

# Glucose and Xylose Productions From Oil Palm Empty Fruit Bunch by Hydrolysis With Enzyme and Acid Using Response Surface Methodology.

Santat Sinjaroonsak (✉ [santat.s@pnu.ac.th](mailto:santat.s@pnu.ac.th))

Princess of Naradhiwas University <https://orcid.org/0000-0002-5937-6358>

Aran H-Kittikun

Prince of Songkla University <https://orcid.org/0000-0001-6383-7973>

Thanongsak Chaiyaso

Chiang Mai University <https://orcid.org/0000-0003-2835-5578>

Wasana Suyotha

Prince of Songkla University <https://orcid.org/0000-0003-3755-2880>

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## Research Article

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## Abstract

Oil palm empty fruit bunch (EFB) is a major cellulosic waste from a palm oil mill. The use of EFB for bioconversion to fuel and valuable products is possible because this biomass is a cheap, renewable and abundantly available. This study was aimed to produce sugars from the alkaline peroxide pretreated EFB (APEFB) by hydrolysis with a commercial enzyme (iKnowzyme acid 2XL cellulase) in comparison with hydrochloric acid. Response surface methodology (RSM) was applied to improve the hydrolysis process. For an enzymatic hydrolysis, the optimum enzyme dose of 40 U/g APEFB and the liquid to solid ratio of 10 ml/g APEFB were investigated at 150 rpm and 50°C for 120 h. After saccharification, glucose and xylose obtained were 65.71 g/l (0.66 g/g APEFB) and 2.14 g/l (0.02 g/g APEFB), respectively. Many acids (acetic, formic, hydrochloric (HCl), nitric, orthophosphoric and sulfuric acids) were used to hydrolyze APEFB. The result showed that HCl was the best acid to produce glucose and xylose from APEFB with low furfural and hydroxymethylfurfural productions. The optimum HCl concentration and temperature for APEFB saccharification were 5.85% (w/v) acid at 114°C for 90 min. The glucose, xylose, furfural, and hydroxymethylfurfural obtained under these conditions were 10.70 g/l (0.11 g/g APEFB), 15.30 g/l (0.15 g/g APEFB), 2.34 g/l (0.02 g/g APEFB) and 0.67 g/l (0.007 g/g APEFB), respectively.

## Novelty

This report shows that the cheap carbon source from empty fruit bunch can be used for sugar productions replacing the commercial carbon source. It can be reduced the cost of an expensive substrate as well as can be reduced the waste from palm oil industry. Moreover, the sugar production by using agro-industrial wastes will be helpful and beneficial to the environment. EFB can be used as a potential source for glucose and xylose productions which could be further used for the biorefinery process to produce biofuel and value chemicals.

## Introduction

Recently, the depletion of the fossil fuel stock in the world has occurred. The utilizing of biomass as a raw material for the production of renewable energy is needed. Lignocellulosic biomass is the most abundant renewable materials on earth. The conversion of lignocellulosic waste feedstocks to value products such as chemicals and fuels has been reported. It is a clean and green technology which is renewable, low cost and intensive in nature [1–3].

Palm oil is one of the most important agricultural products of Southeast Asian countries. In general, the basic palm oil processing operations have a lot of wastes including of 11–13% palm pressed fiber (PPF), 20–28% empty fruit bunch (EFB) and palm oil mill effluent (POME) 0.6–1.05 m<sup>3</sup> ton<sup>-1</sup> fresh fruit bunch (FFB) [4–6]. EFB mainly composed of cellulose 35–59%, hemicellulose 21–28%, and lignin 11–26% [7–10]. Previously, EFB had a little commercial value and became a disposable problem because of the bulk density thus occupying large storage volume [8]. A little amount of EFB has been used as a composting material by the landfilling method, which is very costly [11, 12]. In addition, it is largely being burned instead of being used productively, thus causes environmental pollution [13–15].

EFB may serve as a potential raw material for the productions of chemical and energy following the concept of biorefinery. One possible approach towards the utilization of EFB is to hydrolyze into fermentable sugars and is further converted into ethanol and other value-added products. Many researchers had been utilized EFB to produce many materials such as activated carbon, bio-oil, citric acid, composting, enzymes, sugars, paper pulp and ethanol [9, 10, 16–23].

The conversion of EFB to fermentable sugars required an initial delignification pretreatment followed by hydrolysis by a chemical method or an enzymatic method. Hydrolysis of cellulose produces mostly glucose whereas hydrolysis of hemicellulose produces mostly xylose. These sugars are generally used in separate fermentation process for a production of valuable products. In this study, glucose and xylose productions from the pretreated EFB was optimized by hydrolysis with the commercial enzyme (iKnowzyme acid 2XL cellulase) and acids using response surface methodology with the central composite design.

# Materials And Methods

## Raw material and pretreatment

EFB was supplied by Thai Tallow and Oil Co., Ltd., Surat Thani, Thailand. The EFB biomass was prepared by sun-drying and grinding to a particle size of 2 mm. The pretreatment was done by soaking 50 g EFB in the solution of 15% (w/w) NaOH with 3% (v/v) H<sub>2</sub>O<sub>2</sub> 500 ml for 4 h and then autoclaving for 5 min at 121°C [10]. After cooling, the materials were neutralized by washing with tap water several times and rinsed in distilled water. After that, the pretreated EFB were dried at 105°C for 8 h. The alkaline peroxide pretreated EFB was stored in a plastic bag and kept at room temperature until use. The compositions of cellulose, hemicellulose, and lignin in the alkaline peroxide pretreated EFB were 74.46, 15.72 and 6.40% (w/w), respectively [9].

## Chemicals and enzyme

Hydrochloric acid (37%), nitric acid (95%) and sulfuric acid (96%) were purchased from ACI Labscan Ltd., Bangkok, Thailand. Acetic acid (99%), formic acid (99%) and orthophosphoric acid (85%) were purchased from Ajax Finechem Pty. Ltd., Australia. iKnowzyme acid 2XL cellulase was obtained from Reach Biotechnology Co. Ltd, Bangkok, Thailand.

## Enzyme assay and protein determination

The activity of cellulase was determined by adding 0.5 ml of enzyme source to 1.0% carboxymethylcellulose (CMC) (Fluka) in 0.5 ml of 50 mM sodium acetate buffer (pH 5.0) and incubated by shaking at 500 rpm and 50°C for 10 min. The quantity of the liberated reducing sugars was measured by the DNS method [24] using glucose as a standard. One unit of the cellulase was assayed as the amount of enzyme that liberated one micromole of glucose equivalent per minute under the experimental settings. The activity of xylanase was determined by preparing 0.5% (w/v) beechwood xylan (Sigma) dissolved in 50 mM sodium acetate buffer (pH 5.0) 0.5 ml. The amount of reducing sugar released after shaking at 500 rpm and 50°C for 10 min was evaluated by DNS method [24] and xylose was used as a standard. One unit of xylanase activity was described as the amount of enzyme that released one micromol of xylose under the assay conditions. Protein content was determined by the Lowry method [25] using bovine serum albumin (BSA) as a standard.

## Enzymatic hydrolysis

The time course of an enzymatic hydrolysis of APEFB was carried out in a 250 ml flask in an incubator shaker at 150 rpm and 50°C for 120 h. In the typical hydrolysis reaction, 5 g of the APEFB was added to 45 ml 50 mM acetate buffer pH 5.0 with Tween 80 0.3 g/l and potassium metabisulfite 0.02 g/l. After adding the enzyme solution (iKnowzyme acid 2XL cellulase), the samples were collected at a specific time interval and the reaction was stopped by heating at 90°C for 10 min [26]. Then, the samples were diluted in distilled water and filtrated (0.2 µm) prior to analysis for glucose and xylose by high performance liquid chromatography (HPLC).

## Acid hydrolysis

Acid hydrolysis of 5 g APEFB at 120°C was carried out in a 250 ml flask with 50 ml 0.5% (w/v) acid. The acid solution consisted of 0.5% (w/v) of acetic acid, formic acid, orthophosphoric acid, hydrochloric acid, nitric acid or sulfuric acid. The samples were detected periods time in the variety of 0–90 min. The samples were separated by filtration method and the filtrate was analyzed for furfural, hydroxymethylfurfural (HMF), glucose and xylose by HPLC. The acid which gave the highest sugar production was selected for further study.

## Central composite design

The hydrolysis of APEFB for sugar production by the enzyme and the selected acid, was optimized by the response surface methodology (RSM). Central composite design (CCD) was set to optimize the parameters for sugar production.

The experimental ranges and levels of independent process variables for the sugar produced from the APEFB are shown in Table 1. Two factors chosen for an enzymatic hydrolysis were enzyme dose and liquid to solid ratio. While two factors for an

acid hydrolysis were acid concentration and hydrolysis temperature. The factors were examined at five different levels according to the equation (1):

$$Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 \quad (1)$$

Where  $Y_i$  was the predicted response (glucose or xylose);  $X_1$  and  $X_2$  were independent variables,  $\beta_0$  was the regression coefficient at the center point;  $\beta_1$  and  $\beta_2$  were the linear coefficients;  $\beta_{12}$  was the second order interaction coefficients and  $\beta_{11}$  and  $\beta_{22}$  were the quadratic coefficients. The created regression model was calculated by evaluating the values of regression coefficients, analysis of variance (ANOVA),  $p$ - and  $F$ -values. The coefficient of determination,  $R^2$  was calculated for the value of fit of the polynomial template equivalence. The statistical software used to detect the investigational model as well as to create a regression model to expect the optimal combinations contemplating the outcomes of linear, quadratic and the interaction on total sugar concentration is the Design-Expert version 7.0.0 (Stat-Ease Inc. Minneapolis; free trial).

### Sugars, furfural, and HMF analysis

Glucose and xylose concentrations were determined by HPLC (Agilent serie 1200, Agilent, USA) with Transgenomic™ CarboSep CHO682 column (7.8 x 300 mm, 7-micron, Teknokroma Anlítica, Spain) using RI detector. Water was used as the mobile phase; the running temperature was operated at 80°C and the flow rate was operated at 0.4 ml/min [27]. Standard glucose and xylose were run in the same condition. All the standard sugars and samples were filtered through 0.22 µm nylon membrane prior to analysis. The furfural and HMF were determined by HPLC with LiChrospher®RP-18 (5 µm; 125 x 4 mm) column (Merck KGaA, 64271 Darmstadt, Germany) combined with UV 276 nm detector (Rezende et al. 2011). Water and acetonitrile in the ratio of 90:10 (v/v) was used as the mobile phase with the temperature at 25°C and 1.0 ml/min of the flow rate. Standard furfural and HMF were run in the same condition.

## Results And Discussion

### Enzymatic hydrolysis

#### Time course of an enzymatic hydrolysis

The iKnowzyme acid 2XL cellulase used in this study had cellulase activity of 16,000 U ml<sup>-1</sup> and xylanase activity of 570 U ml<sup>-1</sup> with a protein content of 278.5 mg ml<sup>-1</sup>. Time course of sugar production from the hydrolysis of APEFB by the iKnowzyme acid 2XL cellulase is shown in Fig. 1. The results show that glucose and xylose concentrations were slightly increased when increasing the hydrolysis time. The maximum glucose (24.65 g/l) and xylose (1.77 g/l) were obtained at 120 h.

#### Statistical modeling for an enzymatic hydrolysis

The APEFB (5 g) was hydrolyzed by adding to 45 ml 50 mM acetate buffer pH 5.0 with Tween 80 0.3 g/l and potassium metabisulfite 0.02 g/l. The enzyme dose and liquid to solid ratio were parameters used in this experiment. The conditions for hydrolysis were done at 150 rpm at 50°C for 120 h. The results found that the maximum glucose (65.61 g/l) and xylose (2.22 g/l) concentrations were observed in the run number 7 and 3, respectively (Table 2). The quadratic models in terms of coded variables were shown in equation (2) and (3), where  $Y_1$  represented glucose concentration and  $Y_2$  represented xylose concentration as a function of the enzyme dose ( $X_1$ ) and liquid to solid ratio ( $X_2$ ).

$$Y_1 = 19.85 + 2.90X_1 - 2.09X_2 - 0.06X_1X_2 - 0.02X_1^2 + 0.04X_2^2 \quad (2)$$

$$Y_2 = 2.77 - 0.04X_1 - 0.06X_2 + 1.05E-003X_1X_2 + 6.52E-004X_1^2 - 1.89E-004X_2^2 \quad (3)$$

The response function and experimental data of the regression analysis were performed and the second order model for all responses was evaluated by ANOVA (Table 3 and 4). The greater the  $F$ -value indicates that the factors explain adequately the variation in the data about its mean, and the estimated factor effects are real. The results showed that the value of the adjusted

R<sup>2</sup> determination for glucose and xylose productions was also very high (99.30% and 96.63%, respectively) which indicated a high accuracy of the model [28].

Estimations of glucose and xylose concentrations over independent variables, enzyme dose (X<sub>1</sub>) and the liquid to solid ratio (X<sub>2</sub>) in terms of response surfaces are shown in Fig. 2. The maximum concentrations of glucose (65.61 g/l) and xylose (2.13 g/l) were observed by using the enzyme dose and liquid to solid ratio of 40 U/g APEFB and 10 ml of liquid per g of APEFB (100 g/l), respectively for 120 h. Shamsudin et al. [29] report that glucose and xylose concentration of 8.75 and 3.75 g/l were obtained after hydrolysis the steam pretreated EFB (50 g/l) with Celuclast 1.5L (25 U/g EFB) at 50°C for 24 h. In another study, glucose concentration (17.5 g/l) was obtained after hydrolysis the water pretreated EFB (25 g/l) with 70 U/g EFB at 50°C for 48 h [30].

To confirm the results, the hydrolysis of APEFB by the enzyme was carried out in triplicate under the most and least optimized conditions. The results are shown in Table 5. The highest glucose and xylose concentrations obtained were 65.71 g/l (0.66 g/g APEFB) and 2.14 g/l (0.02 g/g APEFB), respectively when hydrolysis using the iKnowzyme acid 2XL cellulase 40 U/g APEFB and the liquid to solid ratio of 10 ml/g APEFB (100 g/l APEFB) with shaking at 150 rpm and 50°C for 120 h. It was found that the sugars concentrations obtained from enzymatic hydrolysis of APEFB of the experimental tests were similar to the sugar concentrations in the prediction models.

## Acid hydrolysis

### Effect of type of acids

The effect of various acids on the hydrolysis of APEFB to produce glucose and xylose was done by using 0.5% (w/v) of acid solutions at 120°C. The results are shown in Fig 3. Glucose, xylose, furfural and HMF concentrations were increased when increasing the time for hydrolysis. The highest glucose concentration (4.93 g/l) and xylose concentration (15.12 g/l) were obtained when hydrochloric acid was used for hydrolysis. The highest furfural concentration was obtained after hydrolysis the APEFB with nitric acid (1.65 g/l), while the highest HMF was obtained with sulfuric acid (0.41 g/l). The results show that the hydrolysis of APEFB with acid gave xylose concentration higher than glucose. Chong et al. [7] found that the highest yield of glucose (2.75 g/l) and xylose (24.14 g/l) was obtained after hydrolysis the EFB with 6% sulfuric acid at 120°C for 15 min. Rahman et al. [31] report that the maximum xylose, glucose and furfural concentrations of 29.4, 2.34 and 0.8 g/l were observed after hydrolysis the EFB by using 6% sulfuric acid at 120°C for 15 min. The ultrasonic pretreated EFB hydrolysis using 2% sulfuric acid at 100°C for 45 min provided glucose and xylose of 2.0 and 23.2 g/l, respectively [32].

### Statistical modeling for acid hydrolysis

Since hydrolysis of APEFB by HCl provided highest sugar productions and less furfural and HMF, it was further used to optimize the sugar productions. The experimental ranges and levels of the independent process variables including the HCl concentration and temperature for hydrolysis of APEFB are shown in Table 5. The design of the acid hydrolysis experiments including dependent variables, Y<sub>3</sub> (glucose), Y<sub>4</sub> (xylose), Y<sub>5</sub> (furfural) and Y<sub>6</sub> (HMF) are also given in Table 6. The release of glucose and xylose in the hydrolysate after acid hydrolysis was dependent on experimental operating conditions. The maximum concentrations of glucose and xylose obtained were 10.75 and 15.86 g/l, respectively when the HCl concentration, temperature and reaction time were 6% (w/v) at 110°C and 90 min, respectively. The lowest furfural and HMF concentrations (1.41 and 0.51 g/l) occurred when the reaction was carried out with 6% HCl at 96°C for 90 min.

The quadratic models in terms of coded variables are shown in equation (4) – (7), as a function of reaction acid (HCl) concentration (X<sub>3</sub>) and temperature (X<sub>4</sub>).

$$Y_3 = -223.31 + 17.11X_3 + 3.24 X_4 - 0.09 X_3X_4 - 0.57X_3^2 - 0.01X_4^2 \quad (4)$$

$$Y_4 = -300.42 + 16.72X_3 + 4.70X_4 - 0.11X_3X_4 - 0.45X_3^2 - 0.02X_4^2 \quad (5)$$

$$Y_5 = 10.87 - 3.33X_3 - 0.047X_4 + 0.02X_3X_4 + 0.15X_3^2 - 3.08E-005X_4^2 \quad (6)$$

$$Y_6 = -15.13 + 0.74X_3 + 0.24X_4 - 8.99E-04X_3X_4 - 0.005X_3^2 - 1.03E-003X_4^2 \quad (7)$$

To fit the response function and experimental data, regression analysis was performed and the second order model for all response was evaluated by ANOVA. The results are presented in Table 7–10. In this experiment, the value of  $R^2$  of glucose, xylose, furfural, and HMF production from APEFB were 93.97, 95.52, 96.85, and 94.16%, respectively. These values indicate a high degree of correlation between the experimental and the predicted values. The results show that the values of the adjusted  $R^2$  determination were also very high (87.94%, 91.03%, 93.71% and 88.32% for glucose, xylose, furfural and HMF productions, respectively) which indicate a high accuracy of the model [28].

Estimations of sugar concentration (glucose and xylose) and by-products (furfural and HMF) from the hydrolysis of APEFB over independent variables (acid concentration ( $X_3$ ) and temperature ( $X_4$ )) in term of response surfaces are shown in Fig. 4 and 5. The maximum concentration of glucose (10.56 g/l) was obtained by conducting hydrolysis experiment with 5.85% HCl at 114°C for 90 min while the maximum concentrations of xylose (15.86 g/l) was obtained by hydrolysis with 4% HCl at 120°C for 90 min. On the other hand, maximum concentration of furfural (3.68 g/l) and HMF (0.69 g/l) was obtained by hydrolysis with 7.32% HCl at 120°C for 90 min but the minimum concentration of furfural (1.92 g/l) and HMF (0.38 g/l) was obtained by hydrolysis with 4% HCl and at 100°C for 90 min. Thamsee et al. [33] reported that the maximum glucose and xylose concentrations of 1.80 and 33.16 g/l was obtained when hydrolysis the EFB with 4.0% sulfuric acid at 119°C for 60 min. While the maximum glucose and xylose concentrations (1.2 and 11.3 g/l, respectively) were obtained by using a combined of 0.5% sulfuric acid and 0.2% phosphoric acid at 160°C for 10 min [34].

Based on the models, the numerical optimization was carried out with considering each value of response in four conditions. The results are shown in Table 11. The best results of glucose and xylose concentrations obtained by the experiment were 10.70 g/l (0.11 g/g APEFB) and 15.30 g/l (0.15 g/g APEFB), respectively after hydrolysis of the APEFB with 5.80% HCl at 114°C for 90 min. The highest concentrations of furfural (3.81 g/l) and HMF (0.74 g/l) were obtained after hydrolysis with 7.32% HCl at 120°C. Most diluted acid and high temperature was found to generate by-products such as furfural and HMF [31]. Furfural and HMF presented in the hydrolysate higher than 0.2 g/l were potential inhibitors to microbial metabolism. They inhibited the fermentation process by causing cell morphological change or ultimate death of the microorganism [35]. To keep the concentration of furfural and HMF in the hydrolysate at a low level it is necessary to run the hydrolysis reaction at less severe conditions.

## Conclusion

EFB from palm oil mill can be used for glucose and xylose productions either by an enzymatic hydrolysis or an acid hydrolysis. The APEFB hydrolysis with iKnowzyme acid 2XL cellulase provided glucose and xylose concentrations 6 time higher than hydrolysis by using HCl. The enzymatic hydrolysis of APEFB using iKnowzyme acid 2XL cellulase has a number of advantages over the acid hydrolysis. The enzymatic hydrolysis is a very promising method for saccharification of APEFB. The hydrolysis is performed under mild conditions. The glucose obtained from enzymatic hydrolysis was higher than using acid hydrolysis. However, the xylose concentration obtained from acid hydrolysis was higher than using an enzymatic hydrolysis. In addition, the using of acid was harmful to the environment and gave by-products such as furfural and HMF. This experiment showed that EFB can be used as a potential source for glucose and xylose productions which could be further used for the biorefinery process to produce biofuel and value chemicals.

## Declarations

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**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Ethical approval:** This article does not contain any studies with human participants or animals performed by any of the authors.

**Informed consent:** Informed consent was obtained from all individual participants of this study.

**Data availability:** All data generated or analyzed during in this study are included in this published article and its supplementary information files.

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## Tables

**Table 1** Experimental ranges and levels of independent process variables for the hydrolysis of alkaline peroxide pretreated oil palm empty fruit bunch (APEFB) by iKnowzyme acid 2XL cellulase and hydrochloric acid

Independent variable	Symbol	Levels				
		- $\alpha$	-1	0	+1	+ $\alpha$
<u>Enzymatic hydrolysis</u>						
Enzyme dose (U/g APEFB)	$X_1$	15.86	20	30	40	44.14
Liquid:solid (ml/g APEFB)	$X_2$	6.59	10	15	20	23.41
<u>Acidic hydrolysis</u>						
Acid concentration (% w/v)	$X_3$	3.17	4	6	8	8.83
Temperature (°C)	$X_4$	96	100	110	120	124

**Table 2** The arrangement of CCD for enzyme concentration and liquid to alkaline peroxide pretreated oil palm empty fruit bunch ratio used in RSM study for glucose and xylose productions

Run	Variable level		Response parameter g/l	
	Enzyme* dose; U/g APEFB ( $x_1$ )	Liquid: solid; ml/g ( $x_2$ )	Glucose ( $Y_1$ )	Xylose ( $Y_2$ )
1	30.00 (0)	15.00 (0)	41.50	1.84
2	15.86 (- $\alpha$ )	15.00 (0)	25.00	1.75
3	44.14 (+ $\alpha$ )	15.00 (0)	<b>52.24</b>	<b>2.22</b>
4	30.00 (0)	7.93 (- $\alpha$ )	62.95	2.05
5	40.00 (+1)	20.00 (+1)	33.16	1.93
6	30.00 (0)	22.07 (+ $\alpha$ )	25.67	1.64
7	40.00 (+1)	10.00 (-1)	<b>65.61</b>	<b>2.13</b>
8	20.00 (-1)	10.00 (-1)	41.93	1.88
9	30.00 (0)	15.00 (0)	40.37	1.81
10	20.00 (-1)	20.00 (+1)	21.58	1.48
11	30.00 (+1)	15.00 (0)	43.37	1.82

\* iKnowzyme acid 2XL cellulase

The number inside bracket is coded value and the number outside bracket is actual value

**Table 3** Analysis of variance (ANOVA) for glucose production from alkaline peroxide pretreated oil palm empty fruit bunch by iKnowzyme acid 2XL cellulase

Source	SS	DF	MS	F-value	p-value
Model	2142.69	5	428.54	285.33	< 0.0001
Residual	7.51	5	1.50		
Lack of fit	2.92	3	0.97	0.42	0.7577
Pure error	4.59	2	2.30		
Total	2150.20	10			
R <sup>2</sup>	0.9965				Adjust R <sup>2</sup> = 0.9930

SS = Sum of squares, DF = Degree of freedom, MS = Mean squares

**Table 4** Analysis of variance (ANOVA) for xylose production from alkaline peroxide pretreated oil palm empty fruit bunch by iKnowzyme acid 2XL cellulase

Source	SS	DF	MS	F-value	p-value
Model	0.44	5	0.089	58.41	0.0002
Residual	7.58E-003	5	1.52E-003		
Lack of fit	7.16E-003	3	2.39E-003	11.17	0.0833
Pure error	4.27E-004	2	2.14E-004		
Total	0.45	10			
R <sup>2</sup>	0.9832				Adjust R <sup>2</sup> = 0.9663

SS = Sum of squares, DF = Degree of freedom, MS = Mean squares

**Table 5** The validation of the developed quadratic model and optimum parameter point for glucose and xylose production from alkaline peroxide pretreated oil palm empty fruit bunch by iKnowzyme acid 2XL cellulase at various conditions

Conditions	Enzyme dose (U/g APEFB)	Liquid: solid ratio (ml/g)	Concentration (g/l)			
			Predicted		Experiment	
			Glu	Xyl	Glu	Xyl
1	<b>40.00</b>	<b>10.00</b>	<b>66.46</b>	<b>2.15</b>	<b>65.71 (-1.1%)</b>	<b>2.14 (-0.5%)</b>
2	21.75	18.09	26.71	1.61	21.78 (-18.5%)	1.62 (+0.6%)
3	37.37	15.75	25.66	1.61	30.12 (+17.4%)	1.81 (+12.4%)
4	39.51	10.00	65.98	2.14	65.62 (-0.6%)	2.15 (+0.5%)

Where; Glu = glucose, Xyl = xylose

Data in the parenthesis showing % error obtained

Bold values indicate the selected optimum condition solutions for desired results

**Table 6** Effect of HCl concentration and temperature on the hydrolysis of alkaline peroxide pretreated empty fruit bunch

Run	Variable level		Response parameter g/l			
	Acid concentration; % (w/v) ( $x_3$ )	Temperature; °C ( $x_4$ )	Glucose ( $Y_3$ )	Xylose ( $Y_4$ )	Furfural ( $Y_5$ )	HMF ( $Y_6$ )
1	6 (0)	110 (0)	10.50	15.18	2.39	0.81
2	6 (0)	96 (- $\alpha$ )	7.45	10.46	<b>1.41</b>	<b>0.51</b>
3	6 (0)	124 (+ $\alpha$ )	9.03	14.44	2.86	0.66
4	6 (0)	110 (0)	<b>10.75</b>	<b>15.26</b>	2.34	0.83
5	4 (-1)	120 (+1)	8.58	15.43	2.23	0.62
6	6 (0)	110 (0)	<b>10.10</b>	<b>15.86</b>	1.95	0.77
7	8 (+1)	120 (+1)	6.61	10.15	4.61	0.64
8	3.17 (- $\alpha$ )	110 (0)	6.45	13.49	2.47	0.41
9	4 (-1)	100 (-1)	3.32	8.16	1.88	0.33
10	8 (+1)	100 (-1)	8.68	11.33	2.85	0.43
11	8.83 (+ $\alpha$ )	110 (0)	5.63	11.33	4.15	0.32

The number inside bracket is coded value and the number outside bracket is actual value

**Table 7** Analysis of variance (ANOVA) for glucose production from the hydrolysis of alkaline peroxide pretreated empty fruit bunch by hydrochloric acid

Source	SS	DF	MS	F-value	p-value
Model	49.08	5	9.82	15.58	0.0045
Residual	3.15	5	0.63		
Lack of fit	2.93	3	0.98	9.05	0.1011
Pure error	0.22	2	0.11		
Total	52.23	10			
R <sup>2</sup>	0.9397			Adjust R <sup>2</sup> = 0.8794	

SS = Sum of squares, DF = Degree of freedom, MS = Mean squares

**Table 8** Analysis of variance (ANOVA) for xylose production from the hydrolysis of alkaline peroxide pretreated empty fruit bunch by hydrochloric acid

Source	SS	DF	MS	F-value	p-value
Model	66.45	5	13.29	21.31	0.0022
Residual	3.12	5	0.62		
Lack of fit	2.84	3	0.95	6.79	0.1310
Pure error	0.28	2	0.14		
Total	69.57	10			
R <sup>2</sup>	0.9552			Adjust R <sup>2</sup> = 0.9103	

SS = Sum of squares, DF = Degree of freedom, MS = Mean squares

**Table 9** Analysis of variance (ANOVA) for furfural production from the hydrolysis of alkaline peroxide pretreated empty fruit bunch by hydrochloric acid

Source	SS	DF	MS	F-value	p-value
Model	8.88	5	1.78	30.78	0.0009
Residual	0.29	5	0.058		
Lack of fit	0.18	3	0.057	1.03	0.5263
Pure error	0.11	2	30.78		
Total	9.17	10			
R <sup>2</sup>	0.9685			Adjust R <sup>2</sup> = 0.9371	

SS = Sum of squares, DF = Degree of freedom, MS = Mean squares

**Table 10** Analysis of variance (ANOVA) for HMF production from the hydrolysis of alkaline peroxide pretreated empty fruit bunches by hydrochloric acid

Source	SS	DF	MS	F-value	p-value
Model	0.33	5	0.066	16.12	0.0042
Residual	0.021	5	0.004		
Lack of fit	0.018	3	0.006	5.72	0.1523
Pure error	0.002	2	0.001		
Total	0.35	10			
R <sup>2</sup>	0.9416			Adjust R <sup>2</sup> = 0.8832	

SS = Sum of squares, DF = Degree of freedom, MS = Mean squares

**TABLE 11** Effect of acid concentration and temperature on the hydrolysis of alkaline peroxide pretreated empty fruit bunch for 90 min

Conditions	HCl concentration (% w/v)	Temperature (°C)	Concentration (g/l)							
			Predicted				Experiment			
			Glu	Xyl	FF	HMF	Glu	Xyl	FF	HMF
1	5.85	114	10.55	15.86	2.34	0.82	10.70 (+1.4%)	15.30 (-3.5%)	2.34	0.67 (-18.3%)
2	4.22	115	9.22	15.72	2.15	0.66	9.11 (-1.2%)	15.53 (-1.2%)	2.23 (+3.7%)	0.63 (-4.5%)
3	4.00	100	4.17	8.92	1.92	0.38	3.22 (-22.8%)	8.07 (-9.5%)	1.86 (-3.1%)	0.30 (-21.1%)
4	7.32	120	8.00	12.61	3.68	0.69	7.55 (-5.6%)	11.15 (-11.6%)	3.81 (+3.5%)	0.74 (+7.2%)

Where; Glu = glucose, Xyl = xylose, FF = furfural and HMF = hydroxymethylfurfural

Data in the parenthesis showing % error obtained

Bold values indicate the selected optimum condition solutions for desired results

## Figures

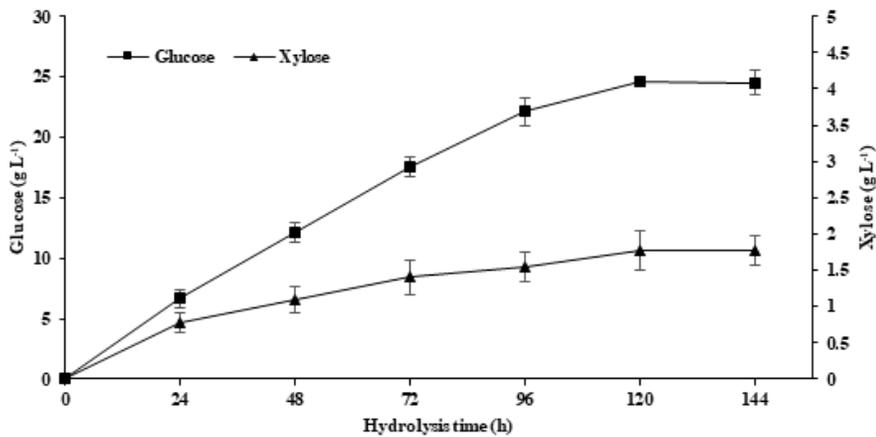


Figure 1

Time cause of glucose and xylose productions from alkaline peroxide pretreated empty fruit bunch using iKnowzyme acid 2XL cellulase with shaking at 150 rpm and 50°C

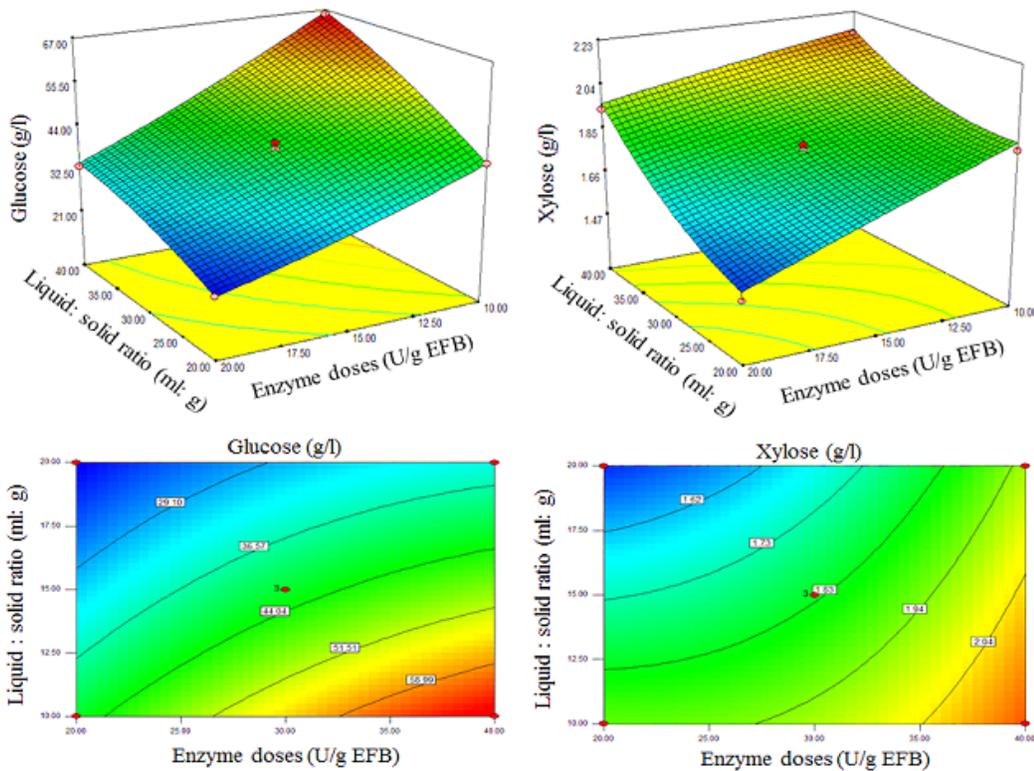


Figure 2

Effect of enzyme dose and liquid to solid ratio on glucose and xylose productions from alkaline peroxide pretreated oil palm empty fruit bunch

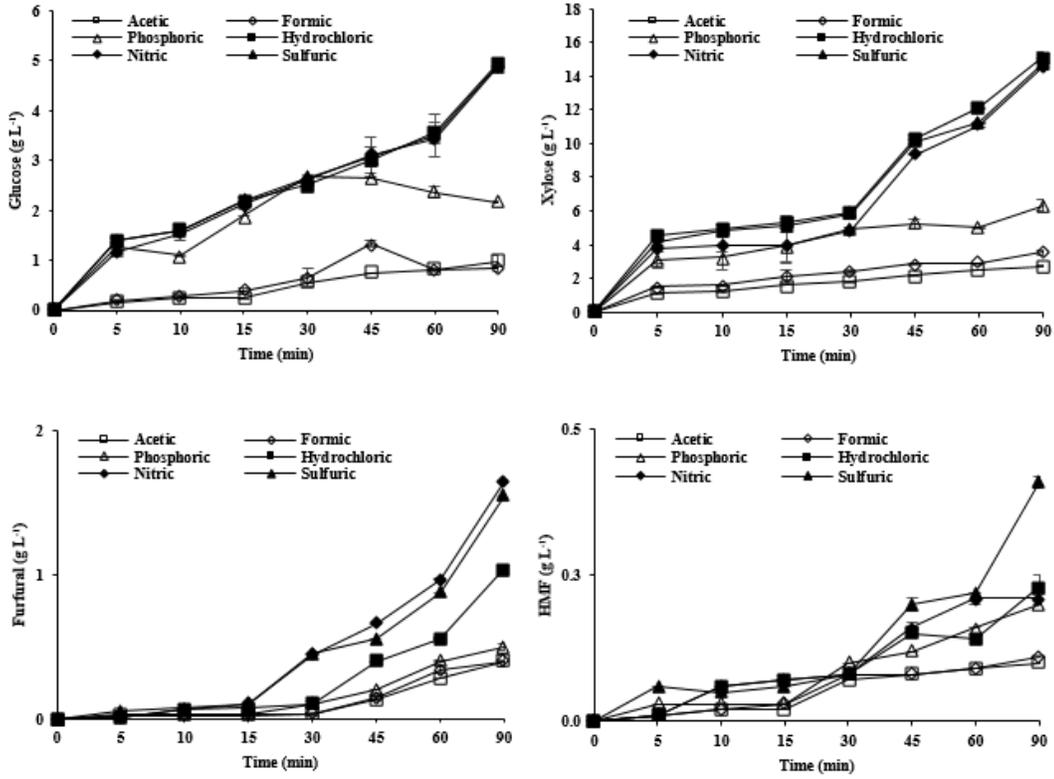
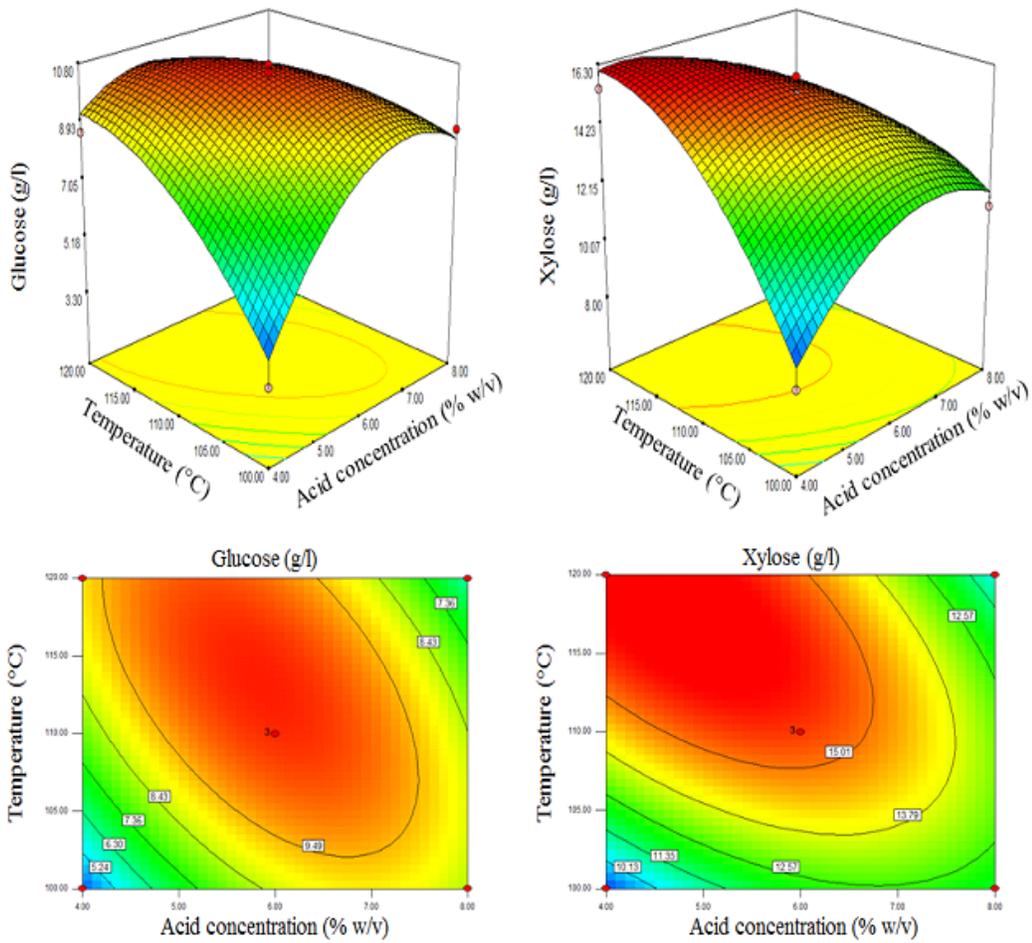


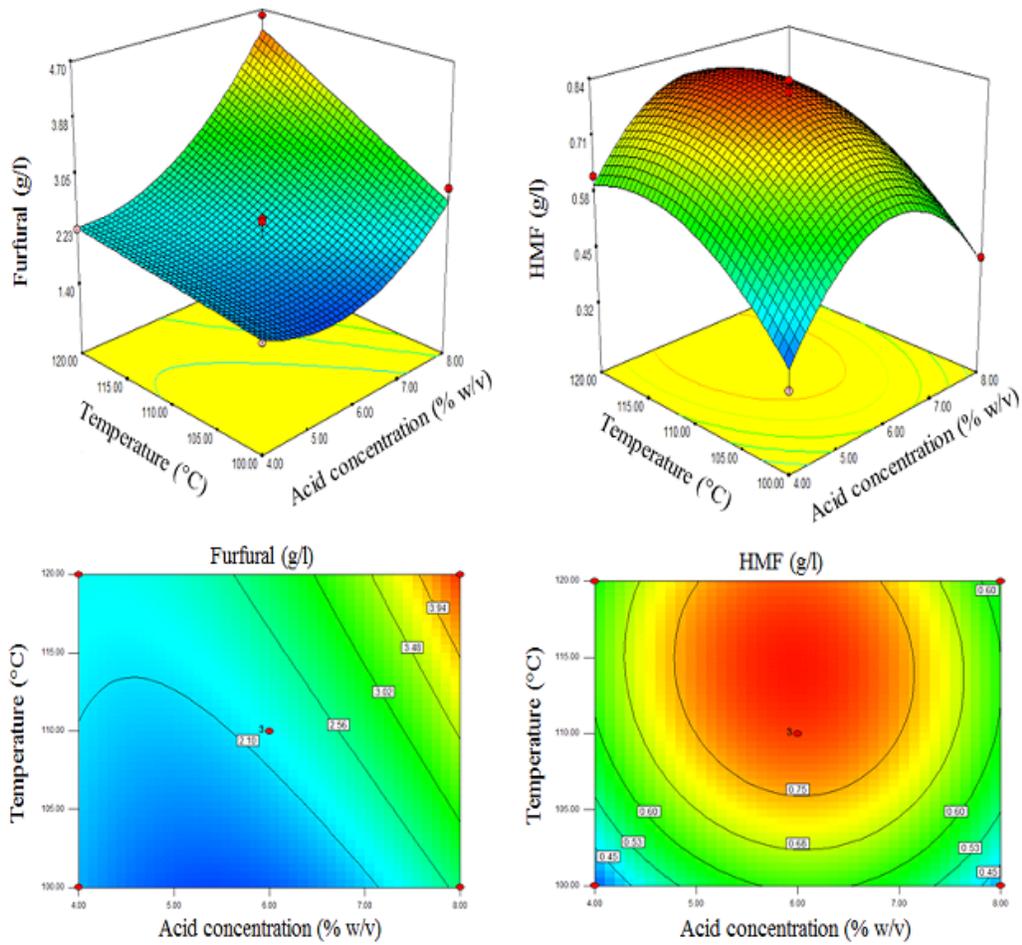
Figure 3

Sugars and by-products productions from alkaline peroxide pretreated empty fruit bunch by different acids at 120°C



**Figure 4**

Effects of hydrochloric acid concentration and temperature on glucose and xylose productions from alkaline peroxide pretreated empty fruit bunch



**Figure 5**

Effects of hydrochloric acid concentration and temperature on furfural and HMF productions from alkaline peroxide pretreated empty fruit bunch