Impact of lignin composition on enzymatic hydrolysis of cellulosic biomass

By

Crystal Oldfield

An Undergraduate Thesis Submitted to

Oregon State University
In partial fulfillment of
the requirements for the degree of

Baccalaureate of Science in BioResource Research,
Bioenergy and Bioproducts Option

May 29, 2015
Signature Page:

APPROVED:

_________________________________   _______________
Dr. Ganti Murthy,   Date
Department of Biological & Ecological Engineering

_________________________________   _______________
Dr. John Simonsen,   Date
Department of Wood Science & Engineering

_________________________________   _______________
Dr. Katharine G. Field, BRR Director   Date

© Copyright by Crystal Oldfield, 5/29/15
All rights reserved

I understand that my project will become part of the permanent collection of the Oregon State University Library, and will become part of the Scholars Archive collection for BioResource Research. My signature below authorizes release of my project and thesis to any reader upon request.

_________________________________   _______________
Crystal Oldfield   Date

Page 2 of 31
Abstract

Lignocellulosic biomass has potential as a renewable resource for global energy and bioproducts production. Biomass consists of three main polymers, cellulose, hemicellulose, and lignin. Cellulose is a homopolymer of glucose, while hemicellulose and lignin are heteropolymers. Lignin is primarily made of syringyl (S), guaiacyl (G) and p-hydroxyphenyl (H) units. Pretreatment of biomass is a critical step to reduce recalcitrance and facilitate further enzymatic hydrolysis. Several studies have demonstrated that the S/G ratio of lignin has a significant effect on the effectiveness of pretreatment. Lignin is known to solubilize, redistribute, precipitate, and coalesce as droplets during conventional pretreatment processes used in production of ethanol from cellulosic biomass. These transformations vary based on the type of the pretreatment process, and are attributed to the differences in the chemical composition and distribution of lignin in biomass. The goal of this study was to quantify the distribution of lignin droplets in order to understand how the S/G ratios of lignin affects enzymatic hydrolysis. Lignin from three biomass sources was isolated using organosolv process to obtain lignins with three S/G ratios. Isolated lignins with different S/G ratios and pure cellulose filter paper were subjected to dilute acid pretreatments (0.75% w/w sulfuric acid concentration) at 120, 150, and 180 °C. Imaging of the pretreated filter paper was done using Scanning Electron Microscopy (SEM) to visually observe the distribution of
the lignin droplets. Wheat straw lignin (with S/G ratio range of 0.50 to 0.69) was observed to produce many large and small droplets of sizes ranging 5.0 µm to well below 0.10 µm in diameter with an increase in droplet amount relative to higher temperatures. Bagasse was coated in a layer of tiny droplets that gave the surface a textured appearance throughout each temperature. Mostly uniform small and large droplets formed on poplar with an increase in amount as temperature increased. Larger droplet size did not correlate to a greater amount of lignin or S/G ratio, however lignin droplets appeared to increase in amount as temperatures increased.

1. Introduction

1.1 Background

Lignocellulosic biomass has great potential as a renewable resource for production of bioenergy energy and bioproduct. For economically viable industrial scale production to take place it is critical to have efficient pretreatments and conversions processes for lignocellulosic biomass. Lignin is known to solubilize, redistribute, precipitate and coalesce as droplets during conventional pretreatment processes (Trajano et al., 2013). These transformations vary based on the type of the pretreatment process, and are attributed to the differences in the chemical composition and distribution of lignin in biomass. The economic viability of biofuels and products depends on efficient enzymatic hydrolysis of cellulose.
The goal of this project was to observe the distribution of lignin droplets in order to understand how different ratios of syringyl (S) and guaiacyl (G) in lignin affect its distribution on biomass after pretreatment. A higher syringyl lignin content and larger percent of lignin, will potentially form more droplets on the cellulose. This project contributes to the understanding of lignin interactions during pretreatment in order to efficiently design enzymatic and pretreatment processes.

1.2 Problem Statement

Pretreatment of lignocellulosic biomass using dilute acid is the common lignocellulose pretreatment method (Alvira et al., 2010). Lignin is known to solubilize, redistribute, precipitate and coalesce as droplets during conventional pretreatment processes (Li et al., 2014). These transformations vary based on the type of the pretreatment process, and are attributed to the differences in the chemical composition and distribution of lignin in biomass. Lignin can inhibit enzymatic cellulose hydrolysis by creating a physical barrier to enzymes, and through lignin-enzyme interactions (Berlin et al., 2005). A higher ratio of syringyl (S) to guaiacyl (G) content of lignin, as well as a higher percent of lignin contained in the biomass, can lower the theoretical yield in the saccharification process (Guo et al., 2009). Droplet size was found to vary by pretreatment conditions when using isolated organosolv lignin from corn stover (Selig et al., 2007). Several studies have observed lignin droplets on just one biomass, such as corn stover (Donohoe et al., 2008; Selig et al., 2007) or wheat straw (Hansen et al., 2011; Zeng et al., 2013). for their lignin droplet observations. It is necessary to fill gaps
in the current research to see how S/G ratios of lignin affect droplet size and area. This information could lead to further understanding of how enzymatic hydrolysis yields and rates are affected by the lignin under dilute acid process conditions.

1.3 Project Objectives

The goal of this project was to understand the differences in the distribution of lignin droplets after dilute acid pretreatment in order to understand how the S/G ratios of lignin affects enzymatic hydrolysis. In order to accomplish this, the specific objectives were: 1. Determine the distribution of the lignin droplets in order to estimate the surface area covered by lignin droplets in pretreated biomass; 2. Determine if S/G ratios affect the size of lignin droplets; 3. Compare the effect of S/G ratios on the hydrolysis rates and final yields during enzymatic hydrolysis of biomass.

2. Literature Review

2.1 Bioenergy

Lignocellulosic biomass is an abundant renewable resource which has potential to contribute to the world’s growing energy needs (Turhollow et al., 2014). The Renewable Fuel Standard (RFS) requires at least 76 GL of cellulosic ethanol in use as transportation fuel by the year 2022. This can be achieved while only utilizing 10% of the estimated supply available for biofuels (Turhollow et al., 2014). The U.S. government has a goal in place for 25% of chemicals to be supplied from biomass by
2030 (Zakzeski et al., 2010). The ethanol that is currently being mixed with gasoline is produced from corn, a food crop. Lignocellulosic biomass in the U.S. has an estimated abundance of 350-450 million tonnes per year and does not compete with food crops, since it comes from agricultural or other residues (Limayem and Ricke, 2012). Plant biomass for bioenergy and chemical products has potential to replace petroleum based products. Lignin contributes to lignocellulosic biomass recalcitrance, and hence pretreatments are necessary to break down the cellular structures of lignocellulosic biomass and reduce biomass recalcitrance (Selig et al., 2007).

2.1.1. Lignocellulosic Biomass and Pretreatment

In order to produce fuel and chemicals, biomass structure must be broken down to facilitate further processing. The physical structure of the biomass can be broken down using mechanical, biochemical or thermochemical conversion to expose the cellulose (Limayem and Ricke, 2012). Once the cellulose is more accessible, enzymes can effectively hydrolyze the sugars. These sugars can then be fermented by microbes to produce ethanol or other bioproducts.

One prevalent pretreatment processes, that was used in this study, is to heat the biomass with a dilute acids (such as sulfuric acid at 0.1-1.5% w/w biomass) from high temperatures (180 °C) to low temperatures (120 °C) with a residence time of 5-90 minutes (Alvira et al., 2010). There is an inverse relationship between the pretreatment temperature and the residence time.
2.2. Feedstocks

Wheat straw (*Triticum aestivum*, var. Thunderbird), sugarcane bagasse (*Saccharum* spp.), and Eastern Cottonwood/Poplar (*Populus deltoides*) are three types of biomass with different lignin S/G ratios used as potential cellulosic feedstocks for the process of bioenergy (Limayem and Ricke, 2012; Zamora et al., 2013). This study focuses on comparing the lignin S/G ratios, lignin droplets, and total percent of lignin of these three feedstocks when pretreated at three different temperatures.

2.3. Chemical Structure

2.3.1. Cellulose

Cellulose is a homopolymer comprised of crystalline, linear, chains of glucose monomers with β (1→4) glycosidic bond configuration. The chains bundle together from the interactions between the hydroxyl groups to form microfibrils. Unlike cellulose, starch has a branched α (1→4) glycosidic bond configuration which is degraded with less energy. Cellulose is very recalcitrant and requires 320 oC and 25 MPa for the structure to turn amorphous (Limayem and Ricke, 2012).

2.3.2. Hemicellulose

Hemicellulose is a branched heteropolymer that includes many types of both hexose and pentose sugars (Limayem and Ricke, 2012). One of the main components in its structure is xylan with a β (1→4) configuration. Dilute acid pretreatment solubilizes hemicellulose and opens up cellulose for the enzymatic pretreatment step.
2.3.3. Lignin

![Chemical structures of lignin monomers](image)

**H Lignin**

**G Lignin**

**S Lignin**

**Fig. 1.** The three monolignols p-coumaryl, coniferyl, and sinapyl alcohols.

Lignin is a complex structure made up of monolignols (Zakzeski et al., 2010). Figure 1 shows the chemical structure of p-coumaryl alcohol (H), coniferyl alcohol (G), and
sinapyl alcohol (S). These monolignols are precursors to hydroxyphenol (H), guaiacyl (G), and syringal (S) that link together in a pseudo random way to form lignin polymers (Vanholme et al., 2008). Lignin transitions between a solid and liquid phase when heated to the melting point (Trajano et al., 2013). The melting point of lignin falls within the ranges of standard pretreatment temperatures of 120 to 180 °C and is dependent on the composition (Selig et al., 2007). Since lignin is hydrophobic, the interactions with water during pretreatment causes the droplet formation (Donohoe et al., 2008). Droplets formed on biomass after pretreatment have been determined to be coalesced lignin. These lignin droplets have been found to form dome or spherical shapes (Donohoe et al., 2008). They can appear textured or clumped together. Researchers suspect that lignin droplet formation may form a physical barrier for enzyme breakdown (Selig et al., 2007). Some enzymes may also end up binding to the lignin instead of coming in contact with the cellulose.

Others who have investigated lignin interactions have found that lignin is necessary not only for the benefit of the living plant where it serves as a defense to pests and pathogens and provides structural support (Limayem and Ricke, 2012). Plants that have been genetically modified to have significantly less lignin developed dwarfism and cell collapse (Bonawitz and Chapple, 2013). Lignin droplets that form after pretreatment are thought to inhibit hydrolysis by binding to enzymes or creating a physical barrier (Li et al., 2014). However, Lignin has also been found to protect the cellulose structure from
changing and becoming more recalcitrant during pretreatment (Bonawitz and Chapple, 2013; Sun et al., 2014). It is important to find the best combinations of amount of lignin, mix of enzymes, and pretreatment conditions in order for bioproducts to become economically viable.

2.4. Cellulases

![Figure 2: Enzyme interaction with cellulose chains.](image)

Enzymatic hydrolysis of cellulose is performed after the dilute acid pretreatment step in which hemicellulose is partially/fully solubilized and cellulose is exposed for greater enzyme interaction. Enzymes need to bind to the cellulose in order to catalyze the
hydrolysis reaction (Guo et al., 2009). Figure 2 depicts cellulose being broken down by several types of enzymes. Exoglucanases can hydrolyze cellulose into cellobiose in only one direction (Dutta and Wu, 2014). They can either move along the cellulose chains from the reducing end or the non-reducing end of the cellulose chains. This movement can be physically blocked by lignin droplets on the cellulose chain. Endoglucanases can hydrolyze at any point on the cellulose chain, but produce various oligosaccharides. Beta-glucosidase is the enzyme that hydrolyzes the cellobiose into two glucose molecules. Lignin binding potential of enzymes has also been found to be higher in lignins with larger Guaiacly (G) content compared to Syringal (S) (Guo et al., 2014).

3. Methods

3.1. Feedstock

<table>
<thead>
<tr>
<th>Biomass</th>
<th>% Glucan</th>
<th>% Xylan</th>
<th>% Total</th>
<th>S/G Ratio Ranges</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat Straw</td>
<td>33.61</td>
<td>19.3</td>
<td>18.1</td>
<td>0.50-0.69</td>
</tr>
<tr>
<td>Bagasse</td>
<td>40.5</td>
<td>22.04</td>
<td>24.4</td>
<td>0.83-1.4</td>
</tr>
<tr>
<td>Poplar</td>
<td>44.6</td>
<td>13.39</td>
<td>27.2</td>
<td>1.30-2.0</td>
</tr>
</tbody>
</table>
Three biomass samples, bagasse, wheat straw, and poplar, with known chemical composition were acquired from National Institute of Standards and Technology (U.S. Department of Commerce). The lignin S/G ratios are in the range of 0.50-0.69 for wheat straw, 0.83-1.4 for bagasse, and 1.3-2.0 for poplars (Lopes et al., 2011; Rahikainen et al., 2013; Sannigrahi et al., 2010; Zeng et al., 2014, 2013).

### 3.2. Organosolv Process

To separate and isolate lignin from the biomass for use with pure cellulose, we used a one-step aqueous ethanol organosolv procedure without presoaking (Hage et al., 2011; Wildschut et al., 2013). 1.2% w/w H$_2$SO$_4$ catalyst and 65% Ethanol was used to make the solution for the organosolv liquid. A solution of ethanol (65 g), DI Water (33.8 g), and H$_2$SO$_4$ catalyst (1.2 g) was made.

The solution (35.6 g) was weighed out and combined with the biomass (4.4 g). A total of 40 g of the biomass, solvent, and catalyst mixture (S/L ratio 1:8) was added to reactor tubes (60 mL volume, 2.54 cm diameter and 30 cm length made of 316 Stainless steel tube and pressure fittings from Swagelok (Solon, Ohio). Reactor temperature was set to 190 °C for 60 minutes. Residue was filtered using 90 mm 1PS silicon treated Whatman filter paper (Whatman GE Healthcare Life Sciences, Buckinghamshire, United Kingdom) in a Buchner funnel. Solid residues were washed 3 times with EtOH/H$_2$O (5:1, 50 mL) at 60 °C. Lignin was precipitated by diluting with water in a 3:1 (water to liquor) ratio. Precipitated lignin was separated by centrifugation.
(10,000 rpm, 15 min), transferred to a crucible, and then dried to a constant weight in an oven at 50 °C until there was no subsequent weight loss.

### 3.3. Dilute Acid Pretreatment of Pure Cellulose

**Table 2.** Pretreatment conditions for reactions performed using each type of isolated lignin; 5 replicates each.

<table>
<thead>
<tr>
<th>w/w acid (%)</th>
<th>Temperature (°C)</th>
<th>Residence Time (min)</th>
<th>Isolated Lignin*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.75</td>
<td>120</td>
<td>40</td>
<td>W, B, P</td>
</tr>
<tr>
<td>0.75</td>
<td>150</td>
<td>20</td>
<td>W, B, P</td>
</tr>
<tr>
<td>0.75</td>
<td>180</td>
<td>10</td>
<td>W, B, P</td>
</tr>
</tbody>
</table>

*W=wheatstraw, B=Bagasse, P=Poplar

Each lignin sample was pretreated along with Whatman #42 ashless pure cellulose filter paper using dilute acid with 0.75% w/w H₂SO₄. Pretreatment conditions were chosen to be representative of those being used in an industrial process to control for the release of sugar degradation products (Alvira et al., 2010). The conditions for each pretreatment can be seen in Table 2. Each lignin type and temperature pretreatment was performed using 5 replicates.

Quartz reactor tubes were cut to 16 cm and one end was sealed using an acetylene oxygen torch. The cellulose, lignin, and acid solution was prepared by weight: 0.23 g liquid and 0.023 g of total solids (10:1 liquid to solid ratio). The total mixture was...
0.25 mL inside a 0.50 mL tube. Individual solids were measured in a 2:1 ratio. 0.01513 g of Whatman #42 filter paper was cut into strips and placed in the reactor tube. Using wire and a glass funnel, 0.0076g of lignin was inserted into the reactor tube containing the filter paper strips to total 0.0227 g of solids. A 0.75% w/w H₂SO₄ acid solution was prepared by weight. The total solution made was 0.36 g of H₂SO₄ and 49.6 g water for 0.75% w/w. 0.23 g of liquids was then added to the reactor tubes using a 1 mL glass syringe.

The ends of the filled reactor tubes were sealed off. The tubes were heated using a sand bath at the conditions seen in Table 2. Tubes were broken open to retrieve the samples. The filter paper solids were retrieved using a syringe and DI water. The mass of the quartz tubes, liquids, and solids were recorded. Solids were then air dried overnight for use as SEM samples.

### 3.3.1. SEM analysis

Scanning Electron Microscopy (SEM) was performed to visually analyze lignin droplets on pretreated pure cellulose filter paper and whole biomass. Samples were prepared by mounting on aluminum stubs with carbon tape. The mounted samples were then coated with a thin layer (8.9 nm average thickness) of gold/palladium alloy for electrical conductivity, using a Cressington 108auto Sputter Coater (Cressington Scientific Instruments Ltd., Hertfordshire WD19, UK). Coating time was 40s for all samples. The images were taken using the FEI Quanta 600F environmental SEM (FEI,
images of each sample were taken in ranges of 300x to 28,000x magnification using a beam voltage range from 10 to 15 kV and spot sizes between 4 and 5. The software ImageJ (version 1.48, (Schneider et al., 2012)) was used to scale droplets and edit images for visual analysis.

### 3.4. Dilute Acid Pretreatment of Feedstock

**Table 3.** Pretreatment conditions for reactions performed using each type of isolated lignin; 5 replicates each.

<table>
<thead>
<tr>
<th>w/w acid (%)</th>
<th>Temperature (°C)</th>
<th>Residence Time (min)</th>
<th>Isolated Lignin*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.75</td>
<td>120</td>
<td>60</td>
<td>W, B, P</td>
</tr>
<tr>
<td>0.75</td>
<td>150</td>
<td>30</td>
<td>W, B, P</td>
</tr>
<tr>
<td>0.75</td>
<td>180</td>
<td>10</td>
<td>W, B, P</td>
</tr>
</tbody>
</table>

*W=wheatstraw, B=Bagasse, P=Poplar

Each whole biomass type was pretreated with dilute acid (0.75% w/w H$_2$SO$_4$) with a solids loading of 10%. 27 g of dilute acid and 3 g of biomass were loaded into stainless steel reactor tubes. Pretreatment conditions can be seen in Table 3. These pretreatment conditions were chosen to investigate droplet formation at three possible temperatures and residence time used in the biomass conversion industry (Alvira et al., 2010). The reactor tubes were heated using a fluidized sandbath at 200 °C in order to reach the desired temperature. A thermocouple fitted inside a reactor tube filled with 30...
mL of water was used to monitor temperature of the reactors in the sandbath. Once the reactors reached the desired temperature, they were quickly (within 2 minutes) placed into a hot air oven, preheated to the desired temperature, in order to maintain temperature through the residence time. Once the residence time had been reached, the reactor tubes were cooled by immersion in ice water. The experiment was performed with three replicates totaling 27 pretreatments.

The slurry from the reactor tubes was emptied using 50 mL of deionized water. The biomass was filtered using 90 mm 1PS silicon treated Whatman filter paper in a Buchner funnel and washed using 20 mL of deionized water. Liquid and solids were stored and frozen for later analysis.

4. Results and Discussion

4.1 Cellulose Filter paper

Earlier research for identifying lignin droplets has typically focused on one biomass type and temperature (Donohoe et al., 2008; Hansen et al., 2011; Li et al., 2014; Selig et al., 2007; Zeng et al., 2013). In this study, three types of biomass, each with a different S/G ratio, which were subjected to three different pretreatment temperatures, were visually analyzed.
Fig. 3. SEM of pure cellulose with lignin isolated from Wheat straw (A-1, 2, 3), Bagasse (B-1, 2, 3) and Poplar (C-1, 2, 3) after dilute acid pretreatment at 120 °C for 40 min (series A, B, C-1), 150 °C for 20 min (series A, B, C-2) and 180 °C for 10 min (series A, B, C-3); all shown with 10 μm scale bar.
Figure 3 shows the entire series of pure cellulose with isolated lignin at each pretreatment temperature: Wheat straw (A-1, 2, 3), Bagasse (B-1, 2, 3) and Poplar (C-1, 2, 3) where 1 is 120 °C, 2 is 150 °C and 3 is 180 °C. Some trends can be seen when looking across each isolated lignin type and across each temperature. Series A shows a mix of large and small droplets that increase in amount as temperature increases. Series B has very small droplets covering the cellulose throughout at each temperature. Series C has large and small droplets that are more uniform in size than in Series A. Series C also has an increasing amount of droplets with increasing temperature.

### 4.1.1 Wheat Straw

![Fig. 4. SEM of pure cellulose with lignin isolated from wheat straw; A-2 was 120 °C for 40 min; A-2 was 150 °C for 20 min; A-3 was 180 °C for 10 min.](image)

As seen in figure 4, the A series of images depicts wheat straw at temperatures of 120 (A-1), 150 (A-2), and 180 (A-3) °C. Of the biomass in this study, wheat straw has
the smallest S/G ratio range of 0.5 to 0.69 (Zeng et al., 2013). It also has the smallest mass fraction of total lignin at 18.1% (NIST). It has been observed that there are a greater amount of droplets at the highest temperature, as well as a mix of larger droplets. Wheat straw at 180 °C had a mix of many small to very large droplets in the image shown at 10,000x magnification in A-3. The larger droplets shown had diameters up to 5.0 µm while the smallest was well below 0.10 µm.

4.1.2 Bagasse

Figure 5 shows the sugarcane bagasse isolated lignin after pretreatment with pure cellulose. This figure illustrates the droplet distribution for temperatures of 120 (B-1), 150 (B-2), and 180 (B-3) °C. The droplet size at 10,000x magnification was very small with many at and below 0.10 µm diameter. The largest droplet in Figure 5, B-2, was around 0.80 µm in diameter. The S/G ratio range is in the middle for bagasse at
0.83 to 1.4 (Lopes et al., 2011; Rahikainen et al., 2013; Sannigrahi et al., 2010; Zeng et al., 2014, 2013). Bagasse also has the medium amount of total lignin compared to the other biomass in the study.

4.1.3 Poplar

![SEM images of pure cellulose with lignin isolated from poplar](image)

**Fig. 6.** SEM of pure cellulose with lignin isolated from poplar; A-2 was 120 °C for 40 min; A-2 was 150 °C for 20 min; A-3 was 180 °C for 10 min.

In Figure 6, Series C of poplar showed droplets sized from 5 µm to less than 0.10 µm in diameter. Image C-3 of poplar pretreated at 180 °C, had many droplets of similar sizes in the realm of 0.5 µm in diameter. Poplar has the most total percent of lignin with 27.2% (NIST) when compared to the other biomass. Poplar also has the highest S/G ratio range of 1.3 to 2.0 (Lopes et al., 2011; Rahikainen et al., 2013; Sannigrahi et al., 2010; Zeng et al., 2014, 2013).
4.2 Whole biomass

![Image 1 of whole wheat straw pretreated at 150 °C for 30 min; Image 1 showing thicker cell wall area with droplets; Image 2 showing concentrated small lignin droplets.](image)

Whole biomass images revealed a complex interaction of lignin droplets with the cell walls compared to the pure cellulose. The lignin in the whole biomass was squeezing out from inside the cells. The lignin was also distributed differently though the cellular structures. The droplets on the pure cellulose were deposited on the surface and did not form in the same way as lignin does in whole biomass. In Figure 7, image 1 has an arrow pointing to a thick cell wall in the vascular area of wheat straw where many lignin droplets are concentrated. Mass of lignin droplets, which have coalesced and formed concentrated areas, were also observed as seen in Figure 7, image 2. In the areas where there are concentrated masses of lignin that have left the biomass,
droplets have been observed to coat the surrounding area. These droplets were very small and coat the biomass as seen in Figure 7, image 2. Figure 8 shows an example of spherical droplets on whole poplar pretreated at 180 °C. Some of them were smooth, while others had small droplets forming on top of each other.

Fig. 8. SEM of whole poplar biomass pretreated at 180 °C for 10 min.
Fig. 9. SEM of whole wheat straw pretreated at 180 °C for 10 min; Image 1 and Image 2 showing the variation of droplets.

Another example of droplet coatings can be seen with a lot textured as in Figure 9, image 1. Figure 9 shows an example of how lignin droplet appearance can vary at the same temperatures depending on the amount of coalesced lignin in a concentrated area. This figure of wheat straw pretreated at 180 °C, also had good examples of flat, dome, and oblong shaped droplets.
Fig. 10. SEM of whole biomass after pretreatment; Wheat straw (D-1, 2, 3), Bagasse (E-1, 2, 3) and Poplar (F-1, 2, 3) after dilute acid pretreatment at 120 °C for 60 min (series D, E, F-1), 150 °C for 30 min (series D, E, F-2) and 180 °C for 10 min (series D, E, F-3); all shown with 10 μm scale bar.
In Figure 10, the pretreated whole biomass is compared in the same way as the pure cellulose from Figure 3. The numbers correspond to the pretreatment temperature with 1 being 120 °C, 2 is 150 °C and 3 is 180 °C. Series D shows wheat straw with many small droplets covering the biomass at each temperature. There was a variation in large and small droplets similar to the pure cellulose (from Series A). Series E is bagasse with many tiny droplets at each temperature and some larger droplets at 180 °C. Again, similarities in droplet sizes between Series E and Series B can also be seen. The droplets of Series F, poplar, appeared larger at each temperature. Just as in the other Series, F and C showed similar droplet distribution and size.

5. Conclusions

5.1 Summary

There was a visual trend in droplet amount and size among the pretreated pure cellulose and isolated lignin samples. Wheat straw was observed to produce many large and small droplets of different sizes with an increase relative to higher temperatures. Bagasse was coated in a layer of tiny droplets that give the surface a textured appearance throughout each temperature. This was especially the case in pretreated whole bagasse. Mostly uniform small and large droplets formed on poplar with an increase in amount as temperature increases. Variability in droplet morphology was observed for each case. Differences observed between lignin droplet behaviors in the whole biomass are due to the complex nature of lignin extrusion from the cellular
structure and the location of lignin within the biomass. At the start of this study, it was expected that a higher syringyl (S) lignin content and larger percent of lignin, would potentially form more droplets on the cellulose. This was not the case, as poplar had the highest amount of lignin and highest S/G ratio. Even though poplar did have a large amount of droplets, bagasse and wheat straw had also developed many that coated the cell walls. Larger droplet size did not correlate to a greater amount of lignin or S/G ratio, however lignin droplets appeared to increase in amount as temperatures increased.

5.2 Limitations
The image analysis of the lignin droplets in this study could not be performed as expected. This was due to the limitations of the lack of automation for image analysis software and time constraints. There were numerous lignin droplets that were much smaller than originally expected, which made quantification not possible for this study. There was not sufficient time available to carry out enzymatic hydrolysis. Without this experiment, this study does not have part of the quantitative data needed to conclude lignin droplet effect on pretreatment and sugar yield.

5.3 Impact and Future Research
Observations on the size and amount of lignin droplets contribute to the overall knowledge needed for designing efficient processes for biochemical production from biomass. A replicate of this study, along with experiments comparing other types of biomass, would be needed for a comprehensive view of lignin droplets. Automation for
the quantification of droplet amount, surface area, and size may be available with future technology.

6 Acknowledgements

I would like to give thanks to the following for their contribution to this project:

Electron Microscope Facility in Linus Pauling at Oregon State University

The USDA funded OSU Bioenergy Education Program

National Science Foundation

Ganti Murthy, PhD.

John Simonsen, PhD.

Kate Field, PhD.

Wanda Crannell

Glen Li, PhD.

Allex McDaniels

Deepak Kumar, PhD.

Haider Jawad Kadhum

S.M. Hossein Tabatabaie
References


