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High Temperature Dilute Acid Pretreatment of Coastal Bermudagrass

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Abstract. *The conversion of lignocellulosic biomass into ethanol is an encouraging technology in the face of concerns over global warming and finite energy resources. In the southeastern United States, coastal bermudagrass shows potential for use as an energy crop for ethanol production. A review of the literature has shown that research has been done on the dilute sulfuric acid pretreatment of coastal bermudagrass at 121°C prior to enzymatic hydrolysis. This study examined dilute acid concentrations of 0.3%(w/w) to 1.2%(w/w) at temperatures from 120C to 180C over residence times of 5 to 60 minutes in an effort to optimize the pretreatment process for sugar production. Bermudagrass was pretreated in a 1:10 mixture with dilute sulfuric acid. The pretreated solids were enzymatically hydrolyzed and the resulting reducing sugars have been quantified using a DNS assay method. Data is still being generated,, but from average total reducing sugar data that has been analyzed, the pretreatment conditions of 1.2% sulfuric acid (w/w) for 30 minutes yields optimum sugar production of 300 mg sugars / gram of un-pretreated biomass. Carbohydrate and lignin content will be measured before and after pretreatment in addition to an estimation of reducing sugars in the pretreatment filtrate in future work.*

Keywords. bermudagrass, ethanol production, dilute acid pretreatment, enzymatic hydrolysis

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Introduction

Consumption of energy for use as transportation fuel in the United States (US) is projected to increase significantly over the next two decades (EIA, 2008). With oil prices rising each year and global warming becoming a prioritized concern, bioethanol is being established as a liquid fuel to replace gasoline. In the United States, corn is the major feedstock for producing bioethanol, but it also competes as a food source (Solomon et al., 2007). As a result, lignocellulosic materials should be examined for use in a second phase of United States bioethanol production. Lignocellulosic materials do not compete as a food source, have more sugar per acre available than corn, and there are many sources to choose from. This variety and density per acre of resources helps to make the feedstock cheaper than starch based feedstocks and it lends to the exploration of new feedstocks and processing technologies (Hemelinck et al., 2005).

Currently, the National Renewable Energy Laboratory (NREL) identifies biomass pretreatment as one of the factors standing in the way of cost effective lignocellulosic bioethanol (Aden, 2007). The most often studied pretreatment technologies include alkaline, dilute acid, steam explosion and ammonia freeze explosion (AFEX). In a summary of studies using different pretreatment technologies on corn stover, dilute acid pretreatment at a temperature beyond 120 C yielded the best results with over 90% of theoretical glucose and xylose recovered (Galbe and Zacchi, 2007). Recovery of both five and six carbon sugars is the benefit of using dilute acid pretreatment and will be important in making the overall process economical if the technology to ferment five carbon sugars matures.

Using dilute sulfuric acid pretreatments, researchers have looked into number of localized waste streams for feedstock sources to ensure the lowest cost. In Spain, Cara et al. (2007) reported on using the waste biomass that results from the pruning of olive trees. Effective pretreatment conditions were found to be 1.0% w/w acid solution at 180 C for 10 minutes. Kalman et al. (2002) and Lloyd and Wyman (2005) both examined the use of corn stover, a major waste product from corn harvesting, as a feedstock source in Hungary and the US. Examining the data from each study, better yields of both glucose and xylose were generated using temperatures higher than 120 C. In Colorado, researchers examined using the normally unused tree bark in hardwood processing from trees local to Colorado and Tennessee for bioethanol production (Torget et al., 1991). The conclusion was mixed, where dilute acid pretreatment worked for aspen bark and at higher residence time and temperature (>120 C) for hybrid poplar bark, but the pretreatment was not effective for sweet gum bark. A group in North Carolina took advantage of the waste management programs required by the state for local hog farms and investigated the plausibility of using rye straw and coastal bermudagrass as feedstocks for lignocellulosic ethanol. Results found coastal bermudagrass gave higher yields of sugar and optimum pretreatment conditions were 121 C with a 1.2% w/w acid concentration for 60 minutes (Sun and Cheng, 2005). Because of the effectiveness of higher temperatures coupled with dilute acid pretreatment as based on reviewing literature, it was decided to examine the effect of dilute acid pretreatment on coastal bermudagrass at temperatures beyond 120 C over a range of residence times and acid concentrations.

Materials and Methods

Harvest and Storage

Coastal bermudagrass was obtained in 2007 from Central Crops Research Station located in Clayton, NC courtesy of Dr. Joe Burns. The collected bermudagrass is stored in a bagged

loose bale at room temperature. As needed, the bermudagrass is ground to particle sizes no greater than 2mm using a mechanical mill and stored in sealed bags at room temperature in the lab until further use.

Composition Analysis: Raw Biomass

Prior to any pretreatment, the biomass (bermudagrass) will be analyzed for its composition. This will allow for a more significant comparison of pretreated biomass versus unpretreated (raw) biomass to help judge the effectiveness of the pretreatment conditions. First, the biomass will be analyzed for extractive components like waxes, chlorophyll, non-structural sugars, and other minor components using the NREL laboratory analytical procedure (LAP) "Determination of Extractive in Biomass". Next the extractive-free biomass can be examined for structural carbohydrates using the NREL LAP "Determination of Structural Carbohydrates and Lignin in Biomass". Last the moisture content and ash content will be found using NREL LAPs "Determination of Total Solids in Biomass" and "Determination of Ash in Biomass" respectively.

Pretreatment

A factorial design is being used to examine sugar yields based on the variation of the pretreatment conditions acid concentration, reaction temperature, and residence time. The values of the design are based on literature reviewed and prior work on bermudagrass done by Sun and Cheng (2005). Sulfuric acid concentrations from 0.3% w/w to 1.2% w/w was examined over a temperature range from 120 C to 180 C and a residence time range of 5 to 60 minutes.

Dilute sulfuric acid pretreatment was performed in specialized stainless steel vessels that can withstand the heat and pressure associated with pretreatments at temperatures higher than 120C. These 306 stainless steel vessels were constructed of 19mm (0.75 inch) inner diameter by 102mm (4 inch) long pipe nipple purchased from McMaster Carr. Both sides were capped with the top side given a lock washer to help better seal the vessel.

For a typical pretreatment, seven of these vessels were washed and dried. Sulfuric acid was prepared on a w/w basis from a 96% stock solution beforehand and stored in a reagent bottle for continued use. Six of the vessels were loaded at a ratio of 10 parts acid to one part raw biomass (30ml acid, 3g biomass). The 7th vessel was loaded with de-ionized (DI) water in the same 10:1 ratio for use as a blank with an internal k-type thermocouple temperature probe.

A Fisher Scientific High-Temp Bath was used to indirectly heat the vessels with silicone oil as heat transfer fluid. A k-type thermocouple temperature probe was submerged in the oil in order to monitor the oil temperature. Once the oil reached the set point temperature, the seven units were carefully added to the bath and the time of this addition was recorded. The internal temperature of the blank vessel was then monitored until it reached the set point at which time it was assumed that all of the vessels were at the exposure temperature and the time was recorded. At this time, the residence time was begun and upon completion of the residence time, the vessels were quickly removed from the oil bath and placed in a bin of cool water where they came down to room temperature almost immediately.

After pretreatment was finished, the vessels were opened up for the filtration step. A GAST vacuum pump was used to pull a vacuum across a P8 filter paper in a standard buchner funnel vacuum flask setup. Each vessel had its contents emptied on to a pre-weighed filter paper associated with that particular sample. The biomass was rinsed with 100ml of water and the filtrate was captured and stored in labeled 15ml storage tubes and frozen at -20C for sugar analysis later. The solids on the filter paper were rinsed with 200 ml of DI water and then placed into an appropriately labeled bag for storage at 4C prior to being used in composition analysis

and enzymatic hydrolysis. These bags were weighed before and after the addition of biomass for use in calculating solid loss later. The filter paper is dried for 24 hours at 105C and then weighed to account for biomass remaining on the filter paper.

Sugar Analysis

An excess of cellulase, Novozymes, NS-50013, and cellobiase, Novozymes NS-50010, was used for the enzymatic hydrolysis step to ensure there was no limitation in sugar production caused by the enzymes. A portion of the retained solid biomass was used for the enzymatic hydrolysis. The liquor (supernatant) resulting from the enzymatic hydrolysis as well as the retained pretreatment filtrate was analyzed for total reducing sugars using a DNS assay. The DNS assay procedure was adapted from the procedure outlined by Ghose (1987) which was adapted from the paper by Miller (1959). The DNS assay is a crude procedure and provided a quick quantification of all the sugar monomers and dimers released from the structural carbohydrates in the biomass.

Total sugar yields were calculated on a per gram of raw biomass basis after accounting for solid loss. Mean total sugar yields, hydrolysis liquor and pretreatment filtrate yields summed, were compared between pretreatment conditions using statistical software (SAS) to assess optimized pretreatment conditions. Additionally, pretreatment conditions for maximized six carbon sugar yield (mostly cellulose) and five carbon sugar yield (mostly hemicellulose) were noted. As a final step, biomass samples that yield maximum sugars were put through a fermentation step to ensure there are no inhibitors resulting from the pretreatment step that keeps the yeast from being able to use the sugars to produce ethanol.

Results and Discussion

Currently only the results shown as shaded in gray in table 1 below have been collected and analyzed for sugar production after an enzymatic hydrolysis step. All other data is still pending.

Table 1. A graphical view of the intended experimental factorial design.

Acid Conc. (% w/w)	Temp. (C)	Time (min)			
0.3	120	5	15	30	60
0.3	140	5	15	30	60
0.3	160	5	15	30	60
0.3	180	5	15	30	60
0.6	120	5	15	30	60
0.6	140	5	15	30	60
0.6	160	5	15	30	60
0.6	180	5	15	30	60
0.9	120	5	15	30	60
0.9	140	5	15	30	60
0.9	160	5	15	30	60
0.9	180	5	15	30	60
1.2	120	5	15	30	60
1.2	140	5	15	30	60
1.2	160	5	15	30	60
1.2	180	5	15	30	60

Of the data analyzed, a maximum sugar yield of just the solid biomass has been identified as a result of the pretreatment conditions of 160 C, 30 minute residence time, and 1.2% w/w acid concentration (see figure 1). Examining 0.6% w/w acid concentration, for a 30 minute residence time over 140 C, 160 C, 180 C draws the conclusion that because the trend is still increasing and temperatures beyond 180 C should be tried until a decline in sugar production occurs (see figure 1).

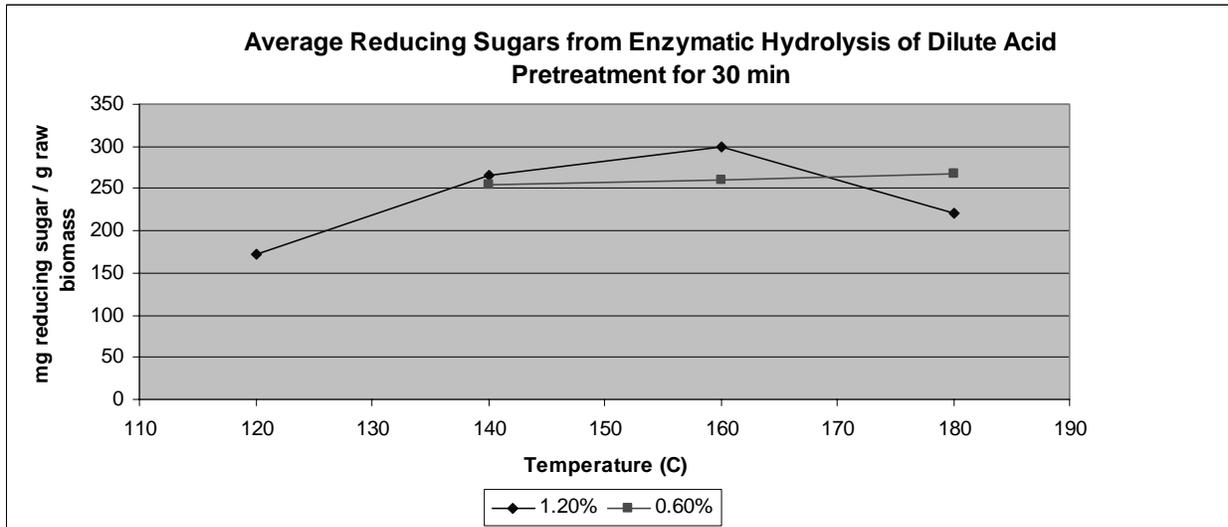


Figure 1. Average reducing sugars from enzymatic hydrolysis liquor as quantified using a DNS assay are shown for acid concentration of 0.6% w/w and 1.2% w/w over a range of different temperatures.

Conclusion

From initial data, temperatures beyond 120 C for a sulfuric acid concentration of 1.2% w/w for 30 minutes results in an increase in sugar yield as hypothesized. A lower acid concentration of 0.6% w/w is being investigated, but current results are inconclusive. Future work must investigate the remaining factorial for enzymatic hydrolysis liquor sugar yields in addition to an estimate of pretreatment filtrate sugar yields for the entire factorial. Currently this data is being generated and analyzed. These full results will provide a clearer view of optimum pretreatment conditions and a relationship between higher temperatures and required acid concentrations for the production of fermentable sugars.

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