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A STUDY ON ALKALI PRETREATMENT CONDITIONS OF SORGHUM STEM FOR MAXIMUM SUGAR RECOVERY USING STATISTICAL APPROACH

Article Highlights

- Sorghum stem was used as a cheap raw material for production of fermentable sugars
- Alkali pretreatment was effective to increase the enzymatic digestibility of sorghum
- Alkali pretreatment conditions of sorghum bicolor stem were optimized using RSM
- At the optimum condition, the glucose yield increased by 7.14 fold

Abstract

Bioethanol production from lignocellulosic biomass provides an alternative energy-production system. Sorghum bicolor stem is a cheap agro-waste for bioethanol production. In this study, response surface methodology (RSM) was used to optimize alkali pretreatment conditions for sorghum bicolor stem with respect to substrate concentration, NaOH concentration and pretreatment time based on a central composite rotary design. The main goal was to achieve the highest glucose and xylose yields after enzymatic hydrolysis. Under optimum conditions of pretreatment (time 60.4 min, solid loading 4.2%, and NaOH concentration 1.7%), yields of 98.94% g glucose/g cellulose and 65.14% g xylose/g hemicellulose were obtained. The results of a confirmation experiment under the optimal conditions agreed well with model predictions. Pretreatment of sorghum bicolor stem at the optimum condition increased the glucose and xylose yields by 7.14 and 3.02 fold, respectively. Alkali pretreatment showed to be a great choice for the pretreatment of sorghum bicolor stem.

Keywords: Sorghum bicolor (broomcorn) stem, enzymatic hydrolysis, response surface methodology, alkali pretreatment.

Over the last few decades, extensive research has been conducted on bioconversion of biomass to fuels and chemicals due to the increasing concerns about environmental protection and energy security [1]. The lignocellulosic biomasses like agricultural residues, municipal and industrial wastes are abundant, and low cost feedstocks that can be converted to products such as fermentable sugars, ethanol and other value-added chemicals [2]. The lignocellulosic materials are composed of cellulose and lignin bound by hemicelluloses chains [3]. Conversion of these feedstocks into fermentable sugars is limited by a number of physico-chemical, structural, and compositional factors [4]. Thus, pretreatment is required to break down the shield formed by lignin and hemicellulose, to disrupt the crystalline structure of cellulose and to decrease the degree of polymerization [5]. Pretreatment makes the cellulose more accessible to enzymatic digestion [6]. Enzymatic hydrolysis of lingocellulosic biomass has been considered as an environmentally friendly method of saccharification that can be a substitute for acid hydrolysis [7].

Optimization studies of processes affected by several factors are difficult and time consuming, especially when the number of variables is large [8]. An efficient approach is the use of statistical methods that provide powerful tools to study and optimize several factors in a process simultaneously [9]. When the number of factors and responses increase, central composite rotary design (CCRD) method is more appropriate because it needs much fewer tests and

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gives almost as much information as the other methods [7,9,10].

Sorghum bicolor (*broomcorn*) is a plant cultivated widely in tropical and subtropical regions. It is used for the fabrication of various brooms and wooden decorative items in Iran. Sorghum stem is a low cost lignocellulosic crop residue that could be converted into fermentable sugars and ethanol. In the current study, sorghum bicolor stem was used as a cheap raw material for production of fermentable sugars. To our knowledge, alkali pretreatment of sorghum bicolor (broomcorn) stem was rarely reported and the main objective of this study was to evaluate the suitability of wasted sorghum stem for production of fermentable sugars using enzymatic hydrolysis. In order to make ethanol production from lignocellulosic materials economically feasible, both hexose and pentose sugars need to be recovered efficiently. Therefore, optimization of pretreatment conditions was conducted for maximum glucose and xylose production. The response surface methodology (RSM) was applied to identify the optimum alkali pretreatment conditions for maximum structural sugars recovery (mainly glucose and xylose) from sorghum stem by analyzing the relationships between a number of parameters that affect the overall process [8]. The main and interaction effects of variables on the yields of liberated sugars were investigated. Based on the obtained response variables, a quadratic model was developed and the optimum condition to attain the highest yield of carbohydrates was predicted and then validated by an extra experiment.

MATERIALS AND METHODS

Substrates and enzymes

The sorghum bicolor (broomcorn) used in this work was obtained from local farmers in Amircola, Babol, Iran. The stems after removal of leaves, hereafter simply referred to as sorghum stem, were used in all experiments. First, the stems were cut to nominally 3-4 cm in length and ground with a food homogenizer (Black & Decker, Model No. FX350, England) and then passed through a 40 mesh screen. The screened materials were stored in tightly sealed plastic bags at room temperature until further use. Total dry content of sorghum bicolor stem was determined by drying at 105 °C for 24 h. The compositional analysis of native sorghum bicolor stem was performed by two stage acid hydrolysis protocol developed by National Renewable Energy Laboratory (NREL) [10]. Two commercial enzymes, *T. reesei* cellulase (Celluclast 1.5L) and β-glucosidase (Novozym 188), were purchased from Novozymes A/S (Bagsvaerd, Denmark) and Sigma (St. Louis, MO) respectively. Activity of cellulase was measured according to the standard procedure provided by the NREL [11]. The activity of Celluclast 1.5L (cellulase) was measured as 45 (FPU)/mL. The β-glucosidase's activity (Novozym 188) was 250 IU/mL reported by the supplier.

Dilute alkali pretreatment

In order to examine the effect of different pretreatment methods on the enzymatic digestibility of sorghum stem, few experiments were conducted. Hydrothermal, dilute sulfuric acid, combined microwave-NaOH and dilute sodium hydroxide pretreatments were examined to select a suitable pretreatment method for the utilized sorghum stem (data not shown). Among these methods, NaOH pretreatment showed to be the most effective with respect to sugars recovery after enzymatic hydrolysis; therefore, it was selected in this work for further investigation. Preliminary experiments were conducted to select the ranges of factors affecting NaOH pretreatment (Table 1). In this set of experiments, the samples containing 1 and 2% NaOH and 4.5% solid loading were pretreated for 30 and 60 min at 121 °C. The yields of glucose after enzymatic hydrolysis of these pretreated samples were considered for evaluation. As shown in Table 1, increase in NaOH concentration and longer time of pretreatment improved the yield of glucose; however it seems that longer time with lower NaOH concentration is superior. Pretreatment at 1% NaOH for 60 min resulted in higher glucose yield compared to that from 2% NaOH and 30 min (Table 1). Consequently, NaOH concentration 0-3% and pretreatment time 15–75 min were selected for further study by CCRD. Working at high solids loadings is advantageous in enzymatic conversion of lignocellulose bio-

Table 1. Results from preliminary experiments

Pretreatment condition		Glucose yield, %
NaOH concentration, %	Pretreatment time, min	
1	30	59.63
	60	84.45
2	30	73.35
	60	91.56

mass as it increases product concentrations. In this work, 10% solid loading was considered as upper limit because the higher solid loadings caused insufficient mixing and increased viscosity of the resultant slurry. Therefore, solid loadings in the range of 2-10% were chosen for the experimental design.

According to the experimental design, the given amount of screened sorghum stem was added into 250 mL flasks containing water solution with desired NaOH concentration to obtained 100 mL slurry. The flasks were placed in autoclave (Hirayama, HV-25, Japan) at 121 °C for different time period according to the experimental design. All flasks were capped tightly in order to prevent evaporation during pretreatment. After autoclaving, pretreated samples were filtered through a Whatman filter paper and thoroughly washed with distilled water until neutral pH was achieved. The samples were also taken from the liquid fraction to be analyzed for the glucose and xylose concentrations. The filtered cakes were then used as the substrates for enzymatic hydrolysis experiments.

Enzymatic hydrolysis

The solid residue of pretreated sorghum was soaked in citrate buffer (50 mM, pH 4.8) and then sterilized in an autoclave at 121 °C for 15 min. The flasks were cooled down to 50 °C before removing them from the autoclave. The cellulase was supplemented with Novozyme 188 to reduce inhibition effect of cellobiose on cellulase. The β-glucosidase enzyme was added at 90 IU/g of dry biomass. Enzymatic hydrolysis was carried out at 50 °C and 150 rpm in a shaker/incubator (IKA, Japan) for 24 h. After hydrolysis, the samples were taken and centrifuged at 13000 rpm for 5 min (Micro Centrifuge, Hermle, Germany) to remove the residue and supernatant was stored at -20 °C for glucose and xylose determination.

Glucose and xylose yields from celluloses and hemicellulose conversion were calculated as follows:

$$\text{Yield}_{\text{Glucose}}(\%) = 100\frac{GH}{GS}\frac{162.2}{180.2} \quad (1)$$

$$\text{Yield}_{\text{xylose}}(\%) = 100\frac{XH}{XS}\frac{132}{150} \quad (2)$$

where *GS* and *XS* are gram glucan and xylan in dry biomass, respectively. *GH* and *XH* are the amount of glucose (g) and xylose (g) present in the aqueous phase of samples after enzymatic hydrolysis.

Analytical methods

All liquid samples from the pretreatment and enzymatic hydrolysis experiments were filtered using a 0.45 μm filter and then analyzed. A HPLC system (Knauer, Germany) equipped with a refractive index (RI) detector (Knauer, Smartline RI Detector 2400, Germany) was used to measure sugar and acetic acid concentrations. An Eurokat H (10 μm) column, 300 mm×8 mm kept at 75 °C and eluted with 0.01 N H_2SO_4 at a flow rate 0.4 mL/min was used for measuring glucose and xylose concentrations. Furfural and hydroxylmethyl furfural (HMF) were determined using HPLC system equipped with Eurospher II (100-5 C18 P, 150 mm×4.0 mm ID) and a UV absorbance detector at 275 nm (Knauer, Smartline UV Detector 2500, Germany). The column temperature was fixed at 25 °C. Water and methanol (20 and 80%) were used as eluent at flow rate of 1 mL min^{-1}.

SEM Analysis

Physical changes and surface characteristics of the native and pretreated sorghum stem were observed by scanning electron microscopy (SEM). Images of the native and pretreated samples were taken using a KYKY-EM 3200 scanning electron microscope (China). The specimens were coated with a gold palladium using a SCD 005 sputter coater (BAL--TEC, Switzerland), mounted with a conductive tape and observed using a voltage of 24 kV.

Statistical analysis

A central composite experimental design (CCRD) for three factors was used as presented in Table 2. A total of 20 experimental runs including 8 tests for factorial points, 6 tests for axial points and 6 replication tests at central points were carried out. The effects of three operating variables of the alkali pretreatment, including solid concentration (X_1), pretreatment time (X_2), and NaOH concentration (X_3), on two response variables, i.e. glucose yield (Y_1) and xylose yield (Y_2) were determined. The independent variables used at five coded levels (-α, -1, 0, +1, +α) [7]. Based on the results of our preliminary experiments and the data from similar lignocellulosic materials available in literature, pretreatment variables were selected in the range of 15-75 min, 2-10% and 0-3% for time, solid loading and NaOH concentration, respectively [12-14]. The response values (glucose and xylose yields) are the average of duplicate experiments.

A second-order quadratic equation was fitted to evaluate the effect of each independent variable to the response [8]:

$$Y = \beta_0 + \sum_{i=1}^{k}\beta_i x_i + \sum_{i=1}^{k}\beta_{ii} x_i^2 + \sum_{i=1}^{k}\sum_{i=1}^{k}\beta_{ij} x_i x_j + \varepsilon \quad (3)$$

Table 2. Observed and predicted values for glucose and xylose yields

Run no.	Actual level of variables			Response			
	Pretreatment time min	Solid concentration % (*w*/*v*)	NaOH concentration % (*w*/*v*)	Glucose yield		Xylose yield	
				Observed	Predicted	Observed	Predicted
1	75	6.00	1.50	97.18	87.90	51.78	54.88
2	60	4.00	0.75	81.12	79.81	61.27	56.04
3	45	2.00	1.50	86.20	81.67	55.96	60.95
4	45	10.0	1.50	39.77	35.69	30.41	30.02
5	30	8.00	2.25	49.90	52.70	36.49	38.28
6	45	6.00	1.50	94.07	91.97	58.95	59.49
7	60	8.00	0.75	56.36	55.70	41.69	39.68
8	60	8.00	2.25	59.33	61.06	44.30	45.68
9	45	6.00	1.50	92.78	91.97	58.37	59.49
10	45	6.00	3.00	59.64	49.20	42.73	41.18
11	60	4.00	2.25	89.85	95.30	62.96	61.94
12	45	6.00	0.00	14.54	20.57	23.62	29.78
13	45	6.00	1.50	92.46	91.97	59.56	59.49
14	30	8.00	0.75	43.51	39.56	35.21	32.78
15	15	6.00	1.50	46.87	51.03	37.18	38.89
16	45	6.00	1.50	92.13	91.97	57.39	59.49
17	45	6.00	1.50	94.07	91.97	58.95	59.49
18	30	4.00	0.75	58.81	51.30	52.26	47.45
19	45	6.00	1.50	93.43	91.97	58.01	59.49
20	30	4.00	2.25	72.38	74.59	54.26	52.85

where Y is the process response, k is the number of the patterns, i and j are the index numbers for pattern, β_0 is the free term called intercept term, x_1, x_2,…,x_k are the independent variables, β_i is the ith linear coefficient, β_{ii} is the quadratic effect, β_{ij} is the interaction effect, and ε is the random error [15]. Design-Expert 7.01 statistical software (Stat-Ease, Inc., Minneapolis, MN, USA) was used for regression analysis of experimental data. Analysis of variance (ANOVA) was used to estimate the statistical parameters.

RESULTS AND DISCUSSION

Characterization, pretreatment and enzymatic hydrolysis

The dry matter content of the sorghum bicolor was measured at 93.09%. The composition of sorghum stem was analyzed with respect to sugars, extractives, acid insoluble lignin, acid soluble lignin and ash [10]. Cellulose was the major component (47.58%) in sorghum stem followed by hemicellulose (24.66%) and acid insoluble lignin (22.98%). The content of acid soluble lignin, ash and extractives were 1.75, 1.93 and 1.1%, respectively. Cellulose and hemicelluloses accounted for about 72% of the total solid content of sorghum stem.

Sorghum stem was selected as a substrate for enzymatic hydrolysis because of its local and abundant availability. It is a lignocellulosic biomass that cannot be hydrolyzed by enzyme in a high yield without pretreatment [7]. In the current study, sorghum stem was pretreated with NaOH prior to enzymatic hydrolysis. Pretreatment of the sorghum stem was performed according to the experimental design presented in Table 2. Analysis of samples from liquid fraction showed the glucose concentration was very low and almost identical in all samples. On the contrary, xylose concentration showed variations in different liquid samples with maximum concentration of 1.05 (g/L), which is about 6.5% of total equivalent xylose content. In overall, higher xylose concentration in the liquor was obtained at more severe pretreatment conditions (longer time and higher NaOH concentration), due to higher fractional hemicellulose dissolution and *vice versa*. Therefore, both glucose and xylose concentrations in liquid fraction were not considered in this study.

The cellulase and β-glucosidase enzymes were used at concentration previously mentioned for hydrolysis of pretreated samples. Xylanase supplementation was not examined since hemicellulase activity was identified in the *Trichoderma reesei* cellulases system by 2D electrophoresis [16]. This fact might probably explain the reason why xylanase addition was not necessary since Celluclast 1.5L is produced from *T. reesei*. The results for glucose and xylose yields as two response variables are displayed in Table 2. The highest value for glucose and xylose yield achieved 97.18% and 62.96% at run numbers 1 and 11, respectively.

Furfural and HMF as inhibitors for the subsequent fermentation step were not detected in substantial level in any samples; concentration of acetic acid was 0.1 g/L. Xylose is liberated into the liquid during hemicellulose degradation and further degraded to furfural at high temperatures. Therefore, the lack of furfural formation is most probably due to stability of xylose under the applied temperature. It is an important advantage of NaOH pretreatment over other pretreatments such as acid pretreatment.

Statistical analysis of the experimental results

In this study, RSM technique was applied to explore the optimum response region of fermentable sugars' yield and to optimize the corresponding variables. The results of CCRD tests are presented along with the observed and predicted responses in Table 2. The responses of the CCRD design were fitted with a second-order quadratic equations (Eqs. (4) and (5)). The equations representing the relationship of the response variables (Y_1 and Y_2) as a simultaneous function of independent variables, can be expressed as follows:

$$Y_1 = -156.785 + 3.742X_1 + 26.38X_2 + 103.58X_3 - \\ -0.103X_1X_2 - 0.173X_1X_3 - 1.69X_2X_3 - \\ -0.025X_1^2 - 2.08X_2^2 - 25.37X_3^2 \quad (4)$$

$$Y_2 = -21.76 + 1.594X_1 + 7.24X_2 + 35.22X_3 - 0.014X_1X_2 + \\ +0.011X_1X_3 + 0.016X_2X_3 - 0.014X_1^2 - \\ -0.875X_2^2 - 10.67X_3^2 \quad (5)$$

Analysis of variance of the quadratic equations for glucose and xylose yields was performed by Fisher's statistical test. Evaluated ANOVA results for glucose and xylose yields shown in Table 3, suggested that the regression was statistically significant. The *p*-value denoting the significance of the coeffi-

Table 3. Results of ANOVA for quadratic models of glucose and xylose yields

Response	Source of variation	Sum of squares	Degree of freedom	Mean square	*F*-value	Probability (*p*)	Significance
$Y_{Glucose}$	Model	10538.8	9	1170.99	25.42	<0.0001	Significant
	X1	1508.35	1	1508.35	32.75	0.0002	
	X2	2135.6	1	2135.6	46.36	<0.0001	
	X3	819.82	1	819.82	17.8	0.0018	
	X12	77.07	1	77.07	1.67	0.2249	
	X13	30.38	1	30.38	0.66	0.4356	
	X23	51.36	1	51.36	1.12	0.3158	
	X_1^2	770.91	1	770.91	16.74	0.0022	
	X_2^2	1741.4	1	1741.4	37.81	0.0001	
	X_3^2	5120.6	1	5120.6	111.17	<0.0001	
	Residual	460.61	10	46.06			
	R2	0.9581					
	Adj R2	0.9204					
	CV	9.68					
Y_{Xylose}	Model	2426.9	9	269.66	17.66	<0.0001	Significant
	X1	234.09	1	234.09	15.33	0.0029	
	X2	963.48	1	963.48	63.09	<0.0001	
	X3	131.10	1	131.10	8.58	0.015	
	X12	1.46	1	1.46	0.096	0.76	
	X13	0.13	1	0.13	0.0085	0.928	
	X23	0.005	1	0.005	0.000327	0.9859	
	X_1^2	253.82	1	253.82	16.62	0.0022	
	X_2^2	308.18	1	308.18	20.18	0.0012	
	X_3^2	906.21	1	906.21	59.34	<0.0001	
	Residual	152.73	10	15.27			
	R2	0.9408					
	Adj R2	0.8875					
	CV	7.96					

cients was important in understanding the pattern of mutual interaction between the variables [3]. The model terms with *p*-values less than 0.05 were considered statistically significant. The *p*-value for both models was < 0.0001. The *F*-values of 25.42 and 17.66 for the yields of glucose and xylose respectively, also suggest that both models were significant. The correlation among the observed experiments results and the predicted values by models can be checked by the determination coefficient (R^2). The high values of R^2 and adjusted R^2 of models show a close agreement between the experimental results and predicted values by the models and demonstrate that the models are well fitted. The coefficient of determination of the models were 0.9581 and 0.9408 for glucose and xylose yields respectively, which further represent that the models were suitable to show the real relationships between the selected reaction variables. A lower value for the coefficient of variation (*CV*) suggests higher reliability of experiments. In this work, the corresponding *CV* values were 9.68 and 7.96% for the yields of glucose and xylose. As shown in Table 3, linear terms (X_1, X_2, X_3) and quadratic terms (X_1^2, X_2^2, X_3^2) are the major significant factors ($P < 0.05$) affecting the glucose and xylose yields. However, the effects of interactive model terms (X_{12}, X_{13}, X_{23}) were not significant.

Figure 1 shows the correlation between predicted and experimental results. This means that the developed statistical models provide an excellent description of the relationship between the independent variables and the responses. Therefore, these models proved to be powerful tools for process optimization [7,8,17].

Effect of parameters on responses

In order to study the interaction among different independent variables and their corresponding effect on the response, counter plots and three dimensional response surface plots were depicted. The responses for the yields of glucose and xylose were depicted by the iso-response contour plots and three-dimensional surface plots in Figures 2 and 3. In these plots, two factors were varied at a time while the other factors were kept at center levels [9]. The shapes of res-

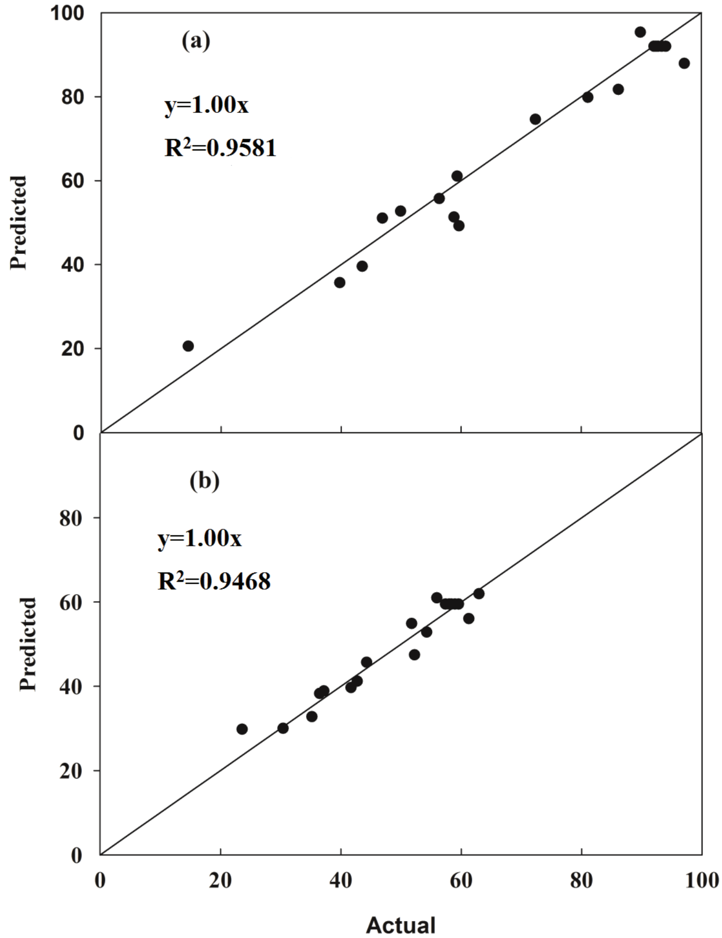

Figure 1. Actual vs. predicted values, a) glucose yield; b) xylose yield.

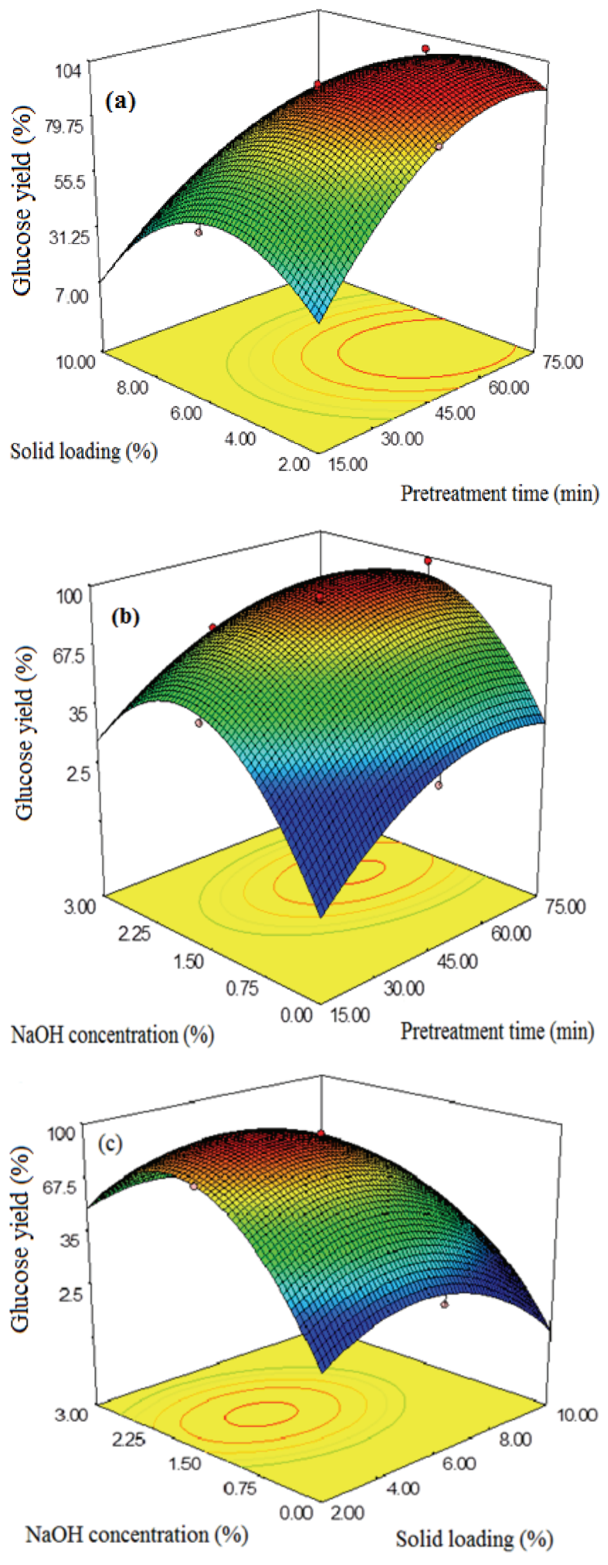

Figure 2. The effect of: a) pretreatment time and solid loading; b) pretreatment time and NaOH concentration; c) solid loading and NaOH concentration on the glucose yield.

ponse surfaces and contour plots indicate the nature and extent of the interaction between various vari-

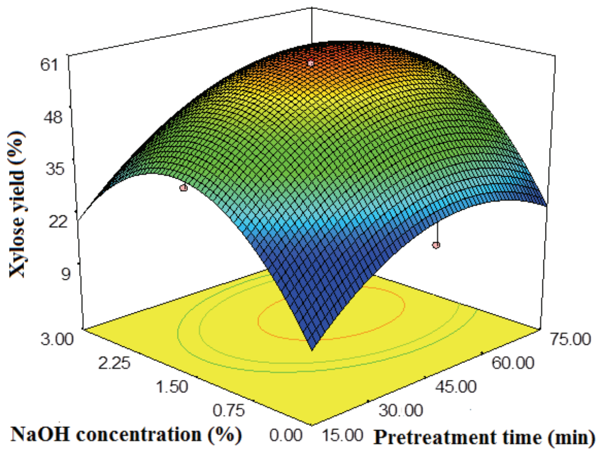

Figure 3. The effect of pretreatment time and NaOH concentration on the xylose yield.

ables [18]. Generally, circular contour plots display that the interactions between parameters were negligible. On the contrary, elliptical ones indicate the evidence of the interactions between the independent variables [19]. Figure 2a represents the effects of solid loading and pretreatment time levels on the yield of glucose when NaOH concentrations were kept at center point. The circular nature of the contour plot indicates that interactive effects between the test variables are not significant, which is in agreement with the results of ANOVA. Solid loading is one of the major factors affecting the conversion rate of enzymatic hydrolysis of lignocellulosic materials. Increasing glucose yield correlated with an increase in solid loading to a point (about 6%) beyond which resulted in a decrease in glucose as shown by Figure 2a. Similar effects of these variables were observed for the second response variable (Y_2). The surface plot indicating the effects of these variables on the xylose yield was similar to Figure 2a (not shown). Gregg *et al.* showed that high solid loading resulted in low hydrolysis yield due to product inhibition, enzymatic deactivation, and a decrease in the reactivity of cellulosic substrate with progress of hydrolysis process [20]. The effect of high solid concentration in enzymatic hydrolysis and SSF has been studied by several groups working with various biomasses [21-26]. Several of these investigations were conducted at less than 10% initial solid loading. Reduction of enzyme efficiency at higher solid loadings appears to be an intrinsic or generic effect of enzymatic hydrolysis at high solid levels. Some researchers suggested that the reason of decreasing enzymatic yield at high solid loadings is due to product inhibition or inhibition by other compounds such as lignin, furfural and HMF [21,22,27]. Others proposed it may be explained by mass transfer limitation or other effects related to the increased content of insoluble solids, such as nonproductive adsorption of enzymes [28]. Kristensen *et al.* (2009), reported that inhibition of enzyme adsorption by hydrolysis products was the main cause of the decreasing yields at higher solid loadings during the enzymatic hydrolysis of lignocellulosic materials [26]. At mid-level solid loading and longer pretreatment time, the optimum yield of glucose could be attained. The effects of NaOH concentration and pretreatment time on glucose yield are illustrated in Figure 2b. Glucose yield was low at zero concentration of NaOH and increased with raise in NaOH concentration up to 1.5%. However, further increases in NaOH concentration decreased the yield of glucose due to higher solid loss. This finding was in agreement with Wang *et al.* [12]. They reported that higher NaOH concentrations and longer times of pretreatment resulted in lower solid recovery and higher lignin removal. The effect of variation in NaOH concentration and pretreatment time on the yield of xylose is similar to the effect of these variables on the glucose yield and therefore, the same results can be derived (Figure 3). The interdependence of NaOH concentration and solid loading on the glucose yield is shown in Figure 2c. The surface plot illustrating the interaction between these variables for xylose yield is similar (not shown). With increase in solid loading from 2 to 6%, glucose yield reached a peak at mid-level NaOH concentration (center point). However, higher amounts of solid loading showed negative effect on the yield of glucose. With an increase in NaOH concentration, the yield of xylose increased gradually at the higher solid loading. An increase in NaOH concentration (0–1.5%) with solid loading from 2 to 6% enhanced the xylose yield.

The results of this study indicate that the yield of glucose improved as pretreatment time increased while the xylose yield slightly decreased at longer pretreatment time because hemicellulose is more vulnerable to chemical pretreatments than cellulose [12]. Overall, the effects of different variables on glucose and xylose recovery were similar. This is an advantage because it allows maximum recovery of both sugars under fairly identical conditions. It is however noteworthy to mention that the second response is slightly more sensitive to the time of pretreatment as the maximum yield was attained at shorter pretreatment time (Figure 3).

Confirmation experiments

The optimal pretreatment conditions for production of fermentable sugars were predicted by Design Expert software. Optimal pretreatment conditions improve cellulose susceptibility tor enzymatic attack thereby maximizing total glucose yield. The optimum operating conditions were: x_1 = 60.4 min, x_2 = 4.2%, x_3 = 1.7% for glucose yield and x_1 = 55.7 min, x_2 = 3.72%, x_3 = 1.68% for xylose yield. At optimum conditions for glucose yield, corresponding glucose and xylose yields were calculated 98.94 and 65.14%, respectively, while the yield of glucose and xylose was calculated 97.74 and 65.54%, respectively at the optimum condition for xylose yield. These results show that the optimum pretreatment conditions are very close to each other, therefore both conditions can be considered as optimal for two responses, which is a great advantage of the applied pretreatment method. In the present work, the optimum conditions for glucose yield were considered for verification experiments. In order to examine the validity of the optimal condition predicted for glucose yield, confirmation experiments were carried out in triplicates. The average results of these experiments were 98.86% (±0.18) and 65.02% (±0.32) for glucose and xylose yields, respectively. The predicted values of the maximum glucose and xylose yields were 98.94 and 65.14% respectively. Since excellent prediction performance was obtained using the models, mean square error (*MSE*) was used to measure the average of the square error between the predicted and observed values. A low values of mean square error (*MSE* = 0.028 and *MSE* = 0.0846 for the glucose and xylose yields, respectively) show that the models were reasonably accurate, and RSM analysis is indeed a useful technique to predict and optimize the pretreatment conditions of lignocellulosic materials.

Several studies were reported about alkali-pretreatment using different lignocellulosic materials [7,12,29–32]. McIntosh and Vancov studied the impacts of varying pretreatment parameters (temperature, time and alkanity) on enzymatic digestion of sorghum bicolor straw. They reported that total sugar release peaked when sorghum straw was pretreated in 2% NaOH at 121 °C for 60 min; representing enzyme saccharification improved by 5.6-fold when compared to the samples pretreated at 60 °C in the absence of alkali [31]. Wang *et al.* used NaOH for pretreatment of costal Bermuda grass. They observed up to 86% lignin removal and optimal NaOH pretreatment condition at 121 °C was reported 15 min and 0.75% NaOH concentration that under this condition, total reducing sugar yield was about 71% of the theoretical maximum [12]. Mirahmadi *et al.* used NaOH for pretreatment of spruce and birch. They reported that alkali pretreatment resulted in a significant reduction of both hemicellulose and the crystallinity of cellulose, which might be responsible for observed improvement in enzymatic hydrolyses of birch (from 6.9 to 82.3%) and spruce (from 14.1 to 35.7%) [33]. Zhang *et al.* studied ability of alkali pretreatment method to increase enzymatic digestibility of cattail cellulose. They observed the highest glucose yield (77.5% of the cellulose) was obtained when the cattails were pretreated with a 4% NaOH solution and when applying a 0.5–4% NaOH solution, approximately 25.5–56% of the lignin was removed [34]. Alkali pretreatment was used for pretreatment of cogon grass by Lin *et al.* They reported that NaOH pretreatment at room temperature led to an increase in cellulose content (38.5 to 60.5% glucan) due to the partial removal of hemicellulose and lignin fractions [35]. These results suggest that NaOH pretreatment is an efficient method for pretreatment of lignocellulosic material however; the optimal condition may vary depending on the materials used.

Finally, the glucose and xylose yields were significantly increased from 13.85 and 21.56% to 98.94 and 65.14%, respectively when sorghum stem pretreated by NaOH at optimum condition. Therefore NaOH pretreatment method was able to effectively increase the enzymatic digestibility of sorghum stem and the glucose and xylose yields after enzymatic hydrolysis were increased by 7.14- and 3.02-fold, respectively. The chemical composition of sorghum stem may vary depending on several factors such as cultivar type, farming inputs and practises, geographical location, seasonal conditions, and stage of harvest [31]. It is noteworthy to mention that total lignin content of sorghum stem used in the present work was much higher, that is, 22.98% compared to only 7% in the reported straw by McIntosh and Vancov, making it more resistant against enzymatic hydrolysis [5,36]. Despite different chemical composition, the results obtained were consistent with those previously reported. This fact suggests that alkaline pretreatment is a suitable choice for pretreatment of agricultural residues. Initial lignin content as the main barrier against enzymatic hydrolysis shows little effect on the efficiency of enzymatic hydrolysis when NaOH pretreated stem is used.

Effect of pretreatment on the composition of sorghum stem under optimal condition

In general, the alkali pretreatments decrease the lignin content, which is responsible for reduced accessibility of enzyme to the hemicellulose and cellulose. Alkali are preferred over other pretreatment methods due to its ability for selective removal of lignin and for less degradation of carbohydrates [37]. NaOH is known to cleave ester linkages between xylan and lignin, leading to increased porosity of the resultant biomass [13,38,39]. Also, Chang and Holtzapple reported partial removal of acetyl and different kinds of uronic acid substitutions on hemicellulose [40]. The compositional analysis of the solid residue after pretreatment at the optimum condition was carried out. Chemical composition, weight loss and lignin removal were characterized. The pretreatment of sorghum stem resulted in 42.13% weight loss mainly caused from solubilization lignin and hemicelluloses; therefore, the cellulose content of the pretreated residue was increased. Residue with cellulose content of 67.24% was obtained at the optimum pretreatment condition. At this condition, 76.45% of lignin content was removed from the raw material. The hemicellulose content for untreated sorghum was about 24.66%, which decreased to 20.31% after the pretreatment. Substantial removal of lignin and partial removal hemicelluloses along with increased porosity might explain the significant enhanced rate of enzymatic hydrolysis. Scanning electron microscope (SEM) images of native and the pretreated sorghum stem are represented in Figure 4. For the untreated sorghum stem, a smooth surface, compact structure and intact morphology was clearly observed (Figure 4a). However, after the pretreatment at optimal condition, the regular structure of sorghum was distorted and the porosity of resultant biomass was significantly increased (Figure 4b).

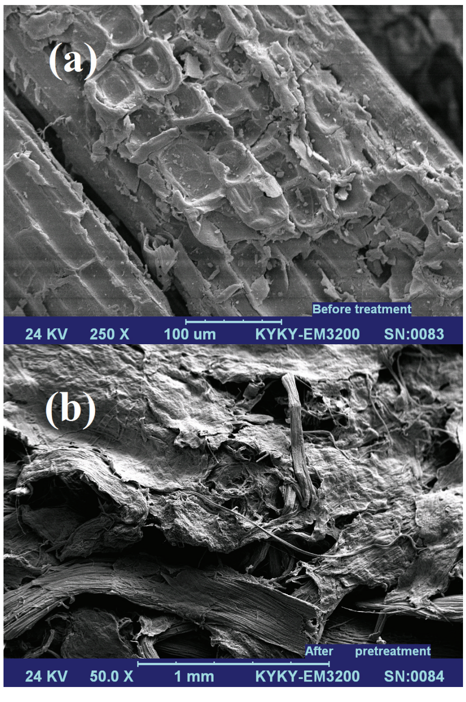

Figure 4. Scanning electron microscopic images of: a) native sorghum stem; b) pretreated sorghum stem.

CONCLUSION

This study is one of the first reports that evaluates the use of sorghum bicolor (broomcorn) stem as a resource to produce fermentable sugars. The conditions for alkali pretreatment of sorghum stem were optimized by using statistical approach. Enzymatic hydrolysis was then performed to evaluate the optimum conditions for maximizing sugar production. Calculated statistical values indicate that the models were very significant. The optimum condition was found as follows: pretreatment time at 60.4 min, solid concentration at 4.2% and NaOH concentration at 1.7% for the glucose yield. The maximum yields of glucose and xylose obtained under the optimum conditions were 98.94 and 65.14%, respectively. The optimization methodology represents a valuable tool for optimization of process factors for alkali pretreatment using sorghum stem as a low cost residue crop.

Nomenclature

Y_2 Xylose yield (%)
β_0 Constant term
CV Coefficient of variation
β_i Coefficients of the linear parameters
GH Amount of glucose present in the aqueous phase after hydrolysis (g)
β_{ij} Coefficients of the interaction parameters
GS Amount of glucan present in dry sorghum (g)
ε Residual
R^2 Coefficient of determination
R^2_{adj} Adjusted coefficient of determination
ANOVA Analysis of variance
XH Amount of xylose present in the aqueous phase after hydrolysis (g)
FPU Filter paper unit
x_i Factor
NREL National Renewable Energy Laboratory
X_i Real value of the independent variable
RSM Response surface methodology
XS Amount of xylan present in dry sorghum (g)
CCRD Central composite rotary design
Y_1 Glucose yield (%)
MSE Mean square error

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NAUČNI RAD

STATISTIČKA OPTIMIZACIJA USLOVA ALKALNOG PREDTRETMANA STABLJIKI SIRKA RADI POSTIZANJA MAKSIMALNOG PRINOSA ŠEĆERA

Produkcija bioetanola iz lignocelulozne biomase obezbeđuje sistem proizvodnje alternativne energije. Stabljika sirka je jeftin agro-otpad pogodan za dobijanje bioetanola. U ovom radu, metodologija površine odziva (RSM) je korišćena za optimizaciju uslova predtretmana stabljiki sirka, i to: koncentracije supstrata, koncentracije NaOH i vreme predtretmana, koristeći centralni kompozitni rotacioni plan. Glavni cilj je bio da se postignu najveći prinosi glukoze i ksiloze posle enzimske hidrolize. Pri optimalnim uslovima predtretmana, tj. vreme 60,4 min, doza biljnog materijala 4,2% i koncentracija NaOH 1,7%, postignuti su prinosi glukoze i ksiloze od 98,94 i 65,14%, redom. Rezultati verifikacionog eksperimenta pod optimalnim uslovima se dobro slažu sa predviđanjima modela. Predtretman stabljiki sirka pod optimalnim uslovima je povećao prinose glukoze i ksiloze za 7,14 i 3,02 puta, redom. Alkalni predtretman je pokazao kao dobar izbor za prethodni tretman stabljiki sirka.

Ključne reči: stabljika sirka, Sorghum bicolor, enzimska hidroliza, metodologija površine odziva, alkalni predtretman.