SWITCHABLE BUTADIENE SULFONE PRETREATMENT OF LIGNOCELLULOSIC BIOMASS

BY

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DISSERTATION

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ABSTRACT

Biofuels and chemicals from lignocellulosic biomass are a renewable and sustainable alternative to traditional fossil fuel sources. However, the reality of commercial lignocellulosic biofuels depends on feasibly overcoming the recalcitrance of the biomass plant cell wall, which consists of lignin and the sugar polymers cellulose and hemicellulose, the source of value-added products. "Pretreatment" is the term that describes the chemical, physical or physicochemical process that breaks the structure of biomass prior to further processing, and represents the most expensive step in the production of biofuels. Towards a more economically feasible and green pretreatment process, this dissertation introduces a new pretreatment method using butadiene sulfone ($C_4H_6O_2S$), a solvent that is inexpensive and recyclable, and effectively pretreats biomass in one step under mild conditions. The production and recovery of butadiene sulfone is available at an industrial scale, thus facilitating a potential transfer of these processes to a large scale lignocellulosic-based operation.

Miscanthus x giganteus, a perennial grass and energy crop, was pretreated with butadiene sulfone in a batch reactor at 90 °C-110 °C for 6-30 h. The ability of butadiene sulfone to "switch" in equilibrium to 1,3-butadiene and sulfur dioxide, allowed for the formation of sulfurous acid in the presence of water. The sulfurous acid hydrolyzed xylan (hemicellulose), removing up to 91% of xylan into the liquid phase as a potential source of value-added products. In the pretreated solids, 90-99% of glucan was preserved, the key substrate for enzymatic hydrolysis of glucan to glucose and downstream fermentation to biofuels and chemicals. The butadiene sulfone in equilibrium solubilized up to 58% of lignin, an important aspect of pretreatment, as lignin is known to inhibit the action of enzymes to release glucose from glucan.

The kinetics of xylan hydrolysis and solubilization mechanisms of lignin from the butadiene sulfone-water pretreatment of *Miscanthus*, were also studied. The hydrolysis of xylan, *via* Brønsted acid catalysis, followed irreversible first-order kinetics with the activation energy determined to be 89 kJ/mol. The solubilization of lignin was attributed to the solvent interactions with the delocalized lignin during or after the hydrolysis of xylan.

Butadiene sulfone was also used to pretreat the solids after the production of furfural from sugarcane bagasse. These furfural residues are rich in glucan, but mostly used as boiler fuel at a commercial-scale. Alternatively, a furfural-based biochemical conversion approach was proposed, consisting in the pretreatment of the glucan-rich furfural residues with butadiene sulfone-water, followed by enzymatic hydrolysis of glucan to glucose for potential production of biofuels. Xylan in sugarcane bagasse was converted in a batch reactor to furfural via sulfuric acid catalysis at 170 °C and 180 °C for 10 min. The furfural yields were comparable to industrial batch production, achieving 38% and 50% of theoretical at 170 °C and 180 °C, respectively. The furfural residues were subjected to pretreatment with butadiene sulfone in the presence of water at 110 °C for 3 h, resulting in the solubilization of 22-28% of the solids, corresponding to the removal of 33-39% of the total lignin in the furfural residues.

Finally, it was demonstrated *via* thermogravimetric analysis that butadiene sulfone can be completely decomposed at 130 °C and 70 min for potential recovery and reformation, as the solvent's decomposition gases are the raw materials for its production.

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To my wife, Daneira, and to the memory of my mother, Vielka, I dedicate this effort.

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CHAPTER 1
INTRODUCTION

Accelerated depletion of non-renewable sources of energy and rising oil prices, have prompted a surge in research to develop biofuels (e.g., ethanol, biodiesel) and chemicals from renewable materials. As the world's largest consumer of crude oil (EIA 2011), the United States has placed utmost importance in the production of biofuels from renewable sources. The U.S government enacted in 2007 the Energy Independence and Security Act (EISA) to "increase the production of clean renewable fuels" and "to move the United States towards greater energy independence and security" (National Research Council 2011). The Renewable Fuel Standard (RFS2) under the EISA act mandates that a minimum volume of biofuels is to be used each year in the fuel supply of the transportation sector (Schnepf 2011). The RFS2 mandate is 36 billion gallons of biofuels consumed annually in 2022, of which no more than 15 billion gallons can be ethanol from corn starch, at least 16 billion must be from cellulosic feedstocks, and at least 5 billion gallons must from other non-corn starch sources (e.g., 1 billion gallons of biomass-derived diesel) (Schnepf 2011).

Even though biofuels from edible biomass, such as corn, are a mature and reliable technology, these feedstocks are not a long-term energy solution, because suitable cropland is not readily available and may compete with food production leading to higher food prices (Huber and Dale 2009). On the other hand, non-food biomass sources (i.e. lignocellulose) are renewable and sustainable, do not interfere with the food chain and can grow on unfertile lands (Huber and Dale 2009). However, while conventional biofuels and biomass-based diesel will meet or exceed the RFS2 consumption mandate by 2022 (National Research Council 2011), cellulosic biofuels will not meet the RFS2 mandate based on current projections released by the U.S.

Energy Information Administration (EIA) 2013 Annual Energy Outlook for 2011-2040 (Fig. 1.1).

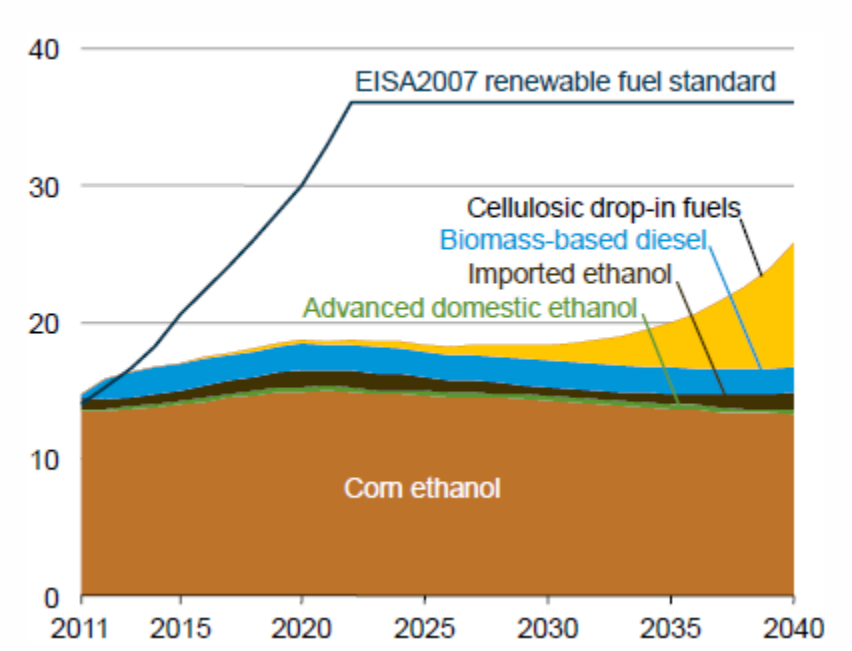

Figure 1.1 The U.S. Energy Information Administration (EIA) 2013 Annual Energy Outlook for 2011-2040, reports that the U.S. consumption of cellulosic biofuels will not meet the EISA 2007 RFS2 targets. The x-axis represents the year and the y-axis represents billion gallons ethanol-equivalent. Reprinted from EIA 2013.

The stagnancy of cellulosic biofuels consumption is due in part to the unavailability of commercial biorefineries to date, policy uncertainty and high production costs, compared to petroleum-based fuels (National Research Council 2011). However, a recent article reports that nine commercial scale facilities in the U.S. are scheduled to be operational in 2013 with an annual production of 215 million gasoline-gallon equivalent by 2014, and each plant has a capacity of at least 20 million gallons per year. The combined production volume would place the cellulosic biofuels RFS2 mandate more than three years behind schedule (Brown and Brown 2013).

Three of these biorefineries follow the "thermochemical" route (i.e., hydrocarbon based-fuels via pyrolysis or gasification) and six follow the "biochemical" route (i.e., enzymatic hydrolysis to ethanol) (Fig. 1.2) (Brown and Brown 2013). The authors argue that the success or failure of these commercial operations will affect the future of the U.S. biofuels policies and the future of the cellulosic biofuels industry.

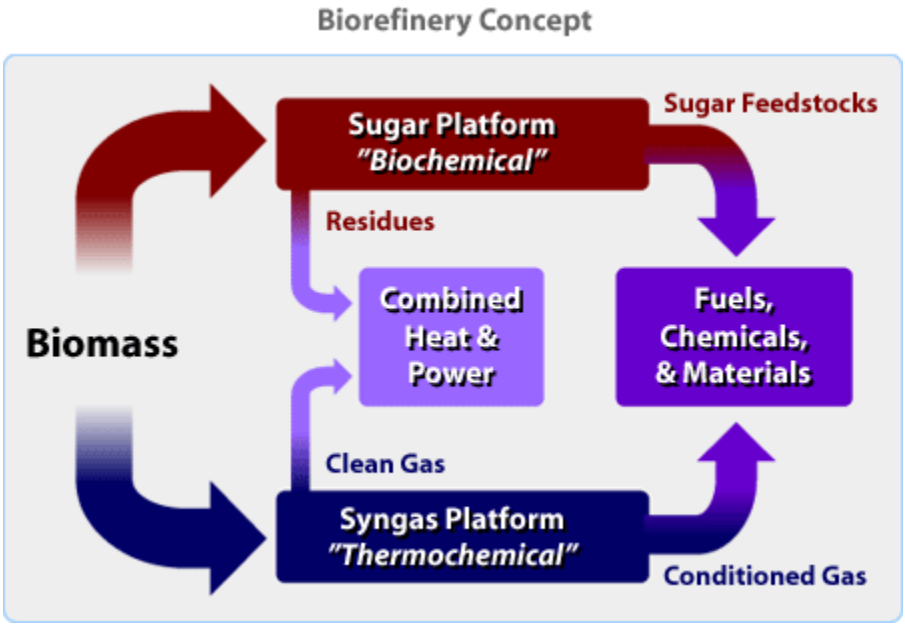

Figure 1.2 Conceptual depiction of a biomass biorefinery by the U.S. National Renewable Energy Laboratory (NREL). Reprinted from NREL 2009.

Notwithstanding the upcoming commercial scale biorefineries, research into cellulosic biofuels remains a very active research area. The scope of the present work sits within the biochemical route which entails overcoming the recalcitrance of lignocellulose (i.e. pretreatment), releasing its sugars via enzymatic hydrolysis, and fermentation into biofuels (e.g. ethanol) (Fig. 1.3). Pretreatment disrupts the recalcitrant and protective nature of the carbohydrate-lignin complex in lignocellulosic biomass that limits the access of enzymes to cellulose (30-50%) and hemicellulose (20-30%) (Yang 2008).

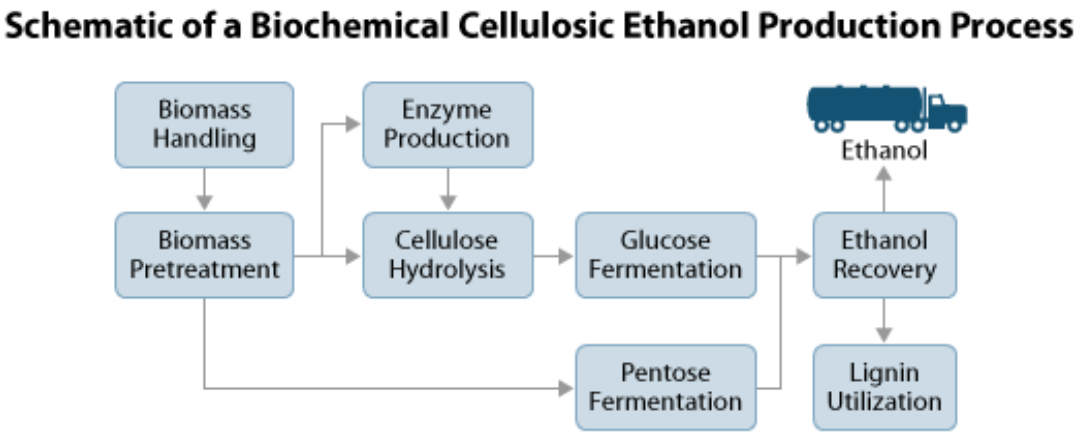

Figure 1.3 Cellulosic ethanol production process. Reprinted from the U.S Department of Energy Biomass Program (EERE 2008).

Pretreatment is the most expensive processing step of the biochemical route, representing 18% of the total projected cost of producing ethanol (Yang 2008). The basis for this projection is the widely studied dilute acid pretreatment. Key costs include expensive construction materials, separation of the liquid and pretreated solids, acid neutralization costs, and costs for removal of enzymatic and fermentation inhibitors (Yang 2008).

The importance of an economically feasible and greener pretreatment process is paramount for the success of the future lignocellulosic biofuels industry. Towards this goal, the objective of this dissertation is to introduce a new pretreatment method using switchable butadiene sulfone, an inexpensive solvent with facile industrial scale production and recyclability. In a previous work, butadiene sulfone was tested by M. Kassner for the pretreatment of corn stover, but the author experienced separation difficulties (Kassner 2008) and was not able to fully assess or confirm the effectiveness of the solvent as a pretreatment option. No other work has explored the possibility of butadiene sulfone for pretreatment.

The hypothesis tested is that butadiene sulfone is able to pretreat *Miscanthus* (energy crop) and sugarcane bagasse furfural residues (lignocellulose waste material) in one step under mild conditions; effectively removing lignin and hemicellulose, and exposing cellulose for downstream processing to value-added products (Fig. 1.4).

The following are the specific objectives of this dissertation:

1) Perform a screening of the polar aprotic solvents sulfolane, butadiene sulfone, dimethylacetamide and n-methylpyrrolidone, for solubilization of biomass and biomass derived feedstocks. (This study led to the discovery of butadiene sulfone as a potential chemical pretreatment).
2) Determine the effect of water concentration, temperature and time on the pretreatment of *Miscanthus* with butadiene sulfone.
3) Investigate the chemical mechanisms for xylan and lignin removal from *Miscanthus* during pretreatment with butadiene sulfone.
4) Evaluate the butadiene sulfone pretreatment effect on sugarcane bagasse furfural residues from the acid-catalyzed conversion of the xylan in the biomass into value-added furfural.
5) Assess the potential recovery of butadiene sulfone.

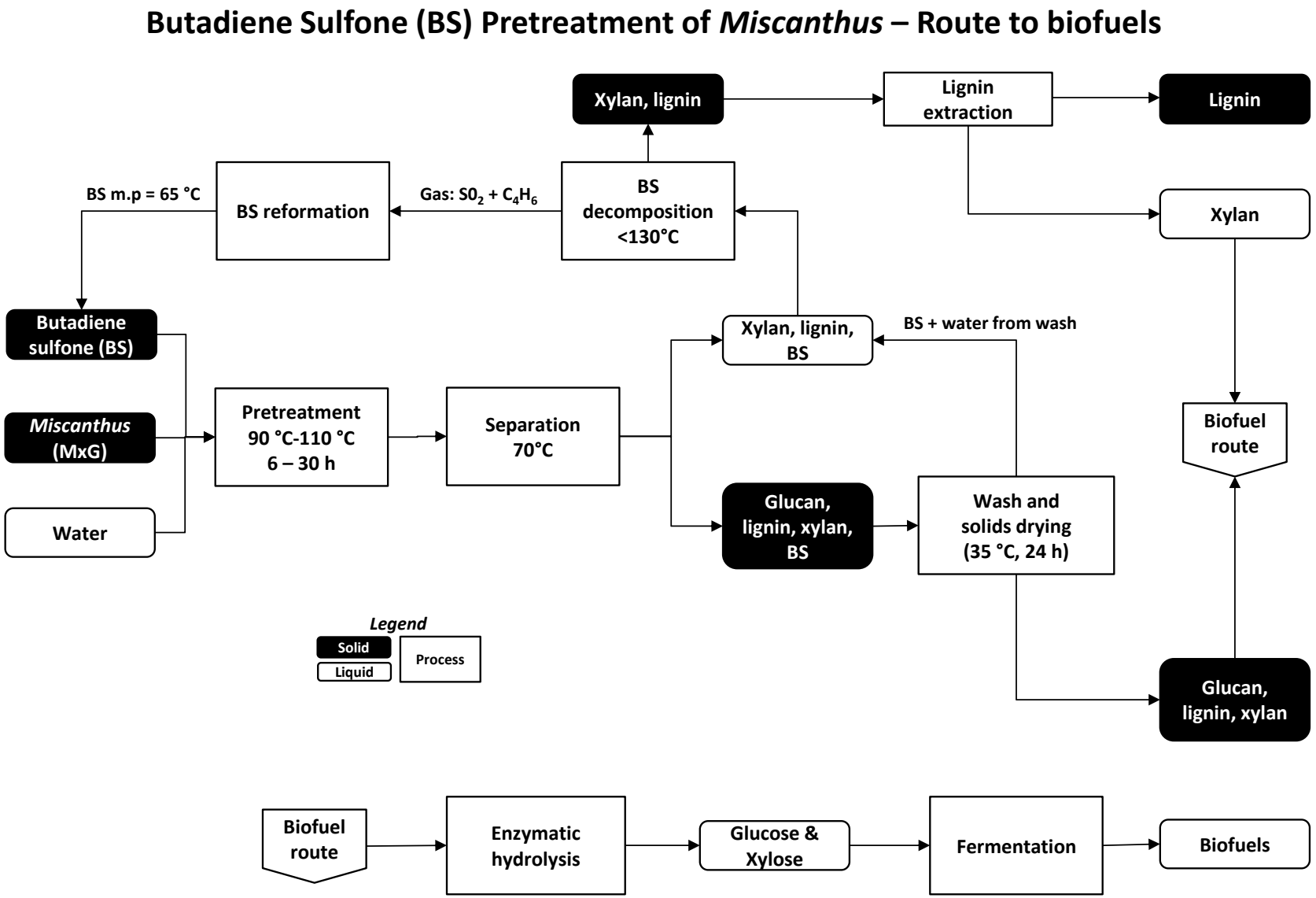

Figure 1.4 Pretreatment of *Miscanthus* with butadiene sulfone: proposed route to biofuels.

CHAPTER 2
LITERATURE REVIEW

2.1 The lignocellulose plant cell wall

Cellulose and hemicellulose are the main sugar polymers in biomass, and together with lignin, represent the key constituents of plant cell walls (Fig. 2.1). The function of cellulose (glucan) (30-50%), which consists of glucose monomers connected by β(1-4) glycosidic bond linkages in a network of hydrogen bonds, is to provide structure to the plant. Hemicellulose (20-30%) is an amorphous and complex mixture of oligosaccharides intertwined among the crystalline cellulose fibers and linked to cellulose via hydrogen bonds. The sugar monomers of hemicellulose are the pentoses xylose and arabinose, and the hexoses glucose, galactose and mannose; and with xylose being the predominant monomer in *Miscanthus* (Brosse et al. 2012), and sugarcane bagasse (Mellinger-Silva et al. 2011), the hemicellulosic component of both feedstocks will be referred henceforth as xylan.

Lignin (10-30%), the third component of importance in biomass, is a non-sugar polyphenolic polymer consisting primarily of *p*-coumaryl, coniferyl and sinapyl alcohol monomers chiefly linked via ether linkages (Morvan et al. 2009; Lu and Ralph 2010). Lignin covalently links to hemicellulose mostly through ester linkages (Chundawat et al. 2011) and protects both cellulose and hemicellulose from enzymatic attack (Mousdale 2008). The primary cell wall of plants primarily consists of cellulose and hemicellulose (Rose 2003) and it is generally accepted that lignin occupies part of the middle lamella and secondary cell wall of the plant (Fig. 2.2) (Sun 2010). Sierra and coworkers refer to lignin in simpler terms as the “glue” for cellulose and hemicellulose; by comparing a plant to fiberglass, where cellulose is analogous

to glass fibers and lignin serving as the epoxy resin (Sierra et al. 2008). The lignin-hemicellulose barrier and the steric hindrance of glucan chains packed tightly in a crystalline morphology, makes biomass resistant to enzymatic attack (Chundawat et al. 2011).

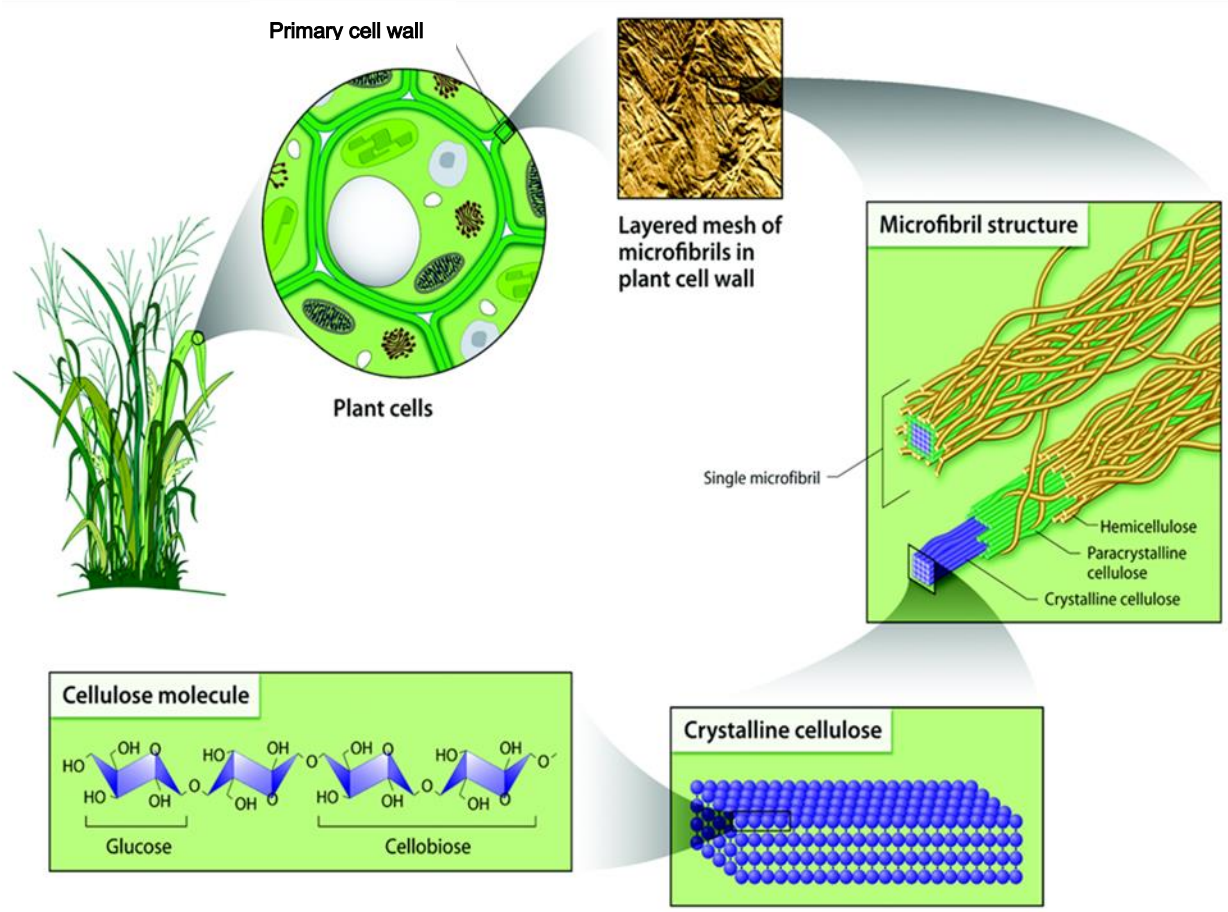

Figure 2.1 Parenchyma of the plant cell wall of lignocellulosic biomass. Lignin is not shown. Reprinted from U.S. DOE Genomic Science 2013.

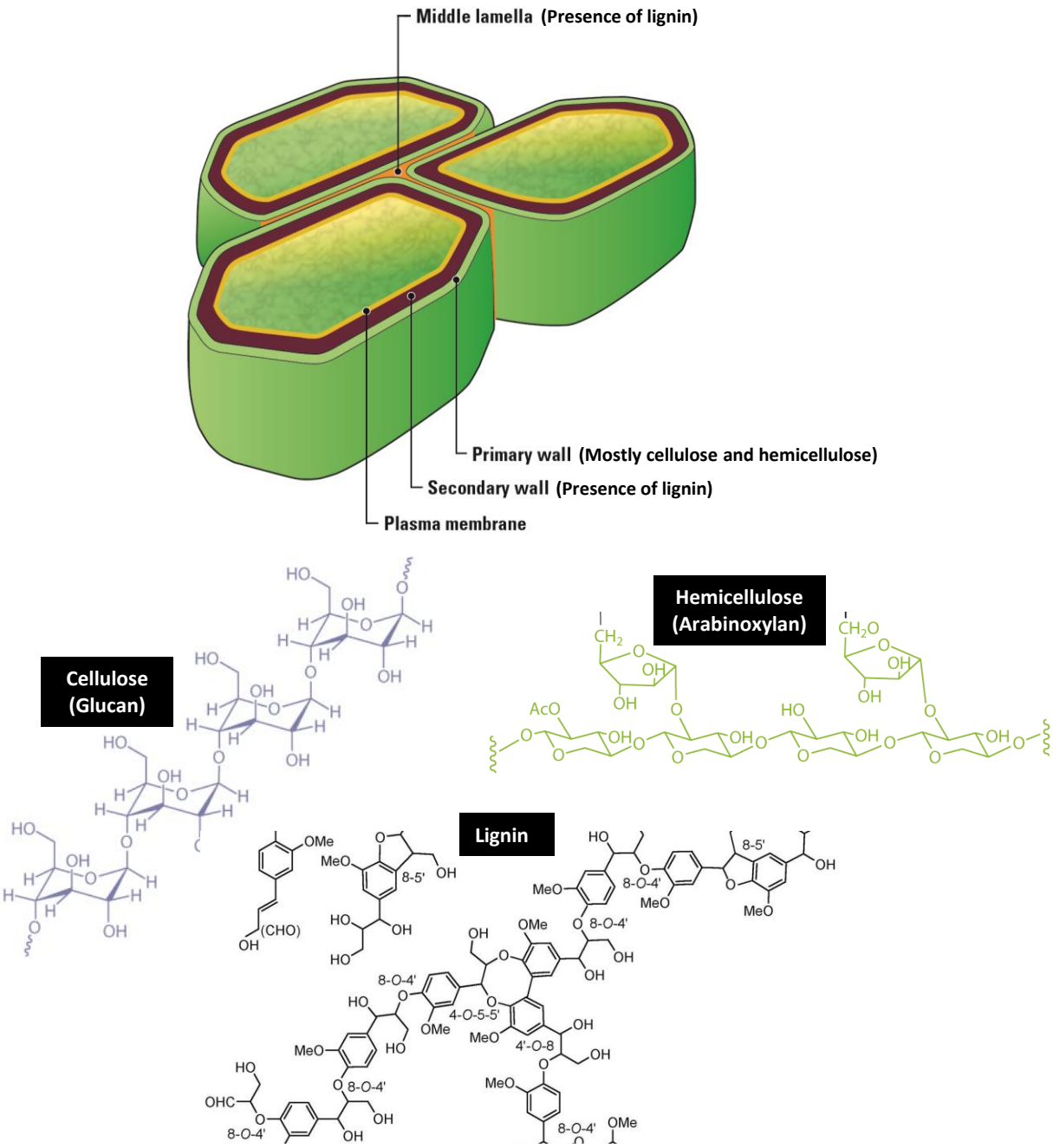

Figure 2.2 Three layers are present in plant cell walls: the primary cell wall, consisting mostly of cellulose and hemicellulose. Lignin is present in the secondary cell wall and the middle lamella (Lu and Ralph 2010; Rose 2003). Adapted from U.S. DOE Genomic Science 2013 and Chundawat et al. 2011.

Miscanthus and sugarcane bagasse

Miscanthus x giganteus, a C4 perennial grass, is an energy crop with low nutrient and water requirements, and more productive in biomass per acre than other energy crops, such as switchgrass, poplar, agave, corn stover and sugarcane bagasse (Table 2.1) (Heaton et al. 2004; Somerville et al. 2010). The chemical composition of the *Miscanthus x giganteus* plant cell wall has been reported to be around 42% cellulose, 25% hemicellulose and 28% lignin (Dee and Bell 2011; Padmanabhan et al. 2011; de Frias and Feng 2013). Sugarcane bagasse is the lignocellulosic waste product from the production of sugar, composed of 40-45% cellulose, 30-35% hemicellulose and 20-30% lignin (Vallejos et al. 2012). Bagasse is either burned in the sugar and ethanol mills for energy production (Vallejos et al. 2012) or used industrially for production of furfural, an important furan compound used as platform chemical (Werpy et al. 2004). The cellulose-rich bagasse furfural residues are burned for energy (Mamman et al. 2008) or used for value-added fuels or chemicals (Win 2005).

Table 2.1 Estimated productivity, water and nitrogen requirements of different energy crops (adapted from Somerville et al. 2010).

Crop	Avg. Productivity (MT ha^{-1} yr^{-1})	Seasonal water requirements (cm yr^{-1})	Tolerance to draught	Nitrogen requirements (kg ha^{-1} yr^{-1})
Corn Stover	3	50-80 (corn)	Low (corn)	90-120
Sugarcane bagasse	10	150-250 (sugar)	Moderate (sugar)	0-100
Miscanthus	15-40	75-120	Low	0-15
Poplar	5-11	70-105	Moderate	0-50
Agave spp.	10-34	30-80	High	0-12

2.2 Pretreatment of lignocellulosic biomass

The recalcitrance of lignocellulosic biomass is the main challenge to overcome in order to use it as a renewable source for chemicals and fuels (Elander et al. 2009). Researchers are attempting to break the complex and protective chemical structure of biomass in order to isolate its fundamental sugar units which can be transformed into biofuels and chemicals (Fig. 2.3). Lignin content in biomass hinders the release of glucose from cellulose by irreversibly adsorbing cellulase enzymes targeted for cellulose (Li et al. 2010). Hence, breaking the lignin seal is vital for effective enzymatic hydrolysis of cellulose (Chang and Holtzapple 2000; Mosier et al. 2005). "Pretreatment" is the term used for the physical, biological, chemical or physicochemical process (Mousdale 2008) of deconstructing the cell wall matrix to remove or alter the lignin and hemicellulose structures (Anugwom et al. 2012a; Anugwom et al. 2012b), and to preserve the cellulose fraction (Sierra et al. 2008). It is the step before the enzymatic hydrolysis of the sugar biopolymers into simple monosaccharides, and fermentation to biofuels.

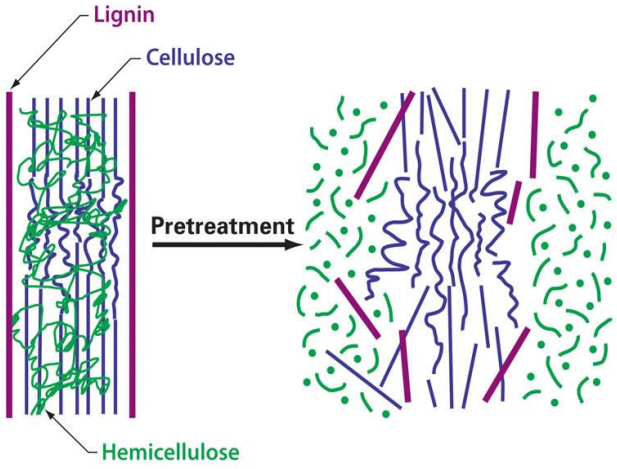

Figure 2.3 Depiction of the effect of pretreatment on the plant cell wall. When biomass is attacked by chemical or biological methods, the structure is broken and exposed. Reprinted from Mosier et al. 2005.

Physical and biological pretreatments

Physical pretreatments (e.g., ball milling) aim at size reduction from ~10-30 mm after chipping to ~0.2-2 mm after grinding to reduce cellulose crystallinity, with the limitation of high energy consumption (Kumar et al. 2009a). Biological pretreatments are based on natural wood-attacking microorganisms that grow on the biomass and have the ability to degrade hemicellulose and lignin, with the main treatments including wood-rotting fungi (Mousdale 2008). Brown rots attack cellulose and white or soft rots attack both cellulose and lignin (Sun and Cheng 2002). However, these methods are limited by long reaction times and low hydrolysis rates (Kumar et al. 2009a; Sun and Cheng 2002).

Physicochemical pretreatments

Physicochemical pretreatments generally combine physical and chemical methods by treating the biomass with or without a chemical under high temperature and pressure, followed by rapid decompression (e.g., steam explosion, ammonia fiber expansion (AFEX), CO_2 explosion) (Kumar et al. 2009a). These techniques are effective for biomass decrystallization and for increasing the surface area. Steam explosion is the most commonly used pretreatment method for biomass (Kumar et al. 2009a), and a modified steam explosion method will be used in one of the first commercial scale cellulosic ethanol facility in the U.S., POET-DSM, for the production of 20 million gallons of ethanol scheduled for early 2014 (Brown and Brown 2013). AFEX is similar to steam explosion, with the exception that biomass is exposed to liquid ammonia for a period of time prior to decompression. As opposed to steam explosion, the ammonia pretreatment has the advantage of producing no fermentation inhibitors and eliminating the need of a water wash (Sun and Cheng 2002).

Chemical pretreatments

Chemical pretreatments are generally more effective at removing a greater amount of lignin and/or hemicellulose (Mäki-Arvela et al. 2011), and in exposing or solubilizing cellulose. These methods include alkali, peroxides, Brønsted acids, organosolv (organic solvent + inorganic acid), ionic liquids (Yang 2008; Mäki-Arvela et al. 2010; Blanch et al. 2011; Brodeur et al. 2011). Alkali pretreatments use mild temperatures (<160 °C) but longer times (hours to weeks) and follow a saponification mechanism of intermolecular ester bonds crosslinking hemicellulose and lignin to remove both biopolymers (Sun and Cheng 2002). Hydrogen peroxide in combination with alkali removes a greater amount of lignin (Wang et al. 2010). In dilute acid pretreatments, hemicellulose can be completely removed from biomass at high temperatures (160-220 °C) and short times (< 30 min) following irreversible first-order kinetics in the hydrolysis to xylose and dehydration into furfural (Blanch et al. 2011); a product that is inhibitory to fermentation, but otherwise a value-added product if a biorefinery concept is considered. Organosolv can fractionate biomass into solid lignin, solid cellulose fibers and an aqueous hemicellulose stream (Blanch et al. 2011). Ionic liquids, salts consisting of an amine heterocycle cation with an organic or inorganic anion, are able to disrupt biomass by dissolving cellulose and/or removing lignin via ionic, π-π, and hydrogen bonding interactions (Li et al. 2010; Mora-Pale et al. 2011).

All pretreatment methods present advantages and disadvantages (Tables 2.2, 2.3) and the reader is referred to comprehensive reviews on the matter (Yang 2008; Mosier et al. 2005; Kumar et al. 2009a; Blanch et al. 2011; Brodeur et al. 2011). However, a common theme is that pretreatment remains the most expensive process step in the production of biofuels and chemicals from lignocellulosic biomass.

Table 2.2 Advantages and disadvantages of current pretreatment methods.

Pretreatment method	Advantages	Disadvantages
Physical (milling)[1]	▪ Reduced cellulose crystallinity	▪ High energy consumption
Biological[1,6]	▪ Lignin and hemicellulose degradation ▪ Low energy requirement ▪ Environmentally friendly	▪ Low hydrolysis rate ▪ Long reaction times
Dilute acid[1, 2,3]	▪ Short reaction times (1-30 min) ▪ Removal of hemicellulose via hydrolysis to xylose ▪ Alters lignin structure	▪ High temperatures (160-220 °C) ▪ Formation of fermentation inhibitors
Hot water[2,3,5]	▪ Short reaction times (1-30 min) ▪ No chemical required ▪ Removal of hemicellulose	▪ High temperatures (160-220 °C) ▪ Formation of fermentation inhibitors
Alkali[1,2,3]	▪ Mild temperatures (25-160 °C) ▪ Removal of lignin and hemicellulose ▪ No formation of fermentation inhibitors	▪ Long reaction times (60 min – weeks) ▪ Salt formation ▪ Chemicals consumption (no recovery)
Organosolv[1,2]	▪ Removal of lignin and hemicellulose	▪ Solvent volatility ▪ High cost for solvent recovery
Ionic Liquids[2,4]	▪ Dissolution of cellulose and/or lignin ▪ Solvents are non-volatile	▪ Expensive solvents ▪ Solvents may be inhibitory to fermentation
Flowthrough[7]	▪ High removal of xylan and lignin	▪ High water and energy use
Steam explosion[1,2,3]	▪ Short reaction times (1-15 min) ▪ Removal of hemicellulose and lignin	▪ High temperatures (180-290 °C) ▪ Formation of fermentation inhibitors
AFEX[1,2,3]	▪ Short reaction times (5-45 min) ▪ Increased surface area ▪ Reduced cellulose crystallinity ▪ No formation of fermentation inhibitors	▪ No removal of hemicellulose or lignin ▪ Not suitable for high lignin biomass

[1] (Kumar et al. 2009a)
[2] (Blanch et al. 2011)
[3] (Chundawat et al. 2011)
[4] (Mora-Pale et al. 2011)
[5] (Khullar, E. 2012)
[6](Sun and Cheng 2002)
[7](Yang and Wyman 2004)

Table 2.3. Preservation of cellulose and removal of hemicellulose and lignin for current pretreatment methods (adapted from Chundawat et al. 2011).

Pretreatment method	% Cellulose preserved	% Hemicellulose removed	% Lignin removed
Dilute acid	85-95	75-95	10-20
Steam explosion	95-99	40-95	40-50
Hot water	90-99	40-55	negative removal[1] - 0[2]
Lime	97-99	3-35	40-50
AFEX	100	0	0

[1] Negative removal of lignin represents an increase in lignin composition after hot water pretreatment as reported by Khullar et al. 2013.
[2] (Nlewem and Thrash Jr. 2010)

2.3 Switchable butadiene sulfone*

Butadiene sulfone (BS) or sulfolene ($C_4H_6O_2S$) is a β,γ-unsaturated cyclic sulfone (m.p. 65°C) (Fadhel et al. 2011) with the unique ability to "switch" from solvent to gaseous 1,3-butadiene and sulfur dioxide in a reversible reaction (Pollet et al. 2011; Drake et al. 1946) that favors BS below 100 °C and the gases above 100 °C (Donaldson et al. 2009). Table 2.4 compares the physical and chemical properties of butadiene sulfone with similar cyclic sulfone structures, sulfolane and piperylene sulfone.

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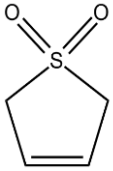
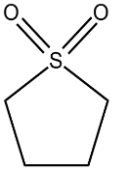
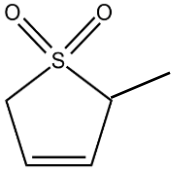
*This section includes previously published material by the author. Reference: J. Atilio de Frias and Hao Feng, *Green Chem.*, 2013, 15, 1067-1078.

Table 2.4. Physical and chemical properties of different cyclic sulfones.

Physical and chemical properties	**Butadiene sulfone** 	**Sulfolane** 	**Piperylene Sulfone** 
Density (g/ml)	1.314[a]	1.261[a]	NA
Molecular wt. (g/mol)	118.15[a]	120.17[a]	~132
Boiling point (°C)	>100[b] (760 Torr)	285.6 [a] (760 Torr)	85[c] (7 torr)
Melting point (°C)	65[c]	28.6[g]	-12[c]
Dipole moment (D)	NA	4.90[e] (30 °C)	5.32[c] (25 °C)
Kamlet-Taft α	NA	0[d]	0[c]
Kamlet-Taft β	NA	0.39[d]	0.46[c]
Kamlet-Taft π*	NA	0.98[d]	0.87[c]
Dielectric constant (ε)	NA	43.39 [f] (30 °C)	42.6[c] (25 °C)

NA (not available)
[a]Chemical Abstract Service (CAS)
[b](Donaldson et al. 2009)
[c](Fadhel et al. 2011)
[d](Marcus 1993)
[e](Lagowski 1976)
[f](Awwad et al. 2002)
[g](Domanska et al. 1996)

In the presence of water, sulfur dioxide forms *in situ* sulfurous acid (Donaldson et al. 2009) which can act as a catalyst in deconstructing lignocellulosic biomass (Kassner 2008), and as such, it can break ester bonds connecting hemicellulose and lignin, with the delocalization of the amorphous structure of hemicellulose and subsequent acid hydrolysis (Chundawat et al. 2011) (Fig. 2.4).

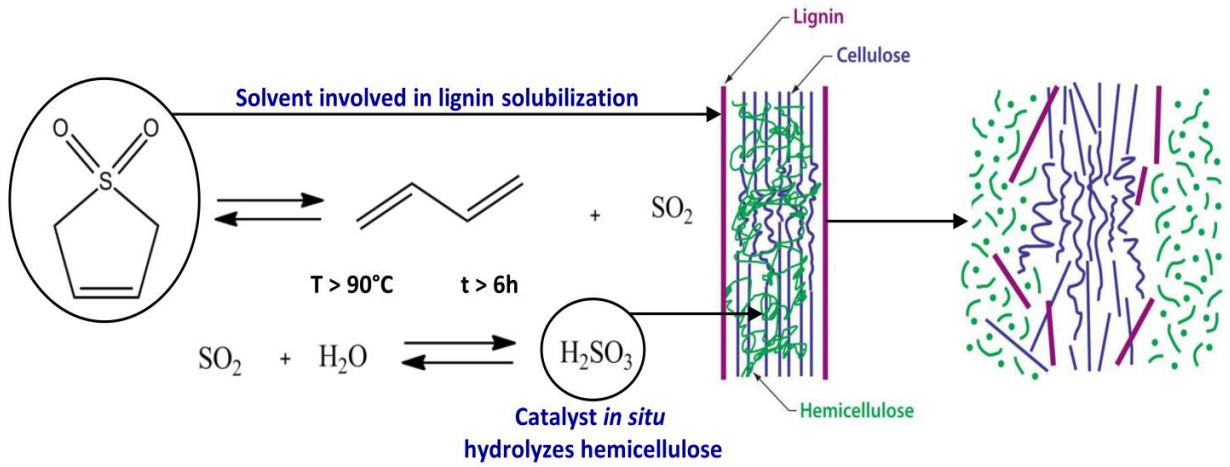


Figure 2.4 Formation of *in situ* sulfurous acid from the decomposition of butadiene sulfone in water at temperatures above 90°C and 6 h. Sulfurous acid acts as Brønsted acid catalyst and butadiene sulfone as organic solvent in the deconstruction of lignocellulosic biomass. Reprinted from de Frias and Feng 2013.

The "switchable" capacity of BS also allows for recovery of the sulfur dioxide and butadiene decomposition gases for recombination into the solvent at potentially high yields. These gases are the raw materials for commercial production of BS and the current large scale availability for both production and recovery of BS (Couper et al. 2005) will allow for a potential transfer of these operations into a biorefinery. The facile and scalable recyclability of BS is an advantage over traditional acid/alkali pretreatments and novel ionic-liquid based pretreatments. The former, would involve neutralization and further processing to remove contaminant salt by-products (Donaldson et al. 2009; Clark 1999) and the latter require difficult recycling processes not yet at large scale operations (Mora-Pale et al. 2011). Another advantage of BS pretreatment is the inexpensive commercial availability of BS, compared for instance, to ionic liquids, which are 5-20 times more expensive than conventional solvents at the laboratory scale (Tadesse and Luque 2011) and require in many cases the addition of an acid-catalyst (Li et al. 2007). For

example, from the Sigma-Aldrich catalog, 500 g of BS (98%) cost ~US$50 and 50 g of the effective ionic liquid 1-ethyl-3-methylimidazolium acetate (97%) cost ~US$868.

In a previous study, M. Kassner used BS in the presence of water to pretreat corn stover at 70 °C for 1-24 h under batch reaction conditions and a 5% solids loading (Kassner 2008). The author showed SEM evidence of biomass alteration (Fig. 2.5); however, no other evidence was presented to demonstrate or confirm the effectiveness of BS as a pretreatment method. As reasons for not pursuing further analysis, the study reported "difficulties" for "separating the BS from the liquid fraction of the biomass sample after pretreatment", due to the solvent's "higher decomposition temperature". As a result, another sulfone was suggested for pretreatment, piperylene sulfone (PS) (m.p. -12°C), with a much lower melting point than BS. However, the author hypothesized the PS method will "likely suffer from previously unexpected separation difficulties" and no other analyses were reported for either BS or PS. The complications in the analysis stemmed from only focusing on analyzing the liquid fraction, even though the study acknowledged there were "many additional aspects that must be examined and studied to determine completely an optimal pretreatment". There have been no other studies exploring the possibilities of butadiene sulfone-water as a lignocellulose pretreatment option.

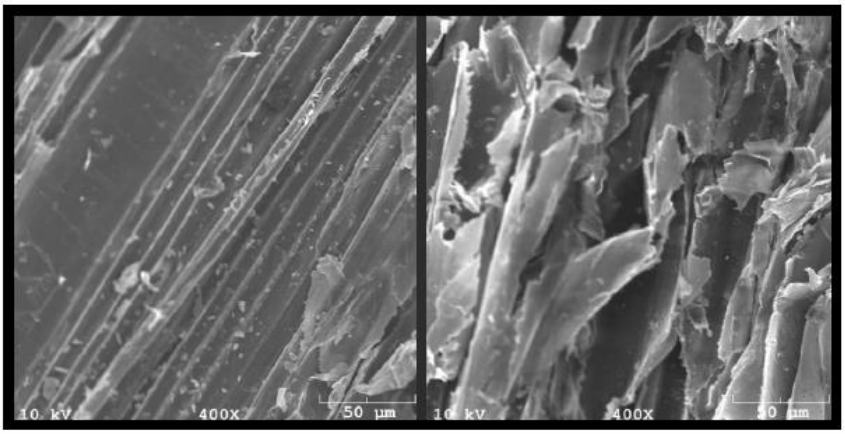


Figure 2.5 SEM micrograph after pretreatment of corn stover with butadiene sulfone-water at 70 °C. Reprinted from Kassner (2008). The image to the right is the pretreated biomass and the image to the left is raw corn stover.

2.4 Conversion of hemicellulose into furfural: a biorefinery concept for value-added utilization of the cellulose-rich residues

The concept of biorefinery encompasses the "integration of biomass conversion processes and equipment to produce fuels, power, and chemicals from biomass" (Mousdale 2008) with the expectation of a sustainable approach for bioenergy production from lignocellulose connecting both upstream and downstream production aspects. In the biochemical biorefinery platform (Fig. 1.2), the downstream processing of lignocellulosic biomass can be defined as the biological or chemical conversion of milled or pretreated biomass for the production of value-added products, whereas the upstream aspects of a biorefinery relates to the logistical issues of feedstock management and sourcing of dedicated energy crops or waste material (Mousdale 2008). A successful implementation of the downstream-upstream connection is paramount for making cellulosic biofuels a reality in the short-to-medium-term.

One option for researchers working on downstream aspects of biofuels production is to select downstream problems with resolved upstream situations. The mature furfural industry, arguably the largest commercial lignocellulosic-based industry in terms of production, has been successful for having a solution in place to the upstream aspects of biomass production by sourcing its raw materials, i.e. corn cobs or sugarcane bagasse, as part of the production/processing of corn and sugar, respectively. In 2005, the world production of furfural was 280,000 tons per year, with China supplying 200,000 tons of furfural from corncobs, followed by the Dominican Republic (32,000 tons) and South Africa (20,000 tons), both from sugarcane bagasse (Win 2005). However, the conventional production of furfural is not an effective biorefinery as only 20-30% of the biomass (hemicellulose) is used to produce furfural and the rest, cellulose and lignin, are under-utilized as boiler fuel (Mamman et al. 2008).

Furfural is part of the top 30 list of value-added chemicals and fuels from biomass developed by the US Department of Energy (DOE) (Werpy et al. 2004), and it is the end product of the acid-catalyzed dehydration of xylose, which is produced from the acid hydrolysis of pentosans (Fig 2.6).

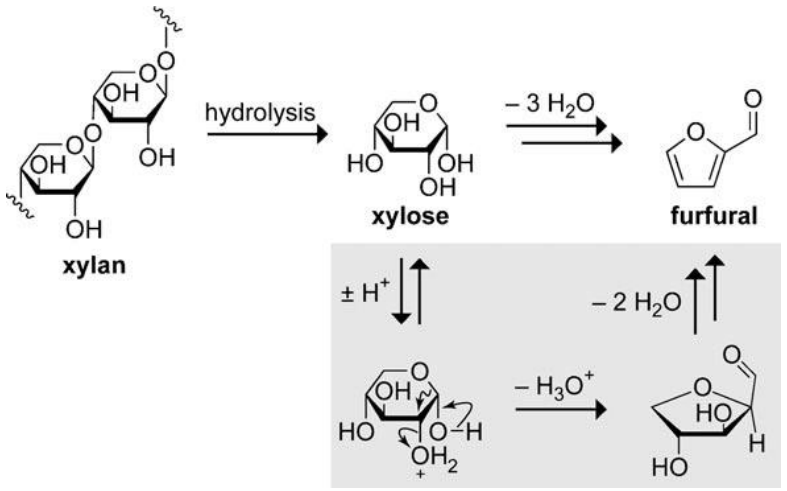


Figure 2.6 Acid catalyzed hydrolysis of xylan (hemicellulose) into xylose with further dehydration into furfural. Reprinted from Binder et al. 2010.

In contrast to the crystalline and recalcitrant cellulose, the pentosan-based hemicellulose fraction is easier to hydrolyze and remove from the plant cell wall due to its amorphous structure and solubility in water at temperatures greater than 80 °C (Gray et al. 2007). The industrial process to produce furfural from biomass pentosans was developed for the first time by Quaker Oats Company in 1921, by using oat hulls from the oatmeal manufacturing process mixed with sulfuric acid at 153°C for 5 hours in pressure cookers, yielding between 40 and 50% of furfural (percentage of theoretical yield) (Zeitsch 2000). Conditions for current industrial batch processes with different feedstocks are 3% w/w sulfuric acid, 170-185 °C and 3 h with furfural yields of 40-50% of theoretical (Mamman et al. 2008). The production of furfural from continuous processes are more commonly used today, such as the "Westpro Modified Huaxia Process" based in China, with yields of 98-99% of furfural from corncobs using fixed bed reactors and continuous dynamic refining (Win 2005). In batch or continuous processes, furfural is separated from the reaction medium as it is formed in order to avoid furfural loss by condensation (reaction with intermediates) or resinification (reaction with itself) (Zeitsch 2000).

More successful biorefinery concepts are converting both the hemicellulose and cellulose fractions into chemicals. The Biofine™ industrial process produces furfural, levulinic acid and formic acid from lignocellulosic biomass *via* dilute sulfuric acid (1-5%) catalysis in a two-stage reaction system at >210 °C and 360 psi (Hayes 2005; Bozell et al. 2000) (Fig. 2.7). At these conditions, the hydrolysis, dehydration and rehydration reactions from cellulose are selective towards levulinic acid and formic acid (Huber et al. 2006). Le Calorie S.p.a., is a commercial scale facility producing levulinic acid from paper sludge, agricultural residue and waste paper, using the Biofine™ process (Le Calorie, S.p.a. 2011). Avantium, in the Netherlands, is

producing 2,5-Furan-dicarboxylic-acid (FDCA) and derivatives using the patented YXY™ chemical catalysis process (Lozowski 2011).

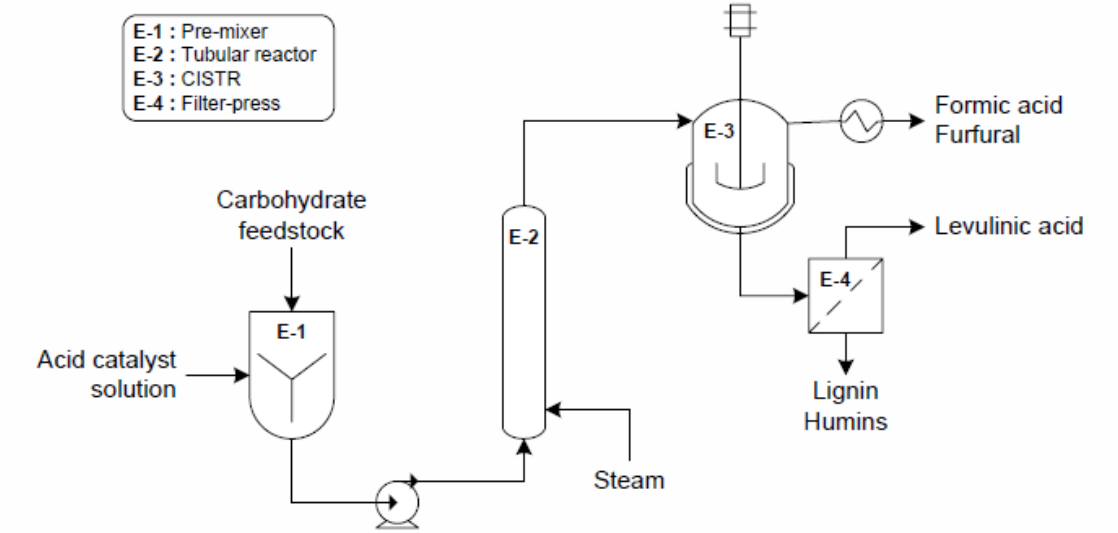


Figure 2.7 Conversion of biomass feedstocks into furfural, formic acid and levulinic acid via the Biofine™ industrial process. Reprinted from Girisuta 2007.

The cellulose fraction in the furfural residues have also been explored as feedstock for enzymatic hydrolysis and fermentation to ethanol (Tang et al. 2011) and lactic acid (Tang et al. 2013). One study using furfural residues from corncobs, reported a maximum glucose yield of 96.8% after 108 h of hydrolysis at an enzyme loading of 25 Filter Paper Units (FPU)/g of cellulose (Sun et al. 2011). Prior to enzymatic hydrolysis, the furfural residues were treated with 1% NaOH and water washed until neutral pH. Moreover, the solids were pretreated with sodium chlorite at 70 °C, removing 54% of lignin, which explains the high enzymatic digestibility. In the same study by Sun et al., it was reported that delignification beyond 30% did not improve significantly the enzymatic hydrolysis. A different study (Tang et al. 2011) performed simultaneous saccharification and fermentation (SSF) of furfural residues from corncobs,

resulting in ethanol yields of 65% (of theoretical) at 38°C and 120 h. The residues in this study were washed but not delignified prior to SSF.

CHAPTER 3

THE PATH TO BUTADIENE SULFONE PRETREATMENT: SCREENING OF POLAR APROTIC SOLVENTS FOR SOLUBILIZATION OF BIOMASS AND BIOMASS-DERIVED FEEDSTOCKS

3.1 Introduction

Several low-cost polar aprotic solvents, with and without lithium salts, were studied for the solubilization of biomass and biomass-derived feedstocks (Appendix A), in particular raw *Miscanthus* and the solid residues after the production of furfural from *Miscanthus.* To the best of our knowledge, the *Miscanthus* furfural residues have not been studied previously for the solubilization with polar aprotic solvents. Compared to *Miscanthus* in its raw state, improved cellulose solubilization may be achieved following the biomass alteration from the acid-catalyzed production of furfural (Appendix B). The objective is to assess the solubility of different feedstocks in several polar aprotic solvents.

The following polar aprotic solvents, with and without lithium salts, were studied for solubilization of biomass and biomass-derived feedstocks: dimethylacetamide (DMA), N-methylpyrrolidone (NMP), and the sulfone-based butadiene sulfone (BS) and sulfolane (S). The following feedstocks were evaluated: raw *Miscanthus*, cellulose-rich *Miscanthus* (solid residues after production of furfural), cellulose (Avicel®), xylan (from birchwood) and lignin (alkali).

3.2 Materials and methods

Unless specified otherwise, all chemicals were used as received. Sulfone-based polar aprotic solvents sulfolane (99%) and butadiene sulfone (98%) were purchased from Sigma-Aldrich (St. Louis, MO) with purities greater than 99% and 98%, respectively. Dimethylacetamide (DMA**)** and n-methylpyrrolidone (NMP) with purities greater than 99% were purchased from Alfa Aesar (Ward Hill, MA) and Acros (Geel, Belgium), respectively. Lithium chloride (certified, 98.5% purity) was purchased from Fisher Scientific (Pittsburgh, PA) and lithium acetate was purchased from Alfa Aesar. Avicel® PH-101 (microcrystalline cellulose), xylan (from birchwood), lignin (alkali), cellobiose (99%), glucose (99%) and xylose (99%) were purchased from Sigma-Aldrich. *Miscanthus x giganteus* was provided by the Energy Biosciences Institute at the University of Illinois at Urbana-Champaign, milled to pass a 1 mm screen and sieved to -20/+80 mesh (Chapter 4).

Feedstocks were solubilized with a polar aprotic solvent, (e.g., sulfolane, butadiene sulfone, dimethylacetamide or n-methylpyrrolidone) to a concentration of 5% solids, with or without the addition of a lithium salt (lithium chloride or lithium acetate) and water. The solubilization was performed in a water bath shaker (New Brunswick Scientific, Classic Series C76, Edison, NJ) at 200 rpm, 70-75ºC and 24 hours. After solubilization, the slurries were vacuum filtered as described in Chapter 4.

The percentage of feedstock dissolved in aprotic solvents/lithium salt systems is defined as follows; based on the solubility studies of raw biomass in ionic liquids by (Padmanabhan et al. 2011):

$$\%\ Feedstock\ dissolved = \frac{m_{before} - m_{after}}{m_{before}} \times 100$$

For cases where there was an increase in weight after a feedstock was treated with a polar aprotic solvent, the *% Feedstock dissolved* was a negative value. Consequently, swelling was defined as:

$$\%\ Feedstock\ swelled\ (\%) = \frac{m_{after} - m_{before}}{m_{before}} \times 100$$

where m_{before} is the mass (g) of feedstock before solubilization/swelling and m_{after} is the mass (g) of feedstock after dissolution or swelling in polar aprotic solvents.

3.3 Results and discussion

Fig. 3.1 shows the solubilization of Avicel®, xylan, lignin, raw *Miscanthus* and the "cellulose-rich *Miscanthus"* (solids after the production of furfural), in butadiene sulfone (BS) or sulfolane at 70 °C, 24 h, without water addition. The swelling of raw *Miscanthus* in DMA/LiCl is shown at 93%. For BS and sulfolane, Avicel® is not soluble and lignin is completely soluble (see discussion in Chapter 5), only 1% of xylan is soluble in BS, and 7% is soluble in sulfolane. The solubility in raw *Miscanthus* at 4% for BS and 5% for sulfolane, represents non-structural elements in the biomass. The "cellulose-rich *Miscanthus*" showed increased solubility at 30% for BS and 26% for sulfolane. The higher solubility of the *Miscanthus* solids after production of furfural is consistent with a more accessible biomass after the acid-catalyzed reaction.

A different experiment was conducted to test the solubility of raw *Miscanthus* in butadiene sulfone at 75 °C and 24 h, but with the addition of water (Fig. 3.2). In the presence of water, butadiene sulfone provided a higher solubility of raw *Miscanthus* at 13% (average), compared with no water addition at around 2% (lower than experiment in Fig. 3.1).

Figure 3.1 Solubilization or swelling of feedstocks in butadiene sulfone, sulfolane or dimethtylacetamide/LiCl, without water addition, at 70 °C and 24 h.

Solubilization of butadiene sulfone (BS) in "Raw *Miscanthus*" with and without the presence of water

Run	Water (g)	As-Is MxG before (g)	ODW MxG (g)	Tare Crucibles (g)	Postfiter (g)	Wt MxG after (g)	% Solubility	**Postfilter observations**
1	0	0.5029	0.4778	28.5148	28.9852	0.4704	**1.5**	
2	2	0.5037	0.4785	19.6074	20.0178	0.4104	**14.2**	Solids have characteristics of pretreated biomass
3	0	0.502	0.4769	28.2176	28.6864	0.4688	**1.7**	
4	2	0.4986	0.4737	23.6682	24.082	0.4138	**12.6**	Solids have characteristics of pretreated biomass

Each run:

10 g BS
0.5 g Raw MxG
T = 75 C
t = 24 h

BS mp = 65 C
Solubility: (Wt before – Wt after) / Wt before
Swelling: (Wt after – Wt before) / Wt before
MxG = *Miscanthus x giganteus*
ODW = oven dried weight

Figure 3.2 Raw data screenshot of the solubilization of raw *Miscanthus* in butadiene sulfone with and without the presence of water at 75 °C and 24 h.

In Fig. 3.3, biomass swelling was observed when raw *Miscanthus* was solubilized with DMA or NMP, with no water addition and in the presence of LiCl or lithium acetate ("LiAc"). The largest swelling was observed with DMA or NMP in the presence of lithium acetate. Swelling is consistent with disruption of the hydrogen bond network in cellulose for solvents like DMA/LiCl with a high hydrogen bond acceptor capacity (as high as $\beta = 2.0$). However, the separation of the solvent from the swelled biomass was foreseen to be an issue. On the other hand, biomass does not swell in butadiene sulfone or sulfolane, consistent with low β numbers for sulfones ($\beta = 0.39$-0.46) (Chapter 5).

Exp 2 - NMP vs DMA, LiCl vs LiAc

Run	NMP or DMA	LiCl or LiAc	Wt MxG before (g)	Tare Crucibles (g)	Postfiter (g)	Wt MxG after (g)	% Solubility	% Swelling
1a	D	Cl	0.2496	27.2045	27.521	0.3165	**0.0**	26.8
1b	D	Cl	0.2512	28.313	28.6395	0.3265	**0.0**	30.0
2a	D	Ac	0.2504	28.5212	28.8366	0.3154	**0.0**	26.0
2b	D	Ac	0.2507	27.6332	28.1494	0.5162	**0.0**	105.9
3a	N	Cl	0.2505	29.27	29.5865	0.3165	**0.0**	26.3
3b	N	Cl	0.2508	28.2317	ND	ND	ND	ND
4a	N	Ac	0.2507	27.0256	27.5181	0.4925	**0.0**	96.4
4b	N	Ac	0.2503	23.677	24.0164	0.3394	**0.0**	35.6

Each run:
5 g NMP or DMA
0.250 g MxG
0.500 g LiCl or LiAc
0 g H2O

MxG = *Miscanthus x giganteus*
NMP =n-methylpyrrolidone, DMA=dimethylacetamide, LiAc = lithium acetate
Density NMP @ 25 C = 1.028 g/ml
Density DMA @ 25 C = 0.943 g/ml
Vol NMP = 4.864 ml (5 g)
Vol DMA = 5.302 ml (5 g)

MxG, LiCl and LiAc dried at 105 C for at least 5 h
T = 75 C
t = 24 h

Solubility: (Wt before – Wt after) / Wt before
Swelling: (Wt after – Wt before) / Wt before

Figure 3.3 Raw data screenshot for the solubilization of raw *Miscanthus* in DMA or NMP with lithium chloride (LiCl) or lithium acetate ("LiAc") at 75 °C and 24 h. No water was added to the system.

Fig. 3.4 shows the effect of solubilization of raw *Miscanthus* with and without the addition of water at different temperatures. At 90 °C, the % of *Miscanthus* solubilized was 25% with water and 6% without water. At 100 °C, the solubilization was 44% with water and 23% without water (see Chapter 4 for butadiene sulfone solubilization methods above 90 °C).

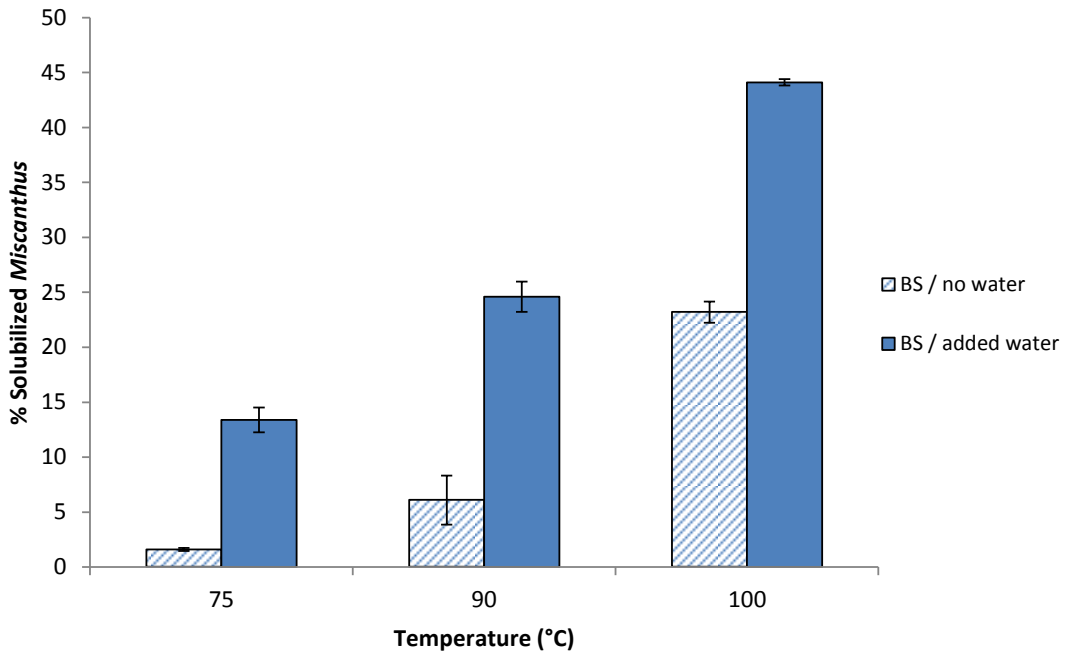


Figure 3.4 % Raw *Miscanthus* solubilized in butadiene sulfone with and without the addition of water at different temperatures.

3.4 Conclusions

The good solubilization of raw *Miscanthus* with butadiene sulfone-water, compared to the lower solubility with sulfolane or swelling with NMP or DMA, prompted the pursuit of more detailed studies with butadiene sulfone. To the extent, that after careful evaluation of the solids after solubilization, and by studying the reaction mechanisms of butadiene sulfone in the presence of water, including the solvent's decomposition and recovery capabilities, we concluded that butadiene sulfone could be in fact an excellent biomass pretreatment method meriting further studies that are now the core of this dissertation.

CHAPTER 4*

EFFECT OF WATER, TEMPERATURE AND TIME ON THE PRETREATMENT OF MISCANTHUS WITH BUTADIENE SULFONE

4.1 Introduction

The presence of water in butadiene sulfone (BS) pretreatment of biomass is the driving force for the formation of sulfurous acid (Kassner 2008). The effect of water concentration, temperature and time on the pretreatment of *Miscanthus* with butadiene sulfone was studied. A minimum ratio of water added : butadiene sulfone was determined to achieve greater than 50% removal of lignin and 70% removal of xylan. Pretreatment temperatures ranging from 90 °C to 110 °C at 6 h to 30 h were evaluated for the removal of xylan and lignin, and the preservation of glucan.

4.2 Materials and methods

Materials

Butadiene sulfone (98%), Avicel® PH-101 (microcrystalline cellulose), xylan (from birchwood), lignin (alkali), cellobiose (99%), glucose (99%) and xylose (99%) were purchased from Sigma-Aldrich (St. Louis, MO) and used as received. *Miscanthus x giganteus* was provided by the Energy Biosciences Institute at the University of Illinois at Urbana-Champaign.

*This chapter includes previously published material by the author. Reference: J. Atilio de Frias and Hao Feng, *Green Chem.*, 2013, 15, 1067-1078.

Pretreatment of *Miscanthus* with butadiene sulfone

Miscanthus was milled to pass a 1 mm screen using a knife mill (Retsch GmbH, model SM 2000, Haan, Germany). The particle size was standardized to a range of -20/+80 mesh sieve (ASTM, U.S. Standard Sieve Series, Soiltest, Inc., Evanston, IL) using a Ro-Tap shaker (model D-4325, Dual Manufacturing Co. Inc., Chicago, IL). Biomass was then packaged in Ziploc® bags and stored in a cold room at 0ºC until use. Following the solids loading used by Kassner (Kassner 2008), 0.5 g of biomass, 10 g of butadiene sulfone, and 0-5 g of deionized water, were added to a 140 ml glass pressure tube and capped with a PTFE plug (#8648-30 and #5845-47, respectively, Ace Glass, Vineland, NJ). The tube was preheated in an oil bath at 80ºC for 15 minutes and transferred to another oil bath on top of an Isotemp® stirring hotplate (Fisher Scientific, Pittsburgh, PA) that provided temperature and stirring control at 90ºC, 100ºC and 110ºC from 6 to 30 h at 600 rpm. After pretreatment, the solution was vacuum filtered using previously tared filtering crucibles (Coors #60531, Golden, CO). During filtration, a heat gun was used to keep the solution liquid (Kassner 2008) at a temperature of 70-75ºC. The filtrate was stored and all remaining solids in the tubes were transferred to the crucibles using at least 50 ml of hot deionized water, which also served as rinsing medium. Crucible and solids were dried for 12 h at 110ºC for gravimetric analysis and subsequent chemical composition analysis.

Analysis of raw and pretreated *Miscanthus* solids

The composition of glucan, xylan and lignin in raw *Miscanthus* was determined according to the NREL Laboratory Analytical Procedures (LAP) "Determination of structural carbohydrates and lignin in biomass" (Sluiter et al. 2008) (Table 4.1). As prerequisite for chemical composition of raw biomass and to avoid analysis interference, moisture content of

biomass was less than 10% and non-structural elements were removed via a two-step extraction with water and ethanol (95% v/v) for 80 and 120 minutes, respectively, with an automatic Soxhlet apparatus (Soxtec™ System HT6 1043 extraction unit and 1046 service unit, FOSS, Hillerød, Denmark). The temperature of the Soxtec oil service unit was 150 °C for water extraction and 140 °C for ethanol extraction. The amount of extractable material was determined gravimetrically and reported as % extractives (Sluiter et al. 2005). The extractive-free solids were dried for 24 h at 30ºC to moisture content of 10% or less prior to chemical composition analysis. The glucan, xylan and lignin content in the pretreated solids (moisture <10%) were determined via the same NREL procedure without the extractives step.

The percentage of *Miscanthus* solubilized represents the total amount of biomass components (dry-basis) dissolved after pretreatment, and it is based on a solubility study of *Miscanthus* in ionic liquids (Padmanabhan et al. 2011):

$$Miscanthus\ dissolved\ (\%) = \frac{m_{before} - m_{after}}{m_{before}} \times 100$$

where m_{before} is the mass of the oven-dried biomass before pretreatment and m_{after} is the mass of the oven-dried biomass after pretreatment. The percentages of xylan and lignin removal, as well as the percentage of glucan preserved after pretreatment of *Miscanthus*, were defined as follows:

$$xylan\ or\ lignin\ removed\ (\%) = \frac{m_{xyl,lig\ raw\ MxG} - m_{xyl,lig\ pretreated\ MxG}}{m_{xyl,lig\ raw\ MxG}} \times 100$$

$$glucan\ preserved\ (\%) = \left(1 - \frac{m_{glucan\ raw\ MxG} - m_{glucan\ pretreated\ MxG}}{m_{glucan\ raw\ MxG}}\right) \times 100$$

where m is the dry-basis amount (grams) of xylan, lignin or glucan in the raw or pretreated *Miscanthus.*

Table 4.1 Chemical composition of *Miscanthus x giganteus.*

Component	***Miscanthus* (% dry basis)**
Extractives[a]	7.7 ± 0.2
Glucan	42.8 ± 0.2
Xylan	22.0 ± 0.4
Lignin[b]	23.9 ± 0.3
Acetate	3.0 ± 0.0

[a]Extractives is the sum of water extractives (3.5 ± 0.1) and ethanol extractives (4.2 ± 0.3).
[b]Lignin is the sum of acid soluble lignin (5.6 ± 0.0) and acid insoluble lignin (18.3 ± 0.2).

X-ray powder diffraction of *Miscanthus* solids

X-ray powder diffraction data for raw and pretreated *Miscanthus* (moisture <10%) were collected at the School of Chemical Sciences (University of Illinois) on a Bruker General Area Detector Diffraction System (GADDS) (Karlsruhe, Germany) equipped with a four-circle diffractometer and multiwire area detector in transmission mode. A Bruker M18XHF22 rotating anode generator operating at 50 kV and 40 mA supplied the Cu Kα graphite monochromatized incident beam. The samples were mounted directly in the beam and rotated during data collection around the phi axis. Two frames were collected for 900 seconds each, integrated, and merged into a 1-dimensional powder pattern from 5-32 degrees 2θ.

Scanning electron microscopy (SEM)

SEM images of dried *Miscanthus* solids (raw and pretreated) were taken with a Philips XL30 FEG Environmental Scanning Electron Microscope (Eindhoven, Netherlands) at the Beckman Institute (University of Illinois) with magnifications of 650×. A Denton Desk II TSC (Moorestown, NJ) turbo-pumped sputter coater was used to coat the dry samples with a thin layer of gold-palladium prior to taking the SEM images.

4.3 Results and discussion

Effect of water content in butadiene sulfone pretreatment of *Miscanthus*

As in traditional acid-catalyzed pretreatment of biomass (e.g., HCl, H_2SO_4), the protons from sulfurous acid, formed by the addition of water to butadiene sulfone, catalyze the break of the xylan-lignin bonds, hydrolyzing xylan into soluble oligosaccharides and xylose, and to a lesser extent, hydrolyzing recalcitrant glucan into glucose. Moreover, unwanted acid-catalyzed dehydration of glucose and xylose can occur in the form of furans (e.g., hydroxymethylfufural and furfural) that participate in condensation reactions with sugars to form insoluble unsaturated carbon or humins (Enslow and Bell 2012).

Fig. 4.1 shows the effect of water addition (ratio of water added to BS) in the removal of xylan and lignin, and the retention of glucan, in the pretreatment of *Miscanthus* with butadiene sulfone at fixed temperature, time and solids loading (100 °C, 24 h, 5% solids loading). A stoichiometric approximation to determine the maximum concentration of sulfurous acid that would form *in situ*, showed that for a water added/BS ratio of 1:10 to 5:10, the acid concentration is between 4% and 5% (w/v). These approximate concentrations are in the higher end of what is commonly used in dilute sulfuric acid pretreatment (0.1% - 6%) (Kumar et al. 2009a; Lu et al. 2007).

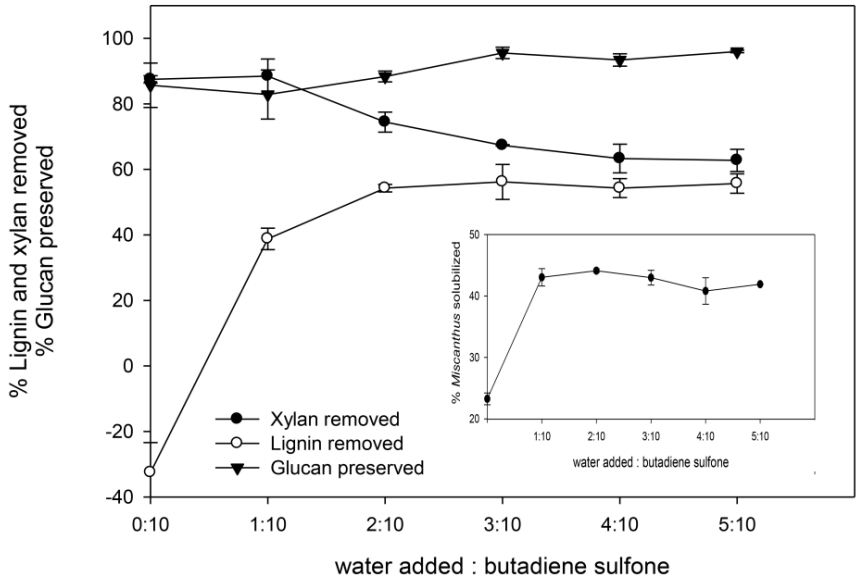


Figure 4.1 Effect of water addition on the removal of lignin and xylan, as well as the preservation of glucan, after pretreatment of *Miscanthus* with butadiene sulfone-water at 100 °C and 24 h.

At no water addition (0:10), the system is not anhydrous, as the moisture content in *Miscanthus* was measured at 7% and BS contains traces of water. The available water in the system proved sufficient to promote the formation of sulfurous acid, which catalyzed the hydrolysis of xylan that removed 88% into the liquid phase. However, a negative value of -33% for lignin removal represents an increase in the acid-insoluble Klason (gravimetric) lignin composition in the pretreated solids, due to the presence of tar-like insoluble carbon compounds (i.e., humins or pseudo-lignin) formed via condensation of furans and sugars. Recent work demonstrated via chemical and spectroscopic analysis that the acid catalyzed dehydration of carbohydrates yields large amounts of unsaturated carbon that increases the Klason lignin content in acid pretreated biomass (Sannigrahi et al. 2011). Based on the increase in Klason lignin in the pretreated solids at no water addition, the formation of humins from sugar degradation may have inhibited any solubilization of lignin in BS.

At a ratio of 1:10 water added/BS (Fig 4.1), the amount of xylan removed from *Miscanthus* was 89%, just 1% higher as when no water was added, but also, 39% of lignin was removed under these conditions. While the hydrolysis or dehydration of carbohydrates is acid-catalyzed, the removal of lignin is mostly due to the solvent effect of BS. This "positive" removal of lignin suggests a significant decrease in unsaturated carbon or humins, which allowed BS to effectively dissolve the delocalized lignin during or after the proton-catalyzed hydrolysis of the ester linkages between xylan and lignin.

The decrease of insoluble carbon from sugar degradation at a ratio of 1:10 water added/BS, may signify that the addition of water is disfavoring the dehydration of carbohydrates following Le Chatelier's principle (Binder and Raines 2010). Binder and Raines observed that gradual increases in water concentration to a solution of cellulose, HCl and the ionic liquid 1-ethyl-3-methylimidazolium chloride at 105°C for 12 h, increased the yield of glucose and decreased the yield of the corresponding dehydration product hydroxymethylfurfural (Binder and Raines 2010).

As the water added/BS ratio increased from 1:10 to 5:10 in the BSW-*Miscanthus* mixture, the amount of xylan removed decreased to a 63% removal at 5:10; while the amount of glucan preserved in the solids increased from 86% to 96% at 5:10. Also, the removal of lignin plateaued to 54-56%, when the ratio was greater than 2:10 (Fig. 4.1). Increasing water added/BS ratios may have catalytically shifted the equilibrium from H_2SO_3 to $SO_2 + H_2O$, since water has a catalytic effect in the dissociation of H_2SO_3 favoring $SO_2 + H_2O$ (Voegele et al. 2002). This effect may explain the decreased acid catalyzed removal of xylan and increased preservation of glucan from 1:10 to 5:10. A ratio of 2:10 was used in subsequent experiments as the minimum ratio that allowed the removal of greater than 50% of lignin and 70% of xylan.

Comparable results of xylan and lignin removal from *Miscanthus* have been achieved with organosolv pretreatment, in which an organic solvent (e.g., ethanol, acetone) is mixed with an inorganic acid catalyst (e.g., HCl, H_2SO_4) (Kumar et al. 2009a). A previous study on organosolv pretreatment of *Miscanthus* reported the removal of 73% of the xylose in raw *Miscanthus* and 71% of lignin after a two-step process which consisted of a heated presoak of the biomass in 0.15 M H_2SO_4 for 17 h followed by ethanol-water-H_2SO_4 reaction at 170 °C for 60 min (Brosse et al. 2009). BSW pretreatment of *Miscanthus* at 100 °C and 24 h achieved lower delignification but higher xylan removal with the advantage of a one-step approach without added catalysts. Table 4.2 compares BSW pretreatment with other acidic pretreatment techniques: dilute acid, organosolv and ionic liquids (with added acid). For dilute acid, general ranges for different conditions were provided, whereas for organosolv and ionic liquids, specific studies that used *Miscanthus* were referenced.

Table 4.2 Comparison of butadiene sulfone-water (BSW) pretreatment with other techniques.

Condition	**Dilute acid[1]**	**Organosolv[3]**	**Ionic liquids[5]**	**BSW*[6]**
Reaction phase	Aqueous	Aqueous	Organic	Organic
Reaction stages	1	2	2	1
Temperature (°C)	160-220	<125-170	80-124	90-110
Time	1-30 min	a. 15 h b. 60 min	a. 6 h b. 120 min	6-30 h
Chemicals (e.g.)	H_2SO_4	0.8 EtOH/ H_2SO_4	[EMIM]Cl / H_2SO_4	Butadiene sulfone
Acid concentration	[2] < 4% w/w	a. 0.15 M, b. 0.9%	add 3.3 M H_2SO_4	*In situ*

Table 4.2 (cont.)

Condition	Dilute acid[1]	Organosolv[3]	Ionic liquids[5]	BSW*[6]
E for xylan hydrolysis (kJ/mol)** [6]	66-172	*n/a*	114	89
Xylan removal	75-95%	73%	50%	91%
Lignin removal	10-20%	71%	77%	58%
Glucan preserved	85-95%	95%	91%	90-99%
Crystallinity index	Increase	*n/a*	Decrease[4]	Decrease

**BSW (butadiene sulfone-water)*
***Activation energy*
[1](Chundawat et al. 2011)
[2](Kumar et al. 2009a)
[3](Brosse et al. 2009)
[4](Mora-Pale et al. 2011)
[5](Dee and Bell 2011)
[6](de Frias and Feng 2013)

Effect of temperature and time on the pretreatment of *Miscanthus* with BSW

A ratio of 2:10 (water added/butadiene sulfone) for the pretreatment of *Miscanthus* at 100 °C and 24 h, allowed for removal of 54% of lignin and 74% of xylan (Fig 4.1). Using the same ratio, BSW pretreatment of *Miscanthus* was performed at 90 °C-110 °C from 6 to 30 h. SEM micrographs reveal severe morphological changes experienced by *Miscanthus* from its fibrous raw state through a gradual disruption of the lignocellulosic structure by BSW pretreatment at 110 °C from 6 to 30 h (Fig. 4.2). Concomitant with delignification and xylan removal from the plant cell wall, the *Miscanthus* structure in the micrographs is noticeably altered from 6 to 12 h, when over 60% of xylan and 50% lignin had been removed (Fig 4.3C and D). At 18 h and 24 h, as delignification and increased removal of xylan continued, the biomass fibers aggregate

(Donohoe et al. 2011) with a cardboard-like appearance to the naked eye. At 30 h, cavities and pores are formed within the surface matrix and the fiber structure is no longer noticeable.

Fig. 4.3 shows the overall solubilization of *Miscanthus*, the removal of xylan and lignin and the preservation of glucan. A maximum of 48% of *Miscanthus* was solubilized at 110 °C and 24 h (Fig. 4.3 A), consisting in 50% removal of total lignin and 91% of xylan (Fig. 4.3C and D). Removal of 58% of lignin was achieved at 100 °C and 30 h (Fig. 4.3C) and most of the glucan (90-99%) was preserved at 90 °C-100 °C from 6 to 30 h, and at 110 °C for 18 h or less (Fig. 4.3B). Glucan preservation in the solid fraction is important to maximize the production of glucose via enzymatic hydrolysis.

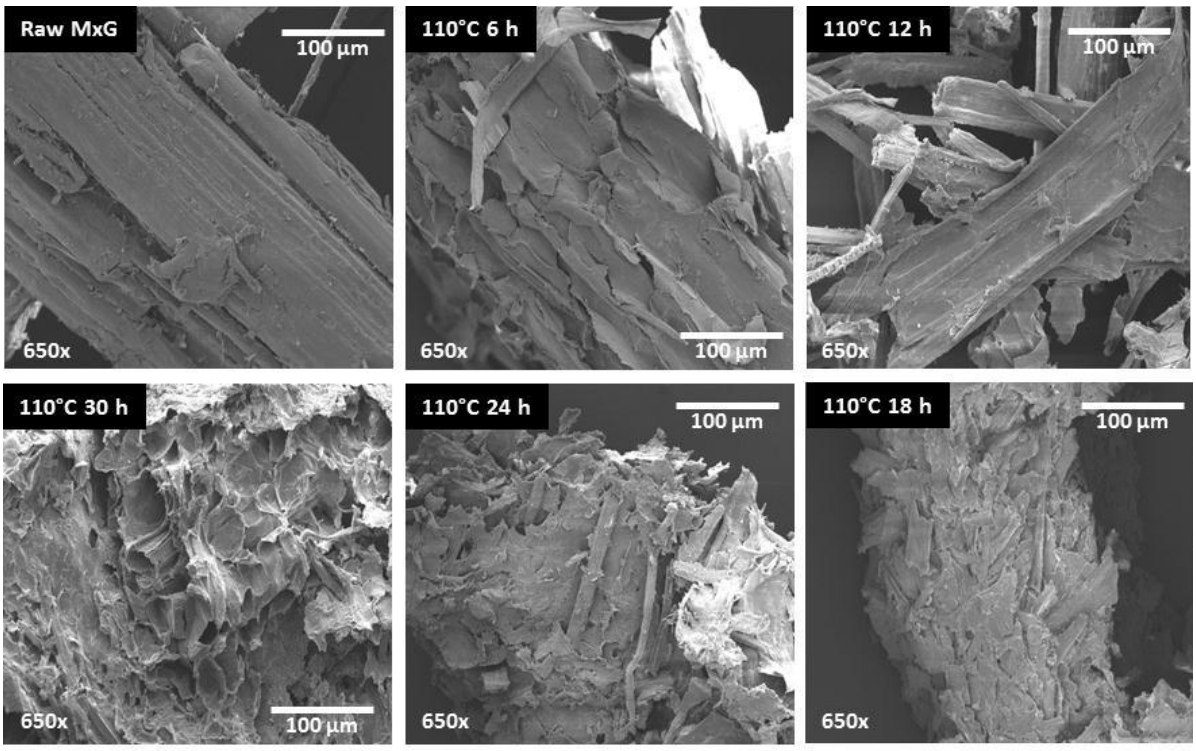


Figure 4.2 SEM micrographs of raw and pretreated *Miscanthus* with butadiene sulfone-water at 110 °C from 6 to 30 h. All micrographs are magnified to 650×.

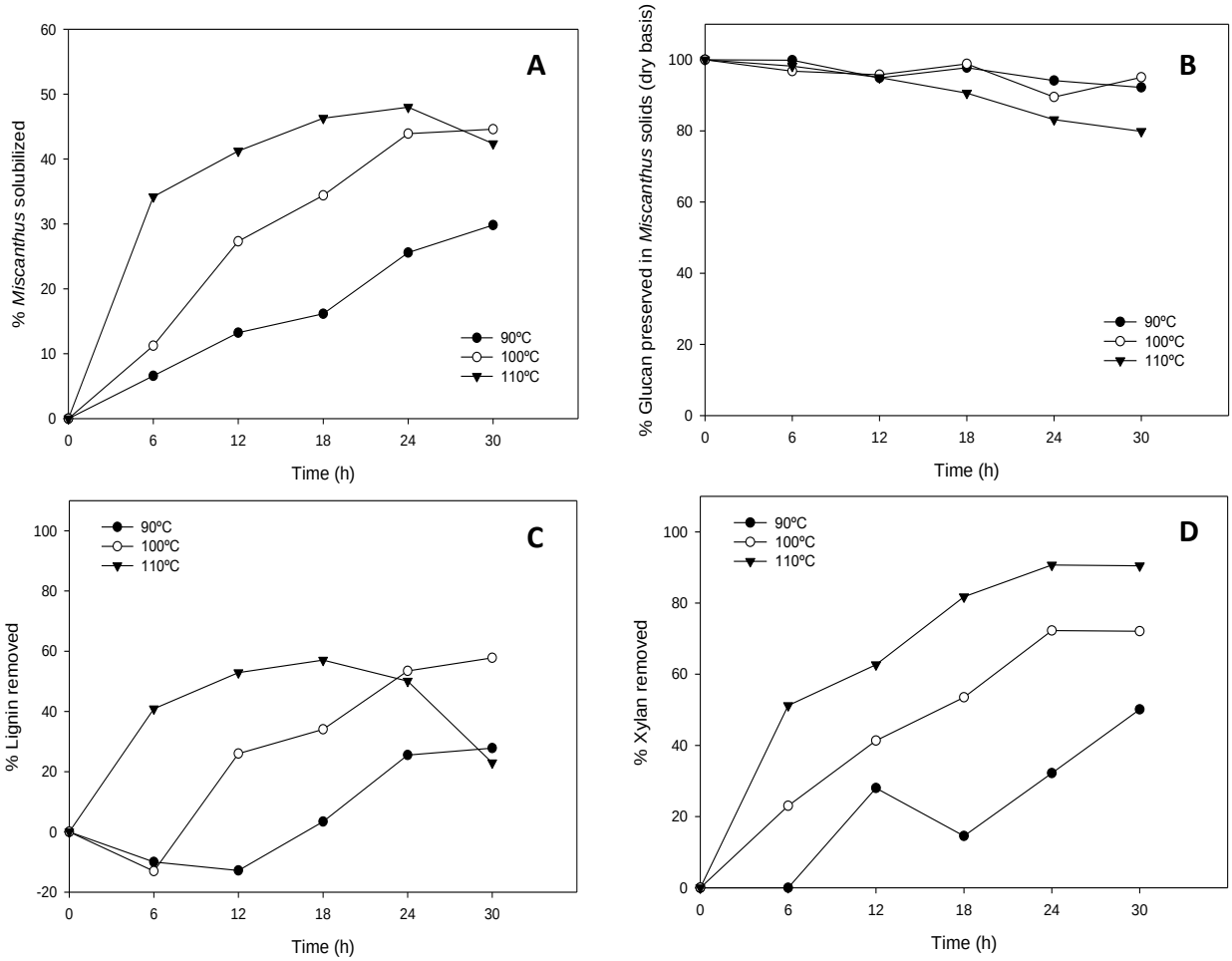


Figure 4.3 (A) % *Miscanthus* solubilized after reaction with butadiene sulfone-water (2:10 water added/butadiene sulfone ratio) at 90 °C, 100 °C and 110 °C. Under the same conditions: (B) Glucan preserved in *Miscanthus* solids, (C) removal of lignin from *Miscanthus* and (D) removal of xylan. All yields are expressed on a dry basis.

At 90 °C for 6-12 h, and 100 °C for 6 h, Fig. 4.3 C shows negative values for total lignin removal due to an increase in acid soluble lignin (ASL) composition from the raw material, with insignificant change in Klason lignin. The increase in ASL during the initial stages of BSW pretreatment can be explained by the redistribution of lignin moieties (i.e., syringyl, guaiacyl and p-hydroxyphenyl), biasing ASL high at the beginning and decreasing towards near-constant values with longer pretreatment time (Yasuda et al. 2001). This trend in ASL content is shown in Fig. 4.4 at 90 °C-110 °C from 6 to 30 h, in agreement with the work by Yasuda, Fukushima

and Kakehi who analyzed the ASL content of beech wood following a sulfuric acid (72%) treatment (Yasuda et al. 2001). They observed that the amount of ASL was highest during the initial stages of treatment (5.1%) and decreased with extended treatment time (240 min) to a constant value (~2.5%), due to a rapid dissolution of syringil lignin concurrent with degradation reactions to yield both ASL and Klason lignin with syringyl nuclei.

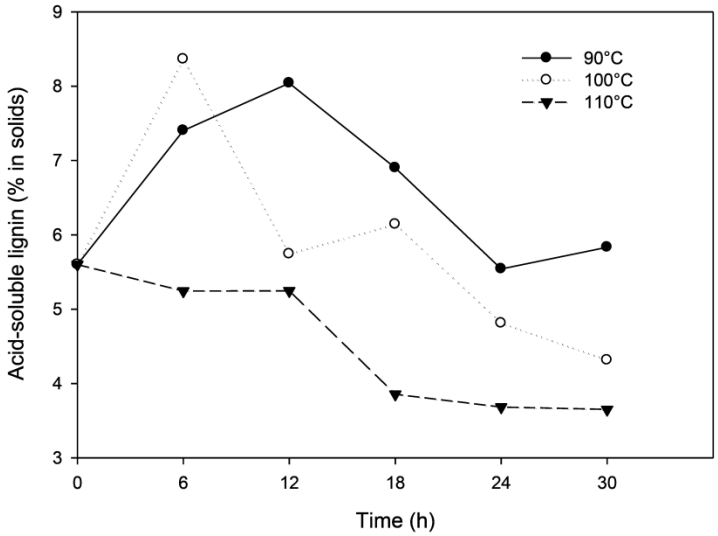


Figure 4.4 % Acid-soluble lignin in solids after pretreatment of *Miscanthus* with butadiene sulfone-water at 90 °C-110 °C from 6 to 30 h.

At 110 °C and t >18 h it is important to note the decreasing tendency in delignification (Fig. 4.3C), due to the increase in gravimetric Klason lignin as a result of the presence of diene polymers in the pretreated solids. Dienes are formed via polymerization of butadiene with or without sulfur dioxide, and their presence is undesirable, as it causes loss of solvent and may inhibit enzymatic hydrolysis and fermentation. The x-ray diffraction patterns in Fig. 4.5 show a distinct peak near $2\theta = 20°$ from pure diene polymers, which does not overlap with any of the two peaks for raw *Miscanthus*. A third peak can be seen in the pretreated *Miscanthus* overlapping with the peak from the pure diene polymers at $2\theta = 20°$, an indication of diene polymers formation within the *Miscanthus* solids after pretreatment at 110 °C for 24 h and 30 h

(Fig. 4.5 C and D). This confirms that the decreasing trend in lignin removal at 24 h and 30 h is due to the increased presence of diene polymers within the pretreated solids. At 12 h and 18 h insignificant formation of diene polymers within *Miscanthus* solids was observed (Fig. 4.5A and B), while achieving a high removal of xylan and lignin (Fig. 4.3C and D). The relevance of this observation is important since diene inhibitors, like 4-t-butylcatechol or ferrocene (Bando et al. 2011) may be avoided under milder reaction conditions.

The near-absence of the diene polymers confounding peaks at 110 °C for 12 h and 18 h (Fig. 4.5A and B) allowed us to assess the effect of BSW pretreatment on the crystalline structure in *Miscanthus* at these conditions. Biomass crystallinity is considered to be one of the key factors, in addition to presence of lignin, degree of polymerization and surface area, which affect the enzymatic hydrolysis of glucan (Li et al. 2010; Zhang and Lynd 2004; Kumar et al. 2009b). The degree of crystallinity of raw and pretreated *Miscanthus* was determined by the "crystallinity index" equation (CrI) (Segal et al. 1959):

$$\mathrm{CrI} = \frac{(I_{002} - I_{\mathrm{am}})}{I_{002}} \times 100$$

where I_{002} is the intensity of the peak at $2\theta = 22°$ and I_{am} is the intensity of the valley at $2\theta = 18°$. I_{002} denotes both crystalline and amorphous material and I_{am} denotes only amorphous material (Xie et al. 2012). CrI for raw *Miscanthus* was calculated to be 68%, whereas CrI for BSW pretreated *Miscanthus* was calculated to be 65% and 63% for 12 h and 18 h of reaction time, respectively. This decreasing trend in CrI is in contrast with dilute acid pretreatment of softwoods, which increases the CrI from the biomass raw state due to the hydrolysis of the amorphous cellulose; leaving biomass with a more crystalline fraction (Kumar et al. 2009a; Blanch et al. 2011). Since the crystallinity in lignocellulosic biomass is impacted not only by cellulose, but also by lignin and hemicellulose (Zhang and Lynd 2004), the CrI decreasing trend

by BSW pretreatment can be explained by the higher removal of lignin (>50%) compared to typical lignin removal yields by dilute acid pretreatment (10-20%) (Chundawat et al. 2011). Future studies on enzymatic hydrolysis of *Miscanthus* after BSW pretreatment can determine if an inverse correlation exists between CrI and enzymatic digestibility of the glucan fraction.

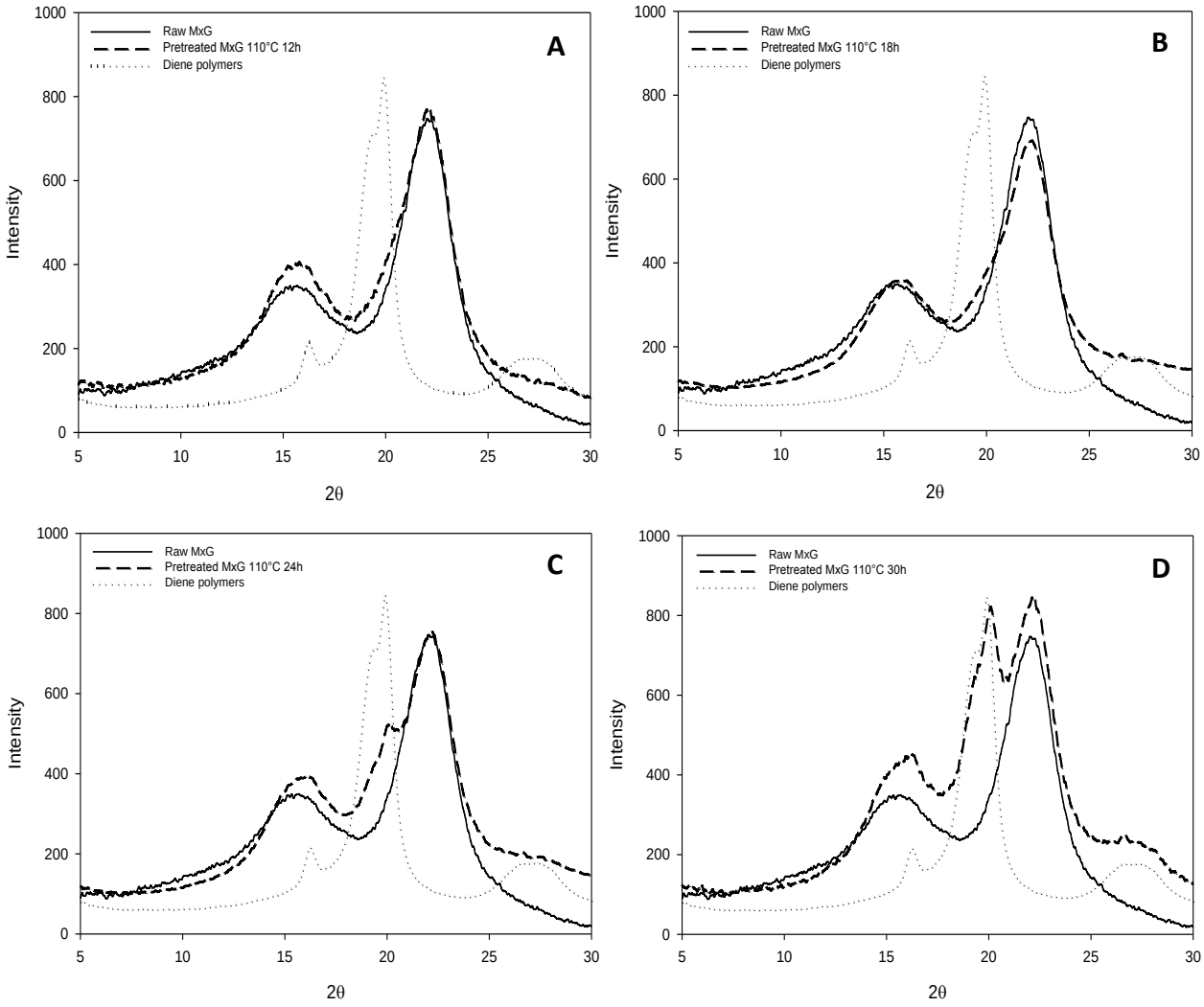


Figure 4.5 X-ray diffraction patterns of raw *Miscanthus* (straight line), isolated diene polymers after pretreatment (dotted line) and pretreated *Miscanthus* (dashes) at 110 °C for 12 h (A), 18 h (B), 24 h (C) and 30 h (D). Isolated diene polymers have a diffraction peak near 2θ = 20°. Pretreated *Miscanthus* solids with peaks at 2θ = 20° indicate the presence of diene polymers (C and D). A and B show no significant presence of diene polymers in the pretreated *Miscanthus* solids.

4.4 Conclusions

This chapter presented evidence for the pretreatment of *Miscanthus* with switchable butadiene sulfone and water that resulted in high removal of both xylan (91%) and lignin (58%) and minor degradation of glucan (90-99% preserved), the key substrate for enzymatic hydrolysis and fermentation towards biofuels and chemicals. Butadiene sulfone (BS) decomposition gases can polymerize into unwanted diene polymers during pretreatment, but X-ray diffractograms of the pretreated *Miscanthus* showed that the presence of dienes in the solids can be minimized if reactions conditions are lower than 110 °C and less than 18 h.

CHAPTER 5*

REMOVAL OF XYLAN AND LIGNIN FROM MISCANTHUS: SOLUBILIZATION MECHANISMS AND KINETICS OF XYLAN HYDROLYSIS

5.1 Introduction

The kinetics of hemicellulose hydrolysis from lignocellulosic biomass have been studied since the 1950s, based on the model by Kobayashi and Sakai consisting of irreversible fast and slow reacting hemicellulose hydrolyzed to monomeric xylose followed by degradation reactions and products. Other related models more accurately include the formation of oligomers prior to xylose (Mäki-Arvela et al. 2011; Wyman et al. 2005). The difference between the fast and slow rates is based on the decreased hemicellulose hydrolysis rate after 70% conversion (Jacobsen and Wyman 2000), but the key difficulty in implementing these models is the determination of the slow and fast hemicellulose fractions (Carrasco and Roy 1992; Bhandari et al. 1984; Shen and Wyman 2011; Yat et al. 2008). This has resulted in disparate fast/slow proportion determinations among researchers (Shen and Wyman 2011). The hemicellulose hydrolysis kinetic model by Kobayashi and Sakai has been simplified for batch systems to an irreversible first-order kinetic dependency for the hydrolysis of hemicellulose into xylose (Yat et al. 2008; Cahela et al. 1983).

$$hemicellulose \xrightarrow{k1} xylose \xrightarrow{k2} degradation\ products$$

*This chapter includes previously published material by the author. Reference: J. Atilio de Frias and Hao Feng, *Green Chem.*, 2013, 15, 1067-1078.

However, hemicellulose is a complex polysaccharide with a diverse composition of hexose and pentose groups, depending on the biomass. In *Miscanthus*, hemicellulose consists of an arabinoxylan backbone with mostly xylose monomers (Brosse et al. 2012), therefore, a more accurate way to represent the simplified kinetic model in the pretreatment of *Miscanthus* with BSW, is to correspond xylose to its corresponding polymer, with dehydration of xylose into furfural:

$$xylan \xrightarrow{k1} xylose \xrightarrow{k2} furfural$$

Results in this study suggest that butadiene sulfone solubilizes lignin from *Miscanthus* as a result of the lignin delocalization by the *in situ* sulfurous acid hydrolysis of the xylan-lignin ester bonds.

5.2 Materials and methods

Materials

Butadiene sulfone (98%) was purchased from Sigma-Aldrich (St. Louis, MO) and used as received. *Miscanthus x giganteus* was provided by the Energy Biosciences Institute at the University of Illinois at Urbana-Champaign, milled to pass a 1 mm screen and sieved to -20/+80 mesh (Chapter 4).

Kinetic analysis of xylan hydrolysis

The kinetic analysis of xylan hydrolysis from *Miscanthus* was performed under a first-order assumption in a constant-volume batch reactor, using the integral method (Fogler 1999) to verify the linearity of the concentration-time data, with the limit $C = C_0$ at $t = 0$:

$$-\frac{dC}{dt} = kC \qquad \rightarrow \qquad \ln\frac{C_0}{C} = kt$$

where k is the specific reaction rate in h^{-1} and C is the xylan concentration expressed as xylose residues in mmol/cm^3. The moles of xylose residues represent the amount of xylan divided by 0.88, the anhydro correction factor (Sluiter et al. 2008). The molecular weight of xylose is 150 g/mol.

The Arrhenius temperature dependence on the specific reaction rates was correlated as follows:

$$k(T) = Ae^{-E/RT}$$

where A is the pre-exponential factor, E is the activation energy in kJ/mol, R is the gas constant = 8.314×10^{-3} kJ/mol-K and T = absolute temperature (K).

5.3 Results and discussion

Mechanisms for xylan and lignin removal from *Miscanthus* with BSW

The protons in equilibrium with sulfurous acid follow the same mechanism as the protons in dilute acid pretreatment, by catalyzing the hydrolysis of the ester linkages connecting the xylan side chains with the lignin-ferulate units, provoking the *in situ* delocalization of both xylan and lignin, and the subsequent hydrolysis of xylan (Morvan et al. 2009; Chundawat et al. 2011; Donohoe et al. 2011). This allows both the BSW and dilute acid pretreatments to remove >90% of xylan from biomass (Fig. 4.3D); achieved at lower temperatures and longer times (90-110 °C, 6-30 h) for the butadiene sulfone-water (BSW) and higher temperatures and shorter times (160-220 °C, 1-30 min) for the dilute acid pretreatment (Chundawat et al. 2011). On the other hand, while traditional acid one-step hydrolysis only extracts 10-20% of lignin from the solid to the liquid phase (Chundawat et al. 2011), BSW solubilizes >50% of lignin (Fig. 4.3C).

This indicates that butadiene sulfone must be involved in lignin solubilization, during or after the acid-catalyzed ester cleavages between xylan and lignin.

The π-interactions between BS and the aromatic moieties of lignin, as well as the capacity of the sulfone group in BS to accept hydrogen bonds from hydroxyl groups in the delocalized lignin, are plausible solubilization mechanisms. Ring interactions between solvent and solute are difficult to quantify, but a solvent's capacity to accept hydrogen bonds has been measured for a wide variety of solvents with a temperature dependent scale known as the β Kamlet-Taft parameter (Mäki-Arvela et al. 2010; Kamlet and Taft 1976) (Appendix A). Doherty and coworkers correlated increased lignin extraction from maple wood flour with increasing values of β (0.6 to 1.2) for several ionic liquids at 90 °C, removing 37% of lignin after 24 h using 1-butyl-3-methylimidazolium acetate (β = 1.2) (Doherty et al. 2010). Solvent systems like dimethylacetamide/LiCl (DMA/LiCl) are known to effectively swell and disrupt the hydrogen bond network in cellulose, consistent with β numbers for DMA/LiCl as high as 2.0 (Spange et al. 1998). Contrarily, cyclic sulfones have low β numbers compared to known cellulose solvents or ionic liquids. The β value for BS is not available in literature, but it is likely comparable to similar sulfone structures. For example, sulfolane has a β = 0.39 (Marcus 1993) and piperylene sulfone, a monounsaturated cyclic sulfone like BS, has a β = 0.46 (Vinci et al. 2007). An unsuccessful attempt was made to determine the β value for BS with available spectrophotometric methods (Kamlet and Taft 1976; Thomas 2006; Doherty 2010) and future work should develop new approaches for the determination of β for BS (Appendix C).

Pure lignin was completely soluble in BS (without water addition) at 70 °C and 24 h, while pure xylan, microcrystalline cellulose (Avicel®) and raw *Miscanthus* were practically insoluble under these conditions (Fig. 5.1). The low β numbers for cyclic sulfones explain why

BS is not able to break the intra and inter hydrogen bond linkages of pure glucan, xylan and raw *Miscanthus,* but sufficient to be a factor in the solubilization of pure lignin. This observation supports the claim that during BSW pretreatment of *Miscanthus,* BS is solubilizing lignin being delocalized by the acid hydrolysis of the xylan-lignin ester bonds.

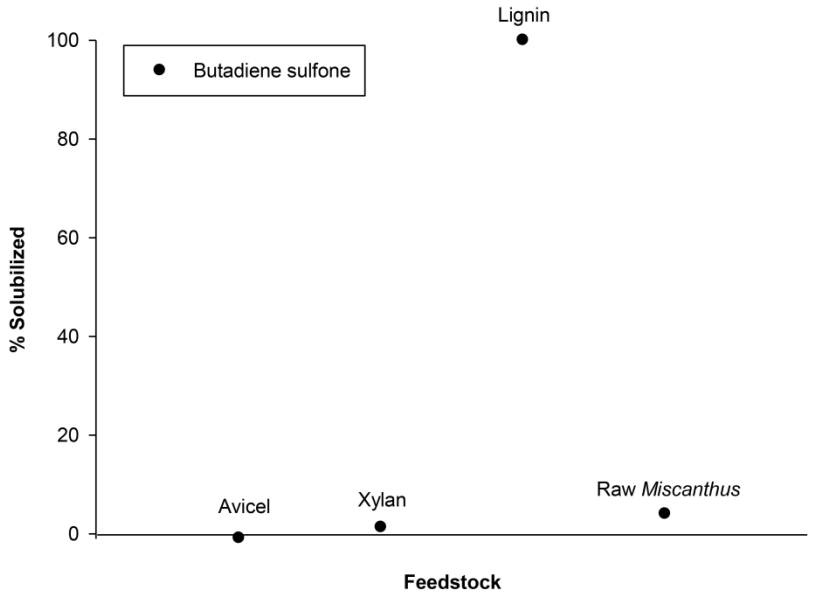


Figure 5.1 Solubilization of different feedstocks (Avicel®, xylan from birchwood, lignin, alkali and raw *Miscanthus*) in butadiene sulfone (without water addition) at 70 °C and 24 h. The minor solubility for raw *Miscanthus* (4%) represents non-structural elements.

First-order kinetics for xylan hydrolysis from *Miscanthus* via BSW pretreatment

The *in situ* production of sulfurous acid during BSW pretreatment of *Miscanthus,* catalyzed the hydrolysis of xylan to xylose in a first-order rate, based on the removal of xylose residues from xylan between 90 °C-110 °C and 6-30 h (Fig. 5.2). The system showed excellent correlation at 100 °C (R^2 = 0.97) and 110 °C (R^2 = 0.95) and average correlation at 90 °C (R^2 = 0.79), as no xylan hydrolysis was observed from 0 to 6 h at 90 °C (Fig. 5.2A). Interestingly, the

results show that $k_{100\,°C} = 2.4k_{90\,°C,}$ and $k_{110\,°C} = 1.9k_{100\,°C,}$ closely following the rule of thumb of double reaction rate for every 10 °C increase in temperature for specific combinations of temperature and activation energy (Fogler 1999). Fig. 5.2B shows the Arrhenius plot from 90 °C-110 °C (363 K-383 K) (R^2=0.99) with the activation energy calculated as 89.1 kJ/mol for the hydrolysis of xylan in *Miscanthus*. This activation energy is 22% lower than the one determined by Dee and Bell (114 kJ/mol) for the first-order hydrolysis of hemicellulose in *Miscanthus* dissolved in the ionic liquid 1-ethyl-3-methylimidazolium chloride with added H_2SO_4 (Dee and Bell 2011). In their work, *Miscanthus* was first dissolved in the ionic liquid for 6 h at 378 K, followed by a decrease in reaction temperature (373 K) and initiated with added 3.3 M H_2SO_4 from 10-120 min. Table 5.1 compares previous experimental determinations of the activation energy for the hydrolysis of xylan/hemicellulose in softwoods via Brønsted acid hydrolysis under organic or water phases.

A unique aspect of the acid-catalyzed first-order hydrolysis of xylan in *Miscanthus* with BSW is the fact that the concentration of sulfurous acid may not remain constant during the course of pretreatment. Some of the SO_2 does not react with water for the formation of acid and may participate in unwanted diene polymerization, as observed during the reaction at 110 °C (Fig. 4.5). In that case, it is possible for the concentration of sulfurous acid to increase with reaction time, which would invalidate first-order kinetics (Donaldson et al. 2009). However, Donaldson and coworkers used butadiene sulfone-water for the *in situ* acid-catalyzed hydrolysis of β-pinene and reported excellent correlation (R^2=0.95) in the rate plots, indicating first-order behavior despite the decomposition of BS and potential increase in acid concentration (Donaldson et al. 2009). Similarly, for the hydrolysis of xylan in *Miscanthus*, excellent linearity

was observed at 110 °C (R^2=0.95), even with the formation of dienes. Based on this observation, the acceptance of first-order kinetics and fairly constant acid concentration is warranted.

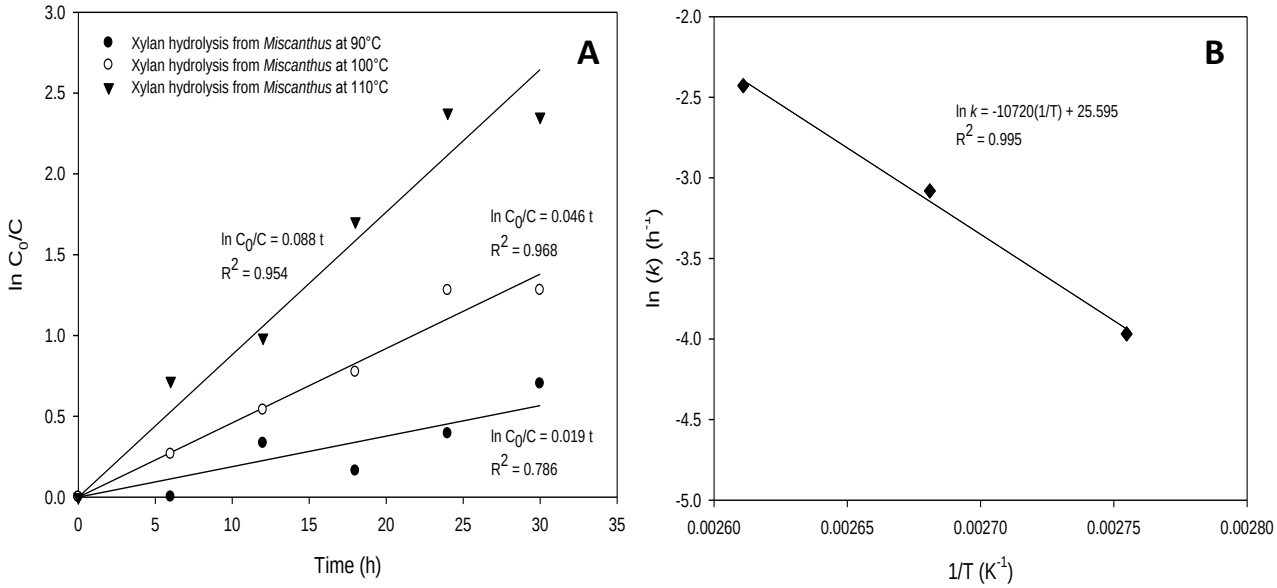


Figure 5.2 (A) First order hydrolysis of *Miscanthus* in butadiene sulfone-water from the *in situ* production of sulfurous acid at 90 °C, 100 °C and 110 °C. Rate of disappearance is based on the concentration of xylose residues (in mmol/cm^3) from 0 to 30 h. The specific reaction rate constant *(k)* is the slope of the curve at each temperature: 0.019 h^{-1}, 0.046 h^{-1}, 0.088 h^{-1} at 90 °C, 100 °C and 110 °C. (B) Arrhenius plot. Determination of the activation energy (E) based on the rate of disappearance of xylose at the same temperatures in *kelvin;* 363 K, 373 K and 383 K. From the slope of the curve, E is 89.1 kJ/mol for the hydrolysis of xylan in *Miscanthus.*

Table 5.1 Activation energy for the irreversible first-order hydrolysis of xylan/hemicellulose to xylose previously reported for different biomass substrates.

$$hemicellulose \xrightarrow{k} xylose$$

Biomass (mesh size)	Solvent	Acid catalyst	Reactor	Temp (°C)	Time	Activation Energy (kJ/mol)
Corn stover (-30/+50)[1]	Water	0.5-1.5% H_2SO_4	Batch	<160-240	<45 min	172
Corn stover (-10/+60)[2]	Water	1-3% H_2SO_4	Flowthrough (8L/min)	90-100	0-180 min	112
Switchgrass (-10/+20)[3]	Water	0.5% H_2SO_4	Batch	<175	<120 min	141
Switchgrass (-10/+20)[4]	Water	0.5% H_2SO_4	Batch	160-190	<120 min	66-115
Miscanthus (~+170)[5]	Ionic Liquid ([EMIM]Cl)	3.3 M H_2SO_4	Batch	80-124	0-120 min	114
Miscanthus (-20/+80)[6]	Butadiene sulfone-H_2O	H_2SO_3 in situ	Batch	90-110	6-30 h	89

[1](Bhandari et al. 1984)
[2](Jin et al. 2011)
[3](Jensen et al. 2008)
[4](Yat et al. 2008)
[5](Dee and Bell 2011)
[6](de Frias and Feng 2013)

5.4 Conclusions

The hydrolysis of xylan in *Miscanthus* from pretreatment with switchable butadiene sulfone in the presence of water, followed irreversible first-order kinetics with an activation energy (89 kJ/mol) that is comparable or lower than other pretreatment techniques, like dilute acid or ionic liquids. The solubilization of lignin was attributed to the solvent (butadiene sulfone) interactions with the delocalized lignin during or after the hydrolysis of xylan.

CHAPTER 6

CONVERSION OF SUGARCANE BAGASSE INTO FURFURAL AND PRETREATMENT OF THE RESIDUES WITH BUTADIENE SULFONE

6.1 Introduction

In a biorefinery concept-based production of furfural from xylan in sugarcane bagasse, using the biochemical route to convert glucan to biofuels (i.e., enzymatic hydrolysis of glucan and fermentation to biofuels), it is important to attain the highest yields of furfural with the maximum preservation of glucan for subsequent enzymatic hydrolysis. The furfural-biochemical route proposed involves the pretreatment of the furfural residues with butadiene sulfone in the presence of water at 110 °C and 3 h (Fig. 6.1). The purpose of the pretreatment is to reduce the total lignin content before the enzymatic saccharification of glucan.

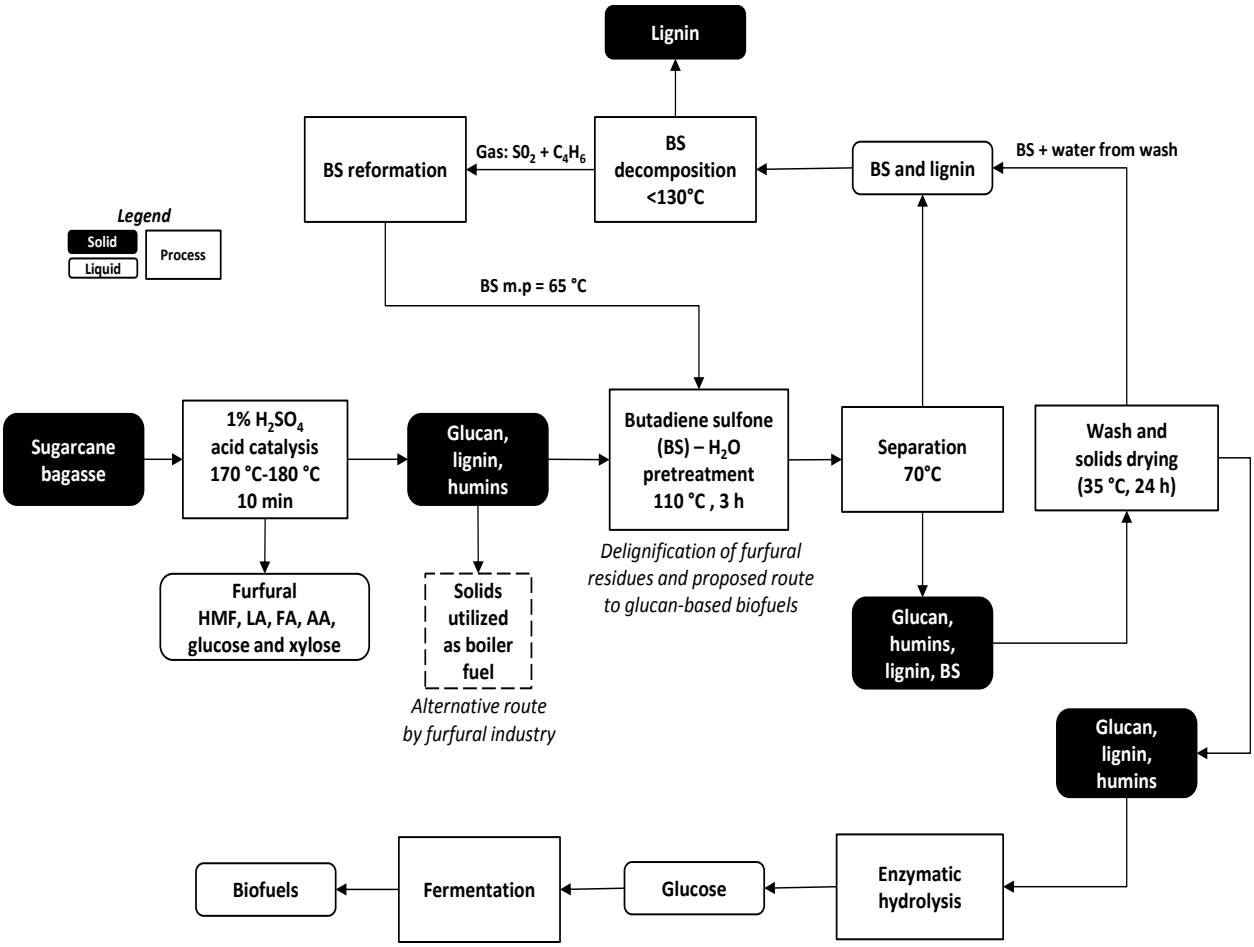


Figure 6.1 Furfural-biochemical route to biofuels from sugarcane bagasse.

6.2 Materials and methods

Materials

Butadiene sulfone (98%), levulinic acid (97%, food grade), formic acid (95%, reagent grade), furfural (purum, 98% GC grade), cellobiose (99%), glucose (99%) and xylose (99%) were purchased from Sigma-Aldrich (St. Louis, MO). Hydroxymethylfurfural (98%) was purchased from Alfa Aesar (Ward Hill, MA) and sulfuric acid (72% ACS reagent grade) was purchased from Ricca Chemical Company (Arlington, TX). All chemicals were used as

received. Sugarcane bagasse was kindly supplied by the Louisiana Sugar Cane Cooperative, Inc. (St. Martinville, LA).

Conversion of sugarcane bagasse into furfural

Sugarcane bagasse (7% moisture content) was knife milled under a 1 mm screen (Retsch GmbH, model SM 2000, Haan, Germany) and the particle size standardized to a range of -20/+80 mesh sieve (ASTM, U.S. Standard Sieve Series, Soiltest, Inc., Evanston, IL) using a Ro-Tap shaker (model D-4325, Dual Manufacturing Co. Inc., Chicago, IL). Biomass was then stored in a cold room at 0ºC until use. The conversion of sugarcane bagasse into furfural was performed in a 1L stainless steel batch reactor (Parr Instrument Company, model 4530, Moline, IL) with temperature control (Parr model 4843) and continuous stirring (Fig. 6.2). Ten grams of biomass (dry weight) were soaked in the reactor glass liner for 24 hours at room temperature in 190 grams of 1% sulfuric acid. After soaking, the liner was placed inside the reactor, which was then tightly sealed and inserted into the heating jacket. The reaction conditions were 170 ºC-190 °C and 10 minutes with stirring at 400 rpm. It took the reactor 35-40 minutes to reach the set temperature, which was controlled to ± 2ºC. Samples (1-2 ml) were taken throughout the reaction every 2 minutes, using a 10 ml sampling cylinder (Swagelok, model SS-4CD-TW-10, St. Louis, MO) connected to a needle valve via a dipping tube into the reactor. After each sampling, nitrogen gas was used to push down any residual liquid in the dipping tube into the reactor, while an ice water bath was used around the sampling cylinder to condense the samples. At 10 min, water was circulated through a serpentine coil inside the reactor to stop the reaction, cooling the slurries down to 40ºC in less than 15 minutes. The liquid samples and slurries were immediately stored in a freezer to minimize unwanted side reactions.

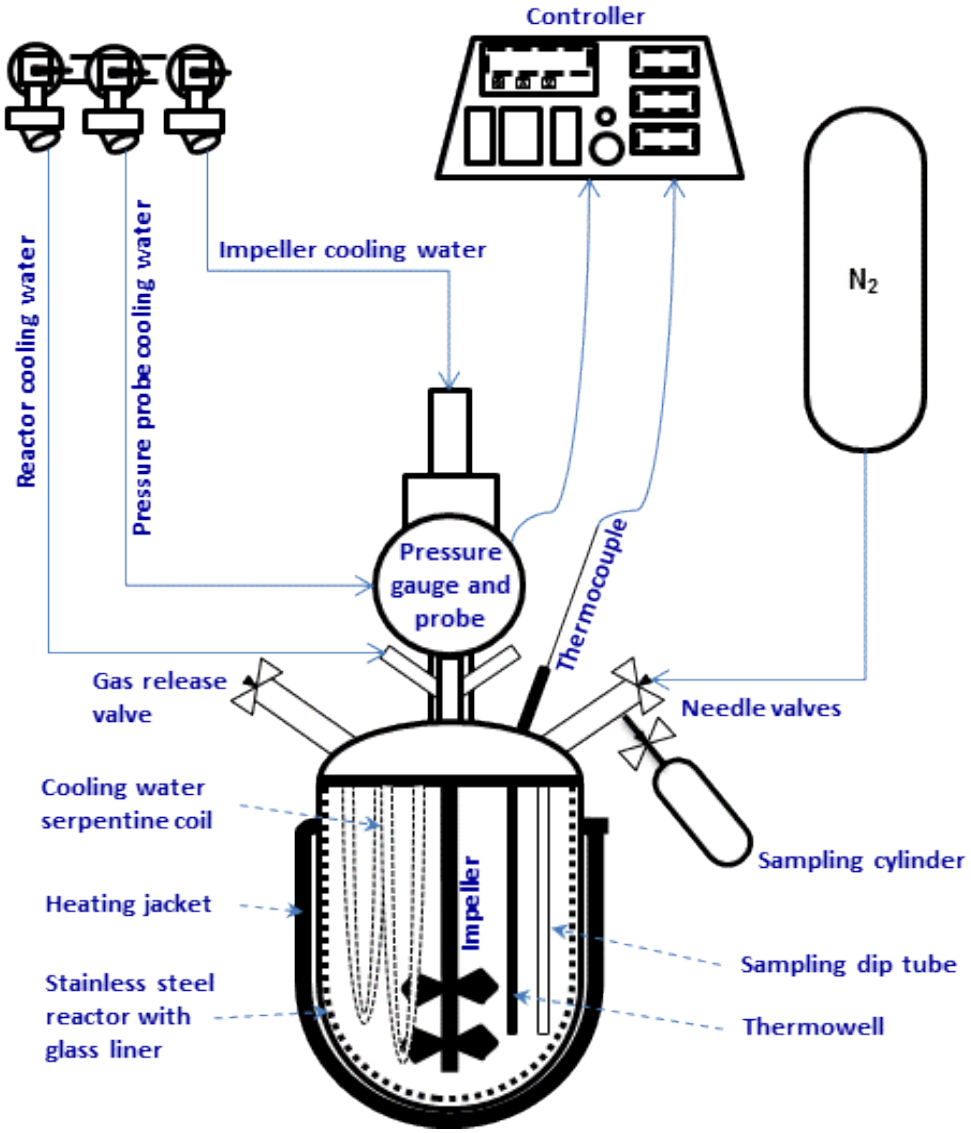


Figure 6.2 Parr® batch rector setup.

Analysis of biomass solids after production of furfural

Glucan, xylan and lignin content in raw sugarcane bagasse was determined according to the NREL Laboratory Analytical Procedures (LAP) "Determination of structural carbohydrates and lignin in biomass" (Sluiter et al. 2008) (Table 6.1). This procedure required a biomass moisture content of less than 10% and the removal of non-structural elements via a two-step extraction with water and ethanol (95% v/v) (de Frias and Feng 2013; Sluiter et al. 2005). The extractive-free solids were dried for 24 h at 35 ºC-40 °C to moisture content of 10% or less prior to chemical composition analysis of raw sugarcane bagasse. To determine the amounts of glucan, xylan and lignin in the raw bagasse, the corresponding composition percentages were multiplied by the oven-dried weight of biomass used.

Table 6.1 Chemical composition of raw sugarcane bagasse.

Component	**Sugarcane bagasse (% dry basis)**
Extractives[a]	4.4 ± 0.1
Glucan	37.2 ± 1.0
Xylan	22.8 ± 0.5
Lignin[b]	26.9 ± 1.7

[a]Extractives is the sum of water extractives (2.3 ± 0.5) and ethanol extractives (2.1 ± 0.4).
[b]Lignin is the sum of acid soluble lignin (5.1 ± 0.0) and acid insoluble lignin (21.8 ± 1.8).

The solids after furfural production were washed five times with deionized water until the pH of slurries was 4-5, and dried for 24 h at 35 °C-40 °C to moisture content <10%, for chemical composition analysis (without the extractives step) and enzymatic hydrolysis.

Analysis of the liquid fraction after production of furfural

Determination of furfural, hydroxymethylfurfural (HMF), levulinic acid and formic acid

Frozen liquid samples were thawed at room temperature, put in a vortex shaker and filtered through a 0.2 µm filter before HPLC analysis with a Waters e2695 separations module (Milford, MA), a Biorad Aminex HPX-87H column (Hercules, CA) at 60ºC and a Waters 2414 refractive index detector at 50ºC. The mobile phase was 0.005 M H_2SO_4 with a flow rate of 0.6 ml/min. The total run time per sample was 50 minutes and the retention times for formic acid, acetic acid, levulinic acid, HMF and furfural were compared to their corresponding standards via a calibration curve for identification and quantification. Yields of furfural, and the combined yields of HMF, levulinic acid (LA) and formic acid (FA) were calculated as follows:

$$Furfural\ (\%) = \frac{[fur]_{HPLC} \times V_s}{m_{xylan\ raw} \times 0.727} \times 100$$

$$HMF + LA + FA\ (\%) = \frac{(\frac{[HMF]_{HPLC}}{0.778} + \frac{[LA]_{HPLC}}{0.716} + \frac{[FA]_{HPLC}}{0.284}) \times V_s}{m_{glucan\ raw}} \times 100$$

where $[fur]_{HPLC}$, $[HMF]_{HPLC}$, $[LA]_{HPLC}$, $[FA]_{HPLC}$ are the individual HPLC concentrations (g/L) of furfural, HMF, LA or FA multiplied by a dilution factor (if applicable). V_s is the total volume of the liquid fraction (L) minus the sample aliquots, and *m* is the dry-basis amount (g) of xylan or glucan in raw bagasse. 0.727, 0.778, 0.716 and 0.284 are the theoretical yield factors from xylan to furfural, glucan to HMF, glucan to levulinic acid, glucan to formic acid, respectively (Zeitsch 2000; Hayes 2005; Kumar et al. 2013).

Determination of xylose and glucose

The amount of xylose and glucose in the liquid fraction after production of furfural from sugarcane bagasse, was determined following the NREL Laboratory Analytical Procedures (LAP) "Determination of sugars, byproducts an degradation products in liquid fraction process samples"(Sluiter and National Renewable Energy Laboratory 2008). Samples were diluted (dilution factor = 5) and filtered through a 0.2 µm filter before HPLC analysis with a Waters e2695 separations module (Milford, MA), a Biorad Aminex HPX-87P column (Hercules, CA) at 80ºC and a Waters 2414 refractive index detector at 50ºC. The mobile phase was water with a flow rate of 0.6 ml/min. The procedure followed in the NREL method corresponded to the analysis for total sugar content (monosaccharides and oligosaccharides). The amounts of xylose and glucose were corrected to their corresponding polymers, xylan and glucan, using the anhydro correction factor of 0.88 for xylose, and 0.90 for glucose (Sluiter et al. 2008). The percentages of xylan to xylose, and glucan to glucose, not dehydrated into furans, were calculated as follows:

$$Xylan\ (\%) = \frac{0.88\ \times [xylose]_{HPLC} \times\ V_s}{m_{xylan\ raw}} \times 100$$

$$Glucan\ (\%) = \frac{0.90\ \times [glucose]_{HPLC} \times\ V_s}{m_{glucan\ raw}} \times 100$$

where , $[xylose]_{HPLC}$ and $[glucose]_{HPLC}$ are the corresponding HPLC concentrations (g/L) of xylose and glucose, V_s is the total volume of the liquid fraction, minus the sample aliquots, $m_{xylan\ raw}$ and $m_{glucan\ raw}$ are the dry-basis amounts (g) of xylan and glucan in the raw sugarcane bagasse.

Determination of humins (pseudo-lignin) from xylan

The percentage of xylan converted to humins (pseudo-lignin), was calculated as:

$$Xylan\ to\ humins\ (\%) = \frac{m_{xylan\ raw} - (0.88 \times [xylose]_{HPLC} \times V_s) - ([fur]_{HPLC} \times V_s/0.727) - m_{xylan\ in\ solids}}{m_{xylan\ raw}} \times 100$$

where $m_{xylan\ in\ solids}$ is the amount (g) of xylan in the solids, which was zero (Table 6.2), after the production of furfural.

Pretreatment with butadiene sulfone of sugarcane bagasse furfural residues

After the conversion of the xylan in sugarcane bagasse into furfural, the washed and dried solids were pretreated with butadiene sulfone-water as previously reported (de Frias and Feng 2013). Pretreatment conditions were 110ºC for 3 h at 600 rpm, and after the reaction, the solids were washed five times with deionized water until the pH of slurries was 4-5, and dried for 24 h at 35 °C-40 °C to moisture content <10% for chemical composition analysis (Sluiter et al. 2008) and enzymatic hydrolysis. Lignin removed after pretreatment was defined as:

$$lignin\ removed\ (\%) = \frac{m_{lig\ fur\ res} - m_{\ lig\ pretreated\ fur\ res}}{m_{lig\ fur\ res}} \times 100$$

where $m_{lig\ fur\ res}$ is the amount of lignin (g) in the furfural residues before pretreatment and $m_{\ lig\ pretreated\ fur\ res}$ is the amount of total lignin (g) after pretreatment of the furfural residues.

Enzymatic hydrolysis of glucan in sugarcane bagasse after furfural production

The saccharification of glucan in the washed and dried bagasse furfural residues before and after pretreatment with butadiene sulfone-water, was performed following the NREL Laboratory Analytical Procedures (LAP) "Enzymatic Saccharification of Lignocellulosic Biomass" (Selig et al. 2008). The NREL method was modified for the utilization of the enzyme products Cellic® CTec2 and HTec2, designed for lignocellulosic materials provided by Novozymes®. Ctec2 is a mix of cellulases and β-glucosidases, and HTec2 consists of endoxylanases (Novozymes). The enzyme loadings used were the optimum recommended by the manufacturer to attain maximum hydrolysis (Novozymes): 0.27 for CTec2 and 0.03 for HTec2, in grams of enzyme per grams of glucan in the pretreated biomass. Aliquots of 0.5 ml were taken at 6 h, 12 h, 24 h, 48 h and 72 h for HPLC analysis of glucose. Glucan digestibility was defined as grams of glucan digested divided by grams of glucan in the biomass (Selig et al. 2008):

$$Glucan\ digestibility\ (\%) = \frac{0.9\ \times [glucose] \times\ V_s}{m_{glucan\ in\ furfural\ residues}} \times 100$$

Where, 0.9 is the glucose-to-cellulose anhydro correction factor, $[glucose]$ is the glucose concentration (g/ml) minus any glucose in the enzyme or substrate blanks, V_s is the total volume of assay volume (ml), minus the sample aliquots, and $m_{glucan\ in\ furfural\ residues}$ is the amount of glucan in the furfural residues before and after pretreatment with butadiene sulfone-water.

6.3 Results and discussion

Conversion of xylan in sugarcane bagasse into furfural

Furfural was produced after the acid-catalyzed hydrolysis of the xylan in sugarcane bagasse to xylose with subsequent dehydration into furfural (Fig. 2.6), in a Parr® reactor at 170 °C-180 °C for 2-10 min, and at 190 °C at 6 and 10 min. At these conditions, glucan was also partially hydrolyzed to glucose with dehydration into hydroxymethylfurfural (HMF) and subsequent partial rehydration of HMF into levulinic acid (LA) and formic acid (FA).

The production of furfural (based on theoretical yield) from sugarcane bagasse is shown in Fig. 6.3. At 2 min, the yields of furfural are 12% at 170 °C and 20% at 180 °C, reaching 38% at 170 °C and 50% at 180 °C when the reaction was stopped at 10 min. At 190 °C, the yield of furfural topped 72% at 10 min. The high yields of the batch lab-scale process at T ≥ 180 °C and t = 10 min under dilute acid conditions (1%) can be explained by the low solids loading (1:20 solid:liquid), biomass particle size (1 mm) and immediate frozen storage of samples.

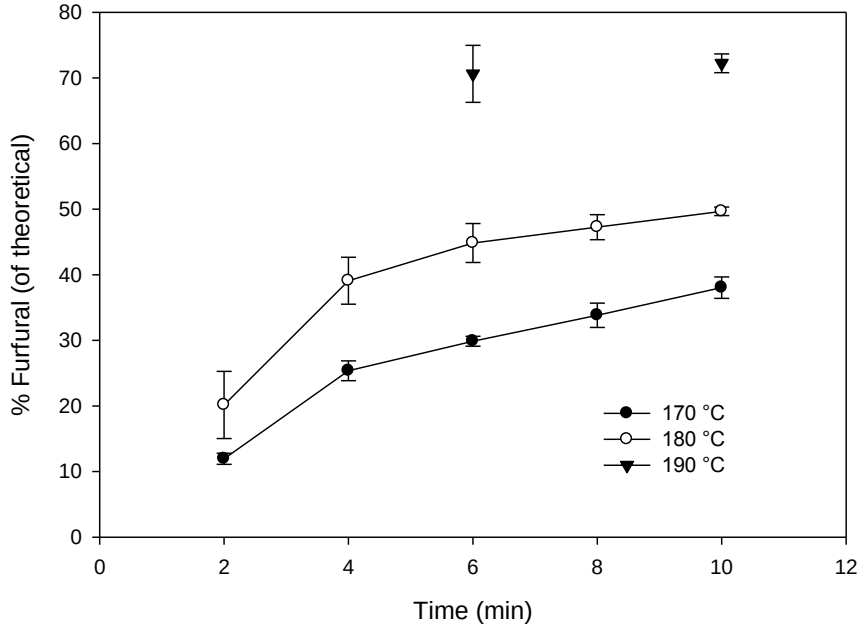


Figure 6.3 Conversion of xylan in sugarcane bagasse into furfural at 170 °C, 180 °C and 190 °C in a 1L Parr® batch reactor. Yields are based on the xylan content (dry basis) in bagasse.

In the industrial batch production of furfural (170-185°C, 3% w/w H_2SO_4, 3 hours) (Mamman et al. 2008), yields do not exceed 40-50% of theoretical, despite reaction severity, due to high solids loadings (1:2 solids:liquids) (Mamman et al. 2008), large particle sizes (20-30 mm) (Zeitsch 2000) and delayed removal of furfural from the reactor. If furfural stays in solution, furfural yields can decrease due to condensation (reaction with intermediates) or resinification (reaction with itself) (Zeitsch 2000). Similarly, in the batch reaction setup used in this study, the furfural yield decreased 6% at 170 °C and decreased 12% at 180 °C, compared to the last sample taken at 10 min, after the reaction ended and the reactor was cooled down.

In industrial conditions, large particle sizes and high solids loading are preferable for an economically feasible process, and improvements have been made to immediately remove furfural from the reactor to avoid loss and increase yields over 50% of theoretical (Zeitsch 2000). Despite the productivity improvements, the left-over solids containing glucan have value as feedstocks, but are mostly under-utilized as boiler fuel (Mamman et al. 2008). Recent industrial initiatives have been successful in utilizing both xylan and glucan for producing furfural and levulinic acid/formic acid, *via* dilute sulfuric acid catalysis (Hayes 2005; Bozell et al. 2000) (Fig. 2.7).

At the furfural production stage it is necessary to minimize the formation of glucose and its degradation products HMF, LA and FA during furfural production. These products are value-added chemicals in other biorefinery schemes, but in the furfural-biochemical route, HMF, LA and FA represent loss of glucan, besides being fermentation inhibitors. The combined yields (based on theoretical) of HMF, LA and FA at 10 min reached 7% at 170 °C, 19% at 180 °C and 48% at 190 °C (Fig. 6.4). Since almost half of the glucan is lost to HMF, LA and FA at 190 °C, the 170 °C and 180 °C conditions were thus explored further for pretreatment with butadiene

sulfone in the presence water with subsequent enzymatic hydrolysis. Both temperature conditions attained average furfural yields (38% and 50%) with less than 20% loss of glucan to HMF, LA and FA.

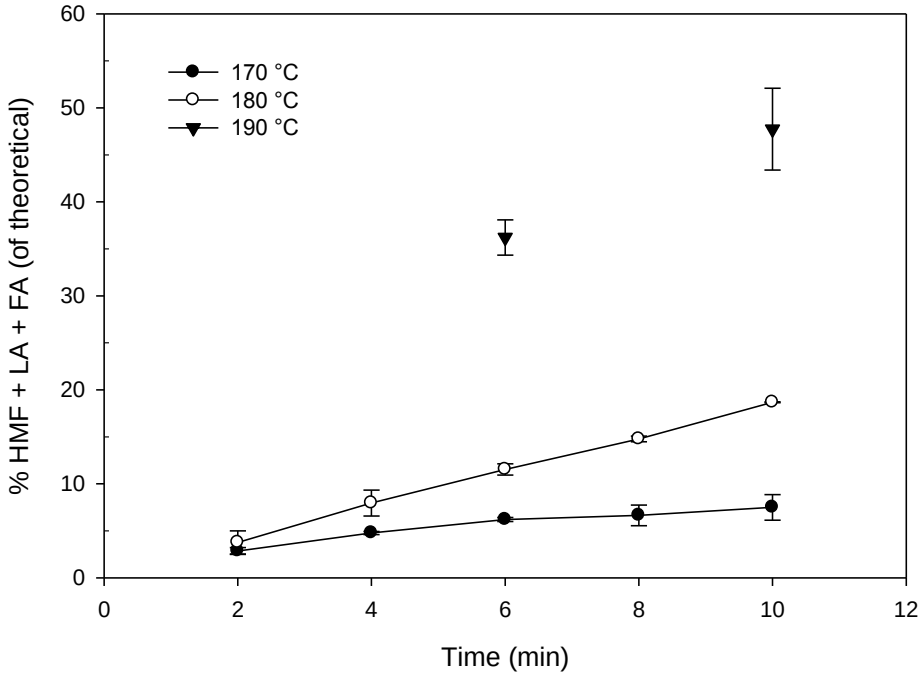


Figure 6.4 Combined yields of hydroxymethylfurfural (HMF), levulinic acid (LA) and formic acid (FA) from glucan in sugarcane bagasse, during conversion of xylan in sugarcane bagasse into furfural.

Acid-catalyzed degradation of glucan during production of furfural

During the production of furfural, the unwanted acid-catalyzed hydrolysis of glucan to glucose with further dehydration into hydroxymethylfurfural (HMF) and rehydration to levulinic acid (LA) and formic acid (FA), is shown in Fig. 6.5. In addition, the 72 h enzymatic hydrolysis of the glucan remaining in the solids, expressed as % glucan digestibility, is overlaid in the figure. After production of furfural at 170 °C, 19% of glucan was converted to glucose, and 8% was converted to HMF+LA+FA. At 180 °C, 25% of glucan was converted to glucose and 23% was converted to HMF+LA+FA. The combined yields of HMF+LA+FA are higher than the

ones reported in the previous section at 10 min, because the analysis was performed after the reactor was stopped and cooled down, which allowed for further glucose dehydration and rehydration of HMF. The glucan lost to glucose and HMF+LA+FA during the acid catalyzed reaction, is consistent with the reaction severity, which provides good furfural yields but degrades the glucan that needs to remain in the solids for enzymatic hydrolysis. In total, 27% of glucan was lost to glucose and HMF+FA+LA at 170 °C, and 47% was lost at 180 °C. The enzymatic glucan digestibility of the solids at 72 h was 73% and 81% at 170 °C and 180 °C, respectively. Previous work on dilute acid pretreatment of biomass have reported increased enzymatic digestibility with increasing temperature or reaction severity (Yang and Wyman 2004; Saha et al. 2005).

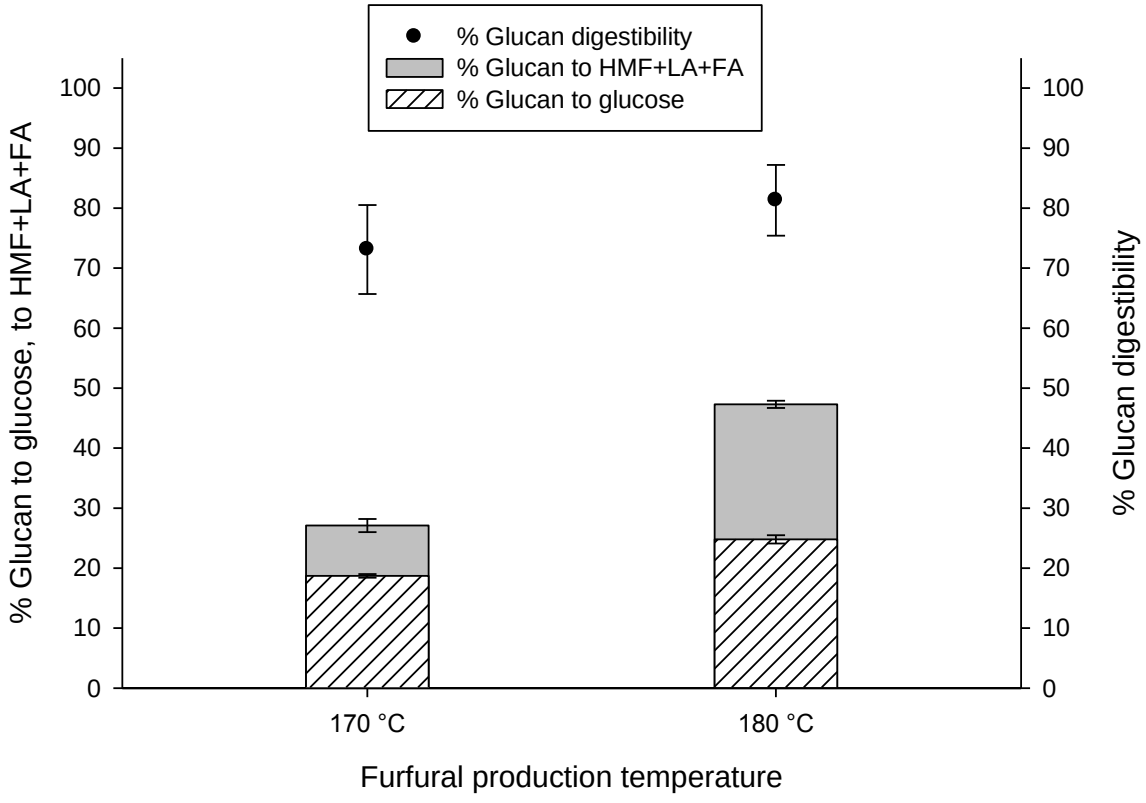


Figure 6.5 Percentage of the glucan in sugarcane bagasse, converted to glucose and HMF+LA+FA after acid-catalyzed production of furfural at 170 °C and 180 °C, and before pretreatment with butadiene sulfone-water.

Formation of humins (pseudo-lignin) during production of furfural

The deconstructed leftover bagasse solids after the production of furfural were rich in glucan, and all of the xylan was completely removed, as shown in Table 6.2. However, an increase in lignin composition was observed due to the formation of humins (pseudo-lignin) which increased the acid-insoluble Klason (gravimetric) lignin composition in the solids (Table 6.2). As previously explained, humins are produced *via* condensation of furans and sugars, and were clearly visible inside the reactor glass liner as tar-like compounds adhered to the liner surface; consistent with observations by Kumar and coworkers who studied the formation of pseudo-lignin from pure cellulose mixed with xylan and xylose after dilute acid pretreatment (Kumar et al. 2013). Other works have studied the formation of pseudo-lignin from lignocellulosic biomass, like hybrid poplar (Hu et al. 2012) and the invasive grass *Typha capensis* (Audu et al. 2012). These studies concluded that the formation of pseudo-lignin not only increases with pretreatment severity, biasing high the Klason lignin value, but also inhibits the enzymatic hydrolysis of cellulose to glucose (Kumar et al. 2013; Hu et al. 2012; Audu et al. 2012).

Fig. 6.6 shows that 36% of the xylan in sugarcane bagasse was converted to humins during the production of furfural at 170 °C, increasing to 51% during production of furfural at 180 °C. The formation of humins from xylan corresponds to an increase in total lignin composition from 26.9 % in raw sugarcane bagasse, to 45.5 % at 170 °C and 53.5% at 180 °C (Table 6.2). These results are in agreement with (Kumar et al. 2013; Hu et al. 2012; Audu et al. 2012) in regards to the increased formation of humins with reaction severity that results in a high lignin bias. Any humins from glucan were not determined as the furfural residues after the reaction were not fully recovered, impeding full closure of the glucan material balance. On the

other hand, the complete removal of xylan after furfural production allowed for full closure of the xylan material balance (Fig. 6.6).

Pretreatment of the bagasse furfural residues with butadiene sulfone

In Chapter 4, it was demonstrated that butadiene sulfone-water (BSW) pretreatment of raw *Miscanthus* removed up to 58% of the total lignin. The removal of lignin from biomass is key for enhanced enzymatic digestibility of glucan, as lignin inhibits the activity of cellulase enzymes (Li et al. 2010). Henceforth, the bagasse furfural residues were pretreated with BSW at 110 °C and 3 h, solubilizing 21.6% and 27.9% of the furfural residues after the production of furfural at 170 °C and 180 °C, respectively (Table 6.2). Since all of the xylan had been removed from sugarcane bagasse after furfural production, the solubilized material was rich in lignin, as observed by the decreased total lignin composition after BSW pretreatment from 45.5% to 35.5 % at a furfural production temperature of 170 °C, and from 53.5% to 42.4% at a furfural production temperature of 180 °C.

The decrease in lignin compositions corresponds to a total lignin removal of 38.6% from the 170 °C furfural solids, and 32.5% from the 180 °C furfural solids. The decreased removal of lignin from the 180 °C solids is probably due to the higher presence of humins compared to the 170 °C solids, as shown in Fig 6.6. In Chapter 4, the formation of humins may have inhibited the solubilization of lignin from *Miscanhtus* after butadiene sulfone pretreatment without water addition. Similarly, in the case of the furfural residues, higher humin content may be the cause of decreased removal of lignin after pretreatment.

Table 6.2 Chemical composition analysis of raw sugarcane bagasse and bagasse furfural residues before and after pretreatment with butadiene sulfone-water (BSW) at 110 °C and 3 h. The percentage of furfural residues solubilized and the percentage of lignin removal are also shown[1].

Solids	Furfural production temp (°C)	Glucan (%)	Xylan (%)	Lignin (%)	% Biomass solubilized after BSW pretreat.	% Lignin removal
Raw sugarcane bagasse	NA*	37.2 ± 1.0	22.8 ± 0.5	26.9 ± 1.7	NA*	NA*
Furfural residues BEFORE BSW pretreatment	170	44.9 ± 1.2	0	45.5 ± 0.3	NA*	ND**
	180	37.7 ± 0.1	0	53.5 ± 1.0	NA*	ND**
Furfural residues AFTER BSW pretreatment	170	66.8 ± 1.5	0	35.5 ± 3.0	21.6 ± 0.1	38.6 ± 5.3
	180	58.5 ± 0.9	0	42.4 ± 2.3	27.9 ± 0.2	32.5 ± 3.6

*Not applicable
**Not determined
[1]All percentages are expressed on a dry basis.

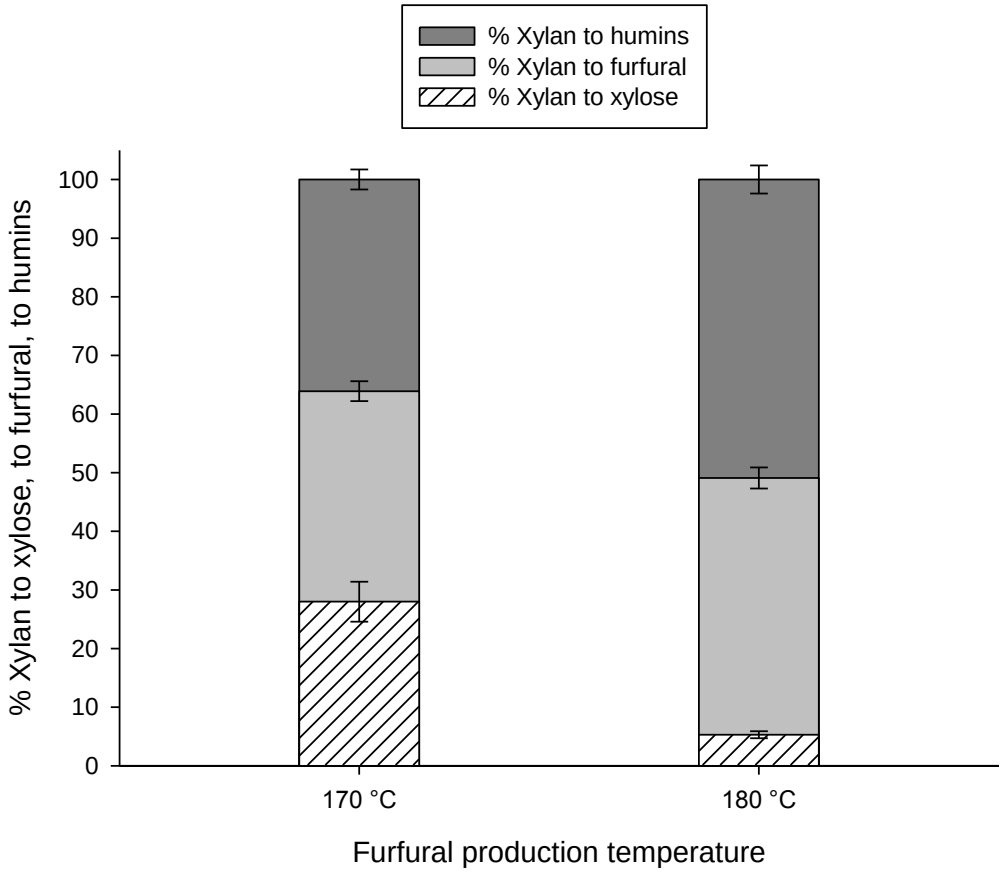


Figure 6.6 Percentage of the xylan in sugarcane bagasse, converted to xylose, to furfural and to humins (pseudo-lignin) after production of furfural at 170 °C and 180 °C, and before pretreatment with butadiene sulfone-water. The percentage of xylan in the solids was zero after the conversion to furfural.

Enzymatic hydrolysis of glucan in the bagasse furfural residues before and after pretreatment with butadiene sulfone

Fig. 6.7A & B shows the enzymatic hydrolysis from 6 h to 72 h of the glucan present in the sugarcane bagasse furfural residues, before and after pretreatment with butadiene sulfone-water (BSW). The glucose released from glucan is expressed as % glucan digestibility. For the furfural residues before BSW pretreatment (Fig. 6.7A), the % glucan digestibility reached 73% and 81% at 72 h, corresponding to the furfural production conditions of 170 °C and 180 °C,

respectively. For the furfural residues after BSW pretreatment at 110 °C and 3 h (Fig. 6.7B), the % glucan digestibility reached 70% and 79% at 72h, for the 170 °C and 180 °C furfural conversion conditions, respectively. In both cases, the higher furfural production temperature, from 170 °C to 180 °C, resulted in higher glucan digestibility; in agreement with previous studies on dilute acid pretreatment of lignocellulosic biomass that reported increased glucan digestibility with increased temperature or severity (Yang and Wyman 2004; Saha et al. 2005). Also, the results suggest that the greater formation of humins (pseudo-lignin) from xylan and/or glucan when the reaction temperature was raised to 180 °C, was not inhibitory to the enzymatic hydrolysis of glucan; contrary to previous work that reported decreased enzymatic digestibility of glucan with higher formation of humins (Kumar et al. 2013; Hu et al. 2012; Audu et al. 2012).

The % glucan digestibility values before and after pretreatment are not directly comparable, as the solids before pretreatment were washed and air-dried once and the solids after pretreatment were washed and air-dried twice; first after the production of furfural and second after pretreatment with butadiene sulfone-water. Several studies have reported that air drying of pretreated biomass solids provokes reduced pore volume and irreversible pore collapse, decreasing the enzymatic glucose release from glucan (Selig et al. 2008; Saddler et al. 1982; McMillan 1994; Jeoh et al. 2007; Sathitsuksanoh et al. 2011). Nonetheless, it is relevant to note that even with the more extensive reduction in pore volume or pore collapse after the second air-dry for the pretreated solids, the glucan digestibility values for the pretreated solids furfural solids were similar to the untreated ones. These results confirm that delignification of biomass is just one of the aspects to ensure high enzymatic digestibility, and accessible surface area (porosity) and degree of crystallinity must also be considered (Li et al. 2010; Zhang and Lynd 2004; Kumar et al. 2009b; McMillan 1994).

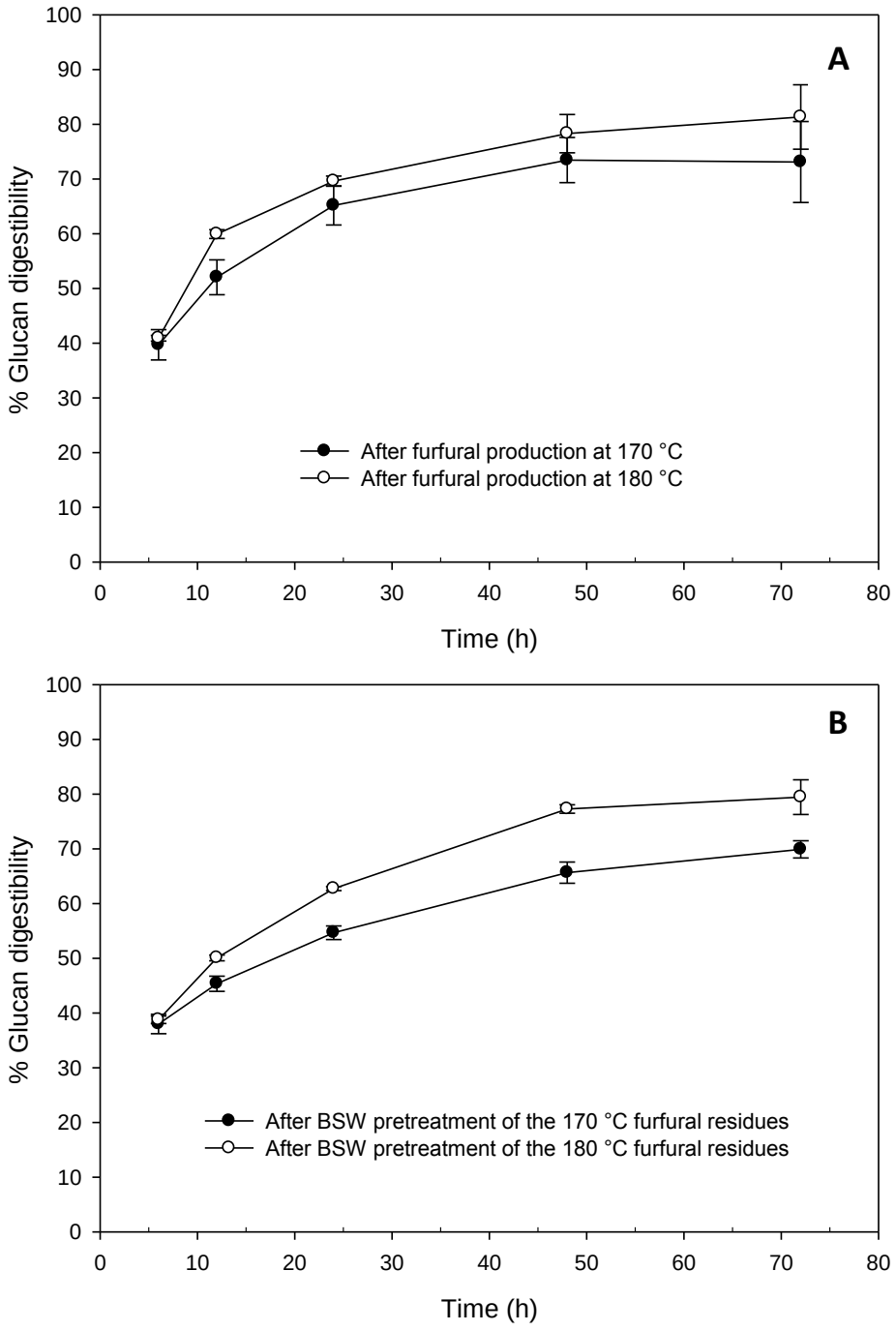


Figure 6.7 (A) Enzymatic hydrolysis from 6 h to 72 h of the glucan in sugarcane bagasse furfural residues after production of furfural at 170 °C and 180 °C, and before pretreatment with butadiene sulfone-water (BSW). (B) Enzymatic hydrolysis from 6 h to 72 h of the glucan in bagasse furfural residues after pretreatment with BSW at 110 °C and 3 h. Glucan digestibility is defined as grams of glucan digested divided by grams of glucan in the biomass (Selig et al. 2008).

6.4 Conclusions

A furfural-based biochemical conversion route was studied by converting xylan in sugarcane bagasse into furfural and by saccharification of the glucan-rich residues, before and after pretreatment with butadiene sulfone-water (BSW), for potential production of biofuels. The furfural yields were comparable to industrial batch production; achieving 38% and 50% of theoretical at 170 °C and 180 °C for 10 min of reaction time. The unwanted formation of humins (pseudo-lignin) during furfural production increased with reaction temperature, as observed by the greater loss of xylan to humins and the increased composition of total lignin, from 170 °C to 180 °C. However, despite the presence of humins, improved enzymatic digestibility was observed from 170 °C to 180 °C before or after BSW pretreatment. Pretreatment of the furfural residues with BSW removed 38.6% and 32.6% of lignin, from the 170 °C and 180 °C solids, respectively. However, the delignification did not result in higher enzymatic release of glucose compared to the untreated furfural residues, as a result of the more extensive drying performed to the pretreated solids which may have further reduced the accessible surface area for enzymatic hydrolysis. Nevertheless, these results demonstrate that butadiene sulfone-water pretreated biomass is enzymatically accessible at potentially high yields and future work should study not only the effect of delignification on enzymatic glucose release, but also the accessible surface are and degree of crystallinity.

CHAPTER 7*
POTENTIAL FOR RECOVERY OF BUTADIENE SULFONE

7.1 Introduction

In chemical pretreatment of lignocellulosic biomass, recovery of chemicals is difficult and not yet at a large-scale (Mora-Pale et al. 2011; Banerjee et al. 2009). On the other hand, the "switchable" capacity of butadiene sulfone (BS) allows for full decomposition from solvent to sulfur dioxide and 1,3-butadiene, which can be recombined back as solvent. BS is manufactured industrially from equimolal proportions of sulfur dioxide and 1,3-butadiene in shell and tube reactors at 100 °C and 300 psig (Couper et al. 2005). Hence, the current availability of large scale operations for both manufacturing and recovery of BS will pave the road to a potential scale-up of BSW pretreatment processes; as the excess BS in liquid and solid phases can be purified via decomposition/reformation.

7.2 Materials and methods

Materials

Butadiene sulfone (98%) was purchased from Sigma-Aldrich (St. Louis, MO) and used as received. *Miscanthus x giganteus* was provided by the Energy Biosciences Institute at the University of Illinois at Urbana-Champaign, milled to pass a 1 mm screen and sieved to -20/+80 mesh (Chapter 4).

*This chapter includes previously published material by the author. Reference: J. Atilio de Frias and Hao Feng, *Green Chem.*, 2013, 15, 1067-1078.

Thermogravimetric analysis (TGA) of butadiene sulfone

Thermogravimetric analysis (TGA) for butadiene sulfone was performed in a Q50 thermogravimetric analyzer (TA Instruments, New Castle, DE) at the Materials Research Laboratory (University of Illinois). 21 mg of pure BS or BS-rich liquid fraction after pretreatment of *Miscanthus* were placed in a 90 µL alumina cup and heated from room temperature to 130 °C for 70 min at a rate of 10 °C/min. A separate gas inlet tube delivered nitrogen gas to the sample. The Q50 includes Advantage software for automatic experimental control and Universal Analysis 2000 software for comprehensive data analysis.

7.3 Results and discussion

Recovery of butadiene sulfone

In Chapter 5, the referenced hydrolysis study of β-pinene with BS by Donaldson et al. (2009), reported the TGA of pure and recycled BS (via hexane extraction) to illustrate that BS can be fully recyclable via solvent decomposition and reformation at 130 °C. In both cases, only 1% of mass remained after the TGA analysis, which the authors attributed to impurities (solvent reformation was not attempted).

In this dissertation, an experiment comparing the TGA of pure BS and the BS-rich liquid fraction after pretreatment of *Miscanthus* with BSW (Fig. 7.1), is presented for the first time in order to assess the decomposition profile of both liquids and determine the amount of xylan derivatives and lignin left after a potential recovery of butadiene sulfone. At 130 °C and 70 minutes, only 0.4% of mass is left from pure BS, whereas 4.1% of solids remained from the liquid fraction. In Fig. 7.1, the 4.1% is the gap between the dashed lines of the BS-rich liquid after pretreatment and the straight line of pure BS, which at ±0.4% represents the proportion of

xylan and lignin solubilized after pretreatment of *Miscanthus* with BSW at 110 °C and 30 h. These results show the potential of recovering BS at 130 °C or less, as higher temperatures would promote unnecessary degradation of xylan and lignin derivatives. Also, depending on the solvent recovery temperature, polymerization inhibitors, like 4-t-butylcatechol and ferrocene (Bando et al. 2011) may be required during recovery of BS to avoid loss of solvent to diene polymers.

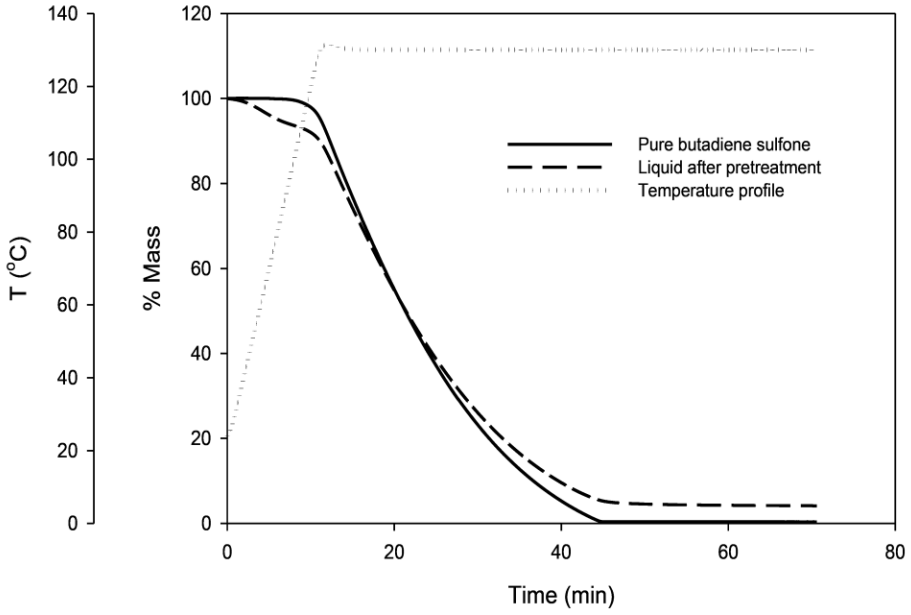


Figure 7.1 Thermogravimetric analysis (TGA) of pure butadiene sulfone (BS) and the BS-rich liquid after pretreatment of *Miscanthus.*

7.4 Conclusions

It was demonstrated via thermogravimetric analysis that the butadiene sulfone present in the liquid after pretreatment can be completely decomposed and potentially reformed, with the additional advantage of isolating the hydrolyzed xylan derivatives and lignin. As in the case of the glucan-rich pretreated solids, these isolated compounds are potential substrates for value-added products.

CHAPTER 8

CONCLUSIONS AND RECOMMENDATIONS

8.1 Conclusions

1. A new one-step pretreatment method for lignocellulosic biomass was developed using switchable butadiene sulfone, representing the first quantitative analysis on the effectiveness of this solvent for pretreatment; through improved reaction conditions, good separation of the pretreated solids from the liquid, and a complete chemical analysis of the pretreated biomass solids that assessed the changes in glucan, xylan and lignin content with temperature, time and water concentration. Also, the kinetics of xylan hydrolysis, the x-ray powder diffraction and SEM imaging of raw and pretreated solids was studied.
2. Pretreatment of *Miscanthus* with butadiene sulfone in the presence of water removed up to 91% of xylan, 58% of lignin and preserved 90-99% of the glucan, the key substrate for enzymatic hydrolysis and fermentation for biofuels and chemicals.
3. The chemical mechanisms for xylan and lignin removal from *Miscanthus* were investigated, concluding that the solubilization of lignin is a result of the solvent interactions with the delocalized lignin during or after the hydrolysis of xylan, which followed irreversible first-order kinetics.
4. A furfural-based biochemical conversion route was proposed for sugarcane bagasse, consisting in the production of furfural and the value-added utilization of the glucan in the residues for downstream processing. The delignification of the furfural residues after

butadiene sulfone pretreatment was determined, and the enzymatic hydrolysis of the solids was evaluated before and after pretreatment.

5. A thermogravimetric analysis demonstrated that butadiene sulfone can be completely decomposed and potentially reformed, since the solvent's decomposition gases are also the raw materials for its production. The solvent's recovery process will also isolate the xylan derivatives and lignin for further value-added processing.
6. Effective one-step pretreatment of lignocellulosic biomass with butadiene sulfone-water under mild conditions, is a step towards a more economically feasible pretreatment process by using a solvent with inexpensive commercial availability and an industrial-scale recovery process; conditions that warrant a potential transfer to a biorefinery.

8.2 Recommendations for future work

Biochemical route assessment from pretreatment of biomass with butadiene sulfone to biofuels

1. Study the effect of delignification, accessible surface area and crystallinity index on the enzymatic hydrolysis of glucan in biomass pretreated with butadiene sulfone.
2. Perform enzymatic hydrolysis and ethanol fermentation of the butadiene sulfone pretreated biomass solids to assess the effect of the pretreatment, in terms of ethanol yields and enzymatic or fermentation inhibition.
3. Assess the pretreatment efficacy of butadiene sulfone with biomass solids loadings greater than 5%, and particle sizes greater than 1 mm.

Reuse and decomposition/recovery of butadiene sulfone

4. Reuse the butadiene sulfone-rich liquid after pretreatment, which contains xylan derivatives and lignin, for other pretreatment experiments.
5. Design a lab scale decomposition and recovery setup for butadiene sulfone after pretreatment of lignocellulosic biomass to determine the recyclability of the solvent, as well as the amount of solvent lost to diene polymers.
6. Determine the chemical composition of the xylan derivatives and lignin residues after decomposition and recovery of butadiene sulfone.
7. Compare the pretreatment efficacy of reused butadiene sulfone (containing xylan derivatives and lignin), recovered butadiene sulfone (via decomposition and reformation) and pure butadiene sulfone.

Fermentation of the glucose released enzymatically from the glucan-rich furfural residues

8. Perform fermentation studies of the glucose released *via* enzymatic hydrolysis of the glucan in the residues after production of furfural from sugarcane bagasse.

REFERENCES

Anugwom, I., P. Mäki-Arvela, P. Virtanen, S. Willför, R. Sjöholm and J. -. Mikkola. 2012a. Selective extraction of hemicelluloses from spruce using switchable ionic liquids. *Carbohydrate Polymers* 87(3): 2005-2011.

Anugwom, I., P. Mäki-Arvela, P. Virtanen, S. Willför, P. Damlin, M. Hedenström and J. -. Mikkola. 2012b. Treating birch wood with a switchable 1,8-diazabicyclo-[5.4.0]-undec-7-ene-glycerol carbonate ionic liquid. *Holzforschung* 66(7): 809-815.

Audu, I. G., N. Brosse, L. Desharnais and S. K. Rakshit. 2012. Ethanol organosolv pretreatment of Typha Capensis for bioethanol production and co-products. *BioResources* 7(4): 5917-5933.

Awwad, A. M., A. H. Al-Dujaili and H. E. Salman. 2002. Relative permittivities, densities, and refractive indices of the binary mixtures of sulfolane with ethylene glycol, diethylene glycol, and poly(ethylene glycol) at 303.15 K. *Journal of Chemical and Engineering Data* 47(3): 421-424.

Bando, S., H. Kanda, Y. Onoda and M. Miyada. 2011. Method for manufacturing sulfolene compound and method for manufacturing sulfolane compound. U.S. Patent Application Publication No. 2011/0288307A1.

Banerjee, S., S. Mudliar, R. Sen, B. Giri, D. Satpute, T. Chakrabarti and R. Pandey. 2009. Commercializing lignocellulosic bioethanol: technology bottlenecks and possible remedies. *Biofuels, Bioproducts and Biorefining* 4(1): 77-93.

Bhandari, N., D. G. Macdonald and N. N. Bakhshi. 1984. Kinetic Studies Of Corn Stover Saccharification Using Sulphuric Acid. *Biotechnology and bioengineering* 26(4): 320-327.

Binder, J. B. and R. T. Raines. 2010. Fermentable sugars by chemical hydrolysis of biomass. *Proceedings of the National Academy of Sciences of the United States of America* 107(10): 4516-4521.

Binder, J. B., J. J. Blank, A. V. Cefali and R. T. Raines. 2010. Synthesis of furfural from xylose and xylan. *ChemSusChem* 3(11): 1268-1272.

Blanch, H. W., B. A. Simmons and D. Klein-Marcuschamer. 2011. Biomass deconstruction to sugars. *Biotechnology Journal* 6(9): 1086-1102.

Bozell, J. J., L. Moens, D. C. Elliott, Y. Wang, G. G. Neuenscwander, S. W. Fitzpatrick, R. J. Bilski and J. L. Jarnefeld. 2000. Production of levulinic acid and use as a platform chemical for derived products. *Resources, Conservation and Recycling* 28(3-4): 227-239.

Brodeur, G., E. Yau, K. Badal, J. Collier, K. B. Ramachandran and S. Ramakrishnan. 2011. Chemical and physicochemical pretreatment of lignocellulosic biomass: A review. *Enzyme Research* 2011(1): 1-17.

Brosse, N., P. Sannigrahi and A. Ragauskas. 2009. Pretreatment of miscanthus x giganteus using the ethanol organosolv process for ethanol production. *Industrial and Engineering Chemistry Research* 48(18): 8328-8334.

Brosse, N., A. Dufour, X. Meng, Q. Sun and A. Ragauskas. 2012. Miscanthus: A fast-growing crop for biofuels and chemicals production. *Biofuels, Bioproducts and Biorefining* 6(5): 580-598.

Brown, T. R. and R. C. Brown. 2013. A review of cellulosic biofuel commercial-scale projects in the United States. *Biofuels, Bioproducts and Biorefining* 7(3): 235-245.

Cahela, D. R., Y. Y. Lee and R. P. Chambers. 1983. Modeling Of Percolation Process In Hemicellulose Hydrolysis. *Biotechnology and bioengineering* 25(1): 3-17.

Carrasco, F. and C. Roy. 1992. Kinetic study of dilute-acid prehydrolysis of xylan-containing biomass. *Wood Science and Technology* 26(3): 189-208.

Chang, V. S. and M. T. Holtzapple. 2000. Fundamental factors affecting biomass enzymatic reactivity. *Applied Biochemistry and Biotechnology - Part A Enzyme Engineering and Biotechnology* 84-865-37.

Chundawat, S. P. S., G. T. Beckham, M. E. Himmel and B. E. Dale. 2011. Deconstruction of lignocellulosic biomass to fuels and chemicals. *Annual Review of Chemical and Biomolecular Engineering* 2121-145.

Clark, J. H. 1999. Green chemistry: challenges and opportunities. *Green Chemistry.* 1(1): 1-8.

Couper, J. R., W. R. Penney, J. R. Fair and S. M. Walas. 2005. *Chemical process equipment: selection and design.* 2nd ed. Burlington, MA, Gulf Professional Publishing-Elsevier.

de Frias, J. A. and H. Feng. 2013. Switchable butadiene sulfone pretreatment of Miscanthus in the presence of water. *Green Chemistry* 15(4): 1067-1078.

Dee, S. and A. T. Bell. 2011. Effects of reaction conditions on the acid-catalyzed hydrolysis of miscanthus dissolved in an ionic liquid. *Green Chemistry* 13(6): 1467-1475.

Doherty, T. V., M. Mora-Pale, S. E. Foley, R. J. Linhardt and J. S. Dordick. 2010. Ionic liquid solvent properties as predictors of lignocellulose pretreatment efficacy. *Green Chemistry* 12(11): 1967-1975.

Domanska, U., W. C. Moollan and T. M. Letcher. 1996. Solubility of sulfolane in selected organic solvents. *Journal of Chemical and Engineering Data* 41(2): 261-265.

Donaldson, M. E., V. L. Mestre, D. Vinci, C. L. Liotta and C. A. Eckert. 2009. Switchable solvents for in-situ acid-catalyzed hydrolysis of β-pinene. *Industrial and Engineering Chemistry Research* 48(5): 2542-2547.

Donohoe, B. S., T. B. Vinzant, R. T. Elander, V. R. Pallapolu, Y. Y. Lee, R. J. Garlock, V. Balan, B. E. Dale, Y. Kim, N. S. Mosier, M. R. Ladisch, M. Falls, M. T. Holtzapple, R. Sierra-Ramirez, J. Shi, M. A. Ebrik, T. Redmond, B. Yang, C. E. Wyman, B. Hames, S. Thomas and R. E. Warner. 2011. Surface and ultrastructural characterization of raw and pretreated switchgrass. *Bioresource technology* 102(24): 11097-11104.

Drake, L., S. Stowe and A. Partansky. 1946. Kinetics of the Diene Sulfur Dioxide Reaction1. *Journal of the American Chemical Society* 68(12): 2521-2524.

EERE. 2008. Schematic of a Biochemical Cellulosic Ethanol Production Process. Washington DC: U.S. Department of Energy - Energy Efficiency & Renewable Energy - Alternative Fuels Data Center. Available at: http://www1.eere.energy.gov/bioenergy/(2013). Accessed in 2008 under U.S. Department of Energy Biomass Program.

EIA. 2011. Top World Oil Consumers, 2011. Washington DC: U.S. Energy Information Administration. Available at: www.eia.gov. Accessed 15 June 2013.

EIA. 2013. Annual Energy Outlook 2013 with Projections to 2040. DOE/EIA-0383. Washington, DC: U.S. Energy Information Administration.

Elander, R. T., B. E. Dale, M. Holtzapple, M. R. Ladisch, Y. Y. Lee, C. Mitchinson, J. N. Saddler and C. E. Wyman. 2009. Summary of findings from the Biomass Refining Consortium for Applied Fundamentals and Innovation (CAFI): Corn stover pretreatment. *Cellulose* 16(4): 649-659.

Enslow, K. R. and A. T. Bell. 2012. The kinetics of Bronsted acid-catalyzed hydrolysis of hemicellulose dissolved in 1-ethyl-3-methylimidazolium chloride. *RSC Advances* 2(26): 10028-10036.

Fadhel, A. Z., P. Pollet, C. L. Liotta and C. A. Eckert. 2011. Novel solvents for sustainable production of specialty chemicals. *Annual Review of Chemical and Biomolecular Engineering:* 2189-210.

Fogler, H. S. 1999. *Elements of chemical reaction engineering.* 3rd ed. Upper Saddle River, NJ: Prentice-Hall PTR.

Girisuta, B. 2007. *Levulinic acid from lignocellulosic biomass.* PhD diss, Groningen, The Netherlands: University of Groningen, Department of Mathematics and Natural Sciences.

Gray, M. C., A. O. Converse and C. E. Wyman. 2007. Solubilities of oligomer mixtures produced by the hydrolysis of xylans and corn stover in water at 180°C. *Industrial and Engineering Chemistry Research* 46(8): 2383-2391.

Hayes, D. J., J. Ross, M. H. B. Hayes, S. Fitzpatrick. 2006. The biofine process: production of levulinic acid, furfural, and formic acid from lignocellulosic feedstocks. *Biorefineries: Industrial processes and products* 1: 139-164.

Heaton, E., T. Voigt and S. P. Long. 2004. A quantitative review comparing the yields of two candidate C4 perennial biomass crops in relation to nitrogen, temperature and water. *Biomass and Bioenergy* 27(1): 21-30.

Hu, F., S. Jung and A. Ragauskas. 2012. Pseudo-lignin formation and its impact on enzymatic hydrolysis. *Bioresource technology* 1177-12.

Huber, G. W. and B. E. Dale. 2009. Grassoline at the pump. *Scientific American* 301(1): 52-59.

Huber, G. W., S. Iborra and A. Corma. 2006. Synthesis of transportation fuels from biomass: Chemistry, catalysts, and engineering. *Chemical reviews* 106(9): 4044-4098.

Jacobsen, S. E. and C. E. Wyman. 2000. Cellulose and hemicellulose hydrolysis models for application to current and novel pretreatment processes. *Applied Biochemistry and Biotechnology - Part A Enzyme Engineering and Biotechnology* 84-8681-96.

Jensen, J., J. Morinelly, A. Aglan, A. Mix and D. R. Shonnard. 2008. Kinetic characterization of biomass dilute sulfuric acid hydrolysis: Mixtures of hardwoods, softwood, and switchgrass. *AIChE Journal* 54(6): 1637-1645.

Jeoh, T., C. I. Ishizawa, M. F. Davis, M. E. Himmel, W. S. Adney and D. K. Johnson. 2007. Cellulase digestibility of pretreated biomass is limited by cellulose accessibility. *Biotechnology and bioengineering* 98(1): 112-122.

Jin, Q., H. Zhang, L. Yan, L. Qu and H. Huang. 2011. Kinetic characterization for hemicellulose hydrolysis of corn stover in a dilute acid cycle spray flow-through reactor at moderate conditions. *Biomass and Bioenergy* 35(10): 4158-4164.

Kamlet, M. J. and R. W. Taft. 1976. The solvatochromic comparison method. I. The β-scale of solvent hydrogen-bond acceptor (HBA) basicities. *Journal of the American Chemical Society* 98(2): 377-383.

Kassner, M. K. 2008. Novel sustainable solvents for bioprocessing applications. PhD diss. Atlanta, GA: Georgia Institute of Technology, Department of Chemical and Biomolecular Engineering.

Khullar, E. 2012. Miscanthus conversion to ethanol: effect of particle size and pretreatment conditions for hot water. PhD diss. Urbana, IL: University of Illinois at Urbana-Champaign, Department of Agricultural and Biological Engineering.

Khullar, E., B. S. Dien, K. D. Rausch, M. E. Tumbleson and V. Singh. 2013. Effect of particle size on enzymatic hydrolysis of pretreated Miscanthus. *Industrial Crops and Products* 4411-17.

Kumar, P., D. M. Barrett, M. J. Delwiche and P. Stroeve. 2009a. Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. *Industrial and Engineering Chemistry Research* 48(8): 3713-3729.

Kumar, R., G. Mago, V. Balan and C. E. Wyman. 2009b. Physical and chemical characterizations of corn stover and poplar solids resulting from leading pretreatment technologies. *Bioresource technology* 100(17): 3948-3962.

Kumar, R., F. Hu, P. Sannigrahi, S. Jung, A. J. Ragauskas and C. E. Wyman. 2013. Carbohydrate derived-pseudo-lignin can retard cellulose biological conversion. *Biotechnology and bioengineering* 110(3): 737-753.

Lagowski, J. 1976. *The Chemistry of Nonaqueous Solvents V4: Solution Phenomena and Aprotic Solvents.* 1st ed. London, UK: Academic Press, Inc.

Le Calorie, S.p.a. 2011. Andover, MA: Bio Development International, Inc. Available at http://www.biodevelopment.us/crnt_lecal.html. Accessed 11 October 2011.

Li, C., Q. Wang and Z. K. Zhao. 2007. Acid in ionic liquid: An efficient system for hydrolysis of lignocellulose. *Green Chem.* 10(2): 177-182.

Li, C., B. Knierim, C. Manisseri, R. Arora, H. V. Scheller, M. Auer, K. P. Vogel, B. A. Simmons and S. Singh. 2010. Comparison of dilute acid and ionic liquid pretreatment of switchgrass: Biomass recalcitrance, delignification and enzymatic saccharification. *Bioresource technology* 101(13): 4900-4906.

Lozowski, D. 2011. Newsfront: The bio-based economy. *Chemical Engineering* 118(8): 14-16.

Lu, F. and J. Ralph. 2010. Chapter 6: Lignin. In *Cereal straw as a resource for sustainable biomaterials and biofuels: chemistry, extractives, lignins, hemicelluloses and cellulose*, 169-207. R. Sun, ed. Amsterdam, The Netherlands: Elsevier.

Lu, X. B., Y. M. Zhang, J. Yang and Y. Liang. 2007. Enzymatic hydrolysis of corn stover after pretreatment with dilute sulfuric acid. *Chemical Engineering and Technology* 30(7): 938-944.

Mäki-Arvela, P., T. Salmi, B. Holmbom, S. Willför and D. Y. Murzin. 2011. Synthesis of sugars by hydrolysis of hemicelluloses- A review. *Chemical reviews* 111(9): 5638-5666.

Mäki-Arvela, P., I. Anugwom, P. Virtanen, R. Sjöholm and J. P. Mikkola. 2010. Dissolution of lignocellulosic materials and its constituents using ionic liquids-A review. *Industrial Crops and Products* 32(3): 175-201.

Mamman, A. S., J. -. Lee, Y. -. Kim, I. T. Hwang, N. -. Park, Y. K. Hwang, J. -. Chang and J. -. Hwang. 2008. Furfural: Hemicellulose/xylose-derived biochemical. *Biofuels, Bioproducts and Biorefining* 2(5): 438-454.

Marcus, Y. 1993. The properties of organic liquids that are relevant to their use as solvating solvents. *Chemical Society Reviews* 22(6): 409-416.

McMillan, J. D. 1994. Pretreatment of lignocellulosic biomass. In *ACS Symposium Series,* 292-324. Washington, DC: ACS Publications

Mellinger-Silva, C., F. F. Simas-Tosin, D. N. Schiavini, M. F. Werner, C. H. Baggio, I. T. Pereira, L. M. da Silva, P. A. J. Gorin and M. Iacomini. 2011. Isolation of a gastroprotective arabinoxylan from sugarcane bagasse. *Bioresource technology* 102(22): 10524-10528.

Mora-Pale, M., L. Meli, T. V. Doherty, R. J. Linhardt and J. S. Dordick. 2011. Room temperature ionic liquids as emerging solvents for the pretreatment of lignocellulosic biomass. *Biotechnology and bioengineering* 108(6): 1229-1245.

Morvan, D., T. B. Rauchfuss and S. R. Wilson. 2009. π-Complexes of Lignols with Manganese (I) and Ruthenium (II). *Organometallics* 28(11): 3161-3166.

Mosier, N., C. Wyman, B. Dale, R. Elander, Y. Lee, M. Holtzapple and M. Ladisch. 2005. Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource technology* 96(6): 673-686.

Mousdale, D. M. 2008. *Biofuels. Biotechnology, Chemistry and Sustainable Development.* 1st ed. Boca Raton, FL: CRC Press-Taylor & Francis Group.

National Research Council (US). 2011. Committee on Economic and Environmental Impacts of Increasing Biofuels Production. *Renewable Fuel Standard: Potential Economic and Environmental Effects of US Biofuel Policy.* Washington, DC: National Academies Press.

Nlewem, K. C. and M. E. Thrash Jr. 2010. Comparison of different pretreatment methods based on residual lignin effect on the enzymatic hydrolysis of switchgrass. *Bioresource technology* 101(14): 5426-5430.

Novozymes A/S. 2010. Cellic CTec2 and HTec2 - Enzymes for hydrolysis of lignocellulosic materials. Bagsvaerd, Denmark: Fuel Ethanol Application Sheet.

NREL. 2009. What is a biorefinery? Golden, CO: National Renewable Energy Laboratory. Available at: www.nrel.gov. Accessed 15 June 2013.

Padmanabhan, S., M. Kim, H. W. Blanch and J. M. Prausnitz. 2011. Solubility and rate of dissolution for Miscanthus in hydrophilic ionic liquids. *Fluid Phase Equilibria* 309(1): 89-96.

Pollet, P., C. A. Eckert and C. L. Liotta. 2011. Switchable solvents. *Chemical Science* 2(4): 609-614.

Rose, J. K. C. 2003. *The plant cell wall, Annual Plant Reviews, Volume 8.* 1st ed. Boca Raton, FL: CRC Press LLC.

Saddler, J., H. Brownell, L. Clermont and N. Levitin. 1982. Enzymatic hydrolysis of cellulose and various pretreated wood fractions. *Biotechnology and bioengineering* 24(6): 1389-1402.

Saha, B. C., L. B. Iten, M. A. Cotta and Y. V. Wu. 2005. Dilute acid pretreatment, enzymatic saccharification, and fermentation of rice hulls to ethanol. *Biotechnology progress* 21(3): 816-822.

Sannigrahi, P., D. H. Kim, S. Jung and A. Ragauskas. 2011. Pseudo-lignin and pretreatment chemistry. *Energy & Environmental Science* 4(4): 1306-1310.

Sathitsuksanoh, N., Z. Zhu, S. Wi and Y. -. Percival Zhang. 2011. Cellulose solvent-based biomass pretreatment breaks highly ordered hydrogen bonds in cellulose fibers of switchgrass. *Biotechnology and bioengineering* 108(3): 521-529.

Schnepf, R. 2011. Renewable fuel standard (RFS): overview and issues. Washington DC: Congressional Research Service Report for Congress.

Segal, L., J. J. Creely, A. E. Martin and C. M. Conrad. 1959. An Empirical Method for Estimating the Degree of Crystallinity of Native Cellulose Using the X-Ray Diffractometer. *Textile Research Journal* 29(10): 786-794.

Selig, M., Weiss, N. and Ji, Y. 2008. Enzymatic Saccharification of Lignocellulosic Biomass. NREL/TP-510-42629. Golden, CO: National Renewable Energy Laboratory (NREL).

Shen, J. and C. E. Wyman. 2011. A novel mechanism and kinetic model to explain enhanced xylose yields from dilute sulfuric acid compared to hydrothermal pretreatment of corn stover. *Bioresource technology* 102(19): 9111-9120.

Sierra, R., A. Smith, C. Granda and M. T. Holtzapple. 2008. Producing fuels and chemicals from lignocellulosic biomass. *Chemical Engineering Progress* 104(8): S10-S18.

Sluiter, A., R. Ruiz, C. Scarlata, J. Sluiter and D. Templeton. 2005. Determination of extractives in biomass. NREL/TP-510-426199. Golden, CO: National Renewable Energy Laboratory (NREL).

Sluiter, A., B. Hames, R. Ruiz, C. Scarlata, J. Sluiter and D. Templeton and D. Crocker. 2008. Determination of structural carbohydrates and lignin in biomass. NREL/TP-510-42618. Golden, CO: National Renewable Energy Laboratory (NREL).

Sluiter, A., B. Hames, R. Ruiz, C. Scarlata, J. Sluiter and D. Templeton. 2008. Determination of sugars, byproducts, and degradation products in liquid fraction process samples. NREL/TP-510-4262311. Golden, CO: National Renewable Energy Laboratory (NREL).

Somerville, C., H. Youngs, C. Taylor, S. C. Davis and S. P. Long. 2010. Feedstocks for lignocellulosic biofuels. *Science* 329(5993): 790-792.

Spange, S., A. Reuter, E. Vilsmeier, T. Heinze, D. Keutel and W. Linert. 1998. Determination of empirical polarity parameters of the cellulose solvent N,N-dimethylacetamide/LiCl by means of the solvatochromic technique. *Journal of Polymer Science Part A: Polymer Chemistry* 36(11): 1945-1955.

Sun, R., X. Song, R. Sun and J. Jiang. 2011. Effect of lignin content on enzymatic hydrolysis of furfural residues. *BioResources* 6(1): 317-328.

Sun, Y. and J. Cheng. 2002. Hydrolysis of lignocellulosic materials for ethanol production: A review. *Bioresource technology* 83(1): 1-11.

Tadesse, H. and R. Luque. 2011. Advances on biomass pretreatment using ionic liquids: An overview. *Energy and Environmental Science* 4(10): 3913-3929.

Tang, Y., L. Bu, J. He and J. Jiang. 2013. l(+)-Lactic acid production from furfural residues and corn kernels with treated yeast as nutrients. *European Food Research and Technology* 236(2): 365-371.

Tang, Y., D. Zhao, C. Cristhian and J. Jiang. 2011. Simultaneous saccharification and cofermentation of lignocellulosic residues from commercial furfural production and corn kernels using different nutrient media. *Biotechnology for Biofuels* 4.

Thomas, C. A. 2006. Reactions and separations in tunable solvents. PhD diss. Atlanta, GA: Georgia Institute of Technology, Department of Chemical and Biomolecular Engineering.

U.S. DOE Genomic Science. 2013. Image Gallery Gateway. Washington, DC: U.S. Department of Energy Genomic Science Program. Available at: http://genomicscience.energy.gov. Accessed 15 June 2013.

Vallejos, M. E., M. D. Zambon, M. C. Area and A. A. Da Silva Curvelo. 2012. Low liquid-solid ratio (LSR) hot water pretreatment of sugarcane bagasse. *Green Chemistry* 14(7): 1982-1989.

Vinci, D., M. Donaldson, J. Hallett, E. John, P. Pollet, C. Thomas, J. Grilly, P. Jessop, C. Liotta and C. Eckert. 2007. Piperylene sulfone: a labile and recyclable DMSO substitute. *Chemical Communications*(14): 1427-1429.

Voegele, A. F., C. S. Tautermann, T. Loerting, A. Hallbrucker, E. Mayer and K. R. Liedl. 2002. About the stability of sulfurous acid (H_2SO_3) and its dimer. *Chemistry - A European Journal* 8(24): 5644-5651.

Wang, B., X. Wang and H. Feng. 2010. Deconstructing recalcitrant Miscanthus with alkaline peroxide and electrolyzed water. *Bioresource technology* 101(2): 752-760.

Werpy, T., G. Petersen, A. Aden, J. Bozell, J. Holladay, J. White and A. Manheim. 2004. Top value-added chemicals from biomass, volume 1: Results of Screening for Potential Candidates from Sugars and Synthesis Gas. DOE/GO-102004-1992. Washington DC: US Department of Energy, Pacific National Laboratory, National Renewable Energy Laboratory.

Win, D. T. 2005. Furfural - Gold from Garbage. *AU J.T.* 8(4): .

Wyman, C. E., S. R. Decker, M. E. Himmel, J. W. Brady, C. E. Skopec and L. Viikari. 2005. Hydrolysis of cellulose and hemicellulose. *Polysaccharides: Structural Diversity and Functional Versatility* 995-1033.

Xie, H., J. Prausnitz and X. Wu. 2012. Determination on crystallinity of ionic liquids pretreated biomass. *Advanced Materials Research* 393-395668-671.

Yang, B. and C. E. Wyman. 2008. Pretreatment: the key to unlocking low-cost cellulosic ethanol. *Biofuels, bioproducts biorefining* 2(1): 26.

Yang, B. and C. E. Wyman. 2004. Effect of Xylan and Lignin Removal by Batch and Flowthrough Pretreatment on the Enzymatic Digestibility of Corn Stover Cellulose. *Biotechnology and bioengineering* 86(1): 88-95.

Yasuda, S, Fukushima, K and Kakehi, A. 2001. Formation and chemical structures of acid-soluble lignin I: sulfuric acid treatment time and acid-soluble lignin content of hardwood. *Journal of Wood Science,* 47, 69-72.

Yat, S. C., A. Berger and D. R. Shonnard. 2008. Kinetic characterization for dilute sulfuric acid hydrolysis of timber varieties and switchgrass. *Bioresource technology* 99(9): 3855-3863.

Zeitsch, K. J. 2000. *The chemistry and technology of furfural and its many by-products.* 1st ed. Amsterdam, The Netherlands: Elsevier.

Zhang, Y. -. P. and L. R. Lynd. 2004. Toward an aggregated understanding of enzymatic hydrolysis of cellulose: Noncomplexed cellulase systems. *Biotechnology and bioengineering* 88(7): 797-824.

APPENDIX A

FURANS AND ACIDS AS PLATFORM CHEMICALS FROM LIGNOCELLULOSIC BIOMASS: A REVIEW

A.1 Introduction

In recent years, the rapid depletion of non-renewable energy sources and the increasing cost of oil have sparked global efforts to find renewable sources of energy and chemicals from non-food feedstocks. This has led to an increased interest in deconstructing the sugar biopolymers present in lignocellulosic biomass: hemicellulose and cellulose. After conversion of these biopolymers into sugar monomers, primarily xylose and glucose, respectively; these sugars can be converted into platform chemicals, like furans and carboxylic acids, from which a wide range of fuels and chemicals can be produced (Winterton 2011).

Furans and carboxylic acids are the main degradation products from sugars. Furans are a group of oxygenated heterocycles derived from C5 and C6 sugars (pentoses and hexoses). Carboxylic acids are aliphatic or aromatic organic compounds with a carboxyl (COOH) functional group. Key furans from biomass are furfural and hydroxymethylfurfural (HMF) and key acids are acetic acid, levulinic acid and formic acid.

Furfural is the only furan mass-produced commercially from lignocellulosic biomass, primarily sugarcane bagasse and corn cobs. It is manufactured from the pentose-based hemicellulose fraction, with the cellulose and lignin fractions being mostly used as boiler fuel (Mamman et al. 2008). As of 2008, the world production of furfural was 280,000 tons per year, all from biomass; with China supplying 74% of the total production capacity. The rest is mostly produced by Dominican Republic and South Africa (Win 2005). Furfural is in the top 30 list of

value-added chemicals and fuels from biomass developed by the US Department of Energy (DOE) (Werpy et al. 2004).

Other furans from lignocellulosics have no current commercial applications, but have the potential to become important platform chemicals. Hydroxymethylfurfural (HMF) is a dehydration product from hexoses, mainly glucose, which is obtained after hydrolysis of the cellulose fraction of biomass. HMF can be transformed into 2,5-dimethylfuran (DMF), a potential gasoline substitute (Román-Leshkov et al. 2007).

HMF rehydrates easily into levulinic acid, which can be esterified to levulinic ester, a potential diesel additive (Mascal and Nikitin 2010b). Levulinic acid has been identified as one of the top 12 potential value-added chemicals from biomass by the US DOE (Werpy et al. 2004). Formic acid is also a rehydration product from HMF. Another acid from biomass, but from the hemicellulose fraction, is acetic acid; formed when acetate groups are released into solution after the catalyzed removal of acetate groups linked to hemicellulose. Acetic acid is in the top 30 list of value-added chemicals from biomass by the US DOE (Werpy et al. 2004).

The hemicellulose fraction of biomass is the easiest fraction to remove from the plant cell wall due to its amorphous structure and solubility in water at temperatures greater than 80°C (Gray et al. 2007). When biomass is treated with dilute acid at high temperatures, the reaction is very selective to conversion to furfural, after hydrolysis of hemicellulose to xylose. This is why it has been industrially favorable to convert hemicellulose from biomass, specifically sugarcane bagasse, corncobs and spent oat (Win 2005).

On the other hand, cellulose is hard to deconstruct due to its crystallinity. The main intermediate product from cellulose is hydroxymethylfurfural (HMF), which is unstable and converts easily under aqueous conditions into levulinic acid, formic acid or into condensation

products (e.g., humins) (Chheda et al. 2007b). The selectivity to certain furans from cellulose is very dependent on the catalyst used and requires a good control of the dehydration reaction (Werpy et al. 2004). These factors, in addition to the low-density nature of lignocellulosic biomass, have impeded a full commercialization of cellulose-derived furans. However, the recent non-renewable energy challenges have prompted an interest in breaking down cellulose to produce furans and carboxylic acids.

Recent studies towards furans and acids have mostly focused on solubilizing and dehydrating glucose or using pure cellulose as starting material. For these reactions, homogeneous and heterogeneous catalysts under inorganic and organic solvents have been used, with temperatures ranging from 150°C-250°C in aqueous and non-aqueous conditions, in single or biphasic reactors (Su et al. 2009), (Román-Leshkov and Dumesic 2009), (Chheda et al. 2007b), (Huber et al. 2005), (Román-Leshkov et al. 2007), (Yin et al. 2011).

Research work on furans and acids from model mixtures of monosugars or pure cellulose provides a fundamental framework for studying catalysis and thermodynamic models (Verevkin et al. 2009) but unfortunately, the work from model sugars does not translate easily to the complex mix of hydrolyzed sugars and derivatives from lignocellulosic biomass after pretreatment. Less common is research work focused on producing furans and acids from lignocellulosic biomass (Yang et al. 2011), (Binder and Raines 2009), (Mascal and Nikitin 2010a). Biomass hydrolyzates consist of complex mixtures of oligosaccharides, monosugars, furans, acids, phenolics and aminoacids, nothing like the model sugar mixtures used in most research work on furans.

One of the most common and inexpensive catalysts used is dilute sulfuric acid (0.5% - 4% w/w). It is used industrially to produce furfural from sugarcane bagasse or corncobs (Mamman et al. 2008). Alternatively, the use of heterogeneous catalysts (e.g., metal halides) or solvents (e.g., ionic liquids or polar aprotic solvents with lithium salts), have been studied in the conversion of biomass into furans and acids at the lab or pilot scale (Binder and Raines 2009), (Yi et al. 2011), (Yang et al. 2011), (Kim and Pan 2010).

Ionic liquids (IL) lead the research for novel solvent applications for dissolution of biomass, due to the "limitless" potential to combine cations and anions to tailor solvent properties (Doherty et al. 2010). An ionic liquid consists of an amine heterocycle cation with an organic or inorganic anion, a key combination that allows an effective dissolution of biomass. Similarly, polar aprotic solvents, when combined with lithium salts, have comparable solvent properties to ionic liquids; with the organic aprotic chain or heterocycle polarized by lithium and forming an ion pair with the salt anion (Binder and Raines 2009). When compared to ILs, the combinations of aprotics and lithium salts have been overlooked as potential solvents for biomass for conversion to furans and acids. A key advantage of the aprotic / lithium salt systems is that they are relatively inexpensive compared to ionic liquids. The ionic liquids that work for dissolution of biomass are currently prohibitively expensive (Simmons et al. 2010).

A.2 Platform chemicals

Furfural

Furfural (Fig. A.1) is derived from the dehydration reaction of pentoses, e.g., xylose. The hemicellulose fraction, which primarily consists of pentose units, is first hydrolyzed to xylose catalyzed by dilute sulfuric acid (<4%), and then dehydrated to furfural under the same acidic conditions (Fig. 2.6). Furfural has many industrial applications, in the production of plastics, pharmaceuticals and agrochemicals (Mamman et al. 2008). It is also used as platform chemical for other products, like furfuryl alcohol, acetylfuran and tetrahydrofuran. The industrial process to produce furfural is described in Chapter 6.

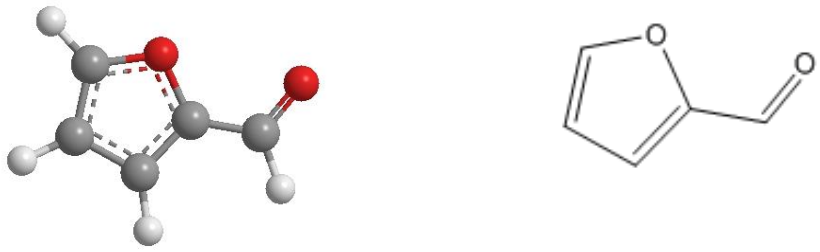


Figure A.1 Furfural is an oxygenated heterocycle with an aldehyde group.

Acetic acid

Acetic acid is a versatile product used in many industrial applications, like the production of vinyl acetate, esters for inks and coatings, and cellulose acetate for photographic films. It is industrially produced from methanol carbonylation, ethylene or alkane oxidation (Fig. A.2).

Figure A.2 Acetic acid is a carboxylic acid.

Acetic acid from biomass is easily produced when acetate groups are released into solution after the acid catalyzed removal of acetate from hemicellulose during the production of furfural (Zeitsch 2000). Acetate comprises around 3-4% of softwood biomass linked via covalent bonds to the hemicellulose chain. Acetic acid is in the top 30 list of fuels and chemicals from biomass developed by US DOE (Werpy et al. 2004).

Hydroxymethylfurfural (HMF)

There is strong research interest in hydroxymethylfurfural (HMF) as a potential platform chemical from biomass and simple hexose sugars. As opposed to furfural, there is no commercialization or industrial production efforts for HMF due to its high manufacturing costs and instability in solution, as it tends to rehydrate easily into levulinic acid, formic acid or into condensation products (Chheda et al. 2007a). Currently, HMF is mostly produced for analytical purposes using fructose as starting material. (Fig. A.3).

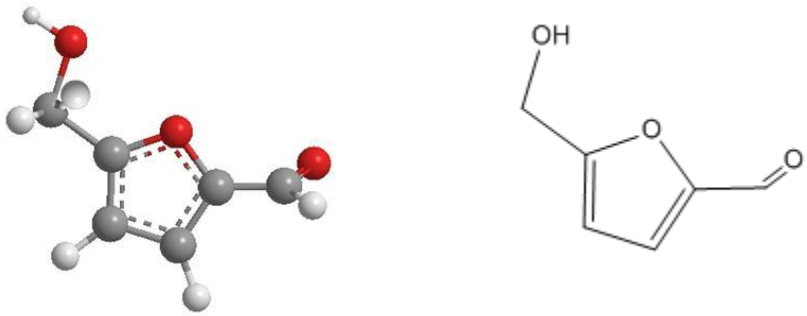


Figure A.3 Hydroxymethylfurfural (HMF) is an oxygenated heterocycle with an aldehyde group and a hydroxymethyl group.

HMF is derived from the dehydration reaction of hexoses, e.g., glucose or fructose. If cellulosic biomass were the starting material, the cellulose fraction, which consists of glucose monomers, would be first hydrolyzed to glucose, isomerized to fructose and finally dehydrated to HMF via acid catalysis (Binder and Raines 2009) (Fig. A.4).

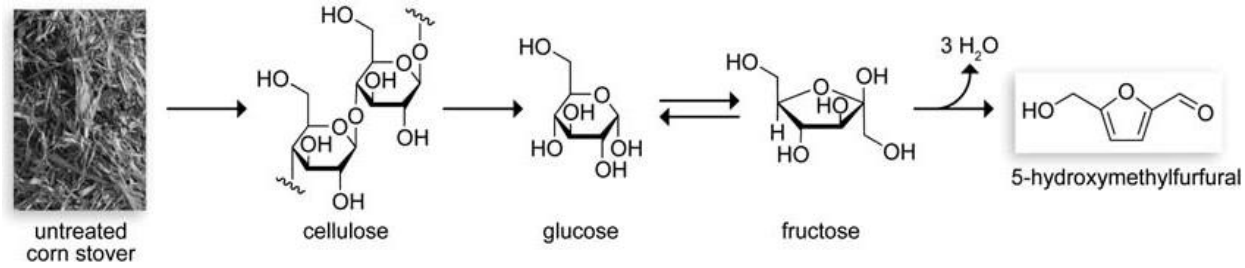


Figure A.4 Route for the synthesis of HMF from lignocellulosic biomass. Reprinted from Binder and Raines 2009.

Binder and Raines produced HMF from lignocellulosic biomass, using untreated and pretreated corn stover, and untreated pine sawdust. They solubilized the biomass in dimethylacetamide (DMA) and lithium chloride (LiCl) using chromium chlorides ($CrCl_2$ and $CrCl_3$) as Lewis acid catalysts, and the ionic liquid 1-ethyl-3-methylimidazolium chloride

[EMIM]Cl as additive (Binder and Raines 2009). Table A.1 shows the reaction conditions, HMF yields and furfural yields.

Table A.1 Synthesis of HMF from lignocellulosic Biomass. Adapted from Binder and Raines 2009.

biomass	solvent	catalyst, mol %	additives, wt %	T (°C)	time (h)	HMF yield (%)[a]	furfural yield (%)[a]
corn stover	DMA-LiCl (10%)	$CrCl_2$, 38	[EMIM]Cl, 10	140	6	16	ND
AFEX corn stover	DMA-LiCl (10%)	$CrCl_2$, 38	[EMIM]Cl, 10	140	6	16	ND
pine sawdust	DMA-LiCl (10%)	$CrCl_2$, 33	[EMIM]Cl, 15	140	5	19	ND
corn stover	DMA-LiCl (10%)	$CrCl_2$, 10; HCl, 10	[EMIM]Cl, 20	140	3	23	ND
corn stover	DMA-LiCl (10%)	$CrCl_2$, 10; HCl, 10	[EMIM]Cl, 40	140	3	24	ND
corn stover	DMA-LiCl (10%)	$CrCl_2$, 10; HCl, 10	[EMIM]Cl, 60	140	3	36	ND
corn stover	DMA-LiCl (10%)	$CrCl_2$, 10; HCl, 10	[EMIM]Cl, 80	140	3	31	ND
corn stover	[EMIM]Cl	$CrCl_2$, 10; HCl, 10		140	3	29	ND
corn stover	DMA-LiCl (10%)	$CrCl_3$, 10; HCl, 10	[EMIM]Cl, 20	140	3	26	ND
corn stover	DMA-LiCl (10%)	$CrCl_3$, 10; HCl, 10	[EMIM]Cl, 40	140	1	39	ND
corn stover	DMA-LiCl (10%)	$CrCl_3$, 10; HCl, 10	[EMIM]Cl, 60	140	2	48	34
corn stover	DMA-LiCl (10%)	$CrCl_3$, 10; HCl, 10	[EMIM]Cl, 80	140	2	47	37
corn stover	[EMIM]Cl	$CrCl_3$, 10; HCl, 10		140	1	42	ND

[a] *HMF and furfural molar yields are based on the cellulose content of the biomass*

The highest yield of HMF was achieved from untreated corn stover at 48%, which is the HMF molar yield based on the cellulose content of corn stover. The reaction conditions were 140ºC and 2 hours, using 10% mol $CrCl_3$ and 10% mol HCl as catalysts and 60% wt of the ionic liquid [EMIM]Cl as additive. Furfural was produced as co-product.

Interestingly, the HMF yields from untreated corn stover were identical to the yields from pretreated corn stover under the same reaction conditions. The authors argued that other biomass components (e.g., lignin and protein) "did not interfere substantially in the process, as yields of HMF based on the cellulose content of the biomass were comparable to those from purified cellulose".

Mascal and Nikitin reported a 69.1% yield of HMF from corn stover via hydration of choromethylfurfural (CMF) (Mascal and Nikitin 2010a). Corn stover was mechanically pretreated to a powder before reaction with concentrated hydrochloric acid (HCl) and 1,2-dichloroethane at 80-100ºC for 3 hours to produce CMF, which was further hydrated into HMF. One aspect to consider in this process is the potential safety hazards posed by the high chemical load of chlorinated organic compounds and concentrated HCl.

Some important by-products can be derived from HMF. One of these products is dimethylfuran (DMF), with an energy content of 31.5MJ/L, similar to that of gasoline (35 MJ/L) and 40% greater than that of ethanol (23 MJ/L) (Román-Leshkov et al. 2007; Binder and Raines 2009). DMF (bp 92-94ºC) is also less volatile than ethanol (bp 78ºC) and immiscible in water. All these properties make DMF attractive for the transportation sector (Binder and Raines 2009). Unfortunately, the production of DMF from lignocellulosic biomass has proven to be energy and chemically intensive. Binder and Raines reported a low yield of 9% for DMF, based on the cellulose content of corn stover. The production of DMF required the conversion of the cellulose in corn stover to HMF, followed by removal of chloride ions from the reaction system via ion-exchange chromatography, in order to avoid contamination of the copper-ruthenium catalyst used in the hydrogenolysis (reaction with hydrogen at 98 psi) of HMF to DMF at 220ºC for 10 h (Binder and Raines 2009).

Levulinic acid

Another derivative of HMF is levulinic acid, a relatively small market specialty chemical (1 million lb/year) which can be used as platform chemical and has important applications in the production of synthetic rubbers, plastics and pharmaceuticals (Bozell et al. 2000). Levulinic acid is a carboxylic acid with a ketone group that has been identified as one of the top 12 potential value-added chemicals from biomass by the US Department of Energy (Fig. A.5) (Werpy et al. 2004).

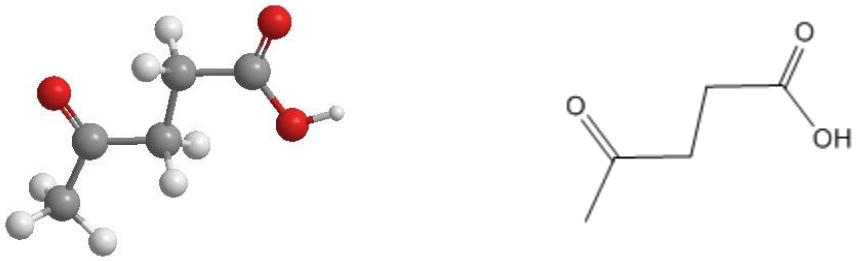


Figure A.5 Levulinic acid is a carboxylic acid with a ketone group.

Levulinic acid is currently produced in various ways, like the acid catalyzed dehydration of carbohydrates via simple rehydration of HMF, or by the petrochemical conversion of maleic anhydride or by acid hydrolysis of furfuryl alcohol, a derivative of furfural. However, the most promising method for producing levulinic acid at a larger scale is by deconstruction of lignocellulosic biomass (Rackemann and Doherty 2011).

Currently, the Biofine™ process (Hayes 2005), (Bozell et al. 2000) converts lignocellulosic biomass into levulinic acid, formic acid and furfural, using a two-stage reaction system catalyzed by dilute sulfuric acid. The first stage consist of a plug flow reactor where the feed is dehydrated to HMF at 210-220ºC, > 363 psi, for 12 seconds in 1-5 % dilute sulfuric acid.

The HMF is removed continuously and supplied to a second reactor (back mix) where HMF is rehydrated to levulinic acid at 190-200ºC, 203 psi, for 20 min. Furfural and formic acid are in the vapor phase at these reaction conditions which allow for easy separation, while the slurry mixtures of levulinic acid and residues are passed to a gravity separator and low pressure distillation columns for product purification. The yield of levulinic acid is ≥ 60%, based on the cellulose content of the lignocellulosic biomass.

At the lab scale, researchers are exploring alternatives for increasing the yields of levulinic acid from biomass. Yan et al. reported levulinic acid yields of 79.6% and 82.7% from bagasse and paddy straw, respectively (Yan et al. 2008). The biomass was mixed with 4.5% hydrochloric acid in a pressurized reactor at 220ºC for 45 min. Chang et al. mixed wheat straw in 3.5% sulfuric acid at 210 ºC to yield 68.8% of levulinic acid (Chang et al. 2007). The yields in these studies are based on the theoretical yield of levulinic acid from the cellulose content in biomass.

An important by-product from levulinic acid is levulinic ester, and researchers have been exploring the potential of the ester derivatives of levulinic acid (Lee et al. 2010), (Mascal and Nikitin 2010b), (Dharne and Bokade 2011). For example, ethyl levulinate (bp 206ºC) is an ester from the reaction of levulinic acid and ethanol at 200°C for 6 hours (Mascal and Nikitin 2010b). It has good lubricity and has been tested in blends with petroleum diesel up to 10% with no change in the cetane number, which is a measurement of the combustion quality of diesel fuel during compression ignition. Moreover, due to the shorter chain of levulinate ester compared to other esters, it has the potential to favorably impact the properties of biodiesel in terms of its cold performance issues (cloud point, pour point, and viscosity) (Mascal and Nikitin 2010b). Higher chain levulinate esters, like butyl levulinate, are also found to be good quality improvers for

diesel (Le Van Mao et al. 2011). The yield of ethyl levulinate from reactions involving the acid-catalyzed thermolysis of cellulose or wood feedstocks in ethanol is in the order of 20% (Mascal and Nikitin 2010b).

A.3 Solubilization and swelling of lignocellulosic biomass in ionic liquids and polar aprotic solvents

After reaction of lignocellulosic biomass with dilute sulfuric acid to hydrolyze hemicellulose to xylose with further dehydration into furfural, the solid residue is rich in cellulose and lignin. With more severe acidic conditions, lignin can be depolymerized further and cellulose could be converted into levulinic acid, the end product from the rehydration of HMF (Huber et al. 2006) and the basis of the Biofine™ process (Hayes 2005). Not content with these harsh conditions, researchers are attempting at relatively milder processes to handle the recalcitrant cellulose-lignin fraction by even avoiding the acidic pretreatment step that removes hemicellulose.

Some alkaline pretreatments, like sodium hydroxide and hydrogen peroxide are able to remove up to 60% of lignin and hemicellulose at mild conditions, 50ºC and 24 hours (Wang et al. 2010), but the reactants are consumed in the process (Gould 1985), (Gupta and Lee 2010). On the other hand, compounds like ionic liquids and polar aprotic solvents, may be recycled (Shill et al. 2011), (Smallwood 2002), and are being studied as chemicals that can solubilize lignocellulosic biomass for the purpose of increasing the accessibility of the plant cell wall towards chemical reactions (Binder and Raines 2009), (Mora-Pale et al. 2011).

In dissolution and swelling of biomass, the intermolecular structures connecting cellulose, hemicellulose and lignin, can be overcome by ionic liquids and polar aprotic solvents; leading to a disruption of the three-dimensional network of lignocellulose (Moulthrop et al.

2005), (Klemm et al. 1998). The consequence is an increase in weight (swelling) or a partial dispersion, with or without solvolysis, of the biomass components in the corresponding solvent system (solubilization). There is a fine line between swelling and dissolution, as the same solvent system can act either as a swelling agent or a solvent, depending on the degree of polymerization and structure of the lignocellulose sample (Klemm et al. 1998).

Ionic liquids

Ionic liquids lead the research as novel solvents for the dissolution or swelling of lignocellulosic biomass. Ionic liquids (ILs) are salts that melt below 100ºC and consist of an amine heterocycle cation with an organic or inorganic anion (Fig. A.6). ILs feature a wide electrochemical window, where cations and anions are inert toward electrochemical oxidation and reduction (Mora-Pale et al. 2011). Studies suggest that anions and cations participate in cell wall and cellulose solubilization, via hydrogen bonding, ionic, and π- π interactions connecting the lignocellulose components (Mora-Pale et al. 2011). For example, the cation of the 1-allyl-3-methylimidazolium chloride ([AMIM]Cl) is able to interact with the polyphenolic structure of lignin and the Cl^- anion forms hydrogen bonds disrupting the structure of cellulose (Mora-Pale et al. 2011). Also, 1-ethyl-3-methylimidazolium acetate ([EMIM]Ac) swells in switchgrass and disrupts the inter and intramolecular hydrogen bonding between cellulose and lignin (Fig. A.7) (Singh et al. 2009).

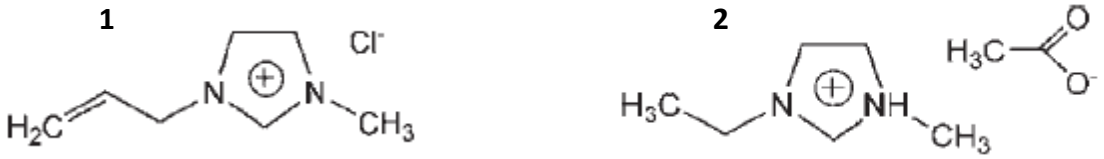


Figure A.6 Ionic liquids 1-allyl-3-methylimidazolium chloride ([AMIM]Cl) (1) and 1-ethyl-3-methylimidazolium acetate ([EMIM]Ac) (2), consisting of an amine heterocycle cation and a chloride (1) or acetate (2) anion. Reprinted from Mora-Pale et al. 2011.

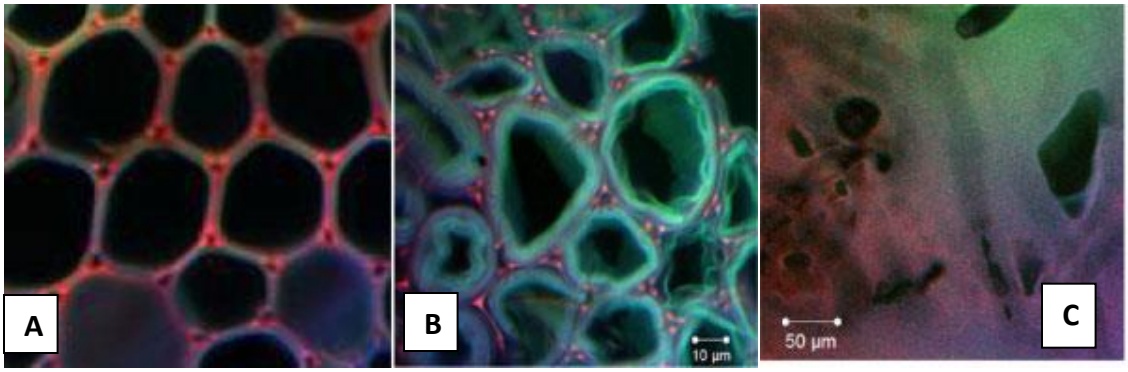


Figure A.7 Parenchyma cell wall of switchgrass showing the intact material (A), and the swelling of the cell wall with the ionic liquid [EMIM]Ac at 10 minutes (B). At 3 hours, the solubilized switchgrass had no coherent structure and all components in the cell wall are solubilized in [EMIM]Ac (C). Images taken via confocal fluorescence and reprinted from Singh et al. 2009.

Padmanabhan et al. studied the solubility and rate of dissolution for *Miscanthus* in ionic liquids (Padmanabhan et al. 2011). The authors screened several ionic liquids with different melting points and states of matter at room temperature (solids and liquids). Solubility was measured as a function of particle size (1-4 mm), temperature (110-130ºC) and time for dissolution (6-30 h) and was defined as:

$$\%\ Miscanthus\ dissolved = \frac{m_{original} - m_{residue}}{m_{original}} \times 100$$

where $m_{original}$ is the mass of *Miscanthus* before dissolution and $m_{residue}$ is the mass of residue after centrifugation and drying.

The results showed that ionic liquids with acetate, chloride and phosphate anions showed the best solubility among the ionic liquids screened, at around 5%, for 8-14 h at 130ºC. For the ionic liquids that showed no solubility, the authors did not determine % of swelling or weight increase. Also, the authors observed that moisture decreased the solubility of *Miscanthus*.

In a different study, Li et al. were able to completely solubilize sugarcane bagasse in 1-ethyl-3-methylimidazolium acetate [EMIM]Ac using a high temperature short time approach for 175ºC and 10 minutes above the glass transition temperature of lignin (150ºC) (Li et al. 2011). However, compared to Padmanabhan et al., much smaller particle sizes were used (<0.25 mm). Regardless, Li et al. were able to solubilize 74% of the lignin and all the sugar biopolymers in the bagasse powder. [EMIM]Ac was recycled by rotary evaporation and vacuum drying.

Mora-Pale et al., pointed at the advantages of ionic liquids as they can solubilize the three-dimensional network of lignocellulose and can be recycled (Mora-Pale et al. 2011). However, the current high costs of the ionic liquids, e.g., make it prohibitively expensive for any potential scale-up of these processes (Simmons et al. 2010).

Polar aprotic solvents

Polar aprotic solvents have been studied to some extent for the non-aqueous solubilization and functionalization of cellulose to develop threads, films, regenerated fibers (e.g., Rayon and Lyocel), and other derivatives with industrial applications (Fidale et al. 2008), (Klemm et al. 1998). However, these solvents have not been sufficiently explored for solubilization of lignocellulosic biomass towards value-added furans and acids.

Polar aprotic solvents are organic compounds that contain no hydrogen atoms connected directly to an electronegative atom (e.g., oxygen) (Klein 2012) (Fig. A.8). As opposed to polar protic solvents (e.g., H_2O), which can solvate both cations and anions, polar aprotic solvents can only solvate and surround external cations (e.g., Na+, Li+) by the solvent's electronegative atoms (Brown and Poon 2011).

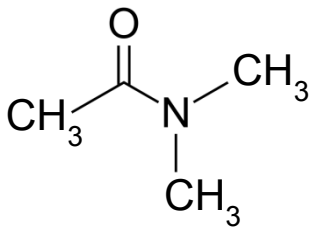


Figure A.8 As a polar aprotic solvent, dimethylacetamide (DMA) has no hydrogen atoms connected to the electronegative oxygen.

The polar aprotic solvent system dimethylacetamide (DMA) / lithium chloride (LiCl) was used by Binder and Raines (2009) to dissolve AFEX pretreated corn stover at 10% solids loading, 75ºC and 24 hours to produce HMF via acid catalysis. The authors reasoned that lithium ions (Li+) are solvated by DMA forming DMA-Li+ macrocations, leaving the Cl- free to disrupt the lignocellulose matrix of corn stover (Fig. A.9). The size of the corn stover samples and the amount dissolved in the solvent system was not specified.

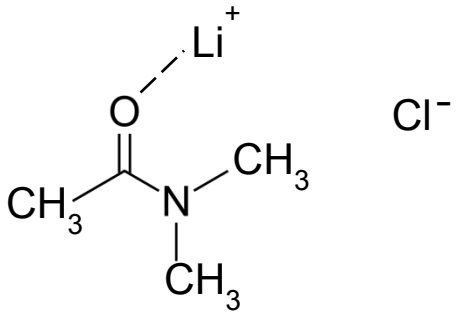


Figure A.9 The polar aprotic solvent dimethylacetamide forms a macrocation with a lithium atom from the LiCl salt. The chloride atom is free to disrupt the lignocellulose matrix (Binder and Raines 2009).

Lu and Ralph (2003) utilized the polar aprotic solvents dimethylsulfoxide (DMSO), tetrabutylammonium fluoride (TBAF) and N-methylimidazole (NMI) without halide salts (e.g., LiCl) to completely solubilize ball-milled (powdered) wood material at room temperature and without degradation of the cell wall components (Lu and Ralph 2003). The authors found that sample sizes of 1 mm or greater did not solubilize the samples, which points to the severe physical pretreatment required for solubilization.

Based on the studies by Lu and Ralph (2003) and Binder and Raines (2009), it appears that some polar aprotic solvents exhibit similar solvation properties to ionic liquids. A key advantage of polar aprotic solvents is cost. A survey of prices of the chemical company Sigma-Aldrich Co., shows that one liter of the aprotic solvent dimethylacetamide (DMA) (99%), costs ~$56. However, one kilogram of the ionic liquid 1-ethyl-3-methylimidazolium acetate [EMIM]Ac (90%), costs ~$900.

A.4 Indicators for solvation energy relationships between solvent and solute: Kamlet-Taft parameters

The intermolecular interactions, i.e., hydrogen bonding and polar interactions, between solvent (ionic liquids, polar aprotics / lithium salts) and solute (cellulose, lignocellulose), can be estimated quantitatively by using solubility parameters. Kamlet and Taft (Kamlet et al. 1983) developed a set of three parameters that quantifies the polarizability and the capacity of a solvent to donate or accept hydrogen bonds.

The Kamlet-Taft parameters are temperature-dependent and measure three scales or polarity in solvents: $\boldsymbol{\pi^*}$ (polarizability or ability of the solvent to stabilize a charge), $\boldsymbol{\alpha}$ (ability to donate hydrogen bonds) and $\boldsymbol{\beta}$ (ability to accept hydrogen bonds) (Doherty et al. 2010; Mora-Pale et al. 2011; Kamlet et al. 1983). Electronegative atoms, like chlorine, are hydrogen bond acceptors, and hydrogen atoms are hydrogen bond donors. The π^*, α and β parameters are determined by UV/Vis spectrophotometry using solvatochromic dyes, which are substances that change color from a change in solvent polarity.

Studies of the Kamlet-Taft parameters in ionic liquids suggest that a high β (hydrogen bond acceptor capacity) is required to effectively dissolve cellulose (Doherty et al. 2010; Mora-Pale et al. 2011; Kamlet et al. 1983). The β parameter for the polar aprotic solvent dimethylacetamide (DMA) is 0.76 (Doherty et al. 2010), however, when 5% lithium chloride (LiCl) is added, the β parameter of the solvent system is 1.90, a 2.5 fold increase (Spange et al. 1998). The β value for the DMA/LiCl system is even higher than the β value reported for the ionic liquid 1-butyl-3-methylimidazolium chloride [BMIM]Cl (0.84) (Brandt et al. 2010).

A.5 Separation of furans and acids from lignocellulose reaction mixtures

Following the acid catalyzed conversion of hemicellulose into furfural, and the subsequent solubilization and conversion of cellulose into HMF or levulinic acid, these products remain in the lignocellulose reaction mixtures and must be separated. In the batch and continuous industrial processes of furfural, the product is in the vapor phase and it is passed through the reboiler of an azeotropic distillation column for separation and purification (Zeitsch 2000). Similarly, in the Biofine™ process (Hayes 2005) for production of levulinic acid, the biomass slurries containing the product are passed through a gravity separator, and the levulinic acid is boiled off under reduced pressures and condensed.

These traditional separation processes, while effective, are limited by their severity. In the biochemical conversion route of lignocellulosic biomass (Chapter 1), some researchers are attempting to remove fermentation inhibitors using polyelectrolyte flocculants and electrodeionization techniques (Carter et al. 2011) (Gurram et al. 2011). In a biomass conversion route based on furans and acids, the fermentation inhibitors from the production of ethanol, i.e., furans and acids, are value-added products in the chemical deconstruction of lignocellulosic biomass for chemicals and fuels.

Carter et al. reported the removal of acetic acid, furfural and HMF from a dilute acid pretreated Ponderosa pine slurry using polyethyleneimine (PEI), a soluble polyelectrolyte flocculant. Using equimolar amounts of PEI to the inhibitors present, 88.3% of furfural and 66.4% of HMF could be removed before enzymatic hydrolysis and ethanol fermentation. Removal temperature and time were not specified (Carter et al. 2011).

Gurram et al. also used acid pretreated Ponderosa pine slurry for removal of acetic acid, HMF and furfural. The authors used resin-wafer electrodeionization (RW-EDI) and were able to

remove 77% of acetic acid, 60% of HMF and 74% of furfural, along with 97% removal of the sulfuric acid used for pretreatment. RW-EDI is an electrochemical ion exchange process developed by Argonne National Lab and Nalco Company to separate fermentation inhibitors (e.g., furans and acids) from ethanol fermentation bioreactors (Gurram et al. 2011). RW-EDI consists of an ion-exchange resin molded into a porous "resin wafer" that allows for better ion transport, pH control and solution flow control during the electrodeionization process. One key advantage of RW-EDI over other electrodeionization techniques is that special function particles can be incorporated into the resin wafers, which can be tuned to enhance performance and consistency (Gurram et al. 2011). These "tunable" properties may prove critical in separating furans and acids as products.

A.6 REFERENCES

Binder, J. B. and R. T. Raines. 2009. Simple chemical transformation of lignocellulosic biomass into furans for fuels and chemicals. *Journal of the American Chemical Society* 131(5): 1979-1985.

Bozell, J. J., L. Moens, D. C. Elliott, Y. Wang, G. G. Neuenscwander, S. W. Fitzpatrick, R. J. Bilski and J. L. Jarnefeld. 2000. Production of levulinic acid and use as a platform chemical for derived products. *Resources, Conservation and Recycling* 28(3-4): 227-239.

Brandt, A., J. P. Hallett, D. J. Leak, R. J. Murphy and T. Welton. 2010. The effect of the ionic liquid anion in the pretreatment of pine wood chips. *Green Chemistry* 12(4): 672-679.

Brown, W. H. and T. Poon. 2011. *Introduction to organic chemistry.* 4th ed. Hoboken, NJ: John Wiley & Sons.

Carter, B., P. Squillace, P. C. Gilcrease and T. J. Menkhaus. 2011. Detoxification of a lignocellulosic biomass slurry by soluble polyelectrolyte adsorption for improved fermentation efficiency. *Biotechnology and bioengineering* 108(9): 2053-2060.

Chang, C., P. Cen and X. Ma. 2007. Levulinic acid production from wheat straw. *Bioresource technology* 98(7): 1448-1453.

Chheda, J. N., G. W. Huber and J. A. Dumesic. 2007a. Liquid-phase catalytic processing of biomass-derived oxygenated hydrocarbons to fuels and chemicals. *Angewandte Chemie - International Edition* 46(38): 7164-7183.

Chheda, J. N., Y. Román-Leshkov and J. A. Dumesic. 2007b. Production of 5-hydroxymethylfurfural and furfural by dehydration of biomass-derived mono- and poly-saccharides. *Green Chemistry* 9(4): 342-350.

Chundawat, S. P. S., G. T. Beckham, M. E. Himmel and B. E. Dale. 2011. Deconstruction of lignocellulosic biomass to fuels and chemicals. *Annual Review of Chemical and Biomolecular Engineering* 2121-145.

Dharne, S. and V. V. Bokade. 2011. Esterification of levulinic acid to n-butyl levulinate over heteropolyacid supported on acid-treated clay. *Journal of Natural Gas Chemistry* 20(1): 18-24.

Doherty, T. V., M. Mora-Pale, S. E. Foley, R. J. Linhardt and J. S. Dordick. 2010. Ionic liquid solvent properties as predictors of lignocellulose pretreatment efficacy. *Green Chemistry* 12(11): 1967-1975.

Fidale, L. C., N. Ruiz, T. Heinze and O. A. El Seoud. 2008. Cellulose swelling by aprotic and protic solvents: What are the similarities and differences? *Macromolecular Chemistry and Physics* 209(12): 1240-1254.

Gould, J. M. 1985. Studies on the mechanism of alkaline peroxide delignification of agricultural residues. *Biotechnology and bioengineering* 27(3): 225-231.

Gray, M. C., A. O. Converse and C. E. Wyman. 2007. Solubilities of oligomer mixtures produced by the hydrolysis of xylans and corn stover in water at 180°C. *Industrial and Engineering Chemistry Research* 46(8): 2383-2391.

Gupta, R. and Y. Y. Lee. 2010. Pretreatment of corn stover and hybrid poplar by sodium hydroxide and hydrogen peroxide. *Biotechnology progress* 26(4): 1180-1186.

Gurram, R. N., S. Datta, Y. J. Lin, S. W. Snyder and T. J. Menkhaus. 2011. Removal of enzymatic and fermentation inhibitory compounds from biomass slurries for enhanced biorefinery process efficiencies. *Bioresource technology* 102(17): 7850-7859.

Hayes, D. J., J. Ross, M. H. B. Hayes, S. Fitzpatrick. 2006. The biofine process: production of levulinic acid, furfural, and formic acid from lignocellulosic feedstocks. *Biorefineries: Industrial processes and products* 1: 139-164.

Huber, G. W., S. Iborra and A. Corma. 2006. Synthesis of transportation fuels from biomass: Chemistry, catalysts, and engineering. *Chemical reviews* 106(9): 4044-4098.

Huber, G. W., J. N. Chheda, C. J. Barrett and J. A. Dumesic. 2005. Chemistry: Production of liquid alkanes by aqueous-phase processing of biomass-derived carbohydrates. *Science* 308(5727): 1446-1450.

Kamlet, M. J. and R. W. Taft. 1976. The solvatochromic comparison method. I. The β-scale of solvent hydrogen-bond acceptor (HBA) basicities. *Journal of the American Chemical Society* 98(2): 377-383.

Kamlet, M. J., J. -. M. Abboud, M. H. Abraham and R. W. Taft. 1983. Linear solvation energy relationships. 23. A comprehensive collection of the solvatochromic parameters, π*, α, and β, and some methods for simplifying the generalized solvatochromic equation. *Journal of Organic Chemistry* 48(17): 2877-2887.

Kim, D. -. and X. Pan. 2010. Preliminary study on converting hybrid poplar to high-value chemicals and lignin using organosolv ethanol process. *Industrial and Engineering Chemistry Research* 49(23): 12156-12163.

Klein, D. R. 2012. *Organic chemistry.* Hoboken, N.J.: John Wiley.

Klemm, D., B. Philipp, T. Heinze, U. Heinze and W. Wagenknecht. 1998. *Comprehensive Cellulose Chemistry Volume 1: Fundamentals and Analytical Methods.* Wiley-VCH.

Le Van Mao, R., Q. Zhao, G. Dima and D. Petraccone. 2011. New process for the acid-catalyzed conversion of cellulosic biomass (AC3B) into alkyl levulinates and other esters using a unique one-pot system of reaction and product extraction. *Catalysis Letters* 141(2): 271-276.

Lee, A., N. Chaibakhsh, M. B. A. Rahman, M. Basri and B. A. Tejo. 2010. Optimized enzymatic synthesis of levulinate ester in solvent-free system. *Industrial Crops and Products* 32(3): 246-251.

Li, W., N. Sun, B. Stoner, X. Jiang, X. Lu and R. D. Rogers. 2011. Rapid dissolution of lignocellulosic biomass in ionic liquids using temperatures above the glass transition of lignin. *Green Chemistry* 13(8): 2038-2047.

Lu, F. and J. Ralph. 2003. Non-degradative dissolution and acetylation of ball-milled plant cell walls: High-resolution solution-state NMR. *Plant Journal* 35(4): 535-544.

Mamman, A. S., J. -. Lee, Y. -. Kim, I. T. Hwang, N. -. Park, Y. K. Hwang, J. -. Chang and J. -. Hwang. 2008. Furfural: Hemicellulose/xylose-derived biochemical. *Biofuels, Bioproducts and Biorefining* 2(5): 438-454.

Mascal, M. and E. B. Nikitin. 2010a. High-yield conversion of plant biomass into the key value-added feedstocks 5-(hydroxymethyl)furfural, levulinic acid, and levulinic esters via 5-(chloromethyl)furfural. *Green Chemistry* 12(3): 370-373.

Mascal, M. and E. B. Nikitin. 2010b. Comment on processes for the direct conversion of cellulose or cellulosic biomass into levulinate esters. *ChemSusChem* 3(12): 1349-1351.

Mora-Pale, M., L. Meli, T. V. Doherty, R. J. Linhardt and J. S. Dordick. 2011. Room temperature ionic liquids as emerging solvents for the pretreatment of lignocellulosic biomass. *Biotechnology and bioengineering* 108(6): 1229-1245.

Moulthrop, J. S., R. P. Swatloski, G. Moyna and R. D. Rogers. 2005. High-resolution 13C NMR studies of cellulose and cellulose oligomers in ionic liquid solutions. *Chemical Communications*(12): 1557-1559.

Padmanabhan, S., M. Kim, H. W. Blanch and J. M. Prausnitz. 2011. Solubility and rate of dissolution for Miscanthus in hydrophilic ionic liquids. *Fluid Phase Equilibria* 309(1): 89-96.

Rackemann, D. W. and W. O. Doherty. 2011. The conversion of lignocellulosics to levulinic acid. *Biofuels, Bioproducts and Biorefining* 5(2): 198-214.

Román-Leshkov, Y. and J. A. Dumesic. 2009. Solvent effects on fructose dehydration to 5-hydroxymethylfurfural in biphasic systems saturated with inorganic salts. *Topics in Catalysis* 52(3): 297-303.

Román-Leshkov, Y., C. J. Barrett, Z. Y. Liu and J. A. Dumesic. 2007. Production of dimethylfuran for liquid fuels from biomass-derived carbohydrates. *Nature* 447(7147): 982-985.

Shill, K., S. Padmanabhan, Q. Xin, J. M. Prausnitz, D. S. Clark and H. W. Blanch. 2011. Ionic liquid pretreatment of cellulosic biomass: Enzymatic hydrolysis and ionic liquid recycle. *Biotechnology and bioengineering* 108(3): 511-520.

Simmons, B. A., S. Singh, B. M. Holmes and H. W. Blanch. 2010. Ionic liquid pretreatment. *Chemical Engineering Progress* 106(3): 50-55.

Singh, S., B. A. Simmons and K. P. Vogel. 2009. Visualization of biomass solubilization and cellulose regeneration during ionic liquid pretreatment of switchgrass. *Biotechnology and bioengineering* 104(1): 68-75.

Smallwood, I. M. N. 2002. *Solvent Recovery Handbook.* 2nd ed. Boca Raton, FL: Blackwell Publishing.

Spange, S., A. Reuter, E. Vilsmeier, T. Heinze, D. Keutel and W. Linert. 1998. Determination of empirical polarity parameters of the cellulose solvent N,N-dimethylacetamide/LiCl by means of the solvatochromic technique. *Journal of Polymer Science Part A: Polymer Chemistry* 36(11): 1945-1955.

Su, Y., H. M. Brown, X. Huang, X. -. Zhou, J. E. Amonette and Z. C. Zhang. 2009. Single-step conversion of cellulose to 5-hydroxymethylfurfural (HMF), a versatile platform chemical. *Applied Catalysis A: General* 361(1-2): 117-122.

Thomas, C. A. 2006. Reactions and separations in tunable solvents. PhD diss. Atlanta, GA: Georgia Institute of Technology, Department of Chemical and Biomolecular Engineering.

Verevkin, S. P., V. N. Emel'yanenko, E. N. Stepurko, R. V. Ralys, D. H. Zaitsau and A. Stark. 2009. Biomass-derived platform chemicals: Thermodynamic studies on the conversion of 5-hydroxymethylfurfural into bulk intermediates. *Industrial and Engineering Chemistry Research* 48(22): 10087-10093.

Wang, B., X. Wang and H. Feng. 2010. Deconstructing recalcitrant Miscanthus with alkaline peroxide and electrolyzed water. *Bioresource technology* 101(2): 752-760.

Werpy, T., G. Petersen, A. Aden, J. Bozell, J. Holladay, J. White and A. Manheim. 2004. Top value-added chemicals from biomass, volume 1: Results of Screening for Potential Candidates from Sugars and Synthesis Gas. DOE/GO-102004-1992. Washington DC: US Department of Energy, Pacific National Laboratory, National Renewable Energy Laboratory.

Win, D. T. 2005. Furfural - Gold from Garbage. *AU J.T.* 8(4): .

Winterton, N. 2011. *Chemistry for sustainable technologies: a foundation.* 1st ed. Cambridge, UK: RSC Publishing.

Yan, L., N. Yang, H. Pang and B. Liao. 2008. Production of levulinic acid from bagasse and paddy straw by liquefaction in the presence of hydrochloride acid. *Clean - Soil, Air, Water* 36(2): 158-163.

Yang, F., Q. Liu, X. Bai and Y. Du. 2011. Conversion of biomass into 5-hydroxymethylfurfural using solid acid catalyst. *Bioresource technology* 102(3): 3424-3429.

Yi, Y. -., J. -. Lee, S. -. Hong, Y. -. Choi and C. -. Chung. 2011. Acid-mediated production of hydroxymethylfurfural from raw plant biomass with high inulin in an ionic liquid. *Journal of Industrial and Engineering Chemistry* 17(1): 6-9.

Yin, S., Y. Pan and Z. Tan. 2011. Hydrothermal conversion of cellulose to 5-hydroxymethyl furfural. *International Journal of Green Energy* 8(2): 234-247.

Zeitsch, K. J. 2000. *The chemistry and technology of furfural and its many by-products.* 1st ed. Amsterdam, The Netherlands: Elsevier.

APPENDIX B

PRODUCTION OF FURFURAL FROM *MISCANTHUS**

Furfural from *Miscanthus* (1 mm particle size, -20/+80 mesh sieve, 7% moisture) was produced in a Parr batch reactor at 190 °C for 10 minutes, by the sulfuric acid catalyzed hydrolysis of hemicellulose to xylose, and dehydration of xylose to furfural (Fig. B.1). The severity of the process also hydrolyzes part of the cellulose fraction into glucose, which is dehydrated to HMF. Some HMF is rehydrated into levulinic acid and formic acid. Yields are not based on theoretical.

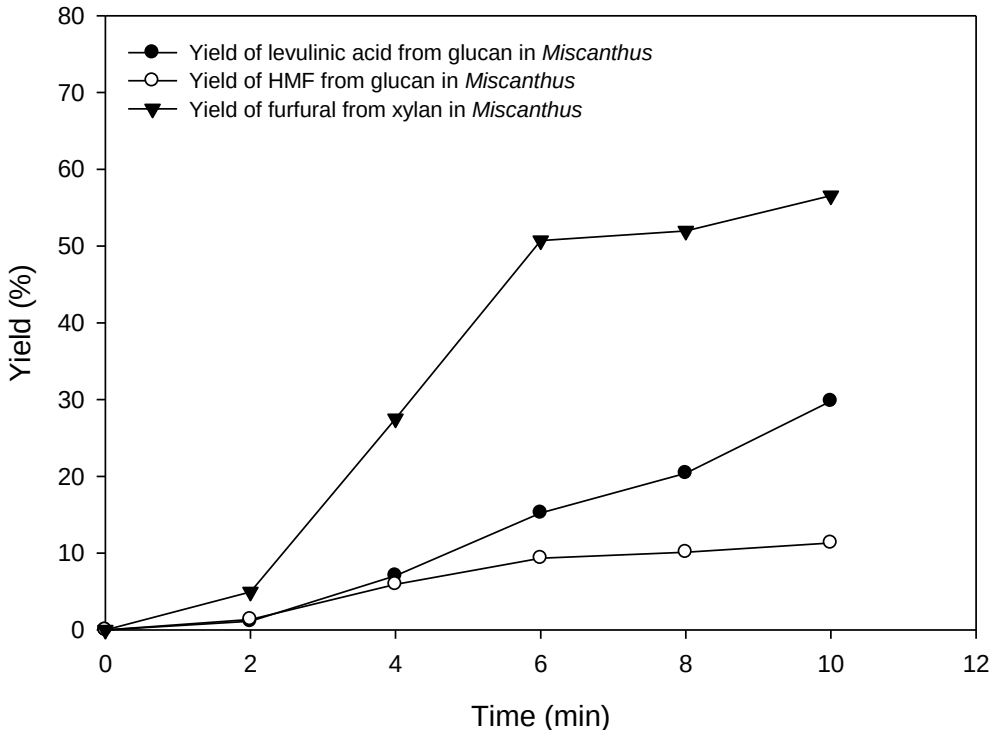


Figure B.1 Yields of furfural, levulinic acid and HMF from *Miscanthus* soaked in 1% H_2SO_4 at 190ºC for 10 min and constant stirring at 400 rpm in a Parr batch reactor. Furfural is produced from xylan, whereas levulinic acid and HMF is produced from glucan. Yield is defined as grams of furan in 100 grams of xylan or glucan and are not based on theoretical.

*The methodology is explained in Chapter 6

Even though the heat-up time in the Parr reactor is 35-40 minutes, when the reaction temperature is reached, the yield of furfural is 5% at two minutes, which means that not much furfural is produced during heat-up. At 6 minutes, the yield of furfural is 51%, reaching 57% at 10 minutes. The yield of HMF stabilizes to 10% at 6 minutes, reaching 12% at 10 minutes. On the other hand, yields of levulinic acid keep increasing to 30% at 10 minutes, as more HMF is rehydrated to levulinic acid. During the furfural production stage, it is desired to minimize the amount of HMF and levulinic acid, to avoid unwanted cellulose degradation to humins (pseudo-lignin), formed via condensation of furans and sugars.

Fig. B.2 shows a clear HPLC chromatogram of sample taken at 10 minutes of the furans and acids analyzed, i.e., formic acid, acetic acid, levulinic acid, HMF and furfural. Quantification was done with a 10 point calibration curve using known concentrations of corresponding reagent grade standards.

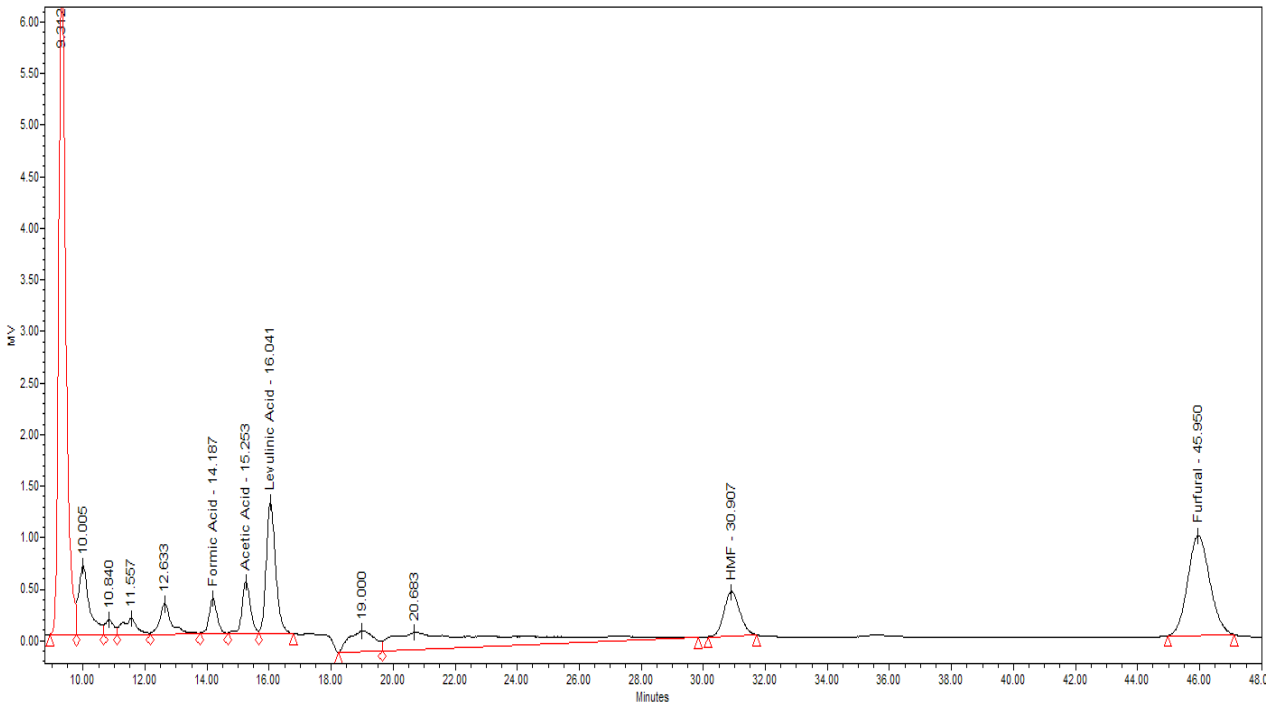


Figure B.2 HPLC chromatogram of sample taken at 10 minutes and 190ºC. The furans and acids analyzed are formic acid, acetic acid, levulinic acid, HMF and furfural.

APPENDIX C

SURVEY OF METHODS FOR THE β KAMLET-TAFT PARAMETER DETERMINATION FOR BUTADIENE SULFONE

As a polar aprotic solvent, the Kamlet-Taft parameters applicable to butadiene sulfone (BS) are π* (polarizability or ability of the solvent to stabilize a charge) and β (ability to accept hydrogen bonds). As an aprotic solvent, α (ability to donate hydrogen bonds) is not applicable to butadiene sulfone and should be zero, if determined. The capacity of butadiene sulfone to accept hydrogen bonds (β) is the most important parameter to assess the interactions between the solvent and the lignocellulose substrate. The Kamlet-Taft parameters for BS are not available in the literature, and we attempted unsuccessfully to determine β for BS.

We first tried the original method proposed by Kamlet and Taft, with the modifications by Doherty (Doherty et al. 2010; Kamlet and Taft 1976) in which the β value is calculated using the maximum UV wavelength of the dyes N,N-diethyl-4-nitroaniline (NNDS) and 4-nitroaniline (4N) in a solution of the solvent analyzed:

$$\beta = \frac{1.035 \times \nu(NNDN)_{max} - \nu(4N)_{max} + 2.64}{2.8}$$

where $\nu(NNDN)_{max}$ and $\nu(4N)_{max}$ are the maximum wavelengths of N,N-diethyl-4-nitroaniline and 4-nitroaniline, in kilokeyser (kK, 10^{-3} cm^{-1}).

The dyes were prepared separately in ethanol in a quartz microcell with a magnetic stir bar and a PTFE stopper (1.3 ml, 4 mm x 10 mm, Agilent Technologies, Santa Clara, CA) following the method by Doherty et al. The ethanol was evaporated under a stream of dry nitrogen and the butadiene sulfone, kept in a water bath at 70 °C, was added at different concentrations. The absorbance spectra were measured between 350 nm and 750 nm using a Cary 300 Bio UV/Vis spectrophotometer with temperature control (Agilent Technologies, Santa

Clara, CA) at 70 °C, and the wavelength at the maximum absorbance was recorded. These parameters are temperature dependent (Doherty et al. 2010), and the maximum wavelength at 70 ºC and 90 °C was determined.

For butadiene sulfone, while N,N-diethyl-4-nitroaniline showed a distinct maximum wavelength, 4-nitroaniline showed three peaks with similar absorbance over a range of 349-278 nm, and the wavelengths shifted significantly over replications. The inconsistencies with 4-nitroaniline absorbance in BS rendered this method useless for BS.

C. Thomas (Thomas 2006) tried to utilize the original Kamlet-Taft method to calculate the β value for piperylene sulfone (PS) (m.p. -12 °C) (BS backbone with methyl group) and also encountered problems with 4-nitroaniline, reporting that "the solvent (PS) absorption obscured the absorbance of 4-nitroaniline". To overcome this issue, the author substituted 4-nitroaniline with the dye pairs N,N-dimethyl-1,4-nitrosoanline and N-methyl-4-nitrosoaniline, using cyclohexane as reference solvent. Using a different equation for β, the author calculated β=0.46 for PS. We tried the method by Thomas to determine the β value for BS, but we found BS to have a low solubility in cyclohexane at 70°C (above BS melting point). Future work should develop new approaches for the determination of the Kamlet-Taft parameters for butadiene sulfone.

REFERENCES

Doherty, T. V., M. Mora-Pale, S. E. Foley, R. J. Linhardt and J. S. Dordick. 2010. Ionic liquid solvent properties as predictors of lignocellulose pretreatment efficacy. *Green Chemistry* 12(11): 1967-1975.

Kamlet, M. J. and R. W. Taft. 1976. The solvatochromic comparison method. I. The β-scale of solvent hydrogen-bond acceptor (HBA) basicities. *Journal of the American Chemical Society* 98(2): 377-383.

Thomas, C. A. 2006. Reactions and separations in tunable solvents. PhD diss. Atlanta, GA: Georgia Institute of Technology, Department of Chemical and Biomolecular Engineering.

APPENDIX D

STUDY OF OTHER CHEMICAL PRETREATMENTS FOR *MISCANTHUS:* ALKALINE PEROXIDE + ACID ELECTROLYZED WATER

D.1 Enzymatic digestibility of cellulose in *Miscanthus* (-50/+80 mesh) pretreated with 1st stage alkaline peroxide (NaOH + 2% H_2O_2) and 2nd stage acid electrolyzed water, versus the enzymatic digestibility of untreated pure cellulose (Avicel®)

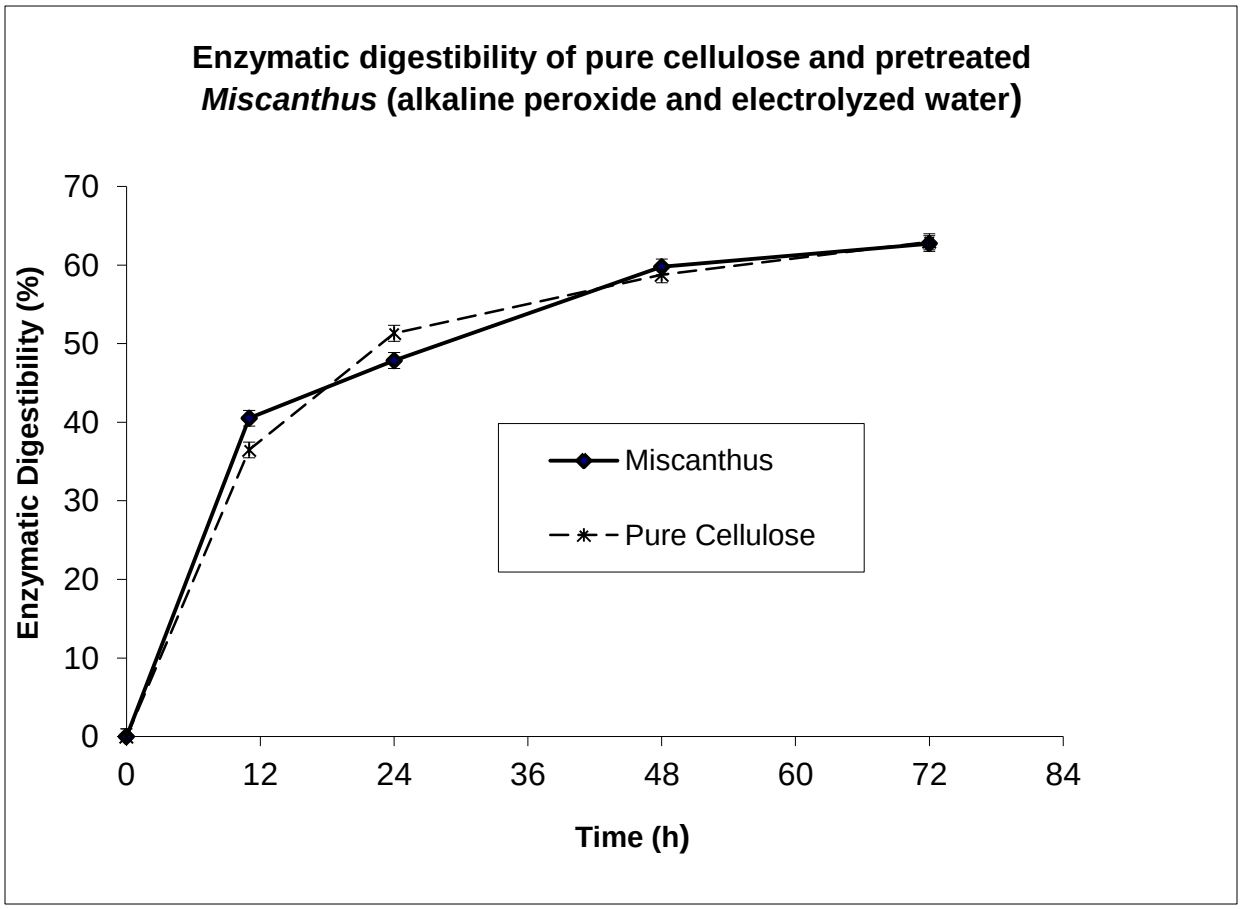


Figure D.1 Enzymatic digestibility of pure cellulose (Avicel®) and pretreated *Miscanthus* with alkaline peroxide and acid electrolyzed water.

D.2 Optimization of the 1st stage pretreatment (alkaline peroxide) of *Miscanthus* based on the enzymatic digestibility of cellulose (glucan). 2nd stage pretreatment (acid electrolyzed water) conditions are constant.

- **Design Variables**
  - H202 (0% - 4%)
  - Temperature (25.5°C, 50.5°C)
  - Time (4h, 24h)

- **Constants**

Optimization Constants		
1st Stage	***2nd Stage***	***Enz. Hydrolysis***
pH = 11.5 (with NaOH)	AEW	Cellulase & B-Glucosidase
S/L = 5%	T = 121 C	pH = 5.0
RPM = 100	t = 20 min	T = 50 C
	S/L = 6.25%	t = 72 h
		S/L = 5%

- **Dependent Variable**
  - Enzymatic Digestibility

- **Design of Experiments**
  - 2^3 factorial with center and axial runs

Run Type	**Run**	**Natural Variables**			**Coded Variables**			**Response Variable**
		H_2O_2 %	**Time**	**Temp**	**x_1**	**x_2**	**x_3**	**% Enzymatic Digestibility**
Factorial	1	0	4	25.5	-1	-1	-1	
Factorial	2	4	4	25.5	1	-1	-1	
Factorial	3	0	24	25.5	-1	1	-1	
Factorial	4	4	24	25.5	1	1	-1	
Factorial	5	0	4	50.5	-1	-1	1	
Factorial	6	4	4	50.5	1	-1	1	
Factorial	7	0	24	50.5	-1	1	1	
Factorial	8	4	24	50.5	1	1	1	
Center	9	2	14	38	0	0	0	
Axial	10	0	14	38	-1	0	0	
Axial	11	4	14	38	1	0	0	
Axial	12	2	4	38	0	-1	0	

Run Type	Run	Natural Variables			Coded Variables			Response Variable
		H_2O_2 %	Time	Temp	x_1	x_2	x_3	% Enzymatic Digestibility
Axial	13	2	24	38	0	1	0	
Axial	14	2	14	25.5	0	0	-1	
Axial	15	2	14	50.5	0	0	1	
Center	16	2	14	38	0	0	0	

- **Response Surface Method**
  - Central Composite Design - Cuboidal
    - 2^3 factorial runs (k = 3)
    - 2 center runs
    - 6 axial runs ($\alpha = 1$)

- **Regression Model**

$y_i = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x^2_1 + b_5x^2_2 + b_6x^2_3 + b_7(x_{1*}x_2) + b_8(x_{1*}x_3)_I + b_9(x_{2*}x_3) + e$

y_i dependent variable ("% enzymatic digestibility")
b_i regression coefficients (i = 0,1..9)
x_1 independent variable "H_20_2"
x_2 independent variable "Temp"
x_3 independent variable "Time"
e error term, part of the observed value not explained by the model

Optimization of 1st Stage Pretreatment

Central Composite Design - Cuboidal

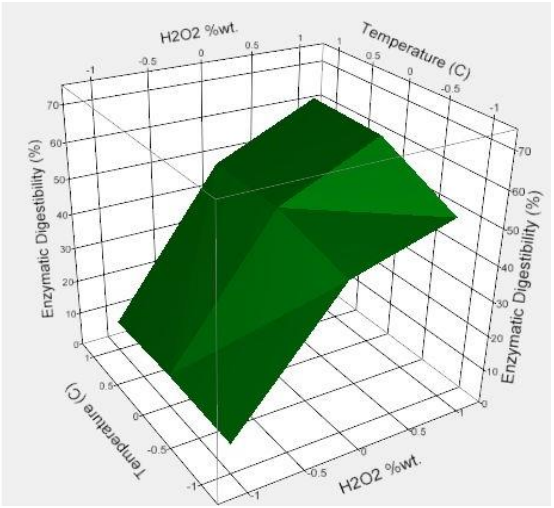
16 runs	Factors	Dependent Variable
8 Factorial	H2O2	Enzymatic Digestibility
2 Center	Time	
6 Axial	Temperature	

Optimization Constants		
1st Stage	***2nd Stage***	***Enz. Hydrolysis***
pH = 11.5	AEW	Cellulase & B-Gluc.
S/L = 5%	T = 121 C	ph = 5.0
RPM = 100	t = 20 min	T = 50 C
	S/L = 6.25%	t = 72 h
		S/L = 5%

Optimization Study of 1st Stage Pre-treatment of Miscanthus (+20/-80 mesh)

								Optimization (Sep. 2009)	
Run Type	Run	Natural Variables			Coded Variables			Response Variable	Enzymatic
		H2O2 %	Time	Temp	x1	x2	x3	Gluc. Conc (g/L)	Digestibility (%)
Factorial	1	0	4	25.5	-1	-1	-1	2.3	**8.6**
Factorial	2	4	4	25.5	1	-1	-1	12.9	**47.5**
Factorial	3	0	24	25.5	-1	1	-1	2.3	**8.6**
Factorial	4	4	24	25.5	1	1	-1	15.2	**55.7**
Factorial	5	0	4	50.5	-1	-1	1	2.4	**8.8**
Factorial	6	4	4	50.5	1	-1	1	16.1	**59.1**
Factorial	7	0	24	50.5	-1	1	1	2.4	**8.7**
Factorial	8	4	24	50.5	1	1	1	18.0	**66.2**
Center	9	2	14	38	**0**	**0**	**0**	13.0	**47.9**
Axial	10	0	14	38	-1	0	0	2.5	**9.3**
Axial	11	4	14	38	1	0	0	16.9	**62.0**
Axial	12	2	4	38	0	-1	0	12.4	**45.4**
Axial	13	2	24	38	0	1	0	14.0	**51.6**
Axial	14	2	14	25.5	0	0	-1	11.7	**43.1**
Axial	15	2	14	50.5	0	0	1	13.4	**49.2**
Center	16	2	14	38	**0**	**0**	**0**	13.6	**50.1**

Optimization Response Surface



Response surface generated with JMP® Statistics and Optimization Software

Response Surface reaches a maximum outside the data range

Inside data range the maximum reaction conditions are:

Enzymatic Digestibility: 66.2%

H2O2 Concentration: 4 % wt.

Temperature: 50°C

Time: 24 hours

Figure D.2 Optimization of the 1st stage pretreatment of *Miscanthus* with alkaline peroxide, based on the enzymatic digestibility of cellulose.

Optimization of the 1st stage Pretreatment - Statistical results

Optimization - Statistical Significance P values at α=0.05		
Linear		
H_2O_2	<0.0001	H_2O_2 has an effect on enzymatic digestibility
Time	0.661	Time has **no** effect on enzymatic digestibility
Temperature	0.0251	Temperature has an effect on enzymatic digestibility
Quadratic		
H_2O_2* H_2O_2	<0.0001	H_2O_2* H_2O_2 has an effect on enzymatic digestibility
Time*Time	0.7847	Time*Time has **no** effect on enzymatic digestibility
Temp*Temp	0.0255	Temp*Temp has an effect on enzymatic digestibility
Cross-product		
H_2O_2*Time	0.0089	H_2O_2*Time has an effect on enzymatic digestibility
H_2O_2*Temp	0.0017	H_2O_2*Temp has an effect on enzymatic digestibility
Time*Temp	0.7767	Time*Temp has **no** effect on enzymatic digestibility

Note: P values must be less than α=0.05 for statistical significance

Statistical analysis performed with SAS® Software