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Co-Solvent Enhanced Production of Platform Fuel Precursors From Lignocellulosic Biomass

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Co-Solvent Enhanced Production of Platform Fuel Precursors From Lignocellulosic Biomass

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of the requirements for the degree of

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by

Charles Miao-Zi Cai

August 2014

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DEDICATION

I would like to dedicate this dissertation to my father Dr. Kevin Cai and mother Hong Miao. They are my pillars of strength and my biggest supporters. I would also like to thank my advisor Prof. Charles E. Wyman for his unfaltering guidance and mentorship throughout my doctoral studies.

ABSTRACT OF THE DISSERTATION

Co-Solvent Enhanced Production of Platform Fuel Precursors From Lignocellulosic Biomass

by

Charles Miao-Zi Cai

Doctor of Philosophy, Graduate Program in Chemical and Environmental Engineering
University of California, Riverside, August 2014
Dr. Charles E. Wyman, Chairperson

Lignocellulosic biomass is the most abundant source of organic carbon on Earth with the highest potential to economically and sustainably replace fossil resources for large-scale production of liquid fuels. However, although lignocellulosic biomass itself is much less expensive than petroleum, its natural resistance to chemical and biological breakdown is the major obstacle that must be overcome for biomass-derived fuels to be economically competitive. This dissertation outlines the inception, development, and application of a novel biomass conversion technology called Co-solvent Enhanced Lignocellulosic Fractionation, or CELF, that applies tetrahydrofuran (THF) as a miscible aqueous co-solvent to greatly augment the dilute acid-catalyzed deconstruction of lignocellulosic biomass to enable production of renewable fuels and chemicals at unprecedented yields. CELF directly enhances the production of primary fuel precursors such as monomeric sugars and secondary fuel precursors such as furfural, 5-HMF, and levulinic acid (LA) from biomass to integrate with downstream catalytic and biological processes to convert the fuel precursors into liquid fuels and renewable chemicals. In this dissertation, three different process configurations for CELF are evaluated. First, coupling CELF with sulfuric acid at higher reaction severities achieved 87% furfural yield from maple wood and produced a glucan enriched solid that was further reacted to LA at 75% yield in a subsequent reaction. Second, coupling CELF with metal halides achieved simultaneous production of furfural at 95% yield and 5-HMF at 51% yield from maple wood and corn stover. Third, reducing the reaction severity of CELF with dilute sulfuric acid drastically improved total sugar recovery, achieving 95% xylose recovery from corn stover after first stage pretreatment and subsequent > 99% glucose recovery after enzymatic hydrolysis of the remaining solids at an enzyme dosage of only 2 mg-protein g-glucan^{-1}. In all cases, CELF was effective at de-lignifying the biomass, dissolving up to 90% of the lignin into the liquid phase. Afterwards, recovery of THF by low temperature distillation caused the dissolved lignin to precipitate as a solid. In addition to enhancing fuel precursor yields, CELF can also serve as a valuable tool to help understand biomass recalcitrance and deconstruction.

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Chapter 1. Introduction

1.1 Overview

The intrinsic energy content stored inside the chemical bonds of the plant cell wall in lignocellulosic biomass is a highly sought-after commodity for renewable energy applications. This is because lignocellulosic biomass remains the most readily abundant source of carbon-based energy that has been a central focus of investigation for the last half-century as a viable replacement for non-renewable fossil fuels to sustain our increasing global energy demands (Lynd et al., 1991). The US Billion-Ton Update[1] predicts that over 1.4 billion dry tons of total biomass residues could be made available by 2030 as feedstock for renewable energy applications (US DOE, 2011). At these optimistic projected crop yields for available agricultural, forestry waste residues and energy crops, renewable liquid fuels from biomass could potentially offset a majority of the liquid fuels demand of the US if the cellulosic residues are fully utilized for the production of biofuels. According to the EIA[2], the US consumed 134 billion gallons of gasoline in 2013, representing 62.5% of the total volume of liquid fuels consumed that year. If bio-ethanol can be produced at a yield of 67 GGE ton-biomass^{-1} (0.67 GGE = 1 gal-ethanol) from the available biomass residues, up to 70% of the gasoline consumption in the US could be replaced by renewable alternatives.

In addition to ethanol, “drop-in” variants of conventional gasoline, diesel, and jet fuels can also be renewably derived from lignocellulosic biomass by both chemical and biological catalysts (Olcay et al., 2013). To date, limited product yields and higher-than-crude production costs for producing biofuels from lignocellulosic biomass have

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[1] US Billion-Ton Update (2011): http://www1.eere.energy.gov/bioenergy/pdfs/billion_ton_update.pdf
[2] US Energy Information Administration: http://www.eia.gov/forecasts/steo/report/us_oil.cfm

prevented its wide commercial adoption in the world market, but technological advances are overcoming these limitations. However, government mandates such as the US Renewable Fuels Standard (RFS) and its analogues in other countries as well as the growing public support for green technologies could help foster the research and development of more effective biomass conversion technologies to improve production yields and reduce cost. Concurrently, it is also crucial to deepen our understanding of the physical, chemical, and biological breakdown of lignocellulosic biomass.

Recalcitrance is a term that refers to the natural resistance of lignocellulosic biomass to chemical or enzymatic breakdown (Lynd et al., 1999). Defeating lignocellulosic recalcitrance is a unique challenge that involves applying both physical and chemical modifications to the complex molecular structure of biomass to improve the accessibility of catalysts and enzymes to the sugars within the biomass. The liberated biomass sugars then become the building blocks for biological and chemical (catalytic) pathways to fuel production. In this context, we defined soluble sugars as primary fuel precursors and acid-catalyzed sugar dehydration products such as furfural, 5-HMF, and levulinic acid (LA) as secondary fuel precursors. These fuel precursors are the primary platform chemicals from which renewable liquid fuels are produced.

Pretreatment is a thermochemical process that facilitates the breakdown of lignocellulosic biomass by destabilizing the glycosidic bonds between sugar molecules as well as the ester and ether bonds between the hemicelluloses and lignin fractions. This improves the reactivity of the solids and accessibility of the sugars to enzymatic or catalytic action. Pretreatment is one of the most important and expensive steps in biomass conversion and is typically integrated with either downstream acid-catalyzed dehydration or enzymatic hydrolysis. The limiting factor of a biomass conversion strategy is pretreatment because insufficient fuel precursor yields at this point would dramatically affect the final yields of all downstream processes.

Figure 1.1 illustrates a mass and energy flow diagram outlining the fate of the major components from a typical poplar wood composition (46% cellulose, 16.3% hemi, 21% lignin, 16.8% other) as they undergo conversion from 1 ton initial biomass to final fuel products at theoretical molar yields by either a catalytic (top pathway) or biological (bottom pathway) route. The most immediate and promising fuel products derived from biomass are dimethylfuran (DMF) from 5-HMF, methylfuran (MF) from furfural, γ-valerolactone (GVL) from levulinic acid, and ethanol from sugars. The boxes represent individual unit operations and the colors of the boxes represent the temperature requirements (red is high temperature, blue is low temperature). The stream thickness represents relative mass contribution. The net lower heating value (LHV) is also shown in parenthesis after the mass number for each labeled stream to keep track of the net energy flow from raw biomass to final fuel products.

In Figure 1.1, the top pathway is completely catalytic, where the hydrolysis and dehydration of biomass polysaccharides occur simultaneously to produce secondary fuel precursors furfural and 5-HMF. Hydrogenation and deoxygenation of furfural and 5-HMF produces up to .273 tons of DMF and .101 tons of MF from 1 ton biomass, respectively. Alternatively, both furfural and 5-HMF could be converted into LA first and then catalytically upgraded to produce up to .407 tons of GVL. Interestingly, the combined energy content (10988 MJ) of DMF and MF products from 1 ton of biomass is higher than the total energy content for GVL (8933 MJ) while also being more energy dense. However, external hydrogen gas input is required for hydrogenation and deoxygenation of secondary fuel precursors and increases the net LHV of the products: MF consumes two moles of H_2 that contributes a net 539 MJ energy input, DMF consumes four H_2 for a net 1872 MJ input, and GVL consumes one H_2 for a net 870 MJ input. Hydrogen must be supplied to the process either from an external standalone process such as aqueous phase reforming of biomass (Huber and Dumesic, 2006) or from a hydrogen donor such as 2-butanol (Bui et al., 2013) or formic acid.

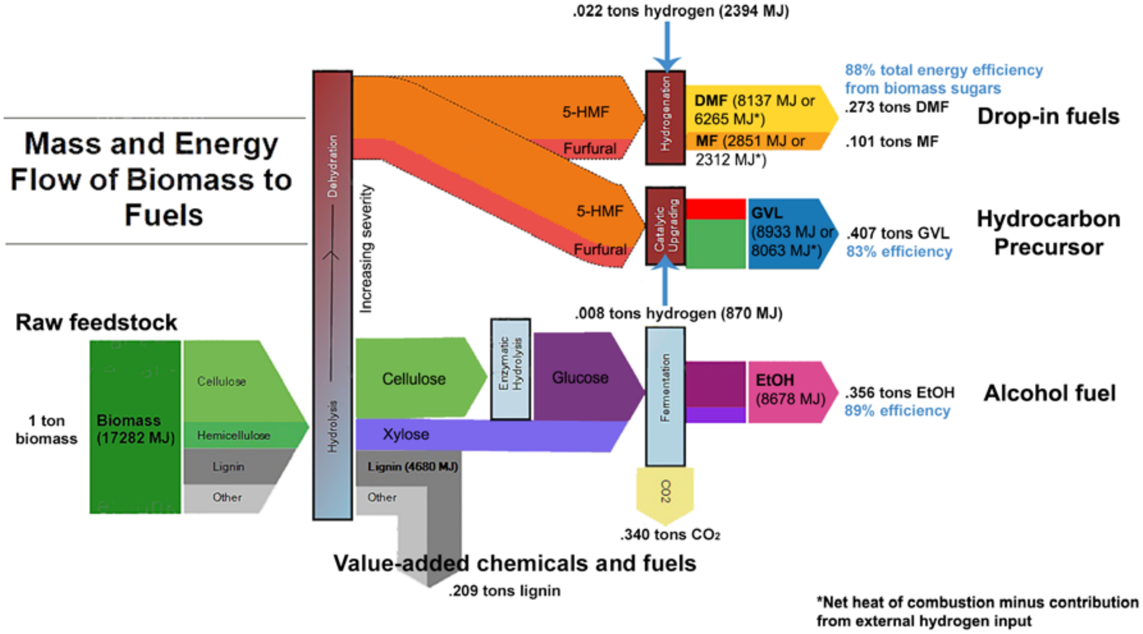

Figure 1.1. A mass and energy flow diagram for potential catalytic and biological conversion routes from biomass to promising renewable liquid fuels at theoretical yields. Thickness of the individual component streams represents their relative mass with respect to 1 ton of initial biomass. Total net energy content, mass, and energy efficiency of each final product stream is shown.

The bottom pathway in Figure 1.1 is more typical for a pretreatment process where hemicellulose sugar monomers are first recovered by pretreatment reaction followed by enzymatic hydrolysis to recover glucose from the remaining cellulose fraction. The sugars can then be fermented by yeast to produce .356 tons of ethanol with net energy of 8678 MJ. The percent net energy efficiency from the sugar fractions in biomass (9700 MJ) is shown in blue and indicates that ethanol is comparable to DMF + MF at 88 - 89% efficiency, and superior to GVL (83%). Lignin also has a significant net energy of 4680 MJ and has a higher energy density than the other sugar fractions. Lignin is often combusted for power and heat generation in a biorefinery, but the chemical and energy potential for lignin is far greater and simple combustion of lignin for heat would be the lowest value proposition

From a value proposition, DMF, MF, and ethanol are the most favorable fuel products due to their higher energy density over GVL and higher energy efficiencies from biomass. However, GVL is still valuable as a promising precursor for producing butane that is useful for the production of various branched or linear alkanes and alkenes for jet and diesel applications. Furthermore, total operating costs for either a biological or catalytic conversion route would need to be considered. Regardless, the final selection of the best fuel product from biomass would be dependent on the overall process efficiency and the product yields that can be achieved in the upstream operations, such as pretreatment. In this functional realm, immiscible and miscible solvents can be uniquely suited to enhance the deconstruction of biomass in aqueous-based pretreatment reactions to support both catalytic and biological conversion pathways.

1.2 Thesis Organization

The chapters in this dissertation are organized chronologically relevant to the development of the novel co-solvent process, CELF. Chapter 2 will begin with a literature review on the production and utilization of furfural, but also sets the stage for research in biofuel production from lignocellulosic biomass. It proposes the need to refine and improve upon older technologies to develop integrated platform technologies that maximize the utilization of biomass by co-production schemes. Chapter 3 evaluates a state-of-the-art biphasic solvent system with the CELF process and discusses the unique solvent properties of THF for the purpose of enhancing furfural and 5-HMF production from biomass. Chapter 4 is the first published paper on the application of CELF at more severe reaction conditions to co-produce furfural and LA from maple wood at high yields. It was also determined in this chapter that both the lignin and remaining solids from the CELF reaction were highly reactive and suitable as valuable co-products of the process. Chapter 5 presents the results of coupling CELF with metal halide acid catalysts to co-produce furfural and 5-HMF in a “one-pot” reaction. Chapter 6 proposes the use of CELF as a novel pretreatment strategy to minimize the need for costly enzymes while still achieving high total sugar yields. CELF pretreatment was also integrated with simultaneous saccharification and fermentation to produce ethanol at high yields and low enzyme loadings. Following up, Chapter 7 compares CELF with several leading pretreatment and catalytic conversion strategies and calculates the operating margin for a CELF biorefinery. Finally, Chapter 8 concludes the dissertation with a summary of the key findings and recommendations for future research.

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Chapter 2. Integrated furfural production as a renewable fuel and chemical platform from lignocellulosic biomass*

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2.1 Abstract

Furfural is a natural precursor to furan-based chemicals and has the potential to become a major renewable platform chemical for the production of biochemicals and biofuels. However, current industrial furfural production relies on relatively old and inefficient strategies that have hindered its capacity, and low production yields have strongly diminished its competitiveness with petroleum-based alternatives in the global market. This mini-review provides a critical analysis of past and current progress to enhance furfural production from lignocellulosic biomass. First, important chemical and fuel products derived from the catalytic conversion of furfural are outlined. We then discuss the importance of developing integrated production strategies to co-produce furfural with other valuable chemicals. Furfural formation and loss chemistries are explored to understand effective methods to improve furfural yields from pentosans. Finally, selected relevant commercial and academic technologies that promise to improve lignocellulosic furfural production are discussed.

2.2 Introduction

Environmental awareness of the need to replace fossil resources with renewable alternatives has fostered research to enhance production of suitable biomass-based platform chemicals (Lynd et al., 1999). In recent years, furfural has received renewed attention as a potential platform for production of biofuels and biochemicals. In a study supported by the US Department of Energy, furfural was selected as one of the top 30 platform chemicals that could be made from biomass with two of its derivatives, levulinic acid and furan dicarboxylic acid, in the top 10 (Werpy and Peterson, 2004). Furfural is a natural dehydration product of xylose, a monosaccharide often found in large quantities in the hemicellulose fraction of lignocellulosic biomass, from which it is almost exclusively produced. In theory, any material containing a large amount of the pentose (five carbon) sugars arabinose and xylose can serve as a raw material for furfural production (Zeitsch, 2000).

Industrial production of furfural was practiced starting in 1921 by the Quaker Oats company using oat hulls, corn cobs, and sugar cane bagasse, but due to limited demand and high maintenance costs (Brownlee and Miner, 1984), yield and production methods have not improved significantly since about the 1980s (Zeitsch, 2000). Currently, about 300–700 Ktons of furfural is produced worldwide annually, the majority coming from China (Mao et al., 2012; Win, 2005). Significant improvements to both yield and production strategy are needed for furfural and its derivatives to compete with petroleum-based products as a renewable alternative. For example, the current price of crude oil is about \$100 per barrel (about \$683 t^{-1} at 38° API gravity), and in order for furfural and its derivatives to be competitive as fuel precursors, its current production cost (Win, 2005) of about \$1000 t^{-1} must be substantially reduced through yield improvements of at least 46% from raw materials such as bagasse or corn cobs.

In this review, furfural and its catalytic products are introduced. The chemistries of furfural formation and furfural destruction are discussed to gain an insight into effective strategies that could improve furfural yields. Potential co-products from lignocellulosic biomass are identified. Finally, several competing technologies relevant to integrated production of furfural with other products from biomass are described.

2.3 Furfural as a renewable chemical and fuel precursor

In addition to attractive thermosetting properties, physical strength, and corrosion resistance (Mamman et al., 2008; Lichtenthaler and Siegfried, 2004) furfural is a natural precursor to a range of furan-based chemicals and solvents, including methylfuran (Sitthisa et al., 2011; Wojcik, 1948), furfuryl alcohol (Wojcik, 1948; Corma et al., 2007), tetrahydrofurfuryl alcohol (Merat et al., 1990; Cass,1948), tetrahydrofuran (Wojcik, 1948; Cass, 1948; Müller, 2000), methyltetrahydrofuran (Wojcik, 1948; Pace et al., 2012), dihydropyran(Brownlee and Miner, 1948; Wojcik, 1948; Cass,1948), and furoic acid (Wojcik, 1948; Hurd et al., 1933). Figure 2.1 outlines some of these potential chemical products from furfural which have high value applications as a fuel or fuel additive (Lange et al., 2012). Hydrogenation of the aldehyde group or furan ring remains the most versatile reaction to upgrade furanic components and can be employed to synthesize hydrocarbon fuels directly from furan derivatives (Wojcik, 1948; Huber and

Dumesic, 2006). Cleavage of the furan ring by hydrogenolysis can produce alcohols such as 1,5-pentanediol.

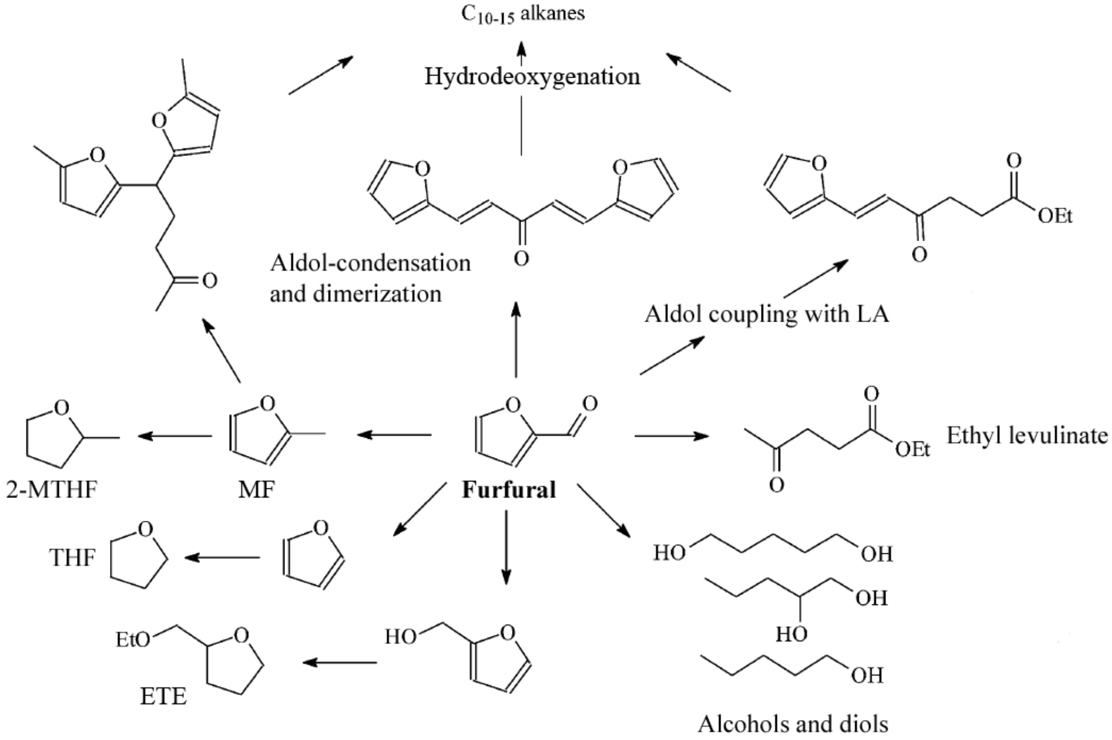

Figure 2.1. Outline of potential chemical and fuel derivatives from furfural by catalytic conversion (Adapted from Lange et al., 2012).

To synthesize longer-chain hydrocarbons from furfural, adduct formation by aldol condensation and dimerization followed by hydrodeoxygenation can produce C8 to C13+ alkanes (Wojcik, 1948; Huber et al., 2005; Barrett et al., 2006; Xing et al., 2010; West et al., 2008; Olcay et al., 2013). In their review of furfural as a potential biofuel, Lange and coworkers (Lange et al., 2012) concluded that removing polar groups and reducing volatility of furan products can yield beneficial qualities for blending applications in diesel, whereas unsaturated aromatic derivatives such as methylfuran (MF) and ethyl furfuryl ether (EFE) have octane values and boiling points suitable as gasoline blendstock.

2.4 Furfural from lignocellulosic feedstocks

Lignocellulosic biomass including agricultural and forestry residues is uniquely suited for large-scale production of renewable fuels and chemicals with the potential for minimal environmental impact when properly managed (Wyman, 2001; Wyman, 2007; Lynd et al., 2008). Lignocellulosic biomass is composed primarily of cellulose, hemicellulose, lignin and watersoluble extractives, in order of typical relative proportions (Wyman et al., 2005; Whetten and Sederoff, 1995). Due to the large amounts of enzymes required to overcome the recalcitrance of lignocellulosic biomass, biological approaches to releasing sugars from the cellulose fraction have been particularly challenged (Sun and Cheng, 2002).

For many acid-catalyzed pretreatment strategies used to extract sugars from hemicellulose and prepare biomass for biological conversion to ethanol, furfural is considered an inhibiting byproduct. However, high severity reaction of lignocellulosic biomass could be employed to produce furfural at higher yields via a purely thermochemical approach. In fact, furfural is produced industrially by acid-catalyzed hydrolysis and dehydration of pentosans in lignocellulosic feedstocks at temperatures ranging from 153 °C to 240 °C. Pentosans are five carbon (C5) polysaccharides contained in the hemicellulose fraction of lignocellulosic biomass and, for many plants other than softwoods, are composed of complex heteromorphous structures of predominantly xylan (C5) and lesser amounts of arabinan (C5), glucan (C6), mannan (C6), galactan (C6), acetic acid, and uronic acids (Sun and Cheng, 2002; Suvorov et al., 2011). Table 2.1 outlines the approximate pentosan content of several plants and agricultural residues potentially suitable for furfural production. Those that have higher pentosan content allow for higher mass yields of furfural and are typically favored in commercial furfural production. Today, bagasse and corn cobs account for more than 98% of all feedstocks used to manufacture furfural because they are relatively high in pentosan content, inexpensive, and readily available from sugarcane and corn processing plants, respectively (O’Brien, 2006).

Table 2.1. Pentosan content of plants and agricultural residues that could be employed for furfural production, listed in order of decreasing pentosan content.

Plant material	**Pentosan content (%)**
Corncobs	35
Almond husks	30
Rye straw	30
Oat hulls	29
Cottonseed hulls	28
Barley straw	25
Birchwood residues after felling	25
Sugarcane bagasse	25
Sunflower husks	25
Wheat straw	24
Flax shives	23
Hazelnut shells	23
Birchwood logs	22
Eucalyptus wood	20
Rice hulls	17
Maple wood	16
Pinewood	8
Peanut shells	3

However, since pentosans only contribute a portion of the total composition of lignocellulose, the sole production of furfural from these residues would be wasteful, inefficient and uneconomic. Cellulose is the largest fraction of lignocellulosic biomass consisting of fibrous bundles of repeating glucan units. As amorphous hemicellulose is far more acid-labile than crystalline cellulose, pentosans are hydrolyzed and dehydrated to furfural much sooner than glucans from cellulose (Saeman, 1945; Saeman, 1949; Fengel and Wegener, 1984; Battista, 1950). Thus, acid-catalyzed production of furfural from biomass often leaves behind a solid residue containing glucan and lignin. Figure 2.2 outlines a diverse range of potential co-products that could be made from lignocellulosic biomass in addition to furfural.

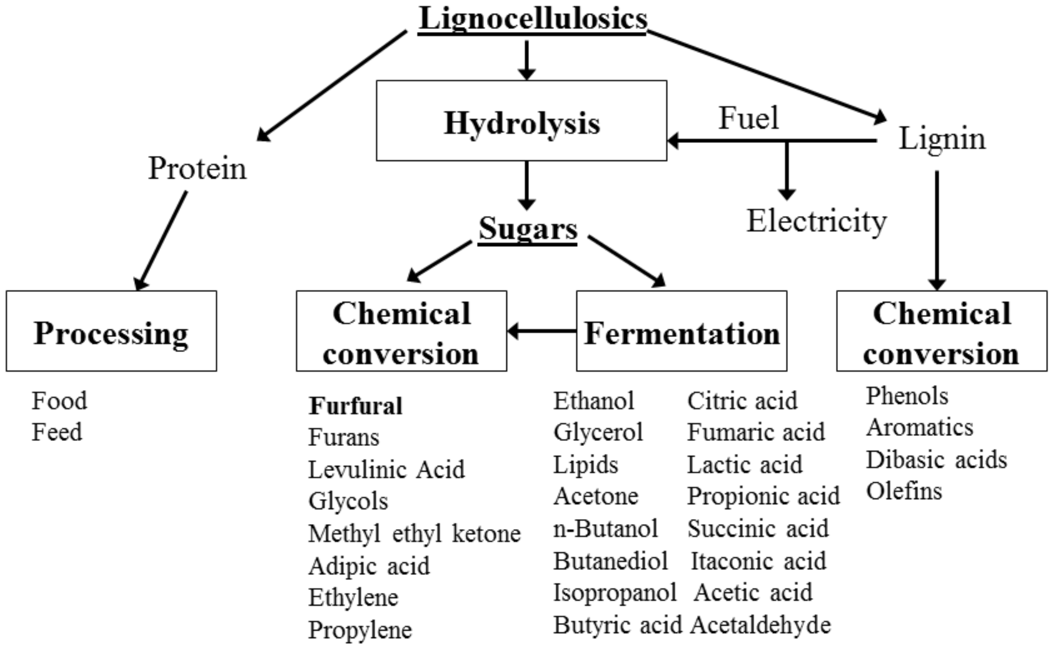

Figure 2.2. Conversion of sugars, lignin, and protein in lignocellulosic biomass for the integrated production of fuels, chemicals, materials, heat, power, food, and feed (Adapted from Wyman, 1990).

Processes that can target production of valuable chemical products from all major fractions of lignocellulosic biomass will greatly improve process economics and could potentially synergistically facilitate both chemical and biological production strategies. Vedernikovs, a Latvian professor and creator of the Vedernikovs furfural process, had stated that furfural production is not economically viable without a low-cost feedstock and coproduction of other higher-value chemicals from the remaining lignin and cellulosic residues (O’Brien, 2006; Vedernikovs et al., 2010). For example, sugars extracted from the glucan-rich residue recovered from furfural production could be used to produce 5-HMF, levulinic acid (Dautzenberg et al., 2011) or bioalcohol (Vedernikovs et al., 2010), and the remaining lignin could be used for the manufacture of aromatics, olefins, dibasic acids, and even carbon fiber (Figure 2.2) (Hutterman et al., 2001; Henriksson et al., 2010; Demirbas, 2006; Baker et al., 2012). Acetic acid and formic acid are also secondary products from biomass (Mao et al., 2012; Yang et al., 2012), which have also been shown to provide catalytic properties to furfural formation. Due to the structural and compositional differences between each fraction, integrated production strategies may benefit from being able to efficiently fractionate biomass to improve co-product yields. Burning solid residues for energy as practiced in many processes that have since been abandoned would be one of the least viable options.

2.5 Furfural formation and destruction chemistry

The formation of furfural from pentosans can be understood in terms of consecutive hydrolysis and dehydration reactions of xylan or arabinan. Following the introduction of a water molecule to break the glycosidic bond (hydrolysis) and form xylose or arabinose, three water molecules are then released from the respective sugars to form furfural (dehydration). Although various open and closed chain sugar intermediates have been proposed (Zeitsch, 2000; Antal et al., 1991; Feather et al., 1972; Marcotullio and De Jong, 2010; Garrett and Dvorchik, 1969), the precise reaction mechanism is still not completely understood (Binder et al., 2010; Marcotullio and De Jong, 2011). As shown in Figure. 2.3, recent thermodynamic simulations by Nimlos and co-workers (Nimlos et al., 2006) favored energetics of the closed-chain formation of a dehydrofuranose intermediate by ring contraction by protonation of the O2 hydroxyl group. This proposed model was determined to have the lowest energy barrier for furfural formation in the presence of a strong Brønsted acid such as H_2SO_4 but does not represent the only valid model, especially in the presence of other catalysts.

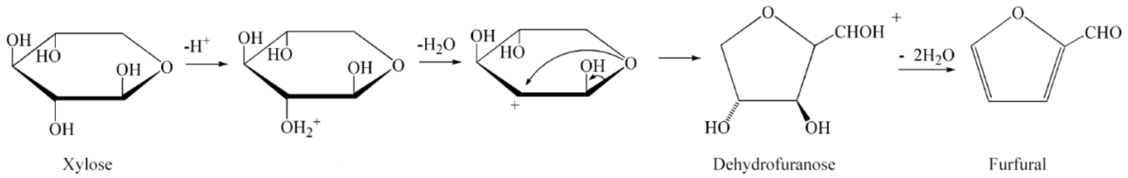

Figure 2.3. Proposed closed-chain mechanism for furfural formation from xylose by O2 hydroxyl protonation and ring contraction to a dehydrofuranose followed by consecutive dehydration (Adapted from Nimlos et al., 2006).

Marcotullio and de Jong (Marcotullio and De Jong, 2011) favored an open-chain mechanism, as shown in Figure 2.4, that involves the rate-limited formation of a 1,2-enediol intermediate (2 in Fig. 2.4) from xylose in the presence of halide salts. They found that the presence of halide ions (such as Cl−, Br−, or I−) in an acidic xylose solution greatly increased the rate of enolization and subsequent dehydration reactions (5, 6, and F in Fig. 2.4) and that furfural selectivity was greatly improved by the presence of a combination of chlorine and iodine (halide) ions that enhance the enolization and dehydration steps, respectively. Similarly, Binder and co-workers (Binder et al., 2010) suggested a mechanism in which xylose undergoes a 1,2-hydride shift in the presence of chromium(II and III) chloride catalysts (Lewis acids) to form xylulose (3 in Fig.2.4), which they found to be more predisposed to form furfural than xylose by way of the proposed open-chain mechanism. Their study suggested that xylulose dehydration could be a more selective pathway to furfural formation and that Brønsted and Lewis acids play uniquely different roles in promoting hydrolysis and dehydration reactions of xylan (Yang et al., 2012). For arabinose, furfural formation rates have been found to be much slower than with xylose and are often neglected in furfural yield calculations due to the relatively small amount of arabinose in lignocellulose (Zeitsch, 2000).

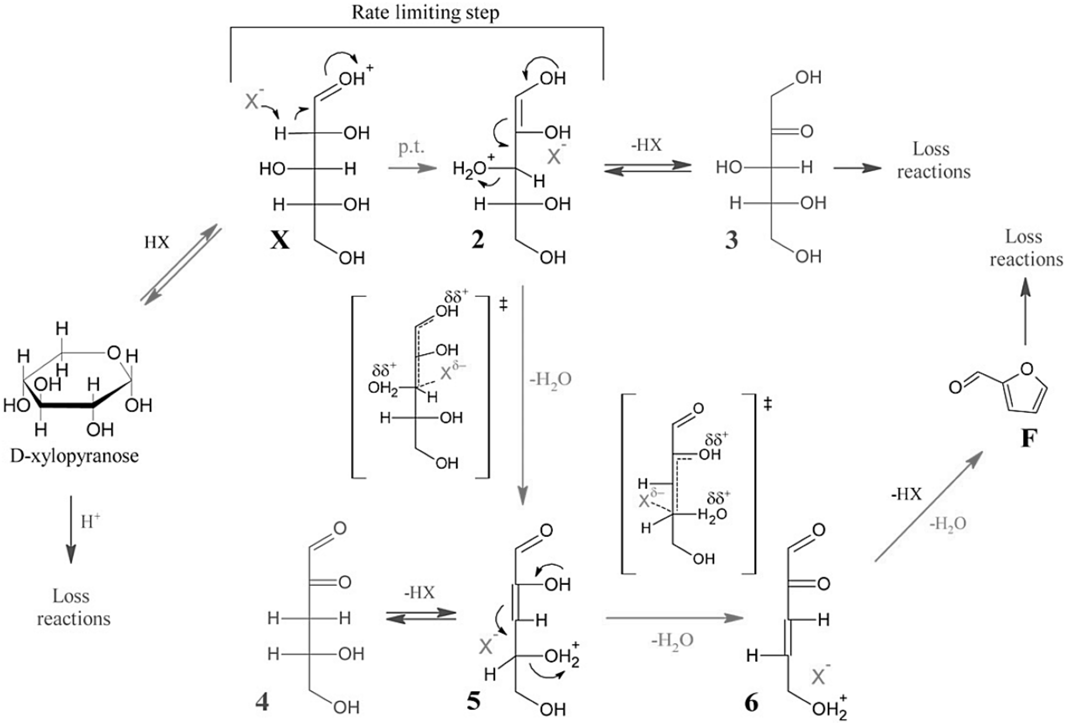

Figure 2.4. Proposed open-chain mechanism of furfural formation by 1,2-enediol formation (2) and dehydration (5 and 6) in the presence of halides, denoted by X- (Taken verbatim from Marcotullio and De Jong, 2011).

Despite differences in the proposed mechanism, the general reaction sequence shown in (Figure 2.5) provides a useful representation of the formation pathway of furfural from pentosans and the competing loss reactions during and after furfural formation. If furfural is allowed to remain in the catalytically active aqueous phase at higher temperatures, hydrolytic fission of the aldehyde group by hydrogen ions at low pH occurs and produces formic acid (Williams and Dunlop, 1948; Dunlop, 1948). Also in Figure 2.5, reaction losses are further complicated by the minor loss of furfural due to self-polymerization (furfural resinification) and the major loss of furfural by cross-polymerization reactions with sugar intermediates (furfural condensation) (Zeitsch, 2000; Marcotullio and De Jong, 2010). Consequently, greater furfural losses occur in the active aqueous phase when higher concentrations of pentoses are present in the solution (Zeitsch, 2000).

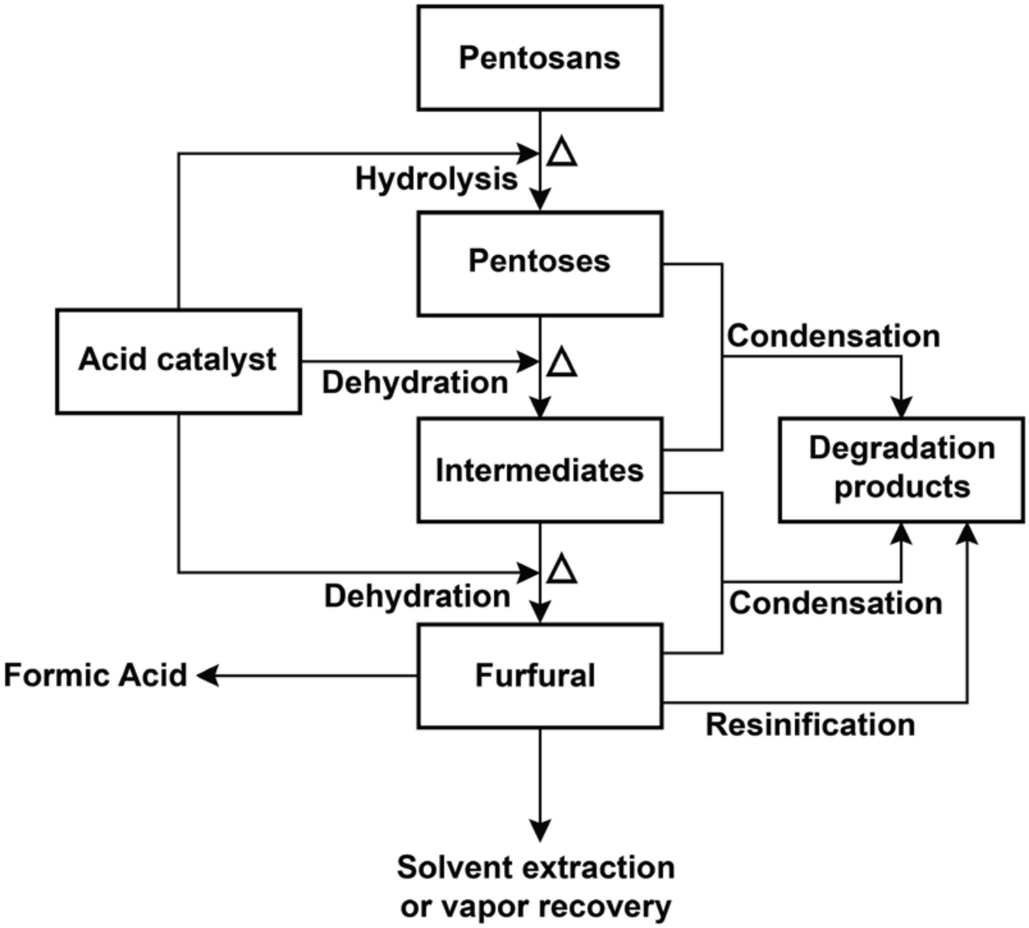

Figure 2.5. Reaction sequences for obtaining furfural from pentosans and loss reactions (Adapted from Vedernikovs et al., 2010).

For furfural destruction, Marcotullio and co-workers (Marcotullio et al., 2009) determined that furfural loss in dilute sulfuric acid at 150–200 °C could be expressed by a first-order model with an activation energy of 125.1 kJ mol^{-1} for an Arrhenius temperature dependence on the hydronium ion activity. However, it was suggested that the same model could not be applied to other acid catalysts such as HCl where both the hydronium and chlorine ions play roles in the degradation kinetics. Several breakdown products were detected, but not all were identified. Although their study confirmed early findings by Dunlop (Dunlop, 1948) regarding the first-order loss approximation, the Dunlop study did not account for variations in the second dissociation constant of sulfuric acid and reported a lower 83.7 kJ mol^{-1} activation energy. Also, unlike Dunlop's findings, formic acid formation and furfural polymer products in the study by Marcotullio and co-workers were found to be minimal.

Inconclusive mechanisms to describe furfural formation and the incomplete understanding of pathways responsible for furfural losses show the pressing need to focus more efforts in this area. Only complete chemical analysis of degradation products produced from both furfural and xylose will conclusively determine the contribution and origin of the degradation species. Determination of accurate formation and loss reaction mechanisms and rates for various catalytic environments and reaction conditions in a process model could help define potentially fruitful routes for continued research and process improvements to enhance yields.

2.6 Improving furfural yields

Industrial furfural yields from sulfuric acid digestion of lignocellulosic residues followed by steam striping have remained at or below 50% (molar) of theoretical. One of the first attempts to improve furfural yields was undertaken by Brownlee (Brownlee, 1938) through application of a two-step process that first hydrolyzed the pentosans in a heated acid solution before the wet matter was subjected to superheated steam that continuously extracted furfural and removed moisture. The resulting increase in the hydrogen ion concentration due to a loss of water and higher temperatures led to a significant reduction in reaction time. The Quaker Oats Company adopted a continuous version of this process to obtain yields of 55% (Zeitsch, 2000).

A more effective strategy to improved furfural yields is to remove furfural from the catalytically active phase soon after it forms (Zeitsch, 2000). In line with this reasoning, boiling or otherwise extracting furfural from the aqueous phase represented the first effective approaches to improving furfural yields. Since furfural forms a minimum-boiling azeotrope with water, separation of furfural vapors can occur by azeotropic distillation in the presence of steam at much lower temperatures. In another technique, by continually boiling the solution, furfural vapors are unable to re-enter solution, and unwanted side-reactions are reduced. Zeitsch developed the SupraYield® process which used slow depressurization at high temperatures (240 °C) to keep the reactants in a boiling state that recovered more furfural, with the result that furfural yields of 50–70% of theoretical could be realized. More recently, Mandalika and Runge (Mandalika and Runge, 2012) applied these concepts in a batch reactive distillation (BRD) approach (Gadewar et al., 2000) in which a continuously heated batch reactor was fitted with a throttle valve that released the vapor contents over the course of the reaction, allowing the solution to boil under depressurization and furfural to escape from the reactor as it was produced, resulting in upwards of 80% yield from wood chips.

Sproull and co-workers (Sproull et al., 1986) proposed that organic solvents such as methyl isobutyl ketone (Weingarten et al., 2010; Chheda et al., 2007), tetrahydrofuran

(Xing et al., 2010; Xing et al., 2011), alcohols, and others can be successfully applied to improve yields by extracting furfural into a separate organic phase during furfural production in biphasic reaction schemes. Amiri and co-workers (Amiri et al., 2010) then compared several extracting solvents and their performance using rice straw and found tetrahydrofuran to achieve higher furfural yields in a biphasic system due to its exceptional extraction efficiency. Such biphasic approaches are advantageous in their ability to capture furfural in the organic phase and protect it from attack by hydronium ions to form degradation products. The biphasic approach can also assist in furfural recovery by avoiding costly distillation from water, particularly with low-boiling solvents such as tetrahydrofuran (b.p. 66 °C). On the other hand, the need for costly recovery operations to recycle the solvent, the reduction of effective solids loading to maintain a distinct organic phase in the reactor, higher operating pressures from their additive properties, safety hazards, and high solvent costs are some of the disadvantages to the biphasic approach. Gürbüz and co-workers (Gürbüz et al., 2012) used lignin-derived alkylphenol solvents to address these issues. They achieved a furfural yield of 75% of theoretical using a xylose solution derived from corn stover hydrolysis in a biphasic solvent system with 0.25 mol L^{-1} HCl and 2-*sec*-butylphenol at a high aqueous/organic mass ratio of 6.67:1. However, saturation of the aqueous solution with NaCl was necessary to increase partitioning of the aqueous and organic phases and further promote the xylose dehydration reaction (Roman-Leshkov et al., 2007). The presence of salts would also elevate the boiling point of water when recovering aqueous co-products and introduce a processing challenge for post-reaction processing of the salt-saturated aqueous phase.

In an effort to find more environmentally friendly alternatives to mineral acid catalysts, the application of solid metal chloride catalysts has shown promise. As mentioned earlier, halide ions, especially Cl^-, appear to promote enolization (step 2 in Fig. 2.4) and improve the selectivity and yield of furfural from xylose (Marcotullio and De Jong, 2011). Acidic metal chlorides such as $CrCl_2$, $CrCl_3$, $ZnCl_2$, $MgCl_2$, $FeCl_3$, and $AlCl_3$ demonstrated the ability to catalyze xylose dehydration and furfural degradation beyond what was achieved using dilute sulfuric acid at the same pH, thereby revealing that the activity of the metal chlorides was not governed solely by its Brønsted activity (Binder et al., 2010; Yang et al., 2012; Liu and Wyman, 2006). In light of this, vom Stein and co-workers (vom Stein et al., 2011) obtained furfural yields of 71% of the theoretical maximum from a xylose solution using $FeCl_3$ as an aqueous-phase catalyst in a biphasic reaction with 2 mol L^{-1} tetrahydrofuran and water to prevent furfural loss. It is worth mentioning that the addition of 20% NaCl was needed to obtain a phase partition to achieve the highest furfural yields and furfural yields decreased to 37% when beechwood hydrolysate was used instead of xylose. More recently, by supplementing $FeCl_3$ with a mixture of seawater (NaCl+water) and acetic acid in a semi-continuous reaction, Mao and co-workers (Mao et al., 2012) obtained a 72% furfural yield of theoretical directly from processed corn cobs. By introducing a weak Brønsted acid with a strong Lewis acid, the hydrolysis of xylan and dehydration reactions to furfural can be enhanced by conversion of xylose to xylulose (Choudhary et al., 2011). Both groups also demonstrated that the catalyst-containing aqueous phase can be recycled without a significant loss of furfural yield.

Heterogeneous solid catalysts can eliminate the need for catalyst recovery altogether. Various zeolite-based catalysts and ion exchange resins (Lam et al., 2011; Agirrezabal-Telleria et al., 2011) with strong Brønsted acidic sites (over Lewis sites) have been shown to improve selectivity towards furfural (Weingarten et al., 2011), whereas the combined presence of Lewis acid sites helped to reduce the energy barrier for xylose to xylulose formation (Choudhary et al., 2011). Many of these catalyst systems, however, may require organic solvents to extract furfural, introducing the issues discussed above, and catalyst deactivation can occur by the coking of the micro-porous surfaces by insoluble polymers and degradation products. Furthermore, since it is not possible to flow solid biomass through these heterogeneous catalysts, the additional pretreatment needed and further discussion are beyond the scope of this paper.

2.7 Integrated furfural production strategies from lignocellulosic biomass

In modern furfural plants, large digesters (about 1.5 × 8m) are used to contain agricultural residues for conversion. Typically, sulfuric acid is used as the catalyst, and steam is employed for digester heating and furfural stripping (Zeitsch, 2000). Due to the corrosiveness of sulfuric acid and the resulting residues, expensive metals are needed for containment, and the process is not environmentally attractive (Zeitsch, 2000; Mao et al., 2012; Weingarten et al., 2011). Heavy furfural losses are also sustained in industrial production methods from by-product formation and inefficient recovery due to a high steam-to-furfural ratio (Zeitsch, 2000; Brownlee, 1927). For Huaxia/Westpro plants currently operating in China, 25–35 tons of steam is consumed per ton of furfural at about a 50% yield of theoretical pentosan content (Win, 2008). Remarkably, these plants are estimated to make $1–2 million in profits each year with the sale of higher value by-products contributing to a significant portion of their revenues (Win, 2008). Thus, integrated production recovers value from a relatively inefficient process.

Table 2.2 lists a number of lignocellulosic furfural production strategies proposed by some commercial and academic research projects along with comparisons to some historical benchmark processes. The furfural production book by Zeitsch (Zeitsch, 2000) describes many of the older furfural-only technologies such as those by Agrifurane, Escher–Wyss, Rosenlew, Supratherm, Stake, Suprayield, and Voest–Alpine and discusses their innovations and disadvantages. This section will focus on more recent advances in the production of furfural integrated with co-production of other products from biomass.

Table 2.2. Selected technologies for the production of furfural and co-products from lignocellulosic biomass to enhance total revenues through integrated processes.

Company/Group /Process	Process Type	Operating Temperature (˚C)	Catalyst	Substrate	Furfural Yield (% theoretical)	Co-products	References
Quaker Oats	Batch/Aqueous	153	H_2SO_4	Oat Hulls	<50%	N/A	Zeitsch, 2000
Quaker Oats	Continuous/Aqueous	N/A	H_2SO_4	Bagasse	55%	N/A	Zeitsch, 2000; Brownlee, 1938
Huaxia/Westpro	Continuous/Aqueous	160-165	H_2SO_4	Corn Cobs	35-50%	Methyl alcohol, acetone, acetic acid, levulinic acid	Zeitsch, 2000; O'Brien, 2006
Vedernikovs	Continuous/Aqueous	188	H_2SO_4	Wood chips	75%	Acetic acid, ethanol	Vedernikovs et al., 2010; Gravatis et al., 2001; Latvian State Institute of Wood Chemistry, 2009
Zeitsch/SupraYield®	Continuous/Aqueous	240	H_2SO_4	N/A	50-70%	N/A	Zeitsch, 2000; O'Brien, 2006
Biofine	Continuous/Aqueous	190-200	H_2SO_4	Paper sludge and waste residues	70%	Levulinic acid, formic acid, char	De Jong and Marcotullio, 2010; Fitzpatrick, 1990; Hayes et al., 2008
Abatzoglou and coworkers	Continuous/Aqueous	190-240	H_2SO_4	Hardwood saw dust	65%	Hexose solution	Abatzoglou et al., 1990
Lignol	Continuous/Organosolv	180	H_2SO_4	Wood chips	>4%	Glucose, xylose, lignin	Pan et al., 2006
de Jong and Marcotullio/MTC	Continuous/Aqueous	180	H_2SO_4	Straw	85% (estimated from model)	5-HMF, cellulosic residues	De Jong and Marcotullio, 2010
Mandalika and Runge	Batch/Aqueous	170	H_2SO_4	Poplar wood chips	80%	Cellulosic residues	Mandalika and Runge, 2012
Alonso and coworkers	Batch/Organic	170	H_2SO_4/Mordenite	Corn stover	81%/87%	Levulinic acid	Alonso et al., 2013
Mao and coworkers	Batch/Aqueous	190	Acetic Acid/$FeCl_3$	Corn cobs	73%	Cellulosic residues, lignin	Mao et al., 2012; Mao et al., 2013

Vedernikovs and co-workers proposed a concept for coproducing hemicellulosic furfural with cellulosic ethanol. The original process achieved furfural yields of 75% by injecting small amounts of strong acid and applying salts to ensure differential catalysis of hydrolysis and dehydration reactions (Gravitis et al., 2001). Further evolution to a two-step hydrolysis process resulted in a claimed seven-fold reduction in degradation of the cellulose residues and provided sufficient material to sustain bioethanol co-production (Vedernikovs et al., 2010). However, the high temperature reactions could increase the enzymatic recalcitrance of the remaining residue, and furfural carry-over could inhibit biological fermentations. In 2006, a commercial-scale furfural plant was built in Iran to Vedernikovs specifications (Latvian State Instiure of Wood Chemistry, 2009).

Another commercially viable furfural process patented in 1990 is the Biofine process (Fitzpatrick, 1990). Primarily designed for production of levulinic acid from hexoses released from paper pulp waste, furfural was also reportedly recovered at 50–70% yield (Hayes, et al., 2008). Their process outline and the furfural yields reported indicated that a furfural stripping step helped reduce degradation reactions. In a commercial plant in Caserta, Italy, paper mill sludge was processed by this process at 3000 t year^{-1}. The acid solution used was recycled, and a large amount of formic acid was also produced due to its equimolar formation with levulinic acid from 5-HMF. Importantly, recovery of furfural and levulinic acid from the reaction by stripping or distillation will require boiling large amounts of water and result in substantial energy demand.

Also in 1990, Abatzoglou and co-workers (Abatzoglou et al., 1990) reported 65% furfural yields and 30% recovery of fermentable hexoses in a single-stage continuous plug-flow reactor using wood sawdust with dilute sulfuric acid as the catalyst. The reactor could handle suspensions of up to 13 wt% sawdust, and at the higher reaction temperatures of 190-240 °C, reaction times were only 20–120 s. Despite lower hexose yields, this work represented some of the earlier efforts to co-produce furfural with cellulose that could be enzymatically hydrolyzed to hexoses. It also demonstrated the successful application of a plug-flow reactor to handle higher solids concentrations, and the reaction could be optimized using smaller scale batch reactions.

Lignol Energy Corporation (since 2001) used an ethanol–organosolv process to fractionate recalcitrant wood chips in their pilot plant in Burnaby, Canada. This process relied on enzymatic hydrolysis of the fractionated cellulose and subsequent fermentation to produce ethanol (Pan et al., 2006). Because they determined that just producing bioethanol may not be economically favorable, production of furfural from the pentoses and other valuable chemicals from the extracted lignin was highly desirable, especially when co-fermentation of pentoses was not possible (De Jong and Marcotullio, 2010). However, their reported furfural concentrations were low when using ethanol, and alternative solvents must be explored to target furfural production from xylans. The acetic acid recovered as a product in this process could also serve a potential catalytic role (Pan et al., 2006).

De Jong and Marcotullio (De Jong and Marcotullio, 2010) proposed a reactor design called the Multi-Turbine-Column (MTC) that continuously hydrolyzed and dehydrated straw while simultaneously stripping furfural product in a counter-current design. Furfural was then extracted by toluene and vacuum distilled at 83 °C to high purity. Up to a 10 wt% straw slurry could be safely loaded into the reactor. Application of a counter-current design minimized product accumulation and reduced cross-polymerization reactions. From their simulation data, furfural yields were estimated to be about 85% in a 10 Kt y^{-1} model (De Jong and Marcotullio, 2010). Anticipated co-products included methylfurfural and cellulose–lignin residues. Although their model demonstrated economic feasibility, important assumptions such as furfural yield, solvent recovery, and energy consumption greatly impacted the economics.

As mentioned earlier, Mandalika and Runge (Mandalika and Runge, 2012) used batch reactive distillation to achieve >85% furfural yields from hybrid poplar, miscanthus, switchgrass and corn stover. The reaction vessel operated isothermally and slowly released the vapors containing furfural product from the reactor head space while inducing internal boiling to reduce loss reactions. A coil submerged in an ice-bath condensed the vapors into a collection beaker for analysis. They found that reactions with pure xylose solutions led to lower yields (75%) than reactions with biomass (>85%) or hemicellulose hydrolysates extracted from biomass (88%). It is likely that the higher initial concentration of xylose in the pure samples triggered greater furfural losses. The co-product of this reaction was porous lignocellulosic residues enriched in cellulose and lignin for potential conversion into fuels and chemicals.

Recently, novel application of a single phase co-solvent system using γ-valerolactone as both a solvent and a potential product was applied to produce levulinic acid from 6.6 wt% corn stover (Alonso et al., 2013). With HCl as the catalyst, open boiling of the reaction media resulted in the highest furfural yields (81%), whereas a closed batch reaction resulted in the highest levulinic acid yields (66%) from corn stover. However, total yields of furfural plus levulinic acid as coproducts were considerably lower due to the inherent differences in recalcitrance between the hemicellulose and cellulose fractions, suggesting that a two-stage reaction may be necessary to obtain high yields of both. The highest furfural yield (87%) was obtained using a solid acid catalyst (Mordenite) at a high loading of 0.9 g catalyst per 1 g of corn stover. γ -Valerolactone also solubilized most of the biomass as well as the lignin, possibly allowing lignin recovery for conversion into products.

The last entry in Table 2.2 refers to recent work by Mao and co-workers (Mao et al., 2013) on the digestion of corn cobs using both acetic acid and $FeCl_3 \cdot 6H_2O$ solid catalyst in a semi batch reactor system. Sieved corn cobs (5–10 mm) were soaked in $FeCl_3$ and saltwater solution and loaded into a tubing-bomb reactor system with a liquid-to-solid ratio of 0.6:1. A mixture of acetic acid and steam was then delivered to the reactor to heat the contents and strip away the furfural product over the course of the reaction. Notably, a 73% furfural yield and 80% delignification were achieved. The remaining cellulosic residue was then steam exploded through a valve in the reactor bottom to produce highly digestible material for enzymatic hydrolysis. The cost and recyclability of the solid catalyst still needs to be determined, and deposition of trace iron compounds on the remaining residues may inhibit enzyme and biological activity.

Such advances in production strategies lend insight into key process features that enhance integrated furfural production. The ability to achieve high yields of furfural and co-products from each major fraction of lignocellulosic biomass is particularly vital. Catalysts that reduce the energy barrier for xylose dehydration will improve selectivity to furfural. Efficient removal or protection of furfural to reduce degradation losses will facilitate process optimization and lower recovery costs and waste treatment. Finally, to have wide-scale application, an integrated lignocellulosic process must be agnostic toward a number of leading feedstocks, and the ability to achieve high yields with more recalcitrant woody feedstocks would further expand the potential impact. It is also vital that the process be capable of handling high solids loadings to keep energy costs as low as possible.

2.8 Concluding remarks

Furfural is a promising renewable platform chemical for production of biochemicals and biofuels from low cost and abundant lignocellulosic biomass. Furfural is a natural precursor to furan-based chemicals and is primarily manufactured from the acid-catalyzed hydrolysis and dehydration of lignocellulosic pentosans at moderate temperatures. Catalytic hydrogenation of furfural remains the most versatile reaction to upgrade furanic components and can synthesize hydrocarbons directly from furfural to serve as fuels. The mechanisms involved in the formation of furfural are still unclear as both open and closed chain intermediates have been proposed in the presence of Lewis or

Brønsted acids. Furfural losses can occur from both resinification and cross-condensation reactions with sugar intermediates.

Modern commercial production is inefficient (25–35 t steam t^{-1} furfural) and suffers from low yields (<50 mol% of theoretical). Various strategies to improve furfural yields were discussed, including the removal of furfural into the vapor phase, the extraction of furfural from the catalytically active aqueous phase using biphasic solvent systems, and application of reusable or recoverable solid catalysts. Emphasis must be placed on the importance of directly using lignocellulosic biomass as the expectation that pure sugars can be extracted is likely to result in excessive costs for the entire process. Finally, recent integrated strategies were discussed in which co-production of other highvalue products in addition to furfural was a central theme to fully utilize all of the feedstock and improve process economics. Overall, advances are still needed to improve our understanding of the underlying chemistries of furfural formation and loss under various catalytic environments to develop efficient production strategies that integrate biomass deconstruction, lignin recovery, sugar dehydration, and product recovery. With success, furfural can become a viable renewable alternative to manufacture many current petroleum-based products at a large commercial scale.

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Chapter 3. Evaluating two different solvent strategies for enhanced furfural and 5-hydroxymethylfurfural production from lignocellulosic biomass*

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3.1 Abstract

Lignocellulosic biomass derived reactive intermediates (RIs) such as furfural, 5-hydroxymethylfurfural (5-HMF), and levulinic acid are potential building blocks and valuable precursors for sustainable production of drop-in hydrocarbon fuels. Currently, achieving high yields of furfural and 5-HMF from lignocellulosic biomass has been challenging for typical aqueous reactions with dilute sulfuric acid. However, significant yield improvements are possible by augmenting acidic aqueous reactions with organic solvents. In this study, we evaluated two different solvent strategies to improve furfural and 5-HMF production from maple wood: a biphasic simultaneous reaction + extraction strategy using methyl isobutyl ketone (MIBK) and a novel single-phase co-solvent reaction strategy using tetrahydrofuran (THF). Both solvent strategies improved furfural yields by 35% over the non-solvent case, but the highest 5-HMF yield (36%) was obtained by using MIBK. Although MIBK achieved excellent extraction efficiency for both furfural (95%) and 5-HMF (91%), incomplete product extraction and solvent recovery will likely hurt process economics. Despite non-ideal P-x behavior, THF is fully miscible in water above 150 °C and helped catalyze sugar dehydration and improve furfural and 5-HMF selectivity. Preliminary investigation on the unique properties of the THF co-solvent system reveals its great tuning potential to surpass the capabilities of the MIBK system.

3.2 Introduction

Declining petroleum supplies around the world have led to the increasing necessity to secure domestic oil supplies and develop an economic and highly effective renewable replacement for fossil fuels (Lugar and Woosley, 1999). Increased public awareness on the imbalance of atmospheric CO_2 emissions from the extensive use of non-renewable fossil fuels has put pressure on industrialized countries to demand the gradual integration of biomass-derived fuels into their transportation sectors (Dautzenberg et al. 2011). A near-term solution is needed to produce transportation fuels renewably without significant cost or alternation of the current infrastructure.

Lignocellulosic biomass in the form of woody crops and agricultural residue is the most abundant resource of organic carbon on Earth and is the only renewable resource that is cheap enough to replace fossil fuels and sustain our energy demands in the transportation sector (Wyman 2007). For the purpose of bioconversion, lignocellulosic biomass is functionally composed of three major polymeric components: cellulose, hemicellulose, and lignin. Cellulose is mostly crystalline in structure and comprised of linear β-1,4 linked glucose units known as glucan. Hemicellulose is amorphous in structure and is often primarily comprised of polymeric chains of β-1,4 linked xylose units known as xylan, but could also contain other polysaccharides not limited to glucan, mannan, and arabinan (Laine 2005). Lignin is a cross-linked heterogeneous complex covalently bonded to hemicellulose involving polymers of phenyl propanol units called monolignols (Whetten et al., 1995). Only the maximum utilization of these three major components from lignocellulosic biomass in the form of fuel products and value-added chemicals will allow production of “drop-in” biofuels to be economical and sustain our current and future energy demands (Cai et al., 2014).

3.3 Production of reactive intermediates from lignocellulosic biomass for biofuel applications

The major routes to converting cellulosic biomass into biofuels include gasification of biomass to syngas and subsequent Fischer-Tropsch synthesis to produce hydrocarbons, pyrolysis and liquefaction of biomass to produce bio-oils, aqueous phase catalytic processing of sugar dehydration compounds to produce gasoline, and sugar hydrolysis by pretreatment followed by enzymatic hydrolysis of solids residue for microbial fermentation to produce ethanol (Yang and Wyman 2008). Process yields, production costs, and feedstock availability are key challenges for these strategies. It is crucial for a biomass conversion strategy to achieve the highest product yields while using the most efficient processes and be compatible for many different feedstock types in order to be economical for fuel applications. For the biological conversion route, raw biomass needs undergo pretreatment in order to maximize the accessibility and utilization of its sugars. Pretreatment technologies, such as with dilute sulfuric acid or high pressure steam, can help to recover sugars from the hemicellulose fraction at high yields and reduce the recalcitrance of the remaining cellulose fraction for enzymatic hydrolysis to glucose. Acid neutralization must follow dilute acid pretreatment and the presence of non-sugar byproducts from pretreatment can inhibit microbial function.

For the aqueous catalytic pathways, reactive sugar intermediates (RIs) such as furfural, 5-hydroxymethylfurfural (5-HMF), and levulinic acid (LA) are useful fuel precursors that can be catalytically hydrogenated and hydrodeoxygenated into 5 to 16 carbon length hydrocarbons by the action of heterogeneous catalysts (Huber et al. 2005). Additionally, hydrogenation of furfural and 5-HMF can also produce aromatic gasoline-range fuel products such as methylfuran and dimethylfuran, respectively. Hydrogenation of LA to furfuryl alcohol followed by ethanolysis can produce ethyl levulinate, a diesel-range fuel (Lange et al., 2009). Although RIs are typically formed as inhibiting byproducts after acid-catalyzed pretreatment, they can be produced at relatively high concentrations from biomass by increasing the reaction severity, in other words, increasing reaction time, temperature, and acid loading in aqueous conditions (Zeitsch 2000). Figure 1 depicts the hydrolysis and dehydration pathways for glucan (common hexose) and xylan (common pentose) to form RIs under acidic conditions.

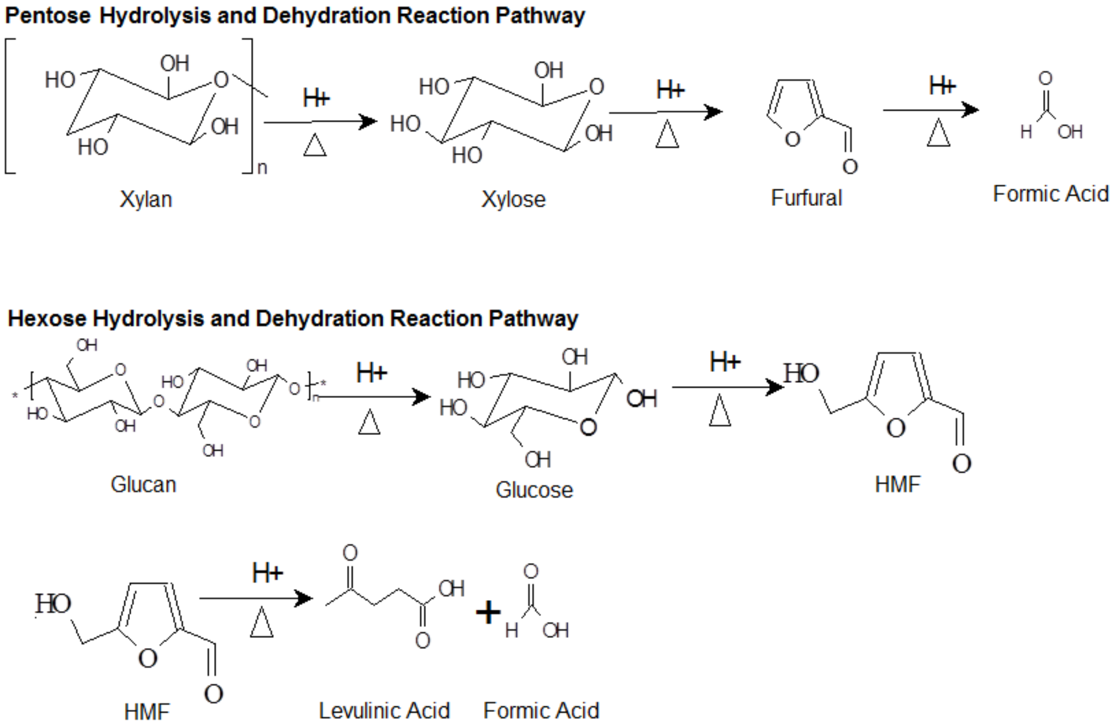

Figure 3.1. Hydrolysis and dehydration reaction pathways for glucan and xylan to form reactive intermediates furfural, 5-HMF, and LA.

In the pentose pathway, xylan is first hydrolyzed to xylose by consuming one water molecule, which is then dehydrated with the release of three water molecules to form furfural. Further reaction of furfural at high temperature aqueous conditions will produce formic acid by decomposition of furfural. Similarly for the hexose pathway, glucan is first hydrolyzed into glucose, which is then dehydrated to form 5-HMF and a net production of two water molecules. 5-HMF is also highly unstable in an aqueous environment and will rapidly hydrolyze to equimolar amounts of levulinic acid and formic acid until the 5-HMF is consumed (Karinen et al., 2011).

Furfural is a heterocyclic aldehyde that is made from agricultural raw materials rich in pentosans and is useful as a furanic precursor. The maximum mass yield of furfural that can be obtained from xylan by virtue of their molar masses is 0.7273 g-furfural g-xylan^{-1}. Since pentosan hydrolysis occurs at a much faster rate than the formation of furfural from a pentose sugar, the kinetics of hydrolysis can be mostly disregarded in the optimization of furfural production (Zeitsch, 2000). Although arabinan, mannan, and galactan can usually account for a small fraction (< 5 wt%) of the total significant pentosan content, its conversion to RIs proceeds more slowly so it is reasonable to approximate that all the pentosan content in most biomass types is xylan (Zeitsch, 2000). Furfural can easily degrade under an aqueous environment by self-resinification or condensation reaction with a sugar intermediate, thus removing furfural from the catalytically active phase by vaporization can prevent these loss reactions from occurring (Zeitsch, 2000). Currently, furfural is produced predominantly in China from various agricultural wastes at reported yields of less than 50%, with higher yields owing to the continuous removal of furfural by steam stripping (Win, 2005).

5-HMF is the 6-carbon analog of furfural with an additional branched carbon and alcohol group on the 4 carbon position. It can be produced by acid catalyzed dehydration from either fructose or glucose with higher yields and faster reaction observed from fructose due to the less stable ring structure of fructose than glucose (Karinen et al., 2011). The highest mass yield of 5-HMF that can be obtained from glucan by virtue of their molar masses is 0.7785 g-5-HMF g-glucan^{-1}. Further hydrolysis of 5-HMF in the aqueous phase leads to LA and formic acid, which are far more stable species with no further acid-catalyzed hydrolysis products of value (Kuster et al., 1990). LA is also considered an important platform chemical for biofuel synthesis with derivatives including gasoline precursor gamma-valerolactone and diesel component ethyl levulinate. (Dautzenberg et al., 2010; Werpy and Peterson, 2004; Lange et al., 2009). LA has a mass ratio to glucan of 0.7167 g-LA g-glucan^{-1} and can be obtained at higher yields than 5-HMF by the hydrolysis of 5-HMF. However, the high boiling point of LA (246 °C) means that its purification and recovery from an aqueous stream would require more energy than with furfural or 5-HMF. Achieving high yields of furfural and 5-HMF from biomass is more difficult than LA so they will be a central focus in this study.

Improving the yields of furfural and 5-HMF requires improving their selectivity from sugars and/or their protection from the catalytically active aqueous phase from further degradation. Extraction of furanics can be performed with immiscible organic solvents such as methyl isobutyl ketone (MIBK), methyl-tetrahydrofuran (MTHF), and larger alcohols (>1-pentanol) and some miscible ones such as acetone and tetrahydrofuran (THF) in a stirred reactor system. MIBK is a promising candidate that is fully immiscible in water and can serve directly as an extracting solvent in a biphasic reaction. On the other hand, miscible solvents such as THF, acetone, and larger alcohols can be fully or partially miscible in water and need to be salted out of solution to form a separate phase by adding in solutes. It has been shown that application of the aforementioned solvents to milled rice straw in a biphasic system can increase furfural and 5-HMF yields over a single phase aqueous reaction system by up to three times (Amiri 2010). However, information on optimization of furanics production from raw cellulosic biomass is limited, particularly compared to the number of studies performed using pure sugars. The present study assesses the capability of THF and MIBK solvents for the production of furfural, 5-HMF, and levulinic acid from raw maple wood as a highly recalcitrant model feedstock. Key observations unique to each system will be noted and used for the future optimization of yields with the selected solvent system.

3.4 Results and Discussion

3.4.1 Biphasic reaction of maple wood using MIBK

For the biphasic reactions, MIBK was supplemented on a 1:1 mass basis to a water solution containing 1 wt% sulfuric acid and 5 wt% loading of milled maple wood chips (~1 mm particle size). Figure 3.2 shows a comparison between typical dilute-acid mixture of maple wood in water (left) and the same mixture with added MIBK as an extracting solvent (right). Although the amount of maple wood (20 g) was identical, the biphasic reaction occupied nearly twice the volume. The resulting mixture was transferred to a 1L Parr® continuously-stirred autoclave reactor and subjected to convective heating by from a fluidized sand bath to a steady reaction temperature of 170 °C. After the reaction, both the organic and aqueous layers were analyzed by HPLC. We determined that 91% of the total 5-HMF produced was extracted into the solvent phase and 9% remaining solubilized in the aqueous phase without any addition of salt. This high extraction efficiency minimized the conversion of 5-HMF to levulinic acid and formic acid as most of the 5-HMF remained protected in the organic phase. Any products in the organic phase are not only protected but are also free from unwanted contaminants and solids that may interfere with necessary downstream separation steps and catalytic processes for biofuel production. At longer reaction times, most of the glucose released from the solids had been consumed and 5-HMF yields steadily increased. Consequently, levulinic acid and formic acid yields remained minimal. The extraction efficiency of furfural in MIBK was 95%, and the formation of furfural was more rapid than the formation of 5-HMF.

Figure 3.2. Left: Mixture containing 5 wt% maple wood chips in dilute acid water solution, used for non-solvent control reactions. Right: Same biomass slurry mixture with added MIBK extracting solvent on a 1:1 mass basis with the aqueous layer.

Figure 3.3 shows the RI yields achieved from a 50 min reaction of maple wood in the biphasic system using MIBK and 1 wt% sulfuric acid compared to a comparable reaction without MIBK at equivalent acid and maple wood loadings. A 50 min reaction was determined to be optimal for furfural production in both the MIBK and non-solvent dilute acid reactions. As shown, the biphasic reaction achieved a 35% increase in furfural yield (85% vs 63%) and nine-fold improvement of 5-HMF yields (27% vs 3%) over the non-solvent case at 50 min. LA yields were similar between the biphasic and single phase reactions. Although the highest 5-HMF yield of 36% was achieved at 60 min reaction, furfural yield had decreased to 70% by then. Optimizing the reaction for the highest furfural production is beneficial because it is the major product of the hemicellulose fraction that is more acid-labile than cellulose from which 5-HMF is produced. Since 5-HMF formation prefers higher reaction temperatures (200 – 210 °C) (Jing et al., 2008), a second reaction at higher temperatures is beneficial to convert the remaining solids to 5-HMF at higher yields. The observed saturated vapor pressure of the MIBK biphasic reaction at 170 ˚C was 138 psig which was only slightly higher than the non-solvent water reaction (105 psig). Further characterization of the MIBK biphasic system was not carried out as the results confirm and agree with the findings of other groups (Chheda, 2007; Amiri et al., 2010, Weingarten et al., 2010).

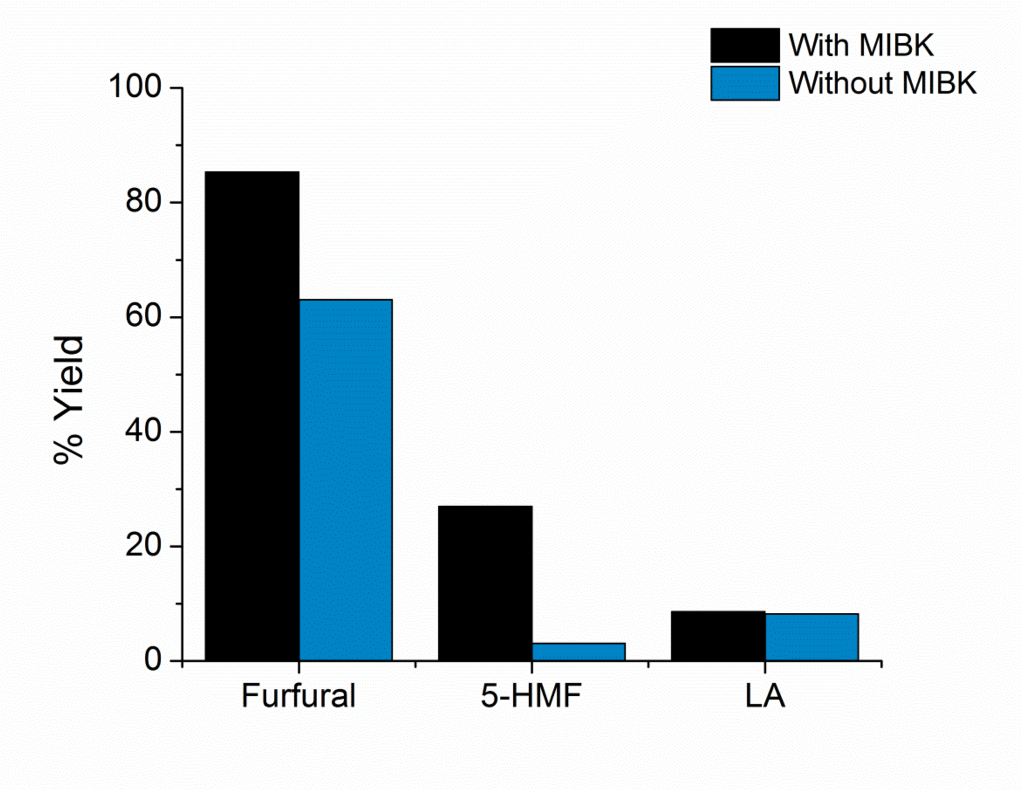

Figure 3.3. Furfural, 5-HMF, and LA yields obtained from biphasic reaction of maple wood with MIBK compared to single phase reaction without MIBK in a standard aqueous reaction as a control. Reaction was optimized for furfural production. Reaction conditions: 800 g Biphasic reaction: 20 g maple wood (5 wt%), 4 g H_2SO_4, 400 g MIBK, 376 g acid water, 170 °C, 50 min.

The major disadvantage of the biphasic system is the reduction of effective solids loading in a reactor of finite volume. Since up to half the reaction volume must now be occupied by the solvent (as shown in Figure 3.2), additional energy must be consumed by heating and stirring the liquid solvent, which could have a higher heat capacity than biomass. Additionally, the extraction efficiency for the RIs in MIBK is crucial because any unrecovered solvent and product remaining in the aqueous stream would be lost or be too expensive to recover in dilute amounts from the aqueous stream. Extracting solvents such as MIBK are typically very expensive to regenerate or replenish and an additional cost must be considered to account for any solvent recovery losses. Thus, the improvement in product yields from a biphasic system must be significant enough to justify the additional process expenses from its implementation. Biphasic reactions have also been found to minimally interfere with aqueous reaction kinetics, and the optimum reaction times will likely mirror that of the non-solvent case (Weingarten, 2010).

3.4.2 Novel single phase co-solvent reaction of maple wood using THF

THF is a unique biomass-derived solvent that is naturally miscible with water over a wide range (Smallwood, 2002). THF is relatively non-toxic and can be synthesized from the hydrogenation of furan. Previous application of THF for biomass conversion has been for biphasic reaction strategies where NaCl or other salts were added to the aqueous mixture to induce a phase separation (Olcay et al., 2013). Here, we apply THF directly as a water-miscible co-solvent to study its application as a solvating or catalytic agent in the acidic aqueous mixture for improving RI yields from raw maple wood. The addition of THF to sulfuric acid containing water was first performed at a 1:1 volume ratio. Similarly to the biphasic reactions and water reactions, 5 wt% of maple wood (40g out of 800g total reaction weight) with co-solvent acid solution was delivered to the Parr® reactor and subjected to heated reaction at 170 °C. It is important to note that the batch of maple wood used in the THF co-solvent reactions was not the same as the ones used in the biphasic reactions with MIBK and cannot be directly compared.

Figure 3.4 shows the resulting furfural, 5-HMF, and LA yields after 40 min reaction from the THF co-solvent reaction compared to a typical dilute acid reaction without THF. As shown, furfural yields (75% vs 56%) were dramatically improved by THF co-solvent reaction over the non-solvent case, up to 20% improvement at 40 min reaction. 5-HMF yield was also improved (13% vs 4%), but not nearly as much as with MIBK in the biphasic reaction. These promising results present the first demonstration that the THF:water co-solvent system can significantly enhance RI yields directly from lignocellulosic biomass without the presence of salts or other phase modifiers. LA yield was also slightly higher for the THF co-solvent reactions suggesting that total sugar conversion was accelerated by presence of THF. Since THF is homogenously mixed in the catalytically active phase, it is highly likely that THF could also influence or enhance the reaction kinetics. The observed saturated vapor pressure of the THF co-solvent reaction at 170˚C was 224 psig which is significantly higher than the non-solvent reaction (105 psig) and much higher than even the biphasic reaction with MIBK (138psig). This is a concern in terms of safety and the additional cost of equipment that would be needed to support the higher pressures. Additionally, THF is a known peroxide former under oxygenated conditions so its storage under a nitrogen blanket is recommended and its constant interface with significant quantities of water as a co-solvent in CELF reduces operating hazards. THF should never be distilled to dryness.

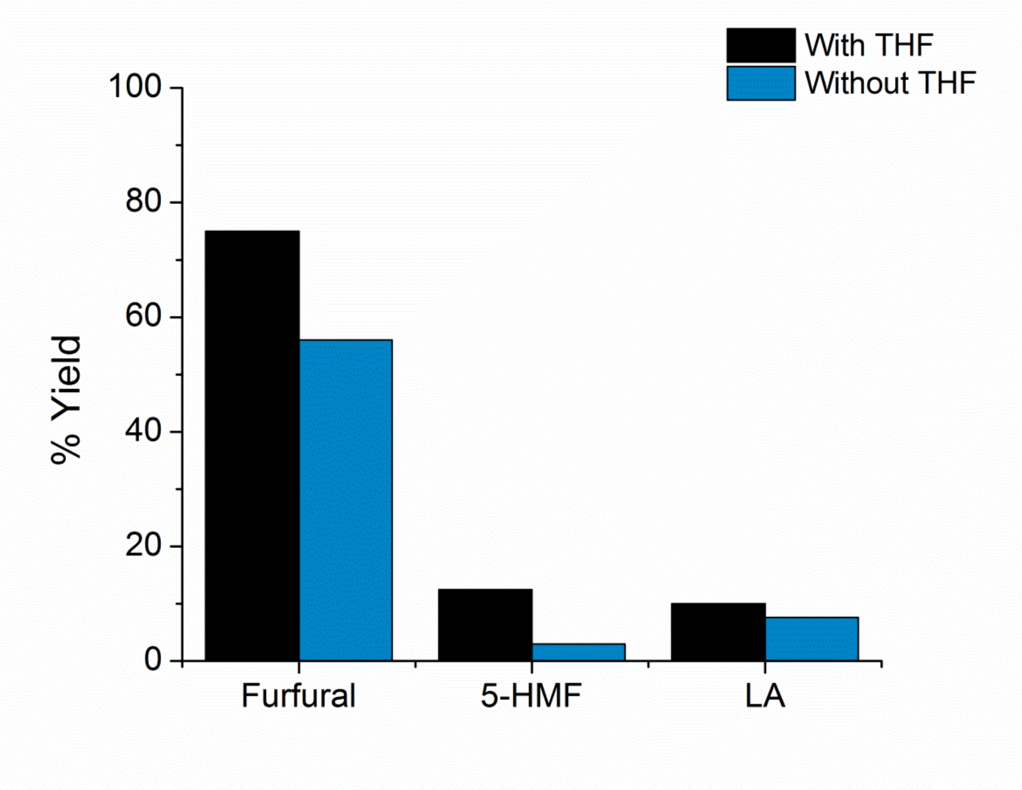

Figure 3.4. Furfural, 5-HMF, and LA yields obtained from single phase reaction of maple wood with THF co-solvent compared to single phase reaction without THF in a standard aqueous reaction as a control. Reaction conditions: 800 g monophasic reaction: 40 g maple wood (5 wt%), 8 g H_2SO_4 (1 wt%), 752 g 1:1 (vol) THF:water mixtures, 170 °C, 1 wt% H_2SO_4, 40 min.

3.4.3 Thermodynamic observations and furfural solubility

Due to the novelty of the application of THF as a miscible co-solvent for the direct conversion of lignocellulosic biomass to RIs, additional thermodynamic data on this system can aid the development and optimization of this system to improve performance. Figure 3.5 shows a three-dimensional plot of the relationship between temperature, gauge pressure, and THF mass fraction, *x*, of the binary mixture of THF and water under isochoric (constant volume) conditions. As shown, the observed gauge pressures from the

Parr® reactor upon closed heating reveals that the resulting system pressures are much higher than expected for a homogeneous binary mixture, reaching gauge pressures upwards of above 400 psig at 0.5 *x* and 200 °C. The gauge pressures were then converted to absolute saturated vapor pressures in Figure 3.6 and plotted against *x* (mass fraction) and temperature. In Figure 3.6, the colored points represent the sum of the individual saturated pressures from each pure component at different temperatures to correspond to the hypothetical vapor pressures expected from a biphasic system containing THF and water. As shown, at the lower temperature of 150 °C, the saturated pressures was very flat over increasing *x* and was very close to expected pressures for a biphasic system at liquid-liquid equilibrium (LLE). At increasing temperatures, the saturated pressures begin to deviate more from the hypothetical two-phase pressures suggesting that the system could still be monophasic as a non-ideal binary homogeneous mixture. It is possible that below 150 °C, a phase split could have occurred where the system became completely biphasic. Increasing temperature moves the system further away from LLE and into a non-idea homogenous system.

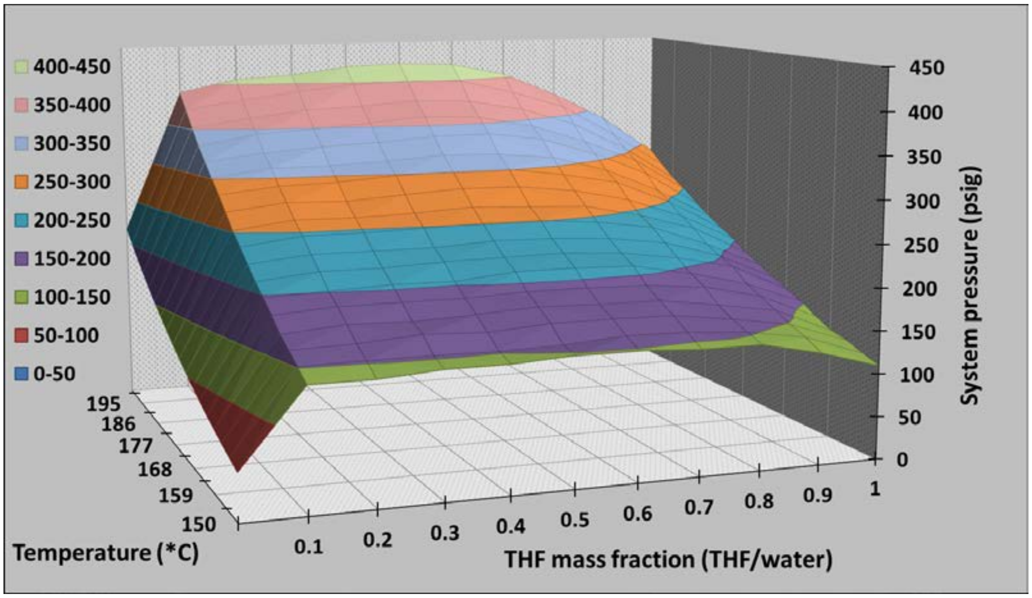

Figure 3.5. 3D plot of the relationship between temperature, gauge pressure, and THF mass fraction of the binary mixture of THF and water in a sealed autoclave reactor. (Mass fraction: 0-pure water; 1-pure THF)

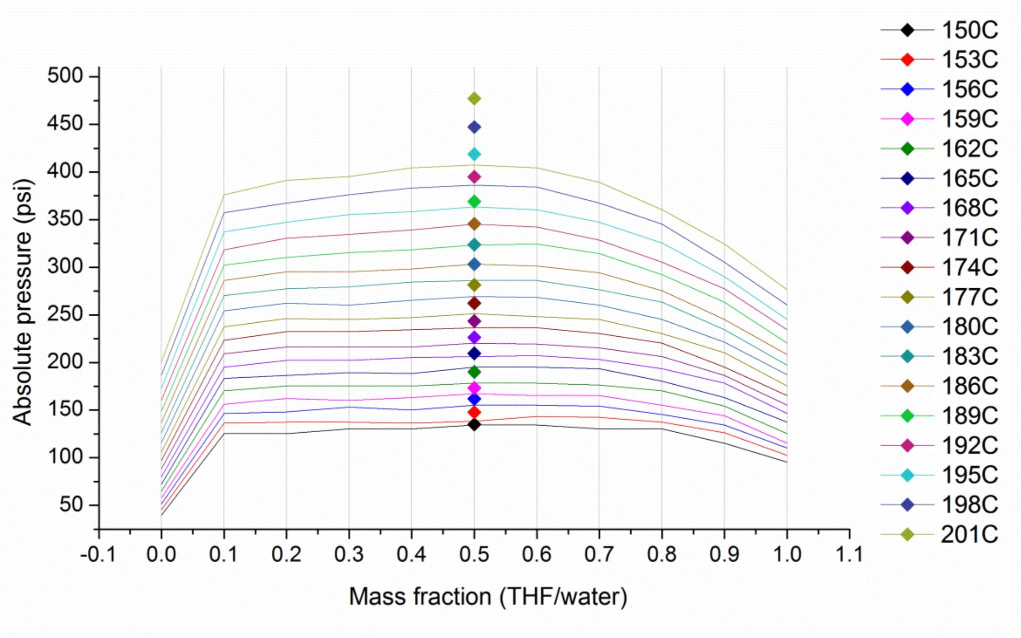

Figure 3.6. P-x diagram of the THF/water binary system at increasing temperatures in isochoric conditions. Points represent the expected saturated pressures of a biphasic system, lines present recorded saturated vapor pressures by varying THF mass fraction and system temperature. (Mass fraction: 0-pure water; 1-pure THF).

As shown in Figure 3.7, evidence of the special LLE phase behavior of THF and water can be explained by a known closed miscibility gap between 75 °C to 140 °C (Matous et al., 1972; Wallbruch and Schneider, 1995). There is also a lack of conformity of this binary mixture to obey ideal fluid properties; evident by a strongly negative deviation of excess volumes between *x* of 0.1 to 0.8 at ambient pressures and strongly positive deviation from Raoult’s law(Schedemann, 2010). Schedemann also observed that artificially increasing head pressure could force the miscibility gap to diminish such that the system remains homogenous. It has also been determined that a phase split can be induced between THF and water with the addition of neat CO_2 gas at lower temperatures (Lazzaroni et al., 2004). As shown in Figure 3.7, Lazzaroni was able to induce a phase split between THF and water at just 25 °C with 1 MPa CO_2 pressure. Further increase of CO_2 pressures to 5 MPa resulted in a broadening of the miscibility gap in terms of *x* (0.1 to 0.9 *x*). These findings suggest that the THF co-solvent system is highly tunable. For example, the system could be maintained in a single phase during heated reaction and then induced to form two phases after reaction at a lower temperature using CO_2 pressure so that the homogeneous catalysts could be recycled in the aqueous stream and RI products could be directly extracted into the organic stream and recovered by lower temperature distillation.

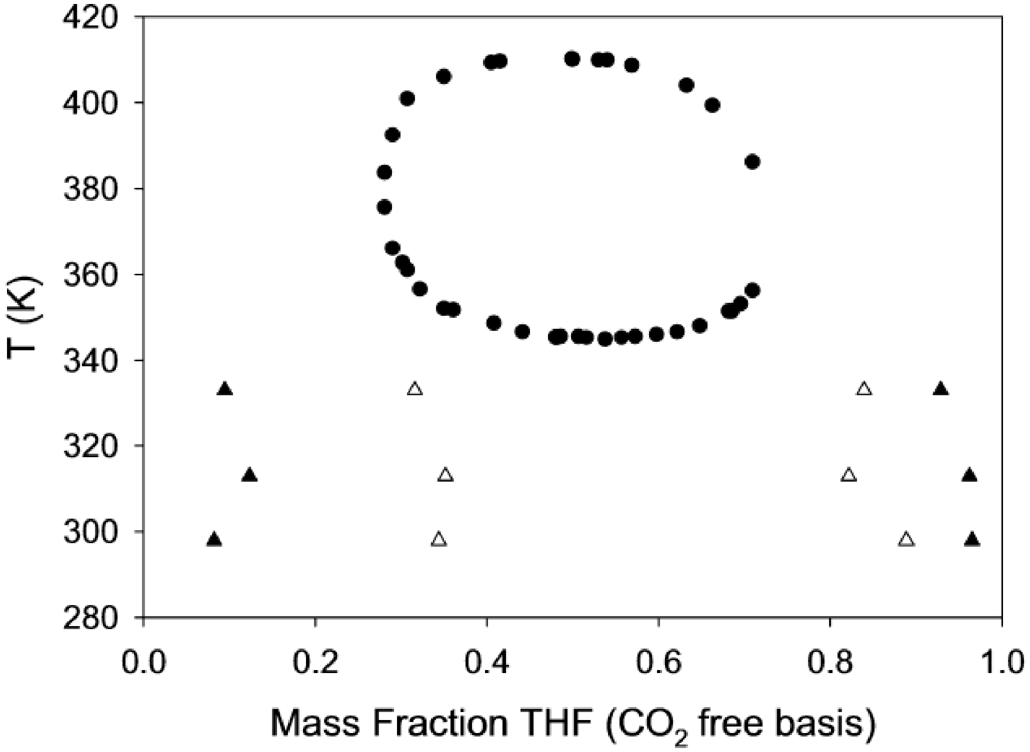

Figure 3.7. LLE for pure THF + water (•), THF + water + 1MPa CO_2 (Δ), and THF + water + 5.2MPa CO_2 (▲). Closed miscibility gap is represented by the circular data points •. (Taken verbatim from Lazzaroni et al., 2004).

The solubility of furfural by measuring its concentration in flasks containing either water or 1:1 THF co-solvent solution was investigated. As furfural has a reported water solubility of 83 g L^{-1} (GESTIS, IFA), we determined that furfural solubility can be significantly improved by adding THF. As shown in Fig. 3.8, we confirmed the maximum solubility of furfural in water to be approximately 83 g L^{-1} at room temperature as we were unable to saturate the water solution further. As shown in Figure 3.9, by increasing furfural concentrations in the THF solutions, we were able to achieve a maximum furfural solubility of 174 g L^{-1}. The high affinity for furfural by THF in solution with water suggests that THF serves a protective function for furfural over the course of the reaction. Interestingly, any further addition of furfural prompted a separation of phases. At 230 g L^{-1} furfural concentration, the furfural was able to fully extract THF out of solution to form two distinct phases: one aqueous and one of furfural with THF. The aqueous layer was still saturated with furfural at the theoretical 83 g L^{-1} solubility, whereas the remaining furfural remained solubilized in the THF organic layer.

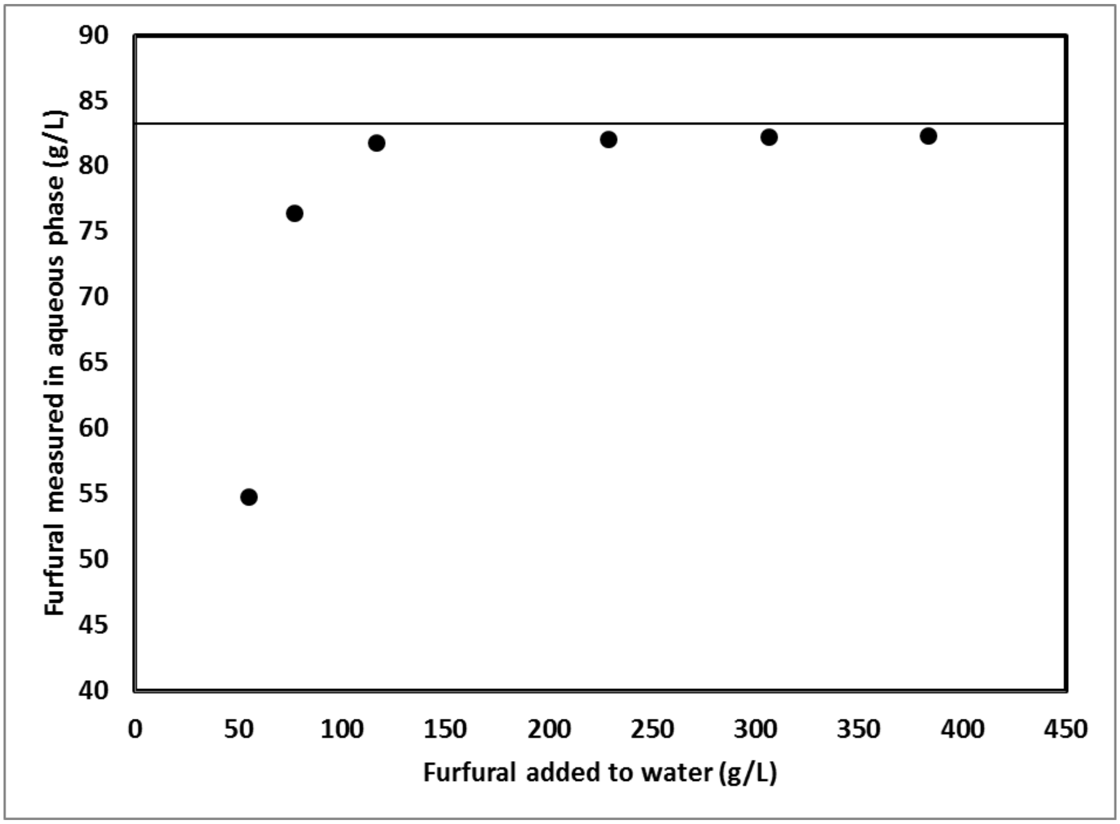

Figure 3.8. Addition of furfural to a water solution to determine the highest solubility of furfural in water at room temperature. The solid line represents the theoretical limit of furfural solubility in water of 83 g L^{-1}.

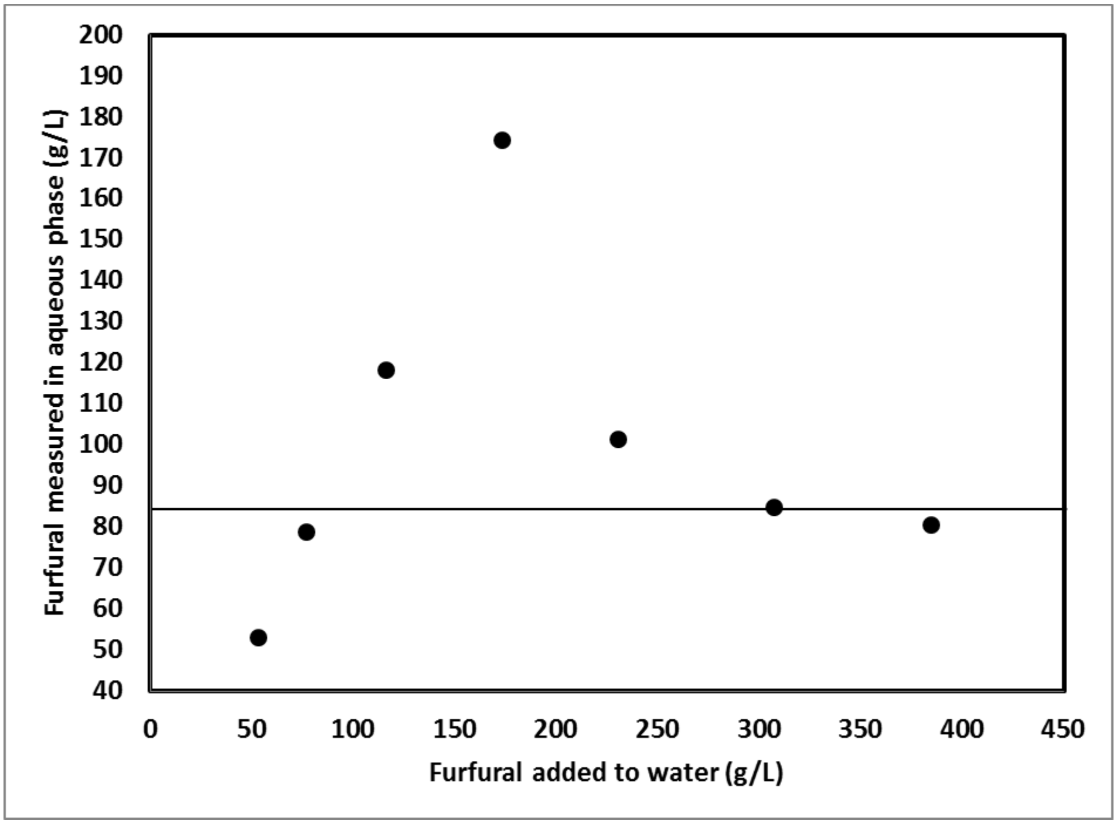

Figure 3.9 Addition of furfural to a 1:1 THF:water solution to determine the solubility characteristics of furfural in this system at room temperature. The solid line represents the theoretical limit of furfural solubility in water of 83 g L^{-1}. The sharp decrease of furfural measured from the aqueous phase after more than 174 g L^{-1} of furfural was added is the result of a phase separation of THF from water.

3.4.4 Sugar destruction kinetics and RI selectivity

From the maple wood reactions, it was suspected that THF could enhance the reaction kinetics of sugar hydrolysis and RI formation. To determine if THF had catalytic properties in an aqueous mixture, acidified solutions (with 1 wt% H_2SO_4) of 1:1 THF:water or just water were prepared each containing either 20 g L^{-1} D-glucose or 10 g L^{-1} D-xylose and were heated in the Parr® reactor at 170 °C to study the disappearance rate of the sugars between the co-solvent and non-solvent system. The glucose and xylose concentrations were selected as they mimicked the expected amounts of glucose and xylose contained inside most types of lignocellulosic biomass at a 5 wt% reactor loading. As shown in Figure 3.10, THF co-solvent appeared to dramatically accelerate the destruction of both glucose and xylose compared to the non-solvent case. A linear fit of a first-order rate expression was then used to model the disappearance of xylose and the calculated rate constants are reported in Table 3.1. To assure that our reaction system was not mass transfer limited and represented accurate kinetics, the rate constants from the non-solvent control reactions were compared and found to be very close to those calculated from an established empirical model (Zeitsch, 2000), as also shown in Table 3.1. These results demonstrated that THF greatly (~2 times) improved the rate of xylose disappearance and suggested that THF had a catalytic effect on xylose dehydration. For glucose, the decomposition of glucose to levoglucosan and significant concentrations of fructose measured from HPLC prevented an accurate calculation of a rate constant. It is assumed that glucose disappearance can be modelled by a pseudo-first order model if the reversible isomerization to fructose can be accounted for (Xiang et al., 2004). Further work will be necessary to build kinetic models to accurately measure both C5 and C6 disappearance in the co-solvent system.

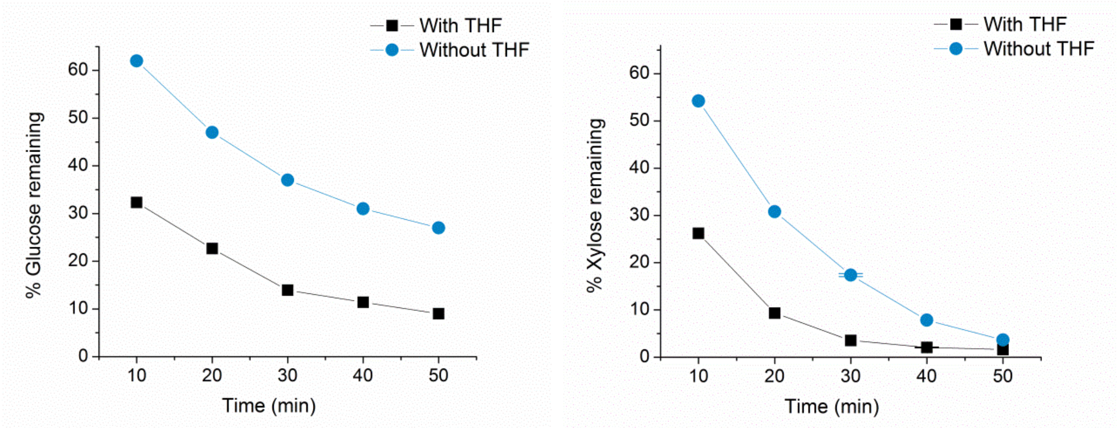

Figure 3.10. Reaction of glucose and xylose solutions with and without THF co-solvent. Left: % glucose remaining from 20 g L^{-1} initial D-glucose concentration. Right: % xylose remaining from 10 g L^{-1} initial D-xylose concentration. Reaction conditions: 170 °C, 1 wt% H_2SO_4 and 1:1 (vol:vol) THF:water concentration (black squares) or just water (blue circles).

Table 3.1. First-order rate constants for the disappearance of D-xylose with and without THF and comparisons to previous literature[a]

Rate Constant	With THF (A/min)	Without THF (A/min)	Literature (A/min)
k[b]	0.109 ± 0.015	0.0557 ± 0.0018	0.0527[c]

[a] Reaction conditions: 10 g L^{-1} D-xylose and 1 wt% H_2SO_4 in batch reactions at 170 °C. THF co-solvent solution contained 1:1 THF and DI water. [b] Rate constant includes a 0.204 M hydrogen ion (A) term. [c] Calculated from Eq. (1) on pg.16 given by K.J. Zeitsch (2000)

Figure 3.11 compares the selectivity of furfural from xylose in both THF co-solvent and non-solvent reactions. For non-solvent reactions, furfural selectivity was initially higher (72% at 10 min), but as the reaction proceeded, selectivity to furfural decreased dramatically to only 59% by 50 min. As more furfural was produced, the formation of irreversible by-products increased. For the reactions with THF, furfural selectivity was initially lower (67% at 10 min) but increased continually over the course of the reaction, surpassing the non-solvent selectivity at 20 min to reach a maximum of 74% by 40 min. Although the exact reason for these differences is not yet clear, the lower initial furfural selectivity for the solvent reactions may be due to increased dehydration rate of xylose resulting in competing xylose intermediates. Subsequently, as xylose was more rapidly consumed in THF co-solvent, furfural selectivity increased as the opportunity for condensation reactions with the intermediate products was reduced. In any event, the overall reduced formation of by-products in the presence of THF resulted in a higher maximum furfural yield from xylose of 73 mol% with THF compared to the 60 mol% yield from the non-solvent system.

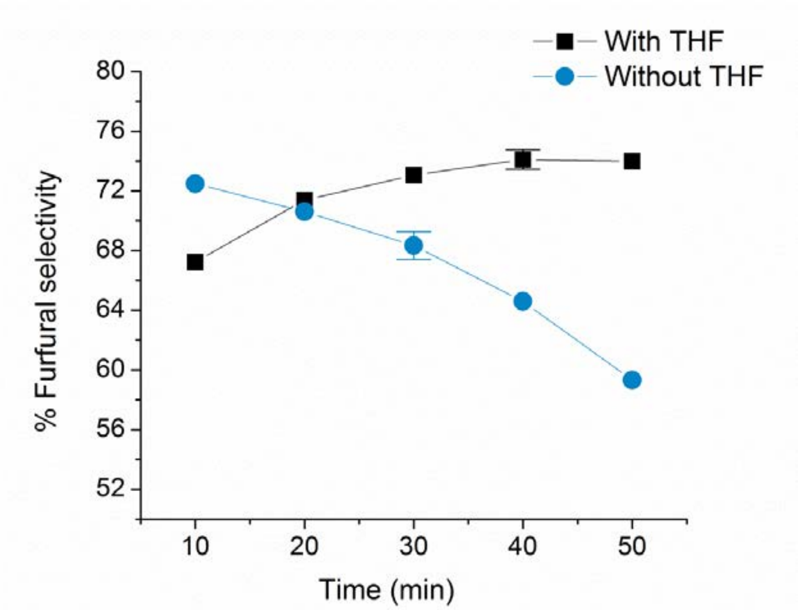

Figure 3.11. Furfural selectivity from reaction of 10 g L^{-1} xylose solution with and without THF co-solvent. Reaction conditions: 170 °C, 1 wt% H_2SO_4 in either 1:1 (vol:vol) THF:water solution or water.

Figure 3.12 compares the selectivity of 5-HMF and LA from glucose in both THF co-solvent and non-solvent reactions. As shown, THF co-solvent improved the selectivity of 5-HMF from glucose by over two times that of the non-solvent reaction. The highest 5-HMF selectivity was observed at the earliest reaction time of 10 min for both cases. 5-HMF is highly reactive in the aqueous environment and quickly hydrolyzes to form LA and formic acid. Consequently, overall LA selectivity is substantially higher than 5-HMF and the opposite trend is observed where LA selectivity as it improves with increasing reaction time. LA selectivity is also slightly lower with THF than without THF due to the greater selectivity towards 5-HMF in THF co-solvent. The resulting selectivity trends of furfural, 5-HMF, and LA provide clues to their relative rates of formation and demonstrate that optimization of co-production strategies may be difficult as their optimum selectivities occur at different reaction times. This means that either a more selective acid catalyst than sulfuric acid is needed to promote co-production of the RIs or independent reactions in the form of a multi-stage reaction strategy will be required. It is also important to consider that the kinetics of xylose and glucose hydrolysis are also vastly different and could complicate optimizing such a process for biomass conversion. Nevertheless, THF shows great promise as a renewable co-solvent useful as a catalytic solvent for enhancing RI yields from both sugar solutions and from lignocellulosic biomass.

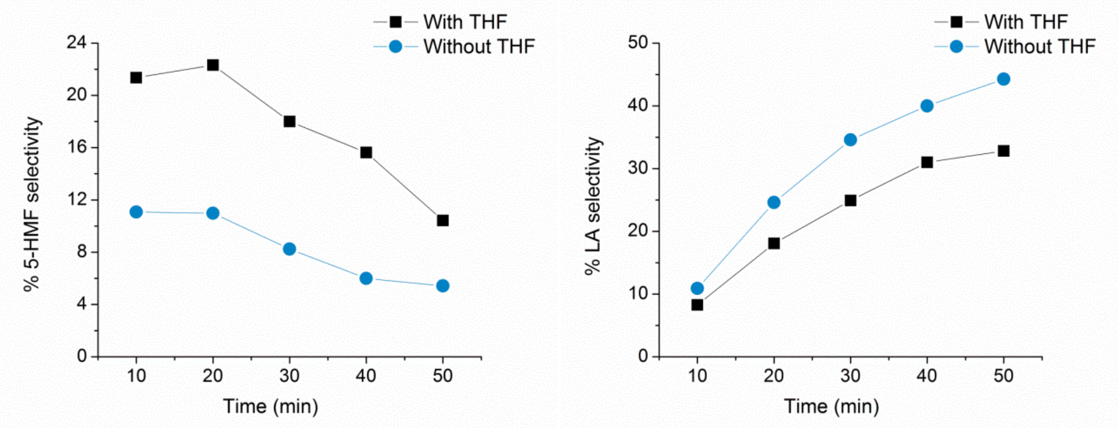

Figure 3.12. 5-HMF (left) and LA (right) selectivity from reaction of 20 g L^{-1} glucose solution with and without THF co-solvent. Reaction conditions: 170 °C, 1 wt% H_2SO_4 in either 1:1 (vol:vol) THF:water solution or water.

3.5 Conclusions

RIs from biomass-derived sugars are important precursors to biofuels production, but they typically suffer from low production yields when produced directly from lignocellulosic biomass. We evaluated two different types of solvent strategies for their potential for enhancing furfural and 5-HMF yields; one employs MIBK as a biphasic extracting solvent and the other employing THF as a water-miscible co-solvent. MIBK demonstrated exceptional extraction efficiency for both furfural and 5-HMF, thus achieving the highest yield of both in this study. However, biphasic reactions are less energetically favorable than single phase reactions and solvent recovery remains a cost concern as MIBK is expensive. A novel single phase reaction strategy using renewable THF was introduced and demonstrated to be effective for enhancing furfural and 5-HMF yields from direct reaction of maple wood. For the first time, we showed that a single phase solution of THF and water combined with sulfuric acid can (1) improve selectivity to furfural and 5-HMF and (2) help catalyze dehydration of the xylose and glucose sugars into furfural, 5-HMF, and LA over the non-solvent case. Temperature-pressure data was collected for the THF-water binary mixture at 150 °C to 200 °C to probe for LLE boundaries and any miscibility gaps at high pressures. As a result, the THF/water co-solvent system was found to be miscible but also highly tunable as miscibility of THF with water could be altered by varying pressure, temperature, mass fraction of THF, and by introducing pressurized CO_2 gas into the head space. THF was also found to have a high affinity for furfural and improved furfural solubility in water more than twofold. Future optimization of the THF co-solvent system on different raw feedstocks will be needed to further improve RI yields to determine possible co-production configurations.

3.6 Materials and methods

Maple wood was provided by Mascoma Corp., NH, and was air dried at 55˚C for one day before reaction. Solid content was measured to be higher than 95% before the maple wood was further milled down to 1 mm size particles. The composition of the resulting maple wood was then determined to be 17.1 ± 0.1wt.% xylan, 41.5 ± 0.5% glucan, 23.5 ± 0.3% Klason-lignin (K-lignin), and 17.9% other unidentified material by following NREL (National Renewable Energy Laboratory) "Determination of Structural Carbohydrates and Lignin in Biomass" procedure (http://www.nrel.gov/biomass/pdfs/42618.pdf). Due to the high temperature and non-specific nature of the reactions to the feedstock, accurate quantification of only xylan, glucan, and K-lignin was possible.

All reactions were performed in a stirred 1L Hastalloy Parr® batch reactor (Parr Instrument Company, IL). The stirring shaft contained twin 6-blade impellers, and stirring rate was held constant at 200 rpms. Heating was provided by a fluidized sand bath with a digital PID temperature controller. Temperature inside the reactor was monitored with an inline K-type thermocouple. The reaction temperature was maintained at 170˚C and fine-tuned by raising or lowering the reactor over the sand bath as necessary, as described in detail elsewhere (Lloyd and Wyman, 2005). Reaction volumes were kept at approximately 800 ml to allow sufficient head space. The solid content of the reaction volume was maintained at 5 wt% with respect to the aqueous phase. 1 wt% H_2SO_4 prepared by dilution of a 72 wt% stock solution was used in each reaction. Histological grade THF (with inhibitor) was purchased from Fisher Scientific (Pittsburgh, PA), and ACS reagent grade MIBK was purchased from Sigma-Aldrich (St. Louis, MO).

For this study, MIBK was added according to an equal mass basis with the aqueous phase and THF solvent was added at a equal volume basis. After sample preparation, the biomass was allowed to soak overnight at 5 ± 3˚C before reaction. A set of control experiments without solvents were also performed at the same 5 wt% loading of maple wood and H_2SO_4 in conjunction with the solvent reactions.

After each reaction, the solids were separated from the liquid by vacuum filtration. Analysis of the liquid fraction was performed on an HPLC (1200 series, Agilent, CA) using an Aminex HPX-87H column (Bio-Rad Laboratories, Hercules, CA) and an eluent rate of 0.6 ml min^{-1} eluent using 5 mM H_2SO_4. The solids were washed several times with water through the vacuum filter until the acidity was 6 pH or higher and analyzed for composition by following the aforementioned NREL procedure (http://www.nrel.gov/biomass/pdfs/42618.pdf).

For the glucose and xylose reactions, 20 g of D-glucose or 10 g of D-xylose (>99% purity, Sigma-Aldrich, St. Louis, MO) was transferred to a 1 L volumetric flask and dissolved with either THF solvent or non-solvent solution containing 1 wt% sulfuric acid concentration. The selected sugar concentrations were selected to mimic the effective amount of glucose and xylose that would be hydrolyzed from a 40 g sample of biomass with a 40% glucan content and xylan content of 17.5% (average of 14-21% typically found in biomass). In this way, the calculated rate constants and selectivities would be relevant to our current and future findings. A first-order approximation of xylose dehydration is represented by Equation (1)

(1) $$Xylose \xrightarrow{k_1} D$$

where D represents degradation products. The expression for the rate of xylose (X) disappearance and calculation of the rate constant are given in Equations (2), (3), and (4). Since acid concentration (A) was held constant throughout our studies, it was combined into the overall rate constant k.

(2) $$\frac{d(X)}{dt} = -(k_1 A)X = -kX$$ (1^{st} order rate law with lumped acid term)

(3) $$\int_{X_{init.}}^{X} \frac{d(X)}{X} = -k \int_0^t dT$$

$$\ln\left(\frac{X}{X_{init.}}\right) = -kt \tag{4}$$

where rate constant k was determined by linear fitting of Equation (4). Results after 30 min were not used in the linear fitting as the xylose solution became too dilute for accurate quantification.

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Chapter 4. THF co-solvent enhances hydrocarbon fuel precursor yields from lignocellulosic biomass*

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4.1 Abstract

A novel single phase co-solvent system using tetrahydrofuran (THF) promotes hydrolysis of maple wood to sugars, sugar dehydration, and lignin extraction simultaneously and achieves higher overall yields of the fuel precursors furfural, 5-hydroxymethylfurfural (5-HMF), and levulinic acid (LA) than previously reported from biomass. In a one-pot reaction, we obtained yields of 86% furfural, 21% 5-HMF, and 40% LA in the liquid phase and over 90% extraction of lignin as a solid powder. The co-solvent reaction also produced a glucan-rich residue that is highly digestible by enzymes for biological conversion to ethanol or further thermochemical reaction to additional 5-HMF and levulinic acid. These findings enable an integrated conversion platform in which THF is both a co-solvent and final co-product to enhance production of fuel precursors for catalytic upgrading to renewable liquid hydrocarbons fuels.

4.2 Introduction

We present here the application of tetrahydrofuran (THF) as a unique co-solvent to enhance yields of the hydrocarbon fuel precursors furfural, 5-hydroxymethylfurfural (5-5-HMF), levulinic acid (LA), and glucose directly from raw maple wood. Wood and other types of lignocellulosic biomass are the most abundant sources of organic carbon on Earth and present the only option to economically and sustainably replace fossil resources for production of liquid fuels for transportation on a large scale (Lynd et al., 2008; Wyman, 2007). Furfural, 5-HMF, and LA are promising platform chemicals (Werpy and Peterson, 2004; Bozell and Petersen, 2010; Dautzenberg et al., 2011) produced from acid-catalyzed dehydration of biomass sugars that can serve as precursors to gasoline, jet fuel, diesel-range alkanes, and fuel blending components compatible with the existing fuel infrastructure (so-called “drop-in” fuels) while also providing energy densities well suited to heavy duty vehicles and aircraft (Cass, 1948; Huber et al., 2005; Roman-Leshkov et al., 2007; Bozell, 2010).

In this context, we define a fuel precursor (FP) as any biomass sugar monomer or sugar dehydration product that can be biologically, chemically, or catalytically converted into fuels and fuel products. Continued efforts to improve the synthesis of furans from sugar streams (Nikolla et al., 2011; Olcay et al., 2013) and their catalytic upgrading to fuel products have made this pathway increasingly viable as an industrially relevant biofuels platform. However, the natural resistance of lignocellulosic biomass to breakdown to monomeric sugars and low yields of their dehydration products are the major obstacles to low cost fuels that must be overcome for biomass drop-in fuels to have impact (Lynd et al., 1999). Thus, effective strategies are needed that can efficiently fractionate raw biomass and achieve high product yields directly from biomass without expensive catalysts or solvents or complex process configurations.

THF is a promising green solvent that is relatively non-toxic and miscible with water over a wide range of reaction conditions. Unlike other miscible solvents and ionic additives used to enhance FP production (Alonso et al., 2013; Binder et al., 2010), THF is low boiling (66 °C), forms an azeotrope with water, and can be synthesized directly from furfural at high selectivities through catalytic decarbonylation to furan followed by ring hydrogenation (Dautzenberg et al., 2011). In previous work, THF demonstrated exceptional properties for extracting furfural and 5-HMF from water mixtures and was successfully used in biphasic reactions to increase their yields from sugar streams and less-recalcitrant biomass (Nikolla et al., 2011; Amiri et al., 2010; Xing et al., 2011) However, biphasic systems are physically limited by the presence of two discernible liquid phases so high initial solids concentrations cannot be achieved, and saturation of the aqueous phase with salts is needed to partition THF from water. Instead of a two phase approach, we show here, for the first time, that THF in a one-pot monophasic reaction can significantly increase fuel precursor yields compared to traditional water-only strategies while promoting deconstruction of biomass beyond what has been possible in biphasic systems.

4.3 Results and discussion

4.3.1 Fractionation of maple wood by THF co-solvent

We selected raw maple wood as a highly recalcitrant model lignocellulosic feedstock for both co-solvent and non-solvent reactions to establish the advantages of this novel application of THF. Consecutive batch reactions at 170 °C were performed using dilute sulfuric acid (1 wt%), which is an abundant and inexpensive strong acid that can be used in low enough amounts at high solids concentrations so that it can be economically neutralized in the waste stream for disposal (Aden and Foust, 2009). From the mass and composition of the remaining solids (per 100 g basis of raw maple wood) presented in Figure 4.1, we observed a significantly higher degree of biomass solubilization in the presence of THF than without. For the reactions containing a 1:1 (vol.) mixture of THF and water, over 90 wt% of the acid insoluble Klason lignin (K-lignin) initially present in maple wood was removed into the liquid phase by 10 min (not shown), producing a solid residue that was highly glucan-rich (>85 wt% glucan). In contrast, the composition of the remaining solids from reactions without THF showed that K-lignin content was only slightly reduced at 30 min but then quickly increased over time due to formation of pseudo-lignin (Sannigrahi et al., 2011) and other acid insoluble degradation products (Fig. 4.1). The THF co-solvent also appeared to catalyze the hydrolysis reaction as the disappearance of glucan from the solids was faster compared to reactions with just water (Fig. 4.1).

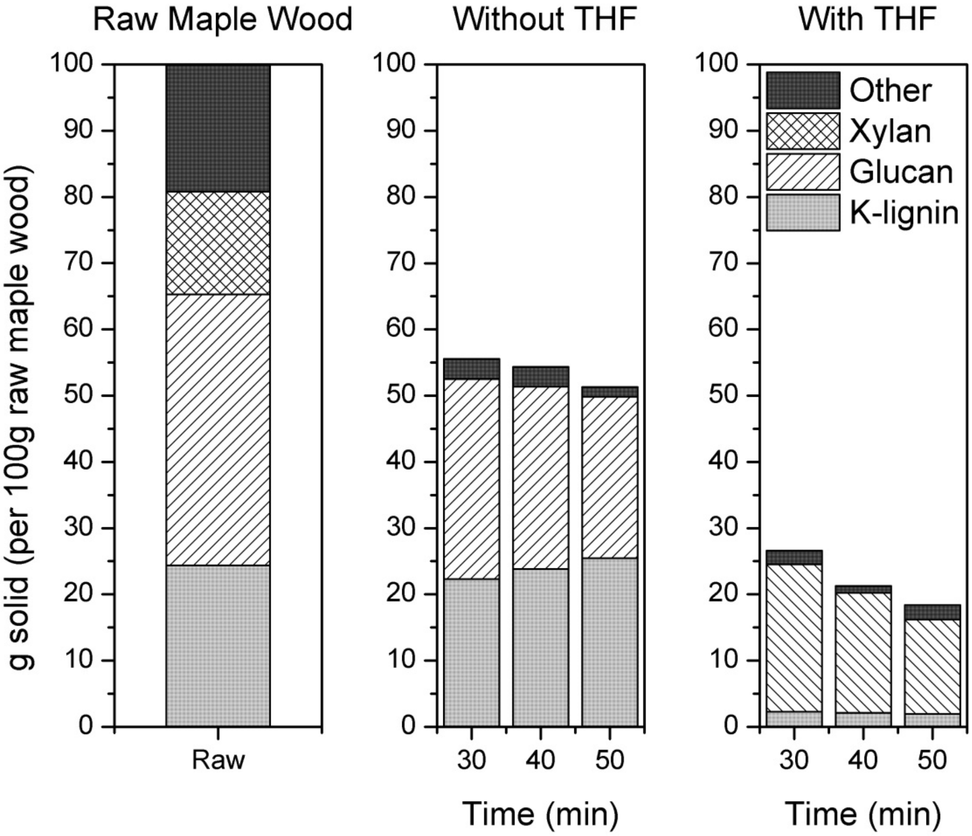


Figure 4.1. Composition of raw maple wood and solids remaining after reaction with and without THF based on 100 g of initial solids. THF dissolved over 90 wt% of the lignin and degradation products that otherwise accumulated in the non-solvent case. Reaction conditions: 5 wt% maple wood and 1 wt% H_2SO_4 in batch reactions at 170 °C. The THF co-solvent solution was at a 1:1 solvent–water ratio.

We were able to easily recover the low-boiling THF from the post-reaction liquor by room temperature vacuum distillation. The removal of THF rapidly precipitated the dissolved lignin in the liquor as a sticky solid residue that could be collected by pouring out the remaining liquid. Further washing of the solid precipitate with diethyl ether followed by oven-drying produced a brown lignin-rich powder largely free of sugars, proteins, and ash (as shown in Fig. 4.5 in the Supplementary Information (SI), Section

4.10). The lignin recovered from the co-solvent reaction readily re-dissolved in THF for catalytic upgrading into valuable aromatics and polymers (Henriksson et al., 2010). FT-IR absorbance spectra (as shown in Fig. 4.6 in the SI) in the fingerprint region of the lignin product extracted by THF matched that of the K-lignin residue that remains from concentrated sulphuric acid extraction (72 wt% H_2SO_4 for 1 h) of raw maple wood very well. Unlike THF extracted lignin, acid extracted K-lignin residue contained a small amount of acid insoluble ash, was darker in color, and did not readily dissolve in either THF or DMSO. Based on previous characterization studies of hard wood lignin (Pandey, 1999), a stronger absorbance band representing carbonyl stretching of unconjugated β-ketones and conjugated acid/esters (Fig. 4.6) indicated a greater oxidation of the lignin structure extracted by THF. Characterization in progress by 2D NMR will provide additional insights on the specific changes in the molecular weight and chemical reactivity of the THF isolated lignin to determine suitable paths for catalytic upgrading.

4.3.2 Characterization of reacted maple wood solids

As shown in Figure 4.2, SEM images of the solid biomass residues that remained after 40 min of reaction for both the co-solvent and non-solvent case showed remarkable differences in the macro surface structure of maple wood. In the reactions with THF (Fig. 4.2), the fibrous structure was nearly completely disrupted, and cell walls collapsed in contrast to the non-solvent case (no THF, Fig. 4.2) where these features were still clearly visible. Removal of hemicellulose and lignin at the high severity conditions applied in the co-solvent reaction and disruption of the surface structure are characteristic of solids that can be more easily hydrolyzed to fermentable glucose with lower enzyme doses than solids from most other pretreatment systems (Yang and Wyman, 2004; Wyman et al., 2005). The results of an enzyme assay of these samples using the commercial cellulase cocktail Accellerase® 1500 is shown in Figure 4.3. As shown, the glucose yield achieved from the enzymatic hydrolysis of THF pretreated maple wood (with THF, Fig. 4.3) was much greater than that for dilute acid-pretreated maple wood (no THF, Fig. 4.3) and raw untreated maple wood (raw, Fig. 4.3). In fact, even at a low enzyme loading of 15 mg-protein per g-glucan, the resulting 72 h glucose yield from the THF pretreated maple wood residue matched that of Avicel® cellulose (95–98% glucan). Thus, the solids could be ideal for biological conversion by such routes as Simultaneous Saccharification and Fermentation (SSF) or Consolidated Bioprocessing (CBP) to produce ethanol after a washing step to remove traces of solvent and inhibitors. Currently, work on applying THF co-solvent as a fractionation and pretreatment strategy to enhance the release of sugar monomers is underway.

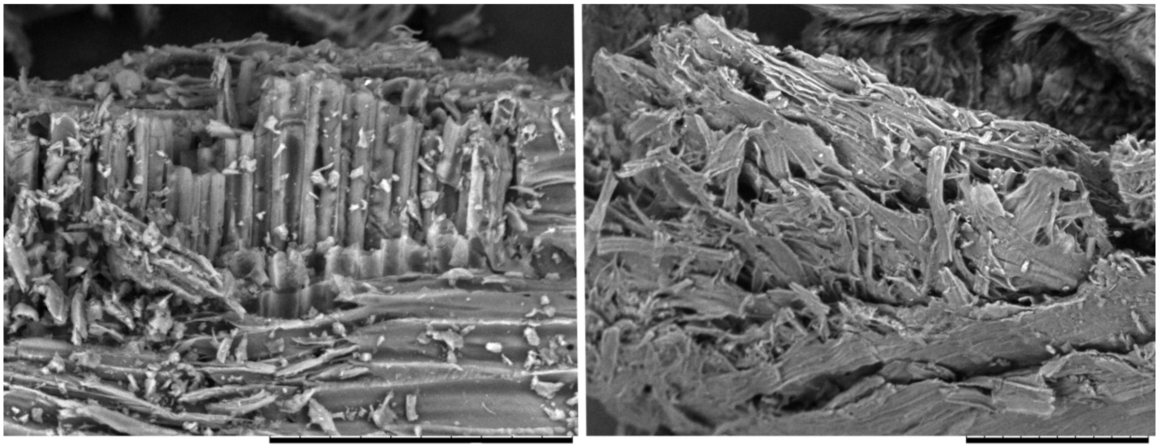


Figure 4.2. SEM images of the maple wood residue after 40 min reaction with (A) no THF and (B) with 1:1 THF co-solvent.

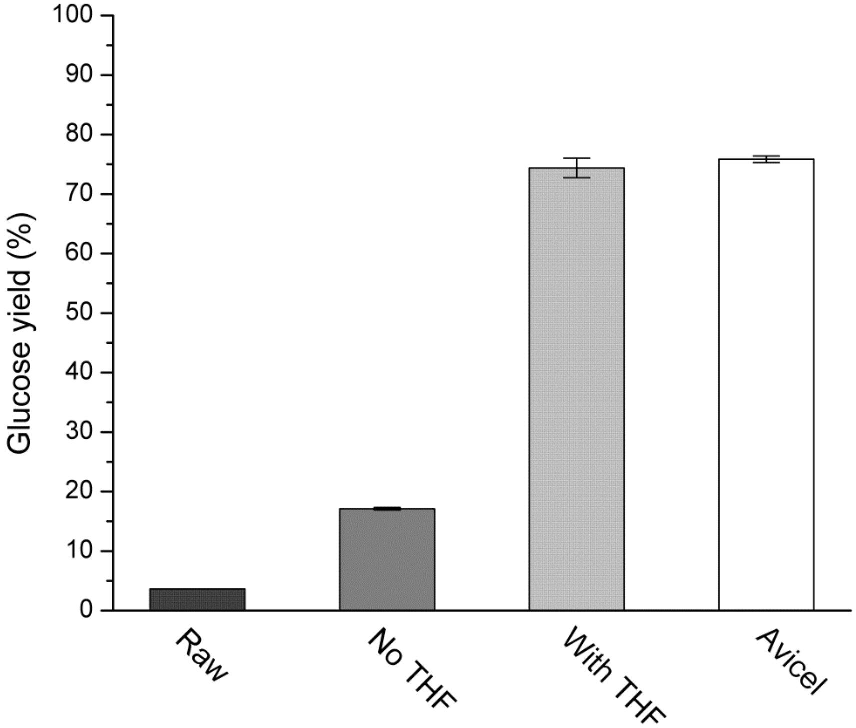


Figure 4.3. Comparison of glucose yields between raw and reacted maple wood samples to Avicel® cellulose after 72 h enzymatic hydrolysis at 15 mg-protein g-glucan^{-1} loading of Accellerase® 1500 cellulase.

4.3.3 THF co-solvent enhances fuel precursor yields

Table 4.1 compares the highest yields of furfural, LA, and 5-HMF obtained from THF co-solvent and non-solvent reactions of raw maple wood chips. Most notably, at 40 min reaction, THF improved furfural yields from 62% to 87% of theoretical compared to the non-solvent case and greatly improved 5-HMF production. Evidence from the concentration profiles of the sugars and sugar products (as shown in Fig. 4.7 in the SI) indicate that THF catalyzed both the hydrolysis of maple wood polysaccharides and the dehydration of C5 and C6 sugars to improve fuel precursor yields. Varying the solvent–water ratio in the co-solvent reactions (Table 4.1, runs 2–4) showed that overall product yields were lower for a 1:3 mixture, the highest furfural yields were realized with 1:1 and 3:1 solutions, and higher yields of LA (32%) and 5-HMF (21%) were obtained from the 3:1 mixture. By carrying the reaction out to 60 min with the 3:1 mixture (Table 4.1, run 5), the LA yield was further increased (40%) with additional biomass solubilisation, but furfural yields began to drop (86%). As 5-HMF readily hydrolyses to form LA and formic acid in the presence of a strong Brønsted acid (Dautzenberg et al., 2011), LA is the primary product from C6 sugars in these reactions. However, we have found that certain strong Lewis acids can increase 5-HMF selectivity, providing an exciting area of future study for various co-production schemes.

Table 4.1. Acid-catalyzed production of furfural, LA, and 5-HMF from maple wood in batch reactions with and without THF addition.

						Yields (of theoretical)		
Run #	Solvent content[c]	Substrate	Acid Catalyst	Time (min)	Reaction Temp. (°C)	Furfural (%)	HMF (%)	LA (%)
1	None[d]	Maple wood[b]	1 wt% H_2SO_4	40	170	62	2.4	7.2
2	1:3 THF:Water	Maple wood[b]	1 wt% H_2SO_4	40	170	76	4.9	8.3
3	1:1 THF:Water	Maple wood[b]	1 wt% H_2SO_4	40	170	87	13	11
4	3:1 THF:Water	Maple wood[b]	1 wt% H_2SO_4	40	170	87	21	32
5	3:1 THF:Water	Maple wood[b]	1 wt% H_2SO_4	60	170	86	21	40
6	None[d]	Maple wood[b]	1 wt% H_2SO_4	120	170	39	2.6	32
7	1:1 THF:Water	Maple wood[b]	1 wt% H_2SO_4	120	170	69	7.6	40
8[e]	None	Maple wood[f]	1.5 wt% H_2SO_4	40	200	-	-	75

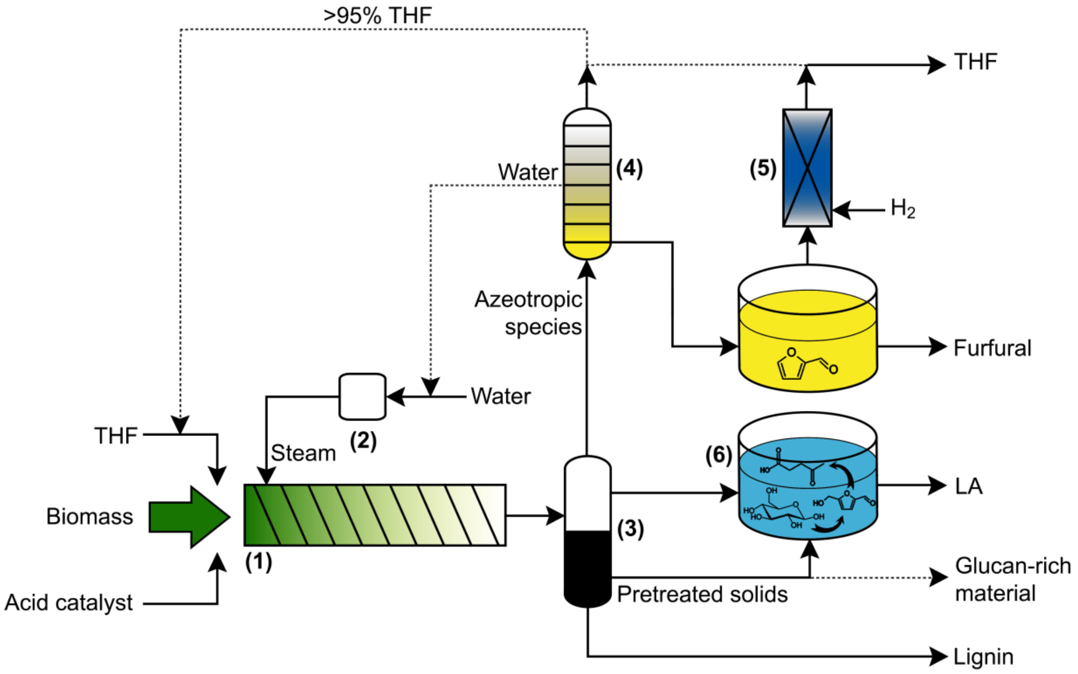
[a] All reactions performed using a 1L Parr reactor and reported yields are % of theoretical maximum. [b] 5 wt% total solids loading. [c] The ratio of solvent to water by volume is as described. [d] These reactions were control reactions without solvent. [e] Production of LA from pretreated maple wood after hemicellulose extraction. [f] 10 wt% total solids loading

Although higher than 3:1 mixtures may increase co-production yields further, excessively high solvent ratios are likely to be detrimental to process competitiveness due to increased recovery costs, heating requirements, and reduced solids loading. As raw biomass often contains significant moisture levels (e.g., 50 wt% for wood), a 1:1 or less mixture is desirable to maximize product concentrations and keep thermal loads as low as possible. In any event, THF as a miscible co-solvent significantly enhanced production of furfural, 5-HMF, and LA over the non-solvent case, achieving yield improvements comparable to our previously reported biphasic reactions with maple wood and MIBK (Zhang et al., 2013). By optimizing reaction conditions for the production of furfural (Table 4.1, run 3), the least thermally-stable fuel precursor, we achieved a maximum overall FP recovery of 87% and 92% (of theoretical) from the C5 and C6 sugars from maple wood, respectively (Material balance shown by Figure 4.8 in the Supplementary information section 4.8).

At longer reaction times (Table 4.1, runs 6 and 7), higher LA yields were obtained at the expense of significant furfural losses in both cases. However, furfural losses were greatly reduced in the co-solvent reaction, providing the initial evidence that THF helped to protect furfural from degradation in the catalytically-active phase. Yet, effective co-production of these fuel precursors was still limited by rapid furfural losses despite increasing the solvent–water ratio. These furfural losses can be attributed to condensation reactions between furfural and intermediate sugar products (Zeitsch, 2000), the slow conversion of furfural to formic acid (Dunlop, 1948), and formation of solid resins and other degradation products (Williams and Dunlop, 1948). Furthermore, since amorphous hemicellulose is more acid-labile than crystalline cellulose, xylan is hydrolyzed and dehydrated much sooner than glucose from cellulose during acid-catalyzed reactions (Wyman et al., 2005). Consequently, achieving high overall yields of fuel precursors from both hemicellulose and cellulose fractions in one vessel is challenging (Lange et al., 2012). Thus, to maximize overall FP yields in a single phase system, preservation of the least stable species is paramount, and separate stages to independently target production of furfural and levulinic acid will be beneficial.

4.3.4 Biorefinery concept for furfural and LA production from biomass

Figure 4.4 illustrates an integrated approach to apply these exciting results in a biorefinery. We propose the direct injection of high-pressure (HP) steam (Fig.4.4, item 2) as an efficient heating method for this system. The Pandia reactor shown (Fig. 4.4, item 1) uses a conveying screw to keep residence times and temperatures reasonably uniform and handle high solids concentrations. Water-volatile species, such as furfural and THF, can be flashed off (Fig. 4.4, item 3) or stripped off by steam into an azeotropic distillation unit for furfural purification, THF recovery, and water recycle (Fig. 4.4, item 4, dotted lines represent recycle streams). The furfural azeotrope (b.p. 97.9 °C) contains about 65% water and can be further purified by a dehydration column. The THF azeotrope (b.p. 65 °C) contains only a small amount of water (4.6 wt%), and since additional drying is unnecessary, its recovery is not energy intensive (Smallwood, 2002). Removal of THF from the liquid phase causes lignin to precipitate so that it can be collected as a bottom product. Mild catalytic upgrading (Fig. 4.4, item 5) of furfural can regenerate any THF losses and produce aromatic gasoline components. Solvent extraction or reduced-temperature vacuum distillation can be used to recover 5-HMF to limit unwanted reactions or the concentrated aqueous solution can be sent directly to a second reactor (Fig. 4.4, item 6) for LA production. The glucan-rich solids remaining from the co-solvent reactions could either be sent to the second reactor or enzymatically hydrolyzed to fermentable glucose.



Process key: (1) High solids screw-type Pandia reactor, (2) High pressure (HP) boiler, (3) Flash of volatiles and liquid-solid separation, (4) Azeotropic distillation to recover furfural, THF, and water, (5) Catalytic upgrading of furfural to THF, and (6) LA production from concentrated sugar solution and cellulose-enriched material . Dotted lines represent recycle and recovery streams.

Figure 4.4. Conceptual process flow diagram for an integrated biorefinery to produce fuel precursors, THF, and lignin products from lignocellulosic biomass using THF as a single-phase co-solvent. Process key: (1) high solids screw-type Pandia reactor, (2) high pressure (HP) boiler, (3) flash of volatiles and liquid–solid separation, (4) azeotropic distillation to recover furfural, THF, and water, (5) catalytic upgrading of furfural to THF, and (6) LA production from concentrated sugar solution and cellulose-enriched material. Dotted lines represent recycle and recovery streams.

Hydrogenation is the most versatile and advocated option to upgrade furfural, 5-HMF, and levulinic acid to hydrocarbon fuel products. At the high yields obtained in the co-solvent reaction, the diversity of potential products from these fuel precursors greatly improves their marketability. Furfural can be catalytically upgraded to furfuryl alcohol (FAlc), tetrahydrofurfuryl alcohol, and dihydropyran (Cass, 1948; Corma et al., 2007; Wojcik, 1948). Methylfuran (MF) and methyltetrahydrofuran (MTHF, P-series fuel) are hydrogenated products of FAlc which have excellent properties for application as gasoline blendstock (Lange et al., 2007). Aldol-condensation and dimerization of furfural adducts followed by hydrodeoxygenation can produce alkanes (Corma et al., 2007) up to tridecanes (Xing et al., 2010). Dimethylfuran (DMF) is a promising fuel product from 5-HMF that is also suitable as a gasoline component (Roman-Leshkov et al., 2007). LA, from the hydrolysis of 5-HMF, is a valuable chemical precursor to levulinate esters, γ-valerolactone (GVL), MTHF, and other fuel-related products (Dautzenberg et al., 2011). LA’s high boiling point (245 °C) makes it difficult to separate by distillation, so maintaining a concentrated product stream, such as with our proposed strategy, will improve separation economics.

4.4 Conclusions

THF as a single phase co-solvent can meet the challenge of increasing FP yields from lignocellulosic biomass that are vital to successful conversion to hydrocarbon, aka “drop-in,” fuels compatible with the existing infrastructure. We have shown that when THF is used as a water-miscible co-solvent, biomass deconstruction, sugar dehydration, and lignin removal are simultaneously promoted, resulting in the highest reported overall FP yields obtained from biomass. Application of other more environmentally-friendly homogeneous or heterogeneous catalysts in our ongoing studies can allow for improved selectivity to furfural and 5-HMF. By integrating this co-solvent system with recent developments in catalytic conversion of these precursors to hydrocarbon fuels, the economic production of sustainable biofuels may be realized.

4.5 Materials and methods

The acid-catalyzed reactions in this study were carried out in solvent and non-solvent water solutions. The co-solvent solution consisted a mixture of THF (>99% purity, Fisher Scientific, Pittsburgh, PA) and deionized (DI) water. Concentrated sulfuric acid (72 wt%, Ricca Chemical Company) was diluted in solution to obtain the indicated acid loadings for each run. The reactions were then carried out in a high-pressure continuously stirred 1 L Parr reactor (Parr Instrument Company, Moline, IL). The reactor temperature was directly measured by an in-line thermocouple (Omega, K-type). All reaction temperatures were maintained by convective heating with a fluidized sand bath (Techne, Princeton, NJ). Mixing was performed by twin 6-blade impellers operating at 200 rpm driven by a top mounted electric motor. At the conclusion of a run, the reactor was cooled by quickly lowering it into a large room temperature water bath. All liquid containing receptacles were made of glass to prevent the loss of furfural and THF that was observed when plastics were used.

Maple wood reactions were carried out using air-dried (<5% moisture content) maple wood chips obtained in upper New York State by Mascoma Corporation (Lebanon, NH). The chips were milled to below 1 mm particle size. Maple wood composition was measured according to the established National Renewable Energy Laboratory (NREL) procedure (version 8-03-2012) in triplicates with a resulting composition of 40.9 ± 0.3 wt% glucan, 15.5 ± 0.2% xylan, 2.1 ± 0.1% mannan, 24.4 ± 0.3% K-lignin, and 17.1% other material. The latter was not further characterized in this study but was expected to consist of minor saccharides, ash, sugar acids, and protein. Because arabinan and galactan were not present in significant quantities (<0.3%), it may be appropriate to treat all maple wood pentosans as xylan and all hexosans as only glucan and mannan. For each run, the biomass was allowed to pre-soak overnight at 4 °C. Contents were then equilibrated to room temperature prior to reaction. Samples were analyzed by HPLC (Agilent 1200 system equipped with a Bio-Rad Aminex® HPX-87H column and RI detector) with an eluent (5 mM sulfuric acid) flow rate of 0.6 ml min^{-1}. Since the HPX-87H column cannot distinguish between xylose, mannose, and galactose sugars, we also equipped our HPLC with an Aminex® HPX-87P column for quantification of xylose and solids composition. Since the HPX-87P column is incompatible with acids, we elected not to use this column for fuel precursor analysis. The solids were then separated from the reaction liquor by vacuum filtration at room temperature through glass fiber filter paper (Fisher Scientific, Pittsburgh, PA). Mass and density of the liquid fraction were measured to calculate accurate yields by the equations below. Due to the difference in density between the solvent and non-solvent solutions, densities were determined by weighing 25 ml of the reacted liquid in a volumetric flask after each reaction. Calculation of the fuel precursor yields is given by equation (1) where the molar equivalents (θ_{FP}) of furfural (equation (2)), LA (equation (3)), and 5-HMF (equation (4)) are individually calculated for their respective sugar sources.

$$\text{(1)} \qquad \%\ \text{FP yield} = \theta_{FP} * \frac{\text{FP}_{\text{prod.}}(\text{g/L}) * \text{mass of liquor}_{\text{prod.}}(\text{g})}{\text{maple wood}_{\text{init.}}\ (\text{g}) * \text{density of liquor}_{\text{prod.}}(\text{g/L})} * 100\%$$

$$\text{(2)} \qquad \theta_{\text{furfural}} = \frac{1.375}{\text{fraction of total xylan}_{\text{init.}}}$$

$$\text{(3)} \qquad \theta_{LA} = \frac{1.396}{\text{fraction of total hexosan}^{a}{}_{\text{init.}}}$$

$$\text{(4)} \qquad \theta_{HMF} = \frac{1.286}{\text{fraction of total hexosan}^{a}{}_{\text{init.}}}$$

[a] Total hexosan = glucan + mannan.

Analysis of the lignin product was performed using an Agilent Cary 630 FT-IR with ZnSe ATR solids analyser (32 scans at 2 cm^{-1} wavenumber resolution). FT-IR spectra were overlaid after baseline correction and absorbances were scaled proportionately by Resolutions Pro software (Agilent) to allow for comparison of relative peak heights. SEM images were taken on a Hitachi TM-1000 Tabletop Scanning Electron Microscope and were generated at the Microscopy Core/Center for Plant Cell Biology at the Institute for Integrative Genome Biology at UC Riverside.

Enzymatic hydrolysis of maple wood and Avicel® cellulose (PH-101, Sigma Aldrich) was performed in 50 mM (pH 4.9) citrate buffer with 3 wt% NaN_3 using

Accellerase® 1500 cellulase cocktail (BCA protein– 82 mg ml^{-1}, DuPont Industrial Biosciences, Palo Alto, CA). Samples were prepared based on 1 wt% glucan loading and 15 mg protein per g glucan in 125 ml shake flasks and were incubated at 50 °C and 150 rpm for 72 h.

4.6 Supplementary information

Tetrahydrofuran (THF) is a versatile chemical that has commercial application as a solvent for the manufacture of plastics and is closely related to 1,4-butanediol (BDO) and γ-butyrolactone (GBL) (Corma et al., 2007). Since THF polymers (PolyTHF and PTMEG) are also major commercial products, further investigation into the application of these products will be of great interest, particularly for the potential production of THF from furfural in this co-solvent strategy. THF owes its excellent solvent properties to the polar oxygen in its ring and dispersive character from the four methylene groups. In a binary mixture with water, the dispersive character of THF increases with increasing THF content in the aqueous phase. THF is also an excellent extracting solvent for furfural and 5-hydroxymethylfurfural (5-HMF), and its affinity for these aldehydes may play a key role in its ability to improve their yields in our single-phase system. THF can also be used to solubilize extracted lignin products from organosolv or ionic-liquid pretreatment strategies or directly used to fractionate raw biomass as with our single-phase process.

Furfural is typically synthesized by acid-catalyzed dehydration of C5 sugars (arabinose and xylose) whereas levulinic acid (LA) can be derived from either C5 (Gürbüz, 2012) or C6 sugars (Dautzenberg et al., 2011) (glucose, galactose, and mannose). Water-soluble Brønsted acids such as HCl and H_2SO_4 have typically been used to catalyze such dehydration reactions (Zeitsch, 2000), but homogeneous and heterogeneous solid acid catalysts have also been applied successfully. Furfural is targeted in this paper as one of the only natural precursors to furan-based chemicals. However, its low commercial yields (~50% of theoretical) from xylan-rich lignocellulosic residues (Zeitsch, 2000) hinder its economic potential, and new approaches are needed to make it economically viable as a fuel precursor. 5-HMF is an important platform chemical that can be produced from the acid-catalyzed dehydration of hexoses. 5-HMF will readily hydrolyze to form equimolar amounts of LA and formic acid (FA) (Dautzenberg et al., 2011) in the presence of a strong acid. Since LA is more stable than HMF in an aqueous environment (Dautzenberg et al., 2011), higher final yields of LA were observed in single phase reactions with sulfuric acid.

Room temperature liquid densities after the 40 min reactions (0.989 kg L^{-1} with 1:1 THF and 1.015 kg L^{-1} without THF at 21 °C) accounted for a 3% difference in the maximum furfural yield. For both solvent and non-solvent reactions in Table 4.1, the highest furfural yield was achieved much sooner (40 min) than the highest LA yield (120 min). Due to the more acid labile amorphous structure of hemicellulose, the rate of hydrolysis of xylan from hemicellulose was much greater than that of glucan from crystalline cellulose (Saeman, 1945). By increasing the ratio of THF to 3:1 (density: 0.935 kg L^{-1}), co-production yields of furfural, 5-HMF, and LA improved with increased solubilisation of biomass without significant furfural losses.

Figure 4.5(A) shows bottles of maple wood and acid solution with and without addition of THF co-solvent. Aside from the additional pigmentation in the solvent mixture, the two appear nearly identical. These mixtures were subjected to batch reactions in order to obtain fuel precursor yields reported in the main article. As shown in Figure 4.5(B), removal of THF from the post-reaction liquid by room temperature vacuum distillation allowed lignin to precipitate as a solid residue. Further washing of the solid residue with diethyl ether produced a brown powdered lignin product largely free of sugars, proteins, and ash, as shown in Figure 4.5(C).

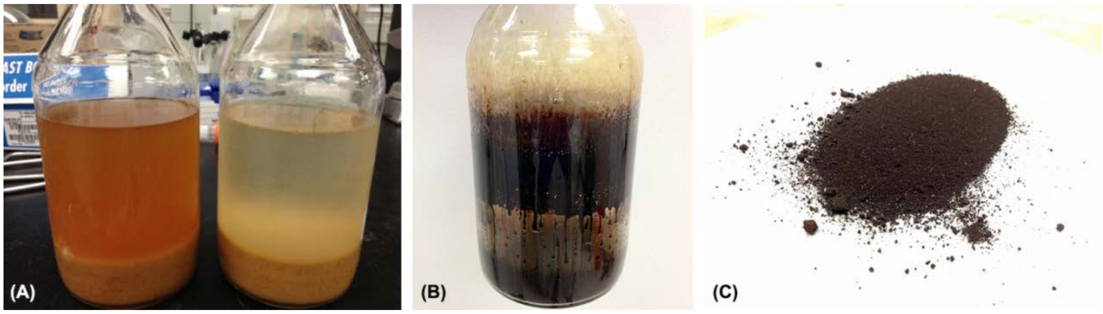


Figure 4.5. (A) Image showing the 1:1 THF co-solvent solution (left) next to water solution without solvent added (right) before reaction. (B) Precipitated lignin and degradation tars accumulated on the glass bottle after recovery of THF and removal of remaining liquids. (C) Isolated lignin powder from co-solvent reaction.

Figure 4.6 shows the FT-IR absorbance spectra in the fingerprint region of lignin product extracted by THF overlaid against that of the K-lignin residue from concentrated sulphuric acid extraction of maple wood. Baseline correction and scaling were applied by Resolutions Pro (Agilent Technologies) to better compare the relative peak heights. Differences in the relative peaks shown indicate potential changes in the lignin chemistry by the two extraction methods. The stronger absorbance seen at 1710 cm^{-1} represents carbonyl stretching of unconjugated β-ketones and conjugated acid/esters and indicates a greater oxidation of the lignin structure extracted by THF. The triple peak in the 1400 to 1500 cm^{-1} range of these samples matched closely and is characteristic of the phenolic hydroxyl groups, C-H deformation, and aromatic skeletal vibrations in hardwood lignin. Peaks between 1100 cm^{-1} and 1330 cm^{-1} represent the C-H and C-O bonds of the guaiacyl and syringyl rings. Peaks present in the 1000 cm^{-1} range represent C-O bonds in the secondary alcohols or potential carbohydrate carry-over residues.

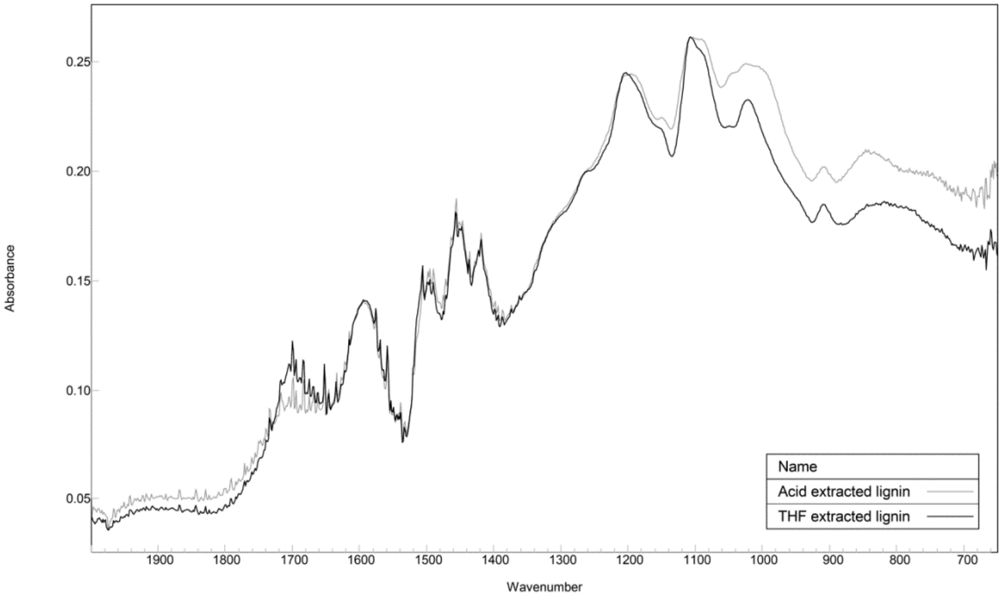


Figure 4.6. FT-IR absorbance spectra of isolated lignin product from THF co-solvent reaction and K-lignin residue from concentrated sulphuric acid extraction.

Figure 4.7(A-D) compares the concentration profiles of the fuel precursors produced from maple wood with and without THF. As shown, the higher initial concentration of glucose with THF suggested that THF enhanced the hydrolysis of cellulose to glucose compared to the non-solvent case (as confirmed by Fig. 4.1 in the paper). Disappearance of glucose and production of LA were also enhanced by the solvent (Fig. 4.7(A) and Fig. 4.7(C) respectively). As for xylose, the HPLC column (Aminex HPX-87H) we used first could not differentiate retention times for xylose, mannose, and galactose monomers present in maple wood, resulting in a lumped concentration profile. Due to the much slower decomposition rate of mannose vs. xylose (Saeman, 1945), we were unable to determine the exact concentration profile of xylose. Instead, we measured xylose concentrations from neutralized reaction samples separately (Aminex HPX-87P) and found that xylose was completely consumed by 40 min with THF and by 60 min for no THF. These results indicated that THF had a catalytic effect on destruction of both glucose and xylose. A more detailed kinetic study is in progress to quantify the improvements in reaction rates.

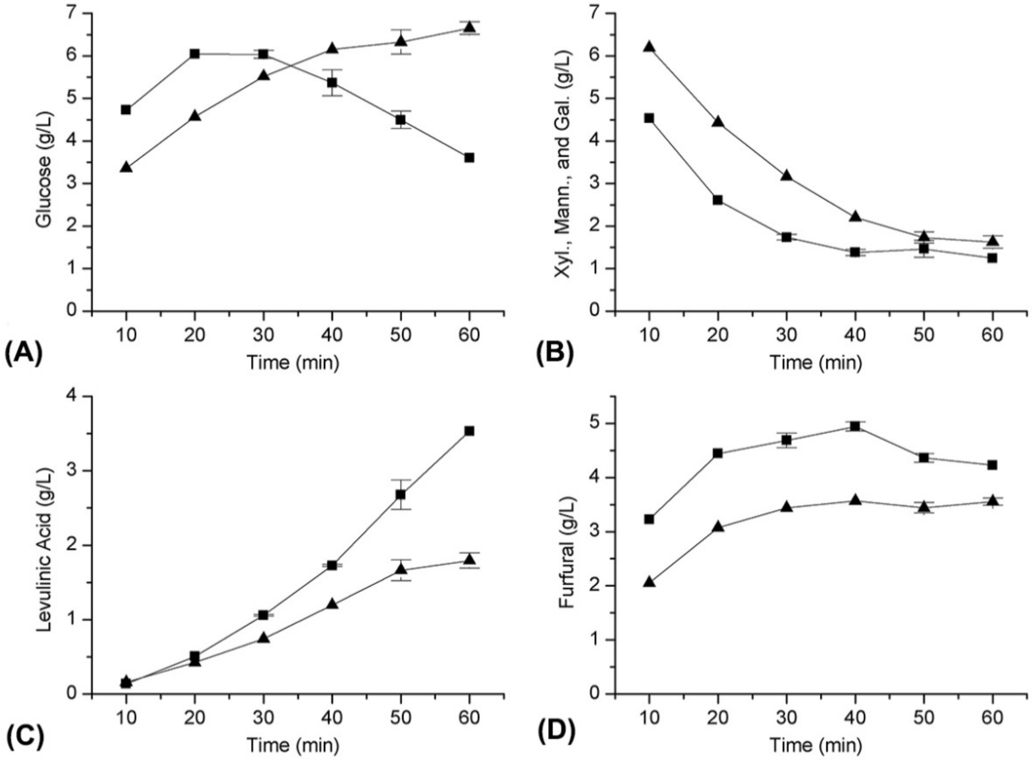


Figure 4.7. Concentration profiles of (A) glucose, (B) xylose + mannose + galactose, (C) LA, and (D) furfural over 60 min of reaction time (■ with THF, ▲ without THF). THF dissolved over 90% (by wt.) of the lignin and degradation tars that otherwise accumulated in the non-solvent case. Reaction conditions: 5 wt% maple wood and 1 wt% H_2SO_4 in batch reactions at 170 °C. The THF co-solvent solution contained a 1:1 ratio of THF and water.

Figure 4.8 shows a material balance of a 1:1 THF co-solvent reaction with maple wood at conditions that were found to be optimal for furfural production (40 min at 170 °C), the least stable fuel precursor. Product masses of both solid and liquid streams were independently quantified for each compound and then combined to calculate the total percent of product recovered from each major lignocellulose fraction (87% for C5, 92% for C6, and 91% for lignin).

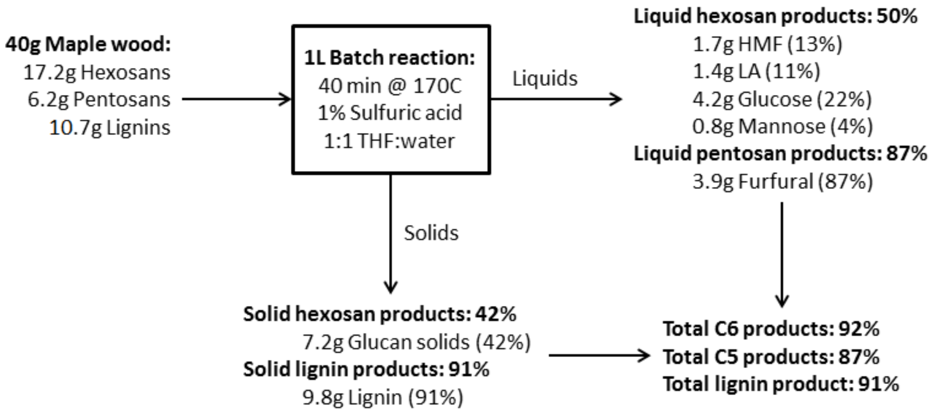


Figure 4.8. Material balance of total products recovered from the hexosan, pentosan, and lignin fractions of raw maple wood after THF co-solvent reaction with yields as percent of the maximum theoretical for the initial biomass composition in parenthesis. Reaction conditions: 5 wt% maple wood and 1 wt% H_2SO_4 in batch reactions at 170 °C. The THF co-solvent solution contained a 1:1 ratio of THF and DI water.

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Chapter 5. Coupling metal halides with a co-solvent to produce furfural and 5-HMF at high yields directly from lignocellulosic biomass as an integrated biofuels strategy*

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5.1 Abstract

Metal halides are selective catalysts suitable for production of the fuel precursors furfural and 5-HMF from sugars derived from lignocellulosic biomass. However, they do not perform nearly as well when applied to biomass even in combination with immiscible extracting solvents or expensive ionic co-solvents. Here, we couple metal halides with a highly tunable co-solvent system employing renewable tetrahydrofuran (THF) to significantly enhance co-production of furfural and 5-HMF from biomass in a single phase reaction strategy capable of integrating biomass deconstruction with catalytic dehydration of sugars. Screening of several promising metal halide species at 170 °C in pH-controlled reactions with sugar solutions and larger 1 L reactions with maple wood and corn stover revealed how the interplay between relative Brønsted and Lewis acidities was responsible for enhancing catalytic performance in THF co-solvent. Combining $FeCl_3$ with THF co-solvent was particularly effective, achieving one of the highest reported simultaneous yields of furfural (95%) and 5-HMF (51%) directly from biomass with minimal levulinic acid formation (6%). Furthermore, over 90% of the lignin from biomass was extracted by THF and recovered as a fine lignin powder. Tuning the volume ratio of THF to water from 4:1 to 1:1 preserved 10% to 31% of the reacted biomass as a glucan-rich solid suitable for further catalytic reaction, enzymatic digestion, or possible pulp and paper production.

5.2 Introduction

Lignocellulosic biomass in such forms as agricultural and forestry residues and herbaceous and woody energy crops is the only sufficiently prevalent sustainable resource for impactful conversion into renewable liquid transportation fuels (Lynd et al., 1991; Wyman and Hinman, 1990). Furthermore, because lignocellulosic biomass sold at \$60/ton is theoretically equivalent in energy cost to oil at about \$20/barrel, it provides the most promising near-term option for achieving low enough costs to alleviate our dependence on fossil resources (Lynd et al., 1999). The conversion of cellulosics into compatible transportation fuels has enormous benefits for addressing global climate change, energy security, rural economic growth and employment, trade deficits, and global competitiveness issues (Lugar and Woolsey, 1999; Lynd et al., 1991). What we term as fuel precursors (FPs) must generally be derived as intermediate platform compounds (Bozell and Petersen, 2010) from lignocellulosic biomass before conversion to “drop-in” liquid transportation fuels and other fuel products is possible. However, the major challenge to realizing this pathway has been to produce primary fuel precursors, such as monomeric sugars, and secondary fuel precursors, such as furfural, 5-hydroxymethylfurfural (5-HMF) and levulinic acid (LA), directly from biomass at the high yields (>80% recovery of C5 and C6 products) essential to low unit costs (Bond et al., 2014) (<\$5 gal-1) without complicated processes, expensive catalysts, and/or high energy demand.

Figure 5.1 outlines a reaction network for the production of ethanol and promising gasoline, jet, and diesel range fuel products from primary and secondary fuel precursors.

As shown, xylose from hemicellulose and glucose from cellulose can be fermented to ethanol or dehydrated with acid catalysts to produce the secondary FPs furfural and 5-HMF. Further 5-HMF hydrolysis results in equimolar formation of more stable products LA and formic acid (not shown). LA can also be synthesized from furfural by a furfuryl alcohol intermediate. These secondary fuel precursors can be catalytically upgraded into potential fuel products by selective hydrogenation over metal-based solid catalysts (Huber et al., 2005). As shown, catalytic hydrogenation of furfural and 5-HMF produces promising gasoline blending products 2-methylfuran (MF, 131 Research Octane Number RON) (Lange et al., 2012) and 2,5-dimethylfuran (DMF, 119 RON) (Roman-Leshkov et al., 2007), respectively. 2-methyltetrahydrofuran (MTHF, 86 RON) (Bozell et al., 2000) can be produced from hydrogenation of LA or MF and ethanol can be produced from sugars by yeast and/or bacteria fermentation (Shaw et al., 2008), both of which are primary components in P-series biofuels. Ethanolysis of LA produces ethyl levulinate (Lange et al., 2009), a diesel blendstock, whereas aldol-addition using acetone and hydrodeoxygenation of secondary fuel precursors with hydrogen can produce longer-chained hydrocarbon fuels of up to 16 carbon lengths for jet and diesel applications (Huber et al., 2005; Xing et al., 2010).

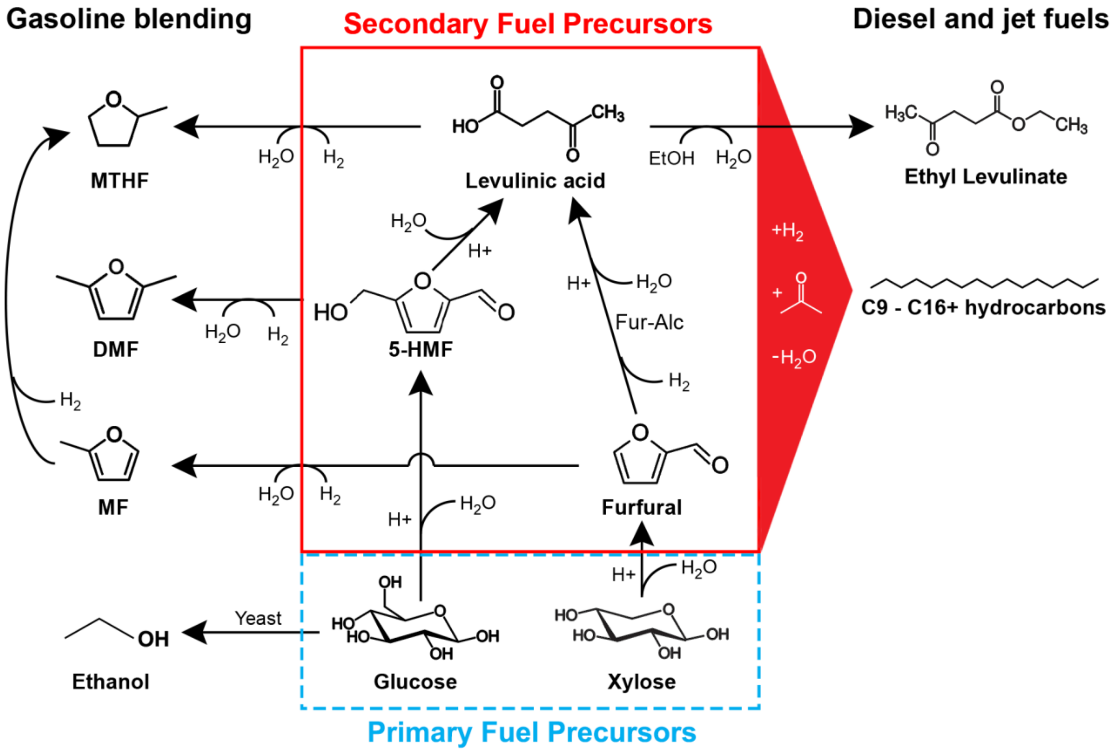

Figure 5.1. Reaction network illustrating primary and secondary fuel precursors for production of ethanol and gasoline, jet, and diesel range aromatic and hydrocarbon fuels. 5-HMF: 5- hydroxymethylfurfural; MF: 2-methylfuran; DMF: 2,5-dimethylfuran; MTHF: 2-methyltetrahydrofuran; Fur-Alc: furfuryl alcohol.

Ongoing advances in catalysis have improved the selective conversion of secondary fuel precursors to so-called drop-in fuel products that are compatible with the existing fuel infrastructure (Olcay et al., 2013; Dutta et al., 2012; Barrett et al., 2006; Huber and Dumesic, 2006), but obtaining high overall fuel precursor yields directly from lignocellulosic biomass has been a long-standing barrier to achieving reasonable product costs of < \$5 gal^{-1} (Cai et al., 2014; Wyman, 1990). Thus, there is a pressing need to develop effective strategies that integrate catalytic conversion with biomass deconstruction to co-produce FPs from both C5 and C6 sugars in order for biomass drop-in fuels to have impact (Cai et al., 2014; Wyman, 1990). Achieving high overall product yields from the major biomass fractions hemicellulose, cellulose, and lignin in an integrated process has the highest potential to enable future biomass-to-fuel technologies (Cai et al., 2014). Various acid catalyzed co-production schemes from biomass have been proposed including co-producing furfural with LA, furfural with 5-HMF, furfural with cellulose, and LA from both furfural and 5-HMF, but many suffer from low yields due to the complex heterogeneous nature of biomass (Lange et al., 2012). For example, furfural and 5-HMF produced early in biomass deconstruction are rapidly degraded before sufficient LA yields from C6 sugars can be achieved. Consequently, LA production and recovery would have to follow furfural removal, thereby necessitating multi-stage reactions with independent product recovery steps, expensive steam stripping to remove furfural, use of corrosive mineral acids, and/or biphasic reactions. Alternatively, co-production of furfural and 5-HMF would appear more desirable as both products could be recovered together by a suitable extracting solvent and simultaneously converted into "drop-in" fuels such as MF and DMF by a single catalyst (Nishimura et al., 2013).

Recently, we demonstrated that tetrahydrofuran (THF) is an exceptionally effective single phase co-solvent for integrated biomass reactions that enhance fuel precursor yields during biomass deconstruction, as well as delignification (Cai et al., 2013). Using just dilute sulfuric acid in a miscible solution of THF and water, we achieved higher overall yields of furfural, 5-HMF, and LA from maple wood than previously reported in a single phase reaction (Cai et al., 2013). However, because sulfuric acid favored furfural and LA production, it became apparent that tuning of this co-solvent system with different catalysts could improve yields for co-production of furfural and 5-HMF. Because aqueous monophasic reactions with dilute mineral acids typically suffer from low 5-HMF yields (< 5%) (Lange et al., 2012; Cai et al., 2013) as it readily hydrolyzes to form LA and formic acid, methyl isobutyl ketone (MIBK) was employed as an extracting solvent in a biphasic reaction (Zhang et al., 2013), but solvent recovery was an issue and the high energy requirements for heating and stirring and limited effective solids loading of a biphasic reaction for large scale fuel production from solid biomass hinder its commercial appeal (Cai et al., 2013). Thus, a single phase reaction is beneficial if a more selective acid catalyst could be used to improve selectivity of biomass glucan to 5-HMF instead of LA.

Metal halides are inexpensive acid catalysts that are well studied for selectively promoting alternate reaction mechanisms of xylose to furfural and glucose to 5-HMF compared to traditional mineral acids (Dutta et al., 2012; Marcotullio and De Jong, 2010; Danon et al., 2014). In analogous pathways, aldose-to-ketose isomerization of glucose to fructose and xylose to xylulose was observed in the presence of certain bi- and trivalent metal cations that can more easily undergo acid-catalyzed dehydration (Danon et al. 2014). Metal cations such as Al^{3+}, Fe^{3+}, and Cr^{3+} also form oxides with water molecules that increase the acidity of the solution as a Brønsted acid (Choudhary et al., 2013). When bound to halide anions such as I^-, Br^-, and Cl^-, the Lewis acid/base pair can further catalyze production of furfural and 5-HMF from aldose sugars through consecutive dehydration reactions that first proceed by enolization to a 1,2-enediol intermediate (Marcotullio and De Jong, 2010). However, evidence also suggests that the strong Lewis acid character of metal halides accelerates several competing loss reactions that could potentially decrease product yields. When used in biphasic (Yang et al., 2012) and expensive ionic-liquid (IL) co-solvent (Siankevich et al., 2014) systems, metal halides demonstrated good performance with sugar solutions but poor performance (19% to 26% 5-HMF, 51% to 66% furfural for biphasic, < 30% for IL) on cellulose and biomass (Saha and Abu-Omar, 2014), necessitating additional biomass pretreatment.

In this paper, we demonstrate that metal halide acid catalysts in combination with THF as a novel miscible co-solvent can significantly improve yields for co-production of both furfural and 5-HMF from lignocellulosic biomass such as maple wood and corn stover from that possible before. In this way, biomass pretreatment and catalytic dehydration of soluble sugars can be performed in a one-pot reaction. We first screened five promising metal salt acid catalysts $AlCl_3$, $CuCl_2$, $CrCl_3$, $FeCl_3$, and $ZrOCl_2$ for sugar conversion and selectivity for furfural, 5-HMF, and LA production by applying our co-solvent system to sugar solutions. We then optimized reaction severity and solvent loadings in 1 L scale biomass reactions with maple wood and corn stover catalyzed by these metal halides to achieve the highest furfural and 5-HMF yields reported from these feedstocks by a single phase reaction strategy. The results reveal how different Brønsted and Lewis activities of metal halide acid catalysts can play a key role in harmonizing the dehydration kinetics of both C5 and C6 sugars and degradation reactions of the final products to maximize overall yields of furanic products for a biorefinery process.

5.3 Results and discussion

5.3.1 Assessment of catalyst performance in sugar reactions

Little is currently known about the application of THF as a water-miscible co-solvent to enhance the production of FPs from biomass sugars. Although strong mineral acids such as sulfuric acid have been used successfully to improve FP yields with THF co-solvent (Cai et al., 2013), metal halide acid catalysts are promising alternatives that are less corrosive, recyclable, and more selective than strong mineral acids (Zhao et al., 2007). Table 1 shows the measured pH for the sugar co-solvent solutions containing 0.1M of each metal halide before titration to 1.6 pH. It is known that metal oxide species form when the metal halides are hydrolyzed by water at elevated temperatures and the formation of OH ligands (as electron pair donors) during hydrolysis of the metal cations increases their acidity (Holovko, 1997). The resulting pH of the solution is related to the first hydrolysis constant of the cationic species (Holovko, 1997), where we found Zr and Fe cations to be the strongest. Metal chlorides are also known to form stable adducts with THF which can influence their ionizability and catalytic activity. As Brønsted acidity typically dominates sugar dehydration kinetics, we elected to normalize the pH of all the sugar solutions to 1.6 using sulfuric acid (close to that of the most acidic metal halide) prior to each reaction. Doing so allowed us to better understand how the relative Lewis strength of each catalyst influenced their selectivity to secondary fuel precursors, the propensity for degradation of final products, and the tunability of the catalysts for optimizing co-production of furfural and 5-HMF from biomass.

Table 5.1. pH of metal halide catalysts in co-solvent solution containing 1:1 THF:water[a]

Catalyst	pH
$CrCl_3 \cdot 6H_2O$	3.13 ± .01
$AlCl_3 \cdot 6H_2O$	2.88 ± .02
$CuCl_2 \cdot 2H_2O$	2.78 ± .01
$FeCl_3 \cdot 6H_2O$	1.90 ± .01
$ZrOCl_2 \cdot 8H_2O$	1.65 ± .01

[a] 0.1M catalyst loading based on each catalyst's anhydrous mass.

In order to characterize catalyst performance with this co-solvent system, we reacted pure glucose and xylose in 1:1 (v:v) THF:water solutions using different metal halides to compare sugar conversion and selectivity toward furfural, 5-HMF, and LA. Metal halides $AlCl_3 \cdot 6H_2O$, $CuCl_2 \cdot 2H_2O$, $CrCl_3 \cdot 6H_2O$, $FeCl_3 \cdot 6H_2O$, and $ZrOCl_2 \cdot 8H_2O$ were selected for this comparison as they have demonstrated high selectivity towards furfural and 5-HMF in previous literature reports (Dutta et al., 2012; Danon et al., 2014; Zhao et al., 2007; Binder et al., 2010; Mao et al., 2012). The sugar co-solvent solutions contained either 20 g L^{-1} glucose or 10 g L^{-1} xylose to simulate likely sugar concentrations from real biomass reactions at 5 wt% solids loading. Each metal halide was added based on their anhydrous catalyst mass to a concentration of 0.1M for each reaction. The sugar solutions were then loaded into Hastelloy tube reactors (10 mL working volume) and heated to 170 °C by a custom designed stainless steel steam chamber. The reaction proceeded until the steam was turned off and the chamber was flooded with cold tap water to quench the reaction. As shown in Figure 5.2A and 5.2B, the conversion of both xylose and glucose was significantly improved by all metal acid catalysts beyond what was possible for sulfuric acid alone in the THF co-solvent system.

The relative performances of the metal halides were also very consistent for glucose and xylose. The most active metals were Cr, Zr, and Al due to their high Lewis acid strength, achieving nearly complete conversion of xylose by 5 min and glucose by 10 min. Cu$^-$ and Fe$^-$ based catalysts were notably slower in sugar conversion, but still achieved near complete conversion after 20 min.

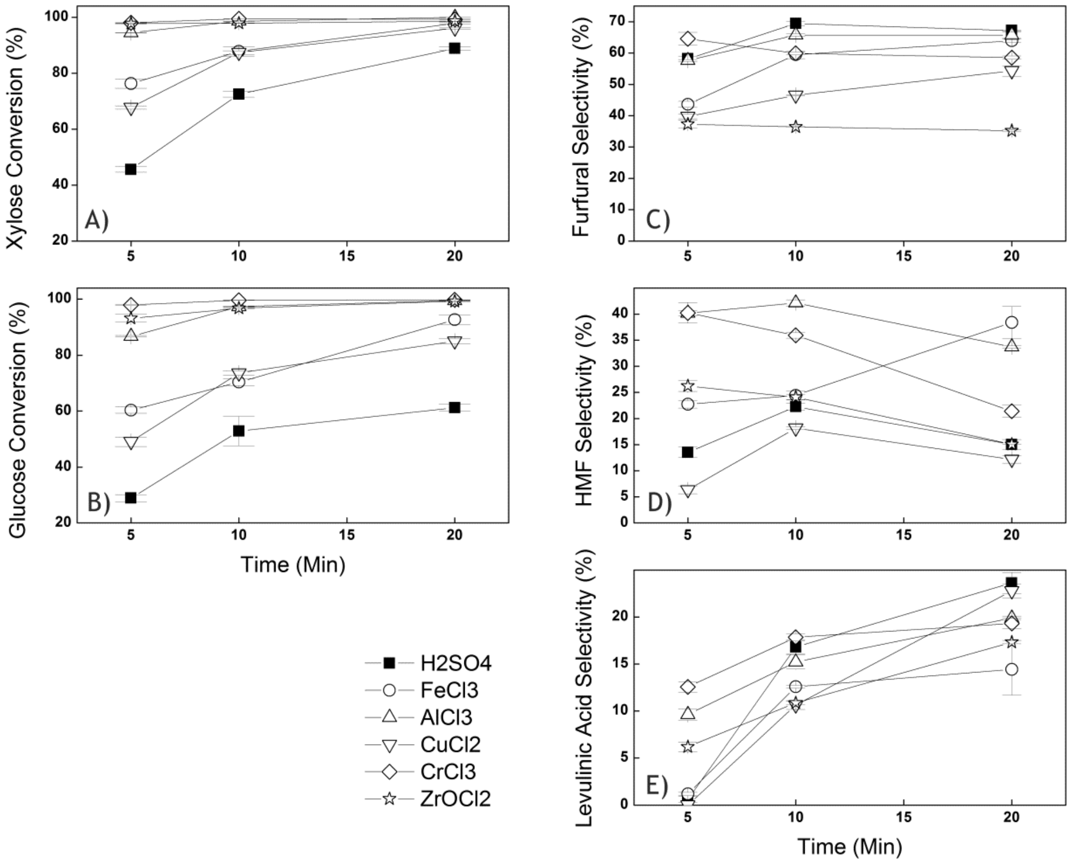

Figure 5.2. Conversions and selectivities for pure sugar reactions with metal halide acid catalysts in THF co-solvent mixture plotted against reaction time. A) xylose and B) glucose conversions and C) furfural selectivity from xylose and D) 5-HMF and E) LA selectivity from glucose. Reaction conditions: 170 °C, 20 g L^{-1} glucose or 10 g L^{-1} xylose, 1:1 THF: water ratio, 0.1M catalyst loading, and normalization of all solutions to pH 1.6 using 72% sulfuric acid. Black squares represent sulfuric acid control also titrated to 1.6 pH. Error bars represent one standard deviation.

In all of the THF co-solvent sugar reactions, we observed accumulation of secondary sugar species whose retention times on the HPLC matched that of fructose and xylulose. Their concentrations also tracked that of glucose and xylose disappearance over the course of the reaction suggesting that aldose-to-ketose isomerization occurred at a faster rate than sugar dehydration (Concentrations shown in Supplementary Information Section 5.7 Figure 5.7 and Figure 5.8). Although it is known that metal halides catalyze an open-chain reaction mechanism that produces ketose sugars with a lower energy barrier for dehydration (Danon et al., 2014), we also found ketose sugars in the sulfuric acid reactions suggesting that THF co-solvent promotes a similar mechanism involving sugar isomerization. This observation supports our earlier findings that THF appeared to co-catalyze C5 and C6 sugar dehydration (Cai et al., 2013) by promoting the more kinetically favorable open-chain dehydration pathway (Danon et al., 2014).

Selectivity to the secondary FPs was then calculated based on their concentrations after each reaction. In terms of furfural selectivity from xylose (Fig. 5.2C), sulfuric acid achieved a maximum of about 70% selectivity at 10 min that bested all other metal salt catalysts. This result suggested that although the Lewis acid character of the metal halides accelerated destruction of sugars, it also promoted competing loss reactions that diminished furfural accumulation in solution attributed to condensation reactions between intermediate sugar species and furanic products to form humins (Danon et al., 2014). Due to rapid sugar conversion, the Cr catalyst achieved the highest furfural selectivity of about 65% earliest at 5 min, whereas $FeCl_3$ required 20 min to reach a maximum furfural selectivity of also about 65%. $ZrOCl_2$ had the lowest selectivity to furfural despite its ability to rapidly degrade xylose, likely due to the high formation of loss products. In the case of 5-HMF selectivity from glucose (Fig. 5.2D), all metal salts except $CuCl_2$ achieved higher selectivities (~40%) than sulfuric acid (~22%) in the co-solvent system, with Al, Cr, and Fe metals performing best. Again, the Fe^- based catalyst required the longest reaction time and continually increased 5-HMF selectivity over the entire time, with it reaching 38% after 20 min. Interestingly, for all catalysts except $CuCl_2$, the maximum selectivity for both furfural and 5-HMF occurred at approximately the same time with the best co-production of the furfurals demonstrated by Al, Cr, and Fe metals. Overall, however, 5-HMF selectivity was significantly lower than furfural selectivity owing to significant loss reactions to both condensation products and LA (Weingarten et al., 2010). This was apparent by the more drastic drop in 5-HMF selectivity than observed with furfural over the course of the reaction for all the metal halides except $FeCl_3$.

In the case of LA selectivity from glucose (Fig. 5.2E), all metal halides demonstrated lower selectivity to LA formation than sulfuric acid, in line with the goal of this study. As LA is produced from the hydrolysis of 5-HMF in this system, $CuCl_2$ and sulfuric acid achieved the highest LA selectivity as their selectivity towards 5-HMF was lowest. By extrapolation of the increasing trend of LA selectivity over longer reaction times, we believe furfural and LA are not compatible co-products on a basis of their formation kinetics. Instead, furfural and 5-HMF can be produced together, whereas LA production would be most effectively targeted in a reaction independent of furfural. The more rapid sugar conversions observed with Cr^-, Zr^-, and Al^- based halides compared to the slower

Fe^- and Cu^- based halides are important differences among these catalysts that can help explain their performance in reacting actual biomass reported in the next section. Quantifiable parameters in the sugar reactions such as sugar conversion, fuel precursor selectivity, and acidity of these metal halide catalysts will impact biomass conversion to achieve high combined furfural and 5-HMF yields.

5.4.2 Co-production of furfural and 5-HMF from maple wood and corn stover

The primary fractions of lignocellulosic biomass of interest for catalytic conversion to platform chemicals are cellulose, hemicellulose, and lignin. Xylan contained within amorphous hemicellulose presents the most readily available source of sugars as it can be completely hydrolyzed at mild to moderate severity reaction (Saeman, 1945). Cellulose, on the other hand, is composed of crystalline polymeric glucose chains that are a primary source of C6 but remains the most recalcitrant sugar fraction to acid hydrolysis and is usually treated with cellulase enzymes after pretreatment to achieve high yields of glucose monomers in solution (Kumar and Wyman, 2013). Because the hemicellulose fraction of lignocellulosic biomass is far more acid-labile than crystalline cellulose, furfural is produced much sooner than 5-HMF and LA (Lange et al., 2012). Thus, an integrated conversion strategy to co-produce furfural and 5-HMF directly from biomass must be tunable to minimize competing side reactions of the least stable products to maximize product yields. For this reason, optimization for high furfural yields is a primary concern as the glucan remaining in the slower solubilizing cellulose fraction can be recovered as a solid product for further biological conversion into glucose or thermochemical conversion into glucose, 5-HMF, and/or LA if not all of it was converted into 5-HMF or LA.

In addition to manipulation of the temperature, time, and acid loadings (combined reaction severity) to optimize yields from a biomass reaction, the THF co-solvent strategy allowed additional tuning by increasing THF concentrations in water to achieve greater selectivity to 5-HMF and increased biomass solubilization (Cai et al., 2013). In this study, we compared the performance of the metal halide catalysts against sulfuric acid in 1 L THF co-solvent reactions with 5 wt% loading of maple wood or corn stover. Table 5.2 lists the secondary FP yields achieved at the reaction conditions found to maximize production of both furfural and 5-HMF for each catalyst. The THF:water ratio was also varied from 1:1 to 7:1 (by volume) to determine the extent of improved product yields and the limit of the single phase regime. The catalysts were loaded on a mass basis similar to commercial operation at a dilute 1 wt% in terms of the total liquid mass contained within the reaction. The heating temperature profile of the reactor shown in the supplementary information (Figure 5.9) demonstrated that total heating time to a stable 170 °C reaction temperature could be achieved in about 5 min.

As shown in Table 5.2 (Runs 1-9), with the exception of $CrCl_3$ and $ZrOCl_2$, metal halide catalysts demonstrated very consistent performance on both maple wood and corn stover, achieving maximum furfural yields close to that of sulfuric acid for a 1:1 THF:water mixture. 5-HMF yields were more comparable among catalysts, but metal halides produced lower LA yields compared to sulfuric acid owing to their increased selectivity to 5-HMF as found for the sugar reactions. Surprisingly, the high furfural

(65%) and 5-HMF (40%) selectivities achieved by $CrCl_3$ from the model sugar solutions were not translated to better furfural yields (43%) from biomass. The results of the sugar reactions shed some light on the poor performance $CrCl_3$ and $ZrOCl_2$ with biomass. In solution, the lower acidity of $CrCl_3$ (pH 3.13) compared to the other metal halides (Table 5.1) may have limited its ability to hydrolyze sugar polymers effectively, particularly without the help of sulfuric acid. Consequently, the excellent xylose conversion performance of $CrCl_3$ (100% in 5 min, Figure 5.2A) may have negatively impacted furfural yields from biomass as furfural losses quickly exceeded furfural production due to much slower release of xylose from biomass. As seen by the rapid drop in furfural selectivity for xylose reactions with $CrCl_3$ (Figure 5.2C), the potential for high furfural yields from biomass suffered from the longer reaction times needed by the biomass reactions. We also suspect that the strong Lewis acid character of $CrCl_3$ was responsible for significant furfural losses. For $ZrOCl_2$, poor furfural selectivity from xylose (37%) resulted in low furfural yields (44%) despite having the highest Brønsted acidity (pH 1.65) of the group. Conversely, $FeCl_3$ was the best performer due to its higher Brønsted acidity, slower xylose conversion rates, and higher furfural selectivity at longer reaction times. $AlCl_3$ and $CuCl_2$ were middle performers owing to their more moderate Brønsted character, with trade-offs between higher 5-HMF yields or high furfural yields, respectively, consistent with their selectivity with the sugar reactions.

Table 5.2. Acid-catalyzed co-production of furfural, 5-HMF, and LA from maple wood and corn stover in batch reactions with THF co-solvent[a]

						Yields (of theoretical)[f]		
Run #	THF:Water[c]	Substrate[b]	Acid catalyst[d]	Time (min)	Solids remaining (%)	Furfural (%)	5-HMF (%)	LA (%)
1[e]	1:1	Maple wood	H_2SO_4	40	21	87	13	11
2	1:1	Maple wood	$FeCl_3$	40	25	85	16	4.7
3	1:1	Maple wood	$CuCl_2$	30	29	83	14	6.3
4	1:1	Maple wood	$AlCl_3$	40	30	58	18	9.3
5	1:1	Maple wood	$CrCl_3$	40	30	43	15	5.9
6	1:1	Maple wood	$ZrOCl_2$	40	39	44	14	11
7	1:1	Corn stover	H_2SO_4	40	19	84	16	11
8	1:1	Corn stover	$FeCl_3$	40	31	85	12	4.0
9	1:1	Corn stover	$ZrOCl_2$	40	43	38	14	12
10[e]	3:1	Maple wood	H_2SO_4	60	1	86	21	40
11	3:1	Maple wood	$FeCl_3$	60	11	97	41	13
12	3:1	Maple wood	$CuCl_2$	60	16	81	22	21
13	3:1	Maple wood	$AlCl_3$	60	16	75	33	8.8
14	3:1	Corn stover	$FeCl_3$	80	14	97	42	12
15	3:1	Corn stover	$CuCl_2$	60	20	89	22	14
16	3:1	Corn stover	$AlCl_3$	60	22	76	36	17
17	4:1	Maple wood	$FeCl_3$	60	10	**95**	**51**	**6**
18	4:1	Corn stover	$FeCl_3$	80	15	**95**	**45**	**7**
19	7:1	Maple wood	$FeCl_3$	60	21	83	43	3

[a] All reactions were performed using a 1L Parr reactor at 170 °C reaction temperature. [b] 5 wt% total solids loading. [c] By volume ratio. [d] All catalysts were loaded at 1 wt% anhydrous content. [e] Data from these runs are reported previously. [f] Furfural yield calculated from raw xylan content, 5-HMF and LA yield calculated from raw glucan content.

In order to investigate the extent of biomass solubilization for each catalyst, maple wood was reacted for 30 min in an 1:1 THF co-solvent mixture and 5 wt% initial biomass loading and 0.1M equivalent catalyst loading in the 1 L Parr reactor. Figure 5.3 shows the composition of raw maple wood solids and the distribution of the major components in the solids remaining after reaction on the mass basis of 100 g of raw maple wood feed. As shown, biomass solubilization with metal halides was reduced compared to sulfuric acid. Also shown, over 90% of the maple wood K-lignin was removed during all metal halide reactions, except with $ZrOCl_2$, leaving behind a substantial amount of glucan-rich solids that contain no hemicellulose and minute amounts of other components. In the 1:1 THF reactions, maximizing lignin free glucan rich solids recovery from the co-solvent reaction is crucial to enhance the economics of this process as the cleanly fractionated solids are suitable as a direct feed to produce fermentable glucose or used to make additional 5-HMF or LA. In the case of $ZrOCl_2$, reduced delignification and the presence of a large non-sugar fraction (labeled ‘Other’ in Figure 5.3) provide an interesting opportunity for future study to help explain why its performance for producing FPs from real biomass was significantly poorer than by other catalysts. The non-sugar fraction may have resulted from accumulation of polymeric degradation products on the solids as the actual glucan remaining in the solids was much lower than from sulfuric acid catalyzed reactions. Composition of this non-sugar fraction still needs to be determined.

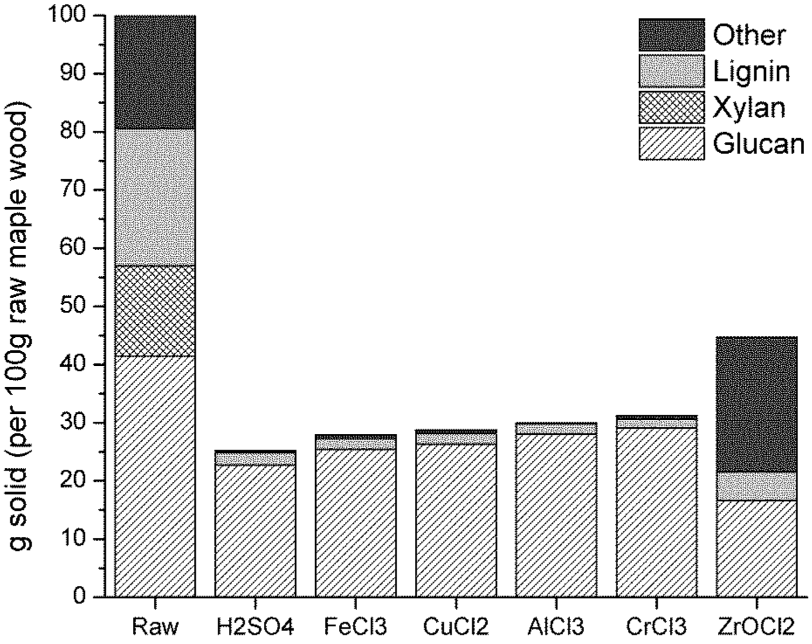

Figure 5.3. Composition of raw maple wood and distribution of major components to the solids remaining after reaction with 1:1 THF co-solvent and various acid catalysts based on 100 g of initial maple wood fed to the systems. Metal halides improved upon sulfuric acid performance by delivering greater amounts of glucan-rich solids for enzymatic conversion to glucose or thermochemical reaction to glucose, 5-HMF, and/or LA. Reaction conditions: 5 wt% maple wood, 0.1M acid catalyst concentration, 1:1 THF:water, 170 °C, 30 min batch reactions. Numerical data and calculated standard deviations are shown in Table 5.3.

Greater biomass solubilization at the higher solvent ratio of 3:1 (as shown in Table 5.2, runs 10-16) can support a reaction strategy that is more focused on furfural and 5-HMF production with less recoverable solids. At a 3:1 THF-to-water volume ratio, the top three performing (Al, Cu, Fe) metal halide catalysts greatly enhanced co-production of furfural and 5-HMF from maple wood and corn stover compared to sulfuric acid. In these reactions, $FeCl_3$ outperformed $CuCl_2$ and $AlCl_3$ in both furfural (97% yield for maple wood and corn stover) and 5-HMF (41% yield for maple wood and 42% for corn stover) production and biomass solubilization (11% solids remaining). $CuCl_2$ was unable to solubilize biomass as quickly as $FeCl_3$, and its lower 5-HMF yields from both maple wood and corn stover reactions could be explained by its lower 5-HMF selectivity (Figure 5.2D) from glucose in the sugar reactions. For $AlCl_3$, tuning the reaction to achieve high furfural and 5-HMF co-production was difficult as the optimal reaction time for furfural was 20 min shorter than for 5-HMF. Thus, higher furfural yields (81%) were achieved with $CuCl_2$ at the expense of greater 5-HMF losses (22% yield) at 60 min and higher 5-HMF yields (36%) were achieved with $AlCl_3$ at the expense of lower furfural yields (76%) at 60 min. Overall, the consistency in performance between corn stover and maple wood in all reactions indicated that the THF co-solvent system may be largely feedstock agnostic and capable of achieving high yields in heterogeneous or mixed feedstock streams, appealing to commercial feasibility.

At a 4:1 solvent ratio (Table 5.2, run 17-18), $FeCl_3$ significantly outperformed sulfuric acid and the other metal halides and achieved the highest reported co-production yields of furfural (95% for both maple wood and corn stover) and 5-HMF (51% for maple wood and 45% for corn stover) from lignocellulosic biomass by a one-pot single phase reaction. The higher solvent ratio was also beneficial to further reducing yields of LA (6 - 7% at 4:1 ratio), the most difficult product to recover due to its high boiling point (245 °C). Thus, $FeCl_3$ proved to be the best metal halide for catalyzing co-production of furfural and 5-HMF in a biomass process using THF as a miscible co-solvent. Its strong acid strength allowed for reasonably fast deconstruction of both maple wood and corn stover, and the close reaction time for optimal furfural and 5-HMF production was beneficial to achieving good yields of both in one pot reaction. As shown in Figure 5.4, the 10% solids remaining after 4:1 co-solvent reaction of maple wood were very rich in glucan and could be recovered for efficient conversion to glucose by enzymes or further thermochemical reaction to 5-HMF and/or LA. A mass balance is shown for this run in the Supplementary Information (Figure 5.10) insuring accountability for 80% of the C6 products and 95% of the C5 products in the soluble and insoluble products after reaction.

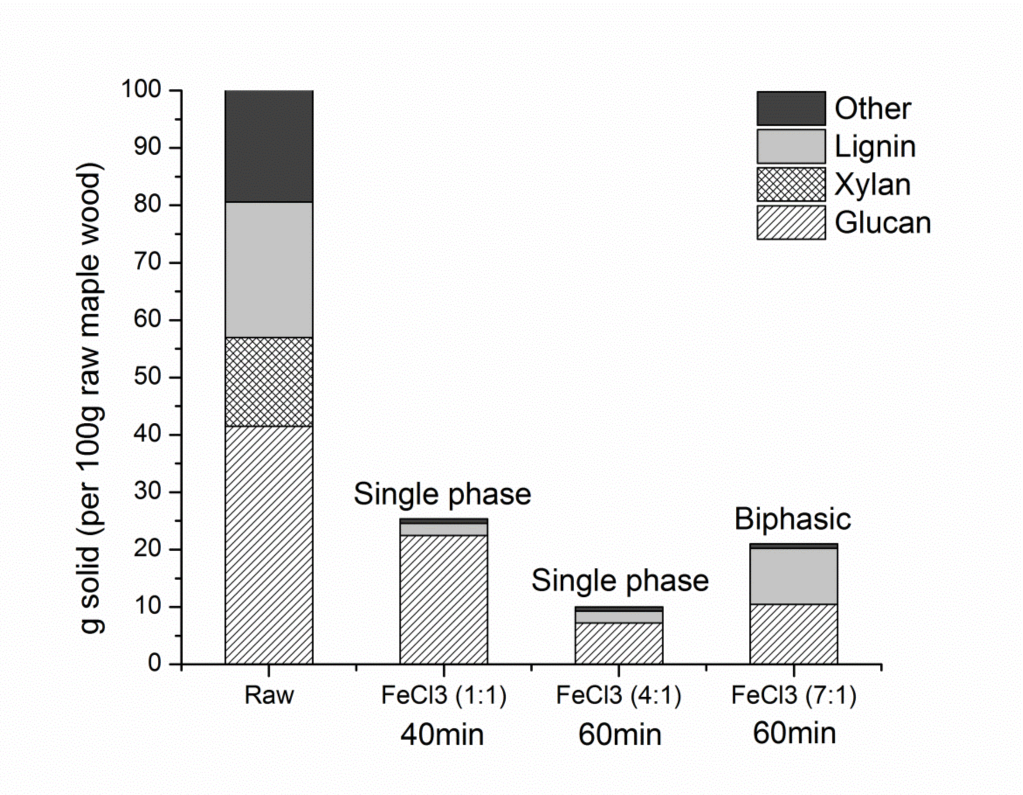

Figure 5.4. Composition of raw maple wood and distribution of major components of the solids remaining after reaction with THF co-solvent with $FeCl_3 \cdot 6H_2O$ at 1:1, 4:1, and 7:1 THF:water volume ratios. Solid mass is based on 100 g of initial maple wood fed to the systems. Suspected phase separation at 7:1 ratio is evident by larger lignin fraction, decreased solids solubilization, and increased remaining glucan fraction after 60 min reaction compared to the 4:1 ratio case. Reaction conditions: 5 wt% maple wood, 1 wt% $FeCl_3 \cdot 6H_2O$ based on anhydrous mass, 170 °C. Numerical data and calculated standard deviations are shown in Table 5.3.

We found that at a 7:1 THF-to-solvent ratio (Table 5.2, run 19) or beyond, the behavior of the reaction suddenly shifted to resemble a two-phase regime. Although we were unable to qualitatively determine this yet (such as by a sight glass in the reactor), the reaction kinetics and composition of the resulting solid material strongly suggested that the system could have become biphasic at such high THF ratios over the course of the reaction. In such a biphasic reaction, the dehydration kinetics of the aqueous phase are largely unaffected by the presence of the extracting solvent (Weingarten et al., 2012). Thus, THF would no longer be able to accelerate biomass solubilization as evident by the much higher solids fraction that remained after reaction (21% for Run 19 vs. 10% for Run 17 in Table 5.2). As shown in Figure 5.4, compositional analysis of this solid fraction also revealed that a larger glucan portion remained unsolubilized and more of the lignin was still intact and not extracted as would be expected at lower concentrations of THF. Reaction pressures increased from 225 psig to 265 psig and was the highest observed and close to the sum of the saturated vapor pressures of water and THF. Nevertheless, high yields of both furfural (83%) and 5-HMF (43%) were achieved with very little LA production (3%), but yields were slightly lower than the 4:1 case. In addition, because biomass often has moisture contents of up to 50% by weight for woods, elevated solvent ratios, such as 7:1 or higher would likely hurt biomass processing economics by requiring larger reactor sizing and consume more heat. The impressive co-production yields achieved by the THF co-solvent system without needing high solvent concentrations (such as >9:1 using GVL) is an important consideration when comparing other recently developed co-solvent systems (Alonso et al., 2013).

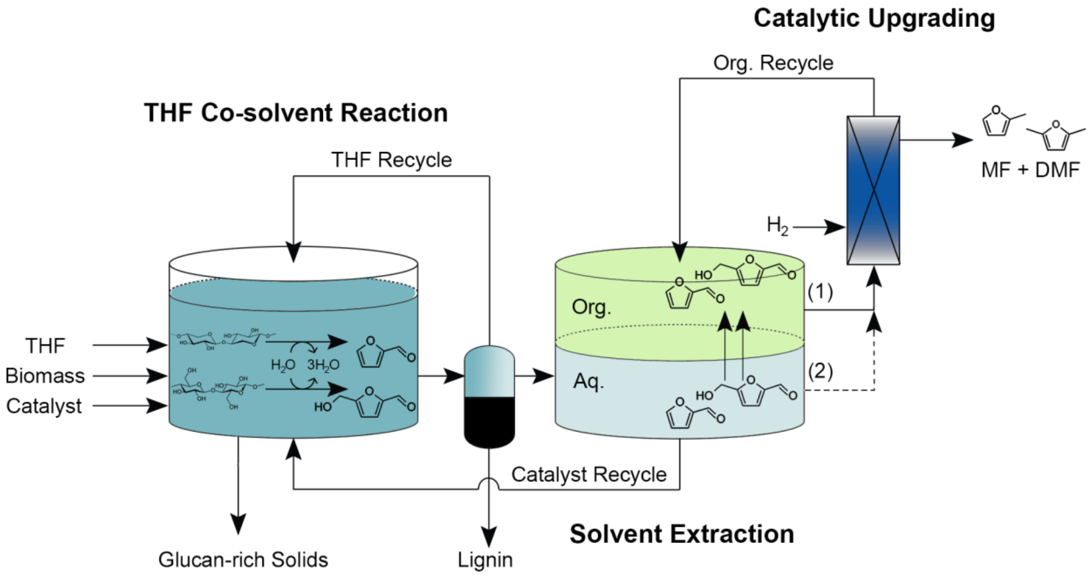

Figure 5.5. Simplified process diagram of the proposed THF co-solvent strategy for direct conversion of lignocellulosic biomass to co-produce furfural and 5-HMF for catalytic upgrading to aromatic fuel products. Furfural and 5-HMF will be extracted by an organic solvent and hydrogenated (blue box, right) to produce aromatic fuels such as MF and DMF. Lignin is precipitated upon recovery of THF. (1) Organic stream containing furfural and 5-HMF (2) Aqueous stream containing metal halide catalyst, furfural and 5-HMF.

Figure 5.5 outlines a proposed integrated THF co-solvent strategy for application of metal halide catalysts to enhance direct conversion of biomass into furfural and 5-HMF followed by two possibilities for their hydrogenation to MF and DMF, respectively. The experimental work in this study was focused on producing high FP yields to be most compatible with leading downstream catalytic upgrading operations. In the process concept pictured in the figure, raw biomass and acid catalyst are loaded into a reactor along with THF co-solvent. Following reaction, high yields of both furfural and 5-HMF are achieved, and the reacted slurry is then collected and filtered to separate the solid residues. As THF is a low boiling solvent (66 °C) and forms a 95.4% azeotrope with water, it could be easily flashed off in a biorefinery to be recovered and recycled (Smallwood, 2002). In fact, we found that room temperature vacuum distillation was sufficient to recover THF from the water phase (Cai et al., 2013).

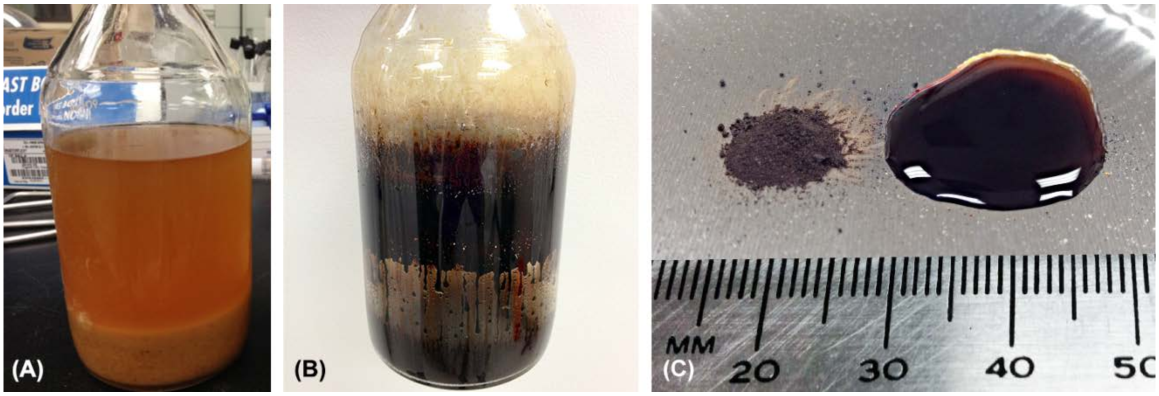

Figure 5.6. (A) 1 L THF co-solvent solution containing 5 wt% maple wood. (B) Precipitated lignin residue after co-solvent reaction, recovery of THF, and water removal. (C) Left, precipitated lignin powder from maple wood after co-solvent reaction with $FeCl_3$ catalyst. Right, same lignin powder shown dissolved in a large droplet of dimethylsulfoxide (DMSO). Metric ruler is shown for reference.

The removal of THF also precipitates extracted biomass lignin as a solid (Figure 5.6B) that can be recovered and rinsed with diethyl ether to produce a very pure lignin powder as shown in Figure 5.6C (left). This powder can in turn be re-dissolved in THF or DMSO (Figure 5.6C, right) and is suitable for catalytic upgrading to valuable chemicals (Henriksson et al., 2010). Afterwards, an appropriate organic solvent (Figure 5.5, Stream (1)), such as MTHF, can be used as an immiscible solvent to extract and concentrate furfural and 5-HMF into the organic layer, leaving most of the trace sugars and contaminants in the aqueous layer. Alternatively, the aqueous stream resulting from THF removal could be fed directly to a catalytic reactor (Figure 5.5, Stream (2)) if desired, depending on the catalyst system chosen for upgrading furfural and 5-HMF. The aqueous stream containing the catalyst could be recycled as $FeCl_3$-containing aqueous streams have been shown to remain effective over several reactions in other systems (Mao et al., 2013). In future studies, we will develop and optimize a high performance catalyst system to achieve high selectivity to final fuel products, such as MF and DMF, from the product stream of the THF co-solvent reaction.

5.4 Conclusions

Metal halides are non-corrosive and highly selective acid catalysts suitable for co-production of furfural and 5-HMF directly from lignocellulosic biomass without a separate pretreatment step. We presented here coupling metal halides with THF as a novel green co-solvent in a highly effective single phase conversion strategy that achieved one of the highest reported co-production yields of furfural and 5-HMF directly from biomass, producing a clean product stream suitable for catalytic hydrogenation to final fuel products. pH-controlled reactions with pure sugar and larger 1 L scale reactions with maple wood and corn stover demonstrated that key differences in the catalytic nature of these metal halides affected conversion of lignocellulosic biomass. Screening of several promising metal halides $AlCl_3 \cdot 6H_2O$, $CuCl_2 \cdot 2H_2O$, $CrCl_3 \cdot 6H_2O$, $FeCl_3 \cdot 6H_2O$, and $ZrOCl_2 \cdot 8H_2O$ on the basis of sugar conversion and selectivity to secondary fuel precursors showed $FeCl_3$ to perform best in the THF co-solvent system owing to its high Brønsted acidity and moderate sugar conversion rate. Surprisingly, $CrCl_3$ did not produce high furfural yields, despite having demonstrated high sugar conversion and selectivity, due to its weaker Brønsted acidity and high Lewis acidity that caused xylose to be rapidly dehydrated and degraded.

At an optimum 4:1 THF:water ratio and 1 wt% $FeCl_3$, the co-solvent reaction achieved 95% yield of furfural and 51% yield of 5-HMF directly from maple wood and similar yields from corn stover after 60 min reaction at 170 °C. Depending on the concentration of THF in the reaction, we could tune biomass solubilization to preserve a glucan-rich solid residue that is suitable for further catalytic reaction, enzymatic digestion, or a potential pulp and paper product. During the co-solvent reaction, THF extracted over 90% of the lignin from biomass that could be recovered as a fine powder. Due to its low boiling point, THF was recovered by room temperature vacuum distillation. Furfural and 5-HMF can be concentrated by an immiscible extracting solvent and the catalyst can be recycled in the aqueous stream. Future study will confirm the recyclability of the catalyst in the aqueous phase and integration with the catalytic conversion of FPs to fuel products.

5.5 Materials and methods

5.5.1 Experimental materials

Reagent-grade THF (> 99% purity, Fisher Scientific, Pittsburgh, PA) was used in all THF co-solvent reactions. The THF co-solvent solution was prepared on a volume basis of increasing the amount of THF additions to realize 1:1 (THF 50% v/v) to 7:1 (THF 87.5% v/v) THF-to-water ratios. Hydrated metal halide catalysts and ≥ 99% pure xylose and glucose sugars were purchased from Sigma Aldrich (St. Louis, MO, US). Due to the high purity of the purchased sugars from Sigma, we use the term pure sugars to designate solutions prepared using these sugars. The hydrate form of each metal halide catalyst ($AlCl_3{\cdot}6H_2O$, $CuCl_2{\cdot}2H_2O$, $CrCl_3{\cdot}6H_2O$, $FeCl_3{\cdot}6H_2O$, and $ZrOCl_2{\cdot}8H_2O$) was used but were loaded based on their equivalent anhydrous mass to achieve 0.1M or 1 wt% catalyst loading. Concentrated sulfuric acid (72 wt% H2SO4) was purchased from Ricca Chemical Company (Arlington, TX) and used to make dilute sulfuric acid solutions. Maple wood chips obtained in upper New York State were provided by Mascoma Corporation (Lebanon, NH), and air-dried Kramer corn stover was provided by the National Renewable Energy Laboratory (NREL, Golden, CO, Lot #33A14). The relatively dry biomass (10-15% moisture) was knife milled to pass through a 1 mm particle size interior sieve using a laboratory mill (Model 4, Arthur H. Thomas Company, Philadelphia, PA). Biomass composition was determined according to the established National Renewable Energy Laboratory procedure (TP-510-42618, ver. 8-03-2012) in triplicates with a resulting composition of 40.9 ± 0.3 wt.% glucan, 15.5 ± 0.2% xylan, 2.1 ± 0.1% mannan, 24.4 ± 0.3% K-lignin, and 17.1% other material for maple wood and

32.7 ± 0.4 wt.% glucan, 20.7 ± 0.2% xylan, 2.6 ± 0.1% arabinan, 16.0 ± 0.1% K-lignin, and 28.0% other material for corn stover. Other materials needed for the biomass composition to total 100% were not characterized in this study but were expected to include minor saccharides, ash, sugar acids, acetate, and protein (Sluiter et al., 2010). Because arabinan, galactan, and mannan were not present in significant quantities and specific quantification of these minor sugars was difficult via HPLC, we elected to treat all quantified biomass pentosans as xylan and all hexosans as glucan.

5.5.2 THF co-solvent sugar reactions

Sugar solutions were prepared in 1:1 THF:water co-solvent mixture containing 20 g L^{-1} glucose or 10 g L^{-1} xylose and 0.1 M (anhydrous) loading of the metal halide catalyst based on total liquid volume. Due to the different acidities of each catalyst, all solutions were normalized to 1.6 pH by titrating with 72 wt% concentrated sulfuric acid. An acidity of 1.6 pH was selected because it was close to the Brønsted acidity of the most acidic 0.1M $ZrOCl_2$-containing mixture (Table 5.1). Pure sugar solutions containing only sulfuric acid were also prepared and titrated to 1.6 pH to directly compare with metal halide acid catalysts as an acid control.

The reactions were carried out in non-stirred 14.3 mL Hastelloy tube reactors (Hastelloy C-276, O.D. of 0.0127 m or 0.5 in.) with a wall thickness of 0.0008890 m (0.035 in.) and length of 0.1524 m (6 in.) to give a working reaction liquid volume of 10 mL. The tube reactors were loaded into a heavy-duty custom steam chamber made of readily available steam rated (to 1 MPa steam pressure) 316 stainless steel 0.102 m (4 in.)

internal diameter fittings (McMaster, Santa Fe Springs, CA). A high-pressure steam boiler (FB-075-L, Fulton Companies, Pulaski, NY) provided steam for rapid and stable heating of triplicate tube reactors. Temperature was monitored by both in-line pressure gauges and two K-type thermoprobes (Omega Engineering Co., Stamford, CT) and controlled by a PID controller via steam pressure. Due to the lengthwise construction of the tube reactors and the application of steam for heating and cold water for quenching, heat transfer was relatively rapid (< 30 sec) even for shorter reaction times (< 10 min) (Trajano et al., 2013). Initial time was defined when a reaction temperature of 170 °C was reached. At the end of the reaction, the steam supply was shut off and the steam chamber was flooded with tap water to stop the reaction.

The liquid content of each reaction tube was transferred into 2 mL glass vials. These samples were centrifuged (2500 rpms for 20 min) and the supernatant was transferred into glass HPLC vials for HPLC analysis by an Agilent 1200 system equipped with a Bio-Rad Aminex® HPX-87H column and RI detector with an eluent (5 mM sulfuric acid) flow rate of 0.6 ml min^{-1}. The calculations for conversion of sugars and selectivity of secondary fuel precursor products are shown below where Ω is the molar equivalence ratio from the starting sugar:

(1) $$\%\text{ Conversion} = [1-\frac{\text{Sugar concentration}_{final}(\text{g/L})}{\text{Sugar concentration}_{initial}(\text{g/L})}]*100\%$$

(2) $$\%\text{ Selectivity} = \text{FP concentration}_{final}(\text{g/L}) * \Omega \,/\, \%\text{ Conversion}$$

(3) $$\Omega_{\text{furfural}} = \frac{1.563}{\text{Concentration of xylose}_{\text{initial}}}$$

(4) $$\Omega_{LA} = \frac{1.552}{\text{Concentration of glucose}_{\text{initial}}}$$

(5) $$\Omega_{5\text{-HMF}} = \frac{1.428}{\text{Concentration of glucose}_{\text{initial}}}$$

5.5.3 THF co-solvent maple wood and corn stover biomass reactions

Corn stover or maple wood solids loadings were calculated based on the total mass of the reaction (800 g) so that each reaction contained 5 wt% solids (40 g dry basis) and 1 wt% acid (7.6 g by anhydrous weight) based on THF:water mixture weight (760 g). Biomass mixtures were then allowed to pre-soak overnight at 4 °C to insure an even distribution of acid catalyst within the biomass pores. Contents were then left in the laboratory for an hour for the temperature to reach about room temperature prior to reaction. The whole biomass slurry was then transferred to a high-pressure continuously stirred 1 L Parr reactor (Parr Instrument Company, Moline, IL) heated by a 4 kW fluidized sand bath (Model SBL-2D, Techne, Princeton, NJ). Mixing was performed by twin 6-blade impellers operating at 200 rpm by a top mounted electric motor, and the reactor temperature was directly measured by an in-line thermocouple (Omega, K-type). At the conclusion of a run, the reactor was cooled by quickly lowering it into a large room temperature water bath. All liquid containing receptacles were made of glass to prevent the loss of furfural and THF that was observed when plastics were used. The solids were then separated from the reaction liquor by vacuum filtration at room temperature through glass fiber filter paper (Fisher Scientific, Pittsburgh, PA). Mass and density of the liquid fraction were measured to complete accurate yield calculations. Due to the difference in density between the co-solvent mixtures and pure water, final densities were determined by weighing 25 mL of the reacted liquid in a volumetric flask after each reaction. Liquid samples were analyzed by an Agilent 1200 HPLC system equipped with a Bio-Rad Aminex® HPX-87H column and RI detector with an eluent (5 mM sulfuric acid) flow rate of 0.6 ml/min. Since the HPX-87H column cannot distinguish between xylose, mannose, and galactose sugar peaks, we also equipped our HPLC with an Aminex® HPX-87P column to differentiate xylose from the other C6 sugars for yield calculations. Since the HPX-87P column is incompatible with acids, we elected not to use this column for fuel precursor analysis. Calculation of the fuel precursor yields is given by Equation (6) where the molar equivalent number (θ) of furfural (Equation. 7), LA (Equation. 8), and 5-HMF (Equation. 9) are individually calculated and divided by the fraction of the total glucan or xylan in the raw material.

$$\text{(6)} \qquad \%\ \text{FP yield} = \theta_{FP} * \frac{FP_{final}(g/L)*\text{mass of liquor}_{final}(g)}{\text{total biomass}_{initial}\ (g)*\text{density of liquor}_{final}(g/L)} *100\%$$

$$\text{(7)} \qquad \theta_{furfural} = \frac{1.375}{\text{fraction of total xylan}_{initial}}$$

$$\text{(8)} \qquad \theta_{LA} = \frac{1.396}{\text{fraction of total glucan}_{initial}}$$

$$\text{(9)} \qquad \theta_{5\text{-}HMF} = \frac{1.286}{\text{fraction of total glucan}_{initial}}$$

For the recovery of extracted lignin, the reaction liquor was transferred to a glass bottle with a screw-on cap that was tapped with a 0.25 in. metal hose barb fitting. The fitting was connected by a flexible hose to a vacuum pump to perform vacuum distillation of THF. The liquor was agitated by a magnetic stir bar on a stir plate as THF was boiled off at room temperature under vacuum. Once the THF was removed from the aqueous liquor, the extracted lignin precipitates from solution as a black resinous solid. The solid lignin residue was then separated from the liquor and crushed to a fine powder by a ceramic mortar and pestle. The powder was then rinsed with water, air-dried, and then rinsed with diethyl ether. The resulting fine lignin powder product is shown in Figure 5.6.

5.6 Supplementary information

Table 5.3. Numerical data and corresponding standard deviation from triplicate runs for compositional data (wt%, dry basis) presented in Figure 5.3 and Figure 5.4. Compositional determination was performed by following the NREL protocol TP-510-42618, ver. 8-03-2012.

Figure 5.3	**Glucan**	**stdev (Glucan)**	**Xylan**	**stdev (Xylan)**	**Lignin**	**stdev (Lignin)**	**Other**
Raw	41.5	0.3	15.5	0.2	23.5	0.3	19.5
H_2SO_4	22.7	0.2	0.0	0.0	2.2	0.1	0.3
$FeCl_3$	25.4	0.2	0.0	0.0	1.8	0.1	0.7
$CuCl_2$	26.3	0.2	0.0	0.0	1.8	0.1	0.6
$AlCl_3$	28.1	0.3	0.0	0.0	1.7	0.2	0.1
$CrCl_3$	29.1	0.3	0.0	0.0	1.5	0.2	0.6
$ZrOCl_2$	16.6	0.2	0.0	0.0	5.0	0.2	23.1

Figure 5.4	**Glucan**	**stdev (Glucan)**	**Xylan**	**stdev (Xylan)**	**Lignin**	**stdev (Lignin)**	**Other**
Raw	41.5	0.3	15.5	0.2	23.5	0.3	19.5
$FeCl_3$ (1:1)	22.5	0.2	0.0	0.0	2.1	0.1	0.8
$FeCl_3$ (4:1)	7.3	0.2	0.0	0.0	2.0	0.1	0.7
$FeCl_3$ (7:1)	10.4	0.2	0.0	0.0	9.8	0.2	0.8

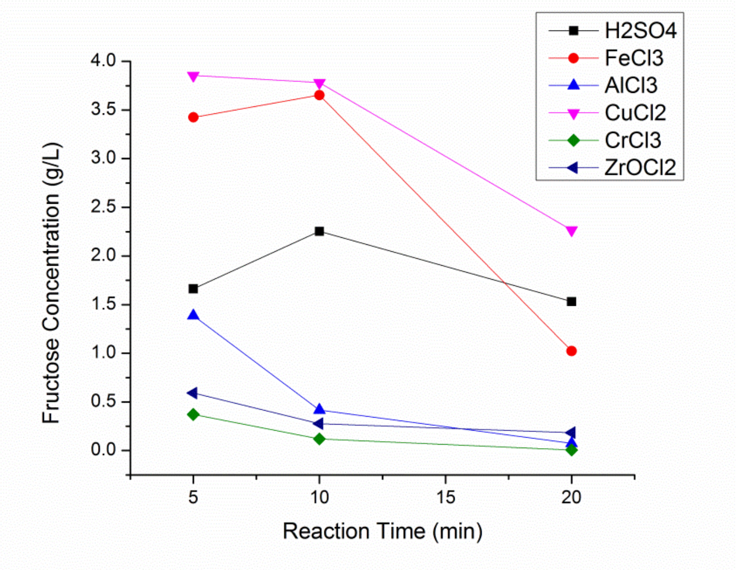

Figure 5.7. Liquid fructose concentration from pure glucose THF co-solvent reactions. Reaction conditions: 20 g L^{-1} glucose, 1:1 THF:Water (vol), 170 °C, 0.1M catalyst loading based on anhydrous mass.

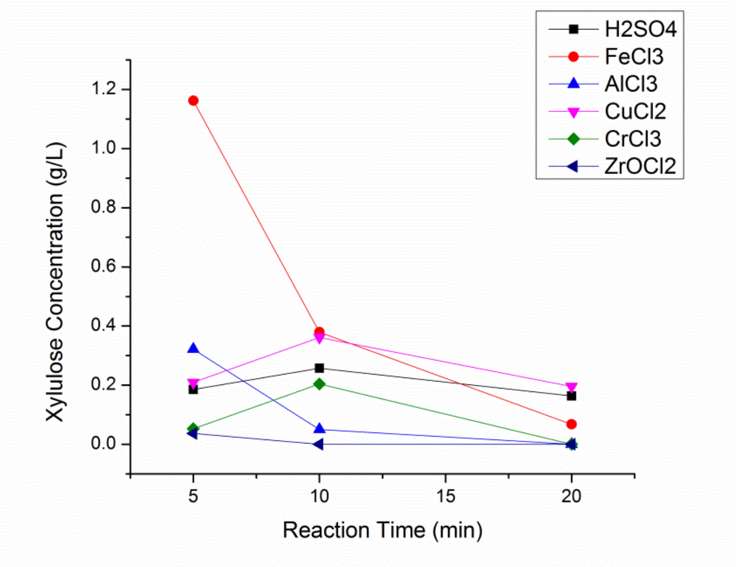

Figure 5.8. Liquid xylulose concentration from pure xylose THF co-solvent reactions. Reaction conditions: 10 g L^{-1} xylose, 1:1 THF:Water (vol), 170 °C, 0.1 M catalyst loading based on anhydrous mass.

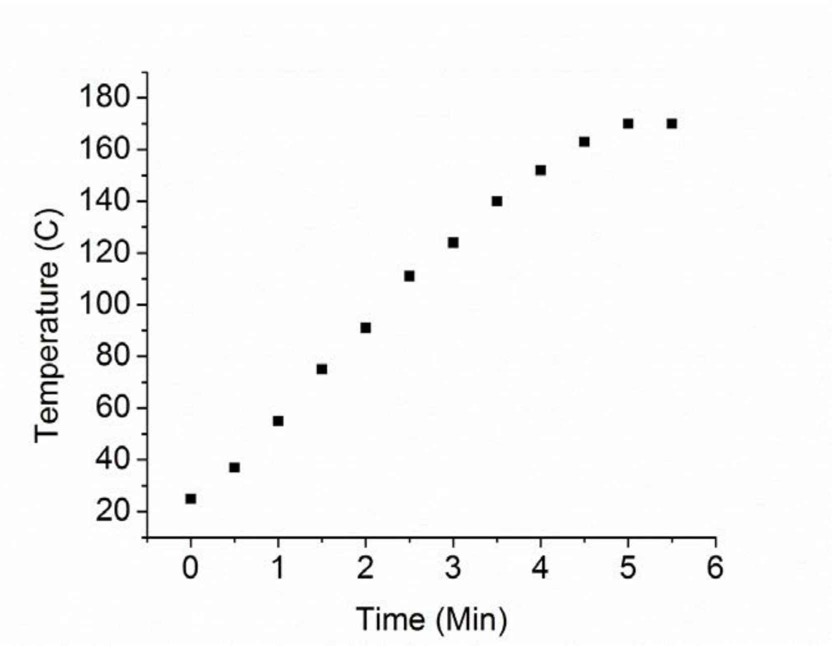

Figure 5.9. Typical heating profile for the 1 L Parr reactor during THF co-solvent reaction with biomass. Reaction temperature of 170 °C is achieved in less than 5 min and is quickly stabilized by raising the reactor over the fluidized sand bath. Overheating is

prevented by spraying water from a spray bottle directly to the outside wall of the reactor. Stabilization of reaction temperature in this fashion takes less < 20 seconds.

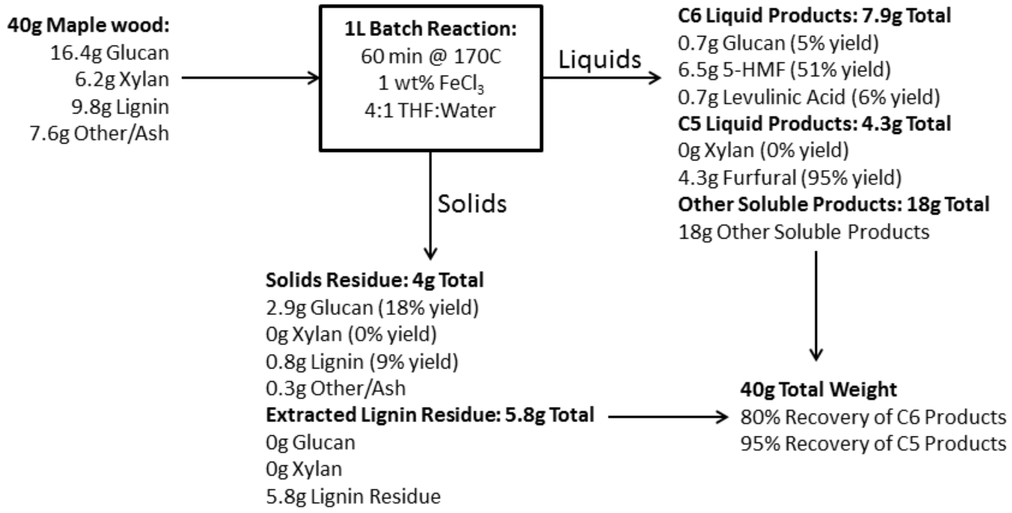

Figure 5.10. Product flow and mass balance diagram describing the mass and yield of products recovered in both the solid and liquid portion after reaction. Data shown for reaction conditions listed in Table 5.2 Run 17 in the main article when the highest furfural and 5-HMF co-production yields were obtained. Total recovery of C6 and C5 products are calculated from the liquid and solid yields.

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Chapter 6. Co-solvent pretreatment (CELF) reduces costly enzyme requirement for high sugar and ethanol yields from lignocellulosic biomass*

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6.1 Abstract

A new pretreatment strategy called Co-solvent Enhanced Lignocellulosic Fractionation (CELF) employs a single phase mixture of THF and water with 0.5 wt% H_2SO_4 to recover most of the hemicellulose sugars and lignin from corn stover in solution and produce a cellulose rich solid that can be saccharified to glucose at nearly theoretical yields. Subsequent evaporation of the highly volatile THF from solution can recover most of the THF and precipitate a nearly pure solid lignin product. The total combined yield of glucose, xylose, and arabinose monosaccharides was 95% of theoretical after a 150 °C CELF reaction followed by subsequent enzymatic saccharification with only 2 mg-enzyme g-glucan-1 (~1.2 FPU). The unusually high rates and extent of saccharification of CELF pretreated corn stover with such low enzyme loadings could be attributed to extensive removal of lignin by THF, as supported by compositional analysis, modeling by fractal kinetics, and SEM imaging. Simultaneous saccharification and fermentation (SSF) of CELF pretreated corn stover solids at low enzyme loadings by Saccharomyces cerevisiae produced twice the amount of ethanol as from solids produced by optimized dilute acid pretreatment of corn stover. Thus, CELF pretreatment provides a significant reduction in enzyme costs for saccharification while achieving the high total sugar yields from both hemicellulose and cellulose fractions necessary for biomass conversion to fuels to be economically viable.

6.2 Introduction

Lignocellulosic biomass, in the form of agricultural, herbaceous, and woody residues and energy crops, promises to provide a sufficient sustainable resource to address global energy demands and reduce dependence on petroleum-based liquid fuels (Wyman, 2007; Wyman and Huber, 2009). Its low cost is also attractive with lignocellulosic biomass at \$60 dry-ton^{-1} equivalent in unit energy cost basis to oil at about \$20 barrel^{-1}(Perlack and Stokes, 2011). Although natural gas production by fracking could provide an abundant near-term alternative to petroleum, its production and use does not substantially mitigate greenhouse gas emissions. Realistic and scalable technologies are particularly needed to capture the energy in lignocellulosic biomass as aromatic, hydrocarbon, and alcohol transportation fuels, because the transportation sector currently consumes about two-thirds of the world's petroleum production (Lynd et al., 2006; Lynd et al., 2008).

Lignocellulosic biomass is comprised of hemicellulose, cellulose, and lignin in a heterogeneous matrix that is recalcitrant; it is structurally durable and resistant to microbial or enzymatic breakdown (Yang and Wyman, 2008; Lynd et al., 1999). This recalcitrance is the major economic obstacle to converting biomass to sugars or other reactive intermediates with high yields at low costs (Wyman, 2007; Lynd et al., 2008). Biomass pretreatment has proven to be essential to overcome the recalcitrance of most biomass materials to downstream biological and chemical processes. Chemical and physical pretreament methods include heat, acid, and/or chemicals, usually in aqueous-based reactions (Yang and Wyman, 2008; Mosier et al., 2005; Kumar et al., 2009). In these two-stage operations, thermochemical pretreatment (Stage 1) opens up the biomass structure for subsequent enzymatic saccharification (Stage 2) by a mixture of cellulase enzymes (“cocktails”) that release sugars from the pretreated solids (Mosier et al., 2005; da Costa Sousa et al., 2009; Wyman et al., 2013). Although modern saccharification enzymes are highly stable and selective, their effectiveness is largely influenced by the efficacy of the pretreatment stage where 1) incomplete removal/relocation of hemicellulose and/or lignin has been shown to impede enzyme function, necessitating higher enzyme loadings that significantly increase costs and 2) ineffective alteration of the polysaccharides remaining in the solids from pretreatment can reduce enzyme access to binding sites, limiting potential sugar yields (Kumar and Wyman, 2013; Arantes and Saddler, 2011). Thus, it appears desirable for an effective pretreatment to remove hemicellulose and lignin to improve accessibility of the pretreated solids to enzymes so that high total sugar yields from both hemicellulose and cellulose can be achieved with low enzyme loadings (Kumar and Wyman, 2013; Zhang et al., 2007).

Currently, leading pretreatment strategies that appear attractive for commercial use include, but are not limited to, those by hydrothermal, dilute acid, solvent (such as ethanol-organosolv or cellulose solvent and organic solvent based lignocellulose fractionation, COSLIF), ammonia (ammonia fiber expansion (AFEX), ammonia recycled percolation (ARP), and soaking in aqueous ammonia), and alkali based processes (da Costa Sousa et al., 2009; Wyman et al., 2013; Karimi et al., 2013). Although each approach has its advantages, incremental implementation costs must be compensated by significant improvements in total sugar recovery and the capability to achieve high yields of fuel products in downstream bioconversion processes. We present here a pretreatment strategy called CELF (Co-solvent Enhanced Lignocellulosic Fractionation) that employs aqueous THF solutions to significantly enhance dilute acid pretreatment, the widely used pretreatment benchmark (Schedemann et al., 2010). THF is a polar aprotic solvent that is miscible with water over a wide range of conditions and concentrations and has the added advantage that it can be sustainably produced from biomass. In its miscible regime at high reaction severities, we found that reacting lignocellulosic biomass with acidified aqueous-THF solutions (1:1 THF-to-water volume mixture) removed lignin from the solid cellulose and solubilized cellulose and hemicellulose to enhance overall sugar dehydration product yields (furfural, 5-hydroxymethylfurfural, and levulinic acid) that could be used as fuel precursors for catalytic reactions (Lloyd and Wyman, 2005; Cai et al., 2013). These results prompted us to examine the features of CELF as a pretreatment technology for lignocellulosic biomass, in which application of lower severity reaction conditions was hypothesized to solubilize most of the hemicellulose sugars with limited breakdown to sugar dehydration products and produce cellulose rich solids that would lend themselves to biochemical conversion such as through simultaneous saccharification and fermentation (SSF) into valuable fuels and chemicals.

6.3 Results and Discussion

6.3.1 Optimizing corn stover pretreatment to maximize overall total sugar yields

The most prevalent sugars found in corn stover are glucose, xylose, and arabinose that represent the total sugars accounted for in this study. To present a valid comparison, we first optimized reaction conditions to achieve the highest total sugar yields (glucose, xylose, and arabinose) from CELF or dilute acid pretreatment of corn stover (Stage 1) combined with subsequent enzymatic hydrolysis of the pretreated solids (Stage 2). Dilute sulfuric acid (0.5 wt%) was used for both CELF and dilute acid pretreatments as it is an inexpensive strong acid that can be neutralized easily at dilute concentrations and is often the pretreatment of choice. We selected a 1:1 THF-to-water volume mixture for all CELF reactions as this concentration was previously found to be effective (Cai et al., 2013) and higher solvent concentrations would require larger reactor volumes and greater energy demands. Furthermore, a reaction temperature of 150 °C was chosen to remain within the miscible range due to a known miscibility gap for THF-water mixtures between 71.8 °C and 137.1 °C (Matous et al., 1972). For optimization, Stage 2 enzymatic hydrolysis of pretreated corn stover solids was performed with Accellerase® 1500 enzyme cocktail (cellulase + β-glucosidase) at an enzyme loading of 15 mg-enzyme g-glucan-1 to yield glucose monomers. Enzyme loadings were based on the glucan composition of the corn stover prior to pretreatment for a fair comparison between the two pretreatments (Mosier et al., 2005).

For CELF, total sugar yields from combined pretreatment and enzymatic hydrolysis were determined over a range of pretreatment times, as summarized in Figure

6.1. As shown, most of the xylose and arabinose was released from Stage 1 whereas a majority of the glucose was obtained from Stage 2, consistent with the typical production of sugars from dilute acid pretreatment. However, in contrast to dilute acid trends in which Stage 2 glucose yields tend to continually increase with pretreatment time, Stage 2 glucose yields from enzymatic hydrolysis of CELF pretreated corn stover remained relatively constant over a range of pretreatment times, as shown in Figure 6.1A. Since little degradation of glucose occurred at the pretreatment conditions applied and enzymatic hydrolysis was effective over a wide range of pretreatment times, the total glucose yield from both stages was nearly 100% of the theoretical maximum and paralleled the trend for Stage 2. On the other hand, the Stage 1 xylose yield peaked at 89% for about a 25 minute pretreatment, with further reaction reducing xylose yields due to degradation. Because most of the xylose was released in Stage 1 and most of the xylose remaining in the pretreated solids was released in subsequent enzymatic hydrolysis, the total xylose yield paralleled the trend for Stage 1. Arabinose yields were fairly constant at about 80% of theoretical over the course of the reaction times. As a result of these trends, the optimal reaction time of 25 min for total combined sugars yield shown in Figure 6.1C was largely dictated by the xylose yield from Stage 1 (Figure 6.1B). This outcome suggested that CELF pretreatment of corn stover could simply be optimized based on xylose recovery in the liquid hydrolzate from pretreatment.

The optimal reaction conditions developed in this study for dilute acid pretreatment of corn stover without THF were consistent with those established by previous work (Lloyd and Wyman, 2005): a 20 min reaction at 160 °C with 0.5 wt% sulfuric acid. Maximizing total sugar yields from combined pretreatment and enzymatic hydrolysis required tradeoffs as sugar yields from enzymatic hydrolysis continually increased with pretreatment time while xylose yields from pretreatment peaked well before the maximum glucose yield was obtained. This contrasts with CELF pretreatment in which xylose was not sacrificed in Stage 1 to realize high enough yields in Stage 2 to achieve the highest possible overall total sugar yields (Lloyd and Wyman, 2005).

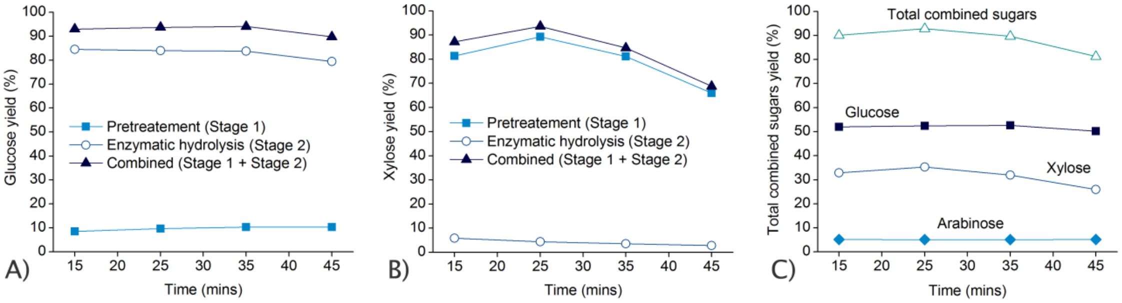


Figure 6.1. Optimization of pretreatment times for CELF pretreatment of corn stover to maximize total sugar (glucose, xylose and arabinose) yields from combined pretreatment (Stage 1) and enzymatic hydrolysis (Stage 2): A) glucose yields, B) xylose yields, and C) total combined glucose plus xylose plus arabinose yields. Reaction conditions: 5 wt% corn stover, 150°C, 0.5% H_2SO_4, and 1:1 THF:water volume ratio. Stage 2 was performed using 15 mg protein g-glucan^{-1} loading of Accellerase® 1500 enzyme based on glucan in corn stover prior to pretreatment.

Compositions of solids from both dilute acid and CELF pretreatments of corn stover at the optimal conditions for the highest overall total sugar yields are shown in Figure 6.2 based on 100 g mass of initial raw corn stover prior to pretreatment. These results show that although the fates of glucan and arabinan were similar for both pretreatments, CELF pretreatment dramatically improved removal of xylan and lignin compared to dilute acid, with remaining component masses of 1.3 g xylan and 3.4 g lignin for the CELF pretreated solids compared to 2.9 g xylan and 15.0 g lignin for the dilute acid pretreated solids on the basis of 100 g of corn stover initially (Figure 6.2). In fact, the reduced solid mass from CELF pretreatment was largely due to extensive delignification not possible with dilute acid pretreatment; pseudo-lignin formation actually increased the K-lignin content in pretreated solids from the latter. As observed previously with maple wood (Cai et al., 2013), THF is highly effective in delignifying biomass, in this case, producing a much more glucan-enriched material than from dilute acid pretreatment (75 wt% vs. 52 wt% glucan, respectively). Corn stover is also known to have a significant fraction of proteins, minerals, and organic acids that remained in similar quantity after both pretreatments. However, the total mass of glucan remaining in the solids was comparable after dilute acid and CELF pretreatments despite a 10 °C difference in temperature at optimal reaction conditions, supporting our early observation that THF can catalyze hydrolysis of biomass sugars (Cai et al., 2013).

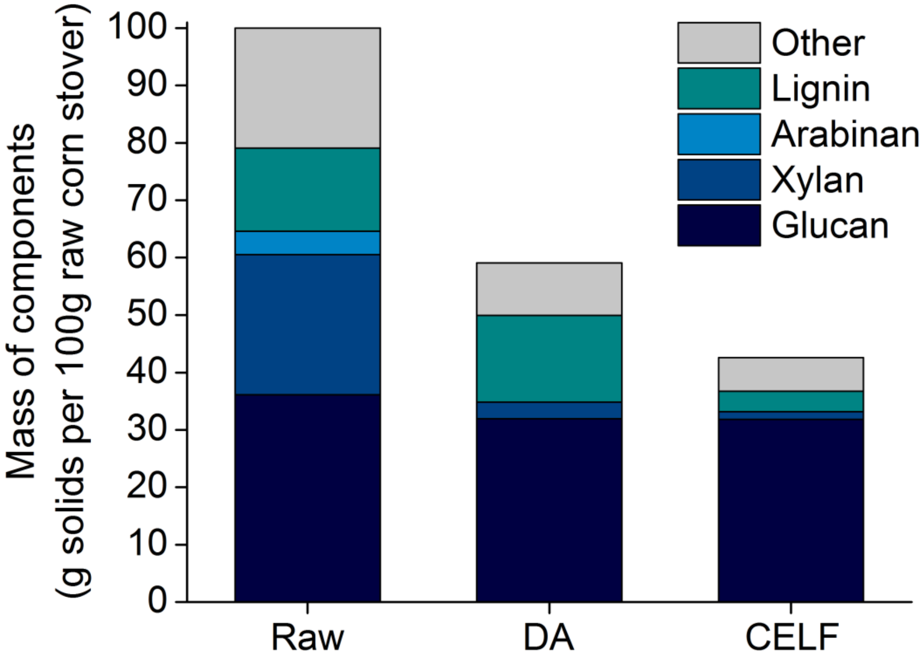


Figure 6.2. Tracking the mass of glucan, xylan, arabinan, lignin, and other compounds left in the solids produced by dilute acid (DA) and CELF pretreatments at conditions optimized for recovery of highest total overall sugar yields. The values shown for dilute acid and CELF are based on the content of each component in 100 g of corn stover prior to pretreatment. Reaction conditions: dilute acid: 160 °C, 0.5% H_2SO_4, 20 min; CELF: 150 °C, 0.5% H_2SO_4, 25 min, 1:1 THF:water volume ratio.

6.3.2 The effect of reduced enzyme loadings on total sugar yields

Because enzymes contribute about 20% of the total cost of producing fermentable carbohydrates from lignocellulosic biomass (Lynd et al., 2008), one of the most important challenges in developing effective pretreatment technologies is reducing the enzyme requirement for high sugar yields. Enzymes have been estimated to cost about \$1.47 gal ethanol^{-1} based on typical yields and conversions (about 70%) for ethanol production from corn stover based on a benchmark dilute acid pretreatment at a moderate loading of 20 mg-enzyme g-glucan^{-1} (Klein-Marcuschamer et al., 2012). Thus, as an example, reducing the enzyme loading by a factor of 4 could significantly lower enzyme costs to a more economical value of \$0.37 gal ethanol^{-1}. Figure 6.3 shows results from Stage 2 enzymatic hydrolysis of solids produced from optimized dilute acid and CELF pretreatments of corn stover for loadings of 30, 15, 5, and 2 mg-enzyme g-glucan^{-1} based on the raw glucan composition. These results show that CELF pretreatment (Figure 6.3B) achieved higher total sugar yields at all enzyme loadings compared to dilute acid (Figure 6.3A). Additionally, CELF pretreated material realized nearly theoretical yields of glucose even at low loadings of 2 and 5 mg-enzyme g-glucan^{-1}, albeit at longer incubation times (16 days and 5 days, respectively). These profiles are in stark contrast to those for dilute acid pretreated corn stover solids that had lower rates of glucan release and incomplete hydrolysis at loadings of 15 mg-enzyme g-glucan^{-1} or less, likely due to cellulose surface blockage by lignin and pseudo-lignin and/or enzyme inhibition/adsorption by lignin and/or pseudo-lignin (Ding et al., 2012; Yang and Wyman, 2006; Kumar et al., 2012; Kumar et al., 2013). The glucose yield profiles vs. enzymatic hydrolysis incubation time also show that long term glucose yields for solids from dilute acid pretreatment dropped with each successively lower enzyme loading, whereas long-term yields climbed to nearly 100% for CELF pretreated material, albeit requiring longer times. Thus, CELF pretreatment reduced the recalcitrance of corn stover to such an extent that a 10-fold reduction in enzyme loading still achieved very high yields and could translate into the enzyme cost potentially dropping to \$0.15 gal ethanol^{-1}, a savings of over \$1.00 gal ethanol^{-1}.

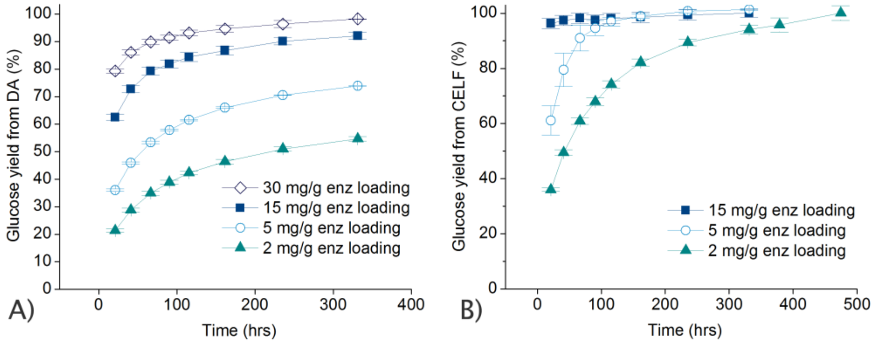


Figure 6.3. Comparison of glucose yields from enzymatic hydrolysis of solids from optimized A) dilute acid and B) CELF pretreatments of corn stover vs. enzymatic hydrolysis time over a range of Accellerase® 1500 enzyme loadings from 2 to 30 mg-enzyme g-glucan^{-1}. Pretreatment reaction conditions were those that gave the highest total combined sugar yields at an loading of 15 mg-enzyme g-glucan^{-1}: dilute acid: 160°C, 0.5% H_2SO_4, 20 min; CELF: 150°C, 0.5% H_2SO_4, 25 min, 1:1 THF:water volume ratio.

Figure 6.4 shows the total combined sugar yields at loadings of 2, 5, and 15 mg-enzyme g-glucan^{-1} for solids from CELF and dilute acid pretreatments at optimized pretreatment conditions, expressed as the sum of glucose, xylose, and arabinose masses obtained from Stage 1 + Stage 2 divided by the initial total mass of these three sugars, normalized on the basis of 100 g total sugars in the initial material before pretreatment. Although xylose (34% to 36%) and arabinose yields (5%) were comparable for both CELF and dilute acid pretreatments as a result of similar sugar release in Stage 1, total combined sugar yields were higher for CELF pretreatment than for dilute acid at all enzyme loadings due to higher glucose release in Stage 2. It is particularly noteworthy that overall sugar yields from CELF pretreated solids reached about 95% even at the low enzyme loading of 2 mg-enzyme g-glucan^{-1}, albeit requiring 14 days to reach this level, while yields from dilute acid solids were only about 70% after 14 days at this low enzyme loading. The apparent rate of glucose release was also higher from CELF pretreated solids, as illustrated by over 95% of the total potential sugar in corn stover being recovered in only 2 days following CELF pretreatment compared to the 14 days needed for dilute acid pretreatment to achieve an 85% total sugar yield at a loading of 15 mg-enzyme g-glucan^{-1}. Even at these longer incubation times for the latter, the lower total combined sugar yields were mostly attributable to incomplete glucan hydrolysis in Stage 2, as xylose and arabinose recovery was comparable to that from CELF pretreatment. A mass balance illustrating the fate of each sugar is shown in the Supplementary Information (SI, Figure 6.9).

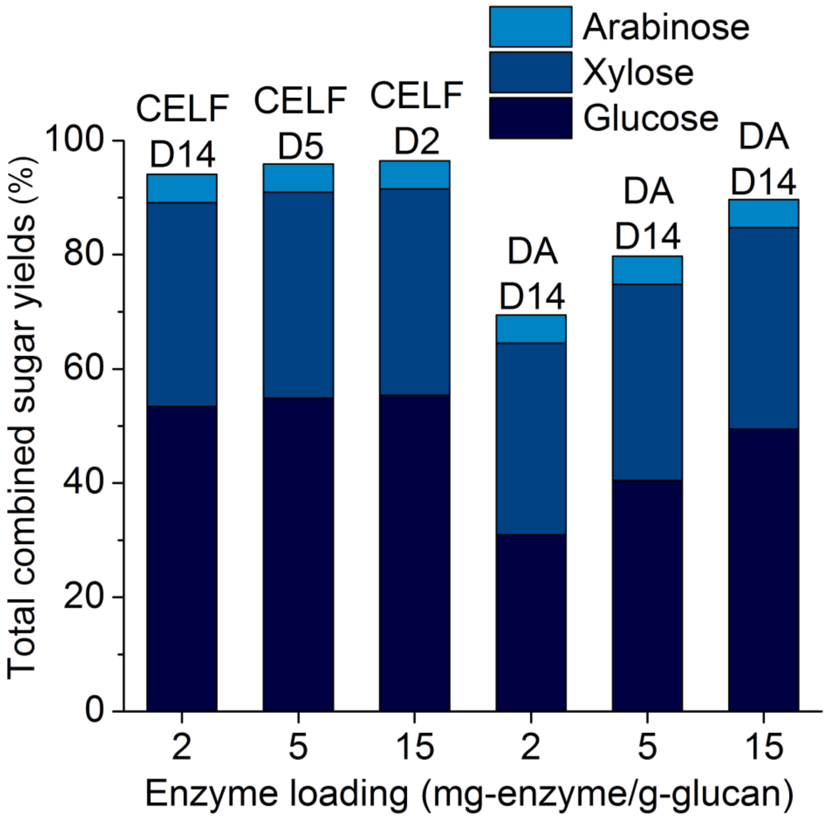


Figure 6.4. Overall Stage 1 + Stage 2 yields of glucose, xylose, and arabinose from CELF and dilute acid (DA) pretreated corn stover solids based on 100 g of total monomeric equivalent of the glucan, xylan, and arabinan content in unpretreated corn stover. The Stage 2 incubation time in days is shown at the top of each bar, e.g., D14 represents 14 days. 100% corresponds to the maximum amount of sugars that could be realized from the total glucan, xylan, and arabinan in corn stover.

6.3.3 Insight gained by a fractal model of enzymatic hydrolysis kinetics

A fractal kinetic model based on an empirical curve fit to the enzymatic hydrolysis results presented provided additional insights into the cause of the sugar yield plateau suffered by dilute acid pretreated corn stover compared to the highly reactive material produced by CELF (fractal model parameters and experimental fit is given in the Supporting

Information SI Table 6.1 and Figure 6.10, respectively). The fractal model used is based on classical first-order kinetics but replaces the rate constant k with a transient rate coefficient $k_t = k_t^{-h}$ that decays over time by a fractal exponent h. The values of the transient rate parameter k_t can be interpreted in terms of both substrate features and enzyme loadings to suggest mechanistic differences in enzymatic hydrolysis of CELF and dilute acid pretreated solids. When k_t is plotted against glucan conversion in Figure 6.5A, differences in the enzyme-substrate interactions can be seen. Specifically, in the higher glucan conversion regime of 60% to 100% where the accessible substrate surface area had been significantly reduced, CELF pretreated corn stover exhibited higher k_t values that dropped more slowly with increasing conversion from 0.039 to 0.030 hr^{-1} (Figure 6.5A) at the lower loading of 5 mg-enzyme g-glucan^{-1}, compared to the larger drop for dilute acid pretreated corn stover at higher loadings from 0.019 to 0.006 hr^{-1} for 15 mg-enzyme g-glucan^{-1} and 0.027 to 0.007 hr^{-1} for 30 mg-enzyme g-glucan^{-1}. This large difference suggests that CELF pretreatment produces substrates that sustain greater accessibility to enzymes over the course of hydrolysis compared to dilute acid pretreatment, resulting in nearly complete digestion of CELF pretreated corn stover solids. From a different perspective, Figure 6.5B depicts the change of reaction rate coefficient k_t with respect to hydrolysis time for both dilute acid and CELF pretreatments. As shown, the temporal progressions of k_t from the CELF pretreated corn stover at 2 and 5 mg-enzyme g-glucan^{-1} loadings were consistently 3 to 4 times greater than values for dilute acid pretreated corn stover at corresponding enzyme loadings, validating the superior enzymatic reactivity of CELF pretreated corn stover. Because dilute acid and CELF removed similar levels of hemicellulose, these results can be attributed to extensive removal of lignin during CELF pretreatment resulting in less cellulose surface blockage and enzyme inhibition than for dilute acid pretreatment. Wang et. al. (Wang et al., 2011) also proposed that a smaller *h* value correlates with less lignin inhibition, and a lower *h* was observed from CELF pretreated corn stover (0.169 at 5 mg-enzyme g-glucan^{-1}) in comparison to dilute acid at the same enzyme loadings (0.607 at 5 mg-enzyme g-glucan^{-1}), as shown in the SI, Table 6.9. Future work with CELF pretreatment will be coupled with fractal kinetic modelling to further investigate how delignification by CELF pretreatment affects substrate and enzyme interactions.

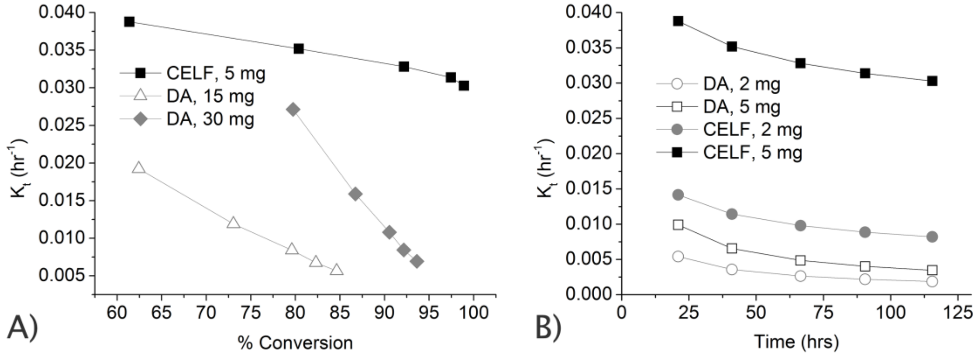


Figure 6.5. Comparison of change in fractal kinetic rate coefficient with respect to: A) percent conversion in the higher glucan conversion regime of 60% to 100% where accessible substrate surface area had significantly decreased for CELF pretreatments at 5 mg-enzyme g-glucan^{-1} (CELF, 5 mg) and dilute acid pretreatments at 15 and 30 mg-enzyme g-glucan^{-1} (DA, 15 and DA, 30 mg respectively) and B) enzymatic hydrolysis time for the dilute acid and CELF pretreatments at low loadings of 2 and 5 mg-enzyme g-glucan^{-1} (denoted as DA/CELF, 2 mg and DA/CELF, 5 mg respectively).

6.3.4 SEM characterization of THF pretreated corn stover

Comparison of the macro- and micro-structure of raw corn stover with solids produced by dilute acid and CELF pretreatments of the same material aids in understanding mechanisms that could account for the extraordinary reactivity of CELF pretreated corn stover with cellulolytic enzymes. Due to the heterogeneous distribution of corn stover particles, particles with common surface features were imaged for comparison. In Figure 6.6A, the outer structure of raw corn stover appears porous, with sheet-like pleats surrounding the open vascular networks used for water and nutrient delivery to the plant. When the corn stover was pretreated with dilute acid, Figure 6.6B shows how the macro-structure of the solids changed by destruction of the sheet-like walls to reveal the porous vascular network underneath, likely a primary feature for improving accessibility for enzyme attack. Since a significant amount of lignin was still present in solids following dilute acid pretreatment, the major features of raw corn stover were still distinguishable. In contrast, Figure 6.6C shows that CELF significantly altered macro and micro-structure features that are vital to efficient deconstruction of lignocellulosic biomass (Kumar and Wyman, 2013), likely due to extensive lignin removal collapsing most of the super-structure so that neither the sheet-like walls nor the vascular channels can be easily distinguished. This drastic destruction and collapse shown in the dried cell-wall structure from CELF pretreatment may be responsible for the substrate’s sustained reactivity to enzymatic hydrolysis. The visible “wrinkling” of material by CELF was likely due to drying of the sample, indicating that the material may be structurally weaker and become readily expanded when rehydrated by water, somewhat like a sponge. Our next steps include applying 13CP NMR to measure crystallinity and Simons' staining and water retention to estimate the accessibility of THF and dilute acid pretreated materials to test these hypotheses.

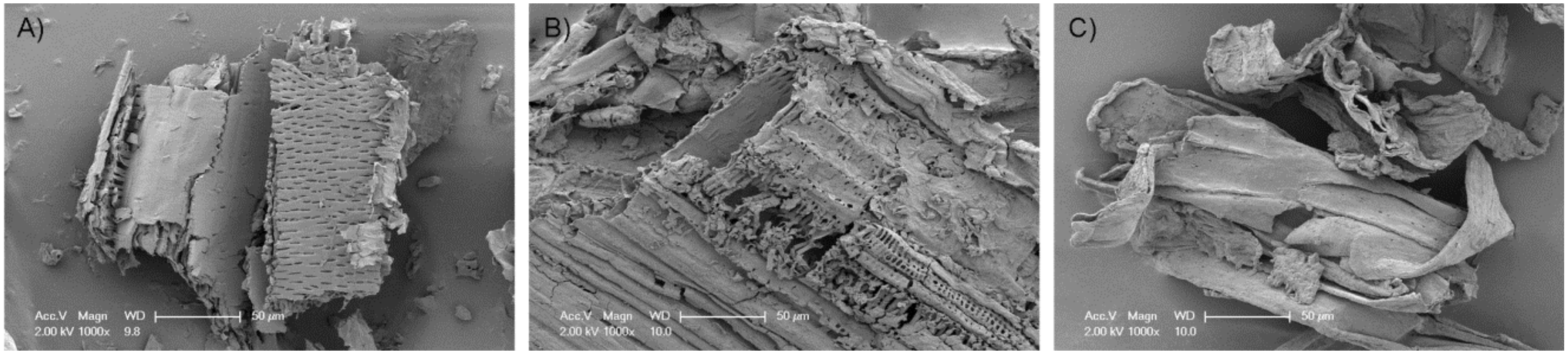


Figure 6.6. SEM images of solids at 1000X magnification from A) raw corn stover, B) dilute acid pretreated corn stover (160°C, 0.5wt% sulfuric acid, 20 min), and C) CELF pretreated corn stover (150°C, 0.5wt% sulfuric acid, 25 min). Scale bar is shown.

6.3.5 Simultaneous saccharification and fermentation (SSF) of pretreated corn stover to ethanol

We applied SSF to both CELF and dilute acid pretreated solids to demonstrate the compatibility of CELF pretreatment with achieving high ethanol yields by fermentation. SSF combines enzymatic hydrolysis of biomass to sugars with sugar fermentation to ethanol in a single step to reduce end product inhibition of enzymes by high soluble sugar concentrations and thereby increase ethanol yields and titers (Schedemann et al., 2010; Andrić et al., 2010). In this study, SSF was applied to pretreated solids in 125 mL shake flasks at 4 wt% glucan loading; each flask contained 50 mL working volume and was fitted with a bubble trap and inoculated with the D5A strain of S. cerevisiae yeast along with Accellerase® 1500. Figures 6.7A and 6.7B present the ethanol yields obtained when SSF was applied to solids produced by dilute acid and CELF pretreatments at optimized conditions compared to yields from an Avicel® cellulose control at loadings of 5 and 15 mg-enzyme g-glucan^{-1}. These results show that although a concern of solvent-based pretreatment such as with ionic liquids can be toxicity of residual solvent in pretreated solids towards enzymes or microbes that would reduce ethanol yields, simply washing CELF solids with water through a vacuum filter was sufficient to achieve high yields via SSF. Furthermore, consistent with our results for just enzymatic hydrolysis of the same solids, higher SSF ethanol yields were obtained for solids from CELF pretreatment at both enzyme loadings. For the higher loading of 15 mg-enzyme g-glucan^{-1}, Figure 6.7A shows that the ethanol yield plateaued at about 90% and 83% of theoretical for solids from CELF and dilute acid pretreatments, respectively, and that the yield rose much more rapidly for CELF pretreated solids. On the other hand, Avicel microcrystalline cellulose proved more recalcitrant than corn stover solids produced by either pretreatment, with ethanol yields still increasing at ~300 hrs fermentation time. These results suggest that, unlike CELF pretreated material, the high lignin content of dilute acid pretreated corn stover and the lower enzyme accessibility of crystalline Avicel® limit the effectiveness of hydrolytic enzymes and reduce overall ethanol yields. The differences are even more dramatic at a more commercially affordable enzyme loading of 5 mg-enzyme g-glucan^{-1}, with yields from CELF pretreated solids peaking at over 90% in only 4 days, while yields for dilute acid pretreated solids are only about 50% after 6 days. Thus, CELF pretreatment gave superior conversion of corn stover to ethanol compared to either of the other two substrates, consistently achieving ~90% or higher ethanol yields at both 15 and 5 mg g-glucan^{-1} enzyme loadings. Future study will optimize SSF ethanol yields from CELF pretreated solids at higher solids concentrations to realize more economically attractive ethanol titers.

Figure 6.7C shows yields of metabolites after 7 days of culture to close material balances on sugars. As shown, more glycerol was produced by conversion of CELF than dilute acid pretreated corn stover solids, likely due to the greater osmotic stress generated by the higher initial glucose concentrations (Nevoigt and Stahl, 1997) or higher ethanol concentrations at the completion of the fermentation. Additional optimization to reduce residual glucose concentrations during culture may further improve ethanol yields. Acetic acid and cellobiose concentrations remained minimal throughout the fermentation for each of the samples. Thus, ethanol yields from SSF were mostly governed by the extent of sugar release by enzymes and not by yeast performance.

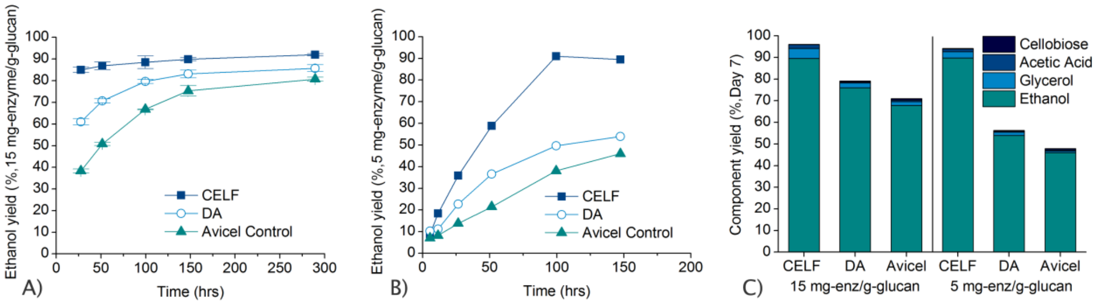


Figure 6.7. Ethanol yields from SSF of solids from dilute acid (DA) and CELF pretreatment of corn stover and Avicel® PH-101 cellulose vs. culture time at A) 15 mg g-glucan-1 and B) 5 mg g-glucan^{-1} loading of Accellerase® 1500 enzyme. C) Cumulative percent yields of SSF meatabolites from a 7 day culture.

Figure 6.8 outlines a possible approach for integration of CELF pretreatment with SSF to enhance ethanol yields from lignocellulosic biomass. As shown, wet biomass combined with THF and sulfuric acid are fed to a plug flow reactor (i.e., Pandia reactor) that is heated to reaction temperature by direct steam injection. After the CELF reaction, depressurizing the contents at the reactor exit rapidly drops the temperature to 100 °C to quench further reaction. The solids from the reactor are then separated from the liquid and washed with water in a countercurrent belt filter to remove soluble inhibitors released in pretreatment while limiting sugar dilution. The liquids are then delivered to a THF recovery unit where the volatile THF can be removed by low temperature distillation. Although THF forms a 95% azeotrope with water, further energy intensive separation is not required in that the co-solvent strategy does not require anhydrous THF (Cai et al., 2013). Furthermore, maintaining THF in contact with water minimizes formation of peroxides that can be explosive. Upon removal and recovery of THF, the dissolved lignin product precipitates as a solid that could potentially be catalytically upgraded to valuable chemicals and fuel products or burned to provide process heat and power. Neutralization and conditioning of the liquid stream by a suitable base (e.g., over-liming with calcium hydroxide) is applied to make the dissolved xylose and arabinose released during pretreatment suitable for biological fermentation. Although Figure 8 shows both the liquid and washed-solid streams feeding the same SSF operation, the liquid stream rich in xylose and arabinose from CELF pretreatment could be fed to one fermentation train, and the cellulose rich solids fed to a separate SSF operation. In the latter case, micro-organisms engineered for fermentation of dissolved xylose and arabinose sugars could be employed in the first fermentor, and conventional yeast such as S. cerevisiae employed for combined enzymatic hydrolysis and fermentation of the cellulose rich solids in the second, with high ethanol yields expected based on the results in this study. Our future work will undertake fermentation of the soluble sugars from Stage 1 to ethanol.

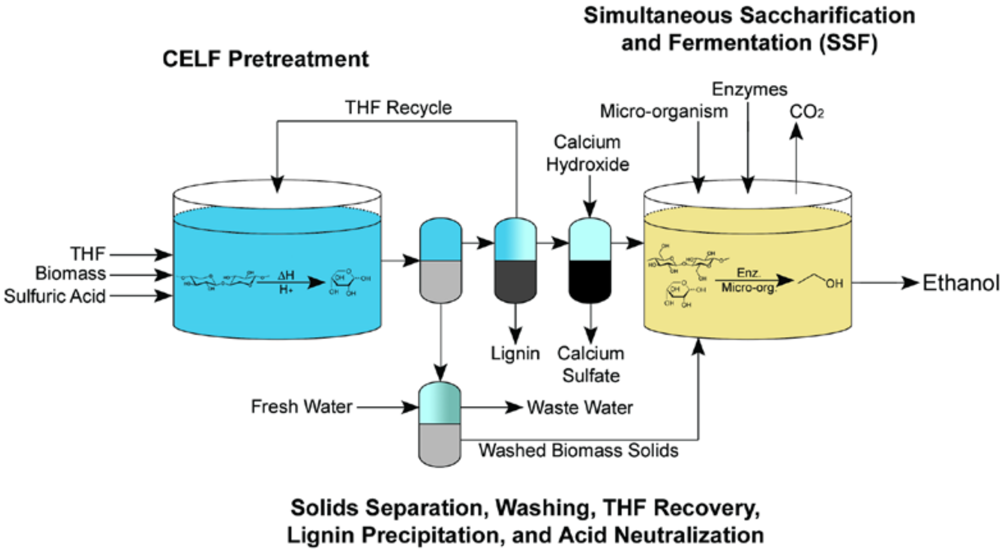


Figure 6.8. Illustration of a simplified block flow diagram for a proposed biomass conversion process that integrates CELF pretreatment with simultaneous saccharification and fermentation (SSF) to produce ethanol.

6.4 Conclusions

THF is a biomass-sourced green solvent with catalytic qualities that promote biomass deconstruction and delignification, and we have shown here that significant augmentation of traditional dilute acid pretreatment by application of THF as a miscible co-solvent represents a significant advancement in pretreatment technology. Optimization of this pretreatment strategy, called CELF, at 150 °C with 0.5 wt % sulfuric acid resulted in total (arabinan, xylan, and glucan) combined (Stage 1 and Stage 2) sugar yields of ~95% of the theoretical maximum at the low enzyme loading of 2 mg g-glucan^{-1}. Unlike dilute acid pretreatment, yields from enzymatic hydrolysis of solids from CELF pretreatment of corn stover remained high over a wide range of pretreatment times, such that optimization of the process could just focus on maximizing xylose recovery in Stage 1. The exceptional sugar yields from enzymatic hydrolysis of CELF pretreated solids also translated into excellent SSF performance with S. cerevisiae D5A of over 90% ethanol yields at an enzyme loading of only 5 mg protein g-glucan^{-1}. A process strategy was outlined such that CELF pretreatment could be integrated with SSF to produce ethanol directly from lignocellulosic biomass and THF could be recycled. Future characterization of the pretreated solids on multiple feedstocks is planned to better understand how CELF alters physiochemical features of biomass and enhances sugar yields.

CELF also gave new insights into promising pretreatment strategies that could enhance enzymatic hydrolysis. Tracing the fate of the primary components in biomass after dilute acid and CELF pretreatments showed that virtually complete lignin removal was the key difference that could account for enhanced enzymatic hydrolysis of the solids from CELF pretreatment. Our fractal kinetic analysis of the experimental enzymatic hydrolysis data also pointed to lignin removal by CELF resulting in higher accessibility and less inhibition towards cellulolytic enzymes than possible for dilute acid pretreatment. SEM images showed that extensive lignin removal by CELF pretreatment apparently altered the vascular and support structures of corn stover dramatically such that the cell walls were completely collapsed and the material appeared “wrinkled” after air-drying.

6.5 Materials and methods

Air-dried Kramer corn stover was provided by the National Renewable Energy Laboratory (NREL, Golden, CO). The composition of the corn stover was determined to be 37.1 ± 0.7% glucan, 25.1 ± 0.3% xylan, 4.2 ± 0.1% arabinan, 14.5 ± 0.4% K-lignin, and 28.0% other materials (Sluiter et al., 2010). Pretreatment reactions were performed in a 1 L Hastelloy Parr® autoclave reactor (236HC Series, Parr Instruments Co., Moline, IL) equipped with a double stacked pitch blade impeller rotated at 200 rpm. The co-solvent mixture contained a 1:1 volume ratio of THF and water. An acid loading of 0.5 wt% sulfuric acid (Ricca Chemical Company, Arlington, TX) was found to maximize total sugar yields from pretreatment and enzymatic hydrolysis combined (Lloyd and Wyman, 2005). Accellerase® 1500 cellulolytic enzyme cocktail was obtained from DuPont Industrial Biosciences (Palo Alto, CA). Protein concentration and activity were reported to be about 82 mg ml^{-1} and 50 FPU ml^{-1}, respectively (Kumar et al., 2013). S. cerevisiae D5A was kindly supplied by NREL. All chemical analyses were based on Laboratory Analytical Procedures (LAPs) documented by the NREL (Golden, CO) (http://www.nrel.gov/biomass/analytical_procedures.html). Liquid samples along with appropriate calibration standards were analyzed by HPLC with an eluent (5 mM sulfuric acid) flow rate of 0.6 ml min^{-1}. As developed and consistently practiced in our laboratory (Lloyd and Wyman, 2005; Zhang et al., 2013), combined total sugar yields (Stage 1 + Stage 2) resulting from each pretreatment were determined as the sum of total mass of soluble glucose, xylose, and arabinose released by pretreatment (Stage 1, reaction) plus the total mass of these three sugars released after saccharification by enzymes (Stage 2, enzymatic hydrolysis). Cellulase enzyme (Accellerase®1500, DuPont Industrial Biosciences, Palo Alto, CA) was loaded on a basis of the mass of glucan in the raw corn stover, as described elsewhere (Gao et al., 2014), and not according to the glucan content of the pretreated material to provide a fair comparison among different pretreatments (Mosier et al., 2005; Wyman et al., 2013; Wyman et al., 2011; Garlock et al., 2011). Additional details about the methods for pretreatment, enzymatic hydrolysis, fractal modeling, SSF, and SEM imaging can be found in Supporting Information.

6.6 Supplemental Information

6.6.1 Detailed materials and methods

Compositional analysis of corn stover was performed according to the established National Renewable Energy Laboratory (NREL) procedure (version 8-03-2012) in triplicates, with a resulting mass composition of 37.1 ± 0.7% glucan, 25.1 ± 0.3% xylan, 4.2 ± 0.1% arabinan, 14.5 ± 0.4% K-lignin, and 28.0% other materials (Sluiter et al., 2010).

Frozen stock of Saccharomyces cerevisiae (D5A) was prepared from plate monocultures that were transferred and cultured in a shaker incubator at 150 RPM and 38°C in yeast peptone dextrose (YPD) media containing 10 g L^{-1} yeast extract, 20 g L^{-1} peptone, and 50 g L^{-1} glucose. After 24 h incubation, 40 wt% glycerol in water solution was added, and 1 mL aliquots of the resulting mixture were transferred to sterile cryovials and placed in a -70 °C freezer. Before each SSF run, the inoculum was prepared by thawing, transferring, and growing the frozen stock on a shaker incubator at 150 RPM and 37 °C for 12 h in a 250 mL baffled flasks with YPD medium. The inoculum was then centrifuged and re-suspended in sterile DI water twice for washing and prepared for inoculation at a 0.5 optical density (O.D.).

6.6.2 Analytical procedures

All chemical analyses were based on Laboratory Analytical Procedures (LAPs) documented by NREL (Golden, CO) (http://www.nrel.gov/biomass/analytical_procedures.html). Liquid samples along with appropriate calibration standards were analyzed by HPLC (Waters Alliance 2695 system equipped with a Bio-Rad Aminex® HPX-87H column and Waters 2414 RI detector) with an eluent (5 mM sulfuric acid) flow rate of 0.6 ml min^{-1}. The chromatograms were integrated by Empower 2 software package (Waters Co., Milford, MA). As developed and consistently practiced in our laboratory (Lloyd and Wyman, 2005; Zhang et al., 2013), combined total sugar yields (Stage 1 + Stage 2) from each pretreatment were determined as the sum of total mass of soluble glucose, xylose, and arabinose released by pretreatment (Stage 1) plus the total mass of these three sugars released by saccharification of the washed pretreated solids with enzymes (Stage 2, enzymatic hydrolysis). Details of the calculation of sugar yields are outlined in the Supporting Information. Due to their greater abundance in corn stover composition, total sugars were considered to include glucose, xylose, and arabinose. Total lignin recovered was calculated from the mass of total unwashed K-lignin precipitated upon recovery of THF solvent whereas delignification was calculated from the percentage of K-lignin remaining in the pretreated material compared to the initial K-lignin content of the raw material.

6.6.3 Pretreatment of corn stover

Pretreatment reactions were performed in a 1 L Hastelloy Parr® autoclave reactor (Yang and Wyman, 2006) 6HC Series, Parr Instruments Co., Moline, IL) equipped with a double stacked pitch blade impeller rotated at 200 rpm. The THF co-solvent mixture for CELF pretreatment contained a 1:1 volume ratio of THF (>99% purity, Fisher Scientific, Pittsburgh, PA) and water. A 0.5 wt% sulfuric acid (Ricca Chemical Company, Arlington, TX) concentration was found to achieve the highest total glucose plus xylose plus arabinose yields from dilute acid alone or CELF pretreatment coupled with subsequent enzymatic hydrolysis (Lloyd and Wyman, 2005). Prior to each reaction, corn stover was added to the acid solution and soaked overnight at 4 °C. Corn stover solid loadings were 5 wt% for both the CELF and dilute acid non-solvent pretreatments. All reactions were maintained at temperature (±2°C) by convective heating with a 4 kW fluidized sand bath (Model SBL-2D, Techne, Princeton, NJ), with the reactor temperature directly measured by an in-line thermocouple (Omega, K-type). The sand bath temperature was set to 340 °C to reduce the heat-up time to under 3 min (Yang and Wyman, 2009). At the conclusion of each reaction, the reactor was cooled by quickly submerging it in a large room temperature water bath. The solids were then separated from the reaction liquor by vacuum filtration at room temperature through glass fiber filter paper (Fisher Scientific, Pittsburgh, PA). Mass and density of the liquid fractions were measured to complete accurate yield calculations. Due to differences in density between the co-solvent mixtures and pure water, final densities were determined by weighing 25 mL of the reacted liquid in a volumetric flask after each reaction. Liquid samples were analyzed by HPLC as previously described.

6.6.4 Enzymatic hydrolysis of pretreated corn stover and avicel cellulose

As noted in the NREL standard protocol (Selig et al., 2005), enzymatic hydrolysis of pretreated materials was performed in triplicate using 125 mL Erlenmeyer flasks with a 50 g total working mass containing 50 mM citrate buffer (pH 4.8) to maintain pH, 0.02% sodium azide to prevent microbial growth, and approximately 1 wt% glucan from pretreated solids or Avicel® PH-101 cellulose (Sigma Aldrich, St. Louis, MO). The cellulase enzyme (Accellerase®1500, DuPont Industrial Biosciences, Palo Alto, CA) loadings were varied from 2 to 30 mg-enzyme g-glucan^{-1} on a basis of the mass of glucan in the raw corn stover, as described elsewhere (Gao et al., 2014), and not according to the glucan content of the pretreated material to provide a useful comparison among different pretreatments (Mosier et al., 2005; Wyman et al., 2013; Wyman et al., 2011; Garlock et al., 2011). Basing enzyme loadings on the raw material is important because a pretreatment should not be penalized for releasing sugars before enzymatic hydrolysis, as total sugar yields from both Stage 1 (pretreatment) plus Stage 2 (enzymatic hydrolysis) should be maximized (Pollet et al., 2014). The flasks containing biomass slurry were placed in a Multitron™ orbital shaker (Infors HT, Laurel, MD) set at 150 RPM and 50 °C and allowed to equilibrate for 1 hour before enzyme addition. Samples of about 0.75 mL were periodically taken into 2 ml centrifuge vials (Fisher Scientific, Pittsburgh, PA) from each flask and centrifuged at 14,000 rpm for 5 min to determine the progress of enzymatic hydrolysis. The supernatant was then transferred into 500 µl HPLC vials (Grace Davison, Deerfield, IL) for HPLC analysis.

6.6.5 Fractal modeling of hydrolysis kinetics

A fractal model based on first order chain breakdown to form glucose was found to best describe cellulose hydrolysis with the rate coefficient k_t related to the hydrolysis time raised to the fractal exponent h (Eqn 1) (Wang and Feng, 2010):

$$\frac{dC}{dt} = k_t C, \ where \ k_t = kt^{-h} \tag{1}$$

The model described by Eqn 2 was used to fit the experimental data from the enzymatic hydrolysis by non-linear regression using MATLAB 7.0 (damped least squares) where $X = \%\ Conversion$ and $t = time\ in\ hrs$:

$$X = 100 * (1 - EXP\left(-k\left(1 + \frac{t^{1-h}-1}{1-h}\right)\right) \tag{2}$$

6.6.6 Cell cultivation and simultaneous saccharification and fermentation

Consistent with NREL standard protocols (NREL LAP, “SSF Experimental protocols-Lignocellulosic Biomass Hydrolysis and Fermentation,” Dowe et. al, 2001), SSF was performed in triplicates in 125 mL flasks with a 50 g working mass containing 50 mM citrate buffer (pH 4.8), 10 g L^{-1} yeast extract (Becton, Dickinson and Company, Redlands, CA), 20 g L^{-1} peptone (Becton, Dickinson and Company, Redlands CA), 40 mg L^{-1} tetracycline (Sigma Aldrich, St. Louis, MO) as an antimicrobial agent, Accellerase® 1500 cellulase (loaded at 5 or 15 mg protein/g glucan), and D5A frozen stock culture. DI water and solids were loaded into flasks (with attached bubble traps) to achieve 4 wt% glucan loading of either the pretreated solid residues or Avicel® PH 101 cellulose, and masses of the whole flask assembly were recorded before autoclaving at 121oC for 30 minutes. Flasks were then cooled, reweighed, and moved into a laminar flow hood (Baker and Baker Ruskinn, Sanford, ME) for aseptic re-addition of pre-sterilized DI water to replenish water loss, yeast extract, citrate buffer, tetracycline, Accellerase® 1500 cellulase, and cell inoculum.

6.6.7 Scanning electron microscope (SEM) imaging

A field emission scanning electron microscope (SEM, Philips XL-30) provided images of the raw, pretreated, and post enzymatic hydrolysis corn stover. Air-dried samples of each were placed on pin stub mounts with carbon tape and sputter coated with platinum in a Cressington 108 Auto (Ted Pella Inc, Redding CA). The surface macro and microstructures of the samples were characterized at an accelerating voltage of 2 kV.

6.6.8 Nomenclature

g: glucan,

x: xylan,

a: arabinan

0: initial

1: stage 1 (pretreatment)

2: stage 2 (enzymatic hydrolysis)

L: liquids from pretreatment

S: solids from pretreatment

$m_{g,o}$ or $m_{x,o}$ or $m_{a,o}$: component mass of sugars from raw corn stover

$m_{g,L}$ or $m_{x,L}$ or $m_{a,L}$: mass of sugars recovered from liquids after pretreatment

$m_{f,L}$ or $m_{h,L}$: equivalent mass of furfural (f) and 5-HMF (h) from their starting sugars

$m_{g,S}$ or $m_{x,S}$: mass sugar remaining from pretreated solids

$m_{g,2}$ or $m_{g,2}$: mass of sugars released by enzymatic hydrolysis

m_o: mass of raw corn stover solids loaded into pretreatments

m_r: mass fraction of solids remaining after pretreatment over solids loaded for enzymatic hydrolysis

R_g or R_x or R_a: sugar composition in the raw biomass

P_g: sugar composition in the pretreated biomass

V_1: total liquids volume remaining after pretreatment

V_2: total liquids volume for enzymatic hydrolysis

$[\text{glucose}]_1$ or $[\text{xylose}]_1$ or $[\text{arabinose}]_1$: sugars concentration from stage 1

$[\text{glucose}]_2$ or $[\text{xylose}]_2$ or $[\text{cellobiose}]_2$ or $[\text{arabinose}]_2$: sugars concentration from stage 2

[biomass]: initial dry biomass concentration loaded into enzymatic hydrolysis or SSF flasks

[ethanol]: ethanol concentration after SSF

[acetic acid]: acetic acid concentration after SSF

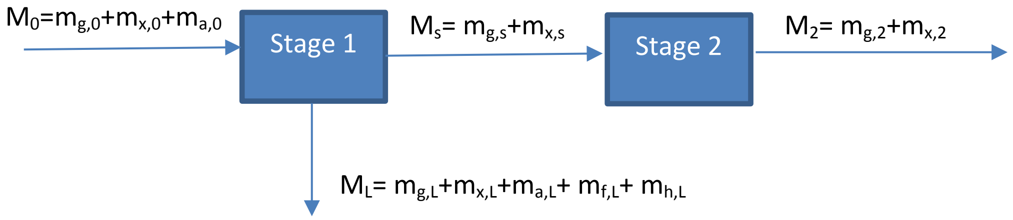
[glycerol]: glycerol concentration after SSF

[cellobiose]: cellobiose concentration after SSF

6.6.9 Calculation of product yields

1) Stage 1 glucose yield $= \frac{m_{g,1}}{m_{g,o}} * 100\% = \frac{\frac{[glucose]_1 * V_1}{1.111}}{m_o * R_g} * 100\%$
2) Stage 1 xylose yield = $\frac{m_{x,1}}{m_{x,o}} * 100\% = \frac{\frac{[xylose]_1 * V_1}{1.136}}{m_o * R_x} * 100\%$
3) Stage 1 arabinose yield = $\frac{m_{a,1}}{m_{a,o}} * 100\% = \frac{\frac{[arabinose]_1 * V_1}{1.136}}{m_o * R_a} * 100\%$
4) Stage 2 glucose yield $= \frac{m_{g,2}}{m_{g,o}} * 100\% = \frac{\frac{([glucose]_2 + 1.053 * [cellobiose]_2) * V_2 * m_x}{1.111}}{m_o * R_g} * 100\%$
5) Stage 2 xylose yield = $\frac{m_{x,2}}{m_{x,o}} * 100\% = \frac{\frac{[xylose]_2 * V_2 * m_x}{1.136}}{m_o * R_x} * 100\%$
6) Stage 2 arabinose yield = $\frac{m_{a,2}}{m_{a,o}} * 100\% = \frac{\frac{[arabinose]_2 * V_2 * m_x}{1.136}}{m_o * R_a} * 100\%$
7) Total combined sugars yield = $\frac{m_{g,1} + m_{g,2} + m_{x,1} + m_{x,1} + m_{a,1} + m_{a,1}}{m_{g,o} + m_{x,o} + m_{a,o}} * 100\%$
8) Glucose Yield (from 1 wt% glucan loading) = $\frac{[Glucose]_2}{1.111 * \mathrm{P}_g * [biomass]} * 100\%$
9) % Ethanol Yield = $\frac{[ethanol]}{0.51 * (1.111 * \mathrm{P}_g * [biomass])} * 100\%$
10) % Acetic Acid Yield = $\frac{[acetic\ acid]}{1.111 * \mathrm{P}_g * [biomass]} * 100\%$
11) % Glycerol Yield = $\frac{[glycerol]}{0.51 * (1.111 * \mathrm{P}_g * [biomass])} * 100\%$

12) $\% \text{ Cellobiose Yield} = \frac{1.053*[cellobiose]}{1.111*P_g*[biomass]} * 100\%$



Stream	**C5**	**CELF**	**DA**	**C6**	**CELF**	**DA**
M_0: Initial CELF = 40 g; DA = 80g	$M_{a,0}$	1.64	3.20	$M_{g,0}$	14.44	28.16
	$M_{x,0}$	9.76	19.03			
M_S: Stage 1-Solids	$M_{a,s}$	0	0	$M_{g,s}$	12.74	24.92
	$M_{x,s}$	0.53	2.24			
M_L: Stage 1-Liquid	$M_{a,L}$	1.28	2.48	$M_{g,L}$	1.35	1.99
	$M_{x,L}$	8.71	16.01	$M_{h,L}$		
	$M_{f,L}$	0.68	0.87		0.10	0.11
Stage 1 Balance $(M_s+M_L)/M_0*100\%$		98.2%	97.2%		98.3%	96.0%
M_2: Stage 2 Liquid hydrolysate	$M_{x,2}$	0.62	2	$M_{g,2}$	12.44	23.77
Stage 2 Balance $(M_2+M_L)/M0*100\%$		**99.0%**	**95.8%**		**96.2%**	**91.0%**

***Note: Stage 2 Balance was not within 100% ± 5% for the C6 DA case because it was not fully digested during enzymatic hydrolysis (solids remained)*

Figure 6.9. Mass balance of C5 and C6 sugars at optimized conditions for CELF and DA pretreatment of corn stover.

Table 6.1. Values of the fractal kinetic parameters, *k* and *h*, for Figures 6.5A and 6.5B, calculated from the non-linear fit of experimental data from Figure 6.3.

Pretreatment (enz. Loading, mg protein/g glucan in raw biomass)	**k**	**h**
DA (2)	0.030	0.545
DA (5)	0.064	0.607
DA (15)	0.166	0.701
DA (30)	0.307	0.797
CELF (2)	0.041	0.350
CELF (5)	0.064	0.169

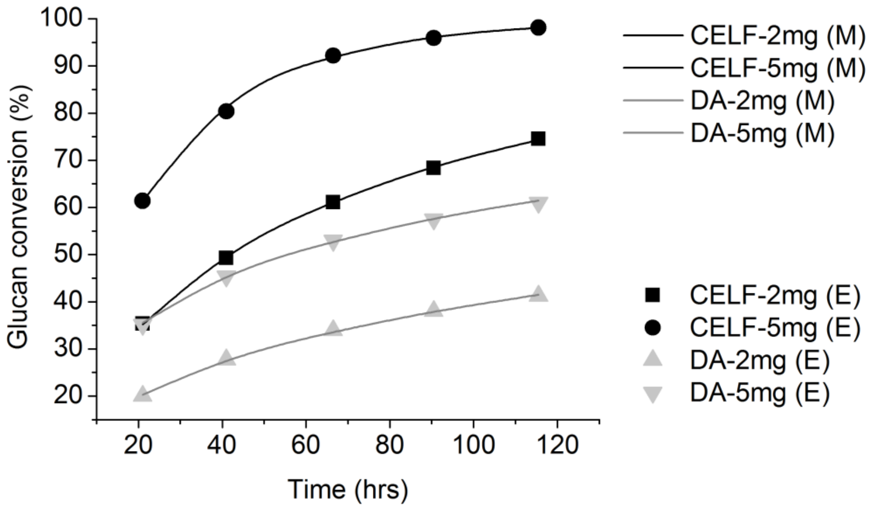


Figure 6.10. Glucan conversion vs. enzymatic hydrolysis time for DA and CELF pretreated corn stover at low enzyme loadings of 2 and 5 mg protein-enzyme g-glucan^{-1}. Points represent actual experimental data from Figure 6.3. Lines represent a non-linear fit of the data points by solving for fractal kinetic rate parameters *k* and *h*.

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Chapter 7. Comparison of CELF process with leading conversion technologies and calculation of the operating margin for a CELF biorefinery

7.1 Abstract

In this study, several leading pretreatment and catalytic processes were compared against CELF in terms of performance, process configuration, and economic feasibility. Key features were described for each process to highlight their advantages and disadvantages. Also, a preliminary plant model was developed for a hypothetical CELF biorefinery based on previous yield data to calculate the plant's operating margin for a scalable 60 ton hr^{-1} reactor set-up. From a sensitivity analysis on input costs and output prices, major driving factors for the plant economics were determined to be product selling price, feedstock cost, solvent recovery efficiency, and capital expenses.

7.2 Introduction

The impact of biomass conversion technologies is determined by both its technological merit and its economics. A successful biomass-to-fuels process should deliver quality end-products at a reasonable market price that are higher performing and/or more economical than previous technologies. Unfortunately, high performing chemical processes are usually more energy intensive and require greater operational and capital expenses, so the additional cost of implementation must be justified by the performance gain. Factors that can influence the potential success of a new biomass conversion process can be posed as the following questions: (1) Does the process improve upon the production yields of the current state-of-art so that utilization of the feedstock is maximized? (2) Are reaction conditions (temperature, time, and system pressure) minimized and solid loadings maximized to reduce energy burden of the process? (3) Are catalysts, solvents, and additives recycled to the fullest extent to reduce material costs? (4) Are high value co-products of the process recovered to improve the profit margin? (5) How is heat energy recycled? Is the energy consumption of the process greater than the energy content of the products? (6) Is the process inherently scalable?

In this study, CELF is compared to several relevant aqueous and solvent-based biomass conversion technologies for either pretreatment or catalytic dehydration. Key features are described for each process to highlight their advantages and disadvantages towards economic feasibility. Also, a preliminary economic model is developed for a hypothetical CELF biorefinery based on previous experimental data to calculate the operating cost margin for a scalable 60 ton hr^{-1} reactor set-up, and a sensitivity analysis is carried out on input costs and output prices.

7.3 Comparison of promising biomass pretreatment technologies

Currently, leading pretreatment technologies suitable for integration with simultaneous saccharification and fermentation (SSF) to produce ethanol are hydrothermal, dilute acid, and ethanol-organosolv pretreatments. Detailed data on multiple feedstocks are available for each pretreatment process as well as economic models evaluating their commercial feasibility. For this study, corn stover was selected as the model feedstock because information was readily available for hydrothermal, dilute acid, and CELF pretreatment of corn stover. For organosolv pretreatment, the model was based on a typical hardwood instead of corn stover. Table 7.1 compares select process details from the leading pretreatment strategies with CELF pretreatment data from Chapter 6.

Table 7.1. Comparison of process details of relevant pretreatment technologies compared with CELF pretreatment. Leading values for each pretreatment type are shown in **bold**.

Process name	*Catalyst*	*Temp. (°C)*	*Solvent*	*Neutralization*	*Solvent Recovery*	*Post-hydrolysis*	*Enzyme Loading (mg protein/g glucan)*	*Xylose yield (%)*	*Glucose yield (%)*	*Delignification (%)*	*Reference*
Hot water	**Auto-catalysis**	190	**Water**	**None**	**None**	Yes	30	82	91	0-5	Mosier et al., 2005
Dilute acid	0.5 wt% Sulfuric Acid	160	**Water**	Lime	**None**	Yes	30	95	90	0-5	Lloyd and Wyman, 2005
Organosolv[a]	1.25 wt% Sulfuric Acid	180	Ethanol/ Water	Ammonia	Multi-stage	Yes	20	82	90	79 - 90	Kautto et al., 2013
CELF	0.5 wt% Sulfuric Acid	**150**	THF/ Water	Lime	Multi-stage	**No**	**2**	**95**	**99**	**80 - 93**	Cai, Chapter 6

[a] Organosolv data retrieved for processing of hardwoods, remaining pretreatments processed corn stover.

Hydrothermal pretreatment is the simplest process involving the use of hot water or steam explosion to catalyze the hydrolysis of hemicellulose in biomass and reduce the recalcitrance of the cellulose fraction for enzymatic saccharification. This simple pretreatment is very cost effective in terms of capital expenses as no additional catalyst, solvent, or neutralizing base is needed (Mosier et al., 2005). Hydrothermal pretreatment relies on “auto-catalysis” where the biomass’ own acetic acid content combined with the self-ionization of water at high temperatures (> 190 °C) cause the acidity in the aqueous reaction to increase, consequently catalyzing the hydrolysis of biomass polysaccharides (Kumar and Murthy, 2011). One disadvantage of hydrothermal pretreatment is the extensive production of xylo-oligomers that require a post-hydrolysis step to hydrolyze the xylo-oligomers to xylose monomers. Afterwards, only an 81% recovery of xylose could be achieved from hydrothermal pretreatment (Mosier et al., 2005). Enzymatic hydrolysis of the pretreated corn stover at 30 mg-enzyme g-glucan^{-1} enzyme loading can produce total glucose yield of 90% from theoretical glucan content. The resulting sugar stream could then be co-fermented by recombinant yeast or bacterium to produce ethanol. Kumar and Murthy estimate a minimum selling price of $2.53 gal-ethanol^{-1} for a 250 Kton-biomass year^{-1} plant (Kumar and Murthy, 2011).

Dilute acid pretreatment is considered one of the most commercially viable pretreatment strategies due to its relatively high sugar yields over hydrothermal pretreatment on multiple feedstock types and moderately more expensive operation (Lloyd and Wyman, 2005). A simplified process diagram of a hypothetical dilute acid pretreatment and SSF operation is shown in Figure 7.1 from an NREL report (Humbird et al., 2011). Additional capital, operating, and material costs are required for dilute acid pretreatment over hydrothermal pretreatment due to the addition of dilute sulfuric acid (0.5 wt%) in the reaction to catalyze the hydrolysis of mostly the hemicellulose sugars and some of the cellulose. The xylose recovery is dramatically improved for dilute acid pretreatment, achieving 95% of theoretical from corn stover. Glucose yields are equally high at 90% of theoretical at equivalent enzyme loadings to hot water (30 mg g-glucan^{-1}, Table 7.1). One disadvantage of dilute acid is the need for an additional neutralization step to detoxify the resulting liquid stream from pretreatment before it can be delivered to SSF operation for ethanol production. This is usually performed by over-liming to produce a gypsum byproduct or by addition of ammonia. Furthermore, sulfuric acid also catalyzes the dehydration of xylose to furfural which is a byproduct to fermentation. As shown in Figure 7.1, anaerobic digestion of the remaining solids produces methane from biogas to supply heating energy to the plant. NREL estimates the minimum selling price for a dilute acid pretreatment-based plant to be \$2.15 gal-ethanol^{-1} for a 773 Kton-biomass year^{-1} plant (Humbird et al., 2011).

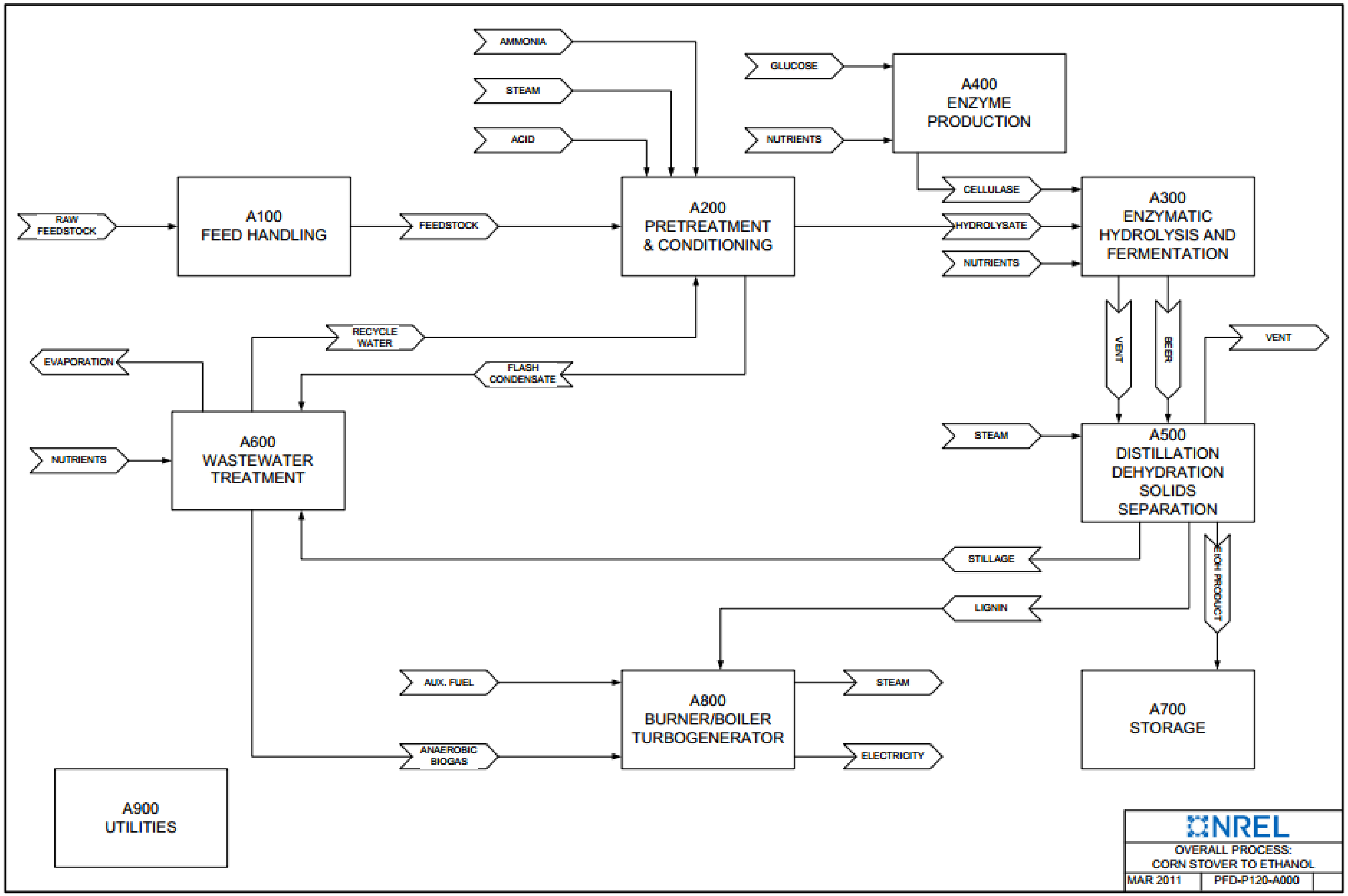

Figure 7.1. Simplified process flow diagram for a dilute acid pretreatment and SSF plant to produce ethanol. (Taken verbatim from Humbird et al., 2011)

Ethanol-organosolv pretreatment is significantly different from either hydrothermal or dilute acid pretreatments in that it utilizes a water-miscible co-solvent such as ethanol to enhance the pretreatment and fractionation of lignocellulosic biomass. As shown in Table 7.1, in addition to a more concentrated sulfuric acid loading (1.25 wt%), a 50 vol% or higher mixture of ethanol and water is also required for the reaction that would necessitate additional recovery operations in order to minimize material costs. Organosolv pretreatment is almost exclusively used with forestry residues such as hardwoods and softwoods due to the more recalcitrant nature of woody biomass over agricultural or grassy biomass that require more powerful pretreatment strategies to achieve high sugar yields. Although dilute acid can demonstrate good performance with corn stover, its performance with woody biomass is dramatically lower in terms of total sugar recovery as the larger crystalline cellulose and lignin fractions of wood reduce enzyme efficiency. Organosolv pretreatment is capable of delignifying woody material and other biomass so that the extracted lignin product can be recovered as a high value by-product instead of being used for combustion in other pretreatment operations. As a result, organosolv requires less enzymes for hydrolysis as the pretreated solids are more digestible by enzymes than dilute acid or hydrothermal pretreatment, thus saving on enzyme costs.

A block diagram of a proposed organosolv process developed by Kautto et al. is shown in Figure 7.2 (Kautto et al., 2013). As shown, several ethanol washing and ethanol recovery steps are needed for the organosolv process that contributes to greater energy consumption than dilute acid pretreatment. Ethanol is also poisonous to microbes at higher concentrations and must be removed before the sugar stream is fermented. A large amount of water is also needed to dilute the liquid product streams containing ethanol to help precipitate and recover the extracted lignin products. This translates to a very high energy demand when separating diluted ethanol concentrations from a large quantity of water. Additional energy from burning fossil natural gas is necessary to maintain organosolv plant operations as the heat generation from plant waste is not sufficient (Kautto et al., 2013). Viell et al. proposed that organosolv pretreatment can only be viable if lignin and other byproducts from the process could be sold at a high value to offset the additional costs of implementation. Limited headroom in process optimization prevents any significant cost savings from process improvements (Viell et al., 2013). Furthermore, the introduction of new process technology such as organosolv and new products (i.e., lignin) requires a high ROI of larger than 24% to mitigate risk of implementation.

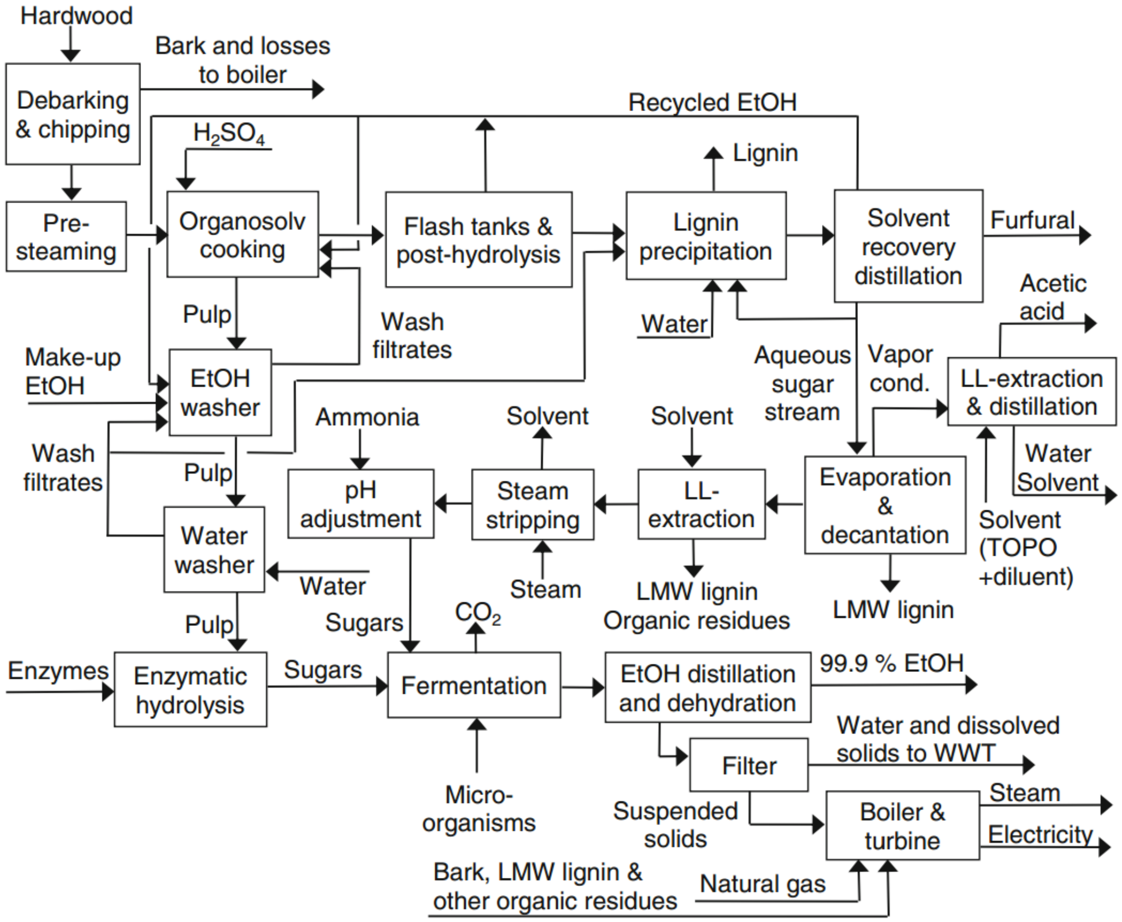

Figure 7.2. Block diagram of a proposed ethanol-organosolv biorefinery. (Taken verbatim from Kautto et al., 2013)

CELF pretreatment is similar to organosolv as it also employs a water-miscible co-solvent to enhance pretreatment and fractionation, however, the similarities end there. CELF uniquely uses THF as a low-boiling solvent to catalyze the aqueous hydrolysis of biomass polysaccharides as well as to extract lignin beyond what is capable with ethanol-organosolv (Table 7.1). Consequently, only dilute concentrations of sulfuric acid (0.5 wt%) is needed and the optimal reaction temperature for the pretreatment of corn stover by CELF is 10 °C lower than dilute acid pretreatment and 30°C lower than organosolv pretreatment (Table 7.1). Furthermore, lignin recovery in the CELF process does not require dilution with water as THF easily boils in aqueous mixture at room temperature under vacuum distillation allowing recovery of THF and subsequent precipitation of the extracted lignin as a highly pure solid powder. THF also forms a lower boiling azeotrope with water than ethanol (65 °C vs. 78.1 °C). The enzymatic digestibility of CELF pretreated solids is also the highest reported out of all other pretreatments, achieving 95% yield of xylose and 99% yield of glucose at enzyme loadings as low as 2 mg g-glucan^{-1}. Unlike organosolv, the CELF pretreated solids can be washed with only water to reduce the acidity and remove residual THF, instead of requiring a separate solvent washing step. These results suggest that CELF pretreatment is less energy intensive and more economically competitive than organosolv. In the reactor, THF is a low viscosity fluid that can potentially support increased solids loadings by lubricating the biomass slurry through an auger-type reactor as the CELF reaction proceeds to solubilize the solids. THF can be renewably regenerated as an end-product by decarbonylation and hydrogenation of furfural, an inhibiting byproduct of the CELF reaction.

7.4 Comparison of promising catalytic conversion technologies

Catalytic dehydration of biomass sugars is a promising alternate route to conversion of lignocellulosic biomass via production of secondary fuel precursors that are catalytically upgraded into “drop-in” liquid fuels. Several promising integrated catalytic dehydration technologies exist such as the MTC reactor, biphasic process using THF, single phase γ-valerolactone (GVL) process, and the single phase CELF process. These sugar dehydration strategies can then be integrated with downstream catalytic reactors to upgrade the fuel precursors to aromatic gasoline blendstock, diesel blendstock, jet fuels, and long-chain hydrocarbons. Key features of each catalytic technology will be mentioned.

The countercurrent MTC reactor, developed by De Jong and Marcotullio, represents a modernized effort to achieve high furfural yields from xylose by a more efficient steam stripping operation designed to prevent furfural loss by removing it from the catalytically active phase as it is produced (De Jong and Marcotullio, 2010). The MTC reactor works by rapidly stirring a heated biomass slurry in a sulfuric acid solution while steam is injected from the bottom of the reactor to simultaneously extract furfural out of the system before it has a chance to degrade. The remaining cellulose-rich material could be used as feedstock for further reaction to produce either levulinic acid (LA) or subjected to enzymatic hydrolysis to produce glucose. Solids loadings are limited to 1:10 solids-to-water mass ratio to prevent clogging of the reactor. The group predicted furfural yield of 85% of theoretical from wheat straw and estimated an economic operating margin of \$8.57M year^{-1} for a 15 wet-ton hr^{-1} reactor capacity selling primarily furfural.

As shown in Figure 7.3, an advanced biphasic reaction strategy employing THF as an extracting solvent has been developed by Bond et al. to produce furfural and LA from biomass for their subsequent upgrade to branched and linear hydrocarbon fuels (Bond et al., 2014). The process first involves a pretreatment step by dilute acid reaction to recover up to 87% of the xylose from the raw biomass. The xylose-rich solution is then delivered to a biphasic reaction containing water, THF, NaCl, and HCl to produce furfural at over 90% yield from xylose (78% furfural yield from biomass). The furfural is simultaneously produced and extracted into the THF solvent phase during the biphasic reaction and delivered to a subsequent catalytic reactor to undergo aldol-condensation to form furfural-acetone-furfural (F-A-F) complexes. The un-hydrolyzed solids from the pretreatment reaction are separately delivered to an aqueous dehydration and hydrolysis reaction at 200 °C with 1.5 wt% H_2SO_4 to produce LA at 75.5% yield. The LA is then recovered and hydrogenated over RuSn/C catalyst to produce GVL. GVL is then decarboxylated over SiO_2/Al_2O_3 catalyst to produce butene isomers. Further oligomerization of the butene isomers over Amberlyst-70 catalyst produces C8+ alkenes. Further hydrogenation and hydrodeoxygenation of F-A-F and alkene products can produce branched or linear hydrocarbon fuels. Due to the elaborate process configuration, expensive recovery operation of the NaCl used to induce a liquid-liquid separation of THF and water, and the costly treatment options for the plant's wastewater stream, estimated selling price of distillate fuel products from this process is \$4.75 gal^{-1} fuel (Bond et al., 2014).

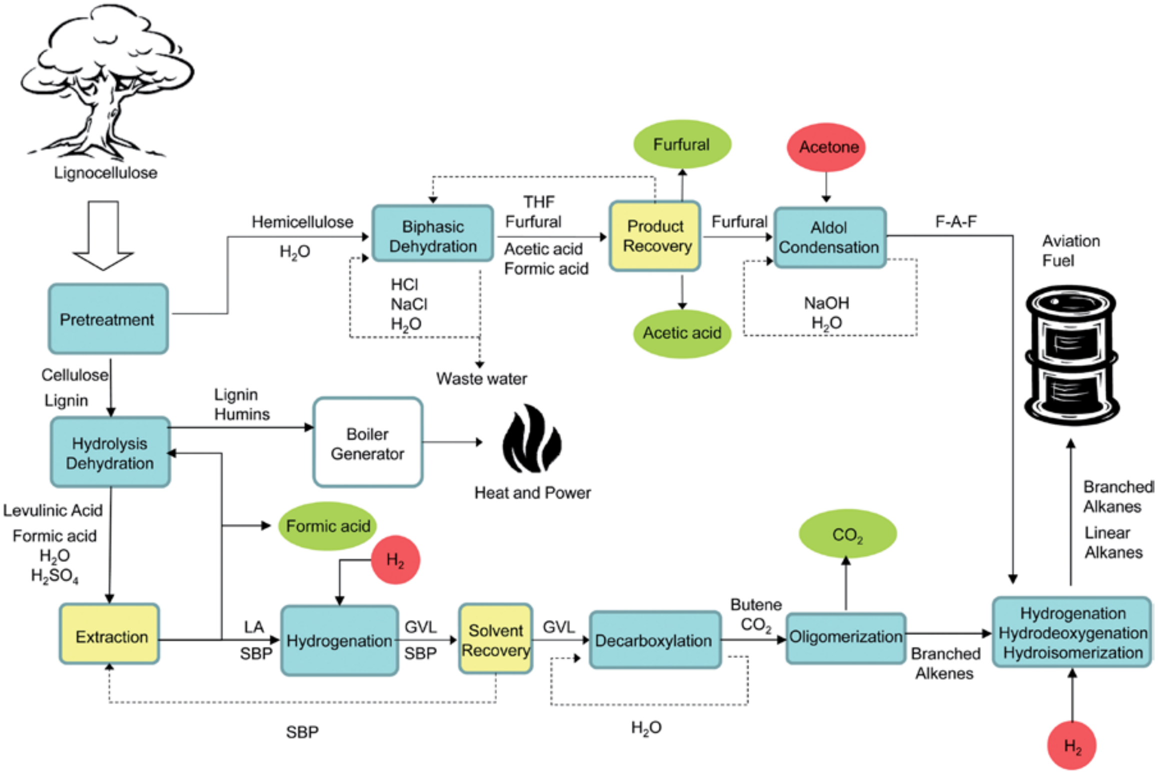

Figure 7.3. Conceptual overview of proposed biphasic THF process for the production of furfural, jet fuels, and acetic acid from lignocellulose. Abbreviations: FAF, furfural–acetone–furfural; GVL, γ-valerolactone; SBP 2-secbutylphenol; LA, levulinic acid; THF, tetrahydrofuran. (Taken verbatim from Bond et al., 2014)

Alonso et al. proposed using GVL as a water-miscible co-solvent to enhance biomass fractionation and catalytic dehydration of sugars to produce furfural and LA. Both furfural and LA are then used to produce additional GVL as a platform fuel precursor for integration with downstream catalytic upgrading. The GVL co-solvent process most closely resembles the functionality of the CELF process with key differences. Like CELF, the GVL-water mixture is capable of solubilizing raw biomass through biomass delignification and fractionation of the remaining holocellulose. The GVL co-solvent reaction conditions are 170 °C with 50 mM H_2SO_4 and produced furfural from corn stover at 81% yield and LA at 15% yield. Similar to CELF, the GVL process could only produce either furfural or LA in one pot at high yields due to their vastly different formation kinetics. However, unlike CELF, the optimal solvent concentration for the GVL process was determined to be 10 wt% water and 90 wt% solvent, which is extremely high for a single phase process, much higher than the 1:1 to 4:1 volume ratio optimal range for THF and water in CELF. Furthermore, GVL has a boiling point of 207 °C, which makes it much more difficult to recover from an aqueous solution containing THF. Nevertheless, GVL can be renewably produced from LA, much like THF could be synthesized from furfural, and is a promising fuel precursor with many different possible paths for upgrading. Future economic evaluation of the GVL process is necessary.

CELF production of secondary fuel precursors achieved the highest product yields of 87% furfural and 75% LA (Chapter 4). Although CELF process can be tuned like GVL to also co-produce 5-HMF at high yields (Chapter 5), the products that could be obtained at higher yields (i.e., furfural and LA) should be the investigated first when developing a plant model for a scale-up commercial demonstration. Considerations on the product selling price, feedstock cost, process yields, material costs, and throughput of the reactor are all important factors that influence the operating budget for a biorefinery. The break-even operating margin is an important first metric for evaluation of the economic viability of the CELF process.

7.5 Economic evaluation of a plant model based on CELF technology

A reasonable point-of-entry for a commercial operation to construct a biorefinery based on CELF technology would be to first produce furfural and LA from agricultural residues such as corn stover. These fuel precursors are preferred as Ag residues typically contain more pentosans for greater production of furfural and both furfural and LA are currently sold in world market with a furfural demand of over 300K tons year^{-1}. Both furfural and LA have a reported market price of approximately \$1000 ton^{-1} or higher, which exceeds the current price of crude oil by over 45%. Higher value chemicals can be produced in the first stages of commercial production to recuperate start-up costs, but as the market becomes saturated with furfural and LA, their selling price could potentially drop to as low as \$500 ton^{-1}.

As shown in Table 7.2, an example cost model to determine operating margin was developed for a hypothetical biorefinery plant based on CELF technology with a single reactor throughput of 60 tons hr^{-1} of wet biomass (50% moisture). This model assumes that THF is added to the biomass slurry at a 1:1 volume ratio with the total water content. The operating margin represents the break-even point for the plant's total operational costs and potential profit margin defined by the difference between the revenues from product sales and the material and utility costs listed in Table 7.2. The two largest contributors for material costs are biomass and THF loss. Solvent recovery is a particularly important factor that can be optimized to significantly reduce total material costs. The 3% THF loss value was determined experimentally at a 1 L reaction scale from

THF loss due to (1) reversible conversion of THF to 1,4-butanediol (BDO) and (2) distillation losses using lab-scale equipment. Both causes of the large assumed THF loss can be minimized with proper equipment to effectively separate THF from water and recover BDO product.

As additional production capacity becomes needed, the calculated operating margin in Table 7.2 can be scaled directly based on the number or throughput of individual reactors. The reactor sizing was determined from a previously-constructed auger press reactor (screw type “French” auger style) contracted by the Quaker Oats company and was used in the very last US commercial furfural plant, located in Belle Glade, Florida, that closed operation in 1997 (Zeitsch, 2000). These reactors were 1.8 m tall and 16 m long and were constructed out of stainless steel that was lined with acid-resistant bricks to withstand sulfuric acid corrosion. The cost model in Table 7.2 assumed a 35 wt% solids loading, year-round plant operation (8760 hrs year^{-1}), a conservative 3 wt% THF recovery loss (based on its miscibility index with water after phase separation), and a feedstock composition of 36% glucan, 24% xylan, and 14% lignin (typical of corn stover).

Table 7.2. Example calculation of the expected economic margin of a CELF biorefinery operating at 60 tons hr^{-1} capacity.

Materials Cost		**mass %/ton feed**	**tons/hr**	**tons/yr**	**$/ton**	**M$/yr**
	Biomass	34.60	20.76	181869	60.00	10.91
	Sulfuric Acid	0.69	0.42	3637	102.27	0.37
	Water	33.91	20.35	178231	0.25	0.04
	THF	30.80	18.57	157433		
	THF Loss (3%)	-	0.55	4856	2086	10.13
	Total Costs					**21.46**
Product Sales		**% yield**		**tons/yr**	**$/ton**	**M$/yr**
	Furfural	85 (of theoretical C5)		26980	1000	26.98
	Levulinic Acid	75 (of theoretical C6)		35193	1000	35.19
	Lignin	91 (mass yield)		23170	500*	11.58
	Formic Acid	50 (of theoretical C5 + C6)		13944	131.15	1.83
	Acetic Acid	3 (mass yield)		5456	600	3.27
	Residues	-		77126	62.56	4.82
	Revenue					**83.68**
Utilities Cost				**tons/yr**	**$/ton**	**M$/yr**
	Steam			40468	14.99	0.61
	Cooling Water			3125322	0.0272	0.09
	Electricity			9053760 kWh	0.068	0.62
	Total Costs					**1.31**
Operating Margin						**60.91**

*Lignin is typically a low value product. However, integration of lignin conversion process to higher value chemicals and fuels could increase its value at the expense of additional processing costs.

A sensitivity analysis for the cost model (from Table 7.2) is shown in Figure 7.4 that compares the influence of biomass feedstock cost and selling price of furfural (FF) and levulinic acid (LA) on the projected operating margin for the plant. As shown, both the selling price of FF and LA as well as the feedstock cost has a significant impact on the operating margin. At an optimistic feedstock cost of \$60 dry ton^{-1}, a 50% reduction in FF/LA selling price from \$1000 ton^{-1} to \$500 ton^{-1} results in a \$31M year^{-1} decrease in operating budget for each 60 ton hr^{-1} reactor. Thus, if market saturation for FF and LA should occur, significant process improvements would be needed to offset the operating budget losses to be able to sell FF and LA near the price point of crude oil (about \$683 t^{-1}, Chapter 2). The estimated capital expenses (CapEx) for the major equipment of a CELF-based plant operating on a single reactor is \$5.6M including installation. Economies of scale are beneficial to biorefineries such as this and the additional CapEx for each subsequent reactor is expected to be reduced.

In looking at other biorefinery models, CELF compares favorably to other technologies in terms of operating costs (Hayes et al., 2008; De Jong and Marcotullio, 2010; Win, 2005), largely due to the reduced consumption of steam and improved product yields compared to other furfural production strategies. For an equivalently sized plant, CELF nearly doubles the operating margin of the MTC reactor. These calculated operating margin and sensitivity analysis is a valuable tool to estimate the impact of key factors on the profitability and commercial success of the CELF process.

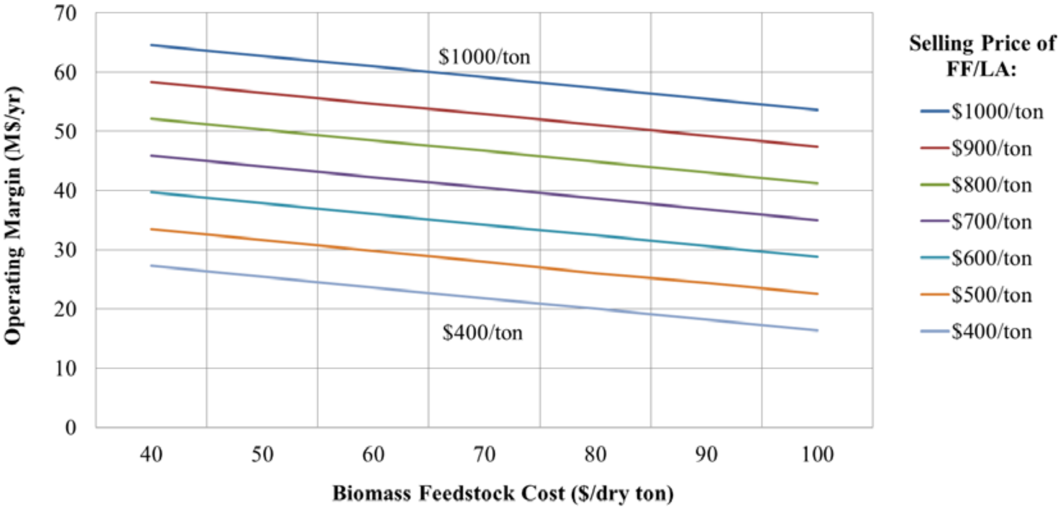

Figure 7.4. The influence of biomass feedstock cost and selling price of furfural (FF) and levulinic acid (LA) on the projected operating margin for a CELF biorefinery operating at 60 tons hr^{-1} throughput.

7.6 Conclusions

Several leading pretreatment and catalytic processes were compared with CELF in terms of performance, process complexity, and economic feasibility. CELF process was determined to be a superior pretreatment technology as it achieved higher sugar yields using lower enzyme dosages than hydrothermal, dilute acid, and organosolv pretreatment strategies. Unlike ethanol in organosolv pretreatment, THF could be easily recovered by room temperature vacuum distillation and can be washed from the pretreated solids with water. Recovery of THF allowed the extracted lignin to precipitate so a large quantity of water would not be needed to dilute the liquid product stream as typically performed in organosolv pretreatment. Optimized reaction temperature for CELF was also the lowest among the other three pretreatments. When compared to other promising catalytic conversion processes, CELF was found to be very similar to the GVL co-solvent process. However, unlike CELF, GVL required a very high 90 wt% solvent concentration in order to achieve decent furfural yields (81%), whereas CELF could obtain 87% furfural yield at a much lower 50 vol% THF concentration in water. The major material and energy costs were calculated for the production of furfural and LA from corn stover for a proposed CELF biorefinery. The break-even operating margin was \$60.9M year^{-1} for a CELF biorefinery operating at a 60 wet-ton hr^{-1} per reactor scale, a furfural and LA selling price of \$1000 ton^{-1}, and a feedstock cost of \$60 dry-ton^{-1}. Major driving factors for the plant economics were the product selling price, feedstock cost, solvent recovery efficiency, and capital expenses.

7.7 References

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Chapter 8. Conclusions and Recommendations

8.1 Concluding remarks

In this dissertation, the inception, development, and application of a novel multi-functional technological platform called CELF (Co-solvent Enhanced Lignocellulosic Fractionation) is reported that applies tetrahydrofuran (THF) as a renewable co-solvent to augment the aqueous processing of lignocellulosic biomass to derive valuable chemical precursors at high yields. CELF is capable of consolidating biomass solubilization, delignification, and sugar dehydration steps into a single reaction and is relatively agnostic to feedstock type. CELF was also used to reveal key insights on the reaction kinetics of sugar destruction and the importance of lignin removal on reducing biomass recalcitrance. CELF can be used for biomass pretreatment to aid in the release of primary fuel precursors such as fermentable sugars or used for catalytic processing of biomass to produce secondary fuel precursors such as furfural, 5-HMF, and LA.

For performing CELF at higher reaction severities (>170 °C, 40 to 60 min), dilute sulfuric acid (1 wt%) was employed to produce furfural (87% yield) and LA (75% yield) from maple wood in a two-step reaction strategy. By replacing sulfuric acid with more selective metal halide acid catalysts, such as $FeCl_3$, CELF was optimized to co-produce furfural (95% yield) and 5-HMF (51% yield) in a single “one-pot” reaction. In both cases, the product yields that were achieved represented the highest reported from biomass thus far. At lower severities (150 °C, 20 to 30 min, 0.5% H_2SO_4), CELF can be combined with enzymatic hydrolysis as a pretreatment strategy to achieve nearly theoretical yields of xylose, glucose, and arabinose from corn stover using enzyme dosages as low as 2 mg-enzyme g-glucan^{-1}. In all cases, CELF augmented the performance of typical aqueous dilute acid reactions.

After CELF reaction, up to 91% of the original lignin content in biomass could be extracted from the solids and dissolved into the liquid phase. Since THF has a low boiling point (66 °C), room temperature vacuum distillation was sufficient to recover THF from water. Afterwards, the dissolved lignin precipitated out of solution as a solid that could then be collected, washed, and crushed into a fine lignin powder. CELF extracted lignin was analyzed by FT-IR and found to contain less impurities such as residual sugars and acid-insoluble humins than lignin from concentrated acid-extracted lignin. CELF lignin was also found to be more oxidized and depolymerized such that it can be readily re-dissolved in polar solvents such as DMSO, methyl-THF, THF, and dioxane. This enables CELF lignin to be highly suitable for catalytic upgrading to valuable chemicals and fuels. CELF can also serve as a unique research tool to study lignin destruction chemistry and the effects of lignin removal on pretreated solids.

Biomass deconstruction is a multi-tiered phenomenon that requires intimate understanding of the structure and destruction chemistries of each biomass fraction. Reaction kinetics play a pivotal role in dictating the possible distribution of end products after thermochemical deconstruction of lignocellulosic biomass. Targeting appropriate co-products based on their selectivity from sugars and on their formation and destruction chemistry is crucial to achieving high overall product yields. It was discovered that different Brønsted and Lewis interactions of various acid catalysts can influence the formation and destruction chemistries of sugar products as well as the rate of sugar hydrolysis. For example, $FeCl_3$ was found to be an ideal acid catalyst for co-producing furfural and 5-HMF from biomass. $FeCl_3$ has a moderate Lewis acidity compared to other metal halides that is beneficial for highly selective production of both furfural and 5-HMF by an open-chain sugar dehydration mechanism. In addition, $FeCl_3$ is also a strong Brønsted acid to achieve rapid sugar hydrolysis and dehydration to minimize competing side reactions. Although THF was not needed to achieve high LA yields, LA formation and furfural destruction kinetics were vastly different which prevented LA from being co-produced with furfural, necessitating a second independent reaction to produce LA. Inexpensive sulfuric acid was deemed sufficient for biomass pretreatment using CELF to produce primary fuel precursors because its strong Brønsted acidity worked well with THF to dissolve hemicellulose and lignin without degrading the sugars.

Preliminary economic assessment of the CELF process looks promising. The primary factors that influence the operating margin for a commercial CELF biorefinery are feedstock cost, selling price, and solvent recovery. Production of furfural and LA is a recommended first step due to their potential for high yield production and an existing global market to sell them. As the supply of furfural and LA becomes saturated, the selling price for both could drop as low as \$500 ton^{-1} to compete in the liquid fuels market. In response, process optimizations and economies of scale would need to offset the loss of operating margin in order for the plant to remain profitable.

8.2 Future Recommendations

Future integration of the CELF process with downstream microbial fermentation and catalytic production of fuels is necessary to demonstrate the commercial potential of this technology. Although we have shown that CELF pretreated solids can be successfully used in fermentation processes such as SSF to produce ethanol, there is still a need to achieve similar results using the liquid products from CELF reaction that are rich in pentose sugars. The liquid product stream would likely contain contaminants that are toxic to microbial fermentation so additional processing to detoxify this liquid stream would be necessary. For a future drop-in fuels strategy, CELF can be combined with a fuel catalyst to immediately hydrogenate or upgrade fuel precursors to fuel products as soon as they are dehydrated from sugars. This concept entails simultaneous production and conversion of fuel precursors to more stable fuel products so that product degradation is minimized. This can be accomplished by either placing the fuel catalyst directly inside the CELF reactor or in a separate downstream reactor where the fuel precursors produced from CELF can be captured and fed to the catalytic reactor in the vapor phase. Reactive distillation is another option to consolidate the catalytic hydrogenation and recovery of fuel products.

Solvent and catalyst recycling strategies must be developed to minimize THF and catalyst losses. Since THF is a switchable solvent, it can be induced to become immiscible with water to serve as an extracting solvent. After CELF reaction, the aqueous phase would contain water-soluble products and the lighter organic phase would contain the extracted furfural and 5-HMF. If the partition coefficient of the induced biphasic system is sufficiently high, heavier aqueous layer can be decanted to recycle the catalyst and the fuel precursors can be directly recovered from THF. The switchable solvent may also be used to recover small amounts of 1,4-butanediol (BDO) from the aqueous phase produced by dehydration of THF during CELF reaction. The reversible formation of BDO from THF is influenced by the concentration and type of acid used. BDO is a valuable chemical precursor in the plastics industry with a market of about 1.5 million tons in 2012 and could be considered a valuable by-product from the CELF process if recovered.

Additional physical and chemical characterization of the solids produced from CELF will also be needed. In particular, the lignin extracted from CELF is valuable and can be characterized by 2D NMR to determine its molecular weight, degree of polymerization (DP), and any exposed functional groups that may be useful for catalytic upgrading to high-value lignin-based products. Instead of combusting CELF lignin for heat energy, which represents the lowest value strategy, several lignin conversion strategies should be explored for increasing the net worth of lignin. Microbial anaerobic digestion of CELF lignin could produce biogas for methane production, pyrolysis of lignin can produce similar products and monomeric lignols, hydrogenolysis/hydrogenation of lignin produces the highest value products such as phenols and gasoline fuels, and oxidation of lignin to produce aldehydes. Alternatively, the lignin could also be modified and applied to enhance the properties of cement and concrete.

Other than using CELF to produce primary and secondary fuel precursors, the CELF pretreated solids can also directly serve as a feedstock for many other purposes. The crystallinity, particle size distribution, and mechanical properties of the glucan-rich pretreated material from CELF could be investigated to determine its suitability for non-fuel applications such as producing paper pulp or nanocellulose. The high purity of cellulose also allows this material to be suitable for pharmaceutical and neutraceutical applications in future research. Due to the highly digestible nature of CELF pretreated material, it is also useful for studying plant recalcitrance and enzyme performance compared to more recalcitrant materials.

In order to understand the physical upper-limits of the CELF process, investigation into increasing the particle size, solids loading, and solvent concentrations should be attempted. Additional scale-up of the CELF process should be performed in a screw-type reactor to study its continuous operation. In preliminary testing, CELF has also demonstrated promising results when used in a flow-through reactor. In this configuration, THF co-solvent solution could be pumped through a tube reactor containing biomass to continuously react and remove biomass components. At moderate temperatures, THF and very dilute sulfuric acid (~5 mM) was sufficient to remove hemicellulose, lignin, and cellulose fractions from poplar wood by flow-through configuration without any additional enzyme needed. Development of more detailed computer plant models is necessary to obtain accurate predictions for the materials and energy consumption for various possible CELF process configurations to determine their economic feasibility. A more detailed economic model and feasibility study would minimize start-up risks.

The future of lignocellulosic biofuels looks bright; advancements in both the engineering of plants and the development of conversion technologies are paving the way to viable solutions for achieving energy independence. In the near term, the economic production of renewable fuels that are compatible with the existing transportation and energy infrastructure is vital. Significant technological, societal, and political milestones still remain to influence the future landscape of bioenergy. For example, an affordable integrated turn-key biorefinery would be hugely beneficial to sustain the energy demands of third-world or developing nations. The adoption, acceptance, and support of green energy by the general public would be needed to discourage the use of fossil resources and encourage energy conservation. New government policies can regulate the registration of new advanced biofuels for their production and use as transportation fuels. For future research in the field of biomass conversion, greater emphasis should be placed on developing tools and methods using real feedstock so that measurable gains in the laboratory can be more impactful to commercial implementation.