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Ethanol production from oil palm empty fruit bunch fiber prepared through pretreatment with sodium hydroxide under optimized conditions

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Abstract

Alkali pretreatment of oil palm empty fruit bunch (OPEFB) using sodium hydroxide (NaOH) was optimized by varying substrate concentration, alkali loading, temperature, and time. A Central Composite Design (CCD) based on the Box–Wilson factorial design was applied to determine conditions that maximized cellulose and hemicellulose and minimized lignin to enhance ethanol production. Under optimized conditions, cellulose reached 65.2%, while lignin and hemicellulose were reduced to 9.4% and 11.8%, respectively. Pretreated OPEFB released high amounts of fermentable sugars, with glucose, xylose and arabinose yields of 43.4 ± 0.2, 28.1 ± 0.1 and 10.2 ± 0.3 mg/g, respectively, representing a clear increase compared with low-alkali conditions. Fermentation of the optimized biomass produced a maximum ethanol concentration of 60.8 g/L.

Introduction

Oil Palm Empty Fruit Bunch (OPEFB) fiber is an essential agricultural lignocellulosic raw resource for the generation of commercially viable and environmentally beneficial composite products (Sunday Noah 2022). Converting lignocellulosic materials into ethanol is difficult because their complex structure and composition make processing challenging (Shrivastava and Sharma, 2023). The OPEFB has an average cellulosic and hemicellulose contribution of 30–50% and 15–35%, respectively, whereas lignin content of 20–30% according to Mardawati *et al.* (2014).
Accessibility to OPEFB's carbohydrate polymers is complicated by its recalcitrance. Enzymatic hydrolysis of lignocellulose is restricted by lignin complexity, cellulose crystallinity, degree of polymerization, acetyl groups in hemicellulose, and biomass particle size and surface area (Zhao *et al.* 2012). Recent studies have highlighted that lignocellulosic recalcitrance is largely governed by lignin distribution, cellulose crystallinity, and lignin–carbohydrate complex linkages, which collectively restrict enzymatic accessibility and sugar release (Kang *et al.* 2025; Yuan *et al.* 2021). Pretreatment is needed to break the tough structure of OPEFB and make the sugar polymers more accessible. This helps in converting cellulose and hemicellulose into simple sugars using enzymes,

which can then be used to produce useful products (Zulkiple *et al.* 2016).

Pretreatment removes the lignin–carbohydrate matrix and increases cellulose accessibility to cellulolytic enzymes, thereby improving hydrolysis. Typically, this refers to a process that uses both heat and a catalyst (an acid or basic) (Fan *et al.* 2025). Alkali pretreatment has gained the most attention as it uses alkaline solutions to remove lignin and uronic acid groups from hemicellulose. This makes it easier for enzymes to access and break down hemicellulose and cellulose (Huang *et al.* 2022). Recent studies show that alkaline pretreatment can solubilize lignin effectively while producing fewer inhibitors than dilute acid or physicochemical methods, which improves fermentation efficiency (Kululo *et al.* 2025).

The effectiveness of alkaline pretreatment depends on the substrate's structure, chemical composition, and treatment conditions. Even though it has been tested on several lignocellulosic materials such as sugarcane bagasse, African oil palm fiber, coffee pulp waste, and Kikuyo grass, most studies mainly aim to optimize the process to improve digestibility (Fuertez-Cordoba *et al.* 2021; Chen *et al.* 2013). Hemicellulose and lignin surrounding cellulose fibers limit the conversion of cellulose into fermentable sugars. Lignin can be eliminated using alkali treatment, which encourages hydrolysis and increases the recovery of glucose from cellulose. The alkali base most frequently used is Sodium hydroxide (NaOH) (Shimizua *et al.* 2018). Alkali pretreatment is advantageous because it operates at lower temperatures and pressures than many other delignification methods. Vinayak Laxman *et al.* (2020) hypothesized that alkaline hydrolysis results from the saponification within ester linkages, which crosslink lignin and other hemicelluloses with xylan hemicelluloses. According to Tsabitah *et al.* (2014), alkaline and diluted acid solutions are often employed for OPEFB pretreatment. Recent biorefinery studies report that alkaline pretreatment improves enzymatic digestibility and glucose recovery and supports sustainable biomass valorization (Sharma *et al.* 2026; Pandey *et al.* 2025).

Efficient experimental designs are needed to study interactions among process variables. Bioprocesses have been effectively modified with Response Surface Methodology (RSM), a mix of statistical tools for creating models, planning experiments, evaluating the impacts of variables and finding the ideal conditions (Sai Datri *et al.* 2023). The pretreatment conditions were effectively improved using the RSM (Rama Mohan, 2024). RSM combined with Central Composite Design (CCD) is widely used for biomass pretreatment optimization because it evaluates variable interactions efficiently while reducing the number of experiments (Sarrai *et al.* 2016; Tsegay *et al.* 2024). This experiment aimed to use RSM, particularly CCD, to identify the optimal pretreatment conditions for enhancing glucose yield from cellulose and reducing lignin in alkali-pretreated OPEFB, leading to ethanol production.

Experimental

OPEFB substrate

OPEFB was collected from a local palm oil processing mill in Songkhla Province, Thailand, in August. The collected OPEFB was then continually cleaned with purified water to eliminate any debris, dust, or other unwanted contaminants. Gathered OPEFB was dried in the oven for 6 to 8 h at 70 to 80 ºC and then ground into a powder for usage.

Design of experiments and statistical analysis

The proposed study employs a factorial CCD (Design-Expert 8.0.6.1, 2016) with replicates at the center points for four factors/variables. Substrate concentration (X_1, % w/v), alkali concentration (X_2, % v/v), pretreatment temperature (X_3, °C), and time (X_4, min) were the pretreatment process variables, with each having low (−1) and high (+1) coded values. Cellulose (Y_1, %), hemicellulose (Y_2, %), and lignin (Y_3, %) yield in OPEFB were the dependent response variables (Y) that were measured. The examined selected factors and their levels in this study are provided in Table 1. The pretreatment conditions were varied using a 2^4-factorial CCD with six replications at the center points (n_0=6), resulting in 30 trials (Table 2). Statistical software (Design-Expert) was employed to generate the second-degree polynomials in

Equation 1 to approximate the dependent variable responses. Using the polynomial function, the variance for each evaluated factor was separated into linear, quadratic, and interaction components (Eq. 1).

$$Y = b_0 + \sum_{i=1}^{k} b_i X_i + \sum_{i=1}^{k} b_{ii} X_{ii} + \sum_{\substack{i=1 \\ i<j}}^{k-1} b_{ij} X_i X_j$$

In the equation above, Y is the expected response, while X are independent variables (substrate, NaOH, temperature and time), and b are regression coefficients (bi—the linear coefficients, bii—the quadratic coefficients, bij—the interaction coefficients). With a probability (P) of 0.05, 0.01, 0.001 the F-value was determined to statistically evaluate the importance of each term in the algebraic expression. The contour maps were built using simple regression and standardized coefficients. The 3-dimensional (3D) graphs were created by changing the other factors within the test range while keeping one variable stable at the center point. Using the Design-Expert numerical optimization tool, the ideal values of four independent parameters for maximal activity were found. Statistical significance of sugar yields and compositional data was evaluated using analysis of variance (ANOVA) at $p<0.05$, and mean comparisons were performed using Tukey's test.

Yeast strain and inoculum preparation

MGYP (malt extract-3, peptone-5, yeast extract-5, glucose-10 and agar-24 g/L) medium slants were utilized to inoculate and maintain a high ethanol-producing yeast strain, such as *Saccharomyces cerevisiae* TBRC13316. The yeast was transferred to MGYP medium present in a conical flask (250 mL) with 100 mL of a basal medium that contained glucose (carbon source) (10 g/L) to generate the inoculum for the fermentation studies. For 12 h, the yeast was cultivated at 35ºC while being shaken in an orbital incubator. The final yeast inoculum concentration for fermentation studies was roughly 1.5×10^8 cells/mL and 10% inoculum was added.

Fermentation of OPEFB substrate

The submerged fermentation (SMF) method for ethanol production utilized a medium with the following (% w/v) composition: yeast extract 0.25, $MgSO_4$ 0.05, 0.1 KH_2PO_4 0.1, $(NH_4)_2SO_4$ 0.25 and pH 5.0. A 250 mL conical flask containing the medium components and saccharified pretreated OPEFB (low and high treated) at a concentration of 1% was sterilized for 15 min at 121ºC. After sterilization, the medium was allowed to cool to room temperature. 10 mL of yeast strain inoculum were then mixed with the sterile medium, and the resulting solution was fermented at 30ºC for 72 h. Fermentation was carried out under oxygen-limited (anaerobic) conditions using static incubation without aeration, and dissolved oxygen was not actively supplied to maintain anaerobic metabolism. Following the fermentation process, ethanol concentration was measured using samples collected at regular intervals, with duplicate samples analyzed for accuracy.

Compositional and ethanol analysis

The components of both untreated and treated OPEFB, including cellulose, hemicellulose, and lignin, were estimated according to the AOAC (2005) standards. Lignin determination was performed as a quantitative gravimetric measurement following standard AOAC fiber analysis procedures, corresponding to acid-insoluble (Klason-type) lignin determination. The reducing sugar concentration and glucose levels were assessed using the 3,5-dinitrosalicylic acid (DNS) technique (Miller 1959). The percentage of xylose and arabinose was quantified using the method developed by Khabarov *et al*. (2006). Calibration curves were prepared using standard solutions for each sugar analyte to ensure quantitative accuracy. Ethanol quantification was performed using an Agilent 6890 system coupled with a flame ionization detector (FID) (Anthony 1984).

Results

Optimization

It is essential to pretreat agricultural feedstocks before enzymatic hydrolysis to achieve optimal results. This investigation found that the lignin, cellulose, and hemicellulose contents of the treated OPEFB biomass are approximately as shown in Table 1.

Table 1. Actual and coded levels of the factors in CCD.

Factor	Name	Low actual	Middle actual	High actual	Low coded	Middle coded	High coded
X_1	Substrate (% w/v)	5	12.5	20	-1	0	1
X_2	NaOH (% v/v)	5	12.5	20	-1	0	1
X_3	Temperature (°C)	60	90	120	-1	0	1
X_4	Time (min)	30	75	120	-1	0	1
Response	Name	Units	obs[a]	Min.	Max.	Mean	Std. Dev
Y_1	Cellulose	%	30	26.1	65.2	40.99	9.78
Y_2	Hemicellulose	%	30	11.8	20.2	17.28	2.16
Y_3	Lignin	%	30	9.4	19.4	16.47	2.06

[a]Observed run values

The most critical phases in the development of an effective, profitable bioprocess include process condition optimization (Rathore *et al.* 2021). Utilising four variables-substrate concentration (5-20% w/v), alkali concentration (5-20% v/v), temperature (60-120°C) and time (30-120 min), the pretreatment design was examined and optimized for the components of the resulting OPEFB. Table 1 shows the selection ranges (minimum and maximum) for each variable and Table 2 includes test results along with experimentally estimated quantities of cellulose, hemicellulose and lignin yield in the resulting processed OPEFB under the above-mentioned conditions.

Table 2 CCD experiments.

Run no.	Substrate (% w/v) X_1	NaOH (% v/v) X_2	Temp. (°C) X_3	Time (min) X_4	Cellulose (%) Y_1	Hemicellulose (%) Y_2	Lignin (%) Y_3
1	5	5	60	30	26.1 (27.2)	19.2(18.3)	18.6(18.3)
2	20	5	60	30	23.6(29.4)	19.8(18.9)	18.2(16.6)
3	5	20	60	30	29.4(30.8)	18.2(18.4)	17.3(17.8)
4	20	20	60	30	26.4(29.5)	20.2(19.2)	18.3(17.6)
5	5	5	120	30	37.6(37.7)	17.6(18.0)	17.9(17.8)
6	20	5	120	30	36.9(37.0)	19.6(19.1)	18.2(19.0)
7	5	20	120	30	41.8(44.6)	16.4(16.3)	15.2(14.9)
8	20	20	120	30	37.9(40.4)	17.8(17.7)	18.1(17.5)
9	5	5	60	120	36.2(36.7)	18.6(18.2)	18.8(18.3)
10	20	5	60	120	34.2(34.5)	19.2(18.7)	17.6(17.8)
11	5	20	60	120	42.4(45.4)	17.6(17.5)	17.8(16.9)
12	20	20	60	120	36.8(39.7)	19.1(18.3)	18.9(17.9)
13	5	5	120	120	50.4(50.4)	14.2(14.6)	13.2(13.8)
14	20	5	120	120	43.7(45.4)	16.3(15.6)	17.8(16.2)
15*	5	20	120	120	**65.2(62.4)**	**11.8(12.3)**	**9.4(9.9)**
16	20	20	120	120	51.8(53.8)	13.3(13.5)	13.6(13.8)
17	20	12.5	90	75	39.2(39.1)	18.6(17.9)	18.2(17.7)
18	20	12.5	90	75	38.9(32.7)	18.2(19.7)	18.4(19.9)
19	12.5	-2.5	90	75	45.6(43.8)	13.9(14.8)	13.2(13.7)
20	12.5	27.5	90	75	60.4(55.9)	12.8(12.7)	10.4(10.9)
21	12.5	12.5	30	75	24.6(18.6)	19.2(20.8)	19.4(20.9)
22	12.5	12.5	150	75	43.8(43.4)	16.4(15.7)	16.8(16.3)
23	12.5	12.5	90	15	38.6(33.1)	19.2(20.0)	17.8(18.2)
24	12.5	12.5	90	165	56.8(56.0)	15.7(15.7)	13.8(14.4)
25[a]	12.5	12.5	90	75	43.6(43.6)	17.6(17.6)	16.2(16.2)
26[a]	12.5	12.5	90	75	43.6(43.6)	17.6(17.6)	16.2(16.2)
27[a]	12.5	12.5	90	75	43.6(43.6)	17.6(17.6)	16.2(16.2)
28[a]	12.5	12.5	90	75	43.6(43.6)	17.6(17.6)	16.2(16.2)
29[a]	12.5	12.5	90	75	43.6(43.6)	17.6(17.6)	16.2(16.2)
30[a]	12.5	12.5	90	75	43.6(43.6)	17.6(17.6)	16.2(16.2)

Std: Standard run order. Value in ( ) shows predicted values. *: Optimal experimental run is indicated by an asterisk

Compared to untreated, the alkali-processed OPEFB has a cellulose content that is 65.2% higher and contains 9.9% less lignin. When comparing the high alkali-pretreated OPEFB to the low-concentration alkali-treated biomass, the hemicellulose percentage was noticeably lower, falling from 19.8% to 11.8%. Additionally, the OPEFB processed with alkali demonstrated that the pretreatment was efficient in revealing cellulose and hemicellulose while removing lignin (Table 2). In run No. 15, where the alkali concentration is 20% and the OPEFB concentration is low (5%), the sample with the highest cellulose and lowest lignin content was observed. At the 15th run, the expected yields of cellulose, hemicellulose, and lignin were 62.4%, 11.8%, and 9.9%, respectively. The generation of more cellulose and the retention of some hemicellulose were affected by the lengthy pretreatment period (120 min) and higher temperature (120ºC). A higher alkali concentration can successfully dissolve lignin. The largest amount of cellulose was obtained during this experimental run; hence, it was taken into consideration.

In run number 2, we observed considerably low levels of cellulose (23.6%) and a high lignin percentage (18.2%). In this experimental run, the hemicellulose content (19.8%) was higher compared to run number 15. The presence of a high substrate concentration (20%) could not be effectively hydrolyzed with a low alkali percentage (5%). Additionally, the process conditions such as a shorter pretreatment duration (30 min) and a low temperature (60 °C) may not have effectively hydrolyzed the OPEFB substrate. As a result, we noted a higher lignin percentage in the pretreated substrate. The predicted levels of cellulose, hemicellulose, and lignin in the second experimental run were 29.4%, 19.8%, and 16.6%, respectively, as shown in Table 3. Alkali treatment increased the removal of lignin from the OPEFB.

A total of 30 experimental runs were conducted using various combinations of the four factors for the RSM based on CCD. Table 2 lists these experimental runs along with the expected outcomes, as indicated by the regression equations (Eqs. 2, 3 and 4). The experimental design was analyzed using analysis of variance (ANOVA) and the F-test to estimate the effects and interactions of each component (Table 3). By performing multiple regression analysis on the trial data, a second-order polynomial equation was generated to accurately represent the yields of cellulose, hemicellulose and lignin.

Table 3. ANOVA of the CCD.

		F-Value			P-value		
Source	df	Y_1	Y_2	Y_3	Y_1	Y_2	Y_3
Model	14	12.34	10.85	10.97	< 0.0001	< 0.0001	< 0.0001
X_1	1	4.16	6.06	6.56	0.0595	0.0265	0.0217
X_2	1	14.86	7.71	11.79	0.0016	0.0141	0.0037
X_3	1	62.24	47.42	29.36	< 0.0001	< 0.0001	< 0.0001
X_4	1	53.21	33.67	20.3	< 0.0001	< 0.0001	0.0004
X_1X_2	1	0.83	0.093	2.06	0.3771	0.7652	0.172
X_1X_3	1	0.57	0.4	7.82	0.4624	0.5344	0.0136
X_1X_4	1	1.31	6.88	1.42	0.2704	0.935	0.2521
X_2X_3	1	0.74	3.43	5.79	0.4042	0.0837	0.0294
X_2X_4	1	1.76	0.64	0.81	0.2045	0.4351	0.3826
X_3X_4	1	0.69	12.72	15.32	0.4183	0.0028	0.0014
X_1^2	1	6.86	3.3	11.28	0.0193	0.0494	0.0043
X_2^2	1	4.54	30.22	24.18	0.05	< 0.0001	0.0002
X_3^2	1	18.24	0.9	9.63	0.0007	0.0585	0.0073
X_4^2	1	0.11	0.19	0.031	0.7487	0.6659	0.8634
Residual	15						
Lack of fit	10						
Pure error	5						
Cor total	29						

*In the above table X_1= substrate, X_2=NaOH, X_3=Temperature, X_4=Time, Y_1=Cellulose, Y_2=Hemicellulose, Y_3=Lignin; *$P<0.05$–significant at 5% level, $^{\alpha}P<0.001$–significant at 1% level, $^{\beta}P<0.0001$ significant at 0.1% level, $^{\#}$not significant

Table 3 presents a statistical analysis of the individual effects of four factors on the model outcome. P-values for time and temperature below 0.05 ($P<0.05$) indicate a significant impact on the conversion of cellulose and hemicelluloses during hydrolysis and throughout the entire process, as shown in Table 3.

P-values for time and temperature below 0.05 ($P<0.05$) indicate a significant impact on the conversion of cellulose and hemicelluloses during hydrolysis and throughout the entire process, as shown in Table 3. Among the factors, temperature emerged as the most influential linear component and significantly interacted with time to affect the outcomes. The p-values for all four parameters were reported to be below 0.05 ($P<0.05$), highlighting the important linear effects of these factors on the yields of cellulose, hemicellulose, and lignin. Furthermore, the analysis of quadratic relationships between the variable components and response yields is demonstrated in Table 3, where the p-values for substrate concentration, alkali percentage, and temperature are also below 0.05 ($P<0.05$). The research findings indicate that these trial factors significantly affect the yields of cellulose, hemicellulose, and lignin, suggesting that the interaction among these factors can be represented as a parabolic relationship. The interaction effects between the factors, particularly the combination of temperature with time and alkali loading, significantly affect the yields of cellulose, hemicellulose, and lignin, as indicated by the P-values below 0.05 ($P<0.05$). The importance of the present model is further underscored by the F-values identified for cellulose (12.34), hemicelluloses (10.85), and lignin (10.97). Such high F-values are unlikely to occur by chance, as they could happen in only 0.01% of cases (Design Expert 2018).

The precision of the model can be assessed using the coefficient of determination (R^2). The R^2 values were 0.9120 for cellulose, 0.9101 for hemicelluloses, and 0.9110 for lignin yield. This indicates that the model can explain over 90% of the variability in the response, leaving only about 10% of the variation unexplained by the independent variables. R^2 values typically range from 0 to 1, with higher values indicating more stable models and better predictive accuracy.

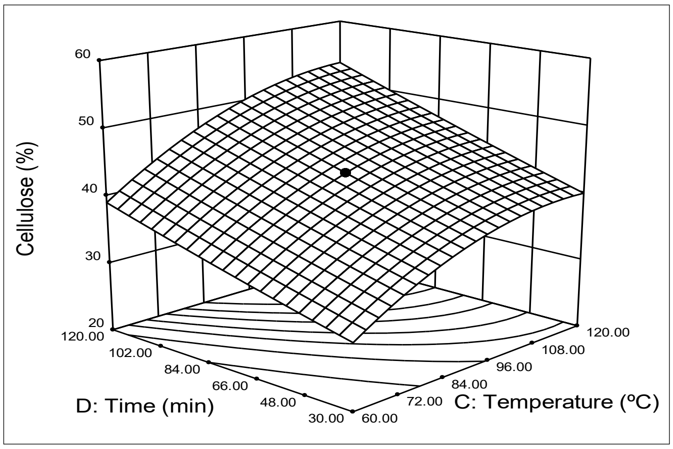

Figure 1: Response surface plot of pretreatment time and temperature on cellulose yield (%) of NaOH-pretreated OPEFB fibre.

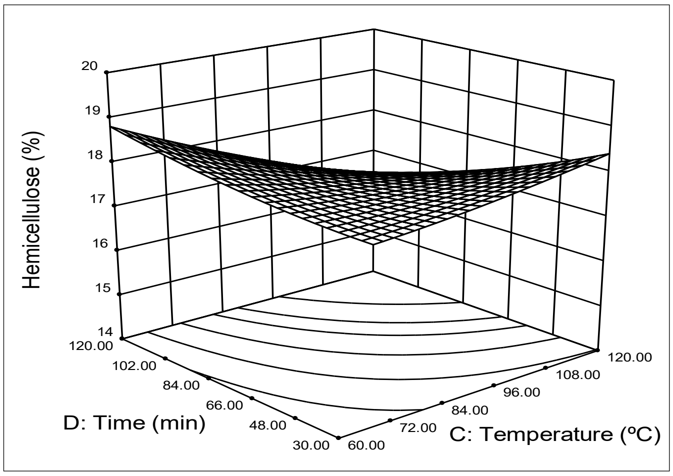

Figure 2: Response surface plot of pretreatment time and temperature on hemicellulose yield (%) of NaOH-pretreated OPEFB fibre.

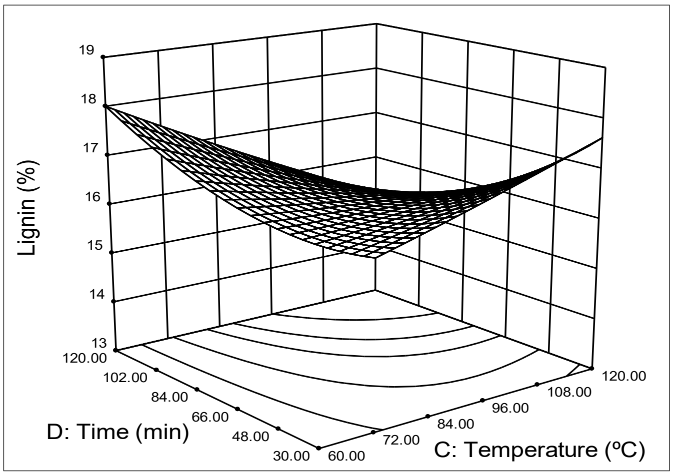

Figure 3: Response surface plot of pretreatment time and temperature on lignin content (%) of NaOH-pretreated OPEFB fibre.

$$\text{Cellulose (\%) } Y_1 = 43.6 - 1.6X_1 + 3.02X_2 + 6.19X_3 + 5.72X_4 - 1.92X_1^2 + 1.56X_2^2 - 3.14X_3^2 + 0.4X_4^2 - 0.87X_1X_2 - 0.73X_1X_3 - 1.1X_1X_4 + 0.83X_2X_3 + 1.27X_2X_4 + 0.80X_3X_4 \quad (2)$$

$$\text{Hemicellulose(\%) } Y_2 = 17.6 + 0.45X_1 - 0.51X_2 - 1.27X_3 - 1.07X_4 + 0.31X_1^2 - 0.95X_2^2 + 0.16X_3^2 - 0.07X_4^2 + 0.06X_1X_2 + 0.14X_1X_3 - 0.01X_1X_4 - 0.42X_2X_3 - 0.18X_2X_4 - 0.81X_3X_4 \quad (3)$$

$$\text{Lignin(\%) } Y_3 = 16.2 + 0.54X_1 - 0.72X_2 - 1.14X_3 - 0.95X_4 + 0.66X_1^2 - 0.97X_2^2 + 0.61X_3^2 + 0.03X_4^2 + 0.37X_1X_2 + 0.72X_1X_3 + 0.31X_1X_4 - 0.62X_2X_3 - 0.23X_2X_4 - 1.01X_3X_4 \quad (4)$$

Table 4. Model validation experiments

No.	X_1	X_2	X_3	X_4	Cellulose (%)	Error	Hemicellulose (%)	Error	Lignin (%)	Error
1	5	20	120	120	65.0(65.4)	0.4	11.2(11.7)	0.5	9.2(9.4)	0.2

*X_1, X_2, X_3, X_4 were OPEFB concentration (%), alkali percentage (%), temperature (ºC) and duration (min)

According to Haaland (1989), a regression model is considered to have a strong correlation if its R^2 value exceeds 0.90. The adjusted R^2 value further refines the R^2 by accounting for the sample size and the number of terms in the model. The current model demonstrates high relevance, indicated by an adjusted determination coefficient of 0.95.

In cases where the model includes multiple factors and the sample size is small, the adjusted R^2 may be significantly lower than the R^2 (Montgomery 2009). For all three response variables such as cellulose, hemicellulose, and lignin the adjusted R^2 values were above 0.80, suggesting a strong agreement between the actual results and the predictions generated by the model.

For the cellulose, hemicellulose, and lignin yields, three-dimensional response surface plots are presented in Fig. 1 - 3. Each graph illustrates the impact of modifying two factors while holding the third factor at zero levels on the response yield. The association between temperature and time on total cellulose yield is shown in Fig. 1. According to Table 3, there is no significant interaction ($P>0.05$) between these two factors. The response surface graphs demonstrate that the greater yield production is influenced by these two factors and the alkali loading.

Fig. 2 illustrates the combined effect of process time and temperature on hemicellulose yield. The relationship between these two variables is significant ($P<0.05$), as seen in Table 3. Both temperature and pretreatment duration increase the hemicellulose yield.

Fig. 3 represents the interactive impact of process time and temperature on overall lignin yield. The removal of lignin is significantly affected by these two variables, as demonstrated in Table 3 ($P<0.05$). Both temperature and pretreatment duration increase the removal of lignin. At higher temperatures, lignin becomes soluble due to increased alkali concentrations.

From the experimental and ANOVA analysis, the most influential factors identified are temperature, time, and alkali loading. Based on these process variables, the response plots were selected.

Validation experiment

Experiments were conducted under the recommended optimal conditions to confirm the results of the optimization. The present method validation (Table 4) is presented. The optimum response yields for cellulose, hemicelluloses, and lignin were 65.0%, 11.2%, and 9.2%, respectively, at an OPEFB concentration of 5%, an alkali percentage of 20%, a temperature of 120ºC, and a duration of 120 min after applying the desirability function. The regression model was suitable and precise in predicting the outcome (Y), as demonstrated by the errors for three responses shown in Table 4, which ranged between 0.2 and 0.5 between the actual experimental and predicted responses.

Sugar yields

Table 5 compares the yield of sugars in pretreated OPEFB with greater alkali concentration (20%) and lower alkali concentration (2%). After 120 min at 120ºC, lignin was removed from OPEFB, increasing cellulose surface availability and raising sugar yields. Pretreated substrates with reduced lignin can effectively saccharify the polysaccharides. With more hemicellulose and cellulose present, the hydrolysate's available aggregate reduced sugar content (50.3 ± 0.8 mg/g) will increase.

Table 5. Sugar yield (mg/g) in pretreated OPEFB.

Substrate	Reducing sugars (mg/g)	Glucose (mg/g)	Xylose (mg/g)	Arabinose (mg/g)
High pretreated	50.3 ± 0.8	43.4 ± 0.2	28.1±0.1	10.2 ± 0.3
Low pretreated	22.5 ± 0.4	16.3 ± 0.1	12.2 ± 0.2	4.8 ± 0.1

Percentage comprised of values determined on a dry-weight basis; data is presented as the mean ± SEM, with n=3.

Pretreated OPEFB with higher alkali loading yielded larger amounts of glucose (43.4 ± 0.2 mg/g), xylose (28.1 ± 0.1 mg/g), and arabinose (10.2 ± 0.3 mg/g) than the substrate treated with lower alkali loading.

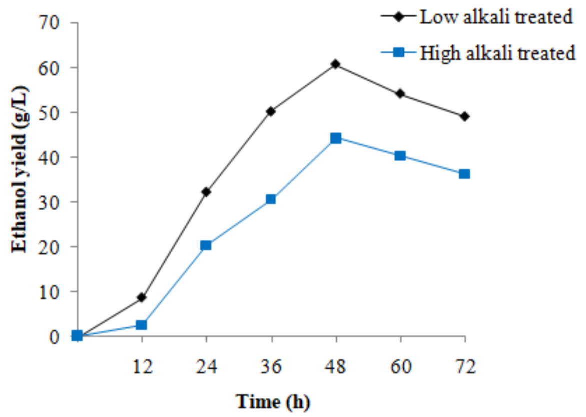

Figure 4: Ethanol yield of *Saccharomyces cerevisiae* TBRC13316.

Fermentation of OPEFB substrate to ethanol

The yeast gradually consumed the sugar during the synthesis of bioethanol, as shown in Fig. 4. The bioethanol yield using OPEFB substrate increased after the first 12 h and remained stable after 48 h. To produce ethanol, fermentation flasks were incubated for a total of 96 h.

After 48 h of analysis, the samples with the highest concentration of ethanol were identified, but a progressive decline in ethanol yield was observed beyond this time. With a substrate loading of 1% w/v, a pH of 5.0, a yeast concentration of 1% v/v, and a fermentation temperature of 37.5ºC, the bioethanol output reached 60.8 g/L at 48 h, compared to 44.3 g/L from lower-treated samples. More ethanol was produced from the OPEFB with higher glucose levels.

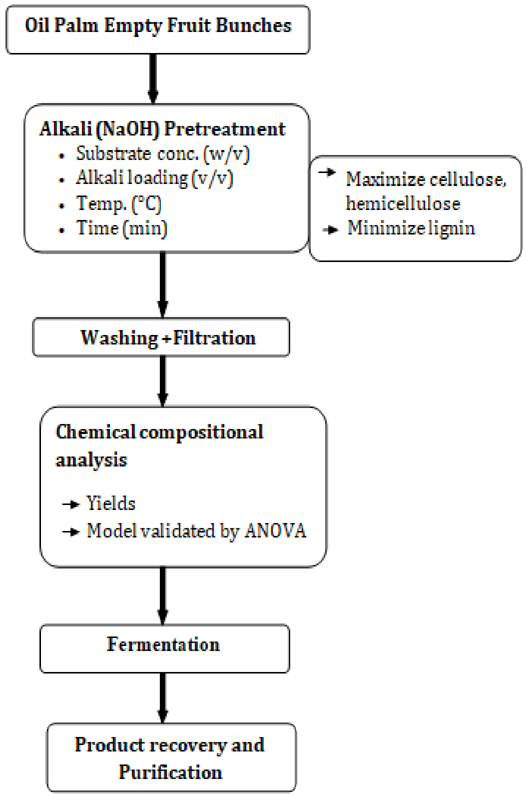

Figure 5: Process flow chart of conversion of OPEFB into ethanol by alkali (NaOH) pretreatment.

The entire process flow is shown in Fig. 5. OPEFB undergo alkali pretreatment to remove lignin and increase sugar release. The resulting sugars are fermented to produce ethanol, which is then purified by distillation.

Discussion

Alkali treatment helps to break bonds within the lignin polymer, disrupting its structure and separating it into crystalline and amorphous regions. This process also removes hemicelluloses from the biomass (Kamdem Tamo *et al*. 2025). As a result, cellulose becomes more accessible to enzymes, facilitating its conversion into glucose. During hydrolysis, complex carbohydrates such as hemicellulose and cellulose are converted into simple sugars. Hemicellulose mainly yields pentose and hexose sugars, while cellulose is primarily hydrolyzed into glucose. Hydrolysis can be carried out using either chemical or enzymatic methods (Anggorowati *et al*. 2021). Thermal pretreatment of oil palm empty fruit bunch (OPEFB) resulted in up to 58% lignin degradation at optimal conditions (1 mm particle size, 210°C, 120 min). Lignin removal improved with decreasing particle size and increasing temperature and time, enhancing suitability for downstream enzymatic processing (Mohammad *et al*. 2020). In the present study, the combined effects of alkali loading, temperature, and time were more influential than temperature alone, confirming that process severity governs biomass fractionation. These findings confirm that alkali pretreatment effectively removed portions of lignin and hemicellulose, thereby enhancing bioethanol production efficiency.

Recent studies have shown that optimized NaOH-based alkaline pretreatment improves cellulose accessibility and enzymatic digestibility of agricultural residues while minimizing carbohydrate degradation. For example, Garcia-Negron *et al*. (2024) reported that combining NaOH pretreatment with complementary pretreatment steps significantly enhanced saccharification efficiency of sweet sorghum bagasse by improving lignin removal and enzyme accessibility. Similarly, Premjet *et al*. (2025) demonstrated that optimized NaOH pretreatment of broom grass biomass at moderate temperature and alkali concentration achieved high lignin removal (≈75%) and improved glucan recovery, resulting in increased enzymatic hydrolysis yields. Additionally, Ha *et al*. (2024) showed that low-temperature, low-concentration NaOH treatment following steam explosion markedly increased cellulose digestibility (≈95.9%) while minimizing water use and wastewater generation. NaOH pretreatment efficiently removed lignin and partially degraded cellulose in wheat straw. The highest conversion (87.2%) was achieved with 4% NaOH at 121°C, demonstrating that a mild alkali concentration and temperature can yield high conversion efficiency (Zheng *et al*. 2018). Our findings are consistent with more recent reports demonstrating that NaOH pretreatment selectively removes lignin and improves carbohydrate accessibility, leading to higher fermentable sugar yields. Raw OPEFB, which has high lignin content and cellulose crystallinity, yields lower amounts of glucose and xylose. In contrast, alkali-pretreated OPEFB exhibits effective delignification and increased sugar release (Zulkiple *et al*. 2016). The present results support these observations and further demonstrate that optimized NaOH pretreatment can achieve substantial lignin reduction while retaining fermentable polysaccharides.

Response surface methodology (RSM) is an effective tool that integrates statistical and mathematical approaches to analyze multiple independent variables and their effects on a response (Kidane 2021). Optimization was performed using statistical software to identify ideal conditions within the tested range. The software evaluates each condition based on desirability and computes a single composite score to determine optimal outcomes (Antony 2014). Previous studies have shown that temperature and alkali loading significantly affect glucan conversion during alkaline pretreatment (Lee *et al*. 2021; Chen *et al*. 2013). Temperature is another key factor influencing hydrolysis efficiency. Higher temperatures enhance delignification and sugar release; however, excessively harsh conditions may lead to lignin condensation, thereby reducing carbohydrate conversion (Yuan *et al*. 2021). Fermentation performance in this study was comparable to previous reports (Ruchala *et al*. 2020). A decline in ethanol yield after prolonged fermentation was observed, which may be attributed to ethanol toxicity and the presence of inhibitory compounds. In this study, specific inhibitors were not directly quantified; therefore, their presence is inferred based on literature. Common inhibitors reported in alkaline pretreatment hydrolysates include phenolic compounds, furfural, and acetic acid (Zhao *et al*. 2013).

From an applied perspective, NaOH pretreatment is attractive due to its relatively low cost, scalability, and compatibility with existing biorefinery infrastructure. Ecologically, improved conversion of OPEFB—an abundant agro-industrial residue—into bioethanol supports waste valorization and reduces environmental burdens associated with biomass disposal. However, chemical recovery and wastewater management remain important considerations for sustainable large-scale implementation.

Conclusions

Current research demonstrates that RSM is an effective tool for optimizing alkali pretreatment conditions to enhance cellulose content and reduce lignin in OPEFB. For maximum hemicellulose and cellulose generation, the ideal pretreatment conditions were 5% substrate concentration**,** 20% alkali concentration, a pretreatment temperature of 120ºC, and a pretreatment time of 120 min. Additionally, alkali-treated OPEFB produced more reducing sugars, resulting in an increase in ethanol yield. These findings confirm the suitability of alkali pretreatment for improving bioconversion efficiency. Future research should focus on scaling up the optimized alkali pretreatment for industrial application and validating its effectiveness on various lignocellulosic residues. Further integration into biorefinery systems and valorization of by-products can improve sustainability and commercial feasibility.

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