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Optimization of Activated Carbon Adsorption for Detoxification of Sugarcane Bagasse Hydrolysate by Response Surface Methodology to Enhance Ethanol Production

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ABSTRACT

Lignocellulosic biomass has significant potential as a renewable resource for biofuel production. Bioethanol is a widely used biofuel derived from agricultural residues such as cassava, rice straw, sugarcane, and sorghum. In this study, sugarcane bagasse was used as the raw material for ethanol production by *Pichia stipitis* TISTR5806. The objective was to examine the effect of activated carbon in removing inhibitory compounds from hydrolysate to enhance ethanol fermentation. Sugarcane bagasse was pretreated with 2.0% v/v diluted sulfuric acid at 121°C for 60 minutes to obtain hydrolysate. Acid hydrolysis generates monomeric sugars and inhibitory compounds, including furfural, 5-hydroxymethylfurfural (5-HMF), phenolics, and organic acids. To mitigate the inhibitory effects and improve ethanol production, this study employed activated carbon for compound removal. A central composite design was used to determine the optimal detoxification conditions for activated carbon, varying its concentration (1–5% w/v), reaction temperature (30–60°C), and reaction time (20–60 minutes). The optimal conditions were identified as 3.0% w/v activated carbon, a reaction temperature of 45°C, and a reaction time of 40 minutes. Under these conditions, activated carbon removed 23.93% of total furans and 61.72% of phenolics. The ethanol yield from the untreated hydrolysate was 0.21 $g_{product}/g_{substrate}$, corresponding to a theoretical yield of 42.44%, with an ethanol production rate of 0.09 g/L·h. In contrast, the detoxified hydrolysate yielded 0.26 $g_{product}/g_{substrate}$, achieving a theoretical yield of 51.30% and an ethanol production rate of 0.28 g/L·h. These results indicate that sugarcane bagasse is a promising substrate for ethanol production and that activated carbon effectively removes inhibitory compounds from hydrolysate, thereby enhancing fermentation efficiency.

1. Introduction

According to the current energy situation, the depletion of fossil fuels (natural gas, oil, and coal) results in many countries facing a lack of energy. Some countries must import and purchase energy from other countries to support their people's daily lives and infrastructure. Currently, the world's population is growing at a rapid pace. Projections suggest it will exceed 9.7 billion by 2050. Asia, in particular, accounts for over 60% of the global population today, and this number is expected to rise to 5.25 billion by 2050 [1]. The combustion of fossil fuels releases large amounts of greenhouse gases, contributing to atmospheric degradation and rising global temperatures. As a result, efforts are underway to explore alternative natural energy sources to address these challenges and ensure future energy security.

Renewable energy is derived from natural resources and abundant, inexpensive materials, making it a viable alternative to fossil fuels. Various types of renewable energy include solar, biomass, biofuels, wind, and hydro power [2]. Bioenergy, a form of renewable energy, depends on the availability of local resources and plant-based materials. Edible biomass sources include sugarcane, beet, corn, rice straw, cassava, and sorghum, all of which have significant potential for energy production, particularly in biofuel generation [3-4].

Bioethanol is an environmentally friendly biofuel. It possesses unique properties, including a high-octane number, a low cetane number, and a high heating value. Additionally, ethanol is commonly blended with gasoline to produce gasohol, such as E20 (80% gasoline, 20% ethanol), which is used as transportation fuel [3]. Bioethanol is primarily produced from agricultural residues. First-generation bioethanol is derived from edible crops, while second-generation bioethanol is produced from lignocellulosic biomass. The next generation of bioethanol is obtained from microalgae [5]. Agricultural residues consist of lignocellulosic materials, which are primarily composed of three major components: cellulose, hemicellulose, and lignin. Cellulose is a homopolymer of glucose linked by glycosidic bonds.

Hemicellulose is a branched heteropolymer composed of pentose sugars (xylose and arabinose) and hexose sugars (glucose, galactose, and mannose). Lignin is a complex aromatic polymer that provides structural support to plants [6-7]. Sugarcane is a promising substrate for bioethanol production due to its high sugar content and widespread availability as a feedstock. Sugarcane bagasse, a solid residue obtained after juice extraction, typically contains 33–36% cellulose, 28–30% hemicellulose, and 17–24% lignin [8]. Pretreatment is a crucial step in breaking down lignocellulosic structures to release monomeric sugars. Various pretreatment methods include physical, chemical, biological, and physicochemical approaches. In this study, sugarcane bagasse underwent both physical and chemical pretreatment to disrupt its structure, increase surface area, and reduce the crystalline nature of cellulose [9]. Chemical pretreatment often involves hydrolysis using acids such as HCl, H_2SO_4, and HNO_3. In this study, sugarcane bagasse was hydrolyzed using dilute H_2SO_4 to obtain hydrolysate. However, acid hydrolysis not only generates monomeric sugars but also produces inhibitory compounds, including furan derivatives (furfural and 5-hydroxymethylfurfural, or 5-HMF), phenolics, and organic acids (such as formic acid and acetic acid) [10]. Furan compounds and organic acids result from the degradation of pentose and hexose sugars, while phenolics originate from lignin degradation. These inhibitory compounds negatively impact yeast metabolism, reducing ethanol production efficiency and leading to lower ethanol yields.

Detoxifying inhibitory compounds is a crucial process for removing these substances from hydrolysate. Various detoxification methods include membrane separation, activated carbon adsorption, overliming, and ion exchange resin. Activated carbon effectively adsorbs toxic compounds from hydrolysate, minimizes sugar loss, and maintains low operational costs, making it a common choice for inhibiting substances [11]. However, some degree of sugar loss is inevitable with all detoxification methods [12].

The adsorption of compounds like furan and phenol on activated carbon is driven mainly by weak electrostatic interactions between the adsorbate molecules and the carbon surface. While the precise mechanism remains unclear, the process can generally be explained through adsorption principles.

Phenolic compounds have weak acidic properties and low molecular weight. Activated carbon prefer to adsorb the ability of low molecular weight organic compounds especially phenols. The adsorption of phenolic compounds is one of the most investigate in liquid phase for activated carbon adsorbent application. Moreover, the factor of AC adsorbent for removing aromatic compounds include pore structure, functional group while the nature of adsorbate depends on functional group, molecular weight, pKa and polarity [13].

Furan compounds adsorption on activated carbon primary involve with physical and chemical adsorption. The physical adsorption including AC surface are between 800-200 m^2/g, pore size diameter between 2-50 nm, the weak acid interaction occurs due to the dipole-dipole or van der Waals force interaction between the furan molecules and surface of carbon, and the temperature prefer more effective at lower temperature. The chemical adsorption including the covalent bond, furan molecules form covalent bonds with reactive sites on the carbon surface. For the electrostatic interaction the oxygen atom in the furan ring can impart a slight polarity to the molecule. The presence of charged functional groups on the surface of the activated carbon, electrostatic interactions between the furan molecules and the surface could contribute to the adsorption process [14].

To enhance ethanol fermentation and achieve a higher ethanol yield, this research focuses on eliminating inhibitory compounds from sugarcane bagasse hydrolysate. The hydrolysate was detoxified using activated carbon and subsequently fermented by *Pichia stipitis* TISTR5806. This microorganism is highly efficient in bioethanol production from renewable resources and is capable of fermenting both pentose and hexose sugars [15]. The optimal conditions for activated carbon detoxification were determined using response surface methodology (RSM) to design and optimize the detoxification process.

Germec et al. [16] conducted a study using Pichia stipitis to convert rice hulls into ethanol through response surface methodology (RSM) optimization for acid treatment and toxin removal. The results indicated that the optimal hydrolysis conditions were 127.14°C, a solid-to-liquid ratio of 1:10.44, an acid concentration of 2.52%, and a hydrolysis time of 22.01 minutes. Activated charcoal was used to detoxify the rice hull hydrolysate, and the highest ethanol yield of 37.55% was obtained from *P. stipitis* ATCC 58785. Prasad et al. [17] used *Pichia stipitis* NCIM3498 for ethanol fermentation from wheat straw. Their study employed 5.0% w/v activated charcoal to mitigate the effects of furfural and 5-HMF. The experimental results demonstrated that activated charcoal successfully removed 84.01% of furfural and 76.42% of 5-HMF. The theoretical yield and ethanol yield coefficients were 87.90% and 0.44 $g_{ethanol}/g_{sugar}$, respectively.

The objectives of this study are to investigate the effectiveness of activated carbon in removing inhibitory compounds. To determine the optimal conditions for inhibitor removal from sugarcane bagasse hydrolysate using response surface methodology (RSM) and to study ethanol production from non-detoxified and detoxified hydrolysate using *Pichia stipitis* TISTR5806.

2. Experimental detail

2.1 Raw materials and pretreatment procedures

Sugarcane bagasse was obtained from a local market in Chiang Mai, Thailand, as shown in Fig. 1. For pretreatment, the raw material was washed with distilled water and dried at 105°C for three days. It was then milled using a crusher machine, reducing the particle size to 0.60 mm. The hydrolysis reaction was carried out using 2.0% v/v dilute H_2SO_4 with a solid-to-liquid ratio of 1:20 at 121°C and 15 psi for 60 minutes in an autoclave. After hydrolysis, a vacuum pump was used to separate the solid and liquid phases, obtaining the liquid hydrolysate. All chemical reagents used in this study were of analytical grade.

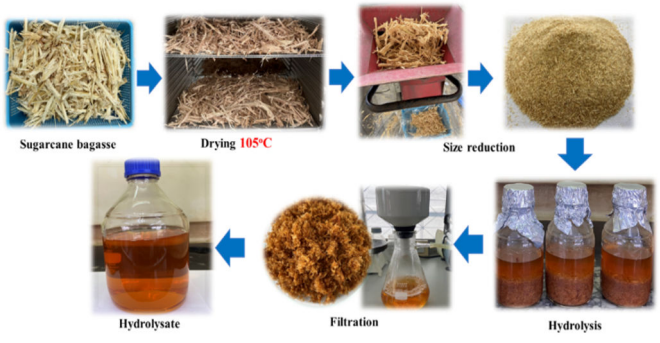

Fig. 1 Process preparation of sugarcane bagasse hydrolysate.

2.2 Response surface methodology for experimental design

In this study, the optimum detoxification conditions were determined using a central composite design (CCD). Design Expert version 13 (trial version) was utilized as statistical software for the design of the experiment. The terms activated carbon concentration (A), temperature (B), and reaction time (C) were selected as independent variables. Response variables comprised reducing sugar and inhibitory compounds (total furans and total phenolics). The experimental design is divided into three levels: minimum, medium, and maximum, as shown in Table 1, for the empirical in term of quadratic model, which can be written as follows:

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 \quad (1)$$

Where Y is the response for variable, β is the regression coefficient. The interaction coefficient are β_{12}AB,β_{13}AC,β_{23}BC. The quadratic coefficients terms are β_{11}A2,β_{22}B2,β_{33}C2. To assess the significance of models on design experts use analysis of variance (ANOVA) as statistical. ANOVA including p-Value indicate the significant of independent variables to each fit model meanwhile R2 present the model adequacy and accuracy. The Quadratic equation was used to predict and determine the optimum detoxification conditions.

Table 1 Central composite design on response surface methodology for detoxifying inhibitors using activated carbon adsorption.

Independent variable	Minimum	Medium	Maximum
(A) Activated carbon concentration (%w/v)	1.0	3.0	5.0
(B) Temperature (°C)	30	45	60
(C) Reaction time (min)	20	40	60

2.3 Detoxification methods

The liquid hydrolysate was detoxified using activated carbon adsorption. Activated carbon (LOBA Chemie) used in this experiment was granular. The specific information including a surface area of 875 m^2/g, iodine adsorption capacity 812.37 mg/g and 4.94% for loss on drying at 120°C. Following the design expert method for 15 trials conditions, as shown in Tables 4 and 5. The optimal detoxification conditions were determined using the central composite design (CCD). These conditions were then applied to remove inhibitory compounds from the hydrolysate before fermentation.

2.4 Microorganism and Inoculum

Pichia stipitis TISTR5806 obtained from Thailand Institute of Scientific and Technological Research (TISTR) Bangkok was used in this experiment. *Pichia stipitis* TISTR5806 was grown in yeast mannitol agar with contains 3.0 g of malt extract, 3.0 g of yeast extract, 5.0 g of peptone, 10.0 g of glucose and 20 g of agar for one lit of distilled water. The microorganisms are in an incubator at a temperature of 30°C and sterilized around. The inoculum contains hydrolysate in a flask of 250 ml sterilized in an autoclave at 121°C for 15 min and adjusted pH to 5.5 [11]. After sterilization, added yeast into flask and shaking 120 rpm in an incubated shaker control temperature 30°C for 24h.

2.5 Fermentation

The fermentation process used 200 mL of hydrolysate as the substrate. The fermentation samples in the flasks contained an initial cell concentration of 5.0 g/L and hydrolysate, both with and without activated carbon adsorption. The pH of the hydrolysate substrate was adjusted to 5.5 and sterilized at 121°C for 15 min before fermentation. All fermentation media were placed in an incubator shaker set to 30°C with a shaking speed of 120 rpm. The fermentation lasted for 96 h, and 10 mL samples were collected every 12 h to analyze cell growth, reducing sugar levels, and ethanol production.

2.6 Analytical methods

The analysis sample comprised of sugar, cell concentration, ethanol, and inhibitory compounds present in the hydrolysate. Spectrophotometric method was applied to analyze sample. Reducing sugar and total sugar were determined by a spectrophotometer. Reducing sugar was determined using dinitrosalicylic methods at 540 nm [18]. Total sugar was determined by phenol-sulfuric acid methods at 540 nm [19]. Total furan compounds were distilled and analyzed using a spectrophotometer at 284 and 320 nm wavelengths. Calculate the concentration using a calibration curve [20]. Total phenolic compounds were analyzed by spectrophotometer at wavelength 760 nm using the Folin-ciocalteu assay method [21]. Cell concentration in the hydrolysate sample was measured by optical density (OD) cell solution using the spectrophotometric method at 600 nm. Calculate dry cell concentration using a calibration curve [22]. The ethanol concentration was determined by a spectrophotometer at 585 nm using the dichromate method [23].

3. Results and Analysis

This study optimized the conditions for activated carbon adsorption detoxification of inhibitory compounds in sugarcane bagasse hydrolysate using response surface methodology to improve ethanol production. It was found that the independent variables—activated carbon concentration, temperature, and reaction time—affected the amount of reducing sugar remaining after detoxification and the elimination of inhibitory compounds. To investigate the effects of inhibitory compounds, present in the hydrolysate on ethanol production, the hydrolysate was fermented using the yeast *Pichia stipitis* TISTR5806 after the detoxification process.

3.*1 Characterization and Chemical compositions of raw materials*

Sugarcane bagasse was used as the raw material in this study. It is a solid residue derived from sugarcane extraction and has significant potential for bioethanol production due to its high sugar content. To enhance its suitability for fermentation, physical and chemical pretreatment processes were applied to reduce moisture content, decrease particle size to increase surface area, and disrupt the crystalline structure of cellulose [8,24]. Following hydrolysis, the liquid hydrolysate was obtained and used as the fermentation substrate. The chemical composition of the sugarcane bagasse hydrolysate, including both sugars and inhibitory compounds, is presented in Table 2.

Table 2 Chemical compositions of sugarcane bagasse hydrolysate.

Parameters	Amount
Total sugar (g/l)	68.73
Reducing sugar (g/l)	57.38
Total Phenolics (µg/l)	21.55
Total Furans (mg/l)	16.84

3.2 Response surface model for detoxification of inhibitory compounds

This study examined the effects of inhibitory compounds on ethanol production and explored detoxification methods to mitigate their impact. Activated carbon adsorption was selected as the detoxification method to remove inhibitors, including furfural, 5-HMF, phenolic compounds, and acetic acid. The experiment was designed using central composite design (CCD) and response surface methodology (RSM) to optimize the detoxification process. Mathematical models were developed to predict the chemical composition of sugarcane bagasse hydrolysate, specifically reducing sugars, total furans, and total phenolic compounds. A quadratic model was employed to estimate response variables after detoxification. These models demonstrated predictive accuracy when compared with experimental results using a quadratic function equation.

The regression models obtained from the RSM design were used to determine the optimal conditions for detoxifying inhibitory compounds. The experimental design included three key factors influencing inhibitor removal: activated carbon concentration (A), temperature (B), and reaction time (C). The quadratic model equation is presented in equations (2-4).

Reducing sugar (g/l)

$$Y_{reducing\ sugar} = 44.3605 - 0.1315A + 0.7536B - 0.4995C - 0.0282AB - 0.0263AC + 0.0033BC - 0.1140A^2 - 0.0095B^2 + 0.0048C^2 \quad (2)$$

Total furans (mg/l)

$$Y_{total\ furans} = 56.9375 - 4.6187A - 0.9578B - 0.1879C + 0.0405AB - 0.0179AC + 0.0009BC + 0.0406A^2 + 0.0069B^2 + 0.0011C^2 \quad (3)$$

Total Phenolics (µg/l)

$$Y_{total\ phenolics} = 32.8197 - 1.4735A - 0.4333B - 0.2602C - 0.0321AB - 0.0246AC + 0.0031BC + 0.1489A^2 + 0.0042B^2 + 0.0009C^2 \quad (4)$$

When Y represents the predicted concentration of response. Variables A, B, and C are activated carbon concentration, temperature, and reaction time, respectively. The analysis of variance (ANOVA) of each model is summarized in Table 3. The response of the reducing sugar model had R^2 = 0.9154, P-value = 0.0313, total furans R^2 = 0.9253, p-value = 0.0235 and total phenolics R^2 = 0.9593, p-value = 0.0056. These models show a p-value <0.05, which demonstrates that these models fit with the experimental data. The independent variables activated carbon concentration, temperature, and reaction time. The interaction between these variables were statistically significant for reducing sugar, total furans and total phenolics as shown in Table 4-5.

Figure 2 shows a 3D response surface plot that shows how the concentration of activated carbon, the temperature of the reaction, and the length of time the reaction lasts affect the response of the reducing sugar concentration. Figure 2A shows the interaction between activated carbon concentration and temperature. As the concentration of activated carbon increases, the reducing sugar concentration decreases. Conversely, as the reaction temperature increases, the reducing sugar concentration increases. Figure 2B depicts the interaction between activated carbon concentration and reaction time, following the same trend as Fig. 2A. Reducing sugar concentration increases with longer reaction times. The analysis of variable effects on reducing sugar concentration revealed that sugar loss occurred at various stages during the detoxification process using activated carbon adsorption [25]. The response surface curve demonstrated that as activated carbon concentration increased, sugar loss occurred. While activated carbon effectively adsorbs inhibitory compounds, it also adsorbs sugar simultaneously, leading to some sugar loss. Reaction temperature and reaction time exhibited similar effects on reducing sugar concentration, as an increase in both variables resulted in higher reducing sugar levels. These findings align with the research conducted by Mustafa Germec et al. [16], who studied the pretreatment of rice hull hydrolysate using dilute sulfuric acid hydrolysis optimized by response surface methodology (RSM).Their results demonstrated that fermentable sugar concentration increased with rising temperature and reaction time. Figure 2C illustrates the interaction between temperature and reaction time.

Table 3 Analysis of variance (ANOVA) of the experimental results of the quadratic model for the concentration of chemical composition in hydrolysate during the detoxification of inhibitory compounds.

Source	Reducing sugar (g/L)			Total Furans (mg/L)			Total Phenolics (µg/L)		
	Sum of Squares	F-value	p-value	Sum of Squares	F-value	p-value	Sum of Squares	F-value	p-value
Model	715.82	6.01	0.0313	978.48	6.88	0.0235	605.88	13.11	0.0056
A-AC concentration	538.2	40.65	0.0014	771.35	48.84	0.0009	495.3	96.44	0.0002
B-Temperature	9.43	0.7124	0.4371	93.88	5.94	0.0588	1.48	0.2881	0.6144
C-Reaction time	12.66	0.9561	0.3731	69.29	4.39	0.0904	80.6	15.69	0.0107
AB	5.73	0.4327	0.5397	11.79	0.7464	0.4271	7.43	1.45	0.2828
AC	8.88	0.671	0.45	4.10	0.2599	0.6319	7.76	1.51	0.2736
BC	7.66	0.5789	0.4811	0.7101	0.045	0.8404	7.11	1.38	0.2923
A^2	1.26	0.0951	0.7702	0.1597	0.0101	0.9238	2.15	0.4181	0.5464
B^2	27.65	2.09	0.208	14.63	0.9264	0.38	5.52	1.08	0.3473
C^2	22.27	1.68	0.2513	1.07	0.068	0.8046	0.7669	0.1493	0.7151
p-value	Significant			Significant			Significant		
R^2	0.9154			0.9253			0.9593		
Remark: P-value < 0.05 Significant									

Remark: F-value = Fisher-Snedecor distribution value, p-value = probability value, R^2=coefficient of determination.

Table 4 Response surface methods for analysis of reducing sugar from detoxification of inhibitor compounds in sugarcane bagasse hydrolysate using dilute sulfuric hydrolysis.

Run	Std.	Factors			Response		
		A	B	C	Reducing Sugar (g/L)		
					Experimental	Predicted	% Reduction
1	8	1	60	20	35.29	34.54	38.50
2	6	1	60	60	39.45	35.94	31.25
3	2	1	30	60	42.33	41.93	26.23
4	9	3	45	40	54.85	53.48	4.41
5	15	0.36	45	40	44.69	44.21	22.12
6	7	3	45	6.36	52.47	50.9	8.56
7	11	5	60	60	38.63	39.56	32.68
8	1	1	30	20	51.91	50.69	9.53
9	3	5	60	20	47.22	48.76	17.71
10	12	3	70.23	40	34.92	36.77	39.14
11	10	5	30	60	28.21	32.36	50.84
12	14	3	19.77	40	46.67	48.02	18.67
13	4	3	45	73.64	40.55	36.62	29.33
14	13	6.36	45	40	49.82	51.26	13.18
15	5	5	30	20	46.95	48.91	18.18

Table 5 Response surface methods for analysis inhibitor compounds from detoxification in sugarcane bagasse hydrolysate using dilute sulfuric hydrolysis.

Run	Std.	Factors			Response					
		A	B	C	Total Furans (mg/L)			Total Phenolics (µg/L)		
					Experimental	Predicted	%Removing	Experimental	Predicted	%Removing
1	3	1	60	20	21.16	19.61	25.65	16.72	17.28	22.41
2	7	1	60	60	19.61	17.14	16.45	16.89	16.28	21.62
3	5	1	30	60	24.57	24.21	45.90	15.36	13.12	28.72
4	15	3	45	40	12.81	12.88	23.93	8.25	8.22	61.72
5	9	0.36	45	40	21.91	25.06	30.11	18.67	20.03	13.36
6	13	3	45	6.36	20.80	17.86	23.52	15.19	13.31	29.51
7	8	5	60	60	4.12	3.10	75.53	0.36	0.34	98.33
8	1	1	30	20	26.57	27.88	57.78	17.99	17.90	16.52
9	4	5	60	20	5.79	8.44	65.62	3.16	5.28	85.34
10	12	3	70.23	40	11.31	12.87	32.84	11.64	10.36	45.99
11	6	5	30	60	3.48	5.32	79.33	1.72	1.04	92.02
12	11	3	19.77	40	23.67	21.69	40.56	10.03	11.47	53.46
13	14	3	45	73.64	7.76	10.28	53.92	3.08	5.14	85.71
14	10	6.36	45	40	3.35	0.22	80.11	0.96	0.23	95.55
15	2	5	30	20	9.08	11.85	46.08	9.26	9.75	57.03

Remark: (A) Activated carbon concentration (%w/v), (B) Temperature (°C), (C) Reaction time (min)

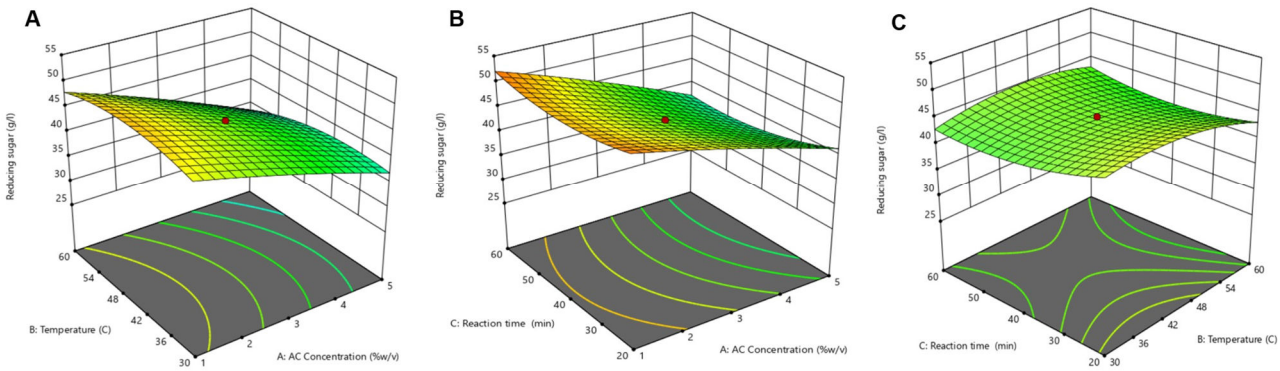

Fig.2 3D response surface curve of reducing sugar on detoxification process. (A) effect of activated carbon concentration and temperature, (B) effect of activated carbon concentration and reaction time and (C) effect of temperature and reaction time.

The reducing sugar concentration increased as the temperature rose slightly, reaching its peak between 42–48°C. However, when the temperature exceeded 48°C and approached 60°C, a partial reduction in sugar content was observed.

The 3D response surface curve for total furans is shown in Fig. 3. Figure 3A illustrates the interaction between activated carbon concentration and temperature on total furan levels. As the concentration of activated carbon increased, the total furans decreased. A rise in reaction temperature also led to a lower total furans. These results indicate that obtaining the lowest total furans after detoxification demonstrates the efficiency of activated carbon in adsorbing these compounds.

Figure 3B presents the interaction between activated carbon concentration and reaction time on total furans. Increasing reaction time followed the same trend as activated carbon concentration and temperature, further reducing total furan levels. These findings suggest that increasing these variables enhances the removal of total furans from the hydrolysate. Fig. 3C illustrates the interaction between temperature and reaction time. The optimal conditions for eliminating total furans ranged between 42–48°C for temperature and 40–50 minutes for reaction time. These trends of the interaction between the independent variables on eliminate total furans related in the table 5. The statistical analysis was analyzed by ANOVA that show in the table 3. The results suggest that p-value of total furans presence less than 0.05 that means this model has significant model. This model illustrates that AC concentration significantly for removing of total furans. This study aligns with the research of Ji-Woo Hong et al., [26] who applied response surface methodology (RSM) to optimize microalgae hydrolysate detoxification using activated carbon. Their results demonstrated that increasing temperature, the ratio of activated carbon to hydrolysate, and reaction time significantly improved the elimination of furfural and 5-HMF in hydrolysate.

Additionally, increasing temperature resulted in a reduction of sugar content. High temperatures break down recalcitrant lignocellulosic structures in cellulose and hemicellulose, releasing large amounts of monomeric sugars (pentose and hexose), which serve as carbon sources for yeast fermentation into ethanol. Furthermore, a longer reaction time facilitates the extraction of reducing sugars from lignocellulosic biomass. The effects of temperature and reaction time align with the findings of Tesfaw, A., and Tizazu, B. Z., [27] to studied the optimization of time and temperature on total reducing sugar extraction from Teff straw biomass. Their results indicated that longer reaction times and higher temperatures led to the decomposition of monomeric sugars, whereas lower temperatures and shorter hydrolysis times made it difficult to extract total reducing sugars from Teff straw.

Figure 3D and 3E display the 3D response surface curves for total phenolics, illustrating the effects of the independent variables on total phenolic removal. As these variables increased, the total phenolic content declines compare with non-detoxified hydrolysate. Under high conditions, phenolic compounds were effectively eliminated. Figure 4C illustrates the interaction between temperature and reaction time. The optimal temperature for total phenolic removal ranged between 42–48°C. These trends of the interaction between independent variables on total phenolics removing consistent with table 5. The statistical analysis, p-value presence less than 0.05 which denote that this significant of the model. This model points out the AC concentration and reaction time significantly on removing total phenolics. Comparison R^2 for these inhibitory compounds and reducing sugar. The R^2 in Table 3 illustrates that among the previous models total phenolics presence the highest R^2 nearest of 1. This analysis elucidates that this model had the highest accuracy. Moreover, activated carbon detoxification display specific on eliminating total phenolics more than total furans.

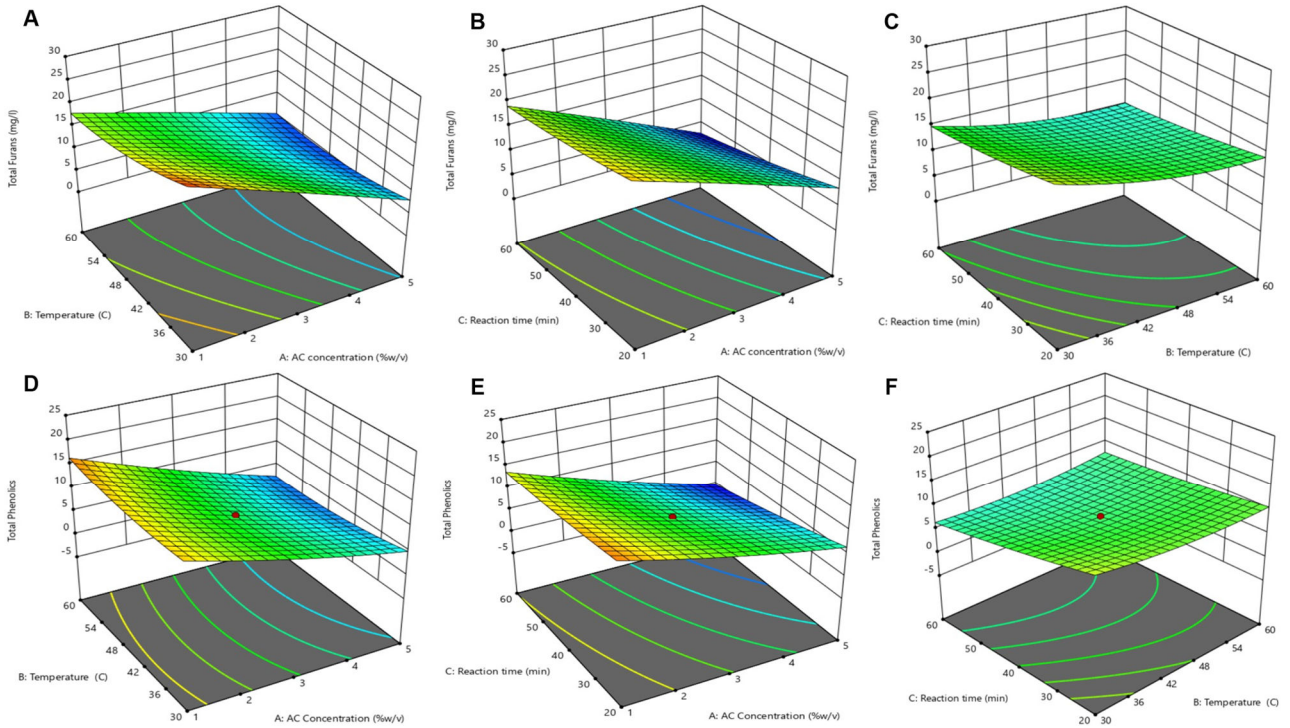

Fig. 3 3D response surface curve of total furans and total phenolics on detoxification process: (A) effect of activated carbon concentration and temperature total furans, (B) effect of activated carbon concentration and reaction time total furans, (C) effect of temperature and reaction time total furans (D) Actual and predicted value on total furans, (D) effect of activated carbon concentration and temperature on total phenolics, (E) effect of activated carbon concentration and reaction time on total phenolics and (F) effect of temperature and reaction time on total phenolics.

The experiment aligning with the study conducted by Yaya, O. L. et al., [28] optimized the acid hydrolysate detoxification of cocoa pod husks. Their research analyzed phenolic compounds and fermentable sugars as response variables and demonstrated that phenolic removal efficiency improved when the ratio of activated charcoal to hydrolysate was higher. Additionally, an increase in activated carbon concentration resulted in a decrease in reducing sugar while enhancing the removal of inhibitory compounds from hydrolysate due to the adsorption properties of activated carbon. Both inhibitors and reducing sugars were adsorbed during the process. Similarly, increasing temperature and reaction time also enhanced the removal of inhibitory compounds.

3.3 Effect of independent variable on detoxification

This study examined the effects of activated carbon detoxification on inhibitory compounds and its impact on ethanol production using response surface methodology (RSM). The experiment selected activated carbon concentration, temperature, and reaction time as independent variables. Total furans and phenolics were measured as response variables to determine the optimal conditions for removing inhibitory compounds that hinder yeast's ability to produce ethanol. RSM utilized the central composite design (CCD) as a tool for experimental design. The three independent variables and their factor levels for reducing sugar and inhibitory compound responses are presented in Tables 4 and 5.

Three different activated carbon (AC) concentrations—1.0, 3.0, and 5.0% w/v—were selected to determine the optimal conditions for detoxifying inhibitory compounds in sugarcane bagasse hydrolysate. The minimum reducing sugar concentration observed in the treated sample was 28.21 g/L at 30°C, 5.0% w/v AC concentration, and a reaction time of 60 minutes. Under these conditions, the highest sugar loss of 50.84% was recorded. Total furans reached their lowest concentration of 3.35 mg/L in the treated sample at 45°C, 6.36% w/v AC concentration, and a reaction time of 40 minutes. Total phenolics had a minimum concentration of 0.96 g/L. Under these conditions, total furans and total phenolics showed the highest removal efficiencies of 80.11% and 95.55%, respectively. The treatment conditions for total furans were like those of total phenolics. Conversely, the highest remaining sugar concentration was 54.85 g/L when the sample was treated at 45°C, 3.0% w/v AC concentration, and a reaction time of 40 minutes. Under these conditions, the lowest sugar loss of 4.41% was observed. The highest concentrations of inhibitory compounds were 26.57 mg/L for total furans and 18.67 g/L for total phenolics when the sample was treated at 30°C and 60°C, with AC concentrations of 1.0% and 0.36% w/v, and reaction times of 60 and 40 minutes, respectively. This experiment indicated that total phenolics were more effectively removed compared to total furans. The other variables in this study were temperature and reaction time. Temperature levels of 30°C, 45°C, and 60°C, along with reaction times of 20, 40, and 60 minutes, were selected to determine the optimal conditions for AC concentration. These independent variables influenced the amounts of sugar and inhibitory compounds. Table 4 showed that increasing AC concentration resulted in a slight decrease in reducing sugar. In contrast, reaction time and temperature had the opposite effect, leading to an increase in reducing sugar concentration. Yildirim O. et al. [29] studied the optimal pretreatment conditions, where acid concentration, temperature, and time were considered independent variables affecting sugar concentration. Their findings indicated that the best conditions for pretreatment and the highest sugar yield from cotton straw were achieved at 121.7°C, 2.28% v/v acid concentration, and 36.82 minutes of reaction time. Conversely, the interaction between temperature and time demonstrated that increasing both variables led to a higher sugar concentration.

The effect of independent variables on inhibitory compounds is presented in Table 5. The results indicate that increasing these independent variables significantly enhanced the removal of inhibitory compounds from the hydrolysate.

According to Gupta V. et al. [30], their study aimed to minimize the generation of inhibitors during rice straw hydrolysis to improve ethanol fermentation. The independent variables in their study included solid loading rate, H_2SO_4 concentration, reaction time, and temperature. Furfural, HMF, and total phenolics were selected as response variables. Their experimental results showed that the optimal pretreatment conditions for minimizing the release of inhibitor molecules resulted in concentrations of 48.60%, 2.32%, and 1.65% per 100 g of solid biomass for furfural, HMF, and total phenolics, respectively.

Previous research indicates that the response variables—reducing sugar concentration and the removal efficiency of inhibitory compounds—are influenced by AC concentration, temperature, and reaction time. Based on the experimental results, the optimal conditions were determined to be the most efficient for the detoxification process of inhibitors [30]. Using Design Expert 13 (trial version), optimal conditions were obtained from the response surface curve. The optimum conditions for reducing sugar were found to be 44.69 g/L at an AC concentration of 3.0% w/v, a temperature of 45°C, and a reaction time of 40 minutes. The optimal parameters for inhibitory compound removal were as follows: total furans were minimized to 12.80 g/L at 3.0% w/v AC concentration, 45°C, and 40 minutes of reaction time, while total phenolics were reduced to 8.24 g/L under the same conditions. These optimal conditions were applied for the detoxification of inhibitors in sugarcane bagasse hydrolysate. The study of optimal detoxification aligns with the research conducted by Deshavath, N. et al., [31] to investigated the optimum hydrolysis conditions for sorghum to maximize pentose production while minimizing fermentative inhibitors. In their study, temperature, reaction time, and acid concentration were selected as independent variables. The optimal conditions were found to be 0.2M H_2SO_4, 121°C, and 120 minutes, yielding a low furfural concentration of 4.6 mg/g.

3.4 Detoxification of sugarcane bagasse hydrolysate

The response surface methodology (RSM) using the central composite design (CCD). The optimal conditions for detoxification were 3.0% w/v activated carbon, a reaction temperature of 45°C, and a reaction time of 40 minutes. The hydrolysate was detoxified under these optimal conditions before fermentation with *Pichia stipitis* TISTR5806. Under these detoxification conditions, the inhibitory compounds were reduced while minimizing sugar loss to approximately 4.41%. Activated carbon effectively removed 23.93% of total furans and 61.72% of total phenolics from sugarcane bagasse hydrolysate. The data on the removal of chemical compositions are presented in Table 6. During detoxification process, the adsorption method by adding adsorbent to pretreat the liquid hydrolysate and combine the inhibitor to precipitate. Activated carbon as an adsorbent which high surface area effective removing on toxic compounds in hydrolysate.

Table 6 Removing of chemicals compositions using activated carbon in sugarcane bagasse hydrolysate at the optimal conditions.

Parameters	Hydrolysate (Non-detoxification)	Hydrolysate (AC Detoxification)	% Removing
Reducing sugar (g/L)	57.38	54.85	4.41
Total Phenolics (μg/L)	21.55	8.25	61.72
Total Furans (mg/L)	16.84	12.81	23.93

The study results indicated that activated carbon had a greater effect on removing total phenolics from the hydrolysate compared to total furans. These findings align with a similar study conducted by Baig, M. Z., and Dharmadhikari, S. M. [32], who investigated the detoxification of cotton stalk hydrolysate using charcoal treatment. Their study demonstrated that activated charcoal effectively removed phenolic compounds and other hydrolysate inhibitors through detoxification.

3.5 Ethanol fermentation

The bagasse was hydrolyzed using dilute H_2SO_4. During acid hydrolysis, monomeric sugars (glucose, arabinose, and xylose) were released from the breakdown of the lignocellulosic structure. However, this process also generated inhibitory compounds, including furans, phenolics, and organic acids, which can hinder microbial metabolism and reduce ethanol production. Activated carbon was used to adsorb the inhibitors before fermentation, thereby enhancing ethanol productivity. The hydrolysate was then used as a substrate for fermentation by *Pichia stipitis* TISTR5806. The results of ethanol fermentation using both detoxified and non-detoxified hydrolysate are presented in Table 7.

Table 7 Parameters of ethanol fermentation from *Pichia stipitis* TISTR5806.

Parameters	Hydrolysate	AC Detoxification
Specific growth rate (h^{-1})	0.0750	0.2115
Initial reducing sugar (g/L)	42.36	38.95
Final reducing sugar (g/L)	30.59	6.24
Reducing sugar consumption (%)	27.78	88.62
Ethanol concentration (g/)	9.18	10.21
Yield ($g_{ethanol}/g_{sugar}$)	0.21	0.26
Theoretical yield (%)	42.44	51.30
Time for maximum ethanol production (h)	96	36
Ethanol productivity (g/L·h)	0.09	0.28

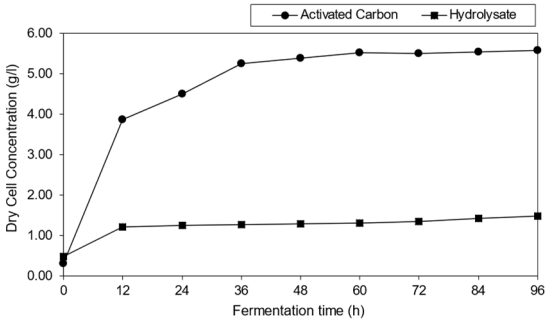

Fig. 4 Dry cell concentration of activated carbon detoxification and hydrolysate on ethanol fermentation from *Pichia stipitis* TISTR5806.

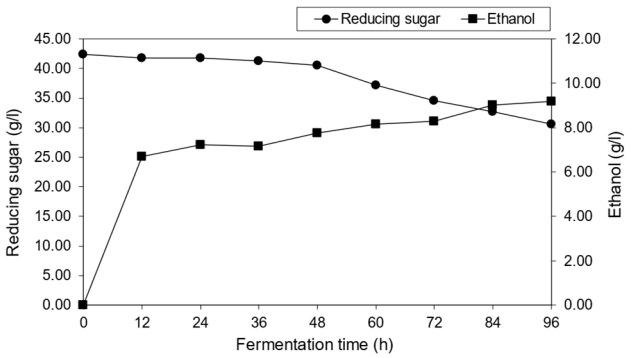

Fig. 5 Ethanol fermentation from sugarcane bagasse hydrolysate by *Pichia stipitis* TISTR5806.

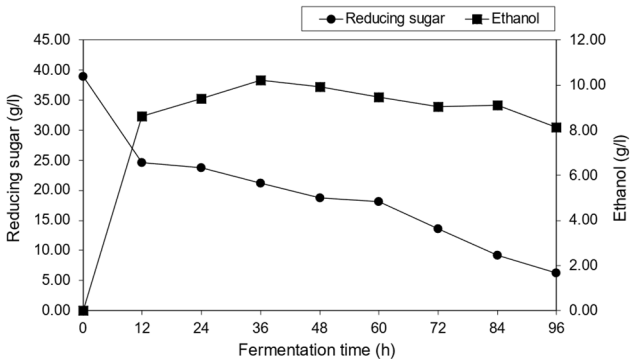

Fig. 6 Ethanol fermentation from sugarcane bagasse hydrolysate on activated carbon detoxification by *Pichia stipitis* TISTR5806.

Ethanol fermentation from non-detoxified hydrolysate is shown in Fig. 5. The reducing sugars in the hydrolysate served as a carbon source for fermentation. In the first 12 hours. At this time, yeast divided cell rapidly approach to exponential phase. Reducing sugars were rapidly consumed, while yeast gradually produced ethanol. Reducing sugars were rapidly consumed, while yeast gradually produced ethanol. After 96 hours, ethanol concentration peaked at 9.18 g/L. At this point, 30.59 g/L of reducing sugars remained, with a conversion rate of 27.78%. Ethanol production reached 0.21 g product/g substrate. The ethanol yield was 42.44%. Ethanol productivity was 0.09 g/L·h. The yeast's specific growth rate during fermentation was 0.0750 h^{-1}.

The hydrolysate was detoxified using activated carbon under optimal conditions determined by response surface methodology (RSM). After detoxification, the reducing sugars in the hydrolysate served as a carbon source for fermentation, as shown in Fig. 6. In the first 12 hours, sugar was rapidly consumed. Sugar levels then declined gradually until 96 hours, leaving 6.24 g/L in the system. The conversion rate of reducing sugar to ethanol was 88.62%. Yeast steadily produced ethanol until 36 hours, reaching a peak concentration of 10.21 g/L. Ethanol levels then declined to 96 hours, when 6.24 g/L remained. The experimental results showed ethanol production of 0.26 $g_{product}/g_{substrate}$. The ethanol yield and theoretical yield were 51.30%. Ethanol productivity was 0.28 g/L·h, while the specific growth rate of yeast was 0.2115 h^{-1}. This study aligns with previous research. Baig, M. Z., and Dharmadhikari, S. M. [33] reported that 4.0% charcoal treatment resulted in an ethanol yield of 0.396 $g_{product}/g_{substrate}$ from cotton hydrolysate. Srinorakutara, T. et al. [33] found a maximum ethanol concentration of 21 g/L under optimal detoxification conditions. Their study determined that 2.5% charcoal powder, pH 5.0, 30°C, 160 rpm

shaking, and a reaction time of 5 minutes were ideal for detoxifying rice straw hydrolysate using *Pichia stipitis* TISTR5806. Brito, P. L. et al. [34] reported an ethanol yield of 0.12 $g_{product}/g_{sugar}$ from activated charcoal detoxification of palm press fiber hydrolysate. Their optimal hydrolysis conditions were 5.0% H_2SO_4, 121°C, and a 60-minute reaction time, determined through RSM. Fermentation of palm fiber hydrolysate with *S. stipitis* NRRLY7124 removed 88.4% of polysaccharides. Activated charcoal eliminated 96% of phenolic compounds and 99% of furfural. Germec, M. et al. [35] studied acid pretreatment and detoxification of rice straw using the Box-Behnken response surface design. Their optimal hydrolysis conditions were 126.5°C, a 1:15 solid-to-liquid ratio, and 5.0% w/v acid. Under these conditions, the reducing sugar concentration was 21.50 g/L. Activated charcoal removed 78.58% of HMF and 94.67% of phenolics. The maximum theoretical ethanol yields were 42.95% and 48.81%, obtained from fermentation with *S. stipitis* (ATCC 58784 and ATCC 58785). These results closely match those of this study.

A comparison of the specific growth rates from previous studies is shown in Fig. 4. Detoxified hydrolysate treated with activated carbon exhibited a higher specific growth rate than non-detoxified hydrolysate. The presence of inhibitory compounds (such as furfural, 5-HMF, phenolic compounds, and organic acids) during the acid hydrolysis reaction reduces the viability of microorganisms and their ability to produce ethanol [36]. Furan compounds inhibit the growth and uptake of microorganisms [37], while phenolic compounds damage cell membrane integrity and suppress the growth of microbial yeast cells [38]. Activated carbon adsorption effectively eliminates these inhibitory compounds, especially phenolic compounds. However, it does not reduce the sugar loss [39], as shown in the experiment results in Table 6. Consequently, detoxified hydrolysate treated with activated carbon yielded higher ethanol concentrations than non-detoxified hydrolysate. The specific growth rate correlates with ethanol yield, indicating that the detoxification process is beneficial for improving ethanol production. However, sugar loss was observed during the process. The optimal detoxification conditions are therefore suitable for enhancing ethanol fermentation.

4. Conclusion

In conclusion, this study demonstrated the effectiveness of activated carbon adsorption in detoxifying sugarcane bagasse hydrolysate by removing inhibitory compounds such as furans and phenolics. The optimal detoxification conditions AC 3.0% w/v at 45°C for 40 min, as determined by RSM, significantly enhanced ethanol fermentation by *Pichia stipitis* TISTR5806, as shown by improved specific growth rates and higher ethanol yields. At this condition could significant remove higher of 61.71% of total phenolics while 23.93% total furans compare with non-detoxify hydrolysate. These findings highlight the potential of activated carbon to improve ethanol production from sugarcane bagasse hydrolysate by reducing the negative impact of inhibitory compounds.

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References

[1] Sadigov, R., Rapid growth of the world population and its socioeconomic results. *The Scientific World Journal*. 2022 (2022) 8110229, doi: https://doi.org/10.1155/2022/8110229.

[2] Ramaraj, R., Unpaprom, Y. and Dussadee, N. Cultivation of Green Microalga, Chlorella vulgaris for Biogas Purification. *International Journal of New Technology and Research*. 2 (2016) 117-122.

[3] Hirani, A. H., Javed, N., Asif, M., Basu, S. K. and Kumar, A. in *Biofuels: Greenhouse Gas Mitigation and Global Warming: Next Generation Biofuels and Role of Biotechnology: A Review on First- and Second-Generation Biofuel Productions*, Ch. 8, Springer India, (2018) 141-154, doi: https://doi.org/10.1007/978-81-322-3763-1_8.

[4] Chia, S. R., Chew, K. W., Show, P. L., Yap, Y. J., Ong, H. C., Ling, T. C. and Chang, J. S., Analysis of economic and environmental aspects of microalgae biorefinery for biofuels production: a review. *Biotechnology journal*. 13 (2018) 1700618, doi: https://doi.org/10.1002/biot.201700618.

[5] Sjulander, N. and Kikas, T., Origin, impact and control of lignocellulosic inhibitors in bioethanol production—A review. *Energies*. 13 (2020) 4751, doi: https://doi.org/10.3390/en13184751.

[6] Olsson, L. and Hahn-Hägerdal, B., Fermentation of lignocellulosic hydrolysates for ethanol production. *Enzyme and Microbial technology*. 18 (1996) 312-331, doi: https://doi.org/10.1016/0141-0229(95)00157-3.

[7] Luo, X., Zeng, B., Zhong, Y. and Chen, J., Production and Detoxification of Inhibitors during the Destruction of Lignocellulose Spatial Structure. *BioResources*. 17 (2022) 1939-1961, doi: https://doi.org/10.15376/biores.17.1.Luo.

[8] Saleh, S. H. and Abd Halim, N. in *Fuel Ethanol Production from Sugarcane: Sugarcane Bagasse Pretreatment Methods for Ethanol Production.* Ch. 4, IntechOpen, (2018) 63-80, doi: https://doi.org/10.5772/intechopen.81656.

[9] Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y. Y., Holtzapple, M. and Ladisch, M., Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource technology*. 96 (2005) 673-686, doi: https://doi.org/10.1016/j.biortech.2004.06.025.

[10] Jönsson, L. J., Alriksson, B. and Nilvebrant, N.-O., Bioconversion of lignocellulose: inhibitors and detoxification. *Biotechnology for biofuels.* 6 (2013) 1-10, doi: https://doi.org/10.1186/1754-6834-6-16.

[11] Luo, X., Zeng, B., Zhong, Y. and Chen, J., Production and Detoxification of Inhibitors during the Destruction of Lignocellulose Spatial Structure. *BioResources*. 17 (2022) 1939-1961, doi: https://doi.org/10.15376/biores.17.1.Luo.

[12] Singh, O. V., Silva, S. and Chandel, A. in *Biofuel Production - Recent Developments and Prospects: Detoxification of Lignocellulosic Hydrolysates for Improved Bioethanol Production.* Ch. 10, IntechOpen, (2011) 225-246, doi: https://doi.org/10.5772/16454.

[13] Dąbrowski, A., Podkościelny, P., Hubicki, Z. and Barczak, M., Adsorption of phenolic compounds by activated carbon—a critical review. *Chemosphere*. 58 (2005) 1049-1070, doi: https://doi.org/10.1016/j.chemosphere.2004.09.067.

[14] Haghseresht, F., Finnerty, J. J., Nouri, S. and Lu, G. Q., Adsorption of Aromatic Compounds onto Activated Carbons: Effects of the Orientation of the Adsorbates. *Langmuir*. 18 (2002) 6193-6200, doi: https://doi.org/10.1021/la025541b.

[15] Huang, C.-F., Lin, T. -H., Guo, G. -L. and Hwang, W. -S., Enhanced ethanol production by fermentation of rice straw hydrolysate without detoxification using a newly adapted strain of Pichia stipitis. *Bioresource technology*. 100 (2009) 3914-3920, doi: https://doi.org/10.1016/j.biortech.2009.02.064.

[16] Germec, M., Kartal, F. K., Bilgic, M., Ilgin, M., Ilhan, E., Güldali, H., Isci, A. and Turhan, I., Ethanol production from rice hull using Pichia stipitis and optimization of acid pretreatment and detoxification processes. *Biotechnology progress*. 32 (2016) 872-882, doi: https://doi.org/10.1002/btpr.2275.

[17] Prasad, S., Malav, M. K., Kumar, S., Singh, A., Pant, D. and Radhakrishnan, S., Enhancement of bio-ethanol production potential of wheat straw by reducing furfural and 5-hydroxymethylfurfural (HMF). *Bioresource Technology Reports*. 4 (2018) 50-56, doi: https://doi.org/10.1016/j.biteb.2018.09.007.

[18] Miller, G. L., Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical chemistry*. 31 (1959) 426-428, doi: https://doi.org/10.1021/ac60147a030.

[19] Nielsen, S. S. *Phenol-sulfuric acid method for total carbohydrates.* Nielsen, S.S. edn, Springer, Boston, MA, 2010, doi: https://doi.org/10.1007/978-1-4419-1463-7_6.

[20] Martinez, A., Rodriguez, M. E., York, S. W., Preston, J. F. and Ingram, L. O., Use of UV absorbance to monitor furans in dilute acid hydrolysates of biomass. *Biotechnology progress.* 16 (2000) 637-641, doi: https://doi.org/10.1021/bp0000508.

[21] Blainski, A., Lopes, G. C. and De Mello, J. C. P., Application and analysis of the folin ciocalteu method for the determination of the total phenolic content from Limonium brasiliense L. *Molecules*. 18 (2013) 6852-6865, doi: https://doi.org/10.3390/molecules18066852.

[22] Myers, J. A., Curtis, B. S. and Curtis, W. R., Improving accuracy of cell and chromophore concentration measurements using optical density. *BMC biophysics.* 6 (2013) 1-15, doi: https://doi.org/10.1186/2046-1682-6-4.

[23] Seo, H.-B., Kim, H.-J., Lee, O.-K., Ha, J.-H., Lee, H.-Y. and Jung, K.-H., Measurement of ethanol concentration using solvent extraction and dichromate oxidation and its application to bioethanol production process. *Journal of industrial Microbiology and Biotechnology*. 36 (2009) 285-292, doi: https://doi.org/10.1007/s10295-008-0497-4.

[24] Martín, C., Galbe, M., Nilvebrant, NO. and Jönsson, L. J. Comparison of the fermentability of enzymatic hydrolyzates of sugarcane bagasse pretreated by steam explosion using different impregnating agents. *Applied Biochemistry and Biotechnology.* 98 (2002) 699-716, doi: https://doi.org/10.1385/abab:98-100:1-9:699.

[25] Ravindran, R. and Jaiswal, A. K., A comprehensive review on pre-treatment strategy for lignocellulosic food industry waste: challenges and opportunities. *Bioresource technology*. 199 (2016) 92-102, doi: https://doi.org/10.1016/j.biortech.2015.07.106.

[26] Hong, J.-W., Gam, D.-H., Kim, J.-H., Jeon, S.-J., Kim, H.-S. and Kim, J.-W., Process development for the detoxification of fermentation inhibitors from acid pretreated microalgae hydrolysate. *Molecules.* 26 (2021) 2435, doi: https://doi.org/10.3390/molecules26092435.

[27] Tesfaw, A. A. and Tizazu, B. Z., Reducing sugar production from Teff straw biomass using dilute sulfuric acid hydrolysis: Characterization and optimization using response surface methodology. *International Journal of Biomaterials.* 2021 (2021) 2857764, doi: https://doi.org/10.1155/2021/2857764.

[28] Yaya, O. L., Appiah, K. E. K., Doudjo, S., Nassirou, A. K. M., Didier, F. G., Benjamin, Y. K., Drogui, A. P. and Tyagi, D. R., Multi-response and multi-criteria optimization of acid hydrolyzate detoxification of cocoa pod husks: Effect on the content of phenolic compounds and fermentable sugars. *Heliyon.* 9 (2023) 1-11, doi: https://doi.org/10.1016/j.heliyon.2023.e15409.

[29] Yildirim, O., Ozkaya, B., Altinbas, M. and Demir, A., 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 (2021) 8882-8899, doi: https://doi.org/10.1002/er.6423.

[30] Gupta, V. C., Singh, M., Prasad, S. and Mishra, B. N., Minimization of Inhibitor Generation in Rice Straw Hydrolysate Using RSM Optimization Technique. *Agriculture.* 13 (2023) 1431, doi: https://doi.org/10.3390/agriculture13071431.

[31] Deshavath, N. N., Mohan, M., Veeranki, V. D., Goud, V. V., Pinnamaneni, S. R. and Benarjee, T., Dilute acid pretreatment of sorghum biomass to maximize the hemicellulose hydrolysis with minimized levels of fermentative inhibitors for bioethanol production. *3 Biotech*. 7 (2017) 1-12, doi: https://doi.org/10.1007/s13205-017-0752-3.

[32] Baig, M. Z. and Dharmadhikari, S. M., Optimization of detoxification with over liming and charcoal treatment for increasing the fermentability of cotton stalk hydrolyzate. Indian Journal of Applied Research. 4 (2014) 453-455, doi: https://doi.org/10.15373/2249555x/july2014/143.

[33] Srinorakutara, T., Subkaree, Y., Boonvitthya, N. and Bamrungchue, N., Optimum Condition of Rice Straw Hydrolysate Detoxification with Charcoal Powder for Cellulosic Ethanol Production by Pichia stipitis TISTR 5806. *Journal of Food Science and Engineering.* 6 (2016) 75-81.

[34] Brito, P. L., de Azevedo Ferreira, C. M., Silva, A. F. F., Pantoja, L. d. A., Nelson, D. L. and dos Santos, A. S., Hydrolysis, detoxification and alcoholic fermentation of hemicellulose fraction from palm press fiber. *Waste and Biomass Valorization*. 9 (2018) 957-968, doi: https://doi.org/10.1007/s12649-017-9882-4.

[35] Germec, M. and Turhan, I., Ethanol production from acid-pretreated and detoxified rice straw as sole renewable resource. *Biomass Conversion and Biorefinery*. 8 (2018) 607-619, doi: https://doi.org/10.1007/s13399-018-0310-1.

[36] Taherzadeh, M. J. and Karimi, K. *Biofuels: Alternative Feedstocks and Conversion Processes.* 1st edn., Academic Press, 2011, doi: https://doi.org/10.1016/B978-0-12-385099-7.00012-7.

[37] Palmqvist, E., Almeida, J. S. and Hahn-Hägerdal, B., Influence of furfural on anaerobic glycolytic kinetics of Saccharomyces cerevisiae in batch culture. *Biotechnology and bioengineering.* 62 (1999) 447-454, doi: https://doi.org/10.1002/(sici)1097-0290(19990220)62:4%3C447::aid-bit7%3E3.0.co;2-0.

[38] Chen, X., Zhai, R., Li, Y., Yuan, X., Liu, Z.-H. and Jin, M., Understanding the structural characteristics of water-soluble phenolic compounds from four pretreatments of corn stover and their inhibitory effects on enzymatic hydrolysis and fermentation. Biotechnology for biofuels. 13 (2020) 1-13, doi: https://doi.org/10.1186/s13068-020-01686-z.

[39] Mussatto, S. I. and Roberto, I. C., Alternatives for detoxification of diluted-acid lignocellulosic hydrolyzates for use in fermentative processes: a review. *Bioresource technology.* 93 (2004) 1-10, doi: https://doi.org/10.1016/j.biortech.2003.10.005