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Optimization of Two-Stage Pretreatment Combined with Microwave Radiation Using Response Surface Methodology

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***Abstract*—**Pretreatment is an essential step in the conversion of lignocellulosic biomass to fermentable sugar that used for biobutanol production. Among pretreatment processes, microwave is considered to improve pretreatment efficiency due to its high heating efficiency, easy operation, and easily to combine with chemical reaction. The main objectives of this work are to investigate the feasibility of microwave pretreatment to enhance enzymatic hydrolysis of corncobs and to determine the optimal conditions using response surface methodology. Corncobs were pretreated via two-stage pretreatment in dilute sodium hydroxide (2 %) followed by dilute sulfuric acid 1 %. Pretreated corncobs were subjected to enzymatic hydrolysis to produce reducing sugar. Statistical experimental design was used to optimize pretreatment parameters including temperature, residence time and solid-to-liquid ratio to achieve the highest amount of glucose. The results revealed that solid-to-liquid ratio and temperature had a significant effect on the amount of glucose.

***Keywords*—**Corncobs, Microwave radiation, Pretreatment, Response Surface Methodology.

I. Introduction

RECENTLY, Second generation biofuels from lignocellulosic biomass have received much attention because this biofuels are renewable energy source derived from natural material which can be used as a substitute for petroleum fuels. Moreover, lignocellulosic biomass is low cost, abundance and non-polluting [1]. Biobutanol is an attractive renewable liquid transportation biofuel. It has a better energy density and performance than ethanol and can be made from more sustainable feedstocks than biodiesel. Hence, biobutanol has the potential to substitute for both ethanol and biodiesel [2].

Corncobs, mainly composed of cellulose, hemicellulose, and lignin. It is one of the potential lignocellulosic biomass that can be used to produce biobutanol by the Acetone–Butanol–Ethanol (ABE) fermentation. Typically, biobutanol production from lignocellulosic biomass requires the enzymatic hydrolysis of carbohydrate polymers into reducing sugar or fermentable sugars. Lignocellulosic biomass consist mainly of cellulose, hemicellulose, and lignin that are closely associated in a complex crystalline structure which results in enzymatic hydrolysis accessibility limited. Therefore, pretreatment is required to alter the structure of lignocellulosic biomass to make cellulose more accessible to the enzymes. Pretreatments of lignocellulose using various alkaline or acidic reagents have been evaluated to improve the accessibility of the enzymes to the lignocellulosic biomass. Alkali pretreatment is used to remove lignin and various uronic acid substitutions on hemicellulose that lower enzyme accessibility [3]. Whereas, acid pretreatment includes concentrated and dilute acids to disrupt the rigid structure of the lignocellulosic biomass and solubilize hemicellulose and expose cellulose for enzymatic hydrolysis [4]. Microwave is an alternative method to improve pretreatment efficiency owing to its high heating efficiency, easy operation, and rapid heating which accelerates the recalcitrant structure disruption. In addition, microwave could be easily to combine with chemical reaction [5].

In this study, response surface methodology (RSM) with a full factorial central composite design (CCD) was applied to optimize the second stage condition of two-stage pretreatment to maximize glucose concentration. RSM is a statistical tool for designing experiments, building empirical models, and evaluating effects of factors [6].

II. Materials and Methods

A. Materials

Corncobs were supplied by Betagro Corporation Limited (Thailand) and stored at ambient temperature. Prior to pretreatment process, corncobs was dried at 105°C for 24 h to remove moisture.

B. First Stage: Microwave/Alkali Pretreatment

Dried corncobs were pretreated with 2 % (w/v) NaOH solution using a solid-to-liquid ratio (SLR) 67:1, g corncobs: L of solution. Then, the mixture of corncobs and NaOH solution was transferred to a microwave oven to treat corncobs samples at 100 °C for 30 min. After that the mixture was filtered to separate solid residues out. The solid residues were thoroughly washed with tap water to neutral pH and

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dried in the oven at 105°C for 24 h Then, the oven-dried samples were stored for further dilute acid pretreatment in second stage of two-stage pretreatment.

C. Second Stage: Microwave/Acid Pretreatment

The solid residues from the microwave/alkaline pretreatment were treated with 1% H_2SO_4 using different SLR (25 to 125). Then, it was transferred to a microwave oven (at 80 °C to 160 °C) for 5 to 25 min.

D. Enzymatic Hydrolysis

The mixture from two-stage pretreatment was mixed with NaOH to adjust the pH of the slurry to 4.8 and then Celluclast 160 µl/g pretreated corncobs (cellulase; Sigma Chemicals, 52 FPU) was added. After that the sample was shaked in the incubator shaker at 50°C for 60 h. The hydrolysate was filtered to separate solid residues out. Then, the liquid fraction was collected for sugar analysis using HPLC (Perkin Elmer LC200) equipped with a refractive index detector and Aminex HPX-87H column under a flow rate of 0.60 ml/min, mobile phase 0.005 M H_2SO_4, and oven temperature 60°C.

E. Response Surface Methodology (RSM)

RSM with a full factorial central composite design (CCD) was employed in this study. The series of experiments designed and conducted are shown in Table I. The metrix corresponding to CCD is presented in Table II. Twenty experiments were carried out with three variables, and each variable varied at five levels for glucose concentration therefore, the concentration of glucose was the dependent variables. CCD was conducted to determine the individual and interactive effects of three parameters on glucose concentration. Equation (1) was used to correlate the dependent and independent variables.

$$Y_i = a_0 + \sum_{i=1}^{k} a_i x_i + \sum_{i=1}^{k} a_{ii} x_i^2 + \sum_{i}^{k} \sum_{j}^{k} a_{ij} x_i x_j \qquad (1)$$

Where Y_i is the response; x_i, x_j are the input variables, which influence the response variable Y_i; a_0 is the offset term; a_i is the *i*th linear coefficient; a_{ii} is the quadratic coefficient and a_{ij} is the *ij*th interaction coefficient.

TABLE I
INDEPENDENT VARIABLES AND THEIR LEVELS IN THE EXPERIMENTAL DESIGN

Independent variable	Symbols	Unit	Code Levels				
			-2	-1	0	1	2
Temperature	x_1	°C	80	100	120	140	160
Time	x_2	min	5	10	15	20	25
SLR	x_3	g/l	25	50	75	100	125

III. RESULTS AND DISCUSSION

A. Optimization of the Glucose Concentration Using RSM

In this research, second stage of two-stage pretreatment was employed to enhance the efficiency of pretreatment. RSM with a central composite design (CCD) was conducted to examine the effect of temperature, time, and SLR of second stage on glucose concentration. The experimental design and results of CCD are summarized in Table II. The polynomial equation explains the glucose concentration of second stage pretreatment (Y_1) as a function of temperature, time, and SLR of two stage pretreatment that is shown in (2).

$$Y_1 = 39.05 + 2.9625x_1 - 0.2131x_2 + 6.43x_3 - 1.2292x_1^2 - 1.9723x_2^2 - 3.2792x_3^2 + 0.93\,x_1x_2 + x_1x_3 - 0.5075x_2x_3 \qquad (2)$$

where x_1 is the second stage pretreatment temperature (°C), x_2 is the second stage pretreatment time (min), and x_3 is the second stage pretreatment SLR (g/l).

TABLE II
EXPERIMENTAL DESIGN AND RESULTS OF THE CENTRAL COMPOSITE DESIGN OF SECOND STAGE OF TWO-STAGE PRETREATMENT

Run	Variables			Response
	Temp. (°C)	Time (min)	SLR (g/l)	Glucose concentration (g/l)
1	140	20	100	45.13
2	140	20	50	31.28
3	140	10	100	47.16
4	140	10	50	27.88
5	100	20	50	21.81
6	100	20	100	32.83
7	100	10	100	37.91
8	100	10	50	24.49
9	160	15	75	37.52
10	80	15	75	30.39
11	120	25	75	28.92
12	120	5	75	30.90
13	120	15	125	35.62
14	120	15	25	14.43
15	120	15	75	37.67
16	120	15	75	40.68
17	120	15	75	38.04
18	120	15	75	38.70
19	120	15	75	37.67
20	120	15	75	37.70

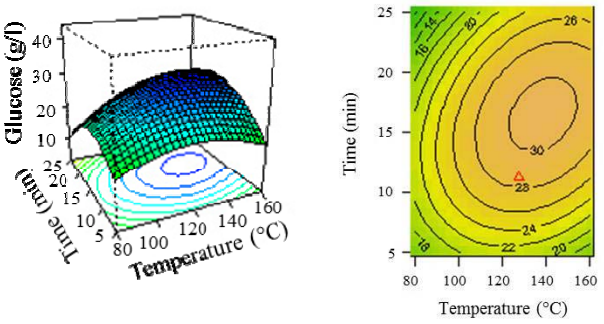
Table III summarizes the statistics for regression including regression coefficient, standard error, t-value, and P-value. The results show that the linear coefficients (a_1 and a_3) are all significant factor because P-value is very low which imply

that these coefficients in the model significantly influence glucose concentration, whereas coefficient of a_2, a_1a_2, a_1a_3, and a_2a_3 do not affect glucose concentration in the study range. In the other word, the temperature and SLR are the most significant factors on the concentration of glucose.

TABLE III
STATISTICS FOR REGRESSION OF THE OPTIMIZATION MODEL

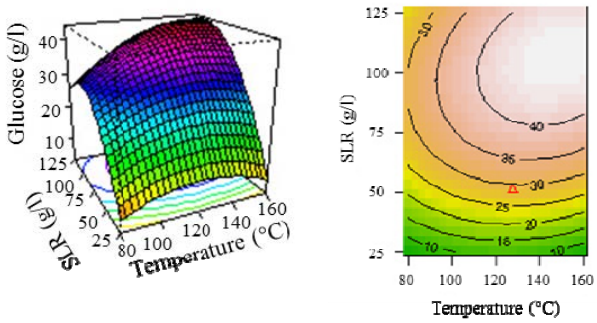
Coefficients	Value	Standard error	t-value	P-value
a_0	39.0536	0.8602	45.401	$< 2\times10^{-16}$
a_1	2.9625	0.3589	8.254	2.50×10^{-8}
a_2	-0.2131	0.3589	-0.594	0.55845
a_3	6.43	0.3589	17.915	5.26×10^{-15}
a_1a_2	0.93	0.5076	1.832	0.07991
a_1a_3	1	0.5076	1.97	0.06099
a_2a_3	-0.5075	0.5076	-1	0.32781
a_{11}	-1.2292	0.34	-3.615	0.00146
a_{22}	-1.9723	0.34	-5.8	6.56×10^{-6}
a_{33}	-3.2792	0.34	-9.644	1.51×10^{-9}

The three-dimensional response surface and two-dimensional contour plots for the concentration of glucose are shown in Figs. 1 to 3 which described the relative effects of two variables on glucose concentration with the third variable maintain constant. The interactive effect between temperature and SLR is important at high temperature and high SLR due to more substrate to produce glucose [7], [8]. In addition, high temperature is a feasible way to disrupt the biomass structure and result in more enzyme accessibility in enzymatic hydrolysis thus glucose concentration will be increased. Nevertheless, under severe conditions (high temperature and long reaction time), carbohydrates are degraded into other compounds, such as furfural and HMF generated in acid condition, resulted in total sugar reduction [9]. Besides, relatively flat response surface is shown in Fig. 1, indicating the effect of temperature and time is lower than that of SLR. The optimization of temperature, time, and SLR of second stage pretreatment for glucose concentration were determined at 156 °C, 16 min, and 106 g/l, respectively, which is calculated by setting the partial derivatives of polynomial equation to zero with respect to the corresponding variables. The predicted maximum glucose concentration from the model was 45.66 g/l while the glucose concentration of 48.58 g/l and 78.71 g/l of reducing sugar including glucose, xylose, and arabinose at the same condition from the experiment was obtained. This was confirmed that experimental result was in agreement with the model prediction.



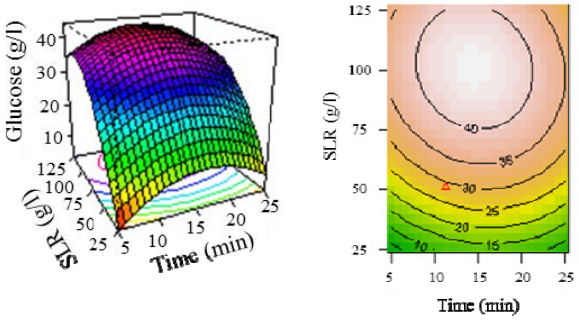
(a) (b)

Fig. 1 Response surface (a) and contour plots (b) for glucose concentration: effects of temperature and time



(a) (b)

Fig. 2 Response surface (a) and contour plots (b) for glucose concentration: effects of temperature and SLR



(a) (b)

Fig. 3 Response surface (a) and contour plots (b) for glucose concentration: effects of time and SLR

B. Chemical Composition of Corncobs

The untreated corncobs were composed of 39.31% cellulose, 34.46% hemicellulose, 10.47% lignin, and others. As seen in Table IV, The Microwave/NaOH or first stage pretreatment decreased lignin content to 3.98 %. The main effect of NaOH pretreatment is to remove lignin from lignocellulosic biomass in order to improve the enzymatic efficiency since lignin inhibits enzymatic hydrolysis. After that, the microwave/NaOH followed by H_2SO_4 or two-stage pretreatment was applied which reduced hemicellulose content to 0.48% because acid pretreatment solubilizes

hemicellulose from solid residual in soluble form [10]. In addition, two-stage pretreatment increased cellulose content to 88.74%, suggesting that cellulose is readily to hydrolize into sugars.

TABLE IV
COMPOSITION OF CORNCOBS BEFORE AND AFTER PRETREATMENT

Method	Chemical Composition (%)		
	Cellulose	Hemicellulose	Lignin
Untreated	39.31	34.46	10.47
Microwave/NaOH	75.54	19.37	3.98
Microwave/NaOH followed by H_2SO_4	88.74	0.48	9.13

C. Surface Morphology by SEM

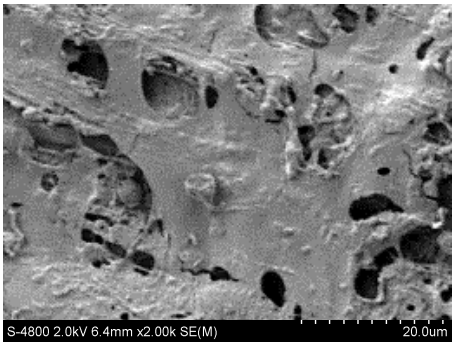
The images of scanning electron microscope (SEM) of the untreated and the pretreated corncobs are shown in Fig. 4. Fig. 4 (a) shows the SEM image of the untreated corncobs has smooth and continuous surface that no pore was observed which may be due to high residual of lignin content. Whereas, the sample pretreated with 2% NaOH pretreatment at 100 °C for 30 min, residual lignin was eliminated, as shown in Fig. 4 (b). The structure was damaged like a sieve or hole at the surface. This indicates that microwave/NaOH pretreatment removed external fibers which increase surface area of the pretreated sample. Moreover, After sample pretreated by microwave/NaOH followed by H_2SO_4 at 156 °C for 16 min, as shown in Fig. 4 (c), the structure was loose and irregular and has a very rough surface because it is easy to damage and solubilize hemicellulose due to the loss of lignin in first stage.

D. BET Surface Area

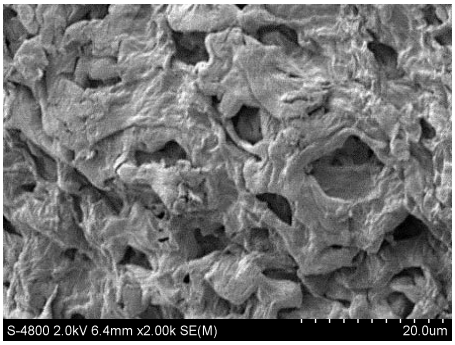
From the SEM images, pretreated corncobs structure was featured by fragmentation and swelling. The fragmentation released small components and enlarged surface area of biomass. On the other hand, the swelling behavior reduced the surface area and scooped the particles interior [11]. From Table V, the surface area of sample pretreated by microwave/NaOH pretreatment was increased from 3.8 to 4.1 m^2/g, it is likely that the increase in surface area caused by fragmentation. The fragmentation increased the surface area of corncobs due to the more small particles formation. In contrast, the surface area of sample pretreated by microwave/NaOH followed by H_2SO_4 pretreatment was reduced from 4.1 to 2.0 m^2/g because of swelling behavior that decreased the surface area of corncobs. The small holes merged into large holes was observed and resulted in the inside of the particles scooped.

TABLE V
BET SURFACE AREA, TOTAL PORE VOLUME, AND AVERAGE PORE DIAMETER OF CORNCOBS

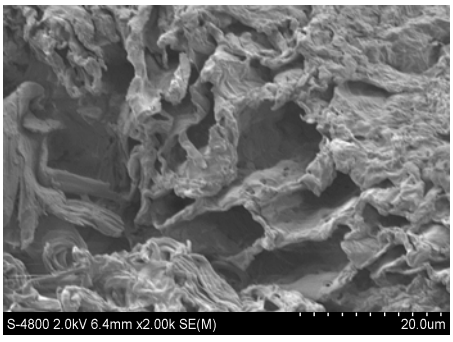
Method	Surface area (m^2/g)
Untreated	3.8
Microwave/NaOH	4.1
Microwave/NaOH followed by H_2SO_4	2.0



(a)



(b)



(c)

Fig. 4 SEM images of untreated corncobs (a), Pretreated corncobs with Microwave/NaOH (b), and Microwave/NaOH followed by H_2SO_4 (c)

IV. Conclusion

The NaOH followed by H_2SO_4 or two-stage pretreatment of corncobs in an environment of microwave radiation prior to enzymatic hydrolysis has been well performed with the response surface methodology (RSM) at a three-variable and five-level CCD. A maximum glucose concentration 48.58 g/l was obtained at the optimal condition of 2% NaOH at 100 °C for 30 min, and SLR 67: 1 for Microwave/NaOH pretreatment and 1% H_2SO_4 at 156 °C for 16 min, and SLR 106 g/l for second stage of two-stage pretreatment. the temperature and solid-to-liquid ratio are the most significant factors on the glucose concentration.

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