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STATISTICAL OPTIMIZATION FOR ALKALI EXTRACTION OF XYLAN FROM SUGARCANE BAGASSE BY SURFACE RESPONSE METHODOLOGY

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Abstract - Xylan extraction from sugarcane bagasse was studied in lab-scale with NaOH and KOH solutions enhanced by thermal autoclaving and statistical optimization using response surface methodology. The raw sugarcane bagasse sample was analyzed for chemical components; cellulose, hemicellulose and lignin of 49.9, 28.6% and 21.5%, respectively. Then the extraction reaction was individually carried out with NaOH and KOH solutions by using statistical experimental design. Three independent variables, namely alkali concentration, extraction temperature and extraction time were investigated. First, NaOH extraction; the maximum experimental extracted xylan of 0.94 g (65.7% of original xylan) was obtained with NaOH solution concentration of 1.75 M, extraction temperature of 75°C and extraction time of 25 min. While KOH extraction; the maximum experimental extracted xylan of 0.78 g (54.5% of original xylan) was obtained with KOH solution concentration of 0.5 M, extraction temperature of 75°C and extraction time of 40 min. The extraction efficiency (%Ee) of xylan extraction from sugarcane bagasse by NaOH and KOH solutions was 18.8 and 15.6%, respectively.

Keywords - Xylan, Sugarcane Bagasse, Alkali Extraction, Response Surface Methodology

I. INTRODUCTION

In recent years, an increasing effort has been made towards a more efficient utilization of renewable agro-industrial residues. Among several potential sources of biomass, the sugarcane bagasse has been one of the most promising industrial residues obtained from the sugar and alcohol industries [1]. Bagasse is an attractive feedstock for the large-scale biological production of biofuel, bioenergy and added value biomolecules, because of the abundance and concentration of low-cost raw materials and the improvement of food security. Among biomass components, hemicelluloses which are mainly composed of xylans, provide an important source of interesting molecules such as xylose and xylooligosaccharides which have potential applications in different areas, notably in chemical, food and pharmaceutical industries [2]. Several technologies were proposed for fractionation or extraction of hemicelluloses from feedstock. Although various physical (comminution, hydrothermolysis), chemical (acid, alkali, ozone, solvents), and biological extraction methods have been investigated over the years [3], thermo-chemical pretreatment of biomass has been the method of choice to enhance most of lignin and extract part of hemicellulose [4]. There are several reported on chemical extraction of biomass with higher xylan extraction; corn cobs (12% NaOH with steam 83% of original xylan by [5], sugarcane bagasse (3% NaOH at 50 1C 74.9% of original xylan and 12% NaOH coupled with steam 85% of original xylan by [6-7], natural grass (12% NaOH coupled with steam 98% of original xylan by [8], green coconut husks 4% KOH coupled with steam 84% of original xylan by [9]. The aim of this study was to investigate optimal alkali extraction of xylan from sugarcane bagasse by using a Central Composite Design (CCD) which is one of experimental design in Response Surface Methodology (RSM) to design of experiments.

II. MATERIAL AND METHODS

2.1 Material

The sugarcane bagasse was obtained from the sugar industry, Rajburi Sugar Co., Ltd., located in the Ratchaburi province, Thailand. The bagasse was washed, dried at 60 °C until constant weight, and triturated in a mill. Soon afterwards, it was separated and selected by two sieves (20-30 mesh). Then the fraction of mesh was collected and rejected bigger particle size fraction. The frection of sugarcane bagasse powder was dried in an oven at 110°C overnight and analyzed for cellulose, lignin and moisture contents according to AOAC methods [10].

2.2 Analytical methods

Certain amounts of alkali extracts were measured and adjusted its pH to 5, then four-fold volume of ethanol (95%, v/v) was added to it and overnight. Afterwards, it was centrifuged at 3000 rpm lasted 10 min and the precipitate was obtained through filtration. Then, certain amounts of sulfuric acid solution (7.0%, v/v) were added to the obtained precipitate. The hydrolysis was lasted 2 h at 100 C. After that it was neutralized to pH 7.0 and filtered. The content of xylose in the solution was analyzed with high performance liquid chromatography (HPLC). Xylose was analyzed using HPLC (HPLC 1100, Hewlett Packard, Germany) employing APS-2 HYPERSIL (NH_2) column of size 250×4.6 mm and RI detector.

Aqueous acetonitrile (75%) was used as the mobile phase with a flow rate set at 0.5 mL/min at 35°C with retention time of 9.924 min [11]. The extraction efficiency was simply calculated according to the following method in order to compare the extraction performance among different extractive methods:

$$Ee = (W_X / W_C) \times 100 \quad \ldots\ldots\ldots\ldots (1)$$

where Ee is the extraction efficiency (%); W_X, the weight of xylan (g); W_C, the weight of sugarcane bagasse (g).

2.3 Experimental methods

2.3.1 Optimization of alkali extraction variables using response surface methodology

Response surface methodology (RSM) is generally used to investigate the combined effects of several variables and to find the optimum conditions for a multivariable system [12]. The central composite design (CCD) is one of the most commonly used response surface designs to study the effects of variables on their response, and subsequently in the optimization studies [13]. Optimization of xylan extraction from sugarcane bagasse residue was studied by using the Design expert software (Trial version 7.0, Stat-Ease, Inc., Minneapolis, USA) with CCD design matrix.

2.3.1.1. Sodium hydroxide extraction

The extraction reaction was carried out in a 150 ml Duran bottle containing 5 g of sugarcane bagasse residue per 50 ml of NaOH solution (1:10 w/v) in triplicates. Three independent variables, namely NaOH solution concentration (A, 1.0-2.5 M), extraction temperature (B, 50-100°C) and extraction time (C, 20-60 min) were used at five coded levels (-α, -1, 0, +1, +α), as shown in Table 1.

Table 1. Coded and real values of variables in the Central Composite design (CCD) – optimisation of xylan extraction from sugarcane bagasse by NaOH

Variables	Code	Variable levels				
		-1.68	-1	0	1	1.68
NaOH conc. (M)	A	0.50	1.00	1.75	2.50	3.00
Temperature (°C)	B	117	50	75	100	33
Time (min)	C	2	10	25	40	50

2.3.1.2. Potassium hydroxide extraction

The extraction reaction was carried out in a 150 ml Duran bottle containing 5 g of sugarcane bagasse residue per 50 ml of KOH solution (1:10 w/v) in triplicates. Three independent variables, namely KOH solution concentration (A, 0.4-1.5 M), extraction temperature (B, 50-100°C) and extraction time (C, 20-60 min) were used at five coded levels (-α, -1, 0, +1, +α), as shown in Table 2.

Table 2. Coded and real values of variables in the Central Composite design (CCD) – optimisation of xylan extraction from sugarcane bagasse by KOH

Variables	Code	Variable levels				
		-1.68	-1	0	1	1.68
NaOH conc. (M)	A	0.00	0.20	0.50	0.80	1.00
Temperature (°C)	B	117	50	75	100	33
Time (min)	C	2	10	25	40	50

The significance of each variable and their interactions, and fitting a predictive model to the experimental responses was based on the following second-order polynomial:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i<j}^{k} \sum_{j}^{k} \beta_{ij} x_i x_j \quad \ldots (2)$$

Here Y is the observed response (xylan extraction efficiency); β_0 is the constant term; i, j and k are integers (in this case i is from 1 to 3, j is from 2 to 3, and k is the total number of factors, 3); β_i, β_{ii}, β_{ij} are, respectively, the coefficients for the linear, quadratic and interactive effects; and x_i and x_j are independent variables or factors, representing the alkali concentration, extraction temperature and extraction time. The experimental data obtained were analyzed for regression and graphical analysis by the statistical software package Design Expert (Trial version 7.0). The fit of the models was assessed from the coefficient of determination R^2 and the adjusted R^2. Experimental validation of the model-based optimum set-point for alkali extraction was performed.

III. RESULTS AND DISCUSSION

3.1 Sugarcane bagasse composition

The sugarcane bagasse sample was analyzed for chemical components. Cellulose, hemicellulose and lignin compositions after cutting into small pieces and drying in an oven at 60°C overnight are contained 49.9% cellulose, 28.6% hemicellulose and 21.5% lignin.

3.2 Optimization of alkali extraction variables using response surface methodology

3.2.1. Sodium hydroxide extraction

The xylan extraction was conducted on RSM designed-experiment using different NaOH concentration, extraction temperature and extraction time while sugarcane bagasse to NaOH solution ratio of 1:10 (w/v) was fixed. The experimental results obtained extraction for xylan extraction from sugarcane bagasse by NaOH are in Table 3. The maximum experimental extracted xylan of 0.94 g (18.8% Ee) was obtained with NaOH solution concentration of 1.75 M, extraction temperature of 75°C and extraction time of 25 min. The significance and effects of each variable on xylan extraction from sugarcane bagasse by NaOH are presented in Table 4. From multiple regression fit to the experimental data, the following second order polynomial model describes the xylan extraction from sugarcane bagasse by NaOH:

$$\text{Xylan (g)} = 2.38 + 0.07C - 9.57B^2 - 8.52C^2 - 5.15AB - 7.23AC - 1.24BC \quad \ldots\ldots\ldots\ldots (3)$$

Table 3. The 2^3 full factorial design with codified values and experimental results obtained for xylan extraction from sugarcane bagasse by NaOH.

Run.	A: NaOH Conc. (M)	B: Temperature (°C)	C: Time (min)	Xylan (g)	Extraction efficiency (Ee; %)
1	1.00	50	10	0.24	4.8
2	2.50	50	10	0.61	12.1
3	1.00	100	10	0.53	10.6
4	2.50	100	10	0.56	11.1
5	1.00	50	40	0.64	12.8
6	2.50	50	40	0.72	14.5
7	1.00	100	40	0.79	15.8
8	2.50	100	40	0.45	8.9
9	0.50	75	25	0.48	9.6
10	3.00	75	25	0.58	11.5
11	1.75	33	25	0.70	13.9
12	1.75	117	25	0.78	15.6
13	1.75	75	2	0.11	2.2
14	1.75	75	50	0.62	12.4
15	1.75	75	25	0.94	18.8
16	1.75	75	25	0.94	18.8
17	1.75	75	25	0.93	18.6

Table 4. Analysis of variance (ANOVA) for the fitted quadratic polynomial model

Term	SS	DF	F value	Prob>F
A	6.18E-03	1	1.76	0.2263
B	4.83E-03	1	1.38	0.2791
C	0.17	1	48.27	0.0002*
A^2	0.2	1	57.66	0.0625
B^2	0.04	1	11.47	0.0001*
C^2	0.41	1	117.82	0.0116*
AB	0.074	1	21.15	0.0002*
AC	0.053	1	15.08	0.0025*
BC	0.017	1	4.9	0.006*
Model	0.82	9	25.8	0.0001*
Residual	0.025	7		
Lack of Fit	0.025	5	147.15	0.2068
Pure Error	6.67E-05	2		
Total	0.84	16		

R^2 = 0.9707; adjusted R^2 = 0.9331;; C.V. (%)=9.50; adequate Precision=16.74; SS, sum of squares; DF, degrees of freedom; * Significant at <0.05

The statistical significance of this model was assessed by Fisher's statistical test (F-test) and by analysis of variance (ANOVA) of this response surface model, Table 4. The model is highly significant, as is evident from the F-value of 25.8 and the very low P-value = 0.0001. This indicates that there is only a 0.01%

chance that an F-value this large could occur byrandom coincidence, as opposed to having an appropriate model. The value of $R^2 = 0.9707$ indicates that only 2.93 % of the total variation remains not explained by the model, so the correlation of experimental and fitted values is excellent. The P-value is used as a tool to check the significance of each coefficient, which helps understand the interactions of factors. In this study, only extraction time (C) was highly significant in the individual effect. In addition, the interaction of NaOH concentration (A), extraction temperature (B) and extraction time (C) representative response surface plots is shown in Fig. 1a-1c.

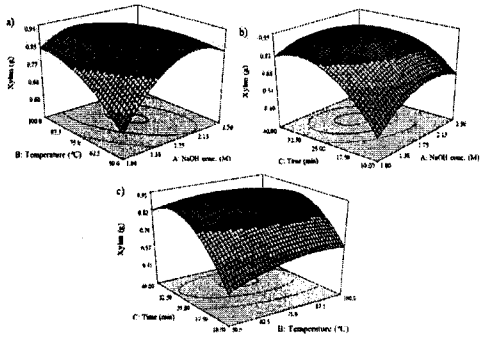

Figure 1. 3D response surface plots for glucose production showing the interaction between (a) NaOH concentration and extraction temperature; (b) NaOH concentration and extraction time; and (c) NaOH concentration and extraction temperature.

In Fig. 1a the interaction plot of NaOH concentration and extraction temperature shows that efficiency of xylan extraction increased remarkably with NaOH concentration lower 2.5 M and temperature lower 100 °C. In addition, when NaOH concentration was reached to 2.5 M, it showed that the efficiency of xylan extraction was little decreased. Moreover, the efficiency of xylan extraction increased with increasing extraction time but lower 40 min in the NaOH concentration lower than 2.5 M (Fig. 1b) and also the same trends of the interaction between extraction temperature and extraction time that the efficiency of xylan extraction remarkably increased with increasing temperature and time but was little decreased when they reached to 100 °C and 40 min, respectively (Fig. 1c).

3.2.2. Potassium hydroxide extraction

The xylan extraction was conducted on RSM designed-experiment using different KOH concentration, extraction temperature and extraction time while sugarcane bagasse to NaOH solution ratio of 1:10 (w/v) was fixed. The experimental results obtained extraction for xylan extraction from sugarcane bagasse by NaOH are in Table 5. The maximum experimental extracted xylan of 0.78 g (15.6% Ee) was obtained with KOH solution concentration of 0.5 M, extraction temperature of 75°C and extraction time of 40 min. The significance and effects of each variable on xylan extraction from sugarcane bagasse by KOH are presented in Table 6. From multiple regression fit to the experimental data, the following second order polynomial model describes the xylan extraction from sugarcane bagasse by KOH:

$$\text{Xylan (g)} = 0.76 + 1.84A - 1.34A^2 - 8.02B^2 - 2.54C^2 + 6.67AB \quad \ldots\ldots\ldots\ldots\ldots (4)$$

Table 5. The 2^3 full factorial design with codified values and experimental results obtained for xylan extraction from sugarcane bagasse by KOH

Run.	A: KOH Conc. (M)	B: Temperature (°C)	C: Time (min)	Xylan (g)	Extraction efficiency (Ee; %)
1	0.20	50	20	0.62	12.4
2	0.80	50	20	0.49	9.8
3	0.20	100	20	0.67	13.4
4	0.80	100	20	0.47	9.4
5	0.20	50	60	0.48	9.6
6	0.80	50	60	0.61	12.2
7	0.20	100	60	0.54	10.8
8	0.80	100	60	0.39	7.8
9	0.00	75	40	0.14	2.8
10	1.00	75	40	0.65	13
11	0.50	33	40	0.52	10.4
12	0.50	117	40	0.48	9.6
13	0.50	75	6	0.24	4.8
14	0.50	75	74	0.46	9.2
15	0.50	75	40	0.78	15.6
16	0.50	75	40	0.74	14.8
17	0.50	75	40	0.71	14.2

The statistical significance of this model was assessed by Fisher's statistical test (F-test) and by analysis of variance (ANOVA) of this response surface model, Table 6. The model is highly significant, as is evident from the F-value of 12.88 and the very low P-value = 0.0014. This indicates that there is only a 0.01% chance that an F-value this large could occur by random coincidence, as opposed to having an appropriate model. The value of $R^2 = 0.9430$ indicates that 5.7 % of the total variation remains not explained by the model, so the correlation of experimental and fitted values is good. In this study, only KOH concentration (A) was highly significant in the individual effect. In addition, the interaction of KOH concentration (A), extraction temperature (B) and extraction time (C) representative response surface plots is shown in Fig. 2a-2c.

Table 6. Analysis of variance (ANOVA) for the fitted quadratic polynomial model

Term	SS	DF	F value	Prob>F
A	0.06	1	21.68	0.0023*
B	0.01	1	1.89	0.2118
C	0.00	1	0.31	0.5942
A^2	0.16	1	60.87	0.0001*
B^2	0.03	1	10.48	0.0143*
C^2	0.12	1	42.86	0.0003*
AB	0.02	1	7.39	0.0298*
AC	0.01	1	3.12	0.1205
BC	0.00	1	0.91	0.373
Model	0.31	9	12.88	0.0014*
Residual	0.019	7		
Lack of Fit	0.016	5	2.67	0.2945
Pure Error	2.47	2		
Total	0.33	16		

$R^2 = 0.9430$; adjusted $R^2 = 0.8698$;; C.V. (%)=9.97; adequate Precision=11.33; SS, sum of squares; DF, degrees of freedom; * Significant at <0.05

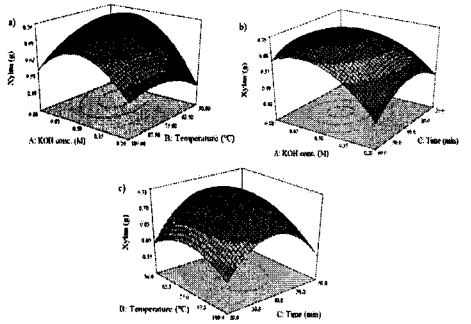

Figure 2. 3D response surface plots for glucose production showing the interaction between (a) NaOH concentration and extraction temperature; (b) KOH concentration and extraction time; and (c) KOH concentration and extraction temperature.

In Fig. 2a the interaction plot of KOH concentration and extraction temperature shows that efficiency of xylan extraction increased remarkably with KOH concentration lower 0.5 M and temperature lower 75 °C. In addition, when KOH concentration was reached to 0.8 M, it showed that the efficiency of xylan extraction was dramatically decreased. Moreover, the efficiency of xylan extraction slightly increased with increasing extraction time lower 40 min but decreased after 40 min in each KOH concentration (Fig. 2b). While the interaction between extraction temperature and extraction time that the efficiency of xylan extraction remarkably decreased with temperature lower and higher to 75 °C and extraction time lower and higher to and 40 min (Fig. 2c). The mutual interactions of the factors can also be assessed from contour plots. If the interactions are negligible, the contours (if not straight lines) will be elliptical with principal axes parallel to the coordinates/factors. In case of significant interactions, the elliptical contours become tilted: the axes of the ellipsoid do not align with the coordinate axes [14]. As described by [15]. when the alkali concentration was lower, the xylan contained in biomass could not fully be dissolved in the solution and thus it resulted in lower efficiency of xylan extraction. With much higher alkali concentration, however, it might result in the degradation of xylan at the experimental conditions. In addition, it was observed that the color of solution operated at 100 °C became darker compared to other experiments. This might be occurred the serious degradation of carbohydrates at the elevated extraction temperature.

CONCLUSIONS

This study proposes on optimization of xylan extraction from sugarcane bagasse by using alkali solution with statistically designed of experiments. The extraction reaction was individually carried out with NaOH and KOH solutions by using statistical experimental design. Three independent variables, namely alkali concentration, extraction temperature and extraction time were investigated. NaOH extraction provided the maximum xylan extraction of 0.94 g (per 5 g sugarcane bagasse; 65.7% of original xylan) while KOH extraction could provide the maximum xylan yield of 0.78 g (54.5% of original xylan). In conclusion, the statistical designed method employed defined the point wherein maximum extraction of xylan could be achieved which was further proved experimentally.

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