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Optimization of Pre-treatment Process of Cocoa Pod Husk Using Various Chemical Solvents

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***Abstract*— The purpose of research is to see the effect of type of reagent (NaOH, H_2O_2 and H_2SO_4) and the condition of pre-treatment of cocoa pod husk towards lignin content after pre-treatment and hydrolysis, reducing sugar and total sugar content. Response Surface Method (RSM) was used to optimize process conditions of pre-treatment (delignification). Hydrolysis for all pre-treated sample were carried out using 3% H_2SO_4 with a ratio of cocoa pod husk to solvent (1:10) for 2 hours at a temperature of 110 ^oC using an autoclave. The chemical pre-treatment with NaOH was optimized by varying the concentrations of NaOH (4-8% (w/v), centre point: 6%), reaction time (60-100 minutes, centre point: 80 minutes) and ratio of biomass to solvent (1:15-1:25, centre point 1:20 w/v). The optimum conditions in this study was at the concentration of NaOH (X_1) of 4% w/v, reaction time (X_2) of 100 minutes; ratio of biomass/solvent (X_3) of 1:25 (w/v). The lignin content after pre-treatment was 15.03% lignin, lignin content after hydrolysis was 19.57%, 11.75% of reducing sugar, and 12.78% of total sugar. The chemical pre-treatment with alkaline peroxide (H_2O_2) was optimized by varying the concentrations of H_2O_2 (4-7% w/v, centre point 5.5% (w/v)), reaction time (40-90 minutes, centre point: 65 minutes), and ratio of biomass/solvent (4-7% w/v). The optimum conditions in this study was at the concentration of H_2O_2 concentration (X_1) of 5.52% w/v, reaction time (X_2) of 61.97 minutes, biomass loading in solvent (X_3) 7% w/v. The lignin content after pre-treatment was 8.759, lignin content after hydrolysis was 25.029%, 8.169% of reducing sugar, and 10.371% of total sugar. The chemical pre-treatment with H_2SO_4 was optimized by varying the concentrations of H_2SO_4 (0.5-1.5% w/v), reaction time (60-120 minutes, centre point: 90 minutes), and ratio of biomass to solvent (1:4-1:6 w/v, centre point 1:5 w/v). The optimum conditions was reached without hydrolysis. The optimum condition this study is at the H_2SO_4 concentration (X_1) of 1.5%, reaction time (X_2) of 120 minutes, ratio of biomass/solvent (X_3) of 6%. The lignin content after pre-treatment was 18.8%, 15.59% of reducing sugar and 20.49% of total sugar.**

***Keywords*— chemical pre-treatment; bioethanol; cocoa pod; response surface method; optimization**

I. INTRODUCTION

Lignocellulosic waste materials including various agricultural residues are the most abundant renewable source of biomass. Among them, cocoa pod is potential biomass feedstock in Indonesia since Indonesia is the third largest cocoa producer in the world. Adzimah and Asiam [1] reported that the weight of cocoa pod husk (CPH) and cocoa beans are 14.71% and 10.93%, respectively. It means is that there is a potential of about 1,067 million tons of CPH a year from cocoa plantation in Indonesia.

Lignocellulosic waste material can be converted into bioethanol. The process of converting lignocellulosic materials into ethanol consists of three stages: pre-treatment (delignification), saccharification or hydrolysis of cellulose into simple sugars, and the fermentation of sugars. Pre-treatment aims to eliminate lignin, reduce cellulose crystallinity, and increased porosity [2].

Good pre-treatment process is a process that can reduce the use of enzymes that are expensive [3]. Cardona and Sanchez [4] states that phase is a major challenge in the conversion of lignocellulosic biomass into bioethanol. Pre-treatment is one of the most expensive processes in the manufacture of bioethanol. Therefore, the efficiency of this process is expected to reduce the cost so that bioethanol can be competitive [5].

An effective and economical pre-treatment should meet the following requirements: (a) production of reactive cellulosic fibre for enzymatic attack, (b) avoiding destruction of hemicelluloses and cellulose, (c) avoiding formation of possible inhibitors for hydrolytic enzymes and fermenting microorganisms, (d) minimizing the energy demand, (e) reducing the cost of size reduction for feedstock's, (f) reducing the cost of material for construction of pre-treatment reactors, (g) producing less residues, (h) consumption of little or no chemical and using a cheap chemical [6].

Pre-treatment can be carried out in physical, physicochemical, chemical, biological or a combination of these ways [7]. Pre-treatments in this study were conducted using chemical solvent (NaOH, H_2O_2 and H_2SO_4). while chemical hydrolysis was performed using H_2SO_4 as a solvent.

Several methods have been Introduced for various chemical pre-treatment of lignocellulosic materials with different types of solvents [8 - 14]. This study focused on chemical pre-treatment for cocoa pod husk as a potential biomass feedstock in Indonesia. The purpose of this research is to see the effect of type reagents, reaction time and conditions of pre-treatment towards lignin content in pretreated biomass, lignin content after hydrolysis reducing sugar and total sugar content of sugar hydrolyzate produced from cocoa pod husk.

II. Material and Method

A. Materials

Cocoa pod was obtained from Cocoa Plantation at Lubuk Minturun, Padang-INDONESIA. Cocoa Pod are washed with water till clean, then cut into small pieces and then dried by solar drying until the moisture content of ± 10%. The dried material is milled with a grinder. Cocoa pod husk was sieved to 40 mesh size.

The composition of the cocoa pod husk used in this study compared with the result from previous research is presented in Table 1.

TABLE I
THE COMPOSITION OF THE COCOA POD HUSK USED IN THIS STUDY

Component	Percentage (%)	*(%)	**(%)
Moisture Content	11.04	14.1	6.75
Ash	7.40	12.3	1.87
Crude Fiber	49.23	-	53.82
Cellulosa	44.69	35.4	28.78
Hemicellulosa	11.15	37.0	8.70
Lignin	34.82	14.7	42.90
Holocellulosa	55.84	74.0	-
Pectin***	10.1 ± 0.3		

source* : Daud *et al,* [13]
** : Syam [15]
***: Vriesmanna et al [16]

Before pre-treatment, the pectin of cocoa pod husk was extracted. Pectin extraction was carried out using citric acid as a solvent [17]. CPHs was weighed as much as 100 grams, then put it into the 3 neck flask and add solvent in accordance with a variable ratio of material and solvent, namely 1: 25, with citric acid as a solvent. Extraction time of 3 hours, pH 2.5 at a temperature of 95 °C. After that the pectin-free sample is dried at the dryer cabinet at a temperature of 60-700 C for 12 hours**.**The material was stored in plastic bags at room temperature until use for pre-treatment.

B. Research Steps

To produce bioethanol there are several stages which includes preparation of raw materials, pre-treatment, hydrolysis, fermentation of sugars, distillation. Figure 1 presented several stages employed in this study.

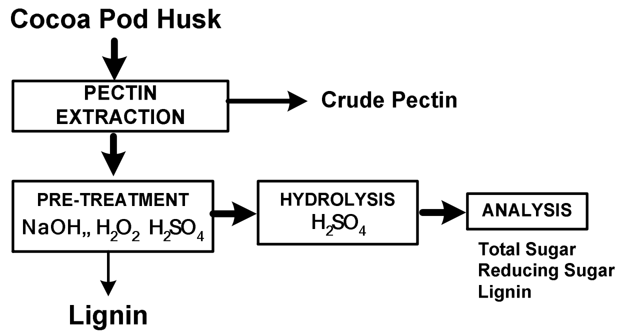

Fig. 1. Research Steps of Pre-treatment Process

C. Pre-treatment

The effects of pre-treatment solvent, sodium hydroxide, alkaline peroxide and sulphuric acid pre-treatment were tested. The effect of pre-treatment parameters including: solvent concentration, reaction time, and solid mass loading in solvent were evaluated. Box-Behnken methodology was used for the design and Response Surface Modelling was applied to determine the effect of the pre-treatment parameters on lignin and sugar content.

Sodium Hydroxide Pre-treatment-Pre-treatment CPHs was pretreated at 121 ^oC in an autoclave. Fine powder coco pod husks is put into the beaker glass. add a solution of NaOH at various concentration of 2.64; 4; 6, 8; and 9.36% w/v, reaction time of 45; 36; 60; 80; 100 and 113.64 minutes, biomass loading ratio to solvent of 1:10; 1:15; 1:20; 1: 25 and 1:30. Cocoa pod husks that have been pretreated was then separated into different phase: solid phase and liquid phase by using a vacuum filter. Solid phase was washed with distilled water until a neutral pH and drying in the oven at temperature of 105 ^oC for 6 hours.

The chemical pre-treatment with NaOH was optimized by varying the concentrations of NaOH (4-8% (w/v), centre point: 6%), reaction time (60-100 minutes, centre point: 80 minutes) and ratio of biomass to solvent (1:15-1:25, centre point 1:20 w/v).

Alkaline peroxide pre-treatment- 5 g of 20 mesh-sized solids were transferred into 100 ml flasks containing 100 ml of alkaline peroxide solutions at pH 11.5. Flask were placed in an orbital shaker at a speed range of 180 rpm and pretreated until desired retention time has been reached. At the end of the retention time flasks were removed from the shaker and the solid and liquid fractions were filtered through 10 µm pore-sized filters. The solid fractions were washed with de-ionized water until they reached neutral pH. After washing, the solid samples were dried in an oven at 50 °C for 48 h and used as feedstockd for hydrolysis.

The chemical pre-treatment with alkaline peroxide H_2O_2 was optimized by varying the concentrations of H_2O_2 (4-7% w/v, centre point 5.5% (w/v)), reaction time (40-90 minutes, centre point: 65 minutes), and ratio of biomass/solvent (4-7% w/v).

Sulfuric acid pre-treatment- Sulfuric acid pre-treatment was performed in an autoclave at 121 °C. After autoclave the remaining solid residues were separated by vacuum filtration using 10 µm pore-sized filters and the solid fractions washed with de-ionized water until they reached neutral pH. Solid recovery yield was calculated as dry weight of water insoluble solid remaining after pre-treatment referred to 100 g of untreated raw material.

The chemical pre-treatment with H_2SO_4 was optimized by varying the concentrations of H_2SO_4 (0.5-1.5% w/v, centre point: 1.0% w/v), reaction time (60-120 minutes, centre point: 90 minutes), and ratio of biomass to solvent (1:4-1:6 w/v, centre point 1:5 w/v).

D. Hydrolysis

Sulphuric acid pre-treatment was performed in an autoclave at 121 °C. Put 3 grams sample into 250 ml Erlenmeyer and added a solution of H2SO4 3% with the ratio of biomass to solvent (1:10) for 2 hours. After that, the liquid phase of hydrolysis reaction result was separated from the solid phase. The liquid phase was used to test reducing sugar and total sugar.

E. Experimental Design

The experimental design selected for this study is a central composite design (CCD) that helps in investigating linear, quadratic, cubic and cross-product effects of the three pre-treatment process variables (independent) on the content of lignin and sugar (response). The three pre-treatment process variables studied are solvent concentration, reaction time, ratio of solid biomass to solvent. Each response of the pre-treatment process was used to develop a mathematical model that correlates the delignified lignin and sugar content to the pre-treatment process variables studied through first order, second order and interaction terms, according to the following second order polynomial equation,

$$y = ß_o + \sum_{j=1}^{3} ß_i x_i + \sum_{ij=1}^{3} ß_{ij} x_i x_j + \sum_{j=1}^{3} ß_{jj} x_j^2$$

where y is the predicted yield of response, xi and xj represent the variables or parameters ßo is the offset term ßj is the linear effect, ßij is first order interaction effect, ßjj is a squared effect.

F. Model fitting and statistical analysis

Design Expert software version 7 (STAT-Ease Inc., Minneapolis, USA) was used for regression analysis of the experimental data to fit the second order polynomial equation and also for evaluation of the statistical significance of the equation developed.

G. Analysis method

Determination of pre-treated lignin after pre-treatment [18]. The isolation of lignin from black liquor was performed using precipitation method using H_2SO_4. The first step, of black liquor was diluted using aquades with a ratio (1: 2). Then stirring using a stirrer using H_2SO_4 20% solution until the pH of the solution to became 2 and heating to a temperature of 60 ° with hot magnetic stirrer. After titration, allow at least 8 hours in order lignin completely precipitated. After lignin precipitated, it is filtered with filter paper and dried in an oven at a temperature of 50-60 ° C for 24 hours and then weighed.

$$\textbf{Lignin (\%)} = \frac{\textbf{Lignin weight (g)}}{\textbf{Cocoa pod husk weight (g)}}$$

Determination of precipitated lignin after hydrolysis-The lignin content in samples after hydrolysis was measured using TAPPI-Standard T 13 m-54.

Estimation of sugars- Total sugars in the filtrate were determined by the method of Dubois et al. [19] and reducing sugars in the filtrate were estimated by using 3, 5 dinitrosalysilic acid method [20].

III. RESULT AND DISCUSSION

A. Optimization of Sodium Hidroxide Pre-treatment

The experimental design matrix and effect of effects of sodium hydroxide pre-treatment of CPHs at different sodium hydroxide concentration, reaction time and solid loading in solvent is shown in Table II.

TABLE II
EFFECTS OF SODIUM HYDROXIDE PRE-TREATMENT OF CPH AT DIFFERENT SODIUM HYDROXIDE CONCENTRATION, REACTION TIME AND SOLID LOADING RATIO TO SOLVENT

Run	NaOH (%)	Reaction time (minutes)	Biomass Loading in Sovent	Lignin*		Reducing Sugar	Total sugar
				I	II		
1	4	60.00	1:15	11.65	22.80	5.19	8.39
2	8	60.00	1:15	13.9	20.53	5.14	7.51
3	4	100.00	1:15	11.7	22.76	10.69	11.21
4	8	100.00	1:15	16	18.43	5.09	6.79
5	4	60.00	1:25	15.97	18.49	8.18	8.44
6	8	60.00	1:25	15.01	17.88	4.73	6.8
7	4	100.00	1:25	16.18	18.23	12.94	13.59
8	8	100.00	1:25	16.68	17.61	4.88	7.42
9	2.64	80.00	1:20	11.55	22.72	9.31	11.37
10	9.36	80.00	1:20	17.01	16.27	5.26	6.41
11	6	46.36	1:20	14.42	19.96	4.09	9.26
12	6	113.64	1:20	14.26	20.15	5.89	8.51
13	6	80.00	1:11.59	10.87	23.72	5.74	7.55
14	6	80.00	1:28.41	14.11	20.21	6.28	7.28
15	6	80.00	1:20	12.8	21.7	6.76	10.02
16	6	80.00	1:20	12.67	21.81	6.68	9.38
17	6	80.00	1:20	12.15	22.25	6.61	9.71

*) I Precipitated lignin after Pre-treatment
II Lignin content after Hydrolysis

Lignin is a chemical component contained in a plant. It is not soluble in most organic solvents. Lignin which protects the cellulose is resistant to hydrolysis since there is aryl alkyl bonds and ether bond [21]. Lignin in cocoa pod sample will inhibit the enzymatic or acid hydrolysis process to convert cellulose into simple sugars. Therefore it is necessary to do a pre-treatment process (delignification) using sodium hydroxide (NaOH). Pre-treatment increased the effectiveness of cellulose hydrolysis and improve the enzymatic digestibility of biomass for fermentable sugars [10].

As seen in Table II, amount of sugars leaving the raw material and entering the liquid fraction changed with respect to the pre-treatment condition [10, 22]. Increasing biomass loading in solvent resulted in decrease especially in precipited lignin and decrease lignin content which remains in biomass [Table II].

Application and Selection of RSM Model- RSM is used to determine the appropriate model in predicting response. The model obtained is used to predict the response levels of precipitated lignin, the lignin content remains after hydrolysis, reducing sugar and total sugar content. The process of selecting the model is based on the description sequential model of sum of square, lack of fit test, and a summary of the model (model summary statistics) and test the statistical base (R-squared) [23]. Table III are presented the analysis of variance of response surface models.

TABLE III
ANALYSIS OF VARIANCE RESPONSE SURFACE MODEL

Source of variance	P values Prob < F			
	Precipitated Lignin[1]	Lignin Content[1]	Reducing Sugar[2]	Total Sugar[3]
Model	0.0099[s]	0.0065[s]	0.0017[s]	0.0048[s]
A-NaOH concentration	0.0037	0.0015	0.0003	0.0004
B-Reaction Time	0.3277	0.5482	0.013	0.134
C-Biomass loading ratio to solvent	0.0028	0.0018	0.240	0.650
AB	0.2404	0.4915	0.013	0.026
AC	0.0374	0.1017	0.114	0.437
BC	0.9242	0.5900	0.876	0.264
A^2	0.0265	0.0105		
B^2	0.0239	0.0262		
C^2	0.5654	0.5396		
Lack of Fit	0.0509[ns]	0.0879[ns]	0.0531[ns]	0.0664[ns]
R^2	0.91	0.90	0.84	0.80

[s)]significant [ns)] non-significant
[1)]after pre-treatment; [2)]after hydrolysis

Response Surface Optimization - Table IV outlines optimization criteria of variables and responses which will be optimized in this study. It can be seen the goal and importance level of each variable and the response to get the solution from variables that produces the optimal response.

TABLE IV
OPTIMIZATION CRITERIA OF VARIABLES AND RESPONSES

Criteria	Goal	Upper level	Lower level	The importance level
Concentration NaOH(%)	*In range*	4	8	3 (+++)
Reaction Time (minutes)	*In range*	60	100	3 (+++)
biomass loading ratio to solvent (%)	*In range*	15	25	3 (+++)
Precipitated lignin after pre-treatment (%)	*maximize*	10.87	17.01	3 (+++)
Lignin content remains after hydrolysis (%)	*minimize*	16.27	23.72	3 (+++)
Reducing Sugar (%)	*maximize*	4.09	12.94	3 (+++)
Total sugar (%)	*maximize*	6.41	13.59	3 (+++)

Variables such as NaOH concentration, reaction time, and the ratio of biomass to solvent is optimized with the goal (target component) in range with the important level 3 (+++). This is due to the possibility of an optimal response is not generated at a central point, but there is the suggested specified value other than central point. In this study, the response of precipitated lignin, total lignin, reducing sugar and total sugar goal is set as maximize because from the delignification process is expected to accrue a maximum precipitated lignin, with maximal reducing sugar and total sugar. while the remaining lignin content expected to be minimal.

After all the variables and the response is determined of its important level and its goal, the program will analyze the optimum solution by looking at the value of desirability. The optimal solution is obtained after being processed by the Design Expert 7 can be seen in Table V.

TABLE V
OPTIMAL SOLUTION ACCORDING TO DESIGN EXPERT 7 SOFTWARE

Concentration NaOH (%)	Reaction Time (minutes)	Biomass loading ratio to solvent (%)	Precipitated lignin (%)	Lignin (%)	Reducing sugar (%)	Total sugar (%)	Desirability
4	100	25	15.03	19.58	11.75	12.77	0.73
4	99.99	24.96	15.01	19.60	11.74	12.77	0.73
4.04	100	25	15.01	19.60	11.67	12.72	0.73

Based on the optimization solution analysis by Design-Expert 7 Software it was found that there were three desirability value displayed Table IX. Desirability intended to reach the value of 1.0. The treatment chosen as an optimum treatment solution by Design-Expert 7 Software is seen in the first solution where NaOH concentration factor (X_1) showed a concentration of 4.00%, and the reaction time (X_2) 100 minutes, biomass ratio to solvent (X_3) of 1: 25 with the desirability of 0.974. These results match with the results from verification in laboratory.

B. Optimization of Alkali Peroxide Pre-treatment

The design of Pre-treatment Process Optimization-The experimental design matrix and effect of alkaline peroxide pre-treatment of CPHs at different sodium peroxide concentration, reaction time and solid loading in solvent is shown in Table VI. Amount of sugars leaving the raw material and entering the liquid fraction changed with respect to the pre-treatment condition.

TABLE VI
EFFECTS OF ALKALINE PEROXIDE PRE-TREATMENT OF RICE STRAW AT DIFFERENT HYDROHEN PEROXIDE CONCENTRATION, REACTION TIME, AND SOLID LOADING RATIO TO SOLVENT

STD	H_2O_2 (%)	Reaction Time (minutes)	Ratio of Biomass loading (%)	Precipitated Lignin (%)	Lignin Content (%)	Reducing Sugar (%)	Total Sugar (%)
1	4	40	4	11	22.9	5.28	12.97
2	7	40	4	10.5	24.29	6.71	8.97
3	4	90	4	11.47	23.35	5.04	6.47
4	7	90	4	10.04	24.85	5.61	7.51
5	4	40	7	9.86	24.8	4.44	7.55
6	7	40	7	6.86	27	10.91	12.77
7	4	90	7	8.43	26.36	7.21	10.03
8	7	90	7	7	27.7	9.39	11.23
9	2.98	65	5.5	9.69	25.25	3.34	9.02
10	8.02	65	5.5	6.73	27	6.89	11.02
11	5.5	22.96	5.5	7.64	26.34	6.49	8.59
12	5.5	107.04	5.5	7.27	25.92	5.94	5.71
13	5.5	65	2.98	13.09	21.5	4.61	16.26
14	5.5	65	8.02	8.48	23.76	11.18	15.06
15	5.5	65	5.5	9.93	24.33	5.33	8.14
16	5.5	65	5.5	9.71	25	5.99	7.19
17	5.5	65	5.5	9.11	24.83	6.46	7.21

Application and Selection the Models- Table VII presented the analysis of variance of response surface models. From Table VII it can be seen that the mathematical model for the all observed response surface is significant. H_2O_2 concentration significantly affected the precipitated of lignin, the lignin content, reducing sugar and total lignin.

Ration of biomass loading in solvent affected significantly precipitated lignin, the lignin content remains after hydrolysis, reducing sugar and total sugars.

TABLE VII
ANALYSIS OF VARIANCE RESPONSE SURFACE MODEL

Source of Variance	P values Prob < F			
	Precipitated Lignin[1]	Lignin Content[1]	Reducing sugar[2]	Total Sugar[3]
Model	0.0008*	0.0031*	0.0024*	0.0434*
A-Concentration of H_2O_2	0.0012*	0.0062*	0.0011*	0.3482
B-Reaction Time	0.4102	0.3258	0.7543	0.1772
C-Biomass loading ratio to solvent	<0.0001**	0.0006**	0.0003**	0.6081
AB	0.7117	0.6983	0.0678	0.8499
AC	0.1764	0.7366	0.0271*	0.1139
BC	0.4599	0.5224	0.3138	0.1303
A^2	0.0880	0.0287	0.4497	0.3717
B^2	0.0099	0.0284	0.4796	0.5816
C^2	0.0147	0.0090	0.0171	0.0025
Lack of Fit	0.3318[ns]	0.1891[ns]	0.2926[ns]	0.0612[ns]
R^2	0.9526	0.9278	0.9331	0.8336

[s)]significant [ns)] non-significant *significant **very significant
[1)]after pre-treatment; [2)]after hydrolysis

Response Surface Optimization-Table VIII outlines optimization criteria of variables and responses which will be optimized in this study. It can be seen the goal and importance level of each variable and the response to get the solution from variables that produces the optimal response.

TABLE VIII
OPTIMIZATION CRITERIA OF VARIABLES AND RESPONSES

Criteria	Goal	Lower level	Upper level	The importance level
Concentration H_2O_2 (%)	*In range*	4	8	3 (+++)
Reaction Time (minutes)	*In range*	60	100	3 (+++)
Ratio biomass loading to solvent	*In range*	15	25	3 (+++)
Precipatated lignin (%)	*maximize*	6.72	13.09	3 (+++)
Lignin content after hydrolysis (%)	*minimize*	21.5	11.18	3 (+++)
Reducing sugar (%)	*maximize*	334	12.94	3 (+++)
Total sugar (%)	*maximize*	6.47	16.26	3 (+++)

Variables such as H2O2 concentration, reaction time, and the ratio of biomass to solvent is optimized with the goal (target component) in range with the important level 3 (+++). This is due to the possibility of an optimal response is not generated at a central point, but there is the suggested specified value other than central point. In this study, the response of precipitated lignin, total lignin, reducing sugar and total sugar goal is set as maximize because from the delignification process is expected to accrue a maximum precipitated lignin, with maximal reducing sugar and total sugar, while the remaining lignin content expected to be minimal.

After all the variables and the response is determined of its important level and its goal, the program will analyse the optimum solution by looking at the value of desirability. The optimal solution is obtained after being processed by the Design Expert 7 can be seen in Table IX.

TABLE IX
OPTIMAL SOLUTION ACCORDING TO DESIGN EXPERT 7 SOFTWARE

H_2O_2 (%)	Reaction Time (minutes)	Ratio Biomass/solvent (%)	Precipitated lignin (%)	Lignin Content (%)	Reducing Sugar (%)	Total Sugar (%)	Desirability
5.52	61.97	7.00	8.759	25.03	8.17	10.37	0.508
5.56	62.46	7.00	8.726	25.02	8.23	10.42	0.508
5.60	61.79	7.00	8.700	25.07	8.26	10.46	0.507

Based on the optimization solution analysis by Design-Expert 7 Software it was found that there were three desirability value displayed Table IX. Desirability intended to reach 1.0. The treatment chosen as an optimum treatment solution is by Design-Expert 7 Software which shows some of the factors that influence, seen in the first solution H_2O_2 concentration factor (X_1) showed a concentration of 5.52%, and the reaction time (X_2) 61.97 minutes, ratio of biomass to solvent (X_3) 7% with the desirability of 0.974. These results match with the results from verification in laboratory.

C. Optimization of Sulfuric Acid Pre-treatment

The experimental design matrix and effect of effects of alkaline peroxide pre-treatment of CPH at different sodium peroxide concentration, reaction time and solid loading in solvent is shown in Table X. Amount of sugars leaving the raw material and entering the liquid fraction changed with respect to the pre-treatment condition.

Application and Selection the Models- Table XI presented the analysis of variance of response surface models. From Table XI it can be seen that the mathematical model for the all observed response surface is significant. H_2SO_4 concentration significantly affected the precipitated of lignin, the lignin content, reducing sugar and total lignin. Ration of biomass loading in solvent affected significantly precipitated lignin, the lignin content remains after hydrolysis, reducing sugar and total sugars.

TABLE X
EFFECTS OF ALKALINE PEROXIDE PRE-TREATMENT OF RICE STRAW AT DIFFERENT HYDROHEN PEROXIDE CONCENTRATION, REACTION TIME, AND SOLID LOADING RATIO TO SOLVENT

STD	H_2SO_4 (%)	Reaction Time (minutes)	Ratio Biomass/solvent (%)	Total Sugar (%)	Reducing Sugar (%)	Lignin Content (%)
1	0.50	60.00	4.00	7.40	5.30	33.03
2	1.50	60.00	4.00	9.96	7.69	28.99
3	0.50	120.00	4.00	9.27	6.77	31.85
4	1.50	120.00	4.00	14.62	6.41	31.98
5	0.50	60.00	6.00	11.63	10.21	23.93
6	1.50	60.00	6.00	18.85	16.32	19.20
7	0.50	120.00	6.00	13.96	11.85	23.09
8	1.50	120.00	6.00	19.92	11.70	22.97
9	0.16	90.00	5.00	6.00	5.20	32.88
10	1.84	90.00	5.00	18.85	16.74	19.77
11	1.00	39.55	5.00	8.96	6.75	31.32
12	1.00	140.45	5.00	14.99	12.51	21.88
13	1.00	90.00	3.32	9.34	7.20	28.28
14	1.00	90.00	6.68	16.56	14.13	20.34
15	1.00	90.00	5.00	16.17	14.67	20.81
16	1.00	90.00	5.00	15.06	13.05	21.45
17	1.00	90.00	5.00	14.77	11.54	23.41

TABLE XI

ANALYSIS OF VARIANCE RESPONSE SURFACE MODEL

Source of Variance	Value of P Prob < F		
	Lignin remains in biomass	Reducing sugar	Total Sugar
Model	0.0040	0.0034	< 0.0001
A-Concentration of H_2SO_4	0.0313	0.0128	< 0.0001
B-Reaction Time	0.3987	0.4811	0.0048
C-Biomass ratio to solvent	0.0018	0.0024	< 0.0001
Lack of Fit	0.1235	0.2762	0.1666
R^2	0.6280	0.6377	0.8840

Response Surface Optimization-Table XII outlines optimization criteria of variables and responses which will be optimized in this study. It can be seen the goal and importance level of each variable and the response to get the solution from variables that produces the optimal response

TABLE XII

DESCRIPTION OF VARIABLES AND RESPONSES OF OPTIMIZATION PROCESS

Criteria	Goal	Lower Level	Upper Level	The Importance Level
Concentration H_2SO_4 (%)	*In range*	0.5	1.5	3 (+++)
Reaction Time (minutes)	*In range*	60	120	3 (+++)
Ratio biomass loading to solvent	*Maximize*	4	6	3 (+++)
Total sugar (%)	*Maximize*	6.00	19.92	3 (+++)
Reducing sugar (%)	*Maximize*	5.20	16.74	3 (+++)
Lignin content remain in biomass. (%)	*Minimize*	19.2	33.03	3 (+++)

Based optimization solution analysis by Design-Expert 7 Software it was found that there were three desirability value displayed Table XIII. Desirability intended to reach 1.0. The treatment chosen as an optimum treatment solution is by Design-Expert 7 Software which shows some of the factors that influence, seen in the first solution H_2SO_4 concentration factor (X_1) showed a concentration of 1.50%, and the reaction time (X_2) 120 minutes, ratio of biomass to solvent (X_3) 6% with the desirability of 0.974. These results match with the results from verification in laboratory.

TABLE XIII

OPTIMAL SOLUTION ACCORDING TO DESIGN EXPERT 7 SOFTWARE

H_2SO_4 (%)	Reaction Time (minutes)	Bimass Loading Ratio (%)	Total sugar (%)	Reducing Sugar (%)	Lignin remained in biomass (%)	Desirability
1.50	120.00	6.00	20.49	15.60	18.87	0.974
1.50	119.54	6.00	20.47	15.59	18.88	0.974
1.49	120.00	6.00	20.41	15.54	18.92	0.973

Based on the optimization solution analysis by Design-Expert 7 Software it was found that there were three desirability value displayed. Desirability intended to reach 1.0. The treatment chosen as an optimum treatment solution is by Design-Expert 7 Software which shows some of the factors that influence, seen in the first solution H_2SO_4 concentration factor (X_1) showed a concentration of 1.50%, and the reaction time (X_2) 120.00 minutes, biomass comparison with solvent (X_3) 6.00% with the desirability of 0,974. These results match with the results from verification in laboratory.

IV. CONCLUSIONS

The NaOH chemical pre-treatment was optimized by varying the concentrations of NaOH (4-8% (w/v), centre point: 6%), reaction time (60-100 minutes, centre point: 80 minutes) and ratio of biomass to solvent (1:15-1:25, centre point 1:20 w/v). The optimum conditions in this study was at the concentration of NaOH (X_1) of 4% w/v, reaction time (X_2) of 100 minutes; ratio of biomass/solvent (X_3) of 1:25 (w/v). The lignin content after pre-treatment was 15.03% lignin, lignin content after hydrolysis was 19.57%, 11.75% of reducing sugar, and 12.78% of total sugar.

The chemical pre-treatment with alkaline peroxide (H_2O_2) was optimized by varying the concentrations of H_2O_2 (4-7% w/v, centre point 5.5% (w/v)), reaction time (40-90 minutes, centre point: 65 minutes), and ratio of biomass/solvent (4-7% w/v). The optimum conditions in this study was at the concentration of H_2O_2 concentration (X_1) of 5.52% w/v, reaction time (X_2) of 61.97 minutes, biomass loading in solvent (X_3) 7% w/v. The lignin content after pre-treatment was 8.76, lignin content after hydrolysis was 25.03%, 8.17% of reducing sugar, and 10.37% of total sugar.

The chemical pre-treatment with H_2SO_4 was optimized by varying the concentrations of H_2SO_4 (0.5-1.5% w/v), reaction time (60-120 minutes, centre point: 90 minutes), and ratio of biomass to solvent (1:4-1:6 w/v, centre point 1:5 w/v). The optimum conditions was reached without hydrolysis. The optimum condition this study is at the H_2SO_4 concentration (X_1) of 1.5%, reaction time (X_2) of 120 minutes, ratio of biomass/solvent (X_3) of 6%. The lignin content after pre-treatment was 18.8%, 15.59% of reducing sugar and 20.49% of total sugar.

This research findings could serve as protocol for pretreatment process in further process design of bioethanol production process from cocoa pod husk using various solvent. Analysis of mass and material balance is important factor to study for practical application of this finding.

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