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Full Length Research Paper

Optimization of xylose production from sago trunk cortex by acid hydrolysis

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Sago trunk cortex is a renewable source for the production of many useful products, such as xylose and xylitol. The potential of bioconversion xylose to xylitol from sago trunk cortex is justifiable as these materials are cheap and widespread sugar sources. Lignocellulose type of residue such as sago trunk cortex structure can break to their monomeric sugars with hydrolysis process. Various hydrolysis temperature and acid concentration at constant temperature were investigated to evaluate the potential maximum xylose concentration in the sago trunk hydrolysate. The objectives of this study were to determine the composition of sago trunk cortex and the effects of sulphuric acid (H_2SO_4) concentration and hydrolysis time on the production of xylose from sago cortex waste. Response surface methodology (RSM) based on central composite design (CCD) was used to optimize the hydrolysis conditions in maximizing the xylose concentration. The optimum hydrolysis time and acid concentration found were 60 min and 8%, respectively. Under these conditions, the xylose concentration achieve was 22.78 g/l. The study provides efficient analysis on optimizing xylose concentration, in order to obtain higher productivity and yield of xylitol.

Keywords*: Sago trunk cortex, xylose, xylitol, response surface methodology, Dilute acid hydrolysis*

INTRODUCTION

In Malaysia, the production of sago starch and the export value has been increasing by 15 to 20% every year. It is reported as the fifth agricultural revenue after pepper, palm oil, cocoa and rubber (Abd. Aziz, 2002, Awg-Adeni et al. 2009). As the demand for sago starch keeps increasing, the production of sago wastes will also increase. Therefore, the sago starch industry is now confronted with waste disposal, such as sago trunk cortex. Due to the presence of hemicellulose contents in sago trunk cortex (20-40%), it can be used as a potential renewable source as its major components of glucose and xylose (Parajo et al., 1998). Xylose is the carbon source for the growth of microorganism for the production of xylitol. Therefore, the production of xylose from this renewable plant residue is essential to enhance the utilization of sago wastes, especially sago trunk cortex.

Bioconversion of lignocellulose to ethanol or xylitol requires the hydrolysis of carbohydrate polymers to their corresponding monomeric sugars prior to fermentation. Lignocellulose hydrolysis has been achieved either by acid hydrolysis (Dominguez et al, 1997; Silva et al, 1998; Roberto et al, 2003) or enzymatic hydrolysis (Kumoro et al, 2008). Acid hydrolysis is the most commonly applied and the method can be used either as a pretreatment preceding the enzymatic hydrolysis or as a method of hydrolysing lignocellulose for the sugars. As a consequence, the amount of sugars recovered from the raw material is dependent on the reaction time, temperature and acid concentration (Taherzadeh and Karimi, 2007). However, acid concentration is the most important parameter affecting sugar yield (Roberto et al., 2003). Apart from the sugar obtained from hemicellulose,

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other by-products, such as furfural, are also produced during the hydrolysis process (Taherzadeh and Karimi, 2007). It is known that furfural, acetic acid and phenolic compound are the potential microorganism inhibitors for the product formation during the fermentation process. Hence, it is necessary to keep the low concentration of by-products in hydrolysate and to run the hydrolysis reaction at less severe conditions (Rahman et al., 2006).

The aim of this study were to determine the composition of sago trunk cortex and the effects of sulphuric acid (H_2SO_4) concentration and hydrolysis time on the production of xylose from sago cortex waste. For this purpose, the response surface methodology (RSM) was employed to optimize the hydrolysis conditions in order to achieve high xylose concentration.

MATERIALS AND METHODS

Raw Material

Sago barks were purchased from a local plantation in Melaka, Malaysia. The cortex (the outer layer) was removed from the barks and the pith (the inner portion), which was then chopped to smaller pieces (25 x 150 mm).

Dilute Acid Hydrolysis

The hydrolysis experiments were performed in 250 ml Erlenmeyer flasks containing different concentrations of sulphuric acid (2 to 8%) (Lavarack et al., 2002). The flasks were loaded with solid to a liquid ratio of 1:8, autoclaved at constant temperature of 121°C and the reaction time was selected at 30 to 60 min (Foyle et al., 2007). After completing the hydrolysis process, the flasks were cooled to room temperature and the solids were removed from the liquid solution using a vacuum pump with a filter paper (Whatman No. 1). The filtered solution which contained xylan (hemicellulose) was neutralized with calcium carbonate, and again, the neutralized solution was filtered to obtain the clear solution. The solution was then analysed for sugar (xylose) and by-products (furfural) determination.

Experimental Design

The response surface methodology (RSM) was used to optimize the hydrolysis reaction parameters for determination of xylose yield from sago trunk cortex. The experimental design and statistical analysis were performed using the Design Expert version 7.1.3 statistical software (Stat-Ease, Inc., Minneapolis, MN). The experiments were based on a central composite rotational design (Cochran and Cox, 1957) with a quadratic model employed to study the combined effect of two independent variables, i.e. acid concentration and hydrolysis time. These two independent variables were coded as x_1 and x_2, respectively. A total of 13 combinations including five replicates of the centre point, four star points were carried out in random order according to a CCD configuration for the two chosen variables. Table 1 shows the experimental values and coded levels of the independent variables planned according to the central composite designs (CCD). In general, CCD contained an imbedded factorial with centre point that is augmented with a group of star point, allowing estimation of curvature. If the distance from centre of the design space to a factorial point is ±1 unit, the distance from the centre of design space to a star point is ±α with α≥1 (Farris and Piergiovanni, 2009). In this study, the value of α was calculated as ±1.414.

These five central points were added to estimate the experimental errors and to investigate the suitability of the proposed model. The two independent variables were coded according to Eq. (1) as follows:

$$x_i = \frac{(X_i - X)}{\Delta X_i} \qquad i = 1,2 \qquad (1)$$

Where x_i and X_i are the dimensionless and the actual values of the independent variable i, X is the actual value of the independent variable i at the central point, and ΔX_i is the step change of X_i corresponding to a unit variation of the dimensionless value. The behaviour of the system can be described as in Eq. (2):

$$y_i = b_0 + b_1 x_1 + b_2 x_2 + b_{12} x_1 x_2 + b_{11} x_1^2 + b_{22} x_2^2 \qquad (2)$$

Xylose concentration was chosen as the response of the design experiments. Where y_i represents the response variable, b_0 is the interception coefficient, b_1 and b_2 are the linear terms, b_{11} and b_{22} are the quadratic terms and x_1 and x_2 represent the variables studied. The Fisher's test for the analysis of variance (ANOVA) was performed on the experimental data to evaluate the statistical significance of the model (Nakai et al., 2007).

Analytical procedure

Xylose and furfural were analysed by high-performance liquid chromatography (HPLC) equipped with UV Visible and Refractive Index detector (Shimadzu, Japan). Xylose was determined by Inertsil NH_2 column at 40°C. Deionized water and acetonitrile were used as mobile phase at ratio of 25:75 at flow rate of 0.5 ml/min. Furfural was analysed by Zorbax Eclipse XDB-C18 column at 60°C with the same flow rate and mobile phase for xylose detection. Concentration of xylose was determined by RI detector, while furfural was quantified by UV detector at 210 nm.

The cellulose, hemicellulose, lignin and ash were determined according to the method presented by Foyle et al. (2007) and the results are summarized in Table 2.

RESULTS AND DISCUSSION

Composition of Sago Trunk Cortex

The result of sago trunk cortex composition is presented in Table 2. The result obtained from the sago trunk cortex was 44.1% cellulose. Likewise, Foyle et al. (2007) in their study revealed 48.28% cellulose from wheat straw.

The most important fraction was the determination of hemicellulose and xylose by using the acid hydrolysis method. This was due to the lower accessibility and solubility of crystalline cellulose compared to the open branched structure in hemicellulose (Parajo et al., 1998).

Table 1. Experimental range and levels of independent process variables to study the hydrolysis of sago trunk wastes

Independent variables	Symbol	Range and levels				
		-α	-1	0	+1	+α
Acid concentration	x_2	0.76	2	5	8	9.24
Hydrolysis time	x_2	23.79	30	45	60	66.21

Table 2. Composition of sago trunk cortex on dry basis

Main fraction	%
Cellulose	44.13
Hemicellulose	21.09
Lignin	23.30
Ash	1.53
Others	9.95

Table 3: Sugar compound detected by HPLC in the sago trunk cortex hydrolysate

Hemicellulose fraction	Sugar recoveries (g/l)
Xylose	24.1
Glucose	2.5
Arabinose	ND
Mannose	ND
Galactose	ND

Notes: ND = Not Detected

In this study, the hemicellulose recovered from the sago trunk cortex was 21%.

Table 3 presents the results from the HPLC analysis of sugar content of the sago trunk cortex hemicellulose hydrolysate. The analysis of sugar content revealed that the sago trunk cortex contained primarily xylose and glucose. Arabinose, galactose, mannose and rhamnose were not detected in this substrate. From 21.09% of hemicellulose portion of the sago trunk cortex, xylose yielded 91.4% of the total sugar (26.6 g/l). Rahman et al. (2006) was successful in obtaining the xylose yield of 90.35% from 24.01% of hemicellulose from oil palm empty fruit bunch. Rice straw contained 25% of hemicellulose and Roberto et al. (2003) was able to obtain the xylose yield of 88%. It is understood that different materials will give different compositions of hemicellulose and its sugar compounds; however, the results will aid in the selection of sago trunk cortex as the substrate for hydrolysis to simple sugars, mainly xylose and glucose, which are potential substrates for various biotechnological processes.

The Effects of Sugar and By-product Formation

The results for the effects of xylose formation to different acid concentrations at different hydrolysis times are shown in Figure 1. It was observed that higher acid concentrations led to higher sugar recovery but longer hydrolysis time caused lower amount of sugar recovery and higher furfural formation (Figure 2). Roberto et al., (2003) found the level of furfural and 5-hydroxymethyl-furfural (HMF) increased with the increase in the reaction time and acid concentration in rice straw hemicellulose hydrolysate. This result showed that sugar degradation occurred in the longer acid environment and this was the basis for assaying pentoses where the furfural production was measured as an indication of the original amount of pentoses presented in the sample (Rivas et al., 2002).

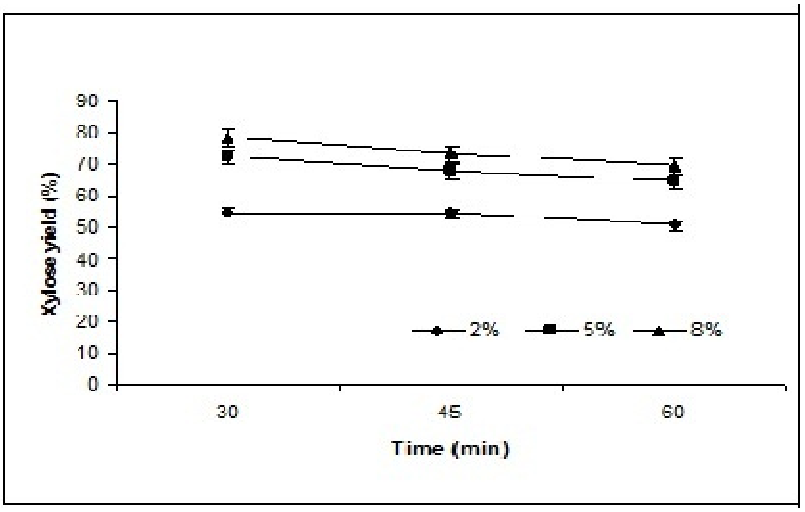

Figure 1: The xylose recovery after the treatment with acid at different concentrations for different hydrolysis times

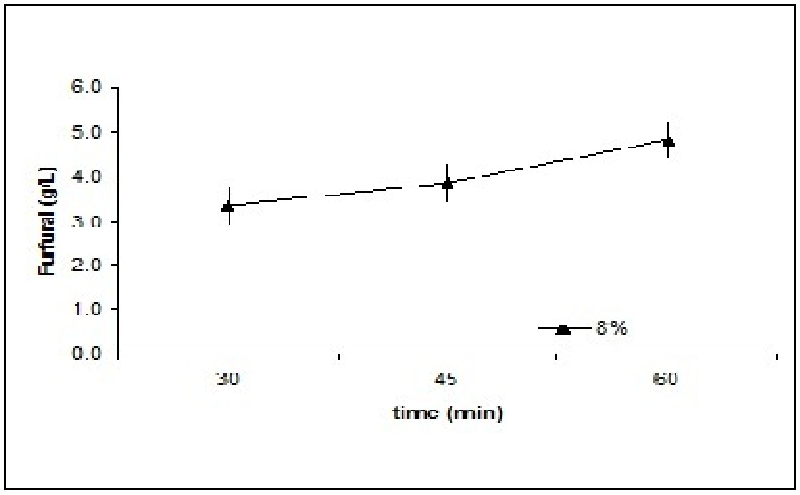

Figure 2: Furfural formation after the treatment with acid at 8% acid concentration

From Figure 1, the xylose yield for acid concentrations of 5% and 8% was closely revealed; however, the higher acid concentrations led to higher amount of furfural, which was known as the inhibitor for the microorganisms in the fermentation process (Palmqist and Hagerdal, 2000a). Thus, it was remarked that 8% of acid concentration gave higher xylose yields and also produced high amount of furfural concentration.

Statistical Analysis

Table 4 presents the experimental results collected by the test planned design by the central composite design (CCD) according to the Design Expert software. Each of the experiment was performed in duplicate and the central point was repeated five times (run 2, 4-6, 13).

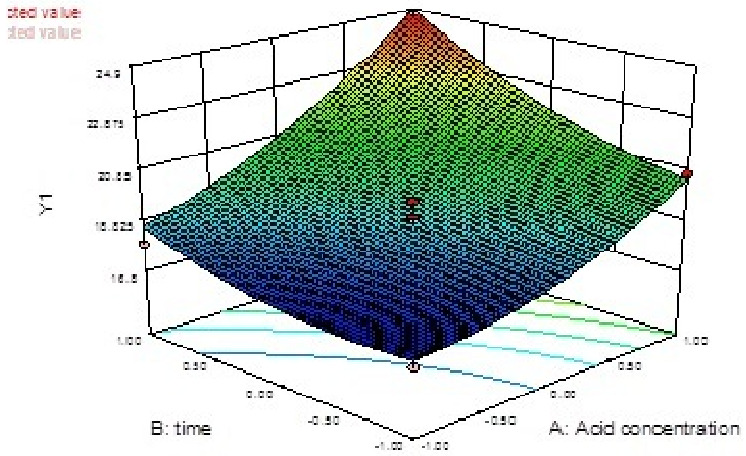

Figure 3. Response surface of xylose concentration at 5% acid concentration and 45 min hydrolysis time

The effects of Response Surface on Xylose Concentration

The effects of two independent variables (i.e. acid concentration and hydrolysis time) on the acid hydrolysis of the hemicellulosic fraction of sago trunk cortex were evaluated. Table 5 presents the analysis of variance (ANOVA) for the model representing the xylose concentration. The model F-value of 47.1 and P value of less than 0.05 implied that the model of xylose concentration was significant. The interaction term between the two factors of acid concentration and hydrolysis time with P value of less than 0.05 indicated that the interaction was significant. This means that there was interaction implied between the acid concentration and hydrolysis time. The linear terms and quadratic terms of the model also showed the significant value. Therefore, all the model terms were related to the regression model that fitted the second order polynomial. The coefficient of determination (R^2) was found to be 0.971, indicating that the interaction of the model was significant at 97.1% of confidence level and only 2.9% of the model could not be explained by the model. The adjusted R^2 value was 95.05% which was good in terms of model. The lack of fit was not significantly related to the pure error. Thus, the model developed for the model of optimum xylose concentration (*Y*1) was expressed by Eq. (3) as follows:

$$Y = 18.93 + 2.42x_1 + 2.42x_2 + 0.71x_1x_2 + 0.74x_1^2 + 0.63x_2^2 \quad (3)$$

Where Y is the xylose concentration, x_1 is the acid concentration and x_2 is the hydrolysis time.

It was evident that there was a quadratic relationship between the independent variables (acid concentration and hydrolysis time) and the response variable (xylose concentration), indicating that all the factors were statistically significant with a good confidence interval.

Figure 3 shows a three-dimensional model of the xylose concentration as a response of acid concentration and hydrolysis time. The figure reveals that the highest xylose concentration can be achieved with the increase of acid concentration with respect to the hydrolysis time at a fixed temperature. As can be seen from the figure, the xylose concentration tended to minimize as low acid concentration and low hydrolysis time implied to the sago trunk cortex. Silva et al. (1998) found that the lower amount of xylose was obtained from *Eucalyptus grandis* at low amount of sulphuric acid at lower immersion time.

The figure clearly presents that the optimum xylose concentration can be achieved at 8% acid concentration and 60 min of hydrolysis time. The predicted value at this condition was 24.895 g/l. Other sets of experiments were conducted to verify the predicted value suggested by the software, and the value obtained was 22.78 g/l. In other studies, by using 6% of H_2SO_4 at 100°C for 60 min for sorghum straw, the result produced 18.27 g/l of xylose (Tellez-Luiz et al., 2002), while 0.5% acid concentration at 10 min hydrolysis time for rice straw resulted in 16.25 g/l of xylose (Karimi et al., 2006), whereas 21.9 g/l of xylose was obtained for oil palm empty fruit bunch at acid concentration of 2% and hydrolysis time at 60 min

Table 4. Experimental design and results obtained by hydrolysis of sago trunk cortex

Run	***Actual Factor*** Acid concentration (%)	Hydrolysis time (min)	***Coded Factor*** Acid concentration (%)	Hydrolysis time (min)	**Xylose concentration (g/L)**
1	8	30	1	-1	20.75
2	5	45	0	0	18.94
3	9.24	45	1.41	0	23.74
4	5	45	0	0	19.62
5	5	45	0	0	18.79
6	5	45	0	0	18.53
7	5	66.21	0	1.41	22.85
8	8	60	1	1	24.65
9	2	30	-1	-1	16.83
10	0.76	45	-1.41	0	17.58
11	2	60	-1	1	17.89
12	5	23.79	-1	-1.41	18.04
13	5	45	0	0	18.75

Table 5. The results of the analysis of variance (ANOVA) for the model representing the xylose concentration according to the central composite design

Source	***Sum of Squares***	***Degree of freedom***	***Mean square***	***F value***	**P-value**
Model	72.10	5	14.42	47.10	<0.0001
Acid	47.0	1	47.00	153.55	<0.0001
Time	17.29	1	17.29	56.50	0.0001
Acid*Time	2.02	1	2.02	6.59	0.0372
$Acid^2$	3.77	1	3.77	12.32	0.0099
$Time^2$	2.75	1	2.75	8.99	0.0200
Residual	2.14	7	0.31		
Lack of fit	1.45	3	0.48	2.82	0.1712
Pure error	0.69	4	0.17		
Correlation total	74.24	12			
Standard Deviation	0.55				
R^2	0.97				

(Rahman et al., 2007). This had proven that the sago trunk cortex could be utilized as a potential source of xylose, which was an intermediate carbon source for the production of xylitol through microbial processes. The acid hydrolysis method was used to synthesize the sugar compound in the sago trunk hydrolysate.

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