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Relatively High-Substrate Consistency Hydrolysis of Steam-Pretreated Sweet Sorghum Bagasse at Relatively Low Cellulase Loading

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Abstract Sweet sorghum bagasse (SSB) was steam pretreated in the conditions of 190 °C for 5 min to assess its amenability to the pretreatment and enzymatic hydrolysis. Results showed that pretreatment conditions were robust enough to pretreat SSB with maximum of 87% glucan and 72% xylan recovery. Subsequent enzymatic hydrolysis showed that the pretreated SSB at 2% substrate consistency resulted in maximum of 70% glucan–glucose conversion. Increasing substrate consistency from 2% to 16% led to a significant reduction in glucan conversion. However, the decrease ratio of glucan–glucose conversion was the minimum when the consistency increased from 2% to 12%. When the pretreated SSB consistency of 12% was applied for hydrolysis, increase in cellulase loading from 7.5 up to 20 filter paper units (FPU)/g glucan resulted only in 14% increase in glucan–glucose conversion compared to 20% increase with cellulase loading varying from 2.5 to 7.5 FPU/g glucan. More than 10 cellobiase units (CBU)/g glucan β-glucosidase supplementation had no noticeable improvement on glucan–glucose conversion. Additionally, supplementation of xylanase was found to significantly increase glucan–glucose conversion from 50% to 80% with the substrate consistency of 12%, when the cellulase and β-glucosidase loadings were at relatively low enzyme loadings (7.5 FPU/g and 10 CBU/g glucan). It appeared that residual xylan played a critical role in hindering the ease of hydrolysis of SSB. A proper

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xylanase addition was suggested to achieve a high hydrolysis yield at relatively high substrate consistency with relatively low enzyme loadings.

Keywords Sweet sorghum bagasse · Steam pretreatment · Relatively high substrate consistency · Enzymatic hydrolysis · Xylanase addition

Introduction

Climate changes and concerns of energy security have driven the global development of bioethanol. As a consequence, global production of fuel ethanol has doubled since 2001 reaching 19,227 million gallons in 2009 [1]. In this context, it is necessary to ensure the availability of sufficient resources and technologies in place to meet this ever-growing demand. Currently, corn and sugarcane are the primary raw materials for bioethanol production [2]. Nevertheless, the use of these first-generation feedstocks is constrained from the viewpoint of food security, environmental and economic implications, which paved the way to search for other alternatives to produce second-generation bioethanol [2, 3]. Among many promising second-generation bioethanol crops, sweet sorghum (*Sorghum bicolor* (L.) Moench) holds great prospects as a sustainable alternative [4, 5]. Many characteristics of this high-yielding sugar crop such as wide adaptability, tolerance to abiotic stresses like drought, water logging, salinity, and alkalinity make it an attractive crop that can be cultivated even in underutilized land in many developing countries [6–8]. In addition, multiple parts of the crop could be potentially used for ethanol production including stalk juice, grain, and bagasse. Despite significant advancements made in the industrial utilization of the stalk juice and the grain for ethanol production, process for the conversion of bagasse fraction of sorghum to ethanol has not yet been developed and commercialized [9–13]. Similar to any other agricultural residues, sweet sorghum bagasse (SSB) contains both cellulose and hemicellulose components which could be potentially used for bioconversion to ethanol. Conversion of SSB to ethanol would allow the overall ethanol productivity of the crop to be considerably enhanced while lowering the production cost [14].

Among many pretreatment processes for the bioconversion of lignocellulosic materials, steam pretreatment has been considered as one of the potential pretreatment processes in terms of numerous technical, economic, and environmental advantages [15–17]. Previous researches have shown that steam pretreatment has been largely effective on agricultural residues including corn stover [18, 19], corn fiber [20], wheat straw [21], barley straw [19], sunflower stalk [22] and hardwoods such as poplar [18], aspen [23], and Eucalyptus [24]. The studies identified the pretreatment conditions which led to efficient recovery of hemicellulose sugars in the liquid fraction simultaneously obtaining a solid fraction highly amenable to subsequent enzymatic hydrolysis. Thus, to assess the susceptibility of SSB to steam pretreatment and subsequent bioconversion, SSB was steam pretreated at the same set of conditions which has been previously shown to be effective on similar agricultural residues like corn stover [18].

Previous techno-economic assessment has suggested that a relatively low enzyme loading and maintaining a high substrate consistency during enzymatic hydrolysis are critical to achieve the economic viability of lignocellulose-based ethanol production. It has been shown that an increase in substrate loading from 5% to 8% (*w*/*w*) can reduce the total production cost by nearly 20% [25, 26]. Thus, in the current study, ease of hydrolysis of steam-pretreated SSB at various substrate consistencies was investigated with a relatively

low range of enzyme loading. Effect of increasing the input levels of both cellulase and β-glucosidase on increasing the efficiency of high-substrate consistency hydrolysis was also investigated.

Moreover, previous studies on steam-pretreated agricultural residues and hardwoods indicated that residual xylan presented in the steam-pretreated solid fraction hindered enzyme hydrolysability [18]. It was found that addition of xylanase significantly increased the enzyme hydrolysability of steam-pretreated lignocellulose, which contained a significant amount of xylan. However, previous studies evaluated the effect of xylanase supplementation during hydrolysis at very low substrate consistency. In the present study, the effect of xylanase addition and effect of resultant removal of xylan in enhancing the high-substrate consistency hydrolysis of steam-pretreated SSB are also investigated.

Materials and Methods

Raw Material

Sweet sorghum cultivar Liaotian I, cultivated on the farm of Shanghai Jiao Tong University, was used for this study. The fresh crop was harvested with leaves stripped to obtain the fresh stalk. The stalks were squashed by a three-roller mill to separate the fresh juice and the bagasse. The bagasse was chopped using a small-scale industry rubbing machine to less than 4 mm. The chopped bagasse was air-dried, and resulting samples had a moisture content of 8%.

Pretreatment

The air-dried bagasse was mixed thoroughly and steam pretreated at the Process Development Unit in the Department of Wood Sciences of the University of British Columbia, Canada. The SSB was pretreated in the conditions of 190 °C for 5 min both with the addition of SO_2 and without SO_2. Prior to steam pretreatment, SO_2 impregnation was done by adding appropriate amount of SO_2 (~5% *w*/*w*) to a plastic bag containing 300 g dry weight of biomass. Once the desired amount of SO_2 was achieved and after thorough mixing, the bag was immediately sealed. The samples were left for approximately 12 h. The bag was reweighted to determine the actual absorbed SO_2. It was found that approximately 70% of the injected SO_2 was absorbed by the SSB in the current work.

The steam pretreatment was done using a 2-L steam gun (Stake Tech II, Stake Technology, Norval, Ontario). After the steam pretreatment, the discharged slurry in a collecting vessel was removed and stored at 4 °C till further use. The water-soluble and water-insoluble fractions were separated by vacuum filtration. The water-insoluble fraction was then washed with approximately 5 L of water and vacuum-filtered to final moisture content of ~70%. An aliquot of wash water was also collected to account for the sugar loss during washing and establish the complete mass balance.

Enzymatic Hydrolysis

The commercial enzymes, cellulose (Spezyme-CP, Genencor-Danisco, Palo Alto, CA) and β-glucosidase (Novzymes188, Bagsværd, Denmark), were used in enzymatic hydrolysis. To examine the effect of xylanase supplementation on hydrolysis of pretreated SSB, Multifect® Xylanase (Genencor-Danisco, Palo Alto, CA) was used. Multifect® Xylanase is

a commercial xylanase derived from a genetically modified strain of *Trichoderma reesei.* Their protein content and activities are listed in Table 1.The water-insoluble solid was enzymatically hydrolyzed in acetate buffer (0.05 M, pH 4.8) in the 150-mL screw-top flasks at 150 rpm and 50 °C for 72 h. Samples (250 μl) were taken periodically during the hydrolysis process and boiled for 10 min to deactivate the enzymes and centrifuged at 1.3×10^5 rpm, 4 °C for 10 min. The supernatants were stored in the −20 °C freezer till further analysis.

Analytical Methods

The raw material and pretreated SSB were analyzed for Klason insoluble lignin and carbohydrates using the Tappi-T-22 om-88 as previously described [18]. The hydrolysate from the Klason analysis was retained and analyzed for soluble lignin by a UV spectrophotometer at 205 nm. The monomeric sugars were measured by using a high-performance liquid chromatography (HPLC; Dionex, ICS-3000, Dionex Corp., Sunnyvale, CA). The HPLC system was mainly integrated with an auto-sampler (Dionex, AS-50), a gradient pump (Dionex, GP50), an anion exchange column (Dionex, CarboPac PA1), and an electrochemical detector (Dionex, ED50). The mobile phase for HPLC analysis was deionized water at a flow rate of 1.0 mL/min, and post-column addition of 0.2 M NaOH maintained optimization of baseline stability and detector sensitivity. The column was reconditioned with 1 M NaOH after each sample. The injection volume in the HPLC was 25 μl, and the column temperature was maintained at 35 °C. The fucose (~0.2 g/L, Sigma) was used as an internal standard. The oligomeric sugars in the water-soluble fraction were hydrolyzed into monomers in an autoclave at 121 °C for 1 h. Thereafter, the monomeric sugars were analyzed with HPLC as mentioned above to calculate the content of oligomeric sugars.

In order to investigate the degree of hydrolysis, the glucose and xylose in the hydrolysate were also analyzed with the HPLC using the same conditions. The influence of solid fraction has been considered in calculation to reduce the overestimation of glucan–glucose conversion as low as possible.

Results and Discussion

Sugar Recovery of SSB After Steam Pretreatment

Composition of the raw SSB indicated that both carbohydrate and lignin contents were in the range of the reported other typical herbaceous residues such as corn stover, rice straw,

Table 1 The protein content and enzyme activities in Spezyme-CP, Novzymes188 and Multifect®

	Protein content (mg/mL)	Cellulase (FPU/mL)	β-glucosidase (CBU/mL)	Xylanase[a] (IU/mL)
Spezyme-CP	133.9	48.6	10.4	535.3
Novzymes188	233.4	N/A	458.4	32.6
Multifect®	37.1	N/A	12.7	2,588.4

N/A not available

[a] Determination of xylanase activity based on Birchwood xylan as the substrate

and barley straw [18, 27]. Especially, the main composition of SSB was similar with corn stover. In addition, xylan represents approximately 90% of the entire hemicellulose presenting in SSB (see Table 2). Generally, in agricultural residues, xylan is acetylated and the acetyl linkages gets partially hydrolyzed during steam pretreatment resulting in the liberation of acetic acid, which acts as an acid catalyst during steam pretreatment and eliminates the need for an external addition of acid catalyst. In order to assess whether external addition of SO_2 was necessary for SSB, steam pretreatment was conducted both in the presence and in the absence of SO_2. Moreover, the suitable pretreatment conditions of corn stover (190 °C and 5 min), obtained in references [18, 28], were directly employed on SSB to estimate whether the similar pretreatment results could be achieved with the similar pretreatment conditions when the substrate compositions were similar.

Results on the composition of the steam-pretreated SSB indicated that significant dissolution of hemicellulose (~70%) occurred during steam pretreatment, leaving a solid fraction containing about 10–12% residual xylan. Similar results were reported previously with corn stover pretreated at the same set of conditions [18, 29]. Based on Table 2, a slightly greater amount of xylan was dissolved when the SSB was steam pretreated using SO_2, resulting in a consequent difference in residual xylan content of the SSB pretreated with and without SO_2. However, the residual xylan content of SSB pretreated with and without SO_2 was 9% and 12%, respectively, compared to 9% and 18% in corn stover at the same set of conditions. This indicated that the degree of acetylation of hemicellulose present in the raw SSB may be higher or the acetyl linkages in the SSB hemicellulose were more susceptible to cleavage during steam pretreatment compared with corn stover. Additionally, when *Brassica carinata* was pretreated at 190 °C for 4 min, 8% residual xylan was also observed after steam pretreatment [30]. Glucan content of the steam-pretreated SSB was also comparable to what has been reported previously with other typical agricultural residues such as corn stover, *B. carinata* pretreated at similar conditions [18, 30].

Regardless of an external acid catalyst used, SSB was found to behave similarly during steam pretreatment in terms of overall sugar recovery and the resulting composition of the solid fraction (Fig. 1). Eighty-seven percent glucose and 72% xylose recovery was obtained at the pretreatment conditions of 190 °C for 5 min with 5% SO_2 impregnation. No noticeable difference was observed in terms of glucan recovery in the presence or absence of SO_2. However, use of SO_2 catalyst appeared to slightly favor higher recovery of xylose.

Table 2 Main composition of raw and steam-pretreated SSB

Composition (%, *w*/*w*)	Raw SSB	Pretreated SSB	
		Without SO_2	With SO_2
Glucan	35.1±1.0	55.7±0.7	54.3±1.2
Xylan	19.4±0.9	12.9±0.3	9.8±0.2
Galactan	1.5±0.1	0.2±0.0	0.8±0.0
Arabinan	1.4±0.0	0.7±0.0	0.6±0.0
Mannan	0.9±0.0	1.0±0.0	1.2±0.1
AIL	18.4±2.8	24.7±0.91	25.8±0.9
ASL	0.2±0.1	1.9±0.0	1.9±0.0
Ash	1.9±0.1	2.7±0.0	2.3±0.0
Extractives	21.2±2.0		

AIL acid-insoluble lignin, *ASL* acid-soluble lignin

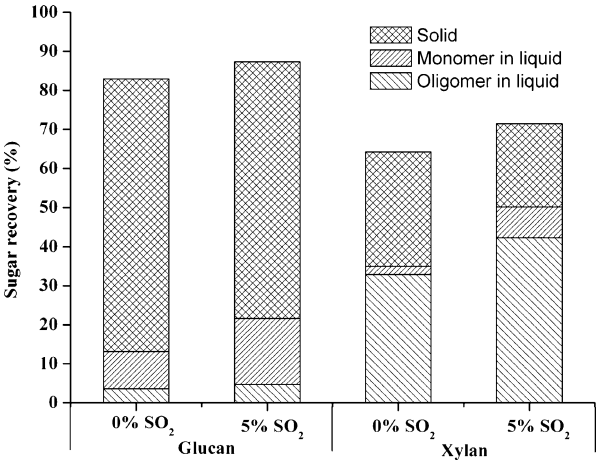

Fig. 1 Sugar recovery of SSB after steam pretreatment with and without SO_2

pH of the water-insoluble fraction resulting from steam pretreatment with and without SO_2 was found to be 2.7 and 3.8, respectively, which indicated that at lower pH, hydrolytic reactions might dominate sugar degradation reactions such as pyrolysis and dehydration as previously observed with softwoods [23, 31]. A slightly lower amount of residual xylan in the solid fraction and higher proportion of monomeric sugars in the liquid fraction resulting from SO_2-catalyzed steam pretreatment also confirmed the dominance of hydrolysis reactions in the presence of an acid catalyst. Significant dissolution of xylan from SSB during steam pretreatment was likely to favor enzymatic hydrolysability of the resulting solid fraction due to a greater exposure of cellulose microfibrils [18]. Moreover, it has also been reported that during steam pretreatment, partial deacetylation of xylan occurred, which favored overall xylan and glucan conversion by cellulolytic and xylanolytic enzymes [32]. Thus, it was next of interest to see how the pretreated SSB responds to the subsequent enzymatic hydrolysis.

Amenability of Steam-Pretreated SSB to Subsequent Enzymatic Hydrolysis

In order to assess the amenability of steam-pretreated SSB towards subsequent enzymatic hydrolysis, hydrolysis was performed at different consistencies ranging from 2% to 16% in shake flasks. The loading of cellulase and β-glucosidase was 10 filter paper units (FPU)/g glucan (corresponding to 27.5 mg of protein/g glucan) and 40 cellobiase units (CBU)/g glucan (corresponding to 20.4 mg of protein/g glucan), respectively. Hydrolysis results indicated that steam-pretreated SSB was highly amendable to enzymatic hydrolysis and comparable to the behavior of other steam-pretreated agricultural residues. When 2% substrate consistency of the pretreated SSB with SO_2 impregnation was for enzymatic hydrolysis, glucan–glucose conversion at 72 h was 70%. It was nearly 10% lower than the previously reported results for the steam-pretreated corn stover at the same pretreatment conditions. However, a higher enzyme loading (15 FPU/g glucan) and a lower substrate consistency (1%) were employed in the previous work for corn stover. These could be the reasons for a higher hydrolysability observed with corn stover.

According to Fig. 2, further increasing the substrate consistency from 2% to 16% reduced the glucan–glucose conversion (72 h) of steam-pretreated SSB from 70% to 49%. Moreover, the hydrolysis rate also slowed down. The similar results could be observed in previous work with various substrates [33–35]. Based on the results of ANOVA, increasing

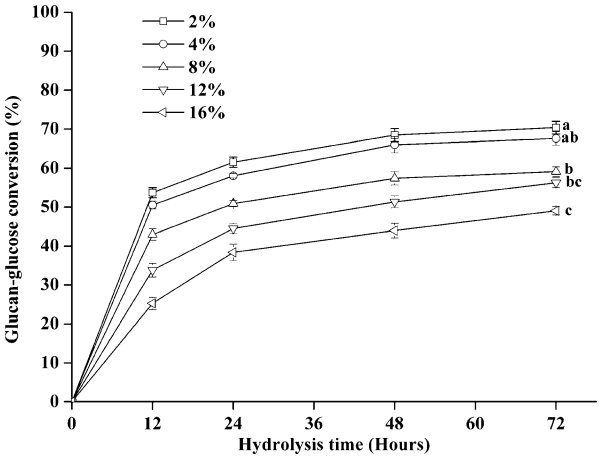

Fig. 2 Enzymatic hydrolysis of pretreated SSB with SO_2 at different substrate consistencies

the substrate consistency had a significant negative influence on the glucan–glucose conversion ($p<0.05$). However, when the decrease ratio of glucan–glucose conversion, which could be defined as the decrease of glucan–glucose conversion with 1% substrate consistency increasing, was considered, it could be found that the decrease ratios were 2.8%/%, 1.9%/%, 1.4%/%, and 1.6%/% as the consistency increased from 2% to 4%, 8%, 12%, and 16%, respectively. These results indicated that the influence of substrate consistency increase on hydrolysis was the minimum when the consistency increased to 12%. Increasing consistency from 2% to 12% could retain glucan–glucose conversion ≥56% and increasing consistency further to 16% reduced the conversion to 49%. When the pretreated SSB without SO_2 impregnation was also hydrolysed in the same conditions with the consistency varying from 2% to 12%, the glucan–glucose conversion of pretreated SSB with 5% SO_2 was 5–13% higher than that without SO_2 impregnation at various substrate consistencies. Moreover, the influence of increasing consistency on hydrolysis was almost similar with the pretreated SSB with SO_2 impregnation. The minimum decrease ratio of glucan–glucose conversion of the pretreated SSB without SO_2 also appeared at the consistency of 12% (data not shown). Thus, it was apparent that 12% could be regarded as a reasonable high substrate consistency for enzymatic hydrolysis for the steam-pretreated SSB. Increasing substrate consistency beyond 12% in shake flasks could start creating significant rheological problems limiting the effective mass transfer during hydrolysis process. In addition, according to the summary in the reference, it could be found that 12–15% total solids (*w*/*w*) were often considered as the upper limit at which pretreated biomass could be mixed and hydrolyzed in conventional reactors [36]. Similar hydrolysis yields (~70%) were reported for steam-pretreated corn stover when the hydrolysis was conducted in shake flasks at 15% substrate consistency with 12 FPU/g glucan enzymes loading after 72 h of incubation [37]. It should also be noted that 55% glucan–glucose conversion was also reported for steam-pretreated olive tree biomass when a higher substrate consistency (20%) hydrolysis was conducted in shake flasks despite a higher enzyme loading applied (15 FPU/g substrate) and low xylan content in the substrates [33]. Previous work has also shown that hydrolysis at consistencies beyond 12% required a special mixing device (peg mixer) to overcome the mass transfer limitations. Only 60% glucan to glucose conversion was obtained with organosolv-pretreated poplar at 20% substrate consistency and 10 FPU/g glucan cellulase even though the laboratory peg

mixer was employed [38]. Thus, the rheological problems and mass transfer limitations could result in lower yields observed with high-substrate consistency hydrolysis in the shake flasks.

Effects of Enzyme Loading on Relatively High-Substrate Consistency Hydrolysis of Steam-Pretreated SSB

As the influence of increasing substrate consistency on enzymatic hydrolysis was minimized at 12% in the shake flasks, the hydrolysis yields in the pretreated SSB with 5% SO_2 were higher than that of without SO_2 impregnation. The pretreated SSB with 5% SO_2 with the consistency of 12% was selected as a relatively high substrate level for further researches on enzyme loading. In order to reduce the enzyme loading for hydrolysis as low as possible, the cellulase loading ranging from 2.5 to 20 FPU/g glucan (corresponding to 6.9 to 55.1 mg of protein/g glucan) was employed with the fixed β-glucosidase supplementation of 40 CBU/g glucan (corresponding to 20.4 mg of protein/g glucan). The results showed that increase in cellulase loading from 2.5 up to 20 FPU/g could enhance the glucan–glucose conversion from 29% to 64% and xylan to xylose conversion from 47% to 79%, respectively (Fig. 3). Obviously, many more available reaction sites on cellulose were occupied when cellulase loading increased and further improved hydrolysis. The Spezyme-CP cellulase had a degree activity of xylanase (535.3 IU/mL), which would result in increasing xylan conversion when the enzyme loading was increased. When the linear regression was made between the glucan–glucose conversion and xylan–xylose conversion, it could be found that the correlation coefficient could be 0.98. Therefore, it could be deduced that the xylan content in SSB will directly affect the conversion of glucan to glucose. Progressive removal of xylan with increased enzyme input facilitates the exposure of greater amount of available surface of cellulose for enzymatic hydrolysis. Based on the ANOVA results, there was a significant increase in glucan conversion ($p<0.05$) with increase in enzyme loading from 2.5 to 7.5 FPU/g glucan (corresponding to about 6.9 to 20.7 mg of protein/g glucan), and there was only a slight improvement with further increasing enzyme loading from 10 to 20 FPU/g glucan (corresponding to about 27.6 to 55.1 mg of protein/g glucan). Therefore, in order to use the cellulase as low as possible in the high-substrate consistency hydrolysis, the cellulase loading of 7.5 FPU/g

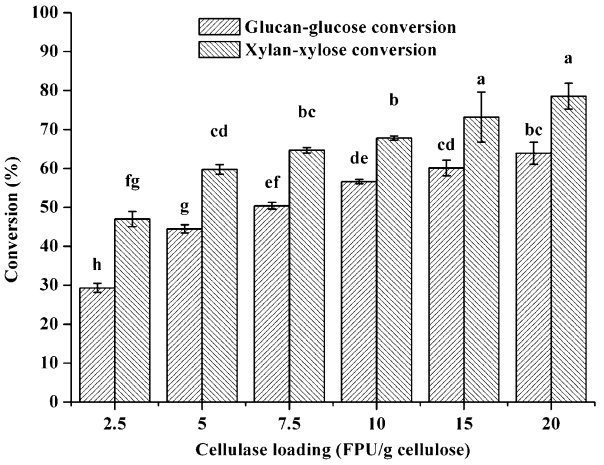

Fig. 3 Enzymatic hydrolysis of steam-pretreated SSB with SO_2 at different cellulase loadings

glucan could be selected as the potential one. It could be noted that even at higher enzyme input such as 20 FPU/g glucan, the steam-pretreated bagasse did not result in considerable hydrolysis conversion.

The accumulation of cellobiose and its consequent inhibition of cellulase activity during high-substrate consistency hydrolysis can be avoided by supplementing additional β-glucosidase, which hydrolyse cellobiose to glucose. Thus, sufficient supplementation of β-glucosidase is very important to obtain higher glucose yield in the biomass-to-ethanol process [35]. However, superfluous loading of β-glucosidase would significantly increase the overall hydrolysis cost. With the objective of determining the minimum required β-glucosidase input, different dosages of β-glucosidase were supplemented ranging from 5 to 80 CBU/g glucan (corresponding to about 2.5 to 40.7 mg of protein/g glucan) while maintaining the cellulase loading constant (7.5 FPU/g glucan). Despite some β-glucosidase activity of the Spezyme, additional supplementation of β-glucosidase was necessary to achieve reasonable hydrolysis at higher consistencies (Fig. 4). It was found that increasing β-glucosidase beyond 10 CBU/g glucan (corresponding to 5.1 mg of protein/g glucan) could not further enhance the hydrolysis conversion levels considerably. The results indicated that 1.3:1 (CBU/FPU) would be the near-optimum ratio of cellulase to β-glucosidase for minimizing the end-product inhibition effects of cellobiose during high consistency hydrolysis. The similar results also could be observed in reference when the amorphous cellulose and crystalline cellulose were hydrolyzed with increasing β-glucosidase loading. Even though the CBU/FPU increased from 2:1 to 16:1, the glucose yield could not be improved obviously [39].

Effect of Xylanase Supplementation on Relatively High-Substrate Consistency Hydrolysis of Steam-Pretreated SSB

Previous studies with corn stover and poplar provided indications that residual xylan content plays a major role in determining the ease of enzymatic hydrolysis of steam-pretreated substrates [18]. It could be found that steam-pretreated poplar containing 4% xylan reached almost complete glucan to glucose conversion compared to 70% hydrolysis conversion observed with the steam-pretreated corn stover having 10% residual xylan. When xylanase was supplemented during hydrolysis, a significant increase in hydrolysis with corn stover could be observed. However, a previous work was conducted with the substrate consistency

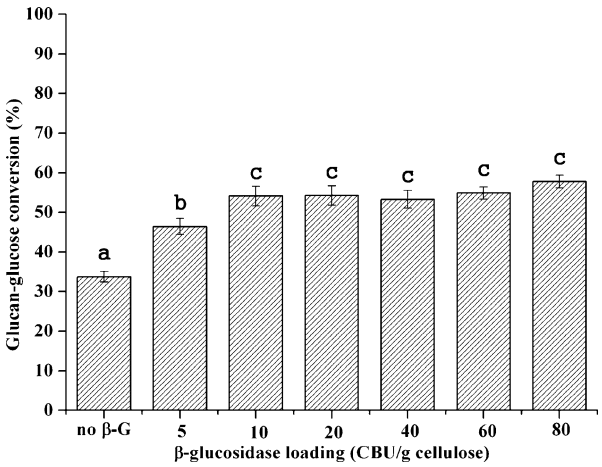

Fig. 4 Enzymatic hydrolysis of steam-pretreated SSB with SO_2 at different β-glucosidase loadings

of 1%. In addition, the above-mentioned results obtained at various cellulase loadings in the present work also gave indications that residual xylan could act as a major hindrance to enzymatic hydrolysis. Thus, in this part, with the objective of investigating the effect of xylan removal on cellulolytic hydrolysis of steam-pretreated SSB at high substrate consistency, the enzymatic hydrolysis with xylanase supplementation was carried out with the substrate consistency of 12%. In addition, according to the above results, the relatively low cellulase and β-glucosidase loading was selected as 7.5 FPU/g glucan (20.7 mg of protein/g glucan) and 10 CBU/g glucan (5.1 mg of protein/g glucan), respectively.

As described in Fig. 3, some increase in hydrolysis yield could be achieved when increase in cellulase loading was from 7.5 to 20 FPU/g glucan. But the increase tendency was slowed down, and only 14% increase of glucan–glucose conversion could be obtained in contrast to the conversion increase of 21% from 2.5 to 7.5 FPU/g glucan. However, addition of xylanase during hydrolysis was found to enhance glucan conversion significantly (Fig. 5a). This was attributed to the enhanced xylan conversion with xylanase supplementation (Fig. 5b) and the resultant removal of xylan in the substrate. In the present

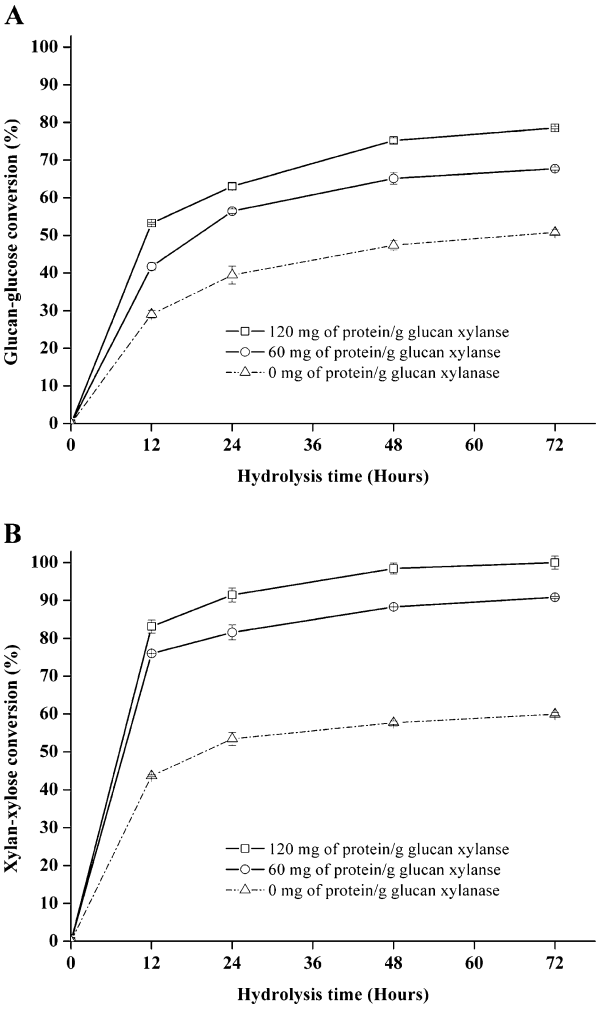

Fig. 5 Effect of xylanase supplementation on **a** glucan to glucose conversion **b** and xylan to xylose conversion

work, the supplementation of xylanase at a level of 60 mg protein/g glucan (4,186 IU/g glucan) increased the glucan conversion from 50% to 68% at the substrate consistency of 12%. The corresponding xylan conversions were 60% and 91%, respectively. Further increasing the supplementation of xylanase to a level of 120 mg protein/g glucan (8,372 IU/g glucan) increased the glucose yield from 50% to 80% at the substrate consistency of 12%. The corresponding xylan conversions were 60% and 100%, respectively. As shown in Table 1, the filter paper activity in Multifect® xylanase was not available, and β-glucosidase was 12.7 CBU/mL. Therefore, the xylanase supplementation with the loading of 60 and 120 mg protein/g glucan could not increase the filter paper activity, but the β-glucosidase loading would be increased to 30–50 CBU/g glucan. However, β-glucosidase loading in 10–80 CBU/g glucan had no significant improvement on hydrolysis (see Fig. 4). According to this analysis, it could be deduced that xylan hydrolysis had a great potential to improve cellulose hydrolysis. The existence of hemicellulose (xylan in agriculture residues) was a steric hindrance impeding the accessibility of cellulase to cellulose. Moreover, the role of effective xylan conversion in improving the cellulose hydrolysis yield was also reported in recent studies [18, 32].

As shown in Table 1, xylanase also existed in the Spezyme-CP cocktail with an activity of 535.3 IU/mL. With steam-pretreated SSB having nearly 10% residual xylan, it has already been observed that increasing cellulase loading from 7.5 to 20 FPU/g glucan could improve the glucose yield from 50% to 64% with xylan–xylose conversion increasing from 54% to 78%. It was apparent that the xylanase in Spezyme-CP was not enough for a complete xylan hydrolysis. In the previous study, it could be found that xylanase loading of 60 mg protein/g glucan was optimum to enhance hydrolysis for pretreated corn stover with 1% substrate consistency, and any further increase did not further enhance hydrolysis yield [18]. In the present work, the pretreated SSB consistency was increased to 12%, and the xylanase supplementation was correspondingly increased to 120 mg protein/g glucan. It was sufficient to reach 80% glucan to glucose conversion corresponding to a 100% xylan conversion even with a relatively low cellulase loading of 7.5 FPU/g glucan. This indicated that increased xylanase protein loading corresponding to a near-complete xylan removal had the potential to enhance hydrolysis yield considerably during high-substrate consistency hydrolysis of xylan-containing substrates. However, the xylanase loading of 120 mg protein/g glucan for hydrolysis was a little high, and it should be given enough consideration on optimization in further work to reduce the dosage as low as possible.

Conclusions

Sweet sorghum bagasse (SSB) behaved in a similar fashion to other typical agricultural residues such as corn stover in steam pretreatment conditions and subsequent enzymatic hydrolysis. The steam pretreatment condition (190 °C for 5 min) was found to be robust enough to pretreat SSB resulting in a reasonable hemicellulose and glucan recovery. Enzymatic hydrolysis showed that the steam-pretreated SSB obtained the maximum glucan–glucose conversion of 70% at 2% substrate consistency. However, at relatively higher consistency (12%) in shake flasks while using relatively low enzyme input levels (cellulase, 7.5 FPU/g glucan and β-glucosidase, 10 CBU/g glucan), the hydrolysis yield dropped to about 50%. However, when an external supplementation of xylanase (120 mg protein/g glucan) coupling with the relatively low enzyme loadings was employed, 80% glucan conversion with a complete xylan hydrolysis was achieved at the relatively high substrate consistency of 12%. Therefore, a proper xylanase supplementation for enzymatic

hydrolysis was suggested to achieve a high glucose yield when the steam-pretreated substrates with high residual xylan were hydrolyzed with a relatively high substrate consistency.

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