KINETIC CHARACTERIZATION OF HOT WATER AND DILUTE ACID PRETREATMENT OF LIGNOCELLULOSIC BIOMASS

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Acidic aqueous-phase pretreatment is a promising approach that has been directed at maximizing intermediates yields (e.g. sugars, sugar degradation products, and lignin) from biomass for fuel and chemical production. This dissertation explores the kinetic fundamentals of biomass hydrolysis in acidic aqueous-phase with different catalysts (e.g. sulfuric acid, metal chlorides), operating conditions (e.g. temperature, time pressure), and equipment configurations (e.g. batch, flowthough).

The kinetic analysis revealed that crystalline cellulose is insusceptible to hydrolysis compared with agarose at low temperature (e.g. 140 °C), while it decomposed rapidly at elevated temperature (e.g. 220 °C). Higher temperature with reduced time was desirable for glucose production whereas lower temperature with prolonged time was preferred for xylose generation. In acidic conditions, furfural and levulinic acid were stable whereas 5-hydroxymethylfurfural was susceptible to decomposition with high rate constant. MgCl₂ can promote the cleavage of C-O-C bond in polysaccharides (e.g. agarose) and enhance the subsequent dehydration reaction to 5-hydroxymethylfurfural. Unlike transition metal chlorides and H₂SO₄, MgCl₂ has little ability to induce retro aldol and rehydration
reactions to generate byproducts like lactic acid and levulinic acid. Mg\(^{2+}\) possessing higher activity than other alkali and alkaline earth metal chlorides (Na\(^{+}\) and Ca\(^{2+}\)) resulted in 40.7% yield and 49.1% selectivity of 5-hydroxymethylfurfural.

Dissolution of biomass was significantly enhanced using acidic hot water flowthrough pretreatment at 200 – 280 °C. Significant cellulose removal accompanied with the transformation of cellulose I to cellulose II and amorphous cellulose were observed when temperature was above 240 °C for water-only and 220 °C for dilute acid. Approximately 100% of the xylan and ~90% of the cellulose were solubilized and recovered. Up to 15% of the lignin was solubilized, while the remaining lignin was insoluble. Over 90% sugar yields were obtained from pretreated whole slurries using less than 10 FPU/g cellulase plus hemicellulase enzyme.

A kinetic model was developed to depict the biomass degradation in flowthrough system. This model predicted the sugar generation more precisely than the conventional homogeneous first-order reaction models. Mass transfer limitations were minimized using 4mm biomass particle sizes with 4g biomass loading at 25mL/min flow rate, produced hydrolyzate slurries with 13g/L potential sugar concentrations.
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Chapter 1

Introduction

1.1. Hot water and dilute acid pretreatment

The decreasing reserves of fossil fuels and the growing greenhouse gas emissions have caused an increase in bioenergy and bioproducts research in recent years. Lignocellulosic biomass typically contains 14.3–68.6 wt. % cellulose, 8.8–22.4 wt. % hemicellulose and 8.4–29.4 wt. % lignin represent a promising carbon-based alternative as an energy source and a sustainable feedstock. Enzymatic hydrolysis of these polysaccharides into fermentable sugars is currently the most viable and scalable option for the deconstruction of biomass carbohydrates, and benefits from mild and environmentally benign operating requirements with little byproduct formation (Saha, 2000; Wingren et al., 2005). However, biomass recalcitrance hampers the efficacy of enzymatic hydrolysis (Kim et al., 2009). Characteristics of cellulose (e.g. crystallinity, degree of polymerization etc.) and the pervasive nature of lignin necessitate biomass pretreatment prior to enzymatic hydrolysis (Brodeur et al., 2011).

Over the years, many pretreatment approaches such as hot water (Allen et al., 1996; Bobleter et al., 1976; Bonn et al., 1983; Homeyer et al., 1988; Kohlmann et al., 1995; Llamsakul et al., 1994; Mok & Antal, 1992a) , dilute acid(Lee et al., 1999; McMillan, 1994b; Torget et al., 1991; Tsao et al., 1982), neutral pH, ammonia fiber expansion (AFEX) (Dale et al., 1996; Foster et al., 2001), ammonia pretreatment (ARP) (Iyer et al., 1996; Kim & Lee, 2005) and lime (Chang et al., 1997; Chang et al., 1998; Karr & Holtzapple, 2000) have been developed to achieve total sugar recovery from lignocellulose. Among these pretreatment approaches,
low pH pretreatment with water-only or dilute acid has been the subject of researches over
years (Yang & Wyman, 2008a). The primary advantage of the hot water and dilute acid
pretreatment conducted around 120 °C—220 °C has been considered as the 80%—90%
hemicellulose sugars recovery during this process (Yang and Wyman, 2009). Furthermore,
hot water or dilute acid pretreatment employed in continuous reactor (e.g. flowthrough
reactor) also achieved 85% lignin removal (Yang and Wyman, 2004). Nevertheless, current
hot water dilute acid pretreatment had insignificant effect on cellulose, at least 20 FPU/g
glucan cellulase needed to obtain desired glucose yields in subsequent enzymatic hydrolysis
of cellulose pretreated with dilute sulfuric acid. Comparatively, pretreatment of biomass at
high pH such as the AFEX technology decrystallized cellulose with amenable enzymatic
accessibility although it removed less hemicellulose and lignin. Hot water and dilute acid
pretreatment operated under elevated temperature (e.g. 270°C) presented the potential of
disrupting and dissolution of cellulose. Even though considerable sugar degradations
compounds (~10% 5-HMF yield) were observed as well, this technology help understand the
degradation pattern of cellulose and also provide new fundamental insights to improve the
efficacy of hot water/dilute acid through disrupting and alteration the total biomass including
xylan, cellulose and lignin for total sugar and lignin generation.

1.2 Biomass degradation in acidic conditions

Hot water or dilute acid shows great potential for degrading lignocellulosic material and
making it accessible to hydrolytic enzymes by disrupting the inter-polymeric association
between lignin, hemicellulose, and cellulose (Laskar et al., 2013b). Understanding the
mechanistic basis of biomass degradation and its reaction kinetics vis-a-vis temperature, reaction time, etc. in hot water pretreatment could provide new insights for optimized pretreatment results.

1.2.1. The decomposition of hemicellulose

Hemicellulose consists of shorter chains with degrees of polymerization ranging from 500–3000, and has a random and amorphous structure with little strength. Xylan is the most common and representative component of hemicelluloses characterized by β-1,4-linkages in the xylose backbone. Approximately 80% of the xylan backbone is highly substituted with side-chains consisting of arabinose or glucuronic acid linked to the O-2 and/or O-3 of the xylose residues, and also by oligomeric side chains containing arabinose, xylose, and sometimes galactose residues (Saulnier et al., 1995), as well as acetyl groups. The mechanisms of hemicellulose depolymerization in hot water and dilute acid pretreatment can be divided into two steps. In the first step, hydronium ions (H$_3$O$^+$) generated from liquid hot water at high temperatures (e.g. 200 °C) accompanied with the proton from acidic source (e.g. H$_2$SO$_4$) catalyze the depolymerization of hemicellulose by hydrolysis of both the β-O-4 glycosidic linkages and acetyl groups. In the second step, hydronium ions from the disassociation of released acetic acid catalyze the reaction improving the overall reaction kinetics (Carvalheiro et al., 2008). In addition, hydrolysis of uronic acid side chains also occurs simultaneously in liquid hot water. Although uronic acids are reported to be resistant to hydrolysis (Conner, 1984), they may also contribute to the overall hydronium ion concentration in pretreatment (Conner, 1984). Apart from xylose, considerable amount of
sugars generated from hemicellulose was in the form of xylooligomers. Furfural is known to result via the primary xylose degradation pathway via dehydration in the presence of proton/hydronium ions in an aqueous medium at subcritical. Saka’s work (Lü & Saka, 2012) indicates that degradation of monomeric sugars could proceed mechanistically in a way that resembles an acid catalyzed dehydration reaction, given the highly furan product distributions reported. With exception of the aforementioned products, xylose also undergoes retro-aldol reactions in the presence of hydroxide ions released from water under elevated temperatures, resulting in products including lactic acid, dihydroxyacetone, glyceraldehyde, methyglyoxal, glycolaldehyde, formaldehyde were observed (Aida et al., 2010). Figure 1.1 summarized the degradation pathway of xylan degradation in acidic conditions. Generally speaking, hemicellulose can be easily hydrolyzed with hot water. The reaction temperature around 200 – 220 °C with saturated pressure could effectively deconstruct most 100% hemicellulose from biomass within 50 min (Diaz et al., 2010; Nitsos et al., 2013; Yang & Wyman, 2004a). Elevating pressure under the similar temperature range led to the relative lower hemicellulose degradation. For example, Allen et al. (Allen et al., 1996) pretreated the sugarcane bagasse in flowthrough pretreatment under 190 – 230 °C with pressure of 5 Mpa and merely obtained 80% hemicellulose removal; Mok and Antal (Mok & Antal, 1992a) reported that they obtained around 90% hemicellulose removal under 200 °C – 230 °C with pressure 34.5 Mpa when treating six woody and four herbaceous biomass species using tubular percolating reactor with water-only. This could be due to decreased solubility of hydrolyzed hemicellulosic fractions when the pressure increased under identical temperatures. The
hydrolyzed xylan mainly took the form of xylooligomers and monomeric xylose. However, Romani et al. (Romani et al., 2010) reported that 95% xylan degradation in batch system under comparable temperature levels merely led to 75% xylan recovery, and the remainder could be in the form of degradation compounds such as furfural. Inasmuch, balancing sugar recovery in light of xylan degradation is a key consideration for the C5 fraction of biomass during pretreatment.

Figure 1.1 The summarized degradation pathway of xylan degradation in acidic conditions.

1.2.2. The decomposition of cellulose

Cellulose is a linearly wound polymer bundle of anhydroglucose with a degree of polymerization between 3,500 to 10,000 units. The β-glucose monomer units are combined together through glycosidic bond between the C1 and C4 positions. Cellulosic biomass is a heterogeneous complex in water solution. There exist a multitude of inter and intra linked hydrogen bonds in cellulose, which exists within a single chain of glucan, and between the adjacent glucan chains respectively. The inter hydrogen bonds are believed as the primary
factor for combining the cellulose chains together forming the crystalline structure (Xiang et al., 2003c). Cellulose hydrolysis starts when an acidic proton interacts with the glycosidic oxygen linking with two sugar monomers, and forms a conjugate acid. The hydrolysis process involves physical interference factors as well (Xiang et al., 2004b). Cellulosic biomass is a heterogeneous complex in water solution. There are lots of inter and intro hydrogen bonds in cellulose, which exists within a single chain of glucan, and between the adjacent glucan chains respectively. The inter hydrogen bonds are believed as the primary factor for combining the cellulose chains together forming the crystalline structure (Xiang et al., 2003c). Apart from the intra and inter hydrogen bonds, the water molecules at the boundary layer of crystalline cellulose can form an “ice-like” structure two to four molecular layers deep (Torget et al., 2000). In this regard, the hydrolysis of crystalline cellulose should be addressed more specifically through looking at disruption of the hydrogen bond with the respect of reaction kinetics in hot water conditions. Cellulosic oligomers and glucose released from cellulose could further decompose into downstream products. The formation of 5-HMF is considered to proceed the primary degradation pathway of glucose in hot water conditions via isomerization glucose to fructose and the subsequent dehydration reactions. 5-HMF is not a stable chemical in acidic conditions, and can be further rehydrared into levulinic acid and formic acid (Girisuta et al., 2007; Shen & Wyman, 2012b). In some weak acidic conditions such as hot water treatment, due to the presence of hydroxide ions, glucose is also susceptible to be cleavage via retro aldol reaction to form varied C3 compounds, which including lactic acid, dihydroxyacetone, glyceraldehyde, methylglyoxal, glycolaldehyde as well as erythrose
(Aida et al., 2007; Antal et al., 1990; Boon & Bobleter, 1983). Furthermore, humins, a kind of darkbrown solid, which could condense from 5-HMF(Patil & Lund, 2011b) and glucose(Girisuta et al., 2007; Shen & Wyman, 2012b), increased the diversity of the streams of glucose degradation compounds. Figure 1.2 summarized the degradation pathway of cellulose in hot water conditions. Generally, conventional hot water pretreatment reported in previous studies had limited effects on cellulose degradation. Mok and Antal (Mok & Antal, 1992a) reported that merely ∼20% cellulose degradation was observed when pretreating biomass with water-only under temperature ranging from 180 °C to 230 °C. Even so, hot water under such temperatures appeared to influence on the structural properties of cellulose. The crystallinity of pretreated biomass was only reported in some cases. Laureano et al. (Laureano-Perez et al., 2005) observed that the crystalline index of untreated biomass with involvement of X-ray diffraction technology was decreased from 50.4 to 44.5 through control pH pretreatment conducted under 195 °C. However, determining the crystallinity of pretreated biomass is not practical, because hemicellulose and lignin lack any regular crystal structure, removing these two compounds while leaving the majority cellulose unchanged could result in the increase of observed crystalline index of pretreated samples compared with untreated one (Kumar et al., 2009). Only a few researchers reported the hot water pretreatment of biomass at temperatures above 230 °C. Hot water flowthrough pretreatment conducted under temperatures around 270 °C with flow rates 12−25mL/min revealed that around 80% cellulose was decomposed (Boon & Bobleter, 1983; Lu et al., 2009a), however, considerable amount of 5-hydroxymethylfufural (5-HMF) (7−10%) was observed as well.
When the water was heated to supercritical condition (>374 °C, >22.1 Mpa), 85%—100% cellulose removal was realized within extremely short residence time (0.24s—0.8s). However, less than 50% cellulose recovery indicated the significant degradation of glucose to decomposed compounds like 5-HMF.

![Summarized degradation pathway of cellulose in acidic conditions.](image)

**Figure 1.2** The summarized degradation pathway of cellulose in acidic conditions.

1.2.3. **Lignin degradation**

Lignin is the third primary component in lignocellulosic biomass, which comprises around 20% of cell wall. Lignin is randomly linked by phenylpropanoid polymer to hold the plant cell wall with a high resistance to attack. Lignin is also a kind of highly branched polymer.
which is mainly composed of three phenylpropane monomers such as coniferyl alcohol, sinapyl alcohol, and \( \rho \)-coumaryl alcohol (Bonini et al., 2008; Whetten et al., 1998). These monomers compose the lignin polymer through covalent bonding between each other, which includes \( \beta \)-O-4, \( \beta \)-5, \( \beta \)-\( \beta \), \( \beta \)-O-5, 5-5, \( \alpha \)-O-4 etc (Holmgren et al., 2009). Under acidic hot water conditions, highly reactive nucleophilic carbonium ion intermediates are formed within the lignin structure. Carbonium ions can then further react, leading to the cleavage of predominant \( \beta \)-O-4 bonds thereby realizing the efficient depolymerization of lignin. However, the carbonium and nucleophiles can also simultaneously lead to the repolymerization reaction (Figure 1.3). Such repolymerization of lignin can be avoided in flowthrough system because the depolymerized lignins are quickly and continuously swept out of the reactor (Laskar et al., 2013a; Trajano et al., 2013). Elevated reaction temperatures facilitate the degradation of lignin. For example, 60\% – 85\% lignin removal was observed under 190 – 230 °C over the reaction time 0 – 300 min in flowthrough reactors (Allen et al., 1996; Mok & Antal, 1992a; Yang & Wyman, 2004a). Continuously increasing reaction temperature (e.g. 270 °C) was reported to enhance the lignin removal around 90\%. Few researches reported the structural characterization of hot water pretreated lignin. Goundalkar et al. (Goundalkar et al., 2010) extracted the maple wood with hot water under 160 °C over 120 min and observed that the extracted lignin was in the soluble form with the structural characterization such as vanillin, coniferyl alcohol and syringaldehyde (Hill et al., 2007). Such soluble lignin were demonstrated as the cellulase inhibitors during the enzymatic hydrolysis (Ximenes et al., 2010), reducing its production or separating from the pretreatment slurries before enzymatic
hydrolysis. However, lignin-derived products such as vanillin, syringaldehyde, coniferaldehyde, etc were well documented as the important value-added chemicals. For example, vanillin was served as a component in the food and flavor industry; syringaldehyde was used as a component in dyes and as a pharmaceutical precursor (Hill et al., 2007). Furthermore, some new innovative catalytic technologies for converting lignin into hydrocarbons are mentioned, which implied the new application field of lignocellulosic biomass apart from cellulosic bioethanol (Laskar et al., 2013a).

![Lignin degradation pathway](image)

**Figure 1.3** The summarized degradation pathway of lignin in hot water and dilute acid

1.3. **Reactor configurations**
The geometries of reactor employed for hot water and dilute acid pretreatment can be predominately classified into batch and continuous systems. Varied batch reactors including mixed Parr reactor, metallic tube reactor, etc were utilized for hot water and dilute acid pretreatment. Mixed parr reactors have been widely used by many researchers. The volume of Parr reactor is often ranging from 25 mL to 5 gallons equipped with magnetic-drive internal stirring to ensure uniform mass and temperature conditions (Figure 1.4), thereby limiting solids concentration to less than 10%. Higher solid concentrations (>10% w/w) are challenging to mix adequately to maintain temperature uniformly (Yang & Wyman, 2009). However, the heating rate of 2—4 °C/min of parr reactor was relatively low because the heating source is an external heating jacket, which implied its limited capability in controlling reaction kinetics (Yang & Tucker, 2013). In this regard, the sugar recovery with hot water in Parr reactor should be obtained to a low value. It was reported that 100% xylan and 12% cellulose was solubilized when pretreating biomass with hot water in Parr reactor, nevertheless, 5.8% 5-HMF and 16.6% furfural formed as well, which significantly reduced the sugar yields (Romani et al., 2010). The metallic tubular reactor with rapid heat-up and cool-down of reactor contents and reproducible pretreatment possess the ability of better control of the reaction kinetics. Such tubular reactor is constructed with stainless steel or Hastelloy tube and Swagelok fittings. Hot sand bath or hot oil bath were often used as heating source for the tube reactor (Tanjore et al., 2011) (Figure 1.5). It was reported that the heating time to the target temperatures and cooling time were only around 3 min and 5 min, respectively (Yang & Tucker, 2013), which could more precisely control the reaction
temperature with less undesired side reactions during the preheating or quenching process. This advantage of tubular reactor was apparent obvious when yielding the sugars (e.g. glucose) from biomass in acidic conditions under relative high temperatures (e.g. 220 °C), where its highest yield was obtained within 5min (Lee et al., 1999). However, the intrinsic properties of batch reactor determined that short reaction time (e.g. less than 1min) still cannot be precisely controlled. For example, Lu and Saka (Lu et al., 2009a) reported that 50% glucose can be rapidly converted to varying degradation compounds within merely 15 s in hot water conditions under temperature 250 °C, which beyond the capability of batch reactor. Furthermore, majority lignin recondensed into the solid phase after dilute acid or hot water pretreatment in batch system, although it can be disrupted during the reaction (Yang and Wyman, 2008).

Due to the limitations of batch system, continuous reaction system was developed in recent years (Yang and Wyman, 2009) with the objective of better controlling the reaction kinetics. Flowthrough reactors are the most acceptable and proved continuous reaction system by previous researchers(Yang & Wyman, 2009). Flowthrough systems employ the flow of hot water in a packed bed of biomass to constantly sweep the soluble or low-molecular-weight products from the reactor (Yang & Wyman, 2009). Reactors with 3.6 mL (0.5 inch ID×6 inch length) or 14.3 mL (0.5 inch ID×6 inch length) total volume have been applied in pretreating the biomass (e.g. corn stover) in hot water condition (Yang & Wyman, 2004a). High-pressure pumps are often used to deliver water in the reaction systems. Before entering into the reactor, the water is preheated to the target temperature in a preheating coil. The outlet tubing can be
used to cool down reaction effluent (Yang & Tucker, 2013) (Figure 1.6). Such designs, as reported by previous researches, are desired for more precisely controlling of the reactions than batch reactors (e.g. batch tubular reactor). In addition, the flowthrough system is considered for allowing researchers to obtain more insightful data regarding the release patterns of hemicellulose, lignin and cellulose during hot water and dilute acid pretreatment, which cannot be realized in other reactor systems including batch tubular reactors, Parr reactors, etc (Yang & Wyman, 2004a). The flow rate in acidic flowthrough reaction often employ ranges of 1–25mL/min (Liu & Wyman, 2003; Mok & Antal, 1992a; Yang & Wyman, 2004a), which resulted in almost 100% total sugar recovery from xylan (xylose+xylooligomers) with negligible degradation compounds (Liu & Wyman, 2003) under temperature ranging from 180 °C–230 °C. Furthermore, such flow rate also led to the effective removal of lignin as well, up to 85% lignin removal was achieved under identical temperature range (Liu & Wyman, 2003; Yang & Wyman, 2004a).

However, when reaction temperature was further elevated to relative high temperature (e.g. 260 °C) for degradation of cellulose (Bonn & Bobleter, O., 1983), the hot water flowthrough pretreatment appeared to result in sugar degradation compounds at the conventional used flow rate range (≤25mL/min). For example, Bobleter and his colleagues (Bonn & Bobleter, O., 1983) applied hot water flowthrough process to hydrolyze air-dried pure cellulose under 260–270 °C at flow rate of 12 mL/min, up to 52% glucose yield was obtained through hydrolyzing cellulose under 265 °C. Nevertheless, sugar degradation compounds, such as 5-hydroxymethylfurfural (5-HMF) (~10%) were also observed. Lu and Saka (Lu et al., 2009a)
found that 79.5% cellulose removal merely resulted in less than 68.7% cellulose recovery after a two-stage (230 °C for 15 min and 270 °C for 15 min) semi-flow hot water pretreatment at flow rate of 10 mL/min under pressure of 10 Mpa. Substantial sugar degradation (e.g. ~6.9% 5-HMF) was observed as well. The cellulose degradation patterns shown by using flowing hot water or dilute acid at higher flow rates (25 mL/min or above) were still unknown. Although flowthrough pretreatment possess the potential of more precise control of the reaction for better sugar recovery compared with batch system through continuously and rapidly delivering the decomposed compounds with flowing water, the considerable water usage compared with relative low solid loading (0.5-1 g) in-turn limited the sugar concentration to a low level (Liu & Wyman, 2003). Because the lack of the comprehensive study of the mass transfer limitation of hot water flowthrough pretreatment in previous studies, current work has focused only on the 0.5-1 g solid loading level (Liu & Wyman, 2003; Yang & Wyman, 2004a). However, given water’s noted advantages, higher solid loadings (e.g. 3 g) warrant investigation to determine whether such biomass to water ratios are significantly mass transfer limited.
Figure 1.4 Schematic of parr reactor system (Esteghlalian et al., 1997).
Figure 1.5 Schematic of batch tubular system (Kim et al., 2005)
Figure 1.6 Schematic of flowthrough system (Liu and Wyman, 2003)
1.4. Kinetic modeling

Various kinetic models regarding lignocellulosic biomass degradation in acidic reaction medium (hot water/dilute acid) have been reported. These models have mainly described the hydrolysis of cellulose and xylan (hemicellulose), where lignin degradation in acidic conditions has been studied to a lesser degree. Saeman first proposed a kinetic model describing acid-catalyzed cellulose hydrolysis to glucose from Douglas fir in batch reactors in 1945 (Saeman, 1945). It was a simplified consecutive pseudo-homogeneous first-order reaction: Cellulose was assumed to be first hydrolyzed into glucose, and then further dehydrated into degradation products 5-HMF and levulinic acid. During subsequent years, Saeman’s model was applied by many researchers to study the acidic hydrolysis of lignocellulosic biomass or polysaccharides (cellulose and xylan) under a wide range of reaction conditions, i.e. acid concentrations (0.05%(w/w)–8%(w/w)) and temperatures (90 °C–240 °C), to predict the glucose concentrations and yields generated from cellulose (Fagan et al., 1971; McKibbins et al., 1962; Mosier et al., 2002; Xiang et al., 2004b). The reaction schemes and equations regarding the hydrolysis of both cellulose and xylan were summarized as follows:

\[
\text{Cellulose } \xrightarrow{k_1} \text{Glucose } \xrightarrow{k_2} \text{5-HMF } \xrightarrow{k_3} \text{Levulinic acid+Formic acid } \xrightarrow{k_4} \text{Degradations}
\]

Scheme 1

\[
dC/dt = -k_1C \tag{1}
dG/dt = k_1C - k_2G \tag{2}
\]
\[
dM/dt = k_2 G - k_3 M \tag{3}
\]
\[
dL/dt = k_3 M - k_4 L \tag{4}
\]

where C, G, M and L represent cellulose, glucose, 5-HMF and levulinic acid, respectively, which can be expressed in the unit of yield(\%) (Gil Garrote, 1999; Lu & Mosier, 2008).

![Scheme 2](image)

\[
dXn/dt = -k_1 Xn \tag{5}
\]
\[
dX/dt = k_1 Xn - k_2 X \tag{6}
\]
\[
dF/dt = k_2 X - k_3 F \tag{7}
\]

where Xn, X, F represent xylan, xylose and furfural, respectively, which can be expressed in the unit of yield(\%) (Gil Garrote, 1999; Lu & Mosier, 2008). \(k_1\) is the rate constant of xylan hydrolysis (min\(^{-1}\)), \(k_2\) is the rate constant of xylose degradation (min\(^{-1}\)), \(k_3\) is the rate constant of furfural degradation (min\(^{-1}\)).

In recent years, extended Saeman’s models involving the hydrolysis of both xylan and cellulose have been developed. McMillan (1992) proposed that xylan displayed a biphasic tendency with one portion of the xylan hydrolyzing quickly and the other at a much slower rate. The intermediates during xylose formation, (i.e. xylooligomers) as well as the major degradation compounds (i.e. furfural) were incorporated into the kinetic model (Garrote et al., 2001; Jacobsen and Wyman, 2002; Nabarlatz et al., 2005) (Garrote et al., 2001) (scheme 3).
Scheme 3

\[
\begin{align*}
\frac{dH_f}{dt} &= -k_{1f}H_f \\ 
\frac{dH_s}{dt} &= -k_{1s}H_s \\ 
\frac{dXO}{dt} &= k_{1f}H_f + k_{1s}H_s - k_{2}XO \\ 
\frac{dX}{dt} &= k_{2}XO - k_{3}X \\ 
\frac{dF}{dt} &= k_{3}X - k_{4}F
\end{align*}
\]

\(H_f, H_s, XO, X\) and \(F\) represent fast-hydrolysis xylan, slow-hydrolysis xylan, xylooligomers, xylose and furfural, which can be expressed in the unit of yield(%) (Gil Garrote, 1999; Lu & Mosier, 2008). \(k_1\) is the rate constant of xylan hydrolysis (min\(^{-1}\)), \(k_2\) is the rate constant of xylooligomer degradation (min\(^{-1}\)), \(k_3\) is the rate constant of xylose degradation (min\(^{-1}\)), and \(k_4\) is the rate constant of furfural degradation (min\(^{-1}\)).

Girisuta et al. (2007) and Shen and Wyman (2012a) proposed a complete cellulose acid-catalyzed degradation process via incorporating 5-HMF, levulinic acid and Humins into the reaction pathway (scheme 4)

\[
\begin{align*}
\frac{dC}{dt} &= -k_1C
\end{align*}
\]
\[
\begin{align*}
dG/dt &= k_1C - k_2G \\
dM/dt &= k_2G - k_3M \\
dL/dt &= k_3M - k_4L
\end{align*}
\]

(14) (15) (16)

C, G, M and L represent cellulose, glucose, 5-HMF and levulinic acid, which can be expressed in the unit of yield(%) (Gil Garrote, 1999; Lu & Mosier, 2008). \(k_1\) is the rate constant of cellulose hydrolysis (min\(^{-1}\)), \(k_2\) is the rate constant of glucose degradation (min\(^{-1}\)), \(k_3\) is the rate constant of 5-HMF degradation (min\(^{-1}\)), \(k_4\) is the rate constant of levulinic acid degradation (min\(^{-1}\)).

Humins are insoluble-solid product during cellulose hydrolysis, which was observed by Patil and Lund (2011) with advanced instruments such as scanning electron microscope (SEM) and infrared (IR) spectra. However, the quantitative analytical approach of Humins was seldom reported in previous studies (Xiang et al., 2004; Patil and Lund, 2011) although humins were symbolically introduced into the cellulose degradation pathway proposed by Shen and Wyman (2012).

All these aforementioned extended Saeman’s models are oversimplifications of the cellulose/xyalin hydrolysis reactions in pretreatment (Conner et al., 1985), which were merely expressed as homogeneous reactions. The hydrolysis of lignocellulosic materials under acidic conditions is a heterogeneous reaction where the mass transfer effects and other physical parameters play important roles in determining the overall reaction rate (Brennan & Wyman, 2004a). The rate constants of many heterogeneous chemical reactions are slower than that obtained in a homogeneous phase, which could be due to the reduction of the potential energy.
surface of the reaction as well as the mass transfer limitations (Brennan & Wyman, 2004a; Dang & Nguyen, 2008). With the consideration of the heterogeneous properties of the lignocellulosic biomass degradation, Brennan and Wyman (2004a) proposed a leaching model incorporating the mass transfer coefficient to describe biomass hydrolysis (e.g. xylan), which was expressed as follows (equations 17-18):

\[
\frac{dC_A}{dt} = -k_c^* \frac{A_s}{V_c^*} (C_A - C_{A\infty})
\]

(17)

\[
\frac{dC_{A\infty}}{dt} = -k_c^* \frac{A_s}{V_t^*} (C_A - C_{A\infty})
\]

(18)

\(C_A\) is the xylan in solids, \(C_{A\infty}\) xylan in solution, \(k_c\) is the mass transfer coefficient (cm/min), \(A_s\) is the surface area of the particles \((m^2/g)\), \(V_c\) is the volume of the solid \((m^3/g)\), and \(V_t\) is the total volume of solution.

However, such leaching model merely focused on the acidic hydrolysis of xylan, while the degradation performance of cellulose and lignin depicted by this model still unknown.

In addition, apart from the intrinsic heterogeneous properties of lignocellulosic biomass, the configuration of the reaction system that could influence the degradation of lignocellulosic biomass (Pronyk & Mazza, 2010a) hasn’t been incorporated in the model. Few models have taken the full effects of mass transfer or geometric nature of reactors into account, thereby limiting their abilities in describing the performance of biomass degradation in hot water conditions. Brennan and Wyman (Brennan & Wyman, 2004a) proposed a leaching model with the addition of mass transfer parameter \((k_D)\) in describing the xylan hydrolysis from solid phase into liquid phase. Pronyk and Mazza (Pronyk & Mazza, 2010b) described xylan
degradation in hot water flowthrough systems. Reactor geometry parameters such as the axial position along the reactor bed (x) and the superficial velocity (u) were incorporated into the model. Because xylan/hemicellulose hydrolysis in acidic conditions was universally investigated with various kinds of reaction models and reactors, we summarized the aforementioned varied model in describing the the xylan/hemicellulose hydrolysis (Table 1.1). In addition, Table 1.2 listed and compared the rate constants and other physical parameters obtained in different models when hydrolyzing xylan/hemicellulose with acidic water in different reactors. The rate constants listed in table 1.2 show that the biphasic model (Model I) and one of the extended Saeman’s models, resulted in significant different xylan/hemicellulose degradation rate constants when pretreating the biomass in different reactors under comparable reaction conditions. Along these lines, Brennan and Wyman (Brennan & Wyman, 2004a) reported that the rate constants of k_{1f} (hydrolysis rate constant of fast-hydrolysis xylan) and k_{1s} (hydrolysis rate constant of slow-hydrolysis xylan) for Model I were 0.813 min^{-1} and 0.227 min^{-1} under 180 °C with water-only in batch reactor, while these two values (k_{1f} and k_{1s}) dropped greatly to 0.020 min^{-1} and 0.118 min^{-1} respectively when the reaction was performed in a glass vial batch reactor under comparable conditions (i.e. 170 °C with 0.05% acid). Additionally, markedly dubious rate constant values were reported in separate trials in a tight temperature range (170 °C−180 °C) for the hydrolysis of xylan in flowthrough reactor configurations. Pronyk and Mazza et al.’s study (Pronyk & Mazza, 2010b) running at flow rate of 100mL/min resulted in drastically lower rate constants (k_{1f} =0.078 min^{-1},k_{1s} =0.004 min^{-1}) than that obtained at flow rate of 10 mL/min (k_{1f} =0.513
min$^{-1}$, $k_{1s} = 0.008$ min$^{-1}$) (Brennan & Wyman, 2004a) when employing the conventional Saeman’s model, thereby contradicting the original mechanistic basis that was proposed. These variations in rate constants suggest the configuration of reactors plays an important role in determining the rate constants of xylan hydrolysis apart from its intrinsic degradation properties. Insofar, the biphasic model proposed based on conventional Saeman’s model describing the chemical reaction displayed low capability in predicting the xylan hydrolysis in reactors with different geometric properties.

Compared with the Model I, the leaching model (Model II) with the effect of mass transfer on xylan/hemicellulose hydrolysis could describe the effects of reactor configuration effectively and reasonably. Yang et al. (Yang et al., 2004) investigated and compared the mass transfer ($k_D$) of xylan/hemicellulose hydrolysis in tube batch, stirred batch and flowthrough reactors based on this leaching model. It was found that the value of $k_D$ obtained via these reactors increased as the following orders: tube batch, stirred batch and flowthrough reactors. In addition, elevating the flow rate in flowthrough reactors also led to enhanced mass transfer rates. For example, the value of $k_D$ of xylan/hemicellulose hydrolysis in flowthrough reactor at flow rate of 1mL/min was 0.240 cm/min, when the flow rate was enhanced to 10 mL/min, the value of $k_D$ increased to 0.660 cm/min.

With the exception of mass transfer coefficient ($k_D$), improved model (Model III) with the addition of the axial position along the reactor bed ($x$) as well as the superficial velocity ($u$) generated reasonable rate constants. The rate constant of xylan hydrolysis at 170 °C with
water-only was obtained 0.082 min\(^{-1}\) with flow rate of 100 mL/min, which was lower than that observed under higher flow rate of 200 mL/min (0.137 min\(^{-1}\)). This flowthrough model fitted the xylose generation from xylan more precisely than Saeman’s model under such conditions. The R\(^2\) for xylose formation based on this flowthrough model was 0.96—0.91, much higher than that for Saeman’s model (0.23—0.25) or biphasic model (Model I) (0.22—0.28), which suggested that the flow rate and reactor geometry played an important role in modulating the xylan degradation pattern.
**Table 1.1** The summarization of varied models in depicting the hydrolysis of xylan/hemicellulose with acidic water in batch and flowthrough reactors

<table>
<thead>
<tr>
<th>Model type</th>
<th>Reactor type</th>
<th>Model equations</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model I</td>
<td>Batch tube</td>
<td>$\frac{dX_n}{dt} = -k_1 X_n$</td>
<td>(Brennan &amp; Wyman, 2004b)</td>
</tr>
<tr>
<td></td>
<td>Glass vial batch</td>
<td>$\frac{dX}{dt} = k_1 X_n - k_2 X$</td>
<td>Lu &amp; Mosier, 2008</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\frac{dF}{dt} = k_2 X - k_3 F$</td>
<td></td>
</tr>
<tr>
<td>Model II</td>
<td>Batch tube</td>
<td>$\frac{dC_A}{dt} = -k_c \frac{A_s}{V_c} (C_A - C_{A,s})$</td>
<td>(Brennan &amp; Wyman, 2004b)</td>
</tr>
<tr>
<td></td>
<td>Stirred batch</td>
<td>$\frac{dC_{A,s}}{dt} = -k_c \frac{A_s}{V_t} (C_A - C_{A,s})$</td>
<td>Yang et al., 2004</td>
</tr>
<tr>
<td></td>
<td>Flowthrough</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model III</td>
<td>Flowthrough</td>
<td>$\frac{dH}{dt} = H \frac{dv}{dz} + v \frac{dH}{dz} - k_1 H$</td>
<td>(Pronyk &amp; Mazza, 2010b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\frac{dO}{dt} = u \frac{dO}{dz} + k_1 x H - k_2 O$</td>
<td>Shao &amp; Lynd, 2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\frac{dP}{dt} = u \frac{dP}{dz} + k_2 O$</td>
<td></td>
</tr>
</tbody>
</table>

1. $X_n$, $X$, $F$ represent xylan, xylose and furfural, respectively, which can be expressed in the unit of yield(%) (Gil Garrote, 1999; Lu & Mosier, 2008). $k_1$ is the rate constant of xylan hydrolysis (min$^{-1}$), $k_2$ is the rate constant of xylose degradation (min$^{-1}$), $k_3$ is the rate constant of furfural degradation (min$^{-1}$).

2. $C_A$ is the xylan in solids, $C_{A,s}$ xylan in solution, $k_c$ is the mass transfer coefficient (cm/min), $A_s$ is the surface area of the particles (m$^2$/g), $V_c$ is the volume of the solid (m$^3$/g), and $V_t$ is the total volume of solution.

3. $z$ (dm) is the reactor length, $v$ (dm/min) is the solid flow rate, $u$ (dm/min) was the liquid flow rate, $H$ is the xylan in solid, $O$ is the solubilized xylan, $P$ is degradation product.
Table 1.2 Comparison of the rate constants and other physical parameters obtained in different models when hydrolyzing xylan/hemicellulose with acidic water in batch and flowthrough reactors

<table>
<thead>
<tr>
<th>Model type</th>
<th>Reactor type</th>
<th>Temperature/Acid</th>
<th>Rate constants</th>
<th>Mass transfer coefficient</th>
<th>Physical parameters</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model I</td>
<td>Batch</td>
<td>180 °C/None</td>
<td>0.813</td>
<td>0.227</td>
<td>-</td>
<td>[1]</td>
</tr>
<tr>
<td></td>
<td>Glass vial batch</td>
<td>170 °C/0.05% acid</td>
<td>0.020</td>
<td>0.118</td>
<td>-</td>
<td>[2]</td>
</tr>
<tr>
<td></td>
<td>Flowthrough, 10mL/min</td>
<td>180 °C/None</td>
<td>0.513</td>
<td>0.008</td>
<td>-</td>
<td>[1]</td>
</tr>
<tr>
<td></td>
<td>Flowthrough, 100mL/min</td>
<td>170 °C/None</td>
<td>0.078</td>
<td>0.004</td>
<td>-</td>
<td>[3]</td>
</tr>
<tr>
<td></td>
<td>Flowthrough, 1000mL/min</td>
<td>170 °C/None</td>
<td>-</td>
<td>-</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>Model II</td>
<td>Batch tube</td>
<td>180 °C/None</td>
<td>-</td>
<td>-</td>
<td>0.150</td>
<td>[4]</td>
</tr>
<tr>
<td></td>
<td>Stirred batch</td>
<td>180 °C/None</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>[4]</td>
</tr>
<tr>
<td>Model III</td>
<td>Flowthrough, 1mL/min</td>
<td>180 °C/None</td>
<td>-</td>
<td>-</td>
<td>0.240</td>
<td>[4]</td>
</tr>
<tr>
<td></td>
<td>Flowthrough, 10mL/min</td>
<td>180 °C/None</td>
<td>-</td>
<td>-</td>
<td>0.660</td>
<td>[4]</td>
</tr>
<tr>
<td></td>
<td>Flowthrough, 100mL/min</td>
<td>170 °C/None</td>
<td>-</td>
<td>0.08</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Flowthrough, 200mL/min</td>
<td>170 °C/None</td>
<td>-</td>
<td>0.13</td>
<td>2.28</td>
<td></td>
</tr>
</tbody>
</table>

1.5. Objective of this study

The objectives of this research include:

(1) To investigate and compare the kinetic fundamentals of acidic degradation of cellulose and xylan derived from plant biomass and agarose derived from algal biomass. To study the kinetic interactions among reaction steps favoring the production of sugars and their degradation compounds.

(2) In particular to enhance 5-HMF production with the novel hydrolysis catalysts.

(3) To enhance the total sugar and lignin yields through disrupting and removing the whole biomass from flowthrough reactor with dilute acid or water-only under elevated temperature (200—280 °C).

(4) To study and compare the degradation pattern of xylan, cellulose and lignin from biomass and the structural alteration of these biomass derived compounds during the dilute acid or hot water flowthrough pretreatment.

(5) To develop a kinetic model to depict the degradation of xylan, cellulose and lignin in flowthrough system.
1.6. Scientific contributions

(1) Cellulose degradation in acidic conditions was predominately temperature dependent along with time or flow rate. Significant cellulose hydrolysis accompanied with the transformation of cellulose I to cellulose II when temperature was above 240 °C for water-only operations, and amorphous cellulose when temperature was above 220 °C in dilute acid (0.05 wt.% H₂SO₄) conditions were observed.

(2) Flowthrough pretreatment under elevated temperatures (≥240 °C for water-only and ≥220 °C for 0.05 wt.% H₂SO₄) achieved total biomass(cellulose, xylan and lignin) decomposition and removal. Approximately 100% of the xylan and ~90% of the cellulose were solubilized and recovered. Up to 15% of the lignin was solubilized, while the remaining lignin was insoluble. Over 90% sugar yields were obtained from pretreated whole slurries using less than 10 FPU/g cellulase plus hemicellulase enzyme.

(3) A kinetic model was developed to describe the biomass degradation at elevated temperatures (240 °C, 0.05 wt.% H₂SO₄) in flowthrough system. Results revealed that mass transfer limitations were minimized using up to 4mm biomass particle sizes with 4g biomass loading at 25mL/min flow rate, which produced hydrolyzate slurries with 13g/L potential sugar concentrations.

(4) 5-HMF yield and selectivity from polysaccharides (agarose) can be significantly improved by MgCl₂ instead of acid (H₂SO₄), which led to 40.7% yield and 49.1% selectivity. MgCl₂ can promote the cleavage of C-O-C bond in polysaccharides (e.g. agarose) and enhance the subsequent dehydration reaction to 5-HMF. However, MgCl₂
has limited catalytic activity to induce retro aldol and rehydration reactions to generate byproducts such as lactic acid and levulinic acid.
1.7. Publications


Lishi Yan, Ava A. Greenwood, Akram Hossain, Bin Yang. A comprehensive mechanistic kinetic model for dilute acid hydrolysis of switchgrass cellulose to glucose, 5-hydroxymethylfurfural and levulinic acid. RSC Advances. Under review.

1.8. References


Liu, C., Wyman, C.E. 2003. The Effect of Flow Rate of Compressed Hot Water on Xylan,


Shen, J., Wyman, C.E. 2012. Hydrochloric acid-catalyzed levulinic acid formation from


Chapter 2

Comparative reaction kinetics of acid hydrolysis of plant biomass and algae biomass in batch system

(This chapter has been submitted to RSC advances)

2.1. Abstract

Dilute acid hydrolysis of polysaccharides from both plant biomass and algal biomass in batch system has been examined as the leading approach to evaluate the performance of the biomass pretreatment technologies on a common basis. A pseudo-homogeneous first-order reaction model that are commonly used to describe the biomass hydrolysis process were employed to obtain an in-depth understanding of degradation of xylan and cellulose from plant biomass (e.g. switchgrass) as well as the algae biomass-derived agarose at temperatures ranging from 140–220 °C with dilute acid (1% (w/w) H₂SO₄). Kinetic analysis revealed that xylan was susceptible to decomposition under tested conditions. The corresponding xylose generation was less temperature dependent. Prolonged time and decreased temperature (e.g. 140 °C and 25 min) was desired for its production. Conversely, temperature was the most significant factor in determining cellulose hydrolysis. The physical obstacle (e.g. crystallinity) of cellulose played an important role in impeding its hydrolysis when comparing the amorphous structures (agarose) and appeared to be negligible at elevated temperatures (e.g. 220 °C). Higher temperature (e.g. 220 °C) was also benefit for the production of glucose, levulinic acid and furfural. The rate constant of 5-HMF degradation was observed 5-9 fold lower than its degradation over the tested temperatures, demonstrating its unstability in acidic
2.2. Introduction

Pretreatment of lignocellulosic biomass with dilute acid in batch system was an extensively used technology for deconstructing lignocellulosic biomass before enzymatic hydrolysis (Lee et al., 1999; Rackemann & Doherty, 2011) was well developed. The hydronium ions (H$_3$O$^+$) released from water at elevated temperatures (e.g. 200 °C) (Kumar et al., 2010a), the acetic acid generated from hemicellulose through the cleavage of its ether bonds (Heitz et al., 1986), as well as the addition of dilute mineral acid (e.g. 1%(w/w) H$_2$SO$_4$) all enhance biomass hydrolysis. Lignocellulosic biomass typically contains 50%–75% cellulose and xylan on a dry weight basis, and the phenylpropanoid biopolymer lignin accounts for the remainder (Mosier et al., 2005). Xylan can be readily hydrolyzed by dilute acid or hot water under moderate temperatures (120 °C to 210 °C). Up to 90% xylan recovery predominately in the form of xylose and xylooligomers have been realized (Yang & Wyman, 2009). However, this temperature range leads to relative low cellulose hydrolysis due to cellulose being highly resistant to mild forms of chemical deconstruction (Himmel et al., 2007; Nitsos et al., 2013). Higher temperatures (170–220 °C) thus are needed for cellulose hydrolysis. Such high temperatures, however, led to the significant degradation of the glucose that is generated as well, which required advanced experimental techniques that can cope with precise control of short reaction time (Lee et al., 1999). Recently, polysaccharides from algal biomass have
been recommended as an alternative feedstock for the large scale production of biofuels and biochemicals because of their substantial abundance, negligible food value, as well as use of non-arable land (Kim et al., 2010)(Ferrell & Sarisky-Reed, 2010). Agarose, mainly composed of galactose and 3, 6-anhydrogalactose (Armisén, 1991; Nijenhuis, 1997) is one of major polysaccharides stored in algal biomass (Fu & Kim, 2010; Kim et al., 2010). For example, the agarose content in *Gelidium amansii* (a red algae species) is approximately 60% – 65% by weight (Kim et al., 2010). Even though agarose exists in a gel form caused by the intra- and inter-molecular hydrogen bonds at room temperature, these hydrogen bonds can be dissociated when the temperature exceeds 90 °C (Nijenhuis, 1997), leaving agarose more susceptible to decomposition at moderate conditions compared with cellulose.

Apart from the formation of sugars, varying degradation compounds including furfural (Lee et al., 1999), 5-HMF (Girisuta et al., 2008; Shen & Wyman, 2012b), levulinic acid (Rackemann & Doherty, 2011), etc were observed using dilute acid or hot water conditions, which significantly consumed the sugar and reduced yields. The toxicity of these compounds is carried through in the subsequent enzymatic hydrolysis. However, these sugar degradation compounds in-turn can act as important platform chemicals that can be utilized to produce a number of bio-chemicals including resins, polymers, pharmaceuticals and flavouring agents (Rackemann & Doherty, 2011; Rosatella et al., 2011). The production of furfural is based on the dehydration of xylose in acidic aqueous phase. Approximately 40%–50% furfural yields have been realized with dilute acid or hot water pretreatments (Mamman et al., 2008). Production of 5-HMF is the sequential degradation product via dehydration of hexose (e.g.
glucose and galactose) (Kim et al., 2010; Rackemann & Doherty, 2011), which is unstable in acidic conditions and can be further hydrolyzed into levulinic acid (Rackemann & Doherty, 2011). It was reported that less than 10% 5-HMF has been obtained in dilute acid (e.g. 3.3% (w/w) hydrochloric acid) treatments of cellulose (Shen & Wyman, 2012b) at the temperatures ranging from 160—200 °C. Levulinic acid is much more stable than its precursor 5-HMF under acidic conditions (Rackemann & Doherty, 2011). However, levulinic acid yields reported in previous studies have not exceed 60% (Girisuta et al., 2007; Rackemann & Doherty, 2011; Shen & Wyman, 2012b).

Investigating the kinetic effects on the degradation performance of polysaccharides from biomass (i.e. xylan, cellulose and agarose) in acidic conditions as well as the generation of sugars and the corresponding sugar degradations could provide new insights into interactions among the various reaction steps that favor the formation of these biomass derived compounds. Developing mathematical interpretation such as kinetic analysis appears to be an advanced approach for obtaining an in-depth mechanistic understanding of the acidic hydrolysis of biomass-derived polysaccharides to sugars and various chemicals. Therefore, the kinetics of the reactions are necessary to design and optimize efficient pretreatment processes. An extensive kinetic mechanism describing the acidic hydrolysis of polysaccharides from biomass (e.g. xylan and cellulose) has been based on the study by Saeman (1945), who investigated the dilute acid hydrolysis of cellulose from Douglas fir in batch reactors. It was a two-step pseudo-first-order irreversible reaction, which could be summarized in scheme 1
Over the years, Saeman’s model has been applied by many researchers to study the dilute acid or hot water hydrolysis of xylan and cellulose hydrolysis (Aguilar et al., 2002; Fagan et al., 1971; Lavaracka et al., 2002; Lee et al., 1999; Mosier et al., 2002; Xiang et al., 2004b).

Other researchers have sequentially extended Saeman’s model with the incorporation of major degradation reactions and compounds from xylose and glucose. Furfural has already been incorporated into kinetic modeling regarding xylan degradation (Garrote et al., 2001; Vazquez et al., 2007). Only a few kinetic models (Girisuta et al., 2008; Shen & Wyman, 2012b) have explained a more complete acid-catalyzed cellulose degradation including the formation of 5-HMF and levulinic acid. A comprehensive kinetic study of dilute acid or hot water hydrolysis of biomass that applies to xylan, cellulose and agarose under a wide range of reaction conditions with accurate description of the reaction profiles for the products of interest is highly desired as the guidance for the design and improvement of the polysaccharides acid hydrolysis.

In this study, we reported a systematic kinetic study on the acidic hydrolysis of xylan and cellulose from plant biomass (e.g. switchgrass) and the algae biomass-derived agarose with 1% (w/w) sulfuric acid and water-only pretreatments under wide temperature ranges of 140—220 °C and residence times from 0—60 min. The widely used pseudo-homogeneous irreversible first order kinetic model was developed to describe the acidic hydrolysis behavior of xylan, cellulose and agarose for understanding the degradation behavior of these biomass.
derived polysaccharides. In addition, the kinetic interactions among the various reaction steps favoring the generation of sugars and their subsequent degradation compounds (5-HMF, furfural and levulinic acid) in acid aqueous medium was investigated.

2.3. Materials and Methods

2.3.1. Feedstocks

Switchgrass was harvested in Richland, Washington in the fall of 2009. It contains 40.2 wt. % glucan, 20.4 wt.% xylan, 2.8 wt. % arabinan, 1.1 wt. % galactan, 0.5 wt. %Mannan, 19.5 wt. % Klason lignin and 6.6 wt. % ash. The compositions of switchgrass were determined based on Laboratory Analytical Procedure (LAP) of “Determination of Structural Carbohydrates and Lignin in Biomass” (Sluiter et al., 2008b). These materials were cut to shorter lengths, then grounded to a particle size of 200-400 µm with a laboratory mill (model:MF10 basic, IKA®works, Inc, NC, Wilmington) and then passed through a 60 mesh to obtain particles added in the reaction. Microcrystalline cellulose (Avicel PH-101, Sigma-Aldrich, St. Louis, MO) and Agarose (BP160, Fisher Scientific, Pittsburgh, PA) were used in this study for comparison. Agarose BP160 contains 98% agarose, which was determined following Laboratory Analytical Procedure (LAP) of “Determination of Structural Carbohydrates and Lignin in Biomass” (Sluiter et al., 2008b). All standard chemicals were purchased from Sigma-Aldrich, St. Louis, Mo.

2.3.2. Experimental methods

Batch tubular reactors (1.27cm OD×15.24cm long with×0.0889cm wall thickness, Hastelloy C-276) (Swagelok Northwest, Richland, WA) were used in this study. The total volume of
each reactor is 14.5mL with 10 mL working volume. 0.2g substrate was loaded into the batch tubular reactor with 10 ml of 1% (w/w) sulfuric acid in water. Reactors were heated to reach target temperature within 1 min in a 4-kW fluidized sand bath (model SBL-2D, Omega engineering, Inc., Stamford, CT) and pretreated for 5 to 60 minutes at temperature of 120 °C to 200 °C. A thermal monitor was connected to the batch reactor to precisely test the targeted temperature before reaction. The average heating speed is 200 °C/min. The reaction was quenched within 1 min by soaking the batch tubular reactor in cold water bath after being subjected to the target reaction temperature for a specified time. After pretreatment the end caps and plugs were removed, pretreated residues were pushed out and separated into liquid hydrolyzate and solid residue by vacuum filtration using a 0.22 µm glass fiber filter (Fisher Science, Pittsburgh, PA) for analysis.

2.3.3. Analytical methods

Glucose, xylose, 5-HMF, furfural and levulinic acid were analyzed through Dionex Ultimate 3000 HPLC equipped with a refractive detector (Shodex RI-101) and a ultimate 3000 autosampler using chromeleon 6.8 software (Dionex, Bannockburn, IL). Bio-Rad Aminex HPX-87H columns (Bio-Rad Laboratories, Hercules, CA) were operated at 65 °C with a mobile phase of 0.005M H₂SO₄ and flow rate 0.6 ml/min. Before analysis, the acid in the liquid hydrolyzate was neutralized with calcium carbonate till pH 5 to 6. The neutralized hydrolyzate was centrifuged and filtered for HPLC analysis (Sluiter et al., 2006). Yields of xylose, furfural, glucose, 5-HMF and levulinic acid were calculated as following (Yan et al., 2013):
Xylose\%(1) = \frac{(W \times MW_{Xyn})}{(W_{Xyn} \times MW_{X})} \times 100\% \\
Glucose\%(2) = \frac{(W \times MW_{Gln})}{(W_{Gln} \times MW_{G})} \times 100\% \\
Furfural\%(3) = \frac{(W_{Fur} \times MW_{Xyn})}{(W_{Xyn} \times MW_{Fur})} \times 100\% \\
5-HMF\%(4) = \frac{(W_{5-HMF} \times MW_{Gln})}{(W_{Gln} \times MW_{5-HMF})} \times 100\% \\
Levulinic acid\%(5) = \frac{(W_{LA} \times MW_{Gln})}{(W_{Gln} \times MW_{LA})} \times 100\% \\

In these equations, $W_{Xyn}$ is the initial weight of xylan (g), $W_{Gln}$ is the initial weight of glucan (g), $W_X$ is the weight of xylose (g), $W_G$ is the weight of glucose (g), $W_{Fur}$ is the weight of furfural (g), $W_{5-HMF}$ is the weight of 5-HMF (g), $W_{LA}$ is the weight of levulinic acid (g). MW is the molecular weight: \( MW_{Xyn}=132,\) \( MW_{Gln}=162,\) \( MW_{X}=150,\) \( MW_{G}=180,\) \( MW_{Fur}=96,\) \( MW_{5-HMF}=126,\) \( MW_{LA}=116.\)

2.3.4. Data analysis

Xylan degradation Xylan(Xn) hydrolysis model with the incorporation of xylose (X) and furfural was developed as follows:

\[
\begin{align*}
Xylan &\xrightarrow{k_1} Xylose &\xrightarrow{k_2} Furfural &\xrightarrow{k_3} Degradation \ compounds \\
\frac{dXn}{dt} &= -k_1X_n \\
\frac{dX}{dt} &= k_1X_n - k_2X \\
\frac{dF}{dt} &= k_2X - k_3F
\end{align*}
\]
where \( X_n, X, F \) represent xylan, xylose and furfural, which were expressed in the unit of yield(%) (Gil Garrote, 1999; Lu & Mosier, 2008). \( k_1(\text{min}^{-1}), k_2(\text{min}^{-1}), k_3(\text{min}^{-1}) \) are the degradation rate constants of xylan, xylose and furfural.

Cellulose degradation

A pseudo-homogeneous irreversible first order reaction model with the incorporation of glucose (G), 5-HMF (M) and levulinic acid (L) as shown in Scheme 3 was used to describe the cellulose(C) hydrolysis process (Saeman, 1945).

\[
\begin{align*}
\text{Cellulose} & \xrightarrow{k_1} \text{Glucose} \xrightarrow{k_2} 5\text{-HMF} \xrightarrow{k_3} \text{Levulinic acid} \xrightarrow{k_4} \text{Degradation compounds} \\
\end{align*}
\]

\[ \frac{dC}{dt} = -k_1C \]  \hspace{1cm} (4)
\[ \frac{dG}{dt} = k_1C - k_2G \]  \hspace{1cm} (5)
\[ \frac{dM}{dt} = k_2G - k_3M \]  \hspace{1cm} (6)
\[ \frac{dL}{dt} = k_3M - k_4L \]  \hspace{1cm} (7)

where \( C, G, M \) and \( L \) represent cellulose, glucose, 5-HMF and levulinic acid, respectively, which were expressed in the unit of yield(%) (Gil Garrote, 1999; Lu & Mosier, 2008), \( k_1(\text{min}^{-1}), k_2(\text{min}^{-1}), k_3(\text{min}^{-1}) \) are the degradation rate constants of cellulose, glucose, 5-HMF and levulinic acid. A MATLAB program was used to fit the parameters in Eqs.5 to 8 and Eqs.13 to 17 to simulate the experimental concentrations of glucose, 5-HMF and levulinic acid over the temperature range of 140–220°C, respectively.

2.4. Results and discussion

2.4.1. Xylan hydrolysis
The hydrolysis of xylan and the release pattern of its degradation compounds were investigated in a series of batch kinetic experiments under temperatures ranging from 140—200 °C, time scope of 0—60min with both 1% (w/w) H₂SO₄ and water-only Pretreatments.

2.4.1.1. Effect of temperature and time on xylan degradation

The results of the xylan degradation are summarized in Figure 1a. Xylan was decomposed significantly over the tested conditions. Under 140 °C, almost total xylan degradation was realized during 30min. When the temperature was elevated to 160 °C or higher, total biomass degradation was achieved within merely 10 min or less. Figure 1b shows the corresponding xylose yields released from xylan. Under lower temperatures between 140—160 °C, the maximum xylose yields obtained were 85.5%—82.9%, respectively. However, continuously enhancing the reaction temperature resulted in a decrease in the xylose yield. The maximum xylose yields obtained under higher temperatures of 180 °C, 200 °C and 220 °C were obtained 76.3%, 52.6% and 16.2%, respectively. Because the reaction rate enhanced with temperature, the reaction time required to reach the highest xylose yields was reduced as the temperature increased. It was noteworthy that lower temperature and prolonged reaction time is more desired for xylose production, the highest xylose yield (85.5%) was obtained under 140 °C for 25 min. The formation of furfural displayed a deviated release pattern compared with xylose under identical conditions (Figure 1c). Under lower temperatures 140—160 °C, the yields of furfural increased as the reaction time prolonged, reached the highest yields of 24.6% and 54.7% with 60min, respectively. Comparatively, higher temperature (180—220 °C) resulted in higher furfural yields with reduced reaction time. The maximum furfural
yields obtained under 180 °C, 200 °C and 220 °C were observed 58.8%, 65.8% and 67.8%, respectively. Although the yields of furfural at 140 °C and 160 °C were lower than those obtained under higher temperatures (180–220 °C), the reaction was incomplete within the tested time course (0–60 min), that could be further reacted to higher furfural yield left at the end of this experiment. A Matlab program was used to fit the parameters in Eqs 1–3 to match the yields of xylan residue and the yields of xylose and furfural (Figure 1a-c). It showed that the model nearly perfectly fitted the experimental data. The correlations (statistical parameters) $R^2$ for xylan residue and the formation of xylose and furfural based on the proposed kinetic model were all ranging from 0.93–0.99 (Table 1). It was noteworthy that the rate constant of xylan hydrolysis ($k_1$), xylose degradation ($k_2$) and furfural degradation ($k_3$) obeyed the following order: $k_1 > k_2 > k_3$ over the tested temperatures (140–220 °C), suggesting that longer reaction time could be desired for both xylose and furfural production. The rate constants depending on the temperature followed an Arrhenius-type equation of the form

$$k = A \times \exp(-\frac{E_a}{RT})$$  \hspace{1cm} (8)

where $k$ (min$^{-1}$) is the rate constant, $A$ (min$^{-1}$) is the pre-exponential factor, $E_a$ (kJ mol$^{-1}$) is the activation energy, $R$ is universal gas constant ($8.3143 \times 10^{-3}$ kJ mol$^{-1}$ K$^{-1}$), $T$ (K) is temperature.

The rate constants obtained in this experiment were also put into the Arrhenius plot, ln(k) vs 1/T (Figure 2.7). The $E_a$ of the xylan hydrolysis, as well as the degradation of xylan and furfural calculated based on equation (8) were 65.8 kJ mol$^{-1}$, 120.5 kJ mol$^{-1}$, and 39.9 kJ
mol$^{-1}$, respectively. This indicated that as the temperature was elevated, the rate constant of xylose degradation ($k_2$) increased more severely than the degradation of xylan ($k_1$). In this regard, considering the aforementioned lower value of $k_2$ than $k_1$, relative lower reaction temperature (e.g. 140 °C) and prolonged reaction time could be beneficial for the xylose generation from xylan. Different from xylose, increasing temperature could have less impact on furfural degradation ($k_3$) due to its relative lower value of Ea, which indicated its relative higher yields under higher temperature.

2.4.1.2. Effect of dilute acid on xylan degradation

The effect of dilute acid on the xylan hydrolysis and the yields of its degradation compounds xylose and furfural are summarized in Figure 2(a–c). It was found that adding 1% (w/w) H$_2$SO$_4$ significantly enhanced the rate of xylan hydrolysis under the tested conditions (Figure 2a). For example, almost total xylan degradation was realized with 20 min at 180 °C using dilute acid, while water-only operation merely resulted in around 50% xylan removal with similar severity (temperature and time). Correspondingly, the addition of dilute acid also enhanced the yields of xylose and furfural. The maximum xylose yields and furfural yields were enhanced significantly with the addition of 1 wt. % H$_2$SO$_4$, which were 76.3% and 58.8% compared with 32.6% and 24.3% for water operation. The kinetic analysis regarding the xylan hydrolysis revealed that the rate constants of xylan degradation ($k_1$) for 1 wt. % H$_2$SO$_4$ operations were much higher than those for water-only operations, whereas the rate constants of xylose degradation ($k_2$) and furfural degradation ($k_3$) with both water-only and 1 wt. % H$_2$SO$_4$ were comparable (Table 2). In this regard, it
appeared that dilute acid played an important role in xylan hydrolysis, whereas has less apparent effects on further degradation of xylose and furfural, thereby resulting in relative higher yields of xylose and furfural. This conclusion was supported by previous research when investigating the degradation of xylan (Möller & Schröder, 2013) and xylose (Oefner et al., 1992) in dilute acid conditions. It was noteworthy that the correlations (statistical parameters) $R^2$ for the yields of xylose and furfural were $0.8572 - 0.9325$ for water-only operations, implying that the proposed kinetic model less precisely fit the experimental data regarding the production of xylose and furfural in water-only conditions. The possible explanation was that there could be considerable amount of xylooligomers generated in water-only conditions (Jacobsen & Wyman, 2002), which affected the mass balance of xylan degradation when using the proposed model in predicting the formation of xylose and furfural. The potential xylooligomers could in-turn reduce the yields of xylose and furfural (Yang & Wyman, 2008a).

2.4.2. Cellulose hydrolysis

2.4.2.1. Effect of temperature and time on cellulose degradation

Cellulose hydrolysis with dilute acid as well as the corresponding generation of glucose, 5-HMF and levulinic acid were plotted versus reaction times from 0–60 min at 140 –220 ºC (Figure 3a-d). Figure 3a shows that the yield of cellulose residues decreases gradually as reaction time is prolonged under relatively low temperatures of 140–160 ºC, where it was observed that more than 70% and 50% over the tested conditions, respectively. It was found that the cellulose degradation was enhanced significantly as the temperature was raised to
180 °C, where 12.1% cellulose residue was obtained after 60 min reaction time. This abrupt increase in cellulose hydrolysis could be attributed to the disruption of the crystallinity of cellulose. Further increasing the reaction temperature to 200 °C and 220 °C resulted in the total dissolution of cellulose at 30 min and 10 min, respectively. Correspondingly, Xiang and colleagues (Xiang et al., 2003a) reported that cellulose was susceptible to decomposition when the temperature was elevated to 215°C in 0.07 (w/w)% H₂SO₄ solution, and therefore led to a significant increase in glucose yield. The sudden change in the cellulose hydrolysis behavior could result from a temperature induced disruption of the hydrogen bonding existing between the cellulose chains. Based on this, temperature which could eliminate the crystalline fraction of cellulose seems to play an important role in determining the efficiency of cellulose hydrolysis to glucose.

The corresponding cellulose degradation compounds including glucose, 5-HMF and levulinic acid are summarized in Figure 2b—c. As shown in Figure 2b, at 140 °C, the glucose yield increases steadily over time, reaching its highest value of 14.4 % at 60 min. When the temperature is increased to 160 °C, the glucose yield is enhanced slowly over time, reaching the highest yield of 19.0% at 40 min, before declining slowly. The glucose yield at higher temperatures (180—220 °C) presented a similar trend as that at 160 °C. However, results implied a higher rate constant of the formation and degradation of glucose. The maximum glucose yields at 180 °C, 200 °C and 220 °C were found to be 31.8%, 32.7% and 43.2% at 15 min, 5 min and 1 min, respectively. These results revealed that relatively higher temperature (e.g. 220 °C) was favored for the formation of glucose compared to relatively lower
temperatures (e.g. 140 °C). The kinetic rate constants calculated based on the proposed model 4-7 revealed that the rate constant of cellulose hydrolysis (k_1) was lower than that of glucose decomposition (k_2) (Table 2), which was more obviously at lower temperatures (e.g. 140 °C). The rate constants obtained in cellulose degradation pathway were also put into the Arrhenius plot, ln(k) vs 1/T (Figure 2.8). The Ea of the degradation of cellulose and glucose calculated based on equation 8 were 103.9 kJ mol\(^{-1}\) and 66.5 kJ mol\(^{-1}\), respectively, indicating that increasing temperature could accelerate k_1 faster than k_2, thereby enhancing the glucose yield, which was in agreement with the kinetic findings reported by Lee and colleagues when investigating the cellulose hydrolysis under comparable conditions (Lee et al., 1999). In this regard, higher temperatures and reduced reaction times could be beneficial for glucose production. However, conducting reaction in batch system under high temperatures within reaction time less than 1min appears to not be feasible because of operational difficulties originating from the rapid reaction rates (Lee et al., 1999). More advanced reactors such as flowthrough reactor (Yang & Wyman, 2004a) could be developed for controlling the reaction more precisely.

The yields of the glucose’s subsequent degradation compounds 5-HMF are shown in Figure 2c. The formation of 5-HMF displayed similar trends as that of glucose, i.e. higher temperature led to higher 5-HMF yield. However, the yields of 5-HMF were extremely low over the tested temperatures (140–220 °C). The maximum 5-HMF yield obtained in this series of experiments was only 5.8% under 220 °C within 2 min.

The kinetic analysis regarding the 5-HMF yield revealed that the rate constant of 5-HMF
formation ($k_2$) was much lower than its degradation ($k_3$). The 5-HMF’s susceptible to be degradation under acidic conditions was also proven by the previous studies. For example, Asghari and Yoshida (2006) reported that the degradation of fructose by 0.03M hydrochloric acid under 240 °C over 2 min resulted in low 5-HMF yields of 9.0%; Chambon (2011) reported that employing solid acids, such as C-SO$_3$H, resulted in less than 10% of 5-HMF yield from cellulose at 190 °C.

Levulinic acid, which originate from the rehydration of 5-HMF, was often considered as the major degradation compounds of 5-HMF (Rackemann & Doherty, 2011). As shown in Figure 2c, under temperatures 140 °C−180 °C, levulinic acid yield increases consistently over reaction times between 0−60min. The highest levulinic acid yields were 6.0%, 22.1% and 59.5% at 60 min, respectively. When the temperature was raised to 200 °C, levulinic acid yields increased significantly over time, reached a maximum value of 59.5% at 30 min, then decreases slowly. Increasing temperature to 220 °C resulted in a maximum levulinic acid yield of 57.8% at 10 min. It was observed that the decomposition rate constant of 5-HMF ($k_3$) was significantly higher than that of levulinic acid decomposition ($k_4$). The ratio $k_3/k_4$ was obtained around 140−300 under the temperature range of 140-220 °C, respectively. Such a high ratio could be served as the possible explanation of the relative high yield of levulinic acid compared with its precursor 5-HMF and glucose. Even though, results indicate that the yield of levulinic acid under experimental conditions did not exceed 60%, which was in agreement with the results reported by previous researchers when using dilute hydrochloric acid to generate levulinic acid from cellulose under similar temperatures 180−200 °C. The
limitation of the levulinic acid production could be attributed to the formation of humins from 5-HMF observed by Patil and Lund (2011b). The humins could consume the 5-HMF thereby decreasing the yield of levulinic acid to some extent. Results suggest that a considerable proportion of 5-HMF was condensed to humans during the reactions, which could contribute to the limited levulinic acid yield.

2.4.2.2. Effect of acid on cellulose degradation

Figure 4a compares the hydrolysis of cellulose with and without the addition of 1% (w/w) H₂SO₄ under temperatures between 180–220 °C. It was found that the cellulose hydrolysis with 1% (w/w) H₂SO₄ displayed much faster rate constants (0.0459-0.4773 min⁻¹) than those with water-only (0.0012-0.0058 min⁻¹) (Table 2) over the tested temperatures. The cellulose residues after dilute acid pretreatment were consequently observed 12.1% at 180 °C over 60 min and negligible at 220 °C even within merely 10 min. In comparison, more than 75% cellulose residue remained in the reactor after hot water pretreatment under temperatures 180–220 °C. Such significant differences between the performance of cellulose hydrolysis with and without the addition of dilute acid suggests that dilute acid plays an important role in disrupting the crystallinity of cellulose under high temperatures.

The rapid hydrolysis of cellulose with dilute acid subsequently led the higher generation of glucose, although it in-turn accelerated the degradation of glucose as well. The kinetic analysis of the experimental results showed that the ratio of cellulose degradation (k₁) to glucose degradation (k₂) for dilute acid operations ranged from 0.2 to 0.9, approximately 2-9 fold higher than those calculated from water-only pretreatment. Such high ratio could be
served as the explanation of higher glucose yield with dilute acid. The lower glucose concentrations in water-only pretreatments result in negligible degradation to the compounds 5-HMF and levulinic acid.

2.4.2.3. The comparison of the hydrolysis of cellulose and agarose

The gradual increasing rate constants of cellulose degradation especially at relative lower temperature (e.g. 140 °C) suggests that the crystalline regions of cellulose, which could be caused by the inter and intro hydrogen bonds in cellulose, impede the degradation of cellulose (Xiang et al., 2003c). In this study, an amorphous structure of polysaccharides, agarose, was used to compare the degradation performance of cellulose under tested conditions (140—200 °C, 0—60min, 1 wt. % H₂SO₄). Agarose is mainly composed of galactose (isomer of glucose) and 3,6-anhydrogalactose is one of the major polysaccharides stored in algal biomass (Armisén, 1991; Kim et al., 2010), which is considered as a kind of polysaccharides with negligible food value. Even though agarose exists in a gel form caused by the intra- and intermolecular hydrogen bonds at room temperature, these hydrogen bonds can be dissociated when the temperature exceeds 90 °C (Nijenhuis, 1997), leaving agarose more susceptible to decomposition. In order to diminish the interpretation of hemicellulose and lignin, we pretreated the microcrystalline cellulose (Avicel PH-101) instead of poplar wood in batch system under identical conditions.

Figure 5 compares the degradation profile of cellulose and agarose in pretreatments at temperature between 140–220 °C. Results revealed that the degradation of cellulose was much slower than that of agarose. For example, the agarose degradation was found to be
negligible under 140 °C within 30min, while over 90% cellulose residue remaining was observed under identical conditions. The kinetic analysis of experimental data reveals that the rate constants of cellulose hydrolysis (e.g. 0.0043 min\(^{-1}\)) were much lower than those of agarose (e.g. 0.1710 min\(^{-1}\)) at lower temperatures (e.g. 140 °C). This result suggests that the recalcitrance of cellulose such as hydrogen bonds play an important role in determining the efficacy of cellulose hydrolysis, requiring higher reaction severity for its decomposition.

Although the diminishment of the cellulose crystalline was realized under high temperatures (e.g. 220 °C) therefore resulting in the considerable cellulose hydrolysis rate constant (0.4773 min\(^{-1}\)) compared with that of agarose hydrolysis (0.5886 min\(^{-1}\)). However, such high temperature in-turn enhanced the degradation of glucose, thereby limiting its yield. In comparison, the agarose’s physical properties determines its significant decomposition when temperatures reached 140 °C, at which the galactose degradation was limited and the corresponding yields were maintained on a relative high level. It was found that the maximum glucose yield was obtained 38.2% at 220 °C, around 30% lower than that observed for galactose (68.3%) at 140 °C.

Similar with galactose, the 5-HMF yield released from agarose was higher than that generated from cellulose. Nevertheless, because 5-HMF is an unstable intermediate in acidic conditions (Shen & Wyman, 2012b), its yields obtained were undesired from both agarose and cellulose. The maximum 5-HMF yields obtained from agarose and cellulose were 17.3% (140 °C, 35min) and 4.7% (220 °C, 2min), respectively. 5-HMF further underwent rehydration reactions to levulinic acid. It was noteworthy that the levulinic acid generated from both
agarose and cellulose showed similar maximum values (around 60%) over the tested severities (temperature and time). This was understandable because the levulinic acid degradation rate constants remained constant among low values (0.0009-0.0161min⁻¹), even under the extreme conditions (220 °C) in this experiment. 220 °C. Results suggest that the physical obstacles of substrate (e.g. crystallinity) had negligible impact on the levulinic acid generation due to its stability in acidic conditions although its production was still limited by the aforementioned humins (section 3.2.1).

2.5. Conclusion

A kinetic model that included a series of pseudo-first order reactions regarding the acidic hydrolysis of plant biomass derived xylan and cellulose as well as algae biomass derived agarose was developed to investigate the degradation performance of these polysaccharides and the interactions among various reaction steps over a wide range of temperatures between 140–220 °C for both dilute acid (1 wt. % H₂SO₄) and water-only pretreatments. The kinetic analysis revealed that xylan was susceptible to hydrolysis under the tested conditions and xylose yield was less effected by temperatures. The rate constant of xylan degradation was much higher than that of xylose production while the activation energy for xylan hydrolysis was lower than that for xylose degradation, demonstrating lower temperature and prolonged reaction time was desired for xylose production. Compared with xylan, cellulose hydrolysis was more prone to be dependent on temperature. The cellulose hydrolysis appeared to be impeded significantly by the physical obstacle (e.g. crystallinity) compared with amorphous structures (agarose) under lower temperature (e.g. 140 °C) and was to decomposition as
temperature was elevated to a higher level (e.g. 220 °C). Elevated temperature (e.g. 220 °C) also favored the hydrolysis of cellulose more than the glucose decomposition, thus was desired for glucose production. Furfural and levulinic acid were stable in acidic conditions with negligible degradation rate constants at reaction conditions. In the contrast, the rate constant of 5-HMF degradation was observed to be 5—9 fold higher than its formation over the tested temperatures, demonstrating its instability in acidic aqueous phase.
2.6. References


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Figure 2.1 Xylan degradation (a) and the yields of xylose (b) and furfural (c) during the 1% (w/w) H₂SO₄ hydrolysis of switchgrass under temperature 140–220 °C during 0–60 min. Black○: 140 °C; Red+: 160 °C; Blue△: 180 °C; Green*: 200 °C; Magenta□: 220 °C.
Figure 2.2 Comparison of xylan degradation (a) and the yields of xylose (b) and furfural (c) with 1% (w/w) H$_2$SO$_4$ and water-only hydrolysis of Switchgrass under temperature 180—220 °C during 0—60 min. Red *: 180 °C with 1% (w/w) H$_2$SO$_4$; Red ○: 220 °C with 1% (w/w) H$_2$SO$_4$; Blue *: 180 °C with water-only; Blue ○: 220 °C with water-only.
Figure 2.3 Cellulose degradation (a) and the yields of glucose (b), 5-HMF (c) and levulinic acid (d) during the 1% (w/w) H$_2$SO$_4$ hydrolysis of swithgrass under temperature 140—220 °C during 0—60 min.

Balck○: 140 °C; Red+: 160 °C; Blue△: 180 °C; Green*: 200 °C; Magenta□: 220 °C
Figure 2.4 Comparison of cellulose degradation (a) and the yields of glucose (b) with 1% (w/w) H$_2$SO$_4$ and water-only hydrolysis of Switchgrass under temperature 200—220 °C during 0—60min. Blue*: 200 °C, water-only; Blue○: 220 °C, 1% (w/w) H$_2$SO$_4$. Red*: 200 °C, water-only; Red○: 220 °C, 1% (w/w) H$_2$SO$_4$. 
Figure 2.5 Comparison of the degradation of cellulose and agarose with 1% (w/w) H$_2$SO$_4$: (a) cellulose degradation; (b) agarose degradation.  Black ○: 140 °C; Blue △: 180 °C; Red □: 220 °C.
Figure 2.6 Comparison of the generation of sugars (glucose and galactose), 5-HMF and levulinic acid from cellulose and agarose. Black○: 140 °C; Blue△: 180 °C; Red □: 220 °C.
Figure 2.7 Arrhenius plot for rate constants in hydrolysis of xylan in dilute acid conditions (140–220 °C, batch reactor, 1% (w/) H₂SO₄).
**Figure 2.8** Arrhenius plot for rate constants in hydrolysis of cellulose in dilute acid conditions (140–220 °C, batch reactor, 1% (w/) H$_2$SO$_4$).
Figure 2.9 Arrhenius plot for rate constants in hydrolysis of agarose in dilute acid conditions (140–220 °C, batch reactor, 1% (w/) H₂SO₄).
**Table 2.1** Kinetic rate constant for the xylan degradation with 1% (w/w) H$_2$SO$_4$ and water-only

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Temperatures (°C)</th>
<th>1%(w/w) H$_2$SO$_4$</th>
<th>Water-only</th>
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<td>140</td>
<td>160</td>
<td>180</td>
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Table 2.2 Kinetic rate constant for the cellulose degradation with 1% (w/w) H₂SO₄ and water-only

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<td>k₃(min⁻¹)</td>
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<tr>
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<td>0.0013</td>
<td>0.0022</td>
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<td>0.0161</td>
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<tr>
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Table 2.3 Kinetic rate constant for the cellulose and agarose degradation with 1% (w/w) H$_2$SO$_4$ and water-only.

<table>
<thead>
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<th>Parameters</th>
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<td>0.4773</td>
</tr>
<tr>
<td>$k_2$(min$^{-1}$)</td>
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<td>0.0706</td>
<td>0.5510</td>
</tr>
<tr>
<td>$k_3$(min$^{-1}$)</td>
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</tr>
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<td>$k_4$(min$^{-1}$)</td>
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<td>0.0161</td>
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</tr>
<tr>
<td>$R_G^2$</td>
<td>0.9423</td>
<td>0.9436</td>
<td>0.9823</td>
</tr>
<tr>
<td>$R_{HMF}^2$</td>
<td>0.9402</td>
<td>0.9258</td>
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Chapter 3

Catalytic conversion of agarose to 5-hydroxymethylfurfural by metal chlorides in hot water batch system

(This chapter has been published in RSC advances)

3.1. Abstract

The production of 5-Hydroxymethylfurfural (5-HMF) from agarose catalyzed by metal chlorides was studied in aqueous phase. A series of metal chlorides, including NaCl, CaCl$_2$, MgCl$_2$, ZnCl$_2$, CrCl$_3$, CuCl$_2$ and FeCl$_3$, were comparatively investigated to catalyze agarose degradation for the production of 5-HMF at temperature 180 $^\circ$C, 200 $^\circ$C and 220 $^\circ$C, catalyst concentration of 0.5% (w/w), 1% (w/w) and 5% (w/w), time 0-50min, and substrate concentration of 2% (w/w). Results revealed that alkali and alkaline earth metal chlorides, including NaCl, CaCl$_2$ and MgCl$_2$, resulted in relatively higher 5-HMF yields from agarose with negligible amount of byproducts, such as levulinic acid and lactic acid, derived from further degradation reactions. 1% (w/w) MgCl$_2$ was the most efficient catalyst among tested metal chlorides for 5-HMF production from agarose and resulted in both the highest yield of 40.7% and highest selectivity of 49.1% at 200 $^\circ$C for 35min. The cleavage of C-O-C bond in agarose with subsequent isomerization of galactose to its ketose was considered as possible mechanism for formation of 5-HMF under MgCl$_2$ catalyzed conditions.

Key words: 5-HMF, agarose, metal chlorides, degradation

3.2. Introduction

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5-hydroxymethylfurfural (5-HMF) is an important chemical building block used to make a wide variety of chemicals (e.g. plastics, polymers, etc.) and fuels that are currently derived from petroleum (Rosatella et al., 2011). However, the current processes of 5-HMF production are primarily dependent on edible monosaccharides such as fructose and glucose (Binder et al., 2010; Rosatella et al., 2011). Using cellulosic biomass as the feedstock for 5-HMF production has been also extensively studied over the years. However, technical challenges in developing an efficient and economic viable process for commercial application still remain (Qi et al., 2011; Rosatella et al., 2011). Our previous studies showed that less than 5% 5-HMF yield was obtained from cellulose with conventional dilute acid approach (Chapter 2). Recently, polysaccharides from algal biomass were recommended as an alternative feedstock for the large scale production of biofuel and biochemicals because of their substantial abundance, negligible food value, as well as use of non-arable land (Kim et al., 2010). Our previous studies revealed that agarose with few hydrogen bonds when temperature above 90°C could be recommended as the feedstock for 5-HMF production (Chapter 2).

Various reaction media, including organic solvent/organic-water biphasic systems, ionic liquids and the aqueous phase, were used for 5-HMF production. The use of organic solvents or ionic liquids as reaction medium has received significant attention because relatively high 5-HMF yield of 50—70 wt. % can be obtained (Román-Leshkov & Dumesic, 2009; Zhao et al., 2007). However, these reaction media present some limitations for the large scale production of 5-HMF. Although the 5-HMF yield was increased in the organic solvent dimethyl sulfoxide (DMSO), 5-HMF is highly soluble in DMSO which has a high boiling
point, thus prevents economical separation of 5-HMF (Musau & Munavu, 1987). Although ionic liquids are reported to enhance the 5-HMF yields, ionic liquids are expensive and require a high level of purity to maintain their physical properties. Thus, industrial applications of ionic liquids are limited (Mikkola et al., 2006; Shamsuri & Abdullah, 2010).

Production of 5-HMF in aqueous phase (i.e. water) instead of these expensive and difficult-to-separate solvents will significantly reduce its production cost. The physical properties of water at elevated temperatures (e.g. 200 °C) are different from those under ambient conditions (Asghari & Yoshida, 2006). For example, the solubility of organic compounds in water can increase at high temperatures (above 100 °C) due to the lower dielectric constant of water (Franck, 1987; Miller & Hawthorne, 1998). Furthermore, protons released from water at high temperatures (Aida et al., 2007; Antal et al., 1990; Bonn & Bobleter, O., 1983) could accelerate the formation of 5-HMF to some extent (Kuster, 1990).

However, the degradation of saccharides (e.g. fructose and glucose) in water-only conditions proceeds with poor selectivity to 5-HMF production, resulting in many byproducts. Byproducts, such as glycoaldehyde, glyceraldehyde, and lactic acid, were generated from the retro aldol reaction of hexose (e.g. glucose and fructose), while others including levulinic acid and formic acid were generated due to the further rehydration of 5-HMF (Aida et al., 2007; Antal et al., 1990; Bonn & Bobleter, O., 1983). Formation of byproducts significantly reduced the 5-HMF yield (Aida et al., 2007; Antal et al., 1990; Bonn & Bobleter, O., 1983).

Catalysts used for 5-HMF production in aqueous phase were mostly limited to mineral acids (e.g. H₂SO₄, H₃PO₄, and HCl) (Asghari & Yoshida, 2006; Shen & Wyman, 2012a), organic
acids (e.g. oxalic acid, ρ-toluenesulfonic acid) (Asghari & Yoshida, 2006), H-form zeolites (Moreau et al., 2000), strong acid cation exchange resins (Rigal et al., 1981; Roman-Leshkov et al., 2006), and solid metal phosphates (Benvenuti et al., 2000; Carlini et al., 1999). However, mineral acid and organic acid possess the catalytic ability of accelerating the rehydration of 5-HMF thereby limiting its yield (Asghari & Yoshida, 2006; Shen & Wyman, 2012a); heterogeneous catalysts, such as H-form zeolites, strong acid cation exchange resins and solid metal phosphates, are often not suitable to catalyze water-insoluble polysaccharides (e.g. cellulose) into degradation products (e.g. 5-HMF) due to mass transfer limitation (Huang & Fu, 2013).

In recent years, metal chlorides were shown effective for transforming saccharides into 5-HMF because they can assist the isomerization of aldose to ketose (e.g. glucose to fructose) (Deng et al., 2012; Yang et al., 2012), which is a key step for 5-HMF production (Binder et al., 2010). In addition, as homogeneous catalysts (Dutta et al., 2012), metal chlorides possess the potential of being easily separated from reaction products such as 5-HMF through semipermeable membranes. For example, Kotraro et al. (Linder, 1988) reported that the use of semipermeable composite membranes for separating organic compounds (molecular weight<300) such as furan from aqueous inorganic salts (e.g. NaCl) containing solutions. Use of CrCl₃ for 5-HMF production was reported by various authors. Qi et al. (Qi et al., 2010) reported that 70% glucose was converted into 5-HMF when 99% glucose was degraded with 1.5% (w/w) CrCl₃ in ionic liquid solvents. Similarly, Li et al. (2009) also presented that 0.5% (w/w) CrCl₃ led to 60% 5-HMF yield from cellulose
substrate in ionic liquid under microwave irradiation. However, with same catalyst (CrCl$_3$) in aqueous phase, the yield of 5-HMF was decreased to around 5% (Peng et al., 2010). Catalysis of cellulose by 0.01 M CrCl$_3$ at 180 °C for 120 min led to 5% 5-HMF yield and 30% levulinic acid yield. (Peng et al., 2010) In addition to CrCl$_3$, several other kinds of metal chlorides were studied for 5-HMF production in aqueous phase. 16.1% 5-HMF yield was achieved when 63% (w/w) ZnCl$_2$ was applied to 5-HMF production from glucose in aqueous phase at 120 °C (Deng et al., 2012). The limited 5-HMF yield from saccharides (e.g. cellulose, glucose) with ZnCl$_2$ was mainly attributed to the formation of byproduct lactic acid during the process (Kong et al., 2008; Rasrendra et al., 2010). Seri et al. (Seri et al., 2002) reported that a 20% 5-HMF yield was obtained from cellulose using 0.25% (w/w) LaCl$_3$ in aqueous phase at 250 °C within 2.5 min, and 5% levulinic acid was simultaneously formed. De et al. (De et al., 2011) applied AlCl$_3$ to catalyze conversion of inulin and starch to 5-HMF. Around 30% 5-HMF yield was obtained from these polysaccharides at 120 °C within 5 min, (De et al., 2011) and byproducts levulinic acid were considered as the cause for limiting the 5-HMF yields (De et al., 2011). These studies of using transition metal chlorides catalyzed reaction suggested low 5-HMF selectivity due to the formation of undesired byproducts such as levulinic acid (Peng et al., 2010) and lactic acid (Rasrendra et al., 2010). Rasrendra et al. (2010) reported poor 5-HMF selectivity (around 3%−27%) when catalyzing glucose under 140 °C for 6h. The use of alkali and alkaline earth metal chlorides for 5-HMF production from mono-, oligo-, or polysaccharides were seldom reported. Nevertheless, Liu and Wyman (Liu & Wyman, 2006) investigated the degradation of xylotriose with 0.8% (w/w) NaCl,
CaCl$_2$ and MgCl$_2$ under hot water conditions at 180 °C and found that the rate constants of xylotriose degradation to furfural (k) using CaCl$_2$ and MgCl$_2$ were around 2.5 fold greater than that with water-only treatment. In addition, Sabesan and Spado (Sabesan & Spado, 2013) obtained 66% furfural yield from biomass with 1 wt. % CaCl$_2$ (160 °C, 30 min, water and THF mixture (1:1) system), which was 18% higher than that without CaCl$_2$. The potential of applying alkaline earth metal/alkali metal chlorides on decomposing polysaccharides (e.g. agarose) to 5-HMF needs more studies. Further efforts are needed to realize enhanced selectivity and yield of 5-HMF in aqueous phase using efficient catalysts. The objective of this study was to investigate effects of different groups of metal chlorides, including alkali metal chlorides, alkaline earth metal chlorides, and transition metal chlorides, on the degradation of agarose to 5-HMF, in order to improve 5-HMF yield with less byproducts in aqueous phase using non-food based feedstocks. The mechanism of agarose to 5-HMF with desired metal chlorides was further discussed.

3.3. Material and methods

3.3.1. Feedstocks, catalysts and standards

Agarose (BP160, Fisher Scientific) was purchased from Fisher Scientific, Pittsburgh, PA. Galactan was defined as the unit of agarose and the purity is 98%. The galactan content was determined following the Laboratory Analytical Procedure (LAP) of “Determination of Structural Carbohydrates and Lignin in Biomass” (Sluiter, 2008). This procedure employed a two-step acid hydrolysis: 1) about 300 mg substrate was placed into a vial and hydrolyzed in
72 wt. % sulfuric acid at 30 °C for 2 hours; 2) the substrate was further hydrolyzed in 4 wt. % sulfuric acid at 121 °C for 1 hour. Sugars in the liquid were determined by HPLC. NaCl, CaCl₂, MgCl₂, ZnCl₂, CrCl₃, CuCl₂ and FeCl₃ (Alfa Aesar, Ward Hill, MA) were used as the catalysts for 5-HMF production. All standard chemicals were purchased from Sigma-Aldrich, St. Louis, Mo.

3.3.2. Experimental methods

Batch tubular reactors (1.27 cm OD × 15.24 cm length, 0.0889 cm wall thickness, Hastelloy C-276) was installed with the accessories purchased from Swagelok Northwest (Richland, WA). The total volume of each reactor was 13.6 ml with 10 ml of working volume. 0.2 g of substrate was loaded into the batch tubular reactor with 10 ml of water, sulfuric acid (e.g. 0.5 wt. %, 1 wt. %, 5 wt. %) or metal chlorides (e.g. 0.5 wt. %, 1 wt. % or 5 wt. %), respectively. Reactors were heated to reach the target temperature within 1 min in a 4-kW fluidized sand bath (model SBL-2D, Omega engineering, Inc., Stamford, CT) and treated at target temperatures. The reactions were quenched within 1 min by immersing the batch tubular reactor in cold water bath after being subjected to the target reaction temperature for a specified time. After cooling the tubular reactor, the caps and plugs were removed, samples were pushed out and separated into liquid hydrolyzate and solid residue by vacuum filtration using a 0.22 µm glass fiber filter (Fisher Science, Pittsburgh, PA) for analysis.

3.3.3. Analytical methods

3.3.3.1. Liquid analysis
HPLC analysis

Galactose, 5-HMF, levulinic acid, formic acid, glyceraldehyde, dihydroxyacetone and lactic acid in aqueous solution were analyzed through Waters HPLC system (model 2695) equipped with a 410 refractive detector and a Waters 2695 auto-sampler using Waters Empower Build 1154 software (Waters Co., Milford, MA). Bio-Rad Aminex HPX-87H column (Bio-Rad Laboratories, Hercules, CA) were operated under 65 °C for separation and quantification of compounds. Mobile phase was 0.005M H$_2$SO$_4$ with flow rate of 0.6 ml/min.

GC-MS analysis

The collected samples were diluted with acetone then analyzed with an Agilent gas chromatography mass spectrometer (GC–MS; GC, Agilent 7890A; MS, Agilent 5975C) equipped with a DB-5MS column (30m × 250µm × 0.25µm). (Bahaffi & Al-lihaibi, 2005) The oven temperature was programmed from 40 °C to 300 °C at a ramping rate of 10 °C /min. Both the initial and final temperatures were held for 5 minutes. The flow rate of the carrier gas (helium) was 1.3 ml/min.

3.3.3.2. Solid analysis

The agarose in solid residue after separation was analyzed based on a standard analysis procedure developed by National Renewable Energy Laboratory (NREL) (Sluiter, 2008). This procedure was a two-step acid hydrolysis: the sample was first treated with 72% (w/w) H$_2$SO$_4$ at 30 °C for 1 h; the reaction mixture was subsequently diluted to 4% (w/w) H$_2$SO$_4$ and autoclaved at 121 °C for 1 hour. The sugars in liquid after this two step procedure were then determined by HPLC. Bio-Rad Aminex HPX-87H column (Bio-Rad Laboratories,
Hercules, CA) were operated under 65 °C for separation and quantification of sugars.

Mobile phase was 0.005M H₂SO₄ with flow rate of 0.6 ml/min.

### 3.3.3. Calculation

The overall reactions include: galactan (galactan is defined as the unit of agarose) to galactose; galactose to 5-HMF; 5-HMF to levulinic acid and formic acid; galactose to glyceraldehyde, dihydroxyacetone or lactic acid. These reactions can be expressed as Eq.1-4, respectively:

1. \[ C₆H₁₀O₅ + H₂O \rightarrow C₆H₁₁O₆ \]  
2. \[ C₆H₁₀O₅ \rightarrow C₃H₅O₃ + 3H₂O \]  
3. \[ C₆H₁₀O₅ + 2H₂O \rightarrow C₃H₅O₃ + CH₂O₂ \]  
4. \[ C₆H₁₀O₅ \rightarrow 2C₃H₅O₃ \]

The yields of degradation compounds were based on the original amount of galactan. Concentrations (g/L) of galactose, 5-HMF, levulinic acid, formic acid, glyceraldehyde, dihydroxyacetone and lactic acid measured by HPLC were converted to yields as a percent of the theoretical maximum, as follows:

**Galactose yield:**

\[ Y_{gal}(\%) = \frac{C_{ac} \times MW_{gal}}{C_{toc} \times MW_{gal}} \times 100\% \]  

**5-HMF yield:**

\[ Y_{5HMF}(\%) = \frac{C_{5HMF} \times MW_{5HMF}}{C_{toc} \times MW_{5HMF}} \times 100\% \]
Levulinic acid yield:

\[ Y_{\text{LA}}(\%) = \frac{C_{\text{LA}} \times MW_{\text{LA}}}{C_{\text{Gan}} \times MW_{\text{Gan}}} \times 100\% \]  

Formic acid yield:

\[ Y_{\text{FA}}(\%) = \frac{C_{\text{FA}} \times MW_{\text{FA}}}{C_{\text{Gan}} \times MW_{\text{Gan}}} \times 100\% \]  

Glyceraldehyde yield:

\[ Y_{\text{Gly}}(\%) = \frac{C_{\text{Gly}} \times MW_{\text{Gly}}}{C_{\text{Gan}} \times (2 \times MW_{\text{Gan}})} \times 100\% \]  

Dihydroxyacetone yield:

\[ Y_{\text{Dih}}(\%) = \frac{C_{\text{Dih}} \times MW_{\text{Dih}}}{C_{\text{Gan}} \times (2 \times MW_{\text{Gan}})} \times 100\% \]  

Lactic acid yield:

\[ Y_{\text{Lac}}(\%) = \frac{C_{\text{Lac}} \times MW_{\text{Lac}}}{C_{\text{Gan}} \times (2 \times MW_{\text{Gan}})} \times 100\% \]  

In these equations, \( C_{\text{Gan}} \) is the initial concentration of galactan; \( C_{\text{Ga}} \) is the concentration of galactose; \( C_{\text{HMF}} \) is the concentration of 5-HMF; \( C_{\text{LA}} \) is the concentration of levulinic acid; \( C_{\text{FA}} \) is the concentration of formic acid; \( C_{\text{Gly}} \) is the concentration of glyceraldehyde; \( C_{\text{Dih}} \) is the concentration of dihydroxyacetone; \( C_{\text{Lac}} \) is the concentration of lactic acid. MW is the molecular weight: MW\(_{\text{Gan}} = 162\); MW\(_{\text{Ga}} = 180\); MW\(_{\text{HMF}} = 126\); MW\(_{\text{LA}} = 116\); MW\(_{\text{FA}} = 46\); MW\(_{\text{Gly}} = 90\); MW\(_{\text{Dih}} = 90\); MW\(_{\text{Lac}} = 90\).

The agarose conversion was evaluated as below:

\[ X = \frac{Y_f - Y_0}{Y_0} \times 100\% \]  

\( X \) is the conversion of agarose, \( Y_0 \) (g) is the initial weight of agarose, \( Y_f \) (g) is the agarose in
solid residue.

The selectivity of degradation compounds from agarose was calculated as below:

$$S = \frac{Y}{X} \times 100\% \quad (13)$$

$S$ is the selectivity of product; $Y$ is the yield of product; $X$ is the conversion of agarose.

3.4. Results and discussion

3.4.1. Catalytic effects of metal chlorides on the products distribution and 5-HMF yield from agarose

The effects of 0.5 wt.%-5 wt.% metal chlorides including alkali metal chlorides (NaCl), alkaline earth metal chlorides (CaCl₂ and MgCl₂), and transition metal chlorides (ZnCl₂, CuCl₂, FeCl₃ and CrCl₃), on the products distribution from 2 wt.% agarose were investigated at varied temperatures ranging from 180-220 °C. Water-only and dilute sulfuric acid treatments were used as references. The 5-HMF yields from agarose catalyzed by these metal chlorides during the time course of 0-50 min were also investigated. In addition, the 5-HMF selectivity obtained by varied metal chlorides and the reference conditions (water-only and dilute sulfuric acid) was also studied.

3.4.1.1. Effects of temperature

Figure 1 shows the effects of 1 wt.% metal chlorides on the distribution of products decomposed from agarose at 180 °C, 200 °C and 220 °C for 30 min. Transition metal chlorides ZnCl₂ and CrCl₃ resulted in various compounds such as galactose, 5-HMF, lactic acid, glyceraldehyde and dihydroxyacetone, which was similar to water-only treatment. In addition to these compounds, CrCl₃ led to the formation of levulinic acid. Results suggested
that transition metal chlorides ZnCl₂ and CrCl₃ could catalyze the degradation process via both reactions of dehydration (Aida et al., 2007) and retro aldol (Rasrendra et al., 2010) to these multiple compounds. Rasrendra et al. (2010) reported that multiple products, including lactic acid and 5-HMF, were observed from the decomposition of glucose in aqueous phase at 140 °C for 6 h using 6.8 wt.% ZnCl₂ and 6.1 wt.% CrCl₂. Levulinic acid yields were reported at 6.1 wt. % using CrCl₂ only. Temperature played an important role in determining the distribution of these degradation compounds from agarose catalyzed by ZnCl₂, CrCl₃, as well as that under water-only conditions. When temperature was raised from 180 °C to 200 °C, galactose yields decreased sharply from 22.6 to 2.7% with ZnCl₂, and from 5.3% to 0.3% with CrCl₃, while the yields of 5-HMF catalyzed with ZnCl₂ and CrCl₃ increased from 15.9% and 9.8% at 180 °C to the highest yields of 20.9% and 12.9% at 200 °C, respectively. The yields of byproducts also increased as temperature was elevated from 180 °C to 200 °C, thus limiting the production of 5-HMF. When the temperature was further raised to 220 °C, 5-HMF yields with ZnCl₂ and CrCl₃ decreased; the yields of byproducts such as lactic acid declined as well; whereas the yields of glyceraldehyde and dihydroxyacetone continuously increased, which further reduced the 5-HMF yield. The distribution of galactose, 5-HMF, and various byproducts under water-only conditions showed a similar trend as when catalyzed by ZnCl₂ and CrCl₃ over the temperature range of 180 °C to 220 °C. However, results indicated both slower formation and degradation of such compounds. Other transition metal chlorides used in this study, including CuCl₂ and FeCl₃, resulted in low yields of galactose (2.2% – 19.4%) and significant quantity of levulinic acid (27.3% – 41.2%), but negligible amount of
5-HMF at all tested temperatures. The decomposition of agarose catalyzed by H$_2$SO$_4$ showed similar pattern of products distribution compared with CuCl$_2$ and FeCl$_3$; only galactose (0 – 8.5%) and levulinic acid (38.6%-40.8%) were observed at temperatures ranging from 180 °C-220 °C. The formation of diverse byproducts (e.g. lactic acid, levulinic acid, etc.) catalyzed by transition metal chlorides contributed to the relatively lower or negligible 5-HMF yield. Temperature (180 °C–220 °C) had negligible effects on the types of generated products when using transition metal chlorides for the agarose degradation. However, temperatures influenced the reaction rates of these degradation products, thereby impacting the yields of 5-HMF and overall byproducts.

Addition of alkali and alkaline earth metal chlorides NaCl, CaCl$_2$ and MgCl$_2$ merely led to 5-HMF and galactose at all tested temperatures. The distribution of galactose and 5-HMF decomposed from agarose catalyzed by NaCl, CaCl$_2$ and MgCl$_2$ over the three temperatures (180 °C, 200 °C and 220 °C) followed a similar trend. At 180 °C, 29.3%-32.5% 5-HMF and 37.5%-38.8% galactose yields were obtained with these alkali and alkaline earth metal chlorides. As temperature increased to 200 °C, 5-HMF yield increased to 31.7%-39.5%, while the galactose yield decreased to 19.1%-23.1%. Yields of 5-HMF and galactose sharply decreased to 19.7%-22.5% and 2.4%-8.5%, respectively, when temperature was further raised to 220 °C. The reduced yield of 5-HMF could be attributed to the formation of humins, which were observed by Patil and Lund (Patil & Lund, 2011a) as the decomposed product of 5-HMF under 135 °C in aqueous phase. The highest 5-HMF yields obtained with NaCl, CaCl$_2$ and MgCl$_2$ were 31.7%, 35.8% and 39.6% at 200 °C, respectively.
3.4.1.2. Effects of catalyst loading

The distribution of degradation products from agarose with metal chlorides was also investigated under different catalyst loadings. The effects of concentrations of the metal chlorides (0.5 wt.%, 1 wt.% and 5 wt.% ), including NaCl, CaCl2, MgCl2, ZnCl2, CrCl3, CuCl2 and FeCl3, as well as H2SO4 treatment (0.5 wt.%, 1 wt.% and 5 wt.%) on degradation of agarose at 200 °C for 30 min are shown in Figure 2. Only galactose and 5-HMF were obtained from agarose using alkali and alkaline earth metal chlorides (NaCl, CaCl2 and MgCl2) over the three catalyst loadings (0.5 wt.% , 1 wt.% and 5 wt.%). Galactose yields decreased with the elevated loading of these alkali and alkaline earth metal chlorides. 5-HMF yields from agarose using these three catalysts increased to the highest value, which were 31.7%, 35.8%, 39.6%, respectively, when loading was increased from 0.5 wt.% to 1 wt.%. As the catalyst loading was further increased to 5 wt.%, the 5-HMF yield decreased sharply. Transition metal chlorides with loadings of 0.5 wt.% , 1 wt.% and 5 wt.% led to various kinds of byproducts. Besides galactose and 5-HMF, 0.5% (w/w) transition metal chlorides ZnCl2 and CrCl3 generated byproducts such as lactic acid, glyceraldehyde, and dihydroxyacetone. Moreover, levulinic acid was also observed with 0.5% (w/w) CrCl3. The increased catalyst loading presented insignificant impact on the types of compounds generated from agarose, whereas had significant influence on their yields. Galactose yield was reduced sharply from 0.5-7.7% to negligible amounts as the loading of ZnCl2 and CrCl3 was raised from 0.5 wt.% to 5 wt.%. In contrast, the yields of 5-HMF and other products catalyzed by ZnCl2 and CrCl3 increased when the catalyst loading was enhanced from 0.5
wt.% to 1 wt.% resulted in the highest 5-HMF yields of 19.0% and 12.9%, respectively. Further increasing the catalyst loading from 1% (w/w) to 5% (w/w) led the yields of 5-HMF and those byproducts to decline significantly. Other transition metal chlorides CuCl₂ and FeCl₃ as well as H₂SO₄ merely resulted in less than 3.5% yield of galactose and a considerable amount of levulinic acid (19.5 – 41.3% yield) over the three tested catalyst loadings (i.e. 0.5%, 1% and 5 wt.%), while 5-HMF was negligible. It was found that the loading of metal chlorides and H₂SO₄ had a minor impact on the variety of degradation compounds from agarose. However, the catalyst loading had evident effects on the distribution of these degradation compounds, thereby leading to different 5-HMF yield.

3.4.1.3. Effects of reaction time

The time courses of 5-HMF yields from agarose by 1 wt. % metal chlorides, water-only and 1 wt.% H₂SO₄ at 200 °C for 0-50 min are shown in Figure 3 Results showed that catalytic reaction with transition metal chlorides CuCl₂ and FeCl₃ led to similar trends as 1 wt.% H₂SO₄, and the highest yield of around 5% 5-HMF was obtained at 5min, and then rapidly decreased. 5-HMF yields with transition metal chlorides ZnCl₂ and CrCl₃ were relatively higher, which were 22.6% at 25 min and 12.9% at 30 min, respectively. However, similar to CuCl₂ and FeCl₃, the 5-HMF yield catalyzed by ZnCl₂ and CrCl₃ declined sharply as reaction time was prolonged. These observations suggested that transition metal chlorides catalyzed 5-HMF production were sensitive to reaction time, which required the reaction system with better control of residence time (e.g. flowthrough reactor). 5-HMF yields catalyzed by alkali
and alkaline earth metal chlorides NaCl, CaCl$_2$, MgCl$_2$, and water–only treatments increased steadily over time, then declined slowly at around 35-40 min. The highest yields of 5-HMF catalyzed by NaCl, CaCl$_2$ or with water-only treatment were 32.8%, 37.2% and 27.4% at 40 min, respectively. MgCl$_2$ resulted in the highest yield of 5-HMF 40.7% at 35 min. Results implied that alkali and alkaline earth metal chlorides (NaCl, CaCl$_2$ and MgCl$_2$) behaved with suitable catalytic activity for the conversion of agarose to 5-HMF, while simultaneously maintaining the relative stability of 5-HMF as reaction prolonged.

3.4.1.4. Comparison of the highest 5-HMF yield with varied metal chlorides and the corresponding selectivity

5-HMF yields catalyzed by metal chlorides (NaCl, CaCl$_2$, MgCl$_2$, ZnCl$_2$, CrCl$_3$, CuCl$_2$ and FeCl$_3$) were optimized under temperature 180-220 °C, time 0-50 min and catalyst loading 0.5% (w/w)-5%(w/w), respectively. Table 1 summarized and compared the highest 5-HMF yields obtained with varied metal chlorides, water-only and H$_2$SO$_4$ treatment. In addition, the corresponding 5-HMF and byproducts selectivity as well as the agarose conversion were also listed and discussed. Results (Table 1) demonstrated that addition of alkali and alkaline earth metal chlorides (NaCl, CaCl$_2$ and MgCl$_2$) led to higher 5-HMF yields than transition metal chlorides (ZnCl$_2$, CrCl$_3$, CuCl$_2$, FeCl$_3$), water-only and H$_2$SO$_4$. Particularly, MgCl$_2$ resulted in the highest 5-HMF yield of 40.7%. The relatively higher 5-HMF yields with alkali and alkaline earth metal chlorides (NaCl, CaCl$_2$ and MgCl$_2$) compared with transition metal chlorides (ZnCl$_2$, CrCl$_3$, CuCl$_2$, FeCl$_3$), water-only and H$_2$SO$_4$ could be attributed to fewer byproducts formed under tested conditions. The corresponding 5-HMF selectivity with varied
metal chlorides listed in Table 1 suggested that alkali and alkaline earth metal chlorides (NaCl, CaCl$_2$ and MgCl$_2$) with fewer byproducts resulted in higher 5-HMF selectivity than transition metal chlorides (ZnCl$_2$, CrCl$_3$, CuCl$_2$, FeCl$_3$), water-only and H$_2$SO$_4$. 5-HMF selectivity obtained using MgCl$_2$ resulted in the highest value of 49.1%. Such selectivity was comparable to the 5-HMF selectivity (40%–53%) obtained from glucose in acidic biphasic systems, as reported by Dumesic and coworkers. (Chheda et al., 2007) In comparison, the considerable selectivity for byproducts (e.g. lactic acid) formed via retro-aldol reaction could serve as a possible explanation of the relatively low 5-HMF selectivity from water-only or ZnCl$_2$ treatment. For example, lactic acid selectivity for ZnCl$_2$ catalyzed reactions was 15.8%, while the corresponding 5-HMF selectivity was merely 24.5%. Using CuCl$_2$, FeCl$_3$ and H$_2$SO$_4$ led to 20.4%–26.9% selectivity toward levulinic acid through further rehydration of 5-HMF, while their selectivity toward 5-HMF didn’t exceed 9.0%. CrCl$_3$ resulted in all byproducts listed in Table 1 with the selectivity ranging from 7.5%–13.7% for each compound, consequently leading to 13.0% 5-HMF selectivity. However, the galactose selectivity for alkali and alkaline earth metal chlorides (NaCl, CaCl$_2$ and MgCl$_2$) catalyzed reaction was maintained on a relatively high level (20.5%–25.2%) compared with some transition metal chlorides (e.g. CrCl$_3$) (0.5%), thus reducing the 5-HMF selectivity. Furthermore, the limited 5-HMF selectivity could be also a consequence of the formation of aforementioned (section 3.1.1) insoluble humans (Patil & Lund, 2011a). The agarose conversion with alkali and alkaline earth metal chlorides (NaCl, CaCl$_2$ and MgCl$_2$), transition metal chlorides (ZnCl$_2$, CrCl$_3$, CuCl$_2$ and FeCl$_3$) were obtained 81.1%–100% (Table 1) under
conditions for achieving the highest 5-HMF yield, which demonstrated the agarose’s susceptibility to decomposition with both alkali and alkaline earth metal chlorides (NaCl, CaCl₂ and MgCl₂) and transition metal chlorides (ZnCl₂, CrCl₃, CuCl₂ and FeCl₃). Different from agarose, when using metal chlorides to hydrolyze cellulose in aqueous phase under 180 °C over 120 min, alkali and alkaline earth metal (e.g. NaCl, CaCl₂ and MgCl₂) showed negligible catalytic ability on cellulose conversion while transition metal chlorides (e.g. CrCl₃, FeCl₃ and CuCl₂) converted cellulose efficiently.

3.4.2. Kinetic analysis of 5-HMF formation from agarose with MgCl₂

MgCl₂ appeared to result in the highest 5-HMF yield and selectivity among various tested metal chlorides under experimental conditions. A kinetic analysis of 5-HMF production from agarose was performed with 1% (w/w) MgCl₂ at a temperature range of 180 °C-220 °C within 50 min. A pseudo-homogeneous irreversible first order reaction model was used to describe the degradation of agarose to 5-HMF with low substrate concentration (2% (w/w)). This model was also used to describe cellulose (0.8% (w/w)-2.4% (w/w)) degradation to 5-HMF and levulinic acid in aqueous phase with dilute acid (Shen & Wyman, 2012a).

Results indicated that when agarose was decomposed with MgCl₂, 5-HMF and galactose were major products without formation of other byproducts, such as levulinic acid, lactic acid, etc. Therefore, the reaction pathway of agarose decomposition in MgCl₂ solution can be simply summarized as follows:

\[
\begin{align*}
\text{Agarose} & \xrightarrow{k_1} \text{Galactose} & \xrightarrow{k_2} \text{5-HMF} & \xrightarrow{k_3} \text{Degradation products (e.g. Humins)}
\end{align*}
\]
Based on the reaction pathway, the kinetic model equations were developed to describe the agarose degradation process as follows:

\[
\frac{dA}{dt} = -k_1 A \quad (1)
\]
\[
\frac{dG}{dt} = k_1 A - k_2 G \quad (2)
\]
\[
\frac{dM}{dt} = k_2 G - k_3 M \quad (3)
\]

Where A, G and M represent the yields of agarose, galactose and 5-HMF, respectively, which were expressed in the unit of yield(%) (Gil Garrote, 1999; Lu & Mosier, 2008). \( k_1 \) (min\(^{-1}\)), \( k_2 \) (min\(^{-1}\)) and \( k_3 \) (min\(^{-1}\)) are rate constants of agarose hydrolyzed into galactose, galactose dehydrated into 5-HMF, and 5-HMF condensed to humins, respectively. Table 2 shows the value of the rate constants increased with reaction temperature. The rate constant \( k_3 \) was lower than both \( k_1 \) and \( k_2 \) over the tested temperatures (180—220 °C), which was especially apparent at 200 °C (i.e. \( k_3 = 0.0399 \text{ min}^{-1} \), \( k_1 = 0.0937 \text{ min}^{-1} \), \( k_2 = 0.0729 \text{ min}^{-1} \)). Results implied that the formation of 5-HMF was much more feasibly than its degradation under such conditions, thus resulted in 5-HMF yield maintaining at a relatively high level (e.g. 40.7%). The Arrhenius equation was also established:

\[
k = A \times \exp \left( -\frac{E_a}{RT} \right) \quad (4)
\]

Where k (min\(^{-1}\)) is the rate constant, A (min\(^{-1}\)) is the pre-exponential factor, \( E_a \) (kJ/mol) is the activation energy, \( R \) is universal gas constant (8.3143 × 10\(^{-3}\) kJ mol\(^{-1}\)K\(^{-1}\)), \( T \) (K) is temperature. The rate constants obtained in MgCl\(_2\) catalyzed agarose degradation pathway were also put into the Arrhenius plot, \( \ln(k) \) vs \( 1/T \) (Figure 3.6).
The activation energy values of the degradation of agarose, galactose and 5-HMF calculated based on Equation 4 were 67.5 kJ/mol, 104.6 kJ/mol and 105.6 kJ/mol, respectively. The lower activation energy for agarose degradation compared with its monomer and 5-HMF indicated the requirement of relatively lower temperature for agarose degradation in 1% (w/w) MgCl₂, which consequently could be favorable for the stability of the released 5-HMF.

### 3.4.3. Postulated mechanism of agarose to 5-HMF catalyzed by MgCl₂

In order to gain more insights into the mechanism of 5-HMF formation from agarose with MgCl₂ treatment, the formation of degradation products derived from MgCl₂ treatments (200 °C, 1% (w/w), 35 min) was further verified by GC-MS analysis. The GC-MS spectra showed that 5-HMF was the dominant compound from agarose catalyzed by MgCl₂, and no levulinic acid from further rehydration of 5-HMF or compounds from retro aldol of galactose were detected (Figure 4). Trace amount of furyl hydroxymethyl ketone (C₆H₆O₃), an isomer of 5-HMF was observed. Formation of this isomer can be explained as a C3 carbonyl and C6 hydroxy group intermolecular closure, while the formation of 5-HMF is caused by the pathway of C2 and C5 ring closure. The reaction mechanism for MgCl₂ catalyzing agarose to 5-HMF in aqueous phase is proposed in Figure 5. It can be anticipated that Mg²⁺ ions (MgCl₂) would be able to coordinate with the oxygen atom of C-O-C bond and subsequently catalyze the cleavage of the C-O-C bond acting as Lewis acid. Coordination of Mg²⁺ ions (MgCl₂) to the oxygen atom of C-O-C in sugars(e.g. sucrose) in aqueous phase has also been supported with IR and ¹³C NMR spectra(Rondeau et al., 2003). Once agarose is hydrolyzed into galactose, magnesium ions from MgCl₂
coordinate with the oxygen atom of the hemiacetal portion of galactose and the closest hydroxyl group to form an enediol intermediate. This leads to the isomerization of galactose to its ketose form, which can be easily dehydrated to 5-HMF. Although other alkali and alkaline earth metal ions, including Na\(^+\) ions and Ca\(^{2+}\) ions, appeared to interact with oxygen atom in sugar (e.g. oxygen atom of C-O-C bond)\(\text{(Rondeau et al., 2003)}\), thereby enhancing the agarose to 5-HMF reactions as compared to water-only operations, Mg\(^{2+}\) ions may possess better activity due to its higher charge density (charge-to-size ratio) than Na\(^+\) ions and Ca\(^{2+}\) ions\(\text{(Rondeau et al., 2003)}\).

Results implied that Mg\(^{2+}\) ions under the tested conditions possess suitable properties for 5-HMF production: Mg\(^{2+}\) ions can promote the cleavage of C-O-C bond of agarose and enhance the subsequent isomerization of galactose to its ketose. Additionally, our results also suggested that Mg\(^{2+}\) ions have little ability to induce the retro aldol reaction of galactose or further rehydration of 5-HMF to levulinic acid, etc, thereby maintaining a relatively higher 5-HMF yield with few byproducts (as depicted in Figure 4).

**3.5. Conclusion**

Metal chlorides had different effects on catalyzing agarose degradation in aqueous phase. The addition of alkali and alkaline earth metal chlorides was found to result in the formation of only galactose and 5-HMF while the addition of transition metal chlorides led to the formation of various degradation products, including levulinic acid, lactic acid, etc. Although temperature and catalyst loading had significant effects on reaction rates and the products distribution, the variety of agarose degradation products catalyzed by these metal chlorides...
was dependent on the intrinsic properties of the catalysts used. Alkali and alkaline earth metal chlorides NaCl, CaCl₂ and MgCl₂ resulted in higher 5-HMF yields and selectivity than transition metal chlorides ZnCl₂, CuCl₂, FeCl₃ and CrCl₃, water-only or dilute sulfuric acid treatments. The addition of 1% (w/w) MgCl₂ was the most favorable additive among the tested metal chlorides, resulting in 40.7% of the 5-HMF yield and 49.1% 5-HMF selectivity at 200 °C within 35 min. The mechanism of the MgCl₂ catalyzed reaction of agarose to 5-HMF was proposed to proceed through assisting the cleavage of C-O-C bond of agarose and accelerating the subsequent isomerization of galactose to its ketose. The specific ability of MgCl₂ in aqueous phase is thought to be the driving force for favoring the 5-HMF formation, while diminishing the generation of undesired byproducts such as levulinic acid, lactic acid, etc.
3.6. References


Figure 3.1 Distribution of agarose decomposition products by various metal chlorides, water-only, H$_2$SO$_4$ treatments under different temperature (180 °C, 200 °C and 220 °C). Conditions: 30 min, 1% (w/w) metal chlorides, water-only or 1% (w/w) H$_2$SO$_4$. Lac: lactic acid; Gly:glyceraldehyde; Dhy: dihydroxyacetone; LA: levulinic acid; 5-HMF: 5-hydroxymethylfurfural; Ga:galactose.
Figure 3.2 Distribution of agarose decomposition products by various metal chlorides, water-only and H$_2$SO$_4$ treatments under different loading (0.5% (w/w), 1% (w/w), 5% (w/w)). Conditions: 200 °C for 30 min. Lac: lactic acid; Gly: glyceraldehyde; Dhy: dihydroxyacetone; LA: levulinic acid; 5-HMF: 5-hydroxymethyl furfural; Ga: galactose.
Figure 3.3 Time course of 5-HMF yield decomposed from agarose by water-only, 1% (w/w) dilute sulfate acid and 1% (w/w) various metal chlorides treatments at 200 °C.
Figure 3.4 Product profiles in hydrolysates from treatments with 1% (w/w) MgCl\(_2\) at 200 °C for 35 min analyzed by GC-MS.
Figure 3.5 Proposed reaction mechanism for Mg\textsuperscript{2+} catalyzing the decomposition of agarose to 5-HMF.
Figure 3.6 Arrhenius plot for rate constants in hydrolysis of agarose with MgCl₂ (180–220 °C, batch reactor, 1% (w/) MgCl₂).
Table 3.1 The highest 5-HMF yield and product selectivity from agarose degradation by various metal chlorides, H$_2$SO$_4$ and water-only treatment

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Conditions$^a$</th>
<th>5-HMF yield (%)</th>
<th>Agarose Conv. (%)</th>
<th>Selectivity</th>
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<tr>
<td></td>
<td></td>
<td>5-HMF</td>
<td>Ga</td>
<td>LA</td>
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<tr>
<td>MgCl$_2$</td>
<td>200°C/1%(w/w)/35min</td>
<td>40.7</td>
<td>82.9</td>
<td>49.1</td>
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<td>CaCl$_2$</td>
<td>200°C/1%(w/w)/40min</td>
<td>37.2</td>
<td>85.3</td>
<td>43.6</td>
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<tr>
<td>NaCl</td>
<td>200°C/1%(w/w)/40min</td>
<td>32.8</td>
<td>81.1</td>
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<tr>
<td>None</td>
<td>220°C/1%(w/w)/20min</td>
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<td>37.2</td>
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<tr>
<td>ZnCl$_2$</td>
<td>200°C/1%(w/w)/25min</td>
<td>22.6</td>
<td>92.1</td>
<td>24.5</td>
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<tr>
<td>CrCl$_3$</td>
<td>200°C/1%(w/w)/30min</td>
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<td>13.0</td>
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<td>CuCl$_2$</td>
<td>200°C/0.5%(w/w)/10min</td>
<td>8.2</td>
<td>91.6</td>
<td>9.0</td>
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<tr>
<td>FeCl$_3$</td>
<td>200°C/0.5%(w/w)/10min</td>
<td>7.7</td>
<td>93.8</td>
<td>8.2</td>
</tr>
<tr>
<td>H$_2$SO$_4$</td>
<td>200°C/0.5%(w/w)/5min</td>
<td>7.1</td>
<td>90.3</td>
<td>7.9</td>
</tr>
</tbody>
</table>

$^a$: conditions to reach the highest 5-HMF yields

Gal: galactose; LA: levulinic acid; Gly: glyceraldehyde; Dhy: dihydroxyacetone; Lac: lactic acid.
Table 3.2 Rate constant of each degradation step of agarose with 1% (w/w) MgCl₂

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Temperature(°C)</th>
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<th></th>
</tr>
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<tbody>
<tr>
<td></td>
<td>180</td>
<td>200</td>
<td>220</td>
</tr>
<tr>
<td>$k_1$ (min⁻¹)</td>
<td>0.0426</td>
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<td>$k_2$ (min⁻¹)</td>
<td>0.0327</td>
<td>0.0729</td>
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<tr>
<td>$k_3$ (min⁻¹)</td>
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<tr>
<td>$R^2_{Aga}$</td>
<td>0.977</td>
<td>0.959</td>
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<tr>
<td>$R^2_{Ga}$</td>
<td>0.947</td>
<td>0.972</td>
<td>0.969</td>
</tr>
<tr>
<td>$R^2_{5-HMF}$</td>
<td>0.976</td>
<td>0.960</td>
<td>0.944</td>
</tr>
</tbody>
</table>

Aga: agarose; Ga: galactose;
Chapter 4

Enhancement of total sugar and lignin yields through dissolution of poplar wood by hot water and dilute acid flowthrough pretreatment

(This chapter has been submitted to Biotechnology for biofuels)

4.1. Abstract

Flowthrough pretreatment provides a promising platform to dissolution of lignocellulosic biomass to generate high yields of fermentable sugars and lignin for biofuels productions. In this study, dissolution of xylan, lignin, and cellulose from poplar wood were significantly enhanced by water-only and dilute acid (0.05 wt.% H$_2$SO$_4$) flowthrough pretreatment when the temperature was raised from 200°C to 280°C over a range of flow rates 10-62.5mL/min, resulting in more than 98% solid removal. Up to 40% of original xylan was converted to xylose in the hydrolyzate and the rest xylan was solubilized into xylooligomers with negligible furfural formation. Up to 100% cellulose was removed into hydrolyzate with the highest glucose yield of 60% and low 5-hydroxymethylfurfural (5-HMF) production. The maximal recovered insoluble lignin and soluble lignin were 98% and 15% of original lignin, respectively. In addition, enzymatic hydrolysis of pretreated whole slurries was characterized under various enzyme loadings with or without Bovine serum albumin (BSA) treatment. More than 90% glucose yield and 95% xylose yield were obtained from enzymatic hydrolysis of dilute acid pretreated whole slurries with 10 mg protein Ctec 2 (9.3 FPU) with
2mg Htec2/g glucan + xylan. Results suggest that kinetics is controlling the flowthrough pretreatment of biomass dissolution with concerning the effects of flow rate.

Key words: Hot water, dilute acid, flowthrough pretreatment; poplar wood; enzymatic hydrolysis

4.2. Introduction

Pretreatment is essential for achieving high yields of desirable products from the biochemical processing of naturally resistant cellulosic biomass (Yang & Wyman, 2008c). The feasibility of many pretreatment technologies has been proven at bench and pilot scales. However, a promising path to improve the technology has been suggested by the use of very dilute acid or even water-only technologies because such approaches could radically reduce the costs of pretreatment (Lynd et al., 1996). Apart from their economic viability, these technologies have several powerful attributes including high yields, high cellulose digestibility, low chemical usage, and fewer safety and environment concerns (Tao et al., 2011). Unfortunately, these alternative approaches typically require awkward process configurations and suffer from high water consumption for biomass dissolution, leading to high heat demands, which is difficult to implement in both pretreatment and product recovery (Yang & Wyman, 2008c). A number of studies over the years have shown that passing liquid hot water with and/or without addition of chemicals (e.g. acid, alkali) (Bobleter et al., 1981a; Bouchard et al., 1991; Lora & Wayman, 1978; Walsum et al., 1996; Weil et al., 1997) through cellulosic biomass at high temperatures produces highly digestible cellulose, high yields of sugars from hemicelluloses (Bobleter, 2005; Mok & Antal, 1992b; Vallejos et al., 2012; Walsum et al., 1996) over 85%
lignin removal (Yang & Wyman, 2004b), and liquid hydrolyzate that appears more compatible with fermentative organisms (Shao & Lynd, 2013). In addition, because lignin has a much lower solubility than xylan, it could be necessary to form lower degree of polymerization (DP) xylan fragments with solubility high enough to compensate for the limited solubility of the attached lignin (Yang & Wyman, 2008a). Thus, lignin-xylan oligomers and their solubility could have significant effects on the rates and yields of hemicelluloses hydrolysis, although little attention has been paid to their interactions (Shigematsu et al., 1995). The nature of lignin should also be considered, as it affects the solubility of lignin-xylan-oligomers (Yang & Wyman, 2008a). Increasing the temperature of hot water flowthrough pretreatments to 225–270 °C within or above saturated steam pressure also solubilizes the cellulose (Bobleter, 2005; Phaiboonsilpa et al., 2010). For example, as early as 1970s and 1980s, Bobleter and his colleagues (Bobleter, 2005) applied hot water flowthrough process to hydrolyze air-dried pure cellulose at 260–270 °C. Up to 52% glucose yield and 10% 5-hydroxymethylfurfural (5-HMF) were obtained through hydrolyzing cellulose under 265°C at flow rate of 12 mL/min. Furthermore, employing a two-stage (230 °C for 15 min and 270 °C for 15 min) semi-flow hot water pretreatment at flow rate of 10 mL/min under pressure of 10 Mpa was found to remove 100% xylan, 89.4% lignin and 79.5% cellulose, respectively. However, substantial sugar degradations, including furfural (~6.9%), 5-HMF (~6.9%), glycoaldehyde (~2.7%), were observed (Lu et al., 2009b). These results from flowthrough pretreatment at elevated temperatures provide invaluable evidence of the deconstruction pattern of biomass, improve understanding of how releases of various
biomass fractions are related and also provide new fundamental insights into hydrolysis kinetics that are not possible in batch operations.

Enzymatic hydrolysis of pretreated whole slurry depending on the technologies and conditions applied, was shown challenging. It was hard to deduce whether altering cellulose microfibrils, removing hemicelluloses, modifying or relocating lignin, or other effects on the substrate as well as minimizing inhibition and deactivation effects on enzyme are responsible for improving enzyme effectiveness (Yang et al., 2011). Various studies reported that enzymatic hydrolysis of cellulose in pretreated solid residues remained in the reactor was improved by employing flow of water and dilute acid through the solids, which enhanced xylan removal, lignin removal, and consequently enzyme accessibility. However, little attention has been paid to enzymatic hydrolysis of flowthrough pretreated whole slurry, including pretreatment hydrolyzate and pretreated solid residues remained in the reactor, in a simplified single step that could lead to lower capital and operating costs (Dutta et al., 2010). Nevertheless, the nature of both soluble and insoluble fractions should be considered (Yang et al., 2011), as it affects the digestibility of pretreated whole slurries. The inhibitory compounds which originate from aromatic compounds, aliphatic acids, furan aldehyde, inorganic compounds and others usually get enriched in the whole slurry produced during pretreatment, and thereby require some form of post-pretreatment detoxification to proceed effectively (Joensson et al., 2013). Bovine serum albumin (BSA) has shown promising in improving cellulase effectiveness with the mechanism attributed to promoting blocking enzymes from non-productive binding(Yang & Wyman, 2006), stabilizing enzyme
(Brethauer et al., 2011), and detoxification of hydrolyzate (Shi & Yang, 2008). Evaluating cellulase effectiveness with BSA and reducing the formation of these inhibitory compounds during pretreatment process are important for improving the efficacy of enzymatic hydrolysis.

In this study, poplar wood was pretreated in a flowthrough system under varying conditions (i.e. temperature 200-280 °C for 0-30 min, sulfuric acid concentration 0-0.05% (w/w), and flow rates 10-62.5mL/min) to assess effects on yields of total mass, xylan, lignin, and cellulose and subsequent enzymatic hydrolysis of pretreated whole slurry to gain a more rational explanation for the kinetic changes in both water-only and dilute acid flowthrough pretreatment performances. In addition, enzymatic hydrolysis of pretreated whole slurries with and without BSA was compared to investigate the efficiency of cellulases on digestion of both cellulosic and xylan fractions in the pretreated slurries under different enzymes loadings.

4.3. Materials and methods

4.3.1. Feedstocks

Poplar wood provided by Forest concepts (Auburn, WA) contains 48.8 wt. % cellulose, 16.8 wt. % xylan and 23.7 wt.% Klason lignin as determined by standard NREL LAPs (Sluiter et al., 2008a). Poplar wood material was grounded with Hammermill (Hammer1067-A-1, Buffalo, NY) at 4500rpm with a 1.59mm screen. Then the particles were collected to pass between Sieve 20 mesh and Sieve 40 mesh to obtain particles over a size range of 0.425—0.850 mm for experiments and analysis. The materials were sealed in heavy-duty zipped bags
and stored at -20°C in a laboratory freezer.

4.3.2. Flowthrough experiment

The flowthrough reactor is 1.3 cm i.d. × 15.2 cm length with an internal volume of 20.2 mL. It is constructed of 316 stainless-steel parts using VCR fittings, including one VCR male union (1.3 cm), two gasket filters (316 stainless-steel, average pore size 5 μm), two VCR glands (1.3 cm × 1.3 cm), two VCR nuts, and two VCR reducing fittings (1.3 cm × 0.3 cm). All reactor parts are obtained from Swagelok Co., Richland, WA. A 0.3 cm stainless-steel thermocouple (Omega Engineering Co., Stamford, CT) is installed at the outlet of the reactor to monitor temperature. Stainless-steel tubing is used as a preheating coil (0.6 cm o.d. × 0.1 cm wall) and to connect the reactor with other system components as well the cooling coil (0.3 cm o.d. × 0.1 cm wall). A high pressure pump (Acuflow Series III Pumps, Fisher) with a flow rate range of 0 to 100 mL/min, a pressure gauge (pressure range 0 to 1500 psi; Cole-Parmer Instrument Co., IL), and a back-pressure regulator (Valve and Fitting Co., WA) are used to control flow through the system. To operate the flowthrough unit, 0.5 g biomass substrate is loaded into the reactor, which is then connected to the system. Distilled water or 0.05% (w/w) sulfuric acid at room temperature is pumped through the reactor to purge air and then used to pressurize the reactor to a set pressure of 225 psi–1245 psi. The loaded biomass is completely wetted by this procedure. The reactors are heated to the target temperature (200–280°C) in a 4-kW fluidized sand bath (model SBL-2D, Omega engineering, Inc., CT). A thermal monitor was connected to the outlet of the flow reactor to precisely control the reaction temperature.
4.3.3. Analytical methods

4.3.3.1. Sugar and sugar degradation products analysis

Glucose, xylose, furfural, and 5-HMF in hydrolyzates of pretreatment and enzymatic hydrolysis were analyzed through Waters HPLC system (model 2695) equipped with a 410 refractive detector and a Waters 2695 autosampler using Waters Empower Build 1154 software (Waters Co., Milford, MA). Bio-Rad Aminex HPX-87H column (Bio-Rad Laboratories, Hercules, CA) was operated under 65°C. Yields of glucose, xylose, furfural, and 5-HMF were calculated as following (Yan et al., 2013):

\[
\text{Glucose}\% = \frac{W_G \times MW_{Gn}}{W_{Gn} \times MW_G} \times 100% \tag{1}
\]

\[
\text{Xylose}\% = \frac{W_X \times MW_{Xn}}{W_{Xn} \times MW_X} \times 100% \tag{2}
\]

\[
5\text{-HMF}\% = \frac{W_{5-HMF} \times MW_{Gn}}{W_{Gn} \times MW_{5-HMF}} \times 100% \tag{3}
\]

\[
\text{Furfural}\% = \frac{W_{Fur} \times MW_{Xn}}{W_{Xn} \times MW_{Fur}} \times 100% \tag{4}
\]

In these equations, \(W_{Gn}\) is the initial weight of glucan (g/100g dw raw biomass), \(W_{Xn}\) is the initial weight of xylan (g/100g dw raw biomass), \(W_G\) is the weight of glucose (g/100g dw raw biomass), \(W_X\) is the weight of xylose (g/100g dw raw biomass), \(W_{5-HMF}\) is the weight of 5-HMF (g/100g dw raw biomass), \(W_{Fur}\) is the weight of furfural (g/100g dw raw biomass). Molecular weight: \(MW_{Gn}=162, MW_{Xn}=132, MW_G=180, MW_X=150, MW_{5-HMF}=126, MW_{Fur}=96\).

Pretreatment hydrolyzate flowing out of the flowthrough system was collected then filtered...
through a 0.45µm polypropylene membrane filter (VWR, Radnor, PA). The filtrate was autoclaved in 4 wt. % sulfuric acid for 1 h at 121°C to breakdown glucose oligomers and xyloooligomers into their monomeric sugars (Sluiter et al., 2006). Sugar standards containing known sugar concentrations are also autoclaved for the same time and at the same acid concentration to estimate hydrolysis loss factors. Yields of soluble glucose oligomers and xyloooligomers were then calculated as below (Zhang et al., 2012):

\[
\text{Glucose oligomers}\% = \frac{W_{TG} - W_G}{W_{OG}} \times 100\% \\
\text{Xylooligomers}\% = \frac{W_{TX} - W_X}{W_{OX}} \times 100\%
\]

(5) (6)

In these equations, \( W_{TG} \) is the total glucose after autoclaving of filtrate corrected for degradation; \( W_G \) is glucose in the pretreatment filtrate before autoclaving; \( W_{OG} \) is the original glucan as glucose in raw biomass; \( W_{TX} \) is the total xylose after autoclaving of filtrate corrected for degradation; \( W_X \) is the xylose in the pretreatment filtrate before autoclaving; \( W_{OX} \) is the original xylan as xylose. The unit of W consistently refers to g/100g dw raw biomass.

Pretreatment hydrolyzate (without filtration, not including solid residue in the reactor) was presoaked with 1 wt. % BSA at pH 4.8 and then followed by enzymatic hydrolysis at 50 °C for 168 hours with a high enzyme loading (100 mg protein Ctec 2 (93 FPU) with 20mg Htec2/g glucan + xylan) that could guarantee maximum glucan conversion. The final glucose concentration after enzymatic hydrolysis was used to determine the total glucan recovery in
pretreatment hydrolyzate. The total glucan recovery by pretreatment was calculated as follows:

\[
\text{Total glucan recovery\%} = \frac{W_{EG}}{W_{OG}} \times 100\% \quad (7)
\]

In this equation, \(W_{EG}\) is the total glucose after enzymatic hydrolysis; \(W_{OG}\) is the original glucan as glucose. The unit of \(W\) consistently refers to \(g/100g\) dw raw biomass.

4.3.3.2. Lignin analysis

Insoluble lignin content was measured by K-lignin method (Templeton & Ehrman, 1995). Soluble lignin was estimated by UV analysis measuring absorbance at 320 nm using similar calculation of acid soluble lignin method (Ehrman, 1996).

The structure characterization of soluble lignin was determined by GC-MS analysis. The pretreated samples were filtered and extracted with dichloromethane (Caldeira et al., 2006), then analyzed with an Agilent gas chromatography mass spectrometer (GC, Agilent 7890A; MS, Agilent 5975C) equipped with a DB-5MS column (30m × 320µm × 0.25µm). The oven temperature was programmed from 45 °C to 250 °C at ramping rate of 5 °C /min. Both the initial and final temperature was held for 5 minutes. The flow rate of carrier gas (helium) was 1.3 ml/min.

4.3.3.3. Enzymes

Commercial preparations of Novozymes Cellic® CTec2 (220mg protein/mL, preserve 200 mg glucose/mL, 205 FPU/mL) and Novozymes Cellic® HTec2 (230mg protein/mL, preserve 180 mg xylose/mL) were generously provided by Dr. Melvin Tucker from NERL for all
hydrolysis experiments. The filter paper activity of CTec2 was determined according to the standard filter paper assay (Adney et al., 1994).

4.3.3.4. Enzymatic hydrolysis

All enzymatic hydrolysis experiments were run in duplicate under standard conditions (50 °C, pH 4.8). The pretreated whole slurries (including solid residue) from flowthrough pretreatment were adjusted to the set pH with 0.1N NaOH. A mixture of Ctec2 and Htec2 enzymes at a ratio of 5:1 based on protein weight was added at three different enzyme loadings: 1) low enzyme loading: the loadings of 3 mg protein Ctec2 (2.8 FPU) with 0.6 mg protein Htec2/g glucan + xylan, 2) medium enzyme loading: 10 mg protein Ctec 2 (9.3 FPU) with 2mg Htec2/g glucan + xylan, and 3) high enzyme loading: 100 mg protein Ctec 2 (93 FPU) with 20mg Htec2/g glucan + xylan, respectively. Liquid samples were taken at 4, 24, 48, 72, 96 and 120 hours and measured directly by HPLC for monomeric sugars. In addition, BSA treatment was conducted for parts of experiments. Prior to enzyme addition to start hydrolysis, the whole pretreated slurries were presoaked with 1% (w/w) BSA 10 mg/L sodium azide for 24 hours.(Yang & Wyman, 2006)

\[
\text{Enzymatic glucose yield\%} = \frac{W_{G2}}{W_{TG} - W_{G1}} \times 100\% \tag{8}
\]

\[
\text{Enzymatic xylose yield\%} = \frac{W_{X2}}{W_{TX} - W_{X1}} \times 100\% \tag{9}
\]

In these equations, \(W_{G1}\) and \(W_{X1}\) are the glucose and xylose released in the pretreatment; \(W_{G2}\) and \(W_{X2}\) are the glucose and xylose released in enzymatic hydrolysis; \(W_{TG}\) and \(W_{TX}\) is the total potential glucose and xylose released after enzymatic hydrolysis of whole pretreated
slurries (including solid residue) with the high enzyme loading (100 mg protein Ctec 2 (93 FPU) with 20mg Htec2/g glucan+xylan) in 168hrs. The unit of W consistently refers to g/100g dw raw biomass.

4.3.3.5. Severity parameters

A severity parameter was used to unify our data obtained at different combinations of temperature and reaction time, which includes the preheating time. The severity Log $R_0$ is defined as:(Overend & Chornet, 1987)

$$
\log R_0 = \log\left[t \times \exp\left(\frac{T-100}{14.75}\right)\right]
$$

(10)

Where $t$ is reaction time in minutes (including the preheating time); $T$ is the hydrolysis temperature in °C.

4.4. Results and discussion

4.4.1. Effects of preheating on removal of total mass, xylan, lignin, and cellulose

Since the flowthrough reactor cannot instantaneously reach reaction temperature upon immersion in a constant temperature of sand bath, the temperature transients that occur as the reactor is heated from ambient to reaction temperature must be considered (Stuhler & Wyman, 2003). A series of experiments for water-only (220—280 °C) and 0.05 wt. %$\text{H}_2\text{SO}_4$ operations (200—250 °C) with flow rate ranging from 10mL/min—62.5mL/min were carried out to determine the removal of xylan, lignin and cellulose as well as the corresponding sugar and lignin recovery in the pretreated slurry during the preheating process. It was found that
the preheating time from room temperature to reach 200 °C, 210 °C, 220 °C, 230 °C, 240 °C, 250 °C, 260 °C, 270 °C and 280 °C were 1.2 min, 1.3 min, 1.6 min, 1.7 min, 2.0 min, 2.2 min, 2.3 min, 2.5 min and 2.8 min, respectively. The average preheating rate to the target temperature was 100 °C/min. It was found that more than 76% xylan and 52% lignin were removed when temperature was preheated to 220 °C within 1.6 min for water-only pretreatment at flow rates 10 mL/min—62.5 mL/min. Before the temperature reached 280 °C, nearly 100% xylan removal accompanied with more than 75% lignin removal was obtained within 2.8 min. Adding acid increased the removal of both xylan and lignin. For example, more than 85% xylan removal and 66% lignin removal were obtained within 1.6 min when the reactor was preheated to 220 °C for dilute acid operations. Overall, Up to 100% of xylan, 49% cellulose and 87% insoluble lignin were removed into the hydrolyzate through the preheating processes of water and dilute acid pretreatment at the tested temperatures. Moreover, most of the dissolved xylan and cellulose for these preheating processes was predominately in the form of oligomers with small amount of xylose and glucose, and negligible degradation compounds such as furfural and 5-HMF for all of the tested pretreatment conditions. In addition, flow rate appears to have limited effects on xylan, lignin, and cellulose removal. For example, there are merely less than 10% xylan removal, 10% lignin removal, and 8% cellulose removal differences were observed when flow rate was elevated from 10mL/min to 62.5mL/min for both of water-only (220–280 °C) and 0.05%(w/w) H_{2}SO_{4} (200–250°C) preheating processes.
4.4.2. Effects of pretreatment severity parameter on removal of total mass, xylan, lignin, and cellulose

Removal of xylan, lignin, and cellulose from poplar wood through flowthrough pretreatment under target temperatures ranging from 220—280 °C for water-only, and 200—250 °C for dilute acid pretreatment, for 0—30 min (including preheating time), and at flow rates of 10mL/min, 25mL/min and 62.5mL/min, were investigated. The pH of each liquid sample was promptly measured upon cooling to room temperature with a pH meter. As shown in Figure 1a for water–only and dilute acid pretreatment, logR0 from 4.0 to 6.0 corresponded to water-only pretreated hydrolyzates with pH value for 4.0 to 3.2, while logR0 from 3.4 to 5.5 corresponded to dilute acid pretreated hydrolyzates with pH value for 2.6 to 2.2 were observed, respectively. These observations suggested that both dilute acid and water-only pretreatment were operated under acidic conditions although the former showed lower pH.

4.4.2.1. Xylan removal

It is known that hemicellulose and lignin are covalently linked in biomass, and the high solubility of hemicellulose oligomers can facilitate their dissolution, thus these soluble compounds can be removed before any further reactions occur (Yang & Wyman, 2008a). Figure 1b and c showed that increasing severities for water-only and dilute acid pretreatment enhanced xylan removal. Almost all xylan was removed when reaction severity logR0 > 4.5 and logR0 >4.2 for water-only and dilute acid operations, respectively. As expected, the most readily hydrolyzed constitute, such as xylan, is partially deacetylated as well as depolymerized in the presence of acidic water (Bouchard et al., 1991). The sulfuric acid
addition increased the rate of xylan removal for flowthrough systems (Figure 1c). Thus, to release analogous xylan removal, lower severity (i.e. lower temperature, less time) was required when acid was added. On the other hand, increasing flow rate from 10 mL/min to 62.5mL/min appeared to have limited effects on xylan solubilization: Only less than 10% xylan removal increased under experimental conditions.

4.4.2.2. Lignin removal

The apparent coupling of lignin and hemicellulose release during flowthrough pretreatment suggests that hemicelluloses-lignin oligomers dissolution rates and solubility limitations play key roles in realizing highly lignin removal (Yang & Wyman, 2008a). Results (Figure 1 b and c) showed that increasing pretreatment severity improved lignin removal for both water-only and dilute acid pretreatment although less portion of lignin than xylan was removed at similar severity. At the lowest severity tested, about 65% and 60% lignin was removed by water-only and dilute acid, respectively. For water-only pretreatment, about 85% lignin removal with flow rate of 10 ml/min and nearly 100% lignin removal with flow rate of 62.5ml/min at logR₀=5.3 were obtained, while for dilute acid pretreatment, nearly 90% lignin removal with flow rate of 10mL/min and 95% lignin removal with flow rate of 25mL/min at logR₀=4.7 were observed. At all comparable values of pretreatment severity, higher flow rate resulted in larger portions of lignin being removed by water-only pretreatment. Results suggested that increasing flow rate from 10mL/min to 62.5mL/min could improve lignin removal by 5-15% for water-only and around 5% for dilute acid, respectively.

4.4.2.3. Cellulose removal
Unlike xylan and lignin, cellulose consists of cellulose Iβ and Iα which are both held together via a network of hydrogen hydrophobic interactions, causing deconstruction of the crystals challenging (Ragauskas et al., 2006). Thus, the removal of cellulose was only 5%–20% at logR₀=4.0–4.7 with flow rates ranging from 10—62.5mL/min for water-only pretreatment, which increased gradually as severity was elevated (Figure 1b). Interestingly, cellulose removal rapidly increased to 40% at 10mL/min and 50% with 62.5mL/min flow rate when severity logR₀ reached 4.8 at 240 °C. As previously reported, cellulose Iβ underwent a transition into amorphous structure when temperature increased to around 220–230°C (Wada et al., 1993). Increasing the temperature of hot water and/or dilute acid flowthrough pretreatments to 220—270 °C within or above saturated steam pressure solubilizes the cellulose (Bobleter, 2005; Phaiboonsilpa et al., 2010). As severity further increased to 6.0 with temperature ranging from 240 to 270 °C, cellulose removal was continuously improved until nearly 100% removal was reached with water-only. The removal of almost entire cellulose also corresponded to more than 98% total biomass dissolution. For dilute acid pretreatment, abrupt enhancement of cellulose removal from 16% to about 50% was also observed but at lower severity of 4.0 and lower temperature of 220 °C. When logR₀ was higher than 4.0 at temperature above 220 °C, cellulose removal was rapidly improved to nearly 100% at logR₀=5.0. Correspondingly, dilute acid batch pretreatment resulted in a sudden departure of the cellulose degradation rate constants from normal Arrhenius pattern occurred around 215 °C under 0.07 wt. % H₂SO₄ loading (Xiang et al., 2003b). We hypothesize that temperature plays an important role among tested factors (e.g. acid
concentration, time) in explaining the effects of acidic aqueous pretreatment on cellulose dissolution and introducing acid can shift the balance among these factors. In addition, flow rate appeared to affect cellulose removal to some extent. For example, increasing the flow rate from 10mL/min to 62.5mL/min for water-only pretreatment resulted in 3%–15% higher cellulose removal at comparable severities (Figure 1b).

4.4.3. Sugars, sugar degradation products and lignin recovery through flowthrough pretreatment

Sugars were recovered in pretreatment hydrolyzate in the form of monomeric and oligomeric sugars, including xylose, xylooligomers, glucose, and glucan oligomers. Insoluble lignin and soluble lignin were measured in the hydrolyzate as lignin recovery. Thus, sugars and lignin recovery profiles were shown in Figure 2–4, respectively, for both water-only and dilute acid pretreatment with flow rate of 10 mL/min, 25 mL/min and 62.5 mL/min at different severity parameters.

4.4.3.1. Xylan recovery

Figure 2a presents xylose and xylooligomers yield from poplar wood by water-only flowthrough pretreatment. Results showed that xylooligomers were predominant recovered xylan in pretreatment filtered hydrolyzate at all tested severities. Higher than 75% xylooligomers yield were observed while xylose yields were found less than 25%. Xylooligomers yield decreased slightly as logR₀ increased while the corresponding xylose yield increased. It indicated that the increased severity could shift the distribution of generated sugars to monomers. In addition, flow rate also played an important role in the
distribution of xylose and xylooligomers in hydrolyzate. Increasing flow rate from 10 mL/min to 62.5mL/min resulted in 10–20% increment of xylooligomers yield while the corresponding xylose yield decreased about 15%. Since xylan removal only changed less than 10% when the flow rate increased from 10 mL/min to 62.5mL/min, results indicated that xylooligomers continued to degrade at lower flow rate instead of being swept out of the reaction system at high flow rate.

Figure 2b revealed that 56.2%–71.2% xylooligomers yield and 7.2%–19.2% xylose yield were obtained at around log\(R_0\)=3.5 with flow rates ranging from 10mL/min to 62.5mL/min for dilute acid pretreatment. As severity increased, xylose yield gradually increased to 39.9%, 35.6% and 26.0% at log\(R_0\)=4.9 with flow rate of 10mL/min, 25mL/min and 62.5mL/min, respectively, then remained similar value when severity was between 5.0 and 5.5. On the contrary, xylooligomer yield climbed to the peak yields of 74.7% and 83.9% around log\(R_0\)=4.3–4.4 with flow rate of 25mL/min and 62.5mL/min, respectively, then it gradually declined as severity increased further. Comparing to water-only pretreatment, adding dilute acid increased the xylose yield (Figure 2b). For example, at flow rate of 25mL/min, the xylose yield was observed 14.7%–35.6% at log\(R_0\)=3.5–5.5, much higher than 6.6%–21.8% xylose yield obtained with water-only at similar severity parameters. On the contrary, xylooligomers yield with dilute acid decreased to 60.8%–74.7% compared with 76.6%–87.0% for water-only. In addition, results showed that with flow rate of 10mL/min, log\(R_0\)=5.9 was necessary to reach the highest xylose yield of 25% for water-only while dilute acid pretreatment yielded similar xylose at log\(R_0\)=3.9. It appeared that lower severity value
was required to reach similar xylose yield for dilute acid than water-only flowthrough pretreatment.

Results indicated that flow rate had more significant effects on xylose and xylooligomer yield for dilute acid: 10–20% increase of xylooligomer yield and 12–20% decline in xylose yield when flow rate was increased from 10mL/min to 62.5mL/min. In addition, results also showed that negligible amount of degradation compounds, such as furfural, was detected under all tested water-only and dilute acid conditions, indicating that almost all the removed xylan was recovered as xylose and xylooligomers in hydrolyzate. For example, 100% xylan removal resulted in 98.2% and 98.8% xylose plus xylooligomers yield for water-only (log\(R_0\)=5.0, 25mL/min) and dilute acid (log\(R_0\)=4.8, 25mL/min), respectively. Most of the high solubility xylooligomers were swept out of the reactor before any further reactions occurred in the preheating procedure, during which the temperature was lower than the target temperature, thus led to low formation of furfural (Yang & Wyman, 2008c).

### 4.4.3.2. Cellulose recovery

Both water and dilute acid at elevated mild temperature could result in the release of acids from hemicelluloses by cleavage of O-acetyl and uronic acid substitutions facilitating the ether linkage cleavage to removal of hemicelluloses and lignin. Increasing the pretreatment severity (e.g. temperature, acid concentration, and reaction time) will lead to the decrystallization of cellulose structure and further release of glucose by cleavage of β(1-4)-glycosidic bonds hence promote the hydrolysis of cellulosic biomass (Bobleter, 2005;
Lu et al., 2009b; Xiang et al., 2004a). In this study, yields of glucose and soluble glucose oligomers in filtered pretreated hydrolysate, which indicated the yields of soluble cellulosic fractions, while the total glucan recovery after enzymatic hydrolysis of unfiltered hydrolyzate which revealed the total glucan available in pretreated hydrolyzate, were comparably investigated. Figure 3a shows that the yields of glucose and soluble glucose oligomers in filtered hydrolyzate and total glucan recovery increased as severity was elevated for water-only pretreatment. Results showed that both glucose and glucose oligomer yields increased gradually as severity increased from 4.0 to 6.0 for all tested flow rates except that glucose oligomers yield showed slightly abrupt increase around log$R_0$=4.8 for flow rate of 25mL/min and 62.5mL/min. The highest glucose yield of 16.2% was achieved at a high severity around log$R_0$=5.8 with flow rate of 10mL/min while the highest glucose oligomer yield of 45.0% was found at log$R_0$=6.0 with flow rate of 62.5mL/min. Correspondingly, although the total glucan recovery increased gradually when log $R_0$<4.8, an abrupt increase was observed at about log$R_0$=4.8 and it continuously rose rapidly to around 95% at log$R_0$ ranging from 4.8–6.0 as temperature was higher than 240 °C. These results indicated that the total glucan recovery was comparable to that of cellulose removal (Figure1b). Furthermore, it was noteworthy that the difference between the total glucan recovery and the sum of glucose and glucose oligomers yields, which implied the yield of removed insoluble cellulosic fractions, also showed abrupt enhancement when log$R_0$ around 4.8 and temperature higher than 240 °C. At log$R_0$=6.0, nearly 100% cellulose removal merely resulted in 50% glucose plus glucose oligomers yield and 1.6% 5-HMF yield (see Table 1) while the total
glucan recovery was about 95% with flow rate of 62.5mL/min. It indicated that when logR₀>4.8, besides glucose and glucose oligomers and the small amount of cellulose in pretreated solid residues, the remainder cellulosic fractions in hydrolyzate was predominately in the form of insoluble cellulosic fractions.

Yields of glucose and soluble glucose oligomers in pretreatment hydrolyzate increased more rapidly with dilute acid than those with water-only at similar severity parameters (Figure 3b). Results suggested that the addition of acid accelerated the hydrolysis rate of cellulose to glucose oligomers, and subsequently to glucose. Glucose yield increased gradually with severity at tested flow rates, then showed steep climbing to the maximum yield of 59.6% with flow rate of 25mL/min at logR₀=4.1−5.5, while soluble glucose oligomer yield continuously increased to the peak yield of 43.3% at logR₀=4.8 with flow rate of 62.5mL/min then declined with all tested flow rates as severity further increased. Within the range of tested severity parameters and flow rates, it was found that the maximum yield of glucose plus soluble glucose oligomers by dilute acid pretreatment reached 86.3%, much higher than that of 50.2% for water-only operation. In addition, glucose yield by dilute acid was much higher than that by water-only pretreatment. For example, with dilute acid pretreatment, 12.3%−59.6% glucose yield was obtained at logR₀=4.1−5.5 with flow rate of 25mL/min. In comparison, under similar conditions (i.e., temperature, time, flow rate), glucose yield reached 0−9.5% for water-only pretreatment. The total glucan recovery pretreated with dilute acid also increased as severity increased and showed abrupt enhancement at lower severity log R₀=4.0 and lower temperature of 220 °C than water-only pretreatment. At logR₀=5.5 with 25ml/min flow rate
with dilute acid, where 100% cellulose was removed, 84.5% glucose plus soluble glucose oligomers yield with negligible 5-HMF was observed and 98.7% original glucan was recovered in pretreatment hydrolyzate. This indicated around 14.2% insoluble cellulosic fractions were formed.

Results showed that soluble glucose oligomers yields increased with flow rate for water-only and dilute acid pretreatment (Figure 3a and b). This could be attributed to the greater amount of water enabling more glucose oligomers to dissolve at higher flow rates thereby limiting their continuous reaction in the solid phase. Meanwhile, the higher flow rate could also rapidly remove dissolved oligomers from the reactor before they can further hydrolyze. On the other hand, lower flow rate increased the portion of glucose in pretreatment hydrolyzate. For example, at logR₀=5.9, when the flow rate decreased from 62.5mL/min to 10mL/min, the glucose yield increased from 4.2% to 16.3%, while the glucose oligomer yield declined from 40.1% to 21.0% with water-only. The total glucan yield increased 10—20% and 5—10% for water-only and dilute acid, respectively, when flow rate was increased from 10mL/min to 62.5mL/min. Thus, flow rate appeared to influence the generation of glucose and glucose oligomers in a manner similar to its effect on the yields of xylose and xylooligomers.

Sugar degradation patterns

It is known that during acidic water pretreatment, the structural components of biomass are converted to water soluble compounds. Liberated oligomers are monomerized and then monomeric sugars can dehydrate into furans (furfural and 5-HMF) (Marcotullio & De Jong, 2010; Rosatella et al., 2011), which in turn can degrade into organic acids, such as levulinic
acid (Song et al., 2013). Formic and acetic acid are released from the dissociation of the hemicellulose/cellulose/lignin inter-polymeric linkages.

As shown in Table 1, at a flow rate of 10 mL/min, 3.1% 5-HMF yield was observed at 240 °C after 10 min water-only, whereas elevating flow rate up to 25 mL/min resulted in negligible 5-HMF yield. Even when the temperature was raised to 270 °C, 5-HMF yield remained negligible with flow rate of 25 mL/min. 0.7% furfural was formed under 250 °C at 10 min when employing a flow rate of 10 mL/min. However, furfural became imperceptible when the flow rate was raised to 25 mL/min and 62.5 mL/min under identical or higher temperatures (e.g. 270 °C). Results indicated that higher flow rates of 25 mL/min and 62.5 mL/min led to both negligible amount of 5-HMF and furfural at elevated temperatures for both water-only (≤270 °C) and 0.05% (w/w) H₂SO₄ (≤240 °C) operations. Comparatively, flow rate of 25 mL/min with relatively lower water consumption appeared to be desired for higher sugar concentration. Results suggested that undesirable decomposition reactions of glucose and xylose to 5-HMF and furfural can be limited by controlling severity parameter and flow rate. In line with this reasoning, it is interesting to note that the yields of 5-HMF and furfural observed under water-only and dilute acid operations under analogous severities were comparable. The yields of furfural was lower than those of 5-HMF under these tested conditions although xylose was much easier to be degraded than glucose (Qian et al., 2005). The possible explanation was that a much higher fraction of xylan was swept out of a reactor in the preheating period due to a greater solubility when temperature, flow rate increased, and acid added.
4.4.3.3. Lignin recovery

Lignin is believed to depolymerize and micellarize under acidic conditions via both homolytic and acidolytic cleavage into low molecular weight lignin globules. As acidic water passes through the material, especially at high flow rates, highly reactive nucleophilic carbonium ion intermediates are formed within the lignin structure, and can react further leading to the cleavage of predominant β-O-4 bonds thereby realizing efficient depolymerization of lignin which can be quickly and continuously swept out of the reactor to limit the repolymerization reaction occurred simultaneously and re-precipitation of the depolymerized lignin at ambient temperature (Laskar et al., 2013a; Trajano et al., 2013). As shown in Figure 4, a large fraction of the recovered lignin in the hydrolyzate during flowthrough reactions was in the insolubilized form for both water-only and dilute acid. For example, insoluble lignin recovery ranged from 59.3% to 87.8% under water-only conditions (25mL/min) when the severity was increased from logR₀=4.1 to logR₀=5.5. In the contrast, adding acid significantly enhanced the insoluble lignin recovery from about 75.6% to 98.0% when logR₀ increased from 4.1 to 5.5. Apart from insoluble lignin, a small fraction of removed lignin was solubilized in the hydrolyzates for both water-only and dilute acid flowthrough pretreatment. For water-only, the yield of soluble lignin was 3.6% at logR₀=4.1, then increased slowly as the severity parameter increased. The highest yield is 11.7% at logR₀=5.7 then decreased when logR₀ continuously increased. By comparison, soluble lignin recovery for dilute acid pretreatment was much less than that with water-only at all severity range. Adding acid resulted in the maximal soluble lignin yield of 5.6% at logR₀=4.7. Flow
rate effected the distribution of the removed lignin to some extent and it was more apparent for water-only pretreatment. Although higher flow rate (e.g. 62.5mL/min) resulted in more lignin removal than lower flow rate (e.g. 10mL/min), it was found that 3%—9% increase in soluble lignin yield was realized when flow rate declined from 62.5mL/min to 10mL/min for water-only. It was plausible that lower flow rate increased the exposure of removed lignin under high temperature for decomposing into low molecular weight compounds.

GC/MS was essentially carried out to determine the chemical components of lignin recovered through water-only and dilute acid flowthrough pretreatment. Among the soluble lignin products, vanillin and syringaldehyde were found as the predominant lignin derived aromatic structures pretreated under both water-only and acid conditions (Figure 4.5 and Table 4.2). These two compounds generally were considered derived from lignin units of coniferyl alcohol (G) and sinapyl alcohol (S). This can be speculated as derived initially from the acidic cleavage of the predominant β-O-4 bonds of lignin to phenylpropanoid structural moieties (e.g. sinapinaldehyde) and further oxidized to vanillin and syringaldehyde (Laskar et al., 2013b; Zhuang et al., 2012). It was noteworthy that dilute acid conditions generated less phenylpropanoids than those with water-only (Table 4.2). For example, no coniferyl alcohol was observed in 0.05% (w/w) H₂SO₄ conditions, suggesting that 0.05% (w/w) H₂SO₄ with relative lower pH was more prone towards the oxidation reactions. Most of these soluble lignin compounds presented in hydrolyzates were considered as inhibitory compounds to biocatalysts in the subsequent bioconversion processes. Such hydrolyzates usually require
some form of post-pretreatment detoxification to proceed effectively (Joensson et al., 2013; Kim et al., 2011).

4.4.3. Effects of enzyme loading and BSA addition on enzymatic hydrolysis of pretreated whole slurries

In this study, whole slurries pretreated under water-only or dilute acid conditions were hydrolyzed by enzymes at different enzyme loadings and enzymatic xylose and glucose yields were investigated. In addition, the enzymatic hydrolysis of pretreated whole slurries with and without BSA was compared to investigate the effects of BSA treatment on digestion of both cellulosic and xylan fractions. Whole slurries pretreated under water-only conditions (i.e., 270 °C, 10 min, 25 mL/min) and dilute acid conditions (i.e., 240 °C, 0.05 wt. % H$_2$SO$_4$, 8 min, 25 mL/min), which resulted in nearly complete biomass removal, highest total monomeric and oligomeric xylose and glucose yield, negligible sugar degradation products, as well as relatively lower liquid consumption, were applied as substrates for enzymatic hydrolysis evaluation.

As shown in Figure 5a–d, results revealed that dilute acid flowthrough pretreatment exhibited overall much better performance in enzymatic hydrolysis than water-only. For example, for water-only pretreated whole slurries, about 70% enzymatic glucose yield was reached within 4hrs at the high enzyme loading and enzymatic glucose yield gradually increased to 95% in 72hrs (Figure 5a). At the medium enzyme loading, only 51.5% enzymatic glucose yield was observed at 4hrs and it improved to about 65% at 120hrs. With the low enzyme loading,
glucose yields were 41.5% and 49.1% at 4hrs and 120hrs, respectively. On the contrary, dilute acid resulted in higher glucose yield of 52.7% at 4hrs and 73.3% at 120hrs with the lowest enzyme loading (Figure 5c). With the medium enzyme loading, about 93% glucose yield was reached within 120hr. At the high enzyme loading, glucose yield was found >90% without BSA within 4hrs. Results indicated that dilute acid pretreatment led to more readily digestible cellulosic derivatives. It could be explained by the fact that the recovered glucan in acid pretreatment hydrolyzate was predominately composed of glucose and soluble glucose oligomers, which totally accounted for 86.3% based on the original glucose in poplar wood. In contrast, only 52.0% yield of glucose plus soluble glucose oligomers was obtained for water-only flowthrough pretreatment, while the rest of removed cellulose (about 50%) was considered as insoluble cellulose derivatives. On the other hand, it was noteworthy that glucose yield within the initial period 4hrs of enzymatic hydrolysis of pretreated whole slurries with various enzyme loading was 41.5%–91.2% for both water-only and dilute acid, then glucose yield gradually increased to 49.1%–100% at 120hrs with prolonged hydrolysis. It indicated that the large portion of soluble glucose oligomers and insoluble cellulose derivatives in pretreatment hydrolyzate were quickly hydrolyzed by enzymes as nearly complete biomass dissolution was achieved and enzymatic hydrolysis of pretreated whole slurries was more effective than hydrolysis of cellulose remained in the pretreated solid residues.

Enzymatic xylose yield of pretreated whole slurries reached 94.1% and 96.8% for water-only and dilute acid, respectively, within 24hrs at the high enzyme loading (Figure 5b). The
medium enzyme loading resulted in 92.2% and 89.2% of enzymatic xylose yield for water-only pretreated whole slurries in 72hrs, respectively. Similar enzymatic xylose yields were found at these lower enzyme loadings for dilute acid pretreated whole slurries. Results suggested that xylooligomers in pretreated whole slurries were effectively hydrolyzed by enzymes even with the low enzyme loading. In addition, it revealed that both water-only and dilute acid flowthrough pretreatment led to high yield of xylose by enzymatic hydrolysis.

It was reported that BSA treatment resulted in substantial improvement of enzymatic glucose yield from enzymatic hydrolysis of solid residues pretreated by various pretreatments (Brethauer et al., 2011; Yang & Wyman, 2006). Effects of BSA treatment on enzymatic hydrolysis of pretreated whole slurries by water-only and dilute acid were investigated. Results showed that enzymatic glucose yield of water-only pretreated whole slurries with high enzyme loading was enhanced to 81.6% at 4hrs which was around 10% higher than that without BSA treatment, and then the enzymatic glucose yield reached about 100% within 48hrs. Addition of BSA improved enzymatic glucose yield about 5–8% with both of the medium and low enzyme loadings for water-only pretreated whole slurries. Comparatively, the effectiveness of BSA treatment was less apparent on enzymatic hydrolysis of dilute acid pretreated whole slurries than that of water-only pretreated slurries. 0–6% increased enzymatic glucose yield was obtained for dilute acid pretreated slurries with BSA treatment, while 1%–10% increase of glucose yields for water-only pretreated slurries. Slight improvement of around 0–5% in enzymatic xylose yield was observed with BSA addition for both water-only and dilute acid pretreated slurries. Results revealed that adding BSA prior to
enzymatic hydrolysis showed less effect on enzymatic xylose yield than enzymatic glucose yield. It was proposed that BSA blocked non-specific binding of cellulases, reduced inhibitory effects of pretreatment generated compounds and stabilized enzymes (Yang & Wyman, 2013). With most of glucan and xylan recovered in pretreatment hydrolyzate in forms of monomers, soluble oligomers and insoluble derivatives in this study, benefits of BSA treatment on improving enzymatic sugar yield were less apparent than that with pretreated solid residues in previous studies (Yang & Wyman, 2006).

4.4.4. Combined total monomer sugar yields through flowthrough pretreatment (stage 1) followed by enzymatic hydrolysis (stage 2)

The pretreated whole slurries after water-only and dilute acid flowthrough pretreatment (stage 1) subsequently underwent enzymatic hydrolysis (stage 2) to maximize mono sugar yield. Figure 6 compared and summarized the sugar yields obtained from stage 1 and stage 2 under water-only (i.e. 270 °C, 10min, 25 mL/min) and dilute acid (i.e. 240 °C, 0.05% (w/w) H₂SO₄, 8 min, 25mL/min) conditions that resulted in the highest total sugar yields (monomers and soluble oligomers), negligible sugar degradation products, nearly complete biomass removal and relatively lower water consumption at Stage 1. The enzyme loading employed during Stage 2 for selected water-only and dilute acid pretreated slurries were 100 mg protein Ctec 2 (93 FPU) with 20mg Htec2/g glucan+xylan (high enzyme loading) and 10 mg protein Ctec 2 (9.3 FPU) with 2mg Htec2/g glucan+xylan (medium enzyme loading) respectively, both of which led to > 90% enzymatic glucose yield and >95% enzymatic xylose yield from corresponding samples.
Results showed that on the basis of 100 g poplar wood, more than half cellulose and nearly all xylan was converted to soluble sugars at Stage 1 for the selected water-only operation: 4.0 g xylose plus 14.7 g xylooligomer, and 7.5 g glucose plus 19.7 g glucose oligomers were obtained. For dilute acid pretreatment, nearly complete polysaccharides solubilization (~100% xylan and ~90% cellulose) led to slightly higher xylose content (6.8 g) accompanied with 12.2 g xylooligomers and much higher glucose content of 31.2 g plus 15.5 g glucose oligomers. Predominate soluble sugar fractions and insoluble sugar fractions were converted into sugar monomers at Stage 2 for both selected water-only and dilute acid operations, resulting in 52.7 g glucose plus 18.6 g xylose, and 50.8 g glucose plus 18.7 g xylose, respectively.

Although the material balance implied slight loss of some mass during pretreatment, the selected flowthrough conditions resulted in more than 93% glucose and 97% xylose yields after stage 1 and stage 2. Particularly, merely less than 10 FPU/g glucan+xylan enzyme was required to reach over 90% sugar yield during stage 2 for dilute acid pretreated whole slurries because around 90% cellulose was solubilized as glucose and soluble glucose oligomers by pretreatment (stage 1). During the pretreatment, almost all lignin was removed together with polysaccharides.

4.5. Conclusion

Poplar wood was pretreated through water-only and dilute acid flowthrough approaches at temperature 200—280°C and it resulted in more than 98% solid removal. Temperature was considered as the most significant factor for cellulose degradation. The cellulose removal
significantly increased as temperature reached 240 °C for water-only and 220°C for dilute acid. Up to 100% xylan and 90% cellulose were hydrolyzed with negligible furfural and 5-HMF formation during pretreatment. Dilute acid pretreatment also resulted in higher yields of recovered xylan and cellulose as monomeric form of sugars in the hydrolyzate than that for water-only pretreatment. However, a larger fraction of recovered lignin was soluble with water-only. The insoluble lignin took account the majority of the original lignin (~90%) while a small amount (~15%) became soluble in the pretreated whole slurries. These results suggested that the pretreatment kinetic parameters, such as temperature, pressure, and residence time, determined the deconstruction and hydrolysis of biomass. Increasing severity enhanced total mass removal, xylan removal, lignin removal, and cellulose removal, and adding dilute sulfuric acid significantly accelerated all of the above. Dissolution of almost all biomass in hydrolyzate was obtained at logR₀ around 6.0 without acid added while a faster rate was achieved with dilute acid (logR₀ around 5.0). In addition, flow rate appeared to have less effect on removal of xylan, lignin, cellulose, and total mass as well as recovery yields although flow rate was associated with reaction time to affect pretreatment kinetics. Because the reaction actually involves the breakdown of solids to form products in solution, mass transfer and solubility effects would merit consideration. Enzymatic hydrolysis of the pretreated whole slurries obtained under desired conditions for water-only (270 °C, 25mL/min, 10min) and dilute acid (240 °C, 0.05 wt. % H₂SO₄, 25mL/min, 8min) revealed that 93%—97% glucose yield and 97%—98% xylose yield were obtained. The pretreated whole slurries under selected dilute acid conditions (240 °C, 0.05%(w/w) H₂SO₄, 25mL/min,
8min) that resulted in much higher soluble glucose plus glucose oligomers yield (~90%) at stage 1 than the water-only operation (270 °C, 25mL/min, 10min) merely required less than 10 FPU/g glucan+xylan enzyme to achieve >90% glucose yield and >95% xylose yield. The inhibitory compounds in the pretreated slurries showed insignificant impact on the performance of enzymes on pretreated whole slurries through BSA testing, especially for dilute acid pretreatment. Results showed that less inhibition to enzymatic hydrolysis of cellulosic fraction pretreated by dilute sulfuric acid was observed. The BSA treatment showed better effect on the digestion of cellulosic fraction than hemicellulosic fraction of the pretreated whole slurries. In addition, the insoluble lignin was recovered from hydrolyzate with low molecular weight (< 1800 Dalton). We also developed catalytic techniques to convert such technical lignin into C7 to C9 range hydrocarbons through a novel hydrodeoxygenation process in our lab (Laskar & Yang, 2013). Overall, both of water-only and dilute acid flowthrough pretreatments of poplar wood followed by enzymatic hydrolysis significantly enhanced momeric sugars and low MW lignin yields. These findings also imply that the fundamental interactions of biomass and water and acid can be applied to understand other aqueous chemical pretreatments — their successes, pitfalls, and best optimization strategies can lend considerable insights into their sensitivities. The new insights gained will lead to obtain even higher yields of fermentable sugars and reactive lignin for biofuels production.
4.6. References


Figure 4.1 Effect of severity parameter (logR₀) on the removal of xylan, lignin and cellulose with water-only and 0.05% (w/w) H₂SO₄. (a) Log R₀ vs pH:

△ water-only condition; ● 0.05% (w/w) H₂SO₄ condition. (b) Log R₀ vs removal of xylan, lignin and cellulose with water-only condition. (c) Log R₀ vs removal of xylan, lignin and cellulose with 0.05%(w/w) H₂SO₄. In (b) and (c): □ xylan removal (10mL/min), ■ xylan removal (25 mL/min); □ xylan removal (62.5mL/min); ▲ lignin removal (10 mL/min), ▲ lignin removal (25 mL/min), Δ lignin removal (62.5 mL/min); ◇ cellulose removal (10mL/min); ● cellulose removal (25mL/min); ○ cellulose removal (62.5 mL/min).
Figure 4.2 Effect of log$R_0$ on xylan recovery with (a) water-only and (b) 0.05% (w/w) H$_2$SO$_4$.

- □ xylose (10 mL/min);
- □ xylooligomers (10 mL/min);
- ▲ xylose (25 mL/min);
- △ xylooligomers (25 mL/min);
- ● xylose (62.5 mL/min);
- ○ xylooligomers (62.5 mL/min).
Figure 4.3 Effect of log$R_0$ on cellulose recovery in (a) Water-only and (b) 0.05% (w/w) H$_2$SO$_4$ conditions. ■ glucose (10mL/min), □ glucose oligomers (10mL/min), ◻ total glucan recovery (10 mL/min); ▲ glucose (25mL/min), △ glucose oligomers (25 mL/min), ◼ total glucan recovery (25 mL/min); ● glucose (62.5mL/min), ○ glucose oligomers (62.5 mL/min), ○ total glucan recovery (62.5mL/min).
Figure 4.4 Effect of logR₀ on lignin recovery under (a) Water-only and (b) 0.05% (w/w) H₂SO₄ conditions. ■ soluble lignin (10 mL/min); □ insoluble lignin (10 mL/min); ▲ soluble lignin (25 mL/min); △ insoluble lignin (25 mL/min); ● soluble lignin (62.5 mL/min); ○ insoluble lignin (62.5 mL/min)
Figure 4.5 The major structure of soluble lignin with water-only or 0.05%(w/w) H₂SO₄ flowthrough pretreatment at flow rate of 25 mL/min within 6 min under (a) 220 °C, water-only; (b) 240 °C, water-only; (c) 260 °C, water-only; (d) 280 °C, water-only; (e) 200 °C, 0.05%(w/w) H₂SO₄; (f) 240 °C, 0.05%(w/w) H₂SO₄

1: Vanillin; 2: Syringaldehyde; 3: Coniferaldehyde; 4: Coniferyl alcohol; 5: Sinapinaldehyde
Figure 4.6 Enzymatic hydrolysis of whole flowthrough pretreated slurries with solid residue at enzyme loading of 3 mg protein Ctec2 (2.8 FPU) with 0.6 mg protein Htec2/g glucan –100 mg protein Ctec 2 (93 FPU) with 20mg Htec2/g glucan with and without BSA treatment: (a) enzymatic glucose yield from water-only (270 °C, 10min, 25mL/min) pretreated slurry; (b) enzymatic xylose yield from water-only (270 °C, 10min, 25mL/min) pretreated slurry; (c) enzymatic glucose yield from 0.05%(w/w) H$_2$SO$_4$ (240 °C, 8min, 25mL/min) pretreated slurry; (d) enzymatic xylose yield from 0.05%(w/w) H$_2$SO$_4$ (240 °C, 8min, 25mL/min) pretreated slurry. 3 mg protein Ctec2 (2.8 FPU) with 0.6 mg protein Htec2/g glucan: low enzyme loading; 10 mg protein Ctec 2 (9.3 FPU) with 2mg Htec2/g glucan: medium enzyme loading; 100 mg protein Ctec 2 (93 FPU) with 20mg Htec2/g glucan: high enzyme loading.
Figure 4.7 Material balance on flowthrough pretreatment (Stage 1) and enzymatic hydrolysis (Stage 2): (a) 270 °C, water-only, 25mL/min, 10min; (b) 240 °C, 0.05%(w/w) H₂SO₄, 25mL/min, 8min. High enzyme loading: 100 mg protein Ctec 2 (93 FPU) with 20mg Htec2/g glucan; Medium enzyme loading: 10 mg protein Ctec 2 (9.3 FPU) with 2mg Htec2/g glucan. XOS: xylooligomers; GOS: glucose oligomers.
Table 4.1 The effect of reaction parameters on the generation of 5-HMF and furfural during water-only and 0.05% (w/w) H₂SO₄ flowthrough pretreatment

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<th>Temp (°C)</th>
<th>Reaction Time (min)*</th>
<th>H₂SO₄ concentration (%)</th>
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<th>Furfural(%)</th>
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* a: including the preheating time
**Table 4.2** The major soluble aromatic compounds detected from flowthrough pretreatment of poplar wood with water-only and 0.05% (w/w) H$_2$SO$_4$.

+: 0—10% relative abundance; ++: 10%—20% relative abundance; +++: 20%—50% relative abundance.

a: Water-only, 25mL/min, 6min; b: 0.05%(w/w) H$_2$SO$_4$, 25mL/min, 6min

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Chapter 5

Characterization of poplar wood chemical transformations during acidic hot water flowthrough pretreated lignocellulosic biomass

5.1. Abstract

Flowthrough pretreatment performed at elevated temperatures ranging from 200 ºC—280 ºC with water-only or 0.05 wt.% H₂SO₄ possesses the potential for disrupting and removing whole biomass. FTIR- spectroscopic analysis of whole pretreated slurries revealed the progressive reduction in the functional groups in biomass. The whole pretreated cellulosic fractions were characterized for crystallinity by X-ray diffraction (XRD). Results showed that the transformation of cellulose I to cellulose II occurred when the reaction temperature reached 240 ºC above for water-only operation. Observations also revealed that the crystallinity of cellulose was significantly reduced as the reaction temperature was raised to 220 ºC or above with 0.05 wt.% H₂SO₄. Enzymatic reactivity of cellulose was significantly improved after it was treated when temperature reached 240 ºC with water-only and 220 ºC with 0.05 wt.% H₂SO₄.

Key words: Pretreatment, Cellulose, FTIR, XRD, IC,

5.2. Introduction

Pretreatment is a significant step for the biochemical conversion of lignocellulosic biomass into ethanol or other chemicals. The typical objective of pretreatment is thought to disrupt the hemicellulose and lignin coating the surface of cellulose microfibrils and reduce the
crystalline nature of the cellulose structure (Alvira et al., 2010; Li et al., 2010a; Li et al., 2010b; Mosier et al., 2005). Among varying pretreatment technologies, acidic hot water pretreatment with water-only or very dilute acid (<0.1) was favored by many researchers due to the lower chemical cost and limited corrosion problems of the equipments (Kim et al., 2001; Liu & Wyman, 2004). In addition, flowthrough system with the proved advantages including rapid removal of generated sugar monomers and oligomers out of flowthrough reactor by fluid flow could reduce the sugar degradations. Acidic hot water flowthrough pretreatment were often conducted under temperatures ranging from 180 °C−230 °C (Bobleter et al., 1981b; Liu & Wyman, 2004; Mok & Antal, 1992b; Yang & Wyman, 2008b; Yang & Wyman, 2004a). It was reported that up to 100% hemicellulose/xylan and 85% lignin removal were realized under such temperature levels. The removed xylan was predominately in the form of xylose and xylooligomers, the distribution of which was impacted significantly by flow rate (Yang & Wyman, 2008a). However, only small amount of cellulose (~22%) was decomposed (Bobleter et al., 1981b; Mok & Antal, 1992b) under such conditions, which was attributed to the resistant properties of crystalline cellulose (Mok & Antal, 1992b). An enzyme loading of 60 FPU/g glucan was required for achieving >90% glucose generated from pretreated cellulose (Yang & Wyman, 2004b). Our previous studies (Chapter 3) conducted the acidic hot water flowthrough pretreatment of poplar wood revealed that reaction temperature above 240 °C for water-only operations and 220 °C for 0.05% (w/w) \( \text{H}_2\text{SO}_4 \) operations led to > 98% dissolution of biomass including the total removal of xylan, lignin as well as cellulose. It was found that the removed xylan was predominately in the
form of xylose and xylooligomers. In regard to the removed cellulosic fractions, only a part
of them was in the form of glucose and glucose oligomers, and the remainder of removed
cellulose was still in the form of cellulosic fractions but with different structural
classification along with glucose and glucose oligomers (chapter 4).
Investigating the physicochemical changes of the acidic pretreated lignocellulosic biomass
properties could provide significant insights in understanding the efficacy of pretreatment for
subsequent enzymatic hydrolysis. The native cellulose possess varying obstacles impeding
enzymatic accessibility. Hemicellulose and lignin wrapped around the cellulose provide extra
rigidity in cell walls but obstacles for enzymatic digestion (Houghton, 2006). FTIR were
often employed to derive structural characteristic features related to performance of
hemi cellulose and lignin depolymerization during pretreatment on physiochemical scale
(Laskar et al., 2013; Kumar). Crystallinity of cellulose is well known as a significant obstacle
during enzymatic hydrolysis (Yang et al., 2011). Transforming the crystalline cellulose to
amorphous structure can be rapidly degraded to cellobiose by cellulases. Thus, previous
studies proposed that hydrolysis rates depended on cellulose crystallinity (Fan et al., 1980;
Lee and Fan, 1983). In addition, its was also reported that the alteration of cellulose I, which
is the considered as the native cellulose with parallel arrangement of chains and high degree
of intra- and intermolecular hydrogen bonding (Kumar et al., 2010b) to cellulose II also
enhance the enzymatic hydrolysis of cellulose. Wada et al. (2010) and Cheng et al. (2011)
reported that the transformation of cellulose I to cellulose II through mercerization or ionic
liquid pretreatment resulted in faster enzymatic hydrolysis rate constant for glucose
production. The most common method for testing the crystallinity and structural features of cellulose is based on the X-ray diffraction (XRD). Kumar et al. (2009 and 2010) applied the XRD approach to investigate the crystalline alteration of cellulose after varied kinds of pretreatment approach. The XRD analysis method was also used to investigate the structural alteration of cellulose such as the transformation of cellulose I to cellulose II (Cheng et al., 2011). The degree of polymerization (DP) of cellulose was found to be related to enzymatic hydrolysis (Hilden et al., 2005., Pala et al., 2007., Hallac, B.B., 2011). The DP reduction of cellulosic fractions should also improve its enzymatic hydrolysis effectiveness through making more ends available to enzyme thereby enhancing the hydrolysis rates and the corresponding glucose yields (Yang et al., 2011). However, there exists a few studies reported that the decreased in DP had less effect on the enzymatic hydrolysis rate (Sinitsyn, et al., 1991, Zhang and Lynd, 2006).

Ion Chromatograph (IC) which was used to analyze the DP of released sugars after pretreatment (Yang and Wyman, 2008) could be served as a approach for the DP analysis of generated sugars.

In this study, we performed a systematic investigations on the flowthrough pretreatment of poplar wood under elevated temperatures ranging from 220 °C—280 °C with water-only and 200 °C—250 °C with 0.05 wt.% H₂SO₄ at flow rate of 25mL/min. The changes in structure characterization of the whole pretreated slurries were tracked by FTIR. Particularly, the structure transformation of the cellulosic fractions, were analyzed via XRD and IC. These physicochemical results were compared with rate constants of enzymatic hydrolysis to
determine the effect of acidic hot water pretreatment under tested conditions on digestibility performance of lignocellulosic biomass.

5.3. Materials and methods

5.3.1. Feedstock

Poplar wood was provided by Dave Lanning from Precision Wood Biomass Feedstock Crumbles™ Material (Auburn, WA). It contains 48.8 wt. % cellulose, 16.8 wt.% xylan and 23.7 wt. % lignin. The compositions of poplar wood is determined based on Laboratory Analytical Procedure (LAP) of “Determination of Structural Carbohydrates and Lignin in Biomass” (Sluiter et al., 2008a). The materials was ground with Hammermill (model: WA-6-HSS, Hammer: 1067-A-1, Max. rpm: 4500, Shutte Buffalo Hammermill, Buffalo, NY 14206) at 4500rpm with a 1.59mm screen. Then the particles were collected to pass between Sieve 20 mesh and Sieve 40 mesh to obtain particles over a size range of 0.425-0.850 mm for experiments and analysis. The materials were sealed in heavy duty zipped bags, and stored at -20°C in a laboratory freezer.

Microcrystalline cellulose (Avicel PH-101) was provided by Sigma-Aldrich (St. Louis, MO).

5.3.2. Acidic hot water flowthrough pretreatment

The flowthrough reactor used in this work were 1.3 cm i.d.×15.2 cm length with an internal volume of 20.2 mL. These units are constructed of 316 stainless-steel parts using VCR (Swagelock Corp.) fittings, including one VCR male union (1.3 cm), two gasket filters (316 stainless-steel, average pore size 5 μm), two VCR glands (1.3 cm ×1.3 cm), two VCR nuts, and two VCR reducing fittings (1.3 cm×0.3cm). All reactor parts are obtained from the
Maine Valve and Fitting Co. A 0.3cm stainless-steel thermocouple (Omega Engineering Co., Stamford, CT) is installed at the outlet of the reactor to monitor temperature. Stainless-steel tubing (316) is used as a preheating coil (0.6 cm o.d. × 0.1 cm wall) and to connect the reactor with other system components as well the cooling coil (0.3 cm o.d. × 0.1 cm wall). The preheating coil was long enough to allow the incoming water to reach the desired temperature before it entered the reactor, as measured experimentally. A high pressure pump (Acuflow Series III Pumps, Fisher) with a flow rate range of 0 to 100 mL/min, a pressure gauge (pressure range 0 to 1500 psi; Cole-Parmer Instrument Co., Vernon Hills, IL), and a back-pressure regulator (Maine Valve and Fitting Co.) are used to control flow through the system.

To operate the flowthrough unit, 0.5g of biomass substrate is loaded into the reactor, which is then connected to the system. Distilled water or 0.05 wt. % sulfuric acid at room temperature is pumped through the reactor to purge air and then used to pressurize the reactor to a set pressure of 225psi—1245psi. The loaded biomass is completely wetted by this procedure. The reactors are heated to the target temperature (200—280 °C) in a 4-kW fluidized sand bath (model SBL-2D, Omega engineering, Inc., Stamford, CT). The setting temperatures of sand bath were 20 °C higher than the target temperatures. The preheating time to reach 220°C, 230 °C, 240 °C, 250 °C, 260 °C, 270 °C and 280 °C were 1.6min, 1.7min, 2.0min, 2.2min, 2.3min, 2.5min and 2.8min, respectively. The average preheating rate is around 100 °C/min.

Flow rate ranging from 10mL/min—62.5mL/min was used in this series of experiments.

5.3.3. Analytical methods
5.3.3.1. Ion Chromatograph

The pretreatment hydrolyzate flowing out of the flowthrough system was collected then filtered through a 0.45 µm polypropylene membrane filter (VWR, Radnor, PA) and then analyzed by the Dionex ICS-3000 Ion Chromatograph (IC) system to quantify the chain lengths of xylooligomers and glucose oligomers over a degree of polymerization (DP) range of 1–30. The IC system consisted of Dionex ED40 Electrochemical Detector (Dionex, Sunnyvale, CA) equipped with a CarboPac PA200 (3mm×50mm) Dionex, Sunnyvale, CA). The column was run at 30 °C with an eluent flow rate of 0.3 mL/min. Two eluents were prepared as the mobile phase in plastic bottles pressured with inert nitrogen gas at 6 to 9 psi pressure, which consisted of 0.1 M NaOH solution and 0.5 M NaOAc containing 0.1 M NaOH solution (NaOAc-NaOH). Xylan oligomers and glucose oligomers concentrations with DPs from 1 to 5 could be calculated by comparison to results with known amounts of standards (xylobiose, xylotriose, xylotetraose, and xylopentaose) and (cellobiose, cellotriose, cellotetraose and celluopentose) obtained from Magazyme International Ireland (Bray, County Wicklow, Ireland) and Sigma-aldrich (St. Louis, MO). However, because such materials were not available for DPs greater than 5, we calculated the concentration of each of these species by taking the ratio of each peak height to the peak height for xylobiose and multiplying this ratio by the measured concentration of the latter according to a procedure we developed previously (Yang & Wyman, 2008a).

5.3.3.2. FTIR
Surface chemical analysis was conducted to determine functional groups’ change on pre-treated biomass using a SHIMADZU IRPrestige-21 Fourier transform infrared Spectrophotometer (Shi-madzu Corp., Japan) with a universal attenuated total reflection. Prior analysis, each sample was homogenized by vortex and pressed against diamond surface by anvil. Spectra were obtained using the triangular apodization in resolution of 4 cm\(^{-1}\) with thirty two scans for each sample from 4000 to 800 cm\(^{-1}\).

5.3.3.3. XRD

The acidic hot water pretreated whole biomass and Avicel slurries and untreated biomass and Avicel were analyzed by XRD. Following the pretreatment, the samples were freeze-dried, and then stored in room temperature prior to XRD analysis. The samples were measured by the general purpose X-ray diffractometer with Philips X’Pert MPD system and a vertical \(\theta-\theta\) goniometer (190 mm radius). The X-ray source was a ceramic X-ray tube with Cu anode. Operating power was 40 kV, 50 mA (2.0 kW). X-ray diffraction pattern of samples obtained after freeze-drying were recorded at room temperature from 10° to 40°. The scan was carried out with a step size of 0.05°.

5.3.3.4. Enzymatic hydrolysis

Novozymes Cellic® CTec2 (220mg protein/mL, preserve 200mg glucose/mL) provided by NREL were used in enzymatic hydrolysis of pretreated Avicel. The pretreated whole cellulosic slurries from flowthrough pretreatment was adjusted to the pH value of 4.8–5.0 and underwent enzymatic hydrolysis in 500mL bottle at 50 °C on the rotary shaker (MaxQ 4000, Thermo Scientific, Waltham, MA ) at 150 rpm. Enzyme loadings of 100 mg protein
Ctec (93 FPU) were used. Samples were taken and filtered periodically at different time points (4, 24, 48, 72, 96 and 120 h), and the filtrates were analyzed for glucose.

Enzymatic glucose yield\(= \frac{W_{G2}}{W_{TG} - W_{G1}} \times 100\% \quad (6)\)

Enzymatic xylose yield\(= \frac{W_{X2}}{W_{TX} - W_{X1}} \times 100\% \quad (7)\)

In these equations, \(W_{G1}\) and \(W_{X1}\) are the glucose and xylose released in the pretreatment; \(W_{G2}\) and \(W_{X2}\) are the glucose and xylose released in enzymatic hydrolysis; \(W_{TG}\) and \(W_{TX}\) is the total glucose and xylose released after enzymatic hydrolysis of pretreated whole slurries with the high enzyme loading in 168hrs; The unit of \(W\) consistently refers to g/100g dw raw biomass.

### 5.4. Results and discussion

#### 5.4.1. Effect of reaction severity on the removal of Poplar wood/Avicel and the corresponding sugar recovery in acidic hot water flowthrough system

The flowthrough pretreatment of poplar wood was operated under temperatures ranging from 220 °C—270 °C for water-only operations (group 1) and 200 °C—240 °C with 0.05 wt.% \(\text{H}_2\text{SO}_4\) (group 2) over reaction time 8—90 min. Both groups of experiments were carried out at flow rate of 25mL/min. Table 1 shows the effects of reaction severity (temperature, time and acid) on the removal of xylan, lignin and cellulose as well as the corresponding sugar recovery after flowthrough pretreatment. It was found that 100% xylan and 83.7%—100% lignin removal were obtained over the tested conditions. The removed xylan was predominately in the form xylose and xylooligomers with negligible degradation compounds.
such as furfural. Compared with xylan and lignin, the cellulose removal under relative lower temperatures (220 °C and 230 °C) was obtained to a less degree. For example, 22.1% cellulose removal were observed under 220 °C over 30min for water-only operations. Prolonging reaction time to 90 min merely enhanced the cellulose removal up to 27.3%. The cellulose removal was enhanced significantly as reaction temperature increased to 240 °C, which led to 52.2%-68.6% cellulose removal after pretreatment of poplar wood over 8-24min, respectively. Continuously increasing reaction temperature (270 °C) or adding extremely dilute acid (0.05 wt.% H₂SO₄) enhanced the efficacy of cellulose hydrolysis. For instance, water-only flowthrough pretreatment under 270 °C for 10 min and 0.05 wt.% H₂SO₄ flowthrough pretreatment under 240 °C for 8 min resulted in 98.7% and 95.2% cellulose removal. However, it was found that the amount of removed cellulose was higher than total amount of soluble cellulosic fraction (glucose and glucose oligomers), which was more apparent for water-only operations. For example, under 240 °C with 12 min for water-only pretreatment, 52.2% cellulose removal from poplar wood merely resulted in 21.0% total glucose plus glucose oligomers yield and negligible 5-HMF production. A subsequent enzymatic hydrolysis of the whole slurries pretreated with overloading enzyme of 100 mg protein Ctec2 (93 FPU) and 20 mg protein led to >95% cellulose conversion to glucose. It indicated that, besides glucose and glucose oligomers detected by autoclaving with 4% H₂SO₄ (Sluiter et al., 2006), the remainder of removed cellulose could exist in the form of cellulosic fractions but with varying structure properties.

5.4.2. Determination of DP of sugar oligomers
Flowthrough pretreatment with water-only or dilute acid resulted in significant biomass removal (~98%) under tested conditions. Apart from the mono sugars (glucose and xylose), large fractions of removed sugar fractions were in the form of soluble oligomers. The analysis of DP distribution of these soluble oligomers appeared to be important in understanding the effect of such leading pretreatment approach on sugar generation. Previous study (Yang & Wyman, 2008a) has proven that IC is an effective instrument for characterizing the DP of the released sugar oligomers from acidic flowthrough pretreatment. The DP distribution data for both xylooligomers and glucose oligomers obtained based on this approach was summarized in Figure 1. Figure 1a revealed that the DP of released xylooligomers was predominately >6 under lower temperatures (220 °C and 230 °C). The DP distribution shifted significantly toward to the lower DP xylooligomers as the temperature increased. When temperature reached 270 °C, 21.1 % xylose and 45.3% short chain xylooligomers (DP 2−6) were obtained, while the xylooligomers with DP>6 were merely observed 31.6%. Adding dilute acid (0.05% (w/w) H₂SO₄) significantly reduced the length of the xyooligomers. The yield of xylose plus short chain xylooligomers (DP 2−6) increased from 35.2%−52.1% to 67.1%−87.5% when adding 0.05% (w/w) H₂SO₄ under identical temperature range (220 °C−240 °C).

Figure 1b shows the DP distribution of removed cellulose after flowthrough pretreatment under tested conditions. Under relative lower temperatures (220 °C−230 °C), less than 15% soluble cellulosic fractions include glucose and shorter chain glucose oligomers (DP 2-6) were observed. Around 12% insoluble longer chain cellulosic fractions as well as substantial
cellulose residue (75%−78%) were observed. Continuously increasing temperatures to 240 °C enhanced the yield of soluble glucose and shorter chain glucose oligomers (DP 2-6) (21.0%). It was found that the yield of glucose and shorter chain glucose oligomers (DP 2-6) reached 50.1% as temperature was elevated to 270 °C. Compared with temperature, adding dilute acid appeared to have more severe impact on the DP distribution of the recovered cellulose. The yields of soluble glucose and shorter chain glucose oligomers (DP 2-6) were obtained 67.0% and 86.3% under 220 °C and 240 °C, respectively, which demonstrated that the majority removed cellulose were obtained in the soluble form.

5.4.3. FTIR analysis of pretreated whole biomass slurries

FTIR spectroscopic analysis was employed to investigate the structural alteration of pretreated whole slurries of poplar wood. The FTIR spectrum that indicates the effects of acidic hot water flowthrough pretreatment with respect to tested conditions on the functional group modification of poplar wood was displayed in Figure 2. The peak around 3348 cm\(^{-1}\) has been previously assigned to the OH stretching, and the samples pretreated at temperatures above 240 °C with water-only (240 °C, 12 min; 270 °C, 10 min) or with 0.05 wt.% H\(_2\)SO\(_4\) (220 °C, 8 min) had the remarkable reduction in the intensity of this peak, which indicated the decrease of the crystallinity in the cellulose (He et al., 2008). The peak at 2900 cm\(^{-1}\) is ascribed to C-H stretching (Kumar et al., 2009), acidic hot water flowthrough pretreatment for poplar wood under tested conditions produced negligible change for each substrate, which implied few methyl and methylene portions of poplar wood were disrupted.
Acidic hot water flowthrough pretreated biomass shows considerable alterations in the region 1800—800 cm⁻¹ of FTIR spectra. The band position at 1040 cm⁻¹ and 1060 cm⁻¹ was associated with C-O stretch, and 1120 cm⁻¹ was attributed to ring stretching frequency of cellulose (Oh et al., 2005). The increased intensity of these peaks with enhanced temperatures or the addition of 0.05 wt.% H₂SO₄ indicated the exposure of cellulose counterpart possibly via intermolecular hydrogen bond cleavage (Laskar et al., 2013b). At the same time, the peak intensity at 1740 cm⁻¹ assigned for C=O in xylan (Pandey & Pitman, 2003) decreased with the enhancement of temperature or the addition of 0.05% (w/w) H₂SO₄, which revealed the degradation of hemicellulose. The peak intensities in the range of 1260–1760 cm⁻¹ were ascribed for lignin regions (El Hage et al., 2010). The biomass pretreated under higher temperature or with the addition of 0.05% (w/w) H₂SO₄ resulted in more significant reduction within the peak intensities in this range.

In regard to the pretreated samples, subsequent change in the band position at 1250 cm⁻¹, 1330 cm⁻¹ and 1500 cm⁻¹ which were assigned to guaiacyl (G)/C=O stretch, syringyl (S)/resinol linkages and aromatic skeletal vibration, respectively (Laskar et al., 2013b) implied the modification and/or degradation on lignin units. The increased temperature or the addition of 0.05% (w/w) H₂SO₄ resulted in the relative significant reduction of these peaks, which could be the consequence of the relative severe modification of the lignin. FTIR results implied that acidic hot water flowthrough pretreatment under high temperature (e.g. 240 °C) with both water-only and 0.05 wt.% H₂SO₄ led to significant xylan disruption and lignin
modification. In addition, the crystallinity of the cellulose appeared to be reduced to some extent.

5.4.4. XRD analysis of the pretreated cellulose

The kinetic results (section 3.1) showed that cellulose was removed significantly during flowthrough pretreatment when reaction temperature was elevated to 240 °C for water-only and 240 °C for 0.05 wt.% H₂SO₄. Investigating the structure properties of the pretreated cellulosic fractions appears to be significant in understanding its subsequent enzymatic hydrolysis efficacy. Since lignin and hemicellulose lack of regular crystal structure, the XRD analysis of the pretreated poplar wood including the hemicellulosic and lignin fractions which influenced the interpretation of the crystalline of cellulose appeared to be difficult. In this regard, we pretreated the microcrystalline cellulose (Avicel PH-101) instead of poplar wood in flowthrough system under identical conditions described in section 3.1. Figure 3a shows the XRD pattern for total cellulosic fractions from flowthrough pretreatment with both water-only under varying tested conditions (section 3.1). The untreated Avicel (control) exhibited several broad diffraction peaks which matched those for cellulose I, and particularly cellulose Iβ, in the database published by the International Centre for Diffraction Data. The pattern also agreed well with one calculated from the cellulose Iβ crystal structure determined by Nishiyama and Langham (2003) and contains a strong peak at 22.5 °2θ corresponding to the distance between hydrogen-bonded sheets. The samples pretreated with water-only at 220 and 230 °C had nearly identical diffraction patterns, showing that no significant structural changes occurred at these temperatures. It was interesting that when reaction temperature
was elevated to 240 °C or above (270 °C), the cellulose Iβ peaks reduced in intensity and new peaks at 12 and 20 °2θ emerged, indicative of cellulose II. At 240 °C some cellulose Iβ remained, but after pretreatment at 270 °C the only crystalline material was cellulose II. The relatively weak cellulose II peaks and the very broad background peak centered around 21 °2θ suggested that a significant fraction of the sample was amorphous. Such structure transition from cellulose I to cellulose II could account for the significant cellulose removal when temperature reached 240 °C or above (e.g. 270 °C) (Langan et al., 2001; Nishiyama Y et al., 2003).

It was reported that both cellulose I and cellulose II with parallel chains and antiparallel chains respectively are stacked through the hydrophobic interaction such as van der Waals force (Wada et al., 2010), which could be calculated to exceed the hydrogen-bonding interactions (French et al., 1993). Such hydrophobic attraction was considered as the main factor to resist enzymatic hydrolysis with cellulase. Comparatively, the hydrophobic force in cellulose II is weaker than that in cellulose I (Wada et al., 2010). In this regard, it can be anticipated that transforming cellulose I into cellulose II with weaker hydrophobic interaction, and to amorphous cellulose, could accelerate the enzymatic hydrolysis rate constant of cellulose. The XRD pattern for total cellulosic fractions from dilute acid flowthrough pretreated cellulose under tested conditions was presented in Figure 3b. No significant change from the untreated control sample was observed following pretreatment at 210 °C, but at higher temperatures the intensity of diffraction peaks reduced significantly, suggesting the disruption of the crystallinity of cellulose. A weak cellulose I peak was
observed for the Avicel sample pretreated at 220 °C, but when the temperature was further elevated to 240 °C the diffraction peaks were replaced by a broad featureless pattern, indicating that the total crystalline cellulose was transformed to an amorphous structure.

5.4.5. **Enzymatic hydrolysis of pretreated cellulose**

The Avicel samples pretreated with both water-only and dilute acid in flowthrough system were hydrolyzed by enzyme to test their amenability for glucose generation. The enzyme loading employed in this study was 93FPU/g glucan. Figure 4a shows the glucose yields obtained from flowthrough water-only pretreated Avicel after enzymatic hydrolysis. Regardless of the pretreatment conditions, the rate constant of glucose generation from all the tested samples appeared to be improved over the control. The glucose yield from untreated Avicel was merely observed 37.1% within 4h, >80 % glucose yield was obtained after 48h enzymatic hydrolysis, and the highest glucose yield was observed 88.9% over 120 h. The efficacy of glucose generation from Avicel pretreated under 220 °C—230 °C with water-only appeared to be enhanced to some extent, which was observed 49.7% and 53.0% during 4h, respectively. The glucose yield increased constantly as the reaction time prolonged, around 90% glucose yield was obtained after 120h enzymatic hydrolysis. Such elevated efficacy of enzymatic hydrolysis could be predominately attributed to the decreased crystalline of cellulose after pretreatment. Avicel pretreated under 240 °C or above (e.g. 270 °C) seemed to result in much higher rate constant of glucose generation compared with that pretreated under lower temperatures (220 °C—230 °C), the glucose yields obtained were 69.5% and 95.4% after 4h and 120 h enzymatic hydrolysis. This was understandable because as aforementioned
(section 3.1 and 3.3), the partial transformation of cellulose I to cellulose II occurred when reaction temperature reached 240 °C or above in water-only flowthrough pretreatment, which contributed to the elevated efficiency of glucose generation. Figure 4b revealed that the glucose yield profile for 0.05%(w/w) H₂SO₄ treatment under 210 °C displayed a much slower rate constant than those at higher temperatures (220 °C—230 °C). It was found that the glucose yields from 220 °C and 240 °C, 0.05% (w/w) H₂SO₄ pretreated cellulose reached and 87.4% and 93.6% after 4h enzymatic hydrolysis. This could be the consequence of the substantial cellulose recovery in the form of glucose and glucose oligomers (82.9%) in pretreatment stage. Particularly, the glucose yield was obtained 55.3%, which accounted for the majority portions based on the original glucose in Avicel.

5.5. Conclusion

The hot water/0.05 wt. % H₂SO₄ pretreatment conducted under temperatures ranging from 200 °C—280 °C enabled the total removal of biomass when temperature was above 240 °C for water-only and 220 °C for 0.05 wt.% H₂SO₄ treatment. The functional groups of hemicellulose and lignin were reduced progressively as the severity enhanced for both water-only and dilute acid (0.05 wt.% H₂SO₄) pretreatment. Temperature play a key role in determining the cellulose degradation. The transformation of cellulose I to cellulose II occurs during the cellulose removal process when temperature reached 240 °C for water-only operations. At 270 °C, all the cellulose I disappeared and only amorphous material and some cellulose II remain. In the dilute acid (0.05 wt.% H₂SO₄) solutions, only the disruption of crystallinity was observed (i.e. no cellulose II). This was almost complete at 220 °C and fully
complete after 8 minutes at 240 °C. Enzymatic hydrolysis of pretreated cellulose revealed that the reactivity of cellulose was significantly enhanced after flowthrough pretreatment at temperature ≥240 °C for water-only and 220 °C for 0.05 wt.% H₂SO₄.
5.6. References


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Figure 5.1 Distribution of degree of polymerization and polysaccharides mass balance for water-only and 0.05% (w/w) H$_2$SO$_4$ hydrolysis of poplar wood. (a) xylan hydrolysis (b) cellulose hydrolysis. a1: 220 °C, water-only, 30min; a2: 230 °C, water-only, 16 min; a3: 240 °C, water-only, 12min; a4: 270 °C, water-only, 10min. b1: 210 °C, 0.05% (w/w) H$_2$SO$_4$, 30min; b2: 220 °C, 0.05% (w/w) H$_2$SO$_4$, 12 min; b3: 240 °C, 0.05% (w/w) H$_2$SO$_4$, 8min.
Figure 5.2 FTIR spectrum of untreated poplar wood (control) and flowthrough (25mL/min) pretreated poplar wood samples with water-only (a) and 0.05%(w/w) H$_2$SO$_4$(b). a1: 220 °C, water-only, 30min; a2: 230 °C, water-only, 16 min; a3: 240 °C, water-only, 12min; a4: 270 °C, water-only, 10min. b1: 210 °C, 0.05%(w/w) H$_2$SO$_4$, 30min; b2: 220 °C, 0.05%(w/w) H$_2$SO$_4$, 12 min; b3: 240 °C, 0.05%(w/w) H$_2$SO$_4$, 8min.
Figure 5.3 XRD pattern of untreated Avicel (control) and flowthrough (25mL/min) pretreated Avicel samples with water-only (a) and 0.05%(w/w) H$_2$SO$_4$(b).

a1: 220 °C, water-only, 30min; a2: 230 °C, water-only, 16 min; a3: 240 °C, water-only, 12min; a4: 270 °C, water-only, 10min. b1: 210 °C, 0.05% (w/w) H$_2$SO$_4$, 30min; b2: 220 °C, 0.05% (w/w) H$_2$SO$_4$, 12min; b3: 240 °C, 0.05%(w/w) H$_2$SO$_4$, 8min.
Figure 5.4 Enzymatic hydrolysis of untreated Avicel (control) and flowthrough (25mL/min) pretreated Avicel samples with water-only (a) and 0.05% (w/w) H₂SO₄ (b).
Table 5.1 The major components removal from Poplar wood/Avicel and the corresponding recovered sugar yields after acidic hot water flowthrough pretreatment

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<th>Time (min)</th>
<th>Xylan removal (%)</th>
<th>Lignin removal (%)</th>
<th>Cellulose removal (%)</th>
<th>Xylose (%)</th>
<th>Xylooligomers (%)</th>
<th>Glucose (%)</th>
<th>Glucose oligomers (%)</th>
<th>Furfural (%)</th>
<th>5-HMF (%)</th>
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Chapter 6

Kinetic modeling of the decomposition of lignocellulosic biomass in acidic hot water flowthrough pretreatment

6.1. Abstract

Dilute acid flowthrough pretreatment has been proven as an effective approach to dissolve biomass with high sugar and lignin recovery. However, low sugar concentration resulted from the relatively low solid to liquid ratio employed still retards its application compared with conventional batch system. In this study, removal of xylan, lignin, and cellulose from poplar wood by dilute acid (0.05 wt.% H_2SO_4) flowthrough pretreatment were found significantly enhanced when operation temperature was raised from 200 °C to 240 °C. The observed removal of xylan, lignin and cellulose correlated with reaction kinetics, flow rate, biomass particle size, and solid loading were described by flowthrough kinetic model with the incorporation of mass transfer coefficient. This model successfully simulated the experimental results from acidic flowthrough pretreatment of poplar wood. The effects of varying flow rates, particle size as well as solid loading on the mass transfer limitation and the sequential biomass dissolution performance was investigated based on this model. These new finding provides a more in-depth understanding on the acidic hot water flowthrough pretreatment of biomass for higher sugar concentration.

Key words: flowthrough pretreatment; dilute acid; kinetic model; mass transfer; reactor

6.2. Introduction

Acidic hot water (water-only or <0.1 wt.% H_2SO_4) flowthrough pretreatment with the
advantage of lower capital and limited reactor corrosions showed promising perspective for the efficient biomass disruption and sugar recovery. Our previous studies (chapter 3 and 4) reported that 0.05 wt. % H$_2$SO$_4$ flowthrough pretreatment under elevated temperature (e.g. 240 °C) resulted in almost total biomass dissolution (~98%) accompanied with total removal of xylan, lignin and cellulose. Maximum 100% xylose plus xylooligomers and 90% glucose plus glucose oligomers were obtained with negligible degradation compounds. The removed lignin was predominately in the insoluble form (85%−100%) after quenched in room temperatures. In addition, the subsequent enzymatic hydrolysis of whole pretreated slurries revealed that over 95% fermentable sugar yields was obtained with 9.3FPU/g glucan cellulase. However, due to the low solid to liquid ratio (e.g. 0.25%) employed in flowthrough system (chapter 3), the concentration of total fermentable sugar didn’t exceed 1.5g/L. Furthermore, the relative small particle size of feedstock (20−40mesh) implied the high energy cost for milling biomass in industrialization (McMillan, 1994a).

Investigating the effects of physical parameters including flow rates, solid loading and particle size through mathematical interpretation such as kinetic modelling appears to be important for obtaining an in-depth understanding of the flowthrough acidic hydrolysis of lignocellulosic biomass in flowthrough system, thereby achieving the improved sugar concentration from biomass with larger particle size for economic viability. The current kinetic modeling used for describing the biomass degradation was based on Saeman’s model (1945), which was developed to investigate the hydrolysis of Douglas fir in batch reactors. During the following years, Saeman’s model was developed to investigate numerous
acid-catalyzed hydrolysis of cellulose and xylan from lignocellulosic biomass (Fagan et al., 1971; McKibbins et al., 1962; Mosier et al., 2002; Xiang et al., 2004b). With the consideration of the heterogeneous properties of the lignocellulosic biomass degradation, a leaching model incorporating the mass transfer coefficient was proposed by Brennan and Wyman (2004a) to describe hemicellulose hydrolysis from corn stover under 180 °C with water-only or 0.5%—1.0% acid in both batch and flowthrough system. It was found that decreasing solid concentration from 10% to 5% or enhancing flow rate from 1mL/min to 10mL/min led to significant enhancement of mass transfer under tested reaction kinetics. However, such leaching model proposed by Brennan and Wyman merely focused on the acidic hydrolysis of hemicellulose, while the degradation performance of cellulose and lignin depicted by this model still unknown. Furthermore, few studies have evolved the leaching model with the systematic consideration of varying reactor configuration (e.g. flow rate, the ratio of solid to reactor volume) and feedstock properties (e.g. particle size s) to investigate the performance of biomass degradation in acidic conditions. The physical parameters such as flow rate, the ratio of solid loading to reactor volume have been reported to effect the mass transfer coefficient during biomass hydrolysis(Yang & Wyman, 2004a) Still, there is concern about the current leaching model’s ability to accurately describe the realeasement of sugars generated from biomass in flowthrough reactor. The biomass degradation in flowthrough system often resulted in considerable amount sugar oligomers(Yang & Wyman, 2008a)(chapter 4 and 5), which was further react to form sugar monomers and the sequential degradation compounds (e.g. fufural and 5-HMF). The distribution of the sugar oligomers
and monomers generated in flowthrough system was also effected significantly by the flow rate (Yang & Wyman, 2008a)(chapter 4 and 5). In this regard, the leaching model may precisely fit the experimental data in flowthrough system but at the expense of accurately describing the true mechanism of biomass hydrolysis(Jacobsen & Wyman, 2002). To alleviate these concerns, researchers should develop more complex models that incorporate some aspects of system configuration and mass transfer coefficient to depict the acidic hydrolysis of biomass as well as the individual components xylan, cellulose and lignin in flowthrough system.

In this study, a flowthrough model which includes the mass transfer coefficient and the aspects of the reactor configuration parameters was developed to describe the performance of dilute acid (0.05 wt.% H₂SO₄) flowthrough pretreatment of poplar wood in terms of xylan, cellulose and lignin degradation and the corresponding products (e.g. sugars) generation. The basic kinetics were determined and used to explain how the hydrolysis of biomass and individual components including hemicellulose, lignin and cellulose is impacted by solid loading, particle size and flow rates in flowthrough system. In addition, the corresponding sugar (monomers and oligomers) generation was predicted by the flowthrough model as well. These kinetic results provide insightful data to overcome the challenge of realizing high sugar concentration when achieving yield and fementability advantages of flowthrough approaches.

6.3. Kinetic model of biomass degradation in flowthrough system

The flowthrough model was proposed to depict the degradation of xylan, cellulose and lignin during the pretreatment process.
6.3.1. Xylan degradation model

Based on the kinetic results obtained in chapter 4, a degradation pathway of xylan degradation in flowthrough system was developed. This assumed that xylan was hydrolyzed into xylooligomers, which was subsequently decomposed to xylose and degradation products (e.g. furfural) (Scheme 1). The kinetic model developed from scheme 1 is described by equation 1—3 to track the degradation of xylan and the corresponding generation of xylooligomers and xylose.

\[
Xylan \xrightarrow{k_m} \text{Xylooligomers} \xrightarrow{k_2} \text{Xylose} \xrightarrow{k_3} \text{Degradation(Furfural)}
\]

Scheme 1

\[
\frac{dX_n}{dt} = \frac{A_s}{V_c} (-k_M) X_n
\]

(1)

\[
\frac{\partial X_O}{\partial t} = \frac{A_s}{V_c} (k_M) X_n - k_2 X_O + \frac{F}{\pi r^3} \frac{\partial X_O}{\partial z}
\]

(2)

\[
\frac{\partial X}{\partial t} = k_2 X_O - k_3 X + \frac{F}{\pi r^3} \frac{\partial X}{\partial z}
\]

(3)

in which \( A_s \) is the surface area of the particles (cm\(^2\)/g), \( V_c \) is the volume of the solid (cm\(^3\)/g), \( F \) is the flow rate (mL/min), \( r \) is the radius of the reactor (cm), \( z \) is the height of the reactor (cm). \( X_n \) is xylan (xylose equivalents) (g), \( X_O \) is xylooligomers (xylose equivalents) (g), \( X \) is the xylose (g), \( t \) is the reaction time (min). \( k_M \) is the mass transfer coefficient (cm/min), \( k_2 \) and \( k_3 \) are the reaction rate constants (min\(^{-1}\)).

The model was applied to predict the xylan hydrolysis during the acidic hot water pretreatment of poplar wood in flowthrough reactor. In the flowthrough reactor used in this
study, the solid flow rate is 0 while the liquid flow rate is ranging from 10—62.5mL/min. The flowthrough reactor had a diameter of 1.3 cm and a reactor height of 15.2 cm. Although the generated sugar (xylose and xylooligomers) is the function of both reaction time (t) and reactor height (z) (equation 2—3), the fixed reactor height used in this study (15.2 cm) revealed that only one variable (i.e. time) indeed determine the sugar generation (Shao & Lynd, 2013). In this regard, the sugar concentrations obtained from two boundaries (inlet and out of 15.2 cm flowthrough reactor) without the internal sugar concentrations were used to fit the model (Shao & Lynd, 2013).

6.3.2. Cellulose degradation model

The kinetic results in chapter 3 showed that merely partial removed cellulose was in the form of glucose, while the remainder was the celloolodextrin (chapter 4 and 5). In this regard, the cellulose degradation pathway was proposed in scheme 2. Equation 4—6 was proposed to depict the cellulose degradation based on scheme 2.

\[
Cellulose \rightarrow^{k_u} Cellodextrin \rightarrow^{k_1} Glucose \rightarrow^{k_2} Degradation (HMF)
\]

Scheme 2

\[
\frac{dC}{dt} = \frac{A}{V_c}(-k_M)C \quad (4)
\]

\[
\frac{\partial C_n}{\partial t} = \frac{A}{V_c}(k_M)C - k_2C_n + \frac{F}{\pi r^2} \times \frac{\partial C_n}{\partial z} \quad (5)
\]

\[
\frac{\partial G}{\partial t} = k_2C_n - k_3G + \frac{F}{\pi r^2} \times \frac{\partial G}{\partial z} \quad (6)
\]
Where $A_s$ is the surface area of the particles (cm$^2$/g), $V_c$ is the volume of the solid (cm$^3$/g), $F$ is the flow rate (mL/min), $r$ is the radius of the reactor (cm), $z$ is the height of the reactor (cm). $C$ is cellulose (glucose equivalents) (g), $C_n$ is xylooligomers (glucose equivalents) (g), $G$ is the glucose (g), $t$ is the reaction time (min). $k_M$ is the mass transfer coefficient (cm/min), $k_2$ and $k_3$ are the reaction rate constants (min$^{-1}$).

The model used to depict the cellulose hydrolysis from poplar wood during acidic hot water flowthrough pretreatment is coincident with that for xylan hydrolysis (section 6.2.1). Based on this, the generation sugars from cellulose is the function of reaction time ($t$) except the reactor height $z$ (Shao & Lynd, 2013).

### 6.3.3. Lignin degradation model

Apart from xylan and cellulose, the lignin degradation pathway in acidic hot water flowthrough pretreatment was also proposed. The lignin degradation pathway (scheme 3) and the corresponding reaction model (equation 7) was proposed as follows:

\[
Lignin \xrightarrow{k} \text{Removed lignin (Insoluble & soluble lignin)}
\]

\[
\frac{dL}{dt} = \frac{A_s}{V_c} (-k_M) L
\]  

(7)

Where $A_s$ is the surface area of the particles (cm$^2$/g), $V_c$ is the volume of the solid (cm$^3$/g), $L$ is lignin (g), $k_M$ is the mass transfer coefficient (cm/min).

The feedstock, flowthrough experiment and the analytical methods were described in Chapter 4.

A MATLAB program was used to fit the parameters in Eqs.1 to 7 to simulate the
experimental data obtained in this set of experiments.

6.4. Results and discussion

6.4.1. The effect of kinetics on biomass degradation in flowthrough system

The degradation the three major compounds in biomass including xylan, lignin and cellulose were investigated in a series of flowthrough kinetic experiments under temperatures ranging from 200–240 °C, time scope of 0–12 min with 0.05 wt.% H₂SO₄. The kinetic parameters including the mass transfer were fit to the degradation of xylan, lignin and cellulose based on proposed flowthrough model under tested conditions. As shown Fig. 1a–c, the model was able to describe the experimental results regarding the hydrolysis of xylan, cellulose as well as lignin degradation.

The xylan hydrolysis was susceptible to be hydrolyzed over the tested conditions. Less than 50% xylan residue was left in the reactor body within 2min flowthrough pretreatment under 200 °C. The xylan residue decreased gradually as the reaction prolonged, which resulted in almost total xylan removal as reaction time reached 12min. The corresponding rate constant (mass transfer) observed under 200 °C was 0.1071×10⁻³ cm/min. The temperature gradients appeared to influence the mass transfer of xylan dissolution significantly. It was noteworthy that the mass transfer of xylan hydrolysis increased from 0.2223×10⁻³ to 0.4593×10⁻³ cm/min as temperature further enhanced from 210 °C to 240 °C.

Compared with xylan hydrolysis, cellulose behaved a much slower hydrolysis pattern. As discussed in chapter 4, temperature played an significant role in determining the dissolution of cellulose under tested conditions. Temperature above 220 °C resulted in the abrupt
removal of cellulose. Results (Figure 1b and Table 1) showed that the mass transfer of cellulose hydrolysis under 200—210 °C was observed $0.0055 \times 10^{-3}$ to $0.0079 \times 10^{-3}$ cm/min, which led to more than 80% and 75% cellulose remained in reactor over 0—12 min. The mass transfer of cellulose hydrolysis enhanced apparently to $0.0558 \times 10^{-3} - 0.1001 \times 10^{-3}$ cm/min when temperature was elevated to 220 °C to 240 °C, which could be the consequence of the disruption of crystallinity of cellulose under this temperature range (chapter 5). Lignin in dilute acid water underwent flowthrough reaction (chapter 4) was removed significantly accompanied with xylan and cellulose. Results revealed that the mass transfer increased gradually from $0.0459 \times 10^{-3}$ to $0.2434 \times 10^{-3}$ cm/min as the temperature increased from 200 °C to 240 °C, resulting almost total lignin removal within 6 min under highest temperature (240 °C).

6.4.2. The effect of particle size, solid loading and flow rate on biomass degradation in flowthrough system

Apart from kinetics, previous studies reported that physical parameters such as solid loading (Jacobsen & Wyman, 2002) and particle size of biomass as well as flow rate should be indicative of a role of mass transfer in governing the biomass degradation in flowthrough system. A series of flowthrough reactions with varied biomass particle size 20—40 mesh to 4 mm, loading 0.5 g—6 g in 20.2 mL reactor, and flow rate 10 mL/min—62.5 mL/min were conducted under 240 °C with 0.05 wt.% H$_2$SO$_4$. The flowthrough kinetic model was used to depict rationally the effect of these physical parameters on the biomass degradation.

6.4.2.1. Effect of particle size
Figure 2a–c shows the degradation of cellulose, xylan and lignin from poplar wood with varied particle size (40–60mesh, 20–40mesh, 10–20mesh, 2mm, 4mm) under 240 °C in 0.05 wt.% H₂SO₄ flowthrough system and the corresponding fitted curves from flowthrough model. The trajectories of the xylan, lignin and cellulose residue versus reaction time are similar for all particle size. It was found that almost 100% xylan and lignin were removed during 4min and 6min respectively with biomass particle size ranging from 40–60mesh to 4mm. Furthermore, 91.2%–97.3% cellulose removal with the tested particle size were achieved when the reaction time was prolonged to 8 min. Table 2 summarized and compared the mass transfer coefficient of the degradation of cellulose, xylan and lignin from poplar wood with particle size ranging from 40–60 mesh to 4mm. The value of surface area (As) of biomass is based on the previous studies (Pu et al., 2013), and the value of particle volume was observed 2.8cm³/g(40–60 mesh), 3.0 cm³/g(20–40 mesh 3.1cm³/g(10-20 mesh), 6.0cm³/g(2mm) and 6.5cm³/g, respectively(4mm).

The mass transfer coefficient of the degradation of cellulose, xylan and lignin were observed between $0.0989 - 0.01741 \times 10^{-3}$ cm/min, $0.4821 - 0.7865 \times 10^{-3}$ cm/min and $0.2396 - 0.4268$ cm/min over the tested particle size, respectively. Results indicated that the larger particle size presented relative lower mass transfer than the smaller particle one. It was noteworthy that the mass transfer in this set experiment with small particle size (40–60 mesh) was 30–40% higher than larger particle size (4mm). Comparatively, previous studies(Kim & Lee, 2002) showed that under lower temperatures ranging from 25 °C–75 °C, 30% difference in particle size (as in 14–20 mesh) caused about 40% difference in estimated
diffusivity of acid into biomass. In this regard, it could be concluded that acidic hot water pretreatment operated under elevated temperature (e.g. 240 °C) was more likely to be temperature dependent than that conducted under lower temperature (e.g. <100 °C), in which the mass transfer limitation caused by the heterogeneous properties of biomass particles could not be overlooked.

6.4.2.2. Effect of solid loading

The effect of solid loading on biomass degradation in flowthrough reactor (20.2 mL) was also investigated. As depicted in Figure 3a–c, under identical reaction conditions (0.05 wt. %H₂SO₄, 240 °C, 25mL/min, 0–8min, 4mm particle size), increasing solid loading from 0.5g–4g resulted in insignificant deviation on the removal of cellulose, xylan and lignin in flowthrough system. However, when the solid loading was continuously elevated to 6g, the efficacy of the degradation of cellulose, xylan and lignin appeared to decline to a relative lower level, which was inconsistent with the chemical reaction control. For example, after 8min 0.05 wt.% H₂SO₄ flowthrough pretreatment for 0.5g–4 g biomass loading merely led to 7.3%–10.2% cellulose left in the reactor. Comparatively, 19.8 % cellulose residue was observed for 0.05 wt.% H₂SO₄ flowthrough pretreatment with 6 g biomass. The mass transfer coefficient presented in Table 3 regarding the degradation of cellulose, xylan and lignin from poplar wood with solid loading ranging from 0.5g–6g revealed that the relative higher solid loading (6g) led to significant mass transfer reduction for these three major compounds degradation as compared to lower solid loading (0.5g–4g) in flowthrough system. In this regard, 4g biomass feedstock in 20.2 mL flowthrough reactor with the ratio
around 20 g/mL appeared to be the maximum solid loading with insignificant mass transfer reduction for flowthrough pretreatment under tested conditions.

### 6.4.2.3. Effect of flow rate

In order to diminish the mass transfer limitation induced by the overloading solids (6g), varying flow rates (10 mL/min – 62.5mL/min) were employed for the flowthrough pretreatment of biomass with elevated solid loading (6g) to investigate their impacts on the mass transfer of the degradation of xylan, cellulose and lignin. Although previous studies (chapter 4) revealed that flow rate had negligible impact on the biomass dissolution with low solid loading (0.5g in 20.2 mL flowthrough reactor) under identical severities, its impact on high solid loading (e.g. 6g) were still unknown. Figure 4a–c shows the degradation of cellulose, xylan as well as lignin in flowthrough system with flow rate 10mL/min, 25mL/min and 62.5mL/min.

2.6%, 4.1% and 5.2% enhancement of xylan, cellulose and lignin degradation were realized as flow rate increased from 10 mL/min to 62.5 mL/min, implying that increasing flow rate played an insignificant role in enhancing the mass transfer under tested conditions. The corresponding mass transfer coefficient ($k_M$) of the xylan degradation increased 21.3% (0.4548 to 0.5527×10⁻³ cm/min) (Table 4) when flow rate increased from 10 mL/min to 62.5 mL/min under 240 °C with 0.05 wt.% H₂SO₄. This result was consistent with the observation by Brennan and Wyman (2004a) when studying the xylan hydrolysis from corn stover in flowthrough system under 180 °C with both water-only and 0.05 wt.% H₂SO₄, where flow rate played a relative important role in governing the mass transfer of xylan.

200
degradation thereby influencing the corresponding xylose and xylooligomers generation. Comparatively, flow rate has less impact on mass transfer of lignin and cellulose hydrolysis. Around 20% and 18% increased in mass transfer coefficient for lignin and cellulose degradation were observed as flow rate increased from 10 mL/min to 62.5 mL/min. Based on this, physical parameters originated from the reactor configurations (e.g. flow rate) appeared to have more impact in controlling the xylan degradation than lignin and cellulose. Because xylan merely account for around 20% in biomass, kinetics (e.g. temperature) still appear to be the key factors in determining the biomass dissolution along with flow rate. Overall, biomass degradation with particle size of 4mm and solid loading of 4 g in reactor body (20.2 mL, solid to reactor volume concentration 20% g/mL) was recommended for the 0.05 wt.% H₂SO₄ flowthrough pretreatment due to the insignificant mass transfer reduction. Elevating flow rate from 10 mL/min to 62.5 mL/min had insignificant impact on the increase of mass transfer coefficient.

6.4. 3. Sugar generation from biomass and kinetic parameters estimation

The sugar generation during the flowthrough pretreatment of the poplar wood under selected conditions with efficient biomass dissolution (240 °C, 0.05 wt.% H₂SO₄, 0–8 min, 10 mL/min–62.5 mL/min) accompanied with desired physical parameters (4mm and 4 g biomass) were depicted based on the flowthrough model. Furthermore, the interaction of reactor geometry and flow rate influencing the sugar production from biomass was investigated as well.
The sugar recovery from xylan was predominately consisted of xylose and xylooligomers (Chapter 2). The yields of xylooligomers and xylose generated from xylan were modeled based on Eq 1–3 described in section 2. Figure 5a shows the yields of xylooligomers with the respect of reaction time at varying flow rates 10 mL/min–62.5 mL/min. 0–41.2% xylose and 0–50.9% xylooligomers were observed with flow rate of 10 mL/min after 0–8 min 0.05 wt.% H$_2$SO$_4$ flowthrough pretreatment. As the flow rate increased, the distribution of xylan recovery shifted from xylose to xylooligomers. 0–39.0% xylose accompanied with 0–57.3% xylooligomers and 0–25.3% xylose plus 0–72.3% xylooligomers were obtained from xylan for operations with 25 mL/min and 62.5 mL/min, respectively. It was found that the total sugar (xylose and xylooligomers) yield obtained at elevated flow rate (25 mL/min and 62.5mL/min) was higher than that with lower flow rate (10 mL/min). This is due to that lower flow rate (10 mL/min) resulted in the formation of furfural to some extent (chapeter 4). Results revealed that the glucose yields decreased from 0–58.3% to 0–39.4% as flow rate increased from 10 mL/min to 62.5 mL/min (Figure 5b). Apart from glucose, the remainder of removed cellulose was considered as the cellulose oligomers or longer DP cellodextrin with amenable structure (Chapter 4 and Chapter 5). A subsequent enzymatic hydrolysis of the removed cellulosic fractions (not including the cellulose residue) at the high enzyme loading of 93 FPU/g glucan revealed almost total removed cellulosic fractions was converted to glucose. As shown in Figure 5, increasing flow rate also led the distribution of removed cellulose shift to cellodextrin. The curve fit of the yields of xylooligomers and xylose successfully described sugar generation from both xylan
and cellulose with varying flow rates (Figure 5). It was noteworthy that the rate constants regarding the sugar ooligomers degradation ($k_2$) and monomers degradation ($k_3$) were comparable under the tested conditions, suggesting that the parameters of flow rate and reactor geometry (e.g. reactor length) incorporated in the flowthrough model played a predominate role in governing the generation of xyooligomers and xylose. Decreasing flow rate or increasing the length of reactor enhancing the exposure of released sugar fractions contributed to the DP reduction of the sugars, while in-turn enhanced the possibility of the dehydration of sugar to furan compounds including furfural and 5-HMF. The flowthrough model was also compared with the conventional pseudo-homogeneous irreversible first order reaction model applied by many researches in describing the biomass degradations (Lu & Mosier, 2008; Saeman, 1945; Shen & Wyman, 2012b) (Figure 6.6). It was found that the flowthrough model predicts the yields of sugar oligomers and monomers generated from biomass much more precisely than the conventional model, which predicts unreasonable yields. The possible explanation was that the conventional model often depict the sugar production in batch system predicts a reduction in mono sugar yields. However, such sugar reduction would not happen in flowthrough reactor, where the released sugars are constantly removed. Overall, the flowthrough model proposed in this study posess the ability to predict the sugar generation from biomass in flowthrough system with the incorporation of parameters reflecting the reactor configurations.

6.5. Conclusion

A flowthrough kinetic model was developed to assess the hydrolysis of poplar wood
in dilute acid flowthrough system under elevated temperatures ($200-240 \, ^\circ C$). This model incorporating the mass transfer coefficient and physical parameters reflecting the reactor configurations successfully simulate the degradation performance of xylan, lignin and cellulose from biomass with varying particle size, solid loading as well as flow rates. Results demonstrated that particle size (up to 4mm) have negligible impact on the mass transfer of biomass hydrolysis in flowthrough system. Up to 4g solid loading in 20.2 mL flowthrough reactor has few reduction in mass transfer coefficient for biomass dissolution, which lead to 13g/L potential sugar concentration. The mass transfer limitation appear to be significant as solid loading increased to 6g. Flow rate played a more important role in governing the mass transfer of xylan degradation than lignin and cellulose. Cellulose hydrolysis with the least influence from flow rate was demonstrated predominately temperature dependent. In addition, this novel flowthrough kinetic model precisely predict the sugar generation from both xylan and cellulose compared with the conventional Saeman’s model which often utilized for batch system. The kinetic analysis based on this flowthrough model provide an in-depth understanding on the acidic hot water flowthrough pretreatment and the perspective for the reaction design for higher sugar concentration.
6.6. References


Figure 6.1. The effect of temperature and time on the degradation of xylan (a), cellulose(b) and lignin (c). 0–8 min, 25 mL/min, 20–40 mesh particle size, 0.5 solid loading in 20.2 mL reactor.

Black ○: 200 °C; Red+: 210 °C; Blue△: 220 °C; Magenta+: 230 °C; Green*: 240 °C.
Figure 6.2 The effect of particle size on the degradation of xylan (a), cellulose (b) and lignin (c). 240 °C, 0.05%(w/w) H₂SO₄, 0–8 min, 25 mL/min, 0.5 g solid loading in 20.2 mL reactor. Green*: 40-60 mesh; Magenta+: 20-40 mesh; Blue△: 10-20 mesh; Red+: 2 mm; Black ○: 4 mm.
Figure 6.3 The effect of solid loading on the degradation of xylan (a), cellulose (b) and lignin (c). 240 °C, 0.05% (w/w) H₂SO₄, 0–8 min, 25 mL/min, 4 mm particle size, 20.2 mL reactor.

Black ○: 0.5 g; Red+: 2 g; Blue△: 4 g; Magenta*: 6 g.
Figure 6.4 The effect of flow rate on the degradation of xylan(a), cellulose(b) and lignin (c).

240 °C, 0.05%(w/w) H₂SO₄, 0—8 min, 25 mL/min, 4 mm particle size, 6 g solid loading in 20.2 mL reactor.

Black ○: 10 mL/min; Red +: 25 mL/min; Blue △: 62.5 mL/min.
Figure 6.5 The generation of xylose and xylooligomers (a) and glucose and glucose oligomers (b) under desired conditions (240 °C, 0.05%(w/w) H$_2$SO$_4$, 0–8 min, 4 mm particle size, 4 g solid loading in 20.2 mL reactor) with flow rate 10–62.5 mL/min.

Blue ○: xylooligomers (10 mL/min); Blue +: xylooligomers (25 mL/min); Blue △: xylooligomers (62.5 mL/min). Red ○: xylose (10 mL/min); Red +: xylose (25 mL/min); Red △: xylose (62.5 mL/min). Black ○: glucose oligomers (10 mL/min); Black +: glucose oligomers (25 mL/min); Black △: glucose oligomers (62.5 mL/min). Magenta ○: glucose (10 mL/min); Magenta +: glucose (25 mL/min); Magenta △: glucose (62.5 mL/min).
Figure 6.6 Comparison of flowthrough model and conventional model (Lu & Mosier, 2008; Saeman, 1945; Shen & Wyman, 2012b) in predicting the sugar generation from xylan (a) and cellulose (b) in flowthrough system. Conditions: 240 °C, 0.05%(w/w) H₂SO₄, 0—8min, 25mL/min, 4mm particle size, 4g solid loading in 20.2 mL reactor.

Black ○: xylooligomers; Black *: xylose; Black △: cellobextrin; Black+: glucose

Blue line: flowthrough model; Red dash line: conventional model.
Table 6.1 The mass transfer of the degradation of xylan, lignin and cellulose for flowthrough pretreatment with varied temperatures.

Conditions: 0–8min, 25mL/min, 0.5g solid loading, 20.2 mL reactor

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<th>Temperature</th>
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<td>Xylan</td>
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<tr>
<td>200 °C</td>
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<td>0.2223</td>
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</table>
Table 6.2 The mass transfer of the degradation of xylan, lignin and cellulose for flowthrough pretreatment with varied particle size.

Conditions: 240 °C, 0.05%(w/w) H₂SO₄, 0—8 min, 25mL/min, 0.5 g solid loading in 20.2 mL reactor

<table>
<thead>
<tr>
<th>Particle size</th>
<th>( k_M \text{(cm/min)} \times 10^{-3} )</th>
<th>Xylan</th>
<th>Lignin</th>
<th>Cellulose</th>
</tr>
</thead>
<tbody>
<tr>
<td>40-60 mesh</td>
<td></td>
<td>0.4821</td>
<td>0.2396</td>
<td>0.0989</td>
</tr>
<tr>
<td>20-40 mesh</td>
<td></td>
<td>0.4593</td>
<td>0.2434</td>
<td>0.1001</td>
</tr>
<tr>
<td>10-20 mesh</td>
<td></td>
<td>0.4225</td>
<td>0.2378</td>
<td>0.1006</td>
</tr>
<tr>
<td>2 mm</td>
<td></td>
<td>0.7888</td>
<td>0.4220</td>
<td>0.1814</td>
</tr>
<tr>
<td>4 mm</td>
<td></td>
<td>0.7865</td>
<td>0.4268</td>
<td>0.1741</td>
</tr>
</tbody>
</table>
Table 6.3 The mass transfer of the degradation of xylan, lignin and cellulose for flowthrough pretreatment with varied solid loading.

Conditions: 240 °C, 0.05%(w/w) H$_2$SO$_4$, 0–8min, 25mL/min, 4mm particle size, 20.2 mL reactor

<table>
<thead>
<tr>
<th>Solid loading</th>
<th>$k_M$ (cm/min) (×10$^{-3}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Xylan</td>
</tr>
<tr>
<td>0.5g</td>
<td>0.7865</td>
</tr>
<tr>
<td>2g</td>
<td>0.7429</td>
</tr>
<tr>
<td>4g</td>
<td>0.6854</td>
</tr>
<tr>
<td>6g</td>
<td>0.5187</td>
</tr>
</tbody>
</table>
Table 6.4 The mass transfer of the degradation of xylan, lignin and cellulose for flowthrough pretreatment with varied flow rate.

Conditions: 240 °C, 0.05%(w/w) H₂SO₄, 0-8 min, 4mm particle size, 6g solid loading, 20.2 mL reactor

<table>
<thead>
<tr>
<th>Particle size</th>
<th>k_M(cm/min) (×10⁻³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Xylan</td>
</tr>
<tr>
<td>10 mL/min</td>
<td>0.4548</td>
</tr>
<tr>
<td>25 mL/min</td>
<td>0.5187</td>
</tr>
<tr>
<td>62.5 mL/min</td>
<td>0.5527</td>
</tr>
</tbody>
</table>
Chapter 7
Conclusion and future work

7.1. Conclusions

The objective of this study was to enhance the intermediates yields (e.g. sugars, sugar degradation products, and lignin) from biomass in acidic aqueous-phase pretreatment with varying catalysts (e.g. sulfuric acid, metal chlorides), operating conditions (e.g. temperature, time pressure), and equipment configurations (e.g. batch, flowthough). Major conclusions from this doctoral research are summarized below.

1. The polysaccharides degradation from biomass and the interactions among various reaction steps in acidic conditions were investigated based on a pseudo-first order kinetic model. Higher temperature with reduced time was desirable for glucose production whereas lower temperature with prolonged time was preferred for xylose generation. Lower temperature and prolonged reaction time was desired for xylose production. Furfural and levulinic acid were stable in acidic conditions with negligible degradation rate constants. In the contrast, the rate constant of 5-HMF degradation was observed 5—9 fold higher than its formation over the tested temperatures, demonstrating its unstability in acidic aqueous phase.

2. The production of 5-HMF was enhanced when employing metal chlorides to catalyze agarose in aqueous phase. The addition of alkali and alkaline earth metal chlorides was found to result in the formation of only galactose and 5-HMF while the addition of transition metal chlorides led to the formation of various degradation products, including
levulinic acid, lactic acid, etc. Alkali and alkaline earth metal chlorides NaCl, CaCl₂ and MgCl₂ resulted in higher 5-HMF yields and selectivity than transition metal chlorides ZnCl₂, CuCl₂, FeCl₃ and CrCl₃, water-only or dilute sulfuric acid treatments. The addition of MgCl₂ was the most favorable additive among the tested metal chlorides, resulting in up to 50% 5-HMF yield. The mechanism of the MgCl₂ catalyzed reaction of agarose to 5-HMF was proposed to proceed through assisting the cleavage of C-O-C bond of agarose and accelerating the subsequent isomerization of galactose to its ketose. The specific ability of MgCl₂ in aqueous phase is thought to be the driving force for favoring the 5-HMF formation, while diminishing the generation of undesired byproducts such as levulinic acid, lactic acid, etc.

3. Flowthrough tests with water-only and dilute acid (0.05% (w/w) H₂SO₄) under elevated temperature 200–280 °C resulted in more than 98% solid removal. Kinetics along with other factors (e.g. flow rate) served as the key factor in determining the removal of biomass. Temperature was considered as the most significant factor for cellulose degradation. The cellulose removal increased significantly as temperature reached 240 °C for water-only and 220 °C for 0.05% (w/w) H₂SO₄. Up to 100% xylan and 90% cellulose were hydrolyzed into their monomers and soluble oligomers with negligible furfural and 5-HMF. Enzymatic hydrolysis of the pretreated whole slurries achieved the simultaneous saccharification of C5/C6 sugars. Flow rate ≥25 mL/min resulting few sugar degradation compounds (furfural and 5-HMF) at elevated temperatures (~270 °C for water-only and ~240 °C for 0.05%(w/w) H₂SO₄) was desired for the sugar production. Insoluble lignin
took account the majority insoluble fractions of the pretreated whole slurries while the soluble lignin was observed less than 15% with the predominately structural formation of vanillin and syringaldehyde. Enzymatic hydrolysis of the pretreated whole slurries obtained under desired conditions for water-only (270 °C, 25mL/min, 10min) and dilute acid (240 °C, 0.05%(w/w) H₂SO₄, 25mL/min, 8min) revealed that 93%–97% glucose and 97%–98% xylose were obtained. The selected dilute acid condition (240 °C, 0.05%(w/w) H₂SO₄, 25mL/min, 8min) with much higher soluble glucose plus glucose oligomers (~90%) than the water-only operation (270 °C, 25mL/min, 10min) merely required less than 10 FPU/g glucan enzyme to achieve >90% glucose yield and >95% xylose yield from whole pretreated slurries. The inhibitory compounds in the pretreated slurries showed insignificant impact on the performance of enzyme on pretreated sugar fractions after BSA testing.

4. A kinetic modeling with the incorporation of physical parameters reflecting the reactor configurations and mass transfer was applied to describe the decomposition of xylan, cellulose and lignin in dilute acid acid flowthrough system under elevated temperatures (200–240 °C). The kinetic analysis regarding the effect of varying particle size, solid loading and flow rate revealed that 4mm biomass particle size with up to 4 g biomass loading (20.2 mL reactor) had insignificant effects on the reduction of mass transfer. Flow rate played a more important role in governing the mass transfer of xylan degradation than lignin and cellulose. Cellulose hydrolysis with the least influence from flow rate was predominately temperature dependent. In addition, this novel flowthrough kinetic model
precisely predict the sugar generation from both xylan and cellulose compared with the conventional Saeman’s model.

7.2. Future research

Based on the studies of this dissertation, areas for additional researches have been suggested as follows:

1. Since flow rate in hot water and dilute acid pretreatment process had insignificant effects on the mass transfer of biomass dissolution under elevated temperatures (200 °C—280 °C), more advanced design of the flowthrough reactor for the enhancement of mass transfer coefficient could be developed. A developed flowthrough reactor equipped with the agitator blade was recommended for the mass transfer enhancement.

2. The microreactor and the advanced heating source (i.e. sand bath) applied in this research allowed fast heat transfer for biomass dissolution even at the elevated temperatures (e.g. 280 °C). Nevertheless, the heat transfer consideration during the pilot scale operations could not be overlooked. How the heat transfer impact the biomass dissolution and the corresponding sugar recovery should be considered in the enlarged reactor.

3. For the acidic hot water pretreated slurries, although insoluble lignin had insignificant effect on the enzymatic hydrolysis of the whole xylan and cellulosic fractions, which was observed in the soluble form, its structure characterization was still worth to be investigated for the conversion to other bioenergy such as jet fuel.

4. An online analytic system should be established to monitor the structural alteration of the
biomass during the aqueous phase biomass dissolution in flowthrough system at elevated temperatures (200—280 °C) for better understanding the degradation mechanism and interaction of xylan, lignin and cellulose.