ZIBELINE INTERNATIONAL™ PUBLISHING

Journal of Wastes and Biomass Management (JWBM)

ISSN: 2710-6012 (Online)
CODEN: JWBMAO

DOI: http://doi.org/10.26480/jwbm.02.2022.102.110

CrossMark

RESEARCH ARTICLE

INDIVIDUAL AND INTERACTIVE EFFECTS OF PEROXIDE PRETREATMENT VARIBLES ON SACCHARIFICATION AND ETHANOL YIELD IN BAGASSE

Rama Mohan Poludasu[a,b*], Piyarath Boonsawang[b]

[a]Nanotechnology laboratory, IFT, Regional Agricultural Research Station, Tirupati, Andhra Pradesh, India- 517502.
[b]Center of Excellence in Innovative Biotechnology for Sustainable Utilization of Bioresources, Biotechnology Program, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkla, Thailand, 90110.
**Corresponding Author Email: rammohanroyal@gmail.com*

ARTICLE DETAILS

Article History:

Received 25 October 2022
Revised 05 November 2022
Accepted 13 December 2022
Available online 21 December 2022

ABSTRACT

The current experiment aimed to find the ideal pretreatment process parameters for maximize the yields of cellulose, fermentable simple sugars, and ethanol from bagasse that had successfully treated with hydrogen peroxide (H_2O_2). The pretreatment process variables like substrate concentration, H_2O_2 concentration, pretreatment time, and temperature were studied individually and in combination to see how they affected the response variables like cellulose, hemicellulose, and lignin content in the pretreated pulp. This was done using the response surface methodology (RSM). The best pretreatment conditions for sugar cane bagasse were found through experiments using a factorial central composite design (CCD). The highest cellulose and hemicellulose yields, which were determined by RSM to be 69.3% and 76.4%, respectively, with a lesser lignin yield (4.8%), were achieved at a substrate concentration of 2%, an H_2O_2 loading of 20%, a temperature of 120 oC, and a pretreatment duration of 120 min. The experimental actual and predicted outcomes were well correlated, demonstrating that the model may be applied to effectively pretreat lignocellulosic biomass. The model has been validated when there is no difference between experimental actual values and anticipated values. The modifications in the chemical structure of bagasse during pretreatment was analyzed using FTIR. Comparatively, samples processed with H_2O_2 had a higher crystallinity (CI = 23.63%) than untreated bagasse (CI = 15.84%). The loss of lignin, which contributed the greatest CrI, increased the proportion of cellulose in treated bagasse compared to untreated bagasse. The bagasse that had a H_2O_2 concentration pretreatment showed the highest reducing sugar yield (58.7 mg/g). Pretreated bagasse had a higher ethanol output (73.88 g/L) than untreated bagasse (45.49 g/L).

KEYWORDS

Bagasse, CCD, H_2O_2, Pretreatment, FTIR, Cri, Saccharification, Ethanol.

1. INTRODUCTION

Lignocellulosic biomass consists of polymers of cellulose, hemicellulose, and lignin bound together in a complex structure. Among these, cellulosic materials are predominantly attractive renewable feedstocks to produce biofuels due to their relatively low price, excessive abundance, and continual supply (Lynd et al., 2002). Lignocellulosic biomass can possibly be transformed into value-added products such as biofuels, primarily bioethanol, biooil, gasoline, and chemicals. Several kinds of conversion technologies exist that follow thermal, thermochemical, and biological routes for lignocellulosic biomass conversion (Nanda et al., 2014). Bio-ethanol can be made from biomass material during the fermentation of sugars resulting from the cellulose and hemicellulose within lignocellulosic substrates, but the biomass needs to be subjected to pretreatment processes to release the sugars necessary for fermentation (Agbor et al., 2011).

Lignocellulosic biomass needs pretreatment to release sugars contained within cellulose fibers fixed in the hetero matrix of plant cell walls. However, lignocellulosic feedstocks need destructive pretreatment to yield a substrate that can be simply hydrolyzed by enzyme-producing microorganisms or by commercial cellulolytic enzymes to unleash sugars for fermentation (Agbor et al., 2011). The harshness of pretreatment conditions is generally compromised to maximize sugar retrieval and is dependent upon what kind of pretreatment process is used. Hemicellulose could be obtained either as a solid fraction or as a mixture of solid and liquid fractions (Chandra et al., 2007). Despite the chance of several pretreatment techniques, the fundamental barrier to the manufacture of bioethanol is the first conversion of biomass to sugars. Pretreatment may be used on a variety of materials, and adding lignocellulosic feedstock should enhance a significant majority of the lignocellulosic components in distinct fractions in a usable form (Agbor et al., 2011).

Numerous pretreatment strategies are continuously being researched and developed. Pretreatment's main objective is to reveal the biomass structure, increase accessible surface area, reduce cellulose crystallinity, and increase porosity, pore diameter, and pore volume (Karunanithy and Muthukumarappan, 2013). With varying degrees of effectiveness, alternative approaches have been tried in significant pretreatment attempts on a variety of biomasses. Among those well-known pretreatment techniques include acid, alkali, hydrothermal (steam, steam explosion, hot water), and thermochemical ammonia fibre expansion (AFEX).

Another essential stage in developing a pretreatment technology that is useful and inexpensive is optimizing the pretreatment process parameters (Karunanithy and Muthukumarappan, 2011; Yildirim et al., 2021). RSM is a convenient statistical application method and is applied in research

Quick Response Code	Access this article online	
	Website: www.jwbm.com.my	**DOI:** 10.26480/jwbm.02.2022.102.110

Cite the Article: Rama Mohan Poludasu, Piyarath Boonsawang (2022). Individual and Interactive Effects of Peroxide Pretreatment Varibles on Saccharification and Ethanol Yield in Bagasse. *Journal of Wastes and Biomass Management, 4(2): 102-110.*

involving complex variable processes. To calculate the impact of two or more independent variables on the dependent variables, multiple regression and correlation analysis are used as statistical methods. Its major advantage is the condensed range of experimental runs needed to provide satisfactory data for a statistically adequate result. Rice straw has been effectively used in RSM's optimization for potential sugar production (Kim and Han, 2012). CCD can be combined with RSM, in which trials are planned by CCD and subsequently optimised by RSM (Dahiya et al., 2005). The RSM has been efficiently applied to pretreating biomass in many researches (Kim and Han, 2012; Zaafouri et al., 2017; Rajendran and Muthukumar, 2012; Yildirim et al., 2021). Despite the almost three decades of research on biomass pretreatment, no efficient conversion process has been formed for the industrial manufacture of biofuels from biomass (De Leon and Coors, 2008).

A proximate analysis of untreated bagasse shows that bagasse contains 42.7% cellulose, 34.4% hemicelluloses, and 18.2% lignin (Mohan et al., 2013). The main goal of the existing study was to use RSM and CCD for finding optimum pretreatment conditions to enrich the cellulose fallow from high sugar and decrease lignin yield in H_2O_2-pretreated bagasse. Hence, the existing study was conducted for to detect the optimum pretreatment conditions and understand the response of individual pretreatment process variables on cellulose, hemicelluloses, and lignin yield in pretreated bagasse through statistical optimization. Further, fermentation was conducted using a pretreated substrate to estimate the performance of the H_2O_2-pretreated bagasse. The current study also gives results describing the feasibility of utilizing the bagasse for ethanol production following H_2O_2 pretreatment.

2. Material and Methods

2.1 Untreated Bagasse

Sugarcane bagasse substrate was taken from local sugarcane bagasse juice sellers, Hat Yai, Songkla, Thailand. The collected bagasse was dried in an air oven incessantly for 6 to 8 hours at 70 to 80 °C and the dried bagasse was milled, grinded (5 mm mesh size by hammer mill), and used as powder.

2.2 CCD (Central Composite Design) Experiment

Table 1: Actual and coded values of the variables in CCD

Factor	Name	Low Actual	Middle Actual	High Actual	Low Coded	Middle Coded	High coded
X_1	Substrate (%, w/v)	2	11	20	-1	0	1
X_2	H_2O_2 (%, v/v)	2	11	20	-1	0	1
X_3	Temperature (°C)	60	90	120	-1	0	1
X_4	Time (min)	30	75	120	-1	0	1
Response	**Name**	**Units**	**obs[a]**	**Min.**	**Max.**	**Mean**	**Std.Dev.**
Y_1	Cellulose	%	30	24.6	69.3	44.4	10.9
Y_2	Hemicellulose	%	30	30.2	76.3	49.82	12.66
Y_3	Lignin	%	30	4.8	19.2	12.88	3.78

[a]Observed run values

Table 2: CCD Experimental Design Matrix With Experimental and Predicted Responses

Std	Substrate (%, w/v)	H_2O_2 (%, v/v)	Temp. (°C)	Time (min)	Cellulose (%)	Hemicellulose (%)	Lignin (%)
1	2	2	60	30	31.2(33.9)	33.8(39.4)	18.2(16.1)
2	20	2	60	30	27.9(33.2)	30.2(35.5)	18.9(18.3)
3	2	20	60	30	35.8(36.5)	37.2(36.9)	16.4(16.9)
4	20	20	60	30	31.4(33.9)	33.6(38.6)	18.6(16.7)
5	2	2	120	30	35.4(39.1)	41.2(44.5)	13.3(12.8)
6	20	2	120	30	30.8(34.0)	34.2(34.8)	15.9(15.9)
7	2	20	120	30	45.6(45.7)	48.4(48.7)	12.6(12.8)
8	20	20	120	30	35.8(38.6)	41.6(44.5)	13.9(13.5)
9	2	2	60	120	37.2(38.8)	44.9(46.1)	12.8(13.2)
10	20	2	60	120	34.6(33.8)	38.5(38.0)	16.9(16.8)
11	2	20	60	120	49.3(45.5)	48.4(47.6)	12.2(12.2)
12	20	20	60	120	37.8(38.5)	44.2(45.0)	12.9(13.4)
13	2	2	120	120	60.9(57.8)	72.1(66.9)	5.20(7.1)
14	20	2	120	120	44.6(48.3)	48.6(53.0)	12.2(11.7)
15	2	20	120	120	69.3(68.4)	76.4(75.1)	4.8(5.4)
16	20	20	120	120	60.2(56.9)	72.4(66.7)	5.4(7.5)
17	20	11	90	75	40.8(42.1)	46.8(47.2)	13.9(13.2)
18	29	11	90	75	35.2(29.9)	39.5(34.9)	17.2(17.5)
19	11	11	90	75	50.4(44.0)	54.6(49.1)	11.6(12.2)
20	11	29	90	75	52.8(55.2)	58.9(60.3)	9.82(8.9)
21	11	11	30	75	24.6(21.9)	30.8(24.5)	19.2(20.7)
22	11	11	150	75	46.8(45.5)	49.2(51.3)	13.3(11.5)
23	11	11	90	75	48.4(39.7)	51.8(42.3)	12.9(15.1)
24	11	11	90	165	58.2(62.9)	65.8(71.1)	8.7(6.1)
25	11	11	90	75	51.4(51.4)	58.6(58.6)	11.6(11.6)
26	11	11	90	75	51.4(51.4)	58.6(58.6)	11.6(11.6)
27	11	11	90	75	51.4(51.4)	58.6(58.6)	11.6(11.6)
28	11	11	90	75	51.4(51.4)	58.6(58.6)	11.6(11.6)
29	11	11	90	75	51.4(51.4)	58.6(58.6)	11.6(11.6)
30	11	11	90	75	51.4(51.4)	58.6(58.6)	11.6(11.6)

[a]Std: Standard run order.

The CCD experiments and statistical method analysis were done in step with the RSM by using Design-Expert 8.0.6.1 (Stat-Ease Inc., Minneapolis, USA) version software package (Design-Expert, 2018). In this study, a factorial CCD with replicates at the centre points was utilized for four variables. The pretreatment method variables were specifically substrate concentration (X_1 %, w/v), H_2O_2 concentration (X_2 %, v/v), pretreatment temperature (X_3 °C) and pretreatment time (X_4 min) at low (-1), middle (0) and high (+1) coded levels. A total of 30 experimental trials or runs that comprised 16 runs for factorial design, 6 runs for axial points, and 8 runs for replication of the central points, were performed. Cellulose (Y_1 %), hemicellulose (Y_2 %) and lignin (Y_3 %) yields are three dependent method variables that were determined in bagasse. In CCD, the selection and levels of the factors tested in the existing study are displayed in Table 1. A 2^4-factorial design model with 8 replications at the centre points results to overall 30 runs (Table 2) were tested to optimize the pretreatment conditions. Regression analysis of process trial information and three-dimensional (3D) graphs of the process factors in the experimental field and optimized values of four absolute variables for maximum activities were determined using the Design-Expert 8.0.6. 1. statistical software package.

2.3 Yeast Strain and Inoculums Preparation

Saccharomyces cerevisiae MTCC174, a yeast strain that is flocculent and tolerant to ethanol was used and the yeast strain was inoculated and maintained on MGYP (malt extract 3, glucose 10, yeast extract 5, peptone 5, and agar 24 g/L) medium slants. The yeast was transferred to MGYP medium in a 250 mL conical flask in addition with 100 mL of basal medium that contained 10 g/L glucose as a carbon source to create the inoculum for the fermentation studies. The yeast was cultured at 35 °C on an orbital incubator shaker for 12 hr. For fermentation, the final yeast inoculum concentration was around $1.5X10^8$ cells/mL, and about 10 % inoculum was added to fermentation experiments.

2.4 Fermentation of Bagasse Substrate

The medium utilized for ethanol fermentation through submerged fermentation (smf) was comprised of yeast extract 0.25 % (w/v), $(NH_4)_2SO_4$ 0.25 % (w/v), 0.1 KH_2PO_4 0.1 % (w/v), $MgSO_4$ 0.05 % (w/v), and pH 5.0. A 250 mL conical flask containing saccharified pretreatment bagasse (1%), in addition with medium constituents, was sterilized at 121 °C for 15 minutes. After sterilization, the medium was permitted to cool to room temperature. Under sterile conditions the medium was added with 10 mL of the yeast strain inoculum, and the combination was subsequently fermented for 72 hours at 30 °C. At regular intervals, samples were obtained, and once the fermentation process was complete, the amount of ethanol produced was estimated.

2.5 Pretreated Bagasse Substrate Saccharification

Experiments were administered in duplicate in 50-mL conical flasks containing 1 gm of low (2 %) and high (20 %) concentration H_2O_2 pretreated bagasse, 0.5 mL of cellulase preparation and Tween-80 in 0.01 M citrate buffer (pH 4.8), supplemented with 1 % (v/v) of penicillin-streptomycin solution (Hi-Media, India) (Mohan et al., 2013). Erlenmeyer conical flasks were incubated at 50 °C on a bench-top rotary shaker (200-220 rpm) until 60-90 hr. The liquid supernatant recovered after centrifuging the samples at 12000 rpm for 10-15 minutes was used to approximate the quantity of sugar in the sample.

2.6 Analytical Method of Analysis

The AOAC (2005) technique, which uses multifunction approaches to divide the contents of cellulose, hemicellulose, and lignin, was applied. The Neutral Detergent Fiber (NDF) method, taking into consideration the cellulose, hemicellulose, and lignin portions, is utilized to evaluate the majority of the fibre cell wall in biomass. Acid Detergent Fiber (ADF) was determined consecutively using the solid residue portion left over from NDF estimation. The hemicellulose was calculated by deducting ADF from NDF (Jung and Vogel, 1992). The material treated by ADF and NDF was also hydrolyzed with 72 % H_2SO_4 to estimate cellulose. Lignin was gained through the ashing of hydrolyzed residue. Utilizing the 3, 5-dinitrosalicylic acid (DNS) technique, the amounts of liberated reducing sugar and glucose were measured (Miller, 1959). The content of arabinose and xylose was valued by the procedure previously described (Khabarov et al., 2006).

2.7 FT-IR Spectroscopy

Untreated and pretreated bagasse FT-IR spectra were estimated by a Fourier transform infrared spectrometer (Spectrum GX-1, PerkinElmer, USA). The tested samples were completely dried at 80°C under vacuum for 12 h before analysis. After that, a small quantity (~1 mg) of sample was combined with potassium bromide (KBr) (300 mg) powder and pressed to create a disc. 100 scans were averaged at a resolution of 1 cm for each sample spectrum, which ranged in size from 4000 to 400 cm^{-1}.

2.8 Crystalline Analyzed by XRD

A Bruker D8 advance diffractometer was utilized to examine the untreated and pretreated bagasse XRD pattern. The Cu K radiation (λ = 0.154nm) was used at 40 kV and 20 mA. The given test sample was scanned, and the recorded intensity in 2θ ranged from 5°to 80°.

2.9 Quantification of Ethanol by Gas Chromatography (GC)

The system employed is a flame ionization detector-equipped Agilent system of type 6890 (FID). The subsequent column chromatographic parameters were utilised for the recognition of ethanol: a graphitized packed column, 5% carbowax 20 M phase, matrix 80/120 carbopack-B, length 6 feet (1.83 m), 2 mm I.D., and 1/4" O.D. In order to release the chemicals, as a carrier gas, nitrogen was employed at a continuous flow rate of 20 mL/min. The fuel gas utilised was hydrogen, which flowed at a steady rate of around 40 mL/min. An internal standard was developed utilizing secondary butyl acetate (Anthony, 1984).

3. Results

Process condition optimization is a part of most acute stages in the progression of an efficient economic bioprocess (Rathore et al., 2021). Conventional and statistical approches are available for optimizing the general process conditions, such as RSM, Taguchi, SX, etc. RSM is an significant mathematical model with an association of statistical methods wherein the interactions among several process variables can be recognised with fewer experimental trials (Bas et al., 2007). The consequence of four Independent process factors on cellulose, hemicellulose, and lignin yield was examined using the RSM method, and the collected data is given (Table 1 and Table 2). The impact of every factor and their associations were concentrated via analysis of variance (ANOVA) and the chi-squared test (X^2) as suitable to the test plan being utilized. The determined regression equation statistical model for the enhancement of pretreatment conditions demonstrated that the cellulose (Y_1 %), hemicellulose (Y_2 %) and lignin (Y_3 %) yields are the functions of the process involving substrate concentration (X_1 %), H_2O_2 concentration (X_2 %), pretreatment temperature (X_3 °C) and pretreatment time (X_4 min). Through employing multiple linear regression analysis on the investigational model data, the later second-degree polynomial equation is effectively signifies the cellulose, hemicellulose, and lignin yields.

$$\text{Cellulose } (Y_1\,\%) = 51.4 - 3.03X_1 + 2.81X_2 + 5.91X_3 + 5.82X_4 - 3.84X_1^2 - 0.44X_2^2 - 4.41X_3^2 - 0.01X_4^2 - 0.5X_1X_2 - 1.12X_1X_3 - 1.09X_1X_4 + 0.99X_2X_3 + 1.0X_2X_4 + 3.43X_3X_4 \quad (1)$$

$$\text{Hemicellulose } (Y_2\,\%) = 58.6 - 3.07X_1 + 2.8X_2 + 6.7X_3 + 7.22X_4 - 4.37X_1^2 - 0.98X_2^2 - 5.16X_3^2 - 0.46X_4^2 + 1.37X_1X_2 - 1.46X_1X_3 - 1.06X_1X_4 + 1.66X_2X_3 + 0.99X_2X_4 + 3.92X_3X_4 \quad (2)$$

$$\text{Lignin } (Y_3\,\%) = 11.6 + 1.07X_1 - 0.84X_2 - 2.31X_3 - 2.24X_4 + 0.96X_1^2 - 0.25X_2^2 + 1.13X_3^2 - 0.23X_4^2 - 0.6X_1X_2 + 0.24X_1X_3 + 0.35X_1X_4 - 0.2X_2X_3 - 0.44X_2X_4 - 0.67X_3X_4 \quad (3)$$

Experimentally predicted levels of cellulose, hemicellulose, and lignin yield in the resultant pretreated bagasse utilizing the above conditions have appeared in Table 3 alongside test data. The goodness of the current model can be tested by the correlation coefficient (R) and coefficient of determination (R2). The R2 was calculated at 0.9110 for cellulose, 0.9131 for hemicelluloses, and 0.9110 for lignin yield (Table 3), showing that the experimental statistical design can clarify above 90 % of the variability in the response, which showed that models for each experimental response variable were well fitted to explain the association between the model involved variables and that only about 10 % of the total variation cannot be assigned to the independent variables. In general, the R2 value is always between 0 and 1.

The nearer the R^2 is to 1.0 the more stable the design and also the higher it predicts the response. Generally, a regression model with an R^2 higher than 0.90 is calculated to have a very high correlation (Haaland, 1989). In the current study, the obtain values of R for all 3 response variables, for cellulose=0.8279, Hemicellulose=0.8319 and for lignin=0.8279, were higher than 0.80, indicating an adjacent agreement between the studied experimental actuals and the predicted theoretical results by the used model equation.

Table 3: Analysis of the variance (ANOVA) of the CCD

		F-Value			P-value		
Source	Df	Y_1	Y_2	Y_3	Y_1	Y_2	Y_3
Model	14	10.97	11.25	10.96	< 0.0001	< 0.0001	< 0.0001
X_1	1	10.66	8.37	11.24	0.0052*	0.0111*	0.0044*
X_2	1	9.14	6.98	6.87	0.0086*	0.0185*	0.0193*
X_3	1	40.46	39.96	51.85	< 0.0001	< 0.0001	< 0.0001
X_4	1	39.21	46.36	48.9	< 0.0001	< 0.0001	< 0.0001
X_1X_2	1	0.19	1.12	2.34	0.6666	0.3062	0.1473
X_1X_3	1	0.98	1.27	0.37	0.3384	0.2776	0.5543
X_1X_4	1	0.91	0.67	0.79	0.3543	0.4259	0.3868
X_2X_3	1	0.75	1.64	0.26	0.3991	0.2197	0.6179
X_2X_4	1	0.77	0.58	1.24	0.3933	0.4586	0.2827
X_3X_4	1	9.06	9.14	2.96	0.0088	0.0085	0.1061
X_1^2	1	19.48	19.47	10.16	0.0005	0.0005	0.0061
X_2^2	1	0.25	0.97	0.72	0.6236	0.341	0.41
X_3^2	1	25.76	27.11	14.22	0.0001	0.0001	0.0018
X_4^2	1	1.44E-04	0.22	0.6	0.9906	0.6476	0.4518
Residual	15						
Lack of fit	10						
Pure error	5						
Cor total	29						

Y_1=Cellulose, Y_2=Hemicellulose, Y_3=Lignin; *P<0.05–significant at 5% level, $^{\alpha}$P<0.001–significant at 1% level, $^{\beta}$P<0.0001 significant at 0.1% level, $^{\#}$not significant

The F-test and the corresponding P-value, along with the factor estimates, are also presented (Table 3). For cellulose yield, the model terms X_3 and X_4 are more significant, with a probability (P) of 99 %. There is a considerable significant interaction between model terms X_3 and X_4, which indicates the good effect of these variables on an increase in cellulose yield in treated bagasse. Concerning hemicellulose yield, model terms X_4, X_2^2 and X_3^2 are critical with the P-value of 99 %, and X_3 is important with the P-value of 95 % (Table 3). Significant interaction between the X_3 and X_4 process variables will influence the hemicellulose yield in pretreated bagasse. Considering lignin yield, the important P-value of 99% is for the model term X_4. 95 % significant P-value was shown by the model terms X_2 and X_3^2 (Table 3). Pretreatment factors and the decline in lignin production in bagasse are not found to interact significantly. According to the findings in ANOVA Table 3, pretreatment temperature and duration both significantly influenced the yields of cellulose and hemicellulose, whereas pretreatment time significantly influenced the yields of lignin. Increasing the temperature induces cleavage of the lignin-carbohydrate bonds. Lower lignin yield was attained at the treated temperature at its higher level (120°C).

3.1 Optimized Pretreatment Conditions

Response surface model plots show the control of four pretreatment variables on cellulose, hemicellulose, and lignin yields in the resulting pretreated bagasse. The outcome denotes that both cellulose and hemicellulose reaction surfaces had the greatest point, with lignin at the restricting point. Even though the process response surface graphs were helpful in showing the way in which to modify the factors for to increase the cellulose, hemicellulose yields and minimizes the lignin yields. The maximum level of cellulose yield was obtained at a elevated temperature (120 °C) and a longer pretreatment retention time (120 min) (Figure 1), and there is considerable interaction noticed between these two variables. Hemicellulose response surface graph implies that the maximum yield of hemicellulose in pretreated bagasse was achieved at the incresed temperature along with a longer pretreatment time (Figure 2). The data was achieved at a higher temperature with a longer pretreatment duration, demonstrating the poor yield of lignin content in bagasse (Figure 3).

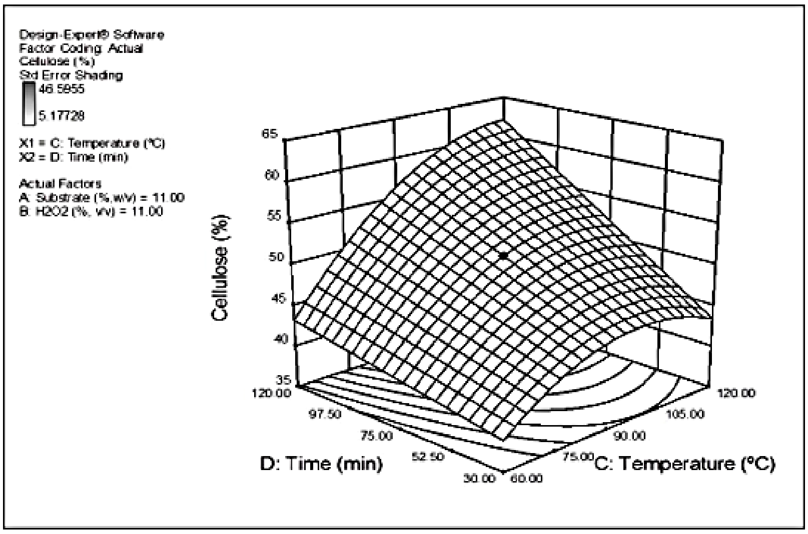


Figure 1: Response surface plot of time and pretreatment temperature (°C) on cellulose yield

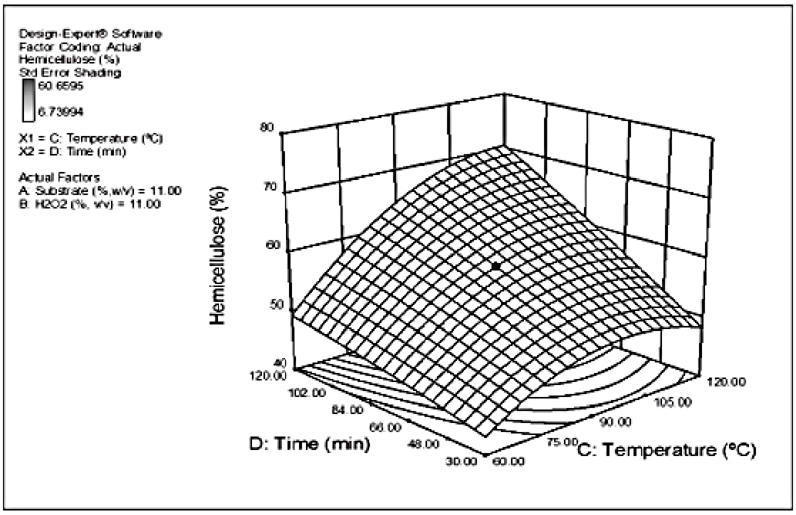


Figure 2: Response surface plot of time and pretreatment temperature (°C) on hemicellulose yield

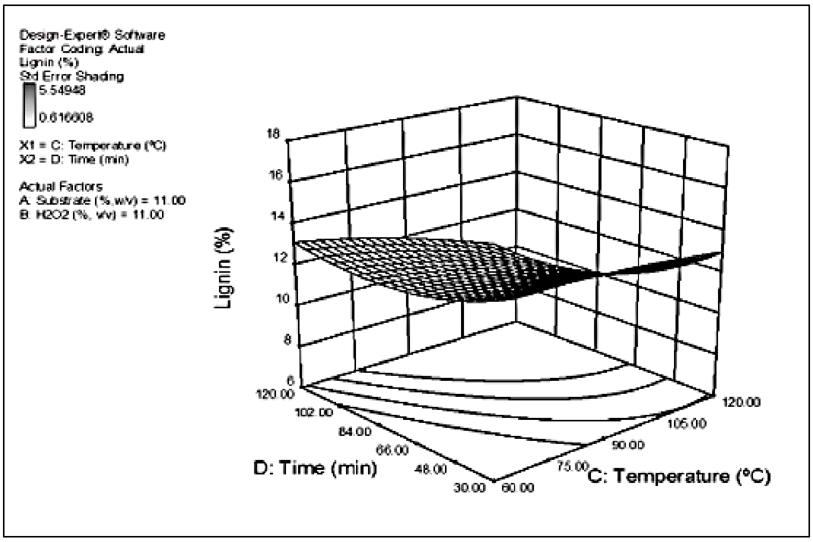


Figure 3: Response surface plot of time and pretreatment temperature (°C) on lignin yield

Under the ideal pretreatment conditions of substrate concentration (20 %), H_2O_2 concentration (2 %), pretreatment temperature (60 °C), and pretreatment time (30 min) the cellulose, hemicelluloses, and lignin yields were 27.9, 30.2, and 18.9 % respectively and minimum yield of cellulose was observed. To ensure accuracy, several tests have been conducted. The outcomes of three replicates were similar to the model regression predicted value, and the current model was verified as to sufficient. Under the model pretreatment conditions of substrate concentration (2 %), H_2O_2 concentration (20 %), pretreatment temperature (120 °C) and pretreatment time (120 min), maximum yields of cellulose and hemicellulose with low lignin yields of 69.3, 76.4, and 4.8 % respectively, were obtained. The maximum response predicted by the model was 68.4, 75.1, and 5.4 %. By comparing the studies, it was discovered that the yields of cellulose and hemicellulose increased from 37.4 to 72.0% and 31.9 to 56.8%, respectively, while the yield of lignin decreased from 16.2% to 4.8. At the experimental temperature of 120 °C and the pretreatment period of 120 min, the highest cellulose content was attained. The highest cellulose percentage of 69.3% achieved shows that the lignin concentration in pretreated bagasse substrate is significantly decreased by pretreatment at a temperature of 120 °C. The hemicellulose content in pretreated bagasse was increased relevant to the cellulose content as the pretreatment time duration was extended. Overall three verification tests, as listed in Table 4, were conducted in the experimental range to evaluate the model's quality. To determine the association between experimental actual and expected values, the validation run results were statistically examined as well. The actual and anticipated R^2 values were fixed to be 0.90, suggesting that the experimental results and predictions are in close agreement and confirming the reliability of the current model.

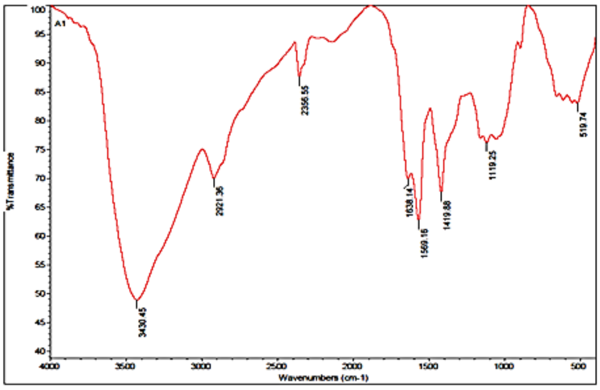
Table 4: Model Validation Experimental Trails

Number	Substrate (%, w/v)	H_2O_2 (%,v/v)	Temp. (°C)	Time (min)	Cellulose (%)	Hemicellulose (%)	Lignin (%)
1	20	20	60	30	30.2(29.2)	32.6(31.0)	18.6(16.9)
2	20	2	120	120	42.1(41.6)	43.6(42.8)	15.8(14.7)
3	2	20	120	120	68.3(66.9)	74.3(73.1)	4.8(4.2)

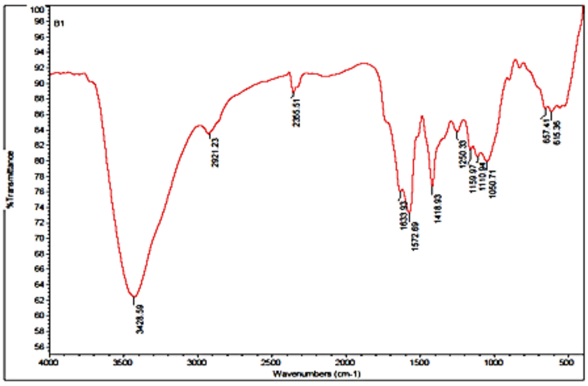
*Values are mean of two replicates

3.2 FTIR and XRD analysis

To recognize the modifications in the complex chemical composition of cellulose, hemicellulose, and lignin during the bagasse pretreatment, semi-quantitative analysis using FTIR has been used. Both untreated (A) and H_2O_2-pretreated (B) samples' infrared spectra were obtained (Figures 4a and 4b). The literature review provided the basis for the allocation made to the observed absorption peaks and bands. The existence of cellulose, hemicellulose, and lignin, the three primary components of lignocellulosic biomass, is associated to the main properties of these spectra. Figures 4a and 4b, which contrast their IR spectra, demonstrate that there are no significant chemical structural changes following bagasse treatment.



(a)

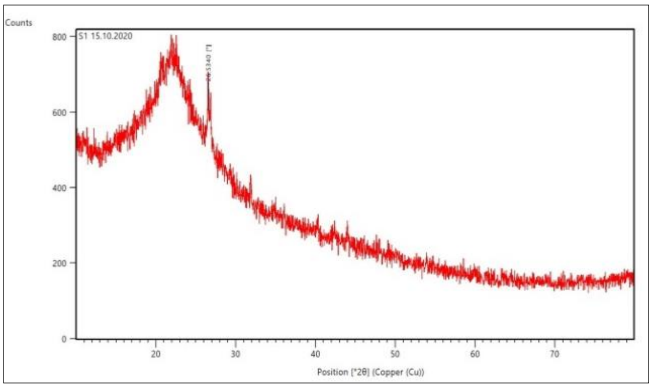


(b)

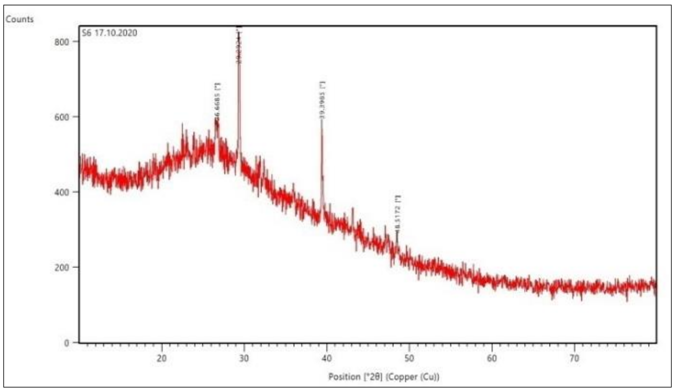
Figure 4: (a) FTIR Spectrum of Untreated sugar cane bagasse; (b) FTIR Spectrum of H_2O_2 treated sugar cane bagasse

The CH_2 and CH_3 groups in cellulose, hemicellulose, and lignin's CH_2 and CH_3 groups might be the reason for the adsorption band at 2920 cm^{-1}. The region between 3100 and 3600 cm^{-1} was where the O-H stretching vibration's spectral peak was discovered. At 3386 cm^{-1}, it was possible to see the characteristics of the OH groups found in lignin and carbohydrates. For the main and secondary OH stretching vibrations, pretreated bagasse displayed a higher absorbance peak than untreated bagasse at 1050 cm^{-1} and 1159 cm^{-1}, respectively. Absorption peak nearer to 2922 cm^{-1} represents the symmetric C-H stretching in the aliphatic methyl group. The peak between 1266–1200 cm^{-1} represents the bending frequency of C–H, O–H, or CH_2, while 1060–1050 cm^{-1} refer to the C–H stretching vibration of C–O. Due to the vibration of silica bonds, a sharp peak was appeared in the 1050 cm^{-1} region. After peroxide treatment, the two peaks in the spectral region between 1,100 and 1,000 cm^{-1} are clearly visible, showing the loss of hemicelluloses. Additionally, the area at 1,247 cm^{-1} shows evidence of hemicellulose clearance. The peaks between 1,200 and 1,000 cm^{-1} are emphasized after the hydrolysis, which proves that the cellulose was hydrolyzed.

Figures 5a and 5b show diffractograms of both untreated (A) and H_2O_2-pretreated bagasse (B). As can be noticed, both samples show the characteristic cellulose diffraction peaks, with the main peak identical to the I002 crystallographic planes. The samples, crystallinity index was calculated accordingly as reported (Rodrigues Filho et al., 2007; Segal et al., 1959). To determine the CrI of samples of bagasse, the difference in intensities (I002) of the amorphous and crystalline cellulose peaks were taken into consideration. In comparison to samples that had received H_2O_2 treatment (23.63%), untreated bagasse showed reduced crystallinity (15.84%). In the current study, the increased crystallinity index in the pretreatment samples is attached to the limited elimination of the hemicellulose component.



(a)



(b)

Figure 5: (a) XRD Spectrum of Untreated sugar cane bagasse; (b) XRD Spectrum of H_2O_2 treated sugar cane bagasse

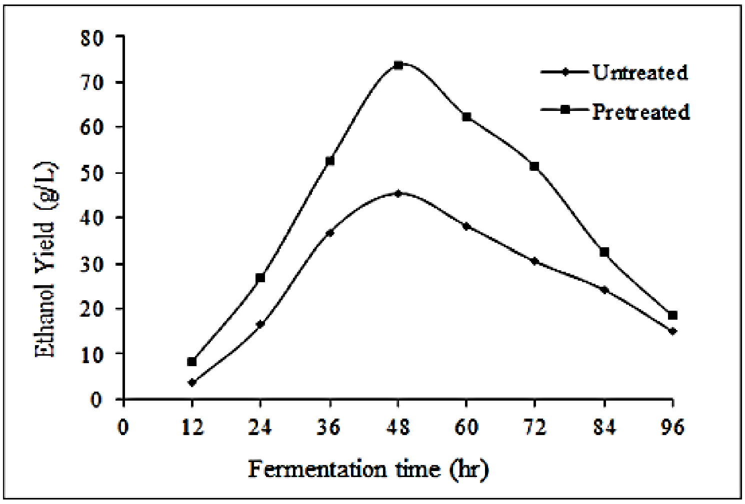


Figure 6: Ethanol yield on untreated and pretreated bagasse

3.3 Pretreated Bagasse Substrate Saccharification

A comparison of low H_2O_2 (2 %) concentration with higher H_2O_2 (20 %) concentration pretreated bagasse and the resulting sugar yields in pretreated bagasse is showed in Table 5. The sugar yields were higher in bagasse pretreated at 120 °C for 120 min, this pretreatment leads to the extraction of lignin, thereby increasing the availability of the cellulose surface. Pretreated bagasse's lower lignin level enables nearly complete saccharification of the polysaccharides. Thus, increased hemicellulose and cellulose content would increase the available aggregate reducing sugar content (58.7±0.4 mg/g) in the hydrolysate.

Table 5: Saccharification yield of sugars (mg/g) in H_2O_2 pretreated bagasse

Substrate	Cellulose (%)	Hemicellulose (%)	Reducing sugars	Glucose mg/g	Xylose mg/g	Arabinose mg/g
Low pretreated	27.9±0.6	30.2±0.6	25.8±0.8	19.8±0.2	12.6±0.1	2.9±0.3
High pretreated	69.3±0.2	76.3±0.2	58.7±0.4	56.4±0.1	48.2±0.2	8.4±0.1

*Composition of percentages calculated from values on a dry-weight basis; Data represents the mean ± SEM, n=3.

According to research findings, following pretreatment, the total availability of reducing sugars and glucose (56.4±0.1 mg/g) from pretreated substrates was increased compared to their relevant carbohydrate content. Reduced lignin concentration was primarily linked to increases in cellulose and hemicellulose production. According to recent findings, the complicated polymer lignin structure may be partially disrupted by H_2O_2 pretreatment, exposing a huge quantity of cellulose surface that is accessible. The pretreated bagasse with higher H_2O_2 loading had higher glucose, xylose, and arabinose yields than the bagasse with lower H_2O_2 loading.

3.4 Ethanol Fermentation

Figure 4 shows the Saccharomyces cerevisiae fermentation of ethanol from both untreated and H_2O_2-pretreated bagasse in smf. According to the results, pretreated bagasse generated more ethanol than untreated bagasse (73.88 g/L vs. 45.49 g/L). The existence of fermentable sugars from the cellulose contained in bagasse substrates was the reason for the variation in ethanol production. Although ethanol production initially grew progressively in both substrates, it remained unchanged after 48 hours.

4. Discussion

RSM is a helpful methodology for examining many independent parameters in response to expected dependent factors because it coordinates statistical and mathematical methodologies (Kidane, 2021). Wet oxidation pretreatment is utilized as an efficient method for opening up the complex crystalline structure of cellulose, solubilizing the hemicellulose fractions, and degrading lignin to CO_2, H_2O, and carboxylic acids (McGinnis et al., 1983). Lignocellulose delignification may also be accomplished by treatment with oxidising agents like H_2O_2, ozone, oxygen, or air. However, the efficacy of delignification is related to the higher reactivity of oxidising agents with the complex aromatic heteropolymeric ring. Besides its impact on the lignin polymer, oxidative pretreatment also alters the hemicellulosic content of the lignocellulose. A substantial fraction of the hemicellulose may have degraded and can no longer be utilised for sugar production (Hermesen et al., 2010). H_2O_2 can enhance the biomass and lignin hydrolysis, and most hemicellulose in biomass can be solubilized (Cai et al., 2022).

In the ongoing study, the pretreatment procedure using 20% (w/v) H_2O_2 at 120 °C for 120 min produced the maximum cellulose yield in bagasse (69.3%). Such conditions led to considerable lignin removal from the resulting bagasse. Further, the pretreatment will also decrease the lignin yield in bagasse. According to present data, a longer pretreatment period (120 min) is required for successful delignification and has a substantial influence on the cellulose yield in pretreated bagasse substrate. As the temperature rises, the yield of hemicellulose increases. Decomposition of the H_2O_2 forms molecular oxygen and more active hydroxyl (HO) and superoxide anion radicals (O_2^-) which afterwards react with lignin polymer in a several ways, thus ensuring delignification by both degradation and dissolution (Sun et al., 1998; Xiao et al., 2001).

Along with temperature, acid concentration, and residence time for pretreatment, some researchers found that adding a solid substrate and the kind of catalyst also had a considerable effect on the bagasse pretreatment rates and yields (Lavarack et al., 2002). In this ongoing study, delignification and a rise in the cellulose content of the resultant bagasse require a higher concentration of H_2O_2 (20%). Bagasse produced the least amount of cellulose when hydrolyzed with H_2O_2 (2%) at 60 °C for 30 minutes (27.9%). The current results support the significance of using a high concentration of H_2O_2 to increase lignin removal at higher temperatures during batch pretreatment of lignocellulosic biomass. Similarly, studies reported that once the substrate was treated at 25 °C with the alkali solution of H_2O_2, nearly half of the lignin found in wheat straw could be solubilized (Gould, 1984).

Accordingly, H_2O_2 removes around 50% of the lignin polymer present in wheat straw and yields a cellulose-rich insoluble residue (a solid) that can be converted from cellulose to glucose (Gould and Freer, 1984). Hypochlorite attacks the lignin network in the occurrence of cellulose (Isroi and Cifriadi, 2018). H_2O_2 was utilized to pretreat the bagasse to achieve 51 % delignification (Irfan et al., 2011). This improved the enzymatic cellulose hydrolysis to 95 % but caused a considerable amount of hemicellulose solubilization. In addition, H_2O_2 was also utilized along with sulfuric acid, ammonia solution, and water for the oak pretreatment by the percolation process and showed considerable improvement in enzymatic hydrolysis (Kim et al., 2001).

Understanding the modifications in the chemical structure of cellulose, hemicellulose, and lignin throughout the bagasse pretreatment requires semi-quantitative analysis using FTIR. Nada and colleagues claim that the peak at 2852 cm^{-1} corresponds to the vibration of O-CH_3 groups in lignin (Nada and colleagues, 1998). The acetyl from hemicellulose may also be connected to this O-CH_3 (alkoxy) group. The C=C double bond is connected to the stretching vibration at 1633 cm^{-1}, which is often existing in the alkyl-aromatic polymer of lignin. Because of these, peak vibrations' associated absorbances are greater after pretreatment indicates the conversion of methoxyl groups into phenolic groups in lignin polymer. Because of high amount of cellulose and hemicellulose, which exhibit maximum values around 1,035 cm^{-1} for C-O stretching and 1,164 cm^{-1} for C-O-C asymmetrical stretching, the peak between 1,200 and 1,100 cm^{-1} is caused (Colom et al., 2003; Pandey, 2005; Pandey, 1999). C-O stretching caused the peak at 1,247 cm^{-1} area, which is a sign of lignin and hemicellulose (Pandey and Pitman, 2003).

The functional groups of OH (3400–3600 cm^{-1}), stretching (CH_2) asymmetry (2921 cm^{-1}), and stretching (C–O–C) of carbohydrate (1159 cm^{-1}) are all observable in the FT–IR spectra of guava leaves. The related cellulose crystal structure is covered by the spectrum area between 3,800 and 3,000 cm^{-1}. The bands of inter- and intramolecular hydrogen bonds also the valence bands of the hydrogen bond with the OH group are all clearly specified by this band (Hinterstoisser and Salmen, 1999). While the peak at 2,918 cm^{-1} is caused by the asymmetrical stretching of CH_2 and CH, which are features of cellulose, the peak at 2,850 cm^{-1} is connected to the symmetric stretch of CH and CH_2 (Ivanova and Korolok, 1989).

Diffractogram studies showed that when the biomass material is exposed to pretreatment, the value of this index rises (Tanahashi et al., 1983). The trend is mostly generated through the elimination of certain lignin polymers and hemicelluloses (amorphous materials), and it was not always the result of modifications to the biomass crystalline structure. As a result of lignin elimination, peroxide pretreatment demonstrated the greatest CrI, which enhanced the cellulose content in bagasse compared to untreated bagasse. It was shown that H_2O_2 pretreatment enhances the removal of hemicellulose in the liquid fraction. According toa study, the yield of cellulose improved when the CrI (67.83%) value was raised in bagasse treated with formic acid and diluted sulfuric acid (Sindhu et al., 2010). In addition, Velmurugan and Muthukumar found that sodium hydroxide-pretreated bagasse had a higher CrI (66%) than native sugarcane bagasse (50%), which was further elevated (up to 70.7%) following sono-assisted pretreatment (Velmurugan and Muthukumar, 2011).

Because of the metabolic stress triggered by the occurrence of ethanol and a decrease in the level of glucose during the fermentation process, the yeast cells were inhibited, as evidenced because of the quantity of ethanol generated in this research further not increased after 48 hours. Likewise, a group researchers used industrial enzymes for biomass saccharification and stated that pretreated rice straw gave higher ethanol production (85 g/L) than untreated (70 g/L) rice straw (Jalil et al., 2010). In this study, it is confirmed that H_2O_2-pretreated bagasse allows a high substrate concentration of 20% to be utilised in the conversion of this biomass into ethanol by a fermentation process. The ethanol yield values attained in this study are similar and occasionally higher than those achieved in other studies using sugarcane bagasse (Sendelius, 2005; Geddes et al., 2011). A detailed study of the trial data indicates that each of the four free variables, individually and collectively, had an influence on the H_2O_2 pretreatment and enzymatic hydrolysis or saccharification of the sugarcane bagasse to ethanol.

5. Conclusion

The findings of this research clearly demonstrate the effectiveness of RSM as a technique for establishing pretreatment trial conditions that would improve the cellulose and hemicelluloses production in the final pretreated bagasse substrate. For optimum hemicellulose and cellulose production, pretreatment process conditions at 2% substrate concentration, 20% H_2O_2 concentration, 120 °C pretreatment temperature, and 120 min pretreatment time were ideal. In parallel, the bagasse that had been formed with H_2O_2 produced more reducing sugars. As observed, pretreated bagasse fermented more efficiently to produce ethanol than untreated bagasse.

Acknowledgments

We are thankful to the Prince of Songkhla University, Hat Yai, for providing funds under the Postdoctoral Fellowship programme and research support by providing laboratories to carry out this research work.

References

Agbor, V.B., Nazim, C., Richard, S., Alex, B., Levin, D.B., 2011. Biomass pretreatment: Fundamentals toward application. Biotechnology Advances, 29, Pp. 675-685.

Anthony, J.C., 1984. Malt beverages and malt brewing materials: Gas chromatographic determination of ethanol in beer. Journal - Association of Official Analytical Chemists, 67, Pp. 192-193.

AOAC., 2005. Official methods of analysis, 18th ed., Pp. 47.

Bas, D., Ismail, H., Boyaci, J., 2007. Modelling and optimization I: usability of response surface methodology. Journal of Food Engineering, 78, Pp. 836-845.

Cai, Z., Zhang, W., Zhang, J., Zhang, J., Ji, D., Gao, W., 2022. Effect of ammoniated fiber explosion combined with H_2O_2 pretreatment on the hydrogen production capacity of herbaceous and woody waste. ACS Omega 7 (25), Pp. 21433-21443

Chandra, R.P., Bura, R., Mabee, W.E., Berlin, A., Pan, X., Saddler, J.N., 2007. Substrate pretreatment: the key to effective enzymatic hydrolysis of lignocellulosics? Advances in biochemical engineering/biotechnology, 108, Pp. 67-93.

Colom, X., Carrillo, F., Nogues, F., Garriga, P., 2003. Structural analysis of photodegraded wood by means of FTIR spectroscopy, Polymer Degradation and Stability, 80, Pp. 543-549.

Dahiya, N., Tewari, R., Tiwari, R.P., Hoondal, G.S., 2005. Chitinase production in solid state fermentation by *Enterobacter* sp. NRG4 using statistical experimental design. Current Microbiology, 51, Pp. 22-228.

De Leon, N., Coors, J.G., 2008. Chapter 7: Genetic improvement of corn for lignocellulosic feedstock production, In: Genetic Improvement of Bioenergy Crops. W. Vermerris (ed.), Springer Science, New York, Pp. 185-210.

Design-Expert® Software, version 8 user's guide, 2018.

Geddes, C.C., Mullinnix, M.T., Nieves, I.U., Peterson, J.J., Hoffman, R.W., York, S.W., Yomano, L.P., Miller, E.N., Shanmugam, K.T., Ingram, L.O., 2011. Simplified process for ethanol production from sugarcane bagasse using hydrolysate-resistant *Escherichia coli* strain MM160. Bioresource Technology, 102, Pp. 2702-11.

Gould, J.M., and Freer, S.N., 1984. High-efficiency ethanol production from lignocellulosic residues pretreated with alkaline H_2O_2. Biotechnology Bioengineering, 26, Pp. 628-631.

Gould, J.M., 1984. Alkaline peroxide delignification of agricultural residues to enhance enzymatic saccharification. Biotechnology Bioengineering, 26, Pp. 46-52.

Haaland, P.D., 1989. Separating signals from the noise. In Experimental design in biotechnology. Marcel Dekker Inc., New York, Pp. 61-83.

Hermesen, P.F.H., Huijgen, W.J.J., Bermudez, L.L.M., Bakkar, P.R.C., 2010. Literature review of physical and chemical pretreatment process for lignocellulosic biomass. Food and Biobased Research (ECN-E-10-013).

Hinterstoisser, B., Salmen, L., 1999. Two-dimensional step-scan FTIR: a tool to unravel the OH-valency-range of the spectrum of Cellulose I. Cellulose, 6, Pp. 251–263.

Irfan, M., Gulsher, M., Abbas, S., Syed, S., Nadeem, M., Baig, S., 2011. Effect of various pretreatment conditions on enzymatic saccharification. Songklanakarin Journal of Science and Technology, 33 (4), Pp. 397-404.

Isroi, I., Cifriadi, A., 2018. The oxidation of cellulose from oil palm empty fruit bunch by using hydrogen peroxide in alkaline condition. Jurnal Selulosa, 8 (2), Pp. 51 – 60.

Ivanova, N.V., Korolok E.V., 1989. IR spectrum of cellulose. Journal of Applied Spectroscopy, 51, Pp. 847–851.

Jalil, R., Ibrahim, W.A., Ali, M.S.M., Hashim, S., Elham, P., Tahar, A., Zahidi, N.F.A., 2010. 7th Biomass Asia Workshop; November 29-December 01; Jakarta, Indonesia.

Jung, H.J., Vogel, K.P., 1992. Lignification of switch grass (*Panicum virgatum*) and big bluestem (*Andropogon gerardii*) plant part during maturation and its effect on fiber degradability. The Journal of the Science of Food and Agriculture, 59, Pp. 166-176.

Karunanithy, C., Muthukumarappan, K., 2011. Application of response surface methodology to optimize alkali concentration, corn stover particle size and extruder parameters for maximum sugar recovery. Biofuel production – recent developments and prospects. Dr. Marco Aurelio Dos Santos Bernardes (Ed.), Intech publishers, Faridabad. Pp. 343-374

Karunanithy, C., Muthukumarappan, K., 2013. Thermo-mechanical pretreatment of feedstocks, T. Gu (Ed.), Green biomass pretreatment for biofuels production, Springer Briefs in Green Chemistry for Sustainability, Springer, Berlin. Pp. 32-33.

Khabarov, Y.G., Kamakina, N.D., Gusakov, L.V., Veshnyakov, V.A., 2006. New spectrophotometric method for determination of furfural and pentoses. Russian Journal of Applied Chemistry, 79, Pp. 103-106.

Kidane, S.W., 2021. Application of response surface methodology in food process modeling and optimization. In (Ed.), Response surface methodology in engineering scince. In tech open, London.

Kim, I., Han, J., 2012. Optimization of alkaline pretreatment conditions for enhancing glucose yield of rice straw by response surface methodology. Biomass and Bioenergy, 46, Pp. 210-217.

Kim, S.B., Um, B.H., Park, S.C., 2001. Effect of pretreatment reagent and hydrogen peroxide on enzymatic hydrolysis of Oak in percolation process. Applied Biochemistry Biotechnology, 93, Pp. 81-94.

Lavarack, B.P., Griffin, G.J., Rodman, D., 2002. The acid hydrolysis of sugarcane bagasse hemicellulose to produce xylose, glucose, arabinose and other products. Biomass and Bioenergy, 23, Pp. 367-380.

Lynd, L.R., Weimer, P.J., van Zyl, W.H, Pretorius, I.S., 2002. Microbial cellulose utilization: fundamentals and biotechnology. Microbiology Molecular Biology Reviews, 66, Pp. 506-77.

Mc Ginnis, G.D., Wilson, W.W., Prince, S.E., Chen, C.C., 1983. Conversion of biomass into chemicals with high temperature wet oxidation. Industrial & Engineering Chemistry Product Research and Development, 22, Pp. 633-636.

Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry, 31, Pp. 426-428.

Mohan, P.R., Pradeep, S., Reddy, O.V.S., 2013. A comparative study on simultaneous saccharification and fermentation of agricultural wastes to bioethanol using two *Saccharomyces strains*. Chiang Mai

Journal of Sciences, 40, Pp. 307-320.

Nada, A.A.M.A., El-Sakhawy, M., Kamel, S.M., 1998. Infra-red spectroscopic study of lignins, Polymer Degradation and Stability, 60, Pp. 247-251.

Nanda, S., Mohammad, J., Reddy, S.N., Kozinski, J.A., Dalai, A.K., 2014. Pathways of lignocellulosic biomass conversion to renewable fuels. Biomass Conversion and Biorefinery, 4, Pp. 157191.

Pandey, K.K., 1999. A study of chemical structure of soft and hardwood and wood polymers by FTIR spectroscopy. Journal of Applied Polymer Science, 12, Pp. 1969-1975.

Pandey, K.K., 2005. Study of the effect of photo-irradiation on the surface chemistry of wood. Polymer Degradation and Stability, 90, Pp. 9-20.

Pandey, K.K., Pitman, A.J., 2003. FTIR studies of the changes in wood chemistry following decay by brown-rot and white-rot fungi. International Biodeterioration and Biodegradation, 52, Pp. 151-160.

Rajendran, V., Muthukumar, K., 2012. Ultrasound-assisted alkaline pretreatment of sugarcane bagasse for fermentable sugar production: Optimization through response surface methodology. Bioresource Technology, 112, Pp. 293-299.

Rathore, A.S., Mishra, S., Nikita, S., Priyanka, P., 2021. Bioprocess control: current progress and future perspectives. Life (Basel), 11 (6), Pp. 557.

Rodrigues Filho, G., De Assuncao, R.M.N., Vieira, J.G., Meirelesm, C.S., Cerqueira, D.A., et al., 2007. Characterization of methylcellulose produced from sugar cane bagasse cellulose: Crystallinity and thermal properties. Polymer Degradation and Stability, 92, Pp. 205-210.

Segal, L., Creely, J.J., Martin, A.E., Conrad, C.M., 1959. An empirical method for estimating the degree of crystallinity of native cellulose using the X-ray diffractometer. Textile Research Journal, 29, Pp. 764-786.

Sendelius, J., 2005. Steam pretreatment optimisation for sugarcane bagasse in bioethanol production. Master of Science Thesis, Department of Chemical Engineering, Lund University, Sweden.

Sindhu, R., Binod, P., Satyanagalakshmi, K., Janu, K.U., Sajna, K.V., Kurien, N., Sukumaran, R.K., Pandey, A., 2010. Formic acid as a potential pretreatment agent for the conversion of sugarcane bagasse to bioethanol. Applied Biochemistry Biotechnology, 162, Pp. 2313-2323.

Sun, R.C., Lawther, J.M., Banks, W.B., 1998. Isolation and characterization of hemicellulose B and cellulose from pressure refined wheat straw. Industrial Crops and Products, 7, Pp. 121-128.

Xiao, B., Sun, X.F., Sun, R.C., 2001. Chemical, structural and thermal characterizations of alkali soluble lignins and hemicelluloses and cellulose from maize stems, rye straw and rice straw. Polymer Degradation and Stability, 74, Pp. 307-319.

Tanahashi, M., Takada, S., Aoki, G.T., Higuchi, T., Hanai, S., 1983. Characterization of explosion Wood.1. Structure and physical properties. Wood Research, 9, Pp. 31-36.

Velmurugan, R., Muthukumar, K., 2011. Utilization of sugarcane bagasse for bioethanol production: sono-assisted acid hydrolysis approach. Bioresource Technology, 102, Pp. 7119-7123.

Yildirim, O., Ozkaya, B., Altinbas, M., Demir, A., 2021. Statistical optimization of dilute acid pretreatment of lignocellulosic biomass by response surface methodology to obtain fermentable sugars for bioethanol production. International Journal of Energy Research, 45 (6), Pp. 8882–8899.

Zaafouri, K., Ziadi, M., Ben Hassen-Trabelsi, A., Mekni, S., Aïssi, B., Alaya, M., Hamdi, M., 2017. Optimization of hydrothermal and diluted acid pretreatments of tunisian luffa cylindrica (L.) fibers for 2G bioethanol production through the cubic central composite experimental design CCD: response surface methodology. BioMed Research International, Pp. 1-14.