Pretreatment and enzymatic hydrolysis of sunflower hulls for fermentable sugar production

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Abstract: Sunflower is a widely adapted crop and can be grown in every temperature region. In the U.S., two million acres were cultivated with sunflowers in 2009. During industrial processing, large quantities of hulls are obtained as a waste product from the dehulling process. This study focused on converting the sunflower hulls into fermentable sugars by dilute acid pretreatment and enzymatic hydrolysis. Raw sunflower hulls are composed of β-glucan (34%±1.1%), lignin (25%±0.95%), xylan and arabinan (27%±1.56%), extractives (13%±2.5%) and traces of ash. Sunflower hulls were first subjected to pretreatment by varying three independent factors: 1) acid concentration (0.5%-2%); 2) reaction temperatures (140-160℃); 3) reaction time (10-30 min). Slurry samples obtained after pretreatment were separated into liquid and solid fractions. Liquid fractions were analyzed for monomeric and oligomeric sugars and inhibitor products by High Pressure Liquid Chromatography (HPLC). Enzymatic saccharification was then performed on pretreated solid fractions to convert remaining cellulose (β- glucan) into fermentable sugars. The results showed an increase in acid concentration and reaction temperature gave high xylose yield in the liquid fraction. However, an increase in reaction time resulted in degradation of xylose into furfural. A quadratic model for xylose yield was formulated based on the experimental results. The maximum xylose yield predicted by the model was 62% at 158℃ for 20 min at 1.75% acid concentration. The maximum β-glucan digestibility of the enzymatic saccharification was 53.5% at 160℃ for 30 min at 2% acid concentration.

Keywords: sunflower hulls, pretreatment, central composite design, enzymatic hydrolysis, lignocellulosic ethanol, biofuel
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1 Introduction

Lignocellulosic biomass, such as forest residue, agricultural residue, yard waste, and wood products, are a great source of energy that may be used for biofuel generation. They store energy from sunlight in their chemical bonds[1]. Lignocellulose material is the most abundant and one of the cheapest materials available in the world for renewable energy production[2].

Lignocellulosic material mainly consists of cellulose, hemicelluloses, and lignin. Cellulose is homo polymer composed of six-carbon sugars. Hemicellulose is a heteropolymer of five-carbon and six-carbon sugars including xylose, arabinose, galactose, and mannose. These carbohydrates can be converted into fermentable

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sugars through pretreatment followed by enzymatic saccharification[3]. Efficient pretreatment methods must be developed to maximize the fermentable sugar yield and to minimize degradation products[4]. Currently, dilute acid pretreatment of lignocellulosic biomass followed by enzymatic saccharification is proven to be one of the most promising and economical processes to obtain fermentable sugars for production of biofuels[5]. Extensive research has been carried out to convert waste products obtained from industrial processing such as bagasse and pulp into lignocellulosic ethanol[6-8]. However, little published data are available about converting sunflower hulls into bioethanol using dilute acid pretreatment and enzymatic saccharification.

The production of sunflower seeds in the United States was approximately 1.5MMT in 2009[9]. Sunflower hulls are obtained as a waste product from the de-hulling process. Sunflower hulls have little commercial value and become a disposal problem because of their low bulk density[10]. The effect of alkali pretreatment on sunflower hulls and stalks has been studied to some extent by researchers, but the effect of dilute acid pretreatment and its outcome on the enzymatic saccharification have yet to be evaluated[10]. The present study was carried out to evaluate these waste hulls as a raw material for lignocellulosic ethanol production.

The primary goal of this research is to evaluate the effectiveness of the dilute acid pretreatment through the removal of xylan from the sunflower hulls to enhance the enzymatic digestibility of cellulose. The pretreatment of sunflower hulls was performed by taking three different factors into consideration: reaction time, reaction temperature, and acid concentration. Based on the experimental results, a model was formulated on the xylose conversion yield. The criteria of optimization were high xylose yield and low inhibitors such as acetic acid, Hydroxymethylfurfural (HMF) and furfural production in the hydrolyzate. Enzymatic saccharification was performed on pretreated solid substrate to evaluate for the resulting fermentable sugar production.

2 Materials and methods

2.1 Biomass raw material

The raw sunflower hulls were obtained from Dahlgren & Company, Inc. (Crookston, MN). The sunflower seeds are passed through the seed mill where seeds open up. To separate the mixture of seeds, hulls were dropped water. The hulls will float on the water and removed easily. The separated hulls were air dried. The size of sunflower hulls was approximately 6-8 mm. Moisture content of the raw sunflower hulls was determined by oven drying at 105℃ for 12 h.

2.2 Compositional analysis

It is necessary to remove the in-organic structural material from the biomass prior to analysis to prevent interference with downstream process of biomass sample. Failure to remove these extractives may result in error in structural sugars values. It also may result in falsely high lignin values when unhydrolyzed carbohydrates condense with acid insoluble lignin. Composition of the original sunflower hulls was measured according to the National Renewable Energy Laboratory (NREL) LAP protocols. Two-stage extraction processes (24 h of water extraction and 8 h of ethanol extraction) were performed to remove extractives such as nitrites/nitrates, proteins, chlorophyll, and waxes (NREL/TP-510-42619). The water and ethanol solvents were oven dried and weighed to account for the overall extractives weight. The source and individual components of these extractives were not verified. After extraction hulls were oven dried for 12 h at 105℃. Then the extractive free hulls were analyzed for structural carbohydrates and lignin based on the NREL LAP protocol (NREL/ TP-510-42618).

2.3 Central Composite Design (CCD)

CCD gives an efficient estimation of quadratic terms and their interactions[11]. Pretreatment of sunflower hulls was performed and analyzed using 20 experiments (including eight factorial points; six axial points and six replicates at the center points). These 20 experiments were generated Minitab 15 software (Minitab, State College, PA) by taking high and low values of the three independent variables. The values of these factors were chosen based on the previous experimental results (data not reported) were summarized in Table 1. The twenty design matrix of the pretreatment conditions including all

the three factors were summarized in Table 4. The analysis of variance (ANOVA) was determined Minitab 15 software by using CCD. The significance of xylose yield in the hydrolyzate (Y_1) was studied by considering three factors variables reaction temperature (X_1), reaction time (X_2), acid concentration (X_3) and the interactions between the factors. The significance of the model was evaluated by the value of R^2. The interval of R^2 is between zero and one. The closer the value of R^2 is to (1) implies the better model fits the sample data. The experimental data was analyzed by Minitab 15 software.

Table 1 Pretreatment factors considered in CCD

Factors	Units	Levels		
		-1	0	1
Reaction temperature (X_1)	℃	140	150	160
Reaction time (X_2)	min	10	20	30
Acid concentration (X_3)	wt%	0.50%	1.25%	2%

The mathematical design equation for each response was a second order quadratic equation given by Equation (1). The coefficients and response surface were determined by Minitab 15 software.

$$Yi = \beta o + \sum \beta i\, Xi + \sum \beta ii\, Xi^2 + + \sum \beta ij\, Xi\, Xj \quad (1)$$

where, Yi is the predicted response variable; βo, βi, βii, βij are constant regression coefficients of the model and $XiXj$ (i=1,2,3; j=1,2,3 $i \neq j$) are the independent variables.

2.4 Pretreatment procedure

The pretreatment of the biomass was performed in a 300-mL internal volume, jacketed batch reactor (manufactured by AutoClave Engineers, Erie, PA). The reactor was made of Hastelloy C-276 to mitigate acid corrosion at high temperatures. Twenty-one grams of dry biomass was added to 200 mL of appropriate amount sulfuric acid solution. The heating source used for the reactor was saturated steam. Saturated steam was drawn into the external jacket of the reactor by opening a three-way valve. The agitation in the reactor was maintained constant at 60 r/min throughout the reaction. The reactor heating kinetics averaged approximately (35±3)℃/min. After the desired temperature was achieved, reaction time was initiated and the temperature in the reactor was maintained constant by operating the 3-way valve manually. The reactor was then cooled by passing the cooling water into the external jacket when the reaction was over. Once the reactor was cooled below 40℃, slurry in the reactor was discharged and collected in a polyethylene bottle for further analysis. The temperature data from the reactor were recorded with the aid of picolog software, throughout the reaction time.

2.5 Analytical procedure

2.5.1 Determination of monomeric sugars in the liquid fraction (Hydrolyzate)

After pretreatment, the slurry samples were vacuum filtered and separated into liquid and solid fractions. The hydrolyzate was then analyzed for monomeric and oligomeric sugars. Prior to analysis, hydrolyzate samples were neutralized by adding calcium carbonate until a pH range of 5.0-6.0 was obtained. The neutralized samples were filtered in order to remove contaminants using a 0.2 μm filter (Millipore, Billerica, MA) into glass vials. The sugar analysis was performed in an Agilent 1200 High Pressure Liquid Chromatography (HPLC) (Palo Alto, CA) fitted with Transgenomic CHO-Pb column (300 mm×7.8 mm). The column temperature was maintained at 80℃. The Refractive Index Detector (RID) temperature was maintained at 55℃ during the analysis. The mobile phase used was DI water. The flow rate was maintained at 0.6 mL/min. The analysis time for each sample was 35 min[12]. Standards were run prior to analysis of the samples. The concentrations of the standards were from 0.5 to 18 g/L. Internal sugar recovery standard with concentration of 4 g/L was run frequently to test for column and RID validity. The standard solutions and sugar recovery standard solution consist of D-(+)glucose, D-(+)xylose, D-(+)galactose, L-(+) arabinose, and D-(+)mannose.

2.5.2 Determination of oligomeric sugars in the liquid fraction (Hydrolyzate)

This step was performed to account for the amount of heterogeneous oligomers that were liberated into liquid fraction in addition to monomeric sugars. Since, these oliogmeric sugars are of little commercial value. It is imperative to break them into homogenous monomers through secondary hydrolysis process. The process includes autoclaving the liquid fraction samples at 121℃ for 60 min. The samples were analyzed in HPLC

system similarly as mentioned in the section 2.5.1. This method is based on the NREL LAP protocol of determination of sugar by products and degradation products in liquid fraction process samples (NREL/TP -510-42623).

2.5.3 Determination of structural carbohydrates and lignin in the pretreated solid residue

The analyses were performed to determine the amount of β-glucan, xylan and lignin retained in the solid fraction after the pretreatment. The solid samples were air dried for 4-5 days at room temperature and milled into 100-mesh particle size. Three hundred milligrams of milled solid biomass was loaded in the pressure tubes manufactured by (Ace Glass Incorporated, Vineland NJ) and three milliliters of 72% sulfuric acid was added to the biomass. The tubes were placed in a water bath at 30℃ for 1 h. Then the acid concentration was reduced to 4% by adding 84 mL of DI water to each pressure tube. These pressure tubes were placed in an autoclave oven at 121℃ for 60 min. The resultant slurry was vacuum filtered by pouring the mixture into porous ceramic crucibles (Coorstek, Oakridge, TN). The liquid fraction was analyzed for the amount of acid soluble lignin (ASL) using UV-VIS spectrometer (manufactured by Thermo Scientific, Waltham, MA), and carbohydrates using HPLC (The samples were analyzed in HPLC system similarly as mentioned in the section 2.5.1). Solid residue retained in the crucibles was oven dried at 105℃ for 12 h to determine acid insoluble lignin content (AIL). Then the crucibles were placed in a muffle furnace at 575℃ for 24 h to and then weighed to determine the ash content. This method is based on the NREL LAP protocol (NREL/ TP-510-42618).

2.5.4 Determination of inhibitor products

Liquid fraction of pretreated samples that were rich in five carbon sugars can be fermented into bio-fuel using pichia stiptis enzyme. The degradation productions such as acetic acid, HMF and furfural are inhibitors during the fermentation process. In order to effectively convert sugars into biofuels, inhibitor products in the liquid fraction should be analyzed. The analysis was performed using Agilent 1200 series HPLC system with the Phenomenex Rezex RFQ column at 80℃. The mobile phase was a 0.01 N sulfuric acid solution. The flow rate was maintained at 1 mL/min[13]. The verification standards for inhibitor products were obtained from Absolute Standards, Inc (Hamden, CT).

2.5.5 Enzymatic saccharification (hydrolysis)

The enzymatic saccharification was performed on washed pretreated solid substrate in a thermal incubator at 50℃ and 250 r/min for 72 h. Compositional analysis of the pretreated solid substrate were performed and the amount of β-glucan retained in the substrate was measured by HPLC analysis. Then the biomass was accurately measured so that 0.1 g (1%) of dry β-glucan was available for enzymatic saccharification. The solid substrate was loaded in 50 mL centrifuge tube and 5 mL of sodium citrate buffer with pH 4.8 and approximately 4.5 mL of DI water was added to the tube. The total volume of the reagents and solid substrate was 10 mL since we used 0.1 g cellulose at 1% solid content. Accellerase 1500 enzyme was supplied by Genencor International (Palo Alto, CA). The reagents and enzyme loading concentration considered to perform enzymatic sacachrification were summarized in Table 2. This procedure and equation mentioned below are from the NREL LAP protocol (NREL/TP 510-42629). After 72 h the liquid hydrolyzate samples were filtered into glass vials and the analysis of β- glucan digestibility were performed by Agilent 1200 HPLC system with Transgenomic CHO-782 Pb column. Since, enzymes convert cellulose into glucose, the cellulose digestibility was measured by integrating the glucose retention peak from the HPLC data. The β-glucan digestibility was calculated by using the following equation.

$$\%\ Digestion = \frac{Grams\ of\ glucan\ digested \times 0.9 \times 100}{Grams\ of\ glucan\ added} \quad (2)$$

Table 2 Enzymatic saccharification conditions

Conditions	Set points
β-glucan loading	1%
Temperature	50℃
Time	72 h
Enzyme loading	40 mg/g of β-glucan
Sodium azide	20 mg/mL

2.6 Combined severity factor (CSF)

Combined severity factor (CSF) combines the experimental effects of temperature and reaction time and pH to enable an easy comparison of results and to facilitate process control. CSF is derived from the observation that reaction rates double for every 10℃ increase in temperature. The denominator value 14.75 is the conventional energy of activation assuming the overall reaction is hydrolytic and the overall conversion is first order[8]. The reference temperature is taken as 100℃ since it is assumed that biomass hydrolysis starts above the reference temperature.

$$CSF = Log_{10}\left[t \times \exp\left(\frac{T_H - T_R}{14.75} \right) \right] - pH \qquad (3)$$

where, t is the reaction time in min; T_H is the reaction temperature in ℃; T_R is a reference temperature (generally considered as 100℃), and pH value.

3 Results and discussion

3.1 Compositional analysis

The major compounds present in hulls were β-glucan (34%±1.1%), followed by lignin (25%±0.95%), and xylan and arabinan (27%±1.56%). Some minor compounds were extractives (13%±2.5%). The amount of ash present in the sunflower hulls was approximately (0.4%±0.012%). The moisture of dry sunflower hulls was approximately (6%±1%). The amount of β-glucan and xylan presented in sunflower hulls is low compared to other biomass materials summarized in Table 3. However, the amount of lignin presented the biomass is high compared with other biomass materials.

Table 3 Compositional analysis of different biomass materials compared to sunflower hulls

Feedstock	β-Glucan	Xylan and Arabinnan	Lignin	Extractives	Ash	References
	% dry weight					
Corn stover	(34.0±0.5)	(21.7±0.5)	(12.3±0.2)	(22.5±0.6)	(4.7±0.2)	[14]
Miscanthus	(48.4±4.8)	(19.0±1.6)	(24.5±0.9)	(6.4±0.2)	(2.4±0.1)	[15]
Switchgrass	(37.8±1.3)	(28.3±0.8)	(21.0±0.2)	(17.0±0.7)	(5.8±0.1)	[16]
Sunflower hulls	(34.0±1.1)	(27.0±1.56)	(25.0±0.95)	(13.0±2.5)	(0.4±0.12)	

3.2 Effect of pretreatment conditions on hydrolyzate composition

The influence of three factors on sunflower hull biomass pretreatment has been studied using CCD in Table 4. A quadratic model was formulated for xylose yield in the hydrolyzate as a response variable (Y_1). Table 5 summarizes the model coefficients obtained from ANOVA table for different measured responses together with statistical significance R[2][17]. The P value was used as tool to check the significance of each coefficient. That the larger the magnitude of T value and smaller the P value the more significant is the corresponding coefficients and their interactions[17]. The model Equation (4) included only the significant coefficients (P <0.05) as summarized from Table 5[18,19]. In addition, R^2 value for the model was approximately 0.986 implying that only 0.014 of the variance in the data was not predicted by the model due to noise. Figure 1 shows good agreement of the predicted and experimental values for percentage xylose yield.

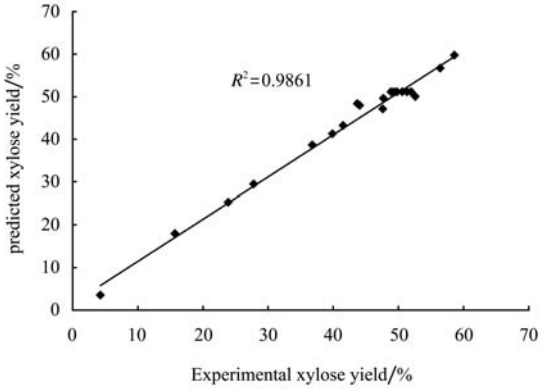

Figure 1 Experimental versus predicted values of xylose yield in the hydrolyzate

The contour plots are the graphical representation of regression Equation (4) that is drawn to illustrate interactive effects of independent variables (X_1 and X_3) on the dependent variable (Y_1). Maximum values are predicted by the surface confined by the ellipse in the contour plots. Elliptical contours are obtained when

there is a perfect interaction between the two independent parameters when the third parameter (X_2) is held constant[17]. The interaction between reaction temperature (X_1) and acid concentration (X_3) was found to be significant when reaction time (X_2) for 20 min was held constant as summarized in Table 5 (P<0.05). The reaction time of 20 min was chosen to plot the contour plots so that high xylose yield could be modeled with minimum amount of inhibitor products in the hydrolyzate samples. The contour plot between the factors mentioned above was analyzed to determine the optimum conditions for xylose recovery in the hydrolyzate Figure 2. The maximum xylose yield predicted by the model was found to be 62% at 1.75% acid concentration at 158℃.

$$Y_1 = 51.02 + 6.20X_1 + 3.92X_2 + 12.36X_3 - 3.60X_1^2 - 13.48X_3^2 + 5.47X_1X_3 \quad (4)$$

Table 4 Xylose yield determined experimentally and theoretically of liquid hydrolyzate and yield of β-glucan after enzymatic hydrolysis of solid residue

Reaction temperature /℃	Reaction time /min	Acid conc† /%	pH	CSF‡	Xylose yield (experimental)/%	Xylose yield (predicted)/%	Glucan digestibility/%
140	10	0.5	1.67	0.5	4.27	3.37	15.8
160	10	0.5	1.89	0.87	27.81	29.35	20.9
140	10	2	1.26	0.91	41.57	43.09	27.8
140	30	0.5	1.71	0.94	15.73	17.91	25.8
150	20	0.5	1.67	1.1	23.97	25.18	18.25
140	20	1.25	1.35	1.12	39.97	41.22	28.3
150	10	1.25	1.28	1.19	44.15	47.83	39.1
160	30	0.5	1.87	1.37	36.81	38.61	31.5
150	20	1.25	1.37	1.4	51.37	51.02	36.3
150	20	1.25	1.35	1.42	49.85	51.02	37.2
150	20	1.25	1.35	1.42	49.40	51.02	39.7
150	20	1.25	1.33	1.44	52.04	51.02	40
140	30	2	1.21	1.44	47.73	49.51	39.35
150	20	1.25	1.31	1.46	48.96	51.02	39.95
150	20	1.25	1.23	1.54	50.64	51.02	45.7
150	30	1.25	1.35	1.59	58.69	59.67	48.52
160	10	1.25	1.06	1.7	56.45	56.62	49.64
150	20	2	1.03	1.74	52.69	49.9	51.3
160	20	2	1.22	1.84	47.69	47.03	51.4
160	30	2	1.1	2.14	43.75	48.33	53.5

Note: † Acid concentration; ‡ Combined severity factor.

Table 5 Analysis of variance table of the coefficients and corresponding P values

Term	Coefficients	Standard error coefficient	T	P
Constant	51.02	0.9146	55.78	<0.001
X_1	6.20	0.892	6.946	<0.001
X_2	3.92	0.9654	4.058	<0.001
X_3	12.36	0.892	13.85	<0.001
X_1*X_1	-3.60	1.551	-2.32	0.04
X_2*X_2	-0.73	1.5684	-0.46	NS
X_3*X_3	-13.48	1.551	-8.69	<0.001
X_1*X_2	-1.32	1.1127	-1.19	NS
X_1*X_3	5.47	1.0124	-5.4	<0.001
X_2*X_3	-2.03	1.1127	-1.83	NS

Note: R^2=0.986, R^2(predicted)=0.86, R^2(adjusted) = 0.972; NS=Not significant.

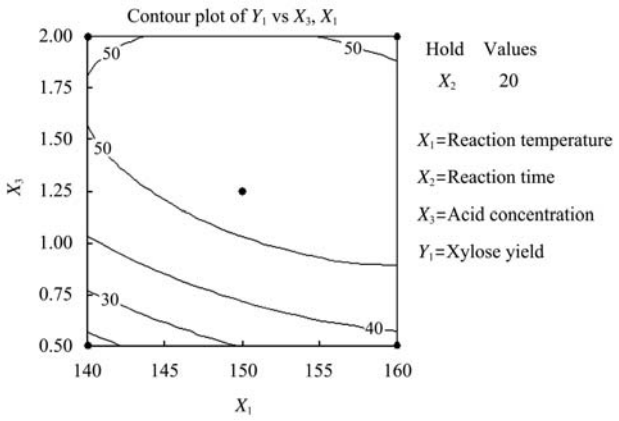

Figure 2 Contour plots for xylose yield in hydrolyzate as a function of acid concentration and pretreatment temperature according to the model (reaction time is 20 min)

3.3 Liquid fraction composition after the pretreatment

During the pretreatment hetero hemicellulose is easily hydrolyzed by dilute acids under moderate conditions. However, more extreme conditions are required to hydrolyze crystalline cellulose.

The success of the pretreatment is commonly evaluated by xylose yield. The xylose yield was analyzed by accounting for monomeric and oligomeric sugars in the liquid hydrolyzate using HPLC. The xylose yield in the hydrolyzate increased from 0.5 CSF to 1.59 CSF as summarized in Table 4. At higher severity factor, xylose yield decreased significantly. This can likely be explained by the formation of furfural due to xylose degradation in the liquid fraction[10]. However, it is interesting to note that at 0.91 CSF the xylose yield was higher compared to 0.94 CSF implying presence of higher acid concentration plays a vital role in hydrolysis of hemicellulose. The maximum xylose yield observed experimentally was 59% at 1.59 CSF. The low xylose yield can be explained by the presence of longer chain oligomers were predominant in the hulls. The dissolution and diffusion rates of longer chain oligomers in the solution are longer compared to shorter ones[20]. Arabinan and galactan accounted for only a small amount of the biomass composition. No mannan was detected in the biomass.

3.4 Evaluation of pretreated solid residue

The composition of solid recovery in terms of β-glucan, xylan and lignin was expressed in Figure 3. Solid recovery varied from 94% at low CSF to 74% at high CSF. Figure 3 shows that the xylan content in the solid fraction decreased as the severity of the pretreatment increased. The minimum amount of xylan retained was less than one percent at CSF of 2.14. β-glucan content increased up to a severity factor of 1.54 but declined slightly at higher severity factor. This can be explained by the degradation of β-glucan into HMF through glucose dehydration, which is attributed to stronger interaction of protons with water than the OH^- atom of the pyranose ring of glucose. This is the critical step in the proposed mechanism for the formation of 5-HMF at high severity factors[21]. Lignin consists of phenolic monolignols such as *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol. The amount of lignin ranged from 33%- 48% in the solid substrate.

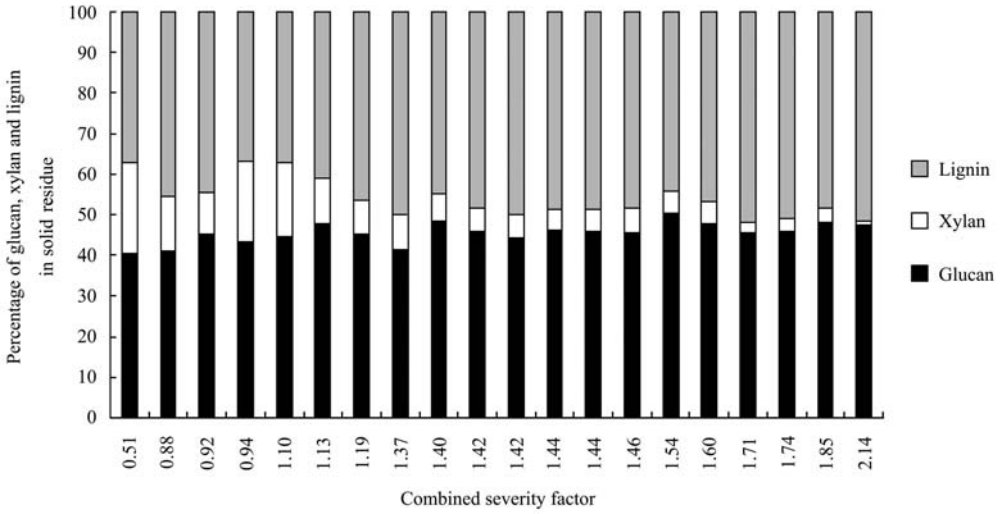

Figure 3 Percentages of β-glucan, xylan and lignin retained in the solid substrate after the pretreatment

3.5 Enzymatic saccharification

The optimum temperature and pH value for sunflower hulls enzymatic saccharification found by other researchers was to be at 50℃ and at pH 4.8[10]. The maximum β-glucan digestibility observed was 53.5% at 2% acid concentration. The digestibility yield of pretreated sunflower hulls was lower compared to popular corn stover biomass. The maximum digestibility observed for corn stover was between 80%-87% when treated at 1.4% acid concentration[22]. The possible explanation is the presence of high lignin content in the solid substrate as evident from Figure 3.

It leads to lignin sites competing against β-glucan sites for enzymes. The enzymes that were adsorbed by the lignin sites became ineffective by forming lignin enzyme complexes. Other researchers proved that there is a quantitatively inverse correlation between lignin content and enzymatic digestibility[23].

3.6 Degradation products

Degradation compounds (acetic acid, furfural, HMF) are known to act as inhibitors for enzyme activity under selected conditions during fermentation process. Acetic acid is liberated from acetyl groups in the xylose fraction; furfural and HMF are products of pentose and hexose degradation respectively[24]. HMF was present in trace amount in the liquid hydrolyzate and there was not much variation in the yields of HMF as shown in Table 6. It implies that hexose sugars degrade at much higher temperatures and at high acid concentration. However, the amount of pentose sugar degradation product ranged from 0 mg/mL at low CSF to 5.58 mg/mL at high CSF. The mechanism for conversion of xylose into furfural involves conversion of xylose into lyxose through isomerization reaction and dehydration of lyxose leads into furfural[25]. The possible explanation for high yields of furfural is that xylose degradation is favored by high reaction temperature and long reaction time. The results obtained on the degradation products were in agreement with the study conducted by Wei et al.[26]. According to their study, xylose decomposes rapidly compared to glucose.

Table 6 Concentration of inhibitor products presented in the liquid hydrolyzate at different CSF

CSF	Acetic acid/mg · mL^{-1}	HMF/mg · mL^{-1}	Furfural/mg · mL^{-1}
0.5	0.41	0	0
0.87	1.44	0.09	0.16
0.91	3.9	0.15	0.15
0.94	1.01	0	0
1.1	1.14	0	0
1.12	3.87	0.16	0.18
1.19	3.92	0.17	0.28
1.37	2.93	0.1	0.56
1.4	4.94	0.18	0.83
1.42	5.13	0.21	0.87
1.42	4.85	0.22	0.8
1.44	5.19	0.19	0.9
1.44	4.89	0.16	0.92
1.46	4.7	0.19	0.77
1.54	5.34	0.2	0.93
1.59	5.61	0.17	1.47
1.7	5.25	0.15	1.45
1.74	5.37	0.16	2.49
1.84	5.23	0.16	2.88
2.14	5.54	0.16	5.58

4 Conclusions

The effects of reaction time, reaction temperature, and acid concentration on the sunflower hulls biomass pretreatment process were studied using Central Composite Design methodology. These three factors and their interactions were statistically analyzed by central composite design methodology for xylose yield. The maximum xylose yield predicted by model was 62% and at 158℃ for 20 min at 1.75% acid concentration. The amount of fermentable sugars formed after the enzymatic hydrolysis showed a linear increase with the severity of the pretreatment. The maximum β-glucan digestibility was observed to be 53.5% at 2.14 CSF. The low digestibility implies that high lignin content in the biomass may be inhibiting the complete hydrolysis of β-glucan during enzymatic hydrolysis. It implies that irreversible adsorption of lignin on to crystalline β-glucan structure was occurring[8]. In order to convert cellulose and hemicellulose effectively into fermentable sugars during enzymatic saccharification, sunflower hulls may need to undergo de-lignification process prior to acid pretreatment. Degradation products were studied on the liquid fraction of pretreated samples. Increase in the severity of pretreatment led to augmentation of inhibitor products such as acetic acid and xylose degradation into furfural. However, the amount of glucose degradation to HMF was relatively low compared with acetic acid and furfural. Other factors worth investigating during the sunflower hulls pretreatment in the future are the effect of particle size, pore volume, and the surface area available. Those factors may play a role in effectively converting cellulose into fermentable sugars for renewable fuels and chemicals production.

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