CONVERSION OF BIOMASS SUGARS TO PLATFORM CHEMICALS

BY HOMOGENEOUS ACID CATALYST

IN ORGANIC WATER MIXTURES.

by

Aidan James Scheffel

A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Environmental Engineering

MONTANA STATE UNIVERSITY
Bozeman, Montana

December 2022

©COPYRIGHT

by

Aidan James Scheffel

2022

All Rights Reserved

TABLE OF CONTENTS

1. INTRODUCTION 1
2. REACTIONS USING PURE SUGARS 5
Summary 5
Materials and Equipment 5
Experimental Methods 6
Results and Discussion 7
Conclusions 15
3. COMPOSITION ANALYSIS ON CANOLA AND INTERMEDIATE WHEATGRASS BIOMASS AND THE EFFECT OF EXTRACTION 17
Contribution of Authors and Co-Authors 17
Manuscript Information 18
Introduction 19
Experimental 20
Materials and Methods 20
Biomass Moisture Content 21
Extractions 21
Biomass Compositions 21
Results and discussion 21
Conclusions 27
4. ACID CATALYZED BIOMASS REACTIONS 28
Summary 28
Materials and Equipment 28
Experimental Methods 28
Results and Discussion 29

TABLE OF CONTENTS – CONTINUED

Conclusions....33
5. CONCLUSIONS....34
REFERENCES CITED....36
APPENDICES....41
APPENDIX A: RStudio code for correlation plots....42
APPENDIX B: Experimental protocol for pH measurements....44

LIST OF TABLES

Table Page

Table 2.1: Comparison of pH strips and probe measurements 12

Table 3.1. Composition of Intermediate Wheatgrass..... 32

Table 3.2. Composition of Froid Canola 32

Table 3.3. Composition of Ras Canola 33

LIST OF FIGURES

Figure Page

Figure 1.1. Possible reaction pathways for the conversion of cellulose to HMF and levulinic acid 2

Figure 1.2. A proposed closed chain mechanism of the dehydration of xylose to furfural 3

Figure 1.3. A possible acyclic pathway for the production of furfural 3

Figure 2.1. Concentration of sulfuric acid versus pH 7

Figure 2.2a-b. Furfural yield from xylose (a) and arabinose (b) 9

Figure 2.2c-d. Xylose (c) and arabinose (d) conversion 10

Figures 2.3a. Correlation plot for xylose reactions in all solvents at varying severities 11

Figures 2.3b. Correlation plot for arabinose reactions 12

Figure 2.5a. Levulinic acid yield from pure glucose. 13

Figure 2.5b. 5-hydroxymethylfurfural yield from pure glucose 14

Figure 2.5c. Glucose conversion in 50/50 organic-water mixtures 14

Figure 2.6. Correlation plot for pure glucose reactions in all solvents 15

Figure 3.1. Amount of sucrose, glucose and xylose in extractives 24

Figure 3.2. Compositions of A) Froid Canola, B) Ras canola, and C) intermediate wheatgrass biomass 26

Figure 4.1. Furfural yield from intermediate wheatgrass in all solvents 30

Figure 4.3. Levulinic acid yield from intermediate wheatgrass in all solvents 31

Figure 4.4. 5-hydroxymethylfurfural yield from intermediate wheatgrass reactions in all solvents 32

LIST OF FIGURES CONTINUED

Figure Page

Figure 4.5. Correlation plot for intermediate wheatgrass reactions in all solvent mixtures 33

ABSTRACT

Lignocellulosic biomass could serve as an alternative to petroleum for the production of platform chemicals that can be upgraded to bio-based plastics, chemicals, and fuels. The polysaccharides present in the cellular structure of biomass can be hydrolyzed into common sugars, which can be further reacted into important chemical intermediates such as furfural, 5-hydroxymethylfurfural, and levulinic acid. However, current commercial processes typically result in low yields. This work aimed to study the conversion of model sugars (glucose, xylose, and arabinose) into platform chemicals using a homogeneous acid catalyst in a variety of organic-water mixtures, and compare those findings to reactions carried out using biomass under the same conditions. Reactions were performed in 50-50 solvent-water mixtures using sulfolane, tetrahydrofuran (THF), or γ-butyrolactone (GBL) as the solvent and using a sulfuric acid catalyst. Intermediate wheatgrass was used in the biomass reactions and its composition was determined using standard NREL methods. Similar trends were observed in both pure sugar and biomass reactions in which the solvent choice had little effect on maximum yield of furfural, HMF, or levulinic acid. However, the THF/water solvent achieved maximum yields at lower severities than the other solvent mixtures indicating that a combination of less time, lower temperature, and/or less acid could be used in the THF reactions compared to the others. Compared to previously published research that found the solvent polarity correlated with product yields of furfural in reactions with no acid present, the effects of solvent properties may be minimized by the presence of the acid catalyst. Higher yields were achieved in biomass reactions than in pure sugar reactions, possibly due to unaccounted for reaction pathways or lowering of degradation reaction rates due to a lower initial reactant weight percent.

CHAPTER ONE

INTRODUCTION

Fossil fuels have long been integrated into nearly every facet of society. They can be found everywhere from clothing and household goods to building materials and energy production. However, fossil fuels such as petroleum and coal are classic examples of nonrenewable resources; they require specific environmental conditions and millions of years to form (Turgeon & Morse, 2022). Despite this, fossil fuel use in the United States, specifically petroleum and natural gas for energy needs has only increased (*U.S. Energy Information Administration – EIA – Independent Statistics and Analysis*, n.d.). To reduce fossil fuel dependence and ultimately curb greenhouse gas emissions, finding alternative feedstocks for products like plastics, chemicals, and fuels is essential. Biomass, and in particular, lignocellulosic biomass, could serve as an important feedstock source for many of these industries; it is both a renewable resource and can act as a carbon sink, helping to offset emissions from other sources (Scurlock & Hall, 1998).

The concept of "green chemistry" or "green chemicals" is not a new one, but it has seen increasing focus in recent years as the need to find fossil fuel alternatives becomes greater and greater in the face of the current climate crisis. For example, ethanol produced from corn stover and other corn byproducts has seen use as a fuel additive as early as 1876 and has seen increased demand since the first Renewable Fuels Standard became law in the United States in 2005 (*History of Ethanol Production and Policy — Energy*, n.d.). Biomass, such as agricultural wastes like corn stover and specifically lignocellulosic biomass such as perennial grasses, contain important structural polymers: cellulose, hemicellulose, and lignin. Cellulose, or linked glucose

monomers, typically makes up between 25-40% of the dry weight of a biomass feedstock. Hemicellulose, primarily composed of glucuronoarabinoxylans (GAXs) can comprise between 10-30% of the dry weight of the feedstock. Lignin, a phenolic polymer can make up between 17-24% of the structure of grasses (B. B. Buchanan et al., 2015; Saake & Lehnen, 2007). This work focuses on upgrading the cellulose and hemicellulose fractions into products that can replace fossil fuels.

Cellulose can be hydrolyzed into glucose monomers that can be dehydrated into an important intermediate, 5-hydroxymethylfurfural (HMF; Figure 1.1). This reaction is generally thought to proceed via fructose, an isomer of glucose (Jiang et al., 2021; Kang et al., 2018a). HMF as a platform chemical can be upgraded to bioplastic precursors, fuel additives, or reacted further via a rehydration reaction into levulinic acid (Román-Leshkov et al., 2007; Sajid et al., 2018), which can serve as an intermediate for gasoline additives and other chemical intermediates used in cosmetics, pharmaceuticals, and pesticide production (Kang et al., 2018). Levulinic acid production from lignocellulosic biomass is typically carried out at high temperatures, often above 150 °C, in the presence of a Brønsted acid. The highest reported yield is 98.9% in a γ-valerolactone (GVL)/water mixture using phosphotungstic acid at 195 °C for 80 min. Reactions in less severe conditions typically range between 50-78% yield, with aqueous systems generally producing comparable yields to organic/aqueous mixtures (Jiang et al., 2021; Li et al., 2019).

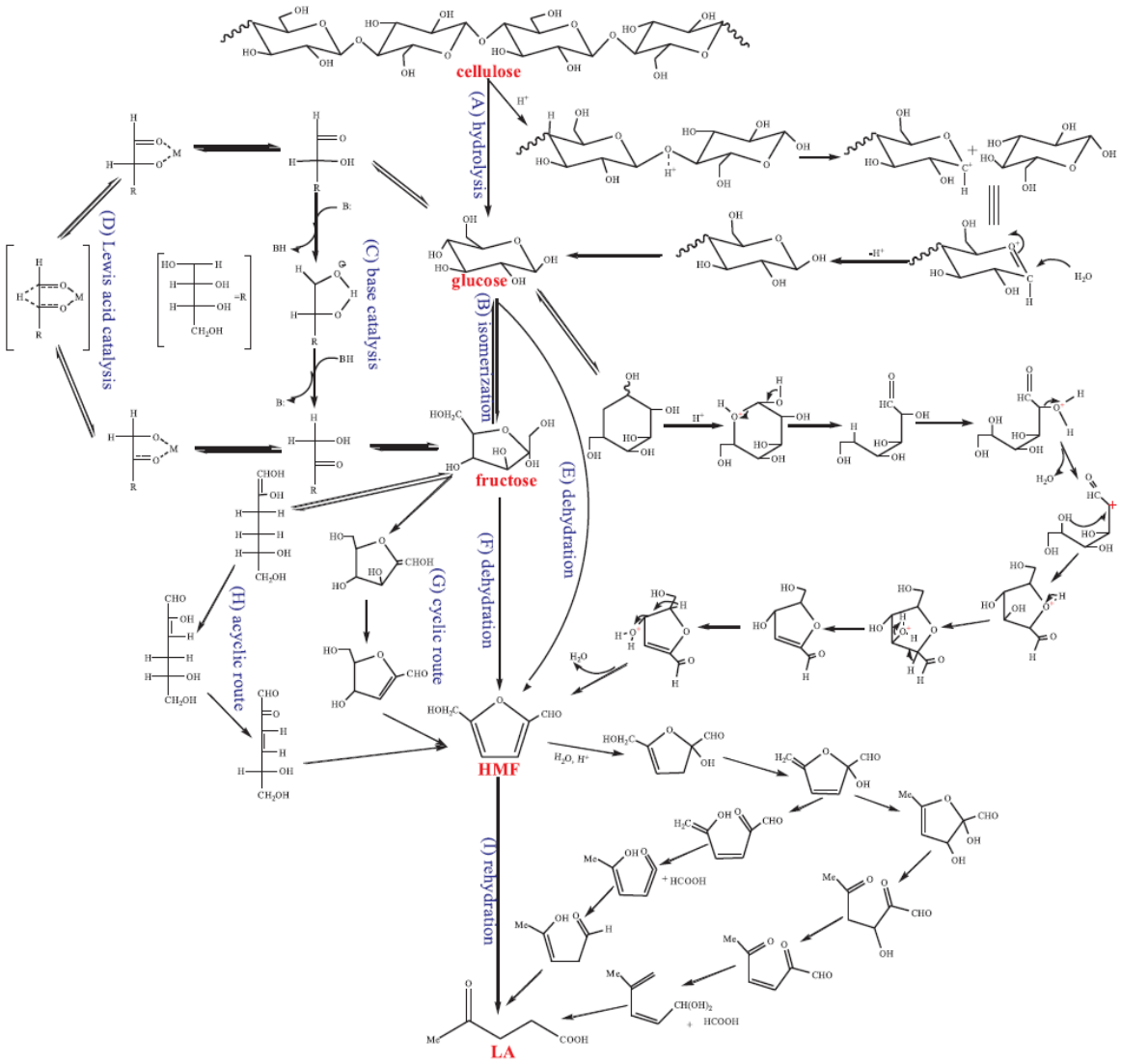


Figure 1.1: Possible reaction pathways for the conversion of cellulose to HMF and levulinic acid (as published in Kang et al., 2018b).

Hemicellulose is composed of a variety of five and six carbon (C5 and C6) sugars such as xylose, arabinose, mannose, and glucuronic acid (Ye et al., 2021) that can be further upgraded after hydrolysis of the biomass. Dehydration of pentoses, such as xylose and arabinose, forms furfural through two possible mechanisms shown in Figures 1.2 and 1.3 (Dussan et al., 2015; Peleteiro et al., 2016; Weingarten et al., 2010). Furfural is a valuable platform chemical that

finds use as a selective solvent in the manufacture of oils and fuels and as an intermediate in the production of solvents like tetrahydrofuran (THF) (Yemiş & Mazza, 2011). Traditional furfural production from biomass involves physical pretreatment of the biomass, a concentrated mineral acid catalyst, and continuous steam extraction of the product. These methods typically result in a low concentration product stream and furfural yields less than 65% (Lee & Wu, 2021a) leading researchers to explore organic solvents and aqueous mixtures for xylose dehydration reactions.

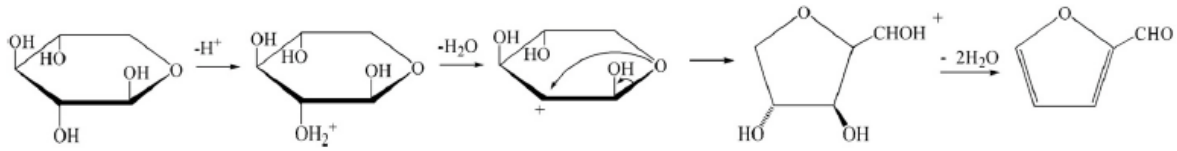


Figure 1.2: A proposed closed chain mechanism of the dehydration of xylose to furfural (as published in Lee & Wu, 2021b).

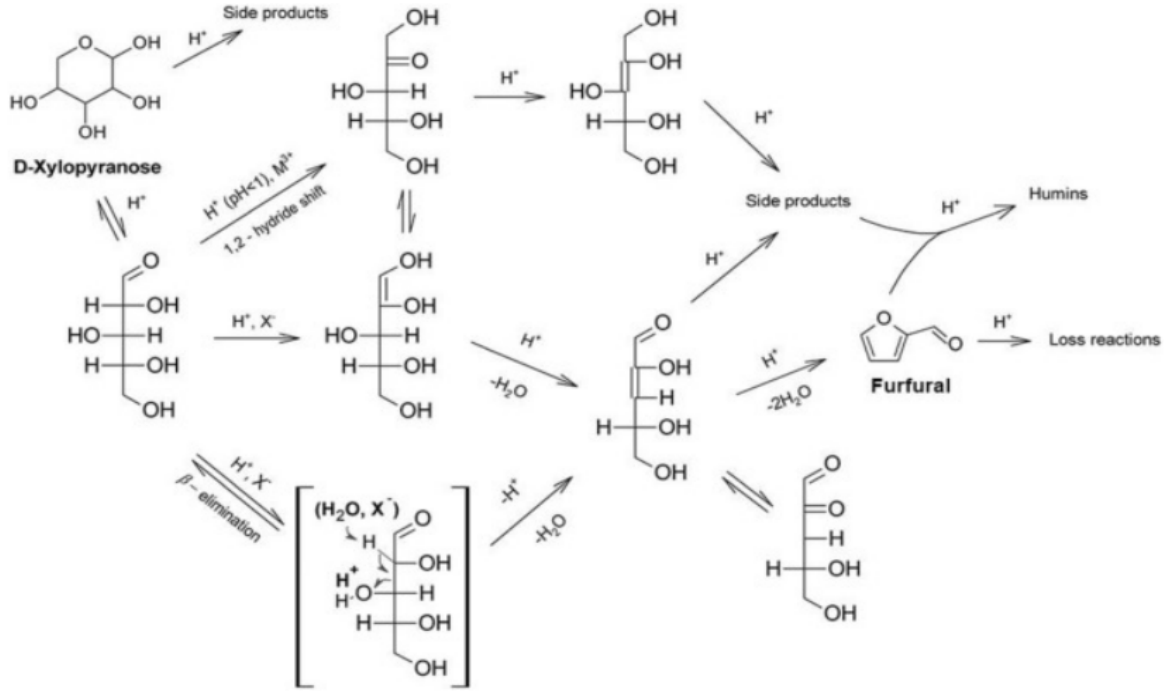


Figure 1.3: A possible acyclic pathway for the production of furfural (as published in Danon et al., 2013).

A previous study by Romo et al. showed that starting from xylose, furfural yield is strongly correlated with properties of the reaction medium, specifically the solvent (Romo et al., 2019). They found that furfural yield positively correlated with the Hansen Solubility Parameter Polarity (HSPP, δ_p) of the solvent in autocatalyzed monophasic reaction systems. Additionally, the reaction coordinate called severity (R_o, Overend and Chornet, 1987) defined as

$$R_o = log_{10}\left[t_{rxn} \exp\left(\left(\frac{T_{rxn} - 100}{14.75}\right)\right)\right] \qquad Eqn.\ 1.1$$

where t_{rxn} is the reaction time and T_{rxn} is the reaction temperature also correlated with furfural yield (Romo et al., 2019). Their work studied the conversion of xylose to furfural in pure aqueous and organic-water mixtures using sulfolane, γ-butyrolactone (GBL), γ-valerolactone (GVL), γ-hexalactone (GHL), and tetrahydrofuran (THF). Correlation plots showed that xylose conversion and furfural yield trended positively with severity. Furfural yield also showed a positive correlation with δ_p, with sulfolane, the highest polarity organic exhibiting the highest yields when compared to lower polarity solvents. In all solvents, furfural yields began to decrease as xylose conversions approached 100% as byproducts formed through resinification reactions increased (Cai et al., 2014; Garrett & Dvorchik, 1969; Peleteiro et al., 2016). Furfural degrades under the same conditions as it is produced, reacting with both reactant pentoses and other furfural molecules to form humic substances and insoluble, resinous compounds, respectively (Danon et al., 2013).

This work aims to further investigate the effect of organic solvents on furfural, HMF, and levulinic acid yields from five and six membered sugars in the presence of a homogeneous acid catalyst. In order to take into account the effect of the acid catalyst on the reaction, a different

form of the severity equation will be used, the combined severity factor (R_{CS}; Chum et al, 1990), which accounts for the pH of the system using

$$R_{CS} = log_{10}\left[t_{rxn}\exp\left(\left(\frac{T_{rxn}-100}{14.75}\right)\right)\right] - pH \qquad Eqn.\,1.2$$

Additionally, the findings will be applied to more complex systems that use biomass as the reactant to determine if pure sugars can be used to predict product yields. As part of the biomass reactions, compositional studies were performed to determine the amount of glucose, xylose, and other fractions within various Montana biomasses such as perennial forage crops (intermediate wheatgrass, smooth brome, and switchgrass), oilseed crops (canola, camelina, and crambe), and others (soybean, juncea, and rapa).

CHAPTER TWO

REACTIONS USING PURE SUGARS

Summary

Reactions utilizing model biomass sugars glucose, xylose, and arabinose were carried out in acid catalyzed reactions systems in aqueous-organic mixtures to determine the effects of solvent parameters and reaction severity on the yield of certain platform chemicals. Additionally, the pH of aqueous-organic mixtures was measured in a range of sulfuric acid concentrations from 0 M to 0.1 M and it was found that pH had a negative correlation with solvent polarity. That is, as polarity increased, the measured pH tended to be lower at a constant concentration of acid. In the absence of acid however, the pH of the solvent/water mixtures did not trend with polarity.

Reactions with glucose, xylose, and arabinose were used to model sugars found in lignocellulosic biomass. Reactions were catalyzed with sulfuric acid and carried out in pure aqueous and 50/50 organic-water mixtures using sulfolane, tetrahydrofuran (THF), and γ-butyrolactone (GBL). At all severities and in all reaction mediums, xylose tended to have a higher furfural yield than arabinose. A maximum furfural yield from xylose (58%) occurred in GBL at a severity of 3.41, while the maximum yield from arabinose (46%) occurred in GBL at a severity of 3.65. This agrees with other studies that have shown that arabinose reacts more slowly and produces significantly lower yields than xylose dehydration to furfural (Danon et al., 2014). The maximum furfural yield achieved by xylose in sulfolane (55%, 3.16 severity) was similar to that found by Romo et al. (2019) at a severity of 5.2. However, furfural yields do not

seem to be correlated with the Hansen Solubility Parameter for polarity (HSPP) as was shown in Romo's study.

In reactions using glucose as the reactant, HMF yields were relatively low at all severities as it reacts to form levulinic acid in acidic solutions (Kang et al., 2018a). Maximum HMF yield of 13% was achieved in both THF (R_o=*2.74*) and sulfolane (R_o=*3.04*) and begins to decrease as levulinic acid formation begins to dominate. Similar to the xylose and arabinose reactions, yields and conversions peak at lower severities in THF when compared to the other solvents. Water shows the highest levulinic acid yield of all solvents, approaching 65% at severities above a severity of 3.5.

Materials and Equipment

Sulfolane (Acros Organics), tetrahydrofuran (THF, Sigma Aldrich), λ-butyrolactone (GBL, Sigma Aldrich), 0.5 M sulfuric acid (Acros Organics), and 95% sulfuric acid (Fisher Chemical) were used as reaction solvents and catalysts. D-glucose (Fisher Chemical), xylose (Sigma-Aldrich), and arabinose (Sigma-Aldrich) were used as reactants. High-pressure glass, 15-mL reaction vessels were used along with Fisher Scientific hot plates and a silicon-based oil bath. K-type thermocouples and Omega data loggers were used to track temperature, and an Orion Sure-Flow pH probe was used to measure pH. Data was analyzed in an Agilent 1100 series HPLC using a Bio-Rad Aminex-87H column, pH 2 tetrafluoroacetic acid mobile phase at 0.6 mL/min, and a column temperature of 50 °C. Reagents were measured using Mettler-Toledo and Ohaus analytical scales.

Experimental Methods

Reactions were performed in 15-mL high-pressure glass reaction vessels with a total reaction volume of approximately 4 mL and a reactant weight percent of approximately 2 wt-%. A stir bar was added to a reaction vessel, followed by 0.08 g of a pure sugar. Next, the organic solvent to be used was pipetted into the vessel followed by sulfuric acid, and lastly water if required to reach the desired catalyst molarity. Each reaction was 50 wt% organic solvent, with catalyst concentrations ranging from 0.02 M to 0.5 M. A glass evaporation dish was filled with high heat resistance silicon oil and a large stir bar and placed on a hotplate. A glass thermometer, and two wire thermocouples were placed in the hotplate and held by a ring stand. One thermocouple was connected to the hot plate while the other went to an Omega data logger set to record temperature every 20 s. The hotplate was set to 190 °C and the stir rate set to 600 rpm. A 3D-printed plate was set atop the evaporation dish to hold up to 6 reactors upright in the oil bath. When the hotplate reached the set temperature as confirmed by the data logger, the reactor(s) was placed in the oil bath for between 15 minutes and 4 hours. When the reaction was completed, the vessel was removed from the oil bath and cooled using pressurized air until the reactor was approximately room temperature. A 1.5 mL sample was then filtered to 0.2 microns and placed in a vial for HPLC analysis. Two injections from each reaction sample were analyzed, and peak values averaged to determine conversion, yield, and selectivity.

The pH of 50/50 organic-aqueous solutions was measured separately from the reactions due to the low volumes present in the reaction vessels and the formation of insoluble products decreasing the stability of measurements. A 100 mL beaker was placed on a stir plate with 25 g DI water and 25 g of either THF, GBL, or sulfolane, along with a stir rod. With the stir rate set to

600 rpm, a pH probe (Orion Sureflow) was held in the solution as known volumes of 0.5M sulfuric acid were pipetted into the beaker. After each addition of acid, the pH was recorded after the probe reached a steady value. A plot of acid concentration against pH with an exponential best-fit line was created in order to calculate the pH of reaction mixtures based on the concentration of sulfuric acid and solvent. Measurements were confirmed using FisherBrand short range pH strips. Details on pH measurement can be found in the appendix.

Correlation plots were generated using Rstudio (see Appendix for code) using yield and conversion data determined from HPLC peak areas. Reaction yields were calculated using

$$Yield = \frac{P}{S_{initial}} \qquad Eqn.\ 2.1$$

where P is the number of moles of product formed as measured by HPLC, and $S_{initial}$ is the number of moles of sugar added to the reaction vessel. Conversion is defined by

$$Conversion = \frac{S_{final}}{S_{initial}} \qquad Eqn.\ 2.2$$

where S_{final} is the number of moles of sugar reactant present after the reaction, as measured by the HPLC, and $S_{initial}$ is the same as above.

Results and Discussion

The pH of several organic-aqueous solutions and pure water is shown in Figure 2.1 and trends downward with increasing solvent polarity (δ_p). At a given acid concentration, in 50-50 organic mixtures, sulfolane ($\delta_p \cong 15$) had the lowest pH, followed by GBL ($\delta_p \cong 14.5$) and pure water ($\delta_p \cong 14.25$), while THF ($\delta_p \cong 9$) had the highest pH. In mixtures of pure organic solvent and water, in the absence of an acid, the pH trends nearly opposite; sulfolane has the

highest pH (8.8), followed by 18.2 $M\Omega$ water (6.0), THF (5.2), and then GBL (4.2). Measurements were also taken using short range pH paper to confirm general trends (Table 2.1). While pH paper did not achieve the same level of distinction between solvents at very low pHs, the paper did confirm the trends seen at lower acid concentrations. It is possible the increased polarity of the solvent mixture increases the solvation of hydrogen ions, making dissociation more likely (Barbosa et al., 1999). Barbosa et al. studied the pH of buffer solutions in THF-water mixtures and found a positive correlation between the Kamlet-Taft polarity parameter (π^*) and the pH of aqueous/organic solutions. However, it should be noted that although π^* and δ_p both are measurements of solvent polarity, they are not directly equivalent. Also, the study by Barbosa et al. only included solvents with polarities of water and lower, which may show a different trend than higher polarity solvents, but increased solvation may be a reason for the trend seen in Figure 2.1. The solvent's effect on pH is further confirmed in the correlation plots shown for each reaction system (Figures 2.3, 2.6, and 4.5) as pH has a negative correlation with HSPP in each.

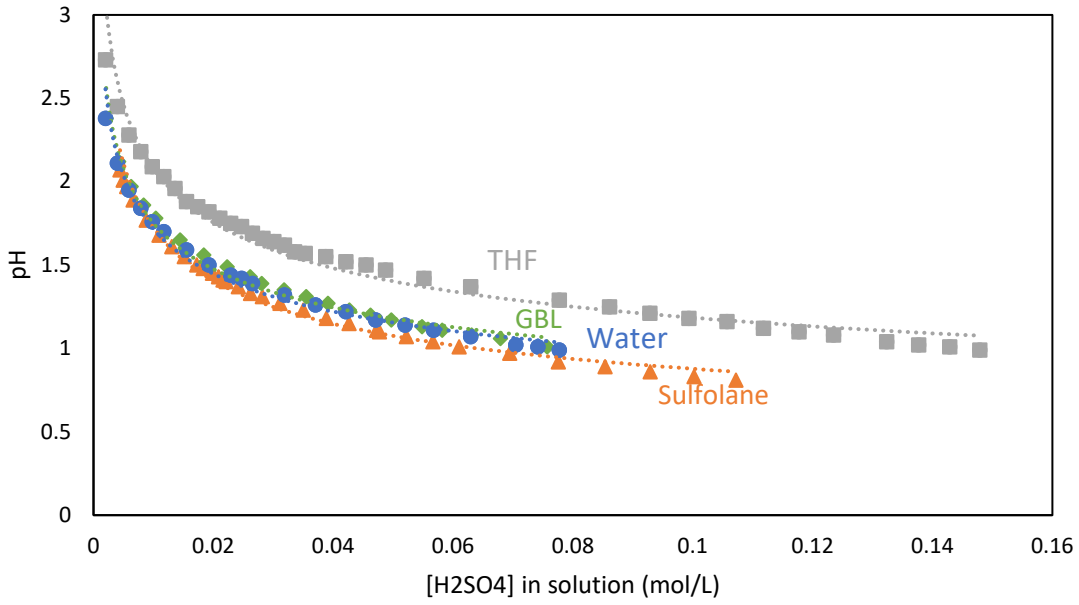


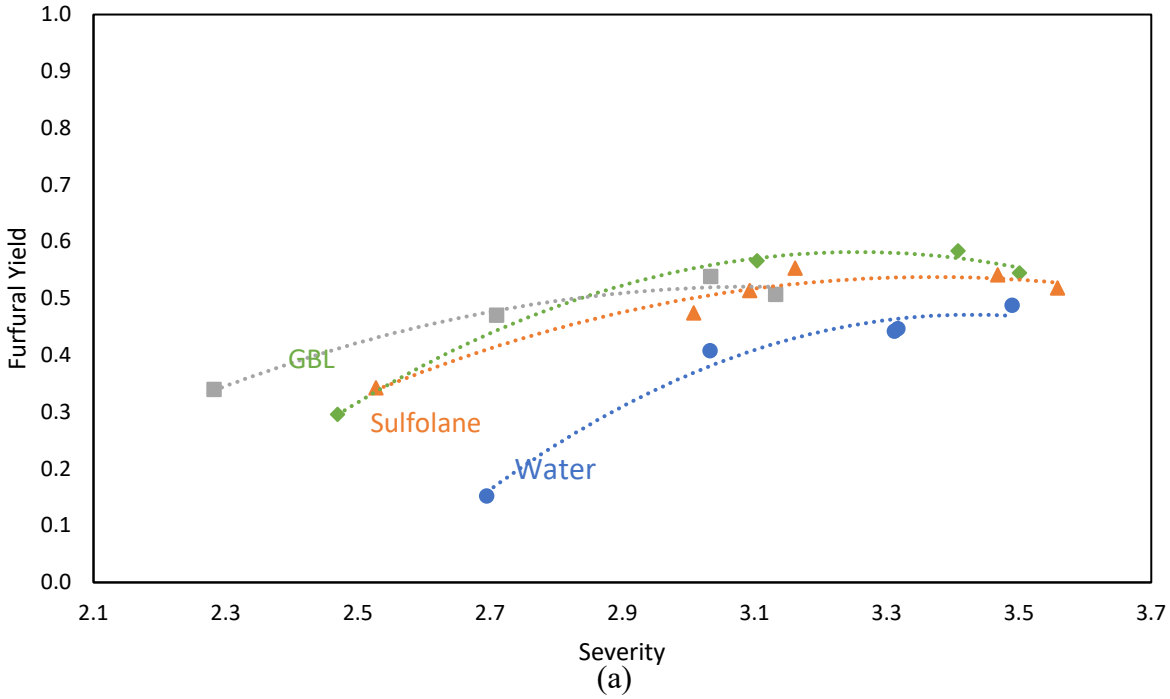
Figure 2.1: Concentration of sulfuric acid versus pH for several organic-aqueous mixtures and water.

Table 2.1: Comparison of pH strips and probe measurements in organic/water mixtures.

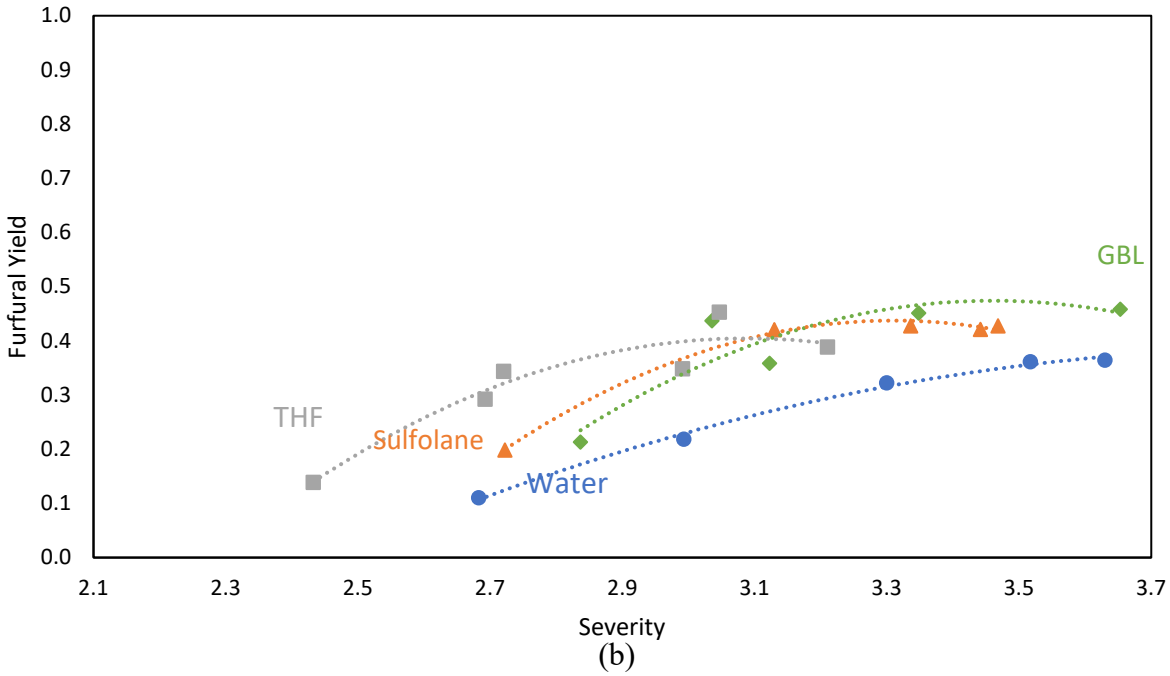
Solvent	**[H2SO4] (mol/L)**	**pH (strip)**	**pH (probe)**
Sulfolane	0.004	3	2.26
GBL	0.004	3.5	2.18
THF	0.004	3.5	2.67
Water	0.004	3	2.15
Sulfolane	0.012	1	1.65
GBL	0.012	1.5	1.68
THF	0.012	2.5	2.01
Water	0.012	2	1.65

Furfural yield from the xylose (Figure 2.2a) reaches a maximum at a severity of approximately 3.1 (3 h, 190°C, 0.1 M SA) and remains relatively constant or begins to decrease at higher severities. This trends with xylose conversion which increases with severity and begins to approach 100% near a severity of about 3.3 (Figures 2.2c). For arabinose, the maximum

furfural yield depended on the solvent ranging from a severity of 3.1 for THF to approximately 3.7 for GBL and water (Figure 2.2b). Compared to xylose, to achieve equal conversion of arabinose, a higher severity was needed (Figure 2.2d). Xylose reactions achieved higher furfural yields (55%) than arabinose reactions (45%) in all solvents. This has been confirmed by previous studies, which have shown that arabinose reacts more slowly and produces significantly lower yields than xylose dehydration to furfural (Danon et al., 2014). The maximum yield from xylose in a sulfolane reaction mixture is similar to that reported by Romo et al. (2019). For both sugars, as the severity increased past the maximum yield, furfural yield tended to decrease as the rate of formation slowed and degradation reactions became more prominent as furfural concentrations begin to increase. The solvent polarity had little influence on the furfural yield, as similar maximum yields were achieved in THF, GBL, and sulfolane. In all cases however, water had a lower yield than all other solvents tested and required a higher severity to achieve similar yields to the organic mixtures. Both yield and conversion reached a maximum at a slightly lower severity in THF than in other solvents but did not result in higher overall yields indicating that the reaction kinetics were faster in the THF mixture.

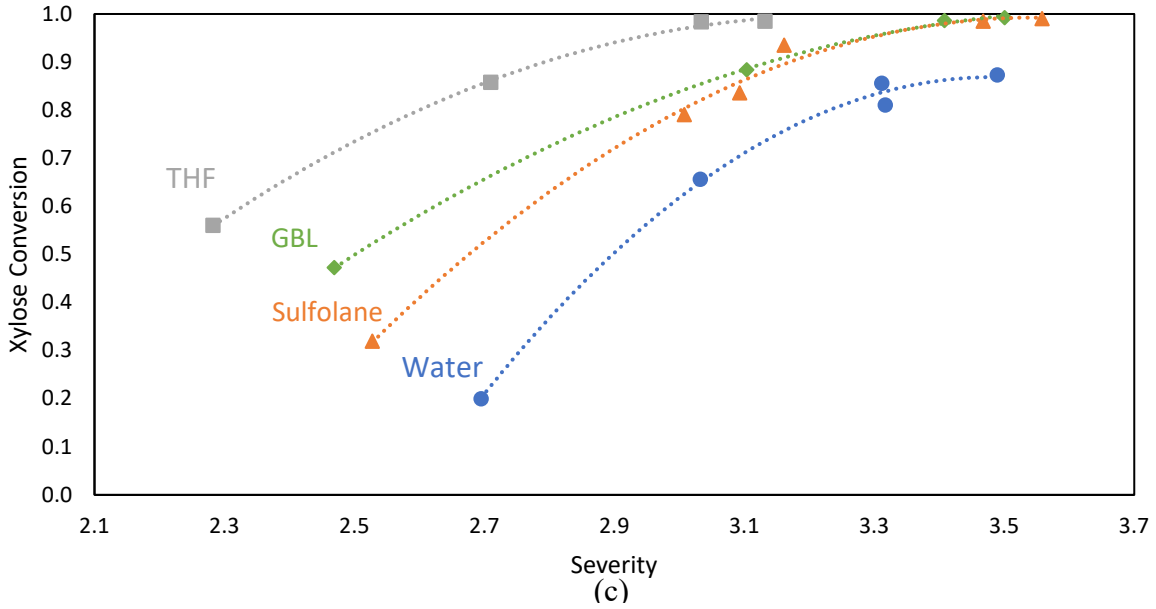


(a)

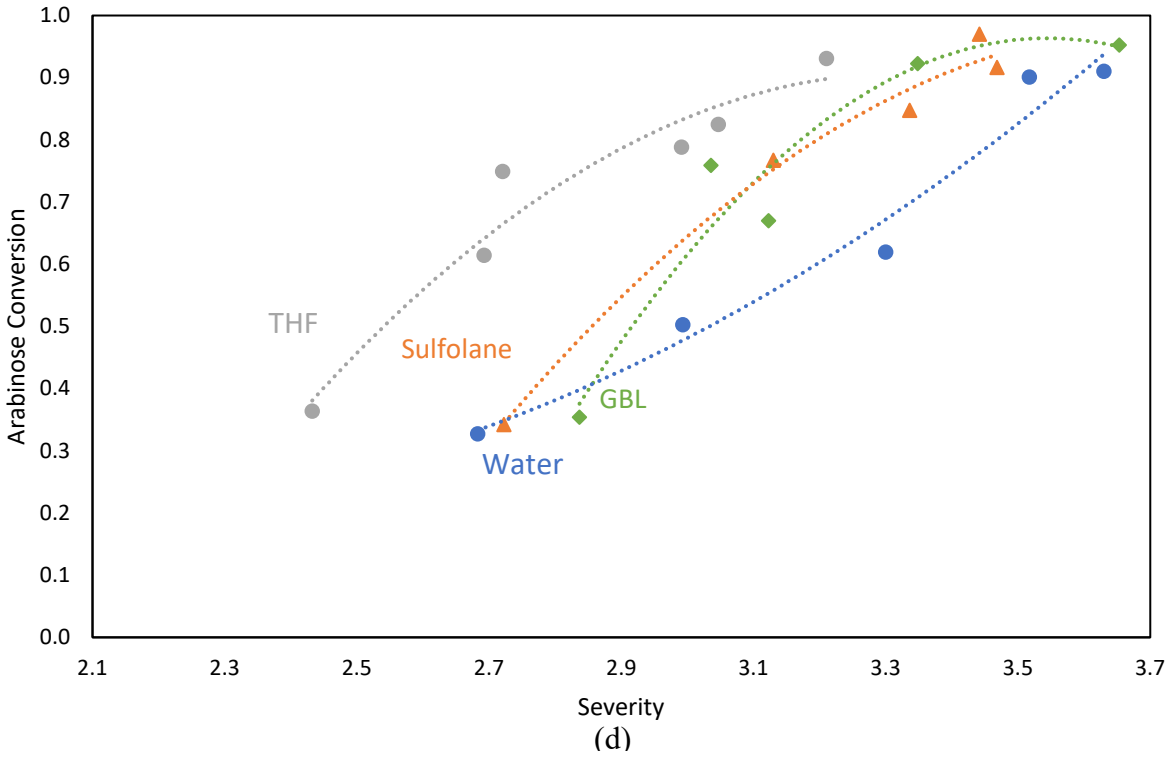


(b)

Figure 2.2a-b: Furfural yield from xylose (a) and arabinose (b) versus reaction severity in 50/50 organic-aqueous mixtures with sulfuric acid catalyst at 190 °C.



(c)



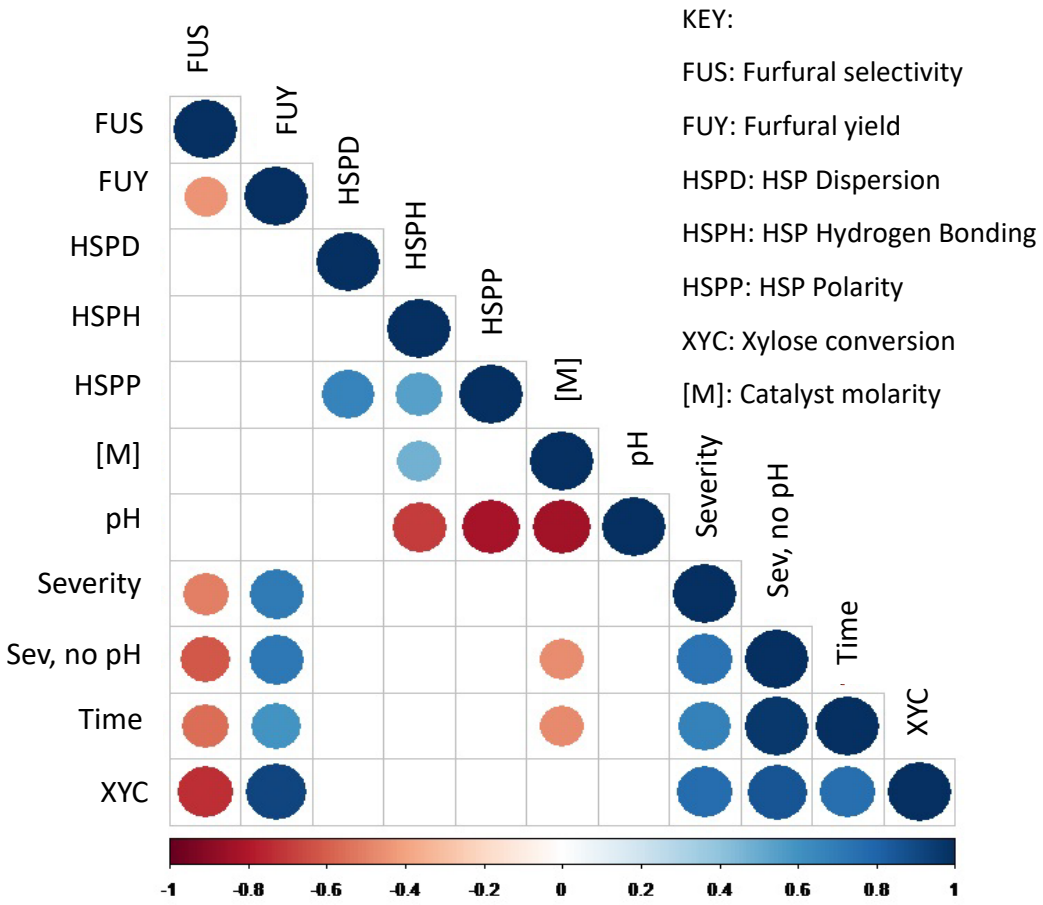
(d)

Figure 2.2c-d: Xylose (c) and arabinose (d) conversion in 50/50 organic-aqueous mixtures with sulfuric acid catalyst at 190 °C.

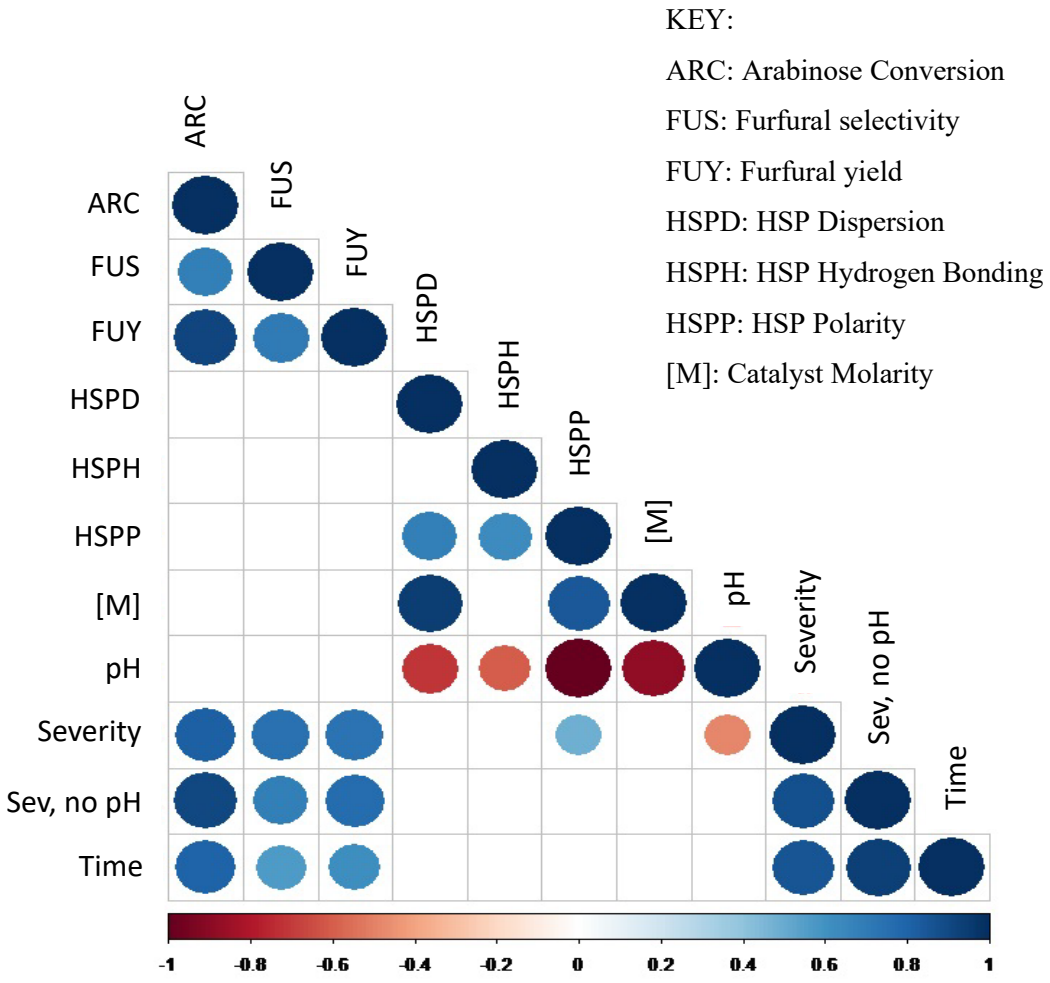
Correlation plots were made in R studio using reaction variables and data (Figure 2.3) for xylose and arabinose reactions. For both sugars, severity negatively correlated with pH, as expected by

$$R_o = log_{10}\left[t_{rxn}\exp\left(\left(\frac{t_{rxn}-100}{14.75}\right)\right)\right] - pH \qquad Eqn.\ 2.3$$

due to the increased concentration of acid catalyst decreasing the pH resulting in a higher severity. Furfural yield showed a positive correlation with severity, time, and temperature in both xylose and arabinose reactions, as would be expected by previous work by Danon et al. (2014) and Romo et al. (2019). Unlike previous work by Romo, no correlation was shown between HSPP and furfural yield at a 5% significance level. It is possible that solvent effects are dampened in acid catalyzed reactions and that incorporating the solvent mixture's pH into reaction severity accounts for the solvent effects.



Figures 2.3a: Correlation plot for xylose reactions in all solvents at varying severities. Positive correlations are shown in blue, negative correlations in red, while the size of the symbol indicates the strength of the correlation. Only correlations above a significance level of 0.05 are shown.



Figures 2.3b: Correlation plot for arabinose reactions in all solvents at varying severities. Positive correlations are shown in blue, negative correlations in red, while the size of the symbol indicates the strength of the correlation. Only correlations above a significance level of 0.05 are shown.

Reactions performed with glucose as the reactant formed HMF, which can be subsequently converted to levulinic and formic acids (see Figure 1.1). As the severity increased, the levulinic acid yields (Figure 2.5a) increased. HMF yields were relatively low at all severities as it is an intermediate to levulinic acid in acidic solutions (Kang et al., 2018a), but the maximum HMF yield of approximately 10% occurs around a severity of 3.0 and begins to decrease as levulinic acid formation begins to dominate.

Similar to the xylose and arabinose reactions, yields and conversions peak at lower severities in THF when compared to the other solvents. Sulfolane shows the highest levulinic acid yield of all solvents, approaching 65% at severities above a severity of 3.5. There appears to be a positive trend between levulinic acid yield and solvent polarity at severities higher than approximately 3.2. Interestingly, nearly all glucose reactions showed measurable quantities of furfural, between 1% and 4% yield. It is known that furfural is a minor byproduct in the hydrolysis of cellulose to glucose and its formation can be promoted in the presence of polar aprotic solvents and acids, but the mechanism through which it forms is not well understood (Asakawa et al., 2019).

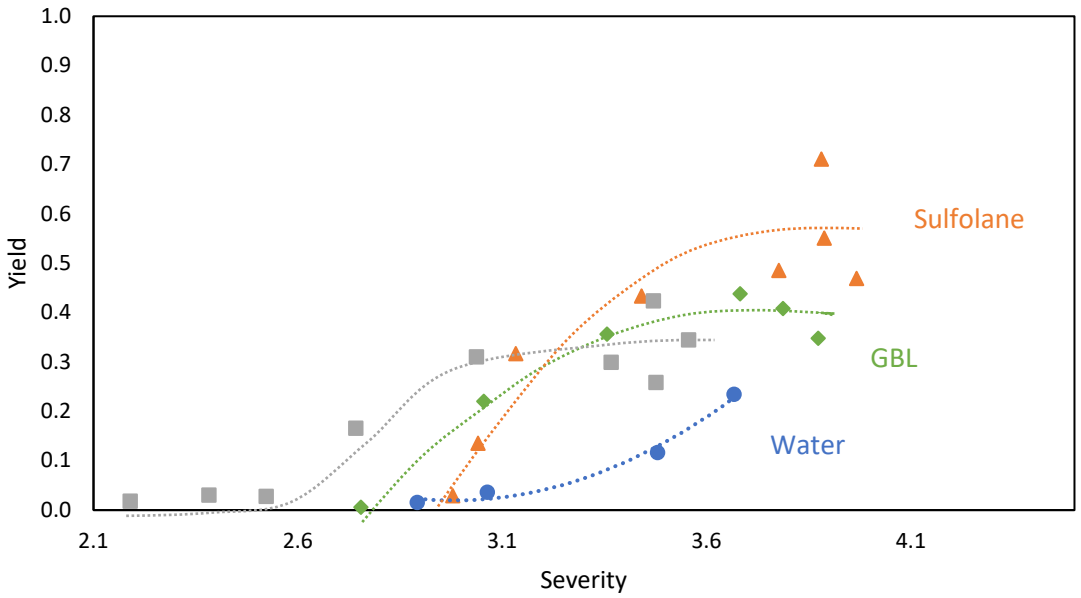


Figure 2.5a: Levulinic acid yield from pure glucose in 50/50 organic-water mixtures at 190 °C with a sulfuric acid catalyst.

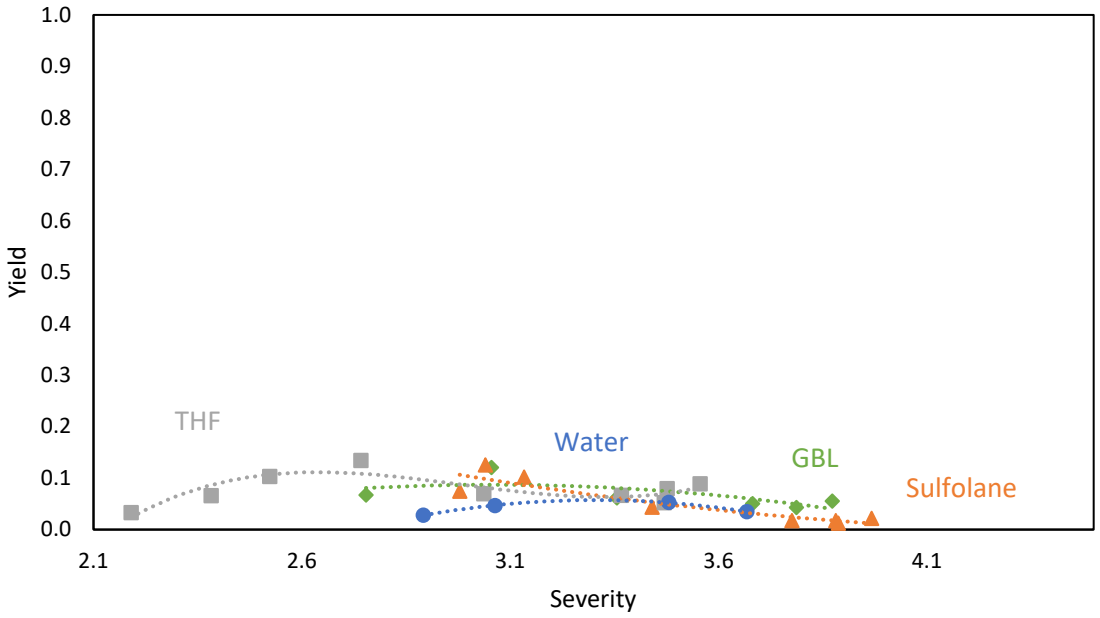


Figure 2.5b: 5-hydroxymethylfurfural yield from pure glucose 50/50 in organic-water mixtures at 190 °C with a sulfuric acid catalyst.

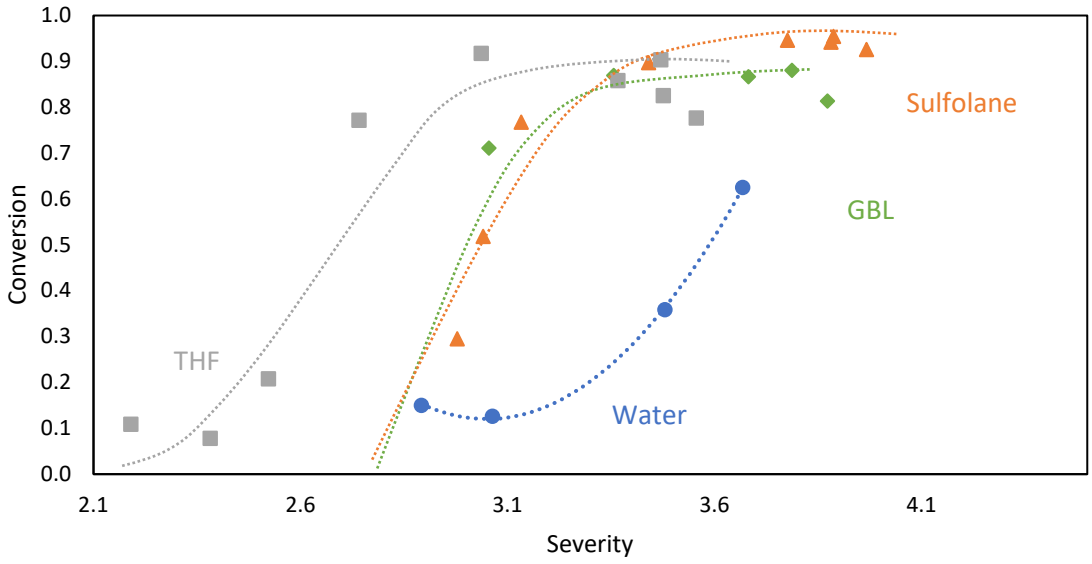


Figure 2.5c: Glucose conversion in 50/50 organic-water mixtures at 190 °C with a sulfuric acid catalyst.

The correlation plot for glucose only reactions (Figure 2.6) only shows significant (greater than 0.05) correlations and has similar findings as those generated for the pentose reactions. For example, there is a positive correlation between sugar conversion and reaction severity and a negative correlation between severity and pH. HMF yield shows a negative correlation with severity, while levulinic acid yield shows a strong positive correlation with severity and a strong negative correlation with pH. Interestingly, levulinic acid also shows a slight positive correlation with the polarity of the solvent reinforcing the visual trend in Figure 2.5a. This could be explained by the pH dependence of the rehydration reaction of HMF to levulinic acid, and the effect of solvent polarity on pH described earlier. Higher polarity solvents lead to lower pH conditions, thus exhibiting higher levulinic acid yields.

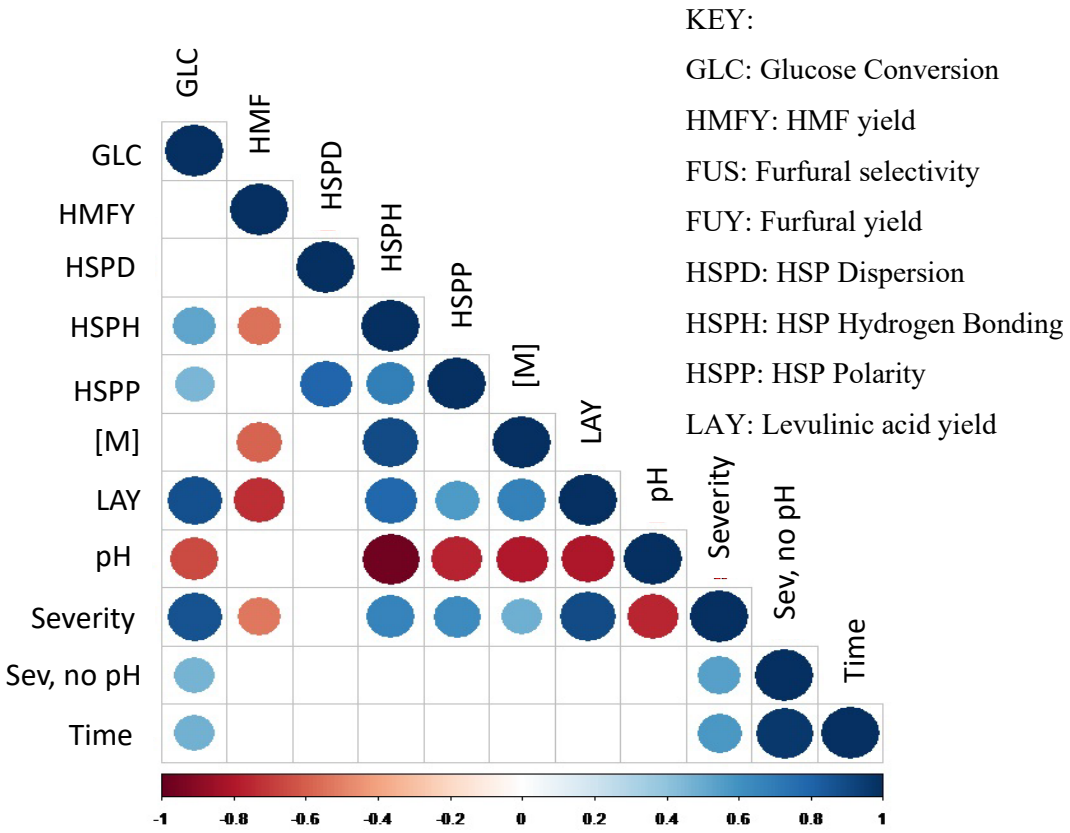


Figure 2.6: Correlation plot for pure glucose reactions in all solvents. Positive correlations are shown in blue, negative correlations in red, while the size of the symbol indicates the strength of the correlation. Only correlations above a significance level of 0.05 are shown.

Conclusions

The formation of furfural, 5-hydroxymethylfurfural (HMF), and levulinic acid in aqueous-organic mixtures was studied over a range of times, temperatures, acid concentrations, and solvent polarity to determine the effects of each on reaction conversion and yields. The solvent polarity affected pH and as polarity increased, pH decreased, possibly due to increased solvation making dissociation of H^+ ions more likely. When pH is incorporated into reaction severity (R_o), product yields follow similar trends to those shown in other studies (Danon et al., 2014; Romo et al., 2019) however, the effects of solvent polarity are less pronounced and may be

absorbed into the pH dependence of severity. It appears that the inclusion of pH into reaction severity may begin to capture solvent effects and more accurately represent the reaction conditions. Solvent polarity does not appear to have a significant effect on furfural yield as each solvent mixture resulted in higher yields than pure water, despite THF having a lower polarity than water.

CHAPTER THREE

COMPOSITION ANALYSIS ON CANOLA AND INTERMEDIATE WHEATGRASS BIOMASS AND THE EFFECT OF EXTRACTION

Contribution of Authors and Co-Authors

Manuscript in Chapter

Author: Lauren M. Johnsrude

Contributions: Lead writing of original draft, supporting writing of review and editing, ran composition experiments.

Co-Author: Aidan J. Scheffel

Contributions: Supporting writing of original draft. Lead writing for review and editing. Ran moisture experiments.

Co-Author: Brett L. Allen

Contributions: Supporting writing and conceptualization. Provided biomass samples. .

Co-Author: Stephanie G. Wettstein

Contributions: Lead on conceptualization, project administration, and resources. Supporting writing.

Manuscript Information

Lauren M. Johnsrude, Aidan J. Scheffel, Brett L. Allen, and Stephanie G. Wettstein

BioResources

Status of Manuscript:

____ Prepared for submission to a peer-reviewed journal

__X__ Officially submitted to a peer-reviewed journal

____ Accepted by a peer-reviewed journal

____ Published in a peer-reviewed journal

North Carolina State University

November 22, 2022

COMPOSITION ANALYSIS ON CANOLA AND INTERMEDIATE WHEATGRASS BIOMASS AND THE EFFECT OF EXTRACTION

Lauren M. Johnsrude,[a] Aidan J. Scheffel,[a] Brett L. Allen,[b] and Stephanie G. Wettstein[a,*]

Knowing the composition of biomass is critical for determining accurate yields of renewable chemicals and fuels; however, nonstructural components can affect the results of standard composition procedures leading to inaccurate reactant amounts. To remove these nonstructural components, water and/or ethanol extractions can be done, but the impact on composition values has not been well-reported. For this study, compositional analysis was performed on as-received canola (*Brassica napus*) and intermediate wheatgrass (*Thinopyrum intermedium*) as well as ethanol, water, and water/ethanol extracted biomasses. Water/ethanol extraction of the intermediate wheatgrass resulted in significantly lower xylose and both acid soluble and insoluble lignin amounts when compared to the as-received analysis. Since sugar was removed during the extractions, it is recommended to use the as-received composition values for glucuronoarabinoxylans; however, the extractives may interfere with the lignin analysis and therefore, the extracted lignin values are likely more reflective of the composition.

Keywords: Composition, extraction, glucose, xylose, lignin, biomass

*Contact information: a: Department of Chemical and Biological Engineering, Montana State University, P. O. Box 173920, Bozeman, MT 59718 USA; b: USDA-ARS, 1500 N. Central Ave., Sidney, MT 59270 USA *Corresponding author: stephanie.wettstein@montana.edu*

Introduction

In recent years, there has been a significant amount of research on upgrading lignocellulosic materials to chemicals and fuels due to theoretical carbon neutrality (Jeswani et al., 2020). Much focus has been on using regional biomass in order to decrease the overall process cost as it has been estimated that the cost of crop straw biomass is between $5,800-$6,500 per kilometer traveled, depending on the type of road used (Cheng et al., 2020). In Montana, wheat-based annual cropping systems are increasingly diversified with food-grade and industrial grade *B. napus* oilseed crops . Perennial forages such as intermediate wheatgrass, are commonly grown as well. These alternative crops allow for harvesting part of the plants, and then potentially upgrading the stover portion to add further value.

The stover and grass portion of lignocellulosic biomass is made up of three main components, cellulose, hemicellulose, and lignin, that can be readily upgraded into chemicals and fuels. Cellulose, comprised of linked glucose monomers can make up 25-40% dry weight of the feedstock. Hemicellulose, primarily glucuronoarabinoxylans (GAXs) in cereals and grasses, can make up 10-30% dry weight of the feedstock. Lignin, a phenolic polymer, is the third main component and comprises between 17-24% of the structure of grasses (B. B. Buchanan et al., 2015; Saake & Lehnen, 2007). Due to this variability in makeup, the precise composition of the plant material must be determined before any further upgrading can be carried out. A common method of quantifying the amount of carbohydrates is performing acid hydrolysis on a sample of biomass to break down the polymeric sugars into monomers and measuring the concentrations of the different sugars through chromatography. The resulting glucose, xylose, and arabinose concentrations can then be scaled to determine the polysaccharide concentrations in the sample

(Sluiter et al., 2012). However, biomass also contains non-structural components, such as non-structural sugars, ash, protein, and salts, that can interfere with sample composition analysis (Sluiter et al., 2008). These nonstructural components can be removed using appropriate solvents so that extractive-free biomass remains for further testing (Sluiter, 2011).

There are various methods for performing extractions on biomass including the National Renewable Energy Laboratory (NREL) NREL/TP-510-42619 procedure (Sluiter et al., 2008) and the Technical Association of the Pulp and Paper Industry (TAPPI) T 204 cm-97 (Buchanan, 2007) method. Both present specific procedures for removing solvent-soluble materials from biomass via a Soxhlet apparatus, which allows the solvent to reflux over the biomass and collect the extracted materials in a flask below the biomass. Water extraction is recommended to remove inorganic material, non-structural sugars and others while ethanol extraction is recommended to remove waxes, chlorophyll, and other minor components. If the extractives are not removed, the hydrophobic extractives may inhibit hydrolysis, and artificially high lignin values may occur due to unhydrolyzed carbohydrates condensing with the lignin (Sluiter et al., 2008).

Previous work (Thammasouk et al., 1997) has shown that both ethanol and water extractions on switchgrass, corn stover, and fescue affected the composition results. Individually, both water and ethanol extractions reduced the Klason lignin values and affected the apparent glucan content in most cases. These extractions are generally seen as giving a more accurate estimate of cellulose in the feedstock by removing noncellulosic, glucose-containing compounds. However, this method would reduce the actual glucose value due to sucrose removal, leading to inflated product yields from glucose if the biomass is used for upgrading reactions. Lower Klason lignin values were hypothesized to be due to the removal of solvent soluble compounds

that would otherwise become insoluble in the acid conditions used in Kalson lignin determinations (Thammasouk et al., 1997). Thammasouk demonstrated that a solvent extraction influenced the compositional analysis of a feedstock, but it is unknown what solvents are best suited for a certain feedstock as relative proportions of solvent-soluble components will vary between species. For example, corn stover was found to have much higher water-soluble extractives than ethanol-soluble extractives. Additionally, Thammasouk et al. did not evaluate the effect of water extraction followed by ethanol extraction, which is recommended for herbaceous biomass in the NREL/TP-510-48087 Summative Mass Closure laboratory analytical procedure (Sluiter, 2011).

In this paper, we present the composition results of two herbaceous biomass species, canola (*Brassica napus*) and intermediate wheatgrass (*Thinopyrum intermedium*), that have undergone ethanol, water, and water/ethanol extractions to determine the effect of extraction on composition.

Experimental

Materials and Methods

The ‘Manska’ intermediate wheatgrass and ‘Hyola 357 Magnum’ canola-quality *B. napus* were provided by the USDA-ARS in Sidney, MT. The canola was harvested at two sites: the Rasmussen (referred to as Ras) and Froid farms. Intermediate wheatgrass was harvested from the Froid farm. The above ground biomass was collected the day before combine harvest in 2012, dried at 55°C for 7 days, and then ground to 1 mm.

Biomass Moisture Content

Prior to analysis, each feedstock was sieved between No. 16 and No. 30 mesh sizes and stored in sealed bags or vials under ambient conditions. A Mettler-Toledo ME204E analytical balance was used to weigh 5 g of biomass onto a metal weigh tray of known weight. The biomass sample was spread evenly on the weigh tray and placed in the Kett FD100 moisture determination balance and heated to the set point of 250°C. The heat automatically shut off when the balance no longer recorded a change in mass and the moisture content was displayed, which took between 4-8 min. The biomass sample was then reweighed on the analytical balance to confirm the moisture content displayed by the FD100.

Extractions

Ethanol extractions were performed according to the TAPPI extraction method T 204 cm-97 (Buchanan, 2007). A 4 g sample of feedstock was weighed to the nearest 0.1 mg, placed in a cellulose thimble, and then set in a standard Soxhlet apparatus glassware. The extractions took place with 150 mL of 95% ethanol. The ethanol reflux was set at approximately 6 cycles/h for a total of 30 total solvent reflux cycles over a 5-hr period. Water extractions were performed similarly to the TAPPI procedure, but instead followed the total extraction time recommended by the NREL/TP-510-42619 laboratory analytical procedure (Sluiter et al., 2008), which was 20-24 hrs until the solvent was clear. Water-ethanol extractions were done by performing a water extraction, drying the biomass, and then performing an ethanol extraction. After each extraction, a small sample (~0.5 mL) was taken for HPLC analysis and the remaining solvent was transferred to a pre-weighed beaker and evaporated to 25 mL over a hot plate. The beaker and its

contents were then dried overnight in an oven at 105°C. The percent extractives was calculated from the dried extractives and the oven-dry mass of the initial feedstock added to the thimble.

Biomass Compositions

Using the NREL/TP-510-42618 laboratory analytical procedure (Sluiter et al., 2012), feedstocks were hydrolyzed in 72% H_2SO_4, diluted with DI water, and then autoclaved for 1 hr. The resulting liquid and solids were separated via vacuum filtration, and the remaining solids in the filtering crucible were heated to 105°C to determine the dry residue weight, and then heated to 575°C to ash the crucible so that the acid insoluble lignin could be calculated. The acid soluble lignin content was determined with a UV-Vis Spectrophotometer to measure the absorbance at 320 nm, which was then converted to a mass using an absorptivity value of 30 L/g*cm using the guidelines for corn stover in the NREL method. The monomeric forms of sugar in the liquid filtrate were quantified with high performance liquid chromatography (HPLC) and sugar recovery standards that were made according to the same laboratory analytical procedure (NREL/TAP 510-42618; Sluiter et al., 2012).

Results and discussion

In order to evaluate the effect of extraction method, water, ethanol, and water followed by ethanol extractions were carried out using the intermediate wheatgrass (Table 3) and canola from both the Froid (Table 4) and Ras (Table 5) sites. The composition data shown in Tables 3 - 5 are on an "as received," oven dried basis. The four different protocols followed were: a) no extraction and the composition determined using NREL/TP-510-42618, b) extraction using ethanol followed by composition analysis, c) extraction using water followed by compositional

analysis, and d) a water extraction as performed in c), followed by an ethanol extraction as performed in b), and then compositional analysis.

Table 3.1. Composition of Intermediate Wheatgrass as Determined by the Analysis of As Received, Ethanol, Water, and Water/Ethanol (W/E) Extracted Methods. Percentages are shown on an As Received Basis and standard deviations are in parentheses where applicable.

Extraction	n/a	EtOH	H2O	W/E
Moisture	4.4	4.4	4.2	4.4
Total glycans	59.9	55.5	59.7	55.2
Glucose	33.5 (0.6)	32.2	36.1	32.7 (1.7)
Xylose	24.2 (0.9)	21	21.1	20.2 (0.3)
Arabinose	2.3 (0.2)	2.22	2.5	2.3 (0.3)
Total Lignin	21.5	17.4	17.8	16.6
AIL	18.6 (0.8)	15.1	16.3	15.3 (0.5)
ASL	2.8 (0.1)	2.4	1.8	1.6 (0.1)
Ash	3.1 (0.2)	4	4.3	3.9
Extractives	n/a	8.3	16.8	19.5
Total mass balance	89	90	103	100

Table 3.2. Composition of Froid Canola as Determined by the Analysis of As Received, Ethanol, Water, and Water/Ethanol (W/E) Extracted Methods. Percentages are Shown on an As Received Basis. Percentages are shown on an As Received Basis and standard deviations are in parentheses where applicable.

Extraction	n/a	EtOH	H2O	W/E
Moisture	4.8	4.8	4.4	5.3
Total glycans	50.3	52.9	60.6	52.4
Glucose	29.7 (0.9)	31.2 (0.4)	36.6 (2.4)	31.6
Xylose	20.0 (0.6)	21.0 (0.1)	23.4 (2.1)	20.3
Arabinose	0.7 (0.03)	0.7 (0.01)	0.7 (0.1)	0.5
Total Lignin	23.2	22.4	26.5	22
AIL	21.3 (0.6)	20.7 (1.1)	25.0 (2.2)	20.7
ASL	1.9 (0.2)	1.8 (0.1)	1.7 (0.2)	1.4
Ash	0.9	1	2	0.1
Extractives	n/a	4.1	10.9	13.5
Total mass balance	79	85	104	93

Table 3.3. Composition of Ras Canola as Determined by the Analysis of As Received, Ethanol, Water, and Water/Ethanol (W/E) Extracted Methods. Percentages are Shown on an As Received Basis. Percentages are shown on an As Received Basis and standard deviations are in parentheses where applicable.

Extraction	n/a	EtOH	H2O	W/E
Moisture	4.9	3.8	4.7	3.9
Total glycans	55.2	57.2	57.3	54.1
Glucose	31.3 (0.5)	32.4	33.4	31.4
Xylose	22.9 (0.2)	23.9	23.1	22
Arabinose	1.0 (0.04)	0.98	0.81	0.72
Total Lignin	21.7	23.5	23.3	23.7
AIL	19.9 (1.1)	21.8	21.9	22.5
ASL	1.8 (0.04)	1.7	1.5	1.3
Ash	1.1	0.2	0.2	5.9
Extractives	n/a	2.2	8	9.8
Total mass balance	83	87	94	97

Removal of extracted materials and their inclusion in the composition improved mass balance closure for each of the tested biomasses for all extractions, which makes sense since the extractives are unaccounted for in the as received method. For intermediate wheatgrass (Table 3), 8.3% of the biomass was extracted using ethanol and 16.5% with water. When a water extraction was performed followed by an ethanol extraction, 19.5% of the biomass was extracted, indicating that approximately 3 wt% of the extractives could only be removed with ethanol. Ethanol extractions typically remove the waxy materials such as sap and resin, (Sluiter, 2011), which are typically not found in high amounts in herbaceous feedstocks. That means that ~5 wt% of the extractives removed with ethanol alone could be removed by water. Water extractions are recommended for herbaceous biomass to remove inorganic materials, proteins, and some carbohydrates, such as sucrose, and will also remove some of the inorganic materials that are extracted with ethanol (Sluiter, 2011). As seen in Fig. 3, ethanol extracted approximately

5 wt% of sugars, but no sugars were detected in the ethanol extraction that followed the water extraction confirming that water removes the majority of easily accessible sugars. For the intermediate wheatgrass biomass, both water and ethanol extractions are important to account extractives.

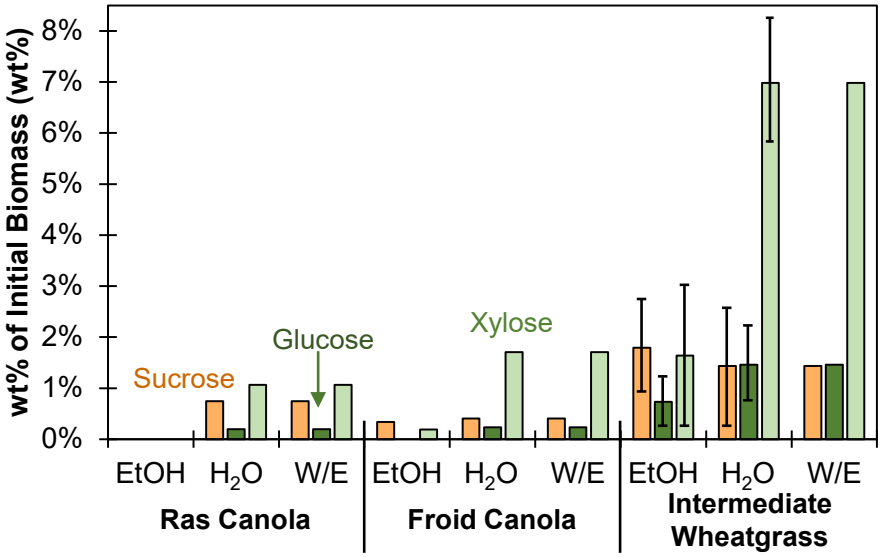


Fig. 3.1: Amount of sucrose (■), glucose (■) and xylose (■) in the extractives after ethanol, water, or water/ethanol extractions of canola and intermediate wheatgrass. Standard deviation error bars were added to the analyses with at least three values.

Both canola samples had a lower amount of extractives compared to intermediate wheatgrass. Ethanol extracted 2.2-4.1% and water extracted 8-10.9%. In the case of the water/ethanol extractions, 9.8-13.5% of extractives were removed, which were all within experimental error. In the case of Froid canola, less than 1.5% of extractives were removed using either water or ethanol. However, this means that of the 4.1% removed by ethanol extraction, 1.5% of that portion was also removed by water, indicating that only 2.6% of extractives were removed with ethanol alone. Due to the small percentage removed, ethanol extraction may not be necessary for accurate compositional analysis of canola. This is confirmed when comparing the

lignin values in Table 4. The total, acid insoluble, and acid soluble lignin values are all within error for the as received and ethanol extracted samples.

The extractives from the intermediate wheatgrass and canola were analyzed for composition although it should be noted that variability in the extractives dryness can lead to variability in these measurements(Milne et al., 1992). All samples, excluding the Ras canola extraction with ethanol, had xylose peaks present ranging from 0.2 wt% to 7 wt% of the initial biomass and most, excluding the ethanol extracted canola samples, had glucose peaks as well, ranging from 0.2 wt% to 2.9% of the initial biomass (Figure 3). The extractions that used water had higher amounts of both xylose and glucose present, which was expected since sugar is more soluble in water compared to ethanol (Alves et al., 2007). It is worth noting that the ethanol extraction after water extraction did not contain detectable amounts of sugars in any sample and therefore, the bars for W/E and H_2O are equivalent in Figure 3. The canola sample had a maximum amount of 2 wt% sugars extracted whereas the intermediate wheatgrass had up to 8.4 wt% of the initial sugars removed during the extraction process. This could indicate that intermediate wheatgrass contains more nonstructural carbohydrates or that the sugars in intermediate wheatgrass are more susceptible to removable and are easier to access than for the canola sample. Nonstructural carbohydrates (NSCs) include important transport molecules such as sucrose and storage carbohydrates such as amylose and amylopectin, which make up starch and concentrations of NSCs have been shown to be highly variable, fluctuating seasonally as well as daily, as sucrose is converted to starch and back (Hartmann & Trumbore, 2016). Amylose and Amylopectin are both insoluble in ethanol; however, a small fraction of amylose can be extracted with hot water (Green, 1975).

Although extractives are useful to quantify, the carbohydrates and lignin content of biomass are typically the fractions of interest and extractives can interfere with the characterization of these materials (Sluiter, 2011). In the case of the carbohydrate and lignin analysis, the composition of both the intermediate wheatgrass and canola were approximately 30% glucose, 20-24% xylose, less than 2% arabinose, and 17-27% lignin depending on extraction method. Water extractions resulted in the highest amount of glucose compared to the as-received compositions (Tables 3 and 4), while changes in content of other sugars depended on the extraction method and biomass.

For the intermediate wheatgrass, t-tests showed that xylose, AIL, ASL, and total lignin were significantly different for the non-extracted and water/ethanol extracted compositions (Figure 4C). For all variables, the non-extracted values were higher than the water/ethanol extracted biomass. The lower lignin values are similar to the results Thammasouk et al. saw in their research (Thammasouk et al., 1997). They determined that the extracted, lower lignin values would be more accurate since the lignin values of the non-extracted biomass likely contained condensed extractives as well, which is also mentioned in the LAP document (Sluiter, 2011). Since extractives make up 20% of the intermediate wheatgrass and ash is 3% (Table 3), that certainly could be the case. The significantly lower xylose percent for the water/ethanol extracted sample can likely be explained by the amount of xylose that was extracted for the intermediate wheatgrass sample, which was 7.0 wt% of the initial biomass (Figure 3). As mentioned previously, the sugars in the intermediate wheatgrass may be more accessible than for the canola sample. For the non-extracted versus extracted canola, there was less of a difference than with the intermediate wheatgrass for glucose and lignin values. Both canola samples had

less extractives and less ash (Table 4), indicating that the lower amount of extractives and ash present may interfere less with the acid hydrolysis than in the case of the intermediate wheatgrass.

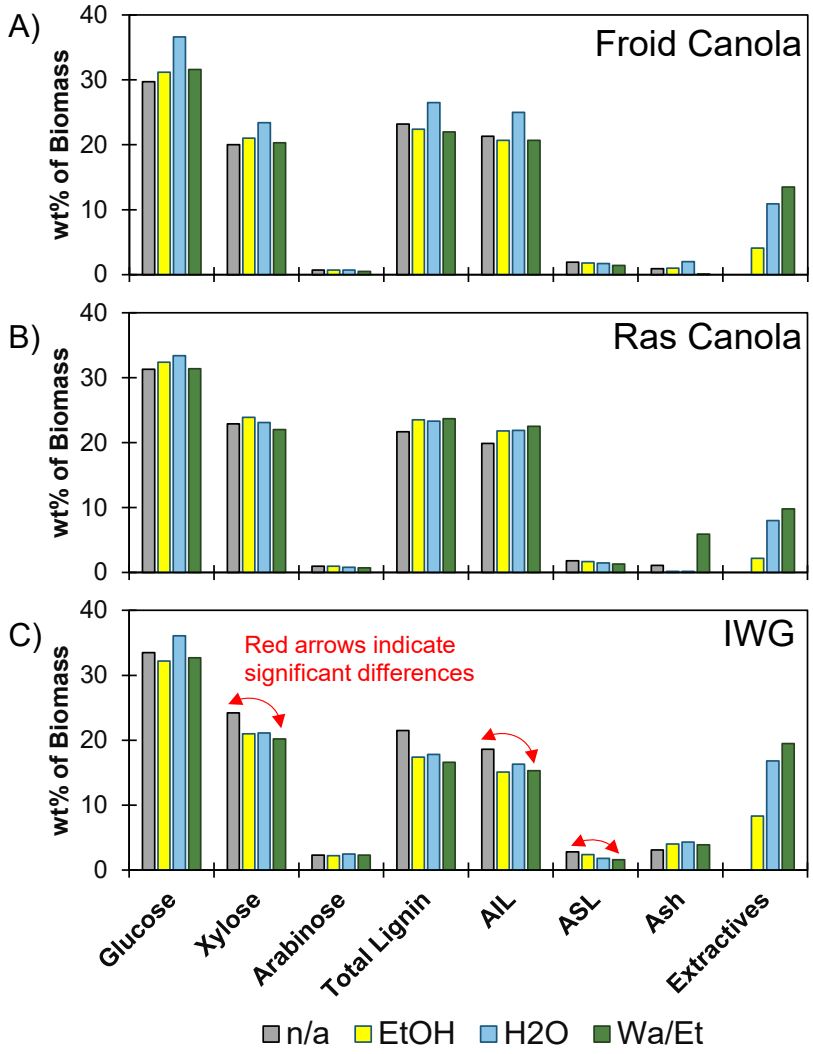


Figure 3.2: Compositions of A) Froid Canola, B) Ras canola, and C) intermediate wheatgrass biomass for non-extracted (■), ethanol extracted (■), water extracted (■), and water/ethanol extracted (■) biomasses. Statistical analysis was only performed on the intermediate wheatgrass due to sample size.

Conclusions

1. Extracting the biomass had a significant effect on the lignin and glucose values of intermediate wheatgrass.
2. If the amount of lignin is desired, it is suggested to perform, at minimum, an ethanol extraction prior to compositional analysis in order to have a more accurate representation of the lignin values of herbaceous biomass.
3. If the percent glucose is desired, it is suggested to perform composition analysis on as received biomass in order to account for the glucose in the non-structural sugars (i.e., sucrose), found in herbaceous biomass.
4. Intermediate wheatgrass was more susceptible to sugar removal during extraction than the canola as indicated by the high amounts of sugars in the extractives after water, ethanol, and water/ethanol extractions.

CHAPTER 4

ACID CATALYZED BIOMASS REACTIONS

Summary

Homogeneous acid catalyzed reactions using intermediate wheatgrass as the reactant were carried out in organic-aqueous mixtures to determine furfural and levulinic acid yields and the effects of solvent properties and reaction conditions. ‘Manska’ intermediate wheatgrass was provided by the USDA-ARS in Sidney, MT and the trends seen were very similar to those found using model sugars (Chapter 2), despite the increased complexity of the reaction system when using biomass. Similar maximum yields were seen in each solvent mixture, and each solvent used demonstrated higher yields than pure aqueous reactions. Maximum furfural yield (68%) was achieved in GBL at a severity of 3.3, while HMF yield was maximized in sulfolane (37%) at a severity of 2.5. The highest levulinic acid yield seen was 96% in sulfolane at a severity of 3.9. With the exception of HMF, reaction mixtures in THF showed higher yields and conversions at lower severities when compared to the other solvent mixtures, another trend that is mirrored from the model sugar reactions in Chapter 2. When considering levulinic acid and furfural, the choice of solvent does seem to matter because they all achieve similar maximum yields. While the trends seen in these reactions seem to mirror those from Chapter 2, the yields themselves are, in general, higher. There were a number of factors that may have contributed to this, but the most likely ones were the lower sugar concentrations seen in the biomass reactions and unaccounted for reacting sugars.

Materials and Equipment

Intermediate wheatgrass was provided by the USDA Agricultural Research Service in Sidney, MT. The above ground biomass was collected the day before combine harvest in 2012, dried at 55°C for 7 days, and then ground to 1 mm. Sulfolane (Acros Organics), tetrahydrofuran (THF, Sigma-Aldrich), gamma-butyrolactone (GBL, Sigma-Aldrich), 0.5 M sulfuric acid (Sigma-Aldrich), were used to form reaction mediums. High-pressure, 15-mL reaction vessels were used along with Fisher Scientific hot plates and a silicon-based oil bath. K-type thermocouples and Omega data loggers were used to track temperature, and an Orion Sure-Flow pH probe was used to measure pH. Data was analyzed in an Agilent 1100 series HPLC using a Bio-Rad HP87X column and pH 2 tetrafluoroacetic acid mobile phase at 0.6 mL/min and a column temperature of 50 °C. Reagents were measured using Mettler-Toledo and Ohaus analytical scales.

Experimental Methods

Reactions were performed in 15-mL high-pressure glass reaction vessels with a total reaction volume of approximately 4 mL and a reactant weight percent of approximately 2 wt%. A stir bar was added to a reaction vessel, followed by 0.08 g of intermediate wheatgrass sieved between mesh sizes #16 and #30. Next, the organic solvent to be used was pipetted into the vessel followed by sulfuric acid, and lastly water if required to reach the desired catalyst molarity. Each reaction was 50 wt% organic solvent, with catalyst concentrations ranging from 0.02 M to 0.5 M. A glass evaporation dish was filled with high heat resistance silicon oil and a large stir bar and placed on a hotplate. A glass thermometer, and two wire thermocouples were

placed in the hotplate and held by a ring stand. One thermocouple was connected to the hot plate while the other went to an Omega data logger set to record temperature every 20 s. The hotplate was set to 190 °C and the stir rate set to 600 rpm. A custom, 3D-printed plate was set atop the evaporation dish to hold up to 6 reactors upright in the oil bath. When the hotplate reached the set temperature as confirmed by the data logger, the reactor(s) was placed in the oil bath and a timer was started. When the timer went off, the reaction was removed from the oil bath and cooled using pressurized air until the reactor was approximately room temperature. A 1.5 mL sample was then filtered to 0.2 microns and placed in a vial for HPLC analysis. Two injections of each reaction were analyzed, and peak values averaged to determine conversion, yield, and selectivity.

The pH of 50/50 organic-aqueous solutions was measured separately from the reactions due to the formation of humins as described in Chapter 2. A plot of acid concentration against pH with an exponential best-fit line was created in order to calculate the pH of reaction mixtures based on the concentration of sulfuric acid and solvent.

Correlation plots were generated using RStudio (see Appendix for code) using yield and conversion data calculated from HPLC peak areas. Reaction yields are expressed as a fraction, shown by

$$Yield = \frac{P}{S_{initial}} \qquad Eqn. 4.1$$

where P is the number of moles of product formed as measured by HPLC, and $S_{initial}$ is the number of moles of sugar in the biomass. Conversion is defined by

$$Conversion = \frac{S_{final}}{S_{initial}} \qquad Eqn. 4.2$$

where S_{final} is the number of moles of sugar reactant present after the reaction is halted, as measured by the HPLC, and $S_{initial}$ is the same as above.

Results and Discussion

Figure 4.1 shows furfural yields from intermediate wheatgrass in all solvents with the yields at each point being an average of all reactions within a severity of 0.05 of that point to improve figure clarity. For example, the yield in sulfolane at a severity of 2.70 is an average of reactions at a severity of 2.65-2.75. The trends observed match those seen in reactions involving model sugars (Chapter 2). In almost all cases, water had the lowest furfural yield at a given severity, while sulfolane and GBL showed comparable yields. At lower severities, THF tended to have a higher yield than the other solvents, but this was less pronounced above a severity of about 2.5. At higher severities, THF showed a lower yield than sulfolane or GBL. The highest maximum furfural yield of 70% occurred in sulfolane, while water achieved a maximum yield of only 46%. For all reaction mixtures, the highest yield occurred at a severity range between 3-3.5. At severities above approximately 3.5 (and around 3.0 in THF), yields began to decrease. Furfural degradation occurs through a variety of mechanisms including polymerization with other furfural molecules and condensation reactions involving xylose molecules (Cai et al., 2014; Danon et al., 2013; Garrett & Dvorchik, 1969; Peleteiro et al., 2016). These degradation products are not detectable by acid column liquid chromatography, but their effect on furfural yield was easily seen.

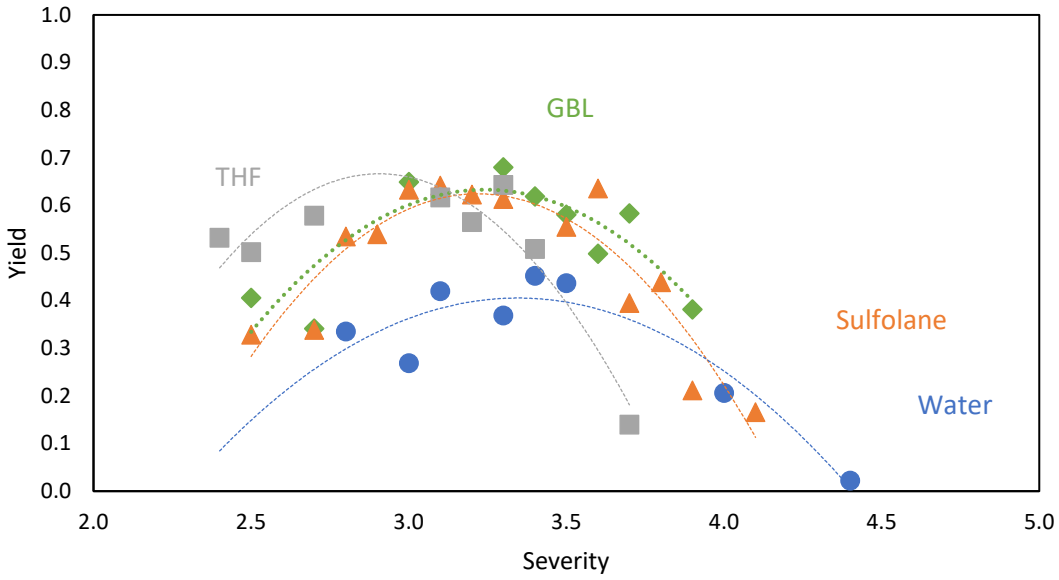


Figure 4.1: Furfural yield from intermediate wheatgrass in all solvents.

Interestingly, the yields achieved with biomass are generally higher than those shown in Chapter 2 in reactions involving pure sugars. Several reasons may be why that is the case. 1) An important consideration for biomass reactions is that the furfural yields are calculated using the number of moles of xylose and arabinose initially present. The sugar content of the biomass was determined by the NREL/TP-510-42618 laboratory analytical procedure (Sluiter et al., 2012) as described in Chapter 3, but it is possible that inaccuracies in the composition determination are leading to inflated yields. However, this would need to be a substantial error in the composition. 2) It is known that hemicellulose is composed of an amalgam of pentoses that may be converted to furfural with a range of activation energies (Hughes et al., 1939). Only accounting for the two largest components, xylose and arabinose, may be underestimating the true number of moles of sugars available for reaction, leading to artificially inflated yields. However, when using a carbohydrate specific column (Zorbax Carbohydrate Analysis), glucose, xylose, and arabinose were the only sugars detected by the HPLC. There are reaction pathways that can convert

hexoses into furfural, however, that are not being accounted for in the furfural yield calculation (Asakawa et al., 2019). This was confirmed when appreciable amounts of furfural were produced in reactions involving pure glucose where HMF and levulinic acid were also produced. Additionally, furfural degradation reactions are a function of both furfural and reactant concentrations in the reaction mixture. It is possible that there is less furfural degradation in biomass reactions because of the lower concentrations of reactant sugars present. Each of the reactions in Chapter 2 was performed at 2 wt% reactant. Biomass reactions were carried out at 2 wt% biomass, which equates to less than 1 wt% of any of the reactant sugars. Each of these factors likely contributes to compounding differences in the yield. However, the reaction severity, R_o, still appears to be the variable with the most correlation to yield.

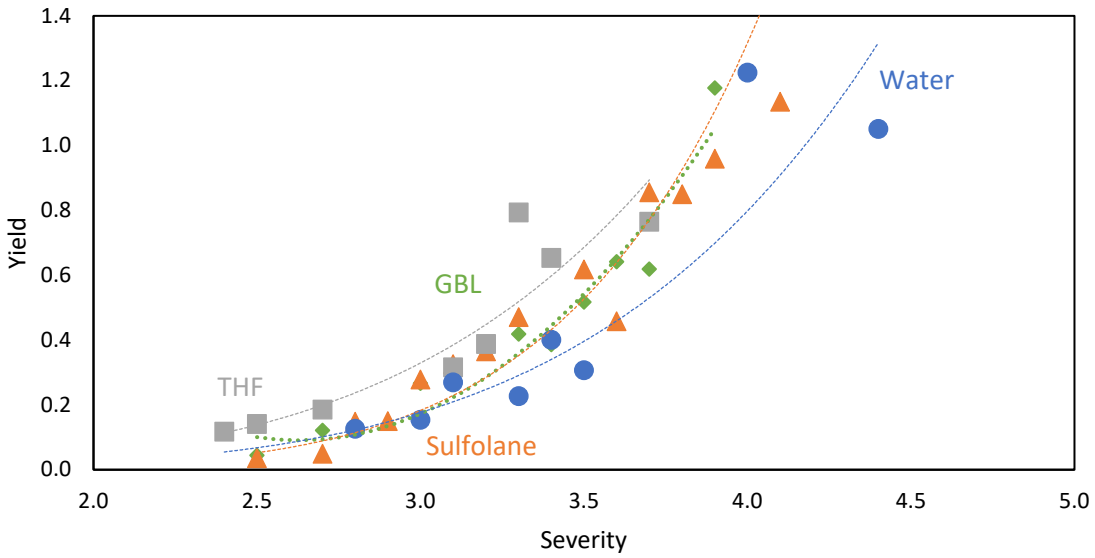


Figure 4.3: Levulinic acid yield from intermediate wheatgrass in all solvents.

At lower severities, levulinic acid yield from intermediate wheatgrass differed from trends seen in Chapter 2 with pure glucose as the reactant. THF had a higher yield than other solvents at a severity less than 3, but the other three solvents had similar yields until severity was

above approximately 3. Above that point, water had the lowest yield, and the other three solvents showed comparable yields throughout. Unlike Figure 2.5a, there was no decrease seen in levulinic acid yields at high severities.

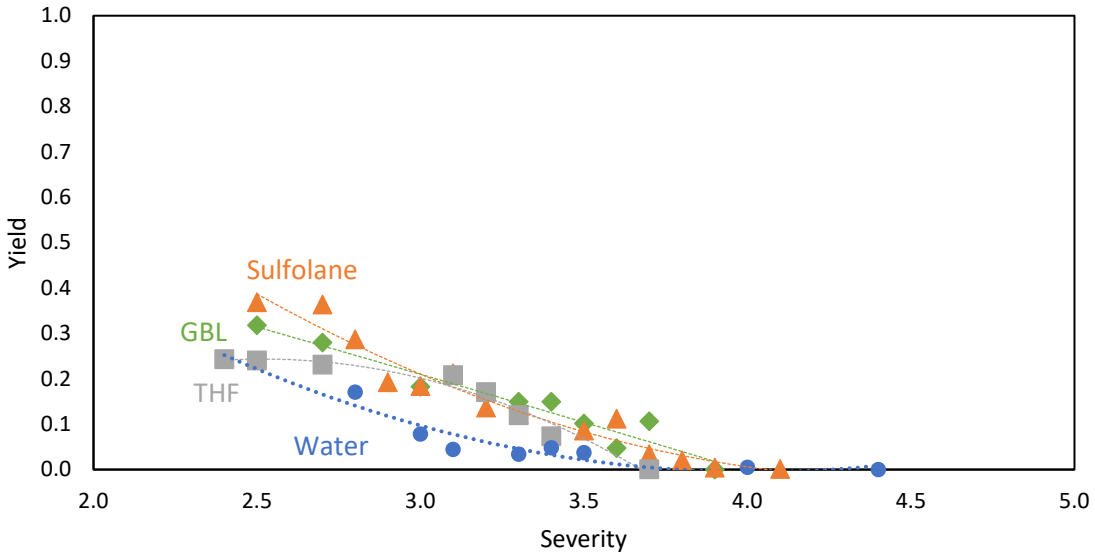


Figure 4.4: 5-hydroxymethylfurfural yield from intermediate wheatgrass in all solvents.

HMF yields shown in Figure 4.4 were significantly higher than those found in reactions using pure glucose. In those reactions, maximum HMF yield was only 13% at a severity of approximately 3, but with biomass the HMF yield was nearly 20% in the organic mixtures and about 8% in water at the same severity. However, as seen with pure sugars, all solvents tended to have a higher yield than water.

A correlation plot for all intermediate wheatgrass reactions is shown in Figure 4.5 and many of the significant correlations seen in pure sugar reactions are also seen in intermediate wheatgrass reactions: severity has a negative correlation with pH, levulinic acid yield has a negative correlation with pH and a positive correlation with severity, and HMF and levulinic acid yield have a strong negative correlation. Again, the solvent Hansen Solubility Parameters

for polarity, dispersion, and hydrogen-bonding (HSPP, HSPD, and HSPH, respectively) showed no significant correlations with product yields, possibly indicating that solvent choice did not have an effect on yield in acid catalyzed reactions. However, in this plot, furfural yield had a slight negative correlation with severity, contrary to what is seen in the correlation plots in Chapter 2. This could have been due to the inclusion of more reactions where furfural degradation was prominent than in Figure 2.3. Furfural yield increased steadily from a severity of 2.5 to approximately 3 in both pure sugar and intermediate wheatgrass reactions. Many more reactions at severities past the peak furfural yield were included in the biomass data (Figure 4.5), where furfural yield shows a negative correlation with severity.

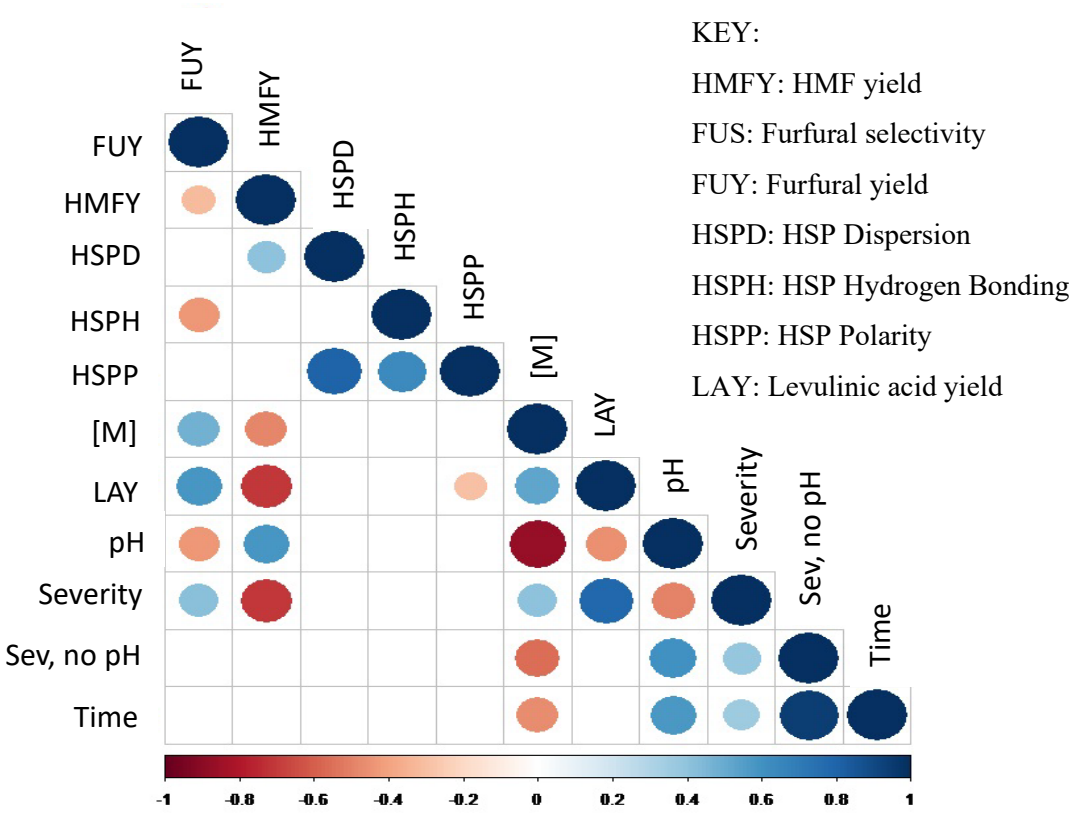


Figure 4.5: Correlation plot for intermediate wheatgrass reactions in all solvent mixtures. All reactions were held at 190 C with sulfuric acid concentrations ranging from 0.01 M to 0.5 M. Positive correlations are shown in blue, negative correlations in red, while the size of the symbol

indicates the strength of the correlation. Correlations below a significance level of 0.05 are not shown.

Conclusions

The formation of furfural, HMF, and levulinic acid from biomass was studied in three organic-aqueous mixtures and pure aqueous systems to determine the optimal conditions for maximum yield. Intermediate wheatgrass was reacted with a heterogeneous acid catalyst, sulfuric acid, in mixtures of THF-water, GBL-water, sulfolane-water, and pure water and it was found that reactions using biomass tended to follow the trends observed in reactions using model sugars. Solvent choice did not seem to have an effect on maximum yield once pH was included in reaction severity. THF tended to have higher yields and conversions than other solvents at lower severities, but this trend disappeared as severity increased past 3.0. It is possible that solvent effects are captured by incorporating the pH of the organic-aqueous mixture into the severity. Product yields from the intermediate wheatgrass tended to be higher than those in model sugars, which is contrary to previous work. This could be due to a lower initial reactant percent leading to fewer degradation products, unaccounted for sugars in the biomass compositional analysis, and unaccounted for reaction pathways.

CHAPTER 5

CONCLUSIONS

The conversion of lignocellulosic biomass into platform chemicals is a promising method to decrease fossil fuel usage moving forward. Lignocellulosic biomass contains cellulose and hemicellulose that can be broken down via hydrolysis into sugars such as glucose, xylose, and arabinose to be reacted further to produce valuable platform chemicals like furfural, HMF, and levulinic acid. The formation of these chemicals is highly dependent on reaction conditions, and a large body of work dedicated to the study of these reaction conditions exists. Much focus recently has been on improving yields through the manipulation of solvent properties and reaction conditions. Previous work proposed that, in mixtures containing organic solvents and water, furfural yield was dependent on the polarity of the mixture (Romo et al., 2019). The Hansen Solubility Parameter: Polarity was found to be positively correlated with furfural yield, possibly due to the stabilization of a carbocation intermediate. Higher polarity solvent mixtures showed higher furfural yields than lower polarity mixtures in auto-catalyzed reactions of pure xylose.

This work investigated the effect of solvent properties on the yields of furfural, levulinic acid, and HMF in acid catalyzed reactions using model sugars and intermediate wheatgrass as reactants. Reactions using model sugars were first studied to extend the findings of Romo et al. to acid catalyzed reactions. Xylose and arabinose were reacted in 50-50 mixtures of sulfolane, tetrahydrofuran (THF), and gamma-butyrolactone (GBL) in water, over a range of severities, using a sulfuric acid catalyst. In these acid catalyzed reactions, solvent effects appeared to be negligible: each organic-aqueous mixture produced higher yields than pure aqueous systems and

required lower severities to reach maximum yields. This was true of both furfural yield from xylose and arabinose, as well as HMF and levulinic acid yields from glucose. Rstudio correlation plots were used to confirm these findings. Yields generally showed no correlation or weak correlations with solvent parameters, but positive correlations with reaction severity. THF typically achieved maximum yield at a lower severity than other solvents and warrants further study to determine possible causes for this phenomenon.

Next, reactions utilizing a biomass reactant were carried out to compare to the results using pure sugar reactants. Intermediate wheatgrass was analyzed using NREL standard methods to determine the composition of structural carbohydrates on an as-received basis. Then, reactions were carried out in the same manner as those with pure sugars: 50-50 solvent-water mixtures using a sulfuric acid catalyst. Similar trends were observed as before: similar maximum yields were achieved in all solvents, and each solvent mixture produced higher yields than water, with the exception of levulinic acid. Water produced a higher maximum levulinic acid yield than THF, albeit at a much higher severity. As before, THF required lower severities to reach maximum yield, which was in general, lower than maximum yields in the other solvent mixtures. Higher percent yields were produced in the biomass reactions than those of pure sugar reactions, contrary to previous studies (Danon et al., 2014). A low reactant weight percent (2%) was used for all reactions in this study. Because intermediate wheatgrass is around 30% cellulose and 25% hemicellulose, this equates to an initial reactant weight percent of less than 1% for all three primary sugars, glucose, xylose, and arabinose. This may have helped limit degradation reactions whose rates depend on both product and reactant concentration.

This study showed that reaction kinetics of acid catalyzed pure sugar and biomass reactions to form furfural, HMF, and levulinic acid are similar, despite the increased complexity associated with a biomass reactant. Solvent choice does appear to have some affect on the reaction kinetics, but it may be dampened by the presence of an acid catalyst. Lignocellulosic biomass remains a viable and attractive option to reduce fossil fuel dependence, however, further study is required to make this a viable process. High yields of furfural and levulinic acid can be achieved from biomass, but not in tandem. Furfural tends to degrade significantly at the high severities that yielded the most levulinic acid.

REFERENCES CITED

REFERENCES CITED

Alves, L. A., Almeida E Silva, J. B., & Giulietti, M. (2007). Solubility of d-Glucose in Water and Ethanol/Water Mixtures. *Journal of Chemical and Engineering Data*, *52*(6), 2166–2170. https://doi.org/10.1021/JE700177N

Asakawa, M., Shrotri, A., Kobayashi, H., & Fukuoka, A. (2019). Solvent basicity controlled deformylation for the formation of furfural from glucose and fructose. *Green Chemistry*, *21*(22), 6146–6153. https://doi.org/10.1039/c9gc02600b

Barbosa, J., Barrón, D., Butí, S., & Marqués, I. (1999). Assignment of pHS values of reference buffer solutions for standardization of potentiometric sensors in THF–water. *Polyhedron*, *18*(25), 3361–3367. https://doi.org/10.1016/S0277-5387(99)00274-0

Buchanan, B. B., Gruissem, W., & Jones, R. L. (2015). *Biochemistry & molecular biology of plants*.

Buchanan, M. (2007). Solvent extractives of wood and pulp (Proposed revision of T 204 cm-97). *TAPPI*.

Cai, C. M., Zhang, T., Kumar, R., & Wyman, C. E. (2014). Integrated furfural production as a renewable fuel and chemical platform from lignocellulosic biomass. *Journal of Chemical Technology & Biotechnology*, *89*(1), 2–10. https://doi.org/10.1002/JCTB.4168

Cheng, W., Zhang, Y., & Wang, P. (2020). Effect of spatial distribution and number of raw material collection locations on the transportation costs of biomass thermal power plants. *Sustainable Cities and Society*, *55*. https://doi.org/10.1016/j.scs.2020.102040

Danon, B., Hongsiri, W., van der Aa, L., & de Jong, W. (2014). Kinetic study on homogeneously catalyzed xylose dehydration to furfural in the presence of arabinose and glucose. *Biomass and Bioenergy*, *66*, 364–370. https://doi.org/10.1016/J.BIOMBIOE.2014.04.007

Chum, H. L., Johnson, D. K., & Black, S. K. (1990). Organosolv Pretreatment for Enzymatic Hydrolysis of Poplars. 2. Catalyst Effects and the Combined Severity Parameter. *Industrial and Engineering Chemistry Research*, *29*(2), 156–162. https://doi.org/10.1021/IE00098A003/ASSET/IE00098A003.FP.PNG_V03

Danon, B., Marcotullio, G., & de Jong, W. (2013). Mechanistic and kinetic aspects of pentose dehydration towards furfural in aqueous media employing homogeneous catalysis. *Green Chemistry*, *16*(1), 39–54. https://doi.org/10.1039/C3GC41351A

Dussan, K., Girisuta, B., Lopes, M., Leahy, J. J., & Hayes, M. H. B. (2015). Conversion of Hemicellulose Sugars Catalyzed by Formic Acid: Kinetics of the Dehydration of D-Xylose,

L-Arabinose, and D-Glucose. *ChemSusChem*, *8*(8), 1411–1428. https://doi.org/10.1002/CSSC.201403328

Garrett, E. R., & Dvorchik, B. H. (1969). Kinetics and mechanisms of the acid degradation of the aldopentoses to furfural. *Journal of Pharmaceutical Sciences*, *58*(7), 813–820. https://doi.org/10.1002/JPS.2600580703

Green, M. M. (1975). Textbook errors, 123 which starch fraction is water-soluble, amylose or amylopectin? *Journal of Chemical Education*, *52*(11), 729–730. https://doi.org/10.1021/ed052p729

Hartmann, H., & Trumbore, S. (2016). Understanding the roles of nonstructural carbohydrates in forest trees - from what we can measure to what we want to know. In *The New phytologist* (Vol. 211, Issue 2, pp. 386–403). Blackwell Publishing Ltd. https://doi.org/10.1111/nph.13955

History of Ethanol Production and Policy — Energy. (n.d.). Retrieved November 7, 2022, from https://www.ag.ndsu.edu/energy/biofuels/energy-briefs/history-of-ethanol-production-and-policy

Hughes, E., Stand, S. A.-J. Res. Natl. Bur., & 1939, undefined. (1939). Quantitative formation of furfural and methylfurfural from pentoses and methylpentoses. *Nvlpubs.Nist.Gov*, *23*. http://nvlpubs.nist.gov/nistpubs/jres/23/jresv23n2p293_A1b.pdf

Jeswani, H. K., Chilvers, A., & Azapagic, A. (2020). Environmental sustainability of biofuels: a review. *Proceedings of the Royal Society A*, *476*(2243). https://doi.org/10.1098/RSPA.2020.0351

Jiang, Z., Hu, D., Zhao, Z., Yi, Z., Chen, Z., & Yan, K. (2021). Mini-Review on the Synthesis of Furfural and Levulinic Acid from Lignocellulosic Biomass. *Processes 2021, Vol. 9, Page 1234*, *9*(7), 1234. https://doi.org/10.3390/PR9071234

Kang, S., Fu, J., & Zhang, G. (2018a). From lignocellulosic biomass to levulinic acid: A review on acid-catalyzed hydrolysis. In *Renewable and Sustainable Energy Reviews* (Vol. 94, pp. 340–362). Elsevier Ltd. https://doi.org/10.1016/j.rser.2018.06.016

Lee, C. B. T. L., & Wu, T. Y. (2021a). A review on solvent systems for furfural production from lignocellulosic biomass. *Renewable and Sustainable Energy Reviews*, *137*. https://doi.org/10.1016/J.RSER.2020.110172

Li, X., Xu, R., Yang, J., Nie, S., Liu, D., Liu, Y., & Si, C. (2019). Production of 5-hydroxymethylfurfural and levulinic acid from lignocellulosic biomass and catalytic upgradation. *Industrial Crops and Products*, *130*, 184–197. https://doi.org/10.1016/J.INDCROP.2018.12.082

Milne, T. A., Chum, H. L., Agblevor, F., & Johnson, D. K. (1992). Standardized analytical methods. *Biomass Bioenergy*, 341–366.

Overend, R. P., Chornet, E., Overend, R. P., & Chornet, E. (1987). Fractionation of Lignocellulosics by Steam-Aqueous Pretreatments. *RSPTA*, *321*(1561), 523–536. https://doi.org/10.1098/RSTA.1987.0029

Peleteiro, S., Rivas, S., Alonso, J. L., Santos, V., & Parajó, J. C. (2016). Furfural production using ionic liquids: A review. *Bioresource Technology*, *202*, 181–191. https://doi.org/10.1016/J.BIORTECH.2015.12.017

Román-Leshkov, Y., Barrett, C. J., Liu, Z. Y., & Dumesic, J. A. (2007). Production of dimethylfuran for liquid fuels from biomass-derived carbohydrates. *Nature 2007 447:7147*, *447*(7147), 982–985. https://doi.org/10.1038/nature05923

Romo, J. E., Miller, K. C., Sundsted, T. L., Job, A. L., Hoo, K. A., & Wettstein, S. G. (2019). The Effect of Solvent Polarity on Autocatalytic Furfural Production Confirmed by Multivariate Statistical Analysis. *ChemCatChem*, *11*(19), 4715–4719. https://doi.org/10.1002/cctc.201900969

Saake, B., & Lehnen, R. (2007). Lignin. *Ullmann's Encyclopedia of Industrial Chemistry*. https://doi.org/10.1002/14356007.A15_305.PUB3

Sajid, M., Zhao, X., & Liu, D. (2018). Production of 2,5-furandicarboxylic acid (FDCA) from 5-hydroxymethylfurfural (HMF): recent progress focusing on the chemical-catalytic routes. *Green Chemistry*, *20*(24), 5427–5453. https://doi.org/10.1039/C8GC02680G

Scurlock, J. M. O., & Hall, D. O. (1998). The global carbon sink: a grassland perspective. *Global Change Biology*, *4*(2), 229–233. https://doi.org/10.1046/J.1365-2486.1998.00151.X

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., & Crocker, D. (2008). *Determination of Structural Carbohydrates and Lignin in Biomass: Laboratory Analytical Procedure (LAP) (Revised July 2011)*. http://www.nrel.gov/biomass/analytical_procedures.html

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., & Crocker, D. (2012). Determination of Structural Carbohydrates and Lignin in Biomass. *TAPPI*.

Sluiter, J., & Sluiter, A. (2011). *Summative Mass Closure: Laboratory Analytical Procedure (LAP) Review and Integration: Feedstocks; Issue Date: April 2010; Revision Date: July 2011 (Version 07-08-2011)*. http://www.nrel.gov/biomass/analytical_procedures.html

Thammasouk, K., Tandjo, D., & Penner, M. H. (1997). Influence of Extractives on the Analysis of Herbaceous Biomass. *Journal of Agricultural and Food Chemistry*, *45*(2), 437–443. https://doi.org/10.1021/jf960401r

Turgeon, A., & Morse, E. (2022). *Petroleum*. National Geographic Society. https://education.nationalgeographic.org/resource/petroleum

U.S. Energy Information Administration - EIA - Independent Statistics and Analysis. (n.d.). Retrieved September 5, 2022, from https://www.eia.gov/totalenergy/data/browser/index.php?tbl=T01.02#/?f=M&start=200001

Weingarten, R., Cho, J., Conner, W. C., & Huber, G. W. (2010). Kinetics of furfural production by dehydration of xylose in a biphasic reactor with microwave heating. *Green Chemistry*, *12*(8), 1423–1429. https://doi.org/10.1039/C003459B

Ye, L., Han, Y., Wang, X., Lu, X., Qi, X., & Yu, H. (2021). Recent progress in furfural production from hemicellulose and its derivatives: Conversion mechanism, catalytic system, solvent selection. *Molecular Catalysis*, *515*, 111899. https://doi.org/10.1016/J.MCAT.2021.111899

Yemiş, O., & Mazza, G. (2011). Acid-catalyzed conversion of xylose, xylan and straw into furfural by microwave-assisted reaction. *Bioresource Technology*, *102*(15), 7371–7378. https://doi.org/10.1016/j.biortech.2011.04.050

APPENDICES

APPENDIX A

RSTUDIO CODE FOR CORRELATION PLOTS

```
        data <-read.csv(file="C:/IWG_thesis_no_deg.csv",header=TRUE,sep=",")
M1<-cor(data)


install.packages("corrplot")
library(corrplot)


#p-values
cor.mtest <- function(mat, ...) {
   mat <- as.matrix(mat)
   n <- ncol(mat)
   p.mat<- matrix(NA, n, n)
   diag(p.mat) <- 0
   for (i in 1:(n - 1)) {
      for (j in (i + 1):n) {
         tmp <- cor.test(mat[, i], mat[, j], ...)
         p.mat[i, j] <- p.mat[j, i] <- tmp$p.value
      }
   }
  colnames(p.mat) <- rownames(p.mat) <- colnames(mat)
  p.mat
}

# matrix of the p-value of the correlation
p.mat <- cor.mtest(data)
head(p.mat[, 1:10])

corrplot(cor(data),
         type="lower",
         method="circle",
         order="alphabet",
         p.mat=p.mat,
         sig.level=0.05,
         insig="blank")
```

APPENDIX B

EXPERIMENTAL PROTOCOL FOR PH MEASUREMENTS

Measuring pH of organic-water mixtures

1. Weigh 25 grams of deionized water into a 100 mL beaker.

2. Weigh 25 grams of organic solvent (sulfolane, GBL, or THF) into the same beaker.

3. Add a stir rod to the beaker and place on a stir plate. Set the stir rate to approximately 600 rpm.

4. Place a pH probe (Thermo Scientific™ Orion™ 8172BNWP or similar) on a ring stand and holder so that the bulb is submerged in the solvent-water solution at least 1 inch.

5. Record the pH of the solution without the addition of any acid.

6. Using a volumetric pipette, add 100 uL of 0.5 M sulfuric acid to the solvent-water solution. Wait for the solution to mix completely and the pH probe to reach a stable reading before recording the pH.

7. Repeat step 6 until the pH is approximately 1, or until pH changes by less than ~0.1 after consecutive additions of acid.

Preparing the probe for measurements

1. After completing pH measurements in an organic-water mixture, the pH probe needs to be properly rinsed before it can be used to measure pH in a different solution.

2. Over an empty beaker or waste receptacle, rinse the bulb of the probe using acetone ensuring that all surfaces are rinsed.

3. Rinse the probe a second time, using deionized water.

4. If using an Orion 8172BNWP probe, flush a small amount of the internal reference solution through the bulb by lightly pressing on the top of the pH probe. Refill the probe with reference solution if it is less than 1 inch above the junction of the probe.

5. Rinse again with deionized water and place in a storage solution until it is to be used again.