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Optimization of Liquid Hot Water Pretreatment and Fermentation for Ethanol Production from Sugarcane Bagasse Using *Saccharomyces cerevisiae*

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Abstract: Sugarcane bagasse can be considered a potential raw material in terms of quantity and quality for the production of alternative biofuels. In this research, liquid hot water (LHW) was studied as a pretreatment process to enhance the digestibility of pretreated material for further conversion into bioethanol. Different variables (temperature, residual time, and acid concentration) were determined to predict the optimized condition. LHW pretreatment showed an impact on the hemicellulose structure. The optimized condition at 160 °C for 60 min with 0.050 M acid concentration reached the highest glucose yield of 96.86%. Scanning electron microscopy (SEM) showed conspicuous modification of the sugarcane bagasse structure. The effect of LHW pretreatment was also demonstrated by the changes in crystallinity and surface area analysis. FTIR techniques revealed the chemical structure changes of pretreated sugarcane bagasse. The prepared material was further converted into ethanol production with the maximized ethanol concentration of 19.9 g/L.

Keywords: sugarcane bagasse; glucose yield; bioethanol-alcohol production; optimization; liquid hot water pretreatment

1. Introduction

Lignocellulose biomass (LB) is predicted to be a substantial carbon-free energy source, which can reduce carbon dioxide (CO_2) emissions and atmospheric pollution. As a result, it represents a viable option for limiting crude oil production, and can be used to produce bioenergy, chemicals, and biomaterials in industry for biorefinery products [1]. Bioethanol is a type of alcohol produced using the sugar alcohols from different biomass sources, mostly identified in starch crops such as sugar beet, corn biomass, rice grain, sugarcane bagasse, and wet sorghum [2]. Moreover, bioethanol sources include sucrose, starch, and

lignocellulose material [3]. Today, a variety of bioproducts are produced according to typical biorefinery processes (physical–chemical reaction, hydrolysis and fermentation) in order to break down the biomass substrate into biofuels and biochemicals. An alternative bioethanol is subsequently refined to blend with fuels for utilization in automobiles and other vehicles. The fuel is typically 90 percent gasoline and 10 percent ethanol. Concerning lignocellulosic resources, corn and sugarcane bagasse are reported to be the most widely used to produce bioethanol [3].

Sugarcane bagasse is an agro-industrial biomass with significant energy resources, which consists of agricultural lignocellulosic biomass. It is a low-cost, renewable, and unique natural resource for renewable energy production or bioethanol production, which can significantly reduce emissions [4]. Sugarcane bagasse is a low-cost agricultural waste product. These biomass materials have a great deal of potential for the development of useful products. Therefore, sugarcane bagasse characteristic data can contribute to the development of sugarcane bagasse valorization in value-added applications. This presents a huge challenge for industry and the environment. Typically, the major components of lignocellulose biomass materials derived from agricultural waste residuals or economic crops contain 40–50% cellulose content, 25–40% hemicellulose content, and 15–30% lignin content depending on the biomass material [5]. In terms of structure, cellulose and hemi-cellulose comprise polysaccharide structures [6]. In general, the cellulose structure is a polysaccharide composed of linear glucan chains consisting of β-(1-4)-glycosidically linked units, featuring numerous crystal structures of small molecules [7]. Bioethanol synthesis requires the hydrolysis of lignocellulose biomass material for glucose release after the pretreatment process to obtain optimal glucose yields.

A previous optimization study of enzymatic hydrolysis from sugarcane bagasse based on organosolv pretreatment for glucose yield used a 1.25% H_2SO_4 solution for 60 min, achieving a high glucose concentration (~28.8 g/L) and yield (~25.1 g/100 g dry matter). In addition, in studies on ethanol production after obtaining glucose yields under optimal conditions, it was found that subsequent hydrolysis occurred using 10% (w/v) substrate in a reaction medium supplemented with xylanase at 300 UI/g and Tween-20 (2.5% w/w) after fermentation for around 24 h with *Saccharomyces cerevisiae*. Ethanol production was achieved with up to 92.8% of the anticipated yield [1]. The optimization of alkaline pretreatment conditions for rice straw was investigated using response surface methodology (RSM) with NaOH concentration (1.0–4.0%), reaction time (30–90 min), and reaction temperature (60–100 °C) as the variables for enhancing glucose yield [8]. The results showed that the maximum glucose yield of 254.5 ± 1.2 g/kg was obtained under conditions of 2.96% NaOH, 81.79 °C, and 56.66 min. Later, Zhang et al. [9] interestingly studied the production of bioethanol and xylooligosaccharides (XOS) from sugarcane bagasse (SCB) by autohydrolysis pretreatment under optimal conditions, and found the maximized optimization for the production of XOS yield of 50.53% at 200 °C for 10 min and conversion into bioethanol yield (66.67%). Milessi et al. [10] studied the hydrothermal and organosolv pretreatments of hemicellulose mainly in the form of oligomers. The results showed that the maximized xylooligosaccharide yield were determined at 185 °C, 10 min at a 1:10 solid: liquid ratio. For both hydrothermal and organosolv liquors, enzymatic conversion to XOs employing an endoxylanase reached approximately 90%. Furthermore, Silva et al. [11] reported that during enzymatic hydrolysis at 30 FPU cellulase g/L treated SCB within 72 h can result in hemicellulose removal of 89.7%, glucan recovery of 97.8% and maximum ethanol concentration of 29.2 g/L. These results are promising particularly when compared with the theoretical ethanol yield, which is equivalent to 58.9%. For an efficient fermentation process to produce ethanol, the main concern is the optimization of the pretreatment process, including various parameters. Therefore, pretreatment of biomass is required to obtain optimal glucose yield conditions before being used for bioethanol production via enzymatic hydrolysis. The aim of this study was to optimize the pretreatment of sugarcane bagasse for the application of fermentation technologies to industrial biorefinery applications. Since sugarcane bagasse is one of the crop residues according to the agricultural sector and sugar

industry in Thailand, the use of by-products and excess materials are of much interest in terms of the economy and consistent with the current situation of biorefineries.

2. Results and Discussion

2.1. Chemical Composition of Sugarcane Bagasse Substrates

The native sugarcane bagasse was mainly composed of cellulose (33.1 ± 0.51) hemicellulose (19.2 ± 0.44), lignin (29.2 ± 0.20), ash (6.1 ± 0.55), and extractives (12.1 ± 0.25%). However, more research into the structural and functional features of extractives at high concentrations is needed.

2.2. Optimization of Reaction Temperature, Residual Time, and Acid Concentration for Glucose Yield

In this study, all parameters were assessed according to the quality and quantity of the solid fraction following liquid hot water pretreatment using the Box–Behnken design matrix shown in Table 1. Along with the results of glucose yield, the experimental values of stable conditions for cellulose, hemicellulose, and lignin content in the remaining solid residue were examined utilizing response surface methodology (RSM).

Table 1. The effect of all response surface method in remaining solid residual were analyzed using the Box−Behnken Design.

Factors			Responses (%)				
T (°C)	Acid Concentration (M)	Time (min)	Cellulose (%) [a]	Hemicellulose (%) [a]	Lignin (%) [a]	Remaining Solid Residual (%) [a]	Glucose Yield (%)
140	0.025	60	26.32	2.11	29.30	72.47	56.07
180	0.025	60	26.34	0.63	22.60	69.87	67.54
140	0.075	60	25.54	0.87	25.76	76.89	68.43
180	0.075	60	23.62	0.27	22.50	56.44	73.69
140	0.050	30	28.89	2.52	28.12	65.46	61.68
180	0.050	30	26.32	1.21	27.65	66.21	77.78
140	0.050	90	26.70	1.90	28.78	79.86	70.32
180	0.050	90	27.61	0.23	24.56	50.45	72.35
160	0.025	30	28.43	2.04	24.00	79.44	64.67
160	0.075	30	25.88	1.50	23.18	69.51	89.58
160	0.025	90	27.83	0.89	25.13	69.35	81.97
160	0.075	90	26.39	0.09	23.35	70.57	76.09
160	0.050	60	29.40	0.59	23.50	67.37	96.86
160	0.050	60	29.43	0.61	23.49	67.43	96.12
160	0.050	60	29.38	0.60	23.54	67.33	97.13
160	0.050	60	29.42	0.64	23.51	67.29	96.72
160	0.050	60	29.46	0.66	23.73	67.44	98.85

[a] Based on relative content of cellulose, hemicellulose and lignin content in remaining solid residual.

The experimental results obtained were analyzed using the Box−Behnken design to assess the relationship among the reaction temperature (°C; X_1), acid concentration (M; X_2), and time (min; X_3). A glucose yield of 56.07–98.85% was obtained from the Box−Behnken design. The resulting optimization of sugarcane bagasse following liquid hot water pretreatment was analyzed using the analysis of variance. It was observed that the optimization of reaction temperature (140–180 °C), residence time (30–90 min), and acid concentration (0.025–0.075 M) for maximum glucose yield (%) could be fitted to a second-order polynomial multiple regression equation. The experimental value calculated (Equation (1)) revealed the suitability of the quadratic model.

$$\text{Glucose yield (\%)} = (-1360.96) + (16.02402\ X_1) + (3153.129\ X_2) + (2.47819\ X_3) - (3.10515\ X_{12}) - (0.00586) - (10.2633\ X_{23}) - (0.04781\ X^2_1) - (-18{,}528.1\ X^2_2) - (-0.00830\ X^2_3) \tag{1}$$

In addition, the predicted values of glucose yield after liquid hot water pretreatment showed that all quadratic models of glucose yield were statistically significant at the 95% confidence interval under optimal conditions. The R-squared value of the glucose yield was greater than 0.90 for all results of this experiment, as shown in Table 2, indicating the high accuracy of the model based on a comparison between predicted and experimental values. It can be seen that the model predicted glucose yield with an accuracy of 99.63% (Figure 1).

Table 2. The significance of all parameters in the regression models of glucose yield on ANOVA analysis.

Source	Sum of Squares	DF	Mean Square	F Value	*p*-Value Prob > F	Comments
Model						
A-Temperature	151.99	1	151.99	233.95	<0.0001	Significance
B-Concentration	176.15	1	176.15	271.14	<0.0001	Significance
C-Time	6.15	1	6.15	9.47	0.0179 [a]	
AB	9.64	1	9.64	14.84	0.0063 [a]	
AC	49.56	1	49.56	76.29	<0.0001	Significance
BC	237.01	1	237.01	364.81	<0.0001	Significance
A*2	1539.83	1	1539.83	2370.18	<0.0001	Significance
B*2	564.62	1	564.62	869.09	<0.0001	Significance
C*2	235.48	1	235.48	362.47	<0.0001	Significance

Note: [a] The non-significant *p*-values.

(**a**)

(**b**)

(**c**)
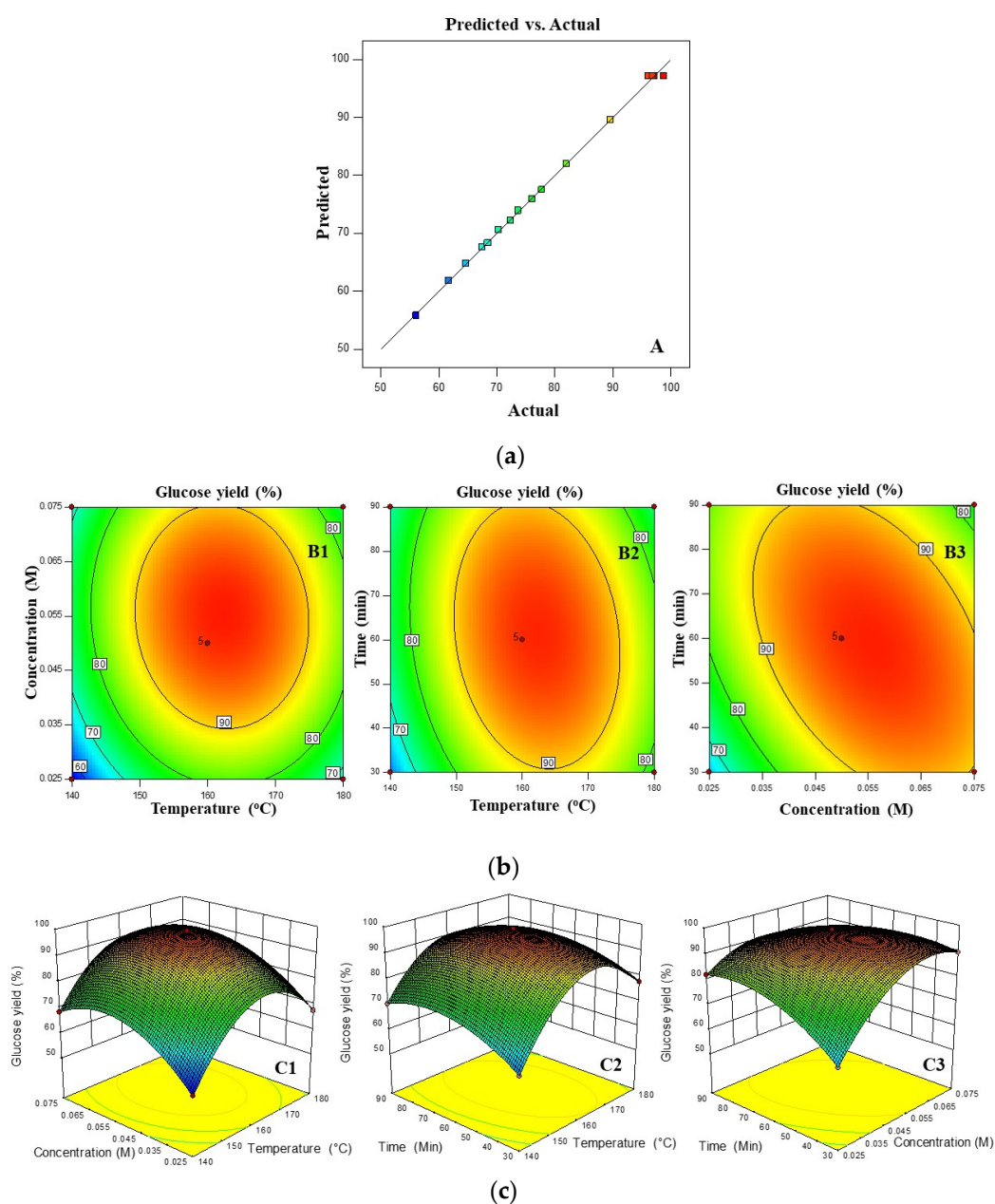
Figure 1. Response surface plot of the LHW pretreatments process: Effect of reaction time (30–90 min), acid concentration (0.025 M–0.075 M) and temperature (140–180 °C) on glucose yield (**a**) Predicted vs. actual data plot (**b**) 2D contour plot (**c**) 3D surface plot on glucose yield under optimization.

2.3. Optimization of Glucose Yield after Liquid Hot Water Pretreatment of Sugarcane Bagasse

The second-order polynomial model obtained in Equation (1) for the target response in this study to optimize the response following LHW pretreatment was determined using Design Expert software 10.0. The results indicated that the optimal glucose yield (96.81%) was predicted under conditions of 0.055 M acid, 162.24 °C, and 57.506 min residence.

Thus, the optimal conditions produced >90% glucose yield. The regression analysis identified the optimal conditions for glucose yield (96.86%) as 0.050 M acid, 160 °C, and 60 min residence, following LHW pretreatment of sugarcane bagasse.

Furthermore, the HMF and furfural in the liquid fraction were discussed. Typically, the hydrolysis of sugarcane bagasse in LHW pretreatment generates by-products in the form of HMF and furfural, especially, under harsh conditions; a temperature above 180 °C marks the initial point to produce by-products. In addition, acid concentrations above 0.1 M also influence the process. Thus, this study determined the level of parameters as lower than the criteria. This resulted in concentrations of HMF and furfural in the ranges of 0.56–0.92 mg/L and 1.1–1.5 mg/L, respectively. It was observed that the concentration of by-products fell within the same ranges. These concentration levels are not inhibitory to *Saccharomyces cerevisiae* or *Candida guilliermondii*, which tolerate HMF and furfural up to 2 mg/mL [12].

2.4. SEM, XRD, BET Surface, and FTIR Characterization of Native Sugarcane Bagasse and Solid Residue after LHW Pretreatment

The physical structure of native sugarcane bagasse was modified compared with the solid residue following LHW pretreatment under suitable conditions, as shown in Figure 2. The surface characterization using SEM revealed an intact, smooth surface and a highly ordered crystalline structure. In addition, it can be seen that the exterior surfaces of cellulose and hemicellulose were densely covered by lignin, thus concealing true cellulose before LHW pretreatment. Following pretreatment under suitable conditions, it can be noted that the lignin and hemicellulose were removed, revealing the structure of cellulose and a rough surface [13]. However, these changes in the microstructure of sugarcane bagasse could increase the surface area of cellulose, thereby facilitating enzymatic hydrolysis and cellulose degradation [14].
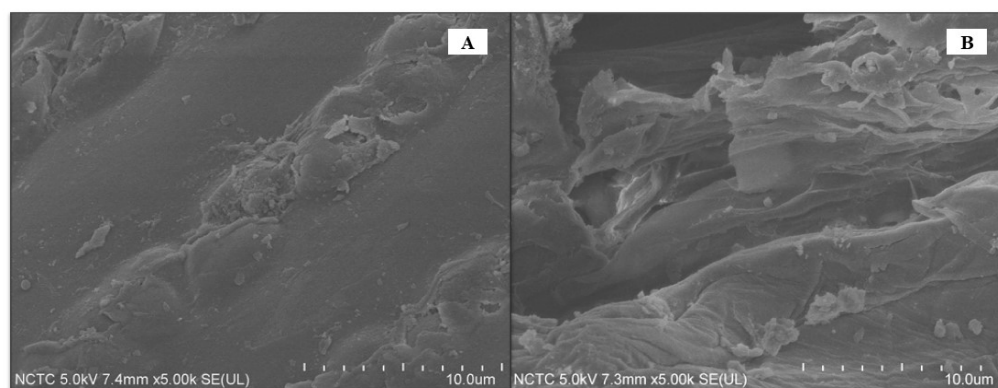
Figure 2. Scanning electron micrographs of (**A**) native sugarcane bagasse and (**B**) solid residue after pretreatment process.

The crystallinity and surface area of the solid residue have a significant impact on enzymatic digestibility [15]. These parameters were directly affected by the efficiency of LHW pretreatment, as shown in Table 3. One can see that a high crystallinity (66.8%) was obtained for the solid residue after LHW pretreatment under suitable conditions (0.050 M, 160 °C, 60 min) in comparison to the native sugarcane bagasse (49.6%). Before pretreatment, the sugarcane bagasse showed a low surface area of 2.1 m^2/g. In contrast, after pretreatment under optimal conditions, the surface area was 10.5 m^2/g. Therefore, the

accessibility of the cellulose surface for enzymatic digestibility was significantly increased when compared with native sugarcane bagasse.

Table 3. Surface area and XRD analysis of native sugarcane bagasse and solid residue after LHW pretreatment under optimal conditions.

Order	Surface Area (m^2/g)	Degree of Crystallinity (%)
Native sugarcane bagasse	2.1	49.6
Solid residuals after LHW pretreatment	10.5	66.8

The FTIR spectra of the native sugarcane bagasse and solid residue after pretreatment are shown in Table 4, revealing the chemical structure changes of lignocellulosic materials. The results showed the O–H stretching of hydroxyl groups (3449–3431 cm^{-1}) in lignin and C–H stretching vibrations of methyl groups (2915–2895 cm^{-1}) [16]. The C–O stretching at 1609–1602 cm^{-1} was attributed to the aromatic ring [17]. The absorption at 1375–1370 cm^{-1} was related to C–H deformation in cellulose and hemicellulose [18]. The intense peak in the range 1429–1428 cm^{-1} was assigned to CH_2 stretching vibrations in cellulose [19]. The vibrations at 1221–1220 cm^{-1} were related to the C–O stretching of syringyl rings in lignin. The band at 1164–1162 cm^{-1} was attributed to the C–O–C vibration of cellulose and hemicellulose [20]. The absorption in the range 1130–1128 cm^{-1} was related to the aromatic structures in lignin [21]. The 1059–1043 cm^{-1} band was related to C–O stretching vibrations in cellulose. Similarly, the peak at ~895 cm^{-1} indicated the C–H–O stretching vibrations of β-(1-4)-glycosidic linkages in cellulose. ln addition, the intensity of the rocking vibration of CH_2 bands in cellulose Iα (~751 cm^{-1}) was apparently increased after LHW pretreatment under optimal conditions [22].

Table 4. FTIR spectra of the native sugarcane bagasse and solid residual after pretreatment process under suitable conditions.

Order	Frequency, (cm^{-1})	Functional Group
1	3449–3431	O–H stretching in phenolic compound
2	2915–2895	C–H stretching vibrations in methyl group
3	1609–1602	C–O stretching in lignin structure
4	1375–1370	C–H deformation in cellulose and hemicellulose
5	1429–1428	CH-2 stretching vibrations in cellulose
6	1221–1220	C–O stretch of syringyl rings in lignin structure
7	1164–1162	C–O–C vibration of cellulose and hemicellulose
8	1130–1128	automatic structure in lignin structure
9	1059–1043	C–O stretch vibrations in cellulose
10	~895	C–H–O stretching vibrations of β-(1-4)-glycosidic linkage
11	~751	CH-2 bands

2.5. Simultaneous Saccharification and Fermentation (SSF)

In the present study, ethanol production was obtained via the SSF process in *S. cerevisiae* (TISTR 5339) yeast using the solid residue after LHW pretreatment as a starting material (cellulose substrate). *S. cerevisiae* was grown in YPD medium supplemented with carbon sources as the cellulose substrate (solid residual). It was previously reported that cellulose is the best source of carbon for ethanol production [23]. After fermentation, the glucose and ethanol production using the solid residue is shown in Figure 3. Using *S. cerevisiae*, the final ethanol production ranged from 4.2–19.9 g/L on a 20–30% dry mass basis at pH 4.5 following 0–96 h of fermentation. The maximum amount of ethanol produced was 19.9 g/L using *S. cerevisiae* yeast for 72 h of fermentation. According to previous research,

Fan et al. [24]. reported the use of various pretreatment strategies (liquid hot water, ethanosolv, dilute acid and alkaline) in the presence of different surfactants for enhancement of glucose production from sugarcane bagasse during enzymatic hydrolysis. The results showed that dilute acid pretreatment could increase the glucose yield from 75.04 to 86.14% in the presence of 1.5% PEG 6000. According to appropriate conditions, the maximum ethanol concentration of 20.17 g/L was obtained via simultaneous saccharification fermentation. Wang et al. [25] studied the effect of LHW pretreated for ethanol production from sugarcane bagasse. The results showed that the pretreated sugarcane bagasse under LHW pretreatment could enhance the ethanol production in both of batch and fed-batch hydrolysis in simultaneous saccharification and fermentation. The ethanol production and theoretical yield obtained from the process of SSF after fed-batch hydrolysis were 55.4 g/L and 88.3% for 72 h, respectively.
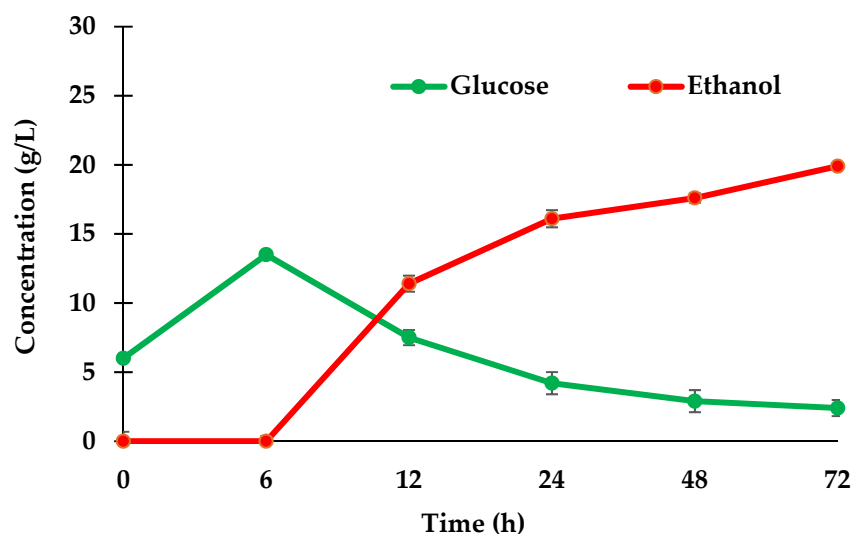
Figure 3. Effect of glucose content on ethanol production of pretreated sugarcane bagasse.

3. Materials and Methods

3.1. Materials

Sugarcane bagasse was obtained from Ban Du, Chiang Rai, Thailand. The sugarcane bagasse was dried at 50 °C for 24 h in a hot air oven. Afterward, it was cut, milled, and sieved to a particle size in the range 1–2.0 mm (Retsch ZM200, Haan, Germany), followed by grinding using 0.061–0.25 mm sieves. The final moisture content of the milled sugarcane bagasse was 5%, as assessed by weight loss after drying in an oven at 105 °C for 5 h with the goal of maintaining constant weight. The prepared sugarcane bagasse was kept in sealed plastic bags at room temperature for the experiments. The composition of sugarcane bagasse was determined according to the National Renewable Energy Laboratory [26]. All chemicals and reagents, including sugar standards, were acquired from major chemical suppliers, such as Sigma-Aldrich (St. Louis, MO, USA), which provided glucose, xylose, arabinose, 5-hydroxymethyl-2-furaldehyde (HMF), and furfural (S.M. Chemical Supplies Co., Ltd., Bangkok, Thailand). Sulfuric acid was purchased from Merck (Merck Ltd. Bangkok, Thailand).

3.2. Liquid Hot Water Pretreatment of Sugarcane Bagasse

The pretreatment of raw sugarcane bagasse was implemented in a stainless-steel reactor with a capacity of 600 mL, heated using an electric jacket with a thermocouple to measure the temperature (Parr Reactor 4560, Parr Instrument Co., Moline, IL, USA). Initially, the pretreatment was carried out using a 1 g/15 mL ratio of native sugarcane bagasse to water with various concentrations of H_2SO_4 acid catalyst (0.025–0.075 M), temperatures (140–180 °C), and residence times (30–90 min). The response time was initiated once the

target temperature was reached. Nitrogen gas (N_2) was flowed into the reactor for purging and adjusting the initial pressure to 20 bar, and the reaction was stirred at 100 rpm to maintain a homogeneous system. At the end of the reaction time, the reaction was quickly quenched in a water bath. The solid cellulose-enriched fraction was separated using Whatman No. 4 filter paper and then washed with ~150 mL of DI water. The pretreated substrate was dried overnight at 70 °C and stored at room temperature for further research. Monomeric sugars and inhibitory products were analyzed by high-performance liquid chromatography (HPLC analysis).

3.3. Experimental Design and Optimization of Glucose Yield Using Box–Behnken Response Surface Design

Box–Behnken response surface methodology (RSM) and statistical analysis were performed using Design Expert software (Trial version 10.0, Stat-Ease, Inc., Minneapolis, MN, USA) to study glucose yield optimization in the sugarcane bagasse residue. Three variables were identified as the critical process parameters for glucose yield from after LHW pretreatment of sugarcane bagasse: reaction temperature (X_1, 140–180 °C), residence time (X_2, 30–90 min), and acid concentration (X_3, 0.025–0.075 M), with three levels of each factor (−1, 0, 1). Therefore, to estimate the model coefficients, 17 experiments were conducted with three replications at the center point, processed in a random order. Box–Behnken design with experimental design is shown in Table 5. The target response was glucose yield, with the response surface regression equation fitted to a second-order polynomial to investigate the interaction effect and optimal circumstances (Equation (2)):

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X^2_1 + \beta_{22} X^2_2 + \beta_{33} X^2_3 \quad (2)$$

where Y is the predicted response; X_1, X_2, and X_3 are the independent variables; β_0 is a constant; β_1, β_2, and β_3 are the linear coefficient terms; β_{12}, β_{13}, and β_{23} are the interaction coefficient terms; and β_{11}, β_{22}, and β_{33} are the quadratic coefficient terms.

Table 5. Box–Behnken design with experimental design of glucose yield.

Run	T (°C)	Acid Concentration (M)	Time (min)
1	−1	−1	0
2	1	−1	0
3	−1	1	0
4	1	1	0
5	−1	0	−1
6	1	0	−1
7	−1	0	1
8	1	0	1
9	0	−1	−1
10	0	1	−1
11	0	−1	1
12	0	1	1
13	0	0	0
14	0	0	0
15	0	0	0
16	0	0	0
17	0	0	0

3.4. Enzymatic Hydrolysis

Enzymatic hydrolysis was performed on the solid residue obtained after pretreatment using commercial cellulase concentrate enzymes (Cellic Ctec2, Novozymes A/S, Bagvaerd, Denmark). The sugar yields from the enzymatic hydrolysis were measured. The enzymatic hydrolysis reaction experiment was performed in 25 mL-Erlenmeyer flasks consisting of 5% *w*/*v* pretreated substrate with 25 FPU/g of enzyme in 50 mM sodium citrate buffer pH 4.8 and 1% *w*/*v* sodium azide. The reaction was carried out under incubation in a rotatory

shaker at 150 rpm, 50 °C, for 72 h. The hydrolysis experiments were performed in triplicate. Profiles of released sugars were analyzed on a high-performance liquid chromatograph (LDC Model 4100, Shimadzu, Kyoto, Japan) equipped with a refractive index detector and an Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA) operating at 65 °C with 5 mM H_2SO_4 as the mobile phase at a flow rate of 0.5 mL/min. Glucose yield was calculated as the percentage of glucose release from enzymatic hydrolysis as a function of cellulose content in the pretreated biomass according to Equation (3).

$$\text{Cellulose hydrolysis (\%)} = \frac{\text{Amount of glucose after hydrolysis (g)}}{\text{Amount of glucose in pretreated material (g)}} \times 100 \quad (3)$$

3.5. Analysis of Aqueous Phase

The soluble product in the liquid phase was interpreted using a liquid chromatograph (LDC Model 4100, Shimadzu, Kyoto, Japan) equipped with a refractive index, UV/Vis detector, and Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA). Fermentable sugar profiles and inhibitory byproducts were obtained at a specific temperature of 65 °C, with a constant flow rate of 0.5 mL/min (5 mM H_2SO_4).

3.6. Characterization of Native Sugarcane Bagasse and Remaining Solid Residue

3.6.1. Scanning Electron Microscopy Analysis

Scanning electron microscopy (SEM; JSM-6301F, JEOL, Japan) was used to examine the microstructure of native sugarcane bagasse and the remaining solid residue after the pretreatment process. The samples were dried and coated with gold for analysis. An electron beam energy of 20 kV was used for analysis.

3.6.2. X-ray Diffraction Analysis

The crystallinity of the native sugarcane bagasse and remaining solid residue was determined by X-ray diffraction (XRD), using an X'Pert PRO diffractometer (PANalytical, Almelo, The Netherlands). The samples were scanned at room temperature in a range of $2\theta = 10°–30°$ with a step size of 0.004° at 500 kV, 30 mA. The crystallinity index (CrI) was determined using the following Equation (4):

$$\text{CrI} = \frac{I_{002} - I_{amorphous}}{I_{002}} \times 100 \quad (4)$$

where I_{002} is the scattered intensity of the main peak of cellulose, which typically lies in the 002 plane, and $I_{amorphous}$ is the scattered intensity of the amorphous portion evaluated in the 101 plane.

3.6.3. BET Surface Area Measurement

The method of Brunauer, Emmett, and Teller (BET) was used to determine the total surface area of materials. Native sugarcane bagasse and the remaining solid residue were analyzed for their BET surface area, using a Belsorp-max TPDpro (BEL Japan, Tokyo, Japan) with a thermal conductivity detector (semi-diffusion type, 4-element W–Re filament) at the National Nanotechnology Center, Thailand.

3.6.4. Fourier-Transform Infrared Spectroscopy Analysis

The chemical composition of native sugarcane bagasse and the remaining solid residue was analyzed using FT-IR measurements on a PerkinElmer instrument (Waltham, MA, USA) with the KBr pellet technique. The measurement resolution was set at 4 cm^{-1} with a mirror velocity of 0.6329 cm/s. Infrared spectra were collected within the range of 400–4000 cm^{-1} from ~32 scans. Commercial cellulose from Sigma-Aldrich was used as a reference.

3.7. Simultaneous Saccharification and Fermentation Process for Ethanol Production

The sample was evaluated in a 1.6 L reactor with a total operating volume of 1.0 L (Biostat$^®$ b 2, B. Braun, Bangkok, Thailand). The fermentation medium for the fermentation process was composed of yeast extract (1 g), $(NH_4)_2SO_4$ (5 g), and $MgSO_4 \cdot 7H_2O$ (0.025 g/L), pH 4.8, with 6.25% of the remaining solid residue under optimal conditions following the liquid hot water pretreatment process. Then, the yeast medium was sterilized using an autoclave at 121 °C for 15 min. The remaining solid residue was predigested using 25 FPU/g Cellic Ctec2 at 50 °C by 6 h of hydrolysis at 300 rpm.

Saccharomyces cerevisiae No. TISTR 5339 was the yeast strain used in this work [27]. The leavening agent cells of this microorganism from inoculated agar plates (30 °C for 24 h) were transferred to 150 mL Erlenmeyer flasks containing YPD medium, consisting of H_3PO_4 and NH_4OH, pH 4.8, incubated in a rotatory shaker at 35 °C, 150 rpm, and fermented for 120 h. The concentrations of sugars (glucose and xylose) and ethanol were analyzed using a high-performance liquid chromatograph equipped with an Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA). The ethanol yield was calculated as a percentage of the potential ethanol yield of consumed glucose, which was 0.511 g/g sugar [26].

4. Conclusions

Liquid hot water pretreatment was identified as an effective method for the pretreatment of sugarcane bagasse. The results indicated that 96.86% glucose yield was predicted under conditions of 160 °C, 60 min residence, and 0.050 M acid after LHW pretreatment using the Box–Behnken response surface design. A high ethanol concentration (19.9 g/L) was obtained via 72 h of the simultaneous saccharification fermentation process. Based on the obtained results, this study provides a methodological framework to optimize the alternative use of sugarcane bagasse for bioethanol production in biorefinery industries. The point of view is considered in term of biomass supply chain, bioenergy manufacturing chain and biofuel trade. In addition, bioethanol is suggested emphasizing sustainability, localness and recycling principles.

Author Contributions: Methodology writing—original draft preparation, P.K.; Conceptualization, N.S.; Visualization, T.K.; Writing—review and editing, N.L.; Supervision, K.W.; Writing—review and Methodology, V.C.; Project administration, K.S.; Software, C.S.; Writing—review and editing C.C.; Methodology, funding acquisition, S.I. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement: Data sharing is not applicable to this article.

Conflicts of Interest: The authors declare no conflict of interest.

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