Optimizing extrusion pretreatment and big bluestem parameters for enzymatic hydrolysis to produce biofuel using response surface methodology

C Karunanithy, K Muthukumarappan

(*Agricultural & Biosystems Engineering Department, South Dakota State University, Brookings, SD* 57007, *USA*)

Abstract: Biomass has been identified as alternative renewable energy resource to replace 30% transportation fossil fuel through biofuels by 2.025. Big bluestem is a warm season native perennial grass warrants attention and studies revealed its potential as energy feedstock. Extrusion pretreatments employed on big bluestem showed a significant improvement on sugar recovery. The current study was undertaken to understand and optimize pretreatment conditions such as barrel temperature (45-225℃), screw speed (20-200 r/min), moisture content (10%-50%wb), and particle size (2-10 mm) for maximum sugar recovery from big bluestem; and to propose a model to predict the glucose, xylose, and combined sugar recovery. Statistical analyses confirmed that all the independent variables included in this study had a strong influence on sugar recovery. A quadratic polynomial model was proposed to predict the glucose, xylose, and combined sugar recoveries from big bluestem, which had high F and R^2 values with low p values. The optimum pretreatment conditions such as barrel temperature 180℃, screw speed 150 r/min, moisture content 20% wb, and particle size 8 mm resulted in maximum glucose, xylose, and combined sugar recoveries of 71.3%, 78.5%, and 56.9%, respectively. Surface area of the optimum pretreated big bluestem increased 68.5% than that of control sample, which is the main cause for increase in sugar recovery.

Keywords: biofuel, extrusion pretreatment, big bluestem, enzymatic hydrolysis, screw speed, barrel temperature, particle size, moisture content, sugar yield

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1 Introduction

Due to ever-increasing demand and escalating cost of fossil fuels coupled with global warming, search for alternative energy resources is in place. Biomass looks attractive due to renewable nature and abundance in supply; moreover, biomass can be grown in many parts of the world with minimum inputs and cheaper costs compared to corn and sugarcane. In general, biomass is composed of cellulose, hemicellulose, and lignin, which are in extremely complex structure and recalcitrant in nature. Mosier et al[1] reported that the enzymatic hydrolysis of untreated native biomass results in less than 20% glucose yield due to the recalcitrant characteristics of the complex substrate. In order to improve the overall economy of the conversion process through higher yield in short time, the substrate accessibility has to be improved through pretreatment.

Among warm season native perennial grasses, switchgrass has received the most attention as biomass energy crop due to yield potential and ability to grow on marginal land with minimum inputs; but several other

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Biographies: **K. Muthukumarappan,** Ph.D, Professor, Agricultural & Biosystems Engineering Department, South Dakota State University, Brookings, SD 57007.

Corresponding author: C. Karunanithy, Post Doctoral Research Associate, Agricultural & Biosystems Engineering Department; Email: Chinnadurai.karunani@sdstate.edu; Ph 605-688-5670; Fax 605-688-6764

species also warrant attention. Big bluestem (*Andropogon gerardii*) is one among them, which has a great potential for biofuels and industrial chemical production[2,3]. Similar to switchgrass, big bluestem is grown from central Mexico to Canada[4]. Big bluestem has higher nitrogen use efficiency, which is an important factor due to ever-increasing fertilizer cost. Big bluestem produces twice the biomass per applied nitrogen more than either switchgrass or Indiangrass[3,5]. Although switchgrass establishes faster, big bluestem has more biomass productivity than switchgrass by the second year. Cost of biomass production is an important factor when determining the feasibility of biofuel crop. The fermentation study utilizing consolidated bio-processing indicated that big bluestem was a superior feedstock than that of sand bluestem and eastern gamagrass[3]. There may be an accelerated development for big bluestem in the biofuel arena in near future. Moreover, big bluestem was listed as one of the herbaceous energy crops on the US Department of Energy website[6]. Based on the above facts and figures, big bluestem was selected for this study.

Several pretreatment methods based on physical, chemical, and biological principles are under investigation. Till date there is no perfect biomass pretreatment method available to produce biofuels from biomass on large scale[7,8]. A viable continuous pretreatment method might be found through extrusion. A few advantages of an extruder to be listed are: moderate temperature, short residence time, high shear, rapid heat transfer, addition or removal of any material during extrusion- all in a continuous process; no solid loss, no formation of potential fermentation inhibitors, no further conditioning are required as in acid or alkali pretreatment. Studies have shown that the extrusion could improve biomass digestibility depending upon the extrusion conditions and addition of chemicals[9-12].

Apart from extruder parameters such as screw compression ratio, barrel temperature, screw speed; biomass moisture content and particle size are also important parameters in biomass conversion. In order to get a measurable sugar recovery from untreated biomass, the size should be approximately 1-2 mm[13]. The particle size requirement depends upon the pretreatment methods. According to a US patent 5,677,154[14], the ethanol production process needs a particle size ranging from 1 to 6 mm of ground biomass. The particle size not only affects the diffusion kinetics, the effectiveness of pretreatment, and the power requirement for size reduction[15-18] but also the hydrolysis rates and rheological properties[19]. Hence, it was decided to investigate the influence of particle size on sugar recovery from big bluestem.

Optimization of pretreatment conditions is one of the most important stages in the development of an efficient and economic pretreatment method. Response Surface Methodology (RSM) is an effective optimization tool and is widely used in various fields. Recently it has been successfully applied to biomass pretreatment by many researchers[20-24]. Earlier extrusion pretreatment studies[25,26] conducted by the authors yielded encouraging results and however, the extrusion factors were not optimized. Therefore, the current study was undertaken with the following objectives: 1) to understand and optimize the influence of extruder parameters such as barrel temperature and screw speed and biomass parameters such as moisture content and particle size on sugar recovery from big bluestem using response surface methodology adopting central composite rotatable design (CCRD); and 2) to propose a mathematical model to predict glucose, xylose, and combined sugar recovery from big bluestem.

2 Materials and methods

2.1 Experimental design

A CCRD with four variables was used to study the response pattern and to determine the optimum combination of barrel temperature, screw speed, moisture content, and particle size for maximizing the sugar recovery from big bluestem. The experimental design was developed using Design Expert 7.1.6[27], which resulted in 30 runs, in addition 6 more center points were added to allow for the estimation of the pure error sum of squares. The 36 experiments (16 factorial, 8 star, and 12 center points) were randomized to maximize the effects of unexplained variability in the observed responses due to extraneous factors. Independent variable levels were selected based on one-factor-at-a-time experiments and previous studies[25,26]. The independent variables were coded according to the following equation:

$$x_i = (X_i - X_0) / \Delta X_i \quad (1)$$

Where, x_i and X_i are the dimensionless and actual values of the independent variable i, respectively. X_0 is the actual value of the independent variable at the center point, and ΔX_i is the step change of X_i corresponding to a unit variation of the dimensionless value. The optimized variables included: barrel temperature (45 to 225℃), screw speed (20 to 200 r/min), moisture content (10% to 50%), and particle size (2 to 10 mm) each at five levels: -2, -1, 0, 1, and 2, as shown in Table 1.

Table 1 Experimental design showing both coded and actual values of variables, observed and predicted responses

Treat	Temp	Speed	MC	PS	Glucose/%			Xylose/%			Combined sugar/%		
					Obsd	Pred	Resl	Obsd	Pred	Resl	Obsd	Pred	Resl
1	1(180)	-1(65)	-1(20)	-1(4)	72.0	72.6	-0.6	88.4	87.3	1.1	58.8	58.8	0.0
2	1(180)	-1(65)	1(40)	1(8)	54.2	53.4	0.8	61.5	62.8	-1.3	45.1	44.8	0.3
3	1(180)	-1(65)	1(40)	-1(4)	57.6	55.2	2.4	78.3	76.5	1.8	47.9	46.0	1.9
4	-1(90)	1(155)	-1(20)	-1(4)	55.7	54.1	1.6	76.4	75.1	1.3	47.6	46.9	0.8
5	-1(90)	1(155)	-1(20)	1(8)	51.9	53.9	-2.0	74.5	76.4	-1.9	44.0	45.3	-1.3
6	0(135)	-2(20)	0(30)	0(6)	44.6	44.6	-0.1	54.5	54.7	-0.2	37.6	37.7	-0.1
7	0(135)	0(110)	0(30)	0(6)	48.0	44.9	3.1	72.2	72.5	-0.3	42.3	40.5	1.8
8	0(135)	0(110)	0(30)	0(6)	43.0	44.9	-1.9	73.3	72.5	0.8	38.7	40.5	-1.8
9	-1(90)	-1(65)	-1(20)	1(8)	46.1	43.0	3.1	66.0	65.9	0.1	38.4	36.8	1.6
10	0(135)	0(110)	0(30)	0(6)	46.6	44.9	1.7	73.0	72.5	0.5	41.1	40.5	0.6
11	0(135)	0(110)	0(30)	0(6)	43.6	44.9	-1.3	73.7	72.5	1.2	38.8	40.5	-1.7
12	1(180)	1(155)	1(40)	-1(4)	47.7	48.4	-0.7	71.9	72.0	-0.1	41.0	41.5	-0.5
13	0(135)	0(110)	0(30)	0(6)	45.5	44.9	0.6	74.4	72.5	1.9	40.2	40.5	-0.3
14	-1(90)	-1(65)	1(40)	-1(4)	50.4	51.3	-0.9	53.5	54.5	-1.0	41.7	42.4	-0.7
15	1(180)	-1(65)	-1(20)	1(8)	64.2	66.2	-2.0	74.6	73.0	1.6	52.9	54.1	-1.2
16	0(135)	0(110)	0(30)	0(6)	44.0	44.9	-0.9	70.5	72.5	-2.0	38.5	40.5	-2.0
17	0(135)	0(110)	-2(10)	0(6)	65.9	68.2	-2.3	87.7	90.2	-2.5	54.1	55.7	-1.6
18	-1(90)	1(155)	1(40)	-1(4)	46.0	43.6	2.4	46.9	48.5	-1.6	40.7	38.9	1.8
19	0 (135)	0(110)	0(30)	-2(2)	46.7	49.4	-2.7	74.0	75.4	-1.4	41.0	43.3	-2.3
20	1(180)	1(155)	1(40)	1(8)	54.2	55.5	-1.3	71.8	71.7	0.1	45.8	46.4	-0.6
21	0(135)	0(110)	0(30)	0(6)	42.3	44.9	-2.6	70.4	72.5	-2.1	39.3	40.5	-1.2
22	0(135)	2(200)	0(30)	0(6)	45.9	48.7	-2.8	61.0	60.7	0.3	40.1	41.7	-1.6
23	-1(90)	-1(65)	-1(20)	-1(4)	53.9	52.1	1.8	77.9	78.0	-0.1	45.6	44.4	1.2
24	0(135)	0(110)	2(50)	0(6)	44.1	44.8	-0.7	55.8	53.3	2.5	38.3	38.4	-0.1
25	0(135)	0(110)	0(30)	0(6)	46.1	44.9	1.2	73.5	72.5	1.0	40.4	40.5	-0.1
26	0(135)	0(110)	0(30)	0(6)	45.8	44.9	0.9	71.3	72.5	-1.2	40.2	40.5	-0.3
27	-2(45)	0(110)	0(30)	0(6)	56.5	58.8	-2.3	65.7	62.6	3.1	47.5	48.2	-0.7
28	0(135)	0(110)	0(30)	0(6)	45.5	44.9	0.6	76.0	72.5	3.5	40.2	40.5	-0.3
29	-1(90)	-1(65)	1(40)	1(8)	45.2	46.8	-1.6	41.2	43.0	-1.8	36.8	38.2	-1.4
30	0(135)	0(110)	0(30)	0(6)	48.0	44.9	3.1	71.6	72.5	-0.9	42.3	40.5	1.8
31	1(180)	1(155)	-1(20)	-1(4)	77.6	75.5	2.1	87.8	86.0	1.8	62.1	60.2	1.9
32	0(135)	0(110)	0(30)	2(10)	47.1	47.3	-0.2	64.5	62.9	1.6	41.1	40.5	0.6
33	1(180)	1(155)	-1(20)	1(8)	81.4	78.1	3.3	86.0	85.0	1.0	63.4	61.6	1.8
34	2(225)	0(110)	0(30)	0(6)	86.3	86.9	-0.6	90.2	93.2	-3.0	67.3	68.2	-0.9
35	-1(90)	1(155)	1(40)	1(8)	50.9	47.9	3.0	49.3	50.4	-1.1	41.9	40.8	1.1
36	0(135)	0(110)	0(30)	0(6)	41.1	44.9	-3.8	70.6	72.5	-1.9	38.4	40.5	-2.1

2.2 Biomass–Big Bluestem–preparation and characterization

Big bluestem harvested in fall 2008 was obtained from a local farm in the form of round bale. The bale was cut opened, the whole big bluestem was fed into a hammer mill (Speedy King, Winona Attrition Mill Co, MN) and was ground using a 2-10 mm sieves to understand the influence of particle size on sugar recovery. The ground samples were stored in an air tight container until used. Compositional analysis of big bluestem such as cellulose, hemicellulose, lignin, and ash was carried out as outlined by Sluiter et al[28,29]. Moisture content of the biomass samples was determined as described by Sluiter et al[30]. The moisture content of ground biomass was adjusted to 10%-50% (w.b) by adding water and equilibrated overnight to determine the effect of moisture content on sugar recovery. Particle size analysis of the raw and optimum pretreated big bluestem samples were carried out using Retsch Camsizer and LS 13 320 particle size analyzer, respectively. The surface area of control and pretreated samples was measured using SA 3,100 surface area and pore size analyzer (Beckman Coulter, CA, USA).

2.3 Extrusion pretreatment

Extrusion was performed using a single screw extruder (Brabender Plasti-corder Extruder Model PL 2000, Hackensack, NJ), which had a 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. A screw with 3:1 compression ratio was selected based on previous study[26]. The single screw extruder was fitted to a 5.6 kW motor, which had a provision to adjust the screw speed from 0 to 210 r/min. The extruder barrel had provisions to control the temperature of the feed and transition zone in both barrel and die sections. The extruder barrel temperature and the screw speed were controlled by a computer connected to the extruder. Extruder feeding was done manually. Compressed air was supplied as a cooling agent along the barrel length. About 500 g of big bluestem was extruded under each pretreatment condition, divided into two batches accounting for variations due to extruder operation, and considered replicates. The mean residence time varied between 30 and 90 s depending upon the screw speed.

2.4 Enzymatic hydrolysis

Enzymatic hydrolysis of pretreated samples (0.3 g dry matter in 10 mL hydrolysis volume) was carried out using 0.1 M, pH 4.8 sodium citrate buffer for 72 h at 50℃ and 150 r/min as described by Selig et al[31]. Based on a survey of literature and a previous study[25], the amount of cellulase (Celluclast 1.5 L, activity 70 FPU/g) enzyme was decided as 15 FPU/g of dry matter. The ratio of cellulase to β – glucosidase (Novo–188, activity 250 CBU/g) was maintained at 1:4 based sugar recovery obtained in an earlier study[25]. All these enzymes were provided by Novozymes (Krogshoejvej, Denmark). After hydrolysis, the samples were kept in boiling water for 10 min to inactivate the enzyme action. The supernatant was centrifuged with 16,060 g force for 15 min and frozen twice before HPLC injection to remove the impurities otherwise which contribute for the pressure increase in HPLC system. Soluble sugars and byproducts were quantified using HPLC (Agilent Technologies, Santa Clara, CA; Bio-Rad Aminex 87 H 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℃ and a sample volume of 20 μL as mentioned by Sluiter et al[32]. The sugar recoveries were calculated using Equations (2) and (3). Glucose and xylose are the major sugars present in the biomass when compared to arabinose. Instead of reporting arabinose separately, it was added with glucose and xylose; reported as combined sugar. The sugar recovery reported in this paper was after the enzymatic hydrolysis of the pretreated samples.

$$Y_i = \frac{S_{ip}}{S_{ir}} * 100 \tag{2}$$

$$Y_c = \frac{\sum S_{ip}}{\sum S_{ir}} * 100 \tag{3}$$

Where, Y_{i} individual sugar recovery, %; Y_{c} combined sugar recovery, %; S_{ip} individual sugar obtained from the hydrolyzate of pretreated samples through HPLC; S_{ir} individual sugar from raw material

2.5 Statistical analysis

The second order polynomial equation was used to describe the effect of independent variables in terms of linear, quadratic, and interactions. The proposed model for the response (Y_i) was:

$$Y_i = b_0 + \sum_{i=1}^{4} b_i X_i + \sum_{i=1}^{4} b_{ii} X_i^{\ 2} + \sum_{i=1}^{3} \sum_{j=i+1}^{4} b_{ij} X_i X_j + \varepsilon \tag{4}$$

Where, Y_i is the predicted response, b_0 is the interception coefficient, b_i, b_{ii} , and b_{ij} are coefficients of the linear, quadratic, and interaction terms, ε is the random error, and X_i is the independent variables studied. Design Expert 7.1.6 software was used for regression and graphical analysis of the data obtained. Statistical analysis of the model was performed to evaluate the Analysis of Variance (ANOVA).

The quality of fit of second order equation was expressed by the coefficient of determination (R^2) and its statistical significance was determined by F test. The effect of each independent variable and their interactions were determined. A number of parameters that were chosen to be included for each model were determined based on significance (α = 0.05) of each model parameter using the F–test. Optimization (maximizing sugar recoveries) of the fitted polynomial was determined using numerical optimization contained in the Design Expert 7.1.6. After optimizing the conditions using RSM, it was validated by extruding the big bluestem at the identified optimum barrel temperature, screw speed, moisture content, and particle size.

3 Results and discussion

3.1 Characterization of big bluestem

The average cellulose, hemicellulose, lignin, and ash content on dry matter basis (%) were determined as 35.0, 18.2, 21.1, and 11.2, respectively. The literature values of cellulose, hemicellulose, lignin, and ash are 29.0–42.5, 18.1–30.4, 17.1–23.8, and 2.8%–8.0%, respectively[2,33-36]. The cellulose and hemicellulose were in agreement with that of the reported values, whereas the ash content was higher in big bluestem used in this study. However, the ash content was in agreement with the values (2.7%–15.7%) of Weimer and Springer[3] for the big bluestem grown in various locations across the USA.

3.2 Effect of barrel temperature, screw speed, moisture content and particle size on sugar recovery

Glucose, xylose, and combined sugar recovery recorded for all treatment combinations are presented in Table 1. Based on the experimental data, the developed quadratic models in terms of coded variables are given (Table 2) for glucose (Y_G), xylose (Y_X), and combined sugar (Y_C) recovery where X_1, X_2, X_3, and X_4 represent barrel temperature (℃), screw speed (r/min), moisture content (% wb), and particle size (mm) of big bluestem, respectively. Similar equations were reported for extrusion pretreatment of corn stover, switchgrass, prairie cord grass[36-38], and miscanthus[39], for dilute acid pretreatment of Douglas fir[20], for concentrated acid pretreatment of pine wood[21], for dilute acid pretreatment of cardoon[22], and for lime pretreatment of sugarcane bagasse[24]. Those equations predict the responses well with high R^2 and low probability values.

Barrel temperature, screw speed, moisture content, and particle size had a strong influence on the sugar recovery from big bluestem and it was confirmed from the p values of linear terms shown in (Table 2). Among the independent variables, temperature had a prominent effect on glucose recovery than that of other variables as evident from their linear term coefficients Table 2. As observed from Table 2, barrel temperature had a positive influence, whereas moisture content had a negative influence on all the sugar recoveries and the observation was similar to that of corn stover, switchgrass[36,37], and miscanthus[39]. The magnitude of the moisture content linear term was lower than that of barrel temperature for glucose and combined sugar recoveries, whereas it was higher for xylose recovery. Not only the linear terms of temperature and moisture content but also their quadratic terms contributed to glucose recovery and combined sugar recovery as evident from Table 2. Again, the difference in magnitude of the quadratic terms explains which variable was dominant for glucose recovery. In addition to temperature and moisture content, screw speed positively contributed to xylose and combined sugar recovery as noted from Table 2. Moreover, the magnitude of screw speed was less than that of other variables. Particle size had a negative impact on xylose recovery and it was higher than that of screw speed. However, quadratic terms of screw speed and particle size were negatively contributed to xylose recovery as seen from Table 2.

Table 2 Coefficient values of the fitted model for different responses

Factor	Glucose				Xylose				Combined sugar			
	Coefft	Std error	F value	P value	Coefft	Std error	F value	P value	Coefft	Std error	F value	P value
Intercept	44.95	0.397			72.53	0.372			40.03	0.387		
Temp(X_1)	7.010	0.536	170.73	< 0.0001	7.648	0.434	310.00	< 0.0001	4.995	0.339	216.17	< 0.0001
SS (X_2)	1.015	0.536	3.58	0.0723	1.505	0.434	12.01	0.0023	1.015	0.339	8.93	0.0070
MC (X_3)	-5.847	0.536	118.81	< 0.0001	-9.210	0.434	449.51	< 0.0001	-4.318	0.339	161.51	< 0.0001
PS (X_4)	-0.504	0.536	0.88	0.3577	-3.123	0.434	51.69	< 0.0001	-0.691	0.339	4.14	0.0546
Temp/SS	0.245	0.657	0.13	0.7123	0.380	0.532	0.51	0.4829	-0.258	0.416	0.38	0.5408
Temp/MC	-4.150	0.657	39.90	< 0.0001	3.163	0.532	35.36	< 0.0001	-2.683	0.416	41.58	< 0.0001
Tem/PS	0.689	0.657	1.10	0.3060	-0.545	0.532	1.04	0.3173	0.742	0.416	3.18	0.0888
SS/MC	-2.431	0.657	13.69	0.0013	-0.771	0.532	2.10	0.1619	-1.477	0.416	12.60	0.0019
SS/PS	2.213	0.657	11.34	0.0029	3.350	0.532	39.64	< 0.0001	1.518	0.416	13.31	0.0015
MC/PS	1.136	0.657	2.99	0.0983	0.157	0.532	0.08	0.7683	0.858	0.416	4.25	0.0516
$Temp^2$	6.979	0.464	225.67	< 0.0001	1.346	0.376	12.81	0.0018	4.537	0.294	237.79	< 0.0001
SS^2	0.431	0.464	0.86	0.3640	-3.709	0.376	97.23	< 0.0001	-0.086	0.294	0.08	0.7729
MC^2	2.882	0.464	38.48	< 0.0001	-0.199	0.376	0.28	0.6013	1.752	0.294	35.47	< 0.0001
PS^2	0.8498	0.464	3.34	0.0816	-0.833	0.376	4.90	0.0379	0.461	0.294	2.45	0.1318

Note: SS- screw speed; MC- moisture content; PS - particle size.

Not only linear and quadratic terms but also interactions terms were contributed to sugar recoveries as evident from Table 2. In order to visualize the interaction effects for glucose recovery, significant interaction response surfaces are shown in Figures 1a-c. As observed from the interactions of moisture with other independent variables, as the moisture content was increased from 20% to 40%, the glucose recovery decreased drastically. However, the moisture content effect was minimized at higher barrel temperature (180℃). This result was comparable to switchgrass, whereas corn stover had a lower sugar recovery than that of big bluestem[36,37]. The sugar recovery increase might be due to insufficient thermal softening at low temperature and utilization of heat for thermal softening at higher temperature and moreover high moisture vaporization. The quadratic terms of temperature and moisture content can be visualized at their low levels (Figure 1a).

The screw speed had a prominent effect than that of moisture content as evident from their interactions depicted in (Figure 1b). The screw speed had insignificant effect on glucose recovery when the moisture content was 40%. Irrespective of screw speeds, moisture content of 20% resulted in high glucose recovery. As noticed from the interaction of screw speed with particle size (Figure 1c) that the screw speed had a strong influence on glucose recovery for particle size of 8 mm as compared to 4 mm. The difference in sugar recovery at screw speed of 65 and 155 r/min for different particle size can be seen from the interaction of screw speed and particle size. The trend might be due to lower lignin content with 4 mm particle and higher mean residence time at 65 r/min. Although the lignin content was high in 8 mm particle size[17], the rate of shear development and utilization was high at 155 r/min. The result was similar to corn stover[36] and lower than that of switchgrass[37]. This was in agreement with the trend reported by Muthukumarappan and Julson[40] for the big bluestem coarse particle extruded in twin screw extruder at 200 and 400 r/min. Thus, the rate of shear development was an important factor than the mean residence time and this observation was in agreement with wheat bran pretreated in a twin screw extruder at a barrel temperature of 150℃[12].

A significant difference on sugar recovery among untreated particle sizes of big bluestem was observed in one-factor-at-a-time (not shown); the extrusion pretreatment erased the differences in sugar recovery among the particle size. Similar results were reported for corn stover pretreated using liquid hot water[13], wheat straw pretreated in wet oxidation[41], and switchgrass pretreated in a single screw extruder[37]. These results show that an effective pretreatment could reduce the need of substrate size reduction, which is the need of the hour thereby energy spent on size reduction could be saved to greater extent. However, Jurisic et al[39] reported a positive effect of particle size on glucose recovery from miscanthus pretreated using a single screw extruder. A glucose recovery of 49%-72% could be obtained depending upon independent variables; it was higher than that of switchgrass[37] and it was lower than that of corn stover[36].

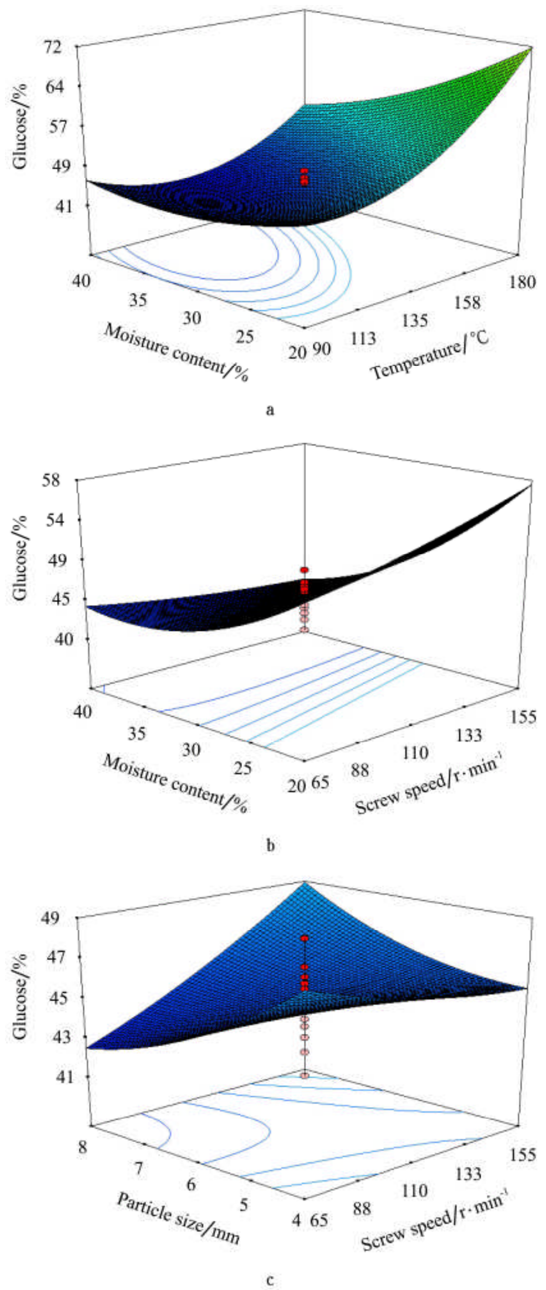


Figure 1 Effect of independent variables interaction on glucose recovery (when other factors fixed at the center point)

The significant interaction effects of different independent variables for xylose recovery response are depicted in Figures 2a-b. As discussed earlier, the rate of shear development and utilization was higher at 155 r/min than did at 65 r/min which attributed to higher xylose recovery. The low rate of shear development and higher lignin content of 8 mm particle size resulted in low xylose recovery when compared to 4 mm size. From the interaction of barrel temperature and moisture content (Figure 2b); it was clear that low moisture and high temperature resulted in better xylose recovery. The result might be due to higher thermal softening at 180℃ than at 90℃ with 20% moisture content. When the moisture content was increased to 40%, most of the barrel temperature might be utilized to vaporize the high moisture of big bluestem instead of thermal softening. The predicted model responses showed that xylose recovery of 76%-88% achievable depending upon barrel temperature, screw speed, moisture content, and particle size. Xylose recovery from big bluestem used in this study was in agreement with corn stover[36], prairie cord grass[38], and higher than that of switchgrass[37].

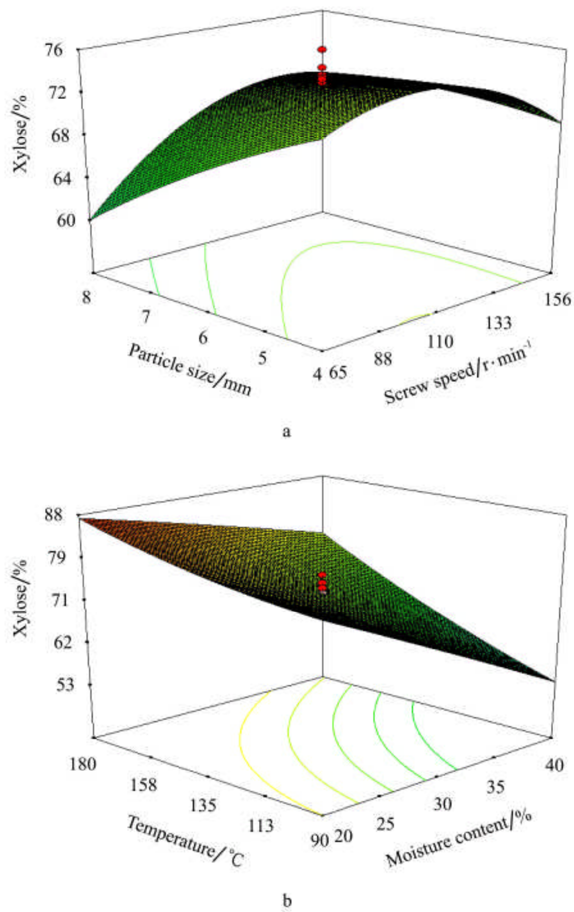


Figure 2 Effect of independent variables interaction on xylose recovery (when other factors fixed at the center point)

Figures 3a-c depict the response surface curves for variation in combined sugar recovery as a function of two independent variables while the other two independent variables were at constant level. The interactions of moisture content with other independent variables were only significant. The possible reasons for difference in combined sugar recovery were similar to the discussion of glucose recovery. Although response surfaces were similar to glucose recovery, the maximum glucose recovery range (49%-72%) was different from combined sugar recovery (49%-59%). The combined sugar recovery prediction was similar to prairie cord grass[38] and lower than that of corn stover and switchgrass pretreated in similar conditions[36, 37].

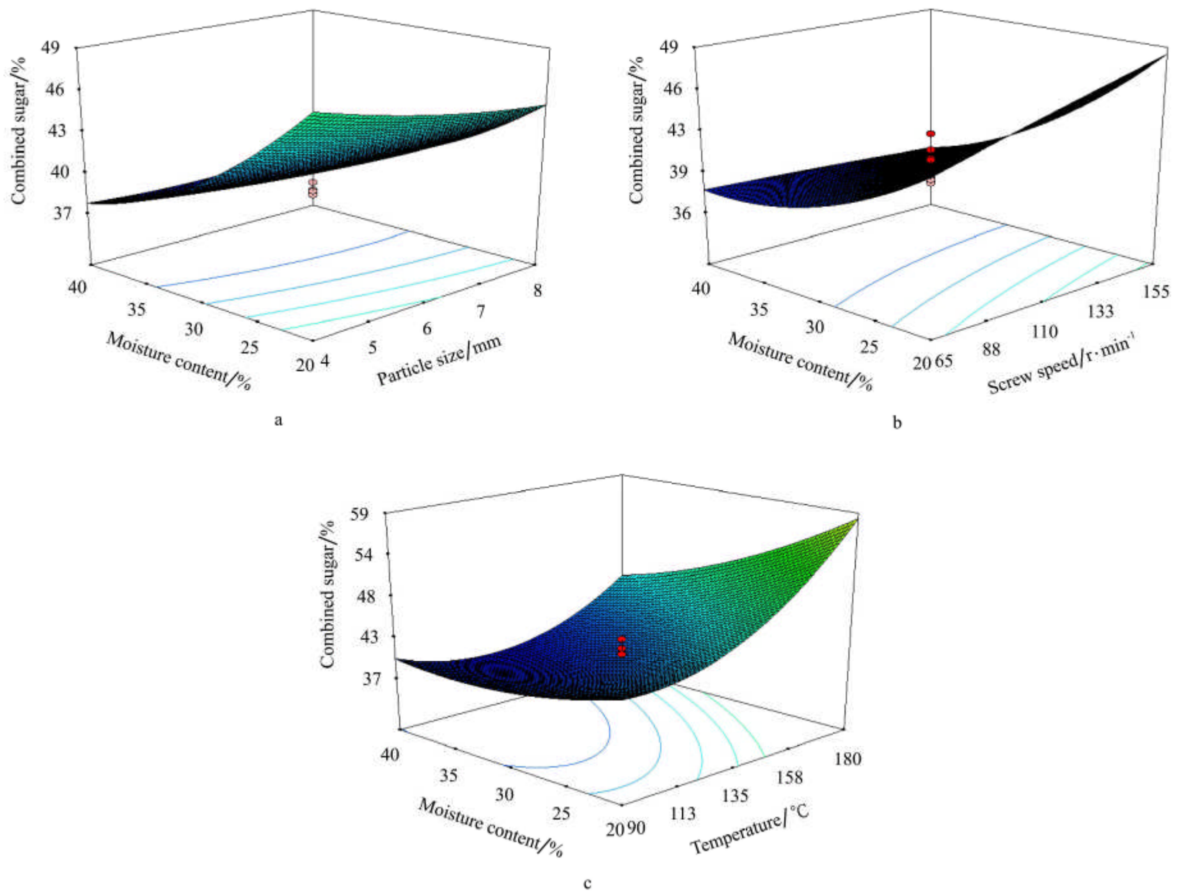


Figure 3 Effect of independent variables interaction on combined sugar recovery (when other factors fixed at the center point)

Shear force development is proportional to screw speed; fiber length reduction might depend on the high shear forces applied to the fiber. As screw speed increased, more (shear force) energy is available for fiber breakages, which change the fiber length and aspect ratio[42]. This process would increase the surface area accessible to hydrolytic enzymes and would result in sugar recovery increase. Another possible reason, an increase in temperature and screw speed would introduce more energy to the material in the barrel, which would enhance the moisture evaporation at the exit[43]; thereby, more disturbance to cell wall structure of the big bluestem. As expected, the moisture content had a negative correlation with sugar recovery. According to Yeh and Jaw[44], friction is the main mode of material conveyance in a single screw extruder. Because water acts as lubricant in the extruder[45], an increase in moisture content resulted in decrease in the friction between the material, screw shaft, and barrel[46] resulted in less disturbance to cell wall of the switchgrass. As a matter of fact, the high moisture content biomass would have low viscosity, which facilitates the flow and reduces the residence time consequently lower the sugar recovery.

3.3 Response surface model evaluation

The experimental design with actual and coded variables, experimental results, and predicted responses by the model equation is given in Table 1. The closeness of predicted and observed values reflects the goodness of fit. The analysis of variance for the data obtained using CCRD are presented in Table 3 along with coefficient of determination (R^2). As observed from the glucose, xylose, and combined sugar F value of the regression (45.10, 72.65, and 52.99) was very high compared to the tabular $F_{14,21}$ value (2.19), which indicated that the model was highly significant. The R^2 is proportion of variability in the response values explained or accounted for by the model[47]. More than 90% of the variation in glucose, xylose, and combined sugar recovery was explained by the proposed quadratic models and was in agreement with R^2 values reported for switchgrass[37] and miscanthus[39]. The higher value of R^2 further suggested that the model was suitable to adequately represent relationship among the selected independent variables. However, a large value of R^2 did not always imply that the regression model was a good one because R^2 would increase with adding a variable regardless of whether the additional variable was statistically significant or not[23,36,37]. Hence, adjusted and predicted R^2 were calculated to check the model adequacy.

Table 3 Analysis of variance of fitted model for different responses

Response	Source	df	Sum of squares	Mean squares	F value	P value	R^2/ Adj R^2 /Pred.R^2	CV(%) / Adeq Precision
Glucose	Regression	14	4,362.92	311.63	45.10	< 0.0001	0.97/ 0.95/ 0.87	5.01/ 25.85
	Lack of fit	10	92.48	9.24	1.93	0.1471		
	Pure error	11	52.58	4.78				
	Residual	21	145.07	6.90				
	Total	35						
Xylose	Regression	14	4,606.82	329.05	72.65	< 0.0001	0.98/ 0.97/ 0.92	3.03/ 36.56
	Lack of fit	10	61.42	6.14	2.00	0.1345		
	Pure error	11	33.68	3.06				
	Residual	21	95.10	4.52				
	Total	35	4,701.93					
Combined	Regression	14	2,055.82	146.84	52.99	< 0.0001	0.97/ 0.95/ 0.89	3.74/ 29.22
	Lack of fit	10	37.44	3.74	1.98	0.1379		
	Pure error	11	20.74	1.88				
	Residual	21	58.18	2.77				
	Total	35	2,114.01					

Table 3 shows that predicted R^2 and adjusted R^2 values for the model did not differ drastically indicating that insignificant terms have not been included in the model. The predicted determination coefficient was in reasonable agreement with the adjusted determination coefficient, which also confirmed the fitness of the model. As evident from Table 3, the regression was significant while the lack of fit was insignificant. The lack of fit measures the failure of the model to represent data in the experimental domain at points which are not included in the regression. Coefficient of variation (CV) is the ratio of standard error estimate to the mean values expressed as percentage and is another measure to evaluate the goodness of the model. As a general rule, the CV should not be greater than 10%[48-50], therefore, the low value of CV (3.0%–5.0%) indicates that the experiments conducted were precise and reliable. "Adeq Precision" measured the signal-to-noise ratio. In general, a ratio greater than 4 was desirable. The ratio of 25.85–36.56 indicated an adequate signal, which implied that the model could be used to navigate the design space[51].

3.4 Optimization and validation of the model

A graphical multi-response optimization technique was applied to determine the optimum combination of temperature, screw speed, moisture content, and particle size of big bluestem for maximum sugar recovery using a single screw extruder. Maximum glucose, xylose, and combined sugar recovery were the desirable responses considered for optimization. The optimum pretreatment condition was determined by superimposing the contour plots of glucose, xylose, and combined sugar recovery responses. As a result of superimposing individual contour plots (Figure 4), the region identified by the shaded area satisfied the maximum sugar recovery from big bluestem.

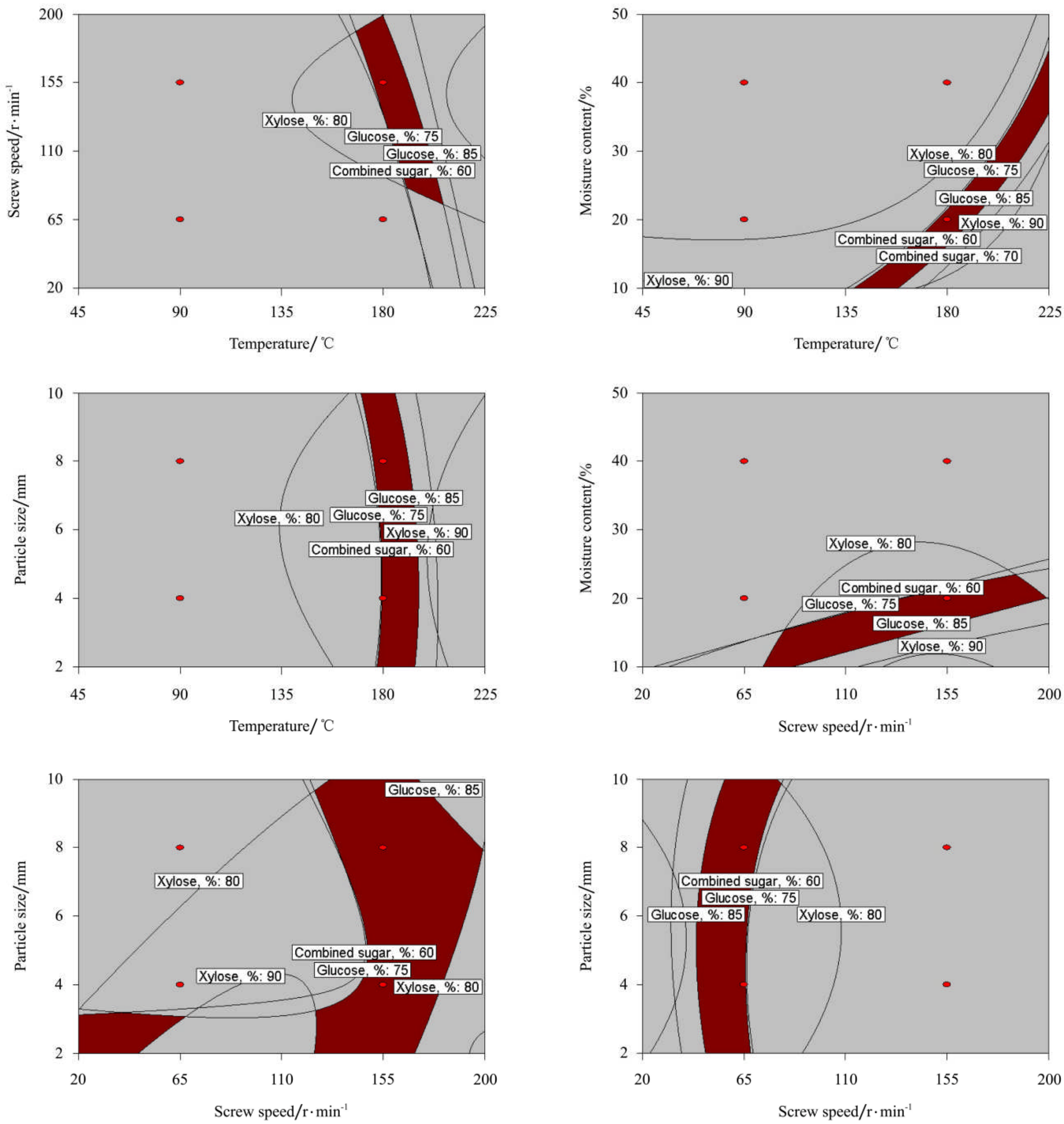
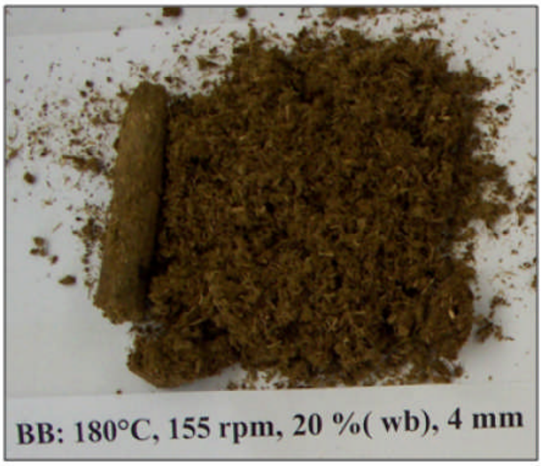


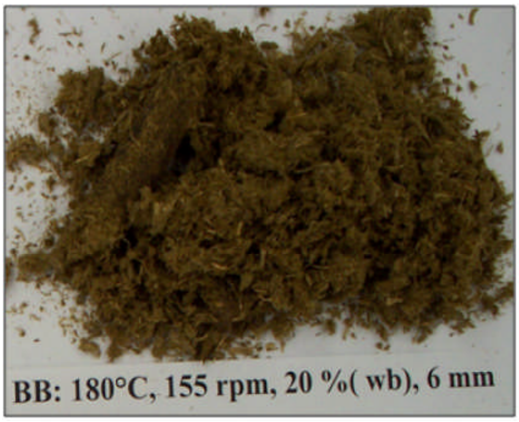
Figure 4 Superimposed contours for sugar recovery responses as affected by temperature, screw speed, moisture content, and particle size

Based on the models, numerical optimization was carried out in Design Expert considering each value of response, 24 solutions were found, and the top ten solutions are shown in Table 4. In order to confirm the predicted results, big bluestem was extruded at three different optimum conditions (solution # 6, 8, and 18) and the samples are shown in Figure 5. The extruded samples were subjected to enzymatic hydrolysis; sugar measurement as explained in the materials and methods. The glucose, xylose, and combined sugar recovery were 71.3%, 78.5%, and 58.9%, respectively, and the result was close to the predicted values; and was 3.5, 2.3, and 2.6 times higher than the control sample. A mass balance diagram is shown in Figure 6 for better understanding (assumption a thumb rule is 50% of the glucose will be converted into ethanol with an efficiency of 90%). A combination of high barrel temperature and screw speed with low moisture content of large particle size changes the fiber length and aspect ratio; hence, increase in surface area for enzymes results in better sugar recovery. In general, more energy is available for fiber breakage as screw speed increased, which changes the fiber length and aspect ratio[42]. Fiber length reduction might not only depend on the high shear forces applied to the fiber but also the utilization of shear, which again depends on barrel temperature, moisture content, and particle size. Particle analysis revealed that about 90% of raw big bluestem passed through 8 mm, whereas 95% of the optimum pretreated corn stover passed through 1.785 mm. The big bluestem (8 mm) had a surface area of 0.469 m^2/g, whereas the optimum pretreated big bluestem had a surface area of 0.790 m^2/g. The optimum extrusion pretreatment conditions increased the surface area about 68.5%. The increase in surface area of big bluestem was higher than that of optimum pretreated switchgrass[37] and lower than that of optimum pretreated corn stover[36] and prairie cord grass[38]. The surface area increase due to extrusion pretreatment of big bluestem was higher than that of switchgrass and lower than that of corn stover pretreated in a single screw extruder[36,37] and that of Douglas fir extruded with ethylene glycol in a twin screw extruder[11]. Other possible mechanisms are increase in pore size[39], reduction in cellulose crystallinity and also deconstruction of hemicellulose chains[12] by which the extrusion aids in increasing sugar recovery.

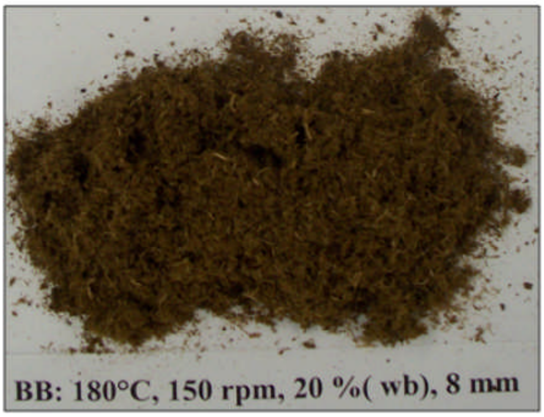
a. Control 8 mm



b. 180℃, 155 r/min, 20%(wb), 4 mm



c. 180℃, 155 r/min, 20%(wb), 6 mm



d. 180℃, 155 r/min, 20%(wb), 8 mm

Figure 5 Big bluestem pretreated at optimum and validated conditions

The optimized sugar recovery was higher than the glucose recovery of 27.2% and 26.8% reported for 20% moisture content big bluestem when extruded in a twin screw extruder at a barrel temperature of 100℃ with 200 and 400 r/min, respectively[40]. The difference might be due to the type of extruder and the pretreatment conditions employed. The present results were higher than the glucose (58%), xylose (66%), and combined sugar (58%) recovery from another study reported for the treatment combination of 150℃ and 200 r/min with 21% moisture content and 4 mm particle size using 3:1 screw compression ratio[25]. The difference in sugar recovery might be due to the extruder and biomass parameters. The maximum sugar recovery from the current study was higher than that of switchgrass[37], miscanthus[39], wheat bran[12], and lower than for corn stover[36]. Titgemeyer et al[33] reported the in vitro degradation of glucose (89%-92%) and xylose (81%-97%) for big bluestem when 1M NaOH was used with N_2 for 24 h at room temperature. The differences in glucose and xylose recovery might be the usage of different alkali concentration, which removed the lignin and the composition of raw big bluestem (variety) used in this study. This optimization study revealed that large particles (8 mm) could be used for biofuels production; thereby the biomass size reduction energy cost can be saved to a greater extent.

Table 4 Solutions for optimal and validated pretreatment conditions

Solution #	Temperature/℃	Screw speed/r · min^{-1}	Moisture content/%	Particle size/mm*	Glucose/%	Xylose/%	Combined sugar/%
1	180	155	20	7.4	77.09	85.66	61.10
2	180	155	20	7.5	77.34	85.46	61.24
3	180	155	20	7.6	77.32	85.39	61.22
4	180	155	20	6.7	76.42	85.99	60.72
5	180	155	20	6.5	76.32	86.13	60.67
6	180	155	20	6	75.89	86.33	60.44
7	180	152	20	7.6	76.75	85.33	60.89
8	180	150	20	8	77.19	85.18	61.17
9	180	148	20	8	76.99	85.14	61.06
10	180	155	20	5.2	75.56	86.37	60.24
Validation							
18	180	155	20	4	72.26	79.56	57.27
6	180	155	20	6	71.89	78.68	57.08
8	180	150	20	8	71.27	78.52	56.88

Note: * particle size refers to sieve size used during grinding.

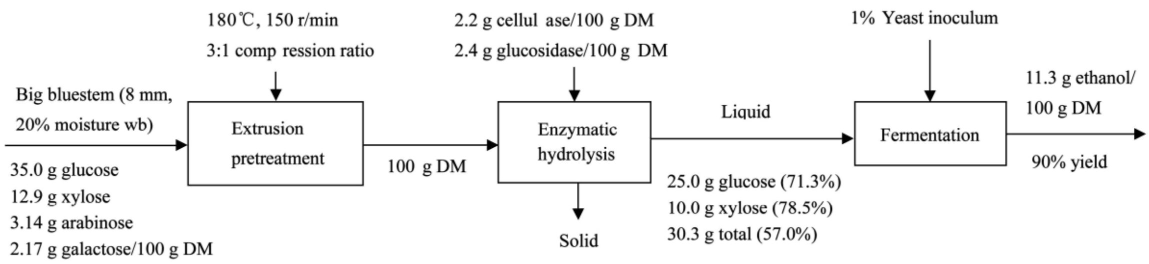


Figure 6 Mass balance diagram extrusion pretreatment followed by fermentation

4 Conclusions

Big bluestem was extruded using a single screw extruder at various conditions based on central composite rotatable design to obtain maximum glucose, xylose, and combined sugar recoveries. Statistical analyses confirmed that the extruder parameters such as barrel temperature and screw speed, feedstock parameters such as big bluestem moisture content and particle size had a significant influence on sugar recoveries. The optimum condition such as barrel temperature of 180℃ and screw speed of 150 r/min, moisture content of 20% wb with 8 mm particle size predicted a glucose, xylose, and combined sugar recoveries of 77.2%, 85.2%, and 61.2%, respectively, and experimentally the recoveries (71.3%, 78.5%, and 56.7%) were confirmed. Moreover, the findings reveal that extrusion can be a feasible pretreatment method and the only concern is cost. In order to improve sugar recovery to near quantitative, a future study will explore combining other methods in the front end of extrusion.

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