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Alkaline Pretreatment of Coastal Bermudagrass for Bioethanol Production

Ziyu Wang
North Carolina State University, Campus Box 7625, Raleigh, NC

Deepak R. Keshwani
University of Nebraska - Lincoln, dkeshwani2@unl.edu

Arthur P. Redding
North Carolina State University, Campus Box 7625, Raleigh, NC

Jay J. Cheng
North Carolina State University, Campus Box 7625, Raleigh, NC

2950 Niles Road, St. Joseph, MI 49085-9659, USA
269.429.0300 fax 269.429.3852 hq@asabe.org www.asabe.org

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Alkaline Pretreatment of Coastal Bermudagrass for Bioethanol Production

Ziyu Wang, Deepak R. Keshwani, Arthur P. Redding, Jay J. Cheng

Department of Biological and Agricultural Engineering, North Carolina State University, Campus Box 7625, Raleigh, NC 27695-7625, USA

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Abstract. *Lignocellulosic materials are regarded as an alternative energy source for bioethanol production to reduce our reliance on fossil fuels. Pretreatment is important for improving the enzymatic digestibility of lignocelluloses to increase the yield of fermentable sugars. Alkaline (sodium hydroxide and lime (calcium hydroxide)) pretreatment of coastal bermudagrass for enhanced reducing sugars recovery was investigated in this study. The effect of NaOH pretreatment at 121°C using 1%, 2% and 3% (w/v) NaOH for 15, 30, 60 and 90 minutes was evaluated first. Lower NaOH concentrations (0.5% and 0.75%) and lower temperatures (50, 80 and 100°C) were then examined. Lime (0.1 g $Ca(OH)_2$/g raw biomass) pretreatment of the biomass was conducted at room temperature, 50°C, 80°C, and 121°C. Total reducing sugars, glucose and xylose were analyzed. The optimal NaOH pretreatment conditions at 121°C for glucose and xylose production are 15 minutes and 0.75% NaOH. However, to maximize total reducing sugars production, pretreatment at 121°C for 30 minutes using 1% NaOH is needed. The highest reducing sugars yield reached up to approximate 86% of theoretical maximum for NaOH pretreatment. Sodium hydroxide is more efficient than lime at 121°C for improved reducing sugars yield. Increasing temperature reduced the optimal pretreatment time at the same lime loading. The reducing sugars production under optimal pretreatment times was enhanced by 8% of theoretical maximum from room temperature to 80°C.*

Keywords. Sodium hydroxide; Lime; Pretreatment; Reducing sugars; Costal bermudagrass

Introduction

Current fuel ethanol production in the United States is mainly based on the fermentation of glucose derived from corn starch, which will compete against the corn-based food and feed production. On the other hand, there are plentiful lignocellulosic materials such as crop residues, grasses, sawdust, solid animal waste and wood chips that can be utilized to substitute the equivalent of 40% of the gasoline in the current US market (Wheals et al., 1999). Lignocellulosic materials can capture CO_2 during growth so that their combustion does not generate net CO_2 (Klass, 1998). Lignocellulosic materials are considered as a potential source for a large amount of low-cost ethanol production.

There are limiting factors to the maximum possible efficiency of the conversion of lignocellulosic materials to ethanol. Enzymatic hydrolysis is hindered by the following substrate-related factors: cellulose contains highly resistant crystalline structure, lignin and hemicellulose surrounding cellulose form a physical barrier, and sites available for enzymatic attacks are limited (Kim et al., 2001). Pretreatment, an important tool for practical lignocellulose conversion processes, is required to alter the structure of lignocellulosic biomass to make cellulose more accessible to the enzymes (Moiser et al., 2005). Pretreatment methods can be physical, chemical, physico-chemical, and biological processes.

Of the promising pretreatment technologies, alkaline pretreatment has received much attention. Saponification of intermolecular ester bonds crosslinking hemicellulose and other components is believed to be the mechanism of alkaline pretreatment (Sun and Cheng, 2002). The major effect of alkaline pretreatment is the delignification of lignocellulosic biomass, thus enhancing the reactivity of the remaining carbohydrates. The lignin contents of the biomass influence the effect of alkaline pretreatment (Fan et al., 1987). Alkaline pretreatments also remove acetyl and different kinds of uronic acid substitutions on hemicellulose, which lowers the extent of enzymatic hydrolysis of cellulose and hemicellulose (Chang and Holtzapple, 2000). Sodium hydroxide effectively enhances lignocellulose digestibility by increasing internal surface area, decreasing the degree of polymerization and the crystallinity of cellulose, and separating structural linkages between lignin and carbohydrates (Fan et al., 1987). The digestibility of NaOH-treated hardwood increased with the decrease of lignin content (Millet et al, 1976). Sodium hydroxide pretreatment was also effective for enhancing the digestibility of wheat straw (Bjerre et al., 1996).

Lime (calcium hydroxide) pretreatment typically mixes the slurry of lime and water with the biomass and then stores the material in a pile for hours or weeks. Studies have been carried out on lime pretreatment of switchgrass at 100°C for 2 h (Chang et al., 1997), wheat straw at 85°C for 3 h (Chang et al., 1998), corn stover at 100°C for 13 h (Karr and Holtzapple, 1998, 2000), and poplar wood at 150ºC for 6 h with 14-atm oxygen (Chang et al., 2001). Adding air/oxygen to the reaction system can significantly improve the delignification of the biomass (Chang and Holtzapple, 2000). Chang et al. (2001) performed oxidative lime pretreatment of poplar wood at 150°C for 6h with 78% removal of lignin and 71% improvement of the glucose yield from enzymatic hydrolysis. Lime (0.5 g lime/g raw biomass) was used to pretreat corn stover in non-oxidative and oxidative conditions at 25ºC, 35ºC, 45ºC, and 55°C. The optimal condition was found to be 55°C for 4 weeks with aeration (Kim and Holtzapple, 2005). Low reagent cost and safety, and the recovery of lime from water as insoluble calcium carbonate by reaction with carbon dioxide benefit the lime pretreatment method (Playne, 1984; Chang et al., 1997).

The swine industry in the southeast of the United States is expanding rapidly. To avoid environmental pollution caused by the swine wastewater, many farmers grow coastal bermudagrass for nitrogen and phosphorus removal to prevent potential pollution of these

nutrients to the nearby watershed. The existence of cropping system makes coastal bermudagrass considered as a potential lignocellulosic feedstock for bioethanol production. Furthemore, the harvested coastal bermudagrass is usually given away or sold at very low price as animal feed. Therefore, there is a great interest to investigate the conversion of coastal bermudagrass into ethanol. The purpose of this study is to investigate the effects of different alkaline (sodium hydroxide and lime) pretreatments on the subsequent hydrolysis and fermentation of coastal bermudagrass for bioethanol production. This research can provide important information on the commercial utilization of coastal bermudagrass for large-scale ethanol production.

Materials and Methods

Biomass Preparation

Air-dried coastal bermudagrass (harvested in June, 2007) was obtained from North Carolina State University Central Crops Research Station in Clayton, NC. The biomass was size reduced to pass a 2-mm sieve using a Thomas Wiley Laboratory Mill (model no. 4) and stored in sealed plastic bags at room temperature until use for characterization and pretreatment.

Pretreatment

All pretreatments were done in an autoclave (for pretreatments at 121°C), a water bath (for pretreatments up to 100°C). Biomass samples were immersed in dilute alkali solutions (solid to liquid ratio of 1:10) in sealed serum bottles. The alkaline reagents used were sodium hydroxide (NaOH) and calcium hydroxide/lime ($Ca(OH)_2$). After pretreatment, the biomass was washed with 200 ml of deionized (DI) water for NaOH pretreatment and 600 ml of deionized water for lime pretreatment, and then refrigerated for enzymatic hydrolysis.

For NaOH pretreatment, three sets of experiments were conducted. The first set of experiments evaluated the effect of pretreatment at 121°C using 1%, 2% and 3% (w/v) NaOH for 15, 30, 60 and 90 minutes. The second set examined pretreatment at 121°C with lower NaOH concentrations (0.5% and 0.75%) for 15 and 30 minutes. The third set examined pretreatment at lower temperatures (50, 80 and 100°C) with the optimal NaOH concentration and pretreatment time obtained at 121°C.

For lime pretreatment, three sets of experiments have been conducted using a lime loading of 0.1g per g of raw biomass. The first set of experiments was done at 121°C for 15, 30 and 60 minutes. The second set of experiments examined pretreatment at room temperature for pretreatment times of 1, 3, 6, 10, 16, 24, 34 and 48 hours. The third set of experiments studied pretreatment at 50°C and 80°C for pretreatment times of 1, 3, 6, 10, 16 and 24 hours.

Enzymatic Hydrolysis

Enzymatic hydrolysis of pretreated biomass was carried out in 250 ml Erlenmeyer flasks in a controlled environment incubator shaker set at 55°C and 150 rpm. 1 g (dry basis) of pretreated biomass was immersed in 0.05 M sodium citrate buffer to maintain a pH of 4.8. Prior to hydrolysis, activity of cellulase and cellobiase was determined to be 76.44 FPU/ml and 283.14 CBU/ml respectively. The dosage of cellulase and cellobiase for all hydrolysis experiments was 40 FPU and 70 CBU per gram of dry biomass. Sodium azide (0.3% (w/v)) was added to the hydrolysis mixture to inhibit microbial growth. The hydrolysis was carried out for 72 hours after which the hydrolyzate was centrifuged and the supernatant was stored at -20°C for sugar analysis.

Analytical Methods

Moisture content of the biomass was measured by drying the sample at 105°C in an oven to constant weight (Sluiter, 2005). The biomass was analyzed for extractives with 2:1 toluene-ethanol mixture in a Soxhlet apparatus with a reflux time of 24 hours (Silverstein, 2004). The extractive free biomass was analyzed for structural carbohydrates and lignin using a two-stage sulfuric acid hydrolysis procedure recommended by the National Renewable Energy Laboratory (Sluiter, 2006). Total reducing sugars in the enzymatic hydrolyzates were determined by the DNS (dinitrosalicylic acid) method using glucose as the standard (Miller, 1959). Monosaccharides (glucose and xylose) from composition analysis and in the hydrolyzates were measured by HPLC using an Aminex HPX-87P column tailored for analysis of hexoses and pentoses in lignocellulosic materials.

Statistical Analysis

Experimental data were statistically analyzed using the GLM procedure in SAS software. For experiments with two factors, results were analyzed to study responses to determine which combination of variables provided highest conversions at the 95% confidence level. For experiments with only one factor, ANOVA analysis of the results was conducted to determine the optimal pretreatment condition.

Results and Discussion

Biomass Characterization

The coastal bermudagrass was analyzed for carbohydrates, lignin, ash and extractives. The weight percentages of each component per gram of dry biomass are presented in Table 1.

Table 1. Chemical composition of coastal bermudagrass.

Component	wt%, dry basis in biomass
Glucan	25.59
Xylan	15.88
Arabinan	1.95
Galactan	1.60
Acid insoluble lignin	15.37
Acid soluble lignin	3.96
Extractives	4.17
Ash	6.60

Glucan was the major component followed by xylan and acid insoluble lignin. There were only a small amount of arabinan and galactan in bermudagrass. No mannan was detected in the biomass. Xylan was the main component of hemicellulose in bermudagrass. From the experimental results, most of the components was less than that based on the previous report except for galactan and ash (Sun and Cheng, 2005). This is because the bermudagrass used in this study was harvested in a different location from where the previous research indicated. Cultivation and harvest time would also affect the chemical composition of the biomass. In addition, the carbohydrates analysis reported here was carried out on extractive free biomass while the previous report used biomass with extractives for sugar analysis.

Sodium Hydroxide Pretreatment

The supernant liquor from the hydrolyzate was quantified for total reducing sugars and monomeric reducing sugars. Figure 1 shows the results from the first set of experiments for NaOH pretreatment at 121°C. Pretreatment with a NaOH concentration of 1% yielded significantly higher reducing sugars than 2% and 3%. Higher NaOH concentrations gave higher total solid loss, thus led to less reducing sugars yield. Extending pretreatment time beyond 30 minutes reduced total reducing sugars production because of high solid loss. Based on this experiment, the highest total reducing sugars yield (~86% of theoretical maximum) was obtained with 1% NaOH for a pretreatment time of 30 minutes. These results prompted the use of lower NaOH concentrations at 121°C. Figure 2 indicates that concentrations lower than 1% yielded significantly lower reducing sugar levels for pretreatment at 30 minutes. But no statistical difference was observed between 0.75% and 1% for pretreatment at 15 minutes.

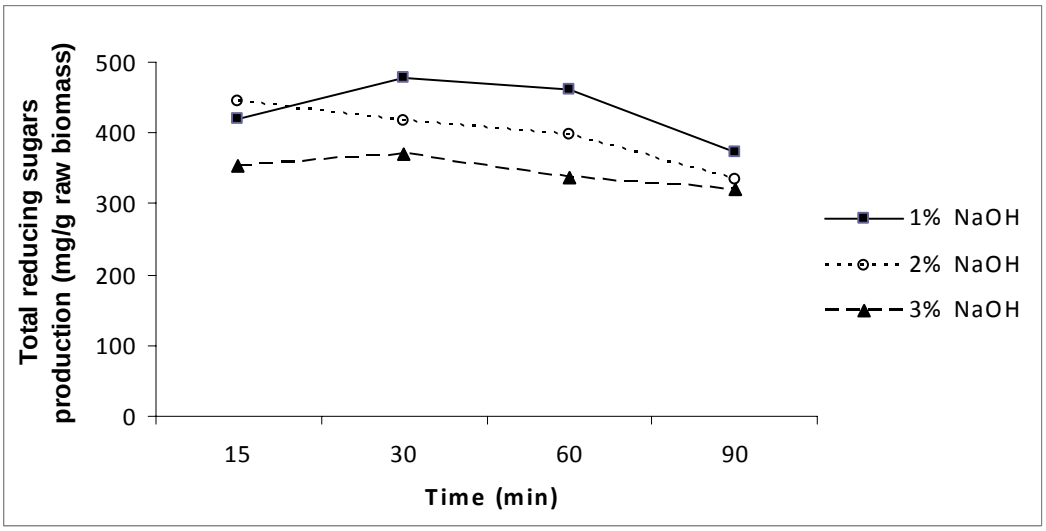

Figure 1. Reducing sugars production for NaOH pretreatment with1%, 2% and 3% for 15, 30, 60 and 90 minutes at 121°C.

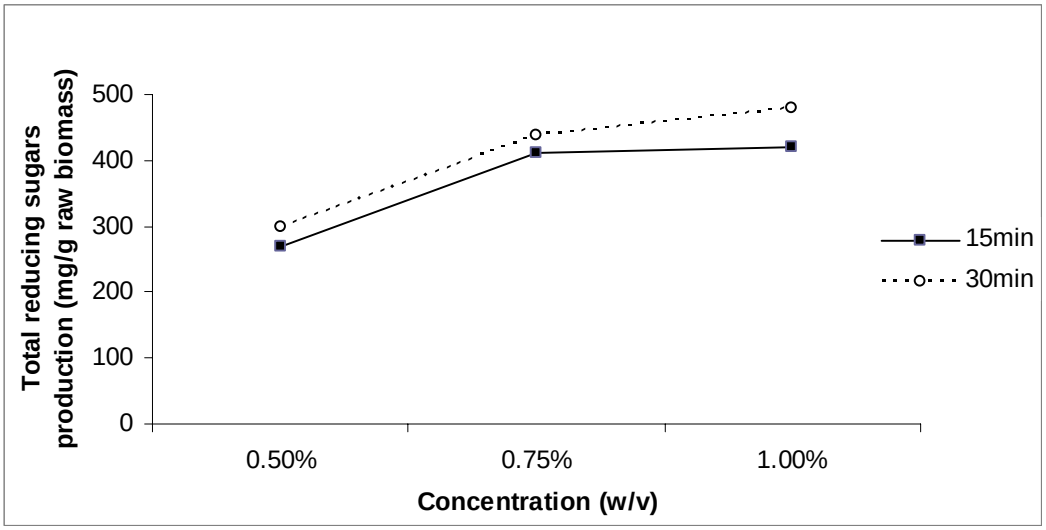

Figure 2. Reducing sugars production for NaOH pretreatment with 0.5%, 0.75% and 1% for 15 and 30 minutes at 121°C.

Analysis of monomeric sugars reveals that glucose and xylose production for pretreatment with 0.75% NaOH were statistically similar to pretreatment with 1% NaOH (Figure 3) for both 15 and 30 minutes at 121°C, while glucose and xylose yield for pretreatment at 0.5% NaOH were significantly lower than pretreatment at 0.75% and 1% NaOH. Based on these results, optimal pretreatment conditions at 121°C for glucose and xylose production are 15 minutes and 0.75% NaOH. But to maximize total reducing sugars production, pretreatment at 121°C for 30 minutes using 1% NaOH is needed. Because of the fairly small amount of galactose and arabinose in the hydrolyzates, glucose and xylose production were considered for optimizing pretreatment conditions based on monomeric reducing sugars yield. In order to make ethanol production from lignocellulosic materials economically feasible, both hexose and pentose in lignocellulose need to be utilized by microorganisms for ethanol production. Therefore, optimization of pretreatment conditions was conducted based on either glucose and xylose production or total reducing sugars production.

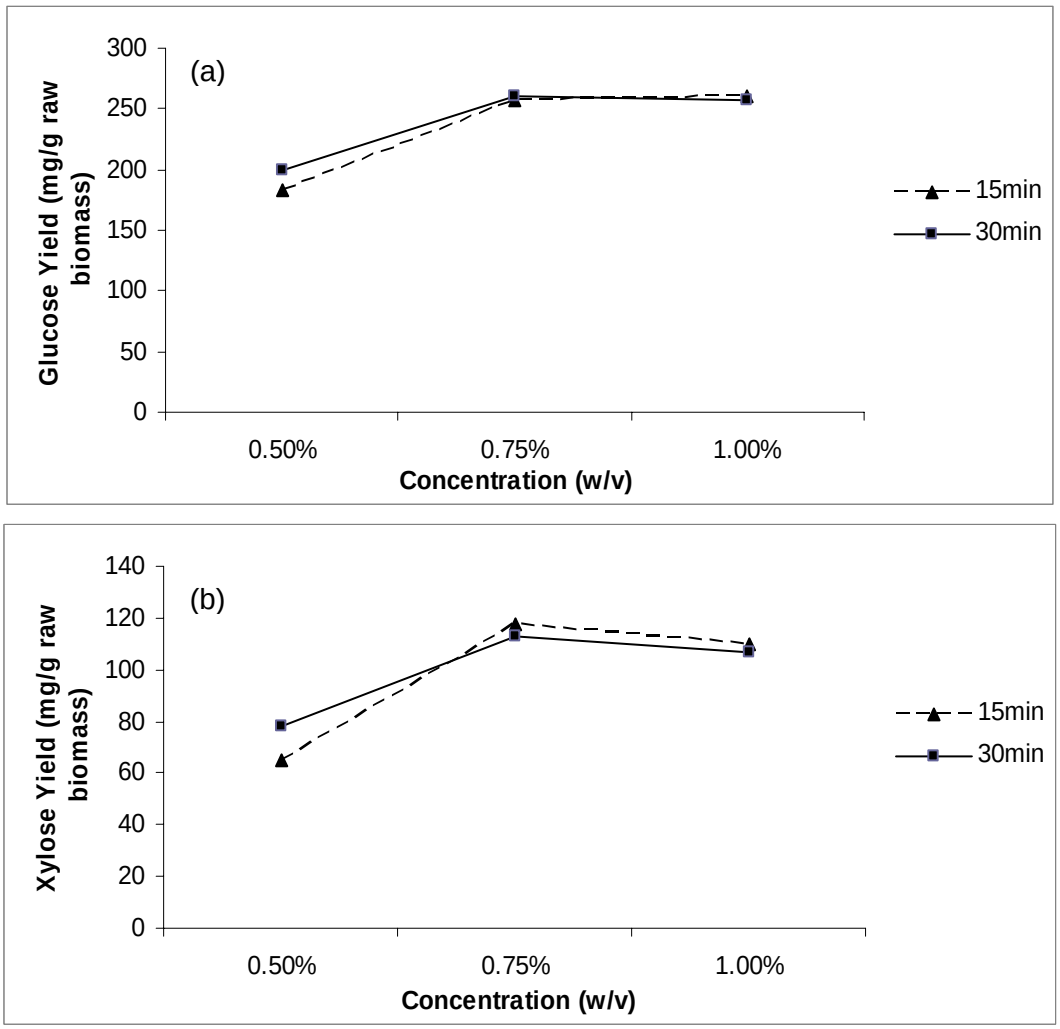

Figure 3. Monomeric sugar production for NaOH pretreatment with 0.5%, 0.75% and 1% for 15 and 30 minutes at 121°C.

Figure 4 shows the effect of temperature on total reducing sugars production for pretreatment with 1% NaOH for 30 minutes. Results indicate that reducing temperature below 121°C significantly lowered production of total reducing sugars. Total reducing sugars production for pretreatment at 80°C was statistically similar to pretreatment at 100°C. As a result of lower

reaction rate during pretreatment at lower temperatures, the biomass structure was not disrupted sufficiently to reach a high sugar conversion.

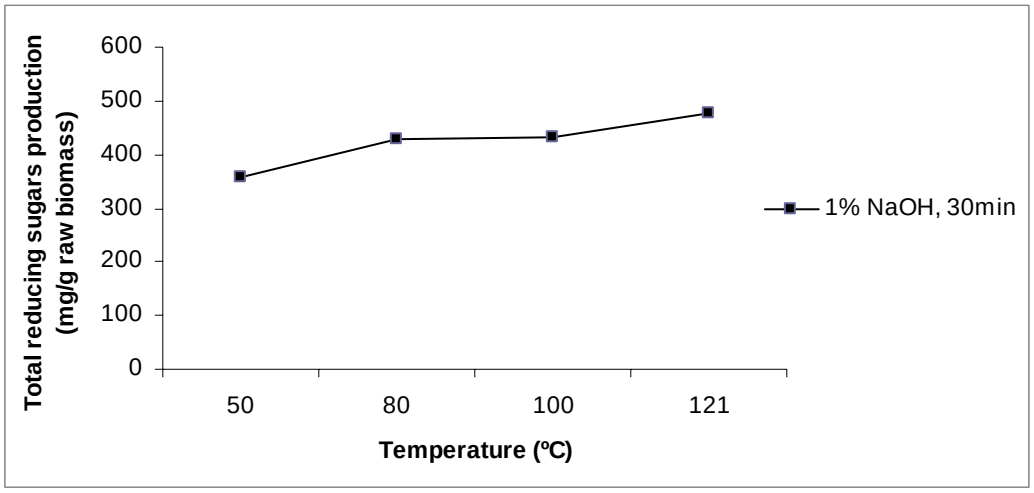

Figure 4. Effect of temperature on total reducing sugars production for NaOH pretreatment with 1% for 30 minutes.

Lime Pretreatment

Unlike NaOH, lime is inexpensive, safe, and can be recoverd by carbonating wash water. Figure 5 shows the results of pretreatment with lime at 121°C in comparison to results from NaOH pretreatment. Overall reducing sugars yields were significantly lower than those of NaOH pretreatment. Because calcium ion carries two positive charges and the surface of molecules in

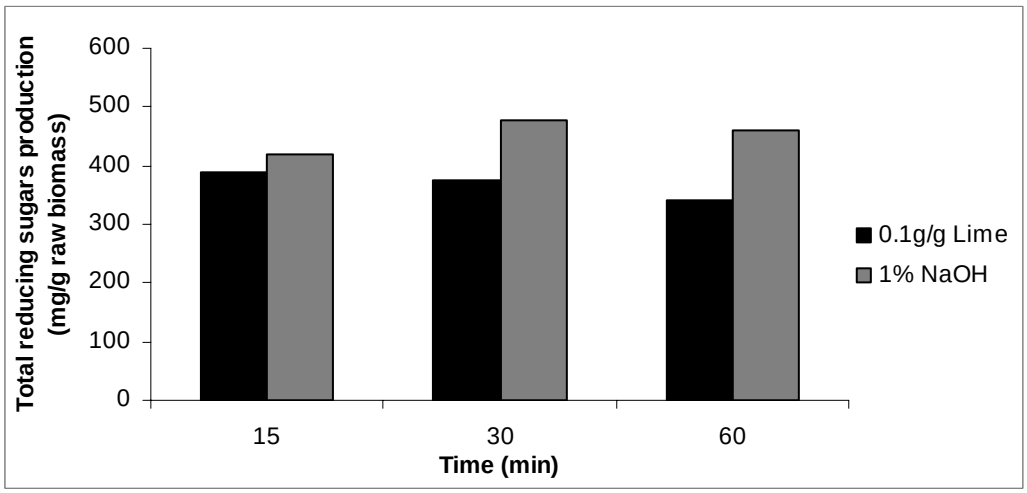

Figure 5. Reducing sugars production for lime pretreatment compared to NaOH pretreatment at 121°C.

the biomass carries negative charges, calcium ion can crosslink components in the biomass to reduce total solid loss and reduce the accessibility of carbohydrates to cellulase enzymes. The experimental results indicate much lower solid loss for lime pretreatment than NaOH pretreatment, which explains the fact that lime did not perform as efficiently as NaOH at 121°C. The next step was the evaluation of lime pretreatment at temperatures lower than 121°C. To date, lime pretreatment at room temperature, 50°C, and 80°C were investigated.

Figure 6 reveals the total reducing sugar production for pretreatment with 0.1 g of lime/g of raw biomass at room temperature. Results indicate that reducing sugars yield was optimized (~72% of theoretical maximum) for a pretreatment time of 34 hours. Extending pretreatment time beyond 34 hours did not increase reducing sugars production. According to data analysis, reducing sugars production was significantly lower than that observed for optimal NaOH pretreatment. Figure 7 shows total reducing sugars production for lime pretreatment at 50°C.

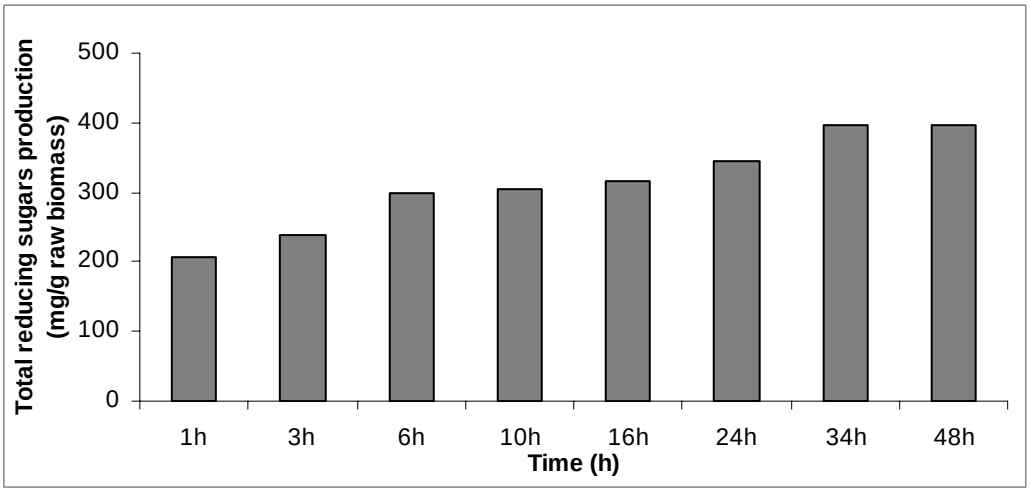

Figure 6. Reducing sugars production for lime (0.1g lime/g raw biomass) pretreatment at room temperature.

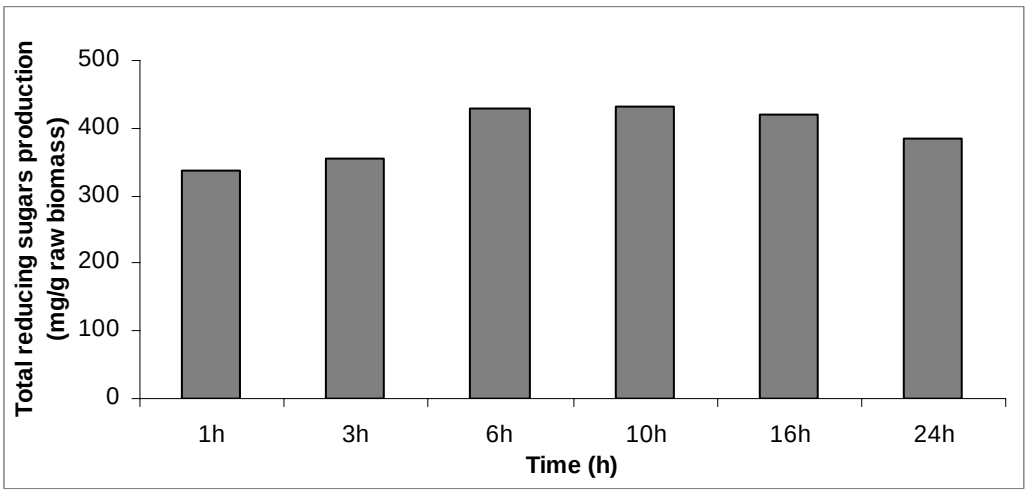

Figure 7. Reducing sugars production for lime (0.1g lime/g raw biomass) pretreatment at 50ºC.

In this case, reducing sugars production was optimized (~78% of theoretical maximum) for a pretreatment time of 6 hours, but was still approximately 8% lower than that for optimal NaOH pretreatment. As shown in Figure 8, reducing sugars production was optimized (~80% of theoretical maximum) for a pretreatment time of 3 hours. Approximate 6% difference in the optimal results between lime pretreatment and NaOH pretreatment exists. With the increase of pretreatment temperature from room temperature to 80ºC, the optimal pretreatment time decreased from 34 hours to 3 hours. This phenomenon indicates that temperature did affect reducing sugars yield for lime pretreatment. Increasing pretreatment temperature could reduce pretreatment time at the same lime loading. It would be interesting to further conduct lime pretreatment at 100ºC.

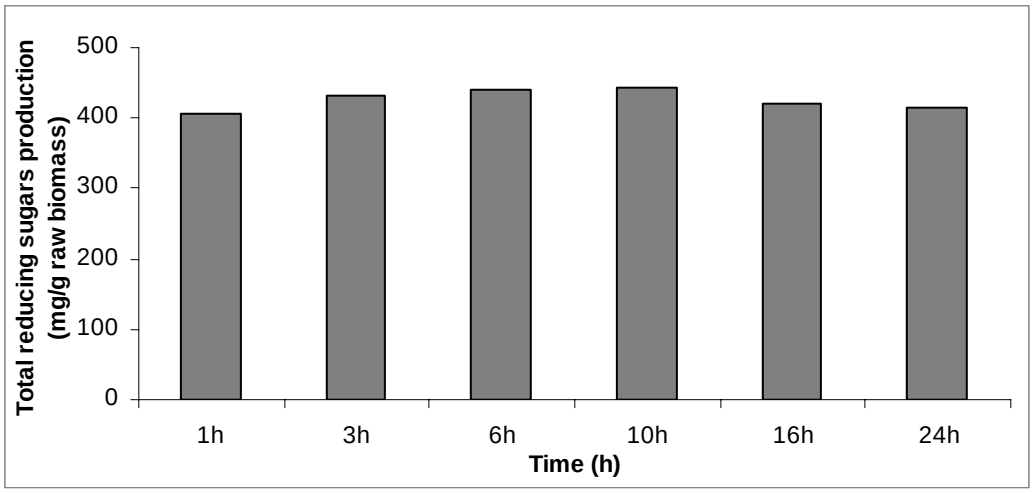

Figure 8. Reducing sugars production for lime (0.1g lime/g raw biomass) pretreatment at 80ºC.

Conclusion

The efficiency of alkaline (sodium hydroxide and lime) pretreatment of coastal bermudagrass for bioethanol production was evaluated in this study. Based on the results to date, sodium hydroxide is more efficient than lime at 121°C for high reducing sugars yield after enzymatic hydrolysis. The optimal NaOH pretreatment conditions at 121°C for glucose and xylose production are 15 minutes and 0.75% NaOH. However, to maximize total reducing sugars production, pretreatment at 121°C for 30 minutes using 1% NaOH is needed. Pretreatment with temperature below 121°C did not perform as efficiently as 121°C pretreatment on reducing sugars production. The highest reducing sugars yield can reach up to approximate 86% of theoretical maximum for sodium hydroxide pretreatment. Lime pretreatment at room temperature did not considerably improve the digestibility of coastal bermudagrass for reducing sugars yield. The optimal reducing sugars production was enhanced by 8% of theoretical maximum from room temperature to 80°C. Increasing temperature reduced the optimal pretreatment time at the same lime loading.

Further study on pretreatment with 0.75% NaOH for 15 minutes at temperatures lower than 121°C need to be conducted to optimize glucose and xylose production with cost reduced. Lime pretreatment at 100°C is necessary for comparison with the results obtained from other temperatures. Additional lime loadings (0.02, 0.05, 0.08g lime/g raw biomass) will be examined for the purpose of cost savings. Finally, enzyme dosing will be optimized during hydrolysis for economical ethanol production from coastal bermudagrass.

Acknowledgements

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