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Minimizing Wash Water Usage After Acid Hydrolysis Pretreatment of Biomass

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Abstract

Dilute acid pretreatment, needed to prepare biomass for saccharification, results in the production of a number of byproducts, which inhibit subsequent enzymatic hydrolysis and fermentation steps. In order to improve saccharification yields in the enzyme hydrolysis step, the pretreated biomass is often rinsed with room temperature water to remove these byproducts. This project sought to find a threshold for wash water usages for conservation of resource use in pilot scale cellulosic biomass processing chains. High-density poplar was pretreated with 1% dilute sulfuric acid at 140 ºC for 40 minutes. After pretreatment the biomass was washed with water volumes equal to 0, 1 ½, or 3 times the biomass volume. The rinsed biomass was then enzymatically hydrolyzed and the concentrations of byproducts and resulting carbohydrates were quantified by high-pressure liquid chromatography (HPLC). Quantification was performed in pretreatment hydrolyzates, rinsing waters and enzyme hydrolyzates. Results show that inhibitory byproducts are highly soluble even in low amounts of wash water, and glucose yields are similar despite halving the amount of water used (3 and 1 ½ water volumes) in the wash step, signifying that the removal of a sufficient number of inhibitory compounds can be accomplished with even at small wash volumes. Specifically, enzymatic hydrolysis (where the washing step has a direct effect) yielded between 3 and 4 grams glucose per gram dry biomass in the 1 ½ and 3 water volumes rinses, respectively, with totals at both conditions equaling between 7 and 8 grams glucose per gram dry biomass, respectively. The rinse step removed similar concentrations of inhibitors in either the 1 ½ and 3 water volume rinsing procedures.

1.1- Introduction

The development of sustainable energy is of growing concern in response to the ever-increasing demand for non-fossil liquid fuels. Second-generation biofuel processes, which produce ethanol

from cellulose sources, are an example of a technology that could be used to fulfill the demand for sustainable renewable energy. To produce fermentable sugars from such biomass, a processing chain, involving multiple steps of variable chemical severity, is required. The most severe and important of these steps is biomass pretreatment, which is necessary to render the cellulose in the biomass susceptible to enzymatic hydrolysis. One of the better understood pretreatment methods is dilute acid pretreatment, also termed acid catalyzed hydrolysis. In this process the biomass is heated in an acidic solution (roughly pH 1.8) to a high temperature for a relatively brief period of time (from 10 to 90 minutes). Pretreating feedstock under these conditions yields biomass that is receptive to enzymatic hydrolysis. However, these pretreatment conditions also yield a number of byproducts formed by side reactions. Organic acids, such as acetic and formic acid, and xylose and glucose degradation products, like furfural and hydroxymethylfurfural, are formed. These byproducts have been shown to have inhibitory effects of later steps of the biomass processing chain, affecting both enzyme and fermentation yields negatively when left in the biomass (Kim 2009).

Despite these problems, dilute acid pretreatment remains attractive as the process and mechanisms are well understood and easily scaled-up when considering industry sized reactor setups (Sannigrahi et. al. 2011). As of 2013, the National Renewable Energy Laboratory (NREL) recommended, in order to remove all undesirable inhibitory byproducts from dilute acid pretreated biomass, a rinsing step with up to twelve times the amount of water to quantity of biomass (Dowe 2001) this added rinsing step increase the amount of water used in the process. Any reduction in this amount of required water in turn will make the whole processing chain more sustainable and cost efficient, as the waste water formed is acidic and costs additional resources to manage. Interestingly, Hodge et al. (2008) reported that the rinsing step can be

performed with as little as three volumes of water, producing fermentable sugar streams with acceptable margins for industrial ethanol production (Hodge et. al 2008). Rinsing with three volumes represents a four-fold water savings over the suggested 12 water volumes. Lowering the rinsing requirements even further would be an interesting proposition for the nascent second generation biofuels industry.

In order to investigate the lower limits of the required water volumes for the rinsing step, experiments were performed with poplar biomass using dilute acid pretreatment with 1 % (v/v) sulfuric acid at 140 ºC for 40 minutes. Three rinsing conditions were tested: no rinsing after pretreatment, which corresponded to the negative control; rinsing with the water volume that corresponded to 1 ½ times the quantity of biomass; and, rinsing with the water volume that corresponded to 3 times the quantity of biomass. Aliquots of pretreatment hydrolyzate, rinse water, and subsequent enzyme hydrolysate were saved and analyzed for their organic acid and carbohydrate concentrations. Mass balance of organic acids and carbohydrates were determined and the effect of rinsing water volumes was examined.

1.2- Objectives

The goal of the investigation was to find out two important parameters:

- To what extent does wash volume effect enzyme hydrolysate yields, and
- Where might an optimum wash volume amount lie for maximizing yields and lowering water costs.

2- Experimental Design & Methodology

2-1 Biomass

The feedstock used in this study was high specific gravity poplar (a clone of *Populus deltoids* with higher density), a common poplar native for Arkansas, which grows with little irrigation (Martin et al. 2011). *P. deltoids* was obtained as described by Djioleu et al. (2012) and Martin et al. (2011) from the University of Arkansas Pine Tree Branch Station. The biomass was received as chips, which were processed through a Wiley Mini Mill (Thomas Scientific, Swedesboro, NJ) to 10 mesh following Torget et al. (1988). Moisture content of the biomass was measured with an Ohaus MB45 Moisture Analyzer (Pine Brook, NJ) to ensure reproducibility. For each run, the biomass was subjected to one of three treatments (0, 1.5, 3 wash volumes) with two replications for each condition.

2-2 Pretreatment

Twenty-five grams (wet basis) of milled biomass was mixed into a 1% solution of H_2SO_4 (v/v) at a loading of 10% solids and a total working volume of 250 milliliters. Pretreatments were conducted in a 1 liter Parr 4525 reaction vessel (Model # 4848, Moline, IL) and heated to 140 ºC for 40 min. The Parr reactor was heated over the course of ten to fifteen minutes with an immediate cool-down time, to prevent the formation of additional byproducts. The liquid slurry was filtered through 0.2 mm Whatman paper using a Buchner funnel, to separate the pretreated solids from the liquid fraction. The liquid fraction containing glucose and xylose released from the pretreatment process was saved for later testing on high-pressure liquid chromatography (HPLC). Moisture content of the solids fraction was quantified by the use of the Ohaus MB45 Moisture Analyzer.

2-3 Wash

The solids fraction of the pretreatment slurry was rinsed with zero, 1 1/2 or 3 volumes of water in proportion with the original loadings of the pretreatment step. The wash was conducted for 5 minutes and mechanically agitated by hand The solids were filtered as described above. The washed liquid fraction was saved for quantification through high pressure liquid chromatography (for both concentrations of sugars and organic acids), and 40 grams of the solid residue was used in the enzyme hydrolysis step. The excess pellet was saved and stored at 4 ºC.

2-4 Enzyme Hydrolysis

The rinsed pretreated pellet was then hydrolyzed using Accellerase®1500 (Genencor, Rochester, NY) enzyme mixture, which contains both endo and exo-cellulases. The enzyme hydrolysis was conducted by loading a 600 ml stirred Parr reactor at 10% solids loading with 200 ml 4.9 g sodium citrate buffer, 20 ml of enzyme, and 180 ml of Millipore filtered water. The reaction was carried out at a low stirring speed and at 50 ºC, following the procedure described by Djioleu et al. (2012). This process was allowed to continue for 24 hours before the enzymatic hydrolysis slurry was collected and saved for HPLC analysis. All experiments were repeated at least two times.

2-5 HPLC Analysis

Two separate HPLC instruments (Waters, Milford, MA) were used to quantify organic acids and carbohydrates in pretreatment hydrolysate, rinse water and enzyme hydrolyzate samples. Each HPLC system required 10 microliter samples, easily obtained from the 100+ ml hydrolysates formed in each step. The carbohydrate HPLC was equipped with a Shodex column (SP0801, Waters, Milford, MA) and precolumn (SP-G) with Millipore filtered water as the mobile phase. Glucose and xylose concentrations were calculated by relating peak area obtained by refractive

index detection to calibration curves. The total amounts of fermentable sugars released during each stage of the process per unit of biomass were calculated by a mass balance.

The HPLC used to quantify degradation products was equipped with a Bio-Rad Aminex column (HPX-87H, Bio-Rad, Hercules, CA) with a mobile phase of 0.002 M H_2SO_4. Using a Waters UV detector, the concentrations of the inhibitory products furfural, acetic acid, formic acid, and hydromethylfurfual (HMF) were then quantified using calibration curves. Total yields of sugars and degradation products per unit of biomass were then calculated for all samples: dilute acid pretreatment hydrolysates, wash waters and enzyme hydrolysates.

3-Results and Discussion

As mentioned in the materials and methods sections, there were three steps in the analyzed process: acid hydrolysis hydrolyzates, wash waters, and enzyme hydrolysis hydrolyzates. All three solutions were analyzed for their sugar and inhibitor concentrations. From the data retrieved by HPLC analysis, total amounts of each analyte were calculated by integration of the peak area. Compounds concentrations obtained during pretreatment, rinse and enzymatic hydrolysis were added together and expressed as a function of the maximum theoretical potential as reported by Martin et al. (2011). Results reporting the concentrations of analytes in acid pretreatments hydrolyzates are presented in figure 1, and are reported as the grams of carbohydrate and byproduct produced per gram of dry biomass. Reporting in this manner allows for comparison between each experiments.

3.1-Pretreatment

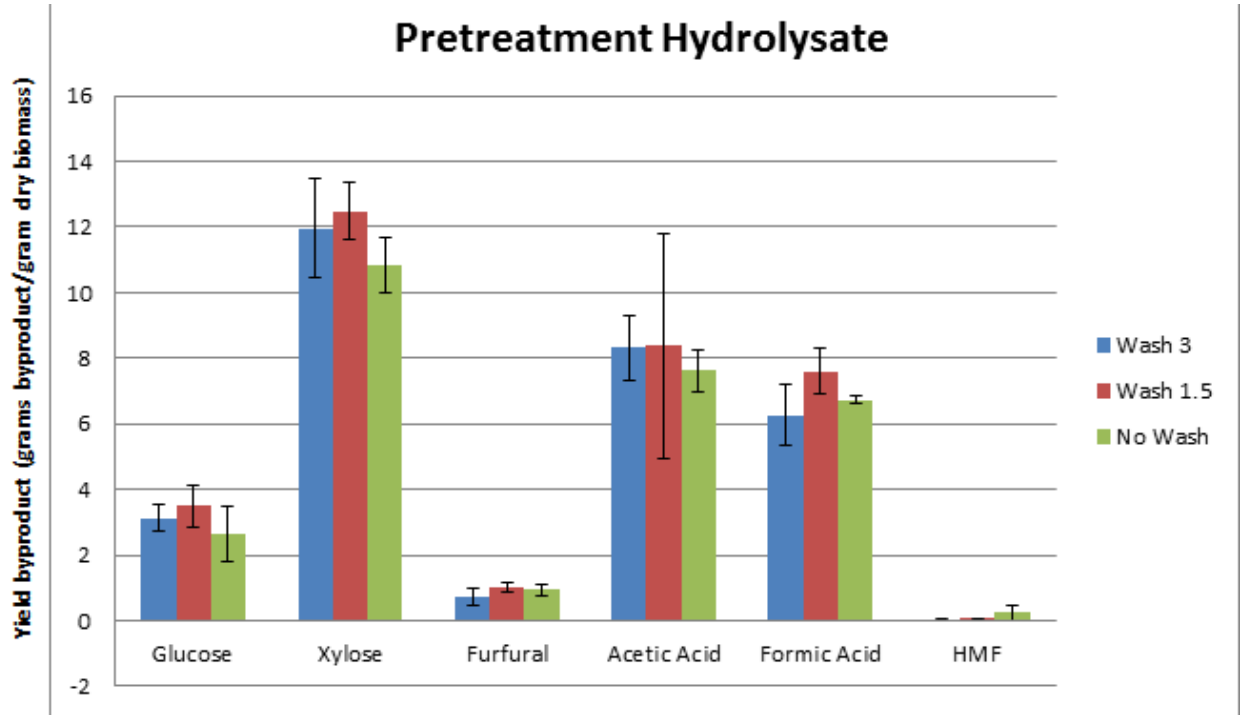

Figure 1. Monosaccharide and inhibitory byproduct yield after 1% dilute acid pretreatment for 40 minutes at 140 ºC, at the conditions of: pretreatment water volume 0 (no wash control); pretreatment water volume of 1 ½ ; and, pretreatment water volume of 3.

The first step was dilute acid pretreatment, and each of the three tested conditions followed the exact same procedure with equal quantities of acid and biomass being used in the reaction. As such, we expect there to be similar amounts of byproducts formed. As expected, the data presented in figure 1 shows that there is no variation between each of the tested conditions.

There were no surprises in these results, which are for the most part uniform. There is no significant difference at an alpha of .05 for a 2-tailed Student's T-test between any of the byproducts between conditions ($t < 1.94$ for all).

3.2- Wash

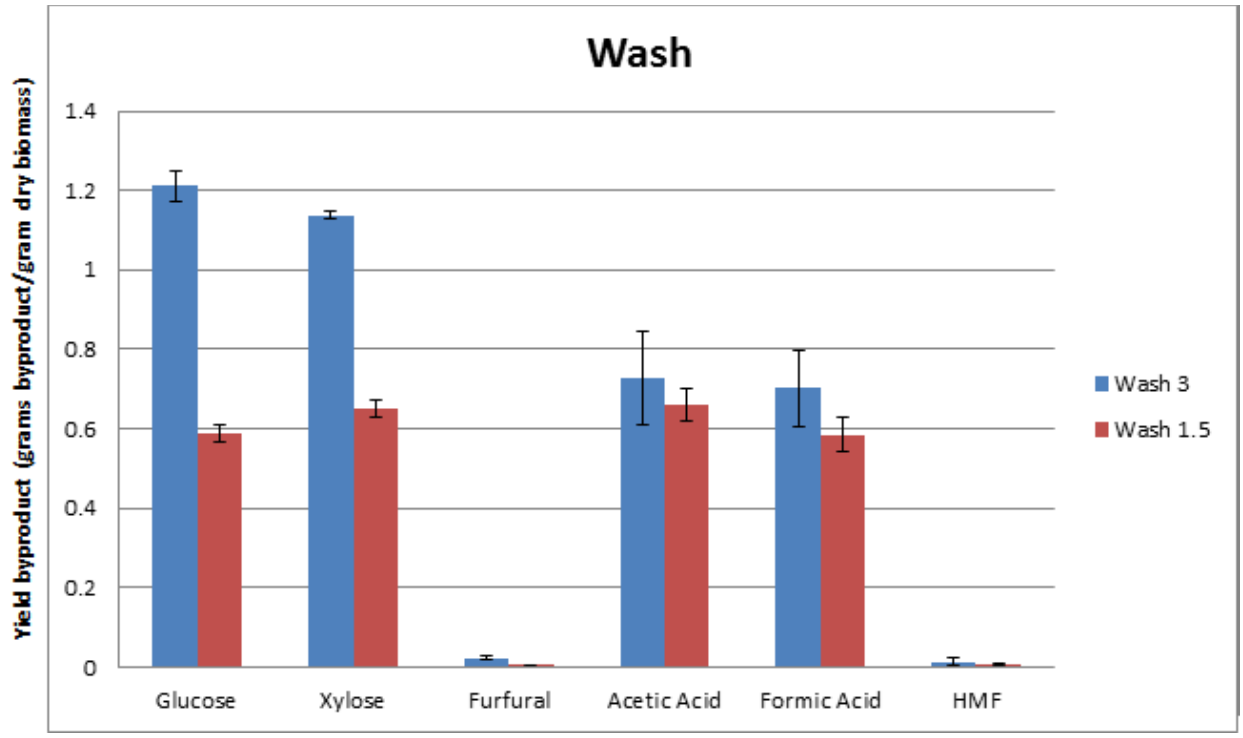

Figure 2. Concentrations of monosaccharides and inhibitory byproducts present in the rinse liquid for: pretreatment water volume of 1.5; and, pretreatment water volume of 3.

The wash step is critical to the process. The quantity of monomeric sugars and inhibitory compounds present in the wash water are presented in figure 2. The pretreated biomass was washed according to the following: 1 pretreatment volume: 1.5 water volumes; and, 1 pretreatment volume: 3 water volumes. The no rinse condition yielded no wash waters; hence no wash water was analyzed. As less water was being used in the 1.5 wash condition, we expected lower total amounts of products in the wash water. This correlation was observed in the sugar yields, which displayed a difference between the two conditions that roughly appeared to be directly proportional with the amount of water used (*i.e.*, twice as much water used, twice as

much glucose detected in wash water). For the inhibitory byproduct yields the totals were within a standard deviation of each other, suggesting no strong correlation between 3 and 1.5 wash and organic acids removed. For the sugars, there is a significant different (glucose $t = 8.32$, xylose $t = 9.19$) at an alpha of .05 for a 2-tailed Student's t-test. For the organic acids at the same conditions, there is not a significant difference ($t < 1.94$ for all).

3.3- Enzyme Hydrolysis

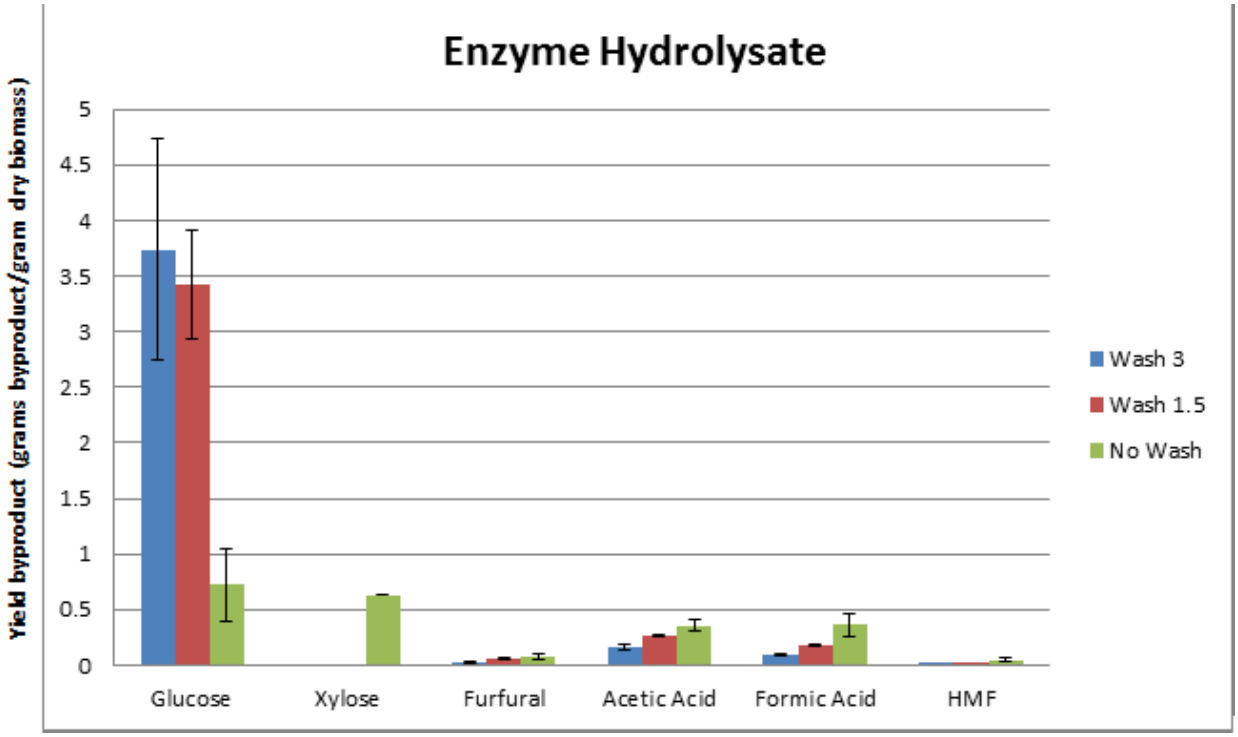

Figure 3. Effect of washing the pretreated biomass (1 pretreatment volume: 0 water volume (no wash); 1 pretreatment volume: 1.5 water volumes; and, 1 pretreatment volume: 3 water volumes) on the concentration of monosaccharides and inhibitory by products present in the enzyme hydrolysis expressed as grams product/gram dry biomass.

Of most interest were the enzyme hydrolysate samples, as these demonstrated the effects of different volumes of water used in the washing step. The concentrations of sugar monomers and inhibitory products as a function of wash are presented in figure 3. The enzyme hydrolyzate had

a volume of 400 ml, which was greater than the volumes of wash used in both 1 ½ and 3 volume rinses. It is important to note this because results presented in figure 3 do not translate in the formation of xylose or inhibitory byproducts during enzymatic hydrolysis, but rather indicate carry over from pretreatment and wash steps. From the data presented in figure 3, not washing the pretreated biomass severely inhibited enzyme hydrolysis, where 0.7 g of glucose per g of biomass processed were reported as compared to 3.5 g of glucose per g of biomass for the rinsed conditions. With regard to sugar monomers, washing with 1 ½ or 3 volumes of water resulted no significant difference at an alpha of .05 in a 2-tailed Student's t-test ($t < 1.94$ for both). It could be necessary to account for the amount of glucose still present in the 1 ½ wash sample that was not removed. It is impossible to determine how much of the glucose present originated from pretreatment or from enzyme hydrolysis. Instead, it was assumed that maximum remaining glucose fraction was present (*i.e.*, it was assumed that the same amount of glucose was present in the 3 wash as in the 1 ½ wash, and the difference in glucose values between the two in the wash fraction was the total glucose 'left behind' in the 1 ½ sample).

3.4- Totals

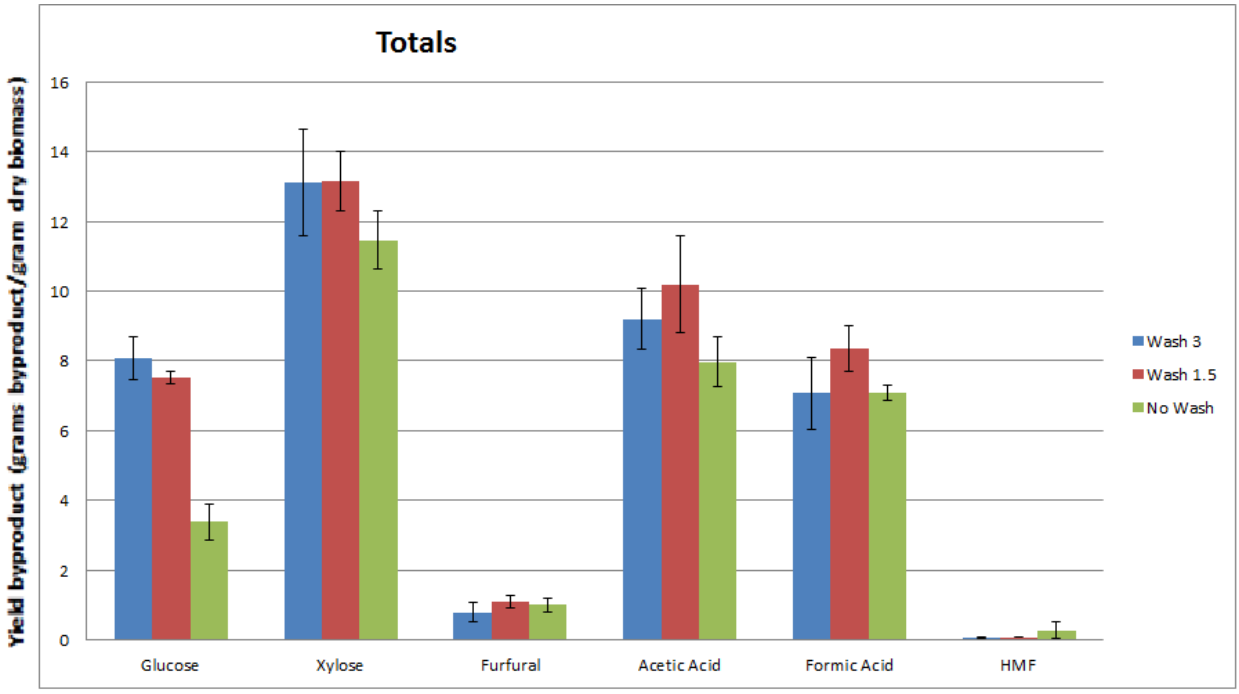

Figure 4. Total concentrations from pretreatment, wash, and enzyme fractions. Monosaccharide and inhibitory byproduct yield, at the conditions of (1 pretreatment volume: 0 water volume (no wash); 1 pretreatment volume: 1.5 water volumes; and, 1 pretreatment volume: 3 water volumes).

Figure 4 presents the overall quantities of sugar monomers and inhibitory products accumulated during the three processing steps. The total inhibitory byproducts were determined to be non-statistically different as they are mostly generated during the uniform pretreatment step. As the wash condition takes place after pretreatment, there is no expected difference in organic acids. Similarly, because xylose is mainly released during pretreatment, all three experimental conditions should result in non-statistically different xylose concentrations. However glucose yields were statistically significantly different, which suggested that washing removes compounds that inhibit enzymatic hydrolysis. Interestingly, known degradation products, such as HMF, formic acid and acetic acid were detected at similarly low concentrations in the three treatments. This suggests that compounds other than those monitored in this work (IE, other than

furfural, acetic acid, formic acid and HMF) might be responsible for the 50% decrease in glucose yields. Compounds that inhibit enzymatic hydrolysis could stem from lignin degradation, and these were not monitored in this work. There might also be effects of washing outside of the removal of inhibitor products, such as some currently unknown process by which the introduction of water could render pretreated biomass more susceptible to enzyme hydrolysis.

4-Conclusions

In each dilute acid pretreatment process, similar amounts of byproducts were yielded at similar initial conditions. Similarly, identical enzyme hydrolysis processes yielded similar data within each condition. The critical step, the wash, showed two important correlations between volume of water used and eventual total yields in the hydrolysis step. This data can be seen in figure 2. The first is that the retrieval of monomeric sugars from the pretreated biomass follows an expected trend of being roughly proportional to the amount of water used. The 1 ½ volumes of wash water yielded about half as much glucose and xylose as the 3 volumes of wash water. The second important correlation showed that the inhibitor byproducts did not follow a strongly proportional relationship with the volumes of wash water used. Likewise, in the subsequent enzyme hydrolysis step it can be seen that glucose yields between the 1 ½ and 3 wash volumes are approximately similar, with the only relationship being a weak one that is proportional to the total wash used. In addition, concentrations of known inhibitory byproducts were particularly low in the enzyme hydrolysis step for all three conditions. The no wash condition yielded notably lower amounts of glucose in the enzyme hydrolysis step, consistent with previous results. This leads to two generalized conclusions. The first is that washing still plays a critical role in achieving good enzymatic hydrolysis sugar yields, though the comparatively low amounts of assumed inhibitory compounds measured in this experiment might indicate that another

unmeasured byproduct (such as lignin byproducts) could be the limited factor. The second is that reducing wash volumes follows a distinct linear trend in retrieving byproducts from the pretreated biomass pellets, but the strength of that relationship in regards to enzyme sugar yields is low. This suggests that less water than the current NREL standard is necessary for enzymatic hydrolysis to be viable at recovering glucose from cellulosic biomass. Further research should be focused on decreasing the wash volumes in additional amounts and testing with a variety of biomass sources in addition to poplar, to see if the trend exists across multiple potential second generation biofuel feed stocks.

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This thesis is approved.

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