Fundamental Study on Kinetics of Hemicellulose Hydrolysis and Bioconversion of Hemicellulose Hydrolysate Mixture into Lactic Acid

by

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Dissertation Abstract

In the first part of this dissertation, the kinetics of hemicellulose hydrolysis in dilute-acid hydrolysis of Kramer Corn Stover (KCS) under high-solid conditions was investigated. Dilute-acid pretreatment is one of the most advanced and widely accepted pretreatment technologies. The National Renewable Energy Laboratory (NREL) has developed a continuous auger-driven pretreatment reactor that can be operated with high-solid charge. It operates at high temperatures and with short residence times resulting in high productivity and sugar concentrations above 10 wt%. We investigated the kinetics of the reactions occurring in dilute-acid pretreatment of KCS, covering the reaction conditions similar to those of the NREL reactor. For this purpose, batch experiments were performed under high solid conditions and the data were put into kinetic model. The conventional bi-phasic kinetic model was initially adopted: two different fragments of hemicellulose to oligomers, to xylose, and to decomposition products. In the model verification process, we observed consistent discrepancies in which xylose and its oligomers were under-estimated during the latter phase of the reactions. It was then speculated that side reactions may have occurred, likely the recombination of xylose and its oligomers with soluble lignin forming lignin-carbohydrate complex (LCC). In subsequent investigations, we positively identified the presence of LCC in the pretreatment liquid by Nuclear Magnetic Resonance (NMR) and by Fourier Transform Infrared (FTIR). The kinetic model was therefore modified incorporating the side reactions. The revised model has shown close and consistent agreement with the experimental data. Although it is a simplified empirical model, the experimental data yielded reliable degree of confidence in the Arrhenius plots for all of the rate constants. This model can thus serve as a useful tool for optimal design and operation of dilute-acid pretreatment reactors in NREL.

Although the continuous reactor is designed to behave as a plug flow reactor (PFR), the residence time distribution (RTD) data obtained from tracer tests have shown that the flow pattern in the reactor deviates significantly from PFR. The kinetic information was put into the reactor performance model incorporating the RTD information. The results were further analyzed to assess the effects of dispersion (RTD) on the performance of the NREL-continuous reactor. The kinetic data, the model procedure, and the output of reactor performance (yield of xylose and oligomers) as it relates to the degree of dispersion are presented, and the strategy to improve the reactor performance is discussed.

In the second part of this dissertation, secondary hydrolysis of hemicellulose liquor from dilute-acid pretreatment of KCS was investigated. Dilute-acid pretreatment often generates substantial amounts of xylose oligomers. Those oligomers need to be hydrolyzed to monomer for it to be effectively utilized in the bioconversion process. A post treatment, secondary hydrolysis, is therefore necessary. Because of the unique features of the NREL-continuous reactor, namely high solid loading, low acid content, and short residence time, the hydrolysis of hemicellulose was limited. The pretreatment liquor (often termed as pre-hydrolysate) obtained from this reactor thus contains relatively high amounts of xylose oligomers, typically in the range of 10-40% of the total sugar. In order to fully utilize the sugars in the pre-hydrolysate, oligomers need to be hydrolyzed to monomers. However, the secondary hydrolysis of this liquor was found to be much more difficult than hydrolysis of xylose oligomers in a clean environment. This investigation was undertaken to verify the factors inhibiting the acid-catalyzed oligomer hydrolysis. For this purpose, the secondary hydrolysis was experimentally studied using rapid-heating small-scale batch reactors. The experiments were done for pre-hydrolysate and for clean xylose oligomers. The comparison of the results indicated that a significant fraction of oligomers

may be bound to lignin fragments, and some of it may also exist in branched form. Formation of LCC was also proven to be a significant factor in oligomer hydrolysis and xylose degradation.

The third part of the dissertation deals with the production of lactic acid from the mixture of pre-hydrolysate and paper mill sludge by simultaneous saccharification and co-fermentation (SSCF). Paper mill sludge is a solid waste material composed of pulp residues and ash generated from pulping and paper making process. The carbohydrate portion of the sludge from a Kraft mill has chemical and physical characteristics similar to those of commercial-grade wood pulp. Because of its high carbohydrate contents and well-dispersed structure, the sludge can be biologically converted to value-added products without pretreatment. In bioconversion of solid feedstock such as paper mill sludge, a certain amount of water must be added to attain fluidity. In this study, hemicellulose pre-hydrolysate, in place of water, was added to the sludge to increase the concentration of the final product. Pre-hydrolysate was obtained by hot-water treatment of pine wood in which the total sugar concentration reached 4 wt. %. The mixture was subjected to SSCF using enzymes (cellulase and pectinase) and Lactobacillus *delbrueckii* (ATCC-10863). Pectinase was added to convert mannose oligomers in the prehydrolysate to monomers. During the SSCF of the mixture, calcium carbonate existing in the paper sludge acted as a buffer keeping the pH to near optimum. The overall product yield on the basis of total carbohydrate content of the initial feed ranged 80-90% of the theoretical maximum. Use of the mixture of pre-hydrolysate and pulp mill sludge as the fermentation feed also increased the product concentration to 60 g of lactate/L.

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List of Abbreviations

KCS	Kramer Corn Stover
RTD	Residence Time Distribution
DP	Degree of Polymerization
SSF	Simultaneous Saccharification and Fermentation
SSCF	Simultaneous Saccharification and Cofermentation
SHF	Separate Hydrolysis and Fermentation
NREL	National Renewable Energy Laboratory
HMF	hydroxymethylfurfural
PFR	Plug Flow Reactor
LCC	Lignin-Carbohydrate Complex
LA	Lactic Acid
LAB	Lactic Acid Bacteria
PLA	Poly-Lactic Acid
MSW	Municipal Solid Wastes
NC	Neutralization Capability
DI water	De-ionized water
SS316	Grade 316 Stainless Steel
AAI	Additional Acid Input
RPMS	Recycled Paper Mill Sludge
RPM	Rounds Per Minute

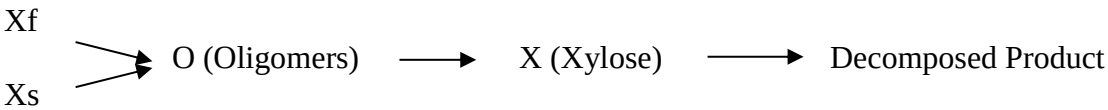
I. Introduction

Biomass is the most important sustainable feedstock for bio-fuels and bio-chemicals today. Lignocellulosic biomass is an abundant and renewable feedstock with an estimated annual worldwide production of 10-50 billion dry tones (Galbe & Zacchi, 2002). Biomass mostly consists of three major components: cellulose, hemicellulose and lignin. From an economic standpoint, it is imperative that all three components be utilized in the biomass conversion process. Hemicellulose is the second largest component in lignocellulosic biomass, representing about 20-35% of biomass. In recent years, bioconversion of hemicellulose has received a great deal of attention due to its amorphous structure that easily hydrolyzes in dilute acid and its practical applications in various agro-industrial processes, such as efficient conversion to fuels and chemicals, digestibility boost for animal feedstock, delignification of paper pulp and improvement in the consistency of beer (Viikari et al., 1993; Wong et al., 1988; Zeikus et al., 1991). The most promising pretreatment option for hemicellulose is dilute acid with high xylose yield which significantly increases susceptibility to hydrolysis for cellulose (Bungay, 1992; Wyman et al., 1993; Lloyd & Wyman, 2005). The dilute-acid pretreatment accompanies hydrolysis of the hemicellulose fraction in the biomass, often termed as prehydrolysis. The prehydrolysis has advanced to the point where the hemicellulose sugars are obtained with yields above 80% and concentrations high enough to be directly used as feed for bioconversion (Springer, 1985; Lee et al., 1978; Nguyen et al., 2000).

The most logical choice for commercial-scale biomass pretreatment reactor is moving-bed (screw-driven) continuous type (Lee et al., 1999). Optimal production of xylose from biomass must be analyzed with respect to reactor yield, product concentration, and reactor cost

considerations (Maloney et al., 1986). Determination of the most desirable processing conditions requires the ability to predict reactor performance from knowledge of hydrolysis kinetics. In particular, the performance of continuous co-current solid-liquid reactors is of interest. NREL has developed an efficient continuous pretreatment reactor that can be operated with high-solid charge and short residence time. In the actual design and operation of the reactor, a number of technical problems have surfaced in the pilot scale testing of the prehydrolysis process. The technical problems stem from several unique features of the process. Firstly, the process is relatively fast, which results in short residence times in the reactor. The reaction is also heterogeneous in that the catalyst is penetrating into the solid reactant. Finally, it is carried out in a moving-bed reactor under high-solid and low-water conditions.

The reaction conditions in this process were beyond the parameters normally explored in conventional processes, particularly with respect to the solid/liquid ratio. It is important to establish the reaction kinetics from which further improvement can be made. The kinetic model of a parallel hydrolysis scheme has been previously developed (Lee et al., 1978; Carrasco & Roy, 1992; Yat et al., 2007; Jacobsen & Wyman, 2002; Jacobsen & Wyman, 2000). This model assumes that xylan is comprised of two different fragments: a fast- and slow-reacting fraction as indicated below. The task in the first part of this dissertation is to generate the necessary kinetics data for these reactions.



Although the NREL reactor is designed to behave as a plug flow reactor (PFR), the residence time distribution (RTD) data obtained from tracer tests have shown that the flow pattern in the reactor deviates significantly from PFR. The kinetic information was put into the

reactor performance model incorporating the RTD information. The results were further analyzed to assess the effects of dispersion (RTD) on the performance of the NREL-continuous reactor.

Dilute acid pretreatment often yields substantial amount of soluble hemicellulose in the oligomeric form (Lloyd & Wyman, 2005; Carvalheiro et al, 2008). The ratio of monomers to oligomers depends on the severity of the pretreatment. More severe conditions are required to achieve maximum monomer yield than was needed to achieve maximum total hemicellulose recovery.

There are not many microbiological cultures that can directly metabolize oligosaccharide to make bio-fuels or chemicals. A post treatment, secondary hydrolysis, is therefore necessary. The post hydrolysis options can be reduced to acid (Saska & Ozer, 1995; Allen et al., 1996; Shevchenko et al., 2000; Duarte et al., 2004; Garrote et al., 2001c; Duarte et al., 2009) or enzyme (Duarte et al., 2004; Vazquez et al., 2001; Walch et al., 1992) catalyzed hydrolysis. Acid hydrolysis typically presents both higher yield and productivity when compared to the enzymatic hydrolysis processes. In contrast to enzymatic hydrolysis, significant monosaccharide degradation reactions may occur during acid post hydrolysis. Examples of such reactions are the degradation of pentoses to furfural, hexoses to HMF, and of both these furans to aliphatic acids such as formic and levulinic acids. Therefore, to obtain a high monosaccharide recovery, a careful optimization of the operational conditions is required.

In the subsequent parts of this dissertation, secondary hydrolysis of hemicellulose liquor from dilute-acid pretreatment of KCS was investigated. This investigation was undertaken to verify the factors inhibiting the acid-catalyzed oligomer hydrolysis. For this purpose, the

secondary hydrolysis was experimentally studied using rapid-heating small-scale batch reactors. The experiments were done for pre-hydrolysate and for clean xylose oligomers. The comparison of the results indicated that a significant fraction of oligomers may be bound to lignin fragments, and some of it may also exist in branched form. Formation of LCC was also proven to be factors significantly affecting the oligomer hydrolysis and xylose degradation.

Lignocellulosic biomass pre-hydrolysate can be converted into bio-fuels or various chemicals with or without mixing with cellulose solids. Lactic Acid (LA) is an important industrial commodity with a large and fast growing market due to its wide applications in cosmetics, pharmaceuticals, textile and food industry (Xu et al., 2006; John et al., 2007; Vickroy, 1985). Its most dominant application is polymerization to biodegradable poly-lactic acid (PLA). Presently, of all the LA produced worldwide, about 90% comes from fermentation while the rest is through chemical synthesis (Hofvendahl & Hahn–Hägerdal, 2000). Most of the fermentation processes utilize starch-derived glucose or sucrose as feedstock (Litchfield, 1996). To reduce the feedstock cost, considerable studies have been pursued on the fermentation of lignocellulosic carbohydrates for lactic acid production (Parajo et al., 1997; Grade et al., 2002; Neureiter et al., 2004).

While a lot of attention and effort have been put into cellulosic LA production, there is another industry struggling in the United States: Pulp and Paper industry. The United States had been the world's leading producer of paper until it was overtaken by China in 2009 (De Sisti, 2012). The decreased demand for paper due to digital information technology and increased competition with cheap supply of foreign papers led American traditional pulp and paper manufacturers to look for new opportunities and industries. Paper mill sludge is a solid waste material composed of pulp residues and ash generated from the pulping and paper making

process. Paper sludge has been a disposal liability for many years (Albertson & Pope, 1999). However, due to its high carbohydrate content and well-dispersed structure, the sludge can be biologically converted to value-added products without pretreatment (Schmidt et al., 1997; Lark et al., 1997; Kang et al., 2010). Utilization of paper mill sludge as feedstock to produce LA has at least two economic advantages. First, the cost of this feed stock is basically zero or even negative considering the cost of disposal is about $20 per wet ton (Kang et al., 2010). Paper sludge is produced at a concentrated site and permanent production location, making the feedstock availability reliable. Second, pretreatment is not necessary to bio-convert paper mill sludge to products. Pretreatment is one of the most costly steps in bio refinery process, thus elimination of pretreatment significantly reduces the production cost of LA. In bioconversion of solid feedstock such as paper mill sludge, a certain amount of water must be added to attain fluidity. In this study, hemicellulose pre-hydrolysate, in place of water, was added to the sludge to increase the concentration of the final product.

The main objectives of the final part of this dissertation are to characterize the chemical and physical properties of paper mill sludge, explore the new scheme of bioconversion process using two feed streams readily available from pulp and paper industry.

II. Literature Review

II.1 Background of lignocellulosic biomass

II.1.1 Types of lignocellulosic biomass

Biomass is the most important renewable energy source today. Lignocellulosic biomass is an abundant and renewable feedstock with an estimated annual worldwide production of 10-50 billion dry tones (Galbe & Zacchi, 2002). Various types of lignocellulosic feedstocks are being used for production of bio-fuels and value-added products, such as lactic acid. Agricultural residues, wood and herbaceous crops, municipal solid wastes and paper mill sludge wastes can all be used as lignocellulosic feedstocks. They have distinct advantages over the first generation feedstocks (starch and sugars) since they have a much higher biomass yield per acre and make use of wastes from various sources (Tilman et al., 2006; Sun & Cheng, 2002; Lavigne & Powers, 2007).

Agricultural Residues

These are the leftovers in the agriculture fields after grains are harvested. Corn stover, wheat straw, and rice straw are the most common agricultural biomass. Compared to the woody biomass, these feedstocks are easier to pretreat and biologically convert to biofuels and chemicals. Table II-1 lists the availability data reported by Perlack et al. (2011). Agricultural residues clearly dominate available biomass resources for bioprocess. As these data show, corn stover is the most abundant agricultural residue. Numerous studies have been focused on corn stover as feedstock to make biofuels and/or value-added products (Sheehan et al., 2003; Öhgren et al., 2006; Lau & Dale, 2009; Varga et al., 2004; Zhu et al., 2007; Sreenath, et al., 2001). Energy crops, such as switch grass and hybrid poplars crops are grown for the purpose of

producing energy and/or chemicals (Keshwani & Cheng, 2009). These crops grow fast and require minimum nutrients. They can be cultivated on fallow, non-agricultural lands.

Table II-1Estimated availability of selected feedstocks

Feedstock type	Estimated availability (million dry t/yr)
Corn stover	215
Other agricultural residues	80
Energy crops	95
Wood residues	129

Woody biomass

Woody biomass is physically larger and structurally stronger and denser than non-woody biomass. Chemically, woody biomass contains higher lignin content compared to other lignocellulosic biomass. There are two types of wood: softwoods such as pine, and hardwoods such as poplar. The fiber in softwoods is longer than that in hardwoods. Hardwoods have a lower level of lignin than softwoods. Also, the degree of polymerization (DP) of softwood lignin is higher than that of hardwood lignin. Softwoods contain more glucomannans than hardwoods, while hardwoods contain more xylans. Softwood species generally show higher recalcitrance toward external chemical and enzymatic treatment compared to the hardwood species (Mabee et al., 2006; Sannigrahi et al., 2010).

Wastes

There is a wide range of biomass materials that are produced as wastes from other processes, operations or industries. Many of these have valuable energy contents that can usefully be exploited. Waste sludge from pulp and paper mills, recycled paper and municipal solid wastes (MSW) are the main examples of this type of biomass. Due to the high percentage of cellulose and low lignin content, these materials are very feasible substrates for bioprocess.

II.1.2 Structure and chemical composition of lignocellulosic biomass

Lignocellulosic biomass is comprised of three main components: cellulose, hemicellulose and lignin. The remainder minor components are ash and extractives. The composition of biomass varies greatly with the type of feedstocks as well as species. Table II-2 gives the average composition of various biomass feedstocks (Sun & Cheng, 2002).

Table II-2 Composition of different biomass feedstocks

Feedstock	wt% of dry biomass		
	Cellulose	Hemicellulose	Lignin
Hardwood	40-55	20-40	18-25
Softwood	45-50	25-35	25-35
Grasses	25-40	25-50	17-30
Straws	30-40	20-30	15-25
Waste paper	60-70	10+20	5-10
MSW	8-15	ND*	24-29

*ND: not determined.

Cellulose

Cellulose is the main constituent in lignocellulosic biomass, accounting for 30-45 wt%. Cellulose is derived from D-glucose units which condense through β (1-4)-glycosidic bonds (Figure II-1). This linkage is different from that for α (1-4)-glycosidic bonds present in starch, glycogen, and other carbohydrates. The cellulose molecule is a linear, unbranched homo-polysaccharide consisting of 10,000 to 15,000 D-glucose units (Goldstein, 1981). The linear chains in cellulose are highly stable and resistant to chemical or enzyme attacks because hydrogen bonding between cellulose chains makes the polymers more rigid. Cellulose molecules have a strong tendency to form intramolecular and intermolecular hydrogen bonds which may give rise to various ordered crystalline arrangements (Nelson et al., 2008). Thus, bundles of

cellulose molecules are aggregated together and form micro-fibrils, in which highly ordered (crystalline) regions alternate with less ordered (amorphous) regions resulting in low accessibility to enzymes. Micro-fibrils build up fibrils and, finally, cellulose fibers (Sjöström, 1993; Zhang et al. 2007).

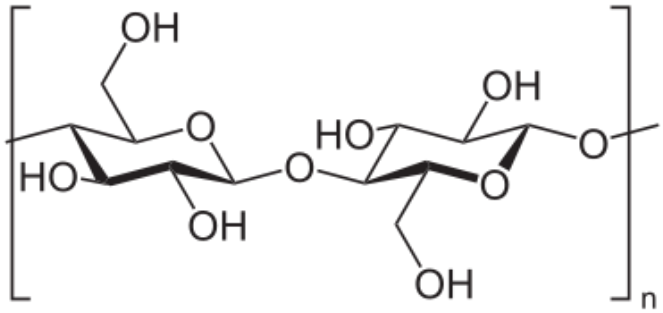


Figure II-1 Schematic structure of cellulose

The degree of crystallinity and degree of polymerization (DP) of cellulose are two important factors in determining its susceptibility to hydrolysis. It has been shown that the amorphous portion can easily be hydrolyzed while the crystalline portion is more resistant to hydrolysis (Fan et al., 1980, 1982; Knappert et al., 1981). The DP is more important when hydrolysis reaction is carried out using enzyme. Certain fragments of enzymes cleave the cellulose by an end-wise mechanism (Puri, 1984).

Hemicellulose

Hemicellulose is the second most common polysaccharides in nature. It represents 20-35 wt% of lignocellulosic biomass. Unlike cellulose, hemicelluloses are not chemically homogeneous; they are highly complex, branched polymers made up of pentoses (xylose, arabinose), hexoses (mannose, glucose, and galactose) and sugar acid. Agricultural residues, such as corn stover and sugarcane bagasse, contain large amounts of xylan, some arabinan, and only very small amounts of mannan. Hardwood hemicellulose mostly contains xylans while

softwood hemicellulose contains large amounts of glucomannan. Xylan is the most abundant hemicellulose, while in softwoods mannan tends to be the most abundant hemicellulose.

The structure of xylan is characterized by a long linear backbone chain of 1, 4-linmked β-D-xylopyranose units (Figure II-2). The frequency and composition of branches are dependent on the source of xylan (Aspinall, 1980). The side chains consist of *O*-acetyl, α-L-arabinofuranosyl, α-1, 2-linked glucuronic or 4-*O*-methylglucuronic acid. The DP of hemicellulose is much lower than cellulose (about 200 vs. more than 10,000). Due to its amorphous structure and low DP, hemicellulose is the most thermal and chemical sensitive amongst the three main components of biomass. During acid pretreatment, hemicellulose is the first to be hydrolyzed (Schell, 2003; Bobleter, 1994).

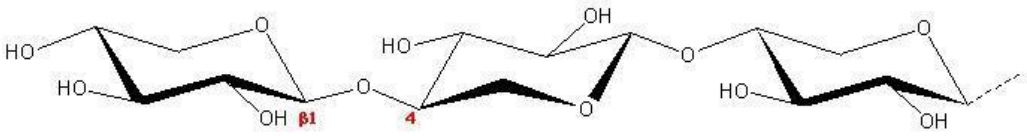


Figure II-2 Schematic structure of cellulose

Lignin

Lignin is the major non-carbohydrate in biomass accounting for 15-25 wt%. Lignin is a complex phenylpropanoid polymer that plays a critical role in giving structural rigidity to hold plant fibers together. The main substituents of lignin, coniferyl alcohol, sinapyl alcohol and p-coumaryl alcohol (Figure II-3) are linked together via β-*O*-4, α-*O*-4, β-5, β-1, 5-5, 4-*O*-5 and β–β linkages (Buranov & Mazza, 2008; Gierer, 1980). The various ratios of the different monomers in different plant species is listed in Table II-3.

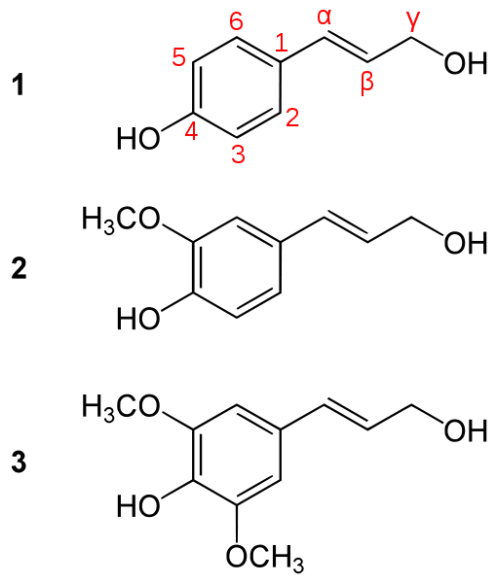


Figure II-3 Three common monolignols

(1) p-coumaryl alcohol (H); (2) coniferyl alcohol (G); (3) sinapyl alcohol (S)

Table II-3 Ratio of the lignin monomers in plant species

Species	H / G / S ratio (%)
Herbaceous plants	(5-26) / (27-54) / (44-67)
Softwood	(2-18) / (82-98) / trace
Hardwood	0 / (22-66) / (44-86)

The lignin must be separated from carbohydrates during biomass conversion to open the protective biomass structure. Therefore, pretreatment is required to improve the susceptibility of the biomass for a subsequent process.

II.2 Pretreatment of biomass

II.2.1 Need for pretreatment and options

The cellulose in lignocellulosic biomass was covered by a lignin-hemicellulose matrix, which makes biomass recalcitrant to direct enzymatic hydrolysis. Additionally, a high degree of crystallinity and polymerization barricade the cellulose from hydrolysis. Therefore, pretreatment is necessary to improve the digestibility of the biomass for a subsequent enzymatic hydrolysis step (Figure II-4) (Mosier et al., 2005). The overall purpose of pretreatment is to break down the shield formed by lignin and hemicellulose, disrupt the crystalline structure and reduce the degree of polymerization of cellulose.

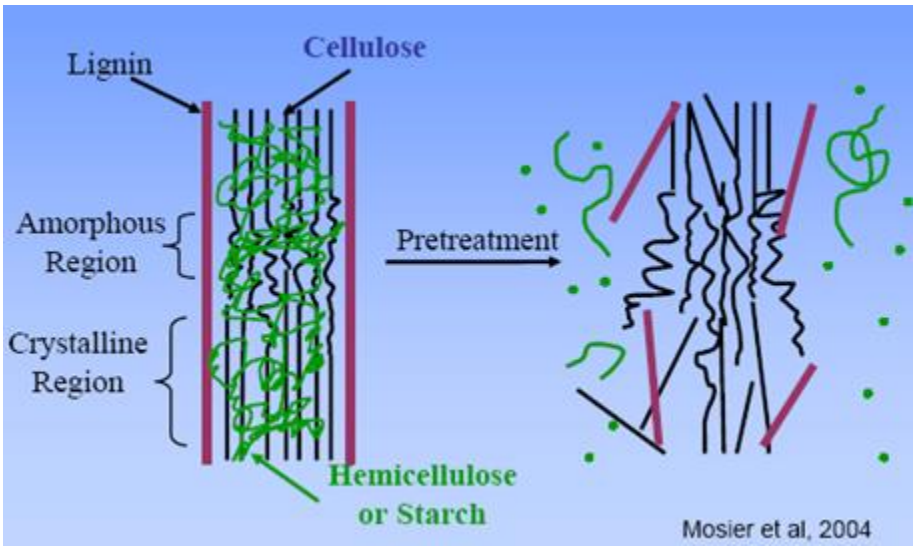


Figure II-4 Pretreatment effect on lignocellulosic biomass (Mosier et al., 2004)

A number of pretreatment technologies based on numerous physical, chemical and biological methods have been developed. Physical pretreatment technologies do not involve the use of chemicals. Chemical pretreatment technologies include: acid pretreatment, alkaline pretreatment and organosolv pretreatment. Biological pretreatment technologies employ lignin degrading microorganisms to improve substrate accessibility to enzymes (Taniguchi et al., 2005).

In order to achieve higher efficiency, some processes are a combination of two or more pretreatment methods (McMillan, 1994).

Physical pretreatment

Mechanical pretreatment is one type of physical pretreatments. It includes process like fine milling and grinding. Mechanical pretreatment reduces the particle size, thus increasing the surface area of biomass. It is also believed that mechanical pretreatment is able to decrystallize cellulose. Another physical pretreatment option is through non-mechanical methods that include irradiation, pyrolysis, steaming, and microwave treatment. The power required for physical pretreatment is usually very high. As a result of its high cost, physical pretreatment is not considered an attractive option in biomass processing.

Chemical pretreatment

Acid pretreatment: Acid pretreatment has a long history of use with most feedstocks because of its high efficiency (Grethlein et al., 1984; Schell et al., 1992; Nguyen et al., 1998; Schell et al., 2003). Various acids such as sulfuric acid, hydrochloric acid, phosphoric acid, and nitric acid can be used in this process (Allen et al., 2001; Kalman et al., 2002; Saha et al., 2005a; Saha et al., 2005b; Kim & Mazza, 2008). The concentrated acid process has relatively high sugar yield with very little sugar degradation. However, it is extremely difficult to recover most of the acid, thus making this process economically unfeasible.

Dilute acid processes (0.5-1.5 wt%, 121-180^{o}C) have been most favored for industrial application, since these processes achieve reasonable high sugar yields from hemicellulose. Lignin is not removed and the cellulose crystallinity is not affected at these pretreatment conditions (Sun & Cheng, 202; Hsu, et al., 2010; Guo et al., 2008; Zhu et al., 2009). Besides the

hydrolysis of hemicellulose, dilute acid pretreatment decreases the DP of cellulose, thus, improves the remaining cellulose susceptibility to enzymes. Since the hemicellulose can be used for the production of biofuels and other value-added products, the liquid stream rich in hemicellulose is added back to the bioreactor (Figure II-5).

Certain levels of furfural and lignin degradation products will be produced during the dilute acid pretreatment process. These are inhibitory to fermentation organisms and/or enzymes. So, a detoxification step is usually needed to both neutralize and detoxify the pretreatment liquor. Another disadvantage of the dilute acid process is that it requires a significant investment in equipment and materials to address acid corrosion.

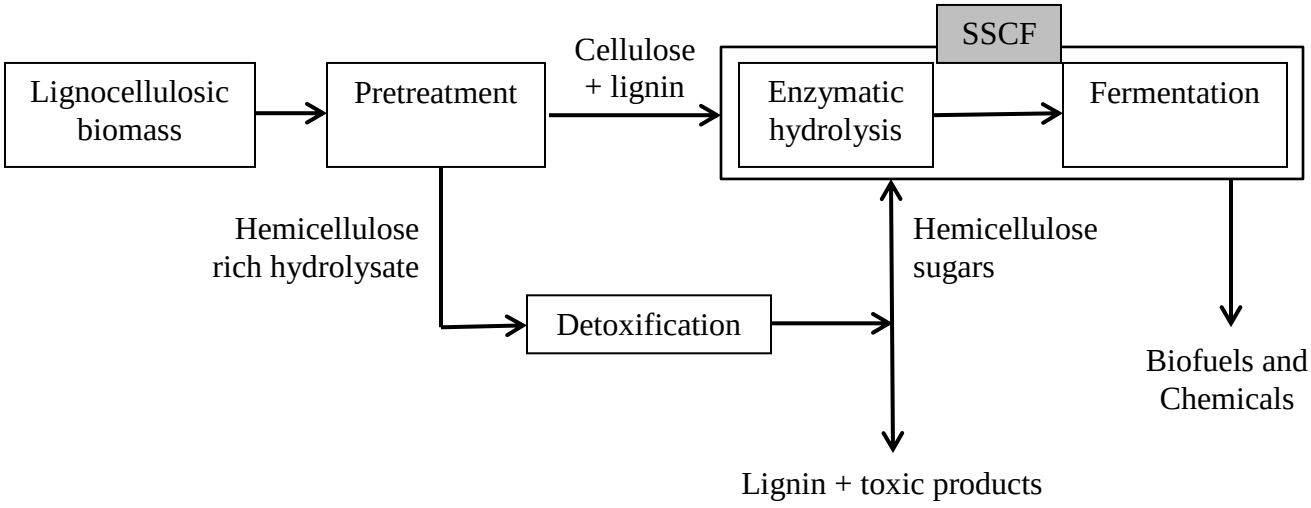


Figure II-5 Process diagram for dilute acid pretreatment of biomass for bioprocess

Alkaline pretreatment: In contrast to the acid process, alkaline pretreatment is more effective for delignification. Only minor amounts of cellulose and hemicellulose are solubilized (Carvalheiro et al., 2008). During alkaline hydrolysis, alkaline induces swelling which leads to an increase in the internal surface area. Next, a decrease in DP and crystallinity occurs with a

consequent cleavage of lignin-hemicellulose bonds. Then, degradation of lignin takes place. Reagents used in alkaline pretreatment can be categorized into two groups: i) sodium hydroxide, potassium hydroxide, or calcium hydroxide; and ii) ammonia. Sodium hydroxide and lime are stronger reagents as compared to ammonia. For lignocellulosic feedstocks with higher lignin content, NaOH is a better delignifying reagent since those feedstocks are more recalcitrant. Ammonia, on the other hand, shows higher efficiencies in the pretreatment of agricultural residues that have low lignin content. Recovery of the reagents is an important part of alkaline pretreatment. The recovery of sodium hydroxide has been well documented in the pulp industry and the high volatility of ammonia makes it easy to recover and reuse. However, the main disadvantage of these alkaline reagents is that they are expensive.

Organosolv pretreatment: As the name indicates, organosolv pretreatment uses organic solvents (ethanol, acetone, carboxylic acid, etc.) as reagents. This process is usually run at a high temperature (e.g., 200^{o}C) and high pressure (Zhao et al., 2009; Hallac et al., 2010). Lignin and hemicellulose can be solubilized while cellulose is protected from solubilization. The advantage of organosolv pretreatment is that the organic solvents are recoverable through distillation and are recycled for pretreatment. However, organosolv pretreatment must be performed under extremely tight and efficient control due to the volatility of organic solvent. Therefore, organosolv pretreatment is not a feasible choice at present.

Biological pretreatment

Biological pretreatment uses lignin degrading microorganisms, such as white, brown and soft rot fungi and bacteria to modify the chemical composition and/or structure of the cellulosic biomass (Kirk and Farrell, 1987). The advantage of biological pretreatment is that the enzymes and microbes are very specific, rarely producing inhibitors or toxins and effectively degrading

lignin. Also, this process requires low energy input and mild environmental conditions. But reaction times tend to be long; anywhere between 5 days and a month. This slow rate, as compared to chemical treatments, has prevented the usage of biological pretreatment in commercial scale plants.

II.2.2 Pretreatment at high solid loading

Any thermo-chemical pretreatment process requires significant amount of water. The reasons are as follows: (1) Biomass is highly water-absorbing and the chemicals used in the pretreatment (e.g. sulfuric acid) are unable to penetrate into the substrate if the substrate is not fully wetted. (2) Excess water is needed to maintain easily-deliverable slurry in continuous pretreatment reactors. For batch reactors, water is required to enable the substrate to be evenly distributed by agitating or shaking. (3) In order to achieve a good heat transfer, the void space between biomass particles needs to be filled with liquid.

A high solid loading is important for process economics because this reduces the reactor size, lowers the cost in heating, and lowers water usage (Hsu et al., 1996). In a previous study, a solid loading of 40% was applied for pretreatment of aspen wood and wheat straw in a small (1/2" o.d. × 4" long) pipe reactor with indirect heating (Grohmann et al., 1986a). Another study involved the loading of 30% solids for pretreatment of corn stover that was applied in a cylindrical reactor with direct steam injection aided by indirect heating (Schell et al., 1992). In a third study, a 100-L pilot-scale horizontal shaft mixer/reactor was used for dilute-acid pretreatment of lignocellulosic biomass at a solids loading of 10-15%, with heating either by steam injection into the reactor jacket or directly into the reactor (Hsu et al., 1996). Shortly afterwards, a solids concentration of 40-50% using a 130-L reactor was reported by the same

group (Kadam and Hsu, 1997). In NREL, the solid loading used in their screw-driven continuous reactor is 30%.

The solid liquid (S/L) ratio is also a minor process variable that affects kinetics. Saeman (1945) reported that S/L ratio over the range of 5 to 20 had a small but definite effect on the rate of cellulose hydrolysis.

II.3 Modeling investigation on acid pretreatment of hemicellulose

Because of the heterogeneity in component and structure, hemicellulose exists in an amorphous state which makes it easier to hydrolyze than cellulose. During the initial phase of acid pretreatment, random attacks by acid on the hemicellulose chains produce oligomers with varying DP. The oligomers continue to break down to monomers. When xylose is subjected to acid for an extended period of time, it will degrade to furfural, reducing the sugar yield and inhibiting the subsequent fermentation process. The overall hydrolysis reaction can be termed as a continual depolymerization process in which the average molecular weight is gradually decreased. The intermediate product, oligomer, is defined as a water-soluble polymer (1< DP <10) (Lee & McCaskey, 1983).

Previous studies in hemicellulose hydrolysis led to various kinetic models. The simplest model describing hemicellulose hydrolysis is based on a two-step first-order reaction (Ranganathan et al., 1985; Bhandari et al., 1984; Converse et al., 1989). According to this model, hemicellulose is hydrolyzed to xylose, which breaks down to degradation products in the second step:

$$Xylan \xrightarrow{k1} Xylose \xrightarrow{k2} Furfural \qquad \text{(II-1)}$$

In 1956, Kobayashi & Sakai (1956) proposed a model in which hemicellulose was categorized into two fragments: fast-reacting xylan and slow-reacting xylan. This modification was based on the fact that the hydrolysis rate decreased dramatically after conversion reached approximately 70%. Since then, most kinetic studies of hemicellulose (Nee & Wen, 1976; Conner, 1984; Grohmann et al., 1986a; Maloney et al., 1985; Maloney et al., 1986; Kim and Lee, 1987; Esteghlalian et al., 1997) have been based on this reaction pattern:

$$\begin{matrix} Fast\text{–}Xylan \\ Slow\text{–}Xylan \end{matrix} \begin{matrix} \overset{k1f}{\longrightarrow} \\ \overset{k1s}{\longrightarrow} \end{matrix} Xylose \overset{k2}{\rightarrow} Furfural \qquad \text{(II-2)}$$

The biphasic behavior of hemicellulose can be explained in many ways. First, a portion of xylan is located in the cell wall and is easily accessible to the reagent, while the remaining xylan is located at a greater depth and is firmly retained within cellulose chains. Second, the slow reaction is due to a part of xylan that is embedded within or attached to the lignin by lignin-carbohydrate bonds (Conner, 1984). Third, the difference in the hydrolysis rate is attributed to the variation in the polymeric structure of xylan as the acetyle and uronic acid ratios to xylose change (Nikitin, 1966). The proportion of slow-reacting hemicellulose is estimated to be 0.20 - 0.32 (Grohmann et al., 1986a; Maloney et al., 1985; Kim and Lee, 1987, Eken-Saraçoğlu et al., 1998). The percentage of fast-xylan in various biomass species from literature is shown in Table II-4.

Table II-4 Fast-xylan fraction in various biomass species

References	Feedstock	Fast-xylan fraction (%)
Simmonds et al. (1955)	Sweetgum	70.0
Kobayashi & Sakai (1956)	Buna	70.0
Springer et al. (1963)	Aspen	60.0
Springer & Zoch (1968)	Aspen, Birch, Elm, Maple	60.0
Veeraraghavan et al. (1982)	Southern red oak	72.0
Conner (1984)	Southern red oak	73.9
	Paper birch	71.6
	Red maple	80.3
	Quaking aspen	76.0
	American elm	84.3
Maloney et al. (1985)	Paper birch	68.4
Grohmann et al. (1986)	Aspen	76.0
	Wheat straw	67.0
Kim & Lee (1987)	Southern red oak	69.7
Esteghlalian et al. (1997)	Corn stover	64.4
	Switchgrass	76.8
	Popler	83.8

A third variation of the basic model is the inclusion of an oligomer intermediate. The measurement of significant amount of oligomers in several studies (Abatzoglov et al., 1986; Chen et al., 1996; Conner & Lorenz, 1986; Jensen et al., 2008) supports the inclusion of the oligomer intermediate in the kinetic model:

$$Hemicellulose \overset{k1}{\rightarrow} \begin{matrix} Fast\text{–}Xylan \\ Slow\text{–}Xylan \end{matrix} \overset{\begin{matrix} kf \\ ks \end{matrix}}{\rightarrow} Oligomer \overset{k2}{\rightarrow} Xylose \overset{k3}{\rightarrow} Furfural \qquad \text{(II-3)}$$

Model II-2 applies to situations where the oligomer to monomer reaction rate is much faster than the formation of oligomers that the latter can be omitted. Model II-3 is best used for reactions under relative mild conditions where buildup of oligomers cannot be neglected, such as in flow through systems (Chen et al., 1996; Jacobsen et al., 2000).

In either case, the reactions are assumed to be pseudo-homogeneous following a first-order dependence on reactant concentrations. The major parameters that affect the kinetic of hemicellulose hydrolysis are temperature and acid concentration. The catalytic activity of acid depends on the type of acid, the L/S ratio and the neutralization capability of the substrate. Each rate constant follows the Arrhenius equation with the addition of an acid term:

$$k = k_0 \times A^n \times e^{-E/RT} \quad \text{(II-4)}$$

where k_0: pre-exponential factor (min^{-1}); E: activation energy (kJ/mol);
n: acid exponent; A: acid concentration (wt%);
R: 8.314×10^{-3} (kJ/mol·K); T: temperature (K).

The concentration of acid should be adjusted according to the feedstock's neutralization capability. Lignocellulosic biomass, such as agricultural residues and forest wastes, has significant levels of mineral content. It is well known that these minerals neutralize some of the acid during dilute acid pretreatment, reducing its effectiveness (Grohmann et al., 1986a; Malester et al., 1992; Springer & Harris, 1985). Increasing the amount of acid used to compensate for the minerals' buffering action is critical especially under high solids loadings, a desired processing condition. However, for sulfuric acid in particular, an equilibrium shift to formation of bisulfate during neutralization can further reduce hydrogen ion concentrations and compound the effect of neutralization. Because the equilibrium shift has a more pronounced effect at lower acid concentrations, additional acid is needed to compensate. Coupled with the effect of temperature on acid dissociation, these aforementioned factors necessitate increased levels of acid to achieve a particular reaction rate; unless, of course, minerals are removed prior to hydrolysis. Neutralization of sulfuric acid by the minerals in biomass reduces the hydrogen ion activity and must be taken into account for models to accurately predict the performance of dilute-acid

hemicellulose hydrolysis. Furthermore, in the case of sulfuric acid, the neutralization products lead to a bisulfate ion shift, further reducing active hydrogen ion. Neutralization and formation of bisulfate can have a particularly significant impact on low acid concentrations or high solids loadings.

II.4 Reactors for pretreatment and reactor residence time distribution

II.4.1 Application of continuous reactor in acid pretreatment

The most logical choice for commercial-scale biomass pretreatment reactor is a moving-bed (screw-driven) continuous type. This type of reactor has a flow-through design, with the solid and liquid traveling through the reactor at the same velocity. The pretreatment reactions proceed as the reagents travel through the reactor. The flow pattern of screw-driven continuous reactors is similar to that of plug flow reactors (PFR). A continuous screw-driven reactor process is capable of providing a unique and continuously stirred thermo-chemical reactor environment in combination with thermo-mechanical energy and dilute acid. This type of reactor can provide high shear, rapid heat transfer, effective pulverization, and adaptability to many different processes.

Thompson and Grethlein (1979) reported a cellulose kinetic study on various biomasses using a PFR system. This system had a tubular reactor, and biomass slurry was pumped through by an external positive displacement pump. An electrical preheater was used for temperature control. The reaction was initiated by the injection of acid at the entrance of the reactor. The residence time was less than 1 minute. The pretreatment conditions covered in this study were 0.5-2.0 wt% sulfuric acid at 200-240^{o}C. They achieved 50% glucose yield, which is a significant improvement from the typical results of a batch reactor. Another example of the application of

PFR is from New York University (Rugg, & Stanton, 1982). A twin-screw extruder was used in this study. The reaction conditions were similar to those in the Thompson and Grethlein study. A number of process studies were done using this type of reactor (Green et al., 1988; Rugg & Brenner, 1982; Rugg et al., 1981). Glucose yields of 50–60% were obtained under similar conditions (232^{o}C, 10-20 s). In order to retain high sugar concentration in the products, it was necessary to use a dense, low water feed into the reactor. Rugg et al. (1981) contend that their twin-screw extruder reactor can accommodate solid feeds with a wide range of solid/liquid ratio (10% for waste paper pulp, 95% for sawdust). The continuous screw-driven reactor in NREL was used for dilute acid pretreatment of corn stover in this study. Conditions were relatively mild with sulfuric acid loading near 1.0% and temperature at 160^{o}C.

II.4.2 Residence time distribution of continuous reactor

The flow pattern of the continuous screw-driven reactor, although similar to that of a PFR, has not been investigated. It is noted, however, that deviation of the flow pattern from a PFR negatively affects the reactor performance. The RTD is commonly used to assess the effects of non-ideal flow in a pretreatment reactor (Janssen et al., 1979; Bounie, 1988; Oberlehner et al., 1994; Puaux et al., 2000). RTD can be used to characterize the mixing and flow within reactors and to compare the behavior of real reactors to ideal models. This is useful, not only for troubleshooting existing reactors, but also for estimating the yield of a given reaction and designing for future reactors. The characteristics of the RTD are defined using several functions and moments. The distribution of residence time is represented by an exit age distribution function E(t), which is defined as $E(t) = \frac{C(t)}{\int_0^\infty C(t)dt}$. The average residence time $\bar{t}$ is called first moment, which is defined as $\bar{t} = \int_0^\infty t \cdot E(t)dt$. The variance of the distribution σ^2 is called the

second moment about the mean, which is defined as $\sigma^2 = \int_0^\infty (t - \bar{t})^2 \cdot E(t)dt$. It indicates the degree of dispersion around the mean.

Residence time distributions are measured by introducing a non-reactive tracer into the system at the inlet. The concentration of the tracer is changed according to a known function and the response is found by measuring the concentration of the tracer at the outlet. Experimental methods to determine RTD include the use of a radioactive tracer (Wolf et al., 1976), a dye (Chen et al., 1995; Weiss & Stamato, 1989), an optical (UV) tracer (Oberlehner et al., 1994) or a magnetic tracer (Werner, 1979).

II.5 Post hydrolysis of pre-hydrolysate

II.5.1 Xylose-oligosaccharides from dilute acid pretreatment

During dilute acid pretreatment, the major reactions are:

1) Oligomers with varying degrees of polymerization are produced by random attacks of acid on the hemicellulose chains,

2) Sugars are further degraded to form monosaccharides and sugar decomposition products such as furfural and HMF,

3) Acetic acid is produced by the cleavage of acetyl groups in biomass, which makes the pH decrease during the prehydrolysis.

So the pretreatment liquor contains both mono- and oligo-sugars along with lignin, acetic acid, and some degradation products. The ratio of monomers to oligomers in the prehydrolysate depends on the severity of the pretreatment. The monomeric sugar yield is mostly affected by acid concentration, while for formation of sugar degradation products, such as furfural is

impacted most by temperature (Roberto et al., 2003; Neureiter et al., 2002). Milder conditions cause less sugar degradation resulting in enhanced hemicellulose recovery. Actually, the partial hydrolysis of hemicellulose can enable a reduction both on energy requirements and on the formation of many relevant sugar degradation compounds, particularly, HMF and furfural.

Oligosaccharides, and particularly xylose-oligosaccharides produced from herbaceous and hardwood feedstock, may have a high added value as marketable products for application in the food, pharmaceutical, and cosmetic industries as specialty chemicals (Nabarlatz et al., 2007; Moure et al., 2006). As these applications are rather restricted in volume, hydrolysis of hemicellulosic oligosaccharides into monomers is almost a compulsory requirement for it to be effectively utilized in the bioconversion process.

II.5.2 Need for post-hydrolysis and options

A post treatment, secondary hydrolysis, is therefore necessary. The post hydrolysis process has been applied to pretreatment hydrolysate obtained from all types of materials: softwood (Shevchenko et al., 2000; Bossaid et al., 2001); hardwood (Garrote et al., 2001a); and herbaceous (Saska & Ozer, 1995; Allen et al., 1996; Duarte et al., 2004; Garrote et al., 2001b; Duarte et al., 2009) catalyzed by H_2SO_4 (conc. from 0.5% to 6.5% w/w) at 100-135^{o}C and reaction time up to 10h (Saska & Ozer, 1995; Shevchenko et al., 2000; Duarte et al., 2004; Garrote et al., 2001a; Garrote et al., 2001b). The post hydrolysis options can be reduced to acid (Saska & Ozer, 1995; Allen et al., 1996; Shevchenko et al., 2000; Duarte et al., 2004; Garrote et al., 2001c; Duarte et al., 2009) or enzymatic (Duarte et al., 2004; Vazquez et al., 2001; Walch et al., 1992) catalyzed hydrolysis.

Acid hydrolysis typically presents both higher yield and productivity when compared to the enzymatic hydrolysis processes. Furthermore, as much of the hemicellulose complex structure is still present in the oligosaccharides (Carvalheiro et al., 2004; Kabel et al., 2002), several enzymatic activities are usually required for the complete hydrolysis (e.g., for hardwood type materials: endoxylanase, exoxylanase, β-xylosidase and accessory activities like acetyl xylanesterase, α-glucuronidase, α-arabinofuranosidase, and feruloyl esterase). As a result, the process is potentially inefficient and uneconomical. Under fully optimized conditions, sugar recovery is around 100% (Saska & Ozer, 1995; Shevchenko et al., 2000; Duarte et al., 2004; Garrote et al., 2001a; Garrote et al., 2001b; Duarte et al., 2009), as compared to standard dilute acid hydrolysis (121 ℃, 4% H_2SO_4 and 60 min). This can be considered a major advantage of the acid post hydrolysis process. Other advantages associated to this process are its high-speed and low catalyst cost.

If the conditions are not carefully chosen, however, significant monosaccharide degradation reactions may occur during acid post hydrolysis. Therefore, to obtain a high monosaccharide recovery, an optimization of the operational conditions is required.

II.6 Lactic acid production from lignocellulosic biomass

II.6.1 Background of bioconversion of biomass into lactic acid

Lactic Acid (LA) is an important industrial commodity with large and fast growing market due to its wide applications in cosmetics, pharmaceuticals, textile and food industry (Xu et al., 2006; John et al., 2007; Vickroy, 1985). Its most dominant application is polymerization to biodegradable poly-lactic acid (PLA). PLA is an environmental friendly material to manufacture plastic (Kharas et al., 1994), and because being able to degrade to harmless lactic acid, it is also

used in drug delivery (John et al., 2007; Schmidt & Padukone, 1997) system or can be used as medical implants (Auras et al., 2011). As a result, the annual worldwide production of LA in 2001 was about 86,000 tons (Bouchoux et al., 2006), 260,000 tons in 2008 and expected to reach 1,000,000 tons in 2020 (Jem et al., 2010). Presently, of all the LA produced worldwide, about 90% was made from fermentation, and the rest was from chemical synthesis (Hofvendahl & Hahn–Hägerdal, 2000).

Chemical synthesis routes produce only racemic lactic acid. The commercial process is mainly based on the hydrolysis of lactonitrile by strong acids. Other possible chemical synthesis routes for lactic acid include oxidation of propylene glycol, base-catalyzed degradation of sugars, reaction of acetaldehyde, carbon monoxide, and water at elevated temperatures and pressures, hydrolysis of chloropropionic acid, and nitric acid oxidation of propylene, etc. None of these routes are technically and economically feasible processes (Holten, 1971; Datta et al., 1995). Most fermentation processes require starch-derived glucose or sucrose as feedstock (Litchfield, 1996). However, a wide variety of carbohydrate sources can be used (molasses, corn syrup, whey, cane or beet sugar). Lactic acid bacteria (LAB) have complex nutrient requirements due to their limited ability to synthesize B-vitamins and amino acids (Chopin, 1993). Corn steep liquor, yeast extract, and soy hydrolysate are normally used to provide nutrients to LAB in the fermentation process. Excess calcium carbonate is added to the fermenters to neutralize the acid produced. The advantages of production of lactic acid through fermentation include low temperature, low energy consumption, and high product specificity as it produces a desired stereoisomer, optically pure L-(+)- or D-(−)-lactic acid (Pandey et al. 2001).

It is very expensive when pure sugars like glucose and sucrose are used as feedstock for production of lactic acid. To reduce the feedstock cost, a considerable number of studies have

been pursued to look at the fermentation of lignocellulosic carbohydrates for lactic acid production (Parajo et al., 1997; Garde et al., 2002; Neureiter et al., 2004; McCaskey et al., 1994; Patel et al., 2004). Bustos et al. (2004) achieved 99.6% of the theoretical lactic acid level in bioconversion of vine-trimming wastes by *lactobacillus pentosus.* Zhu et al. (2007) used corn stover and *L. pensosus* in their study; they got 90% of theoretical maximum yield in batch fermentation and 65% yield in fed-batch, but with a much higher lactic acid product concentration. Iyer et al. (2000) reported an excess of 80% yield of xylose using soft wood hydrolysate and *L. rhamnous*. Garde et al. (2002) reported 95% of theoretical maximum yield from wheat straw hemicellulose hydrolysate by the mixed strains of *L. pentosus* and *L.brevis.* Marques et al. (2008) achieved a yield of 97% from recycled paper sludge by *L. rhamnousus*.

II.6.2 Simultaneous saccharification and fermentation in LA bioconversion

Simultaneous saccharification and fermentation (SSF) is widely used for converting cellulosic material into biofuels or chemicals. Unlike separate hydrolysis and fermentation (SHF), in SSF enzymes are added to the reactor along with the microorganisms. Thus sugar hydrolysis and fermentation is carried out simultaneously. This method was first introduced by Takagi et al. (1977). The main advantage of SSF is that it eliminates the product inhibition of enzymes by sugars and thereby allows for reduced the enzyme loading. The enzyme loading (the amount of enzyme added per unit weight of substrate) is a key variable in the saccharification step since enzymes are the most expensive component in the SSF process. Many investigators measured the effects of enzyme loading on the rates and yields of saccharification and SSF (Wald et al., 1984; Wyman et al., 1986; Spindler et al., 1990; Stockton et al., 1991; Tatsumoto et al., 1988). The results show that the saturated value of enzyme loading is approximately 15-50 FPU / g cellulose. However, when the hydrolysis was carried out alone, much larger levels of enzymes were needed

to obtain reasonable hydrolysis yield (Wald et al., 1984; Wiley, 1985). Marques et al. (2008) found that in the production of LA from recycled paper sludge, the overall yield from SSF was 0.97 g/g compared to 0.81 g/g from SHF. They concluded this might be caused by the reduction of end-product inhibition of hydrolysis as the sugars were quickly consumed by the bacteria when they were released by enzymes. The main disadvantage of SSF is the compromising conditions for hydrolysis and fermentation. For example, the optimum temperature for most LAB (30-40^{o}C) is lower than the optimum temperature for cellulase enzymes (50^{o}C). Therefore, SSF can only be carried out at a much lower temperature than the optimum temperature of cellulase. Thus the fermentation step is usually prolonged in SSF compared to fermentation after separate hydrolysis.

II.7 Development of integrated wood based bio-refinery

II.7.1 Background of paper and pulp making

The pulp and paper industry is comprised of companies that mainly use wood (near 90%) as raw material and produce pulp, paper, board and other cellulose-based products. Some other lignocellulosic non-woody plants can also be used, such as cotton, wheat straw, sugar cane waste, corn stover, bamboo, and linen rags. Pulp can be manufactured using mechanical, chemical (Kraft and sulfite processes), or hybrid methods (Table II-4). The aim of pulping is to break down the bulk structure of the fiber source (chips, stems, or other plant parts) into the constituent fibers. Chemical pulping achieves this by degrading the lignin and hemicellulose into small, water-soluble molecules which can be washed away from the cellulose fibers without depolymerizing the cellulose fibers. The various mechanical pulping methods physically tear the cellulose fibers one from another.

Table II-5 Global pulp production by category (Sixta, 2006)

Pulp category	Production [M ton]
Chemical	131.2
-Kraft	117.0
-Sulfite	7.0
-Semichemical	7.2
Mechanical	37.8
Nonwood	18.0
Total virgin fibers	187.0
Recovered fibers	147.0
Total pulp	334.0

Mechanical Pulp

Historically, pulp has been produced by mechanical processes. Figure II-6 shows the normal process of mechanical pulping. Logs are first debarked mechanically in a revolving drum. After debarking, the logs are reduced to their constituent fibers, either by forcing the log against a grindstone, or by chipping it into small pieces. Most modern mills use chips rather than logs and ridged metal discs called refiner plates instead of grindstones. If the chips are just ground up with the plates, the pulp is called refiner mechanical pulp and if the chips are steamed while being refined the pulp is called thermomechanical pulp. Steam treatment significantly reduces the total energy needed to make the pulp and decreases the damage (cutting) to fibrous pulp (Biermann, 1996; Sjöström, 1993). Mechanical pulps are used for products that require less strength, such as newsprint, paperboards and lightweight coated papers because the fibers have been mechanically shortened and it has high residual lignin content.

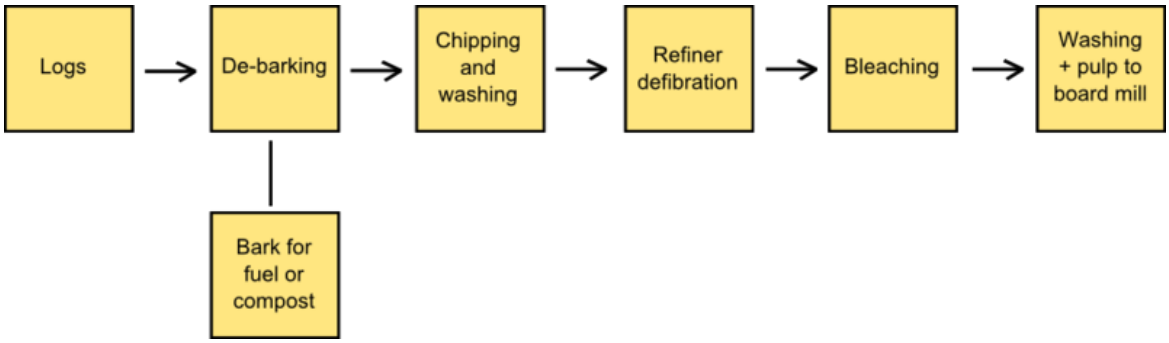


Figure II-6 Mechanical pulping process (Iggesund Paperboard AB, 2008)

Chemical Pulp

Chemical pulp is produced by combining wood chips and chemicals in large vessels known as digesters where heat and the chemicals break down the lignin and hemicellulose while preserving the cellulose fibers. Chemical pulping has lower yield than mechanical pulping but the quality of pulp is better because the fibers are not damaged. The Kraft process is the dominant chemical pulping method, with the sulfite process being second.

Kraft Pulping: The origin of the Kraft pulping process dates back to 1871 (Eaton, 1871). In the Kraft pulping process, wood chips are delignified with a mixture of sodium hydroxide and sodium sulfide. This mixture is called white liquor and can be recovered from a recovery cycle (Figure II-7). The delignification process is usually termed "cooking". The wood chips are cooked in pressurized vessels called digesters. The typical condition of cooking is several hours at around 170^{o}C. Under these conditions most lignin (90~95%), all of it extractive and some hemicellulose, are degraded to yield fragments that are soluble in the strongly alkaline liquid. The remaining solids are collected and washed to produce pulp. At this point the pulp is known as brown stock, because of its color. To make white paper, brown stock is first washed to remove some of the dissolved organic material and then further delignified by beaching.

The combined liquid from the cooking step is called "black liquor''; it contains lignin fragments, carbohydrates from the breakdown of hemicellulose, sodium carbonate, sodium sulfate and other inorganic salts. This black liquor will then be concentrated in multiple effect evaporators to 65-75% solids and transferred to the recovery boiler for burning and recovery of inorganic chemicals (Na_2S, Na_2CO_3), which will in turn be reused in the pulping process. Dissolving these inorganic chemicals from the recovery boiler produces "green liquor". This liquid is then mixed with calcium oxide, which becomes calcium hydroxide in solution, to regenerate the white liquor. Calcium carbonate precipitates from the white liquor and is heated to recover calcium oxide.

$$CaO + H_2O \rightarrow Ca(OH)_2$$

$$Na_2CO_3 + Ca(OH)_2 \rightarrow 2\ NaOH + CaCO_3$$

$$CaCO_3 \rightarrow CaO + CO_2$$

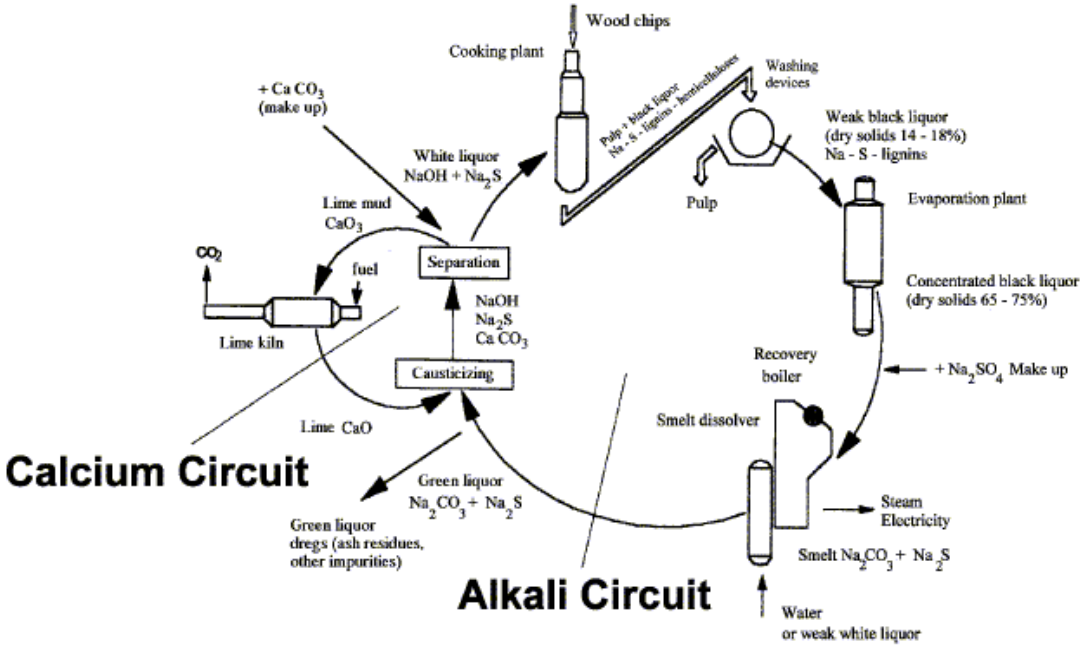


Figure II-7 Recovery cycles of chemicals for a Kraft mill (Prevention, 2001)

Kraft chemical recovery processes consume an estimated 8.04 million BTU/ton of pulp. However, the recovery boilers in the chemical recovery processes can produce 1-2 times more energy in the form of high pressure steam, which is used to reduce the steam usage in the cooking process as well as generate electricity (Martin et al., 2000).

Kraft pulping produces a stronger pulp from a wider variety of fiber sources, compared to other pulping processes. All types of wood, including very resinous types like southern pine, and nob-wood species like bamboo can be used in the Kraft process. The disadvantage of Kraft pulping is that, compared to other pulping process, it produces darker pulp, that needs bleaching to make it white. Bleaching decreases the mass of pulp produced by about 5%, decreases the strength of the fibers and adds to the cost of manufacturing.

Sulfite Pulping: Sulfite pulping evolved from the soda processes developed in the nineteenth century, which used strong bases (alkaline solutions) such as lye to digest wood. In the sulfite pulping process various salts of sulfurous acid are used to extract lignin from wood chips and produce almost pure cellulose fibers. The sulfite process is carried out between pH 1.5 and 5 depending on the counter-ion to sulfite and the ratio of base to sulfurous acid. The pulp is in contact with chemicals for 4-14 hours and temperatures are in the range of 130 to 160^{o}C (Biermann, 1993). The sulfite process does not degrade lignin to the same extent as the kraft process and the lignosulfonates from the sulfite process are useful byproducts. Since the sulfite process is acidic, some of the cellulose was hydrolyzed in the acidic conditions, which causes that sulfite pulp fibers are not as strong as kraft pulp fibers. The yield of pulp (based on wood used) is higher than for Kraft pulping and sulfite pulp is easier to bleach.

Chemi-thermo-mechanical pulp:

The hybrid pulping is mainly chemi-thermo-mechanical pulp or chemi-mechanical pulp making processes involving mechanical action and the use of chemicals. The wood chips are pretreated with sodium carbonate, sodium hydroxide, sodium sulfite, and other chemical prior to a mechanical action. This mechanical action is not as drastic as a mechanical pulp making process. The conditions of the chemical treatment are much vigorous than in a chemical pulping process. It removes extractives and small amounts of hemicellulose but not much lignin. Chemi-thermo-mechanical pulp is stronger than thermomechanical pulp, but requires less energy to produce. Chemi-thermo-mechanical pulp displaces small amounts of chemical pulps in certain grades of paper (Biermann, 1996; Sjöström, 1993).

Recycled Pulp

In the US, approximately 27% of paper is recycled and used in applications which require less brightening, such as newspaper and paperboard. (American Forest & Paper Association, Paper, Paperboard & Wood Pulp: 2002 Statistics, Data through 2001, 2002). Recycled pulp is also called deinked pulp. Deinked pulp is recycled paper which has been processed by chemicals, thus removing printing inks and other unwanted elements. Many newsprint, toilet paper and facial tissue grades commonly contain 100 percent deinked pulp. In many other grades, such as lightweight coated for offset and printing and writing papers for office and home use, deinked pulp makes up a substantial proportion of the furnish.

II.7.2 The Integrated Forest Bio-refinery

The pulp and paper industry in the United States is struggling due to the decreased demand for paper brought on by the advent of digital information technology; as well as increased competition from cheaper foreign products. The United States had been the world's

leading producer of paper until it was overtaken by China in 2009 (De Sisti, 2012). These facts are forcing the pulp and paper industries to find new opportunities in order to improve their competitiveness. The integrated "bio-refinery" process that produces value-added products as supplements in Kraft paper mills is an example of a new opportunity for the pulp and paper industry. A wood-based bio-refinery is a plant that produces multiple value-added products. From the pulp and paper making process, there are several potential opportunities, hemicellulose, black liquor, paper sludge, and waste paper.

Paper mill sludge is a solid waste material composed of pulp residues and ash generated from the pulping and paper making process. Paper sludge has been a disposal liability for many years (Albertson & Pope, 1999). However, due to its high carbohydrate content and well-dispersed structure, the sludge can be biologically converted to value-added products without pretreatment (Schmidt et al., 1997; Lark et al., 1997; Kang et al., 2010). Utilization of paper mill sludge as feedstock to produce LA has at least two economic advantages. First, the cost of this feed stock is basically zero or even negative considering the cost of disposal is about $20 per wet ton (Kang et al., 2010). Paper sludge is produced at a concentrated site and permanent production location, making the feedstock availability reliable. Second, pretreatment is not necessary to bio-convert paper mill sludge to products. Pretreatment is one of the most costly steps in bio-refinery process, thus elimination of pretreatment significantly reduces the production cost of LA.

In the United States, about 80% of pulp capacity is Kraft pulp. During the Kraft pulping process, most of the hemicellulose goes into black liquor that is then burned to recover chemicals and generate steam and electricity (Biermann, 1993). It is difficult to separate the hemicellulose from the black liquor, and the heating value of hemicellulose (13.6 MJ/kg) is only half of that of lignin (27 MJ/kg) (Sixta, 2006). As a result, burning hemicellulose sugar like this can be

wasteful. Studies have shown it is technically feasible to remove part of the hemicellulose before pulping by hot water/steam (Yoon et al., 2008; Yoon et al., 2010), dilute acid (Springer & Harris 1982), or mild alkaline (Al-Dajani & Tschirner, 2008) treatment. Hemicellulose hydrolyzates from water or dilute-acid pretreatment can be utilized to produce value-added products, such as ethanol and xylitol (Buhner & Agblevor, 2004; Mussatto et al., 2005; Walther et al., 2001; Werpy & Petersen, 2004). Hemicellulose pre-extraction has to be done without reducing pulp yield and strength, since the existing ways of pulp and paper-making remain at the heart of wood based bio-refinery (Closset et al., 2005; Mensink et al., 2007).

III. Kinetic Study of Dilute-Acid Catalyzed Hemicellulose Hydrolysis of Corn Stover under High-Solid Conditions

III.1 Abstract

The kinetic model of dilute acid hydrolysis of hemicellulose in Kramer corn stover under high solid loading conditions was investigated in this study. We investigated the kinetics of the reactions occurring in dilute-acid pretreatment of corn stover, covering the reaction conditions similar to those of the NREL reactor. The hydrolysis reactions were performed at various temperatures (155–185^{o}C), acid concentrations (1–2 wt%) and reaction times with a 1/2 solid to liquid ratio. The experimental data was fitted to a first-order biphasic model which assumes that xylan is comprised of two different fragments: fast and slow reacting fraction. Due to the high solid loading condition, significant amount of xylose oligomers was observed during the pretreatment. Thus, the inclusion of an oligomer intermediate in the kinetic model was applied. Kinetic constants were analyzed by an Arrhenius-type expansion with introduction of acid concentration factor. The kinetic model showed overall good agreement with experimental data. However, it under-predicts xylose oligomers at latter stage, particularly under relatively high severity conditions. It was speculated that side reactions may occur, especially recombination of xylose and its oligomers with soluble lignin forming lignin-carbohydrate complex. In subsequent investigation, we have positively identified the presence of lignin-carbohydrate complex during the acid pretreatment by NMR and by FTIR. The kinetic model was therefore modified incorporating the side reactions. The revised model has shown closer agreement with the experimental data.

III.2 Introduction

Corn stover is a renewable, cheap and easily available biomass resource. The hydrolysis of corn stover to produce sugar and conversion to fuels and chemicals has been extensively studied (Sheehan et al., 2003; Kadam & McMillan, 2003; Lau & Dale, 2009; Öhgren et al., 2006; Kazi et al., 2010). Hemicellulose accounts for around 20% of corn stover. Its utilization is an important segment in overall bioconversion process.

Pretreatment is a necessary step in bioconversion process. The overall purpose of pretreatment is to break down the shield formed by lignin and hemicellulose, disrupt the crystalline structure and reduce the degree of polymerization of cellulose (Mosier et al., 2005; Zhang, 2007). Dilute-acid pretreatment is one of the most advanced and widely accepted pretreatment technologies (Torget et al., 2000; Wyman et al., 2005). It can effectively remove and recover most of the hemicellulose, increase the yield of cellulose in subsequent enzymatic hydrolysis.

An exhaustive mechanism of lignocellulosic biomass hydrolysis process in acid environment has not been elucidated. Due to its heterogeneous characteristic with multi-stage mechanism involved, such as diffusion of catalyst and transportation of products. Simplified approaches have been used to understand the complex reaction. Kinetic modeling based on pseudo-homogeneous reactions is a common approach for its ability to accurately reproduce experimental data (Garrote et al., 2001c). The kinetics of hemicellulose hydrolysis catalyzed by dilute acid has been studied comprehensively. The simplest kinetic model that has been proposed is based on the hydrolysis of cellulose (Saeman, 1945). It is a two-step homogeneous pseudo-first-order reaction: xylan is first hydrolyzed to xylose, and the xylose is then degraded to furfural.

$$Xylan \xrightarrow{k1} Xylose \xrightarrow{k2} Furfural \quad \text{(III-1)}$$

An improved model was later proposed by Kobayashi and Sakai (1956), on the basis of observation of biphasic pattern during hemicellulose hydrolysis. Two distinct fractions of hemicellulose that hydrolyze in a parallel mode at different rates: a portion of the hemicellulose hydrolyzes rapidly, while the remainder hydrolyzes more slowly. Since then, most of the kinetic studies have incorporated this modification (Conner, 1984; Maloney et al., 1985; Grohmann et al., 1986a; Kim and Lee, 1987; Esteghlalian et al., 1997; Nabarlatz et al., 2004).

$$\begin{matrix} Fast\text{–}Xylan \xrightarrow{k1f} \\ Slow\text{–}Xylan \xrightarrow{k1s} \end{matrix} Xylose \xrightarrow{k2} Furfural \quad \text{(III-2)}$$

Another common modification of the simple two-step model (eqn. III-1) is the inclusion of a reaction intermediate (xylose oligomer) between xylan and xylose (Abatzoglou et al., 1986, 1992). The intermediate product, oligomer, is defined as the water soluble polymer with DP between 1 and 10 (Lee and McCaskey, 1983). This model is best used for reactions under relative mild conditions or high solid loading conditions where buildup of oligomers cannot be neglected (Abatzoglou et al., 1986; Conner and Lorenz, 1986; Chen et al., 1996; Jensen et al., 2008).

$$Xylan \xrightarrow{k1} Oligomer \xrightarrow{k2} Xylose \xrightarrow{k3} Furfural \quad \text{(III-3)}$$

A more comprehensive model was suggested by Mehlberg and Tsao (1979) in the following pattern:

$$Hemicellulose \xrightarrow{k1} \begin{matrix} Fast\text{–}Xylan \xrightarrow{kf} \\ Slow\text{–}Xylan \xrightarrow{ks} \end{matrix} Oligomer \xrightarrow{k2} Xylose \xrightarrow{k3} Furfural \quad \text{(III-4)}$$

In all cases, the reactions are assumed to be pseudo-homogeneous following a first-order dependence on reactant concentrations. All the rate constants have Arrhenius-type temperature dependence with an expansion of acid concentration term **(**Nee and Yee, 1976; Maloney et al., 1985; Carrasco and Roy, 1992; Nabarlatz et al., 2004; Jensen, et al., 2008**):**

$$k = k_0 * A^n * e^{-E/RT} \quad \text{(III-5)}$$

where k_0: pre-exponential factor (min^{-1}); E: activation energy (kJ/mol);
n: acid exponent; A: acid concentration (wt%).
R: 8.314×10^{-3} (kJ/mol·K) T: temperature (K)

Previous studies have led to various kinetic models with parameters. These models and parameters only apply to specific substrates and cover a narrow range of reaction conditions. According to our preliminary test, none of those models with parameter values gave satisfactory fittings to hydrolysis of corn stover under high solid loading condition. A high solid loading is preferred for process economics because this reduces the reactor size, lowers the cost in heating, and lowers water usage (Hsu et al., 1996). Saeman (1945) reported that the solid to liquid (S/L) ratio over the range of 5 to 20 had a small but definite effect on the rate of cellulose hydrolysis. Solid loading effect was reported as an indicator of deviation from first-order kinetics (Jacobsen and Wyman, 2002). Jensen et al., (2008) suggested that the effect of solid loading could be potentially incorporated in the Arrhenius expression for the kinetic constants.

National Renewable Energy Laboratory (NREL) has developed a continuous auger-driven pretreatment reactor that can be operated with high-solid charge and short residence time. The reaction condition employed in this process is beyond the region normally explored in the conventional processes, especially in the solid/liquid ratio. The purpose of this study is to

investigate the kinetics of the reactions occurring in dilute-acid pretreatment of corn stover, covering the reaction conditions similar to those of the NREL reactor.

III.2.1 Materials and Methods

III.2.2 Feedstock

Kramer Corn Stover (KCS) sample was provided by NREL. This substrate was harvested from the Kramer farm in Wray, Colorado. It was ground and screened, particles with size between 20-60 mesh were collected for the hydrolysis experiments. The ground KCS was air-dried at room temperature to moisture content below 10%. The chemical composition (dry basis) of the KCS was analyzed according to the NREL standard procedure (2008), and this substrate was found to have 37.3 wt% glucan, 20.6 wt% xylan, 3.6 wt% arabinan, 0.6 wt% galactan, 16.03 wt% insoluble lignin, 1.01wt% acid soluble lignin and 6.3 wt% ash.

III.2.3 Neutralization Capacity of Kramer Corn Stover

Since the mineral content of biomass can neutralize some of the acid during dilute acid pretreatment (Grohmann et al., 1985; Springer & Harris, 1985; Malester et al., 1992). The concentration of acid should be corrected according to feedstock's neutralization capability (NC). The NC of KCS was measured using 1N [H+] sulfuric acid solution. Five grams of KCS (dry weight) was put into a beaker with 100 ml DI water. The control group is 100 ml blank DI water. Sulfuric acid solution was added to the beaker gradually while the pH change was monitored in the beaker. After the neutralization capacity has been reached, the [H+] difference would remain constant as the acid addition will increase the [H+] at the same rate in both KCS and the blank.

III.2.4 Dilute Acid Hydrolysis

The acid hydrolysis reactions were carried out in small-scale tubular reactors (provided by NREL) with 4 ml total working volume. The tubular reactors are made out of Hastelloy alloy to prevent formation of metallic ions by sulfuric acid. Some of the metallic ions, especially chromium ion which is one of major component in SS-316, are known to be a strong catalyst for sugar decomposition. Accurate evaluating of kinetic parameters requires a short heat-up period. The reactants must be quickly brought up to desired temperature and remain at isothermal condition. The temperature inside the reactor was measured using a thermocouple probe and a digital indicator. To initiate the reaction, a set of reactors were placed into an oil bath (Haake FS2 model) in which the temperature was pre-adjusted at a level 50℃ higher than the desired reaction temperature. The ramp up time was less than 1 minute. When the center section of the reactor reached the desired reaction temperature, it was transferred into another oil bath pre-set at the desired reaction temperature.

The reaction temperatures were set at 155, 170 and 185^{o}C. The S/L ratio of 1:2 was applied uniformly, equivalent to 33% solid consistency. Three levels of acid loading (after NC correction) were used: 1.0wt%, 1.5wt% and 2.0wt%. The experimental design was set up within practical reaction conditions. For example, the acid level below 1wt% at 155^{o}C was not covered because of low yields; whereas the acid level above 2wt% at 185^{o}C was not tested because of too high xylose degradation. To ensure complete wetting and diffusion of acid through the biomass, the air-dried and screened KCS was presoaked with acid solution for 30 minutes before being placed into the reactor. Each reactor contained 3.0 grams of wet feedstock.

At various reaction times, reactors were collected from the oil batch and were immediately quenched in an ice-water bath to stop the reaction. Because of the high S/L ratio, it

was unable to squeeze out the liquid from the pretreated KCS. Thus, each collected sample was diluted 10-times by DI water. The diluted slurries were filtrated to separate the solid and liquid. The liquid portion was collected for the subsequent analysis of monomer and oligomer sugar components. The oligomer value was determined by taking the difference of xylose monomer values in the liquid after and before autoclave at 4 wt% sulfuric acid and 121^{o}C for 1 hour (eqn. III-6). The solid portion was then washed several times until neutral and was air-dried for the subsequent composition analysis. These data were then used to determine the associated kinetic parameters of the proposed model maximizing the degree of fitness.

$$Xylose\text{-}oligomer = Xylose\ after\ autoclave - Xylose\ before\ autoclave \quad \text{(III-6)}$$

III.2.5 HPLC Analysis

Solid and liquid samples were measured using HPLC equipped with Bio-Rad's Aminex HPX-87P, Aminex HPX-87H column and a refractive index detector. The sugars and degradation products analysis was performed according to the NREL TP 510-42623 and TP 510-42618.

III.2.6 Kinetic Model Development

In the present work, due to the high solid loading conditions, the biphasic model with the inclusion of oligomer as intermediate was applied:

$$\begin{matrix} Fast\text{–}Xylan \overset{kf}{\rightarrow} \\ Slow\text{–}Xylan \overset{ks}{\rightarrow} \end{matrix} Oligomer \overset{k1}{\rightarrow} Xylose \overset{k2}{\rightarrow} Furfural \quad \text{(III-7)}$$

For this kinetic model, the variation of each individual component can be theoretically determined by the following set of differential equations:

$$dX_f/dt = -k_f * X_f \quad \text{(III-8)}$$

$$dX_s/dt = -k_s * X_s \quad \text{(III-9)}$$

$$dO/dt = k_f * X_f + k_s * X_s - k_1 * O \quad \text{(III-10)}$$

$$dX/dt = k_1 * O - k_2 * X \quad \text{(III-11)}$$

$$dF/dt = k_2 * F \quad \text{(III-12)}$$

With initial conditions at $t = 0, X_f = F_f * X_0, X_s = F_s * X_0, O = 0, X = 0, F = 0$.

Where

X_f: fast-xylan	X_s: slow-xylan
O: xylose oligomer	X: xylose monomer
F: furfural	F_f: fraction of fast-xylan
F_s: fraction of slow-xylan	X_0: initial xylan

By solving these linear differential equations with their initial conditions, the time dependent expressions are obtained as follows:

$$X_f(t) = F_f * X_0 * e^{-k_f * t} \quad \text{(III-13)}$$

$$X_s(t) = F_s * X_0 * e^{-k_s * t} \quad \text{(III-14)}$$

$$O(t) = \frac{F_f * X_0 * k_f}{k_1 - k_f}\left(e^{-k_f * t} - e^{-k_1 * t}\right) + \frac{F_s * X_0 * k_s}{k_1 - k_s}\left(e^{-k_s * t} - e^{-k_1 * t}\right) \quad \text{(III-15)}$$

$$X(t) = \frac{F_f * X_0 * k_f * k_1}{(k_1 - k_f)(k_2 - k_f)}\left(e^{-k_f * t} - e^{-k_2 * t}\right) + \frac{F_s * X_0 * k_s * k_1}{(k_1 - k_s)(k_2 - k_s)}\left(e^{-k_f * t} - e^{-k_2 * t}\right)$$

$$- \left(\frac{k_1}{k_2 - k_1}\right)\left(\frac{F_f * X_0 * k_f}{k_1 - k_f} + \frac{F_s * X_0 * k_s}{k_1 - k_s}\right)\left(e^{-k_1 * t} - e^{-k_2 * t}\right) \quad \text{(III-16)}$$

The total amount of xylan remaining in the solids residue can be then expressed as:

$$XR = X_f + X_s = F_f * X_0 * e^{-k_f * t} + (1 - F_f) * X_0 * e^{-k_s * t} \quad \text{(III-17)}$$

Where XR: total xylose equivalents remaining in the solids residue.

The experimental data were fitted to the proposed kinetic model using Nonlinear Regression function in Mathematica. The experimental data of remaining xylan in solids were first fitted to eqn. (17) to determine F_f, k_f and k_s simultaneously. After obtaining nine set of F_f value, the average will be used for subsequent regression. The rate constants were calculated using a maximum likelihood approach, which is based on minimization of error between the experimental data and the kinetic model. After acquiring the rate constants at each set of reaction conditions (temperature and acid concentration), the kinetic parameters (k_0, n and E) of each constant were determined by regression using the linearized Arrhenius equation (eqn. III-18). The accountability of estimated rate constants and parameters were evaluated with statistical analysis.

$$ln(k) = ln(k_0) + n * ln(A) - E/RT \quad \text{(III-18)}$$

III.3 Results and Discussion

III.3.1 Neutralization Capacity of Kramer Corn Stover

Lignocellulosic biomass has a significant amount of mineral content. It is well known that these minerals can neutralize some of the acid during dilute acid pretreatment, reducing its effectiveness (Grohmann et al., 1986a; Malester et al., 1992; Springer & Harris, 1985). The NC has a minor effect at low solid loading and/or high acid loading conditions. However, at high solid concentrations, it has to be considered to evaluate the actual acid level. For sulfuric acid, an

equilibrium shift to formation of bisulfate during neutralization can further reduce hydrogen ion concentrations and compound the effect of neutralization. Because the equilibrium shift has a more pronounced effect at lower acid concentrations, additional acid is needed to compensate (Lloyd and Wyman 2004). Coupled with the effect of temperature on acid dissociation, these effects increase acid requirements to achieve a particular reaction rate unless minerals are removed prior to hydrolysis. Neutralization of sulfuric acid by the minerals in biomass reduces the hydrogen ion activity and must be taken into account for models to accurately predict the performance of dilute-acid hemicellulose hydrolysis.

The hydrogen ions [H+] difference between KCS and blank indicates the neutralization of acid. After the neutralization capacity has been reached the [H+] difference would remain constant. The neutralization point was located at [H+] = 0.013393M per 5g of KCS. Total volume of 1N sulfuric acid solution added at this point is 4.2 ml. Thus the neutralization capacity of this feedstock is:

$$0.013393 * (100 + 4.2)/1000 * 49/5 = 0.01368$$

Every gram of KCS will neutralize 13.68 mg of H_2SO_4. This value will be used to calculate the actual amount of H_2SO_4 required achieving desired acid loading in the dilute-acid pretreatment experiments.

III.3.2 Model Fitness

The experimental data showed that high xylose oligomer concentrations are achieved in all the pretreat conditions under high solid loading. The maximum oligomer concentration ranges from 41 to 53% of initial xylan. The high amount of oligomer indicate that the inclusion of xylose oligomer as reaction intermediate is required to accurate depict the mechanism of

hemicellulose hydrolysis process. The xylose monomer concentration increased with reaction time at the early stage and began to decrease after reaching maximum value at all pretreatment conditions. The only exception is under the low temperature and low acid. These data confirm that the proposed kinetic model (eqn. III-7) is appropriate.

The percentage of the fast-xylan (F_f) in KCS varies between 0.59 and 0.85 at different pretreatment conditions. There was no apparent relationship between the F_f and pretreatment conditions. Therefore, the average value of 0.70 is chosen. This value is close to the reported value (0.65) in the literature for corn stover (Esteghlalian et al., 1997). The percentage of fast-xylan in various biomass species from literature is shown in Table III-1. Figure III-1 depicts the representative reaction pattern of acid hydrolysis of xylan in KCS. The sharp breakage in the plots confirms that the xylan in KCS is biphasic. This finding is in agreement with conventional kinetic model as described previously. One exception was the straight line at lowest temperature and lowest acid concentration (155^{o}C and 1 wt% H_2SO_4). Under this condition, it is believed that 120 minutes of reaction time was not sufficient to remove the entire fast-xylan fraction.

Each set of experimental data were fitted to eqn. (III-13) to (III-17) to calculate k_i for each run. The calculated rate constants for each of the conditions are shown in Table III-2. As we can see from the table, the xylan hydrolysis rates were strictly higher than the xylose degradation rates, which implied all the pretreatment conditions explored favored xylan hydrolysis. The statistical data for all the regression results are shown in Table III-3. As seen from the statistical output, all of the p-Values are far below 0.01, which indicates our regression results are valid. Figure III-2 to III-10 are the experimental and model fitted data profiles. The solid lines are the fitted model data, and the points are experimental data. The model predictions showed overall good agreement with the measured data.

From the experimentally determined rate constants the Arrhenius parameters (pre-exponential factor, activation energy and the acid exponent) were determined by regression using the linearized Arrhenius equation (eq. III-18). The activation energy and acid exponents of k_i determined from statistical are listed in Table III-4. Figure III-11 and III-12 are sample plots for activation energy and acid exponent for k_i respectively. The Arrhenius parameter values are in good agreement with those derived from individual runs.

A direct comparison of the kinetic parameters from this study with the ones from literature is difficult because of the differences in substrates materials kinetic models and experimental conditions. Table III-5 summarizes the comparison of kinetic parameters obtained in this study with the values reported in literatures. The activation energies determined in this study were found to be of the same order as most of the literature values (Kim & Lee, 1987; Maloney et al., 1985; Esteghlalian et al., 1996; Eken-Saraçoğlu et al., 1998). The slow hydrolysis step requires more energy since it has higher activation energy value than the fast hydrolysis step. This is in agreement with the literature values (Kim & Lee, 1987; Maloney et al., 1985; Esteghlalian et al., 1996; Eken-Saraçoğlu et al., 1998). Discrepancy was found between this study and literature results. The activation energies of k_f and k_s are relatively low compare to literature value and the acid exponent are higher. This may be due to the solid loading in this study is much higher than previous studies (Maloney et al., 1985; Esteghlalian et al., 1996; Eken-Saraçoğlu et al., 1998). Also, the formation of xylose oligomers was not taken into consideration as intermediates in most of the previous studies. This can bring significant differences for kinetic parameter values. Of particular significance in this result is that the hydrolysis reactions are more sensitive to temperature and acid concentration than the decomposition reaction; this is indicated

by the magnitude of activation energies and acid exponents. Thus, the higher the temperature and acid concentration, the higher yield of xylose is expected.

III.3.3 Modification of Kinetic Model

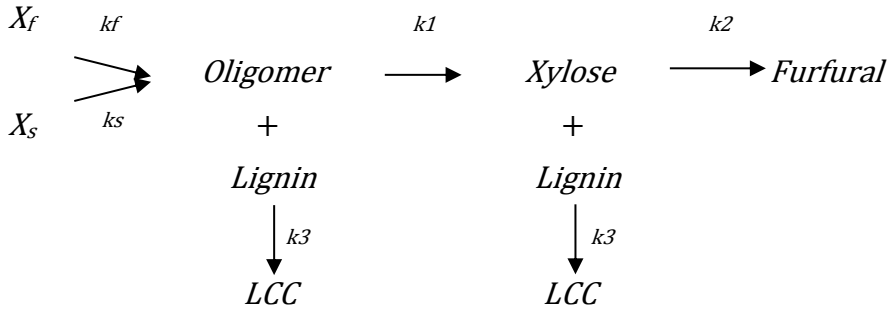
Although the kinetic model (eqn. III-7) accurately predicted the maximum value and initial points of xylose oligomer formation, significant discrepancy was observed at the latter stage of the reaction. The latter phase of xylose oligomer levels was consistently under-estimated for most of the pretreatment conditions. A potential explanation is that the high amount of observed xylose oligomers may be caused by the interaction of xylose monomers/oligomers with the extraneous components released into prehydrolysate during the pretreatment, especially with soluble lignin.

The effect of extraneous components released to the hydrolysate on the hydrolysis of oligomers and/or on the decomposition of xylose was investigated. Parallel experiments were carried out in which xylose oligomers were hydrolyzed under the same experimental conditions such as acid concentration and temperature, but under two different environments: (1) pretreatment hydrolysate as is, (2) pure xylose oligomers without any extraneous components. The pure xylose oligomers were prepared from partial hydrolysis of Beechwood xylan (Sigma X4252). In both cases the total xylose units in the oligomers were set to be identical. The hydrolysis results are shown in Figure III-13. The data clearly indicate that there is a substantial effect of the extraneous components as the yield from pure xylose oligomer was 22% higher than that from pretreatment hydrolysate. We speculated that the yield difference may be caused by formation of lignin–carbohydrate complex (LCC). To look into this closely, the xylose degradation was also investigated. Xylose was put under the hydrolysis reaction conditions with

and without lignin. It was found that xylose degrades more with introduction of external lignin (Figure III-14).

Figure III-15 is a schematic structure of lignin and LCC (Singh, et al., 2005). In order to verify the existence of LCC, lignin sample was extracted from the hydrolysate. Ethyl acetate was used to extract soluble lignin existing in the pretreatment liquor, and this liquid lignin sample was purified by hexane. It was then freeze-dried and subjected to FTIR and NMR test. Both the FTIR (Figure III-16) and NMR (Figure III-17) spectra clearly show that this sample has lignin as well as sugar peaks, a positive proof that at least a part of the extracted solid is LCC. We also analyzed the composition of this LCC sample and found that it contains xylose at level of 15 wt%.

The failure of the proposed model (eqn. III-7) to accurately describe the experimental data of xylose oligomer at latter stage suggests that the formation and hydrolysis of oligomer intermediate may not be described by a single irreversible step. Based on the discovery of LCC formation during pretreatment, we proposed a modified kinetic model with incorporation of LCC:



(III-19)

Because of the complexity of the new kinetic mechanism, and the difficulty in accurately measuring soluble lignin content, the rate constants (k_3) of LCC formation from xylose oligomer and xylose monomer are set to be identical to simplify the regression process. Also, The LCC formed from xylose oligomer and xylose monomer is taken as one entity for the same reason.

$$dX_f/dt = -k_f * X_f \quad \text{(III-20)}$$

$$dX_s/dt = -k_s * X_s \quad \text{(III-21)}$$

$$dO/dt = k_f * X_f + k_s * X_s - k_1 * O - k_3 * O * L \quad \text{(III-22)}$$

$$dX/dt = k_1 * O - k_2 * X - k_3 * X * L \quad \text{(III-23)}$$

$$dLCC/dt = k_3 * O * L + k_3 * X * L \quad \text{(III-24)}$$

$$dL/dt = k_L * SL - k_3 * O * L - k_3 * X * L \quad \text{(III-25)}$$

$$dSL/dt = -k_L * SL \quad \text{(III-26)}$$

$$dF/dt = k_2 * F \quad \text{(III-27)}$$

With initial conditions at $t = 0, SL = 5.05\ gL^{-1}, L = 0, LCC = 0$.

Where

LCC: lignin–carbohydrate complex SL: soluble lignin content in KCS

L: soluble lignin concentration in prehydrolysate

Since this is a non-linear model, there are no analytical solutions for each of the components. Mathcad software was used to perform the non-linear regression based on the differential equations (III-20 to III-27). The Arrhenius parameters for rate constants are shown in Table III-6**.** Since the k_f and k_s values are calculated from the remaining xylan in the solids, it will not affected by the LCC. So the Arrhenius parameters for these two rate constants are the same as before. The modified model resulted in higher R^2-values, indicating a better fitness.

We have also devised a new analytical method to measure xylose-oligomers, which we claim to be more accurate. The oligomer concentration started to increase at the end of acid pretreatment (Figure III-10). This could not be explained by any kinetic model. So we think the way we measure the oligomer may not be accurate. We analyze xylose-oligomers performing secondary hydrolysis (121^{o}C autoclave with 4% sulfuric acid) and taking the difference of xylose monomer values before and after secondary hydrolysis. During the pretreatment, xylose and its oligomers recombine with soluble lignin forming LCC. LCC formation increases as the pretreatment proceeds. According to our analytical method, the portion of xylose monomer that went into formation of LCC is counted as oligomers because when we took the difference after and before autoclaving, that portion is not measured as xylose. Therefore we believe the correct way to calculate xylose-oligomer is:

$$Xylose\text{-}oligomer = Xylose\ after\ autoclave - Xylose\ before\ autoclave - Xylose\ in\ LCC \quad \text{(III-28)}$$

The amount of xylose in LCC can be calculated from the concentration of soluble lignin as we found the soluble lignin in prehydrolysate contains 15 wt% xylose. For sample data taken at 185^{o}C and 2 wt% acid, the fitness of modified kinetic model with the experimental oligomer data is shown in Figure III-18. Figure III-19 is another example of model comparison of xylose monomer. It is clearly shown that the agreement is closer using the modified model.

III.4 Conclusion

The overall kinetic behavior of the xylan in Kramer corn stover was investigated under high solid conditions. The reaction kinetics under high solids conditions is significantly different from that of low solids conditions, the formation of oligomer as reaction intermediate cannot be neglected. And the kinetic parameter values were slightly different from literature values due to the model difference. The kinetic model with no LCC formation showed overall good agreement with experimental data. But under high severity conditions, significant discrepancy was observed. The latter phase of xylose oligomer levels was consistently under-estimated. LCC was proven to be formed during acid hydrolysis which should be considered. Lignin interferes with the xylose oligomer hydrolysis reaction inducing side reactions with the oligomers and with the monomers. The modified model shows better agreement with the experimental data.

Table III-1 Fast-xylan fraction in various biomass species

References	Feedstock	Fast-xylan fraction (%)
Simmonds et al. (1955)	Sweetgum	70.0
Kobayashi & Sakai (1956)	Buna	70.0
Springer et al. (1963)	Aspen	60.0
Springer & Zoch (1968)	Aspen, Birch, Elm, Maple	60.0
Veeraraghavan et al. (1982)	Southern red oak	72.0
Conner (1984)	Southern red oak	73.9
	Paper birch	71.6
	Red maple	80.3
	Quaking aspen	76.0
	American elm	84.3
Maloney et al. (1985)	Paper birch	68.4
Grohmann et al. (1986)	Aspen	76.0
	Wheat straw	67.0
Kim & Lee (1987)	Southern red oak	69.7
Esteghlalian et al. (1997)	Corn stover	64.4
	Switchgrass	76.8
	Popler	83.8
This study	Corn stover	70.0

Table III-2 Fitted kinetic constants during the hydrolysis at various pretreatment condition

T (^{o}C)	wt % H_2SO_4	k_f	k_s	k_1	k_2
155	1	0.0277	0.0070	0.0094	0.0124
170	1	0.1226	0.0076	0.0388	0.0509
185	1	0.2185	0.0300	0.0662	0.1069
155	1.5	0.0803	0.0038	0.0233	0.0134
170	1.5	0.3163	0.0241	0.0650	0.0306
185	1.5	0.5811	0.0372	0.0968	0.0443
155	2	0.2850	0.0106	0.0730	0.0174
170	2	0.7140	0.0559	0.1187	0.0273
185	2	1.6778	0.1265	0.3673	0.0687

Table III-3 Statistical results for kinetic data

a) 1% acid and 155 ℃

	Estimate	Standard Error	P-Value
kf	0.0277	0.00160745	1.21008*10^-12
ks	0.0070	0.00111982	6.35099*10^-6
k1	0.0094	0.000430376	5.75638*10^-16
k2	0.0124	0.00143547	2.96489*10^-9

b) 1% acid and 170 ℃

	Estimate	Standard Error	P-Value
kf	0.1226	0.00507026	6.92769*10^-11
ks	0.0076	0.000879516	3.06763*10^-6
k1	0.0388	0.00231204	1.05821*10^-9
k2	0.0509	0.00351834	5.90937*10^-9

c) 1% acid and 185 ℃

	Estimate	Standard Error	P-Value
kf	0.2185	0.0080913	8.36816*10^-13
ks	0.0300	0.00183991	4.99107*10^-10
k1	0.0662	0.00484218	7.20405*10^-10
k2	0.1069	0.00648817	1.00072*10^-10

d) 1.5% acid and 155 ℃

	Estimate	Standard Error	P-Value
kf	0.0803	0.0038646	3.01745*10^-8
ks	0.0038	0.000479391	0.0000436861
k1	0.0233	0.00086584	6.55386*10^-10
k2	0.0134	0.000735151	1.99364*10^-8

e) 1.5% acid and 170 ℃

	Estimate	Standard Error	P-Value
kf	0.3163	0.0239266	1.63253*10^-8
ks	0.0241	0.00221663	1.42749*10^-7
k1	0.0650	0.00274041	4.39889*10^-12
k2	0.0306	0.00101518	2.07289*10^-13

f) 1.5% acid and 185 ℃

	Estimate	Standard Error	P-Value
kf	0.5811	0.0484428	4.85751*10^-8
ks	0.0372	0.0047867	5.11109*10^-6
k1	0.0968	0.00579479	3.61191*10^-10
k2	0.0443	0.00174082	1.76077*10^-12

g) 2% acid and 155 ℃

	Estimate	Standard Error	P-Value
kf	0.2850	0.0183941	8.51379*10^-8
ks	0.0106	0.00125411	0.0000143307
k1	0.0730	0.00654311	7.78506*10^-7
k2	0.0174	0.00136093	1.65548*10^-7

h) 2% acid and 170 ℃

	Estimate	Standard Error	P-Value
kf	0.714	0.109962	0.00011237
ks	0.0559	0.00514992	1.79021*10^-6
k1	0.1187	0.00708201	1.19929*10^-8
k2	0.0273	0.00260893	1.03694*10^-6

i) 2% acid and 185 ℃

	Estimate	Standard Error	P-Value
kf	1.6778	0.125315	4.73351*10^-8
ks	0.1265	0.0130295	4.91293*10^-7
k1	0.3673	0.0223367	4.4257*10^-10
k2	0.0687	0.00213672	8.9311*10^-14

Table III-4 Fitted Arrhenius parameters for each rate constant k_i

	k_0 (min^{-1})	n	E (kJ/mol)	R^2
k_f	3.28E+11	2.15	105.63	0.99
k_s	1.96E+11	1.68	112.49	0.98
k_1	2.07E+09	2.02	92.088	0.95
k_2	1.47E+08	0.88	82.818	0.94

Table III-5 Comparison of parameters results of this study with the literature values

Reference	Feedstock	Parameter		
		k_0 (min^{-1})	n	E (kJ/mol)
Kim & Lee (1987)	Southern red oak			
	kf	1.04×10^{14}	1.54	120.1
	ks	6.00×10^{12}	1.19	118.0
Maloney et al. (1985)	Paper birch			
	kf	2.67×10^{16}	1	126.6
	ks	16×10^{19}	1	156.5
Esteghlalian et al. (1996)	Switchgrass			
	kf	1.9×10^{21}	0.4	169.0
	ks	4.2×10^{23}	2.0	210.7
	Poplar			
	kf	3.3×10^{21}	0.4	176.7
	ks	3.3×10^{22}	1.5	192.0
	Corn stover			
	kf	6.7×10^{16}	1.5	129.8
	ks	6.9×10^{19}	1.6	167.6
Eken-Saraçoğlu et al. (1998)	Corn cob			
	kf	1.486×10^{10}	1.21	80.34
	ks	2.00×10^{10}	1.86	85.67
	Sunflower seed hull			
	kf	9.642×10^{10}	1.55	92.31
	ks	4.32×10^{9}	1.39	78.35
This study	Corn stover			
	kf	3.28×10^{11}	2.1	105
	ks	1.96×10^{11}	1.68	112

Table III-6 Arrhenius parameters for each rate constant k_i from modified model

	k_0 (min^{-1})	n	E (kJ/mol)	R^2
k_f	3.28E+11	2.15	105.63	0.99
k_s	1.96E+11	1.68	112.49	0.98
k_1	1.88E+09	1.87	92.088	0.97
k_2	1.01E+08	0.91	82.818	0.96
k_3	4.22E+07	1.25	85.364	0.93

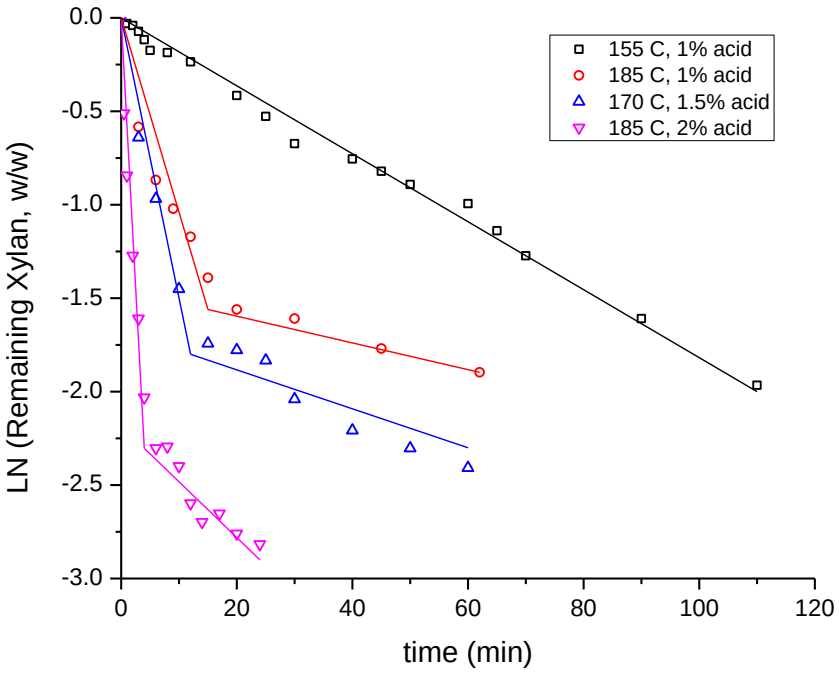


Figure III-1 Hydrolysis of xylan in KCS during dilute acid pretreatments (S/L = 1:2).

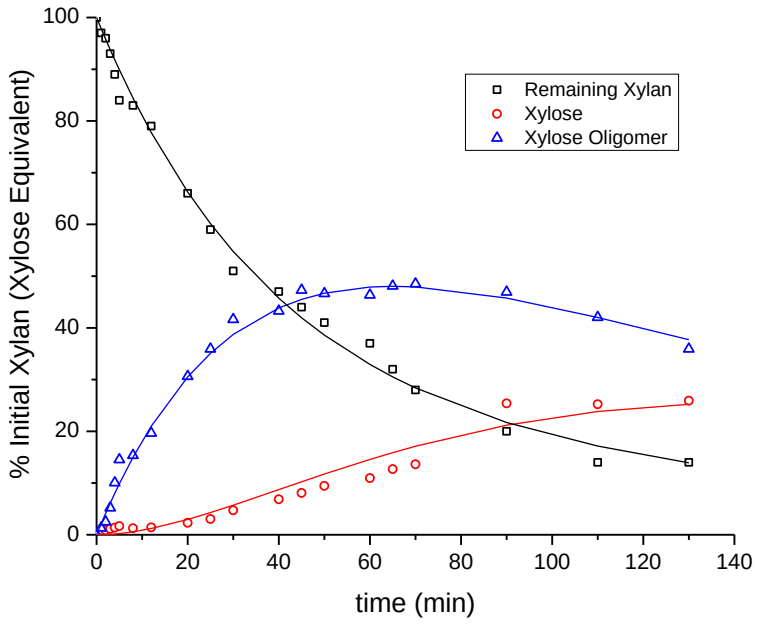


Figure III-2 Experimental and fitted data profiles of KCS hydrolysis (155^{o}C, 1 wt% H_2SO_4)

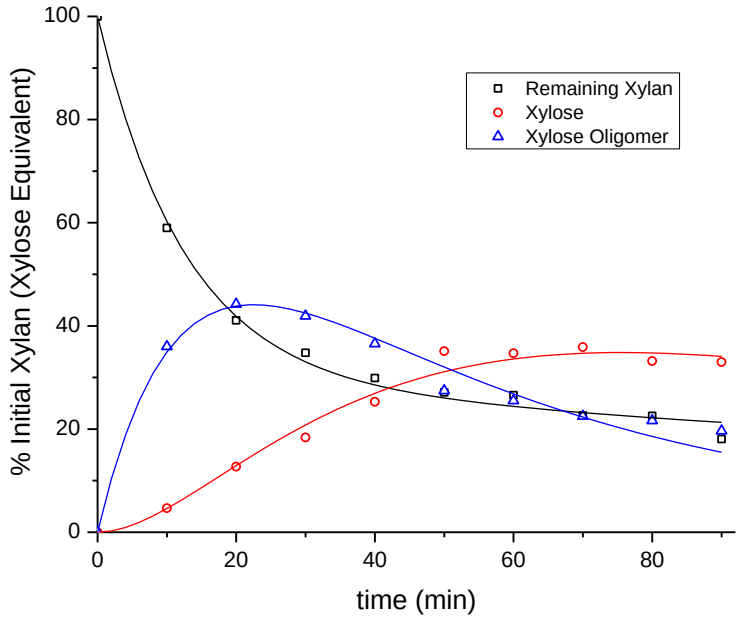


Figure III-3 Experimental and fitted data profiles of KCS hydrolysis (155^{o}C, 1.5 wt% H_2SO_4)

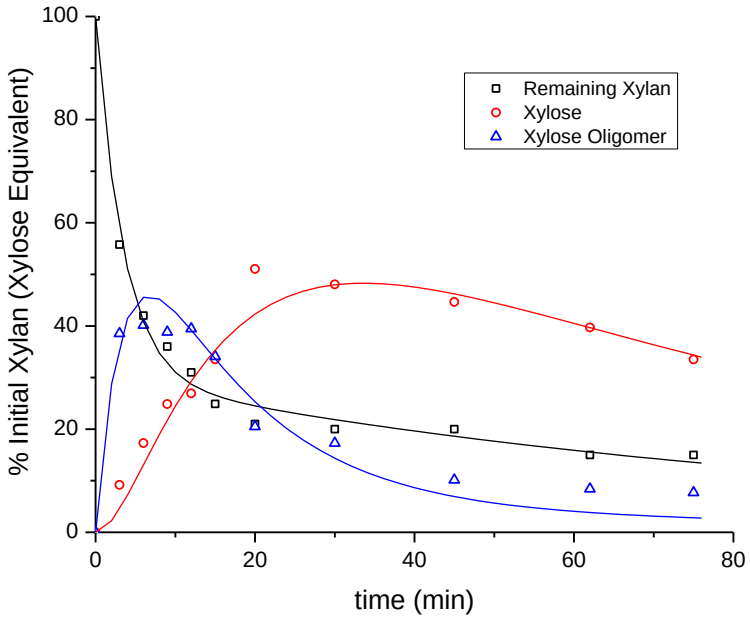


Figure III-4 Experimental and fitted data profiles of KCS hydrolysis (155^{o}C, 2 wt% H_2SO_4)

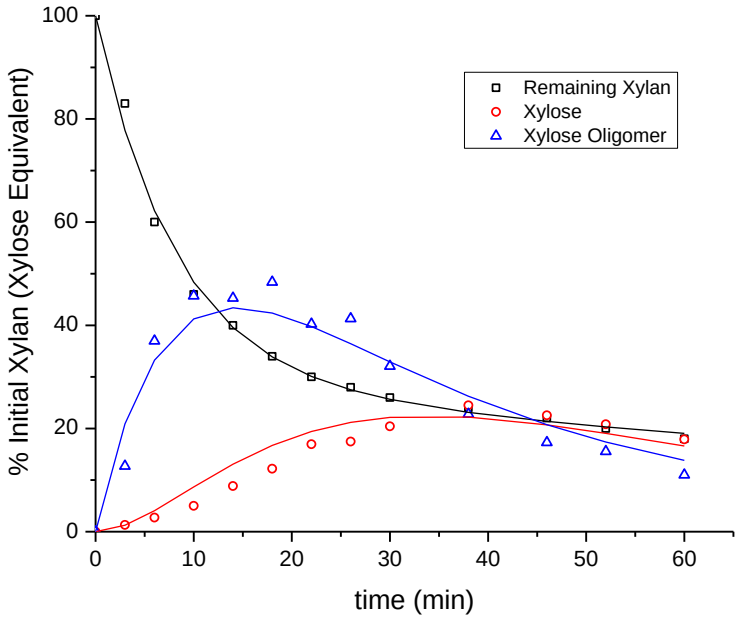


Figure III-5 Experimental and fitted data profiles of KCS hydrolysis (170^{o}C, 1 wt% H_2SO_4)

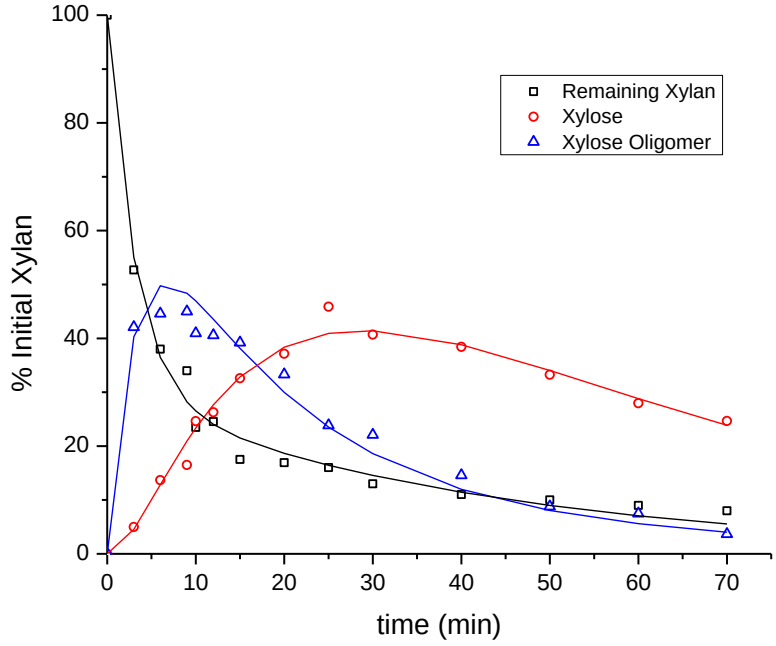


Figure III-6 Experimental and fitted data profiles of KCS hydrolysis (170^{o}C, 1.5 wt% H_2SO_4)

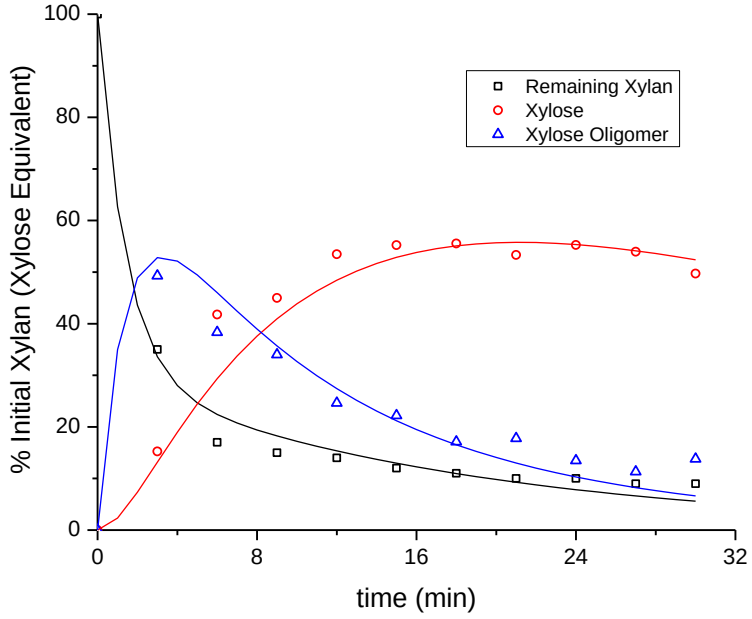


Figure III-7 Experimental and fitted data profiles of KCS hydrolysis (170^{o}C, 2 wt% H_2SO_4)

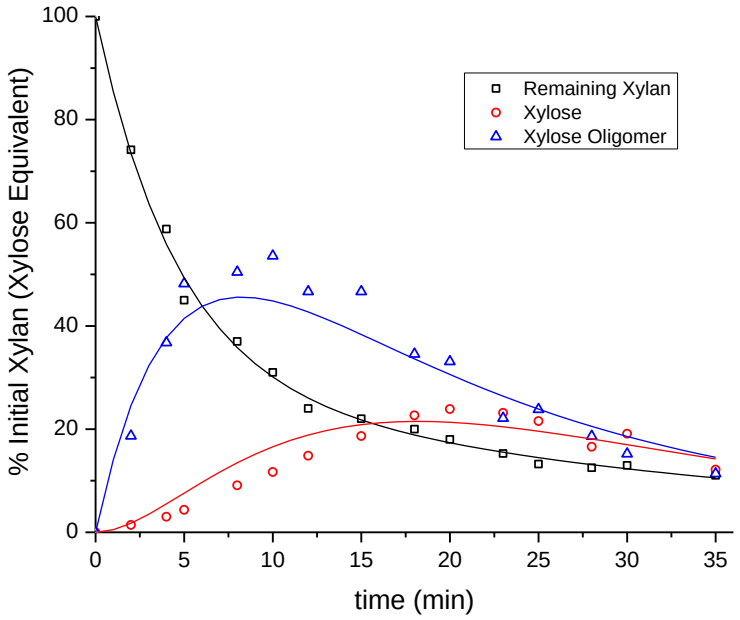


Figure III-8 Experimental and fitted data profiles of KCS hydrolysis (185^{o}C, 1 wt% H_2SO_4)

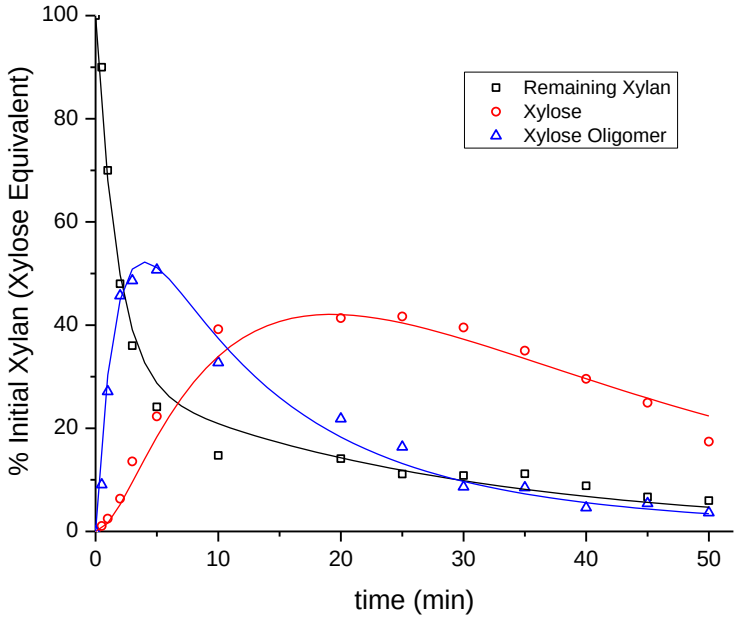


Figure III-9 Experimental and fitted data profiles of KCS hydrolysis (185^{o}C, 1.5 wt% H_2SO_4)

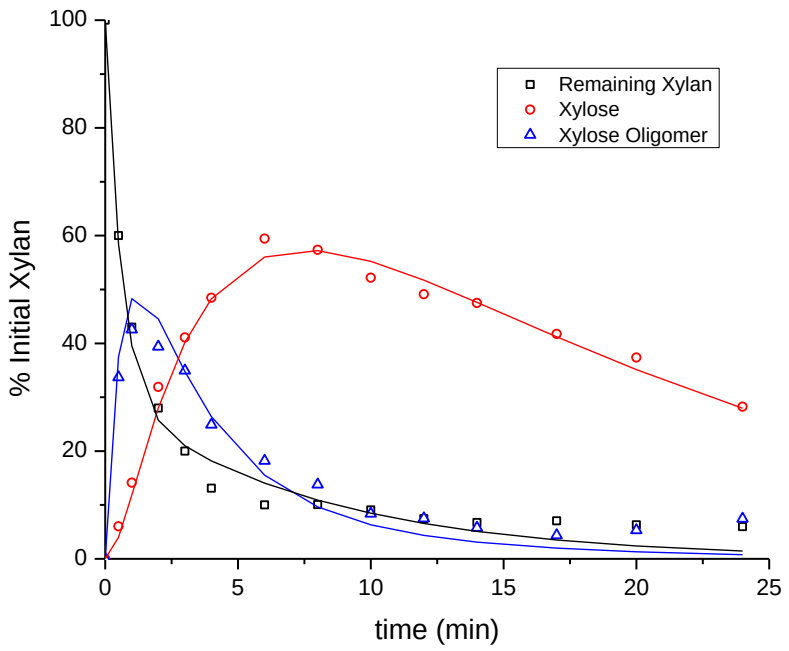
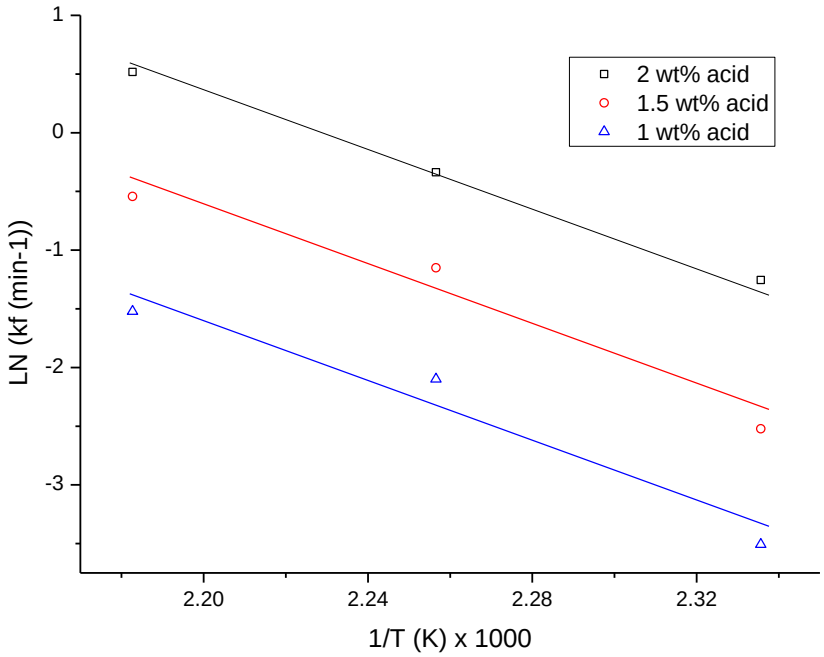
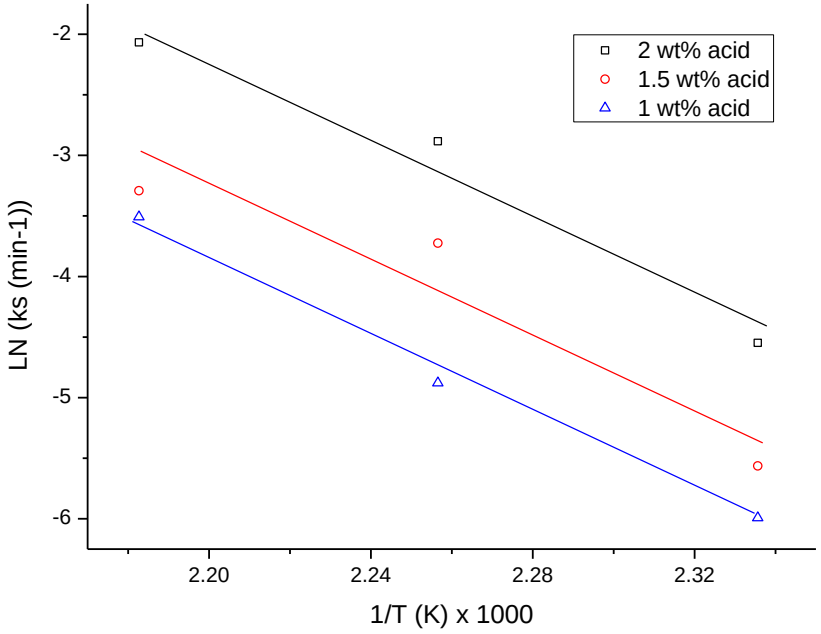


Figure III-10 Experimental and fitted data profiles of KCS hydrolysis (185^{o}C, 2 wt% H_2SO_4)

(a)


2 wt% acid
1.5 wt% acid
1 wt% acid
LN (kf (min-1))
1/T (K) x 1000
1
0
-1
-2
-3
2.20
2.24
2.28
2.32

(b)


2 wt% acid
1.5 wt% acid
1 wt% acid
LN (ks (min-1))
1/T (K) x 1000
-2
-3
-4
-5
-6
2.20
2.24
2.28
2.32

(c)

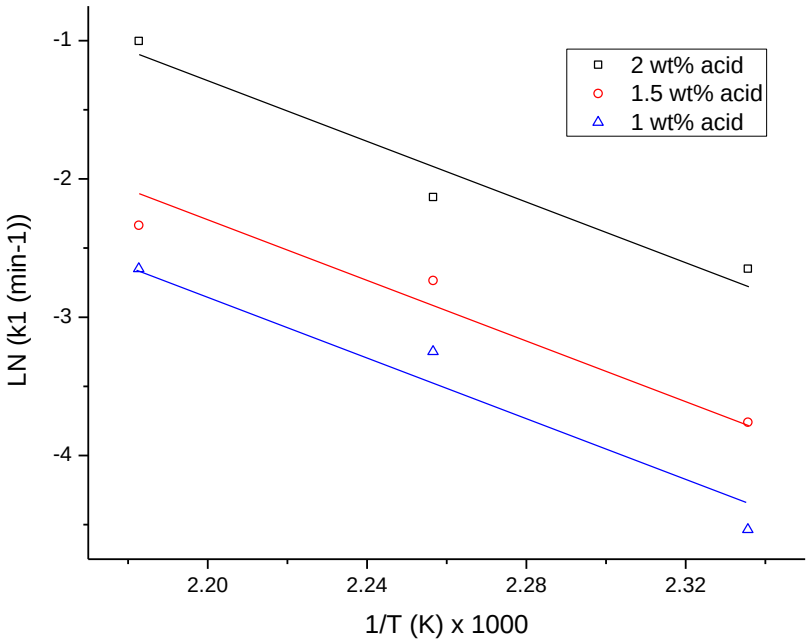


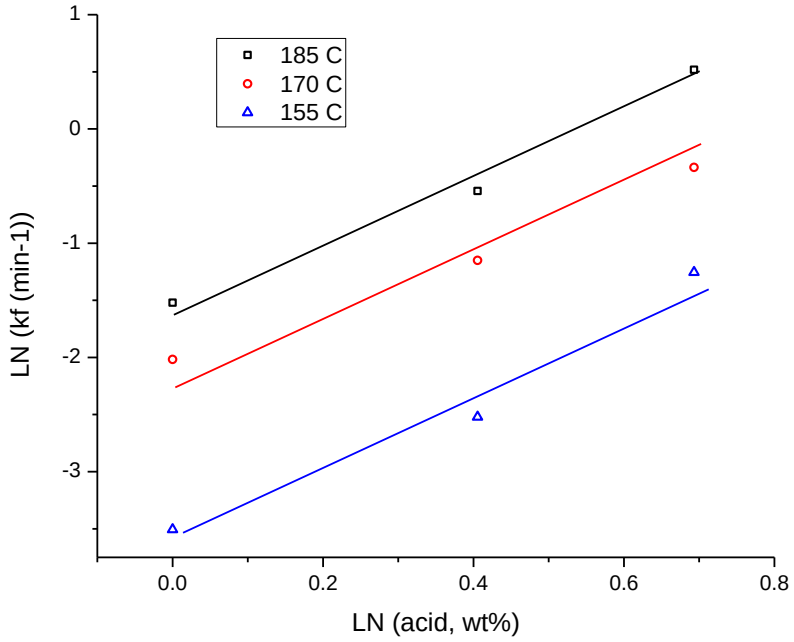
Figure III-11 Activation energy plots for rate constant k_i

(a) activation energy plots for k_f

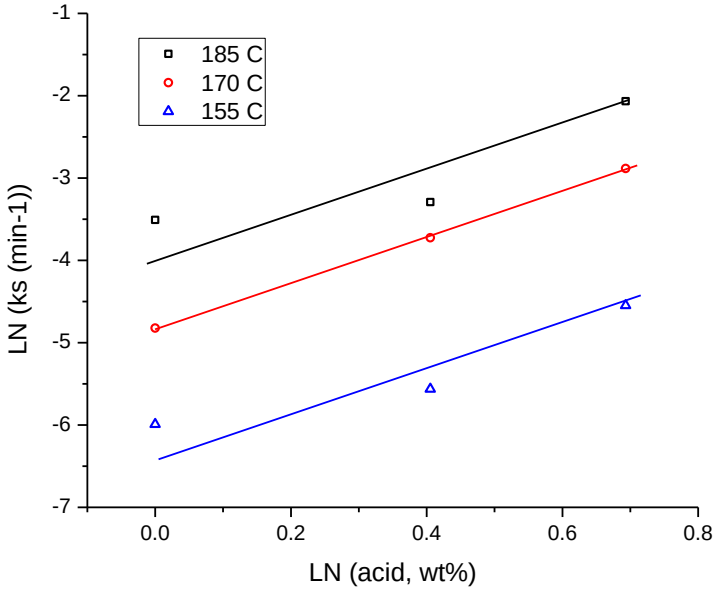
(b) activation energy plots for k_s

(c) activation energy plots for k_1

(a)


185 C
170 C
155 C
LN (kf (min-1))
LN (acid, wt%)
1
0
-1
-2
-3
0.0
0.2
0.4
0.6
0.8

(b)


185 C
170 C
155 C
LN (ks (min-1))
LN (acid, wt%)
-1
-2
-3
-4
-5
-6
-7
0.0
0.2
0.4
0.6
0.8

(c)

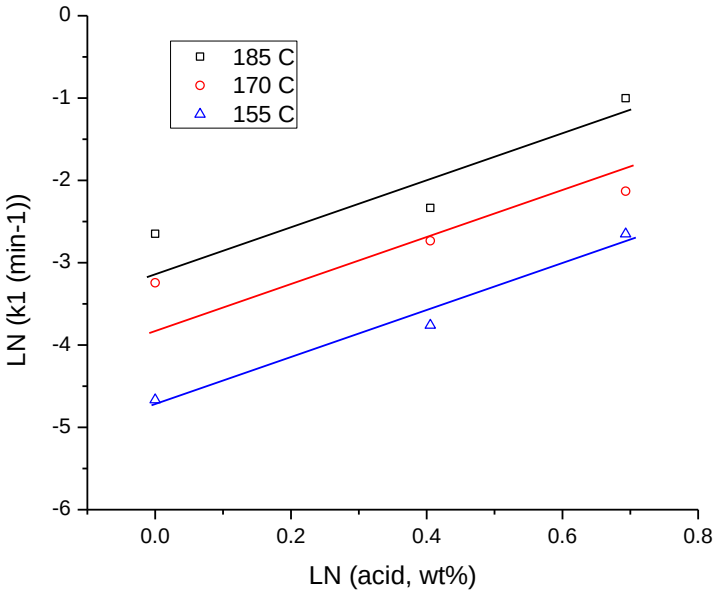


Figure III-12 Acid exponent plots for rate constant k_i

(a) acid exponent plots for k_f

(b) acid exponent plots for k_s

(c) acid exponent plots for k_1

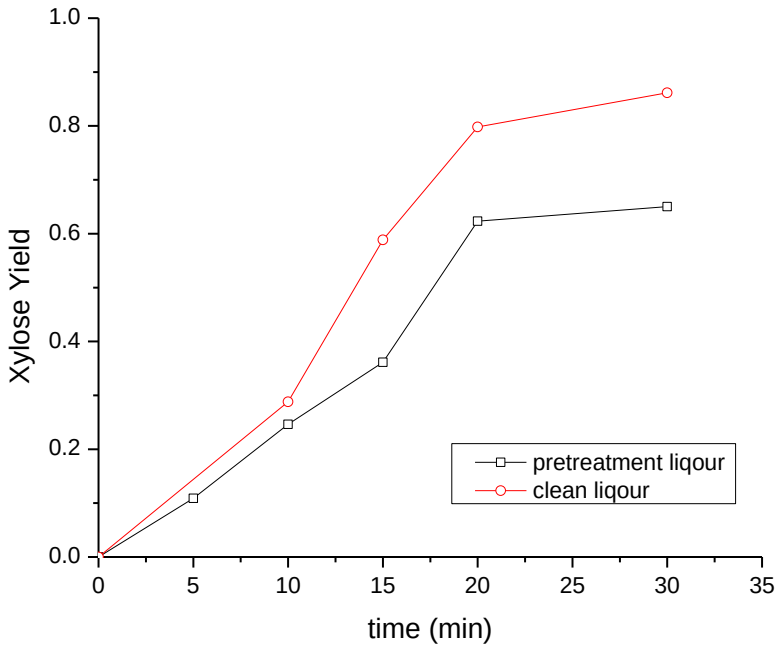


Figure III-13 Xylose oligomer hydrolysis profiles at different environments

(1.5 wt% H_2SO_4 and 155^oC)

□ hydrolysis in pretreatment liquor as is

○ hydrolysis in pure xylose oligomer solution

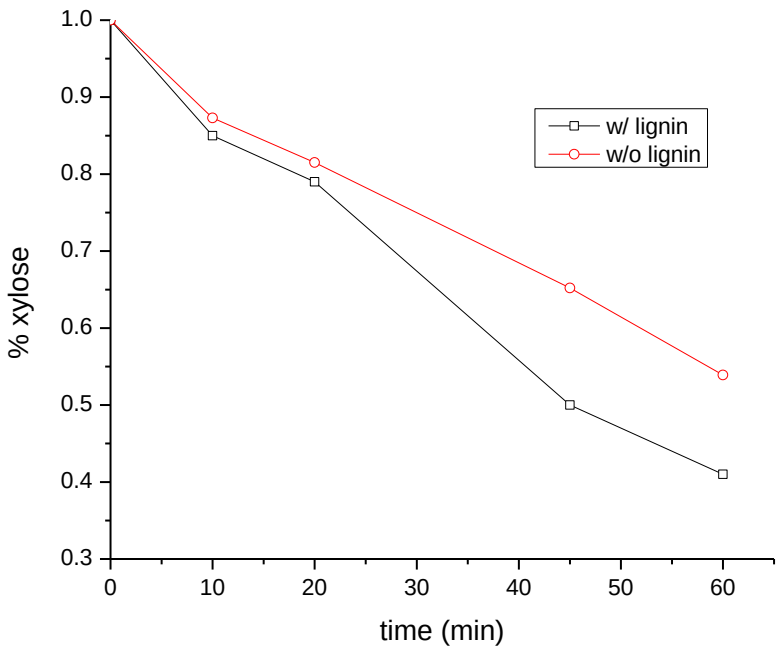


Figure III-14 Xylose degradation profiles at different environments

(1.5 wt% H_2SO_4 and 155^oC)

□ degradation with external lignin

○ degradation w/o addition of lignin

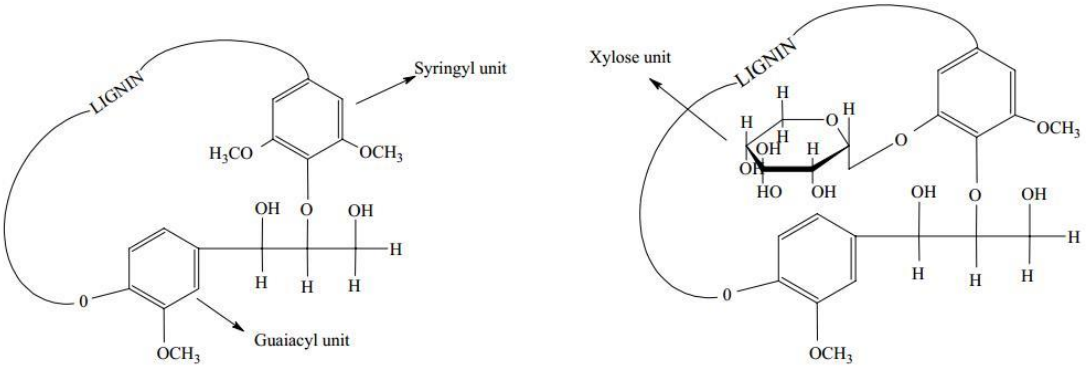


Figure III-15 Schematic structures of lignin and LCC

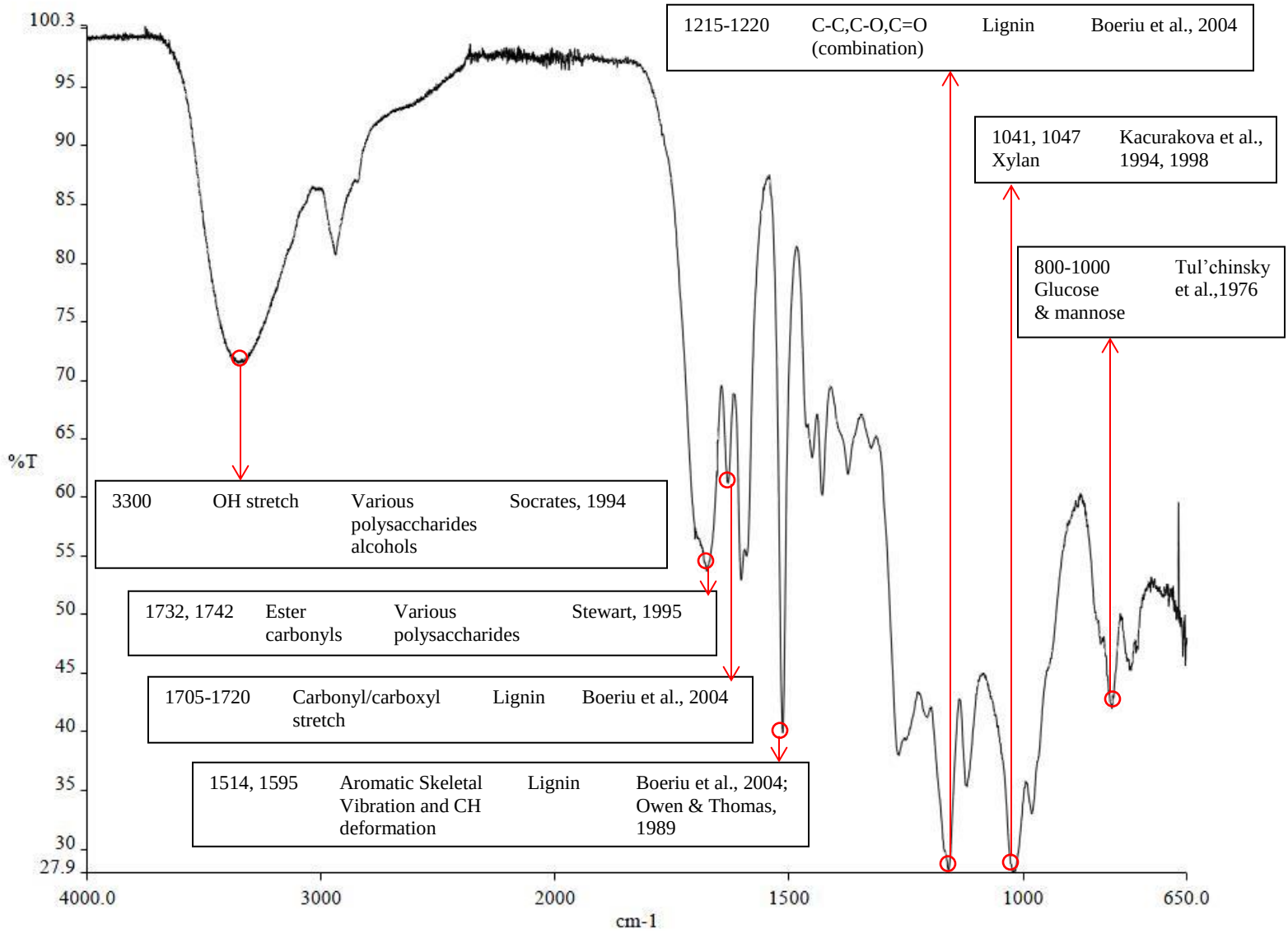


Figure III-16 FTIR spectra of LCC sample

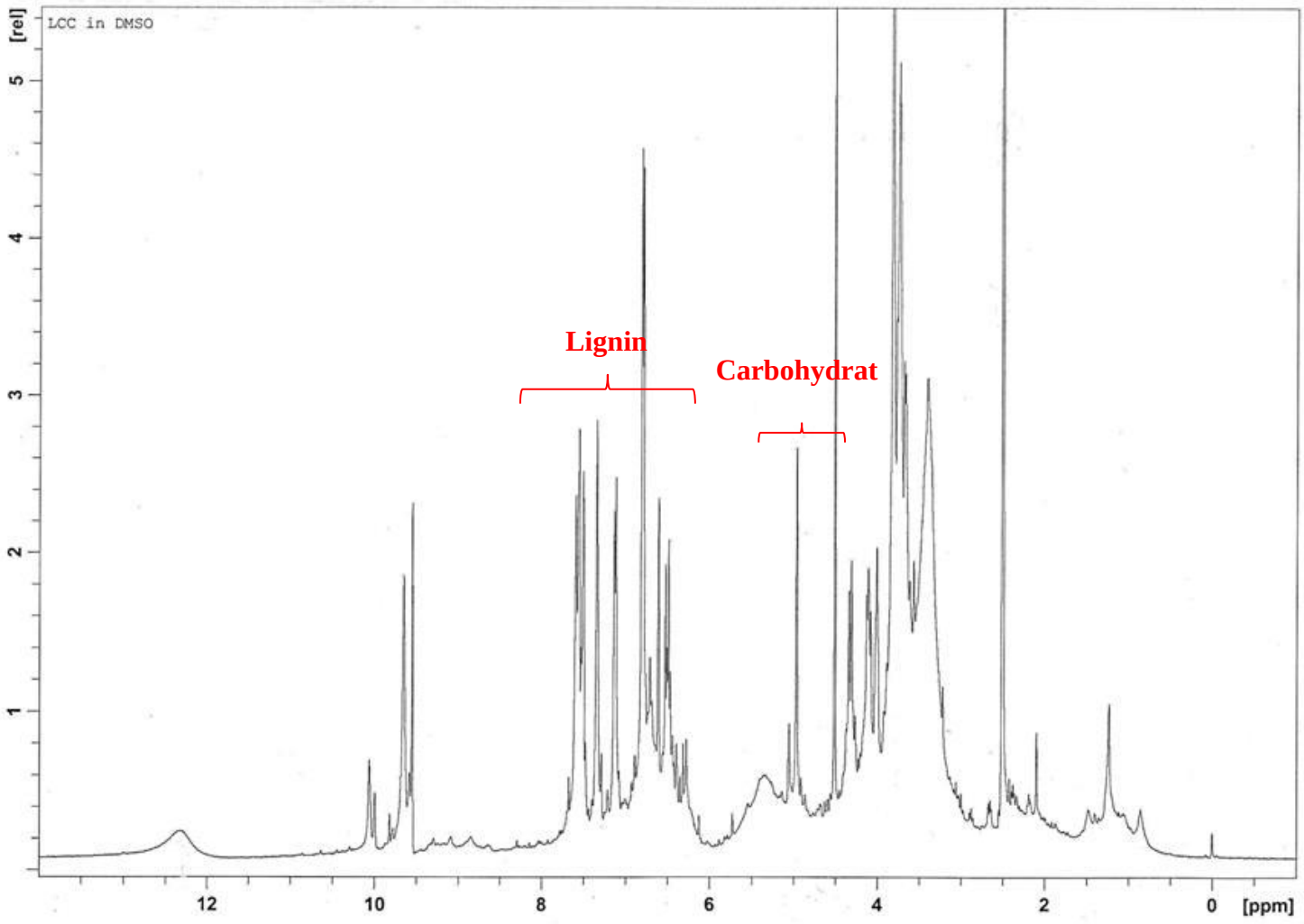


Figure III-17 NMR spectra of LCC sample

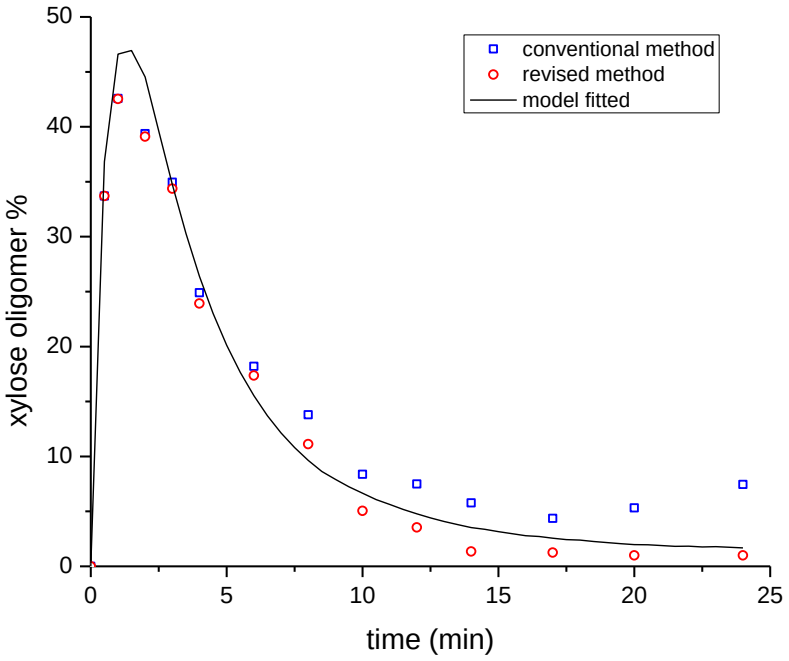


Figure III-18 Modified oligomer calculation

(185^{o}C & 2 wt% H_2SO_4)

□ Oligomer value measured using eqn. (6)
Δ Oligomer value measured using eqn. (28)

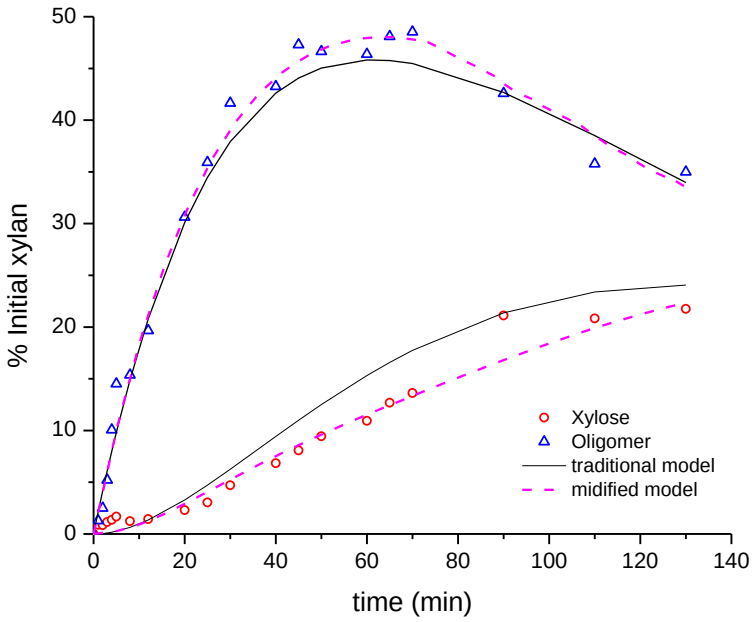


Figure III-19 Modified model fitness

(1 wt% H_2SO_4 and 155^oC)

Solid line: model eqn.7; Dash line: model eqn. 19

IV. Incorporation of residence time distribution in the modeling of NREL screw-driven continuous pretreatment reactor

IV.1 Abstract

In the actual design and operation of the NREL screw-driven reactor, a number of technical problems surfaced in the pilot scale testing of the prehydrolysis process. The technical problems stem from several unique features of the process. The process is relatively fast, which results in short residence time in the reactor. The reaction is heterogeneous in that the catalyst is penetrating into the solid reactant. It is carried out in a moving-bed reactor under high-solid and low-water conditions.

The residence time distribution (RTD) tests were performed to obtain the RTD data. The RTD data were then incorporated with the pre-determined kinetic parameters to compare the xylose yield from NREL reactor and an ideal PFR. The flow pattern of NREL screw-driven continuous reactor is proven be close to that of a PFR by the RTD test, especially when continuous flight and low RPM were applied. The dimensionless variance at this operation condition is 0.045 compared to the theoretical value of 0 for PFR. The reduction in xylose yield due to non-ideal flow in the NREL reactor is 1-3 %.

IV.2 Introduction

Of the many pretreatment technologies, dilute acid pretreatment is preferred since it has been explored extensively in numerous studies and proven to be more scalable than other pretreatment methods (Eggeman & Elander, 2005; Sun & Cheng, 2005; Moiser et al., 2005). The most logical choice for commercial-scale biomass pretreatment reactor is that of a moving-bed (screw-driven) continuous type. This type of reactor has a flow-through design, meaning that the solid and liquid travel through the reactor at the same velocity. The pretreatment reaction proceeds as the reagents travel through the reactor. The flow pattern of a screw-driven continuous reactor resembles that of a plug flow reactor (PFR). A continuous screw-driven reactor process is capable of providing a unique and continuously stirred thermo-chemical reactor environment in combination with thermo mechanical-energy and dilute acid. This type of reactor can provide high shear, rapid heat transfer, effective pulverization, and adaptability to many different processes.

Thompson and Grethlein (1979) conducted a cellulose kinetic study on various biomasses using a PFR system. This system had a tubular reactor, and biomass slurry was pumped through by an external positive displacement pump. An electrical preheater was used for temperature control. The reaction was initiated by the injection of acid at the entrance of the reactor. The residence time was less than 1 minute. The pretreatment conditions covered in this study were 0.5–2.0% sulfuric acid at 200–240^{o}C. They achieved 50% glucose yield, a significant improvement from the typical results of a batch reactor. Another example of the application of PFR is from New York University (Rugg, & Stanton, 1982). A twin-screw extruder was used in this study. The reaction conditions were similar to those in the Thompson and Grethlein study. A number of process studies were done using this type of reactor (Green et al., 1988; Rugg &

Brenner, 1982; Rugg et al., 1981). Glucose yields of 50–60% were obtained under similar conditions (232^{o}C, 10-20 s). In order to retain high sugar concentration in the products, it was necessary to use a dense, low water feed into the reactor. Rugg et al. (1981) contend that their twin-screw extruder reactor can accommodate solid feeds with a wide range of solid/liquid ratio (10% for waste paper pulp, 95 % for sawdust). The continuous screw-driven reactor in NREL was used for dilute acid pretreatment of corn stover in this study. Conditions were relatively mild with sulfuric acid loading near 0.6% and temperature at 155^{o}C.

The flow pattern of the continuous screw-driven reactor, although similar to that of a PFR, has not been investigated. It is noted, however, that deviation of the flow pattern from a PFR negatively affects the reactor performance. The RTD is commonly used to assess the effects of non-ideal flow in a pretreatment reactor (Janssen et al., 1979; Bounie, 1988; Oberlehner et al., 1994; Puaux et al., 2000). RTD can be used to characterize the mixing and flow within reactors and to compare the behavior of real reactors to ideal models. This is useful, not only for troubleshooting existing reactors, but also for estimating the yield of a given reaction and designing for future reactors. The characteristics of the RTD are defined using several functions and moments. The distribution of residence time is represented by an exit age distribution function E(t), which is defined as $(t) = \frac{C(t)}{\int_0^\infty C(t)dt}$. The average residence time $\bar{t}$ is called first moment, which is defined as $\bar{t} = \int_0^\infty t \cdot E(t)dt$. The variance of the distribution σ^2 is called the second moment about the mean, which is defined as $\sigma^2 = \int_0^\infty (t - \bar{t})^2 \cdot E(t)dt$.

Residence time distributions are measured by introducing a non-reactive tracer into the system at the inlet. The concentration of the tracer is changed according to a known function and the response is found by measuring the concentration of the tracer at the outlet. Experimental

methods to determine RTD include the use of a radioactive tracer (Wolf et al., 1976), a dye (Chen et al., 1995; Weiss & Stamato, 1989), an optical (UV) tracer (Oberlehner et al., 1994) or a magnetic tracer (Werner, 1979).

When the RTD data is obtained, it can be incorporated into the reactor modeling along with the predetermined reaction kinetic data to delineate the effects of non-ideal flow on the performance of the NREL pretreatment reactor. The RTD tells us how long the reactants have been travelling in the reactor, but it does not tell us anything about how the exchange of matter between the reactants (i.e., the mixing). The mixing of reacting species is one of the major factors that control the behavior of chemical reactors. But for first order reactions, the space time each molecule spends in the reactor is all we need to predict conversion. In this chapter, the conventional kinetic model will be used, since it is first order reaction and the analytical solution of time progression of each reactant is readily available.

IV.3 Materials and Methods

IV.3.1 RTD Tests

The RTD tests were done in NREL by David Sievers (Sievers et al., 2009). The reactor was operated at ambient temperature without steam addition due to pulse addition and sampled by hand. Water sprayed corn stover (45 wt% solids) was continuously fed through the plug screw feeder at 175 g/min, and material was manually collected as it entered the discharger section. A pulse was introduced into the reactor by pausing the plug screw feeder for 15 seconds and manually adding 15 seconds worth of sodium chloride impregnated corn stover at 60 wt% total solids at the inlet of the plug screw feeder (Material normally fed through the plug screw feeder is dewatered to a level of about 60 wt% solids where excess liquid is discarded). Biomass exiting

the reactor at the discharger was collected in 15-second sample intervals for 10 minutes following the pulse addition.

Each biomass sample was mixed with a small amount of water to enhance sodium chloride extraction (typically 25g biomass with 15 ml water). Liquor from each biomass sample was extracted using a laboratory press and sterile-filtered to remove insoluble solids. The density of the resulting clear liquor was measured and used to calculate residence time distribution curves. Figure IV-1 is the schematic flow chart for this process.

This reactor was operated with two different auger configurations. One configuration utilized the auger with broken flights where rectangular baffles protrude from the bottom of the pipe into the flight breaks to facilitate mixing (Figure IV-2). Following initial experimentation, a new reactor section was designed and installed with a continuous flight auger and anti-rotation bars in place of the baffles in an attempt to achieve better plug flow and reduce severity variance (Figure IV-3).

IV.4 Results and discussion

IV.4.1 RTD Data Analysis

The tracer data indicated that the RTD of the NREL reactor deviated considerably from that of a PFR. It has also shown an unusual non-symmetric tailing profile (Figures IV-4 and IV-5) which normally signifies a local back-mixing or dead region. Operation of NREL's continuous reactor has resulted in lower xylose yields and higher degradation product formation in comparison to the batch reactor operated under the same reaction conditions. The RTD data obtained at NREL were used to determine the mean residence time and the dimensionless variance of RTD, σ^2_θ. The results are sown in Table IV-1 for the two reactor configurations and for two different RPMs (49 and 98). It should be noted that the RPM of the auger had minimal

impact on the mean residence time for both configurations. Doubling the RPM reduced the residence time only by 0.7% for the broken flight reactor and by 5% for the continuous flight reactor.

Intuitively, one would expect the residence time to be inversely proportional to the RPM. It certainly was not the case here. At his time, we have not developed a clear explanation for this phenomenon. It may have something to do with the clogging of the reactor and the fact that the shrinkage or softening of the biomass particles occurs at the extent of it changes with reaction time. The RPM had minimal impact on dimensionless variance for the broken flight reactor; with 49 RPM corresponding to 0.170 and 98 RPM corresponding to 0.184. On the other hand, the RPM effect was significant for the continuous flight reactor; 49 RPM corresponding to 0.045 and 98 RPM corresponding to 0.147. Between the two reactors, the broken flight reactor exhibited the higher variance indicating a higher degree of back-mixing. Based on the theoretical extremes of σ^2_θ, 0 for PFR and 1.0 for CSTR, the mixing pattern of the NREL reactor more closely resembles a PFR.

IV.4.2 Assess the Performance of NREL Reactor Using RTD Data

The batch kinetic model and the data previously obtained in Chapter III were used to delineate the effect of non-ideal flow of the pretreatment reactor. The xylose production from KCS based on the theoretical calculation of a PFR and that of the NREL reactor were compared to see the effect of non-ideal flow.

For PFR, the xylose concentration based upon our previously reported kinetic model with respect to time is:

$$X_1(t) = \frac{F_f * X_0 * k_f * k_1}{(k_1 - k_f)(k_2 - k_f)}\left(e^{-k_f * t} - e^{-k_2 * t}\right) + \frac{F_s * X_0 * k_s * k_1}{(k_1 - k_s)(k_2 - k_s)}\left(e^{-k_f * t} - e^{-k_2 * t}\right)$$

$$-\left(\frac{k_1}{k_2-k_1}\right)\left(\frac{F_f * X_0 * k_f}{k_1-k_f}+\frac{F_s * X_0 * k_s}{k_1-k_s}\right)\left(e^{-k_1 * t}-e^{-k_2 * t}\right) \quad \text{(IV- 1)}$$

The overall xylose concentration (X_2) was then computed by integration of the point-wise xylose concentration of eqn. (IV-1) over the time span of the experimental RTD:

$$X_2(t) = \int_0^t X_1(t) * E(t) dt \quad \text{(IV-2)}$$

Table IV-2 shows the comparison of xylose concentrations from the two cases (PFR and NREL reactor) and the corresponding xylose yields. The results indicate that the reduction in xylose yield due to non-ideal flow in the NREL reactor is 2-3 % for the broken flight reactor and only 1-2% for the continuous reactor. Despite a noticeable degree in deviation of RTD from PFR for the two NREL reactor configurations, the reduction of yield is significant only for the broken flight reactor, and even so, it is less than 5%.

IV.5 Conclusion

The flow pattern of NREL screw-driven continuous reactor is proven be close to that of a PFR by the RTD test, especially when continuous flight and low RPM were applied. The dimensionless variance at this operation condition is 0.045 compared to the theoretical value of 0 for PFR. The reduction in xylose yield due to non-ideal flow in the NREL reactor is 2-3 % for the broken flight reactor and only 1-2% for the continuous reactor.

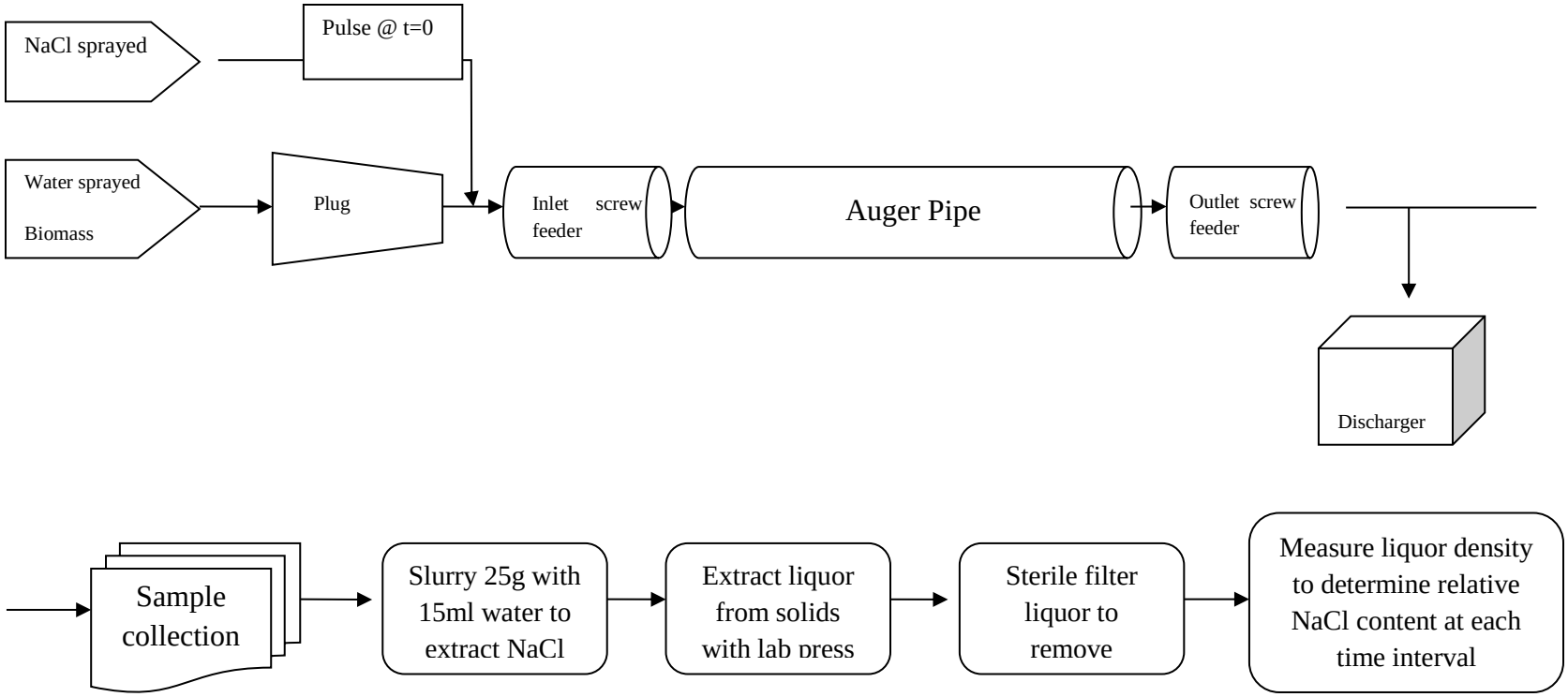


Figure IV-1 RTD test flow chart

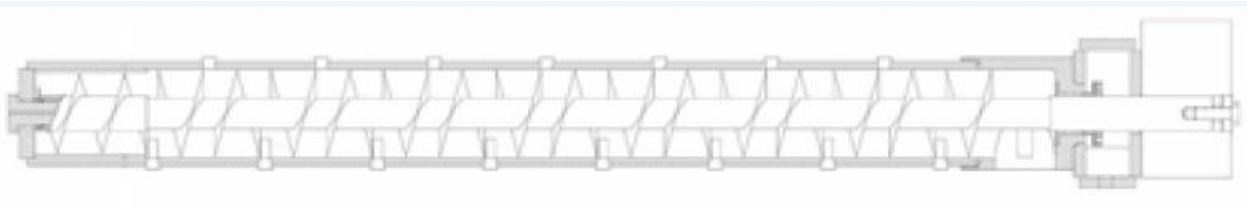


Figure IV-2 Broken Flight Auger Reactor with Baffles

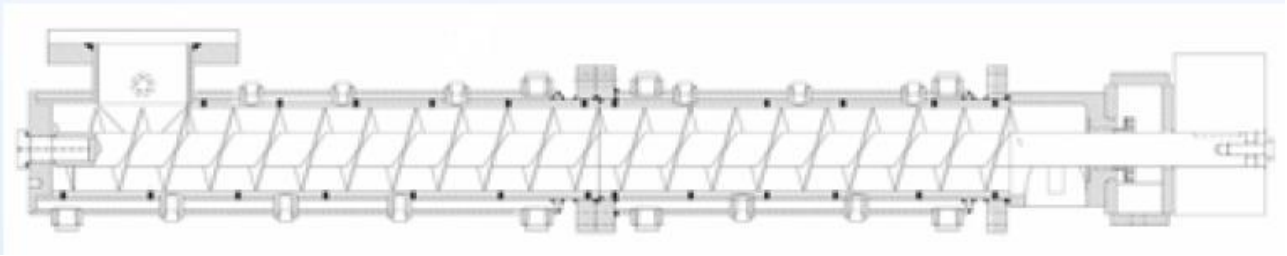


Figure IV-3 Continuous Flight Auger Reactor with Anti-Rotation Bars

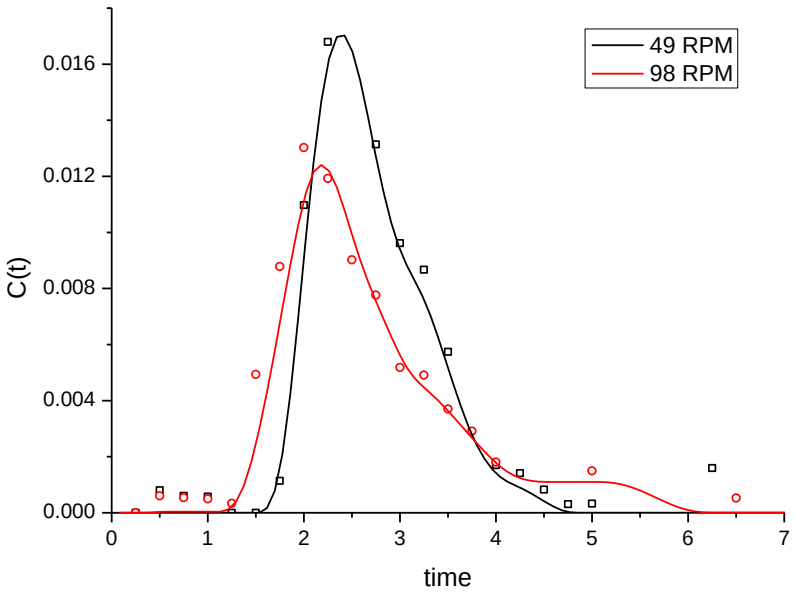


Figure IV-4 RTD profiles for broken flight at different auger speed

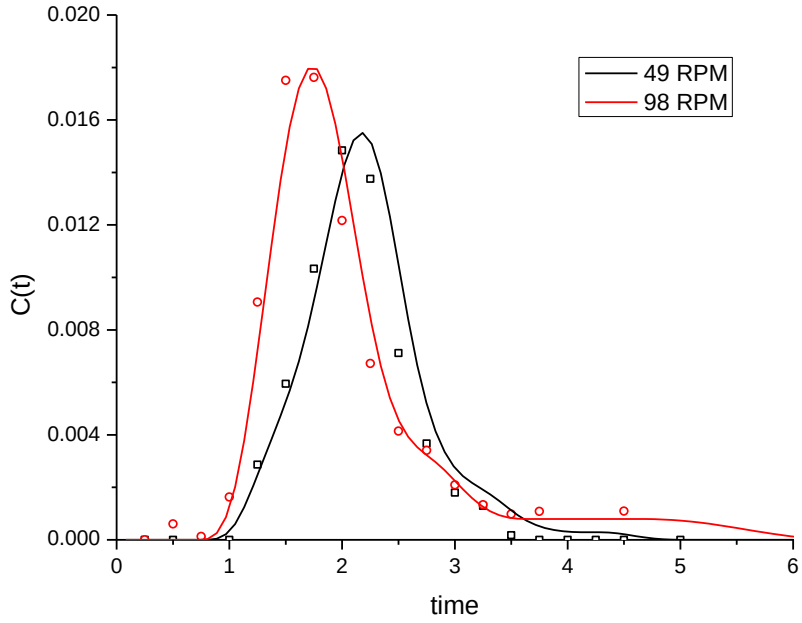


Figure IV-5 RTD profiles for continuous flight at different auger speed

Table IV-1 RTD data for the NREL reactor operated with two different RPM

Mean Residence Time (min)		
Auger Speed	Broken Flight	Continuous Flight
49 rpm	2.73	2.09
98 rpm	2.75	1.99
Dimensionless Variance of RTD, σ^2_θ		
Auger Speed	Broken Flight	Continuous Flight
49 rpm	0.170	0.045
98 rpm	0.184	0.147

Table IV-2 Xylose production comparison between NREL reactor and PFR
(185^{o}C, 2 wt% acid, time equals to mean residence time)

Conditions	Broken Flight @ 49 rpm	Broken Flight @ 98 rpm	Continuous Flight @ 49 rpm	Continuous Flight @ 98 rpm
Xylose from NREL Reactor (g/L)	41.20	42.03	34.69	31.98
Xylose from PFR (g/L)	44.82	45.09	35.33	33.63
Monomeric Xylose Yield from NREL Reactor*	0.343	0.350	0.289	0.267
Monomeric Xylose Yield from PFR	0.374	0.376	0.294	0.280
Total Xylose Yield from NREL Reactor**	0.716	0.728	0.721	0.706
Total Xylose Yield from PFR	0.747	0.747	0.732	0.725

* monomeric xylose yield = $\frac{monomer\ xylose\ in\ prehydrolysate}{initial\ xylan\ (xylose\ equivalent)}$

** total xylose yield = $\frac{monomer+oligomer\ xylose\ in\ prehydrolysate}{initial\ xylan\ (xylose\ equivalent)}$

V. Secondary Hydrolysis of Pretreatment Liquor Obtained from Continuous High-Solids Dilute-Acid Pretreatment of Corn Stover

V.1 Abstract

The prehydrolysate produced from NREL continuous pretreatment reactor contains high levels of hemicellulose sugars, with concentrations surpassing 10 wt%. On the other hand, the short residence time and high solid condition limits the hydrolysis of hemicellulose. The prehydrolysate obtained from this reactor thus contains relatively high amounts of xylose oligomers, typically in the range of 10-40% of the total sugar. In order to fully utilize the sugars in the prehydrolysate, oligomers need to be hydrolyzed to monomers. Dilute acid post hydrolysis experiments were carried out to find the best conditions for post hydrolysis. The objective is to hydrolyze all the xylose oligomers with minimal xylose degradation. At optimal conditions, total xylose recovery is around 95% for all prehydrolysate samples. The degradation is minimal. The dilute acid post hydrolysis was compared with enzymatic hydrolysis. It is found that the dilute acid post hydrolysis gives higher yield with much shorter reaction time.

V.2 Introduction

During dilute acid pretreatment, the major reactions are:

1) Oligomers with varying degrees of polymerization are produced by random attacks of acid on the hemicellulose chains,

2) Sugars are further degraded to form monosaccharides and sugar decomposition products such as furfural and HMF,

3) Acetic acid is produced by the cleavage of acetyl groups in biomass, which makes the pH decrease during the prehydrolysis.

So the prehydrolysate contains both mono- and oligo-sugars along with lignin, acetic acid, and some degradation products. The ratio of monomers to oligomers in the prehydrolysate depends on the severity of the pretreatment. The monomeric sugar yield is mostly affected by acid concentration, while for formation of sugar degradation products, such as furfural is impacted most by temperature (Roberto et al., 2003; Neureiter et al., 2002). Milder conditions cause less sugar degradation resulting in enhanced hemicellulose recovery. Actually, the partial hydrolysis of hemicellulose can enable a reduction both on energy requirements and on the formation of many relevant sugar degradation compounds, particularly, HMF and furfural.

Oligosaccharides, and particularly xylose-oligosaccharides produced from herbaceous and hardwood feedstock, may have a high added value as marketable products for application in the food, pharmaceutical, and cosmetic industries as specialty chemicals (Nabarlatz et al., 2007; Moure et al., 2006). As these applications are rather restricted in volume, hydrolysis of hemicellulosic oligosaccharides into monomers is almost a compulsory requirement for it to be effectively utilized in the bioconversion process.

A post treatment, secondary hydrolysis, is therefore necessary. The post hydrolysis process has been applied to pretreatment hydrolysate obtained from all types of materials: softwood (Shevchenko et al., 2000; Bossaid et al., 2001); hardwood (Garrote et al., 2001a); and herbaceous (Saska & Ozer, 1995; Allen et al., 1996; Duarte et al., 2004; Garrote et al., 2001b; Duarte et al., 2009) catalyzed by H_2SO_4 (conc. from 0.5% to 6.5% w/w) at 100-135^{o}C and reaction time up to 10h (Saska & Ozer, 1995; Shevchenko et al., 2000; Duarte et al., 2004; Garrote et al., 2001a; Garrote et al., 2001b). The post hydrolysis options can be reduced to acid (Saska & Ozer, 1995; Allen et al., 1996; Shevchenko et al., 2000; Duarte et al., 2004; Garrote et al., 2001c; Duarte et al., 2009) or enzyme (Duarte et al., 2004; Vazquez et al., 2001; Walch et al., 1992) catalyzed hydrolysis.

Acid hydrolysis typically presents both higher yield and productivity when compared to the enzymatic hydrolysis processes. Furthermore, as much of the hemicellulose complex structure is still present in the oligosaccharides (Carvalheiro et al., 2004; Kabel et al., 2002), several enzymatic activities are usually required for the complete hydrolysis (e.g., for hardwood type materials: endoxylanase, exoxylanase, β-xylosidase and accessory activities like acetyl xylanesterase, α-glucuronidase, α-arabinofuranosidase, and feruloyl esterase). As a result, the process is potentially inefficient and uneconomical. Under fully optimized conditions, sugar recovery is around 100% (Saska & Ozer, 1995; Shevchenko et al., 2000; Duarte et al., 2004; Garrote et al., 2001a; Garrote et al., 2001b; Duarte et al., 2009), as compared to standard dilute acid hydrolysis (121 ℃, 4% H_2SO_4 and 60 min). This can be considered a major advantage of the acid post hydrolysis process. Other advantages associated to this process are its high-speed and low catalyst cost.

If the conditions are not carefully chosen, however, significant monosaccharide degradation reactions may occur during acid post hydrolysis. Therefore, to obtain a high monosaccharide recovery, an optimization of the operational conditions is required.

V.3 Materials and Methods

V.3.1 Materials

Pretreatment hydrolysate samples were provided by NREL. The pretreatment conditions are listed in Table V-1. The details about the sugar content of these samples are shown in Figure V-1.

V.3.2 Dilute acid hydrolysis Reaction

Reactions were carried out in acid-resistant Nickel-Copper tubular reactors (3/8''x 4'') capped with SS-316 Yor-Lok end fittings. The reactors were filled with 2.0 ml pretreatment liquor. To initiate the reaction, the reactors were placed into a forced-air convention oven in which the temperature was adjusted to a level 100^{o}C higher than the desired point. Reactor temperature was monitored by a thermocouple that extends to the center of the reactor. After the temperature reached the desired point (which normally takes up to 1 minute), the oven temperature was manually decreased to the desired temperature. The two-step procedure was done to minimize the preheating time. After being subjected to specified reaction times, the reactors were quenched in a cold water bath, and the contents were collected. Because the total sugar concentration in the hydrolysate was too high to be accurately measured by the HPLC system, the liquors were diluted before being subjected to the sugar content analysis.

V.3.3 Enzymatic hydrolysis Reaction

Various enzymes were used in the enzymatic hydrolysis test, which include: Spezyme CP (Lot No. 301-00348-257, 59 FPU/ml, 123mg protein/ml), Xylanase (Multifect Xylanase, Lot No. 301-04021-015, 42 mg protein/ml), Pectinase (Multifect Pectinase FE, Lot No. A21-03356-001, 82 mg protein/ml) and β-glucosidase (Novozyme 188, Cat. No. C6150, 655 CBU/ml, 140 mg

protein/ml). The enzyme loading was is 20 mg protein/gram of xylose-oligomer. And the temperature is set to be 50^{o}C.

V.3.4 HPLC Analysis

Sugar analysis for hydrolysate samples were performed according to the NREL TP 510-42623 using HPLC equipped with Bio-Rad's Aminex HPX-87P, Aminex HPX-87H column and a RI detector. The difference of xylose monomer values before and after 121^{o}C incubation was interpreted as the xylose oligomer value.

V.4 Results and discussion

V.4.1 Secondary Dilute Acid Hydrolysis Reactions

For each of the liquor samples, 2–4 levels of temperatures, and 3 levels of acid concentrations (0%, 50% and 100% additional acid input (AAI)) were applied. The experiments were designed to find the optimal condition to convert xylose oligomer to monomer with minimum degradation.

The results of the hydrolysis are presented in Figure V-2 to Figure V-10. The hydrolysis yield is defined as the yield of xylose oligomer to monomer, and the overall yield is total xylose after hydrolysis over total initial total xylose (initial xylose monomers plus oligomers).

The optimum hydrolysis conditions for each sample are listed in Table V-2. For sample A, the optimum condition is 140^{o}C with 100% AAI (final acid concentration is 2.48 wt%) at 8 minutes and the highest hydrolysis and overall yield is 89% and 94% respectively. The xylose degradation during the secondary hydrolysis is 4% at this point. The optimum hydrolysis condition for sample B is 140^{o}C with 0% AAI (final acid concentration is 1.99 wt%) at 20 minutes and the highest hydrolysis and overall yield is 44% and 95% respectively. The xylose

degradation during the secondary hydrolysis is 0.3% at this point. The optimum hydrolysis condition for sample C is 120^{o}C with 0% AAI (final acid concentration is 2.25 wt%) at 19 minutes and the highest hydrolysis and overall yield is 64% and 96% respectively. The xylose degradation during the secondary hydrolysis is 1.1% at this point. Since the initial acid content in sample B and C are high, and the xylose oligomer level is relative low, additional acid are not required for Sample B and C for secondary hydrolysis.

As we can see from the experimental results, at the optimal condition, secondary hydrolysis leads to 95% xylose yield. It appears that the xylose oligomers left in the pretreatment hydrolysate are a resilient fraction. We observed variations in oligomers with concentrations increasing and decreasing throughout the experiment (Figure V-11). This is perhaps an indication that oligomers exist in complex form with other organic components such as lignin to form a lignin-carbohydrate-complex (LCC).

V.4.2 Enzymatic Hydrolysis Reactions

We also attempted enzymatic hydrolysis of the oligomers in the prehydrolysate. The purpose of this study was to see if the mixture of the prehydrolysate and treated solid could be hydrolyzed simultaneously in a single step, simulating the conditions of SSCF. Sample A was chosen for this test because it contains the highest oligomer concentration. Spezyme CP was used for this experiment (Lot No. 301-00348-257, 59 FPU/ml, 123mg protein/ml). The enzyme loading was 20 mg protein/gram of xylose oligomer. Figure V-12 shows the enzymatic hydrolysis profile. With cellulase alone (Spezyme CP), a hydrolysis yield of 42% was achieved after 48 hr. This is far below the acceptable range. This indicates that with normal hydrolysis practice using cellulase alone will not hydrolyze the oligomers effectively. Acid treatment or some other means of secondary hydrolysis is therefore necessary. So, some more commercial

enzymes were then tested: Xylanase (Multifect Xylanase, Lot No. 301-04021-015, 42 mg protein/ml), Pectinase (Multifect Pectinase FE, Lot No. A21-03356-001, 82 mg protein/ml) and β-glucosidase (Novozyme 188, Cat. No. C6150, 655 CBU/ml, 140 mg protein/ml). The enzyme loadings were uniform at 20 mg/gram of xylose oligomer. As shown in Figure V-12, the best performance was obtained with Multifect Pectinase, where the 48h hydrolysis yield was 81%. It is surprising that this yield is higher than that of Multifect Xylanase (59%).

Enzymatic hydrolysis gives a much lower xylose yield (81%) compared to acid hydrolysis (95%). Also, enzymatic hydrolysis requires much longer reaction times (48h vs 20 min). From an economic point of view, acid is a better option for post hydrolysis.

V.5 Conclusion

The total sugars present in the prehydrolysate from NREL continuous reactors are high enough for fermentation. Due to the high solid loading and short residence time, high amount of hemicellulose are still in oligomer form. In order to recover the hemicellulose from prehydrolysate, secondary hydrolysis is a necessary step. At optimal conditions, xylose recovery is around 95% for all three samples used in our study. Xylose monomer/oligomer is reacting with soluble lignin leading to the formation of LCC during the acid hydrolysis process. This leads to recovery less than 100%. Compared to enzymatic hydrolysis, acid hydrolysis is a better option for post hydrolysis of prehydrolysate. Acid hydrolysis produces higher yield and requires shorter reaction time, all at a lower cost.

Table V-1 Pretreatment conditions for hydrolysate samples

Sample Name	Stage 1 Conditions				Stage 2 Conditions				
	T	SA	t	SC	T	SA	t	TSA	SC
XT090403-B (A)	155	0.5wt%	1.5	-	-	-	-	-	-
XT091017 (B)	158	1.0wt%	4.5	-	130	7.5	20	-	-
XT090731-C (C)	155	16.8	5	0.28	130	7.6	20	24.5	0.28

Abbreviations used: T = temperature (^{o}C), SA = biomass sulfuric acid loading (mg H_2SO_4/g dry biomass), t = time (minutes), SC = total solids concentration (mass solids/overall mass) measured in pretreated slurry, TSA = total sulfuric acid loading (mg H_2SO_4/g dry biomass).

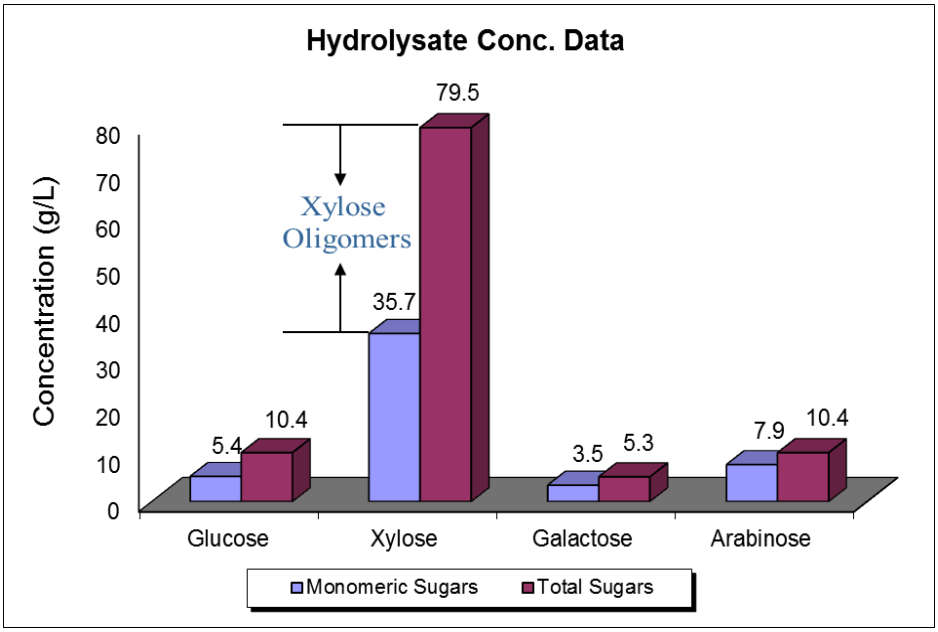
Table V-2 Optimum post hydrolysis condition for pre-hydrolysate samples

	AAI (%)	Final Acid (wt%)	T (^oC)	T (min)	Hydrolysis Yield* (%)	Overall Yield*** (%)	Residual Oligomers (%)	Degradation*** (%)
Sample A	100	2.48	140	8	89	93.6	2.4	4
Sample B	0	1.99	140	20	44	94.8	4.9	0.3
Sample C	0	2.25	120	19	64	96.3	2.6	1.1

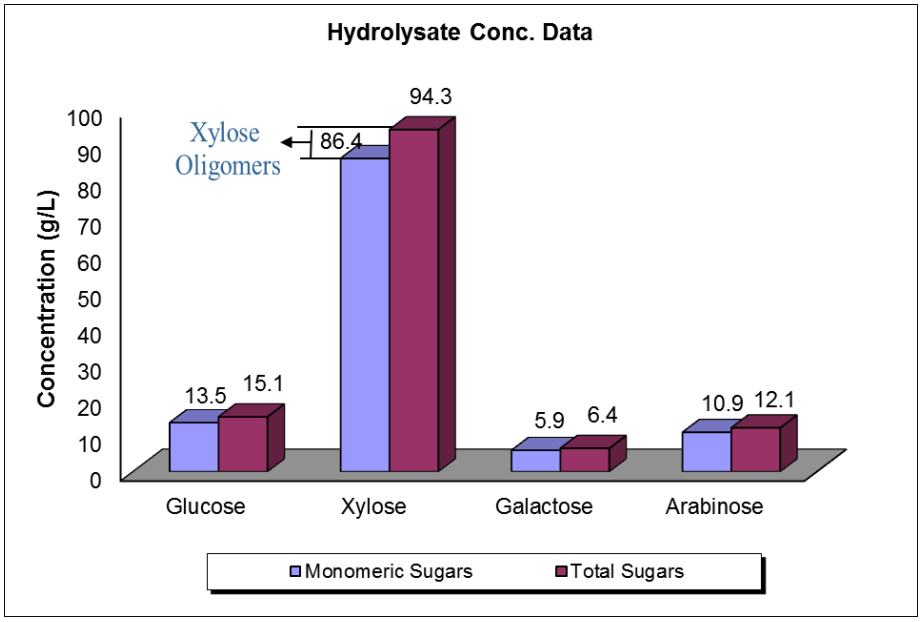
* hydrolysis yield = $\frac{xylose\_produced\_from\_oligomer}{total\_xylose\_oligomer}$

** overall yield = $\frac{monomeric\_xylose\_after\_2nd\_hydrolysis}{initial\_total\_xylose}$

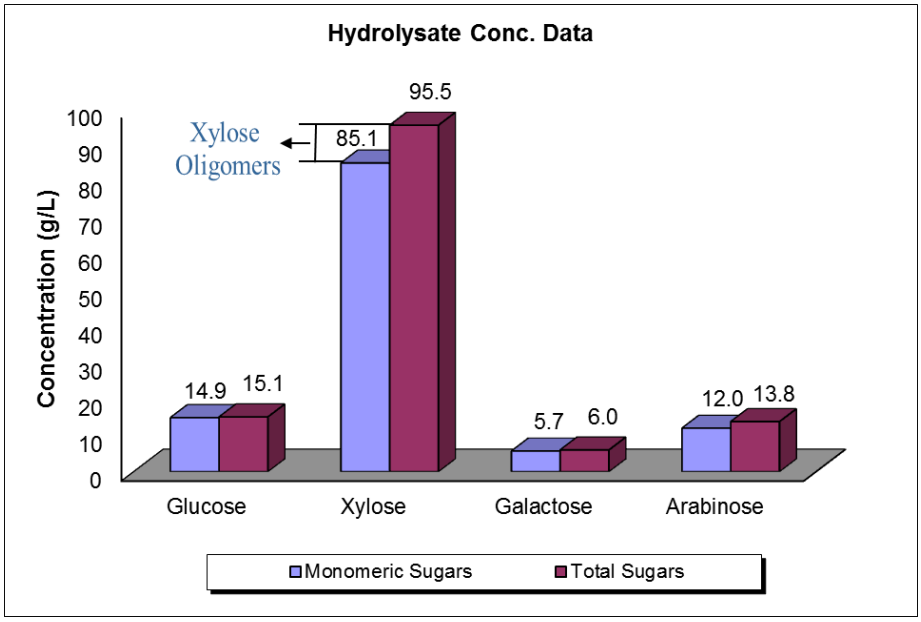
*** degradation = $\frac{initial\ total\ xylose - monomeric\ xylose\ after\ 2nd\ hydrolysis - residual\ oligomer}{initial\ total\ xylose}$


Hydrolysate Conc. Data
Concentration (g/L)
80
70
60
50
40
30
20
10
0
79.5
Xylose
Oligomers
35.7
10.4
5.4
3.5
5.3
7.9
10.4
Glucose
Xylose
Galactose
Arabinose
Monomeric Sugars
Total Sugars

(a)


Hydrolysate Conc. Data
Concentration (g/L)
100
90
80
70
60
50
40
30
20
10
0
94.3
Xylose
Oligomers
86.4
13.5
15.1
5.9
6.4
10.9
12.1
Glucose
Xylose
Galactose
Arabinose
Monomeric Sugars
Total Sugars

(b)



(c)

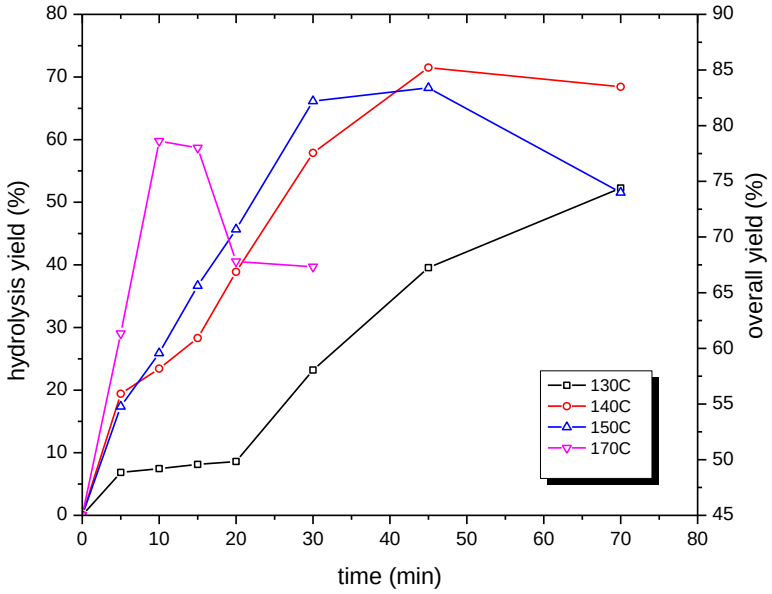
Figure V-1 Sugar content in pre-hydrolysate samples

(a) sample A, acid level in liquor: [H+]=0.254 mol/L (1.24 wt% H_2SO_4)

(b) sample B, acid level in liquor: [H+]=0.406 mol/L (1.99 wt% H_2SO_4)

(c) sample C, acid level in liquor: [H+]=0.460 mol/L (2.25 wt% H_2SO_4)

(a)



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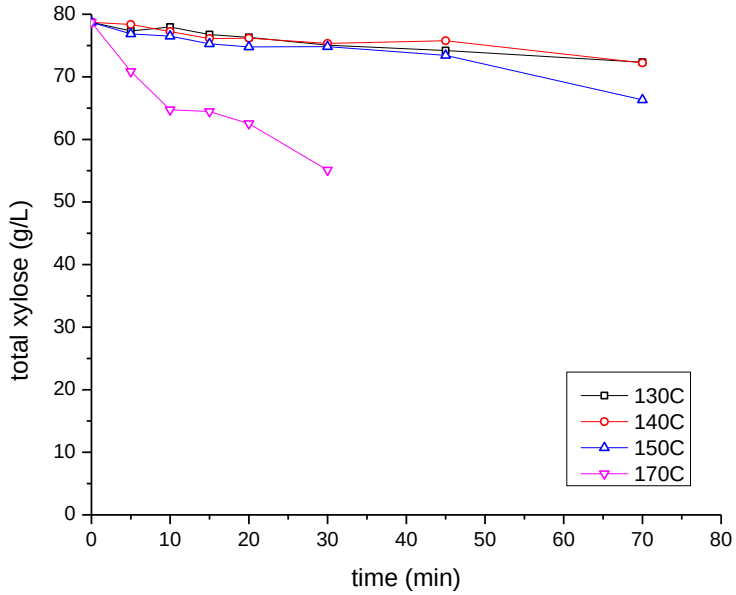
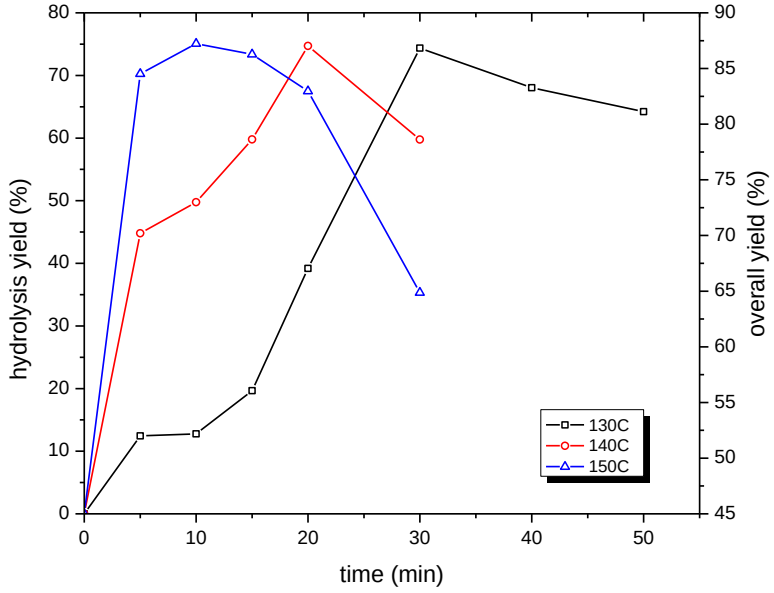


Figure V-2 Secondary hydrolysis profiles for sample A with 0% AAI (1.24 wt% H_2SO_4)

(a) hydrolysis and overall yield of xylose

(b) total xylose decomposition profiles

(a)



(b)

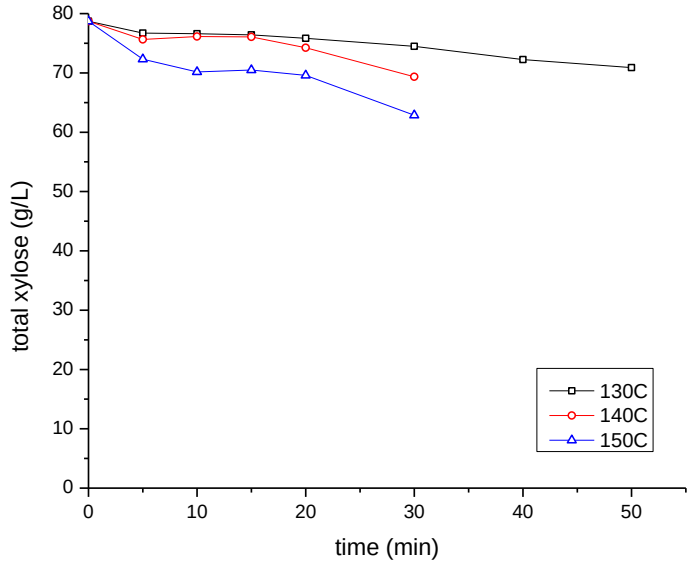
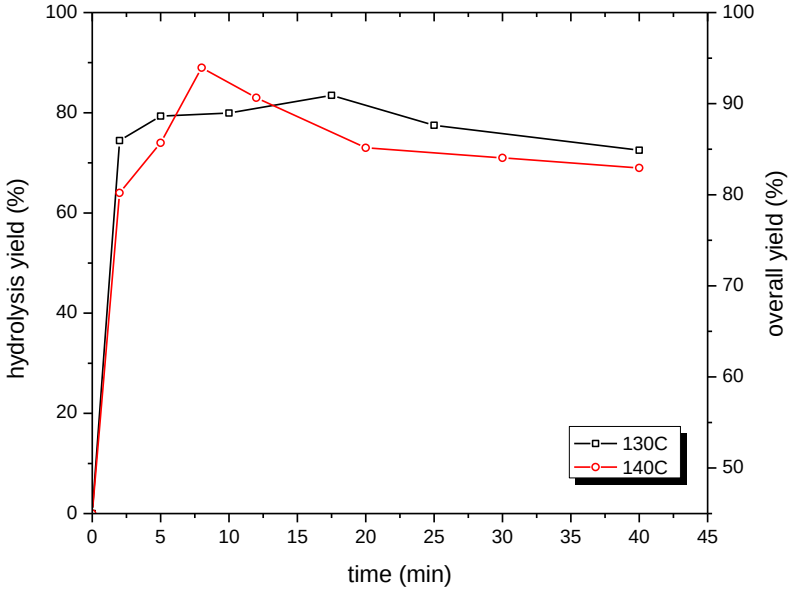


Figure V-3 Secondary hydrolysis profiles for sample A with 50% AAI (1.86 wt% H_2SO_4)

(a) hydrolysis and overall yield of xylose

(b) total xylose decomposition profiles

(a)



(b)

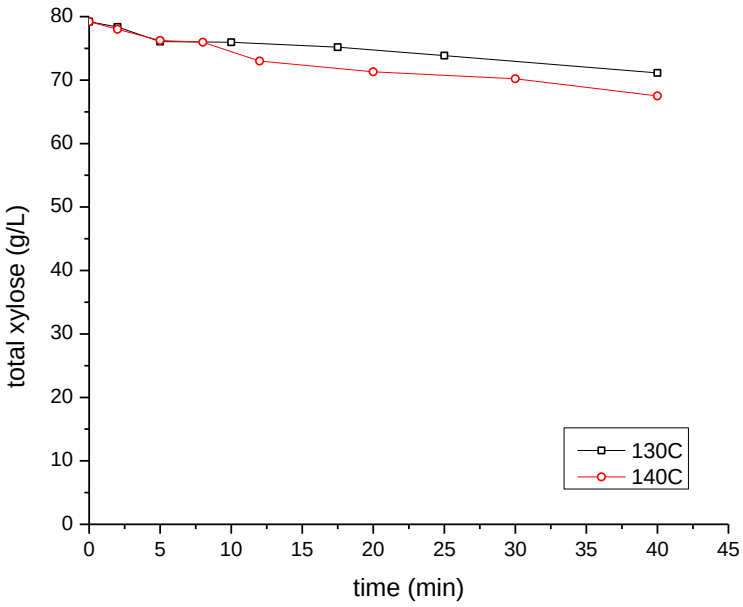
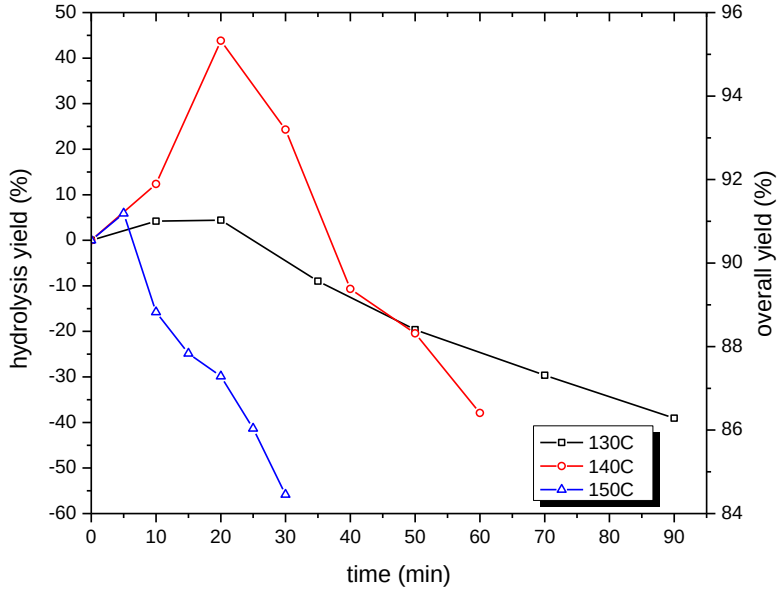


Figure V-4 Secondary hydrolysis profiles for sample A with 100% AAI (2.48 wt% H_2SO_4)

(a) hydrolysis and overall yield of xylose

(b) total xylose decomposition profiles

(a)



(b)

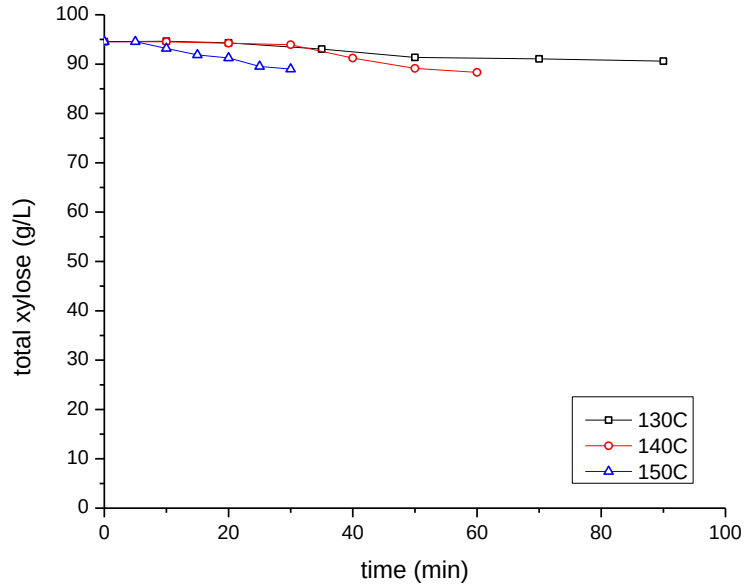
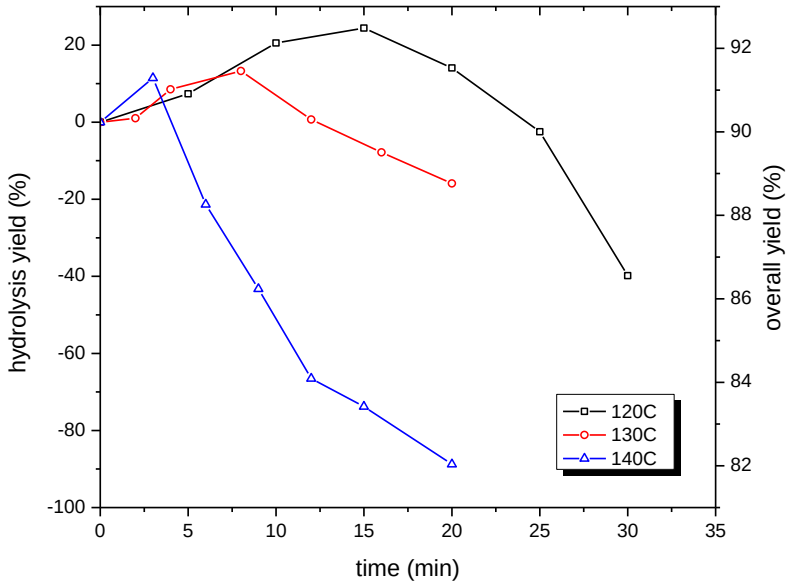


Figure V-5 Secondary hydrolysis profiles for sample B with 0% AAI (1.99 wt% H_2SO_4)

(a) hydrolysis and overall yield of xylose

(b) total xylose decomposition profiles

(a)



(b)

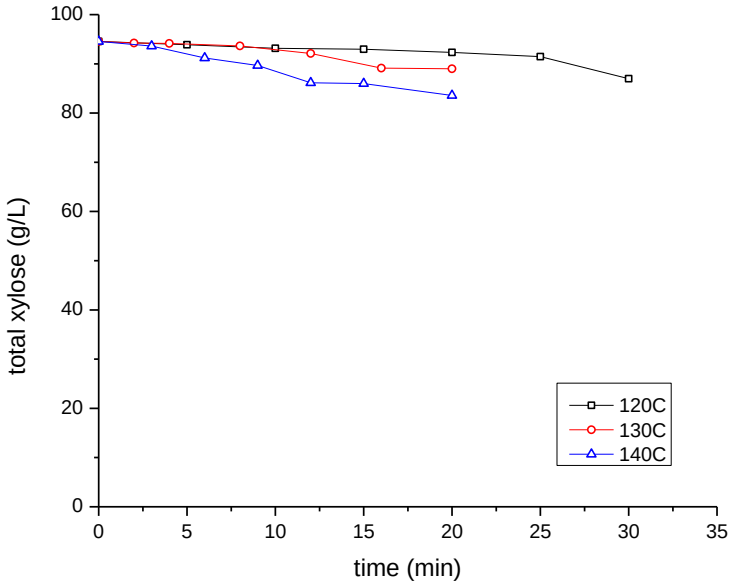
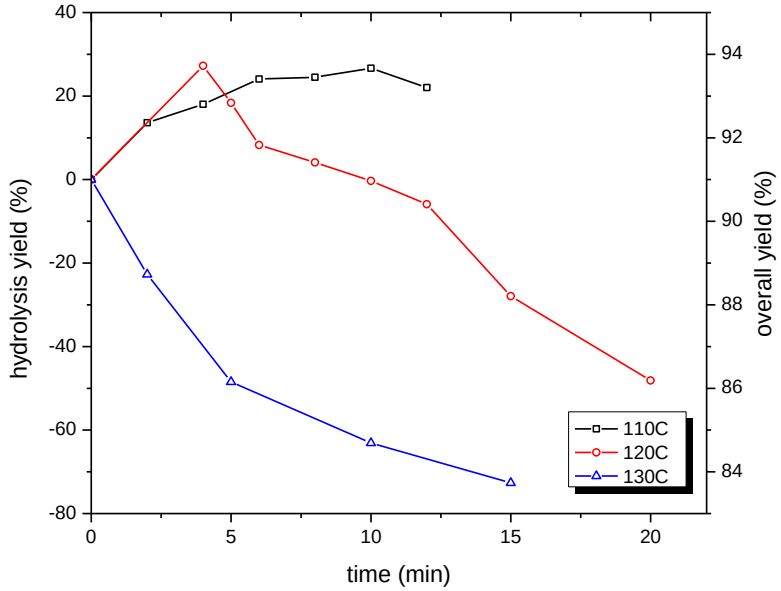


Figure V-6 Secondary hydrolysis profiles for sample B with 50% AAI (2.98 wt% H_2SO_4)

(a) hydrolysis and overall yield of xylose

(b) total xylose decomposition profiles

(a)



(b)

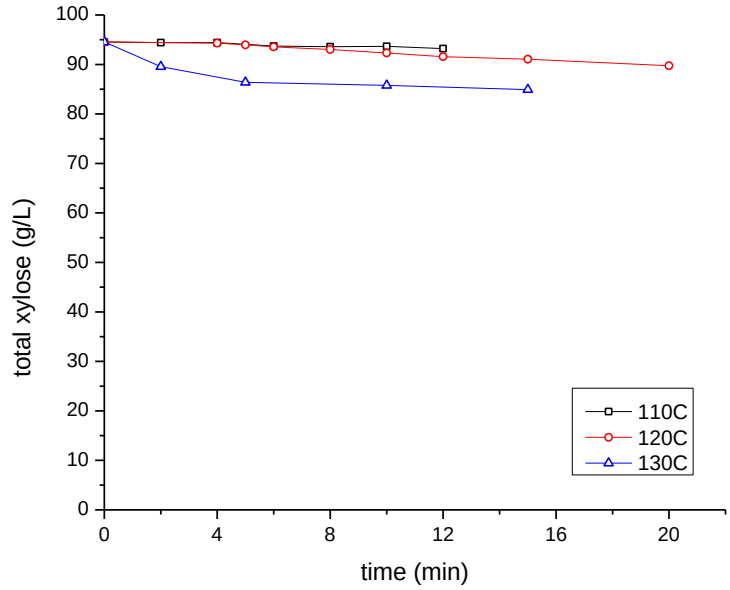
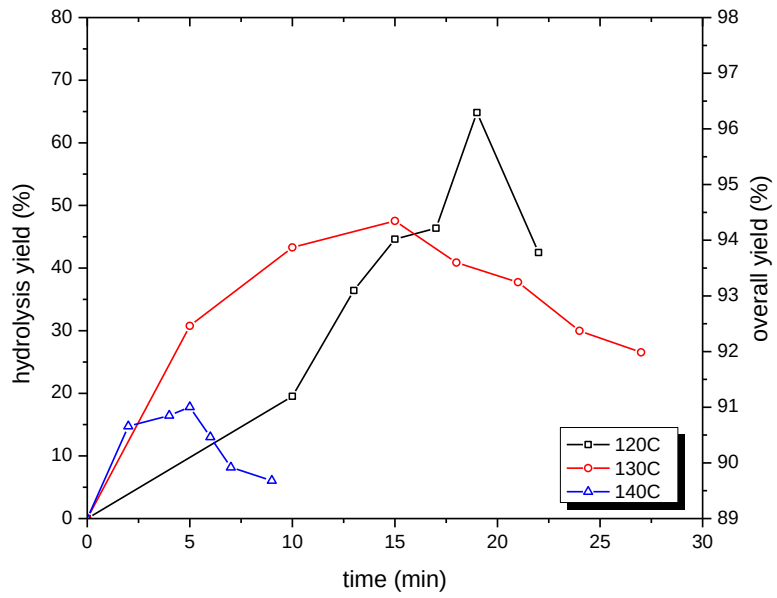


Figure V-7 Secondary hydrolysis profiles for sample B with 100% AAI (3.98 wt% H_2SO_4)

(a) hydrolysis and overall yield of xylose

(b) total xylose decomposition profiles

(a)



(b)

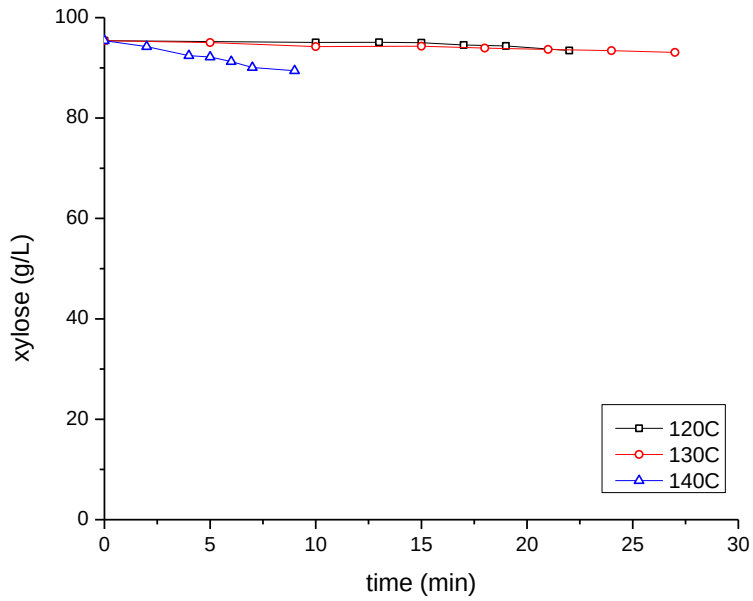
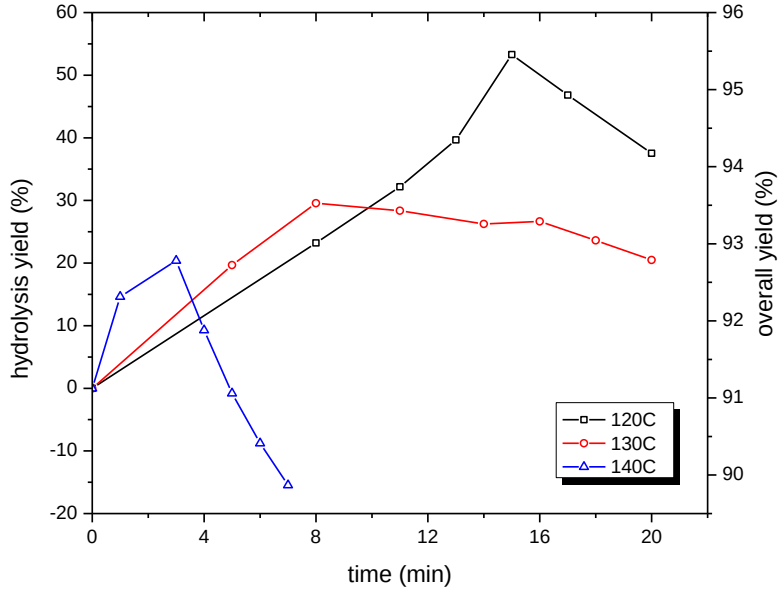


Figure V-8 Secondary hydrolysis profiles for sample C with 0% AAI (2.25 wt% H_2SO_4)

(a) hydrolysis and overall yield of xylose

(b) total xylose decomposition profiles

(a)



(b)

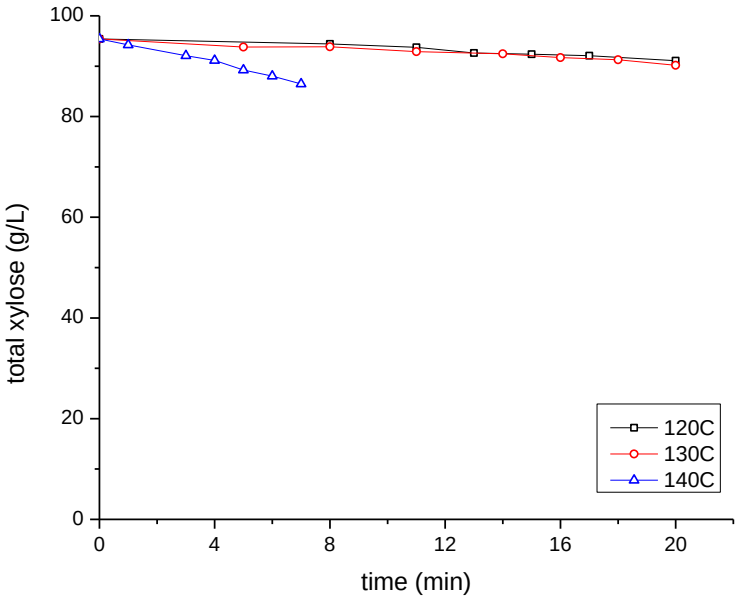
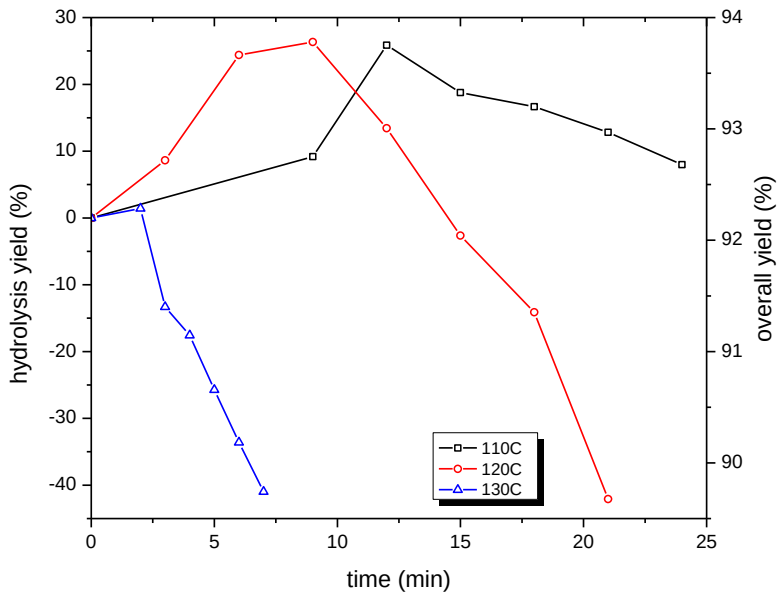


Figure V-9 Secondary hydrolysis profiles for sample C with 50% AAI (3.37 wt% H_2SO_4)

(a) hydrolysis and overall yield of xylose

(b) total xylose decomposition profiles

(a)



(b)

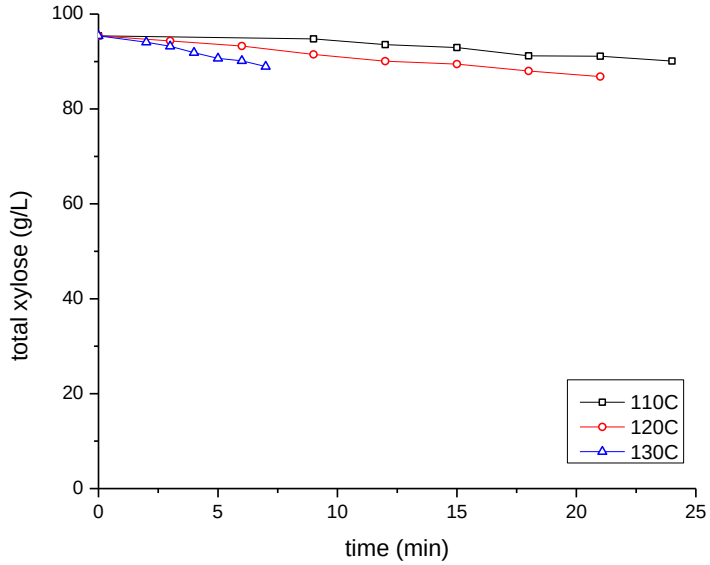


Figure V-10 Secondary hydrolysis profiles for sample C with 100% AAI (4.5 wt% H_2SO_4)

(a) hydrolysis and overall yield of xylose

(b) total xylose decomposition profiles

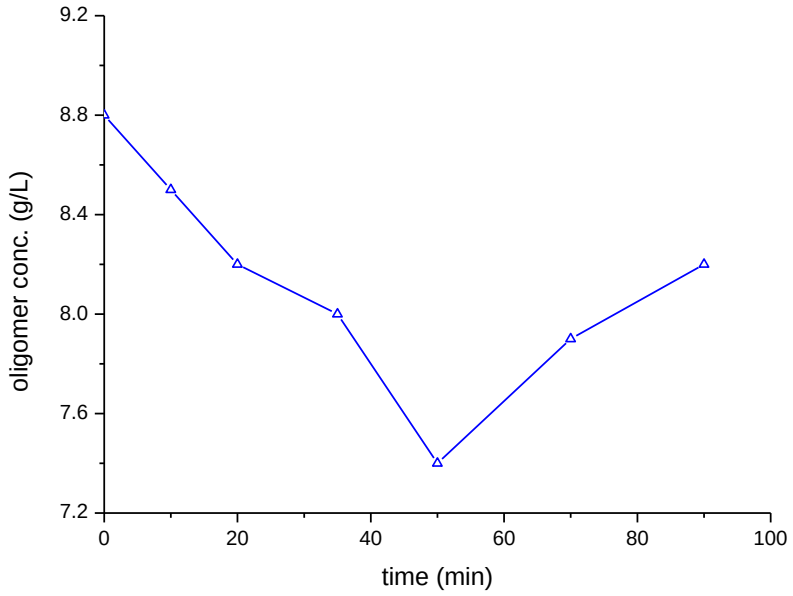


Figure V-11 Oligomer variation during secondary hydrolysis of prehydrolysate (Sample B, Condition: 1.99 wt% acid & 130^{o}C)

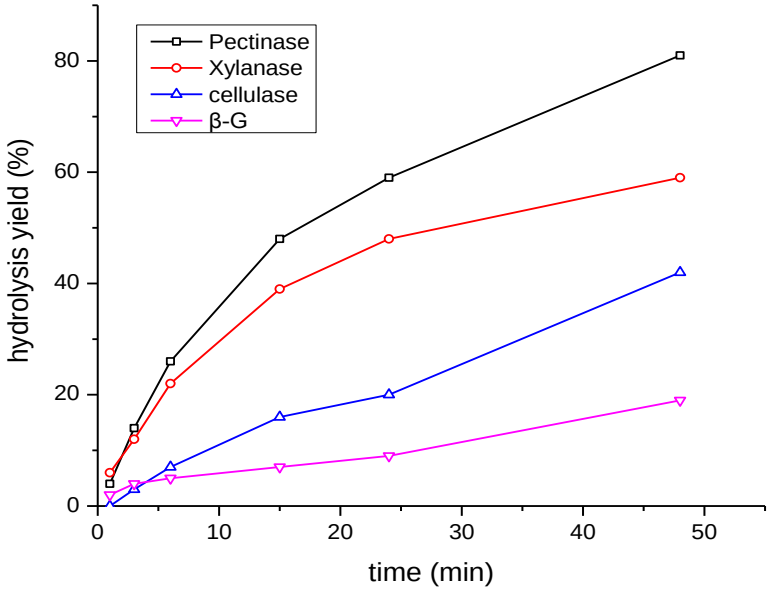


Figure V-12 Enzymatic hydrolysis yield during post hydrolysis of prehydrolysate

(Enzyme loading: 20 mg protein/g-xylose oligomers)

Cellulase: Spezyme CP

β-G: Novozyme -188

VI. Lactic Acid Production from the Mixture of Hemicellulose Prehydrolysate and Kraft Paper Mill Sludge

VI.1 Abstract

Paper mill sludge is a solid waste material composed of pulp residues and ash generated from pulping and paper making process. The carbohydrate portion of the sludge from Kraft/Recycle paper mill has chemical and physical characteristics similar to those of commercial wood pulp. Because of its high carbohydrate content and well-dispersed structure, the sludge can be biologically converted to value-added products without pretreatment. In bioconversion of solid feedstock such as paper mill sludge, a certain amount of water must be present to attain fluidity. In this study, hemicellulose pre-hydrolysate, in place of water, was added to the sludge to increase the concentration of the final product. Pre-hydrolysate was obtained by hot-water treatment of pine wood in which the total sugar concentration reached 4 wt%. The mixture was processed by simultaneous saccharificiaton and fermentation (SSF) using enzymes (cellulase and pectinase) and *Lactobacillus rhamnosus* (ATCC-10863). Pectinase was added to hydrolyze mannose oligomers in the prehydrolysate to monomers. During the SSF of the mixture, calcium carbonate in the paper sludge acted as a buffer, yielding calcium lactate as the final product. External pH control was unnecessary due to the buffer action of calcium carbonate that maintained the pH near optimum for the SSF. The lactic acid yield in the range of 80 - 90% of the theoretical maximum was obtained. Use of the mixed feed of pre-hydrolysate and pulp mill sludge in the SSF raised the product concentration to 60 g of lactate/L.

VI.2 Introduction

Lactic acid is a specialty chemical widely used in cosmetics, pharmaceuticals, textile and food industry (Xu et al., 2006; John et al., 2007; Vickroy, 1985). Strong market growth of lactic acid is projected in production of polymers, especially poly-lactic acid (PLA). PLA is an environmentally friendly polymer used for production of high-grade fabrics and biodegradable plastics (Kharas et al., 1994). Sustained growth in medical-grade polymer such as drug delivery system (John et al., 2007; Schmidt & Padukone, 1997) and medical implants (Auras et al., 2011) is also expected. The worldwide production of lactic acid (LA) in 2001 was about 86,000 tons/year (Bouchoux et al., 2006), 260,000 tons in 2008 and expected to reach 1,000,000 tons in 2020 (Jem et al., 2010). Presently, about 90% of LA produced worldwide is made by fermentation route and the rest by chemical synthesis (Hofvendahl & Hahn–Hägerdal, 2000). Most of the fermentation relies on starch-derived glucose or sucrose as feedstock (Litchfield, 1996). In an effort to reduce the production cost, considerable research work has been conducted in bioconversion of lignocellulosic carbohydrates for lactic acid production (Parajo et al., 1997; Grade et al., 2002; Neureiter et al., 2004).

Lignocellulosic feedstocks are available from various sources. Our main interest was in utilization of wastes materials available from pulp and paper plants. Among the various wastes from pulp and plants, the mill sludge stands out as the most attractive feedstock as it has a number of advantages. First of all, the cost is practically zero or may even be negative. Paper sludge has in fact been a disposal liability for many years, the cost of disposal reported to be about $20 per wet ton (Pope & Albertson, 1999; Kang et al., 2010). It has high carbohydrate content and physical and chemical characteristics amenable for bioconversion. Because of its well-dispersed structure, the paper mill sludge can be processed without pretreatment (Schmidt

et al., 1997; Lark et al., 1997; Kang et al., 2010). Pretreatment is one of the major unit processes in the overall bioconversion process. Its elimination can significantly reduce the processing cost. The paper mill sludge is reliable feedstock available year-round at a single site in a concentrated form making it convenient for further processing. For biofuels application, the quantity of sludge generated from a single paper mill is not sufficient to support a plant of economically-feasible size. It is, however, sufficient for production of a specialty chemical such as lactic acid.

In the US, about 80% of the total pulp capacity is for Kraft pulping. Most of them also operates recycle mill as a supplementary unit. In Kraft process, a large fraction of the hemicellulose is released into black liquor, which is eventually burned in the recovery boiler generating steam and electricity (Biermann, 1993). The heating value of hemicellulose (13.6MJ/kg), however, is only half that of lignin (27MJ/kg) (Sixta, 2006). This underutilized fraction of hemicellulose is another source of bioconversion feedstock. It has been proven that a part of the hemicellulose can be recovered prior to pulping without degrading the quality of the pulp by treatment with hot water/steam (Yoon et al., 2008; Yoon et al., 2010), dilute acid (Springer & Harris 1982), or mild alkaline (Al-Dajani & Tschirner, 2008).

In bio-processing of lignocellulosic feedstocks such as paper mill sludge, a certain amount of water must be present in the bioreactor to maintain fluidity and viable microbial activity. In this work, addition hemicellulose pre-hydrolysate to the sludge, in place of water, was considered as a means to increase the product concentration and to utilize the portion of hemicellulose in the pulp feed that is virtually wasted in pulping process. We explored a novel process scheme wherein the two different carbohydrate resources available from pulp mills are biologically converted to lactic acid. The scope of the work covered characterization of paper mill sludge and the pre-hydrolysate of pulp feed, and technical feasibility of converting them into

lactic acid by way of simultaneous saccharification and fermentation. Of our particular interest was to investigate the feasibility of one-step processing of the mixed feedstock.

VI.3 Materials and Methods

VI.3.1 Materials

Feedstock

Southern pine chips were obtained from Rock-Tenn Company in Demopolis, Alabama. Chips with major defects including bark, knots, and decayed parts were removed prior to screening on a chip class laboratory screen equipped with a stack from top to bottom of 45-mm round screens, 8-mm bar screens, 6-mm bar screens, and 4-mm round screens. The wood fraction passing 45-mm round screens and 8-mm bar screens and retained on 6-mm bar screens was collected, well mixed, and air-dried before use. Composition of southern pine was determined to be: 40.6% glucan, 7.8% xylan, 2.2% galactan, 1.4% arabinan, 9.3% mannan, 30% lignin and 0.5% ash.

The recycled paper mill sludge (RPMS) was collected from the wastewater clarifier unit of a Kraft paper mill, Boise Paper Company (Jackson, AL). The RPMS was washed with tap water three times and thickened to 26.5% consistency using a vacuum filter, and stored at 4^{o} C. The RPMS was analyzed according to the National Renewable Energy Laboratory (NREL) standard procedure (NREL, 2008) to contain 47.6 wt% glucan, 7.5 wt% xylan, 6.6 wt% lignin and 34.5 wt% ash.

Enzymes and Microorganism

Cellic C-Tec2 (Novozymes Cellic CTec2, Batch VCNI0001, 119 FPU/ml) and Pectinase (Multifect Pectinase FE, DuPont-Danisco, Lot No. A21-03356-001, 82 mg protein/ml) were used

in the enzymes hydrolysis test of the sludge and for the SSF experiments. Pectinase was used specifically for hydrolysis of mannose oligomers in the pre hydrolysate. *Lactobacillus rhamnosus* (ATCC-10863) was used as the microorganism in the SSF. Lactobacilli MRS Broth (Acumedia) was used as the growth medium for preparation of inoculum.

VI.3.2 Pre-extraction of Southern pine chips

Hot water treatment was applied to extract hemicellulose sugars from Southern pine chips. In order to avoid damaging the fiber quality, the pre-extraction was done in such a way that the hemicellulose removal is limited to a certain level. In normal batch operation of pre-extraction, the sugar concentration below 2% (w/v) is obtained. In order to increase the sugar content in the extracted liquor, three stage extractions were applied as described in Figure VI-1. This three-stage extraction also simulates the continuous counter-current extraction process, which would be a preferred mode of operation in commercial scale operation. The extractions were conducted in a 500 ml cylindrical bomb digester which was placed inside a M/K laboratory digester filled with water as heat transfer fluid. In each extraction stage, 70 grams of oven-dried wood chips were loaded in the bomb digester. DI water was used in the first-stage extraction. The liquid-to-wood ratio was 5.8 to 1. To determine the optimum conditions, the digester was operated over a broad range of conditions. From the data the operating conditions of 170^{o}C and 60 minutes was applied for generation of the prehydrolysates. The extract obtained from hot-water treatment was drained from the wood chips to collect 70% of the total liquid. The liquor used in the second stage was mixed with the extract liquor from the first stage extraction (70% of the total first stage liquor) and make-up DI water (30% volume of the total first stage liquor) since about 30% of total liquor is absorbed by the extracted wood. The same operation was applied to the third stage; the input liquid consisted of 70% of second-stage liquor and 30% DI water. As wood chips are

extracted, their weight decreases due to the components being dissolved into the extraction liquor (termed as pre-hydrolysate). The wood weight loss data were calculated from the difference between the weight of fresh wood chips and that of thoroughly washed and dried wood residue. The sugar contents of the extract and the reacted residue were determined by the NREL standard procedures (NREL, 2008).

VI.3.3 Detoxification of Prehydrolysate

Over-liming was tested for detoxification of the prehydrolysate by the following procedure. Lime (CaO) was added to the prehydrolysate to increase the pH to 10. The treatment conditions were: 60^{o}C, 30 minutes, 10g CaO/L of prehysrolysate. The pH was readjustment to 5.0 by sulfuric acid, centrifuged to remove precipitates, and the supernatant was used for the SSF test.

VI.3.4 SSF of Prehydrolysate

Erlenmeyer flask (125 ml) was used as the bioreactor with 50 ml liquid volume. The SSF were operated in an incubator shaker (New Brunswick Scientific, Innova-4080) at 37^{o}C with 150 rounds per minute (RPM). $CaCO_3$ was added to the prehydrolysate at the level of 5% w/v for SSF operation as a neutralizing reagent. The prehydrolysate was steam sterilized at 121^{o}C for 15 min. before the SSF. The biological components of the SSF were Pectinase enzyme and *L. rhamnosus*, applied at the level of 15 mg-protein/g-mannan and 3.0% V/V inoculum respectively.

VI.3.5 Enzymatic Digestibility Test of Recycled Paper Mill Sludge

The enzymatic digestibility test of the RPMS was performed according to the NREL Chemical Analysis and Testing Standard Procedures (NREL, 2008). Screw-capped 250 ml Erlenmeyer flasks were used as the hydrolysis reactor. The RPMS samples were suspended in DI

water and put into the flasks to reach a total working volume of 100 ml such that the initial glucan content in the reactor becomes 1% (w/v) and steam sterilized at 121℃ for 15 min. The enzymatic digestibility tests were carried out at 50℃ in an incubator shaker (New Brunswick Scientific, Innova-4080) agitated at 150 RPM. For hardwood pulp, enzymatic hydrolysis was carried out under two different conditions: pH = 4.8 (0.05 M sodium citrate buffer) and with addition of $CaCO_3$ to the level of 0.5 g/g-glucan matching that of sludge without pH control. In all digestibility tests, enzyme loading of 15 FPU Cellic CTec-2 was applied per g-glucan in the feedstock. The enzymes were filter-sterilized. Hydrolysate samples were taken at 6, 12, 24, 48, and 72 h and analyzed for glucose, xylose, and cellobiose. Total released glucose and cellobiose after 72 h of hydrolysis were used to calculate the enzymatic digestibility:

$$\text{Digestibility (\%)} = \frac{Glucose\_released(g) + 1.053 * Cellobiose\_released\ (g)}{1.111 * Glucan\_added\ (g)} * 100 \qquad \text{(VI-1)}$$

Commercial grade hardwood pulp was also put through the same procedure as a control.

VI.3.6 SSF of Recycle Paper Mill Sludge

The SSF tests of the RPMS were carried out with DI water and alternatively with addition of the prehydrolysate. The procedure was similar to that previously described in the section of SSF of prehydrolysate. In the SSF of RPMS alone, the sludge samples were suspended in DI water and put into the flasks to reach a total working volume of 50 ml applying various solid loadings. The slurries were steam sterilized by at 121^{o}C for 15 min. Cellic C-Tec2 was added at different enzyme loadings ranging 5-15 FPU/g-glucan. In the SSF of the RPMS-prehydrolysate mixture, Pectinase and Cellic C-Tec2 were added at various levels. In all SSF experiments, corn steep liquor and yeast extract were added as nutrients for the microorganism

(*L. rhamnosus*) in the amount of 0.5ml and 0.3g respectively. Samples was taken at 12, 24, 36, 48, 60, 72 and 96 h and analyzed for glucose and LA.

VI.3.7 Analytical Methods

The prehydrolysates were centrifuged to separate the solid part from liquid. The liquid was analyzed for monomeric sugars. It was then held for 1 h with 4% sulfuric acid at 121 ℃ in an autoclave to hydrolyze the oligomers to monomers. The total sugar content after autoclave hydrolysis minus the initial monomeric sugar content was taken as the oligomers in the hydrolysate. Determination of sugar content was done according to the standard MREL procedure (NREL, 2008). The solid samples were analyzed for carbohydrates, acid insoluble lignin, acid soluble lignin, and ash following the NREL standard procedures (NREL, 2008). The moisture content was measured by an infrared moisture balance (Denver Instrument, IR-30). Sugars were determined by HPLC using a BioRad-HPX-87P column. LA, acetic acid, and other organic compounds including furfural, HMF, levulinic acid were determined by HPLC using a BioRad-HPX-87H column. A refractive index detector was used with the HPLC. Where applicable, statistical analysis was performed including mean value and standard deviation.

VI.4 Results and Discussion

With the ultimate goal of establishing a bioconversion process utilizing the two different feedstocks available from Kraft paper mills, the experimental work in this investigation was structured to perform the following specific tasks: characterization of the sludge and pre-hydrolysate including the bioconversion compatibility, method to produce pre-hydrolysate from pulp feed, and assessment of the SSF as a bioconversion process converting the mixed feed to lactic acid.

VI.4.1 Partial Extraction of Softwood Hemicellulose

On the basis of our previous study (Kang et al., 2012), the extraction time of 60 minutes was applied to recover as much hemicellulose sugars as possible without degrading the quality the cellulosic fiber. With 3-stage pre-extraction, the total sugar concentration in the hydrolysate was raised to 40.7 g/L, mannose being the largest sugar component at 15.5 g/L (Table VI-1). The overall recovery of hemicellulose sugars was approximately the same as that of each stage. In 3-stage operation, about 38% of total hemicellulose sugars, which is equivalent to 10.7% of total solid, was recovered while the loss of cellulose was only 0.03%.The recovered sugar was obtained mostly in the form of oligomer rather than monomer (Table VI-1). In the hydrolysate, the hexose sugars (mannose, glucose, galactose) had higher ratio of oligomer to monomer than pentoses (xylose, arabinose). Hexose oligomers have been reported to be more stable against acid-catalyzed degradation (BeMiller, 1967). Other organic compounds were also generated during the pre-extraction process. The identifiable organic compounds were acetic acid, acetyl ligand attached to other organic compound, furfural, HMF, levulinic acid (Table VI-2). These compounds also accumulated over the 3-stage processing to the final total concentration of 5.72 g/L (Table VI-2). There are numerous other unidentified compounds in the pre-hydrolysate including lignin degradation products. Over the three-stage pre-hydrolysis, the pH stayed relatively constant at 3.50, 3.43 and 3.40, respectively. It appears that the ash content in the wood is mainly inorganic salt, which act as a buffer when dissolved in water.

VI.4.2 Enzymatic Hydrolysis Test of Recycle Paper Mill Sludge

The washed RPMS was first subjected to enzymatic hydrolysis to produce sugars using Cellic CTec-2. The results were compared with those of pure cellulose. The enzymatic conversion of the sludge was dismal showing less than 20% of glucan digestibility whereas that

of α-cellulose was above 80% under the identical hydrolysis conditions (Figure VI-2). To verify the reason for the difference in the enzyme efficiency between the two substrates, additional hydrolysis tests were conducted using a mixture of α-cellulose and $CaCO_3$, the major inorganic contaminant in the sludge. The α-cellulose was mixed with $CaCO_3$ at the same level as that in the sludge. The hydrolysis profiles of the three substrates shown in Figure 2 clearly indicate that presence of $CaCO_3$ is the main reason for the inhibited enzymatic reaction. This reaffirmed the finding of our previous work on ethanol production from paper mill sludges (Kang et.al, 2012) that ash contained in the sludge interferes with the enzymatic reaction. Specifically, $CaCO_3$ in ash acts as a buffer which maintains the pH two units above the optimum for the cellulose enzymes. Such pH problem can be resolved if the sludge is treated by an acid, thus bringing the pH towards the optimum. Alternatively, one may apply a SSF wherein the end-product is an acid, i.e., lactic acid. With this approach, the acid is internally generated to form calcium lactate as the end-product instead of treating the sludge with external acid. The calcium carbonate in the sludge essentially serves as a pH control reagent in the fermentation process.

VI.4.3 SSF of Recycle Paper Mill Sludge

Since the glucose digestibility is below 20% from enzymatic hydrolysis of RPMS without pH control, separate hydrolysis and fermentation (SHF) was ruled out as a feasible process option. Presence of large amount of $CaCO_3$ in the RPMS makes it extremely difficult to control the pH. SSF was therefore pursued in the subsequent experiments. The SSF of RPMS was first carried out with 5 wt% solid loading applying different enzyme loading, and with 7.5 FPU/g glucan loading applying different solid loading. Higher enzyme dosage gave higher LA yield. At 5% solid loading, when the enzyme dosage increased from 5 to 10 FPU/g glucan, the 72-h LA yield increased from 69% to 88%. At a fixed enzyme loading, the LA yield is seen to have

inverse relationship with solid loading, giving low yield at high solid loading, consequently low LA acid concentration. The decreased LA yield is due to the limited enzymatic hydrolysis at high solid loading, which is well-documented (Wang et al., 2011; Zhang et al., 2011; Cara et al., 2007; Pessani et al., 2011). At a fixed enzyme loading of 7.5 FPU/g-glucan, 5% solid loading led to 82% of LA yield, but the product concentration was only 20 g/L. When the solid loading was increased to 15%, even though the yield decreased from 82% to 59%, the product concentration rose to 44g/L (Table VI-3 and VI-4). The yield and product concentration are both important factors in process economics. The fact that they have inverse relationship between them brings up a delicate situation where a proper balance needs to be taken in the process design and operation. With this in mind, we have explored the use of mixed feed (sludge plus prehysrolysate) in the SSF.

VI.4.4 SSF of Prehydrolysate

Prior to the SSF of mixed feed, the SSF of the prehydrolysate was first investigated. The largest sugar component in the prehydrolysate is mannose which exists mostly in the form of oligomer (Table VI-1). In order to hydrolyze the mannose oligomers to monomer, the cellulase was supplemented with pectinase in the SSF of the pre-hydrolysate. The pectinase used in this study (Multifect Pectinase FE, DuPont-Danisco, Lot No. A21-03356-001, 82 mg protein/ml) was reported to have high mannanse activity (Kang et al., 2011). The cellulase (Cellic C-Tec2) loading was 5 FPU/g Glucan and the Pectinase loading was 15 mg protein/g Mannan.

The prehydrolysate produced from lignocellulosic biomass contains toxic components such as organic acid, furans, and various phenolic compounds originated from lignin that inhibit the fermentation process (Wang et al., 2011; Zhang et al., 2011) as well as the enzymatic hydrolysis (Cara et al., 2007). The SSF of prehydrolysate was tested with and without

detoxification. The results are shown in Figure VI-3. Without detoxification, the SSF produced dismal level of lactic acid, giving less than 5% yield. After detoxification by overliming, the SSF LA yield rose 65% on the basis of total hexose sugars present in the prehydrolysate. The SSF of the prehydrolysate was also carried out without detoxification but with addition of excess amount of $CaCO_3$, which acted as a neutralizing reagent for lactic acid and also as a buffer stabilizing the pH. The pH level stayed near 5.6 throughout the SSF despite continual production of LA, leaving calcium lactate as the end-product. In this method, the lactic acid yield reached 60%, only 5% lower than that obtained from detoxified (overlime-treated) prehydrolysate. Since interaction between $CaCO_3$ and the toxic components in the prehydrolysate at the fermentation temperature of 37^{o}C is quite low, it appears that the negative effect on lactic acid production in the SSF is caused primarily by low pH rather than the direct interaction of the inhibitors with the microorganism and the enzymes. It also indicates that addition of external $CaCO_3$ may not be necessary if the SSF is carried out using the mixed feed of prehydrolysate and RPMS since the sludge contains high amount of $CaCO_3$.

VI.4.5 SSF of the Mixture of RPMS and Prehydrolysate

In order to assess the overall performance and to refine the operating conditions, the SSF of the mixed feed was investigated carrying out experiments under various process conditions. The results of SSF of the mixture are shown in Table VI-6. As shown in the table, the yield is at low solid loading is lower than that of high solid loading. This is contrary to what we observed in the SSF of sludge alone. We believe it is due to the pH effect. Figure VI-4a and b show the pH profiles during the SSF of the mixed-feed. With high solid loading (10%), the pH quickly dropped to 5.8 (a level compatible with the SSF) from the initial vale of 7.2 and stayed at that level throughout the SSF (Figure VI-4b). The reason for initial quick drop of pH is due to

production of lactic acid which eventually forms calcium lactate. The steady pH afterwards is due to the buffer action of the ash content and the presence of calcium lactate and small amount of calcium acetate which collectively act as a buffer. With low solid loading (5%), the pH steadily declined and stayed at 4.2 due to low total amount of ash in the sludge. When the prehydrolysate, the pH of which is 3.3, was added to the reactor that has low solid loading, the acid-alkali balance becomes such that the overall acidity of the prehydrolysate and lactic acid supersede the $CaCO_3$ in the sludge driving the pH to the range of 4.2-4.5 (Fig. VI-4a), which is below the optimum of the SSF. In other words, with low sludge loading, the ash content in the sludge is not sufficient to maintain the pH near optimum level. This eventually led to low lactic acid yield in the SSF.

We have thus proven that the pH problem encountered in the enzymatic hydrolysis of the RPMS is resolved if one uses the mixed feed at high solid loading in the SSF. Unlike the case of the SSF of the prehydrolysate, addition of external $CaCO_3$ is not required when high sludge loading is applied. At high sludge loading, the amount of the ash ($CaCO_3$) contained in the sludge is sufficient to maintain the pH within the operable range of the SSF.

Another important benefit sought in the use of the mixed-feed is to raise the product concentration. Such benefit is also validated by the data presented in Fig. VI-5. It is clearly shown that at a fixed level of solid loading, the LA concentration is much higher for the mixed-feed than that of the sludge alone. With 10 wt% sludge loading and cellulose loading of 15 FPU / g-glucan, the final lactic acid concentration reached 60 g/L for the mixed feed, whereas it is only 40 g/L for the sludge alone. It also proves that the hemicellulose sugars in the prehydrolysates are utilized by the microorganism as well as the carbohydrates in the sludge. On the other hand, the yields were 83% and 92%, for the mixture and the sludge only respectively (Fig. VI-6). The

yields were calculated on the basis of the theoretical maximum wherein the total of hexoses sugars in the sludge and those in the prehydrolysate were taken into consideration in the case of the mixed feed. The fact that the overall yield of the mixed feed is close to that of the prehydrolysate indicate that the presence of the prehydrolysate impedes the SSF of the sludge portion. We speculate that the toxins in the prehydrolysate are inhibiting the enzyme action on the solid as well as the microbial activity.

To refine the process conditions, the SSF of the mixed-feed was further investigated. We chose three process variables as the main parameters affecting the performance of the SSF of the mixed feed: cellulase, pectinase, and solid loading. To identify the significance of the process variables, 4 levels for each of the parameters were applied: solid loading (wt%): 5,7.5, 10, 15; cellulase (C-Tec2) loading (FPU/g Glucan): 5,7.5, 10, 15; and pectinase loading (mg protein/g Mannan): 10, 15, 20, 25. The SSF experiments were carried out following the experimental design of Taguchi (Taguchi 1987; Rao et al., 2008) taking the product yield as the objective function. This method uses orthogonal arrays to organize the parameters affecting the process and the levels at which they should be varied. Knowing the number of parameters and the number of levels, the proper orthogonal array can be selected. These arrays were created using an algorithm developed by Taguchi (Taguchi, 1987). Under this scheme, L'16 orthogonal array was chosen as shown in Table VI-5 (Taguchi, 1987). Total of 16 experimental runs were required in this method as opposed to 64 runs of the exponential design (4x4x4). The results of the 16 experimental runs were organized in Taguchi format as shown in Table VI-6. The performance indexes from each the trial runs were determined to analyze the relative effect of the different parameters. One of such is Ki listed in the table, which is the sum of the yield (objective function) of the four trial runs representing the four levels of a given parameter, i

representing the four levels of that parameter. From this value we can determine the best level for this parameter. Take cellulase loading for example, we applied 4 levels: 5, 7.5, 10 and 15 FPU/g glucan. At 15 FPU/g glucan level, which is the 4^{th} level of the parameter of cellulase loading, the sum of the yield of all trials for this level ($K4_{cellulase}$) is 67.96 + 76.39 + 83.09 + 64.59 = 292.03. Likewise, we calculate the K for all levels of cellulase loading, the highest Ki is K4 = 292.03 which refers to 15 FPU/g glucan. This means the optimum value of cellulose loading is 15 FPU/g glucan within the levels we applied. The R is the difference between the highest K and lowest K value. The larger the R value for a parameter, the larger the effect the variable has on the process. This is because the same change in signal causes a larger effect on the output variable being measured. The highest LA yield observed from the 16 experimental runs was 83% wherein LA concentration was 60 g/L. It was attained at the level of the three chosen parameters: 10% of solid loading, 15 FPU/g glucan of cellulase loading, and 15 mg protein/g mannan of pectinase loading. These are the set of conditions that exhibited highest LA yield within the range of the parameters covered by the 16 runs. It is interpreted as a reasonable operating condition to apply for the proposed SSF of the mixed-feed when the yield is taken as the key performance index. Additional operating conditions need to be considered to determine the true optimum set of operating conditions. From the experiment design, the preferred experiment conditions are determined to be: 10 wt% sludge loading, 15 FPU/g-glucan of cellulase loading and 10 mg protein / g-mannan of pectinase loading. Among the three main parameters, the solid loading and cellulase loading have shown more significant effects on LA yield than pectinase loading as indicted by the R values in Table VI-6.

VI.5 Conclusion

With hot-water treatment of Southern pine, a representative pulp feedstock, 10% of the hemicellulose can be selectively recovered as fermentable sugars without degrading the yield and quality of the pulp fibers. The recovered hemicellulose sugars are 75% hexoses (mannose, galactose and glucose) 25% pentoses (xylose and arabinose); and 62% oligomers and 38% monomers. The prehydrolysate and the pulp mill sludge can separately be converted to lactic acid by SSF using *Lactobacillus rhamnosus* as the microorganism and different set of enzymes for each case: pectinase and cellulase for the prehydrolysate and cellulase for the sludge. The SSF can also be applied for the mixture of these two low-cost feedstocks available from a Kraft pulp mill. The use of the mixed-feed significantly increased the product concentration in the SSF over that of the sludge alone. From the data of multiple SSF runs, it was demonstrated that 83% lactic acid yield with 60 g/L lactic acid concentration is attainable. From the experiment design, the preferred experiment conditions are determined to be: 10 wt% sludge loading, 15 FPU/g-glucan of cellulase loading and 10 mg protein / g-mannan of pectinase loading. Among the three parameters, solid loading and cellulase loading have shown more significant effects on lactic acid production, while the pectinase loading only has minor effect. During the SSF process, calcium carbonate existing in the RPMS acted as neutralizing reagent keeping the pH near optimum level resulting calcium lactate as the end-product.

Table VI-1 Sugar Content in the Liquid Obtained through Three-stage Pre-extraction S/L ratio = 5.8:1, 170°C and 60min for each stage (From Kang, et.al, 2012)

	Monomeric Sugars (g/L)					Oligomeric Sugars (g/L)					Total Sugars(g/L)
	Glu	Xyl	Gal	Ara	Man	Glu	Xyl	Gal	Ara	Man	
First Stage	0.40	1.14	0.73	1.46	0.55	1.60	2.46	2.07	0.27	8.15	19.32
Second Stage	1.07	2.65	1.59	2.27	1.60	2.88	3.02	3.60	0.33	12.97	31.99
Third Stage	2.41	3.76	2.61	3.54	3.20	2.97	2.65	3.93	0.10	15.53	40.70

Table VI-2 Degradation Compounds Obtained through Three-Stage Pre-extraction S/L ratio = 5.8:1, 170oC and 60min for each stage (From Kang et.al, 2012)

	Acetic acid (g/L)	Acetyl ligand (g/L)	Levulinic acid (g/L)	HMF (g/L)	Furfural (g/L)
First Stage	0.75	0.25	0.07	0.20	0.37
Second Stage	1.58	0.37	0.12	0.57	0.98
Third Stage	2.61	0.48	0.20	1.02	1.41

Table VI-3 LA production at 5% solid loading and different enzyme loading after 72hr

Enzyme Loading FPU/g-glucan	Lactic Acid (g/L)	Acetic Acid (g/L)	LA yield %
5	16.97	2.57	69.62
7.5	20.56	2.23	83.17

Table VI-4 LA production at 7.5 FPU/g-glucan and different solid loading after 72hr

Solid Loading w/v	Lactic Acid (g/L)	Acetic Acid (g/L)	LA yield %
5%	20.15	2.12	82.51
10%	39.34	2.79	79.41
15%	43.95	2.84	59.26

Table VI-5 Experiment design via Taguchi methods

Experiment	Solid Loading (w/v%)	Cellulase Loading (FPU/g Glucan)	Pectinase Loading (mg protein/g Man)
1	5	5	10
2	5	7.5	15
3	5	10	20
4	5	15	25
5	7.5	5	15
6	7.5	7.5	10
7	7.5	10	25
8	7.5	15	20
9	10	5	20
10	10	7.5	25
11	10	10	10
12	10	15	15
13	15	5	25
14	15	7.5	20
15	15	10	15
16	15	15	10

Table VI-6 Results of SSF of Mixture

Experiment	Solid Loading	Cellulase Loading	Pectinase	LA concentration (g/L)	96hr Yield
	w/v%	FPU/glucan	mg protein/manan		
1	5	5	10	31.74	62.23
2	5	7.5	15	34.70	68.25
3	5	10	20	34.04	67.15
4	5	15	25	34.25	67.96
5	7.5	5	15	38.15	60.94
6	7.5	7.5	10	41.82	67.05
7	7.5	10	25	45.45	73.13
8	7.5	15	20	47.82	76.39
9	10	5	20	39.83	54.59
10	10	7.5	25	48.55	66.81
11	10	10	10	56.12	77.56
12	10	15	15	59.63	83.09
13	15	5	25	41.72	43.38
14	15	7.5	20	48.61	50.54
15	15	10	15	55.55	57.76
16	15	15	10	62.12	64.59
K1	265.60	221.15	**268.99**		
K2	277.51	252.65	265.58		
K3	**282.05**	275.60	259.49		
K4	216.27	**292.03**	247.36		
R	65.78	70.88	21.63		

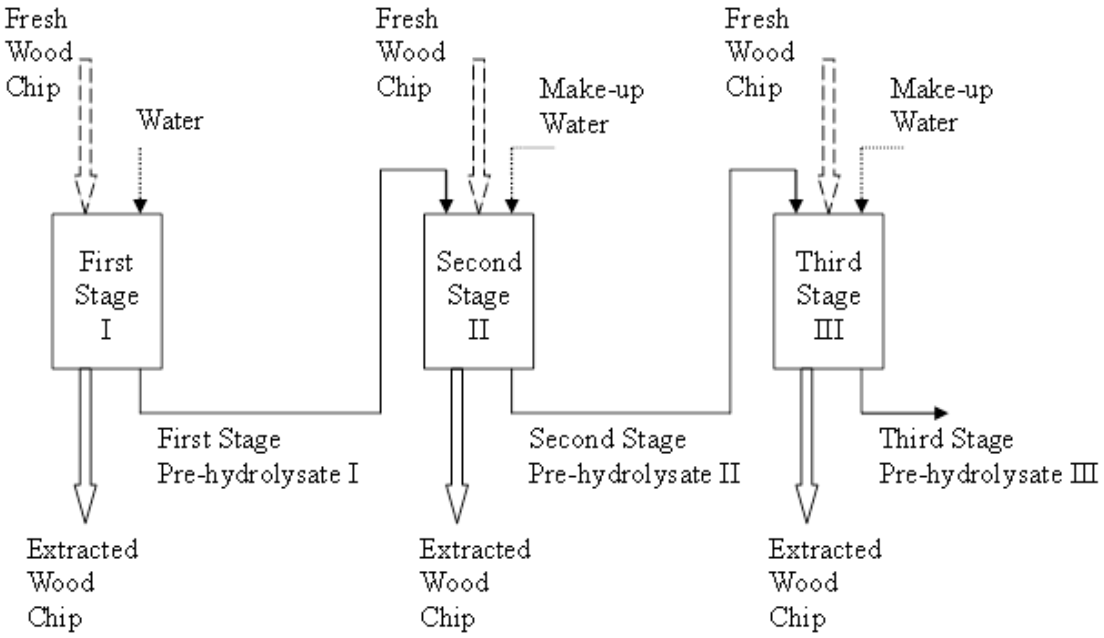


Figure VI-1 Proposed Three-Stage Pre-extraction Process

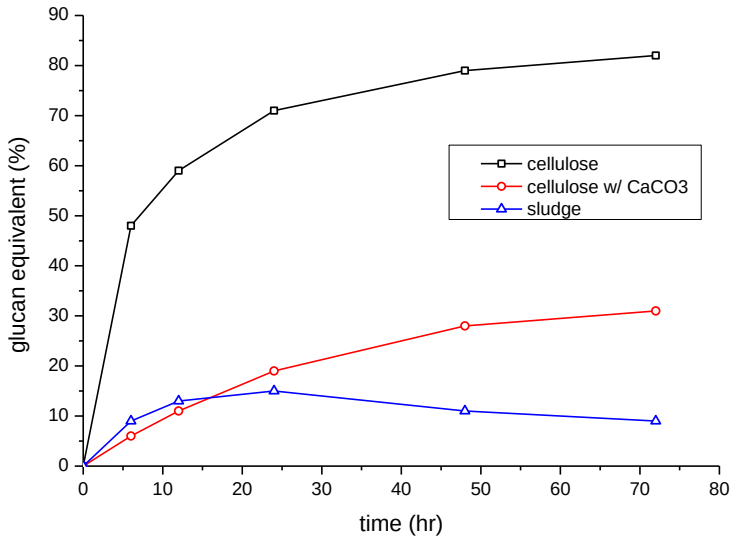


Figure VI-2 Enzymatic Hydrolysis of RPMS, α-cellulose, α-cellulose plus $CaCO_3$ (solid loading: glucan content equals to 1% (w/v), 50^{o}C, enzyme loading: 15 FPU/g glucan)

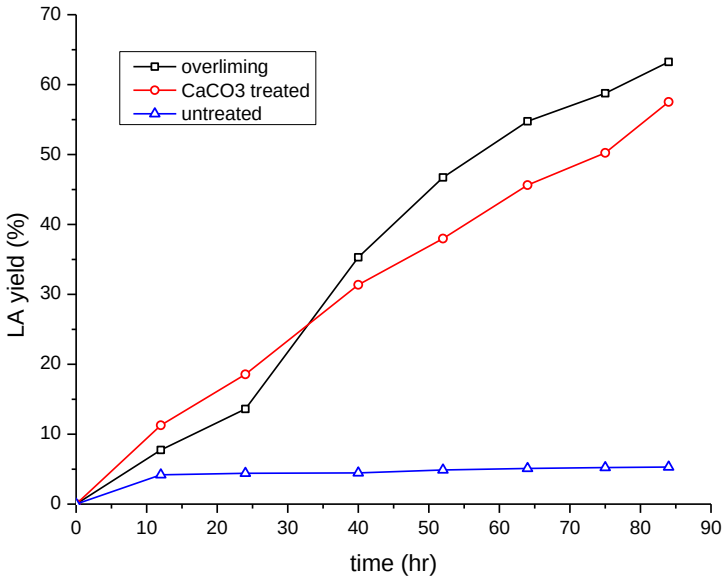
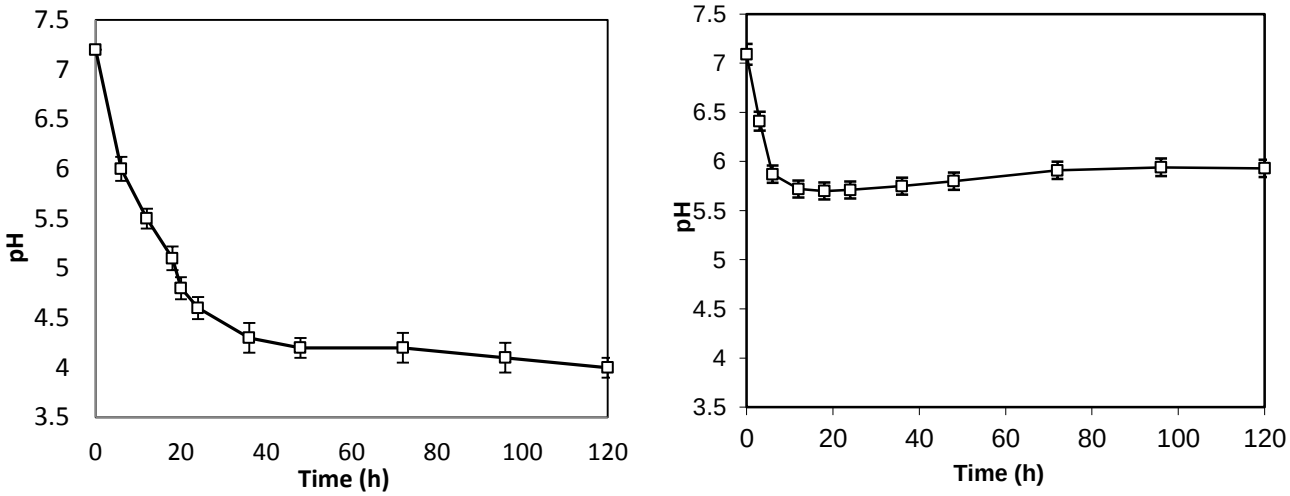


Figure VI-3 LA yield profiles for SSF of prehydrolysate

(Ctec2: 5 FPU/g glucan, Pectinase: 15 mg protein/g mannan, 37^{o}C and 150 RPM)



(a) (b)

Figure VI-4 pH profiles during SSF of the Mixture

a) solid loading: 5%, cellulase(C-Tec2): 15 FPU/g glucan, pectinase: 25 mg protein/g mannan

b) solid loading: 10%, cellulase(C-Tec2): 15 FPU/g glucan, pectinase: 15 mg protein/g mannan

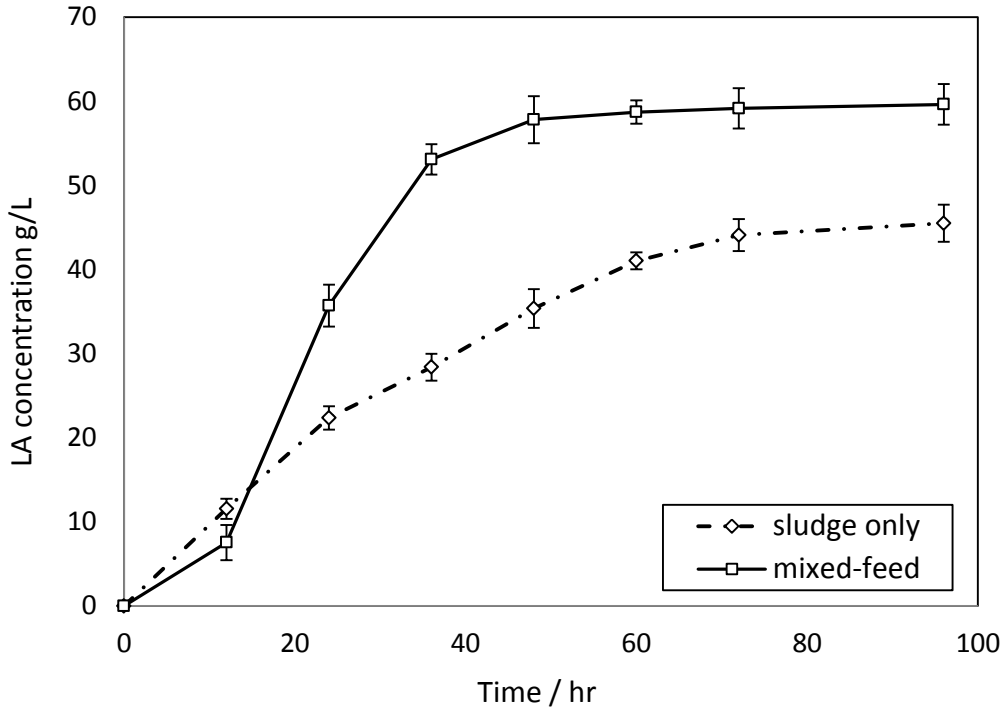


Figure VI-5 LA concentration profiles during SSF of different feedstock
(5% solid loading, cellulase(C-Tec2): 10FPU/g glucan, pectinase: 20 mg protein/mannan)

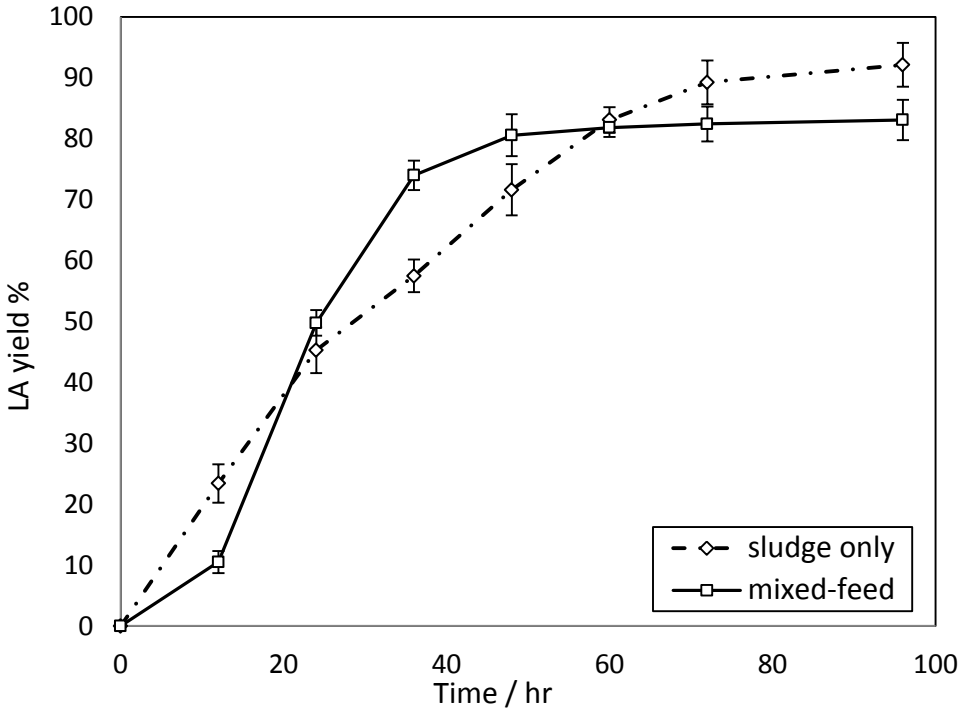


Figure VI-6 LA yields during SSF of different feedstock

(5% solid loading, cellulase(C-Tec2): 10FPU/g glucan, pectinase: 20 mg protein/mannan)

VII. Future Work

Additional work is needed to further prove the LCC formation during the dilute acid pretreatment process. Pure xylose monomers and oligomers are suggested to react with lignin model compounds. The lignin model compounds will be extracted from solution after the reaction and subjected to FTIR and NMR. If the FTIR and NMR spectra show that the extracted sample contains both lignin and carbohydrates peaks, that means we do have LCC formation during the pretreatment and hydrolysis step.

Penetration of acid into the biomass structure and its dispersion in the reactor can become a significant factor in the overall rate process of dilute-acid pretreatment. Since the residence time in the screw-driven reactor is relatively low, the acid within the biomass may not be uniformly distributed within that residence time period. The non-uniform transient acid profile can significantly affect the overall pretreatment reaction. The extent of such effect can be mathematically modeled in such a way that a dimensionless parameter, something equivalent to a Thiele modulus in catalytic reaction theory, is used as an index indicating the overall effect of transient acid diffusion. The dimensionless parameter involves biomass size, reaction kinetic constants, and the acid diffusion coefficient within biomass. Of these entities, the acid diffusion coefficient in biomass structure remains as unknown, and will be experimentally determined. The modeling work will be organized in such a way that the efficiency factor is expressed as a function of biomass size for various treatment conditions. This will provide practical information with regard to the biomass size, whether one needs to reduce the size of feedstock to avoid the negative effect of acid diffusion, if so, to what size for a given reaction condition.

The difference between the diffusion and dispersion is to be noted here. Diffusion refers to intra-particle movement of acid, whereas dispersion refers to the bulk acid movement in the

reactor. One can make a similar argument with regard to dispersion creating no-uniform condition across axial and radial position of the reactor. The dispersion is influenced not only by the transport properties but also by the mechanical movement of particles in the reactor.

Secondly, in chapter IV, the RTD data were used to assess the performance of NREL continuous pretreatment reactor along with the conventional kinetic model. The modified model shows better agreement with experimental data in the latter reaction stage. This modified kinetic model is no longer a first order reaction. For reactions other than first order, RTD data alone is not sufficient to predict conversion. In these cases, the mixing of molecules must be known in addition to how long each molecule spends in the reactor. Consequently, mixing models that represent the mixing inside the reactor must be applied. The overall goal is to use the following equation: RTD data + Kinetics + Model = Prediction. It is our job to choose the model that best combines the conflicting goals of mathematical simplicity and physical realism. For a given reactor, it is very common to test several model assumptions and make a comparison. We are planning to try the following models and find the best one to assess the NREL reactor: Segregation model, Maximum mixedness model, Tanks-in-series model and Dispersion model.

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