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Optimized ultrasound-assisted peracetic acid pretreatment approach for enhanced sugars and decreased inhibitors for bio-ethanol production from oil palm empty fruit bunch fiber

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Abstract. Oil palm empty fruit bunch (OPEFB) biomass, a primary solid waste from the palm oil industry, was pretreated using ultrasonication and peracetic acid (PAA) to optimize sugar yield and minimize inhibitor formation. The response surface methodology, employing a central composite design (CCD), was used to evaluate the effects of PAA concentration, sonication time, and temperature on glucose, xylose, arabinose, furfural, and hydroxymethylfurfural (HMF) yields. Optimal pretreatment conditions were determined to be 10 % PAA concentration, 120 min sonication, and 60 °C, resulting in sugar concentrations of 32.19, 15.83, and 5.92 g/L for glucose, xylose, and arabinose, respectively, with minimal inhibitors (1.86 g/L furfural, 3.2 g/L HMF). Detoxification with activated carbon reduced inhibitory compounds, enhancing fermentation efficiency. Hydrolysate from optimized pretreatment achieved higher ethanol yields (67.5 g/L) compared to untreated OPEFB (56.7 g/L). These findings highlight the potential of ultrasonicated PAA pretreatment for bioethanol production.

Keywords: ultrasonication, peracetic acid, oil palm empty fruit bunch, inhibitors, bioethanol.

Classification numbers: 3.1.1, 3.3.2, 3.8.3.

1. INTRODUCTION

Many developing countries are exploring lignocellulosic biomass (LCB) as a renewable resource for second-generation biofuels [1]. Oil Palm Empty Fruit Bunches (OPEFB), which make up about 23 % of the weight of fresh palm fruit bunches, are the main waste produced by palm oil mills. Using these wastes effectively can benefit both the environment and the economy. OPEFB can be used for chemical production, activated carbon, composites, and bioethanol. Although various pretreatment methods can turn palm oil biomass into valuable feedstock, there are still challenges that need urgent improvement [2].

Key challenges in bioethanol production include efficient pretreatment, cost-effective cellulolytic enzyme production, and effective fermentation of xylose and glucose despite inhibitors [3]. The conversion of lignocellulosic biomass into small compounds like furan

derivatives (e.g., furfural and HMF), organic acids (e.g., acetic acid, formic acid, and levulinic acid), and phenolic compounds is unavoidable with all pretreatment methods, including dilute acid, steam explosion, and alkali [4, 5]. Furfural and HMF, key inhibitors from lignocellulose pretreatment, reduce bioethanol yield and hinder the growth of microbes like *Pichia stipitis* and *Saccharomyces cerevisiae*.

To reduce toxicity during fermentation, efficient methods are needed to remove inhibitors from biomass-pretreated hydrolysates while keeping fermentable sugars like monosaccharides and oligosaccharides [6]. Kuila and Sharma [7] identified several detoxification methods, including physical techniques (like evaporation and membrane treatment), chemical methods (such as neutralization, activated carbon, and ion exchange), and biological methods (enzymatic detoxification using laccase or lignin peroxidase). They also mentioned in-situ and microbial detoxification approaches. However, biological techniques like enzymatic and microbial detoxification provide a good environment for bioethanol fermentation but have low detoxification rates and high costs [8].

Many researchers have studied the potential of activated carbon. Inexpensive activated carbon has unique properties, like a large surface area, high porosity, and specific surface features, making it useful in applications such as adsorption, pollution removal, water treatment, and energy production [9]. Research shows that activated charcoal (AC) can adsorb furfural and HMF, reducing their concentrations. This is due to its porous structure, large surface area (500 to 5000 m^2/g), strong adsorption capacity, and active groups [10]. Sarawan *et al*. [11] found that activated charcoal effectively detoxified acid-pretreated sorghum leaf, removing furfural, HMF, and acetic acid while causing minimal loss of reducing sugars. They also studied how factors like contact time, charcoal concentration, temperature, and pH affect the detoxification process.

Response surface methodology (RSM) is a structured experimental and analytical method used to optimize process conditions by setting specific limits for each factor within the experimental range. Bhattacharya [12] used the central composite design (CCD) within the RSM framework to optimize factors affecting the process. This study evaluates how different variables impact pretreatment and inhibitor removal. The resulting hydrolysate will be used for bioethanol production.

Efficient biomass pretreatment is key to breaking down lignocellulose, improving cellulose, and removing lignin. While traditional methods are costly and harmful to the environment, modern physicochemical methods are cheaper, eco-friendly, and better at separating components, boosting bioconversion efficiency [13]. Enhancing fermentable sugar production from OPEFB pretreatment uses ultrasonication combined with specific chemicals. Studies have used ultrasonication in different ways: Sangadji *et al*. [14] used it alone and Quek *et al*. [15] combined it with deep eutectic solvents (DES). This study is the first to combine ultrasonication with peracetic acid (PAA) to improve OPEFB pretreatment for further processing.

2. MATERIALS AND METHODS

2.1. Preparation of the substrate

OPEFB was collected from the palm oil mill processing facility located in Hatyai, Songkhla. To remove moisture, the bunches were cleaned with regular water and then subjected to a 24-h drying process in an oven at 80 °C. After drying, the OPEFB was milled and sifted to

eliminate any particles larger than 1 mm in size. The processed material was then stored in polyethylene bags at ambient temperature.

2.2. Ultrasound-assisted PAA hydrolysis

The RSM model design was employed to conduct studies on ultrasound-assisted PAA hydrolysis using a 250 mL Erlenmeyer flask containing raw substrate (5 %). Experimental samples were collected at regular intervals and then centrifuged for 10 min at 8000-10000 rpm. After forming pellets, they were thoroughly cleaned and the supernatant was utilised for tests to measure the concentrations of glucose, xylose, arabinose, furfural and HMF. The tests were conducted in duplicate and the collected data were averaged and recorded. The transparent liquid portion (supernatant) was separated and used for subsequent investigations.

2.3. Activated charcoal adsorption

To enhance the sugar levels in the hydrolysates, the substrate with higher sugar content underwent an additional adsorption process using activated charcoal. In the experiments, the collected supernatant was subjected to activated charcoal adsorption to reduce inhibitors in the resulting slurry. Additionally, the adsorption conditions for inhibitors in the resulting liquid component were investigated. For this, in a 250 mL beaker, supernatant was taken and charcoal was added at a ratio of 10:1. After a certain period of time, the supernatant and charcoal were filtered through Whatman quantitative filter paper, Grade-44 to collect the clear portion.

2.4. Vacuum evaporation

To remove approximately 75 % of the initial weight, the untreated, pH-adjusted and fully detoxified hydrolysates were concentrated using evaporation. The Rotary Evaporator Model REV-2000AX system was used for this purpose, which included a vacuum pump and vacuum controller. Either 1M NaOH or HCl solution was used to adjust the pH of the concentrated hydrolysates to 5.5 as needed. Roto-evaporation was applied to the collected clear fraction and the resulting slurry was used to estimate the sugar and inhibitor concentrations.

2.5. Experimental design

To identify the best pretreatment condition RSM was employed. This technique aimed to reduce furfural and HMF levels while maintaining a high sugar yield (Y). Three factors or variables were used to achieve this: pretreatment temperature (X_3) ranging from 40 to 80 ºC, sonication time (X_2) was varying from 30 to 120 min and PAA concentration (X_1) ranges from 5 to 15 %. The experiments were consistently conducted with a solid-to-liquid (S:L) ratio of 1:20. In total, twenty experiments were carried out using the Central Composite Design (CCD), systematically varying the three variables in a predictable manner. Additionally, a central point was evaluated five times to ensure precision and reliability. Design-Expert software [16] was utilized for data analysis.

The quadratic equation (Eq. 1) provides an approximate representation of the correlation between the response variable (Y) and the three variables.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{44}X_4^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{14}X_1X_4 + b_{23}X_2X_3 + b_{24}X_2X_4 + b_{34}X_3X_4 \quad (1)$$

The sugar and inhibitor analyses were conducted based on the experimental designs involving the three variables. The selection range for each variable (minimum and maximum) was utilized in the design, resulting in a total of 20 experiments that were created and performed using the model.

2.6. Experimental analysis

Reducing sugars were identified using the 3, 5-dinitrosalicylic acid (DNS) technique [17]. On UV chromatograms, furfural and HMF were found at 277 and 285 nm, respectively [18]. The quantities of xylose and arabinose were determined following the method described earlier [19]. Gas chromatography was utilised to measure the quantity of ethanol [20]. Statistical analysis was conducted using the GraphPad InStat version 3.1 software. The data were represented using mean values.

2.7. Fermentation of hydrolysates to ethanol

An Erlenmeyer flask with a capacity of 250 mL, containing approximately 10 g/L peptone, 10 g/L yeast extract and 20 g/L glucose, was used to culture *Pichia Stipitis* CBS 6054 yeast in a shaking incubator for 24 h. After the completion of the harvesting process, the cell culture underwent a wash with pure water to eliminate any unwanted residues. The pH was maintained around 6.0 to 7.0 using 1N HCl or NaOH solution. In 250 mL flasks containing washed cells with a dry cell weight of 2.0 g/L, pretreated hydrolysate (10 g/L) was added. To these flasks, 5 g/L urea, 5 g/L yeast extract, 0.5 g/L $MgSO_4.7H_2O$ and 1 g/L KH_2PO_4 were added. The contents were then autoclaved at 120 ºC for 15 min. Fermentation was initiated in an orbital shaker at 30 ºC and 150 rpm and carried out for 72 h. The study aimed to compare the outcomes of two different detoxified hydrolysates (lower and higher ultrasonicated) fermented at a constant temperature with those of non-ultrasonicated hydrolysates.

3. RESULTS AND DISCUSSION

PAA hydrolysis creates inhibitors that disrupt microbial growth, so reducing their impact during optimization is important. The goal of optimization is to maximize sugar production while minimizing inhibitors. Optimization used the desirability function approach to identify the most efficient process parameters. The Box-Behnken design, with three levels and three factors, was used to determine the number of experiments.

Table 1 displays the yields of sugars and inhibitors in untreated OPEFB substrate. The untreated biomass exhibited very low levels of sugars (glucose: 9.10 ± 0.4 g/L, xylose: 3.20 ± 0.3 g/L and arabinose: 0.86 ± 0.2 g/L) and inhibitors (furfural: 0.10 ± 0.1 g/L and HMF: 0.08 ± 0.04 g/L) were detected. This can be attributed to the high crystallinity of the substrate, resulting in low sugar and inhibitor yields. The impact of PAA concentration, sonication time and temperature on glucose, xylose, arabinose, furfural and HMF was investigated and analyzed using RSM. The experimental findings are presented in Tables 2 and 3. For each factor and their interactions, we employed Analysis of Variance (ANOVA) and the $\chi 2$ test, depending on the experimental method. PAA concentration (X_1, %), sonication time (X_2, min) and pretreatment temperature (X_3, ºC) were considered as functions of glucose (Y_1, g/L), xylose (Y_2, g/L), arabinose (Y_3, g/L), furfural (Y_4, g/L) and HMF (Y_5, g/L) based on the regression equation calculated for pretreatment condition optimization. The following quadratic equation was

determined to accurately describe glucose, xylose, arabinose, furfural and HMF using multiple linear regressions on the experimental model data.

$$\text{Glucose (g/L)}: 22.16 + 1.78A_1 + 3.09B_2 + 2.79C_3 - 9.21A_1^2 + 3.6B_2^2 - 2.14C_3^2 + 1.16A_1B_2 + 1.24A_1C_3 + 2.39B_2C_3 \quad (2)$$

$$\text{Xylose (g/L)}: 10.59 + 0.57A_1 + 0.71B_2 + 0.71C_3 - 5.53A_1^2 + 4.27B_2^2 - 7.79C_3^2 + 0.06A_1B_2 + 0.11A_1C_3 + 0.17B_2C_3 \quad (3)$$

$$\text{Arabinose (g/L)}: 3.65 + 0.46A_1 + 0.48B_2 + 0.41C_3 - 1.49A_1^2 + 1.95B_2^2 - 0.97C_3^2 - 0.17A_1B_2 + 0.23A_1C_3 + 0.11B_2C_3 \quad (4)$$

$$\text{Furfural (g/L)}: 1.24 - 1.00A_1 + 0.06B_2 + 0.08C_3 - 0.88A_1^2 + 0.34B_2^2 - 0.31C_3^2 - 0.09A_1B_2 - 0.04A_1C_3 + 0.02B_2C_3 \quad (5)$$

$$\text{HMF (g/L)}: 2.3 + 0.026A_1 + 0.3B_2 + 0.32C_3 - 1.48A_1^2 + 0.19B_2^2 - 8.17C_3^2 - 0.1A_1B_2 - 0.18A_1C_3 + 0.12B_2C_3 \quad (6)$$

Using the above mentioned equations, the expected concentrations of glucose, xylose, arabinose, furfural and HMF in pretreated OPEFB are provided along with test results (Table 3). The model's goodness can be assessed based on many criteria.

Table 1. Sugars yield in untreated OPEFB.

S. No.	Glucose (g/L)	Xylose (g/L)	Arabinose (g/L)	Furfural (g/L)	HMF (g/L)
Untreated OPEFB	9.10 ± 0.4	3.20 ± 0.3	0.86 ± 0.2	0.10 ± 0.1	0.08 ± 0.04

*Values are average of duplicate trials

Table 2. Actual and coded process parameters for CCD model.

Factor	**Name**	**Units**	**Minimum**	**Medium**	**maximum**
A	PAA concentration	%	5	10	15
B	Sonication time	min	30	77.25	120
C	Temperature	ºC	40	60	80
Response	**Name**	**Units**	**Minimum**	**Mean**	**Maximum**
R_1	Glucose	g/L	10.22	18.25	32.19
R_2	Xylose	g/L	4.16	9.779	15.83
R_3	Arabinose	g/L	1.82	3.317	5.92
R_4	Furfural	g/L	0.22	0.808	1.86
R_5	HMF	g/L	0.29	1.648	3.2

* Values are mean of duplicate experiments.

Table 3 shows the experimental results and CCD-based predictions for glucose, xylose, and arabinose yields under different combinations of the three variables. The pretreated biomass yielded a maximum of 32.19 g/L of glucose under the center point conditions of 10 %, 120 min and 60 ºC. Similarly, the highest xylose yield of 15.83 g/L was achieved at the center point conditions of 10 %, 120 min and 60 ºC. Additional center points were tested to calculate experimental error, showing high xylose yields equal to the maximum yield. For arabinose, the greatest yield of 5.92 g/L from the pretreated biomass was obtained at the center point conditions of 10 %, 120 min and 60 ºC. Additional center points were tested to calculate

experimental error, showing moderately high glucose, xylose, and arabinose yields equal to the maximum. Equations (2), (3), and (4) are second-order polynomial equations that describe glucose, xylose, and arabinose yields based on the optimized pretreatment conditions using CCD.

Studies show that ultrasonic treatment improves sugar accessibility. Lee and Ng [21] found that ultrasonic-assisted organosolv pretreatment extracted 41.3 mg of reducing sugars from OPEFB without a NaOH catalyst. Using 100 % sonication power, 50 °C, and 30 % ethanol, this method outperformed organosolv pretreatment with NaOH but without ultrasonication. In another study, sonicated rice straw released 90 % more reducing sugars than untreated rice straw after dilute acid hydrolysis [22].

Table 3. CCD model for experiments.

Std. run	**PAA conc.**	**Time (min)**	**Temp. ºC**	**Glucose (g/L)**	**Xylose (g/L)**	**Arabinose (g/L)**	**Furfural (g/L)**	**HMF (g/L)**
1	5	30	40	10.22 (10.10)	7.44 (7.42)	1.82 (1.78)	0.22 (0.20)	0.29 (0.28)
2	15	30	40	11.63 (11.53)	8.62 (8.54)	2.86 (2.82)	0.32 (0.31)	0.75 (0.73)
3	5	120	40	10.92 (10.85)	8.94 (8.93)	2.96 (2.94)	0.24 (0.22)	0.62 (0.60)
4	15	120	40	12.06 (11.89)	9.12 (9.11)	3.26 (3.24)	0.36 (0.35)	0.97 (0.97)
5	5	30	80	10.98 (10.02)	9.02 (9.01)	2.02 (2.01)	0.39 (0.38)	0.84 (0.83)
6	15	30	80	12.42 (11.23)	9.39 (9.38)	3.92 (3.89)	0.41 (0.41)	0.86 (0.86)
7	5	120	80	16.32 (15.21)	9.95 (9.92)	3.54 (3.52)	0.61 (0.60)	1.93 (1.92)
8	15	120	80	27.34 (26.42)	11.85 (11.79)	4.83 (4.82)	0.46 (0.42)	1.24 (1.23)
9	5	75	60	13.21 (12.16)	4.16 (4.10)	2.02 (2.01)	0.52 (0.51)	0.96 (0.96)
10	15	75	60	16.02 (15.45)	6.23 (6.22)	2.14 (2.12)	0.42 (0.41)	1.08 (1.06)
11	10	30	60	19.92 (18.72)	10.01 (10.00)	2.93 (2.90)	0.89 (0.88)	1.98 (1.97)
12	10	120	60	32.19 (31.28)	15.83 (15.82)	5.92 (5.89)	1.86 (1.84)	3.21 (3.20)
13	10	75	40	18.86 (17.64)	10.21 (10.19)	2.24 (2.21)	0.96 (0.94)	2.01 (2.00)
14	10	75	80	24.51 (23.42)	11.22 (11.20)	2.96 (2.93)	1.12 (1.11)	2.98 (2.97)
15*	10	75	60	21.42 (21.35)	10.6 (10.23)	3.82 (3.78)	1.23 (1.18)	2.21 (2.19)
16*	10	75	60	21.42 (21.35)	10.6 (10.23)	3.82 (3.78)	1.23 (1.18)	2.21 (2.19)
17*	10	75	60	21.42 (21.35)	10.6 (10.23)	3.82 (3.78)	1.23 (1.18)	2.21 (2.19)
18*	10	75	60	21.42 (21.35)	10.6 (10.23)	3.82 (3.78)	1.23 (1.18)	2.21 (2.19)
19*	10	75	60	21.42 (21.35)	10.6 (10.23)	3.82 (3.78)	1.23 (1.18)	2.21 (2.19)
20*	10	75	60	21.42 (21.35)	10.6 (10.23)	3.82 (3.78)	1.23 (1.18)	2.21 (2.19)

*std run: standard run; Values in brackets ( ) represents the predicted values and the values without bracket represents the actual values.

Table 3 shows the actual experimental data and CCD-based predictions for furfural and HMF yields under different process conditions. The highest furfural yield (1.86 g/L) and HMF yield (3.2 g/L) were obtained at center point conditions of 10 % PAA, 120 min sonication, and 60 °C. The optimization using CCD resulted in equations (5) and (6) for predicting furfural and HMF yields. The maximum yields for glucose, xylose, arabinose, furfural, and HMF were 32.19 g/L, 15.83 g/L, 5.92 g/L, 1.86 g/L, and 3.21 g/L, respectively, under these conditions. Higher sugar levels lead to more inhibitors, but the levels of inhibitors were reduced using activated charcoal and evaporation.

Higher sugar yields depend on treatment time, temperature, and catalyst concentration. Zhang et al. [23] found that reducing alkali content from 8 % to 5 % and shortening hydrolysis time from 1.5 h to 30 min can achieve the same degradation rate when using ultrasound. This

improvement is due to ultrasound-induced cavitation, which enhances mass transfer and alkali solubility. Longer sonication times generate more sugar, likely because of improved microjetting and microstreaming. In the present study, the optimal ultrasonication time was 120 min, producing 32.19 g/L of glucose, 15.83 g/L of xylose, 5.92 g/L of arabinose, 1.86 g/L of furfural, and 3.2 g/L of HMF.

Table 4. ANOVA table for response yields.

	Glucose		**Xylose**		**Arabinose**		**Furfural**		**HMF**	
Source	**F-value**	**p-value Prob> F**	**F-value**	**p-value Prob> F**	**F-value**	**p-value Prob> F**	**F-value**	**p-value Prob> F**	**F-value**	**p-value Prob> F**
Model	15.87	< 0.0001	56.24	< 0.0001	17.74	< 0.0001	20.9	< 0.0001	18.6	< 0.0001
A*	6.92	0.0251	16.75	0.0022	18.57	0.0015	4.29	0.9839	0.1	0.753
B*	17.44	0.0019	21.55	0.0009	16.36	0.0023	1.63	0.2305	12	0.0061
C*	16.95	0.0021	25.98	0.0005	14.65	0.0033	3.4	0.095	15.9	0.0025
AB	2.36	0.1553	0.18	0.6795	1.96	0.1922	0.12	0.7355	1.3	0.2807
AC	2.68	0.1328	0.53	0.4819	3.67	0.0843	0.66	0.4365	4.24	0.0666
BC	9.98	0.0102	1.24	0.2906	0.85	0.3782	0.24	0.6372	1.64	0.2296
A^2	43.66	< 0.0001	71.4	< 0.0001	45.09	< 0.0001	77.59	< 0.0001	80.2	< 0.0001
B^2	5.03	0.0488	166.9	< 0.0001	57.89	< 0.0001	8.95	0.0135	0.94	0.3548
C^2	2.36	0.1553	7.37	0.9789	19.14	0.0014	9.45	0.0117	2.43	0.9616
Lack of Fit	34.17		1.64		0.49		0.13		0.6	
Pure Error	1.93		0.3		0.68		0.09		0.04	
-Glucose R^2: 0.9346; Xylose R^2: 0.9806; Arabinose R^2: 0.9411; Furfural R^2: 0.9437; HMF R^2: 0.9495 -Glucose adjusted R^2: 0.8757; Xylose adjusted R^2: 0.9632; Arabinose adjusted R^2: 0.8882; Furfural adjusted R^2: 0.8930; HMF adjusted R^2: 0.9041.										

*Where A-PAA concentration; B-Sonication time; C-Temperature.
*P < 0.05–significant at 5 % level, P < 0.001–significant at 1 % level, P < 0.0001 significant at 0.1 % level, P > 0.05 not significant.

In statistical analysis, the best regression model is determined using parameters like p-value, F-value, predicted R^2, and adjusted R^2 [16]. In this study, the quadratic model had the highest lack-of-fit p-value and predicted R^2, as well as strong adjusted R^2 values, making it the best fit. Key metrics such as F-value, p-value, R^2, and lack-of-fit are essential for evaluating model suitability [24]. The F-test evaluates the significance of mean differences under different operating conditions. The F-values for glucose (15.87), xylose (56.24), arabinose (17.74), furfural (20.9), and HMF (18.6) confirmed the models' reliability. The high F-value of 56.24 for the xylose yield model emphasizes its strength. Additionally, the p-value, related to the F-value, indicates the probability and significance of the regression model [16].

In the ANOVA tables, the model p-values for glucose, xylose, arabinose, furfural, and HMF were very small (< 0.0001), showing that the chance of the large F-values (15.87, 56.24,

17.74, 20.9, and 18.6) being due to noise is less than 0.0001 %. This supports rejecting the null hypothesis, as p-values below 0.05 are considered significant. P-values above 0.1 are not significant and can be ignored. For glucose yield, the terms A, B, C, BC, A^2, B^2, and C^2 had p-values below 0.05, indicating their significant impact. For xylose and arabinose yields, terms A, B, C, A^2, and B^2 were significant. For furfural yield, terms A^2, B^2, and C^2 were significant, while for HMF yield, terms B, C, AC, and A^2 showed significance. Non-significant terms were removed from the models. The lack-of-fit test is another key factor in ANOVA. A good model should have an insignificant lack-of-fit (p-value > 0.10) [16]. All models in this study met this condition, indicating a good fit for the proposed regression models.

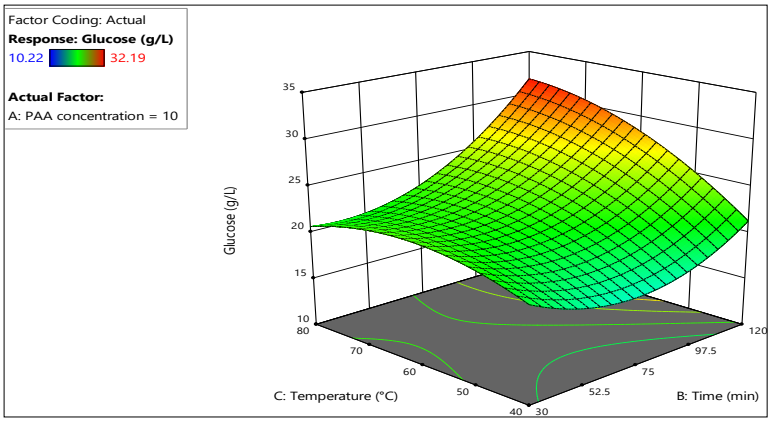

Figure 1. Interaction between temperature and sonication time on glucose yield.

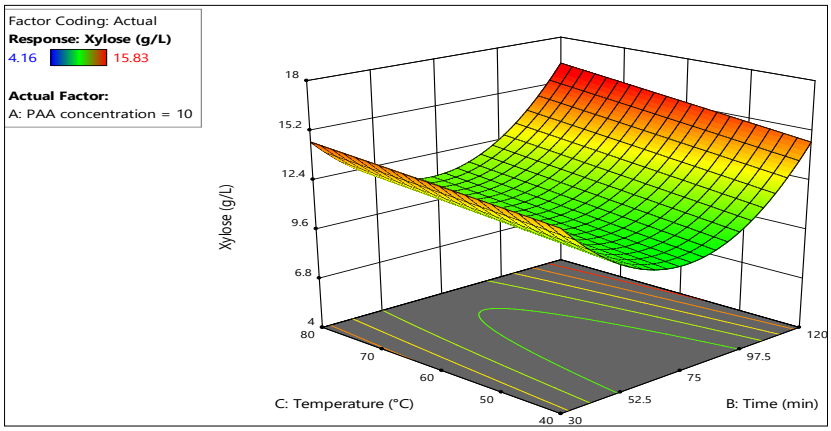

Figure 2. Interaction between temperature and sonication time on xylose yield.

The determination coefficient (R^2) and correlation coefficient (R) are used to assess how well the model performs (Table 4). The R^2 values for glucose, xylose, arabinose, furfural, and HMF are 0.9346, 0.9806, 0.9411, 0.9437, and 0.9495, respectively. These high values show that the model fits well and explains the relationships between the variables. Only 10 % of the variation is unexplained by the independent variables. An R^2 value closer to 1.0 indicates a stronger and more accurate model. A regression model is typically considered to have a very strong correlation if its R^2 value is greater than 0.90 [25]. In this study, the R values for all response variables-glucose (0.8757), xylose (0.9632), arabinose (0.8882), furfural (0.8930), and

HMF (0.9041)-were all higher than 0.80. This indicates a strong correlation between the experimental and theoretical values predicted by the model.

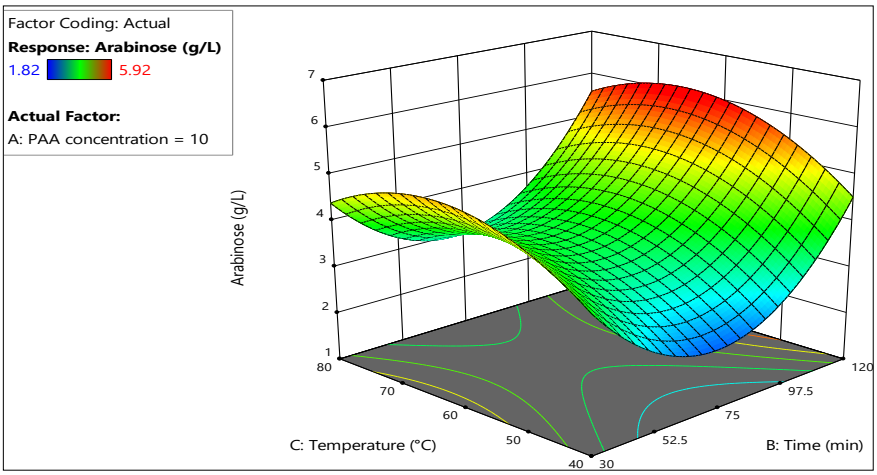

Figure 3. Interaction between temperature and sonication time on arabinose yield.

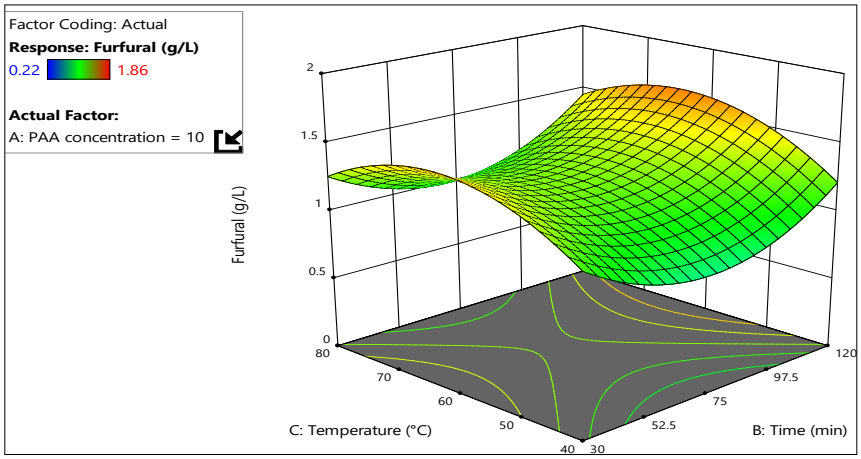

Figure 4. Interaction between temperature and sonication time on furfural yield.

3D response surface graphs show how three process parameters affect the results in pretreated OPEFB. Figure 1 illustrates the effect of temperature and sonication time on glucose yield, with PAA concentration fixed at 10 %. As both temperature and sonication time increase, glucose yield improves, reaching a maximum of 32.19 g/L at 80 min of pretreatment. A decrease in these factors lowers the glucose yield, showing their significant impact. Figure 2 shows that the maximum xylose yield (15.83 g/L) occurs at a process temperature of 60 °C and sonication time of 120 min, with PAA concentration kept at 10 %. Figure 3 shows that maximum arabinose yield (5.92 g/L) is achieved with a 120-min sonication time, 60 °C temperature, and 10 % PAA concentration. Sugar production is more affected by temperature than duration. The combined effects of time and temperature are positive and statistically significant, leading to higher sugar yields. Figure 4 highlights that furfural yield increases with longer sonication time (120 min) and higher temperature (60 °C) with 10 % PAA. Figure 5 shows that HMF yield also increases with

the same conditions. For both furfural and HMF, PAA had less effect than time and temperature, which played a more significant role.

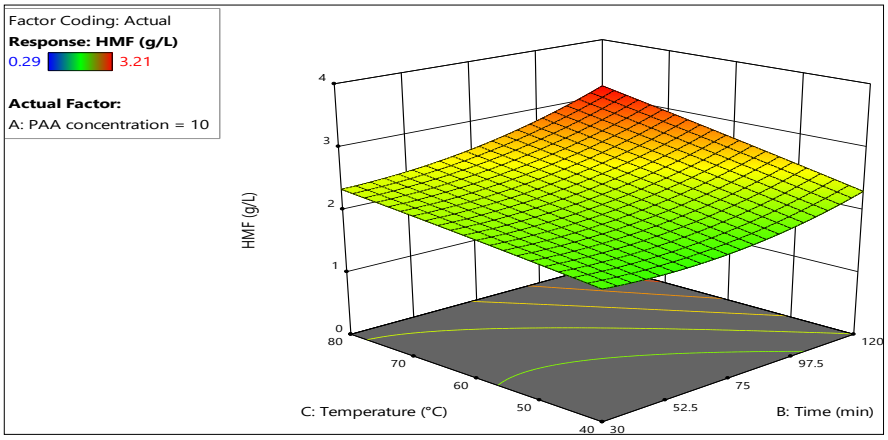

Figure 5. Interaction between temperature and sonication time on HMF yield.

Fang and Yang [26] suggested that detoxification can be improved by increasing the carbon surface area with active functional groups and microporosity through chemical treatment of activated carbon. In this study, using a 10:1 charcoal to supernatant ratio reduced inhibitor yields in the liquid hydrolysate. A higher percentage of activated charcoal was used for detoxification, and the results showed a clear correlation between the amount of activated carbon and the effective removal of contaminants. This suggests that using even more activated carbon could further enhance detoxification. Our findings also support a direct relationship between the amount of activated carbon used and the elimination of pollutants, suggesting that higher carbon loading could further enhance detoxification.

Most trials showed that evaporation was essential for the complete removal of furfural, with HMF also supporting this. Studies [27, 28] indicate that evaporation can almost entirely eliminate both furfural and HMF. Furfural was the dominant furan derivative, while HMF, a breakdown product of hexoses, was present in much smaller amounts. Pentoses, the main monosaccharides, primarily break down into furfural, and they were more efficiently broken down than hexoses, despite the higher concentration of pentoses. This led to significantly higher furfural production compared to HMF. Optimized conditions resulted in fewer inhibitory compounds in the OPEFB hydrolysate.

Table 5. Model validation experiments.

Std. run	PAA Conc.	Sonication time (min)	Temp. ºC	Glucose (g/L)	Xylose (g/L)	Arabinose (g/L)	Furfural (g/L)	HMF (g/L)
1	5	75	60	12.61	3.81	2.21	0.42	0.76
2	10	120	60	31.26	14.89	5.02	1.63	3.12
3	10	75	60	20.22	10.02	3.22	1.03	2.02

*Values are mean of two replicates

Three validation trials were carried out under optimal conditions to assess the accuracy of the design. The results, shown in Table 5, indicated the highest sugar yields at 10% PAA, 120

min of sonication, and 60 °C: glucose (31.26 g/L), xylose (14.89 g/L), and arabinose (5.02 g/L). These findings validated the accuracy and reliability of the model.

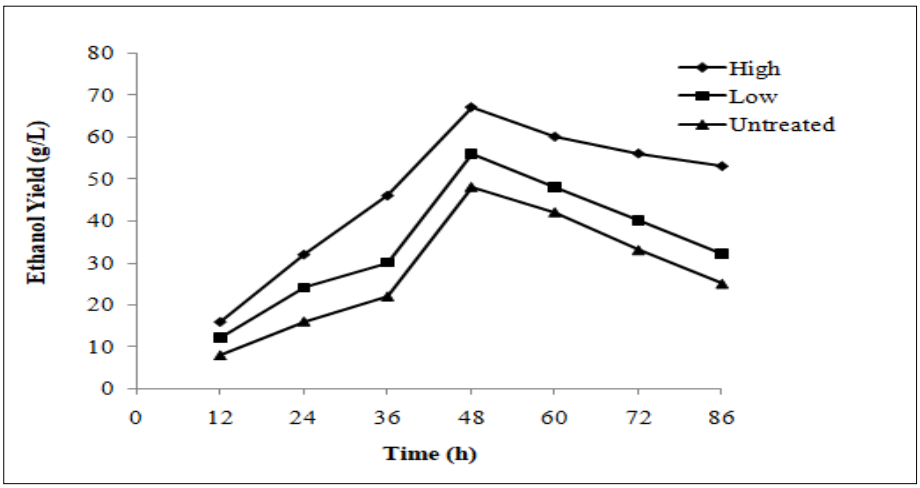

Figure 6. Ethanol yield (g/L) by yeast on different hydrolysates.

Table 6. Comparing ethanol yields between the present study and prior research.

No	Substrate	Pretreatment type	Yeast Strain	Ethanol yield	Reference Study
1	Glucose	Ultrasonication	*Saccharomyces cerevisiae*	30.79 %	[29]
2	Rice straw	Ultrasound-assisted acid	*Saccharomyces cerevisae*	11 g/L	[30]
3	Sugarcane bagasse	Ultrasound-assisted acid	*Saccharomyces cerevisae*	8.11 g/L	[31]
4	Pretreated OPEFB	Ultrasound-assisted Peracetic acid	*Pichia Stipitis* CBS 6054	67.5 g/L	Present study

Sonication during fermentation notably enhanced both fermentation performance and ethanol production. Two types of hydrolysates were tested: one with high detoxification and the other with low detoxification (Figure 6). The high-detoxification hydrolysate, with higher sugar content, produced the highest ethanol yield (67.5 g/L), followed by the low-detoxification ultrasonicated hydrolysate (56.7 g/L). In comparison, the non-ultrasonicated hydrolysate yielded the lowest ethanol (53.1 g/L). Belal [30] found that using acid pretreatment combined with ultrasound, followed by enzymatic saccharification, resulted in the highest ethanol concentration of 11 g/L after seven days of fermentation with *S. cerevisiae*. The study highlighted the positive effect of ultrasonication on rice straw pretreatment, which greatly improved fermentation efficiency. Table 6 shows ethanol yields from different substrates using ultrasonication and its combination with various chemicals. The results of this study were compared with findings from other research.

The findings show that detoxification techniques enhanced fermentation performance, while fermentation of undetoxified hydrolysate resulted in lower ethanol production. Detoxifying OPEFB and using activated carbon significantly improved fermentation, leading to

higher ethanol yields. Only the hydrolysate detoxified with 10 % PAA pretreatment, 120 min sonication, and 60 °C temperature produced ethanol.

4. CONCLUSIONS

The study demonstrates that RSM is a practical approach for adjusting pretreatment conditions to augment fermentable sugar production while reducing the presence of inhibitory compounds in the final pretreated OPEFB. A 10 % PAA concentration, a 120-min sonication period and a pretreatment temperature of 60 °C were identified to be the ideal pretreatment conditions for achieving the largest sugar yield and the lowest inhibitory chemical yield. To further minimize the inhibitory compounds in the obtained substrate and increase fermentation efficiency, activated charcoal adsorption will be applied. Rotational evaporation will be employed in addition to other methods to concentrate the material and further lessen the inhibitory substances. Studies will be done on yeast's ability to ferment these concentrated samples.

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Conflict of interest. The authors state that they have no conflicts of interest.

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