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Methods of Lignin Extraction to Enable its Integration as a High Value Feedstock in a Biorefinery

A Thesis submitted in partial satisfaction
of the requirements for the degree of

Master of Science

in

Chemical and Environmental Engineering

by

Aakash Darshan Parikh

September 2017

Thesis Committee:

Dr. Charles Wyman, Chairperson

Dr. Philip Christopher

Dr. Ian Wheeldon

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The Thesis of Aakash Darshan Parikh is approved:

Committee Chairperson

University of California, Riverside

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CHAPTER 1

Introduction

1.1 Overview

Industrialization combined with population growth has caused a significant spike in the energy demand. Fossil fuels have been the traditional energy source that serves about 80% of the energy demand in the form of coal, natural gas, and oil, as shown in Figure 1.1. However, these fossil fuel reserves are diminishing and eventually will be insufficient to meet energy demands. Moreover, use of these fossil resources negatively affects the environment and is a major contributor to global climate change and air quality issues (Goldemberg 2007) . Thus, rapid shift to sustainable energy sources such as solar or wind, is a necessity.

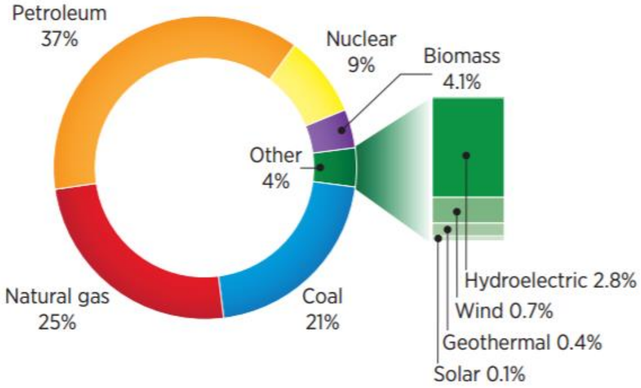

Figure 1.1 Contribution of various energy sources as of 2009 (Taken verbatim from US billion-ton update).

Lignocellulosic biomass, an omnipresent carbon-source, presents the only known viable alternative to fossil resources for large scale sustainable production of liquid fuels that our society relies on so heavily. Thus, it has been investigated to replace oil based fuels and chemicals and most importantly gasoline (Lynd et al. 1991). Over 1.4 billion dry tons of biomass residues in the form of energy crops and agricultural and forest residues has been projected to be available in the US by 2030, with the potential to produce enough fuels to offset a large portion of fossil energy use. Also, it will play a key role in decarbonizing the transportation sector and meeting the ambitious 2 °C target agreed at the COP21 meeting (Lynd et al. 2017). However, overcoming natural resistance of lignocellulosic biomass to conversion into fuels due to its structure is a challenge that is currently addressed by some type of physical or chemical modification for biological routes favored for biomass conversion into fuels. As illustrated in Figure 1.2, this pretreatment opens up the biomass structure to make cellulose accessible to enzymes for breakdown to monomers. However, because pretreatment is one of the most expensive steps in biological processing of cellulosic feedstocks to fuels, developing an efficient and a low-cost pretreatment technology is crucial to achieving commercial scale production of cellulosic fuels and chemicals (Mosier et al. 2005). In this regard, it is imperative that maximum amounts of sugars are captured from the biomass during pretreatment and subsequent enzymatic hydrolysis as sugar yields govern the final yields of all downstream high value products.

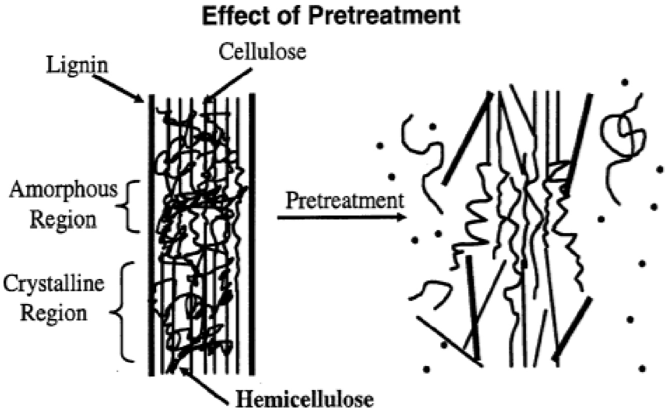

Figure 1.2 Breakdown of biomass structure into cellulose, hemicellulose and lignin (Taken verbatim from Mosier et al. 2005).

Of the numerous pretreatment technologies that have been tried over the decades, dilute sulfuric acid catalyzed pretreatment is considered the most promising technology mainly because of high hemicellulose sugar recovery in monomeric form and high yields of sugars from pretreated solids by enzymes (Torget et al. 1991). Although other pretreatments such as steam explosion, organosolv, ammonia fiber expansion(AFEX), and ionic liquids offer some important advantages, they also suffer from limitations that must be overcome to be cost competitive at large scale (Sousa et al. 2009). In this study, we present a novel pretreatment technology designated as Co-solvent Enhanced Lignocellulosic Fractionation (CELF) that employs THF as a miscible co-solvent in water with a dilute acid. In one configuration, CELF can solubilize up to 90% of the lignin and nearly all of the hemicellulose to produce a highly digestible solid comprising of over 90% cellulose that is amenable for complete saccharification to fermentable glucose using 90% less cellulolytic enzyme loadings than conventional dilute sulfuric acid (DSA) pretreatment. For example, at milder conditions of 150 °C and 25 mins than typically needed for dilute acid pretreatment alone with the same amount of sulfuric acid catalyst, up to 95% of the theoretical yield of glucose, xylose, and arabinose was obtained from raw corn stover needing only only 2 mg enzyme g glucan^{-1} of Accellerase® 1500 from DuPont. Thus, CELF technology demonstrated a considerable advantage over DSA pretreatment in enabling production of ethanol from biomass at lower costs (Nguyen et al. 2015).

Interestingly, under more severe conditions of 170 °C and 60 mins with $FeCl_3.6H_2O$ as the catalyst, a 95% yield of furfural (FF) and 51% yield of 5-hydroxymethyl furfural (5-HMF) was obtained from maple wood (Cai et al. 2014). Both FF and HMF can be catalytically upgraded to a myriad of industrially relevant solvents and fuel products. For example, furan-2,5-dicarboxylic acid, used in the production of polyesters, is produced via oxidation of HMF (Bozell & Petersen 2010). Furfural can be catalytically hydrogeoxygenated to methylfuran (MF) which has high research octane number (131) and low water solubility (7 g L^{-1}), thus may be suitable as a blendstock in gasoline (Sitthisa et al. 2011). Analogously, HMF can be hydrodeoxygenated to dimethylfuran (DMF), another potential gasoline blendstock. Therefore, in addition to the ability for CELF to produce sugars for biological fermentation, the reaction conditions can also be tuned to produce FF and HMF at high yields for their catalytic conversion to liquid transportation fuels, as illustrated in Figure 1.3.

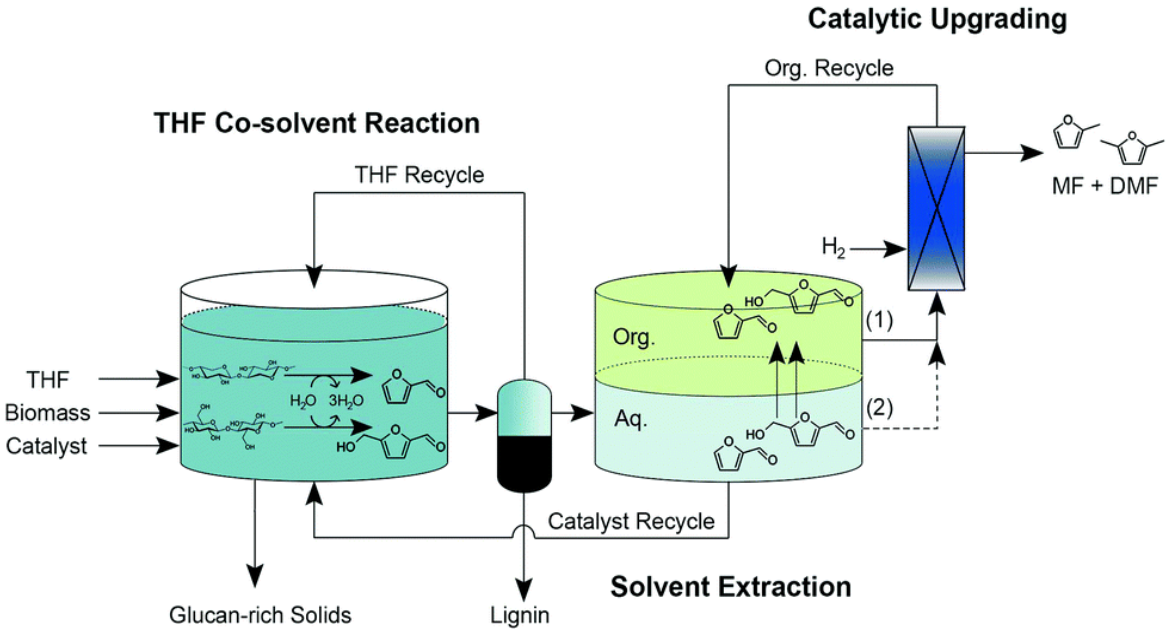

Figure 1.3 Production of methylfuran (MF) and dimethyfuran (DMF) from FF and HMF, respectively, that can be produced with high yields by CELF pretreatment of biomass (Taken verbatim from Cai et al. 2014).

A salient feature of CELF pretreatment is the ability to solubilize lignin during pretreatment and subsequently recover it from solution by a variety of options. Converting lignin into high value products is vital to making CELF cost effective and can accelerate development of a profitable biorefinery when combined with effective conversion of cellulose and hemicellulose to ethanol or other products (W. Wang et al. 2017). One potential pathway for employing CELF to produce furan based gasoline blends is illustrated in Figure 1.3.

1.2 Thesis organization

In this thesis, I sought to develop and evaluate lignin extraction strategies for different process configurations involving CELF pretreatment and choose the most appropriate strategy based on lignin characteristics and overall process economics. In line with this objective, Chapter 2 focuses on optimization of CELF pretreatment to produce FF and HMF from poplar wood using a metal halide catalyst. After time and temperature are optimized to maximize FF and HMF production, extraction strategies are applied to recover lignin for characterization by NMR and Gel Permeation Chromatography (GPC). Chapter 2 includes a technoeconomic analysis of a process for production of MF and DMF from FF and HMF using a non-noble metal catalyst system. Lignin extraction is integrated with this process, and a sensitivity analysis is employed to identify key process parameters affecting overall process economics. In Chapter 3, the goal is to improve the fermentability of sugars in the CELF hydrolyzate by applying a detoxification strategy to remove potential inhibitors to yeast. The detoxification strategy employed, uses an organic solvent to extract THF and soluble phenols from the hydrolyzate, and the sugars remaining in the aqueous phase are then fermented to ethanol by a genetically modified *S. cerevisiae* yeast. Chapter 4 in the thesis follows with an evaluation of two distinct lignin extraction methods for lignin recovery yields, molecular weight, and inter-unit linkages: 1) diluting the hydrolyzate with water 2) boiling the hydrolyzate. These extraction strategies are applied to the lignin produced by two different CELF reaction strategies: one focused on maximizing ethanol yields and the other directed at maximizing production of furans.

Finally, chapter 5 concludes the thesis with a summary of key findings and recommendations for future research.

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CHAPTER 2

Process Strategy to Integrate Co-production of Methylated Furans from Poplar and Recovery of Lignin for Valorization*

***** This chapter was written under the guidance of Dr. Charles Cai who developed the CELF technology at UCRiverside. Nikhil Nagane, a PhD student at UCRiverside, performed the optimization of time, temperature and solvent ratios for CELF reaction with data shown in Figure 2.2 and 2.3. This chapter includes data collected in collaboration with University of Tennessee Knoxville (UTK) as a part of US Department of Energy EERE Bioenergy Technology Office. Dr. Xianzhi Meng from Department of Chemical and Biomolecular Engineering at UTK performed GPC and HSQC NMR characterization of all the lignin samples with data shown in Tables 2.1 and 2.2.

2.1 Introduction

Developing methods to cost effectively convert lignocellulosic biomass to liquid transportation fuels is crucial in the wake of current climate change and to meet the ambitious 2 °C target laid down by the Conference of Parties (COP21) for 2022 (Lynd et al. 2017). The consumption of lignocellulosic feedstocks is expected to increase due to abundant availability, low cost and due to decrease in the fossil fuel reserves. In addition, cellulosic biomass can act as a long-term approach to reduce greenhouse gas (GHGs) emissions and recycle atmospheric CO_2, thereby decarbonizing the environment (Sharma et al. 2017).

Raw biomass typically consists of 25-35% cellulose, 20-30% hemicellulose and 25-35% lignin, and it is vital to utilize all three fractions for a biorefinery to be economically feasible. Hemicellulose and cellulose have the potential to be converted into a myriad of platform chemicals of which the major ones are furfural(FF), hydroxymethylfurfural(HMF), and levulinic acid(LA), which are formed by dehydration of monomeric glucose and xylose (Q. Wang et al. 2017). Furfural can be a precursor for variety of chemicals such as tetrahydrofuran (THF), methyltetrahydrofuran (MTHF) and methylfuran (MF), whereas HMF can be converted to 2,5-dimethylfuran (DMF), a gasoline alternative or 2,5-bis(hydroxymethyl)-furan, an additive in polymers (Wojcik et al. 1948,Merat et al. 1990, Pace et al. 2012, Chheda et al. 2007, Dutta et al. 2012). Moreover, lignin, which is the second most abundant polymer in nature, has been traditionally utilized to provide process and export energy by burning. However, due to the presence of aromatic rings and suitable rheological properties, lignin has the potential to be converted into high value chemicals and solvents, thereby increasing biorefinery profitability (Doherty W.O.S., Mousavioun Payam 2011).

In this study, we present a strategy to integrate high yield co-production of FF and HMF directly from poplar wood and simultaneously extract and depolymerize lignin to produce a highly refined solid, in a simple one-pot single phase homogeneously catalyzed system. We achieved high FF and HMF yields using our THF and water based co-solvent mixture along with an $FeCl_3$ catalyst to fractionate biomass. This co-solvent enhanced lignocellulosic fractionation (CELF) caused extensive delignification of biomass and high sugar dehydration product yields by solubilizing cellulose and hemicellulose (Cai et al. 2014). We optimized CELF reaction conditions based on reaction time, temperature, and THF concentration in water to produce FF and HMF in high yields from poplar wood. It has been shown that FF and HMF can be hydrogenated over a Cu-Ni catalyst on a titania support to produce methylfuran (MF) and dimethylfuran (DMF) with high yields (Seemala et al. 2017). The resulting hypothetical biorefinery for integrated production of furans from FF and HMF designed from experimental data, is used to estimate operating margins (OM), and sensitivity analyses (SA) which identifies key cost drivers of the process. Moreover, two distinct strategies for lignin extraction were evaluated based on the lignin recovery, structural characteristics, and downstream processing of the hydrolyzate stream remaining after lignin extraction. Lignin recovered at optimized conditions was characterized by GPC and HSQC NMR for its average molecular weight and inter-unit lignin linkages distribution respectively.

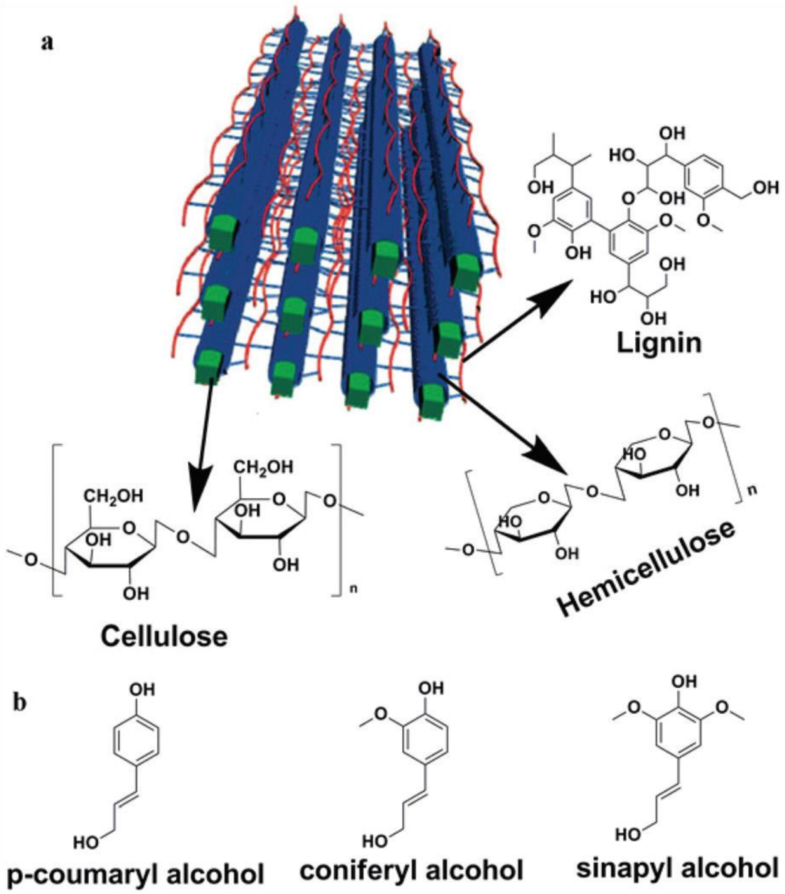


Figure 2.1 a) Primary components of lignocellulosic biomass: Cellulose, Hemicellulose and Lignin. b) Primary monolignol components from lignin (Taken verbatim from Liu et al. 2015).

2.2 Experimental

2.2.1 Materials

Poplar wood was provided by the National Renewable Energy Laboratory (NREL, Golden, CO) and was milled to obtain less than a 1 mm particle size using a laboratory mill (Model 4, Arthur H. Thomas Company, Philadelphia, PA). The composition of poplar wood was measured to be 45±0.5% glucan, 14±0.3% xylan and 22±0.2% K-lignin using NREL laboratory analytical procedure in triplicates. Other materials needed for biomass composition to total 100% were not characterized in this study as small amounts were difficult to quantify using HPLC. All the pentosans were grouped together as xylan and all hexosans as glucan. THF (>99% purity, Fischer Scientific, NJ) was used in all the CELF pretreatment reactions. Hydrated ferric chloride catalyst was purchased from Sigma Aldrich (St.Louis, MO, US).

2.2.2 Analytical procedures

Liquid samples were analyzed by an Agilent 1200 HPLC system with a Bio-Rad Aminex HPX-87H column and RI detector along with appropriate calibration standards and with an eluent flow rate of 0.6 ml min^{-1}. The eluent used was 5mM sulfuric acid. The chromatograms were integrated using Empower 2 software package.

2.2.3 Poplar wood pretreatment

All pretreatment reactions were performed in a 1litre Hastelloy Parr reactor (236HC Series, Parr instruments Co., Moline, IL) equipped with a double-stacked pitch blade impeller rotated at 200 rpm. The THF co-solvent mixture for each reaction was prepared by volume addition of THF to water starting from 1:1 (THF 50% v/v) to 7:1 (THF 87.5% v/v). Biomass solid loadings were 5wt% (40 g) based on dry weight and were calculated based on the total mass of the reaction mixture. A 1wt% loading of $FeCl_3.6H_2O$ catalyst was added based on its equivalent anhydrous mass and was calculated based on THF-water co-solvent mixture weight (760 g). Then, the contents of the reaction were soaked overnight at 4 °C. All the poplar wood reactions were heated using a 4kW fluidized sand bath (Model SBL-2D, Techne Princeton, NJ), and temperature was controlled to within ±1 °C measured by an in-line thermocouple (Omega, K-type). The sand bath was preheated to 380 °C to maintain heat-up time less than 4 mins. At the end of each reaction, the reactor was quenched in a large water bath at room temperature. The solids were then separated from the hydrolyzate using vacuum filtration through a glass fiber filter paper (Fischer Scientific, Pittsburg, PA). The final mass and density of liquid fractions were measured for mass balance and yield calculations. The liquid fractions were analyzed by HPLC.

2.2.4 Lignin extraction

Two strategies were applied to extract lignin from CELF hydrolyzate produced by vacuum filtering the contents after the reaction. In the first method, lignin was precipitated by selectively removing THF by boiling the hydrolyzate at 90 °C. The precipitated lignin was then filtered, washed and dried to obtain pure powder. In the second method, we precipitated the lignin by diluting the CELF hydrolyzate 5 times with water on a mass basis. Precipitated lignin was then recovered by filtering, washing, and drying.

2.2.5 Cellulolytic enzyme lignin(CEL) isolation

Cellulolytic enzyme lignin (CEL) was isolated from poplar by a method adapted from the literature. In brief, biomass samples were milled and screened to a 0.42 mm using a Wiley mill (Thomas Scientific, Swedesboro, NJ) and then Soxhlet-extracted with Toluene/ethanol for 8 hrs. The extractives-free samples were ball-milled in a porcelain jar with ceramic balls via Retsch PM 100 (Newton, PA) at 600 rpm for 2.5 hrs. The ground powder was then subjected to enzymatic hydrolysis in acetate buffer (pH 4.8, 50 °C) using CTec 2 and HTec 2 (1:1), as the enzyme (150 mg protein loading/g biomass) for 48 hrs. Next the residue was isolated and hydrolysed one more time with freshly added enzyme and buffer. To remove any remaining enzyme, the recovered solids were treated with Streptomyces griseus protease (Sigma-Aldrich) at 37 °C overnight followed by deactivation at 100 °C for 10 mins. The enzyme-treated lignin rich residue was then extracted twice with 96% p-dioxane/water mixture at room temperature for 48 hrs. The extracts were combined, rotary evaporated to reduce the volume under reduced pressure (55 °C), and freeze dried to recover cellulolytic enzyme lignin.

2.2.5 Lignin molecular weight analysis

Oven-dried lignin samples (~20 mg) were acetylated with a 2.0 mL acetic anhydride/pyridine (1:1, v/v) mixture stirred at room temperature for 24 hrs. After that, 25 mL of ethanol was added to the reaction and left for 30 mins. The solvent was then removed by rotary evaporation under reduced pressure (35 °C). The addition and removal of ethanol was repeated at least twice until only a trace of acetic acid was left in the acetylated sample. Samples were dried at 45 °C overnight in a VWR 1400E vacuum oven and then dissolved in THF at a concentration of ~1 mg mL^{-1} prior to the GPC analysis. The molecular weight distributions of the acetylated lignin samples were analyzed on a GPC SECurity 1200 system operated on Agilent HPLC 1200 with four Waters Styragel columns (HR1, HR2, HR4, and HR6) and an UV detector (270 nm). Polystyrene narrow standards were used to prepare the calibration curve, and THF was used as the mobile phase at a flow rate 1.0 mL min^{-1}.

2.2.6 HSQC NMR analysis of lignin samples

2D ^{13}C-^{1}H HSQC NMR spectra of lignin samples were acquired with a Bruker Avance 400 MHz spectrometer. A standard Bruker heteronuclear single quantum coherence pulse sequence was used with the following conditions: 210 ppm spectral width in F1 (^{13}C) dimension with 256 data points and 13 ppm spectral width in F2 (^{1}H) dimension with 1024 data points, a 90° pulse, a $^{1}J_{C\text{-}H}$ of 145 Hz, a 1.5 s pulse delay, and 32 scans. ~50 mg of dry lignin samples was dissolved in deuterated DMSO. The central DMSO solvent peak was used for chemical shifts calibration (39.5 ppm, 2.50 ppm). The relative lignin monomer compositions and interunit linkage abundance were estimated semi-quantitatively using volume integration of contours in HSQC spectra. For monolignol compositions of S, G, H, and PB measurements, the $S_{2/6}$, G_2, $H_{2/6}$, and $PB_{2/6}$ contours were used with G_2 integrals doubled. The C_α signals were used for contour integration for the estimation of interunit linkages such as β-O-4, β-β, and β-5. Data processing was performed using Top Spin 2.1 software (Bruker BioSpin).

2.3 Results and discussion

2.3.1 Optimization of CELF reaction conditions for FF and HMF production

The reaction times and temperatures were optimized to obtain the highest HMF and FF yield individually at a 3:1 THF:water ratio. As shown in Figure 1, the highest HMF yield of 58.82% was achieved at 180 °C and 20 mins reaction time, whereas the highest furfural yield of 85% was achieved at 170 °C and 40 mins reaction time. Although the highest solid conversion yield was observed at 190 °C and 200 °C, it did not result in the maximum yields for FF and HMF, as the dehydration of xylose and glucose is much faster than their hydrolysis and both are susceptible to further degradation (Cai et al. 2013). Since poplar consists of 45% glucan which is the source for HMF, optimum reaction conditions for maximum HMF production were chosen for the overall process.

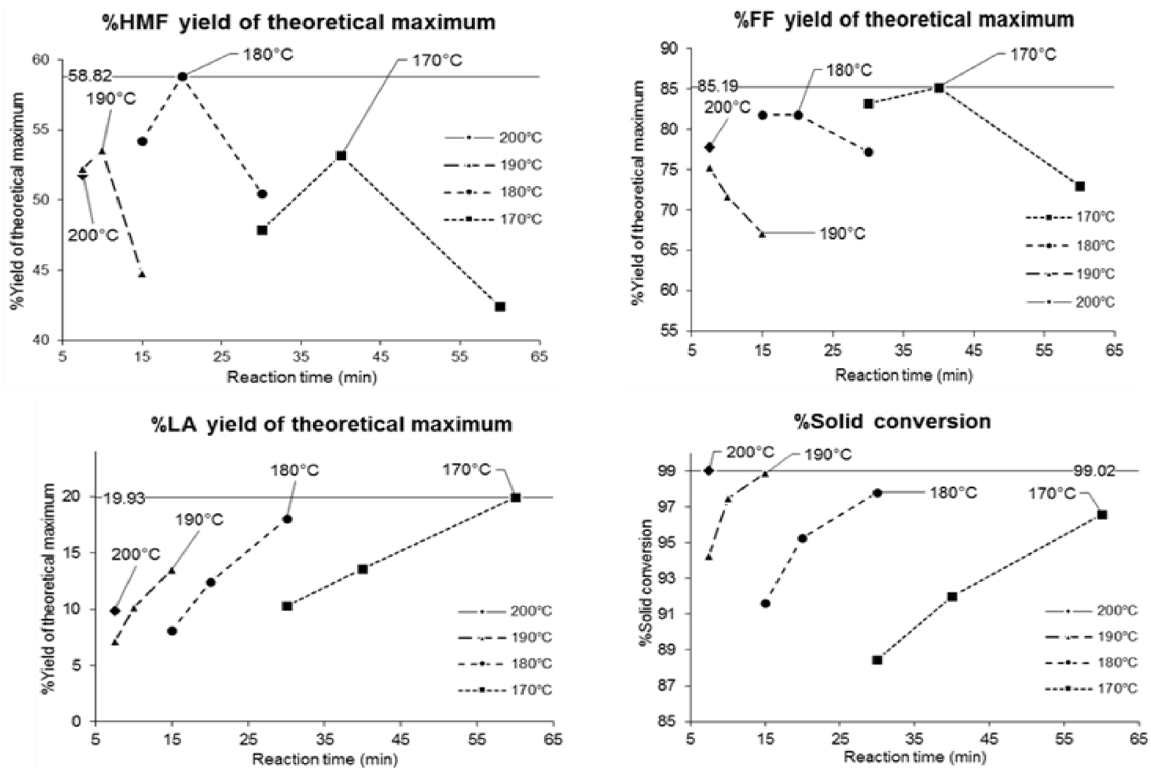


Figure 2.2 Determination of temperature and reaction time for maximum yields of HMF, FF, and LA for poplar wood at a 3:1 THF:water, 5wt% solids loadings, and 1wt% $FeCl_3.6H_2O$.

Once the time and temperature were optimized, THF concentration in water was optimized with increasing THF by volume. Figure 2.2 shows the trend of HMF, FF, LA and sugar yields of the reactions with increasing THF concentration in water. Maximum yield of FF (93.5%) and HMF (66%), was observed at 4:1 THF:water co-solvent ratio. Moreover, yields for LA decreased with increasing THF concentration, suggesting that THF could be reducing degradation of HMF to LA in water. Beyond 4:1 THF:water solvent ratio, yields for HMF and FF dropped, likely due to a phase separation of THF and water at higher temperatures that shifted from THF's role from enhancement as a co-solvent to serving primarily as an extracting solvent. This is further evidenced by the reduced solid conversion yields at the higher THF concentrations in water.

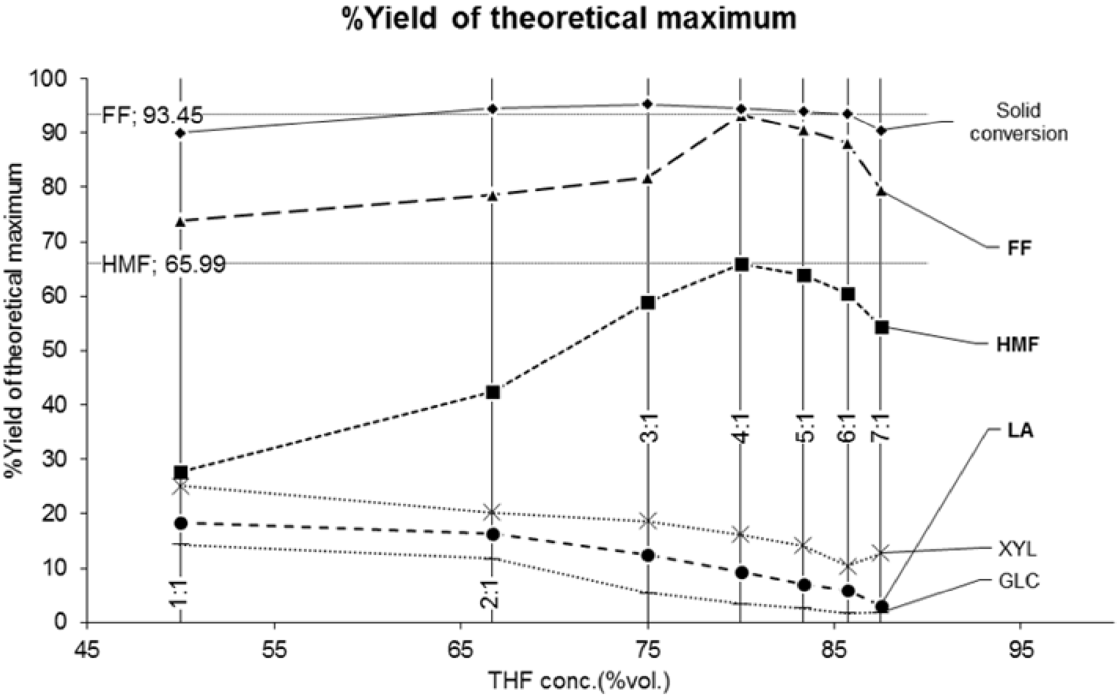


Figure 2.3 The effect of THF concentration in the co-solvent phase on HMF and FF yields from poplar wood at 180 °C for 20 mins, 5wt% biomass solids loading, and 1wt% ferric chloride. Note: FF-furfural, HMF – 5-hydroxymethylfurfural, LA – levulinic acid, XYL – xylose and GLC – glucose.

2.3.2 Characterization of lignin after CELF reaction

The lignin was extracted from CELF hydrolyzate prepared at the conditions optimized for producing FF and HMF by two methods: boiling hydrolyzate to remove THF and diluting hydrolyzate with water. The molecular weight of the extracted lignin was then measured by Gel Permeation Chromatography (GPC). The characteristics of the lignin samples extracted after CELF reaction were then compared to Cellulolytic Enzyme Lignin

(CEL) extracted from poplar wood. The GPC results in Table 3.1 shows that CELF reaction causes high degree of depolymerization as the average molecular weight (M_w) of the extracted CELF lignin (1320 and 1245 g mol^{-1}) was much lower than that of the CEL poplar sample (14352 g mol^{-1}). Moreover, the lignin molecular weight obtained by boiling showed greater depolymerization. This outcome could be due to evaporation of a small quantity of water during THF boiling resulting in precipitation of low molecular weight lignin, thereby lowering the average molecular weight. The polydispersity index (PDI), that indicates the uniformity of the fragments size, suggested that lignin extracted after CELF had a significantly higher uniformity (PDI is 1.6) compared to CEL poplar sample (PDI is 6.5).

Table 2.1 Gel permeation chromatography data. M_w is the weight average molecular weight, M_n is the number average molecular weight, and PDI is the polydispersity index.

Sample	Method type	Mw	Mn	PDI
CELF lignin	Diluted	1320	823	1.6
	Boiled	1245	770	1.61
CEL poplar sample	Enzymatic	14352	2192	6.5

The inter-linkage distribution suggests that CELF pretreatment caused significant bond breakage as no β-O-4, β-β and β-5 linkages were found in lignin from either boiling or dilution. Lignin obtained by boiling had similar properties to the native lignin in terms of syringyl (S) and guaiacyl (G) subunits. The S/G ratio for both the CEL poplar sample and the lignin from boiling was almost 1.28; however, the much greater G subunits and lower S subunits in lignin from the dilution method resulted in a higher S/G ratio of 2.74.

Table 2.2 Lignin inter-unit linkages measured using HSQC NMR.

Samples	Method type	Lignin subunits			PB	Lignin inter-unit linkages		
		S	**G**	**S/G**		**β-O-4**	**β-β**	**β-5**
CELF lignin	Diluted	73.3	26.7	2.74	6.7	0	0	0
	Boiled	54.9	45.1	1.22	12.6	0	0	0
CEL poplar lignin	Enzymatic	54	42.1	1.28	14.8	56.8	4.3	6.8

2.3.3 Evaluation of technoeonomics of hypothetical biorefinery based on experimental data

An economic analysis was performed on a hypothetical biorefinery that produces MF, DMF, LA, and lignin based products from poplar wood. The process flow diagram in Figure 2.4 summarizes the overall process based on CELF pretreatment of biomass to produce FF, HMF, and LA using $FeCl_3$ catalyst. Pretreatment is followed by neutralization of $FeCl_3$ by calcium hydroxide to form calcium chloride and iron (III) hydroxide. After neutralization, THF is recovered by distillation that simultaneously precipitates water insoluble lignin. In order to obtain a pure stream containing FF and HMF for catalytic upgrading, they are extracted by an organic solvent system consisting of toluene and dioxane. Since HMF is more polar than furfural, toluene is able to extract only trace amounts of HMF in the organic phase (Sousa et al. 2009). Thus, dioxane was added to extract remaning amount of HMF in the organic layer. The organic phase, containing FF and HMF was then fed to a reactor along with a solid Cu-Ni/Ti catalyst under hydrogen pressure. FF and HMF were hydrogenated to MF and DMF, respectively, at 200 °C and 24.95 bars Yields from FF and HMF to methylated furans are set at 72.7% and 84.1%, respectively, based on results reported for a novel Cu-Ni/Ti catalyst (Seemala et al. 2017).

Levulinic acid was then distilled and separated and rest of the stream was passed on to waste water treatment.

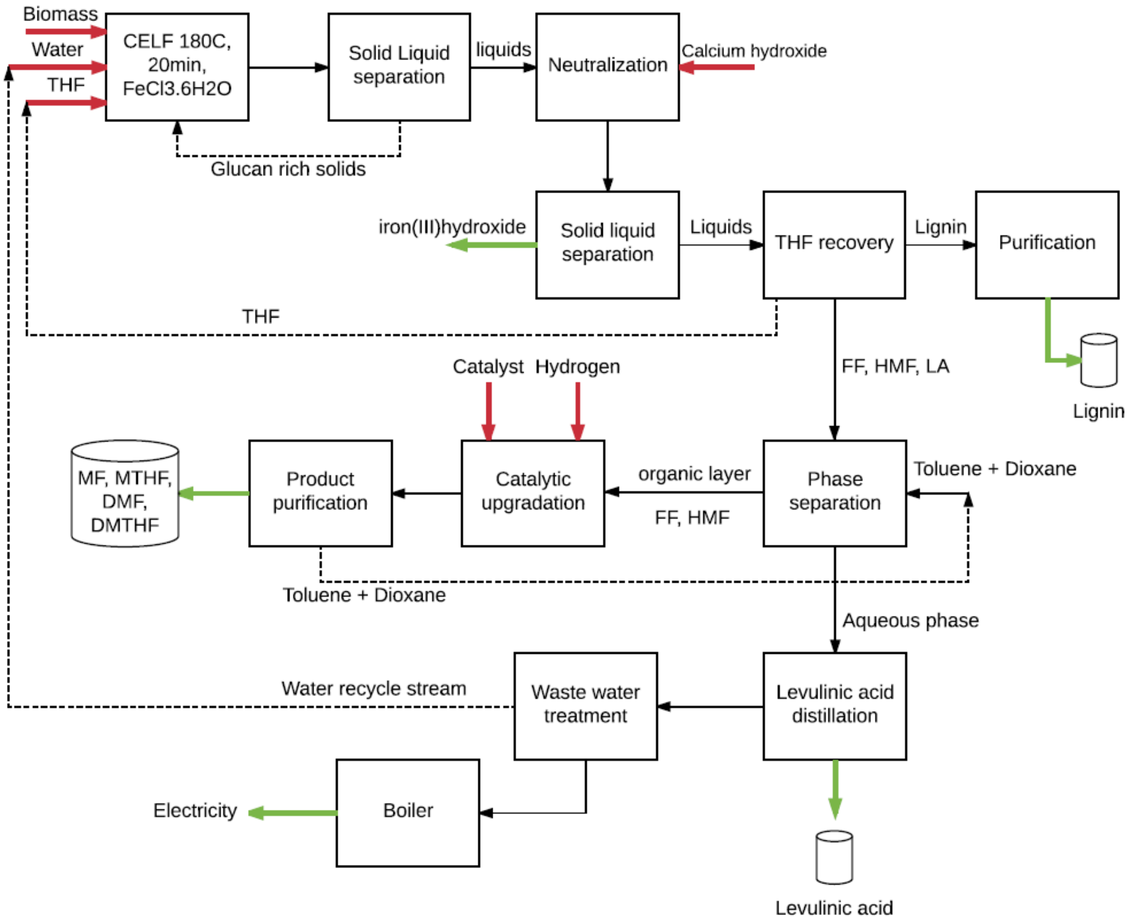


Figure 2.4 Process flow diagram of CELF process for producing the fuel precursors FF and HMF and their further upgrading to furan based gasoline blend stocks.

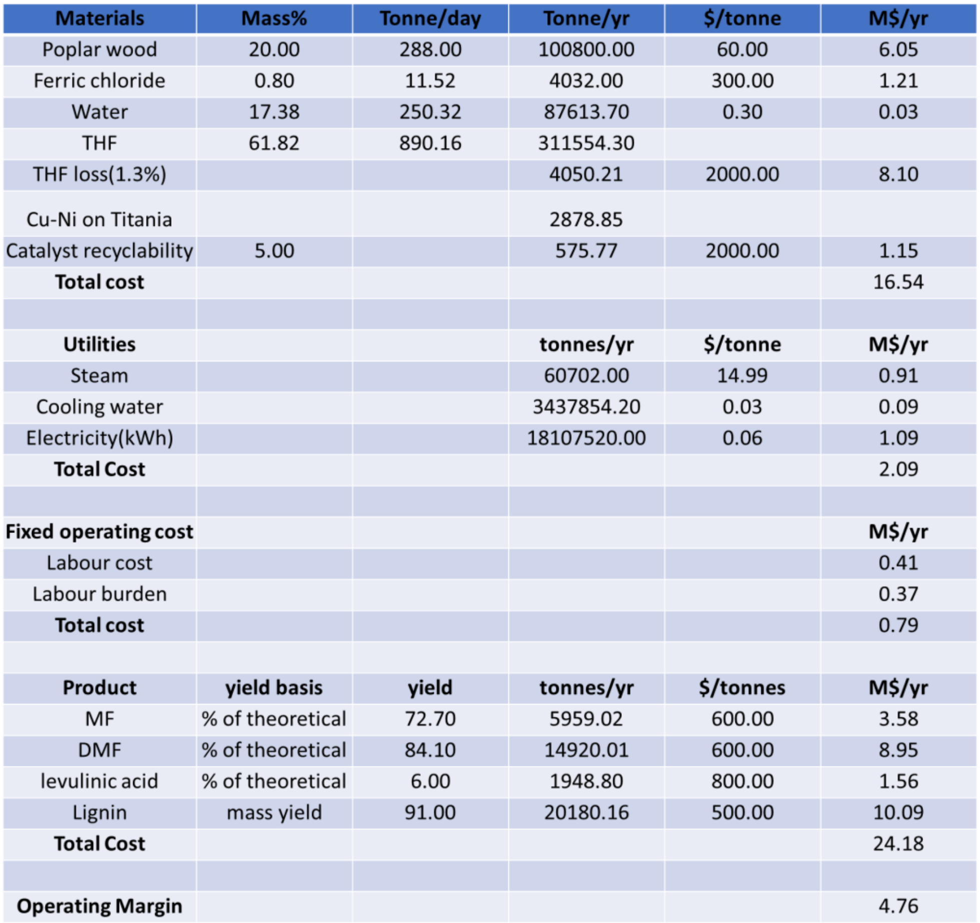
The model was assumed to process 12 dry tonnes hr^{-1} of biomass at a solids loading of 20 wt%. THF was added to water in 4:1 ratio by volume with 1wt% $FeCl_3$ catalyst. The operating margin for this process is caculated as the difference between revenue from product sales and operating costs. The selling price of furan products was based on a gasoline price of \$1.75 gal^{-1} and density of 0.77 kg litre^{-1} . The lignin selling price was set at \$500 dry tonne^{-1}, as it could be expected if it can be used to produce high value products such as polymers and not burned for process energy. THF losses were assumed to be 1.3 wt% based on expected distillation recovery efficiencies and breakdown of some of it to 1,4-butandiol(1,4-BDO). Table 2.3 summarizes the assumptions for the process parameters used to calculate the base case operating margin.

Table 2.3 Key process parameter values used as base case. assumptions

Parameters	Base value
Plant capacity	60 dry tonnes hr^{-1}
Feedstock cost	\$60 dry tonne^{-1}
Biomass solids loading	20wt%
THF loss	1.3wt%
Lignin selling price	\$500 tonne^{-1}
Furan selling price	\$600 tonne^{-1}

Table 2.4 summarizes the calculated operating margin for the biorefinery based on the base case assumptions for the process parameters. The total operating cost was calculated by adding the raw material costs, utility costs, and fixed operating costs. The operating margin was then determined by subtracting the total operating cost from the revenue generated from product sales.

Table 2.4 Operating margin analysis for the base case scenario assumptions.



Materials	Mass%	Tonne/day	Tonne/yr	$/tonne	M$/yr
Poplar wood	20.00	288.00	100800.00	60.00	6.05
Ferric chloride	0.80	11.52	4032.00	300.00	1.21
Water	17.38	250.32	87613.70	0.30	0.03
THF	61.82	890.16	311554.30		
THF loss(1.3%)			4050.21	2000.00	8.10
Cu-Ni on Titania			2878.85		
Catalyst recyclability	5.00		575.77	2000.00	1.15
Total cost					16.54
Utilities			**tonnes/yr**	**$/tonne**	**M$/yr**
Steam			60702.00	14.99	0.91
Cooling water			3437854.20	0.03	0.09
Electricity(kWh)			18107520.00	0.06	1.09
Total Cost					2.09
Fixed operating cost					**M$/yr**
Labour cost					0.41
Labour burden					0.37
Total cost					0.79
Product	**yield basis**	**yield**	**tonnes/yr**	**$/tonnes**	**M$/yr**
MF	% of theoretical	72.70	5959.02	600.00	3.58
DMF	% of theoretical	84.10	14920.01	600.00	8.95
levulinic acid	% of theoretical	6.00	1948.80	800.00	1.56
Lignin	mass yield	91.00	20180.16	500.00	10.09
Total Cost					24.18
Operating Margin					4.76

Table 2.5 reports operating margins calculated for different scenarios applied to identify key cost drivers for the process. The feedstock cost includes such items as material receiving and handling and transportation in additon to raw material costs. A lignin selling price of $1,000 tonne^{-1} was applied based on the market value of monomeric phenolic substitutes, whereas a $300 dry tonne^{-1} value is based on the price for concrete additives

(Jablonsky 2015). The best case operating margin obtained was 22.91 M$ yr^{-1}, whereas for the worst case scenaris applied, the company would lose 13.01 M$ yr^{-1} .

Table 2.5 Operating margins calculated for different scenarios.

Parameter	Base value	Worst case	Best case
Feedstock cost (dry tonne^{-1})	$60	$80	$40
THF loss	1.3 wt%	2.5 wt%	1 wt%
Lignin selling price (dry tonne^{-1})	$500	$300	$1000
Furan selling price (tonne^{-1})	$600	$400	$800
Operating Margin (M$ yr^{-1})	4.76	-13.01	22.91

Table 2.6 reports 20% adjustments in each parameter to increase the operating Figure 2.5 shows how the operating margin is affected by these changes. From Figure 2.5, we can conclude that operating margin is most sensitive to furan selling price, as a 20% increase resulted in a 53% operating margin improvement. Since furan selling price is governed by an ever fluctuating gasoline market price, there is significant risk for the biorefinery as even a small change in gasoline price would have a major impact on the operating margin. From all the parameters evaluated, feedstock cost had the least effect on the operating margin. Reducing THF losses by 20% increased the operating margin by 34%, indicating the importance of achieving high THF recovery.

Table 2.6 Key process parameters after a 20% improvement in their values from the base case (PDT = per dry tonne).

Parameters	Base value	20% change
Feedstock cost (PDT)	60	48
THF loss%	1.3 wt%	1.04 wt%
Lignin selling price (PDT)	500	600
Furan selling price (PDT)	600	720

Furthermore, even though the operating margin is less sensitive to THF losses than changes in other parameters, achieving low losses is still extremely crucial for the process. In particular, the sensitivity analysis in Figure 2.6 shows that a THF loss greater than 2.06 wt% (recovery is 97.94 wt%) results in a negative operating margin. Thus, it is evident that the economics of the hypothetical biorefinery severely hinges on THF capture and recycle, and attention must be given to effective strategies for THF recovery and reducing conversion to 1,4-butanediol (1,4-BDO).

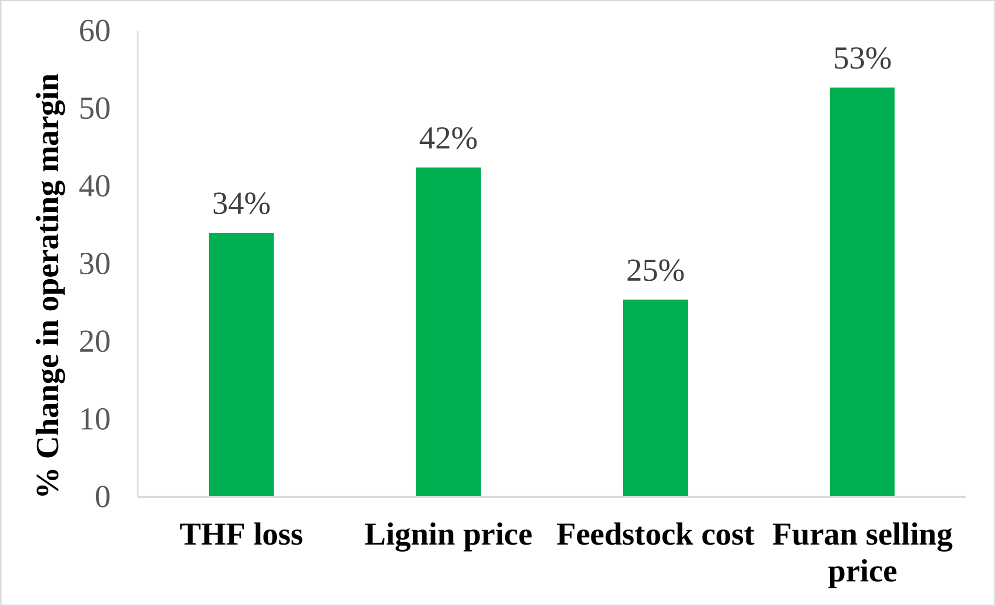


Figure 2.5 Effect of individual 20% changes in process paramaters to improve operating margin.

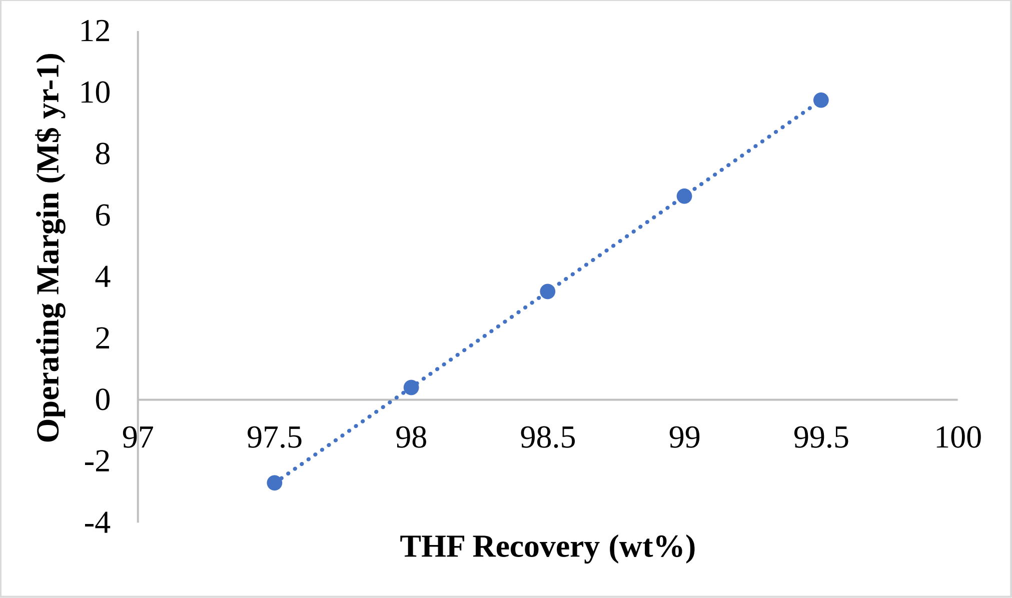


Figure 2.6 Effect of THF recovery on operating margin.

2.4 Conclusions

The CELF process is a promising technology for a biorefinery to produce gasoline blend stocks because of the high yields of the intermediates FF and HMF in a one pot reaction at 180 °C with short residence time of 20 mins. Recovery of THF along with simultaneous precipitation of lignin by distillation avoids the need for large quantities of water to isolate lignin, the traditional approach for lignin recovery following organosolv pretreatment.

Characterization of lignin showed that CELF pretreatment broke all the lignin ether bonds and to produce a highly depolymerized low molecular weight (1245 g mol^{-1}) product. This highly depolymerized lignin can be highly amenable to catalytic upgrading to high value products, thereby making profitable utilization of all three major fractions of biomass promising.

A techno-economic analysis of the process predicted a promising operating margin at a 12 dry tons hr^{-1} plant capacity. At the base case scenario with 1.3wt% THF loss and \$600 ton^{-1} furan selling price, the operating margin was 4.76 M\$ yr^{-1}. Application of a sensitivity analysis indicated that process profits are extremely sensitive to the furan selling price that is dependent on crude oil prices. Because the latter are highly volatile, a biorefinery faces significant risks. Other major cost driving factors of the overall process are THF recovery efficiency and lignin selling price.

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CHAPTER 3

Detoxification of CELF Hydrolyzate Using Organic Solvent to Improve Overall Ethanol Yields*

*****This chapter includes data collected in collaboration with Abhishek Patri, PhD student from Department of Chemical and Environmental Engineering at UCRiverside. Abhishek Patri performed all the fermentation studies and FCR assays with data shown in Figures 3.2, 3.3 and 3.4 and Table 3.2

3.1 Introduction

The USA dependence on imported oil makes it susceptible to volatile oil prices and causes economic uncertainties. Moreover, the transportation sector is the major consumer of oil, is virtually totally dependent on oil, and contributes about two-thirds of greenhouse pollutants such as carbon monoxide (Wyman 1994). Thus, production of fuels such as ethanol from plentiful, inexpensive lignocellulosic biomass could provide a important alternative to oil that could lower the USA dependency on foreign oil, reduce greenhouse gas emissions, and increase domestic employment. Commercial success of cellulosic ethanol, however, relies heavily on a low cost pretreatment step to disrupt the biomass structure and exposes sugar polymers for deconstruction to sugars with the high yields essential to low fuel costs (Lynd et al. 1991).

Following pretreatment opening up the biomass structure, enzymes can access cellulose, the major carbohydrate polymer, to break it down into glucose that many organisms can readily ferment. Although five carbon sugars from hemicellulose are not fermentable to ethanol with high yields by native organisms, yeast and bacteria have been genetically modified to co-ferment C5 and C6 sugars to make the process economical (Mosier et al. 2005). High-temperature pretreatment at acidic conditions can form sugar degradation products in the hydrolyzate, mainly furfural (FF) and 5-hydroxymethylfurfural (HMF) from polysaccharides and phenolic compounds from breakdown of lignin (Klinke et al. 2004).

HMF, which is obtained from hexose degradation, inhibits cell growth and fermentation by *S. cerevisiae* at only 1 g L^{-1} concentrations (Mussatto & Roberto 2004). In

S. cerevisiae, FF, a dehydration product of xylose, causes chromatin, actin and membrane damage that inhibit fermentation due to reactive oxygen species accumulation (Almeida et al. 2007, Dunlop 1948). 4-hydrobenzoic acid and vanillin are some of the major lignin degradation products in hydrolyzates from poplar and willow (Palmqvist & Hahn-Hägerdal 2000b). Even at low concentrations, these lignin degradation products are more toxic to microorganisms than FF and HMF. *S. cerevisiae* xylose metabolism was inhibited completely by vanillin at 5 g L^{-1} (Palmqvist & Hahn-Hägerdal 2000a). Thus, inhibition by degradation products makes it imperative to integrate a detoxification step prior to fermentation to reduce their concentrations and to thereby mitigate their inhibitory effects on fermenting organisms. Detoxification can be by chemical, physical, or biological means. Activated charcoal, ion exchange resins, solvent extraction, evaporation, and over-liming with calcium hydroxide are the most widely adopted detoxification techniques (Carvalheiro et al. 2005, Technology 2004, Millati et al. 2002, Huang et al. 2008)(Huang et al. 2008)(Huang et al. 2008)(Huang et al. 2008)(Huang et al. 2008)(Huang et al. 2008)(Huang et al. 2008)(Huang et al. 2008). However, because introducing a detoxification step could account for up to 22% of the ethanol production cost and significantly impact process economics, a cost effective strategy is needed (von Sivers et al. 1994).

This study sought to develop an effective detoxification strategy so that high ethanol yields are realized by fermenting the hydrolyzate generated after Co-Solvent Enhanced Lignocellulosic Fractionation (CELF) pretreatment of Alamo switchgrass, a senescent energy crop. CELF pretreatment employs THF to extensively solubilize lignin and hemicellulose in the liquid hydrolyzate and leave behind extremely accessible glucan rich solids for further enzymatic hydrolysis. Conditions have been optimized to achieve high ethanol yields from glucan rich solids produced by CELF and recover most of the hemicellulose sugars in the liquid hydrolyzate. In particular, simultaneous saccharification and fermentation (SSF) of CELF solids has achieved 90% ethanol yields with *S. cerevisiae* D_5A with only 2 mg protein g glucan^{-1} enzymes (Nguyen et al. 2015). Furthermore, it is possible to achieve industrially relevant titers of >50 g L^{-1} ethanol at ~90% yields from corn stover. These results allowed CELF-SSF strategy to achieve ethanol titers greater than 50 g L^{-1} reduces energy demand and capital and operating costs associated with ethanol separation in downstream product recovery (Nguyen et al. 2016). However, the high ethanol yields must also be achieved from fermentation of the CELF hydrolyzate that is rich in C5 sugars to reach commercially relevant ethanol yields from the overall process.

In this study, organic solvents were employed to extract THF and phenols from CELF hydrolyzate with the goal to increase total process ethanol yields. Three different solvents, methyl isobutylketone (MIBK), methyl tetrahydrofuran (MTHF), and toluene, were selected for extraction of inhibitors from the hydrolyzate based on boiling point and water solubility that are favorable for inhibitor extraction and solvent recovery. The maximum THF concentration that would allow fermentation was first ascertained based on its addition to model sugar solutions. To ascertain the inhibitory effect of phenols, the hydrolyzate was boiled to remove THF after detoxification and then fermented by *S. cerevisiae*.

3.2. Materials and methods

3.2.1 Materials

THF (>99% purity, Fischer Scientific, NJ) was used in all the co-solvent pretreatment reactions. 1:1 THF-to-water co-solvent mixture was prepared on a volume basis. Concentrated sulfuric acid and MIBK were also purchased from Fischer Scientific (NJ). Alamo Switchgrass was provided by the National Renewable Energy Laboratory (NREL). Its composition was measured to be 38.8 % glucan, 26.8% xylan, 21.6% lignin, 1.8% arabinan, and 1.3% ash using NREL Laboratory Analytical Procedures (LAP). Toluene, Folin-Ciocalteu reagent (FCR), and sodium carbonate were purchased from Sigma Aldrich (St. Louis, MO). 2-MTHF was obtained from Acros, NJ. All chemicals were used without purification.

3.2.2 Pretreatment reaction

A 0.5wt% sulfuric acid solution was produced by adding 3.8 g of sulfuric acid to a THF-water mixture to give a total mass of 760 g, to which 40 g of switchgrass on a dry basis was then added to give a 5wt% slurry of switchgrass solids. Next, this 800 g biomass slurry was soaked overnight at 4 °C to ensure acid penetration. This slurry was transferred to high-pressure continuously stirred 1L Parr reactor (Parr Instrument Company, Moline, IL) heated by a 4kW fluidized sand bath (Model SBL-2D, Techne, Princeton, NJ). Temperature was monitored by an in-line thermocouple (Omega, K-type), stirred by twin 6-blade impellers at 200 rpm. After a set time at temperature, the reactor was lowered into a water bath to quench the reaction. Heat-up, reaction, and cool down times were measured using a stop-watch. The solids were then separated from hydrolyzate by vacuum filtration through glass fiber filter paper at room temperature (Fischer Scientific, Pittsburgh, PA).

3.2.3 Organic solvent selection

The organic solvents were selected for detoxification based on the following criteria: water solubility and boiling point temperature. Table 3.1 summarizes the above-mentioned parameters for three solvent candidates chosen for detoxification: MIBK, toluene, and MTHF. All three solvents have low water solubility and form two distinct phases with water. Because MIBK and toluene have high boiling points (117 °C and 110.6 °C respectively), they can be separated from THF which has boiling point of 66 °C (Ullman's encyclopedia, 1989).

Table 3.1 Organic solvents shortlisted based on their boiling point and water solubility at 20 °C.

Solvent	**Boiling point (°C) (at standard pressure)**	**Water solubility (g of solvent per 100g water) (at 20 °C)**
MIBK	117	1.9g
Toluene	110.6	5.2g
MTHF	80	4g
THF	66	Fully Miscible
Water	100	-

3.2.4 Liquid- Liquid Extraction

The reaction mixture collected after the CELF reaction was filtered to separate glucan rich solids from the liquid hydrolyzate. This hydrolyzate was then mixed with an organic solvent in 1:1 ratio on a mass basis in a 1 L separatory funnel. Next, the mixture was allowed to settle in a separatory funnel for 5 mins, at which time two distinct phases formed. Both phases were collected and stored separately in glass receptacles to avoid any furfural and THF loss that was observed in plastic containers.

3.2.5 Phenolic assay

The Folin-Ciocalteu (FCR) assay that quantifies the amount of phenolics present in terms of gallic acid equivalent was applied to the aqueous layers to quantify the soluble phenols released by lignin depolymerization. In this assay, aliquots from the aqueous layer were diluted 4 times, and 1 ml of these diluted samples was added to 60 ml of distilled water in a 100 ml volumetric flask. To this, 5 ml of FCR was mixed and allowed to stand for 5 mins. Next, 15 ml of a 20% (w/w) sodium carbonate solution was added to the mixture, and enough distilled water was then added to bring the total volume up to 100 ml. Absorbance was measured after 2 hrs at 760 nm in a 1 cm cuvette (Singleton et al. 1998).

3.2.6 Fermentations of the detoxified CELF hydrolyzate

All fermentation solutions were prepared in a sterile laminar flow hood. *S. cerevisiae* cells were grown from frozen glycerol stock on pure glucose for 24 hrs to reach an optical density (OD) of 8. After cell growth, enough cells were collected from the growth flask to produce an OD of 0.5 in each fermentation flask. The cells were then washed and centrifuged before being resuspended in sterile DI water for inoculation of fermentation flasks. Each fermentation flask was then filled with 5 mL of 10X YP (yeast peptone) (as per NREL protocol), 2.5 ml citric acid buffer (pH 4.8), 0.5 ml of 4 g L^{-1} tetracycline, and 1 ml of inoculum. Appropriate amounts of hydrolyzate or sugar control were added to the flask to bring the final liquid volume in the flask to 50 ml. A bubble trap consisting of a looped tube containing 10 ml of DI water was attached to the fermentation flasks to allow release of carbon dioxide while preventing access to invading organisms. Flasks were incubated at 37 °C and 130 rpm, with samples taken at 24 hrs intervals for 7 days. Yields were calculated as percentage of theoretical maximum ethanol concentration.

3.2.7 Process flow diagram of CELF pretreatment and downstream detoxification and THF recovery

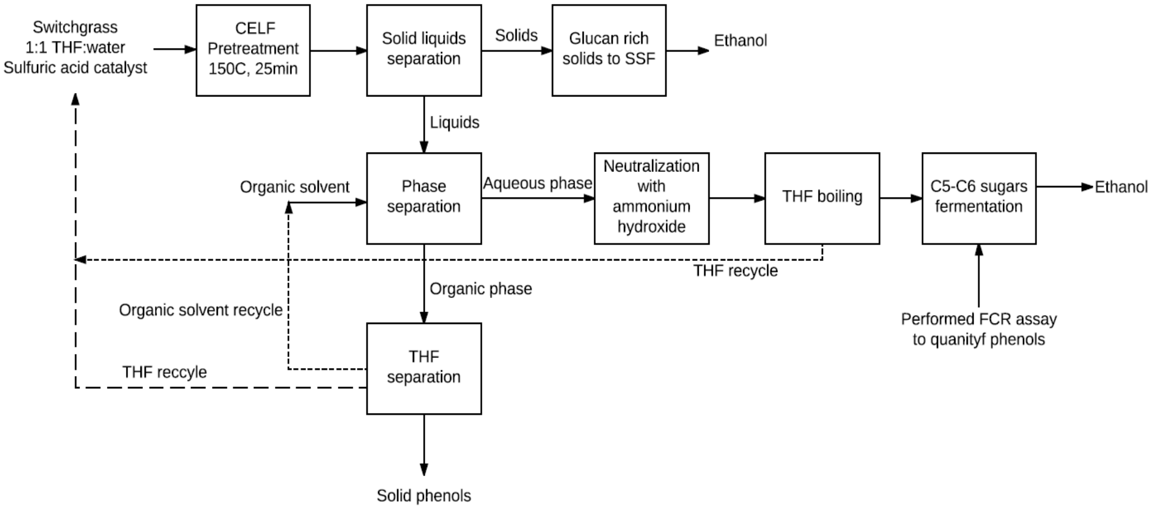

Figure 3.1 Process flow diagram of CELF pretreatment and downstream detoxification and fermentation of hydrolyzate.

CELF pretreatment of Alamo switchgrass at 150 °C for 25 mins produced glucan rich solids and a C5-rich liquid hydrolyzate that also contained most of the lignin. These glucan rich solids were separated from the liquid for conversion to ethanol by SSF. The liquid hydrolyzate obtained after the reaction was detoxified by one of the organic solvents to form two distinct layers. The organic layer and the aqueous layer were the separated using a separatory funnel. The aqueous layer was neutralized with ammonium hydroxide to prevent loss of sugars during boiling at acidic condition and then boiled at 80 °C for 12 hrs in a water bath to remove THF. The resulting liquid containing C5 and C6 sugars was fermented by *S. cerevisiae* yeast to produce ethanol.

3.3 Results and discussion

3.3.1 Effect of THF on fermentation of model sugar compounds

Figure 3.2 shows the inhibitory effect of THF on fermentation of the model sugar compounds glucose (3 g L^{-1}) and xylose (40 g L^{-1}) by *S. cerevisiae*. Thus, one can see that a maximum ethanol yield of 85% of the theoretical maximum was achieved in 96 hrs for a THF concentration of 7 g L^{-1} compared to 72 hrs when at concentrations below 7 g L^{-1}. At a THF concentration of 8 g L^{-1}, only 40% of theoretical maximum yield was achieved in 120 hrs.

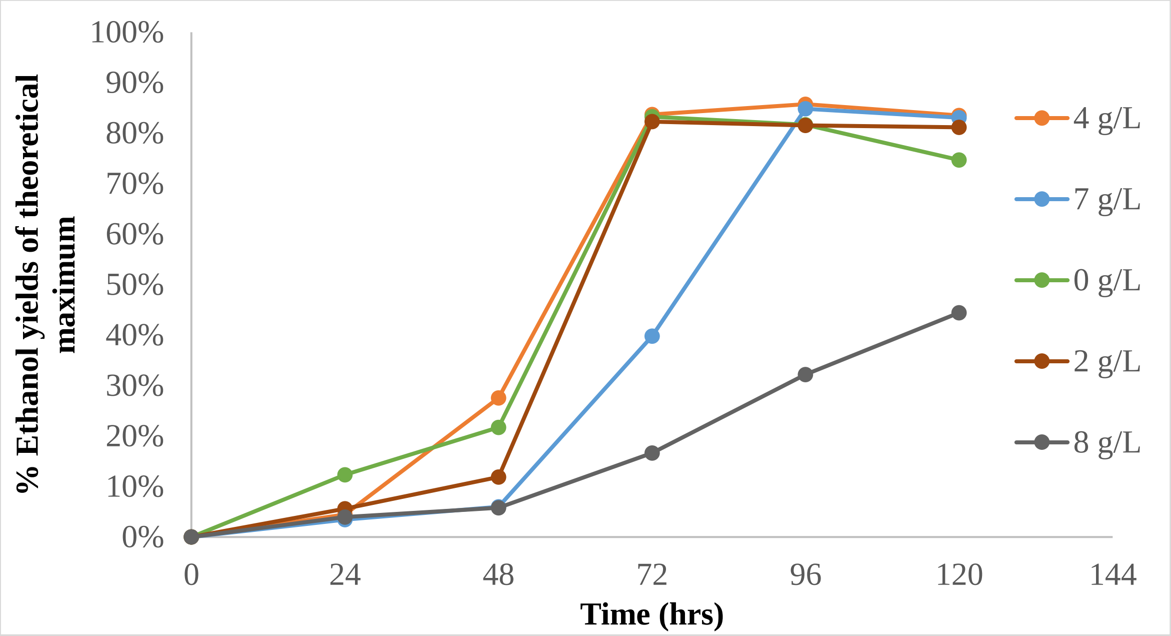

Figure 3.2 The effect of THF on fermentation ethanol yields expressed as percent of theoretical maximum from solutions of pure glucose and xylose.

3.3.2 Effect of extractive solvents on the ethanol yields from CELF hydrolyzate

The CELF hydrolyzate was boiled at 80 °C for 12 hrs to bring the THF concentration below 7 g L^{-1} to reduce the inhibitory effect of THF on fermentation enough to gauge the effect of phenols. Figure 3.3 demonstrates that the rate of fermentation of boiled hydrolyzate compared to that for fermentation of the sugar control. These results show that an 84% ethanol yield was achieved in 72 hrs for the sugar control, whereas fermentation of boiled hydrolyzate took 168 hrs to reach an 73% ethanol yield. Thus, inhibitors other than THF present in the hydrolyzate affected the rate of fermentation.

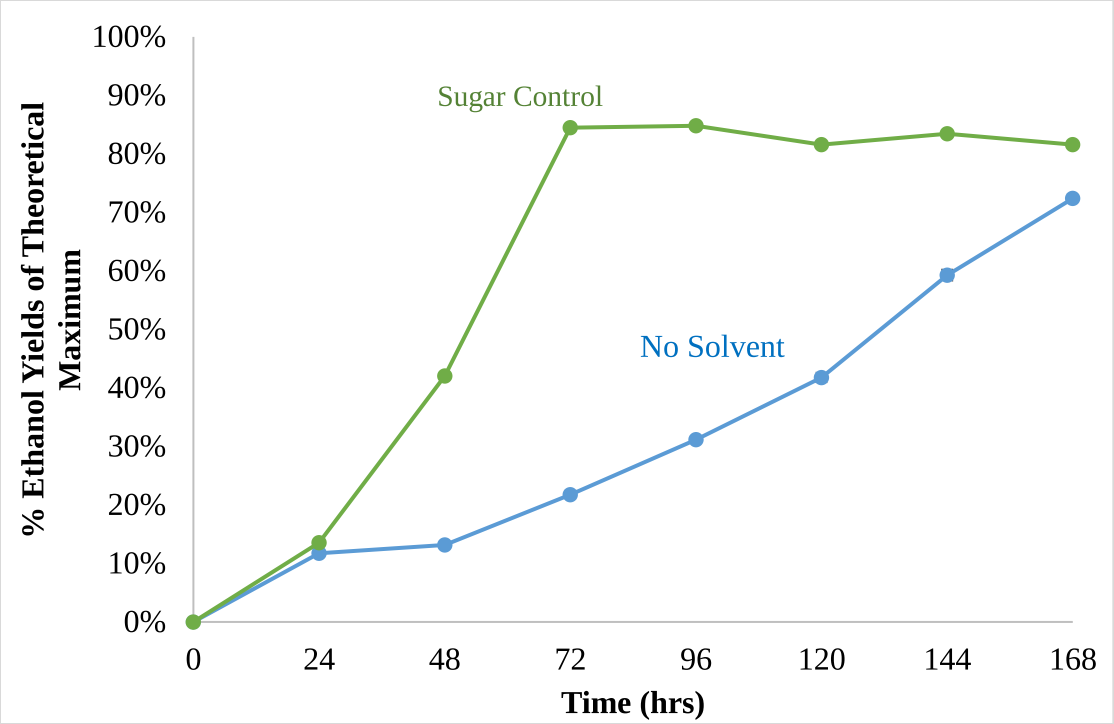

Figure 3.3 Comparison of ethanol yields and rates from fermentation of sugar solution controls and boiled hydrolyzate.

Figure 3.4 shows the effects of detoxification of the CELF hydrolyzates by organic solvents on the fermentation rate. MIBK had the most beneficial effect on the rate of fermentation, in that a 91% ethanol yield was achieved in 24 hrs. Although a 95% ethanol yield was realized for extraction with toluene, it took 140 hrs to reach this yield. Yields from fermentation of hydrolyzate that had not been treated with organic solvent took 168 hours to reach only 70% of the theoretical maximum. No ethanol resulted from fermentation of MTHF treated hydrolyzate.

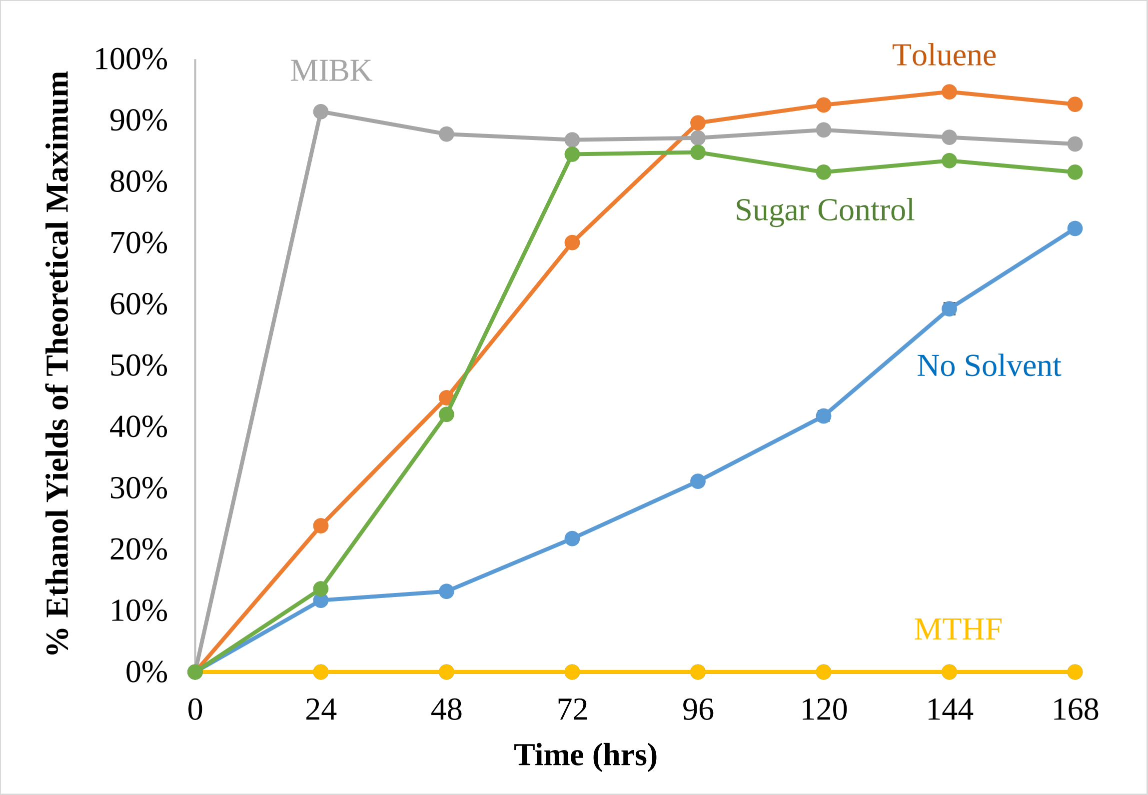

Figure 3.4 Fermentation ethanol yields (expressed as % of theoretical maximum) of CELF hydrolyzate post-LLE with four organic solvents and no LLE (liquid-liquid extraction).

The effect of lignin phenolics on fermentation of model sugar compounds and sugars from different feedstocks was investigated to try to understand factors responsible for MIBK and toluene treated hydrolyzate reaching higher fermentation rates and yields. As shown in Table 3.2, hydrolyzate treated with MIBK had lower FCR absorbance than hydrolyzate without any solvent extraction, suggesting higher fermentation rates due to lower phenol concentrations. A similar trend was seen for hydrolyzate treated with toluene. Although hydrolyzate treated with MTHF had lower phenol concentrations than in untreated hydrolyzate, the large quantity of THF left in the flask resulted in no fermentation. This result is consistent with the effect of THF on fermentations of ideal sugar solution controls in that the THF concentration of 30 g L^{-1} for the MTHF extracted liquid was well above the concentrations shown in Figure 3.2 that slowed fermentations.

Table 3.2 Flask concentrations of THF, glucose + xylose, and FCR absorbance for CELF hydrolyzate and post-LLE CELF hydrolyzate using four organic solvents.

Solvent	**Flask THF (g L^{-1})**	**Flask sugar conc (g L^{-1})**	**Flask FCR Absorbance**
No solvent	1	42	3.3
Toluene	5	39	2.9
MTHF	30	35	1.7
MIBK	1	42	2.1

3.4 Conclusions

Because THF concentration above 7 g L^{-1} were shown to reduce the rate of fermentation of pure sugar solutions, the organic solvents MIBK, toluene, and MTHF were used to increase ethanol yields by extracting THF prior to fermentation. Fermentation of MIBK treated sugar solutions reached 91% of the theoretical maximum ethanol yields in 24 hrs while 168 hrs was required to realize 95% yields from toluene treated solutions. Lignin breakdown products also contributed to inhibition of fermentations of actual CELF hydrolyzate, and both MIBK and toluene extractions proved to be effective extraction vehicles. However, fermentation rates were much faster for MIBK treated CELF hydrolyzate. Although extraction with MTHF removed lignin breakdown products effectively, too much THF was left in the hydrolyzate to achieve high yields.

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CHAPTER 4

Development and Evaluation of Techniques to Isolate Lignin Solubilized by Co-Solvent Enhanced Lignocellulosic Fractionation (CELF)*

***This chapter was written under the guidance of Dr. Charles Cai who invented CELF technology at UCRiverside. This chapter includes data collected in collaboration with University of Tennessee Knoxville (UTK) as a part of US Department of Energy EERE Bioenergy Technology Office. Dr. Xianzhi Meng from Department of Chemical and Biomolecular Engineering at UTK performed GPC and HSQC NMR characterization of all the lignin samples with data shown in Tables 4.2 and 4.3.

4.1 Introduction

It is important to utilize sustainable energy sources to satisfy the ever-increasing energy demand. Moreover, with the negative effects of petroleum based fuels on the environment, biomass based products have been studied. Cellulosic biomass such as forest waste, paper mills and energy crops, has been documented as source for liquid transportation fuels and a myriad of chemicals (Himmel et al. 2007). Cellulosic biomass contains cellulose, hemicellulose, lignin and some extractives in variable quantities. About 40-50% of biomass is comprised of cellulose, a crystalline glucose polymer, about 15-30% hemicellulose, an amorphous matrix of branched oligomers of xylose with galactose, glucose, mannose, and arabinose, that can be fermentable by microorganisms (Li & Zhao 2008). However, a third major component that is lignin comprises of 15-30% of biomass that is difficult to process through biological methods but can contain up to 50% of the total carbon content (Mckendry 2002). Altering lignin is crucial to bioprocessing because it hinders enzymatic hydrolysis of polymeric sugars and also inhibits downstream fermentation of monomeric sugars. Hence, it is advantageous to dislocate lignin from the polysaccharides in biomass to make the latter more accessible to deconstruction to sugars. (Chen & Dixon n.d.,Tan et al. 2009).

The structure of lignin is illustrated in Figure 5.1. In most conceptual process designs for biological conversion of cellulosic biomass into ethanol, lignin is inefficiently burned to produce process heat and electricity at an effective value of lower than \$25 tonne^{-1}. However, lignin from biomass processing has also been investigated in higher value applications such as an additive for concrete, fertilizers, rubber, and surfactants (Stewart

2007). Polymeric components in lignin can be catalytically broken down to oligomeric or monomeric lignols to be used as a precursor for polymer synthesis and derivatized for application in polymer blend systems to produce polypropylene, polyurethane, low density polyethylene, and polyesters (Pucciariello et al. 2004, Canetti et al. 2006, Hatakeyama et al. 2005, Kunio et al. 1991, Doherty et al. 2011, Luong et al. 2012). Furthermore, aromatic components in lignin can be catalytically hydrodeoxygenated to a class of aromatic solvents similar to benzene, toluene, and xylene (BTX). Producing high value chemicals from lignin instead of just burning it can be important in improving the profitability of a biorefinery (Yang & Wyman 2008, Nguyen 1991).

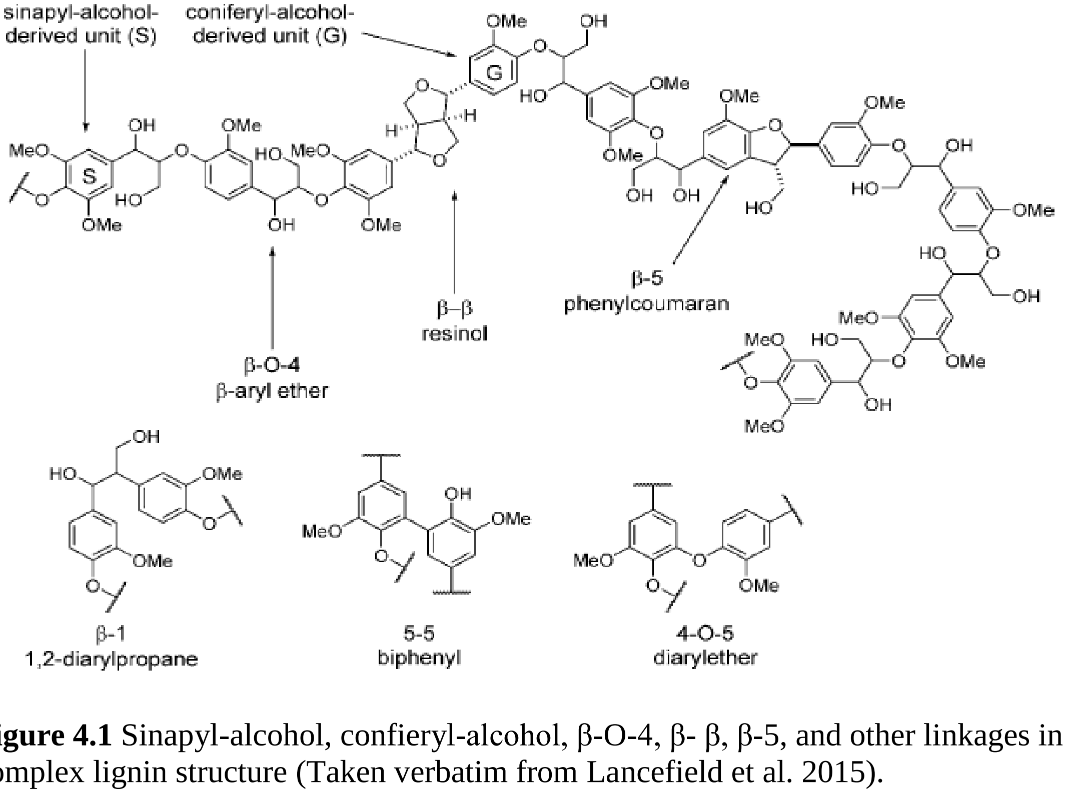

Figure 4.1 Sinapyl-alcohol, confieryl-alcohol, β-O-4, β- β, β-5, and other linkages in complex lignin structure (Taken verbatim from Lancefield et al. 2015).

In the paper and pulp industry, acid precipitation and alum induced precipitation of lignin from black liquor is practiced to recover lignin after the pulping step (Sun et al. 1999, Luong et al. 2012). However, methods to capture and recover lignin effectively from cellulosic biomass is governed by the type of pretreatment used. Lignin can also be extracted by white-rot fungal degradation, however it is economically unfeasible mainly due to long cultivation times required for biological pretreatment (Hatakka 1983). In dilute acid pretreatment, sulfuric or other organic acid is used to break down the hemicellulose to sugars leaving behind acid-insoluble residues called Klason-lignin (D Humbird et al. 2012). From ethanol-based Organosolv pretreatments, lignin is precipitated by diluting the hydrolyzate with water (Zhao et al. 2009). Similar dilution based technique is applied to gamma-valerolactone (GVL) based pretreatment where, GVL is used as a miscible co-solvent with water to facilitate sugar and lignin solubilization (Luterbacher et al. 2015). However, large capital cost is associated with downstream waste water processing of the dilute hydrolysate remaining after lignin extraction. In addition, high boiling point of GVL (208 °C) presents a challenge for its separation from water and any distillation methods of separation will likely suffer high energy costs, thus preventing GVL boiling from being a cost-effective solution to remove it from water to precipitate the extracted lignin. Thus, a process is needed that extracts pure lignin while rendering the carbohydrate fractions amenable for effective downstream processing (Lee et al. 2009). In this study, lignin was extracted from the liquid hydrolyzate generated by Co-Solvent Enhanced Lignocellulosic Fractionation (CELF) pretreatment which employs THF as a co-solvent in water (Cai et al. 2013). THF is a low boiling point solvent (66 °C) which can be easily boiled out from water to precipitate the solubilized lignin. Thus for CELF, two potential lignin recovery strategies were explored to remove THF from water and precipitate lignin from solution: 1) diluting the hydrolyzate with water (dilution method), and 2) evaporation of THF from the CELF hydrolyzate (boiling method). These methods were applied on two CELF hydrolyzate streams that were generated based on the following objectives: 1) maximum yields of furfural (FF) and hydroxymethylfurfural (HMF) from poplar wood (termed Fuel precursor or FP case) and 2) maximum yields of hemicellulose and cellulose sugars from maple wood (termed sugars case). The lignin samples isolated by each of the process configurations were then characterized by Gel Permeation Chromatography (GPC) and HSQC NMR for molecular weight distribution and inter-unit lignin linkages distribution. The structural properties of the lignin extracted from CELF were then compared to that of enzymatically extracted lignin.

4.2 Materials and Methods

4.2.1 Materials

THF (>99% purity, Fischer Scientific, NJ) was used for all CELF pretreatment reactions in a 1:1 or 4:1 THF-to-water ratio co-solvent solution prepared on a volume basis. Concentrated sulfuric acid was purchased from Fischer Scientific (NJ) and diluted with water to make solutions for reactions. Hydrated ferric chloride ($FeCl_3.6H_2O$) catalyst was purchased from Sigma Aldrich (St. Louis, MO, US). Poplar wood and maple wood were provided by the National Renewable Energy Laboratory (NREL, Golden, CO). Compositional analysis of biomass was performed according to the established NREL procedure in triplicates. The composition for poplar wood was measured to be 45.91±0.5% glucan, 14±0.3% xylan, and 22.88±0.2% K-lignin whereas for maple wood it was 40.3±0.3% glucan, 13.72±0.1% xylan, and 24.66±0.2% K-lignin. Other materials needed for the biomass composition to total 100% were not characterized in this study but were expected to be sugars, acetate, and protein in small quantities that are difficult to quantify via HPLC. All pentosans were grouped together as xylan, and all hexosans as glucan. All chemicals were used without purification.

4.2.2 Pretreatment

Biomass solid loadings (poplar and maple wood) were based on total mass of reaction mixture (800 g), thus a 5wt% solids loading corresponded to 40 g of biomass on dry basis. The 0.5wt% (3.8 g) sulfuric acid was based on THF-to-water mixture weight (760 g). For metal halide catalyst, hydrated ferric chloride was used ($FeCl_3.6H_2O$), however the loading was based on anhydrous mass to realize 1wt% (7.6 g). The mixture of biomass with acid and solvent was soaked overnight at 4 °C to ensure acid penetration into the pores. The contents were then transferred to high-pressure continuously stirred 1L Parr reactor (Parr Instrument Company, Moline, IL) heated by a 4kW fluidized sand bath (Model SBL-2D, Techne, Princeton, NJ). Temperature was monitored by an in-line thermocouple (Omega, K-type), and an electric motor-powered twin 6-blade impeller at 200 rpm. At completion of the target reaction time, the reactor was quenched by lowering it into a room temperature water bath. The solids were then separated from the reaction liquor by vacuum filtration through glass fiber filter paper at room temperature (Fischer

Scientific, Pittsburgh, PA). Density and mass of the hydrolyzate were measured using 25 ml volumetric flask for mass balance and yield calculations. The solids were then dried in the 40 °C oven until moisture content was suitable for compositional analysis.

4.2.3 Precipitation of CELF lignin by boiling of hydrolyzate for THF removal

In this method outlined in Figure 4.2, CELF lignin was precipitated by boiling the hydrolyzate at a temperature that preferentially boils out THF and precipitates the lignin fraction solubilized in THF. The hydrolyzate was boiled in 100 ml flasks in triplicates. Each flask, containing 50 g of hydrolyzate, was heated on a stir plate at 90 °C for 6 hrs inside a fume hood. After 6 hrs, approximately 15 g L^{-1} of THF was present in the flask. The flasks were left overnight for 15 hrs to allow the remaining THF to evaporate at room temperature and minimize water loss by heating. The precipitated sticky dark resinous solid lignin was washed with water and diethyl ether to remove non-lignin soluble impurities. The purified lignin was then dried in a 40 °C oven for 15 hrs, and dry weight was measured by a moisture content analyzer.

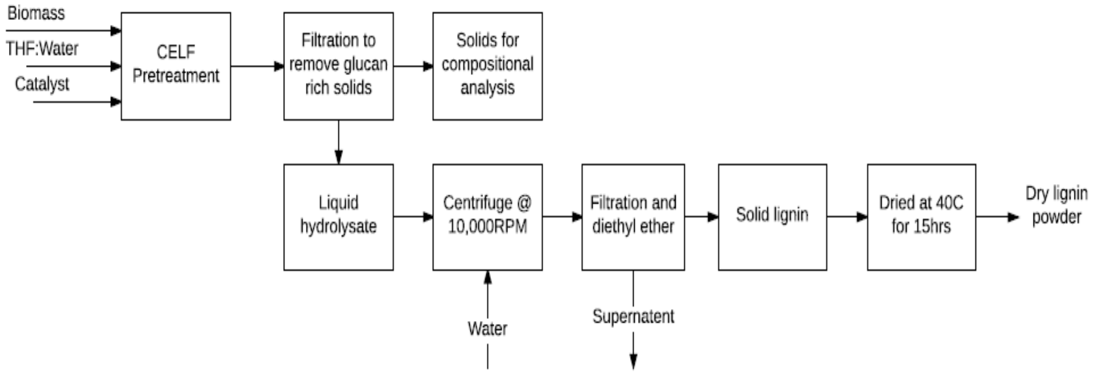

Figure 4.2 Schematic diagram of the boiling off THF to precipitate lignin

4.2.4 Precipitation of CELF lignin by dilution of hydrolyzate with water

In this method outlined in Figure 4.3, lignin was precipitated by diluting the CELF hydrolyzate with water. First, 200 g of deionized water was added to 500 ml polypropylene centrifuge tubes. To this, 50 g of hydrolyzate was added for an effective 5x dilution by mass. This caused the THF soluble lignin to precipitate in the tube. The precipitated lignin was then subjected to centrifugal water washes at room temperature at 10,000 rpm. After the water washes, the lignin was then washed with diethyl ether to remove the non-polar soluble impurities. The washed lignin was then dried along with the filter paper in the 40 °C oven for 15 hrs and final weight was taken.

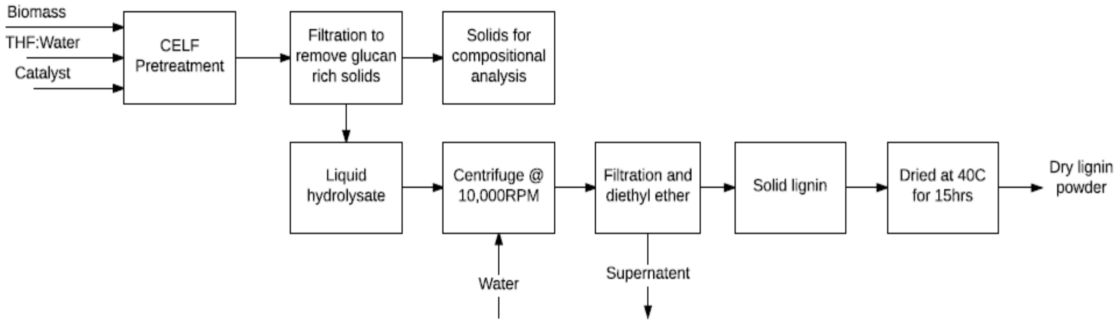

Figure 4.3 Schematic diagram of the dilution method to precipitate lignin.

Figure 4.4 Lignin precipitated on the flask bottom by dilution of 1 part of hydrolyzate with 4 parts of water by weight.

4.2.5 Cellulolytic enzyme lignin (CEL) preparation

Cellulolytic enzyme lignin(CEL) was recovered from poplar wood and maple wood by a method adapted from the literature (Carvalheiro et al. 2005). In brief, biomass samples were milled and screened to 0.42 mm using a Wiley mill (Thomas Scientific, Swedesboro, NJ) and then Soxhlet-extracted with toluene/ethanol for 8 hrs. The extractives-free samples were ball-milled in a porcelain jar with ceramic balls using a Retsch PM 100 (Newton, PA) at 600 rpm for 2.5 hrs. The ground powder was then subjected to enzymatic hydrolysis in acetate buffer (pH 4.8, 50 °C) with the enzymes CTec2 and HTec2 (1:1 ratio), at a total loading of 150 mg protein/g biomass for 48 hrs. Next the residue was isolated and hydrolysed one more time with freshly added enzyme and buffer. To remove any remaining enzyme, the recovered solids were treated with Streptomyces griseus protease (Sigma-Aldrich) at 37 °C overnight followed by deactivation at 100 °C for 10 mins. The enzyme-treated lignin rich residue was then extracted twice with 96% p-dioxane/water mixture at room temperature for 48 hrs. The extracts were combined, rotary evaporated to reduce the volume under reduced pressure (< 55 °C), and freeze dried to recover cellulolytic enzyme lignin.

4.2.6 Lignin molecular weight analysis

Oven-dried lignin samples (~20 mg) were acetylated with 2.0 ml acetic anhydride/pyridine (1:1 v/v) mixture and stirred at room temperature for 24 hrs. After that, 25 ml of ethanol was added to the reaction and left for 30 mins. The solvent was then removed by a rotary evaporator under reduced pressure (35 °C). The sequence of ethanol addition and removal was repeated at least twice or until only a trace of acetic acid was left in the acetylated sample. Samples were dried at 45 °C overnight in a VWR 1400E vacuum oven and then dissolved in THF at a concentration of ~1 mg ml^{-1} prior to the GPC analysis. The molecular weight distribution of the acetylated lignin samples was analyzed on a GPC SECurity 1200 system operated on Agilent HPLC 1200 with four Waters Styragel columns (HR1, HR2, HR4, and HR6) and an UV detector (270 nm). Polystyrene narrow standards were used to prepare a calibration curve. THF was used as the mobile phase at a flow rate 1.0 ml min^{-1}.

4.2.7 HSQC NMR analysis

2D ^{13}C-^{1}H HSQC NMR spectra of lignin samples were acquired on a Bruker Avance 400 MHz spectrometer. A standard Bruker heteronuclear single quantum coherence pulse sequence was used at the following conditions: 210 ppm spectral width in

F1 (^{13}C) dimension with 256 data points and 13 ppm spectral width in F2 (^{1}H) dimension with 1024 data points, a 90^{o} pulse, a $^{1}J_{C-H}$ of 145 Hz, a 1.5 s pulse delay, and 32 scans. ~50 mg of dry lignin samples was dissolved in deuterated DMSO. The central DMSO solvent peak was used for chemical shifts calibration (39.5 ppm, 2.50 ppm). The relative lignin monomer compositions and interunit linkage abundance were estimated semi-quantitatively using volume integration of contours in HSQC spectra. For monolignol compositions of S, G, H, and PB measurements, the $S_{2/6}$, G_2, $H_{2/6}$, and $PB_{2/6}$ contours were used with G_2 integrals doubled. The C_α signals were used for contour integration for the estimation of interunit linkages such as β-O-4, β-β, and β-5. Data processing was performed using Top Spin 2.1 software (Bruker BioSpin).

4.3 Results and discussion

4.3.1 Comparison of extraction methods based on lignin process yields

Figures 4.5 and 4.6 present pictures of the dried lignin precipitated by the boiling and dilution methods applied to the sugars and fuels precursor cases, respectively with reaction conditions illustrated in Table 4.1. The effectiveness of the two precipitation methods was first compared based on process yields computed by the following equation:

$$\text{Process yields} = \frac{\text{grams of dry lignin obtained after extraction}}{\text{grams of total lignin in raw biomass}}\ x\ 100$$

As shown in Figure 4.7, boiling the hydrolyzate resulted in higher lignin process yields than diluting hydrolyzate. This trend was observed for both the reaction conditions: sugars case and fuel precursors case. For the boiling method, 76% and 60% yields were achieved for the fuel precursors and sugars case, respectively, whereas yields from dilution were 35% and 46% for the fuel precursors and sugars case, respectively. The higher yields from the boiling method could be due to precipitation of water soluble lignin due to water evaporation during boiling. Increasing the concentration of fuel precursors or sugars as a result of boiling also makes the hydrolyzate more compatible with economical downstream processing. On the other hand, diluting the hydrolyzate with water reduced the concentration of these constituents, thereby increasing capital costs for downstream processing and especially separation.

Table 4.1 Reaction conditions for CELF optimized for maximizing sugar yields (sugars case) and maximizing fuel precursor (FP case) from biomass feedstock (FS).

Reaction	**FS**	**Temp**	**Solvent ratio**	**Catalyst used**	**Catalyst quantity**	**Reaction time**
Sugars case	Maple wood	160 °C	1:1	Sulfuric acid	0.5wt%	15min
FP case	Poplar wood	180 °C	4:1	$FeCl_3.6H_2O$	1wt%	20min

Figure 4.5 Lignin precipitated by diluting (left) and boiling (right), for sugars case.

Figure 4.6 Lignin precipitated by dilution (left) and boiling (right), for fuel precursors case.

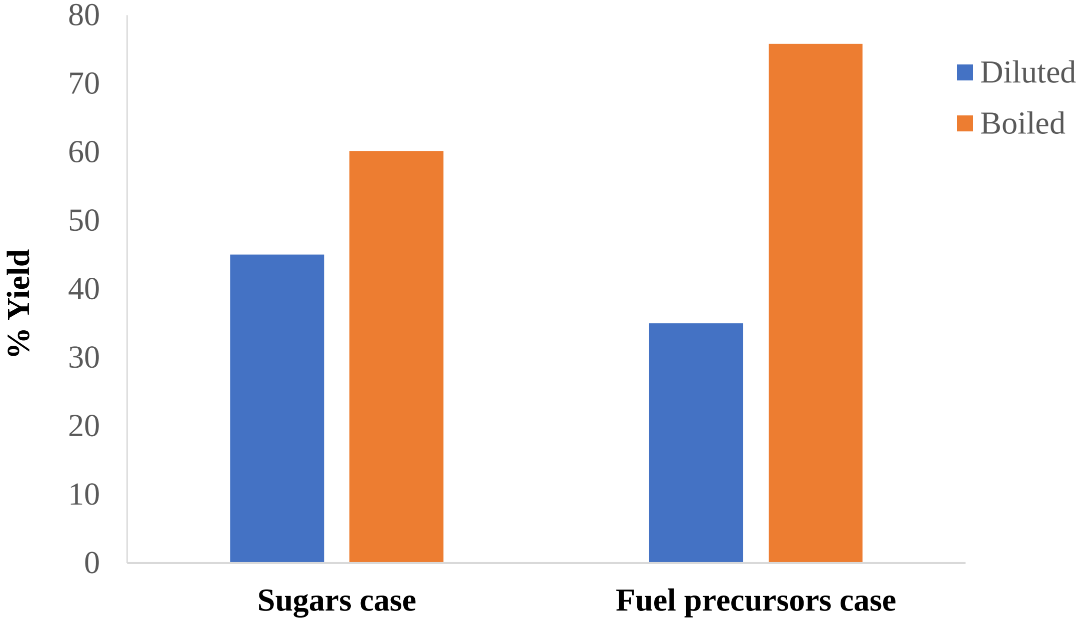

Figure 4.7 Lignin recovery yields from application of dilution and boiling methods to liquid in CELF hydrolyzate prepared at conditions that maximized sugar or fuel precursor recovery.

4.3.2 Comparison of structural characteristics of extracted lignin

Lignins precipitated from CELF hydrolyzate prepared at both reaction conditions were compared based on the average molecular weight (MW) and polydispersibility index (PDI) measured via Gel permeation chromatography (GPC). These lignins were compared to the characteristics of CEL MW (Cellulolytic Enzyme Lignin Maple Wood) and CEL PW (Cellulolytic Enzyme Lignin Poplar Wood) extracted from raw biomass by just using enzymes. The GPC data in Table 4.2 shows that CELF lignin had a lower molecular weight than CEL samples, indicating significant depolymerization during CELF pretreatment. Lignin precipitated by boiling has a lower M_w (1546 and 1245 g mol^{-1}) than that from the dilution method, 1651 and 1320 g mol^{-1}, respectively, for both CELF reaction conditions.

Since THF and water form an azeotrope, boiling of THF was accompanied by water loss that caused a fraction of low molecular weight water-soluble lignin to precipitate and lower the average molecular weight of the extracted lignin samples. Moreover, the low polydispersibility index (PDI) is the ratio of weighted average molecular weight (M_w) and number average molecular weight (M_n) and indicates the uniformity of fragments size. The low PDI for lignins extracted from CELF indicates that lignin fragments exhibit high uniformity in terms of molecular weight distribution for both preparation methods.

Table 4.2 Gel Permeation chromatography (GPC) data for lignin samples. M_w is the weighted average molecular weight, M_n is the number average molecular weight, and PDI.

Sample	Method type	M_w	M_n	PDI
CELF lignin - sugars case	Diluted	1651	976	1.69
	Boiled	1546	887	1.74
CEL MW	Enzymatic	11100	4760	2.33
CELF lignin - FP case	Diluted	1320	823	1.6
	Boiled	1245	770	1.61
CEL PW	Enzymatic	14352	2192	6.5

Lignin inter-unit linkages (β-O-4, β-β and β-5) and subunits (syringyl and guaiacyl) were quantified using HSQC NMR. The syringyl (S) to guaiacyl (G) ratio of the extracted lignin samples was then computed for comparison. As seen in Table 4.3 for the sugars case, the dilution method resulted in an S/G ratio of 1.76 that is closer to the value of 1.56 for CELF MW than the S/G ratio of 2.8 that resulted from boiling. Thus, dilution precipitated more syringyl subunits compared to guaiacyl subunits. There was substantial β-O-4 bond breakage with only 14.4% and 13.4% of bonds remaining for dilution and boiled precipitated lignin compared to 55.5% in CEL MW.

For the case of fuel precursors (FP), the lignin extracted by boiling had an S/G ratio of 1.22 that is similar to that for the CEL PW sample value of 1.28, whereas lignin precipitated by dilution had a higher S/G ratio of 2.74. The absence of β-O-4, β-β, and β-5 linkages in lignin precipitated by both methods from CELF hydrolyzate optimized for fuel precursor production showed that these conditions resulted in significant lignin depolymerization.

Table 4.3 Lignin inter-linkage and sub-unit distribution measured by HSQC NMR. Lignin extracted from CELF optimized for sugars production is termed the sugars case and lignin extracted from CELF optimized for fuel precursor production is termed the FP case.

Samples	**Method**	**Lignin sub-units**			**PB**	**Lignin inter-unit linkages**		
	type	**S**	**G**	**S/G**		**β-O-4**	**β-β**	**β-5**
Sugars case	Diluted	63.7	36.3	1.76	0	14.4	2.53	0.34
	Boiled	73.8	26.2	2.82	0	13.4	3.39	0
CEL MW	Enzymatic	61	39	1.56	0	55.5	6.2	4.9
FP case	Diluted	73.3	26.7	2.74	6.7	0	0	0
	Boiled	54.9	45.1	1.22	12.6	0	0	0
CEL PW	Enzymatic	54	42.1	1.28	14.8	56.8	4.3	6.8

4.4 Conclusions

From the process yields and characterization data of CELF generated lignin, boiling the hydrolyzate is a better strategy to precipitate lignin compared to diluting the hydrolyzate with water in terms of higher process yields and higher degree of depolymerization. Moreover, the hydrolyzate stream after lignin extraction is expected to be more amenable to downstream treatment in that the liquid left after applying the dilution method has a lower concentration of fuel precursors or sugars that would incur higher capital and energy costs to process.

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CHAPTER 5

Conclusions and Recommendations

5.1 Conclusions

In Chapter 2 of the thesis, conversion of poplar wood to fuel precursors using CELF pretreatment was optimized, and a suitable lignin recovery strategy was developed downstream of CELF pretreatment. Operation of CELF pretreatment at 180 °C for 20 mins with a 4:1 THF:water ratio resulted in a maximum yield of FF and HMF of 93.5% and 66% of the theoretical maximum, respectively, using $FeCl_3.6H_2O$ as the catalyst.

Of the two methods developed to recover acid insoluble lignin after the CELF reaction, it was concluded that boiling the hydrolyzate to remove THF was better than diluting the hydrolyzate with water. The boiling method produced 76% lignin yields whereas the diluting method only achieved 35% yields. In addition, the hydrolyzate stream remaining after dilution would require higher capital and energy costs for product recovery due to the low concentration of fuel precursors. Analysis of the lignin by Gel Permeation Chromatography (GPC) showed that lignin extracted after CELF reaction had lower average molecular weight compared to enzyme extracted lignin (CEL PW), the latter resembling native lignin in biomass. The average molecular weights of the lignin produced by the boiling and diluting methods were 1320 and 1245 g mol^{-1} compared to 14352 g mol^{-1} for the CEL PW sample. Moreover, lignin characterization by HSQC NMR indicated that the ether linkages β-O-4, β-β, and β-5 were completely severed by the CELF reaction indicating high degree of depolymerization. Furthermore, a high uniformity in the low molecular weight CELF lignin fragments resulted as illustrated by a low polydispersibility index of 1.6.

The lignin precipitation methods developed in Chapter 2 were also applied to the liquid produced by CELF pretreatment that had been optimized to for sugar yields from maple wood. It was observed that low average molecular weight lignin was recovered by both precipitation methods. The average molecular weight of lignin precipitated by boiling of 1546 g mol^{-1} was lower than that of 1651 g mol^{-1} for lignin extracted by dilution. This outcome could be caused by water evaporation during THF boiling resulting in precipitation of low molecular weight lignin. Lignin extracted by dilution had a S/G ratio of 1.76 that resembled the 1.56 S/G ratio of CELF MW. The high S/G ratio of 2.8 from boiling indicated that more syringyl subunits were precipitated compared to guaiacyl subunits. Furthermore, there was substantial β-O-4 bond breakage during CELF reaction, with only 14.4% and 13.4% of the bonds remaining in lignin recovered by dilution and boiling compared to 55.5% for CEL MW.

A preliminary technoeconomic analysis was applied to a process that integrated CELF pretreatment and lignin recovery with downstream catalytic conversion of FF and HMF to MF and DMF, respectively, for use as gasoline blend stocks. At the base case scenario, the operating margin for the hypothetical biorefinery was projected to be 4.76 M\$ yr^{-1} assuming a THF loss of 1.3wt% and furan selling price of \$600 dry tonne^{-1}. This analysis showed that overall process profitability was very sensitive to the furan selling price. For example, a 20% increase in the selling price of furans from \$600 dry tonne^{-1} to \$720 dry tonne^{-1} caused a 53% increase in the operating margin. Even though THF solvent recovery and lignin selling price had less impact on profitability of the refinery compared to furan selling price, achieving the base case values is vital for the process to have a positive operating margin. In particular, a THF loss greater than 2.06wt% resulted in a negative operating margin. Thus, different strategies are needed to prevent THF loss during distillation and minimize its conversion to 1,4-butane-diol. A portion of furfural formed after CELF could also be channeled to produce THF to offset some of the solvent loss, if economically viable.

In Chapter 3, the fermentability of CELF hydrolyzate was investigated, and a detoxification strategy was developed to improve fermentation yields and rates. THF, a key ingredient for CELF pretreatment, was found to slow the rate of fermentation of C5 sugars by *S. cerevisiae* at a concentration above 7 g L^{-1}. The organic water-immiscible solvents toluene, MIBK, and MTHF were chosen as having a combination of favorable properties for extraction of inhibitors from CELF hydrolyzate and evaluated for their abilities to remove THF, soluble phenols, and other fermentation inhibitors. In an investigation of the inhibitory effect of phenols, fermentations of hydrolyzate boiled to reduce the THF concentration below 7g L^{-1} required 168 hrs to reach 70% of theoretical maximum ethanol yields while the same hydrolyzate treated with the organic solvent MIBK achieved 87% ethanol yields in just 24 hrs. Although hydrolyzate treated with toluene had the highest ethanol yield of 95%, 168 hrs of fermentation time was required to reach this level. MTHF was not able to improve rates and yields due to poor removal of THF. Application of a FCR assay revealed that hydrolyzate treated with toluene and MIBK that achieved the highest fermentation yields also had the lowest phenolic concentration. Thus, using an organic solvent such as MIBK and toluene is a promising strategy to detoxify CELF hydrolyzate by removing THF and phenols, thereby improve the total ethanol yields of the process.

5.2 Recommendations

CELF has been shown to be a promising pretreatment to support biomass conversion into fuels and chemicals. However, an initial technoeconomic analysis indicates that commercial profitability will be largely governed by the ability to recover THF. Hence, effective solutions to recover and recycle at least 97.94% of THF need to be developed. Conversion of FF to THF could potentially be developed to offset any THF losses incurred during recovery for recycle and reaction to 1,4-butanediol.

Boiling the hydrolyzate to remove THF, precipitated uniform low average molecular weight lignin with 76% yields. However, although monomeric phenols formed by lignin depolymerization are valued at about \$1000 dry tonne^{-1}, they could not be recovered. Thus, further investigation and process development is required to recover these soluble monomeric phenols to improve profitability of the overall process.