

ONE AND TWO STAGE AUTOHYDROLYSIS PRETREATMENTS FOR ENZYME HYDROLYSIS OF COASTAL BERMUDA GRASS TO PRODUCE FERMENTABLE SUGARS

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Coastal Bermuda grass (CBG) is an agricultural residue with considerable potential as a feedstock for lignocellulosic-based ethanol. The treatment of biomass with water at high temperature, termed autohydrolysis, can be used to recover sugars in the filtrate and to improve enzyme digestibility of the pretreated solids. The effect of a two-stage autohydrolysis process with respect to total sugar recovery relative to a one stage process was investigated. CBG was subjected to lab scale one-stage (150, 160, and 170 °C) and two-stage (150/170 °C and 160/170 °C) isothermal autohydrolysis processes followed by enzyme hydrolysis on the residual solids with different loadings (5 to 30 FPU/g). Two-stage autohydrolysis (160/170 °C) solubilized 94.2% of the hemicellulose based on the original CBG material but only 17.7% of the cellulose and 30.4% of the lignin. Increases in the severity factor (a combination of time and temperature) of autohydrolysis pretreatments decreased the recoverable carbohydrates and total solids. Two-stage autohydrolysis enhanced enzyme digestibility of the cellulose in pretreated solids relative to one-stage autohydrolysis, especially at higher values of FPU/g. The overall total theoretical sugar recovery achievable by the two stage process was 57.8% and for the one stage process only 51.6% with 30 FPU/g. This marginal increase would have to be considered relative to increased complexity of operations when deciding whether to implement one or two stage autohydrolysis.

Keywords: Autohydrolysis; Coastal Bermuda grass; Ethanol; Enzymatic Hydrolysis; Fermentable sugars; Severity factor

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INTRODUCTION

Options for the production of biofuels from lignocellulosic materials are being explored comprehensively in order to substitute for the usage of petrochemical-based fuels and products. Lignocellulosic materials including agricultural, industrial, and urban residues are potential sources to produce low-cost energy and fuels (US-DOE 2005). Coastal Bermuda grass (*Cynodon dactylon*) is a perennial grass widely grown in the southeast and midwest US states. It has been shown to have considerable potential as a feedstock for sugar production, since it has a carbohydrate content of around 57% and relatively low acid-insoluble lignin content (20%) (Sun and Cheng 2005; Lee et al. 2009, 2010a, 2010b).

Pretreatment processes, which facilitate the removal of recalcitrant structure

within the biomass, considerably improve enzyme conversion of lignocellulosics to soluble sugars (Coughlan 1992). Autohydrolysis pretreatment, also termed hydrothermal processing, has been applied to recover sugars in the prehydrolyzates and to improve enzyme digestibility of autohydrolysis-treated solids (Garrote et al. 1999a; Vegas et al. 2004; Lee et al. 2009, 2010a). During the autohydrolysis process, hydronium ions generated from water and acetic groups released from hemicelluloses cause autohydrolysis reactions to take place with the lignocellulosic linkages, converting some of the polysaccharides into oligomers and also to a much lesser extent, monomers (Garrote and Parajo, 2002; Lee et al. 2009). However, the yields of fermentable sugars from prehydrolyzates and enzyme hydrolyzates depend highly on the severity of the process, which often is expressed as a severity factor (SF). The severity factor represents the combined effects of reaction temperatures and reaction time to compare the autohydrolysis process on the feedstocks (Overend and Chornet 1987). In the case of wheat straw, there were no significant observations of solubilization of xylan and lignin until reaching a severity factor of 3.25 (Carvalho et al. 2009). After the point of 3.25 severity factor, 97% of the original xylan and 14.6% of the original lignin were solubilized with higher severities, suggesting that the severity factor of 3.25 was a critical point for the breakage of lignocellulosic linkages (Carvalho et al. 2009).

In our previous study with one-stage autohydrolysis pretreatment on CBG, hemicellulose was easily solubilized at less severe conditions (3.24 and 3.25 severity factors) relative to cellulose (Lee et al. 2009). It was also shown that the efficiency of enzymatic hydrolysis of the pretreated solids was significantly enhanced with increased temperature and time (up to 3.84 severity factor), resulting in substantial reductions of enzyme requirement for the same extent of carbohydrate conversion (Lee et al. 2009). These results suggested that a combination of a mild pretreatment to extract hemicelluloses followed by a harsher pretreatment to increase the enzymatic digestibility of the residue might be beneficial for the full recovery of sugars from lignocellulosic materials. A similar idea was employed on sugar cane bagasse (Morjanoff et al. 1982), in which sugar cane bagasse was subjected to a mild first stage pretreatment to extract hemicellulose, followed by a much more severe second pretreatment to improve enzyme hydrolysis. However, extremely high severity second stage pretreatments resulted in poor enzymatic digestibility. Herein, one-stage and two-stage autohydrolysis processes were evaluated on CBG with respect to the overall conversion into fermentable sugars.

EXPERIMENTAL

Biomass

Coastal Bermuda grass (CBG) was harvested in early 2007 from Cunningham Research Station (Kinston, NC). The stalk with 10% initial moisture content (MC) was shredded to a particle size of about 2-4 inches. The samples were stored in sealed plastic bags and kept in a cold room prior to experiments. The shredded biomass was further ground in a Thomas Wiley Laboratory Mill (Model No. 4, Thomas Scientific, Philadelphia, PA) to pass through a 20 mesh screen and retained on a 40 mesh screen for compositional analysis.

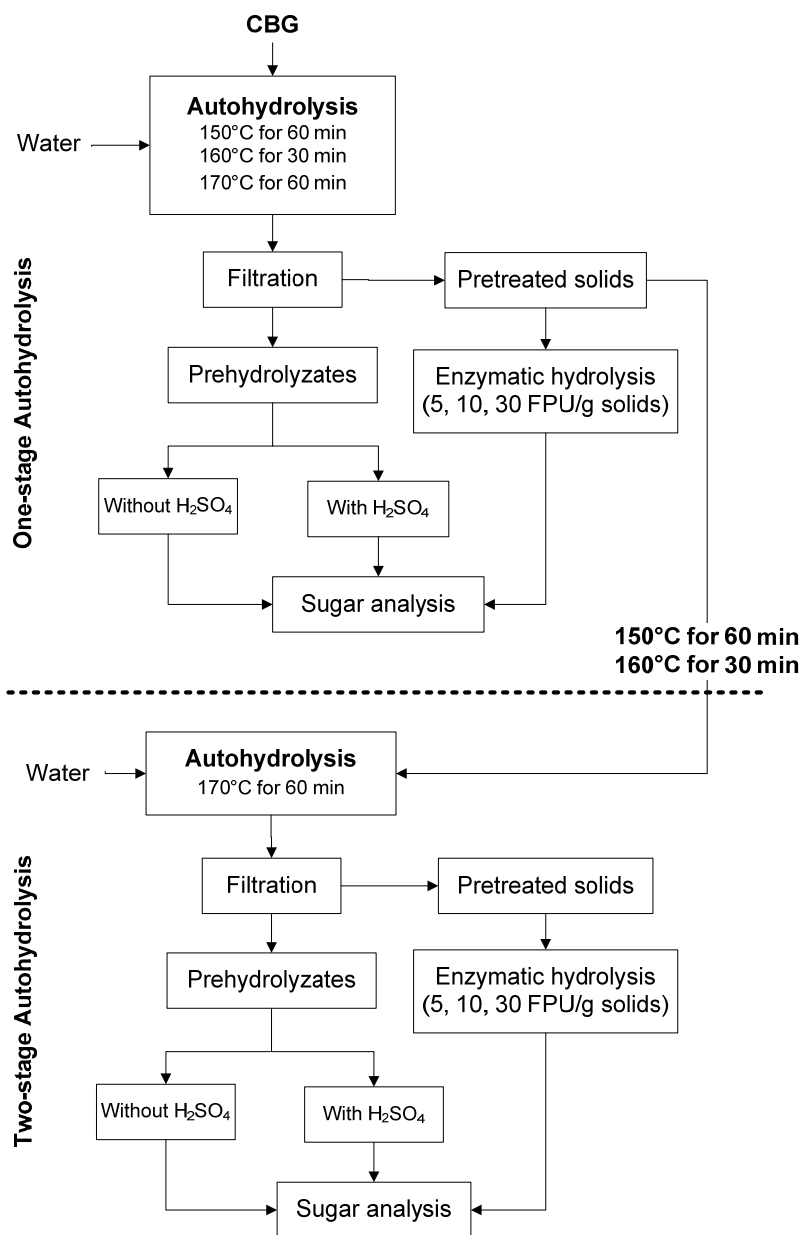


Fig. 1. Experimental process flow diagram of one and two autohydrolysis pretreatments

Autohydrolysis

The autohydrolysis pretreatments were performed in a 1.5 L stainless steel rotating bomb digester (Thermcraft, Winston-Salem, NC). The shredded CBG sample (100 oven dry (o.d)) was mixed with water in the reactor in order to achieve a final solid to liquid ratio of 1:6 and heated to the desired temperatures in the range 150 to 170 °C for 30 to 60 min. The experimental design was set according to the results obtained in

previous experiments, where maximum of hemicellulose solubilization was achieved at 150 °C for 60 min (corresponding to a severity factor of 3.25), following by 160 °C for 30 min (a severity factor of 3.24), whereas residues after autohydrolysis pretreatment at 170 °C for 60 min (3.84 severity factor) showed more enzyme digestibility (Lee et al. 2009). After cooling, the pretreated samples were drained from the filtrate using cheese cloth. The separated prehydrolyzates filtrates were analyzed for solids contents, pH, and sugar compositions. For two-stage autohydrolysis pretreatment, the pretreated solids from 150 °C for 60 min and 160 for 30 min were again mixed with water in the reactor with the same solid to liquid ratio of 1:6 and heated to 170 °C for 60 min. The pretreated samples were cooled to room temperature and filtered through cheese cloth. The separated filtrate was tested for solid content, pH, and sugar composition. Figure 1 shows the scheme employed in this work for sample processing and analysis.

The effects of temperature and time on one-stage autohydrolysis process was calculated based on the severity factor (SF) (Overend and Chornet 1987), and the two-stage process was modified from the severity factor as follows:

$$SF \text{ of one-stage} = \log_{10} \left[t_1 \times \exp \left(\frac{T_1 - 100}{14.75} \right) \right] \quad (1)$$

$$SF \text{ of two-stage} = \log_{10} \left\{ \left[t_1 \times \exp \left(\frac{T_1 - 100}{14.75} \right) \right] + \left[t_2 \times \exp \left(\frac{T_2 - 100}{14.75} \right) \right] \right\} \quad (2)$$

where t_1 is time (min) and T_1 is the reaction temperature (°C) for a one-stage autohydrolysis pretreatment, and t_2 and T_2 for a two-stage autohydrolysis process. The value of 14.75 is an empirical parameter related to activation energy and temperature.

Compositional Analysis

The total mass recovery yields and total lignin (combined of acid soluble and acid insoluble lignin contents) of untreated, and autohydrolysis-treated CBG were determined by use of the National Renewable Energy Laboratory's (NREL) Laboratory Analytical Procedures (LAP 001, 003, 004) (Ehrman 1994; Ehrman 1996; Templeton and Ehrman 1994). Aliquots of 10 ml from each filtrate were dried at 105 °C over night to determine the solids content (mg/mL). The filtrates were subjected to a hydrolysis with 4% w/w H₂SO₄ for 1 h at 121 °C to convert oligomers into monomers. All filtrates were filtered through 0.2 µm nylon filter before sugar analysis.

The hydrolyzate after the Klason lignin determination was collected and analyzed for sugar concentrations. Mono-sugars in the hydrolyzates were determined using an ion chromatography (IC) system (ICS 3000, Dionex Corp., CA) equipped with an AS auto sampler, a GP40 gradient pump, a high-pH anion exchange column (CarboPac™ PA1, Dionex Corp., Sunnyvale, CA), and an ED40 electrochemical detector. Aliquots (20 µL) were injected on the column after passing through a 0.2 µm nylon syringe filter (Millex®, Millipore Corp., Billerica, MA). The column was eluted with deionized water at a flow rate of 1.0 ml/min. The column was re-conditioned using 0.2 M NaOH after each analysis. To optimize the baseline stability and detector sensitivity, 0.4 M NaOH was

added to a post-column at a rate of 0.1 ml/min. L-(-)-Fucose (F2252, Sigma, Saint Louis, MO) was used as an internal standard and sugar contents were quantified by comparison with standards. All chemical composition experiments were performed in duplicate. The cellulose and hemicellulose contents were calculated using following equations 3 and 4 (0.9 is the correction coefficient for hydration, Iyer and Lee 1999).

$$\% \text{ cellulose} = \frac{\text{glucose released (g)} \times 0.9}{\text{sample dry weight (g)}} \times 100 \quad (3)$$

$$\% \text{ hemicellulose} = \frac{\text{hemicellulose released (g)} \times 0.9}{\text{sample dry weight (g)}} \times 100 \quad (4)$$

where it is assumed that the predominant source of glucose is from cellulose and the hemicellulose released (g) is the summation of arabinose, rhamnose, galactose, xylose, and mannose detected with the IC system.

Solids recovery was calculated as a percentage of the total recovery after pretreatment based on the initial sample (dry weight). Cellulose and hemicellulose reductions were estimated as the ratio of cellulose and hemicellulose remaining in the solids recovered to those in the untreated CBG samples, respectively.

Enzymatic Hydrolysis

Enzyme hydrolysis of pretreated solids was carried out with a mixture of three commercial enzymes, cellulase from *Trichoderma reesei* (NS-50013, 700 EGU (endo-glucanase unit)/g, 75 FPU/g), β -glucosidase from *Aspergillus niger* (NS-50010, 250 CBU (cellobiase unit)/g), and xylanase (NS-50014, 750 FXU (fungal xylanase unit)/g) provided by Novozymes A/S (Franklinton, NC, USA) (Lee et al. 2009, 2010b). The cellulase loadings were 5, 10, and 30 FPU/g pretreated solid. The dosage of β -glucosidase and xylanases supplementation constituted 30% of the volume of cellulase added.

Five grams of pretreated sample (dry weight) were supplemented with enzyme solutions and then added to a 50 mM acetate buffer (pH 4.8) to achieve a 5% w/v final solids loading. Sodium azide (0.3%, w/v) was used in the mixture to inhibit microbial contamination. Samples were incubated at 50 \pm 2 $^{\circ}$ C in a shaker bath at 180 rpm for 48h. Hydrolyzed samples were filtered through a pre-weighed filter paper (Whatman No. 4, Whatman International Ltd, Maidstone, UK).

Aliquots of the supernatants were recovered for sugar analysis. The residual solids were dried in a convection oven at 105 $^{\circ}$ C and weighed to calculate percentage weight loss. Also, the chemical compositions of the residual solids were measured, as described in the compositional analysis section. All experiments were carried out in duplicate and the average and range reported.

RESULTS AND DISCUSSION

Effect of One- and Two-Stage Pretreatment on the Chemical Composition of CBG

The chemical composition of CBG may vary depending on its variety and growing conditions. Severity factors, solid recovery yields, and the compositions of the pretreated solids from raw CBG, one- and two- stage autohydrolysis pretreatments are summarized in Table 1. Severity factor is a method used to compare different pretreatments based on both time and temperature history of the pretreatments (Overend and Chornet 1987). In this study the SF value of one-stage autohydrolysis process was calculated based on equation 1. However, the method to calculate the SF value of a two-stage process to reflect the severity of the pretreatment is complex. One may consider that the two-stage process is a combination of reaction ordinates of the first-stage ($R_{0,1}$) and second-stage ($R_{0,2}$), (e.g., $\log((R_{0,1} + R_{0,2}))$), whereas one may consider the two stages as independent treatments (e.g., $\log(R_{0,1}) + \log(R_{0,2})$). Here, we assumed that the 1st and 2nd stages are dependent and thus calculated the severity factor based on equation 2. It is reasonable to consider that the second stage pretreatment effectiveness depends on phenomena occurring in the first stage. The SF values calculated by equation 2 are also more in line with other research (Carvalho et al. 2009; Garrote et al. 1999b).

Table 1. Composition of the Raw Material and of the Solid Residue after Autohydrolysis Treatments as Grams of Component Recovered per 100g of Raw Material

	Raw	One-stage			Two-stage	
		150°C-60 min	160°C-30 min	170°C-60 min	150°C-60 min + 170°C-60 min	160°C-30 min + 170°C-60 min
SF*	-	3.25	3.24	3.84	3.94	3.94
Total Lignin	23.3±0.1	19.2±0.0	19.2±0.6	23.1±0.0	17.3±0.5	16.2±0.1
Cellulose	30.4±0.9	33.6±0.6	29.8±0.3	31.8±1.0	29.1±0.3	25.0±0.1
Hemicellulose	29.3±1.6	17.4±0.3	13.1±0.3	4.5±0.2	2.7±0.0	1.7±0.0
Ash	4.8±0.1	0.6±0.0	1.1±0.1	1.0±0.0	0.7±0.1	0.8±0.1
Others ¹⁾	12.2±0.0	8.1±0.0	9.1±0.6	4.7±0.0	3.4±0.0	5.1±0.3
Yields ²⁾	100	78.9±0.9	72.2±0.2	65.1±0.2	53.1±2.8	48.4±0.3
* Severity factor						
¹⁾ Calculated by difference						
²⁾ Solid recovery yield (g of solid residue recovered after treatment/100g raw material, o.d.)						

The CBG used in this work contained 60% by weight of total carbohydrates, which consisted of glucan 30.4%, hemicellulose 29.3% (xylan 22.6%, and 6.7% of other sugars). The yields in solids decreased from 78.9% to 48.4% with increasing severity. Hemicellulose solubilization and byproduct formation, including furfural and hydroxymethylfurfural (HMF) generation from carbohydrates, were the main contribution associated to the weight loss of the pretreated solids (Palmqvist and Hahn-Hagerdal 2000; Carvalho et al. 2009). As expected, autohydrolysis mainly affected

hemicellulose components, resulting in 94.2% solubilization of the original hemicelluloses at the most severe condition. In contrast to hemicelluloses components, glucan remained essentially unchanged in the pretreated solids. The maximum degradation of glucan occurred under the most severe condition with 17.7 % of the original glucan being solubilized. The recovery of lignin showed a difference between one- and two-stage autohydrolysis pretreatments. Up to a severity of 3.25, 17% of the original lignin was solubilized, but at the higher severity of 170 °C an increase in lignin recovery was observed. This increase is a typical lignin behavior for the autohydrolysis processes and could be associated with condensation of lignin with sugar (pseudo-lignin) (Heitz et al. 1991; Garrote et al. 1999b), re-distribution of lignin-related compounds (Kristensen et al. 2008; Lee et al. 2010a) or side-reaction of aggregation of lignin with protein under higher temperature (Garrote et al. 2007). However, higher severities with two-stage autohydrolysis led to an increase in lignin degradation (solubilization of 30.4% of the original lignin).

Effect of Autohydrolysis Pretreatment Conditions on Acidity (pH)

As expected, higher severities led to higher acidity (lower pH), as shown in Fig. 2. During the autohydrolysis process part of the acetyl esters present in the feedstock are cleaved to produce acetic acid, involving depolymerization of hemicelluloses and celluloses (Garrote et al 1999a; Garrote and Parajo 2002). In addition, hydronium ions generated from water autoionization and from the ionization of acidic species (e.g. formic and levulinic acids) further catalyze a series of autohydrolysis reactions (Palmqvist and Hahn-Hagerdal 2000). Two-stage autohydrolysis leads to more pH reduction. The results indicate that in the second stage, more of the acetyl ester covalently linked with xylan backbone is cleaved (Kabel et al. 2002).

Sugars in Autohydrolysis Filtrate

In order to examine solubilization of the oligo-sugars and mono-sugars from one- and two-stages autohydrolysis pretreatment, the filtrates were collected and analyzed as shown in Table 2. More severe conditions were able to significantly increase the amount of mono-sugars in the autohydrolysis filtrate (Table 2a). Before acid-treated hydrolysis (Table 2a), arabinose was the main component of filtrate for lower severities, whereas xylose was the main monosaccharide for higher severities. This is similar to our previous results (Lee et al. 2009). Compared to one-stage autohydrolysis at 170 °C for 1h (1.26g of mono-sugars/100g raw material), two-stage at 170° C for 1 h with pretreated solids at 150 °C or 160 °C produced more mono-sugars (5.6 and 4.5 g of mono-sugars/100g raw material, respectively). Overall mono-sugar recovery from one-stage autohydrolysis was in the range of 3.6 to 5.0% based on reduced carbohydrate, whereas two-stage autohydrolysis recovered 12.5 to 18.3% of mono-sugars (Table 3). There was no correlation of mono-sugars versus severity factor.

In this study, the filtrates were further hydrolyzed using 4% sulfuric acid (Table 2b). It was assumed that all of the oligo-sugars were converted into their mono-sugars with this treatment. The major effect caused by autohydrolysis pretreatments was the generation of oligomers from hemicellulosic polysaccharide. After acid hydrolysis, the mono-sugars in the filtrate were 4.92 to 14.2 g, depending on the conditions. The two-

stage autohydrolysis at 150/170 °C generated the highest level of mono-sugars at 14.2 g, followed by two-stage autohydrolysis at 160/170 °C.

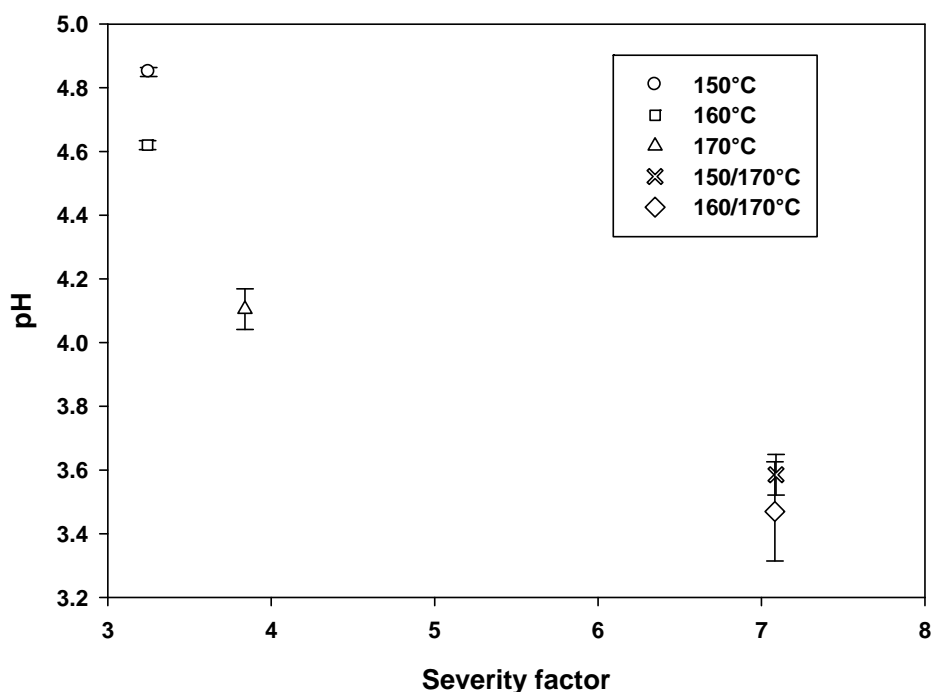


Fig. 2. The pH of the prehydrolyzate under autohydrolysis conditions

Table 2a. Sugars in Prehydrolyzate Filtrate before Acid-treated Hydrolysis

		SF*	Ara	Rham	Gal	Glu	Xyl	Man	Sum
One-stage	150°C for 60 min	3.25	0.40	0.00	0.01	0.06	0.01	0.00	0.48
	160°C for 30 min	3.24	0.49	0.01	0.03	0.09	0.04	0.01	0.67
	170°C for 60 min	3.84	0.19	0.01	0.13	0.09	0.83	0.01	1.26
2 nd stage	150/170 (2 nd stage)	3.84	0.42	0.01	0.23	0.35	4.10	0.02	5.13
	160/170 (2 nd stage)	3.84	0.15	0.00	0.12	0.44	3.14	0.01	3.86
Two-stage	150/170 combined	3.94	0.81	0.01	0.24	0.42	4.11	0.02	5.61
	160/170 combined	3.94	0.66	0.01	0.15	0.50	3.18	0.02	4.53

*Severity factor

Sugars are reported in g/100g (o.d.) of original raw CBG

Table 2b. Sugars in Prehydrolyzate Filtrate after Acid-treated Hydrolysis

		SF*	Ara	Rham	Gal	Glu	Xyl	Man	Sum
One-stage	150°C for 60 min	3.25	0.73	0.10	0.34	2.63	1.04	0.08	4.92
	160°C for 30 min	3.24	1.09	0.12	0.62	3.64	3.68	0.15	9.31
	170°C for 60 min	3.84	0.23	0.02	0.43	4.41	6.38	0.10	11.57
2 nd stage	150/170 (2 nd stage)	3.84	0.28	0.01	0.22	2.66	6.29	0.03	9.49
	150/170 (2 nd stage)	3.84	0.08	0.01	0.09	1.60	3.22	0.01	5.02
Two-stage	150/170 combined	3.94	1.03	0.12	0.57	5.10	7.24	0.11	14.17
	160/170 combined	3.94	1.14	0.13	0.67	4.93	6.37	0.17	13.41

*Severity factor

Sugars are reported in g/100g (o.d.) of original raw CBG

It is of interest to determine what percent of the decrease in carbohydrates from the solids after extraction were detected as sugars in the filtrate. Percentages lower than 100% indicate degradation of mono-sugars to furfural, HMF, or other by-products, especially under more severe conditions. The percent sugar recovery in the filtrate achieved a maximum of 51.4% for the one-stage autohydrolysis at 150 °C for 60 min (Table 3). In contrast, two-stage autohydrolysis at 160/170 °C was able to preserve only 36.9% of the total solubilized sugars (Table 3).

Table 3. Sugar Recovery from Filtrate¹⁾

	One-stage			Two-stage	
	150°C - 60 min	160°C - 30 min	170°C - 60 min	150°C-60 min + 170°C-60 min	160°C-30 min + 170°C-60 min
Reduced carbohydrate in pretreated solids (g) ²⁾	8.7±0.4	16.8±0.3	23.4±0.2	27.9±0.1	33.0±0.1
Total sugar in filtrate (g) ³⁾	0.4±0.0	0.6±0.0	1.2±0.1	5.1±0.2	4.1±0.1
% recovered ⁴⁾	5.0	3.6	4.9	18.3	12.5
Total sugar in hydrolyzed filtrate after 4% H ₂ SO ₄ (g) ⁵⁾	4.5±0.1	8.5±0.1	10.5±0.2	12.9±0.1	12.2±0.2
% recovered ⁶⁾	51.4	50.4	45.0	46.2	36.9

¹⁾ Average and range of duplicates reported. Sugar was expressed as oligo-sugar forms
²⁾ Calculated by (carbohydrate content of raw material – carbohydrate content of pretreated solid at each condition). Carbohydrate content is a summation of cellulose and hemicellulose
³⁾ Mono-sugars detected in the prehydrolyzate and reported in oligo-sugar form
⁴⁾ Calculated by total sugar in filtrate³⁾ / reduced carbohydrate in pretreated solids²⁾ as a %
⁵⁾ Sugars (oligo-sugars were converted to mono-sugar form by acid hydrolysis) expressed in oligo-sugar form
⁶⁾ Calculated by total sugar in hydrolyzed filtrate after 4% H₂SO₄⁵⁾ / reduced carbohydrate in pretreated solids²⁾ as a %

Effect of Autohydrolysis Condition on Enzymatic Hydrolysis

Autohydrolysis significantly improved the cellulose and hemicellulose conversions with subsequent enzymatic hydrolysis. One-stage autohydrolysis produced 41.9 to 56.9 % of theoretical cellulose conversion at 30 FPU/g, as shown in Fig. 3. Higher severity factor increased the enzymatic conversion of cellulose to glucose. Enzyme loading greater than 10 FPU/g pretreated solids did not significantly increase the cellulose conversion with one-stage autohydrolysis. Two-stage autohydrolysis has a higher theoretical cellulose conversion of 67 to 75% at 30 FPU/g compared to the one-stage autohydrolysis. However, one and two stage processes were similar for FPU values of 10 or lower.

Similar to cellulose conversion, higher severity conditions increased xylan to xylose conversion (Fig. 4). The maximum hemicellulose theoretical conversion of 85.3% was measured for the 160/170 °C two-stage autohydrolysis for 30 FPU/g. Also, the effect of enzyme loading above 10 FPU/g had a more significant effect on theoretical hemicellulose conversion for the two stage autohydrolysis processes than the one-stage autohydrolysis processes (Fig. 4). Similar to the cellulose conversion, the theoretical

hemicellulose conversion increased significantly with severity factor at 30 FPU/g but not at 5 FPU/g.

The maximum concentration of glucose and xylose obtained after enzymatic hydrolysis (30 FPU/g) was determined to be 2.0 ± 0.1 and 0.2 ± 0.0 g/l for the sample with two-stage pretreatment at 150/170 °C.

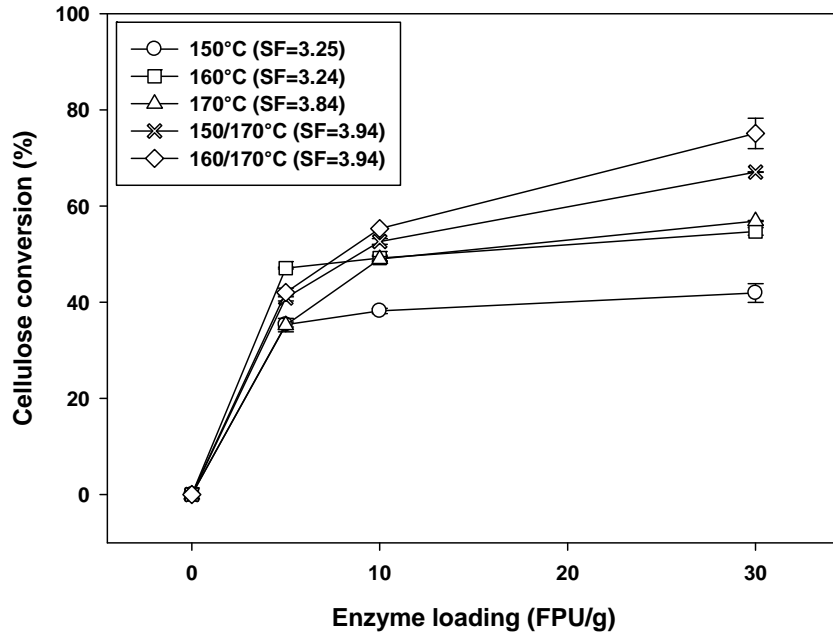


Fig. 3. Cellulose conversion versus severity factor and enzyme loading

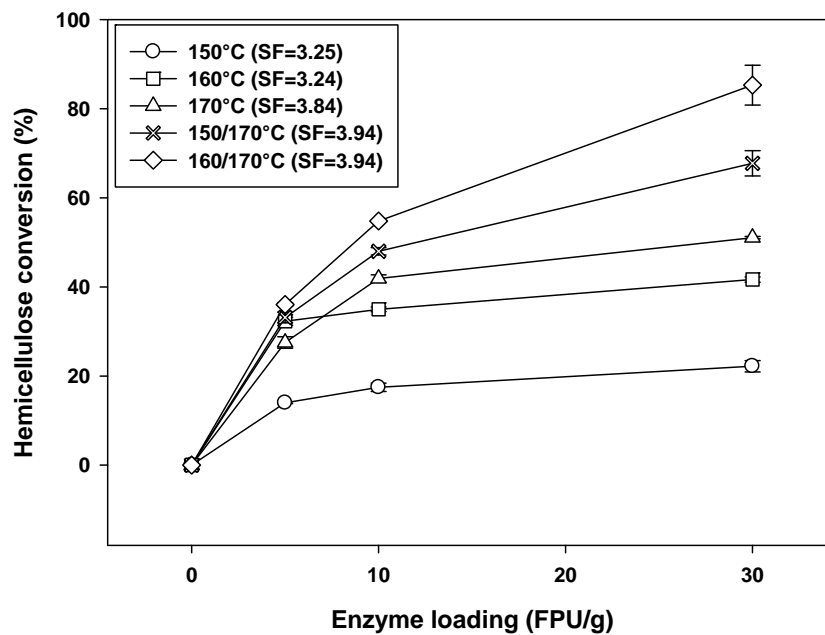


Fig. 4. Hemicellulose conversion versus severity factor and enzyme loading

Material Balance

A material balance of the autohydrolysis pretreatment process as well as the subsequent enzymatic hydrolysis was performed for all pretreatment conditions (Table 4). The material balance was able to track greater than 98% of the solid recovery through the process for the lowest severity condition. However, significantly lower recovery of materials was determined for the two-stage processes with higher severity, with a minimum of 85.8% recovery for the 160/170 °C conditions. Again, this indicates that higher severity conditions produced byproducts including acetic acid, formic acid, furfural, and HMF. Also, the solid recovery measurement was carried out with 105°C oven drying method, so that some volatiles were released during the measurement. The solids recovery and the total sugar recovery were similar but not the same as our previous research on CBG under the same single-stage pretreatment conditions (Lee et al. 2009). This is not unusual, as the two studies utilized raw materials harvested at different times and had slightly different material handling and storage procedures. However, the main trends with increasing severity factor are in agreement for both studies.

The total amount of sugar detectable in the filtrate after acid treatment increased with increased severity. This caused less sugar generation from hemicellulose with enzymatic hydrolysis with the two-stage autohydrolysis processes relative to the single-stage autohydrolysis processes. However, the glucose generation from enzymatic hydrolysis increased in general with higher severity. The two-stage autohydrolysis process at 150/170 °C with a 30 FPU enzyme charge provided the highest theoretical sugar recovery, in total 37.9 g of sugar, which is 57.8% of the theoretical sugar yield (Table 4). In contrast, a simpler one-stage 170 °C process provided 51.6% of the theoretical sugar yield. The total sugar yield versus process complexity would have to be considered when deciding whether to implement one or two stage autohydrolysis.

CONCLUSIONS

1. Autohydrolysis pretreatment of CBG most strongly affected the solubilization of hemicelluloses relative to cellulose and lignin.
2. Increases in the severity of the extraction conditions of CBG increased the amount of hemicellulose solubilized.
3. Increases in the severity factor of autohydrolysis pretreatments of CBG decreased the recoverable carbohydrates and total solids
4. Two-stage autohydrolysis enhanced the enzyme digestibility of the cellulose in pretreated CBG solids relative to one-stage autohydrolysis, especially at higher values of FPU/g.

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Table 4. Material Balances from Autohydrolysis Pretreatments Followed by Enzyme Treatment

AH	Temp (°C)	Time (min)	SF	Pretreated Solids (g)	Filtrates (g)	Recovery (%)	Sugars in Filtrates (g)			FPU	Enzyme Hydrolyzate (g)			Sugar recovery	
							G ¹⁾	H ²⁾	T ³⁾		G ¹⁾	H ²⁾	T ³⁾	(g) ⁴⁾	(%) ⁵⁾
One-stage	150	60	3.25	78.9	19.9	98.9	2.63	2.28	4.92	5	13.05	2.75	15.80	20.72	31.55
										10	14.08	3.27	17.35	22.27	33.91
										30	15.51	4.29	19.8	24.72	37.64
	160	30	3.24	72.2	25.7	97.9	3.64	5.67	9.31	5	15.37	4.75	20.12	29.43	44.82
										10	16.01	5.23	21.24	30.55	46.52
										30	17.74	6.02	23.76	33.07	50.36
	170	60	3.84	65.1	30.7	95.8	4.41	7.16	11.57	5	12.32	1.29	13.61	25.18	38.34
										10	17.19	2.15	19.34	30.91	47.07
										30	19.91	2.43	22.34	33.91	51.64
2 nd stage	150/170	60	3.84	-	18.94	-	2.66	6.38	9.49	-	-	-	-	-	-
	160/170	60	3.84	-	10.85	-	1.60	3.41	5.02	-	-	-	-	-	-
Two-stage	150/170	60/60	3.94	53.1	39.2	92.3	5.10	9.08	14.17	5	13.22	0.94	14.16	28.33	43.14
										10	17.08	1.41	18.49	32.66	49.73
										30	21.77	1.99	23.76	37.93	57.76
	160/170	30/60	3.94	48.4	37.4	85.8	4.93	8.48	13.41	5	11.55	0.64	12.19	25.60	38.98
										10	15.29	0.97	16.26	29.67	45.18
										30	20.75	1.61	22.36	35.77	54.47

¹⁾ G: Glucose; ²⁾ H: Xylose and other mono-sugars; ³⁾ T: total sugars; ⁴⁾ Sum of sugars in filtrate + hydrolyzate
⁵⁾ % of sugar recovery (g) / 65.67 (g).