Chapter 7

Optimization of Delignification and Enzyme Hydrolysis of Steam Exploded Oil Palm Trunk for Ethanol Production by Response Surface Methodology

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Additional information is available at the end of the chapter

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1. Introduction

The inevitable depletion of the world's petroleum supply and the increasing problem of greenhouse gas effects have resulted in an increasing worldwide interest in alternative nonpetroleum-base source of energy. As the transportation sector is practically entirely depending on oil and as it is responsible for half of the total CO_2 emission [1], the increasing in market share of renewable biofuels includes ethanol fuel. The uses of ethanol fuel will significantly reduce net carbon dioxide emission once it replaces fossil fuels because fermentation-derived ethanol is already a part of the global carbon cycle. However, to enhance the market position of the biofuel the production cost should be reduced. Nowadays, the raw material and enzyme production are the two main contributors to the overall costs, thus using high cellulose containing agricultural residues as feedstock agricultural could result in cost reduction. The techniques employed to produce bioethanol from agricultural residue materials or lignocellulosic materials are subjected to the same economical demands as the more traditional sugar and starch processes, as the price of bioethanol must be competitive with that of petrol. Conversion of lignocellulosic materials to monomeric sugars and finally ethanol must thus be performed at low cost, while still achieving high yields. This can be done by developing processes that require limited amounts of the material chemicals, yeast and enzymes. To convert lignocellulosic materials to monomeric sugars, they must pretreat by different methods, such as dilute acid, steam explosion, ammonia fiber explosion (AFEX) and dilute alkali. All of these methods can change lignocellulosic structure and enhance the enzymatic saccharification of cellulose to hexose sugar.

The bioconversion process from lignocellulose biomass to ethanol consists basically of three steps: pre-treatment, enzymatic hydrolysis and fermentation. Pretreatment is a necessary step to facilitate the enzymatic attack of lignocellulosic materials. Steam explosion is recognized as an efficient pre-treatment method in ethanol production [2]. The raw material is treated at high pressure steam followed by suddenly rapid reduction in pressure resulting in substantial breakdown of the lignocellulosic structure, hydrolysis of the hemicellulosic fraction, depolymerization of the lignin components and defibration [3]. Therefore, the accessibility of the cellulose components to degrade by enzymes is greatly increased. The process of ethanol production from lignocellulosic material is shown in Figure 1.

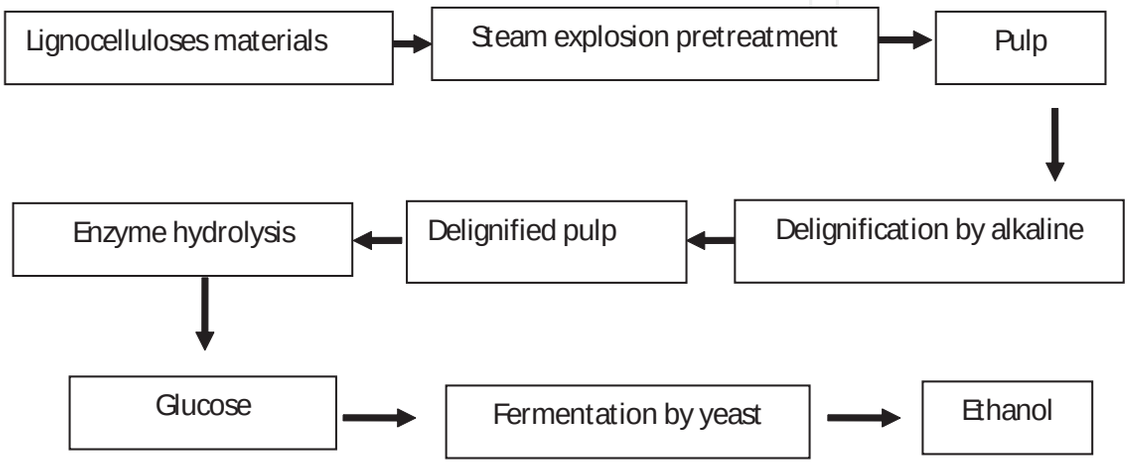

Figure 1. Flowchart of procedure for ethanol production

Numerous experimental studies of ethanol production from biomass have been carried out. A review article by Kaur *et al*. [4] examined the steam explosion of sugarcane bagasse as a pretreatment for ethanol production. In order to identify the optimum conditions of steam explosion, a range of operating temperatures at 188 – 243ºC and residence times at 0.5 - 44 min were applied. The results showed that pretreatment with steam explosion followed by enzyme hydrolysis had high efficiency in converting monosaccharide sugar to ethanol. Nunes *et al.* [5] reported the steam explosion pretreatment and enzymatic hydrolysis of Eucalyptus wood. The comparison under conditions of acid and non acid impregnation of wood before steam explosion was experimented. The results demonstrated the same solubilization effect of both experiments. Ballesteros *et al*. [6] reported that simultaneous saccharification and fermentation (SSF) process for ethanol production from various lignocellulosic woody (poplar and eucalyptus) and herbaceous (*Sorghum* sp. bagasse, wheat straw and *Brassica carinata* residue) materials had been assayed using the thermotolerant yeast strain. Biomass samples were previously treated in a steam explosion pilot plant to provide biomass with increased cellulose content relative to untreated materials and to enhance cellulase accessibility. SSF experiments were performed in laboratory conditions at 42ºC for 160 hours. The results showed that eucalyptus, wheat straw and sweet sorghum bagasse gave ethanol concentration at 17, 18 and 16.0 g/L respectively, in 72 hours of fermentation. Montane *et a*l. [7] studied the steam explosion of wheat straw. A fractionation of wheat straw components in a two-step chemical pretreat-

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ment was proposed. Hemicellulose was hydrolyzed by dilute H_2SO_4, allowing a substantial recovery of xylose. Lignin was removed by means of a mild alkaline/oxidative solubilization procedure, involving no sulphite or chlorine and its derivatives. The use of diluted reagents and relatively low temperatures was both cheap and environmentally friendly. The pretreated material was nearly pure cellulose, whose enzyme hydrolysis proceeded fast with high yields, that leading to high glucose syrup of remarkable purity. Ballesteros *et al.* [8] investigated the enzyme hydrolysis of steam exploded herbaceous agricultural waste (*Brassica carinata*) at different particle sizes. The objective of this work was to evaluate the effect of particle size on steam explosion pretreatment of herbaceous lignocellulosic biomass. Hemicellulose and cellulose recovery and effectiveness of enzyme hydrolysis of the cellulosic residue was presented for the steam-exploded agricultural residue (*Brassica carinata*) with different particle sizes. The parameters tested were: particle size (2-5, 5-8 and 8-12 mm), temperature (190 and 210°C), and residence time (4 and 8 min). The composition analysis of filtrate and water insoluble fibre after pretreatment and enzyme digestibility data were presented. The results showed that larger steam exploded particle (8-12 mm) resulted in higher cellulose and enzyme digestibility. The use of small particles in steam explosion would not be desirable in optimizing the effectiveness of the process improving economy. Punsuvon *et al*. [9] studied the fractionation of chemical components of oil palm trunk by steam explosion. The results showed optimal conditions for pretreatment at temperature 214°C for 2 min of steam explosion. Ohgren *et al*. [10] reported the ethanol fuel production from steam-pretreated corn stover using SSF at higher dry matter content. This study was performed on steam-pretreated corn stover at 5, 7.5 and 10% water-insoluble solids (WIS) with 2 g/L hexose fermenting *Saccharomyces cerevisiae*. The results showed that SSF at 10% WIS gave 74% of ethanol yield based on the glucose content in the raw material. Ruiz *et al*. [11] studied the steam explosion pretreatment prior to enzymatic hydrolysis of sunflower stalks. The stalks were subjected to steam explosion pretreatment in the temperatures ranging between 180ºC and 230ºC. The steam-exploded pulp was further hydrolyzed by enzyme. The result showed that after 96 hours of enzymatic reaction, a maximum hydrolysis yield of 72% was obtained after pretreatment at 220ºC, corresponding to a glucose concentration of 43.7 g/L in hydrolysis media. With regard to the filtrate analysis, most of the hemicellulosic derived sugars released during the steam pre-treatment were in the oligomeric form. The highest recovery was obtained at 210ºC of pretreatment temperature. Moreover, the utilization of hemicellulosic-derived sugars as a fermentation substrate would improve the overall bioconversion of sunflower stalks into ethanol fuel.

1.1. Response Surface Methodology (RSM)

Response surface methodology is an empirical statistical technique employed for multiple regressions analysis by using quantitative data. It solves multivariable data which is obtained from properly designed experiments to solve multivariable equation simultaneously. The graphical representation of their function was called response surface, which is used to describe the individual and cumulative effect of the test variables and their subsequent effect on the response. The effect of the variables on the response is investigated using second-order polynomial regression equation. This equation, derived using RSM for the evaluation of the response variables, is as follows:

$$Y = b_o + \sum_{i=1}^{4} b_i x_i + \sum_{i=1}^{4} b_{ii} x_i^2 + \sum_{i<j=1}^{3} \sum^{4} b_{ij} x_{ij}$$

Where Y is the response, b_o, b, $b_{ii\ and}$ b_{ij} are regression coefficients for intercept, linear, quadratic and interaction terms, respectively. The x_i and x_{ij} are uncoded values for independent variables. An analysis of variance (ANOVA) is performed to determine the lack of fit and the effect of linear, quadratic and interaction terms on the response. Many researches have used RSM in optimization process as these examples. Roberto *et al*. [12] studied the dilute acid hydrolysis to recover xylose from rice straw in a semi-pilot reactor. Rice straw is consisted of pentose that could be used as a raw material for the production of many useful compounds. One of these was xylitol, with a potential application in the food and medical areas. The interest in biotechnological processes employing lignocellulosic residues was increased because this material was cheap, renewable and widespread sugar sources. The objective of the study was to determine the effects of H_2SO_4 concentration and reaction time on the production of sugars (xylose, glucose and arabinose) and on the reaction byproducts (furfural, HMF and acetic acid). Dilute sulfuric acid was used as a catalyst for the hydrolysis of rice straw at 121ºC in a 350-L batch hydrolysis reactor. Rationale for conducting this study was determined based on a central composite statistical design. Response surface methodology (RSM) was adopted to optimize the hydrolysis conditions aiming to attain high xylose selectivity. The optimum condition was 1% H_2SO_4 concentration for 27 min. This condition gave 77% of xylose yield and 5.0 g/g of selectivity. Kunamneni *et al*. [13] applied the response surface to optimize the enzymatic hydrolysis of maize starch for higher glucose production. Doses of pre-cooked α-amylase, post-cooked α-amylase, glucoamylase and saccharification temperature were examined to produce maximum conversion efficiency and all values were selected for optimization. Full factorial composite experimental design and response surface methodology were used in the experiment design and result analysis. The optimum values for the tested variables were: 2.243 U of pre-cooked α-amylase /mg solids, 3.383 U of post-cooked 3.383 U of α-amylase /mg solids, 2.243 U of glucoamylase /mg solids at a saccharification temperature of 55.1ºC. The maximum conversion efficiency of 96.25% was achieved. This method was efficient because only 28 experiments were necessary for the assessment and also the model adequacy was very satisfactory.

1.2. Oil palm trunk

The oil palm tree (Elaeis guineensis) is indigenous to the tropical forests in weat Africa. The oil palm tree has become one of the most valuable commercial cash a crop due to the palm oil is used as a raw material in many industries such as soap, cosmetic, detergent, vegeTable oil and biodiesel. Nowaday almost 80% of the world oil palm plantation is centered at Southeast Asia, with most of it occurring in Indonesia (5.44×10^6 hectares) and Malaysia (4.85×10^6 hectares). Additionally, there are 260,000 hectares planted in Thailand, with smaller areas in the Philippines and some recent planting in Cambodia and Myanmar [14]. Oil palm trunks are available only when the economic lifespan of the palm is reach at the time of replanting. The average age of replanting is approximately 25 years. The main economic criteria for felling are the height of the palm, reaching 13 m or above and the diameter of the felled trunk is around 45 cm to 65 cm. More than 15 million tons of oil palm trunks per year are replanted in the world [15]. The increase of oil

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palm trunk every year can create massive pollutions thus development technology for value-added products are need for this raw material. There are many need uses of potential value-added products made from oil palm trunk such as particleboard, laminated board, plywood, fiberboard and furniture [16]. Oil palm trunk can also be used for making paper [17]. It can also be used as raw material in ethanol production, too [9].

The objectives of this research are performed according to central composite design (CCD) and response surface methodology (RSM) to optimize and compare the condition for delignification and hydrolysis of steam-exploded oil palm trunk prior to ethanol fermentation to understand the relationship between the critical factor involved in enzymatic degradation of pulp and conversion to ethanol.

2. Materials and methods

2.1. Raw material and microorganism

The steam-exploded pulp obtained from oil palm trunk was prepared by steam explosion treatment. An amount of 150 g of dry oil palm trunk chip sample was placed in 2.5 L batch digester (Nitto Koatsu Company, Japan). Heating was accomplished by direct steam injection into the digester and the temperature of steam at 214°C for 2 min. This condition was previous work by Punsuvon *et al.* [9]. It could briefly explained that oil palm trunk chip was steamed at temperatures varying between 214 and 220ºC for 2 and 5 minutes. The optimization of the pretreatment condition was 214ºC and 2 minutes that gave the highest glucose yield after enzyme hydrolysis. In this studied, the explosive discharge of the digester contents into a collecting tank was actuated by rapidly opening a value. The combined pulp slurry was collected and washed with hot water (80°C) at total volume of 2 L for 30 min. The pulp was filtered and dried at room temperature for using as raw material in alcohol production study.

Saccharomyces cervisiae TIRS 5339 obtained from TISTR, Thailand was used in this study. It was maintained on a medium containing 20.0 g/l glucose, 20.0 g/l peptone and 10.0 g/l yeast extract at 4ºC and subcultured every month at 30ºC. The growth medium of the yeast consisted of 10.0 g/l yeast extract, 6.4 g/l urea, 2.0 g/l KH_2PO_4, 1.0 g/l $MgSO_4$-7H2O and 2.0 g/l glucose at pH 5.5 [18].

2.2. Alkaline delignification of steam-exploded pulp

The water-insoluble cellulose pulp obtained from steam explosion was delignified with potassium hydroxide. The reactions were carried out in a beaker under various maintained temperature. Before RSM was applied on alkaline delignification, approximate conditions for glucose content in pulps, namely concentration of pulp, concentration of alkaline solution, reaction time and temperature were determined by varying one factor at time while keeping the other constant. The initial step of the preliminary experiment was to select an appropriate amount of concentration of pulp. Five different concentrations of pulp (3, 6, 9, 12, 15 %w/v) were examined. The other three factors, concentration of alkaline solution, reaction time and

temperature, were kept constant at 20%w/w, 60 min and 80ºC, respectively. Based on the glucose content in pulp after delignification, the optimum concentration of pulp was chosen. The second step of the preliminary experiment was to determine the concentration of alkaline solution. The glucose content in pulp was analyzed using the optimum condition of pulp chosen in the previous step. The alkaline solution concentration varied from 2 to 30% w/w while holding reaction time and temperature at 60 min and 80ºC. The third step of the preliminary experiment was to determine the reaction time. Using the concentration of pulp, concentration of alkaline solution, reaction time from the previous steps, alkaline delignification was studied under various reaction times from 15 to 90 min. The final step was to select an appropriate temperature by using the concentration of pulp, concentration of alkaline solution, reaction time from the previous step. The temperature varied from 30 to 100ºC. Based on these results the five level of each process variable were determined for RSM. The independent variables of RSM experiments were shown in Table1. Delignified pulp was recovered by filtration, washed several times with distilled water, died and then analyzed for glucose content. These pulps were ready to be used as the substrate for enzymatic hydrolysis.

2.3. Enzyme hydrolysis

Delignified pulps were hydrolyzed by cellulase (Celluclast 1.2L, Novozymes A/S Denmark) in flasks. The hydrolysis was performed in 0.05M sodium citrate buffer (pH 4.8) at 150 rpm of shaking. The dependent variables of experiments were shown in Table 2. All enzymatic hydrolysis liquor was analyzed for glucose content by High Performance Liquid Chromatography (HPLC).

2.4. Inoculums and ethanol fermentation

S. cerevisaie was initiated in the maintenance medium at 30ºC. The yeast was grown for 48 h at 170 rpm on a rotary shaker at 30°C. A 2.5 % w/v inoculums was used for subsequent subcultures. Ethanol fermentation was evaluated at 30°C in 150–ml Erlenmeyer flasks containing 100 ml fermentation media. The yeast fermentation medium consisted of the hydrolysis liquor containing 50g/l glucose, 2.0 g/l $KHPO_4$, 1.0 g/l $MgSO_4.7H_2O$, 10.0 g/l yeast extracts and 6.4 g/l urea at pH 5.5. The flasks were sealed with a one-hole rubber stopper, which a glass tube was connected to an air lock filled with 40% sulfuric acid solution. Ethanol content from fermentation was analyzed by Gas Chromatography (GC).

Independent variable	Symbol	Coded variable levels				
		α	-1	0	1	α
Concentration of pulp, %w/v	X_1	3	6	8	12	15
Concentration of alkaline solution, %w/w	X_2	2	8	14	20	26
Reaction time, min	X_3	15	30	45	60	75
Temperature, ºC	X_4	35	50	65	80	95

Table 1. Independent variables and their levels for central composite design in optimization of alkaline delignification of steam-exploded pulp

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Independent variable	Symbol	Coded variable levels				
		α	-1	0	1	α
Reaction time, h	X_1	10	30	50	70	90
Temperature, °C	X_2	28	35	42.5	50	57.5
Enzyme loading, Filter Paper Unit (FPU)/g substrate	X_3	5	30	55	80	105
Concentration of pulp, %w/v	X_4	1	2	3	4	5

Table 2. Independent variables and their levels for central composite design in optimization of enzyme hydrolysis of delignified pulp

2.5. Experimental design

A central composite design was employed the response, namely percentage of glucose for alkaline delignification, and percentage of glucose yield for enzyme hydrolysis. The independent variables of alkaline delignification were X_1, X_2, X_3 and X_4 representing concentration of pulp, %w/v, and concentration of alkaline solution, % w/w, reaction time, min and temperature, °C, respectively. The independent variables of enzyme hydrolysis were X_1, X_2, X_3 and X_4 representing reaction time, h, temperature, °C, enzyme loading, FPU/g substrate and concentration of pulp, %w/v, respectively. Each variable to be optimized was coded at five levels: - α, -1, 0, +1 and + α. This gives a range of these variables of alkaline delignification (Table 1) and enzyme hydrolysis (Table2). Six replication runs at the centre (0, 0, 0) of the design were performed to allow the estimation of the pure error.

2.6. Statistical analysis

The data obtained by carrying out the experiment according to central composite design were analyzed by SPSS package (version 12.0). The response surface was expressed at the following second-order polynomial equation:

$$Y = b_o + \sum_{i=1}^{4} b_i x_i + \sum_{i=1}^{4} b_{ii} x_i^2 + \sum_{i<j=1}^{3} \sum^{4} b_{ij} x_{ij}$$

Where Y is the response (percent glucose, %), x_i and x_{ij} are uncoded independent variables, b_o is constant, b_i is linear term coefficients, b_{ii} is quadratic term coefficients. b_{ij} is cross-product term coefficients. SPSS package was used for regression analysis of variance (ANOVA) and response surface methodology was performed using STATISTICA Software. Response surface plots were developed using the fitted second order polynomial equation obtain from regression analysis holding one of the independent variables at a constant value corresponding to the stationary point and changing the other two variables.

2.7. Glucose determination

The glucose content of hydrolysis liquid was analyzed by High Performance Liquid Chromatography (HPLC, Shimadzu, Kyoto, Japan) with refractive index detector. An AMINEX HPX-87C carbohydrate analysis (Bio-Rad, Hercules, USA) was used as column. The mobile phase

was deionied water with flow rate 0.6 ml/min. The injection volume was 20 μl and the column temperature was maintained at 80˚C. The glucose content of the solid residue was determined based on monomer content that was measured after two steps of acid hydrolysis. The first step hydrolysis was performed with 72% (w/w) H_2SO_4 at 30ºC for 60 min. In the second step, the reaction mixture was diluted to 4% (w/w) H_2SO_4 with distilled water and subsequently autoclaved at 121˚C for 1 h. This hydrolysis liquid was then analyzed for glucose content as described above. All analytical determinations were performed in duplication.

2.8. Ethanol determination

The ethanol content in fermented solution was analyzed by gas chromatography (GC). GC analysis was carried out with an Agilent Technologies (Santa clara, CA) 6890 gas chromatograph equipped with a flame ionization detector and a HP-5 (Bonded 5%phenyl, 95% dimethylpolysiloxane) capillary column (30 m x 0.32 mm ID, 0.25 μm film thickness). The temperature program was an initial temperature of 150ºC, increased to 190ºC at 10ºC/min then 15ºC/min to 250ºC, and held for 15 min. The injector and detector temperature were 250 ºC and 300ºC, respectively. Standard and sample were injected by using the split mode ratio of 50:1.

3. Results and discussion

3.1. Chemical components of oil palm trunk and steam exploded oil palm trunk pulp

The Figure 2 (a) showed oil palm trunk chip before steam explosion pretreatment and Figure 2 (b) showed steam exploded oil palm trunk pulp obtained after steam explosion.

Figure 2. Oil palm trunk chip (a) and steam exploed oil palm trunk pulp (b)

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The chemical components of steam exploded oil palm trunk pulp were 40.54% of cellulose, 9.36% of hemicelluloses, 38.46% of lignin and 8.56% of extractive in ethanol/benzene. This pulp obtained after steam explosion was used as raw material for optimization study in ethanol production.

3.2. Model fitting for optimization of alkaline delignification

The complete design matrix together with the values of both the experimental and predicted responses is given in Table3. Central composite design was used to develop correlation between the NaOH and KOH delignification variables to the percentages of glucose yield. The percentages of glucose yield were found to range between 41.4-49.8% for KOH delignification and 38.0-49.9% for NaOH delignification. Runs 17-23 at the center point were used to determine the experiment error. For both reponses of NaOH delignification and KOH delignification, the quadratic model was selected, as suggested by used software. The final empirical models in terms of coded factors are given by equation (1) and Equation (2) in Table 4. Where X_1, X_2, X_2 and X_4 were the coded values of test variables that represented pulp concentration, concentration of NaOH or concentration of KOH, reaction time and temperature, respectively. The variables X_1X_2, X_1X_3, X_1X_4, X_2X_3, X_2X_4 and X_3X_4 represented the interaction effects of pulp concentration and concentration of NaOH or concentration of KOH, pulp concentration and reaction time, pulp concentration and temperature, respectively. The quality of the model developed was evaluated based on the correlation coefficient R^2. The R^2 for the two obtained equations were found to be 0.875 and 0.890 in NaOH and KOH delignification, respectively. This indicates that 87.5% and 89.0% of the total variation in both delignifications were attributed to experimental variables studied. The R^2 of 0.875 and 0.890 were considered as the good fit of the models.

The adequacy of the two models was further justified through analysis of variance (ANOVA). The ANOVA for the quadratic models for the two reponses is listed in Table 5. The Fisher's test (F-test) carried out on experimental data make it possible to estimate the statistical significance of the proposed model. The F-test value of the models being 16.69 and 10.88, respectively for glucose in pulp obtained after NaOH and KOH lignifications, with a low probability value ($p<0.01$), we can conclude that they were statistically significant at 99.9% confidence level. It should be noted that p-value indicates the statistical significance of each parameter. It is based on hypothesis that a parameter is not significant, thus the more effect is significant. From Table 5, it was showed that the two models (both p-value<0.01) were adequate to predict the glucose in pulp obtained after NaOH and KOH lignifications within the range of studied variables.

Response surface contour plots of the RSM as a function of two factors at the time are helpful in understanding both the main and the interaction effects of these factors. The effects of concentration of pulp and concentration of NaOH on the percentage of glucose are shown in Figure 3 (a). Figure 3 showed that the concentration of pulp and NaOH could increased percent glucose in pulp after NaOH delignification. The concentration of pulp higher than 13.50% (w/w) had no significant effect on the amount of percent glu-

cose in pulp. Response surface plot indicated the optimized condition at 12.50% w/v of pulp concentration and 21.50% (w/v) NaOH that gave 48.10% of percent glucose remained in pulp after NaOH delignification. Figure 3 (b) showed that increasing temperature and concentration of pulp could increased percent glucose in pulp after NaOH delignification. The temperature higher than 80ºC had no significant effect on the percent glucose in pulp after NaOH delignification. Response surface plot indicated the optimized condition at 12.5% w/w of pulp concentration and 80ºC that gave 47.95% of percent glucose remained in pulp after NaOH delignification. Figure 3 (c) showed that increasing time and concentration of pulp could increased percent glucose in pulp after NaOH delignification. The time longer than 67 min had no significant effect on the percent glucose in pulp after delignification. Response surface plot indicated the optimized condition at 12.5% w/v of pulp concentration and 65 min that gave 50.23% of percent glucose in pulp after NaOH delignification.

Response surface contour plots of the RSM on the effects of concentration of pulp and concentration of KOH on the percentage of glucose are shown in Figure 4 (a).Figure 4 (a) a showed that increasing concentration of pulp and KOH concentration could increased percent glucose in pulp. The pulp concentration higher than 12.5% (w/v) had no significant effect on percent glucose in pulp. Likewise, the KOH concentration higher than 23.4% (w/w) had no significant effect on percent glucose in pulp after KOH delignification. Response surface plot indicated the optimized condition at 10% (w/v) of pulp concentration and 23.5% (w/w) of KOH concentration that gave 49.04% of percent glucose in pulp after KOH delignification.

Figure 4 (b) showed that increasing concentration of pulp and temperature could increased percent glucose in pulp. The temperature higher than 80°C had no significant effect on percent glucose in pulp. Likewise, the concentration of pulp higher than 12% (w/v) had no significant effect on percent glucose in pulp after KOH delignification. Response surface plot indicated the optimized condition at 78°C of glucose in pulp after KOH delignification.

Figure 4 (c) showed that increasing time and concentration of pulp could increased percent glucose in pulp. The time longer than 60 min had no significant effect on percent glucose in pulp. Likewise, the concentration of pulp more than 12% (w/v) had no significant effect on percent glucose in pulp after KOH delignification. Response surface plot indicated the optimized condition at 12% (w/v) of pulp concentration and 70 min of reaction time that gave 48.35% of percent glucose in pulp after KOH delignification.

The summary result from combination of each response surface plot showed that the optimum condition for NaOH delignification was obtained from 11% (w/v) pulp concentration, 21% (w/w) NaOH concentration, 65 min reaction time and 78ºC temperature with the maximum glucose at 47.50% remaining in the pulp. The optimum condition for KOH delignification was 12% (w/v) pulp concentration, 23% (w/w) KOH concentration, 65 min of reaction time and 80ºC of temperature with the maximum glucose at 49.50% remaining in the pulp.

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Run	X_1	X_2	X_3	X_4	Glucose (%), Delignification			
					Experimental		Predicted	
					KOH	NaOH	KOH	NaOH
1	1	1	-1	-1	48.3	45.2	48.7	45.1
2	1	1	-1	1	46.4	49.9	46.6	49.2
3	1	1	1	-1	42.0	43.6	42.3	43.4
4	1	1	1	1	49.2	49.0	49.6	49.8
5	1	-1	-1	-1	48.1	42.3	48.6	42.3
6	1	-1	-1	1	43.2	40.0	43.2	40.2
7	1	-1	1	-1	47.9	47.4	47.6	47.4
8	1	-1	1	1	49.3	49.5	49.3	49.5
9	-1	1	-1	-1	44.2	38.4	44.7	38.2
10	-1	1	-1	1	46.4	30.8	46.5	30.6
11	-1	1	1	-1	39.8	40.8	39.9	40.6
12	-1	1	1	1	47.2	42.1	47.6	42.7
13	-1	-1	-1	-1	44.1	48.1	44.6	48.5
14	-1	-1	-1	1	41.4	49.3	41.5	49.3
15	-1	-1	1	-1	46.3	38.0	46.7	38.1
16	-1	-1	1	1	49.0	48.2	49.0	48.7
17	0	0	0	0	48.3	43.3	48.2	43.6
18	0	0	0	0	47.4	44.9	47.3	44.3
19	0	0	0	0	48.6	44.0	48.0	44.5
20	0	0	0	0	46.4	44.9	46.9	44.6
21	0	0	0	0	46.3	44.3	46.0	44.3
22	0	0	0	0	48.5	43.8	48.2	43.2
23	0	0	0	0	48.1	43.2	48.3	43.6
24	α	0	0	0	49.5	39.8	49.6	39.7
25	-α	0	0	0	44.3	47.2	44.3	47.9
26	0	α	0	0	48.4	48.9	48.2	48.4
27	0	-α	0	0	45.7	43.9	45.0	43.7
28	0	0	α	0	49.8	48.6	49.5	48.8
29	0	0	-α	0	47.3	42.7	47.4	42.7
30	0	0	0	α	49.4	49.0	49.7	49.9
31	0	0	0	-α	43.7	44.7	43.3	44.9

Table 3. Design and response of the central composite design for glucose (%) obtained from NaOH and KOH delignification

Dependent variable	Predictive	R^2
Glucose (%) NaOH (Eqs.1)	$37.112 - 0.166\ X_1 - 0.959X_2 + 0.511X_3 + 0.104X_4 - 0.074X_12 - 0.005X_22 + 2.706X_32 + 001X_42 + 0.133X_1X_2 - 0.011X_1X_3 + 0.001X_1X_4 - 0.005X_2X_3 - 0.005X_2X_4 - 0.006X_3X_4$	0.875
Glucose (%) KOH (Eqs.2)	$60.266 + 0.574X_1 - 1.398X_2 - 0.320X_3 + 0.091X_4 - 0.030X_12 - 0.001X_22 + 0.006X_32 - 002X_42 + 0.133X_1X_2 - 0.008X_1X_3 + 0.006X_1X_4 - 0.004X_2X_3 + 0.015X_2X_4 + 0.013X_3X_4$	0.890

Table 4. The linear regression of dependent for alkaline delignification of glucose

	Source	Degree of freedom	Sum of square	Mean square	F-value	P-value
NaOH	Model	14	10005.23	714.65	15.65	0.0078
delignification	Residual	16	728.64	45.54	-	-
	Lack of fit	10	530.43	53.04	1.60	-
	Pure error	6	198.21	33.04	-	-
	Total	30	10733.87			
	R^2	0.875				
KOH	Model	14	7935.29	566.81	10.88	0.0071
delignification	Residual	16	833.06	52.07	-	-
	Lack of fit	10	523.45	52.34	1.01	-
	Pure error	6	309.61	51.60	-	-
	Total	30	8768.35			
	R^2	0.890				

Table 5. Analysis of variance (ANOVA) for the fit of experimental data to response surface models

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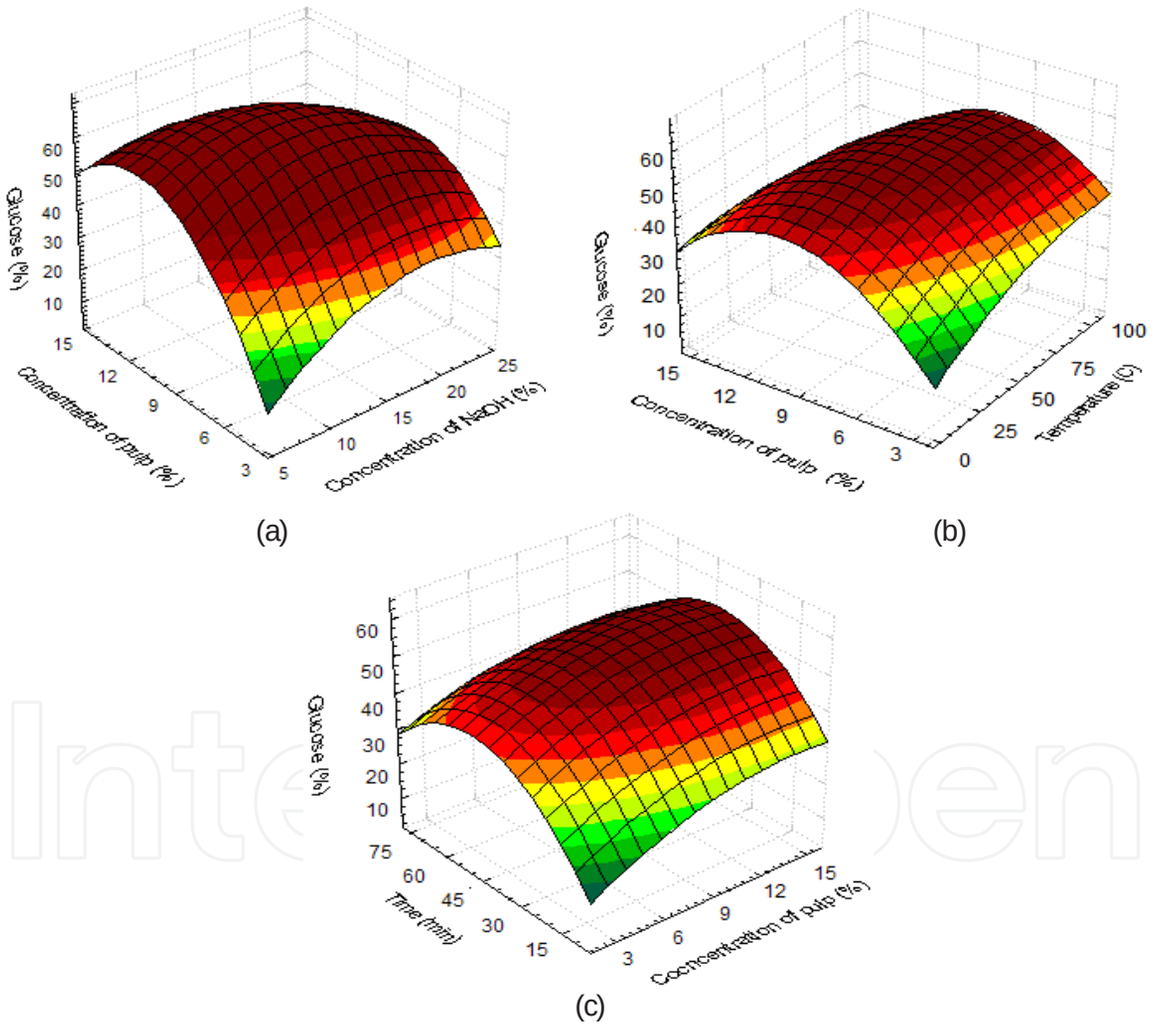

Figure 3. Response surface plots of glucose as a function of concentration of pulp and concentration of NaOH (a), concentration of pulp and temperature (b), time and concentration of pulp (c), other fixed variables

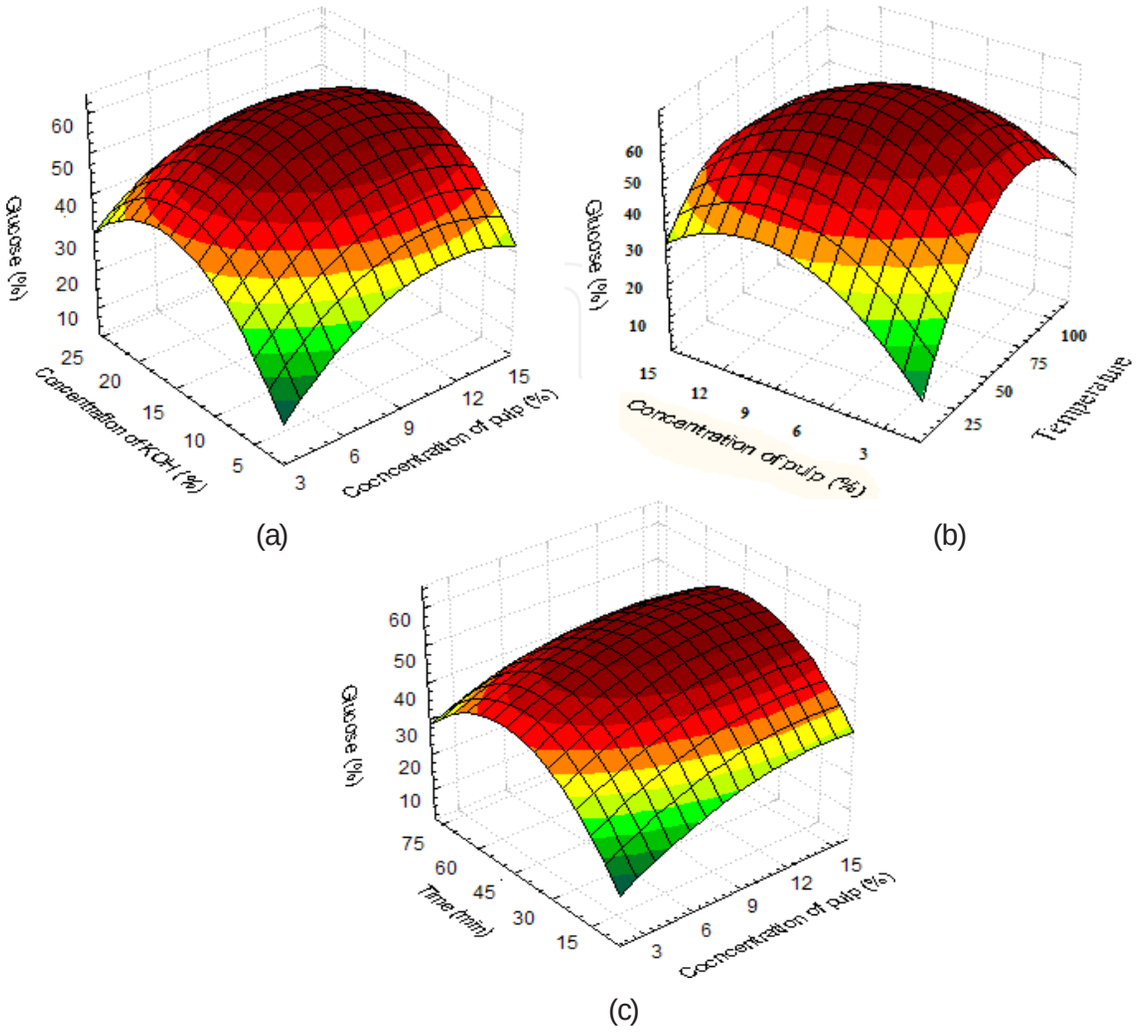

Figure 4. Response surface plots of glucose as a function of concentration of pulp and concentration of KOH (a), concentration of pulp and temperature (b), time and concentration of pulp (c), other fixed variables

3.3. Model fitting for optimization of enzyme hydrolysis

The complete design matrix together with values of both experimental and predicted responses is given in Table 6. Central composite design was used to develop correclation between enzyme hydrolysis of both pulps obtained after NaOH or KOH delignification to percentage of glucose yield were found to range between 6.30-80.10% for pulp obtained after KOH delignification and 10.30-89.80% for pulp obtained after NaOH delignification. Runs 17-23 at the center point were used to determine the experiment error. For both responses of enzyme hydrolysis, the quadratic model was selected, as suggested by used software. The final empirical models in terms of coded factors are given by Equation (3) and Equation (4) in Table 7. Where X_1, X_2, X_2 and X_4 were the coded values of test variables reaction time, temperature, enzyme loading and pulp concentration, respectively. The variables X_1X_2, X_1X_3, X_1X_4, X_2X_3, X_2X_4 and X_3X_4 represented the interaction effects of reaction time and temperature, reaction time and enzyme loading, reaction time and pulp concentration, temperature and enzyme

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loading, temperature and pulp concentration and enzyme loading and pulp concentration, respectively. The quality of the model developed was evaluated based on the correlation coefficient R^2. The R^2 for the two obtained equations were found to be 0.867 and 0.935 in enzyme hydrolysis of pulp obtained after NaOH and KOH delignification. This indicated that 86.70% and 93.50% of total variation in both enzyme hydrolysis were attributed to the experimental variables studied. The R^2 of 0.867 and 0.935 were considered as the good fit of the models.

The adequacy of the two models was further justified through analysis of variance as the same as delignification reaction in previous studied. The statistical result showed that the two models had p-values less than 0.01 that indicated these two models were adequate to predict the percentage of glucose yield in enzyme hydrolysis within the range of the studied variables.

In addition, response surface contour plots of the RSM on the effects of reaction time and concentration of pulp on the percentage of glucose in enzyme hydrolysis are shown in Figure 5 (a). Figure 5 (a) showed that increasing time and pulp concentration could increased percent glucose yield in hydrolyzed solution. The pulp concentration higher than 4.5% (w/v) had no significant effect on percent glucose yield in hydrolyzed in solution. Response surface plot indicated the optimized condition at 3% (w/v) of pulp concentration and 65 h of reaction time that gave 81.01% of percent glucose yield in hydrolyzed solution obtained after NaOH delignification. Figure 5 (b) showed that increasing time and temperature could increased percent glucose yield in hydrolyzed solution. The temperature higher than 50ºC had no significant effect on percent glucose yield in hydrolyzed solution. Response surface plot indicated the optimized condition at 44ºC of temperature and 65 h of reaction time that gave 81.33% of percent glucose yield hydrolyzed solution obtained after NaOH delignification. Figure 5 (c) showed that increasing time and enzyme loading could increased percent glucose yield in hydrolyzed solution. The enzyme loading higher than 65 (FPU/g substrate) had no significant effect on percent glucose yield in hydrolyzed solution. Response surface plot indicated the optimized condition at 54 (FPU/g substrate) of enzyme loading and 50 h of reaction time that gave 91.36% of percent glucose yield in hydrolyzed solution obtained after NaOH delignification. Figure 6 (a) showed that increasing time and pulp concentration could increased percent glucose yield in hydrolyzed solution. The reaction time longer than 75 h had no significant effect on percent glucose yield in hydrolyzed solution. Response surface plot indicated the optimized condition at 3% (w/v) of pulp concentration and 65 h of reaction time that gave 92.08% of percent glucose yield in hydrolyzed solution obtained after KOH delignification. Figure 6 (b) showed that increasing time and temperature could increased percent glucose yield in hydrolyzed solution. The temperature higher than 50ºC had no significant effect on percent glucose in hydrolyzed in solution. Response surface plot indicated the optimized condition at 50ºC of temperature and 65 h of reaction time that gave 88.91% (w/v) of percent glucose yield in hydrolyzed solution obtained after KOH delignification. Figure 6 (c) showed that increasing time and enzyme loading could increased percent glucose yield in hydrolyzed solution. The enzyme loading higher than 85 (FPU/g substrate) had no significant effect on percent glucose yield in hydrolyzed solution. Response surface plot indicated the optimized condition at 54.5 (FPU/g substrate) of enzyme loading and 66 h of reaction time that gave 88.44% of percent glucose yield in hydrolyzed in solution obtained after KOH delignification.

Run	X_1	X_2	X_3	X_4	Glucose (%), Enzyme hydrolysis			
					Experimental		Predicted	
					KOH	NaOH	KOH	NaOH
1	1	1	-1	-1	80.1	86.2	79.8	86.0
2	1	1	-1	1	69.5	75.4	69.0	75.3
3	1	1	1	-1	74.2	80.0	74.5	80.0
4	1	1	1	1	54.4	63.9	55.4	64.2
5	1	-1	-1	-1	37.6	46.7	37.4	46.2
6	1	-1	-1	1	16.8	23.0	16.1	23.2
7	1	-1	1	-1	30.3	35.4	30.9	35.1
8	1	-1	1	1	14.5	20.6	14.3	20.0
9	-1	1	-1	-1	74.4	80.3	74.4	80.8
10	-1	1	-1	1	63.8	71.6	63.3	70.2
11	-1	1	1	-1	70.1	76.4	70.4	76.3
12	-1	1	1	1	53.8	58.6	53.6	58.2
13	-1	-1	-1	-1	34.9	41.4	34.7	41.3
14	-1	-1	-1	1	12.2	18.8	12.5	18.8
15	-1	-1	1	-1	20.1	27.3	20.1	27.3
16	-1	-1	1	1	4.4	10.5	4.5	10.9
17	0	0	0	0	74.2	89.8	74.2	89.2
18	0	0	0	0	73.3	79.3	73.3	79.2
19	0	0	0	0	74.8	80.7	74.8	80.7
20	0	0	0	0	75.2	80.4	75.2	80.1
21	0	0	0	0	75.3	81.3	75.3	81.6
22	0	0	0	0	75.9	81.6	75.4	81.9
23	0	0	0	0	75.3	81.4	75.2	81.8
24	α	0	0	0	77.0	83.7	77.3	83.4
25	-α	0	0	0	6.3	10.3	6.3	10.0
26	0	α	0	0	11.7	15.4	11.7	15.4
27	0	-α	0	0	45.1	50.5	45.1	50.5
28	0	0	α	0	73.0	77.6	73.0	77.6
29	0	0	-α	0	15.2	21.3	15.3	21.3
30	0	0	0	α	74.4	80.2	74.8	81.4
31	0	0	0	-α	62.2	79.8	62.4	79.7

Table 6. Design and response of the central composite design for glucose (%) obtained from enzymatic hydrolysis

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Dependent variable	Predictive	R^2
Glucose yield (%) NaOH (Eqs.3)	$-49.956 + 2.654X_1 + 20.429X_2 + 1.310X_3 - 6.477X_4 - 0.021X_12 - 0.228X_22 - 0.012X_32 - 1.134X_42 - 0.005X_1X_2 + 0.001X_1X_3 + 0.003X_1X_4 + 0.002X_2X_3 + 0.225X_2X_4 + 0.002X_3X_4$	0.867
Glucose yield (%) KOH (Eqs.4)	$-51.956 + 2.654X_1 + 20.332X_2 + 1.410X_3 - 6.477X_4 - 0.021X_12 - 0.224X_22 - 0.012X_32 - 1.835X_42 - 0.004X_1X_2 + 0.001X_1X3 + 0.003X1X4 + 0.002X2X\ 3 + 0.225X2X4 + 0.003X3X4$	0.935

Table 7. The linear regression of dependent for enzyme hydrolysis of glucose yield

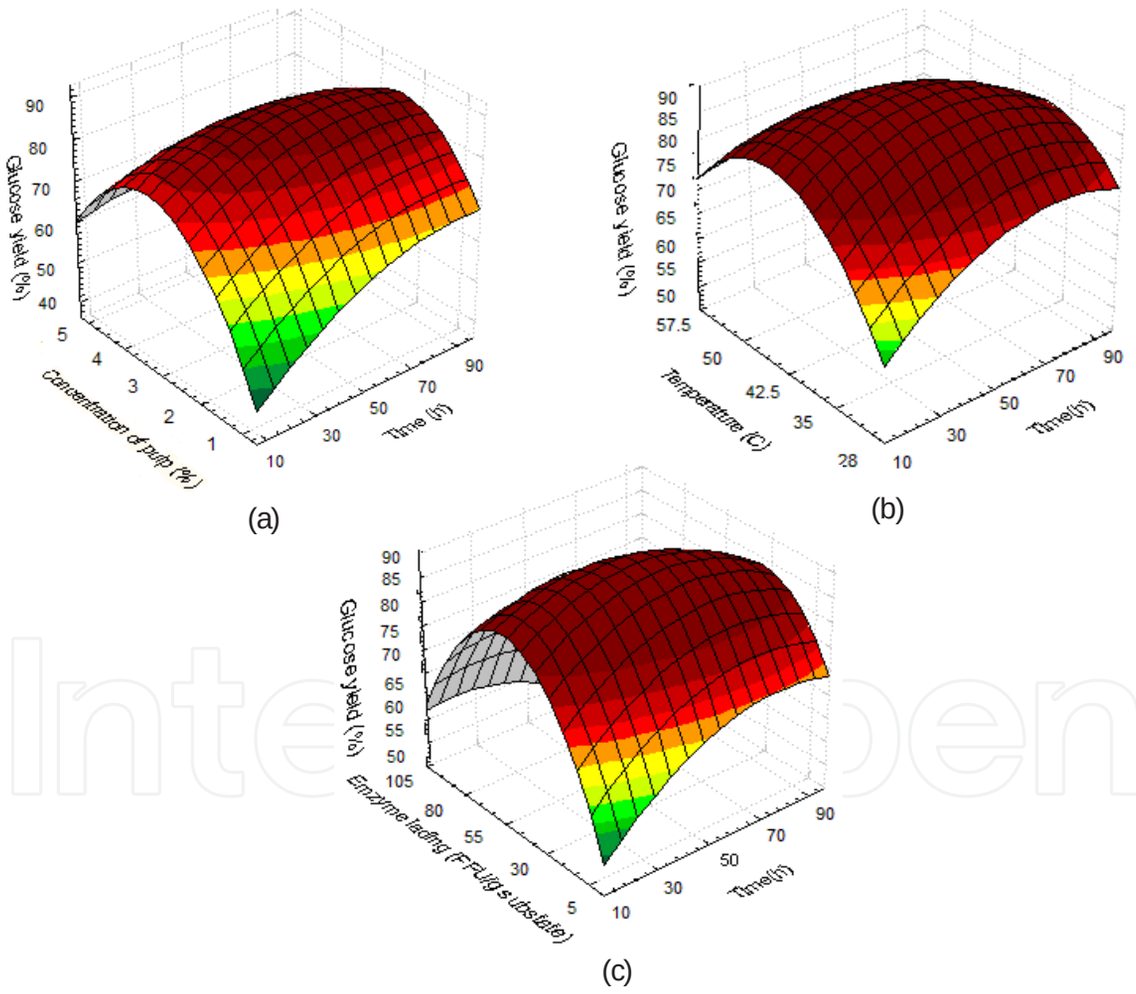

Figure 5. Response surface plots of glucose yield as a function concentration of pulp and time (a), temperature and time (b), enzymatic loading and time (c), other fixed variables

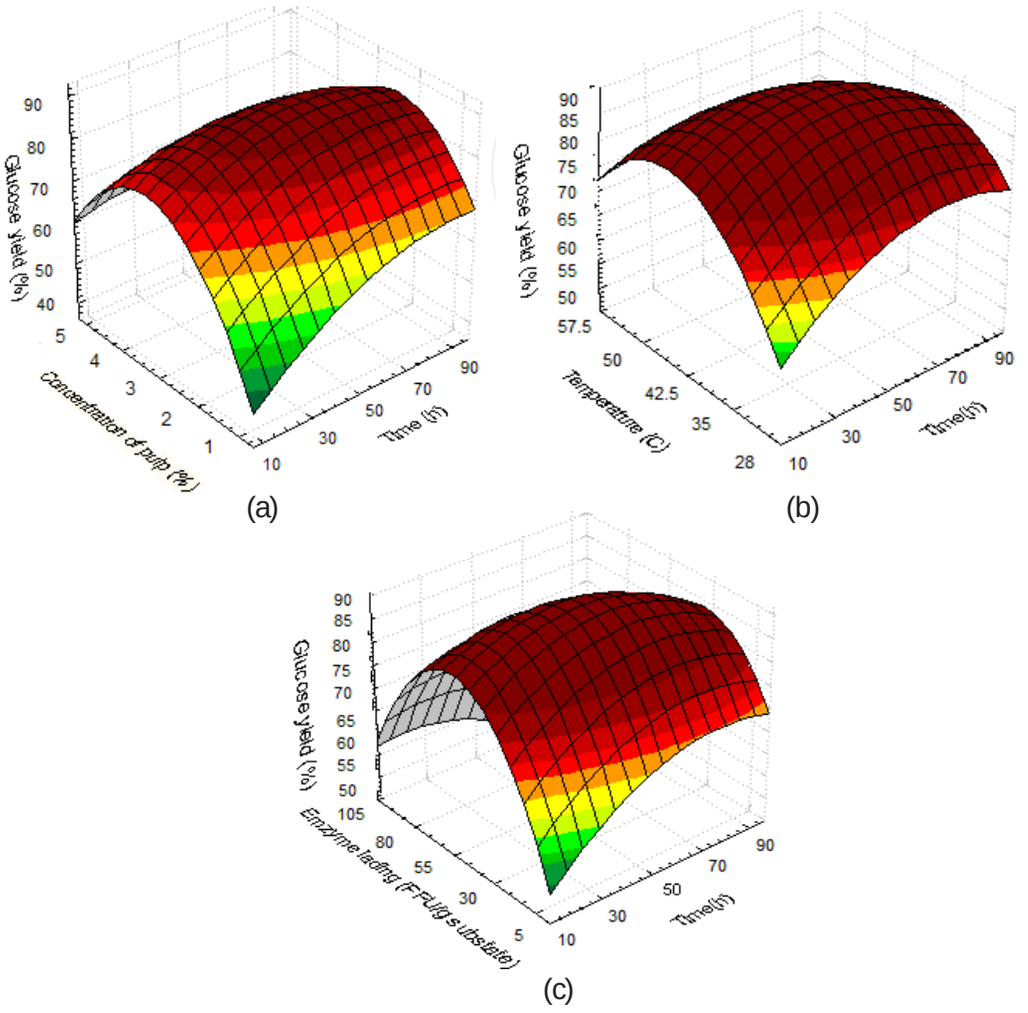

Figure 6. Response surface plots of glucose yield as a function concentration of pulp and time (a), temperature and time (b), enzymatic loading and time (c), other fixed variables

The summary result from combination of each response surface plots showed that the optimum condition of enzyme hydrolysis for NaOH and KOH delignification were 54 FPU/g substrate, 65 FPU/g substrate enzyme concentation, 50 h, 60 h reaction time, 50ºC reaction temperature and 2.5% pulp concentration, respectively. The maximum glucose contents were 47.50% and 48.00% from NaOH and KOH delignification, respectively. The maximum glucose yield obtained were 85%, 81% and 75% from NaOH and KOH delignification and without delignification respectively. Both optimization conditions in enzyme hydrolysis of delignification pulp by NaOH and KOH used to hydrolyze undelignification pulp, the result showed that percent glucose yield was lower than those from delignified pulp about 8-10%.

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3.4. Ethanol fermentation

3.4.1. Production of ethanol from pure glucose

S. Cerevisiae TISTR 5339 was grown in YMB containing 50 g/l glucose, 20 g/l peptone and 10 g/l yeast extract. The experiment was performed at room temperature. The result of ethanol production was shown in Table 7.

Time (h)	Residual glucose (g/l)	Consumed glucose (g/g)	Ethanol (g/l)	Ethanol yield (%)
0	50.00	0.00	0.00	0.00
6	40.52	9.48	4.62	18.12
12	33.21	16.79	8.05	31.57
18	25.16	24.84	12.15	47.65
24	17.38	32.65	16.08	63.06
30	10.30	39.70	19.46	76.31
36	3.21	46.79	21.92	85.96
42	0.00	50.00	21.81	85.53
48	0.00	50.00	21.75	85.29
54	0.00	50.00	21.04	82.51

Table 8. Production of ethanol from 50 g/l of pure glucose by S. Cerevisiae TISTR 5339

$$\text{Ethanol yield} = \frac{\text{ethanol from experiment}}{\text{Theoretical ethanol}} \times 100$$

Table 8 showed the production of ethanol from 50 g/l of pure glucose by S. Cerevisiae TISTR 5339. The result showed that within 36 h of fermentation, the highest ethanol concentration was obtained at 21.92 g/l. The ethanol yield from calculation was 85.96%. This result indicated that S. Cerevisiae TISTR 5339 has 85.96% in capability to change pure glucose to ethanol.

3.4.2. Production of ethanol from hydrolyzed solution of NaOH and KOH delignified pulp and nondelignified pulp

The hydrolyzed solutions from three samples of pulp were concentrated to 50 g/l of glucose concentration. S. Cerevisiae TISTR 5339 was applied in the same amount as production of ethanol from pure glucose but pure glucose was replaced with the three hydrolyzed solutions. The fermentation solutions were analyzed by GC for ethanol concentration. The results were shown in Table 9.

Sample	Ethanol concentration (g/l)	Ethanol yield (%)
Nondelignification*	16.25	65.0
KOH delignification	16.42	65.7
NaOH delignification	16.35	65.4

*glucose obtained from steam exploded pulp that directly employed for ethanol production without delignification with NaOH or KOH.

Table 9. Comparison of resulting ethanol concentration (g/l) and yield (%) after 36 h fermentation

Comparison of resulting ethanol after 36 h fermentation of NaOH and KOH delignified pulp was shown. The obtained ethanol concentration and yield from non delignified pulp was 16.25 g/l and 65%, respectively. The fermentation of KOH delignified gave ethanol concentration and yield at 16.42 g/l and 65.7% whereas those obtained from NaOH delignified pulp were 16.35 g/l and 65.4%, respectively. Alkaline delignification process showed no significant influence on the fermentation process. When the ethanol yield obtained from pure glucose and hydrolyzed solution (Table 8 and Table 9) were compared, it showed that the three hydrolyzed solutions gave lower ethanol yield due to the three hydrolyzed solution contained toxic substances such as furfural, 5-hydroxy methyl furfural, phenolic compound and acetic acid derived from steam explosion process. All substances can inhibit the fermentation of S. Cerevisiae TISTR 5339.

4. Conclusion

From this study on optimization of alkaline delignification and enzyme hydrolysis on steam exploded oil palm trunk followed by yeast fermentation to produce ethanol, an encouraging results were obtained. We concluded that delignification of steam explode pulp with NaOH was more influenced than delignification with KOH on term of less alkaline concentration consuming and lower reaction temperature. The comparison enzyme hydrolysis condition for both delignified pulp showed that NaOH delignified pulp gave higher the percentage of glucose yield than KOH delignified pulp. After optimizing the both delignification (NaOH and KOH) and both enzyme hydrolysis parameter by RSM, the highest ethanol yield of 65% were obtained from both fermentations with 50 g/l of glucose raw material.

The empirical quadratic models successfully predicted the percentage of glucose in pulp after delignification and the percentage of glucose in hydrolyzed solution after enzyme hydrolysis and they used in the development of better estimation tools. In addition, response surface plot in three-dimension obtained from the empirical quadratic models can show the interaction effect of two variables on the studied response and the optimum values of the selected variables are obtained from response surface plot, too.

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