EUROPEAN JOURNAL OF MATERIALS SCIENCE AND ENGINEERING
Volume 5, Issue 4, 2020: 234-245 | www.ejmse.ro | ISSN: 2537-4338

EJMSE

DOI: 10.36868/ejmse.2020.05.04.234

OPTIMIZATION OF HYDROLYSIS CONDITIONS USING CENTRAL COMPOSITE DESIGN TO CONVERT CORN STOVER TO BIOETHANOL

Ketema BEYECHA HUNDIE[1,*], Abraham BEKELE BAYU[1]

[1]School of Chemical Engineering, Jimma University, Institute of Technology, Jimma, Ethiopia.

Abstract

The goal of the research was to find out optimum hydrolysis parameters to convert corn stover to bioethanol using response surface methodology. The pretreatment of corn stover was administered using alkali potassium hydroxide. The results of the pretreated indicated that the important elements of corn stover were cellulose, hemicellulose, and lignin, 35.23, 23.5, and 16.3% respectively. Additionally, cellulose is the main component of cornstover that is sufficient for biofuel production. Experimental layout and statistical evaluation were carried out using the response surface technique. Acid concentration at (1.5-2.5%, w/w), particle dimension (0.15-0.25 mm), temperature (125-145 °C) and time (30-80 min) were used to evaluate the hydrolysis parameters. The result indicates that the optimum hydrolyzed parameters were, acid concentration; 2.334 (w/w %), particle size; 0.153 mm, temperature; 144.976 ^{0}C, and time; 77.233 minutes. The optimized independent variables were derived from the quadratic model and selected primarily based on the highest desirability. Under these conditions, the yield of glucose and xylose was 48.69 and 33.091% respectively. After 48 h of fermentation time, 27.1 g of ethyl alcohol concentration was investigated (this is equivalent to 92.07% of theoretical yield of ethyl alcohol at optimized conditions).

Keywords*: biomass, alkali pretreatment, response surface methodology, biofuel*

Introduction

Energy price, energy security, trade deficits, environmental and pollution, could be improved, if the production of bioethanol, will be carried out from lignocellulosic biomass [1]. Since biofuel is considered a renewable energy source, compared to fossil fuels, it is necessary to replace fossil fuel with renewable energy [2]. The use of bioethanol as a shipping fuel has been believed to be an appropriate substitution for nonrenewable based energy [3]. However, currently bioethanol production is highly food crops based energy [4]. To avoid these conflicts via food-based, the use of crop residues are believed as feedstock for biofuel production as a renewable resource. The most available resources worldwide are second-generation biofuels. These advanced biofuels do not compete with food-security and environmental pollution compared to fuels derived from the first generation [3].

Currently, there is a great deal of research being carried out to convert this lignocellulosic biomass to biofuels for replacement of fossil fuel [5]. Lignocellulosic biomass is considered the most alternative available resource and renewable source of energy that has been highly used as a feedstock to produce bioethanol [6].

Since, lignocellulosic biomass contains cellulose, hemicellulose and lignin compositions constituting a complex structure which limits response media to get in near contact with cellulose; hence this biomass is not effectively changed into bioethanol [7]. Pre-treatment, hydrolysis, fermentation, and product separation are the main steps for bioethanol production from

Corresponding author: ktmbych2001@gmail.com or ambo bara2012@gmail.com

lignocellulosic biomass[8]. Therefore, effective pretreatment and hydrolysis are sequential step to obtain highly fermentable sugar.

With a common object of causing biomass greater susceptible to hydrolysis, several types of pretreatment techniques have been employed on a global scale with acid and alkaline methods as the gold standard for lignocellulosic material decomposition [9]. One of the best viable process options mainly to its effective pretreatment and comparatively simple process is applying alkaline for biomass pretreatment. The main reason for alkali pretreatment is that due to selectively removal of lignin without any degrading sugars. In addition, it enhances the porosity and surface area, by favoring hydrolysis conditions[10].

Hydrolysis is another essential step to obtain good fermentable sugars from lignocellulosic biomass. Concentrated and dilute acid hydrolysis, as well as the enzyme are used for hydrolysis of lignocellulosic materials [11]. Previously several studies have used dilute acid pretreatment that indicates high sugar yields from pretreated and next hydrolysis of the pretreated sugar [12]. However, attention was to identifying hydrolysis conditions that will result in the highest yields of sugars from the hydrolysis stage using H_2SO_4 as a hydrolyzer. Dilute or concentrated acid can be employed, to hydrolysis cellulose and hemicellulose present in the lignocellulosic biomass to simple sugars while the remaining lignin, can be used as boiler fuel and generate extra electricity [1].

For the commercial application of the quality product, optimizations of hydrolysis variables are very important and this could be attained by empirical or statistical analysis. One of the generations of statistical and mathematical techniques that are beneficial for formulating, and optimizing process parameters is the response surface method. The primary advantage of this method is, used to minimize the number of experimental trials and selects the optimum point of parameters, and is used to evaluate multiple variables and their interactions [13].

Several researchers have been done on bioethanol production form corn stover. Even though, particle size as well as dilute acid hydrolysis is not included in the previous study in the hydrolysis step to obtain optimum dependent as well as independent parameters. Therefore, the objective of the present study was, to prove hydrolysis parameters (acid concentration, particle size, temperature, and time) using the response surface method (RSM) to convert corn stover biomass to bioethanol.

Materials and Methods

Sample preparation

The corn stover was collected from the farmers in Jimma (Gibe; Ethiopia) and saved for evaluation. It was cleaned, shredded, and dried using an electric oven at 60 °C for one day at a moisture content of 10 %. By varying the dimensions of the sample below 4mm (0.1−0. 25 mm), it was milled using a laboratory mill, sieved and stored in cool material at 25^0C for the next step.

Alkali pretreatment

Potassium hydroxide (KOH) was used to pretreat the corn stover under optimum pretreatment conditions. The sample pretreatment was carried out at 10% (w/w) solids loading by taking 2% KOH at 121 °C for 26min in sterilizer [10]. Then the sterilized result percolated to classify the firm relaxation and the separated. The firm rest very washed using deionized water to remove the residual alkali until the pH of the residue turned to neutral.

Compositional analysis

Thereafter, the samples were stored at 25 °C for subsequent steps; 30g of the sieved sample was taken to a laboratory for physicochemical evaluation consistent with strategies defined utilizing (AOAC 2012) and ASTM test.

Hydrolysis

The hydrolysis was conducted based on a previous study with modification[14]. Using H_2SO_4 concentration (1.5–2.5 w/v %), temperature (125–145 °C), hydrolysis time (30–80 min) and particle size (0.15 to 0.25 mm). Cellulose and hemicellulose were decomposed to obtain monosaccharide sugars by fixing to 10v/w of liquid to material ratio, in the 250-ml volumetric flask. After completion of hydrolysis time, the liquefied phase of the hydrolyzate was chilled, percolated, gathered, and then conformed to bring pH to five by using targeted H_2SO_4 with 2N KOH, and the combination developed for the subsequent step.

The consequences of hydrolysis time, temperature, H_2SO_4 concentration, particle size, and as well as their interaction effects were studied using Design-Expert version 11.1 software. The summary of the design is indicated in Table 1 including coded and real value.

Table 1. Central Composite Design to optimize hydrolysis of corn stover

Factor	Units	Minimum	Maximum	Level		
A - Acid concentration (wt %)	wt.%	1.5	3.5	1.5(-1)	2.5 (0)	3.5(+1)
B - Particle dimension (mm)	mm	0.15	0.25	0.15(-1)	0.2(0)	0.25(+1)
C - Temperature (°C)	^{0}C	125	145	125 (-1)	135 (0)	145(+1)
D - Time (min)	min	30	80	15(-1)	52.5 (0)	80(+1)

The yield of glucose, xylose, and ethanol had been chosen as responses for evaluations. Thirty numbers (30) of experiments with central points have been executed according to Eq. (1):

$$R = 2^V + 2\times V + C_P \quad (1)$$

Where:
R – the total number of runs;
V - several variables;
C_P - the number of the center point.

Measurement of reducing sugars

A standard stock solution of glucose and xylose was developed according to [15] including modification. Sugar concentration and absorbance relations were determined using Lambert's-law at 490 nm from hexoses group glucose and at 480nm from pentose groups" xylose. The sugar concentration present within the sample solution was determined using the standard graph of Eq. (2):

$$Y = mx + b \quad (2)$$

Where:
y - stands for absorbance;
x – concentration;
m – slope;
b – intercept.

For the concentration of the unknown sample (C) and sugar, yield Eqs. (3−4) were developed.

$$C\ (mg/ml) = (\text{Absorbance of unknown sample} - \text{intercept})\ /\text{slope} \quad (3)$$

$$\text{Sugar yield} = (\text{gram of sugar}) - \text{intercept})/\ \text{gram of raw material used} * 100 \quad (4)$$

Microorganism and fermentation

Where the ethyl alcohol is directly produced from the metabolism activity of the zymosis agent, zymosis is one critical stage in ethyl alcohol production. Hydrolyzate, in this process, is

introduced to a specific zymosis agent (yeast or bacteria) according to the suitability to digest the respective sugar bond. In the course of zymosis, the sugars discharged during hydrolysis are fermented into ethanol, in alignment with the formation of carbon oxide. The pH was adjusted during zymosis by adding 2 N KOH. The process was started in a 250 mL flask with a working volume of 150 mL at pH 6.5 solution and 35 °C under optimized conditions [16]. S. cerevisiae was obtained from Holeta agricultural research center. The Strains were cultivated in a 200 mL flask with 150 mL working volume of the vaccination medium containing Agar; 3.5g/L, sugar (glucose); 15g/L, sugar (Pentose); 10g/L, Magnesium sulfate; 0.25g/L and Ammonium sulfate; 1.5 g/L.

The samples were withdrawn periodically to investigate ethanol production and sugar utilization at different fermentation times (12, 24, 36, 48, and 72 hours).

The ethyl alcohol yield (Y) and maximum theoretical amount (Y_m %) have been calculated by the use of Eqs. (5a and 5b) respectively:

$$Y\% = \text{amount of ethyl alcohol obtained/ amount of sugar used } *100 \quad (5a)$$

$$Ym\ \% = 1.96* (\text{gram of ethyl alcohol / gram sugar})/*100 \quad (5a)$$

Analysis

Centrifuge is necessary after fermented sugar and the solution had been centrifuged for 10 min at 8,000 rpm to classify the supernatants. The supernatants have been percolated later on separation and evaluated for sugars and ethanol determination by using high-performance liquid chromatography (HPLC) using refractive index (RI) detectors.

Results and Discussion

Pretreatment and composition analysis

To evaluate the chemical compositions of the corn stover, the pretreatment was carried out using dilute KOH. According to the result obtained after pretreatment, cellulose, hemicellulose, and lignin were the main components of corn stover (Table 2).

Table 2. Corn stover composition analysis

Compositions (%)	Current study	Past study and References [17]	[18]
Cellulose	35.23	35.4	35.52
Hemicellulose	23.5	24.65	21.36
Lignin	16.3	16.15	14.23
Ash content	3.8	3.5	3.11
Protein	4.85	5.01	4+-0.4
Insoluble	1.83	-	-

The result indicates that the compositions of the samples are similar to the compositions investigated by Liu et al.2018; D. Aboagye et al., [17, 18**]** in Table 2 with a small variation. However, the duration required for the plant to grow, differences in plant origin, air conditions, or different causes could have resulted in the variation in their contents [18]. The minor components have also been confirmed with the data reported by Aguilar-reynosa et al. [13]. The result indicated that for agricultural biomass-conversion to bioenergy, corn stover could be considered as a good source of sugars. It also shows appreciable amounts of crude protein, which could supply a nitrogen reservoir in any biomass-conversion process.

Measurement of reducing sugar

A standard stock solution of glucose and xylose were prepared according to [15] and Lambert's-law. From the plots, sugar concentration and absorbance were obtained, and the

produced sugar was investigated by varying process variables on hydrolysis stage. The results of glucose and xylose concentration with their absorbance are indicated in Fig.2.

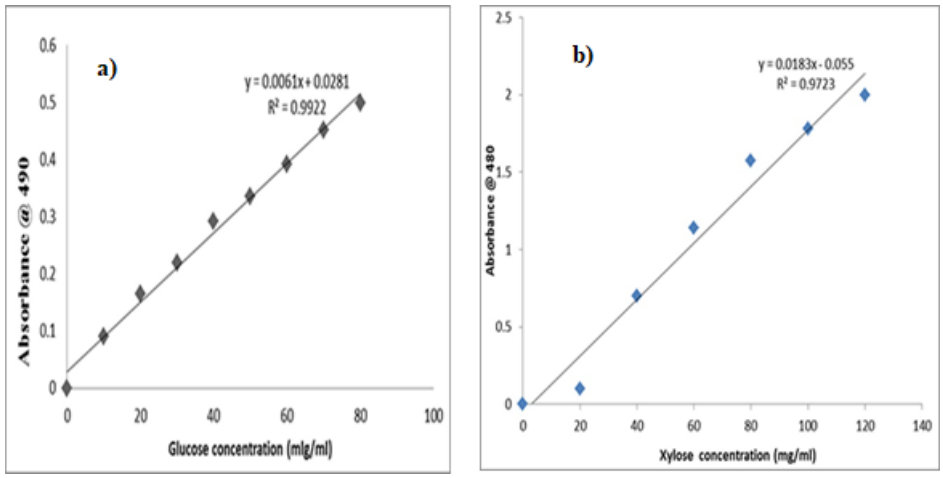

Fig. 1. Graphs of: a) glucose v-s its absorbance; b) xylose v-s its absorbance

This plots were used to determine the concentrations of unknown samples using Eq., Y = 0.0061x +0.02807: R^2 = 0.992 for glucose and Y = 0.0183x -0.055; R^2 = 0.969 for xylose Eq. (1−4) as results indicated in Table 5.

Where:

R^2 - coefficient of determination.

Model validation and Statistical data evaluation

Table 3, indicates hydrolysis parameters and responses, calculated by, equation (3), (4), and (5a) respectively.

Table 3. Experimental design showing independent variables, actual and predicted values for responses (Glucose, Xylose, and Ethanol)

Run	Factors				Glucose (%)		Xylose (%)		Ethanol (%)	
No.	A (Wt.)	B	C	D	E.value	P.value	E.value	P.value	E.value	P.value
1	1.5	0.15	125	80	39.99	40.19	27.74	28.00	40.49	40.56
2	2.5	0.25	145	30	41.50	41.43	29.04	28.52	42.80	42.66
3	2	0.2	155	55	46.80	46.50	31.30	30.97	44.78	44.72
4	2.5	0.15	145	80	48.80	49.06	33.05	32.88	47.50	47.36
5	1.5	0.15	145	30	43.89	44.02	29.01	28.70	42.99	43.54
6	2	0.1	135	55	43.60	43.30	30.12	30.50	44.60	44.51
7	1.5	0.25	125	30	38.00	37.90	24.08	23.99	38.51	38.58
8	2	0.2	135	55	42.75	42.70	32.60	32.86	45.40	45.45
9	3	0.2	135	55	42.78	42.46	31.10	31.44	44.43	44.09
10	2	0.2	135	55	42.45	42.70	33.09	32.86	45.50	45.45
11	2.5	0.15	125	80	39.50	39.62	29.80	29.64	41.04	41.42
12	2	0.2	135	55	42.67	42.70	33.01	32.86	45.60	45.45
13	2	0.2	135	55	42.80	42.70	32.98	32.86	45.50	45.45
14	1.5	0.25	125	80	35.60	35.63	23.87	23.77	39.01	39.16
15	2	0.3	135	55	34.98	34.86	24.40	24.08	39.23	38.88
16	1.5	0.25	145	80	38.34	38.46	28.03	28.46	43.50	44.19
17	2	0.2	135	105	42.40	42.14	28.40	27.95	45.30	44.60
18	2	0.2	135	5	40.80	40.65	25.50	26.01	43.30	43.56
19	2.5	0.25	145	80	41.50	41.70	30.00	30.38	42.80	43.12
20	2.5	0.15	145	30	45.08	45.30	30.43	30.72	47.05	46.90

Run	Factors				Glucose (%)		Xylose (%)		Ethanol (%)	
No.	A (Wt.)	B	C	D	E.value	P.value	E.value	P.value	E.value	P.value
21	1.5	0.15	145	80	47.80	47.78	30.00	30.13	46.60	46.50
22	1.5	0.25	145	30	38.01	38.18	26.98	27.34	41.70	41.24
23	2	0.2	135	55	42.76	42.70	32.50	32.86	45.70	45.45
24	2	0.2	135	55	42.79	42.70	33.00	32.86	45.00	45.45
25	2.5	0.25	125	30	39.01	39.28	24.00	24.06	39.99	40.01
26	1.5	0.15	125	30	38.90	38.98	28.10	27.91	40.30	39.98
27	2.5	0.25	125	80	36.98	37.01	24.52	24.58	37.60	38.09
28	2	0.2	115	55	36.89	36.77	24.00	24.39	36.50	36.12
29	2.5	0.15	125	30	38.39	38.40	29.50	28.82	42.99	43.34
30	1	0.2	135	55	39.89	39.79	28.90	28.62	41.91	41.81

The degree of variation between the anticipated and experimental value of the yield has been plotted in Fig. 2. The small amount of variation shows that the anticipated values are near to the experimental values. There is a high correlation value of R squared (Table 4) between the anticipated and experimental values for each yield which shown that the anticipated values and experimental values are in fair correspondence. It means that the data fitted well with the model and gave a convincingly good agreement of response for the expression process in the range studied.

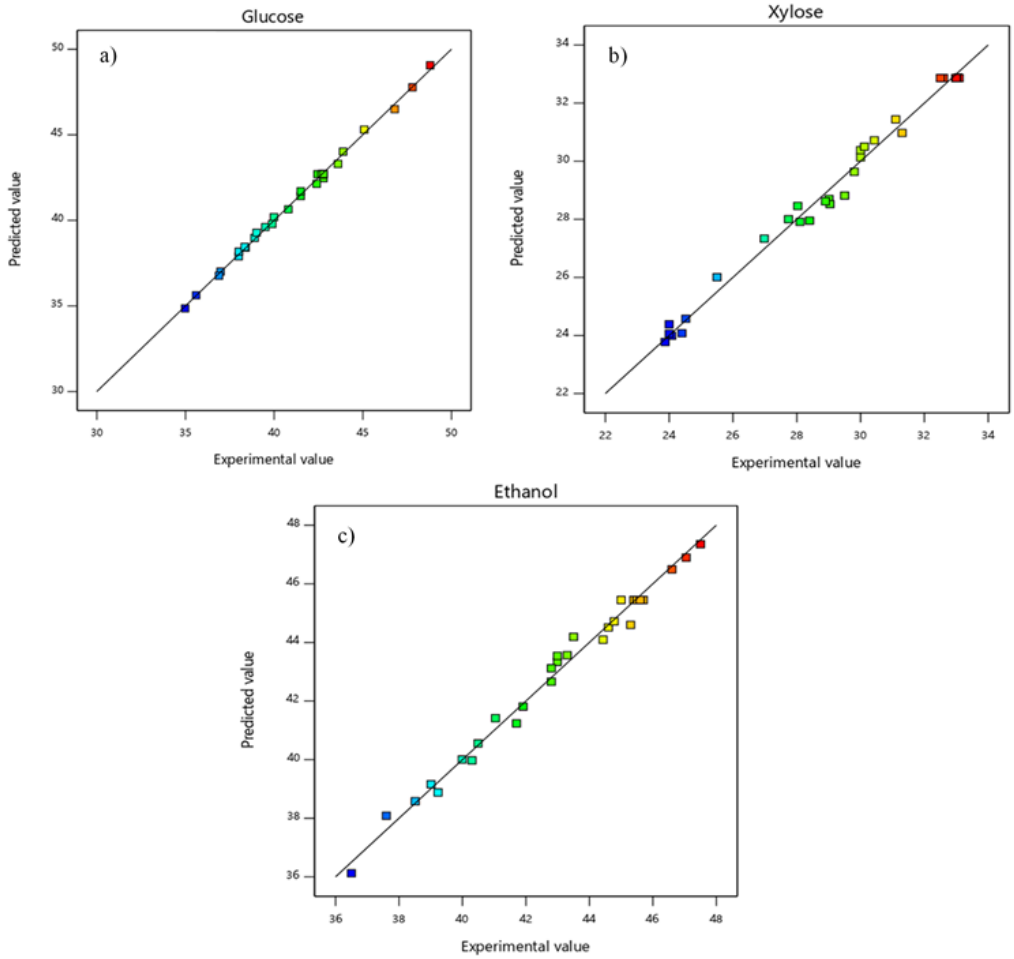

Fig. 2. Predicted versus the actual value of: a) glucose; b) xylose; c) ethanol on the yield

The anticipated optimum yield of glucose; 49.06 %, xylose; 32.88 %, and ethanol; 47.36 % were investigated at a time of 80 min, the particle size of 0.15 mm, the temperature of 145 ^{0}C, and acid concentration of 2.5 wt%. Under those most suitable conditions, the experimental values were 48.80, 33.09, and 47.50 % for glucose, xylose and ethanol respectively, which are in corresponding with those anticipated by computation. The small magnitude of the deviation indicated in Fig. 2, shows that the anticipated values are close enough to the actual values and regression analysis was evaluated for their significance as shown in Table 4. This represents the accuracy of the model selected for the anticipated values.

Table 4. Different responses for an adjusted model using analysis of variance

yield (Responses)	Source	SS	DF	MS	F -Value	P-Value
	Model	336.87	13	25.91	476.10	0.0001
Glucose	Lack of fit	0.7833	11	0.0712	4.07	0.0667
	Residual	0.8708	16	0.0544		
	Model	280.88	14	20.06	99.22	0.0001
Xylose	Lack of fit	2.73	10	0.2727	4.45	0.0566
	Residual	3.03	15	0.2022		
	Model	247.23	12	20.60	114.60	0.0001
Ethanol	Lack of fit	2.76	12	0.2301	3.90	0.0716
	Residual	3.06	17	0.1798		

SS= sum of the square, DF=degree of freedom, MS =mean square

The F and P-value tested the statistical effluences of all the terms of the model. The larger F-values for all responses are significant corresponding to small p-values below 0.05, indicating that the model was statistically significant. Additionally, all independent variables had a significant influence on the response. Therefore, all independent process variables should be controllable. The calculated regression coefficient (in terms of coded) and variance analysis of the anticipated model and all R values (R^2, R^2adj, and R^2pre) have been used, to check the suits of the model [19] shown in (Table 5).

Table 5. ANOVA (Analysis of Variance) for statistical data analysis

Model Parameters	Glucose	Xylose	Ethanol
R^2	0.9974	0.9893	0.9878
Adjusted R^2	0.9953	0.9793	0.9792
Predicted R^2	0.9878	0.9431	0.9517
Std. Dev.	0.2333	0.4497	0.4240
Mean	41.19	28.97	42.92
C.V. %	0.5664	1.55	0.9879
Model Precision	89.1131	28.6470	40.2360

The results also proved that the proposed regression model for yields of the response was satisfactory (Table 5) and the familiarity between the observed and anticipated value was indicated with all R squared values. The Pred- R^2 has reasonably confirmed with the Adj-R^2; i.e. the distinction is much < 20 % (Table 5).

Meanwhile, a very small value of the coefficient of variation (CV) (0.566%) clearly shown a very strong degree of accuracy and a great deal of reliability of the experimental values [20].

F-value of lack of fit (Table 4) for this model is insignificant which shows that the anticipated model is suitable for predicting optimum conditions to hydrolysis corn stover for bioethanol using H_2SO_4 as hydrolyzer.

In conclusion, the central composite design suggested the quadratic model equation is appropriate for the estimation of the similarity among the parameters. The quadratic polynomial model was formulated, to estimate the yield of glucose (Y_G), xylose (Y_X), and ethanol (Y_E) as a function of acid concentration (A), particle size (B), hydrolysis temperature (C), and hydrolysis time (D).

$$Y_G = 42.70 + 0.67A - 2.11B + 2.43C + 0.37D + 0.49AB + 0.47AC - 1.19BC - 0.87BD + 0.64CD - 0.39A^2 - 0.90B^2 - 0.27C^2 - 0.33D^2 \quad (6)$$

$$Y_X = 32.86 + 0.71A - 1.61B + 1.65C + 0.49D - 0.21AB + 0.28AC + 0.18AD + 0.64BC - 0.08BD + 0.34CD - 0.71A^2 + -1.39B^2 - 1.30C^2 - 1.47D^2 \quad (7)$$

$$Y_E = 45.45 + 0.57A - 1.41B + 2.15C + 0.26D - 0.48AB - 0.62AD - 0.23BC + 0.59CD - 0.62A^2 - 0.94B^2 - 1.26C^2 - 0.34D^2 \quad (8)$$

The magnitude and sign of their coefficients could explain the importance of the variables and their effects.

Effect of hydrolysis variables on the yield

The best method to identify whether or not individual or interaction can affect the yield is to indicate individual and interaction plots.

Acid concentration (A), hydrolysis temperature (C), and time (D) had a positive influence on the yield, whereas particle size (B) caused a negative influence on each yield. According to Eq. (6) via (8), the temperature had highly significant, while time had the lowest effect on the yield of glucose, ethanol, and xylose respectively. However, magnitudes of the linear terms of the temperature were substantially higher than those of acid concentration and time. The quadratic terms of individual variables had also negatively contributed to the individual yield.

The yield of glucose was influenced by the interaction between, A-B, A-C, and C-D positively. In contrast, B-C and B-D caused a negative contribution to yield the yield of glucose.

Surfaces plots derived from the quadratic equation are indicated in Fig.3, to represent the consequence of hydrolysis parameters on the yield of glucose. While the interaction between B versus D had a negative influence on the yield of glucose, the interaction between C and B had a greater significance. The temperature and particle size have the biggest role in yield. Higher temperatures, lead to a higher yield of glucose, than at lower temperature [21].

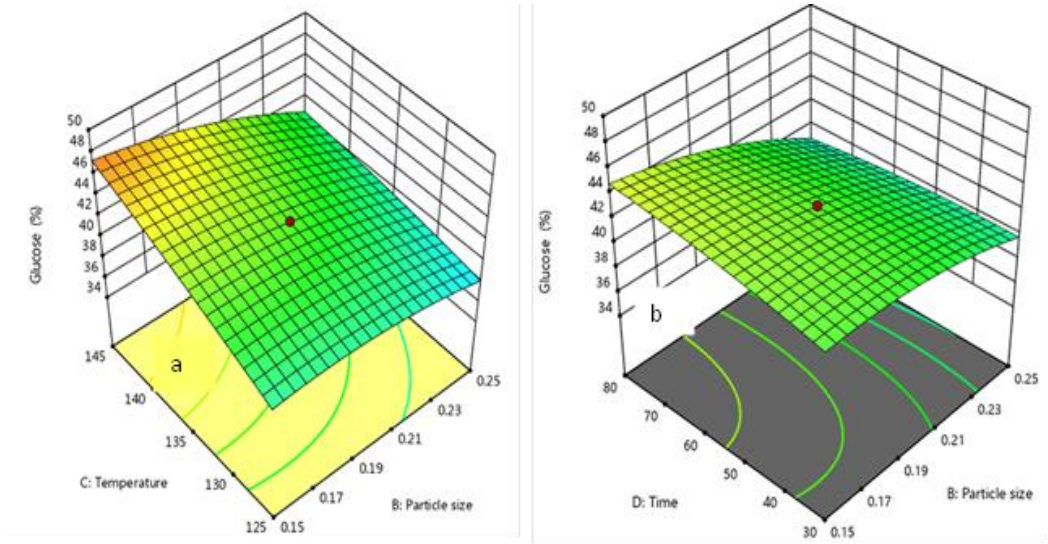

Fig. 3. Effect of interaction between: a) particle size and temperature;
b) time and particle size on the yield of glucose fixing other variables

It was indicated that the effectiveness of the biomass-hydrolysis process is expected to be its dependence on both particle dimension and the biomass quantity. At lower biomass, a diminish in particle dimension is expected to bring up the speed of hydrolysis reaction, since the rate of reaction is influenced by the sugar accessibility and small size of the molecule for better yield, while the bigger size of the molecule indicates a small amount of yield [22].

Under this condition the maximum yield of glucose was observed; 48.80% at acid concentration; 2.5 wt.%, particle size; 0.15 mm, hydrolysis temperature; 145 °C and hydrolysis time; 80 min.

The yield of xylose was also stroked positively by the interaction between, A-C, A-D, B-C, and C-D and negatively by interaction between, A-B and B-D. In this case, the most influential factors were the interaction between, B-C and C-D respectively as shown in Eq. (7) and Fig.6.

The higher yield of xylose (33.09 %) was recorded, at an acid concentration of 2 wt%, the particle size of 0.2 mm, the temperature of 135 °C, and time of 55 min via the real value.

Hydrolysis temperature and acid concentrations had also highly influenced the yield of xylose; this means, at a lower temperature, it is sensitive to acidic concentration. At elevated temperature, the yield starts to shrink since the transition of xylan to pentose sugar is, favored at the beginning as temperature raise. However, under severe conditions, it will be converted to furfur aldehyde [16].

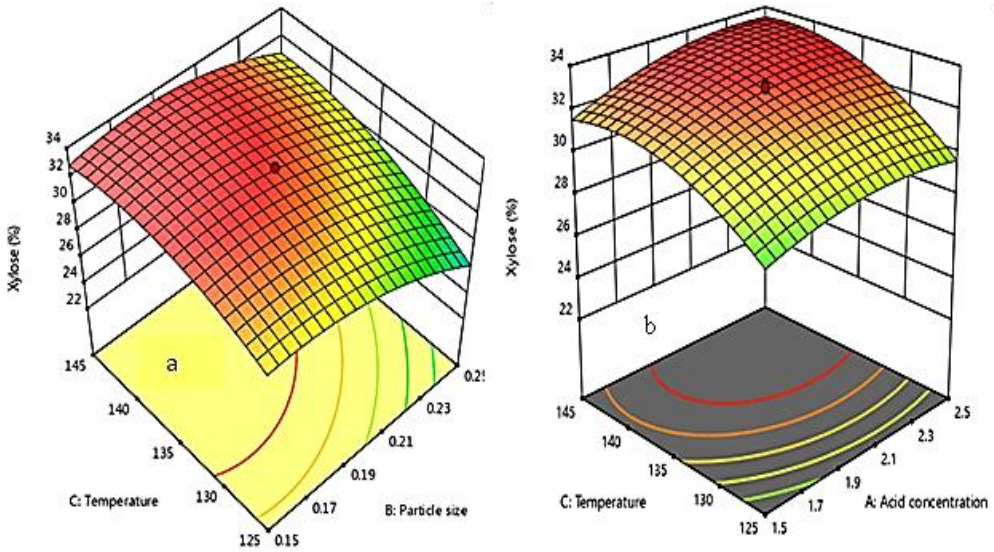

Fig. 4. Effect of interaction between: a) particle size and temperature; b) temperature and acid concentration on xylose yield by fixing others.

The yield of ethanol was strongly affected by hydrolysis temperature and particle size respectively. The interaction between C-D had a positive effect, while A-B, A-D, and B-C had a negative contribution to its yield. However, the yield of ethanol was strongly affected by the interaction between A and D, and C and D respectively see Eq. (7) and shown Fig.6.

Optimization of hydrolysis conditions

The ANOVA analysis of optimum parameters was mathematically carried out primarily depend on glucose, xylose, and ethanol yields. The primary goal of the research was to determine the best effective hydrolysis parameters to convert corn stover to bioethanol. The optimized variables were derived from the quadratic equation recommended by central composite design

and chosen primarily based on the best possible desirability. Table 8 suggests the comparison between expected and observed results of triplicated experiments. The best-anticipated values of glucose, xylose, and ethanol yield were investigated at acid concentration; 2.334 wt.%, temperature; 144.976 ^{0}C, particle dimension; 0.153 mm and time; 77.233 min. At these optimum points, the anticipated yield of glucose, xylose, and ethanol was found to be 48.71, 33.096, and 47.054%, respectively at the desirability of value a unity (1.00). The robustness of the model built via the statistical data was established by the small variation between the experimental and the anticipated result.

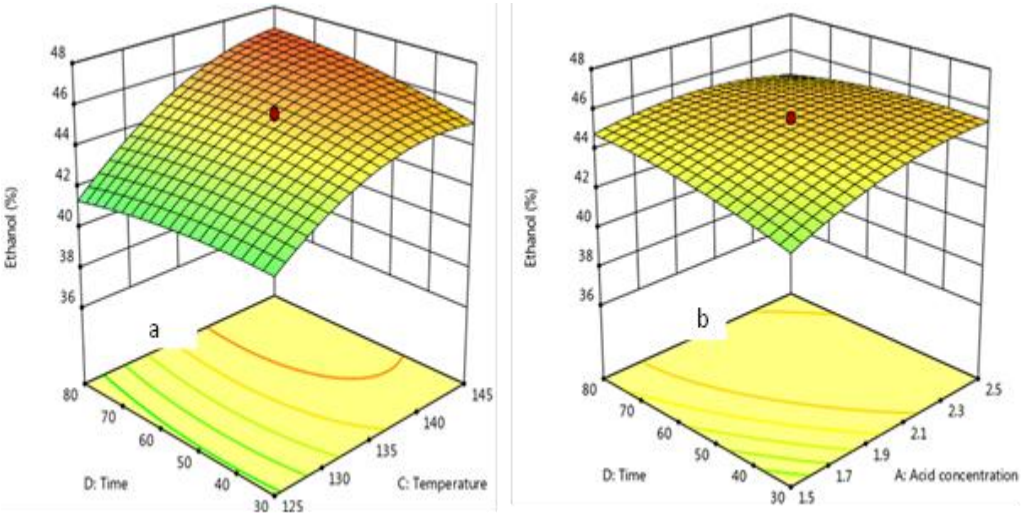

Fig. 5. Yield of ethanol: a) time versus acid concentration; b) time versus temperature fixing other variables

Table 5. Model verification of optimized yield (%) of, glucose, xylose, and ethanol.

Responses	Desirability	Experimental	Predicted	Error
Glucose	1.00	48.69	48.67	0.0004
Xylose	1.00	33.091	33.086	0.0002
Ethanol	1.00	47.050	47.031	0.0004

The model confirmation and the existence of the optimal point show that there was the best indication of the predicted through observed value. Central composite design performed numerical optimization of yield by setting the desired goal for each parameter and response. The optimum combinations of process parameters were selected to attain the highest yield of the responses.

Ethanol Fermentation

During zymosis and cell development yeast, S. cerevisiae is held at 30 °C and five pH solutions [23]. The conversion of the sugars generated later on hydrolysis was fermented to ethyl alcohol up to 72hrs. The sugars were observed by using high-performance liquid chromatography (HPLC), using refractive index (RI) detector and the best result was achieved at 48 h. At this time, 27.1 g/L of ethyl alcohol concentration was achieved from 57.601 g of monosaccharide sugars theoretically equivalent to 92.07% of ethyl alcohol. Additionally, Y. S. Kim et al. [21] investigated 89% of the theoretical yield of ethyl alcohol from 25 g/L of fermentable sugar at optimized conditions. Therefore, the use of dilute H_2SO_4 and RSM, in the present research

indicated that a great achievement method for hydrolysis of corn stover, since efficient ethyl alcohol was produced compared to the previous study.

Conclusion

The alkaline pretreatment was carried out for corn stover analysis. The results of the pretreated material shown that the major components of corn stover were cellulose, hemicellulose, and lignin, 35.23%, 23.5%, and 16.3% respectively.

The main finding was, investigation of hydrolysis parameters using the response surface method.

The optimal conditions were achieved with 2.334 wt % of acid concentration, 0.153 mm of particle size, 144.976 °C of hydrolysis temperature and 77.233 min of hydrolysis time. Under these conditions, the highest yield of glucose 48.69 %, xylose 33.091 %, and 92.07% of ethanol were achieved.

Therefore, alkali pretreatment, acid hydrolysis and response surface methodology are effective techniques for achievement of the maximum yield of sugars from cornstover biomass for renewable energy production.

For agricultural biomass-conversion to bioenergy, corn stover could be considered as a good source of sugars.

Acknowledgment

We like to acknowledge the Jimma Institute of Technology, School of Chemical Engineering, the Ethiopian paper and pulp industry, and all lab technicians for their support in different ways.

References

[1] Wyman, C.E. *Ethanol from lignocellulosic biomass: Technology, economics, and opportunities.* **Bioresour. Technol.** 1994**, 50,** 3–15, doi:10.1016/0960-8524(94)90214-3.

[2] Lal, R. *Soil quality impacts of residue removal for bioethanol production*. **Soil Tillage Res.** 2009**, 102**, 233–241, doi:10.1016/j.still.2008.07.003.

[3] De Bari, I.; Cuna, D.; Di Matteo, V.; Liuzzi, F. *Bioethanol production from steam-pretreated corn stover through an isomerase mediated process*. **N. Biotechnol.** 2014**, 31**, 185–195, doi:10.1016/j.nbt.2013.12.003.

[4] Kim, S.; Dale, B.E. *Global potential bioethanol production from wasted crops and crop residues.* **Biomass and Bioenergy,** 2004**, 26,** 361–375, doi:10.1016/j.biombioe.2003.08.002.

[5] Chen, W.; Wu, F.; Zhang, J. *Potential production of non-food biofuels in China*. **Renew. Energy,** 2016**, 85**, 939–944, doi:10.1016/j.renene.2015.07.024.

[6] Choudhary, J.; Singh, S.; Nain, L. *Thermotolerant fermenting yeasts for simultaneous sacchari fi cation fermentation of lignocellulosic biomass.* **EJBT** 2016**, 21**, 82–92, doi:10.1016/j.ejbt.2016.02.007.

[7] Zhao, Y.; Damgaard, A.; Christensen, T.H. *Bioethanol from corn stover – a review and technical assessment of alternative biotechnologies.* **Prog. Energy Combust. Sci.** 2018**, 67**, 275–291, doi:10.1016/j.pecs.2018.03.004.

[8] Aditiya, H.B.; Mahlia, T.M.I.; Chong, W.T.; Nur, H.; Sebayang, A.H. *Second generation bioethanol production: A critical review*. **Renew. Sustain. Energy Rev.** 2016**, 66,** 631–653, doi:10.1016/j.rser.2016.07.015.

[9] Aguilar-Reynosa, A.; Romaní, A.; Ma. Rodríguez-Jasso, R.; Aguilar, C.N.; Garrote, G.; Ruiz, H.A. *Microwave heating processing as alternative of pretreatment in second-generation biorefinery: An overview*. **Energy Convers. Manag.** 2017**, 136,** 50–65,

doi:10.1016/j.enconman.2017.01.004.

[10] Seok, J.; Lee, Y.Y.; Hyun, T. *Bioresource Technology A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass.* **Bioresour. Technol.** 2015, doi:10.1016/j.biortech.2015.08.085.

[11] Sun, Z.Y.; Tang, Y.Q.; Iwanaga, T.; Sho, T.; Kida, K. *Production of fuel ethanol from bamboo by concentrated sulfuric acid hydrolysis followed by continuous ethanol fermentation.* **Bioresour. Technol.** 2011**, 102,** 10929–10935, doi:10.1016/j.biortech.2011.09.071.

[12] Lloyd, T.A.; Wyman, C.E. *Combined sugar yields for dilute sulfuric acid pretreatment of corn stover followed by enzymatic hydrolysis of the remaining solids.* **Bioresour. Technol.** 2005**, 96**, 1967–1977, doi:10.1016/j.biortech.2005.01.011.

[13] Chen, R.; Jin, C.; Tong, Z.; Lu, J.; Tan, L.; Tian, L.; Chang, Q. *Optimization extraction, characterization and antioxidant activities of pectic polysaccharide from tangerine peels.* **Carbohydr. Polym.** 2016**, 136,** 187–197, doi:10.1016/j.carbpol.2015.09.036.

[14] Kim, Y.S.; Jang, J.Y.; Park, S.J.; Um, B.H. *Dilute sulfuric acid fractionation of Korean food waste for ethanol and lactic acid production by yeast.* **Waste Manag.** 2018**, 74**, 231–240, doi:10.1016/j.wasman.2018.01.012.

[15] Khedr, F.G.; Tohamy, E.Y.; Darwish El-Gamal, A.; Abouelwafa, A.M. *Bioconversion of Rice Straw into Bioethanol by Enzymatic Hydrolysis of Bacillus Subtilis.* **IOSR J. Pharm. Biol. Sci. (IOSR-JPBS** 2019**, 14**, 9–29, doi:10.9790/3008-1404040929.

[16] Avci, A.; Saha, B.C.; Dien, B.S.; Kennedy, G.J.; Cotta, M.A. *Response surface optimization of corn stover pretreatment using dilute phosphoric acid for enzymatic hydrolysis and ethanol production.* **Bioresour. Technol.** 2013**, 130,** 603–612, doi:10.1016/j.biortech.2012.12.104.

[17] Liu, G.; Zhang, Q.; Li, H.; Qureshi, A.S.; Zhang, J.; Bao, X.; Bao, J. *Dry biorefining maximizes the potentials of simultaneous saccharification and co-fermentation for cellulosic ethanol production.* **Biotechnol. Bioeng.** 2018**, 115**, 60–69, doi:10.1002/bit.26444.

[18] Aboagye, D.; Banadda, N.; Kambugu, R.; Seay, J.; Kiggundu, N.; Zziwa, A.; Kabenge, I. *Glucose recovery from different corn stover fractions using dilute acid and alkaline pretreatment techniques.* **J. Ecol. Environ.** 2017**, 41**, 1–11, doi:10.1186/s41610-017-0044-1.

[19] Zhang, X.; Chen, J.; Mao, M.; Guo, H.; Dai, Y. *Extraction optimization of the polysaccharide from Adenophorae Radix by central composite design.* **Int. J. Biol. Macromol.** 2014**, 67**, 318–322, doi:10.1016/j.ijbiomac.2014.03.039.

[20] Prakash Maran, J.; Sivakumar, V.; *Thirugnanasambandham, K.; Sridhar, R. Microwave assisted extraction of pectin from waste Citrullus lanatus fruit rinds.* **Carbohydr. Polym.** 2014**, 101**, 786–791, doi:10.1016/j.carbpol.2013.09.062.

[21] Andrew, A.N.; Henrietta, I.; Elvis, A.; Ogbeide, S.E.; Okieimen, C.O. *Modelling and optimisation of dilute acid hydrolysis of corn stover using box-behnken design.* **J. Eng. Sci. Technol.** 2014**, 9,** 443–454.

[22] Sarkar, N.; Ghosh, S.K.; Bannerjee, S.; Aikat, K. *Bioethanol production from agricultural wastes: An overview.* **Renew. Energy** 2012**, 37**, 19–27, doi:10.1016/j.renene.2011.06.045.

[23] Li, X.; Kim, T.H.; Nghiem, N.P. *Bioethanol production from corn stover using aqueous ammonia pretreatment and two-phase simultaneous saccharification and fermentation (TPSSF).* **Bioresour. Technol.** 2010**, 101,** 5910–5916, doi:10.1016/j.biortech.2010.03.015.

Received: November 06, 2020
Accepted: December 08, 2020