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Influence of Extruder Temperature and Screw Speed on Pretreatment of Corn Stover while Varying Enzymes and Their Ratios

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Abstract Pretreatment is being the first and most expensive step, it has pervasive impacts on all other steps in overall conversion process. There are several pretreatment methods using physical, chemical, and biological principles which are under various stages of investigation. Extrusion can be used as one of the physical pretreatment methods towards biofuel production. The objective of this study was to evaluate the effect of barrel temperature and screw speed on sugar recovery from corn stover, to select a suitable enzyme combination and its ratio. Corn stover was pretreated in a single screw extruder with five screw speeds (25, 50, 75, 100, and 125 rpm) and five barrel temperatures (25, 50, 75, 100, and 125 °C). In order to select a suitable enzyme combination and ratio, different levels of cellulase and β-glucosidase, multienzyme complex and β-glucosidase were used during saccharification of pretreated corn stover. From the statistical analysis, it was found that screw speed and temperature had a significant effect on sugar recovery from corn stover. Higher glucose, xylose, and combined sugar recovery of 75, 49, and 61%, respectively, were recorded at 75 rpm and 125 °C. This pretreatment condition resulted in 2.0, 1.7, and 2.0 times higher than the control sample using 1:4 cellulase and β-glucosidase combination.

Keywords Biomass · Pretreatment · Extrusion · Screw speed · Temperature · Enzymatic hydrolysis · Sugar recovery

Introduction

Environmental impact of fossil fuel and their inevitable depletion have led to intense research on the development of alternate energy sources throughout the world. Brazil and the US are the world leaders, and together they produce about 60% of the world's ethanol exploiting sugarcane and corn, respectively. The US corn ethanol process is a quite matured

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technology with little possibility of process improvement [1]. In order to meet the Department of Energy's strategic goal of 60 billion gallons of ethanol, in addition to corn ethanol, greater amounts of biomass needs to be converted into biofuels [2]. Biomass appears to be an attractive feedstock because of its renewability and positive environmental impacts resulting in no net release of carbon dioxide (CO_2) and very low sulfur content. The US Department of Agriculture and Department of Energy have estimated that the US has the resource potential to produce over 1 billion tons of biomass annually [3], thus accounting for close to 30% displacement of current fossil fuel usage (about 80 billion gallons).

Unlike corn grain where the major carbohydrate is starch, biomass is composed of 40–50% cellulose, 25–35% hemicellulose, and 15–20% lignin [4]. Pretreatment is one of the key elements in the bioconversion of biomass into biofuels. The purposes of pretreatment are to disorganize the biomass structure, to reduce the cellulose crystallinity, increase the surface area, and pore size. Pretreatment is the first and one of the most expensive steps in conversion of biomass to biofuels [5], it has pervasive impact on downstream processing steps [6]. Thus, more attention must be given to gain insight into interactions among these operations and applying that insight to advance biomass conversion technologies that reduce costs [7].

There are several pretreatment methods using physical, chemical, and biological principles which are under various stages of investigation throughout the world. Physical pretreatment methods include size reduction, steam explosion, and hydro-thermolysis. The power requirement of size reduction of agricultural materials depends on the final particle size and the biomass characteristics [8]. Costs and energy requirements for particle size reduction increase geometrically with decreasing particle size. It has been reported that the grinding process can account for one-third of the energy requirements of the entire process [9]. The elevated temperature used in hydrothermal pretreatment usually leads to degradation of carbohydrates. Chemical pretreatment using acids or bases promote the hydrolysis and improve enzymatic hydrolysis by removing hemicellulose or lignin. The most commonly used acid and base is H_2SO_4 and NaOH, respectively. Though dilute sulfuric acid achieves glucose yields over 90% through enzymatic hydrolysis [10]; it requires costly construction material, high pressures, neutralization, and conditioning of hydrolysate prior to biological steps, slow cellulose digestion by enzymes, and nonproductive binding of enzymes to lignin [10–12]. Formation of degradation products and release of natural biomass fermentation inhibitors are other characteristics of acid pretreatment [5]. Sodium hydroxide and other bases are too expensive and very difficult to recover and recycle to make them viable for producing fuels and chemicals [10]. The mode of action in ammonia fiber explosion (AFEX) pretreatment is simultaneous reduction of lignin content and removal of some hemicellulose while decrystallizing cellulose [5]. The cost of ammonia and ammonia recovery are critical for AFEX [13]. Ammonia-based solvents, aprotic solvents, metal complexes, and wet oxidation also reduces cellulose crystallinity and disrupts the association of lignin with cellulose, as well as dissolves hemicellulose. These methods, while effective, are too expensive to be practical when measured against the value of glucose, i.e., approximately $0.11/kg [5]. Biological pretreatments employ microorganisms to disturb the biomass structure; they are time-consuming processes and result in low sugar recovery. No perfect pretreatment method has been established for biofuel production from biomass on commercial scale [14].

Extrusion is widely used technique in snack food, feed, and plastic industries. It differs from other food-processing methods in which several unit operations are performed

simultaneously. In extrusion, the materials are subjected to heating, mixing, and shearing, resulting in physical and chemical changes during the passage through the extruder barrel [15]; it can be used as one of the novel pretreatment methods for biomass towards biofuels production. The hypothesis is that extruder screw speed and barrel temperature may disturb the biomass structure thereby increase the accessibility of cellulose for enzyme action.

The second most expensive input in biomass conversion is the enzyme loading, which can amount to as much as 60% of the process cost [16–18]; hence, enzyme usage should be as low as possible. Enzymatic hydrolysis of cellulose is typically carried out by cellulases—a complex system consists of three enzymes (endoglucanase, exoglucanase, and cellobiase) that act synergistically. Endoglucanase randomly cleaves cellulose chains to form glucose, cellobiose, and cellotriose. Exoglucanase attacks the nonreducing, cellobiase cleaves cellobiose units into glucose units. Glucose formation from pretreated corn stover catalyzed by cellulase is subject to product inhibition. The cellobiase/β-glucosidase supplementation with cellulase enzyme was necessary to eliminate the inhibition effect of cellobiose [19]. When β-glucosidase removes cellobiose results in the absence of product inhibition [20, 21], the hydrolysis can be achieved at reduced enzyme levels. Complementation with β-glucosidase was necessary to reach an appropriate β-glucosidase activity for hydrolysis. With complementation of β-glucosidase, glucose recovery of 78.5–81.2% was recorded for corn stover pretreated using wet oxidation (60 g/L of corn stover, 195 °C, 15 min, 12 bar O_2, 2 g/L of Na_2CO_3 pH 9.3) whereas, without β-glucosidase, it was only 57.8% [22]. A wide range of enzyme dosage, ratios, and hydrolysis conditions have been reported for corn stover depending on the pretreatment as presented in results and discussion section. Comparison of sugar recovery from different pretreatment methods is very difficult due to variation in enzyme activity, dose, ratio, and conditions employed during hydrolysis. Hence, there is a need to determine a suitable enzyme combination and its dose for economic reasons and for the purpose of comparison too.

The present study was undertaken for two reasons as explained above. The first objective is to evaluate the effect of screw speed and barrel temperature on sugar recovery from corn stover. The second objective is to select a suitable enzyme combination and its ratio for the enzymatic hydrolysis of pretreated corn stover.

Materials and Methods

Biomass—Corn Stover

According to Kadam and McMillon [23], about 80–100 dry tons of corn stover/year can be utilized for ethanol production in the US. It has been estimated that approximately 256 million dry tons of corn stover will be available in the year 2030 due to collection technologies improvement and a steady yield increase [3]. Corn stover is considered as one of the potential biomass and most studied material too; thus, corn stover was selected for this study. Corn stover obtained from a local farm was ground in a hammer mill (Speedy Jr, Winona Attrition Mill Co, MN) using a 4-mm circular sieve for further pretreatment. The moisture content was determined as described by Sluiter et al. [24] in NREL/TP-510-42621. The moisture content of ground corn stover was adjusted to 21% (wb) based on glucose yield through enzymatic hydrolysis of samples from a preliminary study. Compositional analysis of corn stover was carried out as outlined by Sluiter et al. [25, 26] and shown in Table 1.

Table 1 Chemical composition of corn stover on dry matter basis (%) reported in literature.

Glucose	Xylose	Arabinose	Galactose	Mannose	Lignin	Ash	References
40.9	21.5	1.8	1.0		16.7		[29]
36.0	19.8	2.8	1.3		19.7		[58]
41.3	27.9[b]				22.1	6.3	[51]
39.0	20.1	2.0			21.5	6.8	[52]
40.2±0.3	21.7±0.2				20.8±0.6		[59]
37.1	19.8	2.5	1.6	1.4	20.7	5.2	[60, 61]
41.6±0.3	27.7±0.4	3.6±0			20.2±0.2	4.0±0	[62]
37.8	21.3	1.6	1.4	3.8	17.8	7.8	[30]
36.8	21.7	2.6	0.68	0.3	17.2		[31]
36.7±0.2	21.2±0.2	3.8±0	1.1±0	0.3±0	14.4±0.1[a]	6.1±0.1	[63]
36.1	21.4	3.5	2.5	1.8	17.2	7.1	[32, 33]
36.8±1.2	22.2±0.8	5.5±0.9	2.9±1.0		23.1±0.7		[64]
37.5	20.8	2.7	1.6	0.8	17.6	6.7	[65]
37.1-39.8	24.4-28.2[c]	6.5-7.1			19.5-20.3	4.3-10.2	[43]
36.8	22.2	5.5	2.9	0.3	21.2	6.5	[34]
36.1	21.4	3.5	2.5	1.8	17.2	7.1	[34]
42.2	19.6	2.9	1.1		20.8		[66]
36.9	24.7	3.2	1.7	0.8	19.9		[67]
34.1	22.8	4.2			11.4		[68]
16.3±1.9	10.2±0.7	1.8±0.6		2.0±0.1	20.2±1.1	7.2±0.2	This study

[a] Acid insoluble lignin

[b] Hemicellulose

[c] Xylose+galactose

Extrusion Pretreatment

Extrusion was performed in a single screw extruder (Brabender Plasti-corder Extruder Model PL2000, Hackensack, NJ), which had a compression ratio of 3:1, barrel length to screw diameter ratio (*l*/*d*) of 20:1. In order to have a smooth biomass (plug) flow into the die section, the screw discharge end was fitted with a conical metal piece. The single screw extruder was fitted to a 7.5-hp motor, which had a provision to adjust the screw speed from 0 to 210 rpm. The screw speed of the extruder was maintained at 25, 50, 75, 100, and 125 rpm during the extrusion of samples. The extruder barrel had provisions to control the temperature of the feed and transition zone in both barrel and die section. The transition zone and die section temperature of barrel was maintained at 25, 50, 75, 100, and 125 °C for different screw speeds. The temperature inside the barrel and the speed of the screw were controlled by a computer; and feeding to the extruder was done manually. Compressed air was supplied as a cooling agent along the barrel length.

Enzymatic Hydrolysis

The enzymatic hydrolysis were conducted in hungate glass tube (Bellco glass, Inc, NJ, USA) with 0.3 g dry weight of pretreated corn stover in a solution of citrate buffer (0.05 M, pH 4.8) and sodium azide (0.02 g/l) to maintain constant pH and inhibit microbial

contamination, respectively. In order to select an enzyme combination and ratio, multienzyme (NS50012, activity 100 FBG/g), cellulase (NS50013, activity 70 FPU/g) with β-glucosidase (NS50010, activity 250 CBU/g) in the ratio of 1:1 and 1:4 was added to the pretreated corn stover. The amount of cellulase was maintained at 15 FPU/g of dry matter, β-glucosidase amount was maintained at 15 and 60 CBU/g dry matter, respectively, for 1 and 4 ratios. All these enzymes were provided by Novozyme. Multienzyme complex consists of arabinase, β-glucanase, cellulase, hemicellulase, pectinase, and xylanase, which has the ability to liberate bound materials and can degrade a variety of non-starch polysaccharides. Hydrolysis was carried out for 72 h at 50 °C and 150 rpm as described by Selig et al. [27] in NREL/TP-510-42629. After hydrolysis, the samples were kept in boiling water for 10 min to inactivate the enzyme action. The supernatant was centrifuged at 13,000 rpm for 15 min and then frozen twice before injecting into the HPLC to remove the impurities which contribute to the pressure increase in the HPLC system. Soluble sugars and byproducts were quantified using HPLC (Agilent Technologies, Santa Clara, CA; Bio-Rad Aminex 87H column Hercules, CA) with a mobile phase of 0.005 M H_2SO_4 at a flow rate of 0.6 ml/min at 65 °C and a sample volume of 20 μl as mentioned by Sluiter et al. [28] in NREL/TP-510-42623. Ground corn stover was also subjected to enzymatic hydrolysis and analyzed as the control. The sugar concentration obtained from chromatogram was divided by dry weight of corn stover taken for enzymatic hydrolysis in order to calculate the percentage of different sugar with respect to total biomass. Glucose and xylose were the major sugars present in the biomass as compared to arabinose. Instead of reporting arabinose separately, it was added with glucose and xylose and reported as combined sugar. Authors observed that xylose and mannose eluted at the same retention time and appeared as a single peak due to the capability of 87H column; hence, the xylose reported here is the combination of xylose and mannose. Acetic acid and glycerol were the byproducts found in the pretreated samples and their concentration was reported in grams per liter.

$$Y_{\mathrm{i}} = \frac{S_{\mathrm{ip}}}{S_{\mathrm{ir}}} \times 100 \tag{1}$$

$$Y_{\mathrm{c}} = \frac{\sum S_{\mathrm{ip}}}{\sum S_{\mathrm{ir}}} \times 100 \tag{2}$$

Y_{i} individual sugar recovery, %
Y_{c} combined sugar recovery, %
S_{ip} Individual sugar obtained from pretreated samples through HPLC
S_{ir} Individual sugar from raw material

Statistical Analysis

The moisture-balanced corn stover was extruded using a screw with 3:1 compression ratio at varying screw speeds of 25, 50, 75, 100, and 125 rpm and barrel temperatures of 25, 50, 75, 100, and 125 °C. This resulted in 25 treatment combinations (i.e., 5×5=25). Each treatment run was divided into two batches and the samples collected were considered as replicates. The collected data were analyzed with proc GLM procedure to determine the main, interaction, and treatment effect using SAS 9.1 (SAS Institute, Cary, NC) using a type I error (α) of 0.05.

Results and Discussion

Characterization of Corn Stover

The corn stover composition such as glucose, xylose, arabinose, mannose, lignin, and ash content on dry matter basis (%) was determined and given in Table 1. In general, glucose is referred as cellulose and xylose, arabinose, galactose, and mannose are combined together termed as hemicellulose. The corn stover used in this study had glucose and xylose content was far below (50%) than the values reported in most of the studies whereas, arabinose and mannose content was comparable with those studies. The corn stover used in this study had no galactose in contrary to the literature values (0.68–2.9%). The lignin content was higher than the values reported by Torget et al. [29], Yang and Wyman [30], Zhu et al. [31], Kim and Lee [32], Kim et al. [33], and Öhgren et al. [34] and comparable with other studies listed in the Table 1. The lower glucose and higher lignin content offers more resistance for any pretreatment method. Lignin plays a critical role in biomass utilization. Lignin restricts the degradation of structural polysaccharides through enzymatic hydrolysis, thus limiting the bioconversion of grasses into either animal products or fuels and industrial products [35, 36]. In general, the chemical composition of any biomass varies from place to place depending upon the agronomic practices, season, biomass maturity, and varieties.

Effect of Screw Speed on Different Sugar Recovery

Cellulose and hemicellulose, which typically make up two-thirds of cell wall dry matter, are polysaccharides that can be hydrolyzed to sugars and then fermented to ethanol. Process performance, i.e., ethanol yield from biomass, is directly related to cellulose, hemicellulose, and individual sugar concentration in the feedstock. The difference in different sugar content of pretreated samples was due to the influence of screw speed and temperature during extrusion. Ammonia fiber explosion is a process which uses a quick pressure reduction after soaking the biomass with liquid anhydrous ammonia solution [37]. Extrusion and explosion are similar processes. It is well known that when material passes through the extruder barrel, high pressure is developed and when the extruded material comes out of the die, it experiences low pressure, thus exploding. This system does not directly liberate any sugars, but allows cellulose and hemicellulose more amenable to hydrolytic enzymes and reduce to simple sugars.

The main effect of screw speed on glucose, xylose, and combined sugar recovery using different enzymes such as cellulase, multienzyme, and β-glucosidase at different ratios are depicted in Fig. 1. As the screw speed was increased from 25 to 125 rpm, the glucose, xylose, and combined sugar recovery decreased by 25, 4, and 21%, respectively, but further increase in screw speed increased the sugar recovery as evident from Fig. 1a. Karunanithy et al. [38] reported a similar trend for prairie cord grass pretreated in a single screw extruder. Screw speed had a strong influence on sugar recovery from corn stover. As observed from Fig. 1a, the xylose recovery differed only for the screw speed between 100 and 125 rpm. This result showed that the mean residence time was more critical than rate of shear development. A different sugar recovery pattern was noticed (Fig. 1b), when the amount of β-glucosidase was increased by four times while cellulase amount was kept constant. When the screw speed increased from 25 to 125 rpm, the glucose, xylose, and combined sugar recovery were also increased from 48.9, 34.4, and 37.9 to 57.0, 41.5, and 44.9%, respectively (Fig. 1b). A maximum glucose, xylose, and combined sugar recovery of 58.4, 41.0, and 45.7%, respectively, were achieved at a screw speed of 100 rpm. The

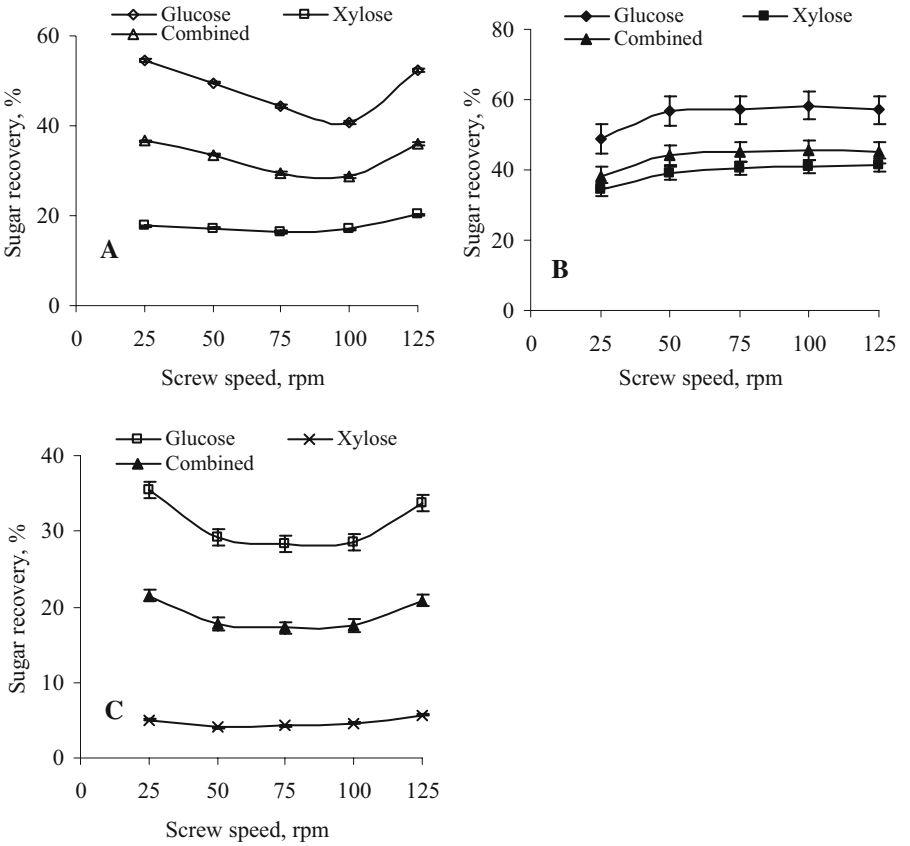

Fig. 1 Effect of screw speed on sugar recovery from corn stover (**a** 1:1 cellulase and β-glucosidase, **b** 1:4 cellulase and β-glucosidase, and **c** 1:1 multienzyme and β-glucosidase)

results suggested that rate of shear development was more important than the mean residence time. However, the increase in sugar recovery was not statistically significant across the screw speed as seen from the error bars except xylose recovery at 25 and 50 rpm.

The sugar recovery decreased by 18.0, 18.8, and 17.7%, respectively, when the screw speed was increased from 25 to 50 rpm while multienzyme and β-glucosidase (1:1) employed during enzymatic hydrolysis (Fig. 1c). The change in glucose and combined sugar recovery was not statistically different between the screw speeds of 50–100 rpm, whereas xylose recovery had significant difference. The sugar recovery trend was similar across the screw speeds when cellulase or multienzyme was used with β-glucosidase at 1:1 ratio. These two enzyme combinations resulted in a maximum sugar recovery at the lowest screw speed studied indicating that the mean residence time was an important factor. Among the enzymes and ratios studied, multienzyme with β-glucosidase gave the lowest sugar recovery and cellulase with β-glucosidase produced comparable results between their ratios. This might be due to fewer amounts of cellulase present in multienzyme than cellulase enzyme. The higher sugar recovery was noted at 1:4 cellulase and β-glucosidase over 1:1 cellulase and β-glucosidase, showed that the insufficient amount of β-glucosidase to breakdown the dimers to monomers. In general, the glucose recovery decreased as the screw speed was increased from 25 to 100 rpm (Fig. 1a and c); one possible reason could

be the behavior of lignin. The rate of shear development at 100 rpm might be sufficient to soften the lignin whereas further increase in screw speed might be responsible for recondensation of lignin. However, it needs to be confirmed through thermo-gravimetric analysis (TGA).

Effect of Extruder Barrel Temperature on Different Sugar Recovery

Figure 2 represents the effect of barrel temperature on sugar recovery while varying the enzymes and their ratios. As inferred from the Fig. 2 that the barrel temperature had a significant influence on sugar recovery from corn stover. In general, an increase of barrel temperature had a negative influence on glucose, xylose, and combined sugar recovery as seen from Fig. 2a. The sugar recovery decreased with an increase in barrel temperature from 25 to 75 °C, further increase to 100 °C slightly increased the sugar recovery, and however, the sugar recovery recorded was less than at 25 °C. The glucose, xylose, and combined sugar recovery decreased by 20.8, 27.1, and 20.5%, respectively, when the barrel temperature was increased from 25 to 125 °C, while cellulase and β-glucosidase was used at 1:1 ratio. The sugar recovery trend was different when β-glucosidase amount was increased by four times while cellulase amount was kept constant. Glucose, xylose, and

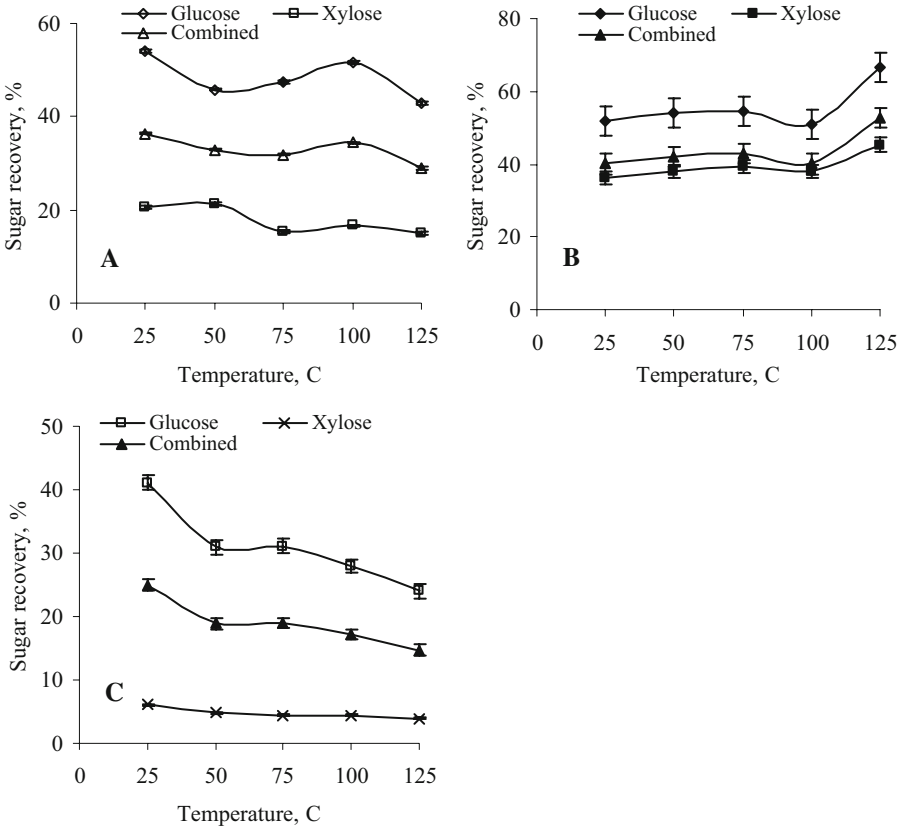

Fig. 2 Effect of barrel temperature on sugar recovery from corn stover (**a** 1:1 cellulase and β-glucosidase, **b** 1:4 cellulase and β-glucosidase, and **c** 1:1 multienzyme and β-glucosidase)

combined sugar recovery exhibited a similar trend with an increase of barrel temperature (Fig. 2b). A similar trend was reported for prairie cord grass, big bluestem, and switchgrass when extruded in a single screw extruder [38, 39]. As the barrel temperature increased, the sugar recovery was also increased; however, the change in sugar recovery was not significant between the barrel temperatures of 25 to 100 °C, further increase of barrel temperature showed a significant difference. One possible reason for the decrease in glucose recovery at 100 °C might be thermal softening of corn stover whereas further increase in barrel temperature might have facilitated the recondensation of lignin, as mentioned before it needs further confirmation through thermo-gravimetric analysis. The glucose, xylose, and combined sugar recovery increased from 51.7, 36.2, and 40.1 to 66.5, 45.3, and 52.8%, respectively, when the barrel temperature was increased from 25 to 125 °C while cellulase to β-glucosidase at 1:4 ratio was employed. As observed from the Fig. 2a and b that cellulase and β-glucosidase (1:4) had higher sugar recovery than 1:1 cellulase to β-glucosidase regardless of the barrel temperatures. This result indicated that β-glucosidase was not enough when 1:1 cellulase to β-glucosidase ratio was used during hydrolysis.

A decreasing sugar recovery trend was observed when multienzyme and β-glucosidase (1:1) was used during enzymatic hydrolysis for the barrel temperature increase (Fig. 2c). However, the decrease in glucose and combined sugar recovery were not significant between the barrel temperatures of 50 to 100 °C. A drastic decrease in glucose and combined sugar recovery was observed between the temperatures of 25 and 50 °C when compared to other barrel temperatures. The glucose, xylose, and combined sugar recovery decreased by 41.7, 36.7, and 41.0%, respectively, when the barrel temperature was increased from 25 to 125 °C. Multienzyme and β-glucosidase combination resulted in lower sugar recovery than cellulase and β-glucosidase combination (1:1). The sugar recovery pattern was more or less similar between multienzyme with β-glucosidase and cellulase with β-glucosidase as evident from Fig. 2b and c. The average glucose, xylose, and combined sugar recovery increase was 1.5, 3.6, and 1.7 times higher for cellulase with β-glucosidase compared to multienzyme with β-glucosidase with 1:1 ratio. Based on the results, higher barrel temperature is required to obtain higher sugar recovery from corn stover.

Interaction and Treatment Effect on Sugar Recovery from Corn Stover

The *p* values for interaction analysis of screw speed and barrel temperature for different enzymes and ratios are given in Table 2. In general, extruder parameters such as screw speed, barrel temperature, and their interactions affected the sugar recovery from corn stover. The interaction effect was significant when cellulase or multienzyme was used with β-glucosidase at 1:1 ratio, whereas, interaction was not significant for the hydrolysis with 1:4 cellulase and β-glucosidase ratio. Statistical analyses across the treatment combinations are presented in Table 3 for different enzymes and ratios studied. The extruder barrel temperature and screw speed had a significant influence on sugar recovery regardless of the enzyme combinations and their ratios. In general, the sugar recovery decreased irrespective of the barrel temperatures when cellulase or multienzyme was used with β-glucosidase at a ratio of 1:1. The maximum sugar recovery was recorded at different treatment combinations depending on the enzyme combinations and ratios. However, the maximum glucose and combined sugar recovery was recorded at a screw speed of 25 rpm and barrel temperature of 25 °C. This indicated that the mean residence time was more important than the rate of shear development and the friction developed at lower temperature might be sufficient to

Table 2 Interaction effects (p value) of screw speed and barrel temperature on sugar recovery.

Source	1 Cellulase: 1 β-glucosidase			1 Cellulase : 4 β-glucosidase			1 Multienzyme: 1 β-glucosidase		
	Glucose	Xylose	Combined	Glucose	Xylose	Combined	Glucose	Xylose	Combined
SS	<0.0001	<0.0001	<0.0001	0.4934	0.0665	0.3158	<0.0001	<0.0001	0.0015
Temp (*T*)	<0.0001	<0.0001	<0.0001	0.0757	0.0167	0.0239	<0.0001	<0.0001	<0.0001
SS×*T*	<0.0001	<0.0001	<0.0001	0.9112	0.7808	0.8454	<0.0001	<0.0001	<0.0001

disturb the cell wall structure of corn stover. The highest glucose, xylose, and combined sugar recovery of 74.6, 49.2, and 60.9%, respectively, were recorded at a screw speed of 75 rpm and barrel temperature of 125 °C when cellulase and β-glucosidase was used at a ratio of 1:4. The highest glucose, xylose, and combined sugar recovery was 1.96, 1.70, and 2.0 times higher than the control sample.

Present results were higher than the results of Muthukumarappan and Julson [40] for Indian grass (31.7%) and big bluestem (35.5%) extruded in a twin screw extruder with 20% moisture content and screw speed of 400 rpm. The difference might be due to the type of extruder, nature of biomass, and enzymatic hydrolysis conditions including enzyme loading. The sugar recovery from 1:4 cellulase and β-glucosidase combination was in agreement with Dale et al. [41] for corn stover extruded at 40–50 °C in a twin screw extruder after 24 h of enzymatic hydrolysis. In this study, the maximum combined sugar concentration recorded as 17.39 g/L whereas, de Vrije et al. [42] reported monomeric sugar as 23.6 g/L for miscanthus. The difference might be due to type of screw extruder, conditions employed, NaOH pretreatment after extrusion, and inherent nature of biomasses.

A wide range of enzyme dosage and hydrolysis conditions have been reported for corn stover depending on pretreatment used are presented in Table 4. Considering the cellulase enzyme dose, the highest glucose recovery obtained in this study was comparable with hot water [43], ammonia recycle percolation-ARP [32], and AFEX [44]; slightly less than steam [34]; and less than dilute acid [45], lime [46], hot water [47], pH controlled liquid hot water [48], compressed hot water [49], hot water-ARP [47], and ammonia soaking [32], pretreatment at room temperature. The maximum xylose recovery recorded in the present study was in agreement with lime [46] and hot water [43] while it was less than the values reported in pretreatments such as pH controlled hot water [50], compressed hot water [49], steam [34], low liquid ammonia recycle percolation [33], aqueous ammonia soaking at room temperature [32], and AFEX [44]. The combined sugar recovery was comparable with acid [51], lime [52], and AFEX [44]. The difference in sugar recovery might be due to the mechanisms involved in the pretreatment methods, variation in enzymes, their dose and activity, hydrolysis conditions, the corn stover composition, solubilization of hemicellulose, and delignification (45–81%).

Byproduct Formation

Glycerol and acetic acid were the byproducts found in a few pretreated corn stover samples. The concentration of glycerol was in the range of 0.04–0.07 g/L recorded at a barrel temperature of 100 °C with screw speeds of 25 and 50 rpm. The maximum concentration of acetic acid was 0.02 g/L obtained at a screw speed of 50 rpm and barrel temperature of 100 °C. It has been reported that furfural and HMF in the range of 1.23–3.99 and 0.2 g/L, respectively, from Japanese beech, oil palm empty fruit bunch fiber, eucalyptus, and

Table 3 Effect of screw speed and temperature on sugar recovery from corn stover.

Screw speed, rpm	Temperature, °C														
	25	50	75	100	125	25	50	75	100	125	25	50	75	100	125
	1 Cellulase: 1 β-glucosidase					1 Cellulase : 4 β-glucosidase					1 Multienzyme: 1 β-glucosidase				
Glucose															
25	64.9^{a}	59.3^{c}	50.0^{gf}	49.6^{g}	48.9^{g}	45.1^{ab}	60.3^{ab}	52.2^{ab}	39.5^{b}	47.5^{ab}	53.3^{a}	42.8^{bc}	23.3^{f}	37.7^{cd}	20.2^{f}
50	62.2^{b}	54.5^{d}	44.4^{i}	52.0^{ef}	34.8^{k}	53.6^{ab}	47.6^{ab}	61.7^{ab}	48.4^{ab}	72.1^{a}	46.7^{ab}	26.1^{ef}	18.7^{f}	32.6^{de}	21.4^{f}
75	41.0^{j}	31.6^{l}	49.6^{g}	53.6^{de}	46.3^{h}	50.7^{ab}	53.7^{ab}	47.9^{ab}	58.1^{ab}	74.6^{a}	35.7^{cd}	23.6^{f}	37.9^{cd}	23.1^{f}	20.8^{f}
100	43.7^{i}	26.1^{m}	43.3^{i}	50.1^{fg}	40.6^{j}	57.0^{ab}	57.2^{ab}	53.5^{ab}	53.7^{ab}	70.6^{ab}	37.6^{cd}	22.8^{f}	37.0^{cd}	23.2^{f}	21.6^{f}
125	58.5^{c}	57.5^{c}	49.3^{g}	52.9^{de}	43.2^{i}	52.2^{ab}	52.7^{ab}	58.0^{ab}	54.7^{ab}	67.5^{ab}	32.2^{de}	39.1^{cd}	38.5^{cd}	22.9^{f}	35.8^{cd}
Xylose															
25	20.4^{cd}	22.4^{b}	15.2$^{i–l}$	14.9$^{i–l}$	15.5$^{h–k}$	27.3^{d}	40.6$^{a–d}$	37.2$^{a–d}$	30.6^{cd}	36.2$^{a–d}$	6.6^{a}	6.6^{a}	3.3^{g}	5.1$_{cd}$	3.2^{gh}
50	22.6^{b}	19.7^{de}	13.4^{l}	16.7$^{g–i}$	13.6$^{j–l}$	36.2$^{a–d}$	34.8$^{b–d}$	41.0$^{a–d}$	36.1$^{a–d}$	47.2^{ab}	6.1^{ab}	4.0$^{e–g}$	2.4^{h}	4.4$^{d–f}$	3.3^{g}
75	15.9$^{h–j}$	21.9^{bc}	14.9$^{i–l}$	15.3$^{i–l}$	13.9^{kl}	37.$^{9a–d}$	37.4$^{a–d}$	36.6$^{a–d}$	42.0$^{a–c}$	49.2^{a}	6.1^{ab}	3.7^{fg}	5.0^{cd}	3.8$^{e–g}$	2.4^{h}
100	18.6^{ef}	20.8$^{b–d}$	13.8^{kl}	17.3$^{f–h}$	14.3$^{j–l}$	40.1$^{a–d}$	38.9$^{a–d}$	39.2$^{a–d}$	38.6$^{a–d}$	48.2^{ab}	6.3^{a}	3.9$^{e–g}$	5.0^{cd}	3.8$^{e–g}$	4.0$^{e–g}$
125	24.8^{a}	21.2$^{b–d}$	19.4^{de}	18.5$^{e–\ g}$	17.2$^{f–h}$	39.7$^{a–d}$	37.5$^{a–d}$	42.4$^{a–c}$	42.2$^{a–c}$	46.0^{ab}	5.3$^{b–d}$	5.8$^{a–c}$	6.3^{a}	4.6^{de}	6.3^{a}
Combined sugar															
25	43.2^{a}	40.5cd	33.6$^{f–h}$	32.8$^{g–i}$	32.5^{hi}	33.5^{c}	46.2$^{a–c}$	40.7$^{a–c}$	31.6^{c}	37.8^{bc}	31.5^{a}	26.1$^{a–c}$	14.4^{de}	22.8^{bc}	12.5^{e}
50	42.5^{ab}	37.2e	29.4^{k}	34.6^{fg}	24.3^{mn}	41.1$^{a–c}$	37.4$^{a–c}$	48.2$^{a–c}$	38.2^{bc}	55.8^{ab}	28.1^{ab}	15.9^{de}	11.4^{e}	19.8^{cd}	13.1^{e}
75	23.5^{no}	25.4^{lm}	32.9^{g}-i	34.9^{f}	30.5^{jk}	40.0$^{a–c}$	41.5$^{a–c}$	38.1^{bc}	45.5$^{a–c}$	60.9^{a}	22.1^{bc}	14.5^{de}	22.6^{bc}	14.4^{de}	12.1^{e}
100	31.3^{ij}	22.1^{o}	29.2^{k}	33.9$^{g–h}$	26.9^{l}	44.2$^{a–c}$	43.1$^{a–c}$	42.0$^{a–c}$	41.9$^{a–c}$	56.4^{ab}	23.1^{bc}	14.2^{de}	22.3^{bc}	14.3^{de}	13.5^{e}
125	41.2^{bc}	39.2^{d}	33.9$^{g–h}$	35.4^{f}	30.4^{jk}	41.5$^{a–c}$	41.0$^{a–c}$	45.5$^{a–c}$	43.7$^{a–c}$	52.9$^{a–c}$	19.9^{cd}	23.7^{bc}	23.7^{bc}	14.5^{de}	22.2^{bc}

Superscripts with same letter within column are not significantly different for each sugar under each enzyme combination

Table 4 Various methods used for pretreatment of corn stover along with enzyme dose and reported results.

Pretreatment	Pretreatment condition	Cellulase FPU	β-glucosidase CBU	Glucose %	Xylose %	Total %	Ref
Acid	1% HCl, autoclaving for 60 min	25[a]	ANR	32.4			[51]
Acid	5% H_2SO_4, autoclaving for 60 min	25[a]	ANR	46.2		>65	[51]
Dilute acid	140 °C, 40 min , 0.98% H_2SO_4	7[b]	14[b]	88			[45]
		15[b]	30[b]	90			
		60[b]	120[b]	93			
Alkali	10% NaOH, autoclaving for 60 min	25[a]	ANR	79.4			[51]
Chemical	1% NaOH(1 day)[b] 1% H_2SO_4, autoclaving for 60 min	25[a]	ANR	95.7		89.7	[51]
Lime	55 °C, 4 weeks with aeration	15[b]	40[b]	91.3	51.8		[46]
Lime	0.075 g $Ca(OH)_2$; 5 g H_2O for 4 h at 120 °C	10[a]	28.4 IU[a]	60	47	53%	[52]
Lime	0.075 g $Ca(OH)_2$; 5 g H_2O for 4 h at 120 °C	25[a]	28.4 IU[a]	88.0	87.7		[52]
Wet oxidation	195 °C, 15 min, 12 bar O_2, 2 g/L of Na_2CO_3 pH 9.3	5[a]	ANR	71			[22]
		25[a]		85			
Hot water	220 °C, 30 min, 5.0 mL/min, 2.5 MPa	15[b]	30[b]	93.3			[47]
		60[b]	120[b]	95.0			
Hot water	190 °C, 15 min, at pH between 4.3 and 6.2	15[b]	65 IU[b]	64–70	53–59[c]		[43]
pH controlled liquid hot water	160 °C and pH above 4.0	7[b]0	ANR	58			[50]
pH controlled liquid hot water	190 °C, 15 min	7.5[b]	15[b]	75	75		[48]
		15[b]	30[b]	90	80		
		60[b]	120[b]	93	80		
Compressed hot water	200 °C, 24 min, a flow rate of 10 mL/min	15[b]	30[b]	95	96		[49]
Steam	190 °C, 5 min, 3% SO_2 for American	15[b]	37.5[d]	83.2	70.5	78.5	[34]

Table 4 (continued)

Pretreatment	Pretreatment condition	Cellulase FPU	β-glucosidase CBU	Glucose %	Xylose %	Total %	Ref
	corn stover						
Steam	190 °C, 5 min, 3% SO_2 for Italian corn	15[b]	37.5[d]	87.9	63.3	78.6	[34]
Steam	190 °C, 5 min with 2% H_2SO_4	25[b]	27 IU[a]	81		73	[62]
Hot water-Ammonia recycle percolation-	190 °C, 30 min of water treatment and 170 °C, 60 min of ARP at 2.3 MPa, 5 ml/min of flow rate	15[b]	30[b]	93.6			[35]
		60[b]	120[b]	84.8			
Ammonia recycle percolation	15 wt.% of ammonia, 170 °C, 90 min, 5 mL/min of flow rate, 2.3 MPa	15[b]	30[b]	71.7			[47]
		60[b]	120[b]	93.4			
Low liquid ammonia recycle percolation	170 °C for 10 min, 3.3 mL of 15 wt.% NH_3/g of corn stover	7.5[b]	15[b]	86	71		[33]
		15[b]	30[b]	90	78		
		60[b]	120[b]	95	86		
Aqueous ammonia soaking	At room temperature for 10 days	15[b]	30[b]	86	72		[32]
		60[b]	120[b]	92	84		
Ammonia fiber explosion	60% moisture, 1 corn stover:1 NH_3, 90 °C, 5 min	7.5[b]	20[b]	75	50		[44]
		15[b]	40[b]	81	55		
		60[b]	16[b]0	90	70		
Ammonia recycle process	15 wt.% of ammonia, 170 °C, 90 min, 5 mL/min of flow rate, 2.3 MPa	10[b]	37 IU[b]			84–93	[59]
		60[b]	37 IU[b]			92–99	

FPU filter paper unit *CBU* cellobiase unit, *IU* international unit, *ANR* added but not reported

[a] Per gram of dry matter

[b] Per gram of cellulose

[c] xylose + galactose

[d] % volume of cellulase

sugarcane bagasse when compressed hot water and acid hydrolysis pretreatment employed [53–56]. The formation of furfural and HMF might be due to acid coupled with higher temperature and prolonged pretreatment time. Interestingly, no furfural and HMF were found in any of the pretreatment conditions studied, which were in agreement with other extrusion pretreatments performed on different biomasses [41, 42, 57]. It could be noted that no byproducts were found at a screw speed of 75 rpm and barrel temperature of 100 °C, which happened to be the treatment combination produced the highest sugar recovery from corn stover.

Conclusions

This experiment was conducted to understand the influence of screw speed and barrel temperature on sugar recovery from corn stover. When different enzymes and ratios were employed during hydrolysis, it was confirmed that screw speed, temperature, and their combinations significantly influenced the sugar recovery. Based on the highest glucose (75%), xylose (49%), and combined sugar recovery (61%), corn stover could be pretreated at a screw speed of 75 rpm with barrel temperature of 125 °C. This pretreatment condition yielded the highest glucose and xylose recovery with 1:4 cellulase and β-glucosidase applied during hydrolysis, which was 1.96 and 2.0 times higher than the control sample.

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