OPTIMIZATION OF ALKALI, BIG BLUESTEM PARTICLE SIZE, AND EXTRUDER PARAMETERS FOR MAXIMUM ENZYMATIC SUGAR RECOVERY USING RESPONSE SURFACE METHODOLOGY

Chinnadurai Karunanithy* and Kasiviswanathan Muthukumarappan

Extrusion can be a viable continuous biomass pretreatment that industry can adopt readily due to its uniqueness (including pretreatment time less than 90 s) over other pretreatment methods. The current study was undertaken to evaluate the combined effect of alkali soaking and extrusion of big bluestem to improve the sugar recovery to nearly quantitative. In order to evaluate the combined effect of alkali soaking and extrusion on the performance of enzymatic saccharification, big bluestem (2-10 mm) was soaked in different alkali concentrations (0.5-2.5 % w/v NaOH) for 30 min at room temperature and then extruded using a lab scale single screw extruder at various barrel temperatures (45-225°C) and screw speeds (20-200 rpm). Statistical analyses confirmed that all the independent variables considered had a significant effect on sugar recovery. A proposed quadratic model to predict sugar recovery had high F and R^2 values with a low p value, and adequately represented the relationship among the independent variables on sugar recovery. The optimum pretreatment condition found was the following: 90°C barrel temperature, 155 rpm screw speed, 2.0% alkali concentration, and 4 mm particle size resulted the maximum glucose, xylose, and combined sugar recovery of 90.1, 91.5, and 89.9%, respectively.

Keywords: Biomass; Pretreatment; Enzymatic hydrolysis; Byproduct; Screw speed; Barrel temperature

*Contact information: Agricultural & Biosystems Engineering Department, South Dakota State University, Brookings, SD 57007, *Corresponding author: Chinnadurai.karunani@sdstate.edu*

INTRODUCTION

Long-term economic and environmental concerns have provided incentive for research in renewable sources of liquid fuels to replace fossil fuels. Conversion of lignocellulosic biomass to biofuels is a viable option to improve the energy security and also to reduce greenhouse emissions (Wyman 1999). Wang et al. (2007) reported that cellulosic ethanol has the potential to cut greenhouse gas emissions by 86%. Lignocellulosic materials are the most abundant renewable resources on earth (Lynd et al. 2005). Corn stover and switchgrass are the most researched biomass in the US. These two biomasses alone may not be sufficient to meet the fuel requirement. There are other warm season grasses grown in most part of the USA that have comparable yield and better nitrogen use efficiency (Perry and Baltensperger 1979; Weimer and Springer 2007), and big bluestem is one among them. Moreover, big bluestem is one of the herbaceous energy crops listed in the Department of Energy's website. Studies have shown that big bluestem has a greater potential for biofuel production (Weimer and Springer 2007; Jefferson et al. 2004) as wells as bio-oil production (Tiffany et al. 2006), and it has low nitrogen content, which is a beneficial factor towards biofuel production (Weimer and Springer 2007). In general, more than 90% of the dry weight is composed of cellulose, hemicellulose, lignin, and pectin (Yat et al. 2008). 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 fuel and industrial products (Brown 1985; Jung and Deetz 1993).

According to Gibbons et al. (1986), ethanol production from biomass is quite different from the process used for corn grain, because the carbohydrates in biomass are more difficult for hydrolytic enzymes to access than the starch in grain. Pretreatment of biomass is the first step in the conversion process, and it constitutes an additional step compared to corn ethanol process. The purposes of pretreatment are, to disorganize the crystalline structure of macro and microfibrils, to modify the pores for the enzyme access, to increase the surface area, and to reduce the cellulose crystallinity (Galbe and Zacchi 2002). An effective pretreatment should be an inexpensive process and use simple equipment, avoid loss of carbohydrates, preserve the pentoses, and avoid the formation of inhibitory byproducts (Sun and Cheng 2002). There are several physical, chemical, physiochemical, and biological methods introduced for the pretreatment of lignocellulosic biomass. Physical methods include milling, irradiation (γ, microwave, and electron beam), and pyrolysis; chemical methods include acid, alkali, and organosolv pretreatments. Physiochemical methods are such as steam explosion, AFEX, CO_2, and SO_2 explosion. Biological methods employ microorganisms and enzymes derived from them. It has been reported that these methods have not yet demonstrated either technical or economic feasibility in large scale processing (Taherzadeh and Karimi 2007).

Extrusion has potential to be a viable continuous biomass pretreatment that industry can adopt readily due to its uniqueness over other pretreatment methods. High shear, rapid heat transfer, effective mixing (disturbance to cell wall), adjustable temperature, short residence time, adaptability to process modification, and above all continuous operation are the advantages of extrusion. A few extrusion pretreatments showed a significant improvement on sugar recovery from corn stover, switchgrass, prairie cord grass, big bluestem, Indian grass (Dale et al. 1999; Muthukumarappan and Julson 2007; Karunanithy and Muthukumarappan 2009a-c; 2010a-d,f), miscanthus (de Vrije et al. 2002; Jurisic et al. 2010), and Douglas fir (Lee et al. 2009) through enzymatic hydrolysis. No furfural and HMF were reported in any of the above studies; however, acetic acid and glycerol were reported in low concentration (Karunanithy and Muthukumarappan 2009a-c; 2010a-h). Karunanithy et al. (2009a-c) reported that the screw speed and barrel temperature had a significant effect on sugar recovery from different biomass types such as corn stover, big bluestem, switchgrass, and prairie cord grass. Karunanithy and Muthukumarappan (2010c) achieved a glucose, xylose, and combined sugar recovery of 57, 87, and 66%, respectively, for the following pretreatment conditions: of 50°C barrel temperature, 50 rpm screw speed, 4 mm big bluestem particle size with 15% moisture content using 3:1 screw compression ratio. Recently, these authors optimized the extrusion pretreatment conditions (180°C, 155 rpm, 20% moisture content, and 8 mm particle size) for big bluestem and recorded a glucose, xylose, and combined sugar recovery of 75, 85, and 60%, respectively (2009b). These results showed that there still is room to improve the sugar recovery from big bluestem when extrusion is combined with other pretreatment methods.

Between acid and alkali options, alkali is preferred due to its ability to remove lignin and to cause less degradation of carbohydrates. Also, no washing is required after pretreatment, and the addition of alkali would not result in corrosion problem in the extruder as compared to acid. Miller and Hester (2007) reported that acid-resistant stainless steel such as AL6XN would be required for the fabrication of extruder screw and barrel so that acid could be incorporated during extrusion. Furthermore, the selection of pretreatment should be compatible with the selected hydrolysis method (Taherzadeh and Karimi 2007). Morrison (1988, 1991) reported that NaOH is known to cleave ester linkages and to solubilize some of the hemicellulose as well as some of the lignin. Among different alkalis employed on big bluestem, NaOH showed higher glucose retention, no p-coumaric acid, and comparable *in vitro* digestibility (Titgemeyer et al. 1996). Based on the above facts NaOH was selected for this study.

Extruder parameters such as barrel temperature, screw speed, and screw compression ratio are important factors responsible for thermal softening, rate of shear development, residence time, and pressure. Each of the forgoing factors is known to affect sugar recovery (Karunanithy et al. 2008; Karunanithy and Muthukumarappan 2009a-c; 2010a-f). The size reduction of biomass has become an integral part of biomass pretreatment. It has been reported that three to six-fold size reduction (ca. 1 to 2 mm) is required to observe measureable sugar recovery from untreated biomass (Zeng et al. 2007); again the particle size requirement depends upon the pretreatment methods. Particle size affects many aspect of pretreatment, including its overall effectiveness, diffusion kinetics (Kim and Lee 2002; Chundawat et al. 2007), the enzymatic hydrolysis rate, the rheological properties (Chundawat et al. 2007; Desari and Bersin 2007), lignin removal, the sugar yield (Hu et al. 2008), and the power requirement for size reduction (Cadoche and Lopez 1989; van Walsum et al. 1996; Mani et al. 2004; Bitra et al. 2009). It is a well known that alkali acts as delignification agent at low concentration without degrading the carbohydrates. Hence, the extruder barrel temperature, screw speed, big bluestem particle size, and alkali (NaOH) concentration were the independent variables selected for this study.

Pretreatment conditions need to be optimized for the development of an efficient and economical pretreatment method. The traditional one-factor-at-a-time approach is time consuming; moreover, the interactions between independent variables are not considered. A statistical technique called response surface methodology (RSM) is used for the modeling and optimization of multiple variables, which determines optimum process conditions by combining experimental designs with interpolation by first- or second-order polynomial equations in a sequential testing procedure. RSM considers many factors, and their interactions affecting the response can be identified with fewer experimental trials than in a one-factor-at-a-time experiment. RSM has been widely used in various fields such as food processing, food science, and biotechnology. Recently, RSM has been successfully applied to biomass pretreatment by many researchers (Miller and Hester 2007; Schell et al. 1999; Ballesteros et al. 2007; Araque et al. 2008; Lin and Saka 2008; Rabelo et al. 2009). Earlier extrusion pretreatment studies conducted by the authors yielded encouraging results; however, the extrusion factors including alkali concentration were not optimized. The specific objectives of this study are: 1) to understand and optimize the effect of extruder parameters such as barrel temperature and screw speed, particle size, and alkali (NaOH) concentration for maximum sugar recovery using RSM and adopting a central composite rotatable design (CCRD), and 2) to develop a mathematical model to predict the glucose, xylose, and combined sugar recovery from big bluestem.

MATERIALS AND METHODS

Experimental Design

A central composite rotatable design (CCRD) with four independent variables was used to study the response pattern and to determine the optimum combination of barrel temperature, screw speed, alkali concentration, and particle size for maximizing the sugar recovery from big bluestem. The CCRD combines the vertices of the hypercube whose coordinates are given by a 2^n factorial design with star points. The star points provide the estimation of curvature of the nonlinear response surface. The experimental design was developed using Design Expert 7.1.6, which resulted in 30 runs. In addition, six more center points were added to allow for the estimation of the pure error sum of squares. A total of 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 (not shown). The independent variables were coded according to the following equation:

$$x_i = (X_i - X_0)/\Delta X_i \tag{1}$$

where x_i and X_i are the dimensionless and actual values of the independent variable i, X_0 is the current 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 variables optimized included barrel temperature (45 to 225°C), screw speed (20 to 200 rpm), alkali concentration (0.5 to 2.5% w/v), and particle size (2 to10 mm) each at five levels: -2, -1, 0, 1, and 2, as shown in Table 1. Many alkali pretreatments were carried out with high alkali concentration and prolonged period to achieve high sugar recovery. In this study low range of alkali concentrations were selected, since alkali soaking was followed by thermo-mechanical/extrusion pretreatment, in addition to avoid sugar degradation and washing of the pretreated solid.

Biomass Preparation

Big bluestem obtained from a local farm was ground in a hammer mill (Speedy King, Winona Attrition Mill Co, MN) using 2, 4, 6, 8, and 10 mm sieves to understand the influence of particle size on sugar recovery. The compositional analysis of big bluestem was done following the Sluiter et al. (2008a-b) procedure. Alkali (NaOH) solutions of different concentrations (0.5, 1.0, 1.5, 2.0, and 2.5 % w/v) were prepared. It was found that big bluestem required seven times the amount of solution relative to its dry weight for complete soaking, resulting in a solid loading rate of 12.5%, which was similar to that of prairie cord grass (Karunanithy and Muthukumarappan 2009c). The different particle size big bluestem was soaked in different alkali concentrations as given in the experimental design (Table 1) for 30 min at room temperature. The black liquid was drained using a cheese cloth followed by manual squeezing to remove excess moisture. Moisture content of the biomass samples was determined as described by Sluiter et al (2008c). The moisture content of alkali soaked samples was in the range of 75-78% wb.

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 3:1compression ratio was selected based on a previous study (Karunanithy and Muthukumarappan 2010c). 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 extruder barrel had provisions to control the temperature of the feed and transition zone in both barrel and die section. 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. Once the barrel temperature stabilized, about 500 g of biomass was extruded under each pretreatment condition. Tests were each divided into two batches in order to account for variations due to extruder operation, and these were considered replicates. The mean residence time varied between 30 and 90 s depending upon the screw speed.

Enzymatic Hydrolysis

Enzymatic hydrolyses of pretreated samples (0.3 g in 10 mL hydrolysis volume) were carried out using 0.1 M, pH 4.8 sodium citrate buffer for 72 h at 50°C and 150 rpm, as described by Selig et al. (2008). Based on the literature survey and previous study (Karunanithy and Muthukumarappan 2010a), the amount of cellulase (Celluclast 1.5 L, activity 70 FPU/g) enzyme was chosen as 15 FPU/g of dry matter. The ratio of cellulase to β-glucosidase (Novozyme 188, activity 250 CBU/g) was maintained at 15 FPU/60 CBU, based on an earlier study (Karunanithy and Muthukumarappan 2010a). All of these enzymes and their activities were provided by Novozyme (Krogshoejvej, Denmark). After hydrolysis, the samples were kept in boiling water for 10 min to inactivate the enzyme action. The supernatant was centrifuged with a force of 16060 g and then frozen twice before injecting it into the HPLC to remove the impurities that contribute to the pressure increase in the HPLC system. Soluble sugars and byproducts were quantified by HPLC (Agilent Technologies, Santa Clara, CA; Bio-Rad Aminex 87H column, Hercules, CA) using RI detector 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. (2006). The sugar concentration obtained from the chromatogram was divided by dry weight of biomass taken for enzymatic hydrolysis in order to know the percentage of different sugars with respect to total biomass; sugar recovery was calculated using Equations 2 and 3 shown below. The sugar recovery reported in this paper was after the enzymatic hydrolysis of the pretreated samples. Glucose and xylose are 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. Acetic acid was the only byproduct found in the hydrolyzate of the pretreated samples, and its concentration was reported in gram per liter.

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

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

In these equations Y_i is the individual sugar recovery (%), Y_c is the combined sugar recovery (%), S_{ip} is the individual sugar obtained from the hydrolyzate of the pretreated samples through HPLC, and S_{ir} is the individual sugar from compositional analysis of raw material.

Statistical Analysis

Once the extrusion pretreatments are performed, the most commonly used 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 is the linear term, b_{ii} is the quadratic term, b_{ij} is the interaction term, 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 (α =0.05) was determined by the F test. The individual effect of each variable and also the effects of the interaction were determined. Optimization (maximizing sugar recovery) of the fitted polynomial was determined using numerical optimization contained in the Design Expert 7.1.6. After optimizing the pretreatment conditions using RSM, validation was done by extruding the corn stover at two different optimum conditions of barrel temperature, screw speed, alkali concentration, and particle size from the numerical solution, depending on the particle size, due to availability of standard sieve size.

RESULTS AND DISCUSSION

Solid Loss and Washing of Pretreated Big Bluestem

Wyman (1996) indicated that the amount of weight lost following chemical pretreatment of residue was due to lignin removal. A solid loss of 40.3% has been reported for big bluestem when 1M NaOH was used with N_2 for 24 h at room temperature (Titgemeyer et al. 1996). The solid loss and delignification varied from 22.6 to 67.3 and 40.0 to 96.0%, respectively, depending upon the alkali used and the pretreatment conditions employed on corn stover (Kaar and Holtzapple 2000; Gupta 2008; Varga et al. 2002). Hu et al. (2008) reported a lignin removal of 65% with 0.1 g NaOH/g switchgrass pretreated using radio-frequency dielectric heating for 60 min at 90°C. Hu and Wen (2008) also studied the combined effect of alkali and microwave pretreatment and reported about 50 to 98% lignin removal depending upon the alkali loading (0.05 to 0.3 g/g switchgrass). Keshwani (2009) reported about 25 and 57 % solid loss, 58 and 83% lignin removal for switchgrass pretreated in a microwave oven with 1% NaOH for 5 min and 3% NaOH for 20 min, respectively. Recently, Tenlep et al. (2009) achieved 71 and 76% of delignification for switchgrass and prairie cord grass, respectively, when pretreated in 1% NaOH at 200°C and 1500 psi for 10 min. Considering the solid loss (17%) and delignification (25%) during alkali soaking, the sugar recovery was calculated and shown in mass balance diagram under results and discussion. The reason for lower solid loss and delignification is due to the use of room temperature treatment, when compared to the above mentioned methods. The solid loss from big bluestem was in agreement with corn stover (15%) and prairie cord grass (20%) (Karunanithy and Muthukumarappan, 2010g; h). However, delignification from big bluestem was lower than that of corn stover (40%) and prairie cord grass (35%) soaked in a similar alkali concentration (Karunanithy and Muthukumarappan, 2010g; h).

Many researchers (Tanaka et al. 1979; Titgemeyer et al. 1996; Kaar and Holtzapple 2000; Dawson and Boopathy 2007) have reported washing of the biomass with different medium after alkali pretreatment; however, it may not be necessary if the alkali concentration is low enough. According to Novozymes biomass kit, most of the enzymes have optimum activity between a pH of 4.5 and 6.5 within the range 45 to 70 °C. When different biomasses were soaked with different alkali concentration, the samples prepared for enzymatic hydrolysis (after adding citrate buffer, DI water, and enzymes) had pH values of 4.8 to 5.4, which is well within the range of Novozyme's recommendations (pH of 4.5 to 6.5 for Celluclast 1.5 L and 2.5 to 6.5 for Novozyme 188). This observation reveals that washing can be eliminated when alkali concentration is low enough, which is an added advantage of alkali soaking-extrusion pretreatment. Depending upon the alkali concentration and particle size, a sugar recovery of 35 to 45% was recorded, and the sugar range was in agreement with corn stover and prairie cord grass (Karunanithy and Muthukumarappan 2010g; h). However, the washed samples had lower sugar recovery (due to loss of sugar about 5-7%) than that of unwashed samples. The size-reduced raw big bluestem went through the same enzymatic hydrolysis and considered as control.

Effect of Independent Variables on Sugar Recovery

The observed sugar recovery for all the treatment combinations is presented in Table 1. The developed quadratic models in terms of actual variables are given below (Equations 5 to 7) 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 (°C), screw speed (rpm), alkali concentration (%w/v), and particle size (mm) of big bluestem, respectively. Similar equations were reported for dilute acid pretreatment of Douglas fir (Schell et al. 1999) and cardoon (Ballesteros et al. 2007), hot water pretreatment of Japanese beech (Xin and Saka 2008), lime pretreatment of sugarcane bagasse (Rabelo et al. 2009), extrusion pretreatment of big bluestem (Karunanithy and Muthukumarappan 2009b), alkali-extrusion of corn stover (Karunanithy and Muthukumarappan 2010g), and alkali-extrusion of prairie cord grass (Karunanithy and Muthukumarappan 2010h), switchgrass (Karunanithy and Muthukumarappan 2010f), and prairie cord grass (Karunanithy and Muthukumarappan 2009c). Those equations predict the responses well with high R^2 (more than 0.9) and low probability values (0.001 to less than 0.0001).

$$Y_G = -83.83 + 0.538X_2 + 75.31X_3 - 0.002X_1X_2 + 0.0613X_1X_4 - 0.0956X_2X_4 - 1.2685X_3X_4 - 0.0011X_1^2 + 0.0015X_2^2 - 19.03X_3^2 - 0.654X_4^2 \quad (5)$$

$$Y_X = -79.93 + 0.2844X_2 + 97.40X_3 + 0.0276X_2X_3 - 0.0381X_2X_4 - 0.001X_1^2 - 0.0005X_2^2 - 28.53X_3^2 - 1.0807X_4^2 \quad (6)$$

$$Y_C = -82.36 + 0.5X_2 + 72.9X_3 - 0.0013X_1X_2 + 0.0308X_1X_4 - 0.0637X_2X_4 - 0.0011X_1^2 - 20.45X_3^2 - 0.8374X_4^2 \quad (7)$$

The regression coefficient, standard error, F and p values are shown in Table 2. Among the independent variables, only alkali concentration and screw speed had a significant effect on sugar recoveries. As observed from the equations 5 to 7, screw speed and alkali concentration had a positive influence on sugar recoveries, and this observation was in agreement with corn stover (Karunanithy and Muthukumarappan 2010g), switchgrass, and prairie cord grass (Karunanithy and Muthukumarappan 2009c). Similar screw speed trends were reported for miscanthus (Jurisic et al. 2009), corn stover, big bluestem (Karunanithy and Muthukumarappan 2009b), and switchgrass (Karunanithy and Muthukumarappan 2010f) when pretreated in a single screw extruder. The rate of shear development is more critical than that of residence time. According to Dreiblatt (2003), the rate of shear development increases with screw speed. Moreover, increase in screw speed increases the available energy/shear force for fiber breakage and changes the fiber length and aspect ratio in turn increase the surface area thereby sugar recoveries. Extrusion pretreatment of miscanthus (Jurisic et al. 2009) and corn stover (Karunanithy and Muthukumarappan 2010e) studies showed that particle size had a strong impact on glucose recovery; however, it was not observed in this study. Although a significant difference on xylose and combined recovery among untreated particle sizes of big bluestem was observed in one-factor-at-a-time (not shown); the extrusion pretreatment

Table 1. Experimental Design Showing both Coded and Actual Values of Variables, Observed and Predicted Responses

					Glucose, %			Xylose, %			Combined sugar, %		
Treat	Temp	Speed	Alkali	PS	Obsd	Pred	Resl	Obsd	Pred	Resl	Obsd	Pred	Resl
1	0(135)	0(110)	0(1.5)	0(6)	64.5	65.6	-1.1	84.6	85.1	-0.5	71.0	73.8	-2.8
2	1(180)	-1(65)	1(2.0)	-1(4)	54.7	52.3	2.5	78.4	76.9	1.5	65.7	62.1	3.6
3	0(135)	0(110)	0(1.5)	0(6)	66.7	65.6	1.1	83.7	85.1	-1.4	76.3	73.8	2.5
4	1(180)	-1(65)	1(2.0)	1(8)	79.2	79.0	0.2	86.9	86.8	0.1	80.6	78.6	2.0
5	0(135)	0(110)	0(1.5)	0(6)	67.0	65.6	1.4	87.0	85.1	1.9	75.3	73.8	1.6
6	0(135)	0(110)	0(1.5)	0(6)	64.4	65.6	-1.3	83.5	85.1	-1.6	73.7	73.8	-0.1
7	1(180)	1(155)	1(2.0)	-1(4)	69.8	71.3	-1.4	90.9	92.5	-1.6	76.8	80.1	-3.3
8	-1(90)	-1(65)	-1(1.0)	-1(4)	40.7	39.6	1.1	51.3	54.6	-3.3	45.3	45.7	-0.4
9	0(135)	0(110)	0(1.5)	0(6)	65.1	65.6	-0.6	85.5	85.1	0.4	74.3	73.8	0.5
10	-1(90)	1(155)	-1(1.0)	-1(4)	69.5	71.4	-1.9	63.3	63.1	0.2	67.0	69.3	-2.3
11	0(135)	0(110)	0(1.5)	2(10)	54.0	56.2	-2.2	64.6	66.8	-2.2	57.6	60.6	-3.0
12	0(135)	2(200)	0(1.5)	0(6)	86.5	86.2	0.3	82.6	86.1	-3.4	86.9	85.9	0.9
13	1(180)	1(155)	-1(1.0)	1(8)	47.8	48.9	-1.1	55.9	53.7	2.2	52.7	51.8	0.9
14	-1(90)	-1(65)	1(2.0)	-1(4)	55.1	55.6	-0.5	75.2	77.1	-1.9	60.7	61.8	-1.1
15	0(135)	0(110)	0(1.5)	0(6)	64.0	65.6	-1.6	83.5	85.1	-1.6	71.1	73.8	-2.7
16	0(135)	0(110)	2(2.5)	0(6)	59.7	61.9	-2.2	83.2	85.4	-2.2	69.4	72.3	-3.0
17	0(135)	0(110)	0(1.5)	0(6)	64.8	65.6	-0.8	87.6	85.1	2.5	74.4	73.8	0.7
18	0(135)	0(110)	0(1.5)	0(6)	66.8	65.6	1.2	84.8	85.1	-0.3	73.8	73.8	0.0
19	-2(45)	0(110)	0(1.5)	0(6)	56.6	57.0	-0.4	77.0	76.1	0.9	65.4	65.3	0.1
20	0(135)	0(110)	0(1.5)	0(6)	63.7	65.6	-1.9	83.4	85.1	-1.7	72.4	73.8	-1.4
21	-1(90)	1(155)	-1(1.0)	1(8)	46.6	46.7	-0.1	49.5	51.1	-1.6	51.1	53.2	-2.1
22	1(180)	-1(65)	-1(1.0)	1(8)	70.0	67.8	2.2	55.5	57.7	-2.3	60.9	61.4	-0.5
23	-1(90)	-1(65)	1(2.0)	1(8)	61.3	60.2	1.0	82.2	82.9	-0.7	67.9	67.3	0.7
24	0(135)	-2(20)	0(1.5)	0(6)	68.9	69.9	-1.0	78.9	75.6	3.2	65.1	67.3	-2.2
25	0(135)	0(110)	0(1.5)	0(6)	67.3	65.6	1.7	86.0	85.1	0.9	73.4	73.8	-0.4
26	0(135)	0(110)	0(1.5)	0(6)	64.5	65.6	-1.2	84.0	85.1	-1.1	71.3	73.8	-2.4
27	1(180)	-1(65)	-1(1.0)	-1(4)	32.5	36.0	-3.5	50.4	51.9	-1.5	42.0	43.5	-1.5
28	0(135)	0(110)	-2(0.5)	0(6)	32.7	31.3	1.4	29.8	27.8	2.0	36.0	34.3	1.6
29	0(135)	0(110)	0(1.5)	-2(2)	55.6	54.1	1.5	70.8	68.9	2.0	61.8	60.2	1.7
30	-1(90)	1(155)	1(2.0)	-1(4)	90.9	90.8	0.2	93.8	91.6	2.2	92.1	90.1	2.1
31	-1(90)	-1(65)	-1(1.0)	1(8)	49.2	49.4	-0.2	58.1	56.3	1.8	55.6	52.6	3.0
32	1(180)	1(155)	-1(1.0)	-1(4)	52.9	51.5	1.3	62.2	61.5	0.6	57.7	56.8	0.9
33	-1(90)	1(155)	1(2.0)	1(8)	62.9	61.0	1.9	85.4	83.6	1.8	73.8	72.5	1.3
34	2(225)	0(110)	0(1.5)	0(6)	55.6	55.9	-0.3	77.4	78.5	-1.1	62.8	64.2	-1.4
35	0(135)	0(110)	0(1.5)	0(6)	68.5	65.6	2.9	87.6	85.1	2.5	78.3	73.8	4.5
36	1(180)	1(155)	1(2.0)	1(8)	64.8	63.6	1.3	91.9	88.7	3.2	75.7	73.7	2.0

Obsd- Observed Pred- Predicted Resl- Residual

Table 2. Coefficient Values of the Fitted Model for Different Responses

	Glucose				Xylose				Combined sugar			
Factor	Coefft	Std error	F value	P value	Coefft	Std error	F value	P value	Coefft	Std error	F value	P value
Temp	-0.275	0.404	0.465	0.5026	0.581	0.502	1.34	0.2599	-0.282	0.539	0.273	0.6064
SS	4.08	0.404	102.02	<0.0001	2.605	0.502	26.95	<0.0001	4.653	0.539	74.49	<0.0001
AC	7.651	0.404	358.76	< 0.0001	14.388	0.502	821.79	< 0.0001	9.503	0.539	310.69	< 0.0001
PS	0.515	0.404	1.625	0.2163	-0.523	0.502	1.08	0.3092	0.1045	0.539	0.0376	0.8481
Temp*SS	-4.04	0.494	66.94	< 0.0001	0.279	0.615	0.20	0.6538	-2.553	0.66	14.94	0.0009
Temp*AC	1.72	0.494	0.03	0.8634	0.620	0.615	1.01	0.3246	0.6407	0.66	0.941	0.3429
Temp*PS	5.51	0.494	124.38	< 0.0001	1.036	0.615	2.84	0.1066	2.772	0.66	17.63	0.0004
SS*AC	0.859	0.494	3.01	0.0974	1.483	0.615	5.82	0.0250	1.712	0.66	3.148	0.0905
SS*PS	-8.59	0.494	302.16	< 0.0001	-3.425	0.615	31.04	< 0.0001	-5.73	0.66	75.39	< 0.0001
AC*PS	-1.27	0.494	6.57	0.0181	1.000	0.615	2.65	0.1184	-0.353	0.66	0.286	0.5985
$Temp^2$	-2.28	0.349	42.81	< 0.0001	-1.954	0.43	20.22	0.0002	-2.252	0.47	23.26	< 0.0001
SS^2	3.11	0.349	79.30	< 0.0001	-1.065	0.43	6.00	0.0231	0.716	0.47	2.349	0.1403
AC^2	-4.76	0.349	185.07	< 0.0001	-7.133	173.86	269.30	< 0.0001	-5.112	0.47	119.87	< 0.0001
PS^2	-2.61	0.349	55.92	< 0.0001	-4.322	0.43	98.91	< 0.0001	-3.350	0.47	51.45	< 0.0001

SS- screw speed AC- alkali concentration PS – particle size erased the differences in sugar recovery of alkali soaked biomass having different particle sizes. Similar results were reported for wet oxidation of wheat straw (Pedersen and Meyer 2009), hot water treatment of corn stover (Zeng et al. 2007), and extrusion pretreatment of switchgrass (Karunanithy and Muthukumarappan 2010d). These results show that an effective pretreatment could reduce the need of substrate size reduction; thereby energy spent on size reduction could be saved to a greater extent. As expected, an increase in alkali concentration had a positive influence across the levels of other independent variables due to delignification. Alkali concentration had a more prominent effect on sugar recoveries than that of either screw speed or particle size, as evident from their magnitudes (Equations 5 to 7). Among the interactions, few were statistically significant for sugar recovery from big bluestem. Although extruder parameters such as barrel temperature and screw speed were insignificant as independent variables, their quadratic terms were significant for all the sugar recoveries.

Not only linear and quadratic terms but also interactions terms contributed to sugar recoveries, as is evident from Table 2 and Equations 5 to 7. The predicted glucose recovery responses for significant interactions of independent variables are shown in Fig. 1a-d. As the screw speed and barrel temperature increased, the glucose recovery was also increased. However, screw speed showed a different behavior relative to glucose recovery across the barrel temperature as is evident from Fig.1a. The increase in barrel temperature had a positive effect on glucose recovery at low screw speed, whereas the effect was negative at high screw speed (155 rpm). The glucose recovery increase might be due to a high rate of shear development and its utilization. The low temperature had a strong impact on glucose recovery compared to high temperature due to high residence time with low screw speed. The alkali-soaked big bluestem had a moisture content in the range of 75 to 78%. The low temperature might be insufficient to remove the moisture; on the other hand high barrel temperature removed high moisture, thus resulted in high glucose recovery. At low temperature the rate of shear development plays an important role, whereas at high temperature the mean residence time plays a significant role on glucose recovery.

The interaction of particle size with barrel temperature, screw speed, and alkali concentration had a significant effect on glucose recovery. The particle size exhibited different glucose recovery patterns depending upon the interaction with other independent variables. Friction is the main mode of material conveyance in a single screw extruder (Yeh and Jaw 1998). In general, the increase in barrel temperature and particle size resulted in high glucose recovery due to high moisture evaporation and high resistance offered by large particles, thereby increasing the friction. The barrel temperature had a positive effect on glucose recovery when the particle size was an 8 mm, but the effect was reversed for 4 mm particle size, as is evident from Fig. 1b. Similarly, the particle size effect on glucose recovery was positive at 180°C and negative at 90°C. The mixed glucose recovery behavior might be due to the high moisture content, its vaporization, and thermal softening.

As mentioned earlier, the increase in glucose recovery with an increase in concentration of alkali was possible because of high delignification. The effect of alkali concentration on glucose recovery was more than that of particle size, as evident from Fig. 1c. The screw speed interaction with particle size exhibited different trends depending upon their levels (Fig. 1d). The increase in particle size had high glucose recovery at a screw speed of 65 rpm, whereas the glucose recovery decreased at 155 rpm. The quadratic effects can be easily visualized from the curved shape surface plots in Fig. 1a-d. In general, high barrel temperature, screw speed, and alkali concentration would result in 69 to 99% glucose recovery from big bluestem. The maximum model predicted glucose recovery from big bluestem was lower than for corn stover, switchgrass, and prairie cord grass (Karunanithy and Muthukumarappan, 2009c; 2010g; h), which might be due to differences in the characteristics of the biomasses.

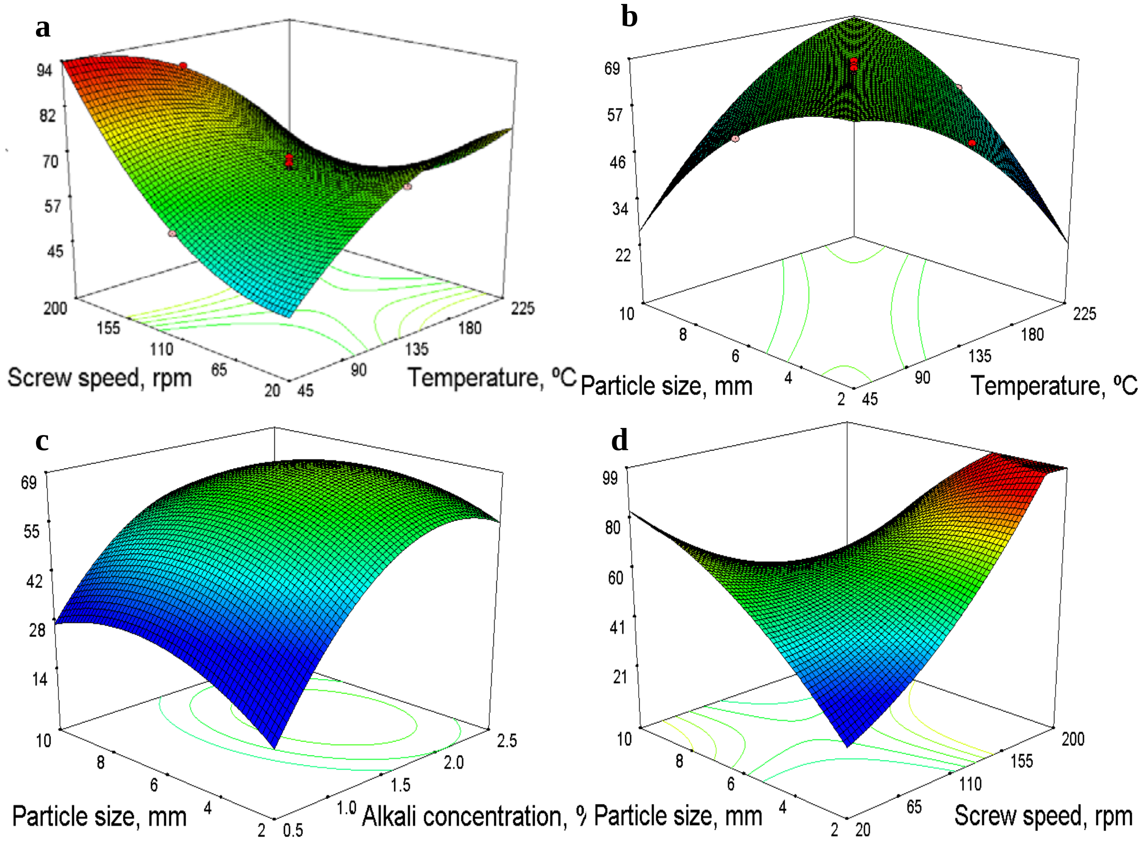

Fig. 1. Interaction effect of independent variables on glucose recovery % (when other factors fixed at the center point: 135ºC, 110, rpm, 1.5% w/w, and 6 mm)

The xylose recovery response for various significant interactions predicted by the proposed model is depicted in Figure 2a-b. When the screw speed interacted with alkali concentration or particle size, it had a strong influence on xylose recovery. Alkali concentration had a stronger impact on xylose recovery than that of screw speed, as noted in Fig. 2a. The alkali concentration had a similar effect on xylose recovery across the screw speeds (Fig. 2a). The interaction of screw speed with particle size showed mixed behavior on xylose recovery. The screw speed had a positive contribution to xylose recovery for a particle size of 4 mm, and it was reversed for an 8 mm particle size (Fig. 2b). Similarly, the particle size influence was positive at 65 rpm, and it was negative at 155 rpm. The mixed behavior of xylose recovery might be attributed to high shear development rate and its utilization by small big bluestem particle size. The inference was that high screw speed and high alkali concentration would be required for the xylose recovery to reach the range 94 to 97%. Near-quantitative xylose recovery was not achieved, and a possible reason could be the loss of hemicellulose due to alkali soaking. Xylose recovery range was comparable to that of corn stover, switchgrass, and prairie cord grass pretreated under similar conditions (Karunanithy and Muthukumarappan 2009c; 2010g; h).

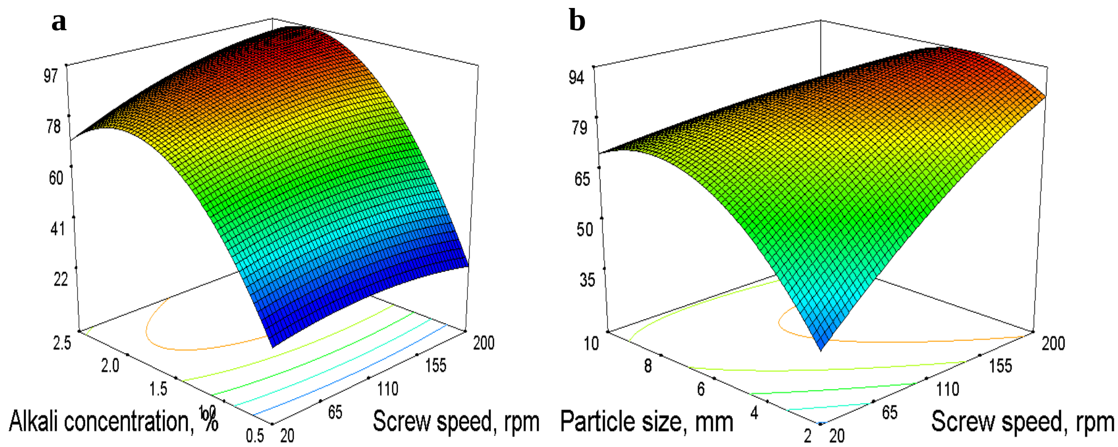

Fig. 2. Interaction effect of independent variables on xylose recovery % (when other factors fixed at the center point: 135ºC, 110, rpm, 1.5% w/w, and 6 mm)

The significant interaction of independent variables on combined sugar recovery response predicted by the model is shown in Fig. 3a-c. As observed from the figure, the interaction of alkali concentration with other independent variables was insignificant. The interaction of screw speed with barrel temperature or particle size (Fig. 3a) indicated that the high screw speed resulted in high sugar yield, which showed that rate of shear development was more important than the mean residence. The effect of screw speed on combined sugar recovery was similar across the barrel temperatures; similarly the effect of temperature on combined sugar recovery was similar regardless of screw speeds. The predicted surface (dome) for the interaction of barrel temperature with particle size (Fig. 3b) showed that high combined sugar recovery was possible at a temperature of 140 to 160°C and 5 to 7 mm particle size. The interaction of screw speed and particle size (Fig. 3c) produced mixed behavior for combined sugar recovery, and it was similar to glucose recovery (Fig. 1d). In general, high screw speed, and barrel temperature were required for combined sugar recovery of about 90%. Although the glucose recovery differed between big bluestem and corn stover, switchgrass, and prairie cord grass, the combined sugar recovery was comparable among the biomasses (Karunanithy and Muthukumarappan 2009c; 2010e).

The maximum glucose, xylose, and combined sugar recovery of 90.9, 93.8, and 92.1%, respectively were recorded from big bluestem particle size of 4 mm soaked in an alkali concentration of 2% and extruded at a barrel temperature of 90°C and screw speed of 155 rpm (treatment# 30). As screw speed increased, more energy is available for fiber breakages, which change the fiber length and aspect ratio. According to Baillif and

Oksman (2009), fiber length reduction might depend on the high shear forces applied to the fiber during the extrusion, which is similar to pelletizing. In general, high shear rate can be achieved with high screw speed, large screw diameter or with a small gap between the screw and barrel. Moreover, screw speed is directly proportional to shear rate, which produces linear effect when the extruder is flood fed as in the case of single screw extruder (Dreiblatt 2003). The sugar recovery from the present study was higher than the sugar recoveries reported earlier for big bluestem pretreated in a single screw extruder at different pretreatment conditions (Karunanithy and Muthukumarappan 2010a; c). The increase in sugar recovery might be due to combined effect of delignification and extrusion. These authors achieved 75, 85, and 60% of glucose, xylose, and combined sugar recovery, respectively, at the optimized condition of 180°C barrel temperature, 155 rpm screw speed, and particle size of 8 mm with 20% moisture content.

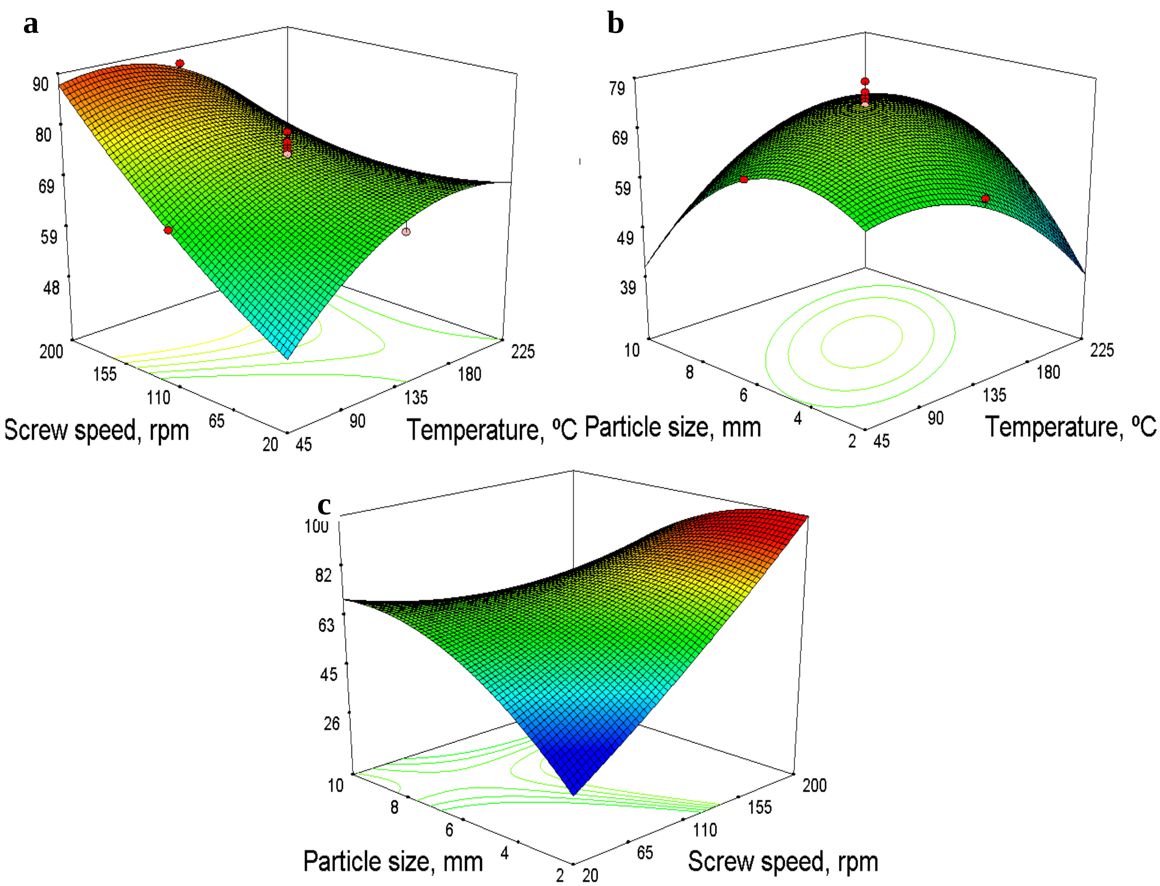

Fig. 3. Interaction effect of independent variables on combined sugar recovery % (when other factors fixed at the center point: 135ºC, 110, rpm, 1.5% w/w, and 6 mm)

A comparable glucose (88%), xylose (90%), and combined sugar (90%) recovery was reported for the treatment combination of 180°C, 155 rpm, 20% moisture content, and 8 mm corn stover particle size (Karunanithy and Muthukumarappan 2009b). Similar glucose, xylose, and combined sugar recoveries of 86.5, 84.5, and 86.0%, respectively, were realized for the treatment combination of 180°C, 155 rpm, 2% alkali concentration, and switchgrass particle size of 4 mm (Karunanithy and Muthukumarappan 2009c). These authors obtained a maximum glucose (97.5%), xylose (95%), and combined sugar (94%) for the treatment combination of 180°C, 155 rpm, 2% alkali concentration, and prairie cord grass particle size of 8 mm (Karunanithy and Muthukumarappan 2009c). The difference in sugar recovery might be due to the inherent nature of biomasses including their chemical composition. de Vrije et al (2002) reported a 77% delignification, 69% glucose, and 38% of xylose and arabinose conversion from combined pretreatment of miscanthus in a twin screw extruder (100 rpm and 100°C) and alkali (NaOH 12% and 70°C). In fact, a higher alkali concentration not only removes the lignin but also degrades the carbohydrates. Hence, the low sugar recovery reported for miscanthus might be due to degradation of carbohydrates and inherent characteristics of biomass. A comparable sugar recovery was reported for switchgrass pretreated in a combination of alkali (0.1 g NaOH/g)- radio frequency heating at 90ºC for 60 min with 20% solids loading (Hu et al. 2008); NaOH-microwave retreatment of switchgrass (Hu and Wen 2008; Keshwani 2009). Recently, Lee et al (2009) extruded Douglas fir in a twin screw extruder at 50 rpm and 40°C, and they reported 62.4% cellulose to glucose conversion when ethylene glycol was added as a cellulose affinity additive. The difference in glucose recovery might be due to delignification, hydrolysis of hemicellulose, type of extruder, pretreatment conditions such as screw speed, temperature, particle size, and the inherent characteristics of biomasses.

Response Surface Model Evaluation

The predicted and observed responses along with coded and actual variables are presented in Table 1.The closeness of the predicted and observed responses reflected the goodness of fit. Analysis of variance of the observed data, p value (α = 0.05), and coefficient of determination (R^2) of the regression model using CCRD are presented in Table 3. The F value of glucose, xylose, and combined sugar was very high compared to the tabular $F_{14, 21}$ value (2.19), which indicated that the model was highly significant. As noted from Table 3, the regression model was significant, whereas the lack of fit was not significant for glucose and combined sugar recovery. These results indicate that the proposed model is a good one. In addition, the coefficient of determination was also close to one, which also reflected on the adequacy of the model to represent the real relationship among the barrel temperature, screw speed, alkali concentration, and particle size on different sugar recovery. However, a large value of R^2 does not always imply that the regression model is good one, because R^2 would increase with adding a variable regardless of whether the additional variable was statistically significant or not (Karunanithy and Muthukumarappan 2009b; c; 2010e-h; Xin and Saka 2008). Hence, adjusted and predicted R^2 were calculated to check the model adequacy.

The predicted determination coefficient was in reasonable agreement with the adjusted determination coefficient, which also confirmed the fitness of the model. The proposed models explain about 90% of the variations in sugar recoveries. Another measure to evaluate the goodness of the model is coefficient of variation (CV), which is the ratio of standard error estimate to the mean values expressed as percentage. As a general rule, the CV should not be greater than 10% (Cocharan and Box 1957; Linko et al. 1984; Vainionpaa and Malkki 1987). A low value of CV (3.2-3.9%) showed that the

Table 3. Analysis of Variance for 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	5204.88	371.77	94.93	< 0.0001	0.98/ 0.97/ 0.93	3.23/ 46.57
	Lack of fit	10	55.10	5.51	2.23	0.1018		
	Pure error	11	27.13	2.46				
	Residual	21	82.23	3.91				
	Total	35	5287.12					
Xylose	Regression	14	7794.56	556.75	92.08	< 0.0001	0.98/ 0.97/ 0.92	3.26/ 40.77
	Lack of fit	10	98.47	9.84	3.80	0.0192		
	Pure error	11	28.48	2.58				
	Residual	21	126.96	6.04				
	Total	35	7921.52					
Combined	Regression	14	4847.31	346.23	49.62	< 0.0001	0.97/ 0.95/ 0.88	3.93/ 32.69
	Lack of fit	10	93.33	9.33	1.93	0.1478		
	Pure error	11	53.18	4.83				
	Residual	21	146.51	6.97				
	Total	35	4993.82					

experiments conducted were precise and reliable. “Adeq Precision” measured the signal-to-noise ratio. The larger the ratio, the better the prediction; a ratio greater than 4 was desirable. The ratio of 32.69-46.57 indicated an adequate signal, which implied that the model can be used to navigate within the design space (Liu et al. 2009).

Optimization and Validation of the Statistical Model

The interactions discussed in the above section were dealt with in an individual sugar recovery. Maximum glucose, xylose, and combined sugar recovery were the desirable responses considered for optimization. Hence, overlay contour plots superimposing glucose, xylose, and combined sugar recovery responses are depicted in Fig. 4. The shaded region shows a wide range of options to select the barrel temperature (45 to 170°C), screw speed (110 to 200 rpm), alkali concentration (1.4 to 2.5%), and particle size (2 to 7 mm) for maximum glucose (75 to 90%), xylose (80 to 95%), and combined sugar (80 to 95%) recovery from big bluestem. Based on the models, numerical optimization was carried out using Design Expert. Considering each response, 35 solutions were found, and the top ten are shown in Table 4. In order to confirm the predicted responses, big bluestem was extruded at three different optimum conditions (solution # 1, 19, and 32), as given in Table 5, considering wide ranges of temperature, screw speed, and particle size, and the validated samples are shown in Fig. 5. The extruded samples were subjected to the enzymatic hydrolysis and sugar measurement as described in Materials and Methods. The glucose, xylose, and combined sugar obtained were 90.1, 91.5, and 89.9%, respectively; the values were very close to the predicted values, and they were 4.4, 2.7, and 4.0 times higher than the control sample. Considering the energy requirement for heating the extruder barrel, the pretreatment condition of 90°C, 155 rpm, 2% alkali concentration, and 4 mm particle size was determined to be the optimum. The optimized pretreatment condition for big bluestem (90.1%) had higher glucose recovery than that of Douglas fir (59.2%) and eucalyptus (81.5%) pretreated with hot compressed water followed by extrusion, which might be due to high delignification (alkali soaking) and high shear force (155 rpm).

Although the optimum pretreatment condition differed from switchgrass, sugar recovery was in agreement with the values reported for switchgrass, and the present result was higher than the sugar recovery obtained from prairie cord grass (Karunanithy and Muthukumarappan 2009c; 2010h). The sugar recovery comparison of control, alkali soaked, and alkali soaked and extruded big bluestem is shown in Fig. 6 for better understanding. A well known fact is that the cellulose microfibril bundles encased by hemicellulose and lignin provide significant recalcitrance to enzyme accessibility. Hence, loosening this complex structure through the partial removal of lignin by alkali soaking is very effective to enhance the enzymatic hydrolysis and to facilitate the fibrillation, as noticed in Fig. 6. This observation was in agreement with Lee et al. (2010) who pretreated Douglas fir and eucalyptus wood with hot compressed water followed by extrusion. They also reported that the extruded products had finer fibrous morphology than that of hot compressed water treated woods due to high shearing force in the extruder when the materials pass through the narrow clearance between the screws and barrel (Blechschmidt et al. 2004). This observation would be true if the morphology of the alkali soaked and extruded big bluestem are examined. In general, the yield of ethanol from glucose is 0.51 g/g, and if the fermentation efficiency is 90%, Fig. 7 depicts the mass balance of alkali soaking-extrusion pretreatment of big bluestem followed by fermentation. The difference in sugar recovery and optimum conditions might be attributed to the inherent nature of biomasses, including their chemical compositions.

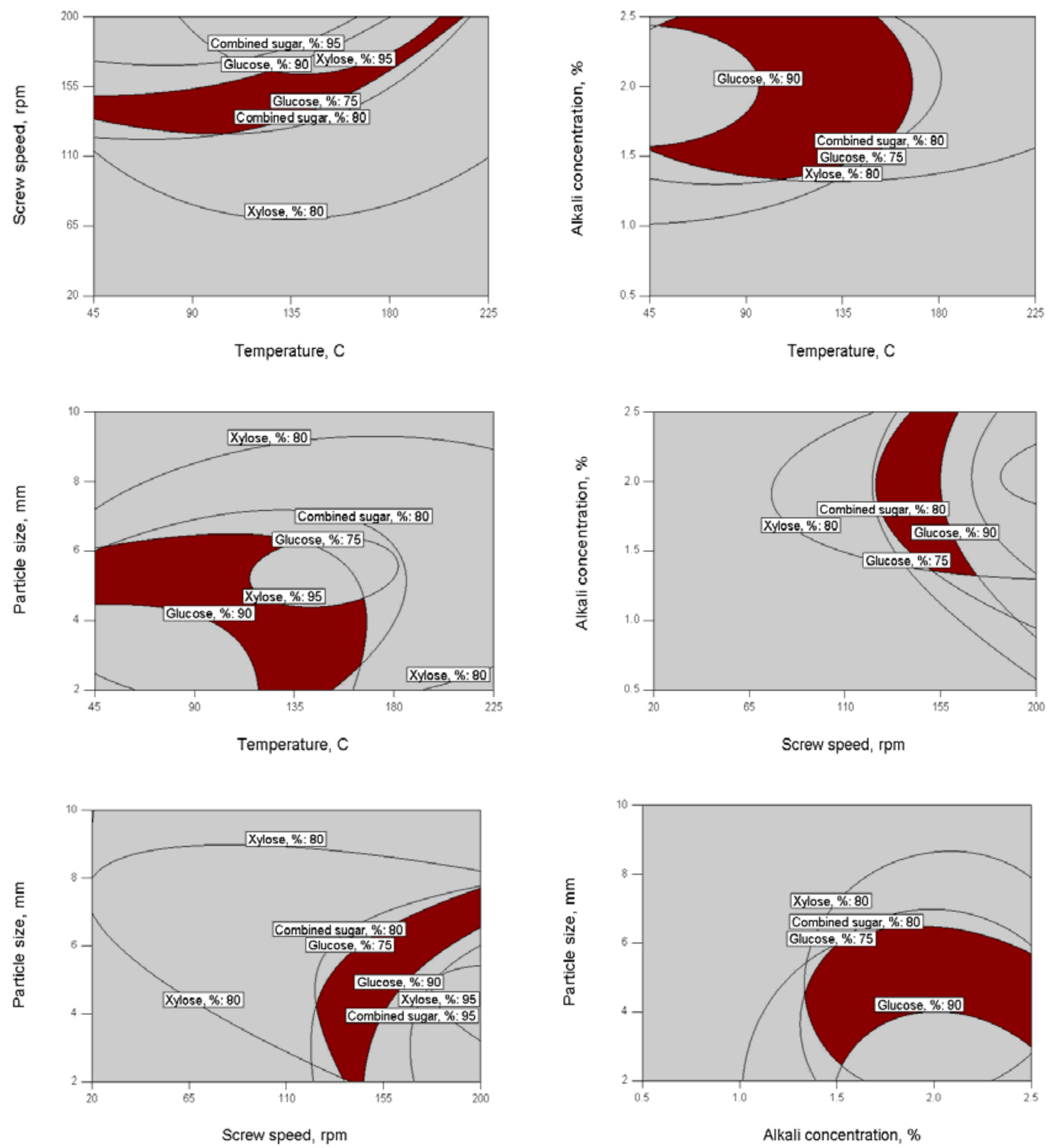

Fig. 4. Superimposed contours for sugar recovery responses as a function of temperature, screw speed, alkali concentration, and particle size of big bluestem

The glucose, xylose, and combined sugar recoveries of 90.5, 81.5, and 88%, respectively, were achieved for the optimum pretreatment condition of 180°C barrel temperature, 118 rpm screw speed, 2% alkali concentration, and 6 mm switchgrass particle size. The differences in alkali concentration could be explained by the lower amount of lignin present in big bluestem (21.1%) in comparison to switchgrass (24.7%).

Table 4. Solutions for Optimal and Validation Conditions

Solution #	Temperature, °C	Screw speed, rpm	Alkali conc., %	Particle size, mm	Glucose, %	Xylose, %	Combined sugar, %
1	90	155	2	4	90.76	91.61	90.05
2	91	155	2	4	90.63	91.72	90.03
3	95	155	2	4	90.10	92.10	89.95
4	96	155	2	4	89.97	92.19	89.93
5	92	155	1.9	4	90.40	91.72	89.92
6	93	155	1.9	4	90.31	91.82	89.92
7	102	155	2	4	89.28	92.59	89.78
8	98	155	2	4	89.00	92.70	89.73
9	90	155	1.9	4	89.70	91.67	89.61
10	90	153	2	4	89.36	91.31	89.18
Validation							
1	90	155	2	4	90.12	91.45	89.89
19	90	150	2	4	87.23	89.76	86.78
32	180	65	2	8	78.24	85.86	78.15

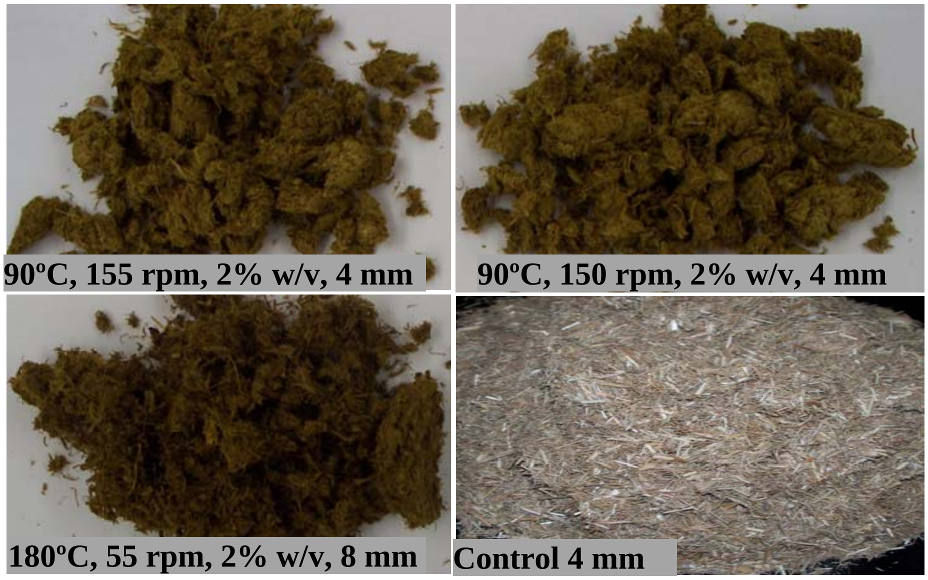

Fig. 5. Big bluestem pretreated at optimum and validated conditions

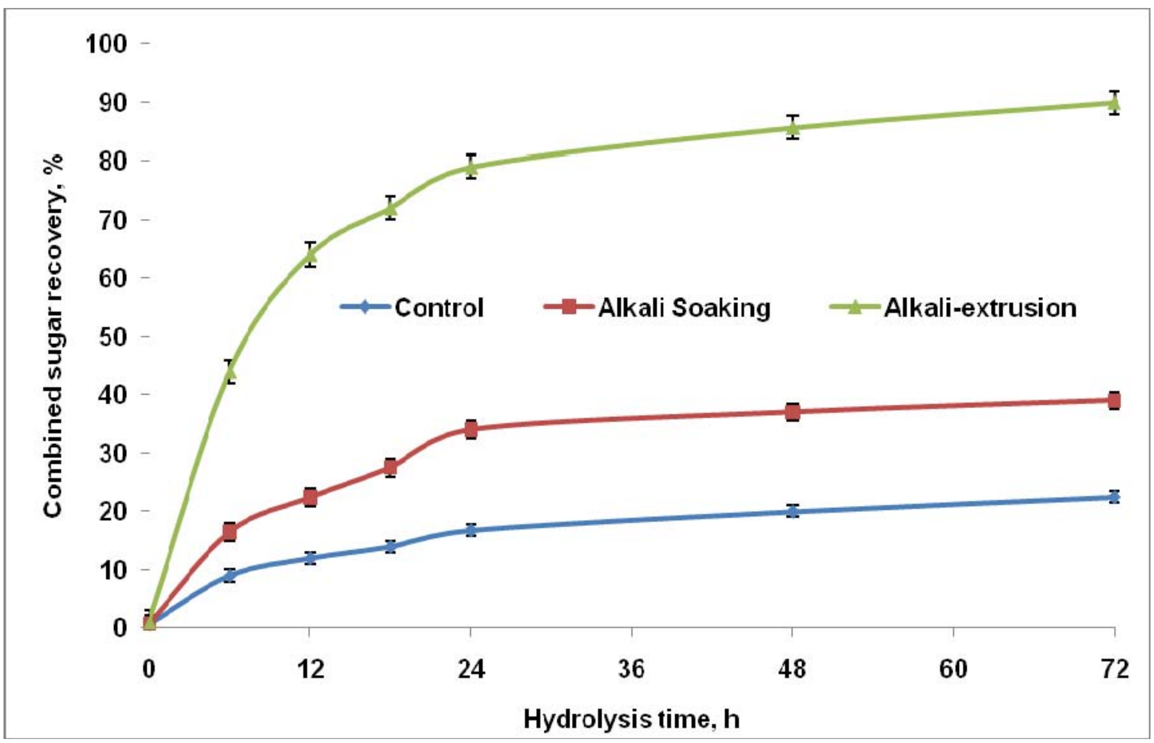

Fig. 6. Comparison of sugar recovery profile from control, alkali soaked, and alkali soaked-extruded big bluestem

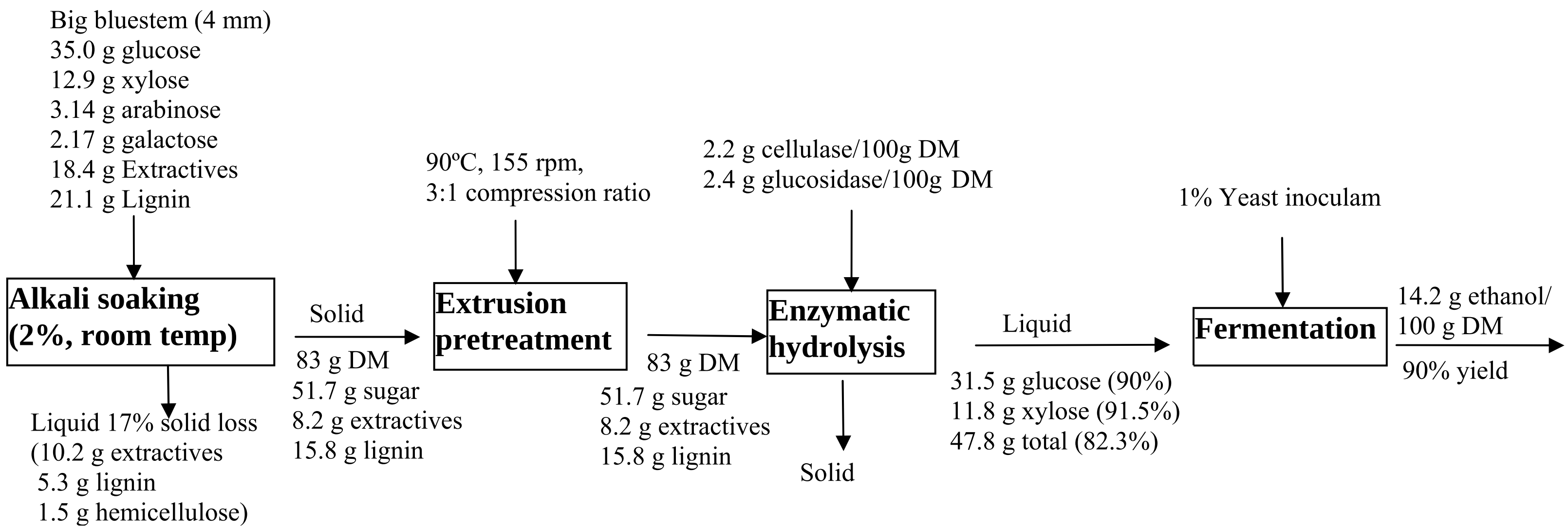

Fig. 7. Mass balance diagram of alkali soaking-extrusion pretreatment of big bluestem followed by fermentation

However, a more important determiner of biomass digestibility is the specific types of phenolic acids that constitute the non-core lignin of lignocellulosic biomass (Jung and Deetz 1993). These phenolic acids are involved in the ester linkages between hemicellulose and lignin. The major non-core lignin phenolic acids are p-coumaric acid (CA) and ferulic acid (FA). According to Burritt et al (1984) and Jung (1989), the ratio of CA to FA present in non-core lignin has a strong negative impact on biomass digestibility. Big bluestem has higher CA to FA ratio compared to switchgrass (Jung and Shalitajones 1990; Jung and Vogel 1992). Esterified and etherified p-coumaric and ferulic concentration were generally higher in switchgrass than big bluestem (Jung and Vogel 1992). Thus, it is reasonable to expect a higher alkali requirement for the pretreatment of switchgrass in comparison to big bluestem. However, the model predicted 2% alkali concentration as optimum for big bluestem and switchgrass.

Byproducts Formation

The severity of pretreatment can be expected to affect the formation of potential fermentation inhibitors. Furfural, HMF, and acetic acid are produced from pentose, hexose, and acetyl groups in hemicellulose of biomass, depending upon the pretreatment temperature, time, and pH conditions. Acetic acid was the only byproduct found in most of the pretreated big bluestem samples in the range of 0.06 to 0.10 g/L. The highest acetic acid (0.10 g/L) resulted at a barrel temperature of 135°C, a screw speed of 110 rpm, an alkali concentration of 1.5%, and a particle size of 6 mm. The acetic acid range recorded in this study was lower than the range reported for switchgrass and prairie cord grass in a similar study (Karunanithy and Muthukumarappan 2009c); big bluestem and corn stover extruded without alkali soaking (Karunanithy and Muthukumarappan 2009b; 2010a-c); and 0.04 g/L from big bluestem pretreated at 100°C and 150 rpm using 3:1 screw compression ratio and 21% moisture content (Karunanithy and Muthukumarappan, 2010c). The difference in acetic acid concentration might be due to acetyl content of biomasses. It was noticed that acetic acid concentration was 0.08 g/L for the optimized conditions (90°C, 155 rpm, 2.0%, and 4 mm). Acetic acid concentration of 7.3 g/L from a dilute acid pretreatment of Dougls fir (Schell et al. 1999); 5 g/100g from dilute acid pretreatment of cardoon (Ballesteros et al. 2007); 0.28-1.12% (wt) from compressed hot water pretreatment of Japanese beech (Xin and Saka 2008); moreover these pretreatments produced furfural and HMF also. The acetic acid formation and its concentration confirmed that the degradation of hemicellulose in a small amount. Sodium hydroxide is known to cleave ester linkages and to solubilize some of the hemicelluloses as well as some of the lignin (Morrison 1988, 1991). Lai (2001) reported that NaOH had higher reactivity with hemicellulose than cellulose due to amorphous characteristics of hemicellulose. The acetic acid concentration recorded in this study was far below than the inhibition limit (Taherzadeh et al. 1997). It has been reported that particle size of rice straw and silvergrass influenced the formation of acetic acid during dilute acid pretreatment (Guo et al. 2008); it was not observed in this study.

Glycerol was found in none of the pretreatment combinations in the current study, in contrast to earlier results (Karunanithy and Muthukumarappan 2009a-c). In addition, no furfural and HMF were found in any of the pretreatment combinations, in agreement with results for corn stover, switchgrass, and prairie cord grass pretreated samples

(Karunanithy and Muthukumarappan 2009a-c; 2010a-h). This also was in agreement with extrusion pretreatment employed on corn stover, miscanthus, Douglas fir, big bluestem, switchgrass, and Indian grass (Dale et al. 1999; Muthukumarappan and Julson 2007; de Vrijie et al. 2002; Lee et al. 2009; 2010). The uniqueness of extrusion pretreatment is the absence of potential inhibitors such as furfural, HMF, and formic acid; though acetic acid was found, it was at a very low concentration that would not affect the fermentation process.

CONCLUSIONS

Alkali-soaked big bluestem was extruded using a single screw extruder under various conditions based on a central composite rotatable design to obtain maximum glucose, xylose, arabinose, and combined sugar recovery. Statistical analyses revealed that the extruder screw speed, alkali concentration, and particle size had significant effects on sugar recovery. The proposed quadratic model to predict sugar recovery had high F (50-95) and R^2 values (more than 0.97) with low p value (<0.0001), demonstrating an adequate relationship among the independent variables studied on sugar recovery from big bluestem. The optimum pretreatment conditions, namely a barrel temperature of 90°C, screw speed of 155 rpm, and alkali concentration of 2.0% w/v with 4 mm particle size, resulted in a glucose, xylose, and combined sugar recovery of 90.1, 91.5, and 89.9%, respectively, and this was also confirmed through validation.

ACKNOWLEDGEMENTS

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