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Sodium hydroxide pretreatment and enzymatic hydrolysis of coastal Bermuda grass

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Abstract

Coastal Bermuda grass was pretreated with NaOH at concentrations from 0.5% to 3% (w/v) for a residence time from 15 to 90 min at 121 °C. The pretreatments were evaluated based on total lignin removal and production of total reducing sugars, glucose and xylose from enzymatic hydrolysis of the pretreated biomass. Up to 86% lignin removal was observed. The optimal NaOH pretreatment conditions at 121 °C for total reducing sugars production as well as glucose and xylose yields are 15 min and 0.75% NaOH. Under these optimal pretreatment conditions, total reducing sugars yield was about 71% of the theoretical maximum, and the overall conversion efficiencies for glucan and xylan were 90.43% and 65.11%, respectively.

Keywords: coastal Bermuda grass, NaOH pretreatment, glucose, xylose, reducing sugar

1. Introduction

Pretreatment is required to disrupt the structure of lignocellulosic materials during cellulosic ethanol production, because the extensive interactions among cellulose, hemicellulose and lignin, and the barrier nature of lignin minimize enzyme access to the carbohydrates and result in poor yields of fermentable sugars (Kumar and Wyman, 2009a, 2009b; Mansfield et al., 1999). The major effect of alkaline pretreatments is the delignification of lignocellulosic biomass, thus enhancing the reactivity of the remaining carbohydrates (Kim and Holtzapple, 2005). Several studies (Bjerre et al., 1996; Hamilton et al., 1984; Millet et al., 1976) have shown the potential of NaOH pretreatment on a variety of lignocellulosic materials.

Coastal Bermuda grass (CBG) (*Cynodon dactylon* L.) is a promising lignocellulosic feedstock for the Southeastern US with annual dry matter yields of 6–10 tons per acre (Holtzapple et al., 1994). Previous works related to pretreatment of CBG include high pressure hot water pretreatment (Brandon et al., 2008), ammonia fiber explosion (Holtzapple et al., 1994) and dilute sulfuric acid pretreatment (Sun and Cheng, 2005). To date, no study has investigated the pretreatment of CBG using NaOH. The objectives of this study were to test the effectiveness of NaOH pretreatment of CBG, optimize enzyme loadings for the hydrolysis of pretreated biomass and conduct fermentation tests to evaluate the ethanol yield from the biomass.

2. Methods

2.1. Biomass preparation

Air-dried CBG (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 its use for characterization and pretreatment.

2.2. Pretreatment experiments

CBG samples (5 g per replicate) were pretreated with 0.5%, 0.75%, 1%, 2%, and 3% (w/v) sodium hydroxide solutions (solid to liquid ratio of 1:10) in sealed glass serum bottles in an autoclave (Model 3021, Amsco) for 15, 30, 60 and 90 min at 121 °C. The pretreated biomass recovered by filtration through a porcelain Buchner funnel was washed with 400 ml of deionized (DI) water (80 ml DI water/g raw biomass) which was sufficient to neutralize the pH of the substrate. The wet solids were completely transferred to a preweighed plastic bag, weighed and stored sealed at 4 °C for the enzymatic hydrolysis experiment later. A small portion of the wet pretreated biomass was weighed and dried at 105 °C to determine solid recovery.

2.3. Enzymatic hydrolysis

The activities of cellulases (NS50013 cellulase complex) and cellobiase (NS50010 β-glucosidase) obtained from Novozymes North America Inc. (Franklinton, NC) were determined to be 76.4 FPU (filter paper unit)/ml and 283.1 CBU (cellobiase unit)/ml, respectively (Ghose, 1987).

To evaluate the sugar yields from the pretreatments, the wet pretreated biomass (triplicates per sample) equivalent to 1 g dry biomass was hydrolyzed with excessive dosage of cellulase (40 FPU/g dry biomass) and cellobiase (70 CBU/g dry biomass) in 0.05 M sodium citrate buffer (pH 4.8) with a total liquid volume of 30 ml in an Erlenmeyer flask (250 ml) in a controlled environmental incubator shaker set (Model 25, New Brunswick Scientific). Sodium azide (0.3% (w/v)) was added to the hydrolysis mixture to prevent microbial growth. The hydrolysis was carried out at 55 °C and 150 rpm for 72 h after which the hydrolyzate was centrifuged (Model 5810R, Eppendorf) at 4 °C and 4000 rpm for 15 min and the supernatant was stored at –20 °C for further analysis. Enzymatic hydrolysis of untreated biomass was conducted as a control. Enzyme and substrate blanks both were run in the hydrolysis step and their respective sugar concentrations were approximately zero.

2.4. Analytical methods

Total solids, ash and extractives were measured for raw CBG (Sluiter, 2005a, 2005b; Han and Rowell, 1997). The extractive-free raw and pretreated biomass were both analyzed for lignin including acid soluble lignin and acid insoluble lignin and monomeric sugars (glucose, xylose, galactose and arabinose) (Sluiter, 2006). Total reducing sugars in the enzymatic hydrolyzates were determined by the dinitrosalicylic acid (DNS) method (Miller, 1959). Monosaccharides in the raw and the pretreated biomass and in the hydrolyzates were measured with a high performance liquid chromatography (HPLC) system (Model 1200, Agilent). The HPLC system was mainly equipped with a Bio-Rad Aminex HPX-87P column (300 mm × 7.8 mm), a Bio-Rad Micro-Guard column, and a refractive index detector. The analytical column was operated at 80 °C with Milli-Q water (0.2 μm filtered) as the mobile phase at a flow rate of 0.6 ml/min. Experimental data were statistically analyzed using the GLM procedure with Tukey adjustment in SAS 8.02 software.

3. Results and discussion

3.1. Biomass characterization

Glucan was a major component (25.59 wt%) in CBG followed by xylan (15.88%) and acid insoluble lignin (15.37%). There were only a small amount of arabinan (1.95%) and galactan (1.46%) in CBG. The contents of acid soluble lignin, extractives and ash were 3.96%, 4.17%, and 6.6%, respectively. The other 25.02% may include some organic compounds such as uronic acid and acetyl groups, and other trace components including minerals, waxes, fats, starches, resins and gums (Samson et al., 2005).

3.2. Effect of NaOH pretreatment on compositional changes of CBG

Lignin removal during sodium hydroxide pretreatment of CBG is shown in Table 1. Pretreatment time of 30 min was sufficient to achieve a significant amount of total lignin removal as long as the sodium hydroxide concentration was equal or over 1%. On the other hand, decreasing sodium hydroxide concentration from 1% to 0.5% significantly reduced total lignin removal, but there was no significant difference in lignin removal between 2% and 3% NaOH.

Unlike lignin, glucan content of CBG decreased by a fairly small percentage (less than 10%) during sodium hydroxide pretreatment. Hemicellulose solubilization displayed an obvious difference from cellulose degradation during sodium hydroxide pretreatment of CBG, mainly because hemicellulose is more vulnerable by chemical pretreatments than cellulose (Schmidt and Thomsen, 1998). The results indicate that lignin removal and xylan degradation account for the major parts of total solid loss during sodium hydroxide pretreatment of CBG.

3.3. Overall conversion efficiencies of carbohydrates in CBG

To investigate the optimum conditions for the sodium hydroxide pretreatment of CBG, overall conversion efficiencies of cellulose and hemicellulose based on raw biomass need to be considered. It was observed that, as shown in Figure 1, pretreatment with NaOH of 1% and 2% yielded significantly higher total reducing sugars than that with 3% NaOH. Higher NaOH concentrations and longer pretreatment times during the pretreatment resulted in lower solid recovery, although they had higher lignin removal (Table 1), thus leading to less reducing sugars yield. As shown in figure, hot water pretreatment (control) of CBG at 121 °C did not work at all with regards to enhancing sugar yield. Based on this experiment, the highest total reducing sugar yield (approximately 77% of the theoretical maximum) was obtained with 1% NaOH for a pretreatment time of 30 min. The pretreated CBG with 0.5% NaOH had much lower digestibility than that with 0.75% and 1% NaOH, simply due to the significantly less lignin removal for pretreatments with 0.5% NaOH. Although pretreatments with 1% NaOH gave significantly higher lignin

Table 1. Composition of NaOH-pretreated coastal Bermuda grass at 121 °C.

Pretreatment conditions Time (min)	NaOH concentration (%)	Total solids[a] (g/100 g initial dry biomass)	Composition of solids fractions[a,b] (g/100 g initial dry biomass): Lignin	Glucan	Xylan	Galactan	Arabinan
Raw sample		100	19.33	25.59	15.88	1.46	1.95
15	0.5	78.73	16.93 (12.42%)	25.01 (2.27%)	13.60 (14.36%)	1.03 (29.45%)	1.36 (30.26%)
	0.75	66.47	10.61 (45.11%)	24.86 (2.85%)	12.96 (18.39%)	0.90 (38.36%)	1.21 (37.95%)
	1.0	55.56	6.57 (66.01%)	24.51 (4.22%)	12.35 (22.23%)	0.73 (50.00%)	1.19 (38.97%)
	2.0	49.31	5.35 (72.32%)	23.75 (7.19%)	9.35 (41.12%)	0.43 (70.55%)	0.74 (62.05%)
	3.0	45.87	4.77 (75.32%)	23.64 (7.62%)	7.37 (53.59%)	0.32 (78.08%)	0.55 (71.79%)
30	0.5	80.62	16.81 (13.04%)	25.07 (2.03%)	13.42 (15.49%)	0.95 (34.93%)	1.22 (37.44%)
	0.75	62.98	9.38 (51.47%)	25.01 (2.27%)	12.65 (20.34%)	0.81 (44.52%)	1.16 (40.51%)
	1.0	51.26	4.91 (74.60%)	24.24 (5.28%)	11.84 (25.44%)	0.62 (57.53%)	1.14 (41.54%)
	2.0	44.76	3.46 (82.10%)	23.46 (8.32%)	8.78 (44.71%)	0.37 (74.66%)	0.70 (64.10%)
	3.0	42.45	3.39 (82.46%)	23.36 (8.71%)	6.63 (58.25%)	0.32 (78.08%)	0.60 (69.23%)
60	1.0	50.08	4.57 (76.36%)	23.62 (7.70%)	11.58 (27.08%)	0.55 (62.33%)	0.90 (53.85%)
	2.0	41.94	3.16 (83.65%)	23.38 (8.64%)	8.66 (45.47%)	0.29 (80.14%)	0.55 (71.79%)
	3.0	42.81	2.80 (85.51%)	23.09 (9.77%)	6.34 (60.08%)	0.32 (78.08%)	0.58 (70.26%)
90	1.0	50.75	5.15 (73.36%)	23.43 (8.44%)	11.36 (28.46%)	0.55 (62.33%)	0.89 (54.36%)
	2.0	42.70	3.13 (83.81%)	23.09 (9.77%)	8.64 (45.59%)	0.29 (80.14%)	0.57 (70.77%)
	3.0	40.41	2.82 (85.41%)	23.11 (9.69%)	6.27 (60.52%)	0.32 (78.08%)	0.58 (70.26%)

a Data are means of three replicates.
b Data in parentheses next to the composition number are the % amount of each component removed during pretreatment.

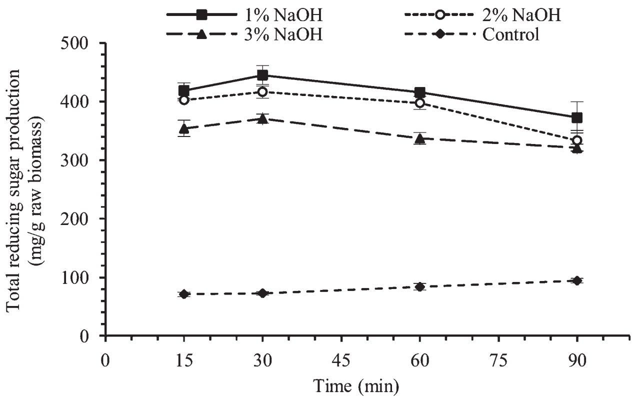

Figure 1. Total reducing sugars production from pretreated coastal Bermuda grass with NaOH of 1%, 2% and 3% (w/v) for 15, 30, 60 and 90 min at 121 °C.

removal than those with 0.75% NaOH, no statistical difference in sugar yield was observed among the pretreatments with 0.75% and 1% NaOH for 15 and 30 min.

Analyses of glucose and xylose yields after hydrolysis were summarized in Table 2. It was noted that the overall glucan conversion efficiency slightly decreased as pretreatment time and NaOH concentration increased. Comparing the total reducing sugar production in Fig. 1 and overall yield of xylose in Table 2, it was noticed that the amount of reducing sugar released after hydrolysis was highly associated with the xylose yield. With the increase of NaOH concentration from 1% to 3%, the overall xylose yield was significantly reduced while the overall glucose yield was fairly constant, thus leading to the decrease of total reducing sugar production. The pretreatment with 0.75% NaOH generated statistically similar overall glucose and xylose yields compared to the pretreatment with 1% NaOH for both 15 and 30 min at 121 °C. However, both glucose and xylose yields for pretreatments with 0.5% NaOH were significantly lower than those obtained for pretreatments with 0.75% and 1% NaOH (Table 2). Therefore, 0.75% NaOH with a pretreatment time of 15 min was determined as the optimal condition for sodium hydroxide pretreatment of CBG at 121 °C.

Table 2. The overall glucan and xylan conversion efficiencies[a] after 72-h enzymatic hydrolysis of NaOH-pretreated coastal Bermuda grass.

Time (min)	NaOH concentration (%)	Temperature (°C)	Glucan conversion (%)	Xylan conversion (%)
15	0.5	121	64.56	35.79
	0.75	121	90.43	65.11
	1.0	121	91.56	60.98
	2.0	121	89.51	46.45
	3.0	121	91.68	37.61
30	0.5	121	70.19	43.18
	0.75	121	91.61	62.66
	1.0	121	90.73	59.27
	2.0	121	87.72	45.41
	3.0	121	87.02	34.85
60	1.0	121	88.43	57.24
	2.0	121	87.37	44.47
	3.0	121	88.36	35.06
90	1.0	121	90.27	46.25
	2.0	121	84.91	45.84
	3.0	121	86.12	35.16
Control[b]			31.22	6.73

a Overall glucan/xylan conversion efficiencies = (g glucose/xylose produced in the hydrolyzate) × (0.9/0.88)/(g glucan/xylan in raw biomass).

b Untreated Bermuda grass sample hydrolyzed with excessive loadings of cellulases and cellobiase.

4. Conclusions

This study showed that sodium hydroxide pretreatment at 121 °C was effective in improving the digestibility of CBG to enhance reducing sugar yield. Removal of lignin from CBG was able to facilitate the digestibility of carbohydrates. The xylan conversion efficiency was more sensitively affected by the variation of NaOH pretreatment conditions than glucan conversion efficiency. However, the study of other pretreatment methods, enzyme loading test, fermentation study, scale-up and economic analysis of the overall conversion process is needed for further conclusions.

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