley, oats, wheat, soybean, rice, hay, and silage), a 51.6-billion rise in crop net income is expected. The long term scenario of the Department of Energy, i.e., production of 5 billion gallons ethanol per year, is also expected to improve the U.S. balance of trade by 7.6 billion dollars.[71] It has been estimated that a new 100-million-gallon ethanol facility would generate about 370 temporary jobs during the construction phase and about 840 permanent jobs during the operational phase.[52]

Despite all benefits and advantages of ethanol as a combustion fuel, its production and use is currently impeded by its higher price over relatively cheap and readily available gasoline in the United States. Using advanced pretreatment techniques and efficient enzymatic bioconversion processes, the price of ethanol produced from energy crops ($42/ton) could be lowered to 0.32/L or $1.22/gal to be competitive with corn-derived ethanol. The goal, however, is to reduce the price to less than $0.18/L ($0.67/gal) so it can compete with gasoline derived from oil at the price of $15/bbl.[79]

Production of greenhouse gases, air pollutants effective in formation of urban "smog", acid rain, and its impact on global climate change, will add to the cost of seemingly cheap gasoline in the United States. The extra expenses, known as externality costs, are spent on health care and other provisions needed for removing the air pollutants from tailpipe gases, filtering the air for residential and work environments, or providing

green landscapes for improving the air quality. A survey by Bernow and Marron[28] indicated that on the basis of existing scientific literature and current environmental data, the externality cost of petroleum is approximately $45 per barrel. This number should be added to the nominal oil price of approximately $15 per barrel, resulting in a truer cost of $60 per barrel.

2.2 Lignocellulosic Biomass

Biomass is defined as all renewable organic matter including plant material in land or in water; animal products and manure; food processing and forestry products, and urban wastes.[5] Lignocellulosic biomass is composed of three major constituents, cellulose, hemicelluloses, and lignin with an approximate ratio of 1.7:1:1.[32] Cellulose forms a skeleton surrounded by a hemicellulose matrix, and both are encrusted by lignin.[27,62] Distribution of these three polymers in different layers of plant cell walls will be discussed later in this chapter.[49]

2.2.1 Chemistry and Structure of Plant Cell Wall Components

Cellulose is a homopolysaccharide of β-D-glucopyranose units linked together by β-(1$\rightarrow$4) glucosidic bonds. The β-linkages force the chain to rotate, and anhydro-cellobiose is the repeating unit of this chain.[2] Cellulose molecules are completely linear and are stiffened by two inter- and four intramolecular hydrogen bonds.[62,74] Figure 2.1 shows
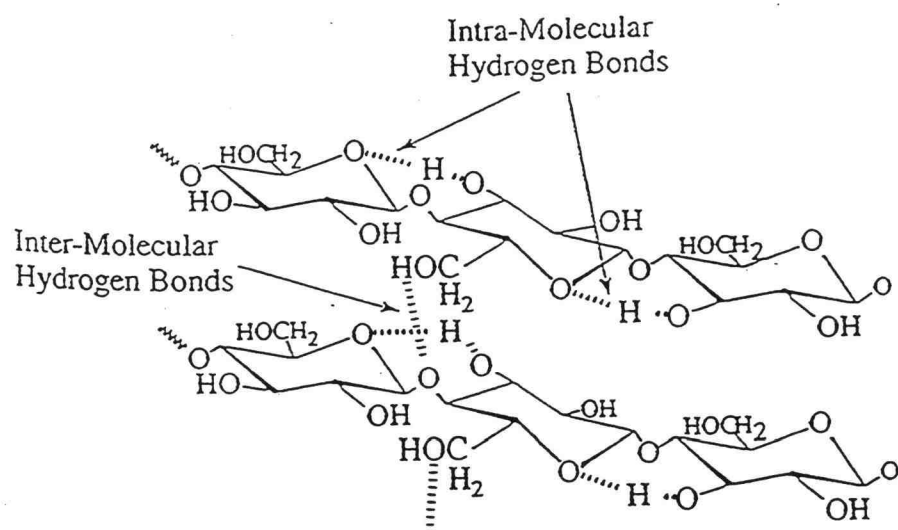
Fig 2.1. Adjacent cellulose molecules showing intra- and intermolecular hydrogen bonding[62]

these two types of hydrogen bonding between adjacent cellulose molecules. Aggregation of a group of cellulose molecules forms the cellulose microfibrils, with alternating crystalline and amorphous regions. In crystalline regions, tens of cellulose molecules are laid parallel in the same direction from non-reducing end to the reducing end, or vice versa, whereas in amorphous regions these molecules are arranged in a random fashion.[22] Microfibrils build up the fibrils and finally the cellulose fibers. Cellulose is insoluble in most solvents and has a high tensile strength as a result of its fibrous structure.[32]

Hemicelluloses are heterogeneous polysaccharides which function as supporting material in the plant cell wall. Unlike cellulose, hemicelluloses are composed of different pentoses and hexoses, namely D-glucose, D-mannose, D-galactose, D-xylose, L-arabinose, and small amounts of L-rhamnose.[62] Hemicelluloses are shorter (compared with cellulose), and are frequently branched either through attachment of short side chains or by more extensive branching. Hemicelluloses may also contain other groups such as D-glucuronic acids, 4-O-methyl D-glucuronic acids, and D-galacturonic acids. The C-2 and C-3 positions of the backbone sugars are partially substituted by O-acetyl groups. Hemicelluloses are less resistant to chemicals (acid and alkali) because of their shorter chains, branched structure, and less hydrogen bonding with other polysaccharides.[62]

In grasses, the main portion of hemicelluloses are xylans with a backbone of 4-linked xylose residues and short side chains of arabinose, glucuronic acid, and 4-O-methyl glucuronic acid residues. A low content of pectic substances (rhamnose and galactose residues) have also been observed in grasses.[2]

In wood, percentage of hemicelluloses vary between 20-30% (dry weight). The composition and structure of hemicelluloses in softwoods are different from those in hardwoods. The major hemicelluloses in softwoods are:[62]

1. Galactoglucomannans: The backbone of this group consists of β-D-glucopyranose and β-D-mannopyranose units linked together by β-(1→4) glycosidic bonds. The single-unit side chain of α-D-galactopyranose residue is attached to the backbone by (1→6) union. The hydroxyl groups at C-2 and C-3 positions of the main chain sugars are partially substituted by O-acetyl groups. Galactoglucomannans are easily depolymerized by acid, especially the bond between galactose side unit and the backbone. The acetyl groups are generally more susceptible to alkali hydrolysis than acid cleavage.

2. Arabinoglucuronoxylan: The β-D-xylospyranose units, partially substituted by 4-O-methyl-α-D-glucuronic acid groups at C-2, make the backbone of this group of hemicelluloses. The backbone also contains α-L-arabinofuranose units, with an average of 1.3 residues per ten xylose units. These side chains are easily hydrolyzed by acids.

3. Arabinogalactan: This group is a minor constituent of wood species, and found in large amounts in heartwood of larches. The backbone of these polymers are built up by (1→3)-linked β-D-galactopyranose units. The side branches of galactopyranose and L-arabinose units are linked to the backbone by (1→6) bonds. There are also a few glucuronic acid residues present in the molecule. Arabinogalactans are water soluble due to their highly branched structure.

Other polysaccharides, such as starch (containing amylose and amylopectin) are also present in soft woods in small quantities.

The principal hemicelluloses of hardwoods are:[62]

1. Glucuronoxylan: This group of polysaccharides, known as O-acetyl-4-O-methylglucurono-β-D-xylan or more conveniently glucuronoxylans, is the major component of the hardwood hemicelluloses. The framework consists of β-D-xylopyranose units linked by $(1\rightarrow4)$ bonds. Most of the xylose residues contain an O-acetyl group at C-2 or C-3. The xylose units of the xylan chain (backbone) are also linked to 4-O-methyl-α-D-glucuronic aid residues by $(1\rightarrow2)$ bonds. The xylosidic bonds (of the backbone) are easily cleaved by acids, whereas the linkages between the uronic acid groups and xylose units are very resistant. Acetyl groups are easily cleaved by alkali.

2. Glucomannan: This group is composed of β-D-glucopyranose and β-D-mannopyranose units linked by $(1\rightarrow4)$ bonds. The mannosidic bonds between the mannose units are more rapidly hydrolyzed by acid than the glucosidic bonds. Glucomannans are easily depolymerized by means of acid.

Other polysaccharides, of the same type occurring in softwoods, are also present in hardwoods. Figure 2.2 shows the chemical arrangement of some of the hemicelluloses.

Lignin is a three dimensional polymer of phenylpropane units. Lignin contains characteristic methoxyl, phenolic hydroxyl, and some terminal aldehyde groups in the side chain. Most of the phenolic hydroxyls are occupied through linkages to the adjacent phenylpropane units. The alcoholic hydroxyl groups and carbonyl groups are introduced into the final lignin polymer during the dehydrogenative polymerization process.[2,62]

In some wood species most of the alcoholic (OH) groups are esterified with p-hydroxy benzoic acid or p-hydroxycinnamic acid. In aspen lignin (hardwood) esters of p-hydroxy benzoic acid are common, whereas in grass, esters of p-hydroxy cinnamic acid are

a)

$$\rightarrow 4\text{-}\beta\text{-}D\text{-}Xyl\,p\text{-}1 \left[\rightarrow 4\text{-}\beta\text{-}D\text{-}Xyl\,p\text{-}1 \atop {2 \atop \uparrow \atop 1 \atop 4\text{-}O\text{-}Me\text{-}a\text{-}D\text{-}Glc\,pA} } \right]_2 \rightarrow 4\text{-}\beta\text{-}D\text{-}Xyl\,p\text{-}1 \rightarrow 4\text{-}\beta\text{-}D\text{-}Xyl\,p\text{-}1 \atop {3 \atop \uparrow \atop 1 \atop a\text{-}L\text{-}Araf } \left[\rightarrow 4\text{-}\beta\text{-}D\text{-}Xyl\,p\text{-}1 \rightarrow \right]_5$$

b)

$$\rightarrow 4\text{-}\beta\text{-}D\text{-}Glc\,p\text{-}1 \rightarrow 4\text{-}\beta\text{-}D\text{-}Man\,p\text{-}1 \atop {6 \atop \uparrow \atop 1 \atop a\text{-}D\text{-}Gal\,p} \rightarrow 4\text{-}\beta\text{-}D\text{-}Man\,p\text{-}1 \atop {2,3 \atop \uparrow \atop Ac} \rightarrow 4\text{-}\beta\text{-}D\text{-}Man\,p\text{-}1 \rightarrow$$
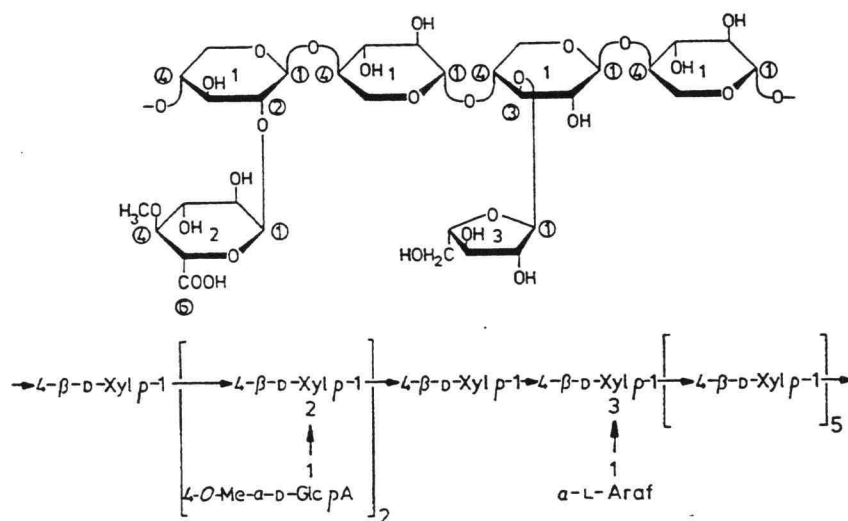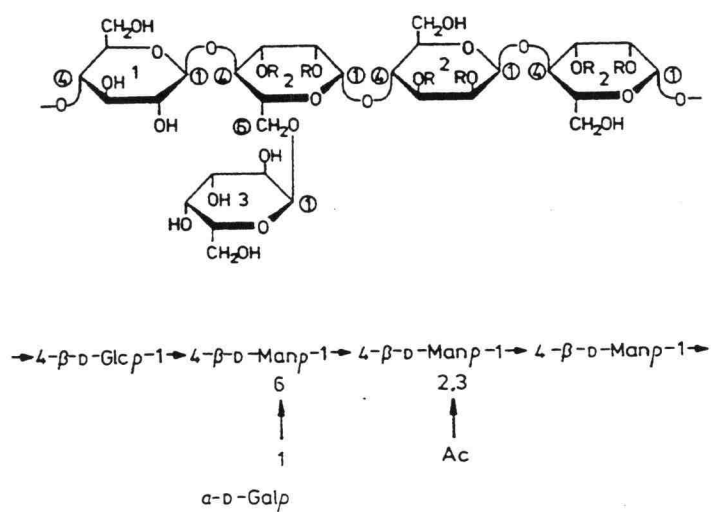
Fig. 2.2. Structure of major hemicelluloses of wood:
a:Galactoglucomannan; b:Arabinoglucuronoxylan[62]

abundant. Figure 2.3 shows the Adler's lignin formula. This formula consists of 16 phenylhydroxypropane units and represents only a segment of the lignin macromolecule.

Lignin is generally classified in three major groups based on the three structural precursors. Guaiacyl lignin, a polymerization product of coniferyl alcohol, is found in almost all softwoods. The guaiacyl-syringyl lignin, a copolymerization product of coniferyl and syringyl alcohols, is the typical lignin of hardwoods. P-hydroxyphenyl lignin and the guaiacyl units have a high proportion in compression wood (which develops under the leaning stems of conifers). Lignin is almost completely insoluble in most solvents.[62]

Nonstructural components will typically make up 5-15% of lignocellulosics, and can include organic acids, soluble carbohydrates, polysaccharides, cutin, silica, tannins, essential oils, and polyphenols.[32]

As mentioned before, cellulose, hemicellulose, and lignin are distributed in different layers of plant cell wall with various proportions rendering different structure and chemical composition to each layer. These layers are middle lamella (M), primary wall (P), outer layer of the secondary wall (S1), middle layer of the secondary wall (S2), inner layer of the secondary wall (S3), and the warty layer (W). Figure 2.4 shows a simplified picture of a woody cell wall.

The middle lamella, comprised of amorphous material, is located between the adjacent cells and binds them together. This layer is highly lignified (in well-grown wood) and has a thickness of 0.2-1 μm.[2,27] The concentration of lignin in the middle lamella is about 70% in softwoods and about 90% in hardwoods.[32]

The primary wall contains cellulose, hemicellulose, pectin, and protein. All these polymers are embedded in lignin. Cellulose microfibrils form an irregular network in the
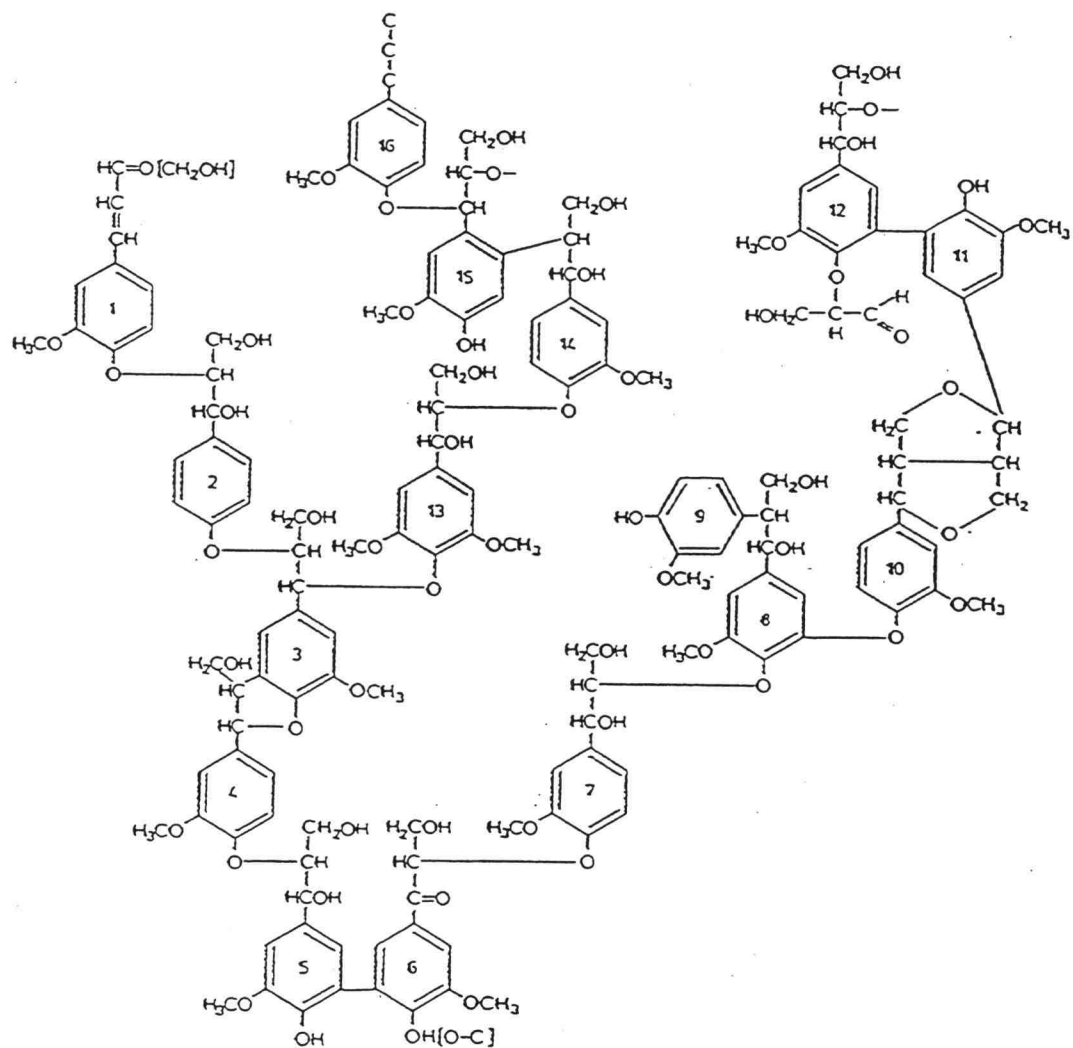
Fig. 2.3. A structural segment of softwood lignin proposed by Adler (1977)[62]
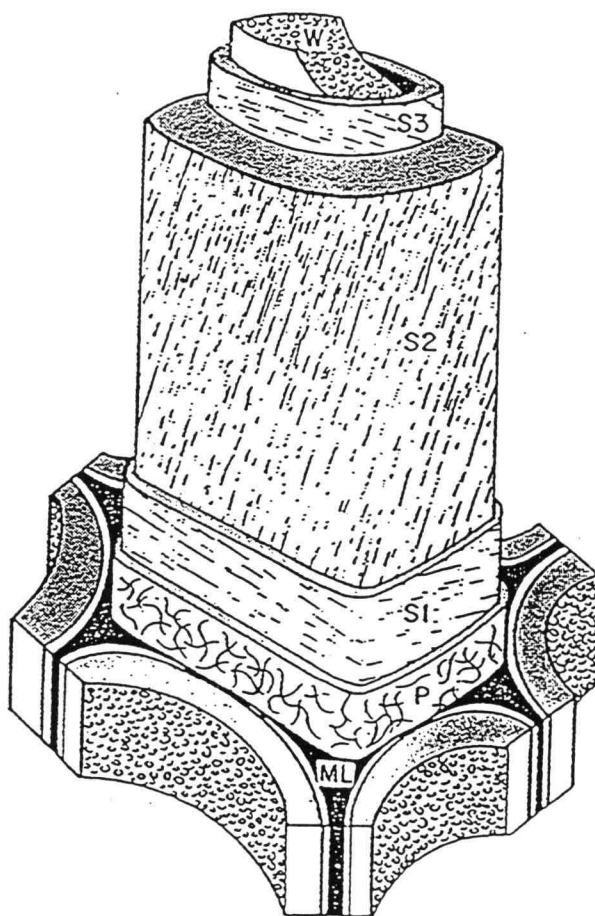
Fig. 2.4. Simplified structure of a woody cell, showing the middle lamella (ML), the primary wall (P), the outer (S_1), middle (S_2), and inner (S_3) layers of the secondary wall, and the warty layer (W)[62]

outer portion of this thin layer (0.1-0.2 µm). In the interior, cellulose microfibrils are perpendicular to the cell axis. The primary wall together with the middle lamella on both sides (one from each cell), often called compound middle lamella, is thin and has a high lignin content.[2,62]

The secondary wall consists of three layers, two thin outer (S1, 0.2-0.3 µm) and inner (S3, 0.1 µm) layers, and a thick middle layer (S2, 1-5 µm) which forms the main portion of the cell wall. In these three layers, lignin and hemicelluloses are kept between parallel microfibrils of cellulose. The lignin concentration in these layers is low. However, 70% of softwood lignin and 90% of hardwood lignin is located in the secondary wall because of its thickness.[2,32,62]

The warty layer (W) is thin, amorphous, and is located in the inner surface of the cell wall in all conifers and in some hardwoods. This layer contains warty deposits with a still unknown composition. The membranous warty layer seems to be formed by condensation of the protoplast with lignin precursors.[62]

2.2.2 Interactions of Cell Wall Components

There are three possible linkages between lignin and hemicelluloses namely ester, ether, and glycosidic bonds. Different groups such as galacturonosyl residues, glucuronic acids, 4-O-methyl glucuronic acid, and hydroxycinnamic acids are suggested to be involved in ester linkages. The linkage between 4-O-methylglucuronic acid group present in xylan and a free alcohol group on lignin is an example of the ester bonds. The free

alcohol group can also be linked to an arabinose or mannose unit present in arabinoxylan and glucomannan by an ether bond. The latter, is the more common, and also more stable type of linkages between lignin and carbohydrates. There are also indications that lignin in the middle lamella and primary wall is associated with the pectic polysaccharides (galactan, and arabinan) through ether linkages. It has been suggested that glycosidic bonds can also unite lignin and polysaccharides. The benzylic and phenyl alcohol groups are known as the probable connection points in these glycosidic bonds. These linkages are easily cleaved by acid.[26,62]

Hemicelluloses can also bind to cellulose by multiple hydrogen bonds. For instance, xyloglucans are found tightly associated with cellulose microfibers and usually require strong alkali for disassociation. An open stretch of unsubstituted glucosyl residues in hemicelluloses allows extensive hydrogen bonding to cellulose. The mixed linked β-glucans with random $(1\rightarrow 3)$ and $(1\rightarrow 4)$ linkages result in a kinked ribbon conformation, whereas long regions (5-11 glucose residues) of uninterrupted $(1\rightarrow 4)$ linkages are more "cellulose like" and form linear ribbons. From chemical fractionation schemes, it is clear that xylans containing various degrees of branching are tightly associated with the cellulose microfibrils.[26]

2.3 Feedstock Pretreatment and Microbial Digestion

Conversion of lignocellulosics to value-added products, such as ethanol, requires specific fermentable sugars (e.g., glucose and xylose) as substrates for downstream

fermentation. Cellulose, the major source of glucose in biomass, is embedded in a hemicellulose-lignin network. This network retards cellulose biodegradation by cellulolytic enzymes. In order to remove the protecting shield of lignin-hemicellulose, and make the cellulose more readily available for enzymatic hydrolysis, biomass must be pretreated. Pretreatment can be carried out by physical means (e.g., high energy radiation, steam explosion, and ball milling), chemical agents (e.g., acid or basic catalysts), or biological treatments.[23] Physical and biological treatments are not generally as effective as chemical pretreatments. Although, they are less problematic in terms of generation of waste and operating costs.

Pretreatment can affect the structure of biomass by solublizing or otherwise altering hemicelluloses, altering lignin structure, reducing cellulose crystallinity, and increasing the available surface area and pore volume of the substrate. During pretreatment, hemicelluloses may be hydrolyzed to their monomeric constituents, and lignin-hemicellulose-cellulose interactions are partially disrupted.[22,32,74]

In chemical processes, the choice of solvent or swelling agent is important. In this respect water has many obvious advantages. Water removes hemicelluloses from lignocellulosics through a combination of dissolution and autohydrolysis at temperatures ranging from 120 °C to 200 °C. It is the cheapest solvent known; is nonflammable and has no fire or explosion hazard. It can also be recovered and used for other downstream processes, such as enzyme production and fermentation. Although water is a good solvent for hydrolysis products, it cannot dissolve lignin and its degradation products to a great extent. These insoluble materials will remain with the pretreated solids and may hinder the availability of cellulose by cellulolytic enzymes.[32]

The ammonia freeze explosion (AFEX) process uses liquid ammonia under moderate pressure (15 atm) and mild temperatures (50-80 °C). The advantage of AFEX process, as compared with steam explosion method, is that in this technique the base-catalyzed degradation of sugars is avoided as a result of low treatment temperatures. During steam explosion treatments, autohydrolysis may produce different degradation products.[23,74]

Organic solvents, such as *n*-propylamine and ethylenediamine (EDA) can remove the lignin and cause swelling of the biomass. The former agent reduces the crystallinity index of the swollen pulp, and has an inhibitory effect on the cellulase enzymes, while the latter, causes a small increase in the crystallinity index with a significant increase in the swelling of the hardwood.[74]

In alkaline-catalyzed pretreatments, the base is usually consumed by neutralization of carboxylic acids and phenolic compounds present in the biomass or formed during the pretreatment reactions. Grohmann et al.[23] has estimated the minimum consumption of sodium hydroxide for pretreatment of wheat straw and aspen wood to be between 5 and 20% based on dry biomass.

In acid catalyzed pretreatments, while hydrolysis of hemicelluloses is the dominant effect, the lignin is also solublized. Although, lignin may recondense and form an altered lignin polymer that can hinder the availability of cellulose. Acid pretreatment requires less catalyst that are also cheaper than alkali and organic solvents. Considering the low cost of common acids such as H_2SO_4, chemical cost can be substantially less than that of base dependent processes.[23,74]

Among all pretreatment procedures, acid pretreatment has always been favored for providing efficient hydrolysis, fast reactions (compared with enzymatic process), and low cost of required hydrolyzing agent (e.g., H_2SO_4).[23] In this method, the catalyst (hydrated hydrogen ion) with a 4-A° size, can reach all the glucosidic bonds of different types of biomass with relatively the same ease.[22] The process of acid pretreatment is also very simple and has many economic advantages particularly in terms of plant cost.[59] This method, however, suffers from problems, such as sugar decomposition and necessity of acid recovery when concentrated acid solutions are used.[1]

An acid-catalyzed pretreatment plant, would consist of a reaction unit (i.e., reactor) and an acid recovery or neutralization unit. A sugar-acid separation unit is also required to separate and wash the sugars from acid, and make it ready for the fermentation process. An appropriate reactor for acid pretreatment process must:[59]

1. have enough capacity to handle the incoming biomass, and provide enough product for the fermentation unit,

2. provide proper residence time required for pretreatment at given reaction conditions, and

3. be corrosion resistant.

The pretreatment reaction conditions, i.e., time, temperature, and acid concentration, are the key parameters for design and operation of such a unit.

Both temperature and concentration of acid in the reactor affect the rate of hydrolysis reaction. Acid, however, is the major cost effective component of such processes, since heating requirements can be inexpensively provided by incineration of either raw biomass or process waste (lignin cake). The pretreatment processes using

concentrated acid are not economically feasible, because of the high cost of consumed or wasted acid, expenses associated with required acid recovery units, and also cost of neutralizing chemicals, such as lime and gypsum.[21,40] Pretreatment of lignocellulosics under harsh conditions will degrade the hydrolyzed monomeric sugars (xylose) to non-fermentable byproducts, such as furfural. The formation of byproducts decreases the sugar yield, and consequently the ethanol production capacity. Byproducts have inhibitory effects on the fermentation microorganisms. Cellulose degradation and lignin condensation are two other undesirable results of severe pretreatment conditions. Cellulose degradation lowers the total ethanol equivalent value, and lignin condensation makes the cellulose less accessible for microorganisms or enzymes. The dilute sulfuric-acid pretreatment at high temperatures uses a relatively cheap acid in small quantities and eliminates the need for an expensive acid recovery unit. In this method, the effect of low acid concentration is augmented by high temperatures (>140 °C) which will result in an effective xylan hydrolysis with less sugar degradation. The reaction conditions, i.e., time, acid concentration, and temperature must be optimized such that the cellulose structure remains intact while hemicelluloses are effectively removed from the solids. Pretreatment at such optimum conditions will limit the degradation of monomeric sugars (pentoses) in the resulting liquid stream and provide a sugar-rich prehydrolysate that can also be used to produce ethanol.

The pretreated solids as well as prehydrolysate liquid contain fermentable sugars that can be converted to ethanol. The solids contain the "available cellulose" that must be reduced to glucose monomers to be metabolized by fermentative microorganisms. The break down of cellulose polymers, known as saccharification, is the most significant

remaining problem for production of ethanol fuel from biomass.[12] Saccharification of cellulose can be carried out either enzymatically or by acid hydrolysis. Although sulfuric acid is cheaper than enzymes, and acid hydrolysis reaction is fast, it requires severe conditions and produces lower yields.[7] Decomposition of glucose during acid hydrolysis is the major problem in this method. It lowers the ethanol production yield as a result of sugar loss, and produces byproducts, such as hydroxymethyl furaldehyde (HMF) which are toxic to ethanologenic microorganisms.[42,59,63] HMF can be readily converted to formic and levulinic acids. Reversion and epimerization are two other undesirable side reactions by which glucose is lost. Reversion is the polymerization of glucose units by which di- and oligosaccharides, such as maltose and cellobiose are formed. Epimerization of glucose monomers results in a mixture of 64% D-glucose, 20% D-fructose, and 3% D-mannose equilibrium solution.[63]

The enzymatic sacharification involves the depolymerization of cellulose by the act of celluslase enzymes. The advantages of this method are mild reaction conditions and large sugar yields, but the cost of cellulase enzyme is very high and conversion rates are low. Crude cellulase systems consist of 1,4-β-D-glucan glucanhydrolase (endonglucanases), 1,4-β-D-glucan cellobiohydrolases (exoglucanases), and β-D-glucoside glucohydrolase (β-glucosidase). The cooperative activity of endo- and exoglucanases hydrolyzes the cellulose to cellobiose, which is then converted to glucose by β-glucosidase. The enzymatic activity of exo- and endo-enzymes is inhibited by the presence of cellobiose, and to a lesser extent glucose. Such inhibitory effects reduce the rate of sugar production, and increase the cost of cellulose bioprocessing.[45] The following equation shows this phenomenon:

$$Cellulose \xrightarrow{\ Cellulases\ } Cellobiose \xrightarrow{\ \beta-glucosidase\ } Glucose$$
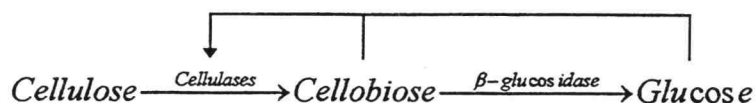
The accumulation of cellobiose may be avoided by using enzymes with higher β-glucosidase activity, supplementing the enzyme broth with additional β-glucosidase, or using yeast mutants that are able to ferment glucose and cellobiose simultaneously.[4,64] Mixed cultures of *Sacharomyces cerevisiae*, a strong glucose fermenter, with a β-glucosidase producing strain, such as *Brettanomyces clausenii*, has also proven to improve the yield of ethanol production considerably.[64] On the other hand, β-glucosidase itself is strongly inhibited by accumulation of glucose (product inhibition) in the reaction mixture. Removal of glucose from the reaction environment will increase the activity of β-glucosidase, hence the breakdown of cellobiose. Lower levels of cellobiose, in turn, increase the activity of cellulase enzymes, and therefore, the rate of the saccharification reaction. The simultaneous saccharification and fermentation (SSF) method can keep the concentration of glucose and cellobiose in the fermentation media at very low levels, and improve the yield of ethanol production.

During the SSF, glucose units, produced from depolymerization of cellulose, are simultaneously converted to ethanol, and hence, removed from the reaction mixture. This effect minimizes the enzyme product inhibition. Besides, the presence of ethanol reduces the potential of microbial contamination in SSF reactors. The SSF process improves both reaction kinetics and process economics. The improvement in process economics results from low enzyme loading requirement, higher ethanol yields, and possibility of using a

single reactor for both saccharification and fermentation reactions. The SSF unit operation is still the major contributor to the cost of ethanol (>20%), and requires further improvement and performance optimization. [45,53]

Saccharomyces cerevisiae, Zymomonas mobilis, klebsiella oxytoca have been successfully used for fermentation of glucose to ethanol with yields well above 70% of theoretical yield. Although the optimal hydrolysis temperature for cellulase enzyme is 45 °C, microbial cells lose their viability at such a high temperature. Temperatures between 37 and 40 °C are the optimum temperatures for the SSF process. Several thermotolerant strains of yeast capable of fermenting glucose at temperatures above 40-48 °C have also been used.[35]

Ethanol does not pose any significant inhibition on β-glucosidase, nevertheless, it may have moderate inhibitory effects on cellulases and cells.[53] Therefore, removal of ethanol form the SSF reactor can increase the yield of ethanol production. It has been suggested to recycle the fermentation broth through a vacuum flash vessel so that ethanol can evaporate at operating temperature (37 °C).[20]

Economic utilization of lignocellulosic biomass necessitates the use of xylose-rich acid prehydrolysate, in addition to cellulose-rich solids, for the production of ethanol. Xylose is the major product of acid hydrolysis of xylan polymers. The acid prehydrolysate also contains small quantities of arabinose, glucose, galactose, mannose and cellobiose.[18] However, the amount of these sugars (except xylose) is too small to be considered for fermentation.[5] The two major concerns in fermentation of pentoses are: selection of yeast or microbial strains which can metabolize pentoses, and inhibitory effect of xylan degradation products on these microorganisms. Bacteria and yeasts as well as fungi can

ferment xylose. The yeast strains *Pachysolen tannophilus, Candida shehatae, Clostridium thermosaccharolyticum, and Pichia stipitis* give the highest product yield compared to bacteria and fungi.[5,51,70,77] These yeast strains usually require very low levels of oxygenation for maximum ethanol production. The exact level of aeration, however, has not been determined. Insufficient oxygenation will result in production of xylitol or may cause the ethanol to be reassimilated.[5] It is also possible to isomerize xylose to xylulose with glucose isomerase, and then use *Saccharomyces cerevisiae* to convert the xylulose to ethanol.[5]

The following byproducts are always present in acid prehydrolysates in different quantities: furfural, acetic acid, phenolics, aromatic acids and aldehydes, sulfite, lignosulfonates, as well as ions from hydrolysis and neutralization.[5] These compounds are toxic to ethanologenic microorganisms and can hinder the microbial fermentation of xylose.[5,51,70,77] Some of these compounds are derivatives of lignin and extractives. For aspenwood, the major derivatives of lignin hydrolysis are vanillin, syringaldehyde, vanillic acid, and syringic acid. In red oak acid prehydrolysates the following additional compounds were also identified: dihydroconiferyl alcohol, syringol methyl ketone, dihydrosinapyl alcohol, and β-oxisinapyl alcohol. The major derivatives of extractives are caproic acid, caprylic acid, pelargonic acid and palmitic acid.[70] Acetic acid and furfural are by far the most produced byproducts of biomass acid hydrolysis, and have the strongest inhibitory effect on the microorganisms.[5,70] Acetic acid is the product of the hydrolysis of acetyl groups on the xyaln backbone, and furfural is produced by degradation of xylose.

Pretreatment of acid prehydrolysate with ion-exchange resins or molecular sieves, use of steam stripping, or providing strain adaptation can remove some of the inhibitory

byproducts form the acid prehydrolysates.[5,70] The ion resins and molecular sieves were found particularly effective in removing acetic acid and furfural.[70] The *S. cerevisiae 354*, if inoculated with pentose fermenting strains, can convert furfural to furfuryl alcohol, hence removing furfural from the fermentation broth.

3. MATERIALS AND METHODS

In this work, three lignocellulosic feedstocks were pretreated: switchgrass, poplar chips and corn stover. Switchgrass and poplar are two of the three species identified as model energy crops in the United States, i.e., switchgrass (*Pancium virgatum*), hybrid poplar (*populus spp.*) and hybrid willow. Corn stover, produced more than 150 million tons every year, is the most abundant agricultural residue in the United States, and after bioconversion can furnish 10% of the nation's liquid fuel requirements.[9] It has been estimated that inclusion of corn stover (with corn cob and kernel) will increase the energy efficiency ratio (energy out/energy in) of ethanol production from 6.7 to 8.8.[14]

Switchgrass is a tall-growing, perennial prairie grass, native to North America. It has shown promising potential as a dedicated biofuel feedstock crop. It is highly adaptable to different climates, is tolerant to drought conditions, and produces high yields with relatively low inputs.[48] It can also offer many environmental benefits, such as soil stabilization, and provide excellent protective cover and nutritive value to wild life. Soil erosion and loss of soil organic matter caused by continuous annual cultivation of row crops (e.g., corn) and subsequent polluting effects on aquatic systems are significantly reduced by planting perennial grasses (e.g., switchgrass). Switchgrass produces dry weight yields 2 to 7 times greater than average forage grass yields under similar conditions, and requires less energy to produce than does corn.[19] High yields of switchgrass may be produced without irrigation and annual cultivation and planting cycle of annual crops, and with nitrogen and phosphorous fertilizer requirements that are typically one-fourth to one-

half of those for corn production. An acre of switchgrass will produce 20.6 times the energy required to produce it if it is transported directly to the ethanol plant.[13]

Hybrid poplar and hybrid willow are the two final candidates of 154 short-rotation woody crops tested as the potential woody feedstoks for ethanol production. Both species can grow in a variety of climatic conditions, and can be used as multi-purpose feedstocks for pulp and paper industry and bioenergy production. Considering high yield (4.6 dry tons/acre/year) and short growth period of poplar (7-8 years), it is expected to provide a continuous feedstock source for ethanol production.[13]

3.1. Experimental Methods and Design

3.1.1 Pretreatment Experiments

Herbaceous (corn stover and switchgrass) and woody (hybrid poplar) feedstocks were provided by the National Renewable Energy Laboratory (NREL). Each feedstock was pretreated with dilute sulfuric acid with final concentrations of 0.6%, 0.9% and 1.2% at three temperature levels (140, 160 and 180 °C). Reaction times varied between 1 and 60 minutes at 140 °C and between 0.5 and 5 minutes at 160 and 180 °C. A matrix of approximately 80 data points (including replicates) was generated for each feedstock. Table 3.1 shows an example of such a matrix. After measuring the moisture content, 30.00 g of feedstock was weighed and stored in sealed plastic bags. The amounts of water and acid solutions for each reaction condition were calculated using the moisture content

Table 3.1. The experimental design for pretreatment of lignocellulosic feedstocks. Squares show the replicates.

C:	T: 140 °C		160 °C		180 °C	
	1	[1]	0.5	[0.5]	0.5	[0.5]
	5	[5]	1	[1]	1	[1]
0.6%	10		1.5		1.5	
	15	[15]	3	[3]	3	[3]
	30		5	[5]	5	[5]
	60	[60]				
	1		0.5		0.5	
	5	[5]	1	[1]	1	[1]
0.9%	10		1.5		1.5	
	15	[15]	3	[3]	3	[3]
	30		5	[5]	5	[5]
	60	[60]				
	1	[1]	0.5	[0.5]	0.5	[0.5]
	5	[5]	1	[1]	1	[1]
1.2%	10		1.5		1.5	
	15	[15]	3	[3]	3	[3]
	30		5	[5]	5	[5]
	60	[60]				

value, and a final solid concentration of 10% (w/w). Below, is a series of such calculations for corn stover (7.7% moisture content):

Weight of feedstock sample (WW) = 30 g

Moisture content (MC) = 7.7%

Solid percentage (SP) = 10%

Acid concentration (C) = 0.6%, 0.9% and 1.2% (w/w)

Feedstock dry weight (DW) = WW . (1- MC/100) = 27.69 g

Total mass (acid+water+substrate) in reactor (TM) = DW/SP = 27.69/0.1 = 276.9 g

Weight of required concentrated sulfuric acid (WCA) = C . TM

$\quad$ $WCA_{0.6\%}$ = 0.006 TM = 0.006 x 276.9 = 1.66 $\qquad$ g of solution A (see below)

$\quad$ $WCA_{0.9\%}$ = 0.009 TM = 0.009 x 276.9 = 2.49 $\qquad$ g of solution B (see below)

$\quad$ $WCA_{1.2\%}$ = 0.012 TM = 0.012 x 276.9 = 3.32 $\qquad$ g of solution C (see below)

In order to avoid large variations in the volume of injected acid at different conditions, it was necessary to prepare three acid stock solutions (solutions A, B and C) with different concentrations. In this way, by using the more concentrated stock solution for higher acid requirements the weight of injected acid remained nearly constant. The concentration of pure sulfuric acid, used for preparing the stock solutions, was measured by titration with NaOH (0.5 N) solution:

Concentration of pure sulfuric acid ($[H_2SO_4]_{96.92\%}$) = 96.92% (w pure $\bar{H}_2SO_4$ / w solution)

Solution A: {50 g $[H_2SO_4]_{96.92\%}$ + 500 g H_2O}

$C_A = (50 \times 0.9692)/(500+50) = 0.088$ g/g

Solution B: {150 g $[H_2SO_4]_{96.92\%}$ + 1000 g H_2O}

$C_B = (150 \times 0.9692)/(1000+150) = 0.126$ g/g

Solution C: {200 g $[H_2SO_4]_{96.92\%}$ + 1000 g H_2O}

$C_C = (200 \times 0.9692)/(1000+200) = 0.161$ g/g

<u>W</u>eight of required <u>d</u>ilute sulfuric <u>a</u>cid (WDA_i) = WCA_i/C_j where i=0.6%, 0.9% or 1.2%

j=A, B or C

$WDA_{0.6\%} = 1.66/0.088 = 18.87$ g

$WDA_{0.9\%} = 2.49/0.126 = 19.76$ g

$WDA_{1.2\%} = 3.32/0.161 = 20.62$ g

Weight of required water (H_i) = TM - (WDA_i + WW)

$H_{0.6\%} = 276.9 - (18.87 + 30) = 228.03$ g H_2O

$H_{0.9\%} = 276.9 - (19.76 + 30) = 227.14$ g H_2O

$H_{1.2\%} = 276.9 - (20.62 + 30) = 226.28$ g H_2O

Thus, the weights of water and acid solutions used at different conditions are almost equal.

The pretreatment experiments were carried out in a Parr reactor model #4563. Figure 3.1 shows a schematic diagram of this reactor. This 600-ml stainless steel reactor
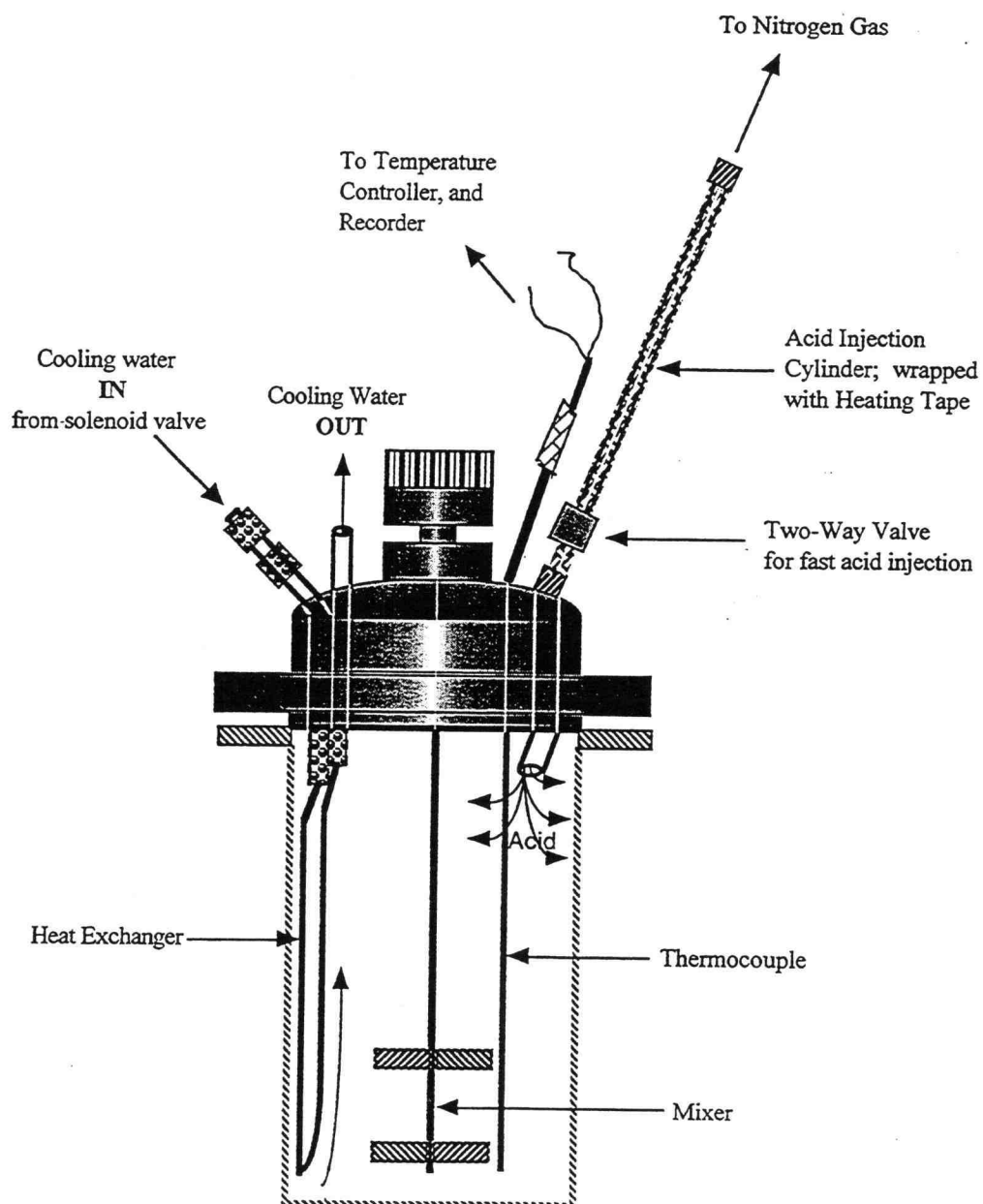
Fig 3.1. Stainless steel reactor (Parr model 4563) equipped with acid injection system, mixer and heat exchanger

was equipped with a glass liner, a heat exchanger, and an impeller type mixer. The glass liner was used to prevent the corrosive effect of sulfuric acid on the stainless steel reactor. The required amount of distilled water was added to the substrate (30.00 g) in the glass liner. The resulting aqueous solution was heated to the desired temperature (140, 160, or 180 °C), and the reaction was initiated by the injection of preheated acid (0.6, 0.9, or 1.2% w/w). The warm-up times for reaching temperatures 140, 160, and 180 °C were 17.5, 21, and 26 min, respectively. The acid injection system consisted of an 8-inch Monel cylinder (I.D. 0.5") which was connected in series between a nitrogen tank and the reactor. The acid injection cylinder was wrapped with a heating tape, and a plug valve separated this cylinder from the reactor. The acid injection pressure exceeded the anticipated internal reactor pressure by 15 psi. The injection system could handle pressures up to 600 psi, corresponding to temperatures above 200 °C. The injection time was about 5 seconds, and time zero for all reactions corresponded with acid injection. Approximately 20g of acid solution was used for each reaction. The exact mass of acid depended on the desired acid concentration in the reactor (see the calculations above). Test runs indicated that 98.5% of the acid loaded into the injection cylinder is delivered into the reactor with a coefficient of variation of less than 1%. The reactor temperature was controlled by a Parr controller model #4831. A solenoid valve adjusted the water flow through the internal heat exchanger, and regulated the temperature at ±1 °C. Each reaction was terminated by running cold water through the internal heat exchanger while simultaneously removing the heating mantel and spraying the external surface of the reactor with cold water. The chosen method allowed constant stirring while cooling, thereby maintaining a relatively

homogeneous environment. This method provided a 20-degree temperature decrease within less than 30 seconds, hence, a 3-fold decrease in the reaction rate. As a result, the reaction was effectively terminated after 30 seconds. The pretreated materials were then filtered (Whatman No. 5) and washed with distilled water (~1500 ml). The total volume of the liquid stream (both filtrate and wash) was recorded, and a 125-ml sample was stored at 4°C until analyzed. The solids residue was dried in oven at 45 °C for 24 hours.

3.1.2 Prehydrolysate Fermentation

Innoculum Culture Preparation: In a baffled flask, 200 ml of medium containing 30 g/l xylose and 10 g/l yeast extract were inoculated with a vial of previously grown and frozen *Pichia stipitis* culture. The pH was maintained between 5.0 and 6.0 by addition of $1N$ NaOH. When the dry cell mass (DCM) reached 5.0 g/l (24 hours), the cells were centrifuged and enough yeast extract (10g/l) was added to make the DCM 50 g/l.

Prehydrolysate preparation: Unwashed, dilute-acid biomass prehydrolysate was neutralized to pH 6.0 with $Ca(OH)_2$. The neutralized prehydrolysate was refrigerated overnight, then sterile-filtered through a 0.22μm membrane. Normally, two prehydrolysate concentrations, 40% and 80%, as well as a 0%-prehydrolysate control solution, were prepared for fermentation.

Fermentation medium preparation: The final fermentation volume was 100 ml. Enough xylose solution (600 g/l), yeast extract (20 g/l), innoculum culture (50 g/l), and water were

added to either 40 ml or 80 ml of prehydrolysate to make the final concentrations in each flask 30 g/l xylose, 10 g/l yeast extract, and 5 g/l DCM. Prior to the addition of the innoculum culture, the pH of the medium was aseptically adjusted to 6.0 ± 0.1.

Fermentation: The pH was maintained between 5.5 and 6.0 by addition of 1N NaOH. Samples were drawn for pH measurement and HPLC analysis at regular intervals (every 6 hours). When the xylose concentration reached a minimum, an endpoint sample was taken and the DCM was again measured.

3.2 Analytical Methods

The neutral monosaccharide composition of the constituent polysaccharide in the initial feedstocks was quantified by an HPLC-based assay of the hydrolysate resulting from a two-stage sulfuric acid treatment. Sugars were separated using a Bio-rad Aminex HPX-87P column, and quantified based on the signal from a Waters 401 refractive index detector using Waters Baseline 810 integration software. The neutral monosaccharide composition of hydrolysates from pretreated residues, and the neutral sugar composition of the soluble phase resulting from pretreatments were also determined by this method. Lignin plus other acid insoluble components were determined as Klason lignin. Moisture and ash contents were determined by standard methods as described in NREL Alternative Fuels Division Chemical Analysis and Testing Standard Procedure No. 005.[16] Acetic acid and furfural concentrations of pretreatment filtrates were determined on a Varian 1400 gas chromatograph equipped with a flame ionization detector.

The basic cations of lignocellulosics can partially neutralize the sulfuric acid and lower the acidity of the reaction mixture.[23,38,65] The neutralizing ability (NA) of each feedstock was determined by measuring the pH of sulfuric acid/distilled water (0.6%, 0.9%, 1.2%) solutions before (pH_{dw}) and after (pH_s) mixing with the feedstock. The proportions of substrate, acid and water were the same as in the pretreatment experiments. The following equation (3.1) was used to correlate the change of pH of acid solution to the neutralizing ability (g H_2SO_4/g dry substrate) of each feedstock.

$$NA = MW_{H_2SO_4} \cdot \frac{[10^{-pH_{dw}} - 10^{-pH_s}]}{Solid\ Concentration} \qquad (3.1)$$

The solid concentration in the reactor is expressed as g dry substrate/L liquid (acid + water).

Sugar concentrations in the fermentation media were measured by HPLC using an Aminex 87-H column and refractive index detection. Dry cell mass was measured by taking a 10 ml aliquot of fermentation broth, centrifuging, washing once with distilled water, centrifuging again, and drying the pellet at 80°C overnight in a tared aluminum dish.

4. KINETIC MODEL DEVELOPMENT

Numerous studies have been directed to model the kinetic behavior of hemicelluloses in woody and herbaceous materials during the acid hydrolysis reaction. The proposed mechanism varies from case to case. Some have proposed a series of two consecutive first-order reactions for xylan hydrolysis and xylose degradation: [7,9]

$$Xylan \xrightarrow{\ k_1\ } Xylose \xrightarrow{\ k_2\ } Byproducts$$
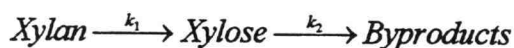
Note that xylan refers to the principal hemicellulose in hardwoods and herbaceous materials, i.e., 4-O-methylglucuronoxylan.

Others assumed more complex and perhaps more realistic mechanisms, such as the following: [54]
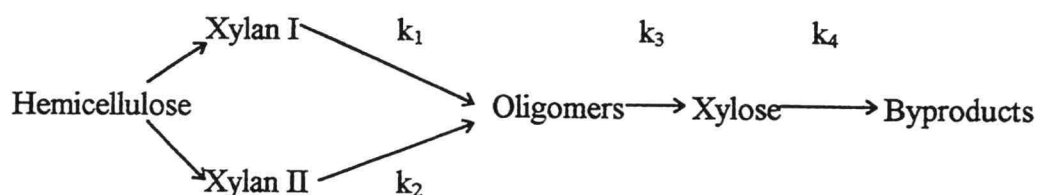
As will be explained later, during the acid hydrolysis reaction, xylan exhibits two distinct reactivities as if it consisted of two distinct fractions, one easy-to-hydrolyze and the other more resistant. In the above model, these two fractions are represented by xylan I and xyaln II. The formation of two xylan fractions are too fast, and concentration of intermediates (oligomers) are too difficult to measure, therefore such detailed mechanisms need to be simplified for modeling purposes.[7,54] It is worth noting here that the xylose

conversion reaction itself is postulated to have a complex scheme. The proposed mechanism takes into account the formation of intermediates, formation of furfural, condensation reaction between intermediates and furfural, and degradation of furfural:[58,67,75]

Xylose $\longrightarrow$ Intermediates $\longrightarrow$ Furfural $\longrightarrow$ Degradation products
$\quad\quad\quad\quad\quad\quad\quad\quad\downarrow$Condensation products $\longleftarrow$
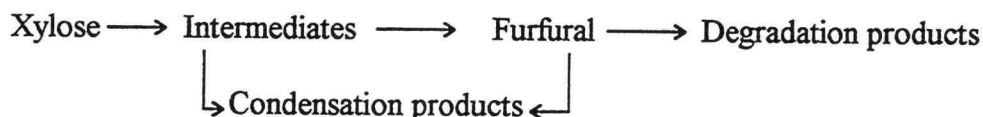
In this study, we used the following reaction scheme which takes into account the biphasic nature of xylan with two distinct rate constants, and assumes a first-order reaction for xylose conversion, and ignores all the other side reactions which will add to the complexity of the kinetic models:

Hemicellulose (Xylan)
$\quad$ Susceptible xylan $\xrightarrow{k_1}$
$\quad$ Resistant xylan $\xrightarrow{k_2}$ Xylose $\xrightarrow{k_3}$ Degradation products
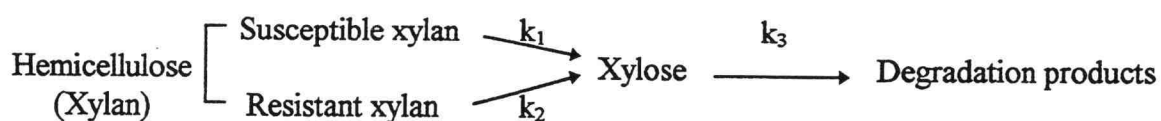
In previous studies the kinetic parameters of hydrolysis rate constants (activation energy and pre-exponential factors) and percentages of fast- and slow-reacting xylan of different feedstocks have been determined, and in some cases the optimum reaction conditions (temperature, acid concentration, time) for maximum xylan removal were identified.[10,23,30,31,39,46] The objective of this study was to develop a unifying kinetic model that could correlate the reaction conditions to pertinent kinetic parameters, such as rate constants, activation energies and percent of susceptible versus recalcitrant hemicellulose

fractions. Such model was used to determine the amounts of xylan remaining in the pretreated solids, net xylose yield in the liquid prehydrolysate, and xylose degraded at any moment during the course of the reaction. Using this model, the optimum pretreatment conditions for maximum xylose recovery in the liquid were also identified. The liquid prehydrolysate obtained from pretreatment of each feedstock at such optimum conditions were fermented, and then, final optimum reaction conditions for maximum ethanol production were identified.

4.1 Xylan Hydrolysis

As mentioned before, results of dilute-acid pretreatment of lignocellulosic feedstocks (hardwoods, softwoods, grasses and wastepaper) indicate that the amount of xylose remaining in biomass changes in a biphasic fashion with time. It has been postulated that such behavior might arise from mass transfer limitations within the biomass structure, hence creating two fractions of xylan, one susceptible and the other more resistant to hydrolyzing agents. Such behavior has also been attributed to the possible structural changes of the hemicelluloses during hydrolysis reaction.[39] For modeling purposes, regardless of the cause of the biphasic nature, xylan is assumed to contain two distinct fractions, i.e., fast- and slow-reacting, and its hydrolysis is represented by the following reaction scheme:[10,23,30,31,39,46]

$$\text{Hemicellulose} \begin{bmatrix} \text{Fast-reacting xylan} \xrightarrow{k_f} \\ \text{Slow-reacting xylan} \xrightarrow{k_s} \end{bmatrix} \begin{array}{c} \text{Xylose} \\ \text{(in liquid)} \end{array} \xrightarrow{k_2} \text{Byproducts}$$

$$(4.1)$$
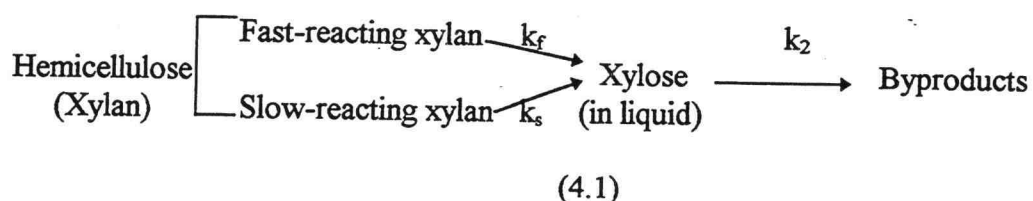
Initially the fast-reacting portion reacts with a high velocity and dominates the other reaction rate. Once this fraction is almost entirely hydrolyzed, the recalcitrant xylan continues to react, though with a much slower rate. The shift form the fast to slow phase is what renders the biphasic nature to the overall reaction, and has been speculated to involve a switch from one rate-controlling step to another. In hydrolysis of susceptible xylan, the rate-controlling step is known to be the hydrolysis of β-(1-4) glycosidic linkages. The rate controlling-step in removal of recalcitrant xylan, however, is not known.[66]

At harsh reaction conditions, high temperatures (e.g., 180 °C), more concentrated acid solutions, or both, we observed that the shift from one phase to another was very abrupt. Under such conditions, the short period (< 1 min) of fast reaction during which the majority of xylan (~85%) was hydrolyzed, was followed by a very slow phase. The latter phase was so slow that no appreciable xylan removal could be observed at typical time intervals used (< 5 min). Thus, it appeared that xylan contained two fractions; "soluble" and "insoluble". This observation led us to the use of a "single rate" model instead of the biphasic model:

```
              k₁              k₂
   ┌─ Soluble ───────→ Xylose ───────→ Byproducts
   │
Xylan
   │
   └─ Insoluble ─┤
```
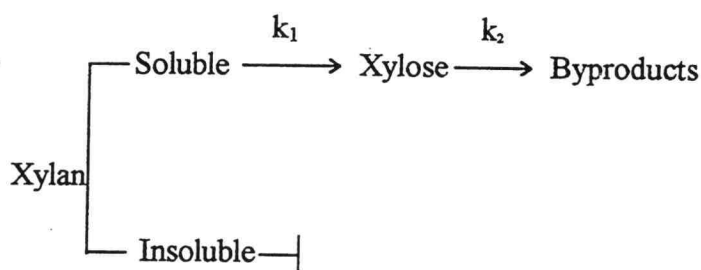
$$X_R = S.e^{-k_1.t} + I$$

where S and I were percentages of soluble and insoluble xylan, X_R was the amount of xylose remaining in the pretreated solids, and k_1 and t represented the reaction rate constant and time, respectively. In this model, xylan was still assumed to have two different fractions, in agreement with the well-established biphasic reaction scheme, nevertheless, there was only one possible reaction and one rate constant (k_1) involved.

The biphasic mechanism (4.1) assumes that both xylan fractions are simultaneously hydrolyzed and reduced to xylose monomers. Upon hydrolysis, different sizes of xylose oligomers are also formed in the liquid phase, concentration of which cannot be easily measured.[7,29,54] The hydrolysis rates of these oligomers are not equal and also differ from that of xylan polymers, hence affecting the rates of overall xylan hydrolysis and xylose degradation. For engineering purposes, it can be assumed that the majority of hydrolyzed sugars in the liquid stream are in the monomeric form and oligomers are instantaneously reduced to produce monomeric xylose.

This kinetic representation (4.1) also assumes that xylose monomers, upon arrival in the liquid stream, are apt to further degradation, with the major degradation product being furfural. The xylose degradation is one of the drawbacks of acid hydrolysis as a

pretreatment method. Reaction conditions desirable for maximum xylan hydrolysis are also very likely to cause a significant portion of monomeric sugars to degrade. The result of degradation reaction is a reduction in yield of fermentable xylose, and formation of byproducts (e.g., furfural) with toxic and inhibitory effects on fermentative microorganisms.[9] In addition to furfural, the byproducts include condensation products of furfural with other intermediates, and degradation products of furfural.[58] Highest yields of furfural were obtained at high temperatures in dilute acid solutions, conditions similar to those used in this study for xylan hydrolysis. Increasing the xylose concentration, however, has an adverse effect on the furfural yield.[58,75]

It should be emphasized here that xylose recovery plays an important role in the economics of the ethanol production process. It is estimated that utilization of lignocellulosic materials may not become economically feasible unless hemicellulosic pentoses are efficiently extracted and fermented to ethanol. Therefore, it is imperative to minimize the extent of xylose degradation, while maintaining a sufficient level of xylan removal from solids. The reaction conditions must be strong enough to effectively remove the xylan form the substrate to produce a cellulose-rich solid phase with increased digestibility, and a xylose-rich liquid prehydrolysate that can be fermented to ethanol. The development of the kinetic model in this section seeks to find the pertinent reaction conditions for maintaining such balance.

In using the biphasic reaction scheme (4.1), hydrolysis of the two xylan fractions and degradation of xylose monomers are assumed to be of first order, and the corresponding reaction rates may be written as:

$$\text{Hemicellulose} \left[\begin{array}{l} \text{Fast-reacting xylan} \xrightarrow{\ k_f\ } \\ \text{Slow-reacting xylan} \xrightarrow{\ k_s\ } \end{array} \right. \xrightarrow{} \begin{array}{c} \text{Xylose} \\ \text{(in liquid)} \end{array} \xrightarrow{\ k_2\ } \text{Byproducts}$$

(Xylan)

$$(4.1)$$
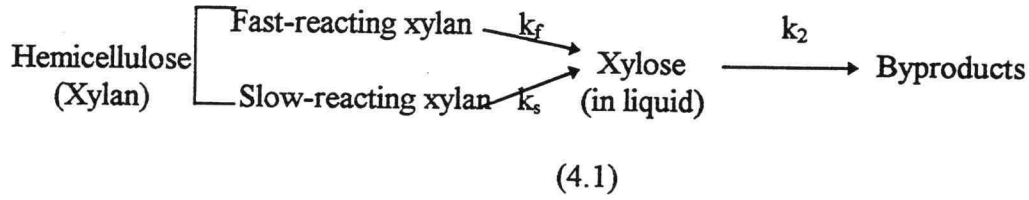
$$\frac{d[xylan]_{fast}}{dt} = -k_f \cdot [xylan]_{fast} \qquad (4.2)$$

$$\frac{d[xylan]_{slow}}{dt} = -k_s \cdot [xylan]_{slow} \qquad (4.3)$$

$$\frac{d[xylose]}{dt} = k_f \cdot [xylan]_{fast} + k_s \cdot [Xylan]_{slow} - k_2 \cdot [xylose]_{liquid} \qquad (4.4)$$

$$\frac{d[Byproducts]}{dt} = -k_2 \cdot [xylose]_{liquid} \qquad (4.5)$$

By integrating equations (4.2) and (4.3):

$$X_f = X_{f_o} \cdot e^{-k_f \cdot t} \qquad (4.6)$$

$$X_s = X_{s_o} \cdot e^{-k_s \cdot t} \qquad (4.7)$$

X_f, X_s: Percentage of fast- and slow-reacting xylan at any time

X_{fo}, X_{so}: Percentage of fast- and slow-reacting xylan at t=0; $X_{so} = 100 - X_{fo}$

k_f and k_s: Rate constants of fast- and slow-reacting xylan hydrolysis (min^{-1})

t: time (min)

Equations (4.6) and (4.7) give the amount of fast- and slow-reacting fractions of xylan remaining in the solids at any moment as a percentage of their initial amounts, X_{fo}, X_{So}, respectively. The total amount of xylan remaining in the solids residue at any moment can be obtained by adding these two portions, i.e., X_f and X_s :

$$X_R = X_f + X_s = X_{f_o} . e^{-k_f t} + X_{s_o} . e^{-k_s t} \qquad (4.8)$$

where X_R is the percentage of total xylose equivalents remaining in the solids residue after pretreatment relative to the initial weight of xylose in the substrate.

The reaction rate constants (k_f, k_s, k_2) are assumed to have an Arrhenius type temperature dependence:[39,46,54]

$$k = A . e^{\frac{-E}{R.T}}$$

A: Pre-exponential factor

E: Activation energy (KJ/mol)

R: 8.3143×10^{-3} (KJ/mol.$^\circ$K)

The pre-exponential factor, A, is known to be dependent upon acid concentration (C_a):

$$A = A_o . C_a^n$$

therefore,
$$k = A_o . C_a{}^n . e^{\frac{-E}{RT}} \qquad (4.9)$$

Other correlations have also been used to express the dependency of reaction rate constants on temperature, acid concentration or both. [24,58]

It is known that both herbaceous and woody materials can potentially neutralize some of the acid solution in which they are impregnated.[24,37,39,65] Since the quantity and concentration of acid affect the kinetics and economics of the pretreatment process, the neutralization ability of the feedstocks must be measured. The neutralization capacity can be used to calculate the effective (actual) acid concentration, the amount of wasted acid, and the amount of acid required to compensate for the neutralization effect.

Wood and grass contain small amounts of soluble organic acids and considerable quantity of carboxylic acid groups (e.g., 4-O-methylglucuronic acid and calcium oxalate). On the other hand, the inorganic cations contained in wood and grass (K^+, Ca^{++}, Mg^{++} and Na^+) are associated with both organic and inorganic anions. Those attached to organic anions are mostly present as 4-O-methylglucuronic salts. Crystalline calcium oxalate is frequently found in wood in small amounts.[24] Some woods, however, contain considerable amounts of this salt, which will also contribute to the neutralization ability of the wood.[24,65] The major inorganic anions are sulfate (SO_4^{-2}), phosphate (PO_4^{-3}), and chloride (Cl^-). Considering the chemical make-up of the feedstock and pH of the acid solution, two types of reactions can affect the acidity of the reaction mixture: 1) replacement of bound inorganic cations with bound hydronium ions (ion exchange); 2) hydrolysis of esters to produce ionized acids.[65] First the ion exchange reaction, at low pH levels (<1.5), the free

organic anion (e.g., 4-O-methylgucuronate) resulting from dissociation of organic salts take up one or more hydronium ions. The resulting acid remains undissociated, because its pK value (=3) is higher than the solution pH (=1.5). The effect is exhibited as an increase in solution pH level. At higher pH levels (>4), however, the produced acid is completely dissociated and no neutralization takes place. As a general trend, the neutralizing capacity of the substrate decreases as the pH increases. The contribution of the salts of inorganic anions (e.g., sulfate and chloride) to the neutralizing ability of the substrate at low pH levels is negligible.[24,65]

The second reaction, the hydrolysis of esters, is also a function of pH. If the pK of the acids produced by the hydrolysis of esters (e.g., acetic, glucuronic) is greater than the pH of the solution in which they are liberated, acids will remain undissociated and do not contribute to the acidity of the solution. The pK values of acetic acid and glucuronic acid are 4.8 and 3.0, respectively, therefore, at pH levels used in this study (<2) the hydrolysis reaction does not affect the acidity of the reaction mixture. At higher pH levels, however, the resulting acids are totally ionized and resulting hydronium ions lower the solution pH.[65] The actual acid concentration in the reaction mixture, C_a, was always lower than the nominal concentration as a result of neutralizing capacity of the feedstocks. The actual acid concentrations (g acid after neutralization/g total mass of reaction mixture) were calculated by equation (4.10). The coefficient (0.1) in equation 4.10 represents the solid ratio in the reactor (g dry substrate/g total mass of reaction mixture) for all pretreatments in this study:

$$C_a = C - (0.1 \times \text{neutralizing ability of the substrate}) \quad (4.10)$$

The neutralizing abilities (g H_2SO_4/g dry substrate) of the three feedstocks are reported in the Results and Discussion section.

As explained before, at each set of reaction conditions (temperature and acid concentration), pretreatments were carried out at different reaction times. The reaction times varied between zero and five minutes at harsh conditions (160 and 180 °C with 0.6%, 0.9% and 1.2% sulfuric acid), and zero to 60 minutes at 140 °C with all three levels of acid concentration. After each pretreatment, the amounts of xylose remaining in the solids residue (X_R), and in the liquid stream were measured using HPLC. All these values were calculated as percentages of equivalent xylose (in solid or liquid) relative to the initial amount of xylose in raw materials.[17] There was no indication of xylan hydrolysis at elevated temperatures (140, 160, 180 °C) when the reaction time was zero. This is in agreement with the observed significant induction period during which the organic esters contained in wood need to be hydrolyzed to form acids.[65]

The kinetic parameters of the biphasic model (eq. 4.8) were calculated by *non-linear regression analyses* on eq. (4.8) using measured values of X_R and corresponding times (t). Thus, for each combination of temperature and acid concentration (a total of nine) one set of (k_f, k_s, and X_{fo}) values were calculated. There was no apparent relationship between the percentage of fast-reacting xylan (X_{fo}) and pretreatment conditions. Therefore, the average value of X_{fo} was used to calculate X_R. The nine sets of k_f and k_s values along with the corresponding temperatures and acid concentrations were

used to determine the kinetic parameters (A_o, n and E) of the reaction rate constants (k_f and k_s) using *linear regression analyses* on transformed Arrhenius equation:

$$\ln(k) = \ln(A_o) + n.\ln(C_a) - \frac{E}{R.T} \qquad (4.11)$$

4.2 Net Xylose Yield in Liquid Prehydrolysate

As previously mentioned, xylose monomers produced by xylan hydrolysis, may be converted to other byproducts, such as furfural. Based on eq. 4.1, the net amount of xylose in the liquid stream (X_L) may be determined by the following differential equation accounting for both formation and possible degradation of xylose:

$$\frac{dX_L}{dt} = k_f.X_f + k_s.X_s - k_2.X_L \qquad (4.12)$$

Using equations (4.6), (4.7) and (4.12):

$$\frac{dX_L}{dt} + k\ X_L = k_f\ X_{f_o}\ e^{-k_f\,t} + k_s\ X_{s_o}\ e^{-k_s\,t} \qquad (4.13)$$

By solving equation (4.13), X_L can be expressed as:

$$X_L = \frac{k_f . X_{f_o}}{k_2 - k_f}(e^{-k_f t} - e^{-k_2 t}) + \frac{k_s . X_{s_o}}{k_2 - k_s}(e^{-k_s t} - e^{-k_2 t}) \qquad (4.14)$$

X_L: Net sugar yield: Percentage of xylose in liquid (relative to initial weight of xylose in starting material)

X_{fo}:   Percentage of fast-reacting xylose at $t = 0$

X_{so}:   Percentage of slow-reacting xylose at $t=0$; $X_{so} = 100 - X_{fo}$

t: time (min)

k_f and k_s: Rate constants of fast and slow reacting xylan hydrolysis (min^{-1})

k_2 is the xylose conversion rate constant (min^{-1}) given by:

$$k_2 = A_{o_2} . C_a^{n_2} . e^{\frac{-E_2}{R.T}} \qquad (4.15)$$

R = 8.3143 X 10^{-3} KJ/mol.^{o}K

T: temperature (^{o}K)

C_a: actual acid concentration (wt %); see equation (10)

As mentioned before, after each pretreatment experiment the xylose concentration in the liquid prehydrolysate (X_L) is measured. It was also noted that in modeling of xylan hydrolysis reaction, nine sets k_f, k_s, and X_{fo} were determined. These values (k_f, k_s, X_{fo} and X_L) along with the corresponding reaction times (t) were used to determine nine k_2 estimates using *non-linear regression analysis* on equation (4.14). The kinetic constants

of k_2 (A_o, n, E) were determined by *linear regression analysis* on equation 4.15 and using the nine k_2 estimates and corresponding temperatures and acid concentrations.

Equation 4.14 reflects the fact that the net yield of xylose in the liquid stream depends on the rate constants of both xylan hydrolysis and xylose degradation reactions. This in turn implies that the overall impact of reaction conditions, i.e., temperature (T) and acid concentration (C), on the net xylose recovery will depend on the relative impacts of these parameters on reaction rate constants. During a pretreatment experiment with optimum reaction time, which is relatively short (< 3 min), hydrolysis of fast-reacting xylan plays the major role in removal of xylan from solids. Therefore, the net xylose yield in the liquid is primarily affected by the magnitude of k_f and k_2. Examining the ratio of these two constants, *selectivity ratio (k_f/k_2)*, with respect to temperature and acid concentration will reveal the effect of reaction conditions on xylose formation and degradation:

$$\frac{k_f}{k_2} = \frac{A_{o,f}}{A_{o,2}} \times C^{(n_f - n_2)} \times e^{\frac{E_2 - E_f}{R.T}} \qquad (4.16)$$

Taking the derivative of this ratio with respect to temperature (T) and acid concentration:

$$\frac{\partial(k_f/k_2)}{\partial T} = \frac{A_{o,f}}{A_{o,2}} \times \frac{C^{(n_f - n_2)}}{R.T^2} \times e^{\frac{E_2 - E_f}{R.T}} \times (E_f - E_2) \qquad (4.17)$$

$$\frac{\partial(k_f/k_2)}{\partial C} = \frac{A_{o,f}}{A_{o,2}} \times C^{(n_f - n_2 - 1)} \times e^{\frac{E_2 - E_f}{R.T}} \times (n_f - n_2) \qquad (4.18)$$

The very last term in each of the above equations determines whether the expression is positive, negative or zero. Three cases can be recognized for each of the above equations (4.17 and 4.18):

Equation (4.17):

    1. if $E_f > E_2$: $d(k_f/k_2)/dT > 0$, therefore, temperature increase favors the xylan hydrolysis reaction.

    2. if $E_f < E_2$: $d(k_f/k_2)/dT < 0$, therefore, temperature increase favors the xylose degradation reaction.

    3. if $E_f = E_2$: $d(k_f/k_2)/dT=0$, therefore, temperature increase affects both reactions equally.

Equation (4.18):

    1. if $n_f > n_2$: $d(k_f/k_2)/dC > 0$, therefore, higher acid concentration favors the xylan hydrolysis reaction.

    2. if $n_f < n_2$: $d(k_f/k_2)/dC < 0$, therefore, higher acid concentration favors the xylose degradation reaction.

    3. if $n_f = n_2$: $d(k_f/k_2)/dC=0$, therefore, higher acid concentration affects both reactions equally.

The following equation can be used to determine the maximum amount of xylose in the liquid stream at a given set of temperature and acid concentration:

$$\frac{dX_L}{dt} = 0 \qquad (4.19)$$

Applying this condition to equation (4.14) gives rise to:

$$\frac{(k_f . X_{f_o})(k_2 - k_f)}{(k_s . X_{s_o})(k_2 - k_s)} = -\frac{1 - (\frac{k_s}{k_2})e^{(k_2 - k_s)t_{max}}}{1 - (\frac{k_f}{k_2})e^{(k_2 - k_f)t_{max}}} \qquad (4.20)$$

where t_{max} is the reaction time (min) at which the net sugar yield in the liquid phase (at a given set of temperature and acid concentration) is maximum. At such conditions, the left hand side of equation (4.20) becomes a constant ($K_{T,C}$), because X_{fo} and X_{so} are constant, and k_f, k_s and k_2 are solely dependent on temperature and acid concentration (not time). Table 4.1 shows the numerical values of $K_{T,C}$ at different conditions for the three feedstocks. It can be shown that at a given set of reaction conditions there is only one (t_{max}) which satisfies this equation. This unique solution may be determined by iteration. The maximum sugar yield (X_L) in the liquid phase may be determined by the following equation which is derived from equations (4.13) and (4.19):

$$X_{L@t_{max}} = X_{f_o} . (\frac{k_f}{k_2}) . e^{-k_f . t_{max}} + X_{s_o} . (\frac{k_s}{k_2}) . e^{-k_s . t_{max}} \qquad (4.21)$$

Table 4.1. The calculated values of $K_{T,C}$ (x 10^{-3}) for a: switchgrass; b: poplar; c:corn stover - to be used with equation 4.20

4.1.a

T→ C↓	140	145	150	155	160	165	170	175	180
0.6%	-6312.0	175.8	82.4	51.5	36.1	26.8	20.6	16.2	13.0
0.7%	546.5	89.6	46.5	30.1	21.4	16.1	12.4	9.8	7.9
0.8%	186.0	51.6	28.6	19.0	13.7	10.3	8.0	6.4	5.1
0.9%	91.5	32.3	18.7	12.7	9.2	7.0	5.5	4.3	3.5
1.0%	52.7	21.4	12.9	8.8	6.5	4.9	3.9	3.1	2.5
1.1%	33.3	14.9	9.2	6.4	4.7	3.6	2.8	2.3	1.8
1.2%	22.4	10.8	6.8	4.8	3.5	2.7	2.1	1.7	1.4

4.1.b

T→ C↓	140	145	150	155	160	165	170	175	180
0.6%	-105.4	3.8	2.0	1.4	1.1	9.0	7.7	6.7	5.9
0.7%	6.2	2.0	1.2	9.1	7.3	6.2	5.3	4.7	4.2
0.8%	2.5	1.2	8.3	6.4	5.3	4.5	3.9	3.4	3.1
0.9%	1.4	8.3	5.9	4.7	3.9	3.4	2.9	2.6	2.3
1.0%	9.4	6.0	4.5	3.6	3.0	2.6	2.3	2.1	1.8
1.1%	6.7	4.5	3.5	2.8	2.4	2.1	1.8	1.7	1.5
1.2%	5.0	3.5	2.8	2.3	2.0	1.7	1.5	1.4	1.2

4.1.c

T→ C↓	140	145	150	155	160	165	170	175	180
0.6%	14.6	9.0	6.1	4.3	3.1	2.3	1.8	1.4	1.1
0.7%	12.0	7.9	5.5	3.9	2.9	2.2	1.7	1.3	1.0
0.8%	10.5	7.1	5.1	3.7	2.8	2.1	1.6	1.3	0.99
0.9%	9.6	6.6	4.8	3.5	2.6	2.0	1.6	1.2	0.96
1.0%	8.9	6.2	4.5	3.4	2.5	2.0	1.5	1.2	0.96
1.1%	8.3	5.9	4.3	3.2	2.5	1.9	1.5	1.2	0.91
1.2%	7.9	5.7	4.2	3.1	2.4	1.8	1.4	1.1	0.89

Finally, the sugar loss (L) can be expressed as the amount of xylose that is neither in solids residue nor in liquid prehydrolysate:

$$L = 100 - (X_R + X_L) \qquad (4.22)$$

By replacing X_R and X_L with equations (4.8) and (4.14), respectively, L can be rewritten as:

$$L = 100 - X_{f_o} \frac{(k_f \cdot e^{-k_2 t} - k_2 \cdot e^{-k_f t})}{(k_f - k_2)} - X_{s_o} \frac{(k_s \cdot e^{-k_2 t} - k_2 \cdot e^{-k_s t})}{(k_s - k_2)}$$

$$(4.23)$$

L: Percentage of xylose loss by degradation reaction
(relative to initial weight of xylose in starting material)

X_R: Percentage of xylose equivalents remaining in the solids residue
(relative to initial weight of xylose in starting material)

X_L: Percentage of xylose in liquid
(relative to initial weight of xylose in starting material)

5. RESULTS AND DISCUSSION

As mentioned before, the warm-up times for reaching the desired temperatures, i.e., 140, 160, and 180 °C, were 17.5, 21, and 24 min, respectively. Such long warm-up times were caused by the presence of two heat transfer barriers: 1) high thickness of the reactor wall, and 2) air gap between the glass liner and inside wall of the reactor. By removing the glass liner the warm-times were lowered to some limited extent, 28% on average. The new warm-up times after removing the glass liner were 12, 15, and 18 min for reaching 140, 160, 180 °C, respectively.

In order to eliminate the need for the glass liner, the reactor has to be made of an acid resistant alloy (e.g., Hastelloy). It is also possible to heat up the reactor internally by passing steam through the internal heat exchanger. Using a Hastelloy reactor with internal steam heating, the heat-up times were lowered to 7.5, 10.5, and 14.5 min for reaching 140, 160, and 180 °C, respectively. These values were about 50% and 30% lower than those obtained in the old reactor with and without glass liner, respectively. Figure 5.1 shows a comparison between the warm-up times using different systems. Although, the new method made it possible to warm up the reactor twice as fast, the warm-up times were still long relative to optimum reaction times (< 1 min). In addition, achieving much shorter warm-up times requires high flow rates of superheated steam (instead of saturated) through the internal heat exchanger. This, in turn, requires modifications in the steam generator.
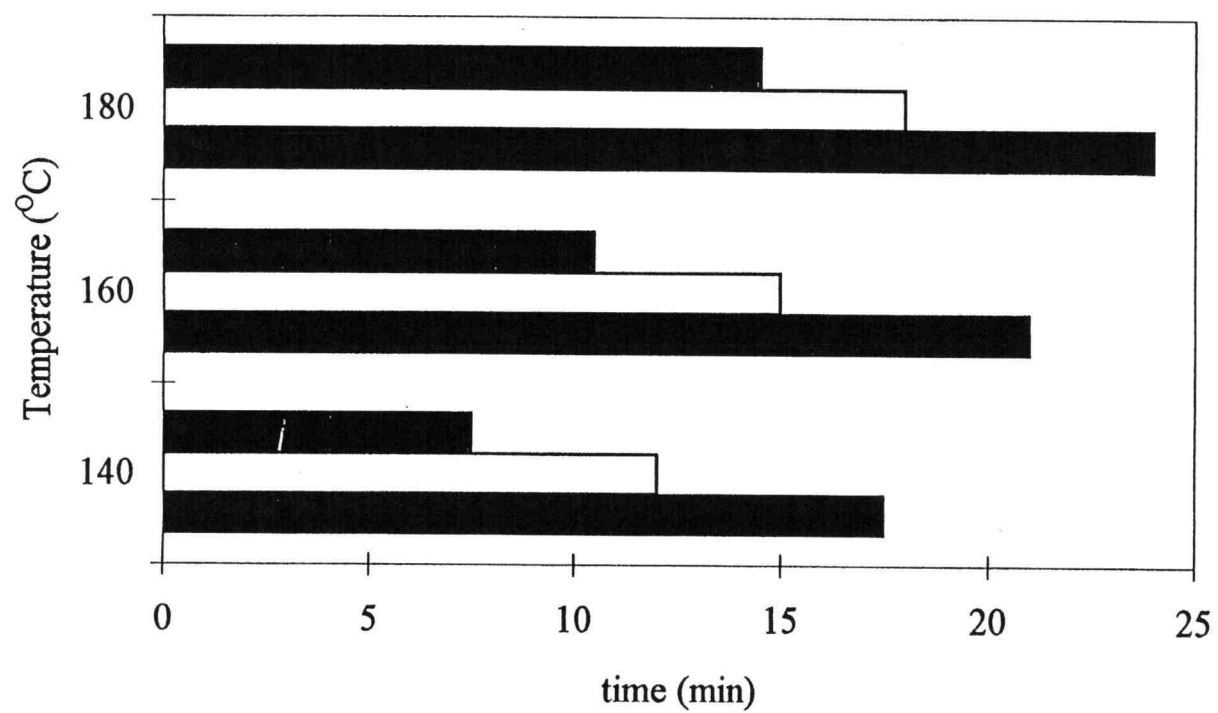
Fig. 5.1 Heat-up times for the batch reactor

■ Mantel+steam w/o glass liner
□ Mantel w/o glass liner
■ Mantel+glass liner

5.1 Acid Hydrolysis of Hemicelluloses

The chemical composition of the three feedstocks, corn stover, poplar and switchgrass, is shown in Table 5.1. The major components of these substrates were glucan, lignin, and xylan. Although the total amount (%) of glycans in all three feedstocks was almost the same, poplar contained 24% and 11% more glucan than switchgrass and corn stover, respectively. The higher glucan content of poplar could conceivably be less accessible, because of its higher lignin content. The percentage of xylan was lower in poplar, but all three feedstocks contained the same percentage of fermentable sugars (glucan+xylan). The herbaceous materials, corn stover and switchgrass, had higher ash contents than poplar. The ash content is a direct representative of neutralizing capacity of a feedstock.

The neutralizing abilities of corn stover, switchgrass and poplar were determined as 43.7 ± 1.2, 25.8 ± 0.8 and 16.7 ± 2.7 mg H_2SO_4/g dry substrate, respectively. Each value is the average of the neutralizing capacity of the unit mass of the substrate measured at three levels of acid concentration. At each level, two measurements were made and averaged. The neutralizing capacity of the feedstocks did not have an apparent relationship with the concentration of acid solution. The higher neutralizing abilities of corn stover and switchgrass were in agreement with their higher ash content. The inorganic cations in these herbaceous materials were perhaps associated with their heavily branched hemicellulose structure. Grass xylans are known to contain L-arabinofuranosyl side chains that may be further coupled to low molecular weight phenolic acids, primarily ferulic and p-coumaric acids, and can be associated with different inorganic cations.[50]

Table 5.1. Compositional analysis of raw biomass samples (wt. %)

Component	Switchgrass		Poplar		Corn Stover	
	Mean	SEM*	Mean	SEM*	Mean	SEM*
Total glycans	56.6	-	58.2	-	59.9	
glucan	32.2	0.29	39.8	0.01	36.0	0.4
xylan	20.3	0.26	14.8	0.03	19.8	0.25
galactan	-	-	-	-	1.3	0.05
arabinan	3.7	0.04	1.2	0.02	2.8	0.06
mannan	0.4	0.02	2.4	0.04	-	-
Klason lignin	19.5	0.3	26.9	0.26	17.8	0.08
Acid soluble lignin	3.7	0.03	2.2	0.06	1.9	0.03
Ash	7.1	0.06	1.3	0.02	7.2	0.01
Uronic acid	1.1	0.01	2.4	0.13	nd[Ψ]	-
Other	12.0	-	9.0	-	13.2	-

* Standard Error of Mean

Ψ not determined

The neutralizing ability of poplar (16.7 mg H_2SO_4/g dry wood) was considerably higher than those reported for other hardwoods, e.g., paper birch (3.5 mg H_2SO_4/g dry wood) and red oak (8-9 mg H_2SO_4/g dry wood).[39] This difference is in agreement with the higher ash content (1.3%) of poplar used in this study relative to those of other hardwoods and other types of poplar. The reported ash contents of different wood species are as follows: 0.7% and 0.6% for two types of hybrid poplar (NE 388 and N11)[69] ;0.8% for aspen wood, 0.4% for sweetgum[23] ;and 0.3% for red oak wood[10]. Springer (1968) has reported lower ash contents for aspen (0.33%), paper birch (0.31%), American elm (0.34%), and red maple (0.25%) woods. The measured pH values of poplar solutions had a large variation resulting in a relatively large standard error of mean. Repeated measurements could not lower this variation. For corn stover, a lower ash content (2%) was reported by Clausen and Gaddy (1983), although the percentages of glucan, xylan, and lignin of their sample were also very different from those found in this study.

High neutralizing capacities, such as those found in this study, have important implications with regard to kinetic parameters and process economics. Corn stover, for instance, could neutralize up to 73% of the sulfuric acid (1.2%) injected into the reaction mixture. Ignoring this effect in kinetic studies could result in obtaining erroneous parameters and yield predictions. From processing standpoint, such high values require the amount of acid to be high enough to exhaust the neutralizing capacity of the feedstock. This increases the process operating cost, and necessitates the use of cheaper acids, e.g., H_2SO_4. The percentage of neutralized acid was much lower for poplar and switchgrass, i.e., 28% and 43%, respectively. Corn stover had a much higher neutralizing capacity than switchgrass, although the ash contents of these two feedstocks were quite similar, 7.2%

and 7.1%, respectively. This difference could be attributed to the difference between the binding strength and valence of the inorganic cations present in each substrate. Harris (1984) has also reported a 40% acid neutralization by red oak wood, and cases in which the net sugar yield was lowered by the neutralizing capacity of wood and herbaceous feedstocks.[24]

The neutralizing abilities in this study were obtained by measuring the pH of raw (untreated) solutions of each feedstock at room temperature. Thus, the effect of reaction time and temperature level on the neutralization reaction was not accounted for, primarily because it was not possible to take liquid samples from the reactor during the course of pretreatment. Nevertheless, the pH of liquid prehydrolysates resulting from pretreatment of corn stover at different conditions (time, acid concentration, temperature) were measured, and analysis of the data indicated that the pH at each level of acid concentration did not depend on the reaction time and temperature, and that the acid neutralization reaction occurred instantaneously. This was also observed in working with untreated solutions in which the pH of acid solutions dropped upon mixing with the feedstock, and did not change even after 30 minutes. The average pH values of prehydrolysates, resulting from pretreatment of corn stover, were in full agreement with those obtained from untreated samples.

The kinetic parameters of the biphasic model (eq.8) and reaction rate constants (k_f, k_s) are presented in Table 5.2. The model generated values using these parameters were in close agreement with the experimental data. The correlation coefficient (R^2) between the experimental and predicted values were 0.97, 0.96, and 0.95 for poplar, switchgrass and corn stover, respectively. The intercept was always statistically insignificant.

Table 5.2. Kinetic parameters of the biphasic model: $X = X_f \cdot e^{-k_f t} + X_s \cdot e^{-k_s t}$ for switchgrass, poplar and corn stover

		Switchgrass	Poplar	Corn Stover
	$X_{fo \, (average)}$:	76.8%	83.8%	64.4%
k_f:	Ao	1.9×10^{21}	3.3×10^{21}	6.7×10^{16}
	E (KJ/mol)	169.0	176.7	129.8
	n	0.4	0.4	1.5
	$X_{so \, (average)}$:	23.2%	16.2%	35.6%
k_s:	Ao	4.2×10^{23}	3.3×10^{22}	6.9×10^{19}
	E (KJ/mol)	201.7	192.0	167.6
	n	2.0	1.5	1.6

The percentage of fast-reacting xylan (X_{fo}) determined at different reaction conditions was virtually independent of reaction conditions, therefore, the average value was used and reported for modeling purposes. The X_{fo} values of corn stover, switchgrass and poplar were comparable to those reported for other lignocellulosic substrates as shown in Table 5.3. The woody feedstock (poplar) had a higher percentage of susceptible xylan (X_{fo}) than herbaceous substrates (switchgrass and corn stover). This was in contrast to the lower xylan content and higher percentage of lignin in this feedstock. Such a difference between woody (aspen) and herbaceous (wheat straw) feedstocks is also reported by Grohmann et al. (1985) with no further explanation (see Table 5.3).

Acid hydrolysis of fast-reacting xylan in corn stover was more sensitive to the concentration of acid than poplar and switchgrass by comparing $\underline{n}$ values (see Table 5.2). This might be due to the difference in chemical bonds and diffusivity of acid into the xylan structure in different feedstocks. This effect was also reflected in potential sugar recovery from this feedstock which will be discussed later.

The activation energy of fast-reacting xylan in poplar was higher than those of switchgrass and corn stover which could be attributed to the difference in chemical composition of hemicelluloses in woody and herbaceous materials. Hardwood hemicelluloses are mainly composed of 4-O-methylglucurono-xylan, whereas in herbaceous materials, arabino-xylans are the principal hemicelluloses.[23,50,62] It is known that the uronic acid side chains stabilize the adjacent backbone linkages.[66] Therefore, the more uronic acid side chains, the more difficult it would be to break down the xylan backbone. The higher uronic acid content (see Table 5.1) of poplar could explain the higher resistance (higher activation energy) of this hardwood to acid hydrolysis, whereas

Table 5.3. Percentage of fast-reacting xylan (X_{fo}) in different types of lignocellulosic feedstocks

Reference	Feedstock	X_{fo} (%)
Conner (1983)	Southern red oak	73.9
	Paper birch	71.6
	Red maple	80.3
	Quacking aspen	76.0
	American elm	84.3
Grohmann et al. (1985)	Aspen	76.0
	Wheat straw	67.0
Maloney et al. (1985)	Paper birch	68.4
Kim and Lee (1987)	Southern red oak	69.7
This study	Corn Stover	64.4
	Switchgrass	76.8
	Poplar	83.8

the herbaceous materials with less uronic acid side chains exhibited less resistance. The initial xylan hydrolysis rates of the three feedstocks at selected temperatures are shown in Table 5.4 to demonstrate the lower rates of xylan hydrolysis in poplar as compared to corn stover and switchgrass.

Table 5.5 summarizes some of the kinetic parameters obtained for other feedstocks along with their references. Although this data are comparable to those found in this study, a direct comparison cannot be made because of the difference between the reaction conditions employed in each study.

The rate of xylose conversion to byproducts (k_2) was thought to be substrate-independent, and literature values were supposed to be used for modeling purposes. It was, however, decided to determine these parameters for each feedstock separately and independently, primarily because the literature data varied widely with reaction conditions, and differed from those reported for pure xylose. Table 5.6 shows the xylose degradation parameters obtained from the pretreatment of poplar, switchgrass and corn stover. Some of the literature values along with their references are presented in Table 5.7.

Our results (Table 5.6) indicate some similarities between the kinetic parameters of xylose decomposition in different prehydrolysates. Activation energies are essentially equal, although the preexponential factors (A_o) and acid dependency exponents (n) are somewhat different (see Table 5.6). Table 5.8 shows the calculated xylose degradation rate constants using the results of this study for different feedstocks at selected conditions. As seen in Table 5.8, there is a very small variation between these values. This discrepancy might be caused by the difference in products of xylan hydrolysis (oligomers) from different feedstocks, and their subsequent effect on xylose degradation. For instance, it is

Table 5.4. The rate constants of fast-reacting xylan hydrolysis (k_f) for switchgrass, poplar and corn stover at selected conditions

Reaction conditions	Switchgrass	Poplar	Corn Stover
(140 °C, 0.6% H_2SO_4)	0.65	0.12	1.19
(140 °C, 0.9% H_2SO_4)	0.77	0.14	2.19
(140 °C, 1.2% H_2SO_4)	0.86	0.16	3.37
(160 °C, 0.6% H_2SO_4)	6.35	1.30	6.84
(160 °C, 0.9% H_2SO_4)	7.47	1.53	12.56
(160 °C, 1.2% H_2SO_4)	8.38	1.71	19.34
(180 °C, 0.6% H_2SO_4)	50.46	11.35	33.60
(180 °C, 0.9% H_2SO_4)	59.34	13.34	61.72
(180 °C, 1.2% H_2SO_4)	66.58	14.97	95.02

Table 5.5. Kinetic parameters of xylan hydrolysis rate constant:

$$k = A_o \cdot C^n \cdot e^{-E/RT}$$

Reference	Feedstock	Parameter		
		A_o	n	E (KJ/mol)
Grohmann et al. (1985)	Wheat Straw: k_f	nd[*]	nd	50.2
	k_s	nd	nd	104.6
	Aspen Wood: k_f	nd	nd	117.2
	k_s	nd	nd	154.8
Kim and Lee (1986)	Southern Red Oak: k_f	1.04×10^{14}	1.54	120.1
	k_s	6.00×10^{12}	1.19	118.0
Maloney et al. (1985)	Paper Birch: k_f	2.67×10^{16}	1	126.6
	k_s	1.16×10^{19}	1	156.5
Bhandari et al. (1983)	Corn Stover	$10^{20}(7.64-3.68/C)$	-	172.2
This study	Switchgrass: k_f	1.9×10^{21}	0.4	169.0
	k_s	4.2×10^{23}	2.0	210.7
	Poplar: k_f	3.3×10^{21}	0.4	176.7
	k_s	3.3×10^{22}	1.5	192.0
	Corn Stover: k_f	6.7×10^{16}	1.5	129.8
	k_s	6.9×10^{19}	1.6	167.6

[*] not determined

Table 5.6. Parameters of xylose degradation rate constant in acid hydrolysis of switchgrass, poplar and corn stover; $k_2 = A_{o2} . C^{n_2} . e^{-E2/RT}$

	Switchgrass	Poplar	Corn Stover
A_{o2}	3.8×10^{10}	8.5×10^{10}	3.7×10^{10}
E_2 (KJ/mol)	99.5	102.0	98.4
n_2	1.45	0.55	0.50

Table 5.7. Kinetic parameters of xylose degradation rate constant:

$$k = A.e^{-E/RT}$$

Reference	Feedstock	Parameter	
		A	E(KJ/mol)
Bhandari (1983)	Corn Stover	$10^{14}(4.60-1.95/C)$	134.4
Mehlberg & Tsao (1979)	Corn Cob	1.36×10^{12}	109.2
Dunlop & Peters (1953)	Pure Xylose	$10^{15}(2.95C_{H+})$	134.4

Table 5.8. The xylose degradation rate constants (k_2) for switchgrass, poplar and corn stover at selected conditions; and with average kinetic parameters: $A_o=5.33 \times 10^{10}$, $E=100$ KJ/mol, and $n=0.83$

Reaction conditions	Switchgrass	Poplar	Corn Stover	k_2 calculated with average parameters
(140 °C, 0.6% H_2SO_4)	0.00	0.01	0.01	0.01
(140 °C, 1.2% H_2SO_4)	0.01	0.01	0.01	0.01
(160 °C, 0.6% H_2SO_4)	0.02	0.03	0.04	0.03
(160 °C, 0.9% H_2SO_4)	0.03	0.04	0.05	0.04
(180 °C, 0.6% H_2SO_4)	0.06	0.11	0.13	0.10
(180 °C, 1.2% H_2SO_4)	0.17	0.16	0.18	0.18

reported that in dilute sulfuric acid solutions of red oak wood at maximum xylose yields, up to 30% of the total xylose was present in oligomeric form.[24] Moreover, the xylose degradation reaction has a complicated scheme, as explained in Kinetic Model Development section, which can affect the parameters of xylose degradation.

In order to examine whether or not the xylose degradation rate constant is truly independent of feedstock, at least for those studied in this work, the xylose degradation kinetic parameters (A_o, E, n) of the three feedstocks were averaged and resultant values were used to calculate a new series of k_2 values which are shown in the last column of Table 5.8. The new values were very close to those specifically determined for each feedstock. It appears that the average values (A_o=5.33X10^{10}, E=100 (KJ/mol), n=0.83) could be essentially assumed substrate-independent and used for calculating the net xylose yield with no major error.

It is worth noting that although the pure xylose degradation rate constant (eq. 5.1), given by Root (1959), can be used under a broad range of reaction conditions it has some limitations. The proposed rate constant depends on the xylose concentration in the reaction mixture. On the other hand, during the xylan hydrolysis reaction, xylose concentration is constantly changing and this model would not be appropriate for predicting the rate of xylose degradation.

$$k = 2.72\alpha.\delta.\gamma.(C_A).e^{[-35.7(473.1-T)(\frac{1}{T})]} \qquad (5.1)$$

k: xylose degradation rate constant

T: absolute temperature (^{O}K)

C_A: acid normality

α: a function of xylose molarity

δ: a function of temperature

γ: a function of temperature and acid normality

Moreover, as the xylan hydrolysis reaction proceeds, the volume of the reaction mixture changes. This in turn changes the values of α and γ, hence making the model less reliable for predicting the xylose decompostion rate under pretreatment conditions.

Figures A.2 through A.10 of the Appendix show the experimental and model-predicted values of xylose remaining in the feedstock solids residue (X_R) after pretreatment at different reaction conditions. These figures show that increasing acid concentration and pretreatment temperature increase the reaction rate as well as ultimate xylan removal level. One can compare the impact of temperature and acid concentration on the xylan hydrolysis reaction rates for corn stover, switchgrass and poplar by examining Figures 5.2, 5.3 and 5.4, respectively. These figures show that pretreatment with concentrated sulfuric acid (~0.9%) at higher temperatures (~ 180 °C) hydrolyzed about 90% of the xylan within the first minute of the reaction period. During such short periods, the cellulose solublization was very small (<10%), and xylose degradation was minimal.

The maximum net sugar yields resulting from pretreatment of switchgrass, poplar and corn stover at different reaction conditions with optimum reaction time ($X_{L\ @\ t=\infty}$ eq. 5.18) are shown in Figures 5.5, 5.6 and 5.7, respectively. An interesting fact revealed by
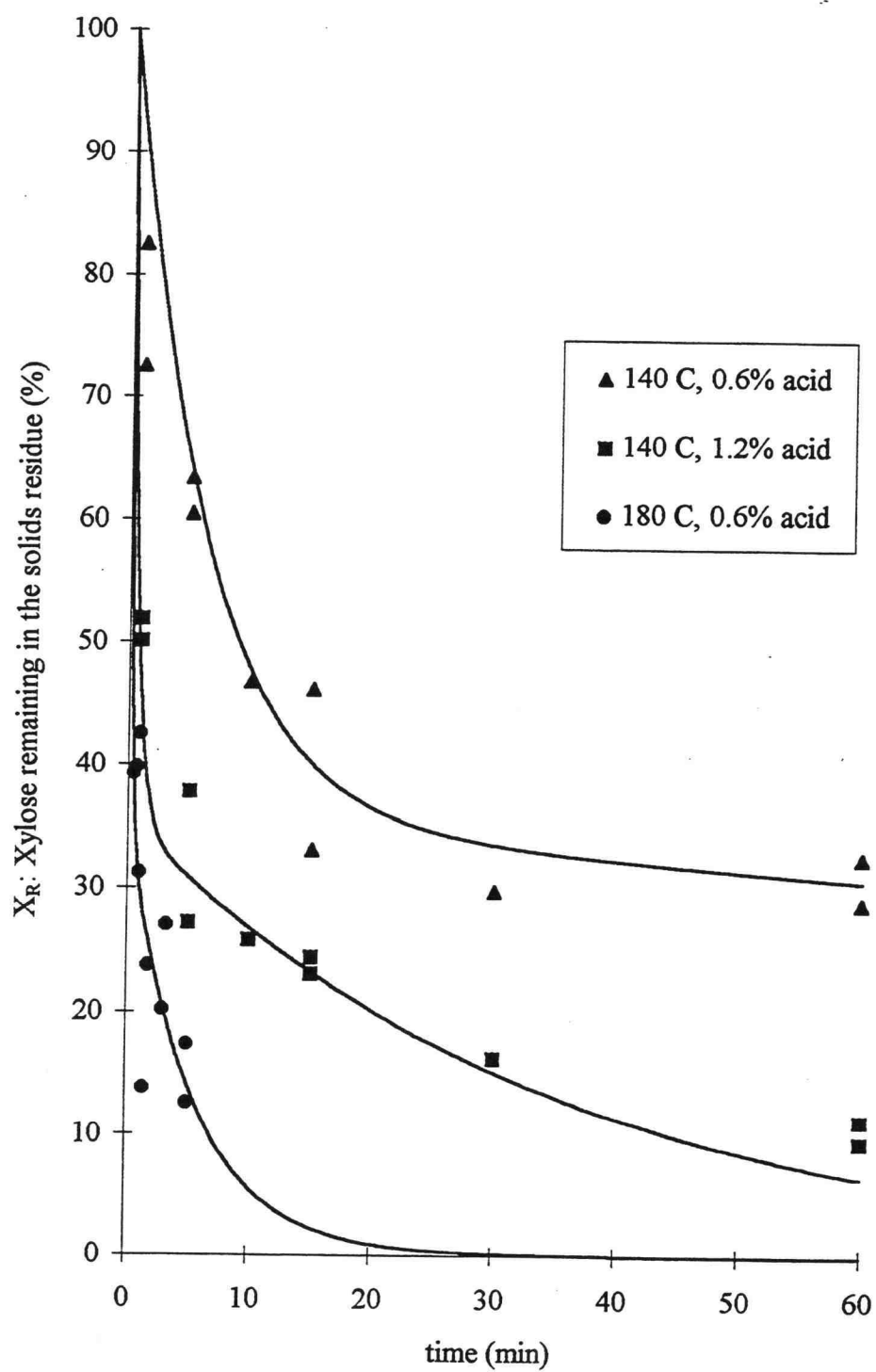
Fig. 5.2. Xylan hydrolysis of <u>corn stover</u>
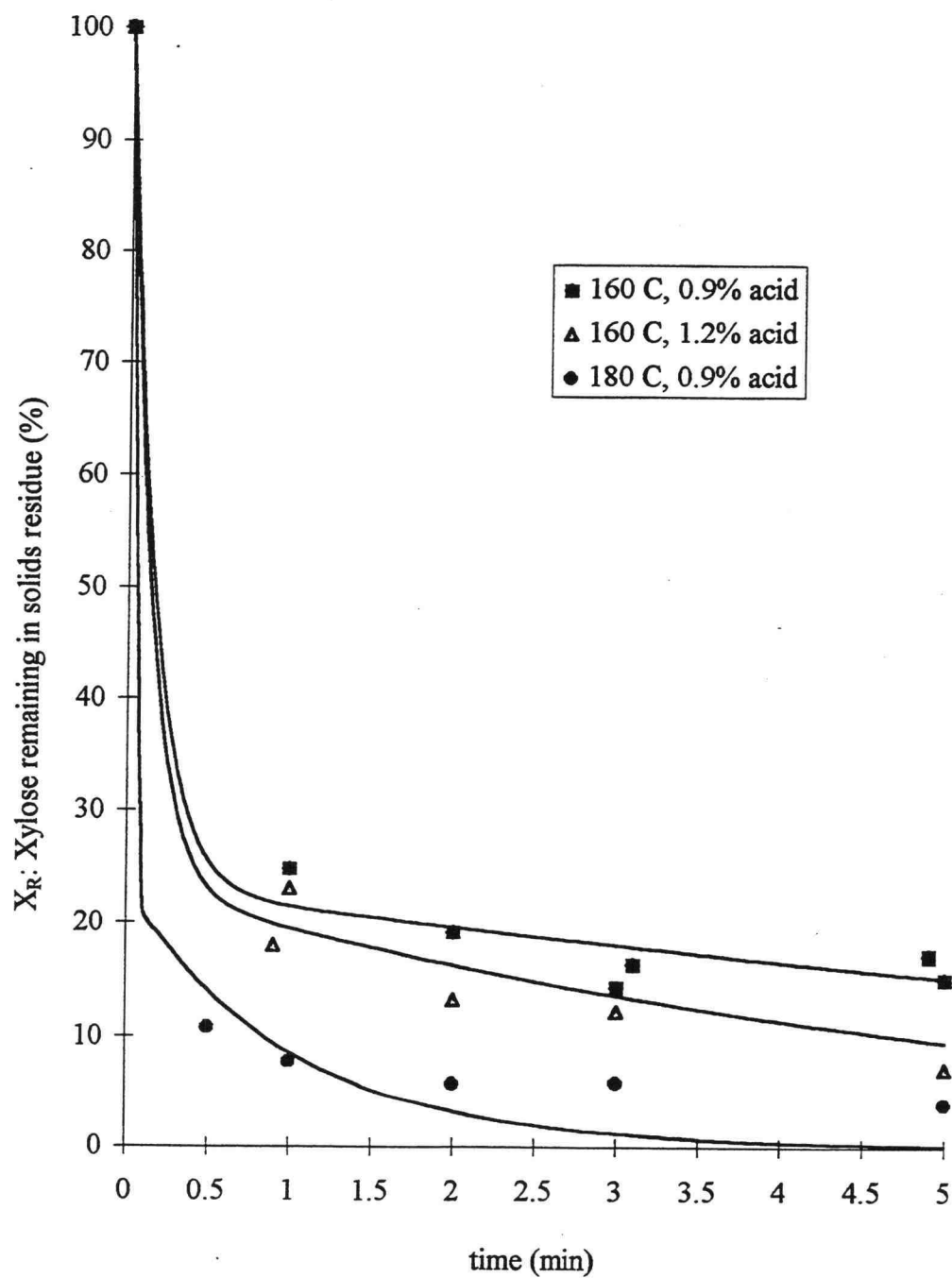solid lines show the model-predicted values

Fig. 5.3. Xylan hydrolysis of <u>switchgrass</u>
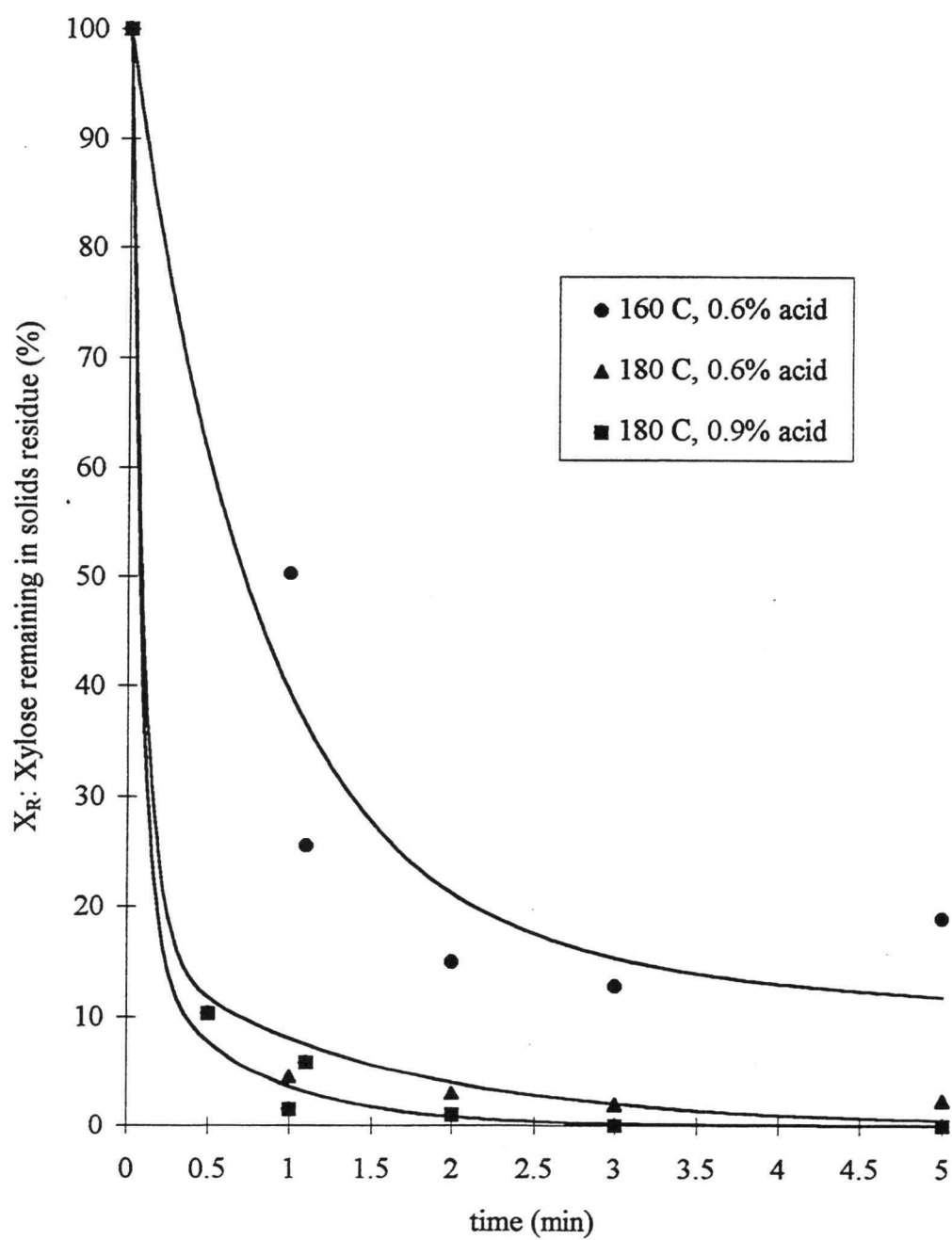solid lines show the model-predicted values

Fig 5.4. Xylan hydrolysis of poplar
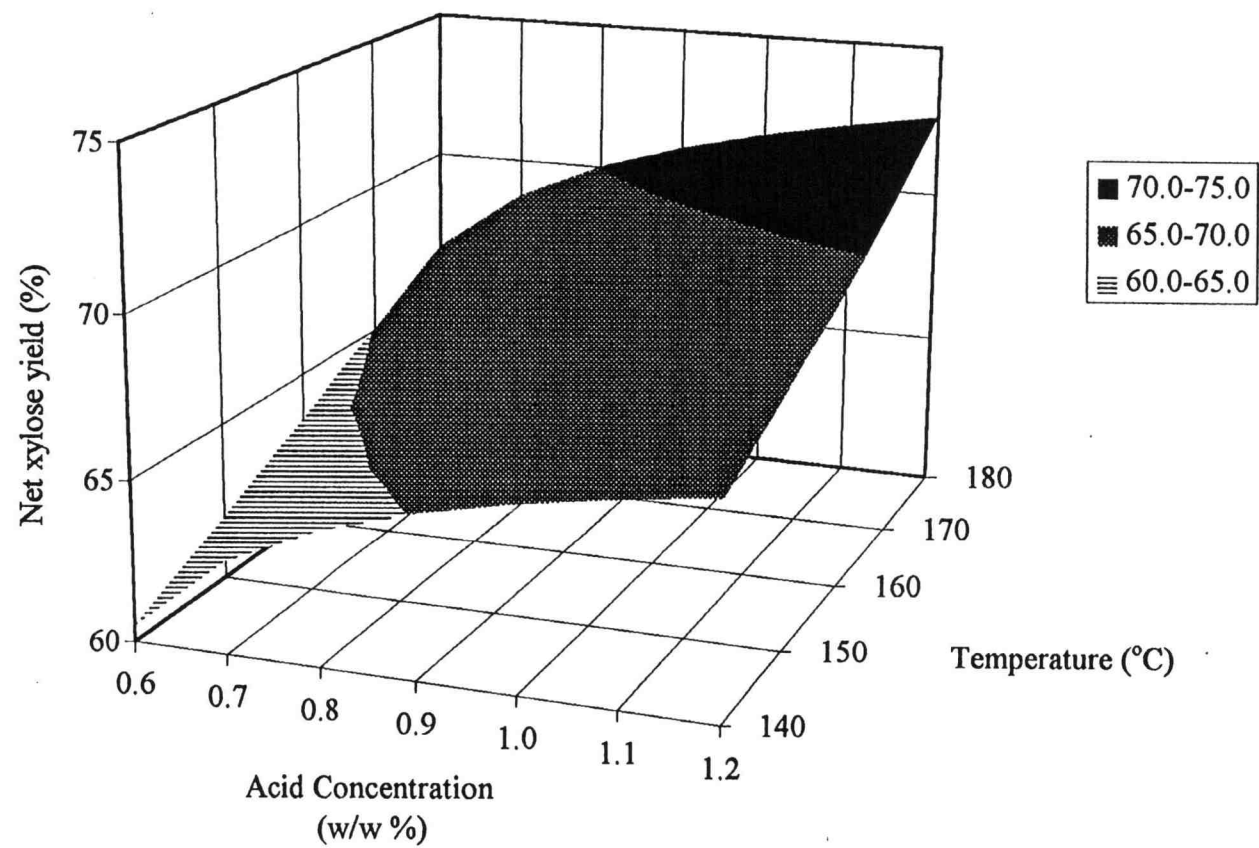solid lines show the model predicted values

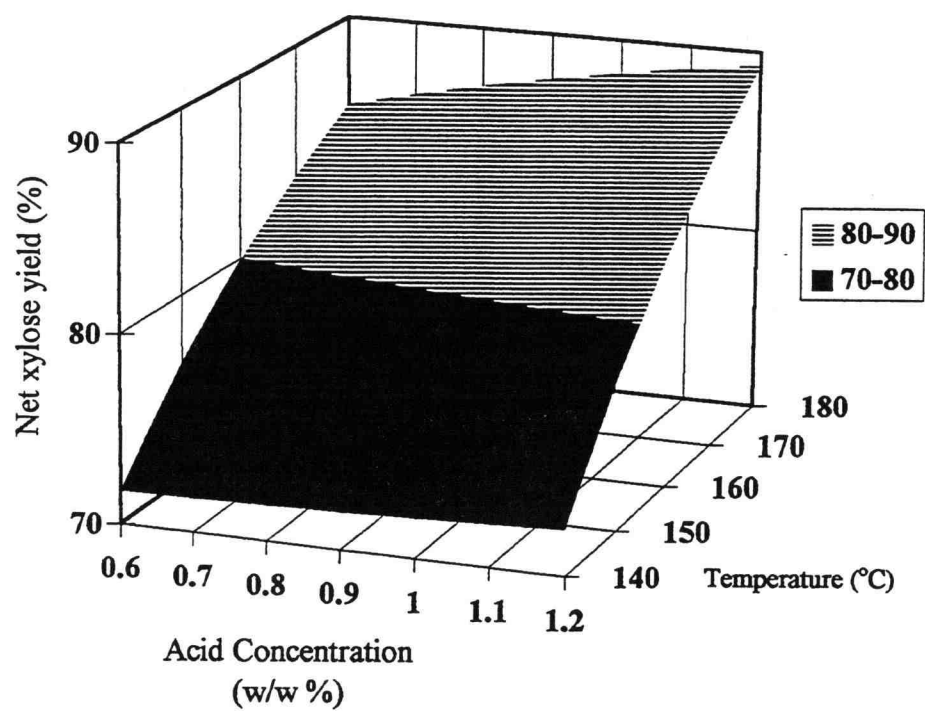**Fig. 5.5.** Net xylose yield in prehdrolysate (%)
<u>Corn Stover</u>
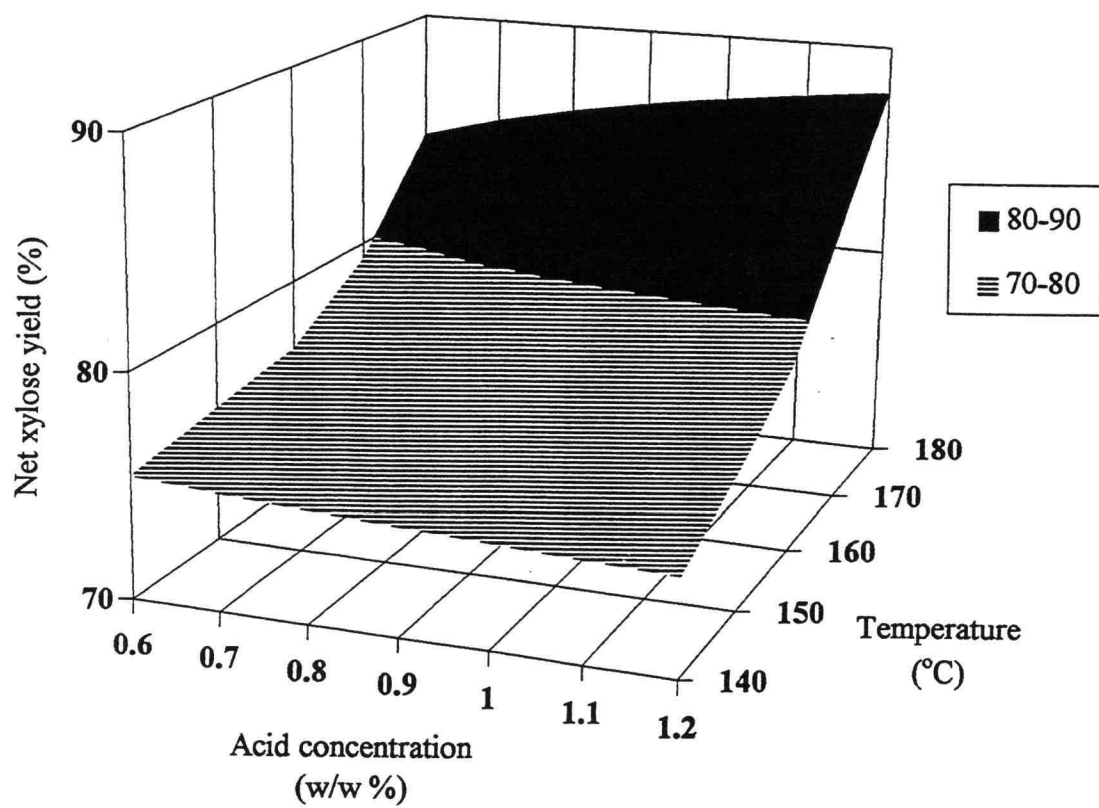
Fig 5.6. Net xylose yield in prehydrolysate (%)
Poplar

Fig 5.7. Net xylose yield in prehydrolysate (%)
<u>Switchgrass</u>

Figures 5.5 and 5.6 is that temperature (not the acid concentration) is the major factor affecting the extent of sugar recovery from pretreatment of poplar and switchgrass. It is only at high temperatures (e.g., 180 °C) that increasing the acid concentration can affect the sugar recovery. In comparison, the xylose yield from corn stover is more sensitive to a change in acid concentration level, especially at higher temperatures. Likewise, the effect of temperature is more pronounced at higher levels of acid concentration.

Figures 5.5, 5.6 and 5.7 show that using more severe reaction conditions produces higher net sugar yields which is in agreement with the results of equations (4.17) and (4.18). For all three feedstocks, E_f is greater than E_2 (see Table 5.2), therefore, higher temperatures favor the xylan hydrolysis reaction, and increases the net sugar yield. On the other hand, for corn stover n_2 is smaller than n_f, therefore, using higher acid concentrations increases the xylan hydrolysis rate constant more than the rate constant of the xylose degradation reaction. For switchgrass and poplar, however, n_2 is larger than n_f, and increasing the acid concentration will speed up the byproduct formation. However, the xylan hydrolysis rate constant (k_f) is so large (compared with k_2; see Tables 5.4 and 5.8) that it offsets the effect of acid concentration, and leave the net xylose yield unaffected.

At temperatures between 170-180 °C with acid concentrations greater than 0.9%, sugar yields from poplar and switchgrass are reasonably high (>80%). The reaction times for obtaining the maximum sugar yield under these conditions are between 0.5 to 1 minute. In case of corn stover, an 80% sugar recovery requires higher acid concentrations (>1.0%) at the same temperature range (170-180 °C), which is not a major difference at such low levels of acid concentration. The net sugar yield from corn stover, in general, is

lower than those produced by pretreatment of poplar and switchgrass at similar conditions. This is in part due to the higher neutralizing ability of corn stover which substantially lowers the acid concentration in reaction mixture.

Fig. 5.8 shows the effect of pretreatment time on the net xylose yield in the liquid stream. These curves are generated using the kinetic parameters of corn stover, and are very similar to those of poplar and switchgrass. At each set of reaction conditions the net xylose yield reaches a maximum which corresponds to the optimum time for that particular condition. Reaction times shorter or longer than the optimum reaction time does not allow for maximum sugar recovery as a result of either insufficient solublization of xylan or dissipation of xylose monomers by degradation to byproducts.

The optimum reaction times for attaining reasonable sugar yields (>60%) are generally short (<5 min). For instance, producing high xylose yields (>80%) at severe conditions (e.g., 180 °C, 1.2% acid) requires reaction times as short as 54 seconds. Figure 5.9 shows the optimum reaction times at different conditions. This figure shows that at temperatures between 160 and 180 °C the optimum reaction times are typically below 5 minutes.

5.2 Fermentation of Prehydrolysate

The liquid prehydrolysates resulting from pretreatment of each feedstock at four optimum reaction conditions were used to study the potential yield of ethanol production, and the inhibitory effect of xylose degradation products on *Pichia stipitis* activity. The optimum pretreatment conditions were as follows:
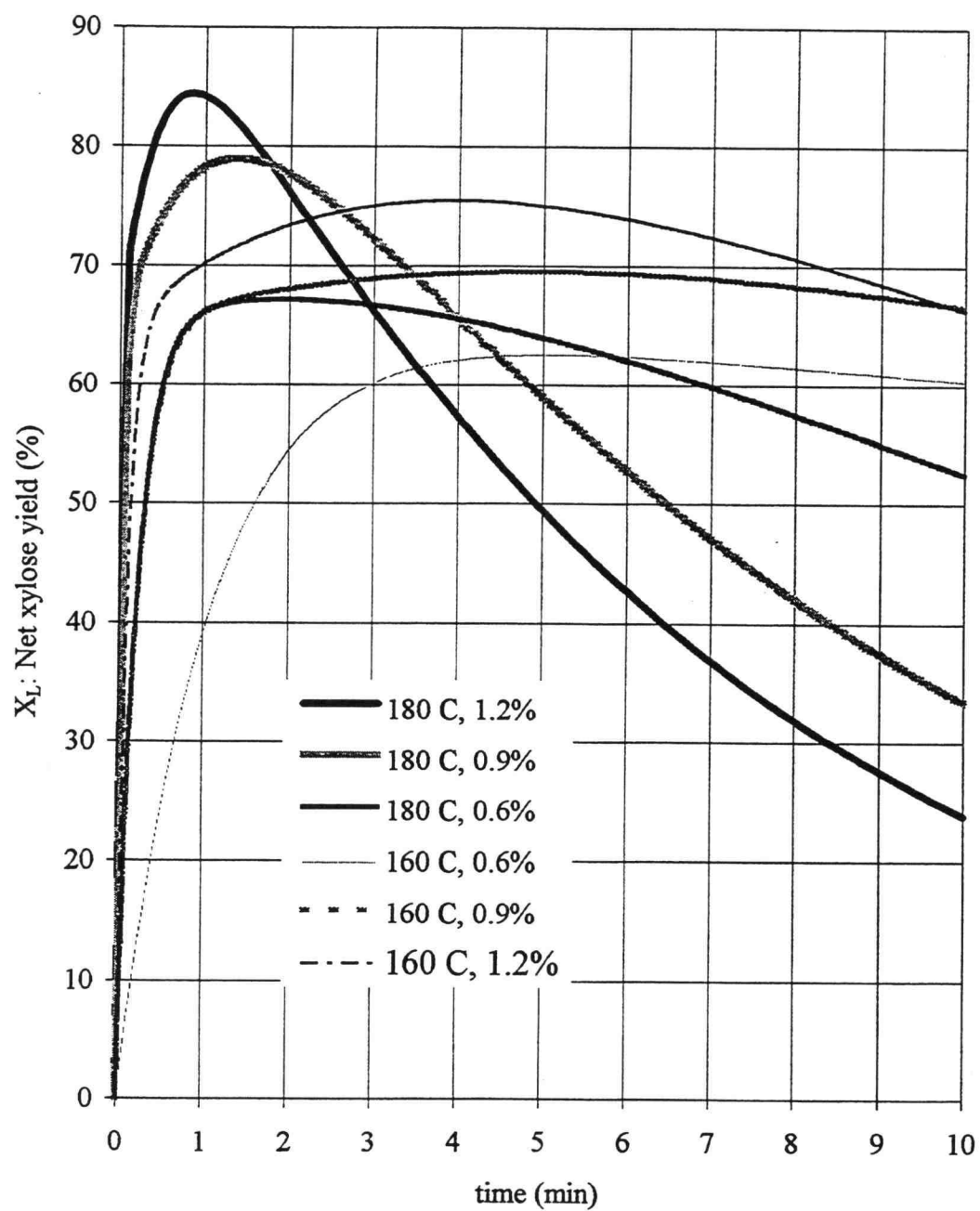
Fig. 5.8. Net xylose yield in the liquid prehydrolysate at different reaction conditions
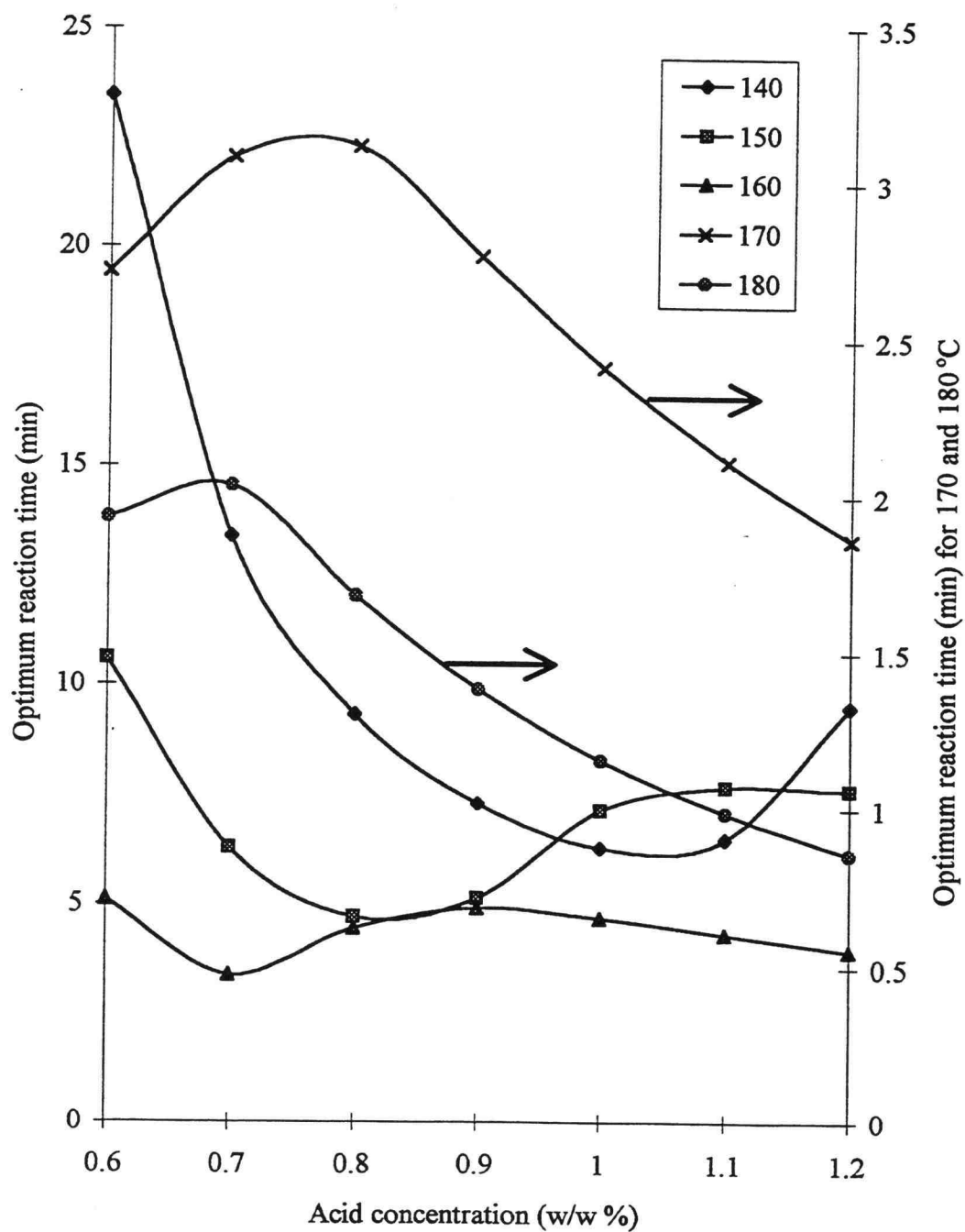
Fig 5.9. Optimum reaction times for maximum net xylose yield in prehydrolysate at different reaction conditions

Switchgrass &
Poplar: 180 °C, 1.1% H_2SO_4, 0.5 min

                180 °C, 1.0% H_2SO_4, 0.5 min

                170 °C, 1.2% H_2SO_4, 1.1 min

                170 °C, 1.1% H_2SO_4, 1.1 min

Corn Stover: 180 °C, 1.2% H_2SO_4, 0.9 min

                180 °C, 1.1% H_2SO_4, 1.0 min

                180 °C, 1.0% H_2SO_4, 1.2 min

                170 °C, 1.2% H_2SO_4, 1.9 min

It should be noted that for switchgrass the reaction times are not exactly the optimum times, although very close. It was decided to use such similar conditions to be able to compare the ethanol yields from the prehydrolysates of the two feedstocks (poplar and switchgrass). The net xylose yields in the "optimum" prehydrolysates are presented in Figures 5.10 and 5.11. The xylose yields in poplar prehydrolysates were higher than those of switchgrass and corn stover. The high neutralizing capacity of the herbaceous feedstocks (corn stover and switchgrass) which substantially reduced the acidity of the reaction mixtures could account for this difference. Figure 5.12 shows the net xylose yield in the above-mentioned optimum prehydrolysates versus the theoritical values predicted by the kinetic model developed in this study. The close agreement between the experimenatal and model-generated values could be seen in this figure (5.12).
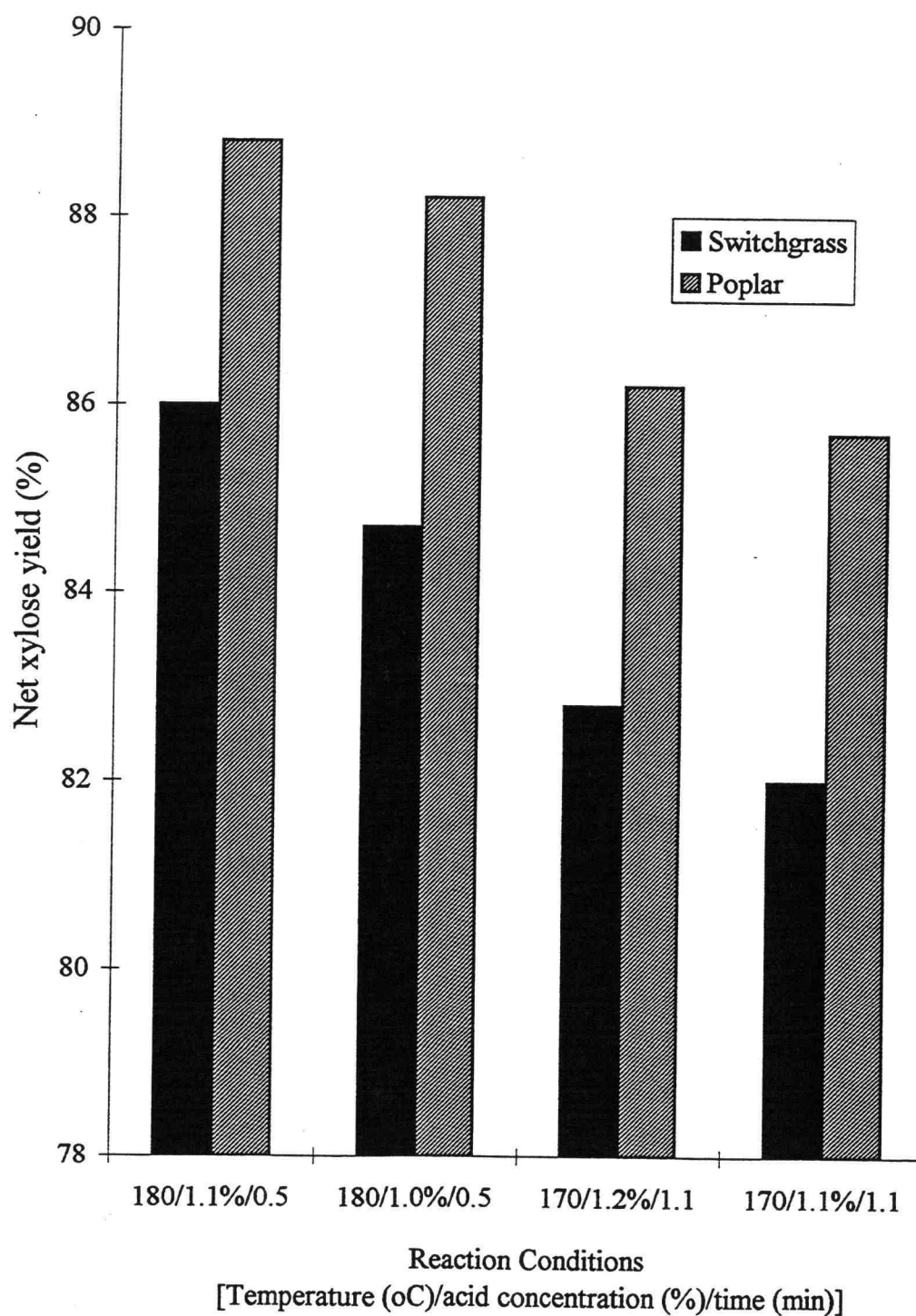
Fig 5.10. Net xylose yield at optimum reaction conditions-poplar and switchgrass
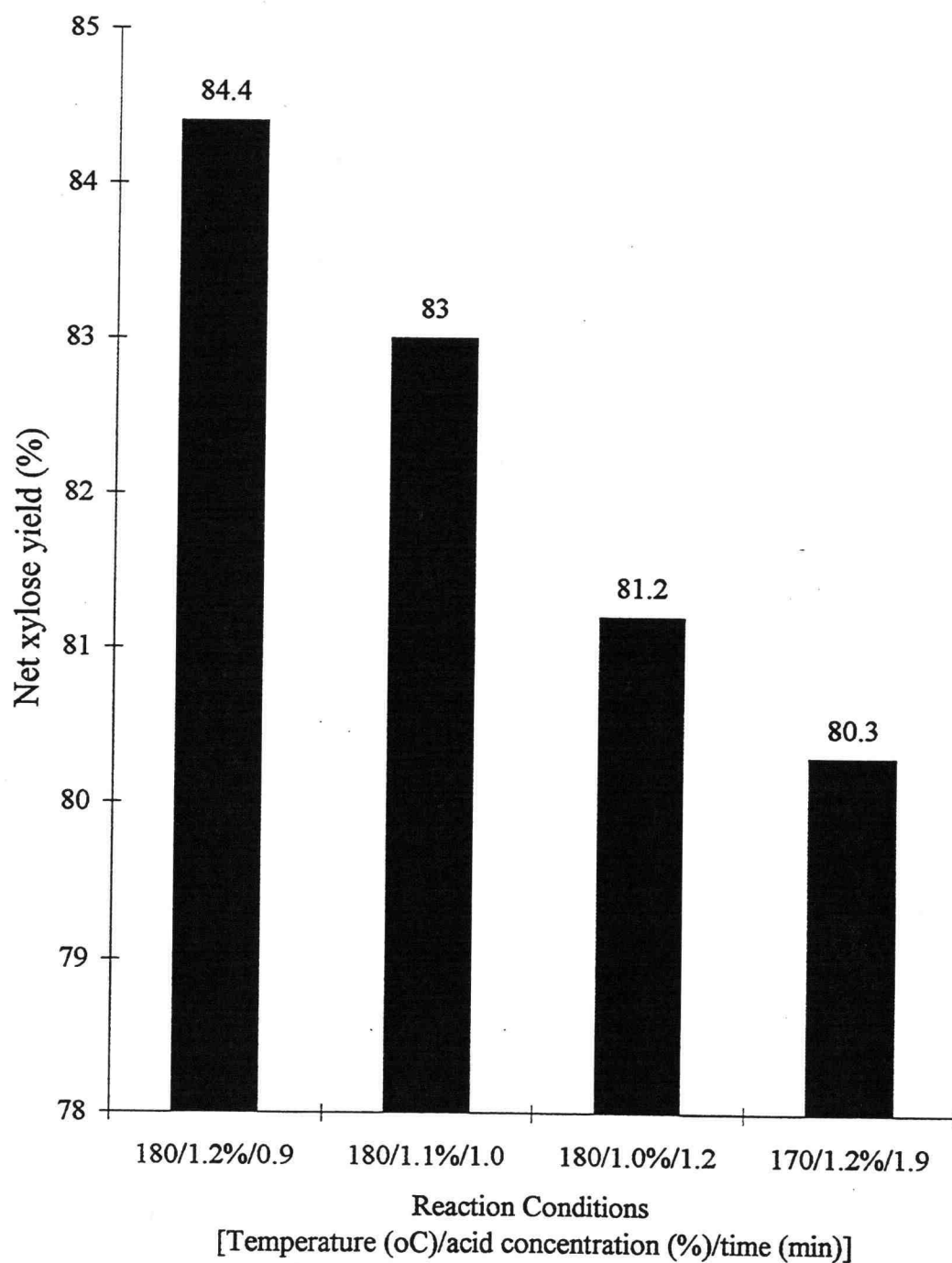
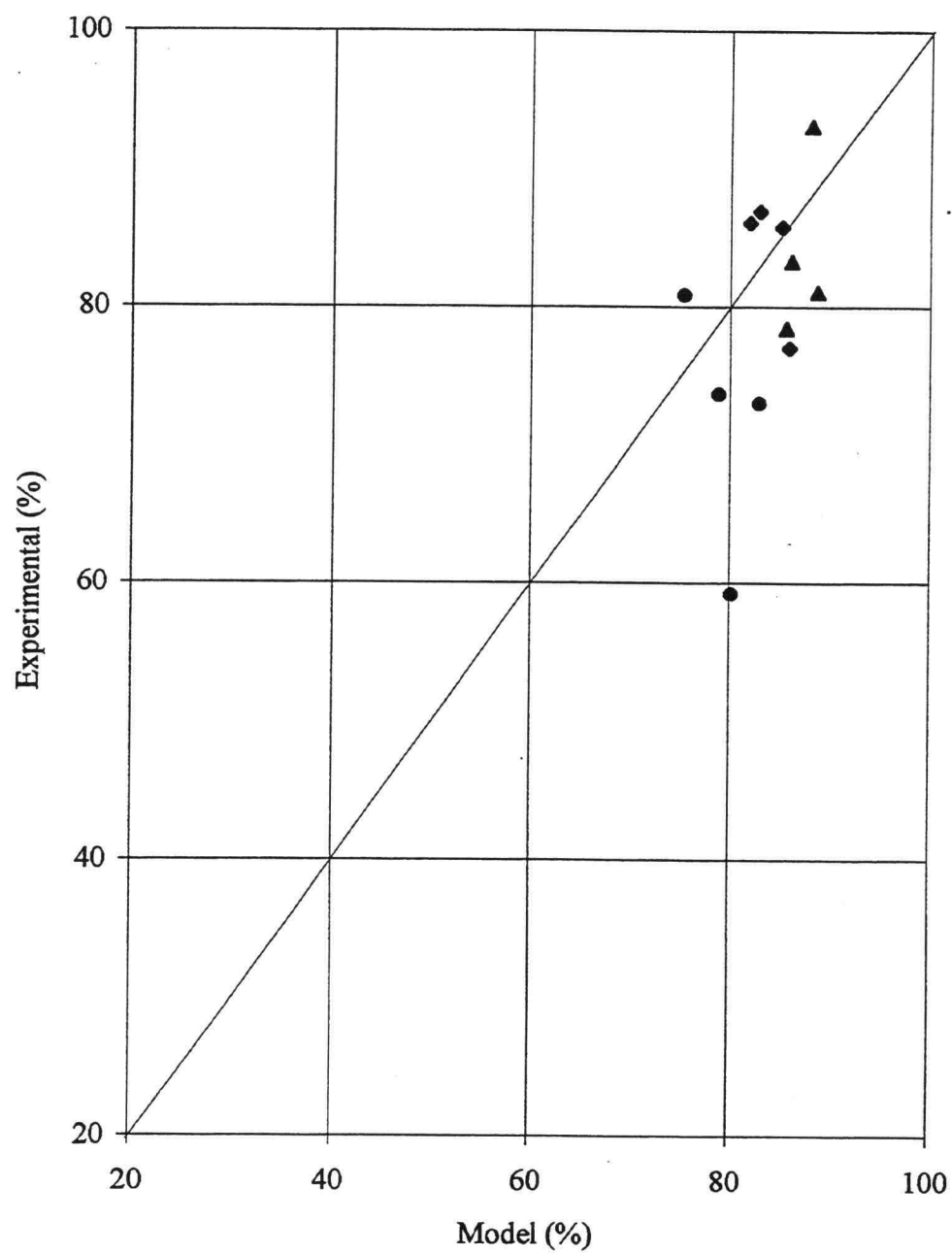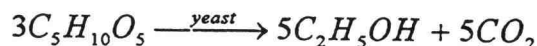**Fig 5.11.** Net xylose yield at optimum reaction conditions-corn stover

Fig. 5.12. Net xylose yield (%) in optimum prehydrolysates
Experimental vs. Model values
Circles: Corn Stover, Triangles: Poplar, Diamonds: Switchgrass

The theoretical yield of ethanol production from xylose, based on the following reaction, is 0.51 (g ethanol/g xylose):

$$3C_5H_{10}O_5 \xrightarrow{\ yeast\ } 5C_2H_5OH + 5CO_2$$

The ethanol production yield (g ethanol/g xylose) ranged from 0.26 to 0.44 for poplar, 0.39-0.47 for corn stover, and 0.36-0.45 for switchgrass. These values are equivalent of 51-86%, 76-90%, and 71-92% of the theoretical yield (0.51) for poplar, corn stover and switchgrass, respectively. It is interesting to note that the highest ethanol yields were produced from corn stover prehydrolysates which contained the least amount of fermentable sugars. The upper limits were always produced in media containing 40% prehydrolysate (PH), whereas those containing 80% PH produced the lower values. The ethanol production yield in control media, containing pure xylose with no prehydrolysate, was about 0.49 (g ethanol/g xylose).

The yields and rates of ethanol production in all 80% poplar PH samples were noticeably low (0.26-0.36). Fermentation times longer than 48 hours were required to obtain the final yields in 80% PH solutions, whereas in 40% PH samples the final yield was attained within 24 hours with no further improvement in the yield (control: 12 hours). For corn stover, fermentation times for attaining maximum ethanol yield in 40% and 80% PH solutions were 12 and 24 hours (control: 12 h), respectively. The maximum ethanol yields in 40% and 80% switchgrass PH samples were attained after 24 and 30 hours (control: 18 h), respectively.

The concentration of acetic acid, a known inhibitor of *Pichia stipitis*, were considerably higher in poplar prehydrolysates (2.6-3.0 g/L) than in corn stover (~1 g/L) prehydrolysates. Switchgrass prehydrolysates contained slightly lower amounts of acetic acid (2.5 g/L) relative to poplar prehydrolysates. The higher acetate concentration in poplar prehydrolysates could be the result of a more heavily acetylated xylan backbone of poplar as compared to those of herbaceous feedstocks. Higher levels of acetic acid can explain the marked inhibition of fermentation of poplar prehydrolysates as compared with corn stover. It cannot, however, account for the differences among the 80% poplar PH samples. The results indicated that the acetate was usually metabolized by the yeast, except in cases with more pronounced inhibition, in which the acetate concentration remained unchanged. Concentration of furfural was only measured in switchgrass prehydrolysates. Initially, furfural concentration was very low and dropped to much lower levels within 12 hours.

According to the unifying model developed in this study, at optimum reaction conditions, the highest levels of byproduct formation will occur in corn stover prehydrolysates (~10.5% of original xylose), followed by those of poplar (~6%) and switchgrass (~4.5%). Although the more pronounced inhibition of the yeast by poplar prehydrolysates, compared to that of switchgrass, could be explained by these results, the substantially higher ethanol yield in corn stover prehydrolysates remained unjustified. The variation of ethanol production yield of different prehydrolysates of each feedstock had no apparent relationship with the model-predicted levels of byproducts in the prehydrolysates. Further studies are required to determine the inhibitory effect of each compound (e.g., furfural and acetic acid) separately. Although, the effects of unknown species resulting

from pretreatment and the overall effect of all byproducts need to be identified by comparing the prehydrolysates obtained at optimum conditions with those resulted from pretreatment at conditions that produce higher levels of byproducts.

BIBLIOGRAPHY

1.   Abasaeed, A.E. and M. E. Mansour. "Thermal Effects on Acid Hydrolysis of Cellulose." Bioresource Technology. (40). 1992: 221-224

2.   Aman, Per. "Composition and Structure of Cell Wall Polysaccharides in Forages." Forage Cell Wall Structure and Digestibility. Ed. H.G. Jung et al. Madison, Wisconsin. 1991: 183-201

3.   Argonne National Laboratory. Environmental Implications of Accelerated Gasohol Production, Preliminary Assessment. ANL/ES-91. ANL, Argonne, IL. 1980

4.   Bailey, R. B. et al. Applied Environmental Microbiology 44 1982:631

5.   Barbel, H. et al. "Ethanolic Fermentation of Pentoses in Lignocellulose Hydrolysates." Applied Biochemistry and Biotechnology 28/29. 1991: 131-144

6.   Bernow, Stephen S., Donald B. Marron. "Valuation of Environmental Externalities for Energy Planning and Operations." Tellus Institute Boston, MA. May 1990

7.   Bhandari, Nadan et al. "Kinetic Studies of Corn Stover Saccharification Using Sulphuric Acid." Biotechnology and Bioengineering 26. 1984: 320-327

8.   Bluhm, George C. et al. "The Economic of Biomass and Biomass-derived Energy and Fuels." Proceedings of 2nd Biomass Conference of the Americas. Portland, OR. Aug 21-24, 1995: 1262-1271

9.   Clausen, E. C. and J. L. Gaddy. "Economic Analysis of Bioprocess to Produce Ethanol from Corn Stover." Biotechnology and Bioengineering Symp. 13. 1983: 495-510

10.   Conner, Anthony H. "Kinetic Modeling of Hardwood Prehydrolysis, Part I: Xylan Removal by Water Prehydrolysis ." Wood and Fiber Sci., 16(2). 1984: 268-277

11.   Day, Donald L. "Biomass Waste." Biomass Handbook. Ed. Osamu Kitani and Carl W. Hall. New York: Gordon. 1989: 142-147

12.   Doran, J. B. et al. "Saccharification and Fermentation of Sugar Cane Bagasse by Klebsiella oxytoca P2 Containing Chromosomally Integrated Genes Encoding the Zymomonas mobilis Ethanol Pathway." Biotechnology and Bioengineering 44. 1994: 240-247.

13.   Downing, Mark. "Is There a Need for Site Productivity Functions for Short-Rotation Woody Crop Plantings?" Proceedings of 2nd Biomass Conference of the Americas. Portland, OR. Aug 21-24, 1995: 207-215

14.   Downing, M. et al. "Energy, Economic, and Environmental Implications of Production of Grasses as Biomass Feedstocks." Proceedings of 2nd Biomass Conference of the Americas. Portland, OR. Aug 21-24, 1995: 288-297

15.   Dunlop A. P. and F. N. Peters. The Furans. New York: Reinhold Publishing Corp. 1953

16.   Ehrman, T. "Determination of Total Solids/Moisture in Biomass." NREL Alternative Fuels Division Chemical Analysis and Testing Standard Procedure, No. 005. 1992

17.   Fenske, John. M.S. Thesis. Oregon State University, 1995

18.   Ferrari, M.D. et al. "Ethanol Production from Eucalyptus Wood Hemicellulose Hydrolysate by Pichia stipitis." Biotechnology and Bioengineering 40. 1992: 753-759

19.   Ferrel, J. E. and L. L. Wright. "Research to Develop Improved Production Methods for Woody and Herbaceous Biomass Crops." Proceedings of 2nd Biomass Conference of the Americas. Portland, OR. Aug 21-24, 1995: 197-206

20.   Ghosh, P. et al. Enzyme Microbial Technology 4. 1982

21.   Grethlein, Hans E. "The Cost of Ethanol Production from Lignocellulosic Biomass Final Report." NREL Library." April, 1993

22.   Grethlein, Hans E. and Alvin O. Converse. "Common Aspects of Acid Prehydrolysis and Steam Explosion for Pretreating Wood." Bioresource Technology. 1991: 77-82

23.   Grohmann, K., R. Torget and M. Himmel. "Optimization of Dilute Acid Pretreatment of Biomass." Biotechnol. and Bioeng. Symp., No 15. 1985: 59-80

24.   Harris, J. F. et al. "Factors Influencing Dilute Sulfuric Acid Prehydrolysis of Southern Red Oak Wood." Progress in Biomass Conversion 5. 1984:101-141

25.   Harris, W. L and Howard Rosen. "Sustainable Biomass Utilization." Proceedings of 2nd Biomass Conference of the Americas. Portland, OR. Aug 21-24, 1995: 1456- 1467

26. Hatfield, Ronald D. "Cell Wall Polysaccharide Interactions and Degradability." Forage Cell Wall Structure and Digestibility. Ed. H.G. Jung et al. Madison, Wisconsin. 1991: 285-314

27. Higuchi, Takayoshi. Biosynthesis and Biodegradation of Wood Components. Orlando: Academic Press. 1985

28. Hughes, Evan E., and G. A. Wiltsee. "Comparative Evaluation of Fuel Costs from Energy Crops." Proceedings of 2nd Biomass Conference of the Americas. Portland, OR. Aug 21-24, 1995: 1291-1301

29. Kamiyama, Yoshi and Yoshio Sakai. "Rate of Hydrolysis of Xylo-Oligosaccharides in Dilute Sulfuric Acid." Carbohydrate Research 73. 1979: 151-158

30. Kim, S. B. and Y. Y. Lee. "Kinetics in Acid-Catalyzed Hydrolysis of Hardwood Hemicellulose." Biotechnol. and Bioeng. Symp. No. 17. 1987:71-84

31. Kobayashi, Tatsuyoshi and Y. Sakai. "Hydrolysis Rate of Pentosan of Hardwood in Dilute Sulfuric Acid." Bull. Agr. Chem. Soc. Japan, 20, No. 1. 1956: 1-7

32. Ladisch, Michael R. "Hydrolysis." Biomass Handbook. Ed. Osamu Kitani and Carl W. Hall. New York: Gordon. 1989: 434-451

33. Ladisch, Michael R. and G. Tsao. Science, 201. 1978:743

34. Lipinsky, E. S. Science, 199. 1978: 645

35. Lynd Lee R., "Production of Ethanol from Lignocellulosic Materials Using Thermophilic Bacteria: Critical Evaluation of Potential and Review." Advances in Biochemical Engineering/Biotechnology, 38. 1989: 1-52

36. Lynd Lee R., et al. "Fuel Ethanol from Cellulosic Biomass." Science, 251. 1991: 1318-23

37. Malester, A. I. et al. "The Effect of the Neutralizing Capacity of Cellulosic Materials on the Kinetics of Cellulose Dilute Acid Hydrolysis." Biological Wastes 26. 1988:115-124

38. Malester, Ilan A. et al. "Kinetics of Dilute Acid Hydrolysis of Cellulose Originating form Municipal Solid Wastes." Ind. Eng. Chem. Res., 31. 1992: 1998-2003

39. Maloney, Mark T., Thomas W. Chapman and Andrew J. Baker. "Dilute Acid Hydrolysis of Paper Birch: Kinetics Studies of Xylan and Acetyl Group Hydrolysis." Biotechnol. and Bioeng., 27. 1985: 355-361

40. Maloney, Mark T. et al. "An Engineering Analysis of the Production of Xylose by Dilute Acid Hydrolysis of Hardwood Hemicelluloses." Biotechnol. Progress, 20, No. 4. 1986: 192-202

41. McNeel, Joseph F. "Technologies for Delivering Forest Biomass: Challenges and Implications." Proceedings of 2nd Biomass Conference of the Americas. Portland, OR. Aug 21-24, 1995: 65-76

42. McParland, J. J. et al. "Kinetics of Acid Hydrolysis of Corn Stover." Solar Energy 28. No. 1. 1982: 55-63

43. Mehleberg R. and G. T. Tsao. "Low Liquid Hemicellulose Hydrolysis of Hydrochloric Acid." Proceedings of 178th ACS National Meeting Washington D.C. 1979

44. Mielenz, J. et al. "Commercialization of Biomass Ethanol Plant Siting Studies." Proceedings of 2nd Biomass Conference of the Americas. Portland, OR. Aug 21-24, 1995: 1494-1504

45. Moritz, J. W. and Sheldon Duff. "Simultaneous Saccharification and Extractive Fermentation of Cellulosic Substrates." Biotechnology and Bioengineering 49. 1996: 504-511

46. Nee, Charles I., Wen F. Yee. "Hydrolysis of Pentosans in Bagasse Pith." J. Appl. Chem. Biotechnol. 26. 1976: 283-287

47. Nishizawa, Kazutoshi. "Degradation of Cellulose and Hemicellulose." Biomass Handbook. Ed. Osamu Kitani and Carl W. Hall. New York: Gordon. 1989: 254-270

48. Parisi, Frederico. "Advances in Lignocellulosics Hydrolysis and in the Utilization of the Hydrolyzates." Advances in Biochemical Engineering/Biotechnology, 38. 1989: 53-87

49. Parrish, D. et al. "Switchgrass as a Biofuels Feedstock for the Upper Southeast." Proceedings of 2nd Biomass Conference of the Americas. Portland, OR. Aug 21-24, 1995: 307-312

50. Penner, Michael, A. Hashimoto, A. Esteghlalian and J. Fenske. "The Acid Catalyzed Hydrolysis of Lignocellulosic Materials." Agricultural Materials as

Renewable Resources. Ed. G. Fuller, T. McKeon and D. Bills. American Chemical Society, Symposium series 647. 1996

51. Perego, Patritzia et al. "Fermentation of Hardwood Hemicellulose Hydrolysate by *Pachysolen tannophilus, Candida shehatae and Pichia stipitis*." Journal of Industrial Microbiology 6. 1990: 157-164

52. Petrulis, M., Sommer, J., and Hines, F. "Ethanol Production and Employment." Agricultural Information Bulletin No. 678. 1993

53. Philippidis, G. P. et al. "Study of the Enzymatic Hydrolysis of Cellulose for Production of Fuel Ethanol by the Simultaneous Saccharification and Fermentation Process." Biotechnology and Bioengineering 41. 1993: 846-853

54. Ranganathan, S. et al. "Kinetic Studies of Wheat Straw Hydrolysis Using Sulfuric Acid." The Canadian J. Chem. Eng., 63. 1985: 840-844

55. Reding, B. and Mark Stumborg, "Development of a System For Long Hauling Chaff to an Ethanol Facility." Proceedings of 2nd Biomass Conference of the Americas. Portland, OR. Aug 21-24, 1995: 100-109

56. Roberto, I.C. et al. "Utilization of Sugar Cane Bagasse Hemicellulose Hydrolysate by *Pichia stipitis* for the Production of Ethanol." Process Biochemistry 26. 1991:15-21

57. Roberts, P. et al. "An Assessment of the Economic and Employment Impacts of the Commercialization of Renewable Technologies in Washington/Oregon." Proceedings of 2nd Biomass Conference of the Americas. Portland, OR. Aug 21-24, 1995: 1484-   1493

58. Root, D. F. et al. "Kinetics of the Acid-Catalyzed Conversion of Xylose to Furfural." Forest Products Journal. May 1959: 158-165

59. Sen, D.C. "Ethanol Fermentation." Biomass Handbook. Ed. Osamu Kitani and Carl W. Hall. New York: Gordon. 1989: 254-270

60. Shapouri, H., J. Duffield. "Estimating the Net Energy Value of Corn Ethanol." Proceedings of 2nd Biomass Conference of the Americas. Portland, OR. Aug 21-24, 1995: 976-985

61. Singh, A., Kumudeswar Das and Durlubh K. Sharma. "Production of Xylose, Furfural, Fermentable Sugars, and Ethanol from Agricultural Residues." Chem. Tech. & Biotechnol., 34A. 1984: 51-61

62. Sjostrom, Eero. <u>Wood Chemistry: Fundamentals and Applications.</u> London: Academic press. 1993

63. Smith, P. C. et al. "Glucose Decomposition at High Temperature, Mild Acid, and Short Residence Times." <u>Solar Energy 28. No. 1.</u> 1982: 41-48

64. Spindler, Diane. D. et al. "The Simultaneous Saccharification and Fermentation of Pretreated Woody Crops to Ethanol." <u>Applied Biochemistry and Biotechnology 28/29.</u> 1991: 773-786

65. Springer Edward L. and John F. Harris. "Procedures for Determining the Neutralizing Capacity of Wood during Hydrolysis with Mineral Acid Solutions." <u>Ind. Eng. Chem. Prod. Res. Dev. 24.</u> 1985: 485-489

66. Springer, Edward, Lawrence L. Zoch. "Hydrolysis of Xylan in Different Species of Hardwoods." <u>Tappi 51, No. 5.</u> 1968: 214-218

67. Sproull, Robert D. et al. "Production of Furfural from Corn Stover Hemicellulose." <u>Biotechnology and Bioengineering Symp. 15.</u> 1985

68. Tolbert, V. R., M. Downing. "Environmental Effects of Planting Biomass Crops at Larger Scales on Agricultural Lands." <u>Proceedings of 2nd Biomass Conference of the Americas. Portland, OR.</u> Aug 21-24, 1995: 1628-1635

69. Torget, R., P. Werdene, M. Himmel and K. Grohmann. "Dilute Acid Pretreatment of Short Rotation Woody and Herbaceous Crops." <u>Appl. Biochem. and Biotechnol., 24/25.</u> 1990: 115-126

70. Tran, Ai V. and Robert P. Chambers. " Ethanol Fermentation of Red Oak Acid Prehydrolysate by the Yeast *Pichia stipitis* CBS 5776." <u>Enzyme Microbial Technology 8.</u> 1986: 439-444

71. U.S. Department of Agriculture, Agricultural Economic Report No. 667. 1995: 12

72. U.S. Department of Energy. <u>Gasohol: Economic Feasibility Study. SAN-1681-TI.</u> Development Planning and Research Associates, Inc. Manhattan, KS. March 1978

73. Waits, E. D. and J. L. Elmore. "Environmental Consequences of Industrial-Scale Fuel-Ethanol Production." <u>Environmental International, 9.</u> 1983: 325-34

74. Weil, Joseph et al. "Cellulose Pretreatments of Lignocellulosic Substrates." <u>Enzyme Microb. Technol., 16.</u> 1994: 1002-4

75. Wenzl, Herman F. J. "The Acid Hydrolysis of Wood." <u>The Chemical Technology of Wood.</u> Academic Press: New York. 1970: 229-239

76. Wilson, J. R. "Organization of Forage Plant Tissues." <u>Forage Cell Wall Structure and Digestibility.</u> Ed. H.G. Jung et al. Madison, Wisconsin. 1991: 1-32

77. Wilson, J. J. et al. "Comparative Fermentability of Enzymatic and Acid Hydrolysates of Steam-Pretreated Aspenwood Hemicellulose by *Pichia stipitis* CBS 5776." <u>Applied   Microbiology and Biotechnology 31.</u> 1989: 592-596

78. Wyman, Charles E. and Norman D. Hinman. "Ethanol, Fundamentals of Production from Renewable Feedstocks and Use as a Transportation Fuel." <u>Applied Bichemistry and Biotechnology 24/25.</u> 1990: 735-753

79. Wyman, Charles E., and Barbara Goodman. "Biotechnology for Production of Fuels,   Chemicals, and Materials from Biomass." <u>Applied Biochemistry and Biotechnology 39/40.</u> 1993: 41-58

80. Wyman, Charles E. "Biomass-Derived Oxygenates for Transportation Fuels." <u>Proceedings of 2nd Biomass Conference of the Americas.</u> Portland, OR. Aug 21-24, 1995: 966-975

APPENDIX

APPENDIX

This Appendix includes Figures A.1 through A.9 showing the amount of xylan reamining in the feedstocks solids residue after being pretreated with 0.6%, 0.9% and 1.2% sulfuric acid at 140, 160 and 180 °C.
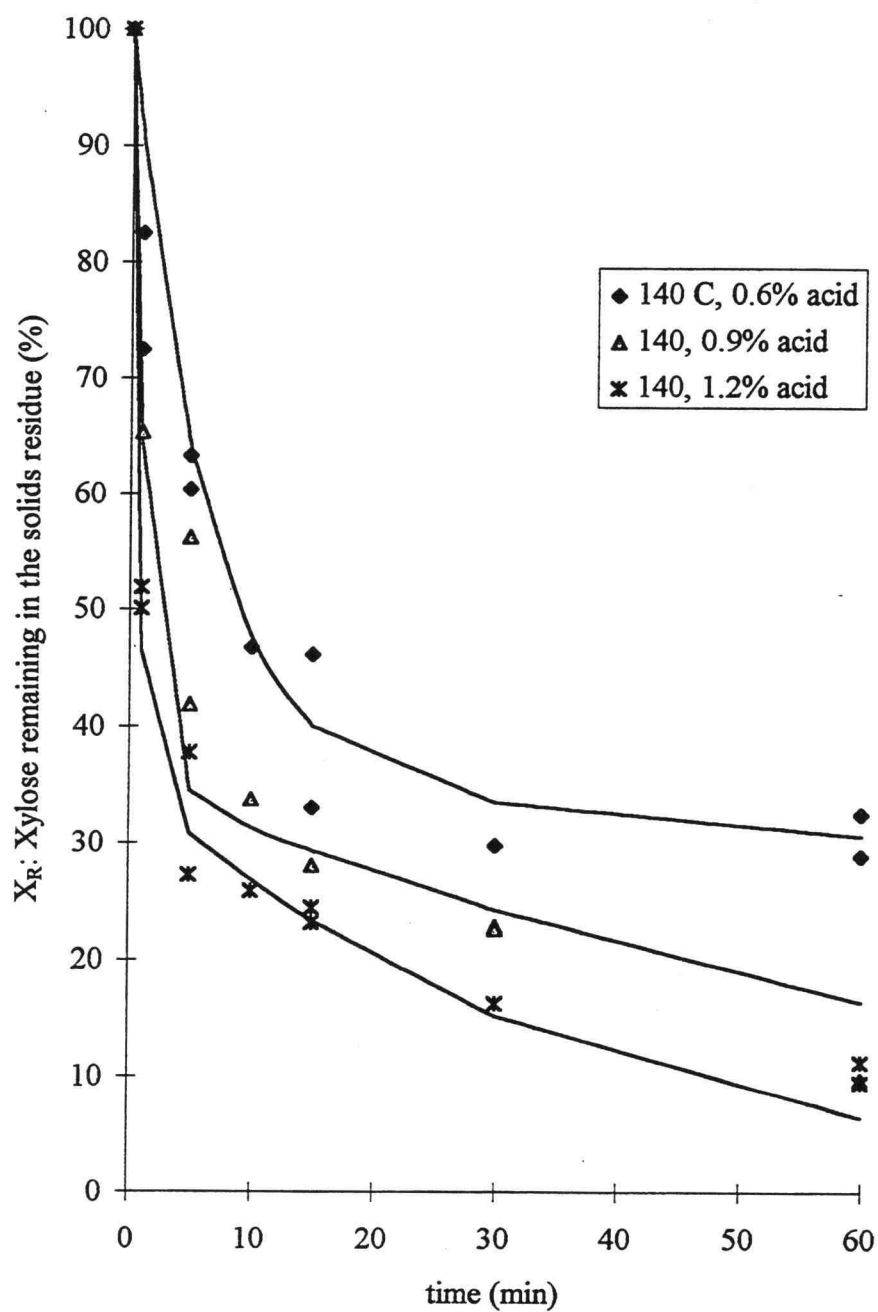
Fig. A.1. Xylan hydrolysis of <u>corn stover</u> at 140 °C
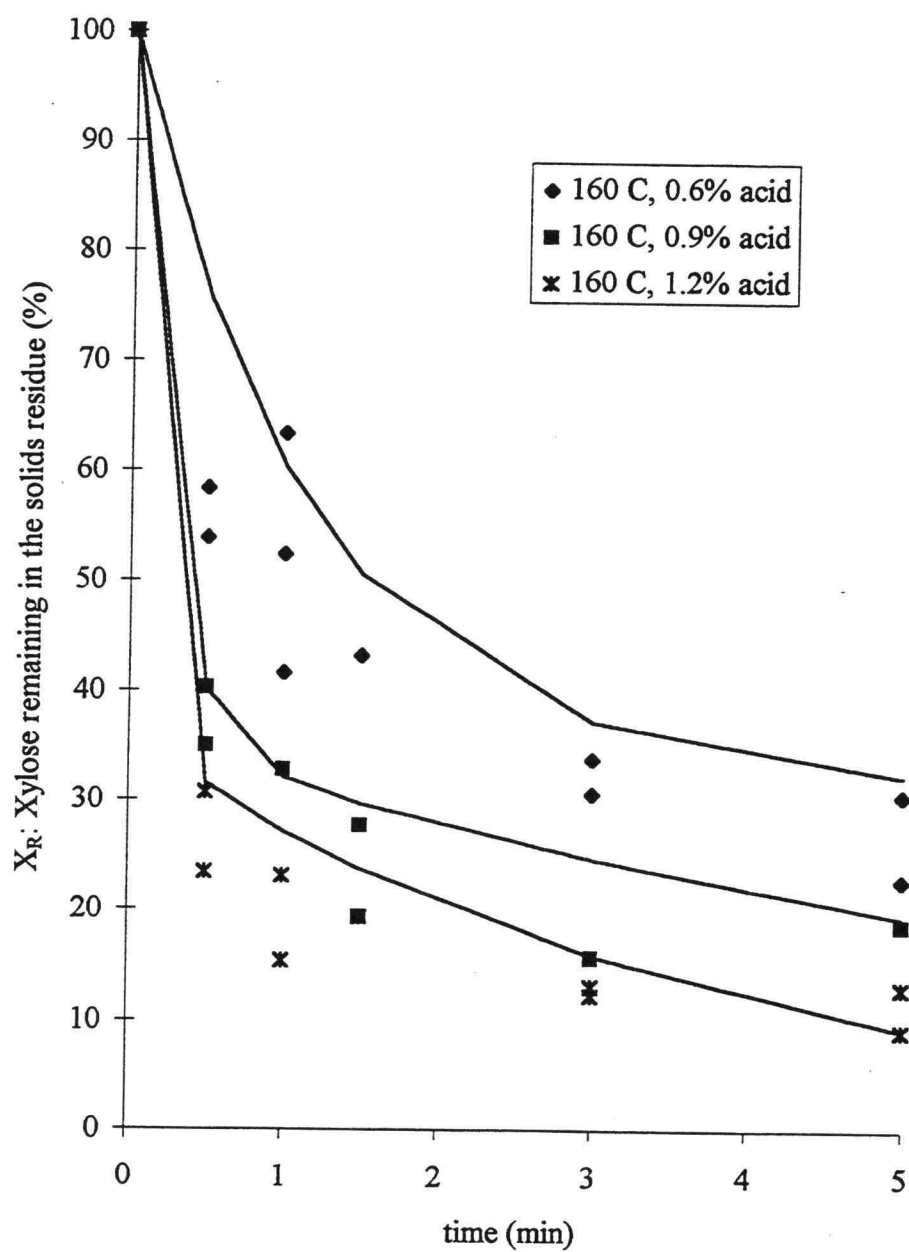solid lines show the predicted values

Fig. A.2. Xylan hydrolysis of <u>corn stover</u> at 160 °C
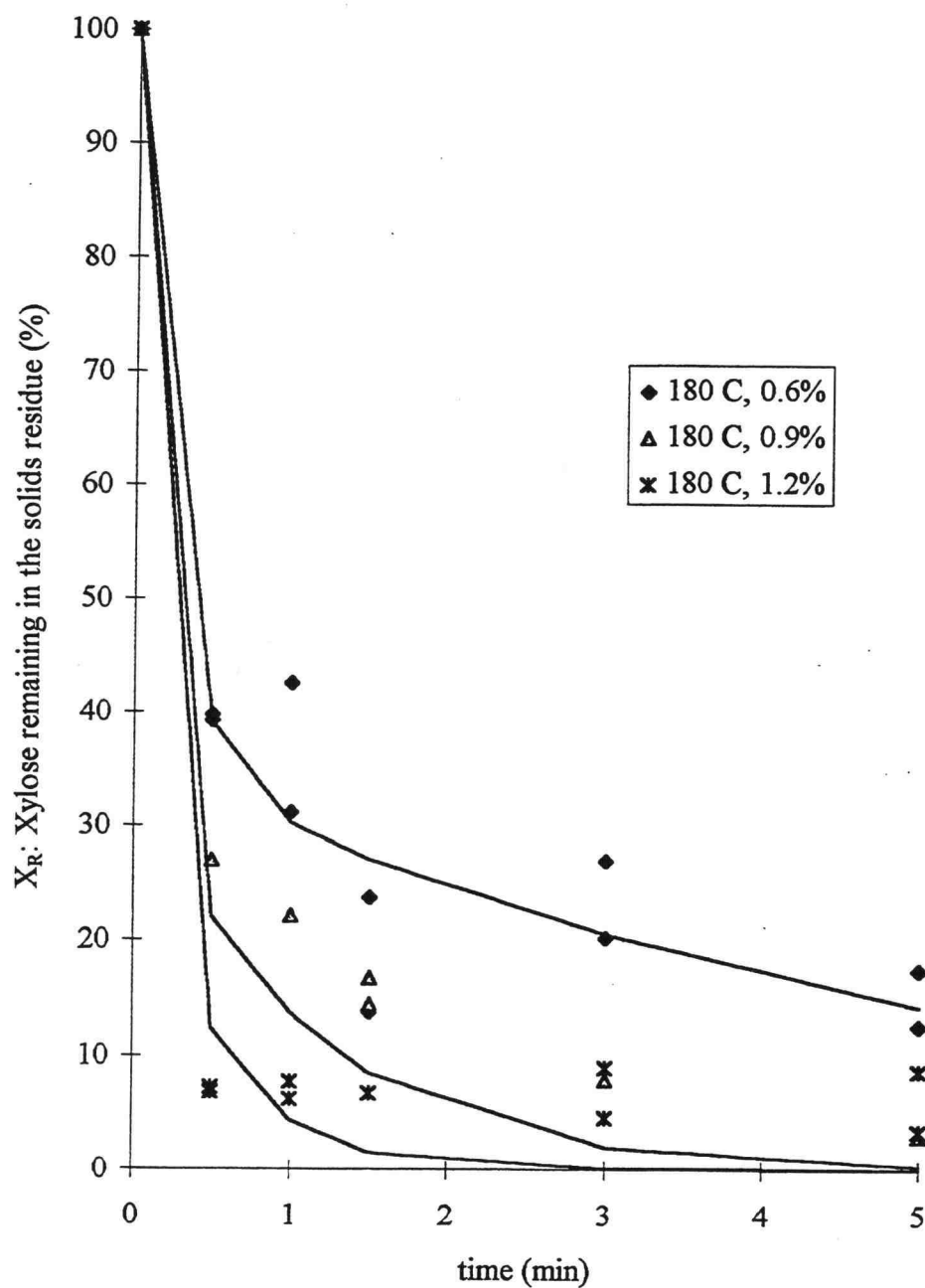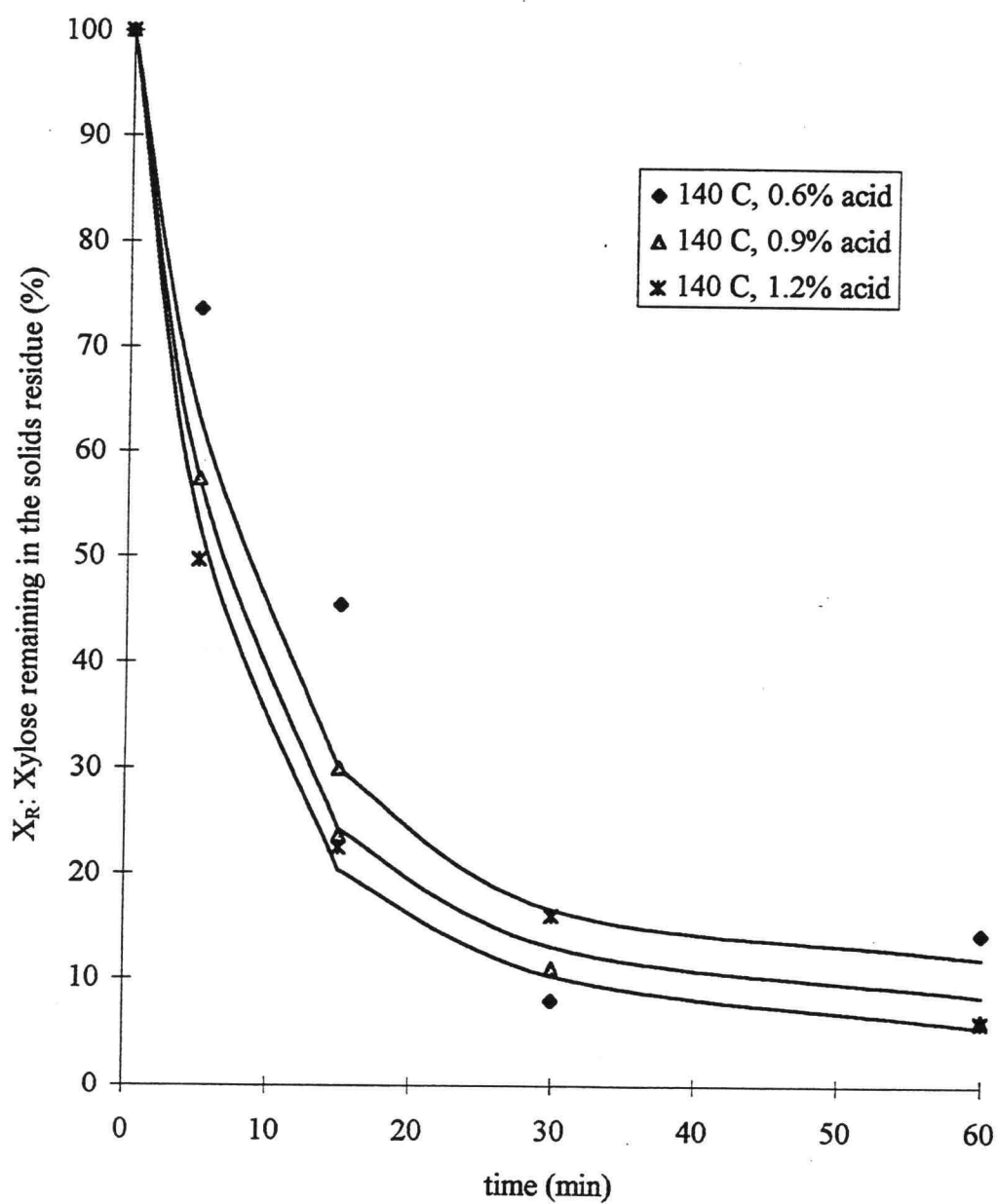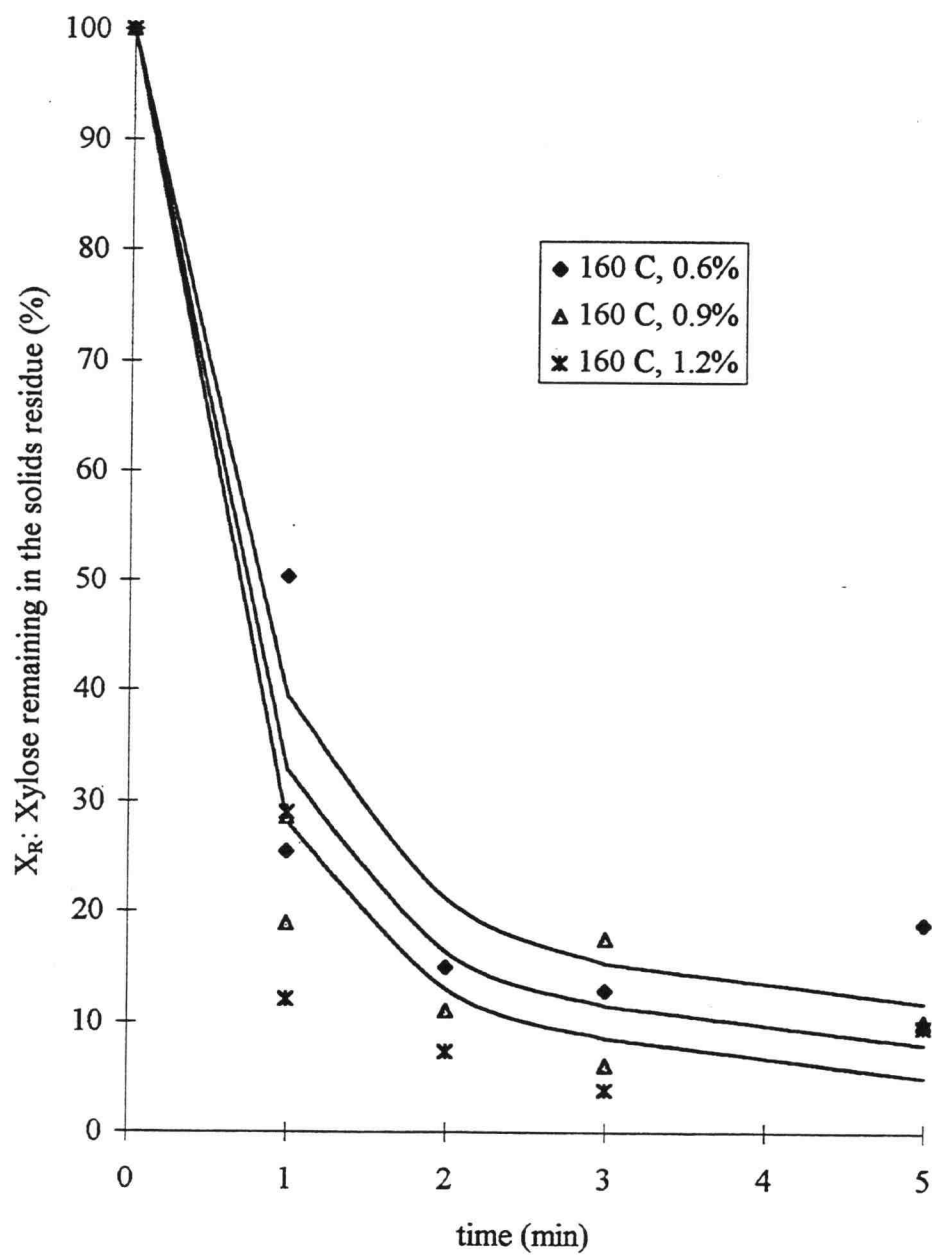solid lines show the predicted values

Fig. A.3. Xylan hydrolysis of <u>corn stover</u> at 180 °C
solid lines show the predicted values

Fig A.4. Xylan hydrolysis of <u>poplar</u> at 140 °C
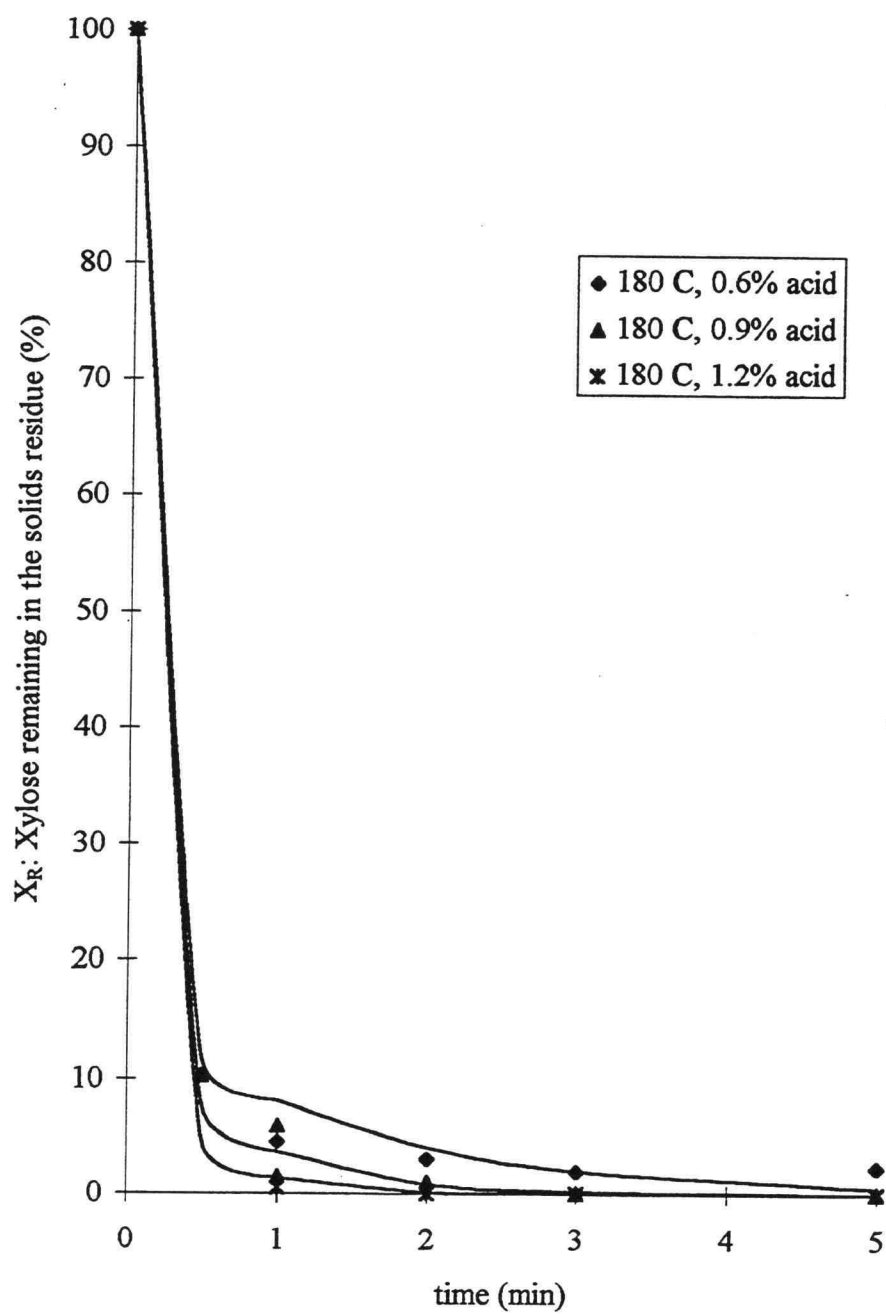solid lines show the model-predicted values

Fig A.5. Xylan hydrolysis of <u>poplar</u> at 160 °C
solid lines show the model-predicted values

Fig. A.6. Xylan hydrolysis of <u>poplar</u> at 180 °C
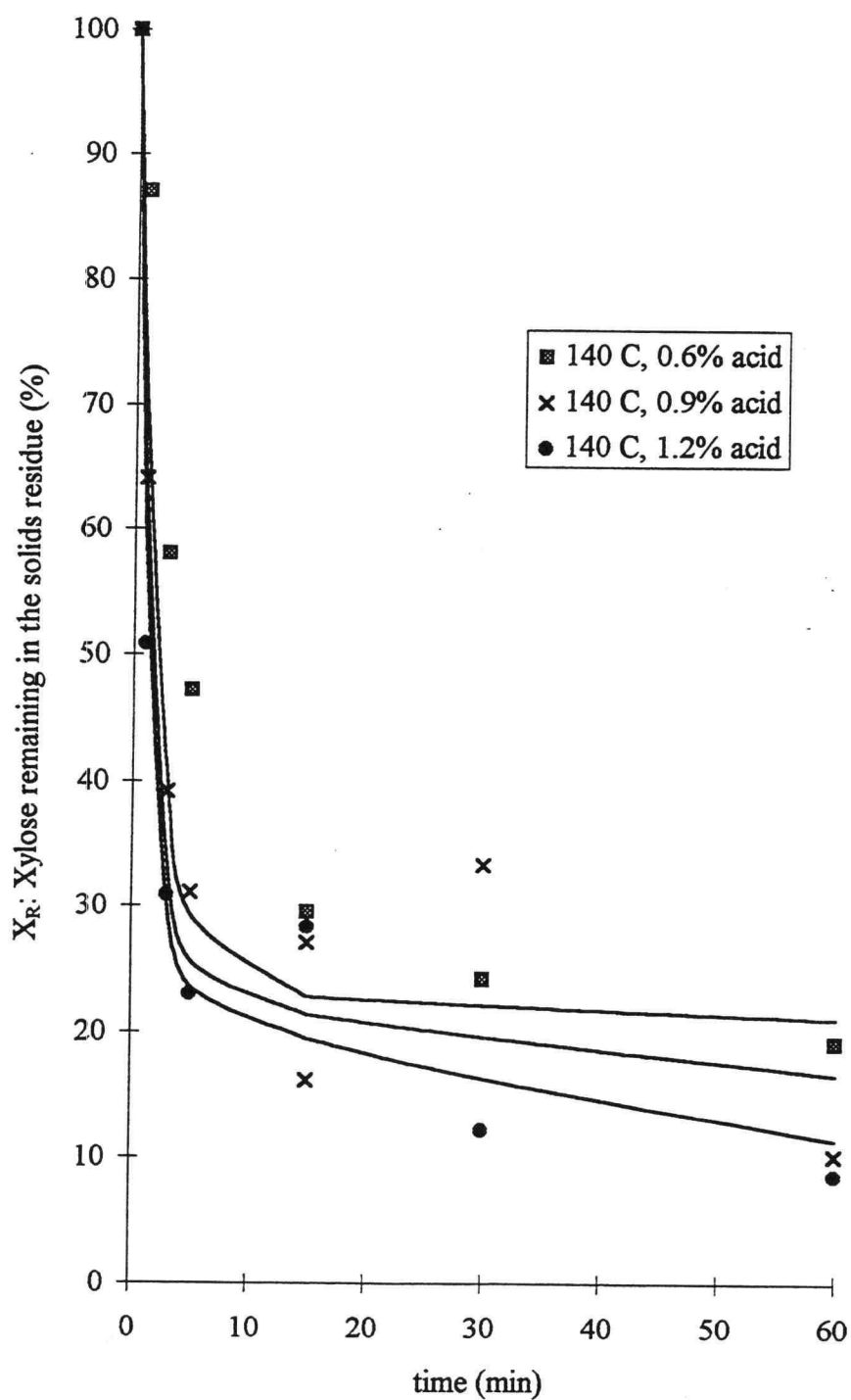solid lines show the model-predicted values

Fig. A.7. Xylan hydrolysis of <u>switchgrass</u> at 140 °C
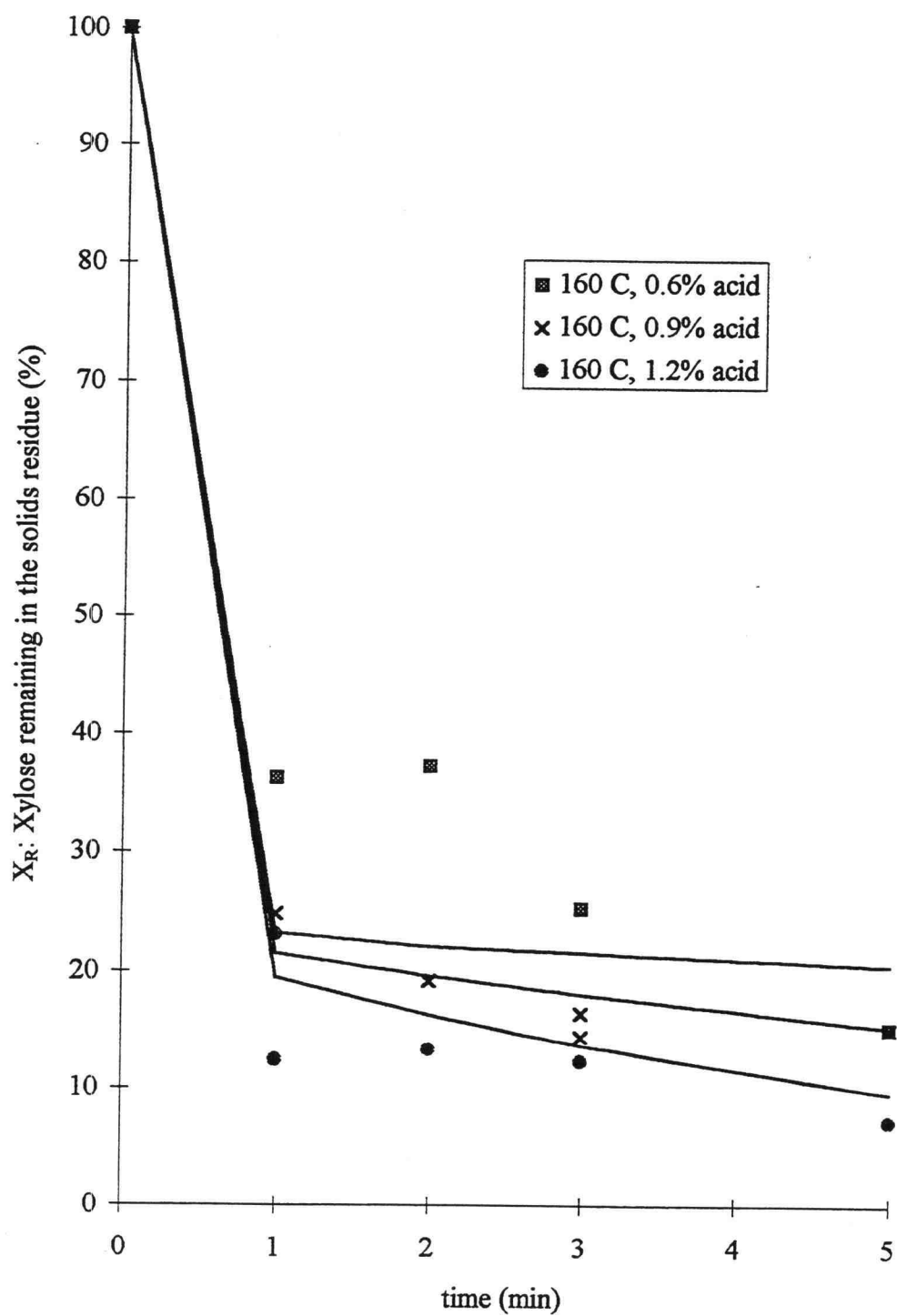solid lines show the model-predicted values

Fig. A.8. Xylan hydrolysis of <u>switchgrass</u> at 160 °C
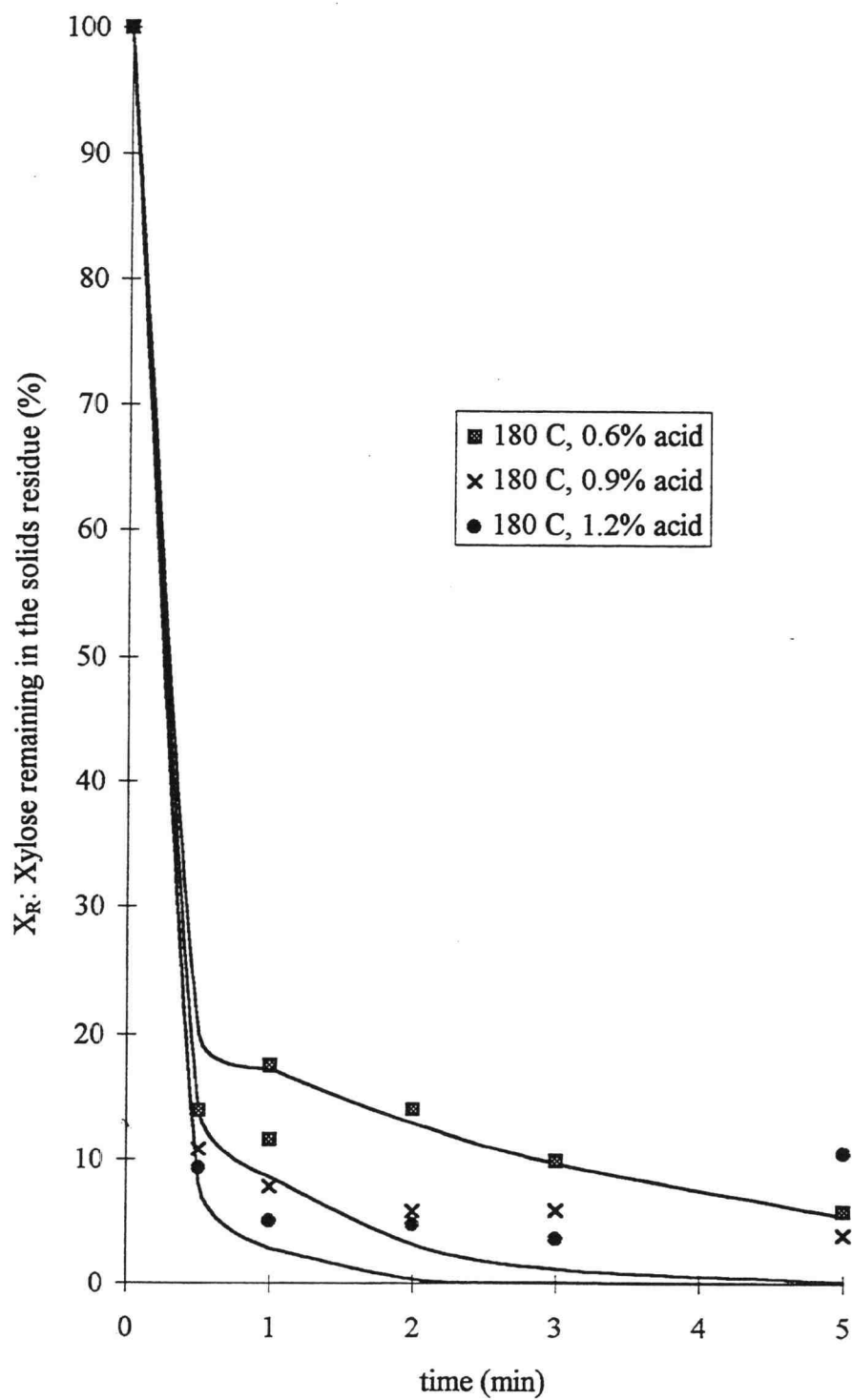solid lines show the model-predicted values

Fig. A.9. Xylan hydrolysis of <u>switchgrass</u> at 180 °C
solid lines show the model-predicted values