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Statistical Optimization of Bioethanol Production from Corn Stover Biomass

Ketema Beyecha Hundie*

Department of Chemical Engineering, University of Jimma Institute of Technology, Jimma, Ethiopia

ABSTRACT

The goal of the study was to find out optimum parameters on conversion of cornstover biomass to renewable fuel. The pretreatment of cornstover was carried out utilizing potassium hydroxide. The result indicated that the important elements of this biomass were cellulose, hemicellulose, and lignin, 34.03, 22.6 and 15.1% respectively.

The experiment and statistical evaluation was carried out using response surface methodology. The optimized variables were derived from the quadratic model and selected primarily based on the highest desirability.

The result shows that, the optimum parameters were, acid concentration; 2.334 (w/w %), particle size; 0.153 mm, temperature; 144.976 ^{0}C and time; 77.233 minutes. At these parameters, the yield of glucose and xylose were, 48.69 and 33.091% respectively. 27.1 g of ethanol concentration was investigated after 48 h of fermentation time at optimized conditions.

Keywords: Acid hydrolysis; Corn Stover biomass; Bioethanol; Statistical optimization

INTRODUCTION

Energy price, energy security, trade deficits, air pollution, and carbon dioxide accumulation to the atmosphere, could be improved, if production of bioethanol, will be carried out from agricultural residues and forest residues, municipal waste and other forms of lignocellulosic biomass. Since biofuel is that the considered as renewable energy sources, compared to fossil fuels, it is necessary to replace fossil fuel by renewable energy . The use of bioethanol as a shipping fuel has been believed to be an appropriate and t he substitution for nonrenewable based energy. substitution of renewable source is appreciably existed as opportunity supply for car fuel, due to environmentally friend and renewable source compared to un renewable source. Globally bioethanol production is highly food crops based production according to prediction of . To avoid this conflicts via food based use and industrial use of crops, only crop residues, is believed as feedstock for biofuel production as renewable resource. Bioethanol, from numerous agricultural byproducts could be produced if an appropriate, pretreatment hydrolysis and fermentation methods could be used. The most available resources on the worldwide are second-generation biofuels [1].

Lignocellulosic biomass is considered the most alternative available resource and renewable source of energy that has been highly used as second-generation feedstock to produce cellulosic bioethanol. Barley straw, wheat straw and the corn stover (cobs, stalks and leaves) are included in these biomass feedstocks. These advanced biofuels do not vie with food based crops and emits minimum amount of GHG, compared to biofuels derived from first generation which are food based product . Enhancing requirement for corn stover as feedstock brings up concerns about agricultural residues and environmental sustainability. Currently, there is a great deal of research being carried out to convert this lignocellulosic biomass to biofuels [2] .

Pretreatment is a crucial step and the cost effective of the whole process in production of bioethanol from lignocellulosic materials . Additionally, ethanol produced from this biomass is considered as relatively low yield product, since the physical and chemical composition of this biomass forms strong native recalcitrance. Therefore to overcome this problem, an appropriate pretreatment and hydrolysis techniques are essential steps. Pre-treatment, hydrolysis, fermentation and the product as separation are the main steps for bioethanol production from second generation lignocellulosic biomass. For reduction of biomass dimension, decompose of hemicelluloses to sugars, and to open up the composition of the cellulose contents

Correspondence to: Ketema Beyecha Hundie, Department of Chemical Engineering, University of Jimma Institute of Technology, Jimma, Ethiopia, Tel: +25142637868; E-mail: ktmbych2001@gmail.com

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pretreatment is first steps Particle size is considered as one of the most important factor in biomass utilization. To achieve the high pretreatment efficiency, particle size utilization is very important and hence used to improve subsequent hydrolysis performance [3].

With a common object of causing biomass greater susceptible to hydrolysis, a number of different types of pretreatment techniques have been employed on a global scale with acid and alkaline methods as the gold standard for lignocellulosic material decomposition. One of the best viable process options due to mainly to its effective pretreatment and comparatively simple process is applying alkaline for biomass pretreatment. The main reason of alkali pretreatment is that due to selectively removal of lignin without any degrading sugars, and enhances porosity and surface area, thereby favoring hydrolysis conditions, alkali pretreatment is considered as alternative choice and additionally it allows incursion of water molecules to the inside layers, and penetrate the attachments carbohydrate [4].

For ethanol production from lignocellulosic biomass after pretreatment, complete hydrolysis is other essential steps to obtain good fermentable sugars. Concentrated and diluted acid hydrolysis, as well as enzyme are considered are used for hydrolysis of lignocellulosic materials . Previously a number of studies have used dilute acid pretreatment that indicates high sugar yields from pretreated and next hydrolysis of the pretreated sugar. However, attention was to identifying hydrolysis conditions that will result the highest yields of sugars from hydrolysis stage using H_2SO_4 as the hydrolyser. 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 [5].

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 a generation of statistical and mathematical technique that is beneficial for formulating, enhancing and optimizing processes parameters is response surface method. The primary advantage of this method is, used to minimize number of experimental trials and selects the optimum point of parameters and used to evaluate multiple variables and their interactions . Several researchers have been done on bioethanol production cornstover. Even though, particle size as well as dilute acid hydrolysis is not included in previous study in hydrolysis step to obtain optimum dependent as well as independent parameters. Therefore, the objective of present study was, investigation of hydrolysis parameters (acid concentration, particle size, temperature, and time) at optimized conditions using response surface method to convert cornstover to bioethanol [6].

MATERIALS AND METHODS

Sample preparation

The cornstover was collected from farm in Jimma (Gibe) and saved for evaluation. It was cleaned, shredded, and dried using electric oven at 60 ^{0}C for one day and half at moisture content of 7 %. By varying the dimensions of the sample below 4 mm (0.10. 25 mm), it was milled using a laboratory mill, sieve analysis was carried out and stored in cool material at 25^{0}C for the next step [7].

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 2.5% KOH at 121^{0}C for 25min in sterilizer . Then the sterilized result becomes percolated to classify the firm relaxation and the separate out fraction. The firm rest very well dampened by using deionized water to remove the residual alkali until the pH of the residue turned into neutral [8].

Compositional analysis

Thereafter, the samples were stored at 25^{0}C for subsequent step; 35 g of the sieved sample was taken to a laboratory for physicochemical evaluation consistent with strategies defined by means of (AOAC 2012) and ASTM test [9].

Hydrolysis

For the extraction of sugar from biomass materials, the operational ranges of process factors are important. Therefore, it is advisable to use non-isothermal conditions or optimum duration of time. This is very essential, if dilute acid will be considered to use . The hydrolysis was conducted based on previously study with modificatio for efficiency conversion of cornstover to sugar with respect to that H_2SO_4 concentration (1.5–2.5 w/v%), temperature (125–145^{0}C), hydrolysis time (30–80 min) and particle size (0.15 to 0.25 mm) using response surface design. Cellulose and hemicellulose were decomposed in order to obtain monosaccharide sugars by fixing to 10 v/w of liquid to material ratio, in 250 -mLvolumetric flask. After and the 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 the 2N KOH, and the combination developed for subsequent step [10].

The consequences of the 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.

Factor	Name	Units	Minimum	Maximum	Level		
A	Acid conc.	wt. %	1.5	3.5	1.5(-1)	2.5 (0)	3.5(+1)
B	Particle size	mm	0.15	0.25	0.15(-1)	0.2(0)	0.25(+1)
C	Temperature	0C	125	145	125 (-1)	135 (0)	145(+1)

D	Time	min	30	80	15(-1)	52.5 (0)	80(+1)

Table 1:Experimental design parameters

Measurement of reducing sugars

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

Y=mx+b (1)

Where: y is stand for absorbance x, for concentration, m for slope and b for intercept respectively.

For concentration of unknown sample (C) and sugar yield Equations. (3 were developed.

C (mg/ml) = /slope (2)

Sugar yield =)/ (3)

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 corbondoxide. The pH was adjusted during zymosis by adding 2 N KOH. The process was started in 250 mL flask with a working volume of 150 mL at pH 6.5 solution and 35^0C under optimized conditions . S. cerevisiae was obtained from Holeta agricultural research center and the Strains were cultivated in a 200 mL flask with 150 mL working volume of the vaccination medium containing Agar; 3.5 g/L, sugar (glucose); 15 g/L, sugar (Pentose); 10 g/L, Magnesium sulfate; 0.25 g/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 (Ym %) have been calculated by the use of Equations. (4a and 4b) respectively:

Y% = amount of ethyl alcohol obtained/ amount of sugar used *100 (4a)

Ym % =1.96* (gram of ethyl alcohol / gram sugar)/*100 (4b)

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 cornstover, the pretreatment was carried out using dilute KOH. According to the result obtained after pretreatment, cellulose, hemicellulose, and lignin were the main components of cornstover. The result indicated also that minor constitutes (Crude protein, ash, and unknown soluble) exist in cornstover. The outcomes of the pretreated and evaluated composition of cornstover are indicated in Table 2.

compositions	**Current study**	**Past Study (Liu et al. 2018)**	**(D. Aboagye et al., 2017)**	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	3.50%	3.11				
Protein (%)	4.85	5.01	4+ -0.4				
Insoluble (%)	1.83	-	-				

Table 2: cornstover composition analysis

The result indicates that, compositions of the samples are similar to the compositions investigated in Table 2 with small variation. However, the duration required for plant to growth, differences in plant origin, air conditions, or different causes could be resulted the variation in their contents. The minor components have also confirmed with the data reported. The result indicated that for agricultural biomass-conversion to bioenergy, cornstover could be considered as a good source of sugars. It also shows appreciable amounts of crude protein, which could supply nitrogen reservoir in any biomass-conversion process.

Measurement of reducing sugar

Standard stock solution of glucose and xylose were prepared according to and Lambert's-law and from the plots, sugar concentration and absorbance were obtained and then produced sugar was investigated by varying process variables on hydrolysis stage. The results of glucose and xylose concentration with their absorbance's are indicated in Figure.1.

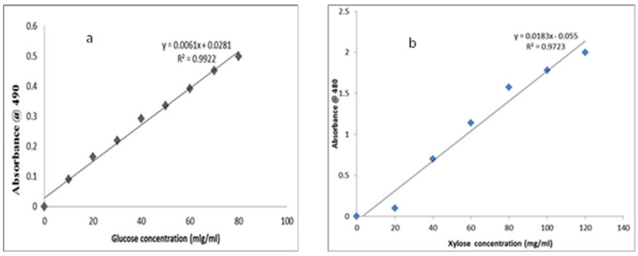

Figure.1. Graphs of glucose v-s its absorbance (a) and xylose v-s its absorbance (b)

The concentrations of unknown samples were determined, from the plots using Eq., Y = 0.0061x + 0.02807: R2 = 0.992 for glucose and Y = 0.0183x -0.055; R2 = 0.969 for xy lose Equations. (14) as results.

Model validation and statistical data evaluation

The degree of variation between the anticipated and experimental value of the yield has been plotted in in Figure. 2. The small amount of the variation shows that the anticipated values are near to the experimental values. There is a high correlation values of R squared 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.

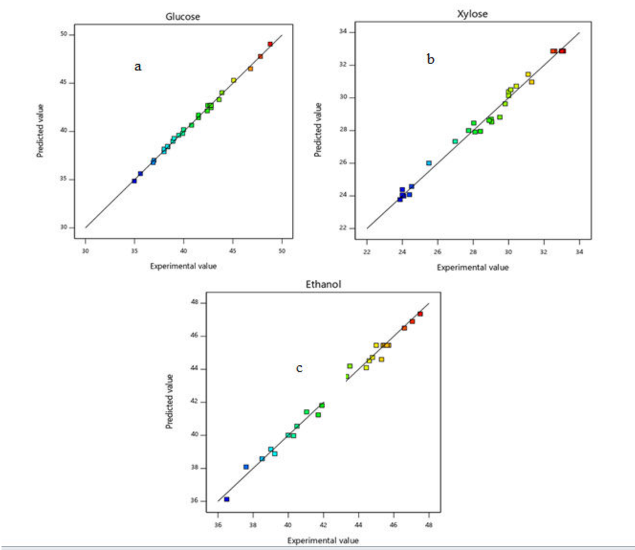

Figure. 2. Predicted versus actual value of glucose (a), xylose (b) and ethanol (c) on the yield.

The anticipated optimum yield of glucose; 49.06%, xylose; 32.88% and ethanol; 47.36% were investigated at time of 80 min, particle size of 0.15 mm, temperature of $145^{0}C$, and acid concentration of 2.5wt%. Under those most suitable conditions, the experimental values were 48.80, 33.09, and 47.50% respectively, which are in corresponding with those anticipated by computation. The small magnitude of the deviation indicated in Figure 2, shows that the anticipated values are close enough to the actual values and regression analysis was evaluated for their significance as shown in.This represents that the accuracy of the model selected for the anticipated values. To determine the significance and accuracy of the model, ANOVA is used as instrumental tool by using Fisher's variance (F-value) and probability (P-value).

The statistical effluences of all the terms of the model were tested by the F and P-value. The larger F-value of glucose, xylose and ethanol are significant corresponding to small p-values below 0.05, indicated that the model was statistically significant. The results showed that, higher F-value and lower p value which in turn show the suitness of model was strongly significant Additionally, all independent variables had significance influence on the response. The calculated regression coefficient (interms of coded) and variance analysis of the anticipated model and all R values (R2, R2adj and R2pre) have been used, to check the suitness of the model.

The results also proved that, the proposed regression model for yields of the response were satisfactory and also the familiarity between the observed and anticipated value were indicated with all R squared values using multinomial design equations. The Pred- R is in reasonable confirmed with the Adj-R ; i.e. the distinction is much of <20% To determine the signal to noise ratio, adequate accuracy (AC) is other important parameter . It is desirable; when adequate accuracy ratio is greater than 4; shows that an adequate signal.

Meanwhile, a very small value of coefficient of variation (CV) (0.566%) clearly shown a very strong degree of accuracy and a great deal of reliability of the experimental values . F-value of lack of fit for this model is insignificant which shows that the anticipated model is suitable for prediction optimum conditions to hydrolysis cornstover for bioethanol using the H_2SO_4 as of the hydrolyser. In conclusion, the central composite design suggested quadratic model equation is appropriate for estimation of the similarity among the parameters. The quadratic polynomial model was formulated, to estimate the yield of glucose (YG), xylose (YX), and ethanol (YE) as a function of acid concentration (A), particle size (B), hydrolysis temperature (C) and hydrolysis time (D).

YG = 42.70 + 0.67A -2.11B + 2.43C + 0.37D + 0.49AB + 0.47AC -1.19BC-0.87BD + 0.64CD -0.39A2-0.90 B2-0.27C2 -0.33 D2 (5)

YX = 32.86 + 0.71A -1.61 B + 1.65C + 0.49 D -0.21AB + 0.28AC + 0.18AD + 0.64BC -0.08BD + 0.34CD -0.71A2 + -1.39B2 -1.30 C2 -1.47D2 (6)

YE = 45.45 + 0.57A -1.41B + 2.15C + 0.26D -0.48AB -0.62AD -0.23BC + 0.59CD -0.62A2-0.94B2 -1.26C2 -0.34D2 (7)

Effect of process parameters 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. (5)

via (7), temperature had highly significant, while time had lowest effect the 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 negatively contributed to yield the yield of glucose. Surfaces plots derived from quadratic equation are indicated in to represent the consequence of hydrolysis parameters on the yield of glucose. While, the interaction between B versus D had negative influence on the yield of glucose, the interaction between C versus B had the greater significance. The temperature and particle size have the biggest role on yield. At higher temperature, lead to higher yield of glucose, than lower temperature. It was indicated that the effectiveness of the biomass-hydrolysis process is expected to be its dependence both particle dimension and on 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 bigger size of the molecule indicates small amount of yield . 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^{0}C and hydrolysis time; 80 min.

The yield of xylose also stroked positively by 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. (6) The higher yield of xylose (33.09 %) was recorded, at acid concentration of 2 wt%, particle size of 0.2 mm, temperature of 135^{0}C and time of 55 min via the real value. Hydrolysis temperature and acid concentrations had also highly influences the yield of xylose; this means, at lower temperature, it is sensitive to acidic concentration. At elevated temperature, the yield starts to shrink since transition of xylan to pentose sugar is, favored at the beginning as temperature raise. However under severe conditions it will be converted to furfuraldehyde.

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 negatively contributed to its yield. However, the yield of ethanol was strongly affected by interaction between A versus D and C versus D respectively

Optimization of hydrolysis conditions

The ANOVA analysis of optimum parameters was mathematically carried out primarily depend on glucose, xylose, and ethanol yield. The primary goal of the research was to determine the best effective hydrolysis parameters to convert cornstover 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. 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 of concentration; 2.334 wt.%, temperature; 144.976^{0}C, and particle dimension; 0.153 mm and time; 77.233 min. At these optimum points the anticipated yield of glucose, xylose and ethanol were found to be 48.71, 33.096 and 47.054%, respectively at 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.

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

Ethanol fermentation

During zymosis and cell development yeast, S. cerevisiae are held at 30^{0}C and at five pH solution . The conversion of the sugars generated later on hydrolysis was fermented to ethyl alcohol up to 72 hrs. 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 monosaccharaide sugars theoretically equivalent to 92.07% ethyl alcohol. Additionally, 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 , in present research indicated that a great of the achievement method for hydrolysis of cornstover, since efficient ethyl alcohol was produced compared to previous study.

CONCLUSION

The pretreatment was carried out utilizing potassium hydroxide. The result shown that, the major components of cornstover biomass were cellulose, hemicellulose, and lignin, 35.23%, 23.5% and 16.3% respectively.

The effect of the parameters has been investigated and the optimal parameters were 2.33 wt% of acid concentration, 0.153 mm of particle size, and 144.98^{0}C of temperature and the 77.23 min of hydrolysis time. The highest yield of glucose and xylose are 48.69 % and 33.091% respectively. Theoretically, the best yield of ethanol was 92.07% after 48h fermentation time at optimized parameters.

Response surface methodology is an efficient technique to optimize the hydrolysis parameters to estimate the maximum yield of sugars (glucose, xylose) and ethyl alcohol for cornstover.

Cornstover biomass is considered as a massive source of sugar for renewable energy production.

Conflict of interest

Regarding the publication of this paper, the authors announce that there is no any conflict of interests.

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