Penerbit Akademia Baru

Journal of Advanced Research in Materials Science

Journal homepage: www.akademiabaru.com/arms.html
ISSN: 2289-7992

Acid Hydrolysis Optimization of Corn Cob As Raw Material for Xylitol Production

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ARTICLE INFO

Article history:
Received 5 June 2018
Received in revised form 4 July 2018
Accepted 2 August 2018
Available online 29 December 2018

ABSTRACT

The aim of this study is to determine the optimum condition of corn cob hydrolysis in xylose production as a raw material of xylitol. The optimum condition was determined with maximization of xylose concentrations, the ratio of xylose-glucose concentrations, and minimization of acetic acid concentrations responses. The method used in this research was experimental research with Response Surface Methodology (RSM), particularly Central Composite Design (CCD), utilizing Design Expert 7.0.0 software which was performed at operating temperature of 121 ºC using various reaction time (10-15 min) and concentration of sulfuric acid (2–6%). The result of this research showed that mathematical models were obtained in order to predict the concentration of xylose, the ratio of xylose-glucose concentration, acetic acid concentration in the hydrolysate and to optimize the process. The optimum condition of acid hydrolysis in multi-objective responses was obtained at 12.54 minutes hydrolysis time and 3.19% usage of sulfuric acid concentration. The optimum of xylose concentration, the ratio of xylose-glucose concentration, and acetic acid concentration found in the hydrolysate was 8.89 g/L; 1.06; and 1.27 g/L, respectively.

Keywords:
Acetic acid, corn cob, hydrolysis, xylose-glucose concentration ratio, xylitol, xylose

1. Introduction

The availability of food in Indonesia is a factor affecting the world's economic growth. In Indonesia, availability of food source is abundant. Biomass is one of the wastes generated from food production. Corn is one of the commodities that has been processed in Indonesia. A corn cob occupies 30% mass of corn which is a waste from corn processing. The availability of corn cob is abundant in Indonesia. Corn cob obtained from corn processing in Indonesia was about 5.7 million tons/year. Most of the corn cobs were being dumped and burned and consequently caused

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pollution. In order to reduce the impact of the corn cob waste to the environment and improve its economic value, it is necessary to process the corn cob.

Utilization of corn cob waste can be done by processing its hemicellulose fraction [1]. Hemicellulose is a polysaccharide which most of its monomer consists of pentosan (xylose). Corn cobs mainly contain 36% of hemicellulose; its hemicellulose content is different depending on its variety. High hemicellulose content of material makes it potential for xylose production [2].

Xylose (C5H10O5) has a wide application in the food industry and can be synthesized into xylitol, bioethanol, organic acid (butanol, acetone, acetic acid, and lactic acid) and single cell protein [3,4]. Xylose at its crystal form is widely used as a low-calorie sugar that serves as a substitute for sucrose, especially for diabetes mellitus patients [5]. Xylose can be extracted from hemicellulose hydrolysis (xylan) either enzymatically or acidly [6]. Acid hydrolysis is more efficient due to its lower production cost, its relatively short hydrolysis time, and its ability to produce higher yield compared to enzymatic hydrolysis. Nevertheless, acid hydrolysis of hemicellulose caused the obtained hydrolyzed to have a salty taste due to the neutralization which produces a high concentration of salt, consequently requiring further purification [7]. Acid hydrolysis also produces decomposition products such as furfural, hydroxymethylfurfural, and acetic acid that can act as inhibitors at the fermentation process if being used as xylitol or bioethanol substrate. It is due to the high temperature and pressure used at acid hydrolysis [8].

Xylitol (C5H12O5) is a sugar alcohol consisting of five carbon atoms. Xylitol is known as a natural sweetener because it can be found in vegetables and fruits. Its sweetness level is as high as sucrose. Xylitol can inhibit the growth of mouth bacteria, making it useful in preventing dental caries, forming plaque, and stabilizing salivary pH [9]. Xylitol also prevents the growth of Streptococcus pneumoniae inside nasopharyngeal which can consequently reduce middle ear infection and sinusitis. Xylitol has a high economic value due to its advantages, making the utilization of agriculture waste containing lignocellulose for xylitol production a great opportunity as an effort of agriculture waste processing [10].

Factors affecting acid hydrolysis efficiency are the type of substrates, substrates concentration, acid type, acid concentration, reaction condition (temperature, pressure, and hydrolysis time) [11]. Hemicellulose hydrolysis efficiency to produce xylose for a raw material for xylitol can be observed based on the product concentration such as xylose, glucose, furfural, and acetic acid. Optimum condition of hydrolysis process is reached when a high concentration of xylose and low concentration of decomposition products are obtained [3,4].

This research aims to determine the optimization models and optimum condition of corn cob acid hydrolysis to produce xylose as xylitol fermentation substrate at various hydrolysis times and H2SO4 concentrations.

2. Experimental

A. Raw Materials

Corn cob used in this research was Unpad hybrid corn cobs obtained from Arjasari of Bandung Regency, West Java, with 15 weeks harvesting time from its planting time. Before being used, the corn cob was dried in an oven blower at 60ºC overnight and ground. Only corn cobs with small size, max 60 mesh, were used. The corn cob composition had been previously determined to be 41.17% hemicellulose, 20.89% cellulose, and 16.26% lignin.

B. Acid Hydrolysis of Corn Cob

Small sized corn cob was mixed in 2-6% H2SO4 solution in 250 mL Erlenmeyer. The ratio of corn cob with the H2SO4 solution used was 1:25 (w/v). Erlenmeyer containing mixed solution was sealed with aluminum foil. The hydrolysis process was operated in an autoclave at 121°C for 10-15 minutes. The variation of hydrolysis time and H2SO4 concentration factor being used were based on RSM (CCD) data interpretation. The hydrolysate was obtained by separating the solid containing corncob waste from the solution through centrifugation and filtration.

C. Fermentation of Hydrolysate

The yeast strain used in this study, D. hansenii ITB CCR85, was obtained from Microbiology and Bioprocess Technology Laboratory of Chemical Engineering ITB. The yeast was grown in glucose yeast extract (GYE) agar for 3 days at 30 °C before further usage [12].

The fermentation process was operated in a batch method in a 700 mL Bioflow 115 (New Brunswick) bioreactor. The bioreactor was equipped with pH, temperature, level, dissolved oxygen (DO), foam and mixing control system. Batch fermentation was executed in 700 mL working volume and mixed at 450 rpm at pH 5 and 30 °C temperature for 96 hours. Inoculum used was 10 % of working volume, which was prepared using 15 g/L xylose. The culture was sampled over time during the exponential growth and the stationary phases.

D. Analysis

Xylose, glucose and acetic acid concentrations in hydrolysate and xylose, glucose, acetic acid, and xylitol concentrations in fermentation liquid were determined using high-performance liquid chromatography (HPLC) with a type of BioRad Aminex HPX-87H column and 0,005 N H2SO4 as eluent. Cell concentration was analyzed by total plate count (TPC) method.

E. Optimization Model

Response Surface Methodology (RSM) is a method that can optimize various processes. Experiment design method of RSM combines mathematic and statistic techniques to make and analyze a response (Y) which is affected by some factors (X) for optimizing its process.

Response (Y) is a function of factors (X). Generally, the correlation between (Y) and (X) can be written as Y=f(X_1, X_2,..., X_3). Optimization using RSM can be done through 2 steps, namely the first-order optimization to find its optimum area, then followed by second-order reaction to find its optimum point. The correlation between response (Y) and factors (X) in first-order optimization model is represented by the following scheme:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \cdots + \epsilon \quad (1)$$

where β is the coefficient of regression. Meanwhile, the correlation between response (Y) and factors (X) in second-order optimization model is represented by the following scheme:

$$Y = \widehat{a_0} + \sum_{i=1}^{k} \widehat{a}_i X_i + \sum_{i=1}^{k} \widehat{a}_i X_i^2 + \sum_{i<j}^{k-1} \sum_{j=2}^{k} \widehat{a_{ij}} X_i X_j + \dot{a} \quad (2)$$

where $\hat{a}$ is the coefficient of regression and $\dot{a}$ shows residual component (error).

3. Result and Discussion

A. Corn Cob Hydrolysis Optimization

Optimization of corn cob acid hydrolysis is discussed into two parts, which are single objective optimization and multi-objective optimization. Validation of optimization model is done in multi-objective optimization.

i) Single Objective Optimization

a) The response of xylose concentration

According to the analysis of variance that had been done in data processing, optimization model in actual value for predicting xylose concentration from hydrolysis time and H_2SO_4 concentration factors design was obtained. Optimization of xylose concentration model is represented by the following scheme:

Xylose Concentration = 2.63 + 0.51HT + 1.28AC – 0.009(HT)(AC) – 0,02(HT)^2 -0.13(AC)^2

where:
HT: Hydrolysis Time
AC: Acid Concentration

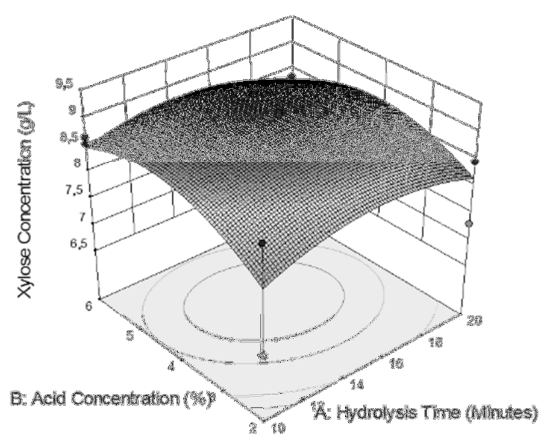


Fig. 1. Effect of reaction time and sulfuric acid concentration on a generalized model for the prediction of xylose concentration

Fig 1. shows the effect of reaction time and sulfuric acid concentration on a generalized model for prediction of xylose concentration where A is the symbol of hydrolysis time and B is the concentration of H_2SO_4 which are presented from 10 – 20 minutes for A factor and 2% - 6% for B factor. According to Fig 1., xylose concentration increased from min 10 until min 15, then decreased from min 15 until min 20. Mean while, xylose concentration increased by the usage of 2%-4% H_2SO_4 concentration and it decreased by the usage of H_2SO_4 concentration higher than 4%. The highest concentration of xylose in hydrolyzed was 9.22 g/L. It was obtained at 15 minutes hydrolysis time with the use of 4% of H_2SO_4 concentration.

Higher concentration of substrate for hydrolysis will make the process of hemicellulose conversion to xylose require longer time. When all xylan groups in hemicellulose hydrolyzed become xylose monomers and the process continues, xyloses produced will react with acid to become decomposition products such as acetic acid and furfural [13]. Increasing acid concentration to accelerate hydrolysis time is ineffective. High concentration of acid used in hydrolysis will make the hydrolyzed become poisonous and corrosive. It will harm the microorganisms in the fermentation process for xylitol production. Furthermore, corrosive hydrolysis reactor is required [11].

b) Response of ratio xilose-glucose concentration

According to the analysis of variance that had been done in data processing, optimization model in actual value for predicting the ratio of xylose-glucose concentration from hydrolysis time and H_2SO_4 concentration factors designed was obtained. Optimization of the ratio of xylose-glucose concentration model is represented by the following scheme:

The ratio of Xylose-Glucose Concentration = $-1.15 + 0.21HT + 0.37AC - 2.75(HT)(AC) - 0.0068(HT)^2 - 0.0043(AC)^2$
where:
HT: Hydrolysis Time
AC: Acid Concentration

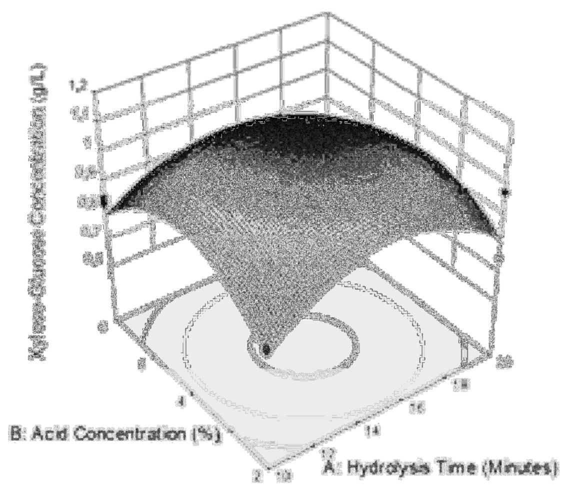


Fig. 2. Effect of reaction time and sulfuric acid concentration on a generalized model for the prediction of the ratio of xylose-glucose concentration

Highest ratio of xylose-glucose concentration in hydrolyzed was 9.22 g/L. It was obtained at 15 minutes hydrolysis time with the use of 4% of H_2SO_4 concentration. It is shown in Figure 2. The ratio of xylose-glucose concentration increased from min 10 until min 15, then it decreased from min 15 until min 20. Meanwhile, the ratio of xylose-glucose concentration increased by the usage of 2%-4% H_2SO_4 concentration and decreased by the usage of H_2SO_4 concentration higher than 4%.

The concentration of substrate being used in hydrolysis was the main factor which determined the concentration of xylose and glucose produced. Hydrolysis has a maximum limit of effectiveness in xylose and glucose production. Unpad hybrid corn cob has 41.17% hemicellulose and 20.89% cellulose according to composition test which had been previously determined. Constituent monomers of hemicellulose are D-glucopyranose, β-D-mannopyranose, β-D-galactopyranose, L-arabinose, and β-D-xylopyranose, while constituent monomers of cellulose are D-glucopyranose [14]. In hydrolysis for xylose production, β-D-xylopyranose units will be converted to xyloses when β-1,4-xylopyranose bonds between D-xylopyranose units are separated because of adduction by water in acidic and high-temperature condition. D-glucopyranose units will be converted to glucose when β-1.4 glycosidic bonds adducted by water in acidic and high-temperature condition.

c) The response of acetic acid concentration

According to the analysis of variance that had been done in data processing, optimization model in actual value for predicting acetic acid concentration from hydrolysis time and H_2SO_4 concentration factors designed was obtained.

Optimization of acetic acid concentration model is represented by the following scheme:

$$\text{Acetic Acid Concentration} = -0.775 + 0.191HT + 0.192AC - 0.0006(HT)(AC) - 0.0054(HT)^2 - 0.0084(AC)^2$$

where:
HT: Hydrolysis Time
AC: Acid Concentration

Fig 3. shows that the longer hydrolysis time occurred and the higher H_2SO_4 concentration used, a higher concentration of acetic acid was obtained in the hydrolysate. Otherwise, the shorter hydrolysis time occurred and the lower H_2SO_4 concentration used, a lower concentration of acetic acid was obtained in the hydrolysate. The lowest concentration results found in hydrolysis was 0.83 g/L. It is shown in Fig 3 at 10 minutes and 2% H_2SO_4 concentration usage of hydrolysis operating condition.

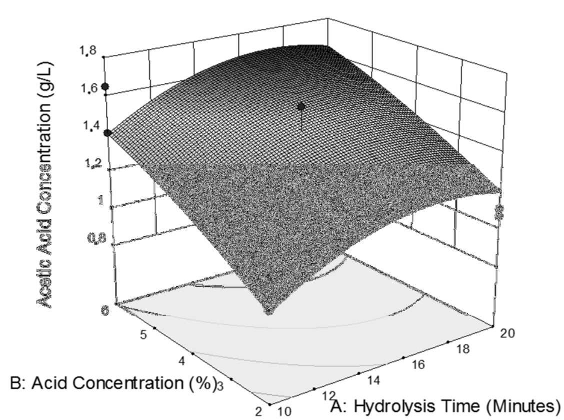


Fig. 3. Effect of reaction time and sulfuric acid concentration on a generalized model for the prediction of acetic acid concentration

Acetic acid is a decomposition product of sugar monomers, such as xylose and glucose, from the hydrolysis process. The decomposition products are formed by hydrolysis reaction in high acidic condition, long time, and high-temperature condition. The hydrolysis time factor affects the amount of decomposition product such as acetic acid the most because the longer hydrolysis time occurred, the more sugar monomer (xylose and glucose) will be reacted with acid and produce acid solutions such as acetic acid [15].

The acetic acid which is contained in hydrolysate is formed from hemicellulose-containing acetyl groups. The acetyl groups will be separated from hemicellulose then bonded by carbon and hydrogen in sugar monomer forming an acetic acid when being reacted in high acidic and temperature condition [7]. The existence of acetic acid in hydrolysate could be an indicator for xylose and glucose decomposition. Moreover, the existence of acetic acid in hydrolysate will be an inhibitor of the microorganism in fermentation for xylitol production [11].

ii) Multi-Objective Optimization

From model (3), (4), and (5), we obtained optimum points for each factor of the whole response. At this solution, hydrolysis time used was 12.54 minutes, while acid concentration factor used was 3.19%. Response value for xylose concentration, the ratio of xylose-glucose concentration, and acetic acid concentration at these optimum points were predicted in the amount of 8.89 g/L, 1.06, and 1.27 g/L, respectively. Meanwhile, model validation result from prediction value obtained at the same hydrolysis operating conditions showed that response value for xylose concentration, the ratio of xylose-glucose concentration, and acetic acid concentration was 7.74 g/L, 0.94, and 1.07 g/L, respectively.

B) Fermentation of Corn Cob Acid Hydrolysate

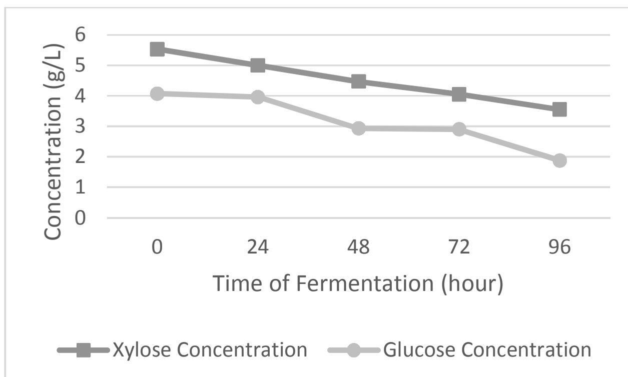
Table 1
Fermentation Results

Information	Result
Xylose Utilization (%)	35.8± 0.23
Glucose Utilization (%)	53.81± 0.30
Final Xylitol Concentration (g/L)	0.200 ± 0.01
Xylitol Yields towards Xylose (g/g)	0.101± 0.01
Biomass result towards Xylose (g/g)	0.101± 0.02
Biomass result towards Glucose (g/g)	0.091± 0.005
Xylitol Yields per Cell Biomass (g/g)	1.000± 0.02
Specific Growth Cell Rate (μ) (g cell/L.hour)	0.017

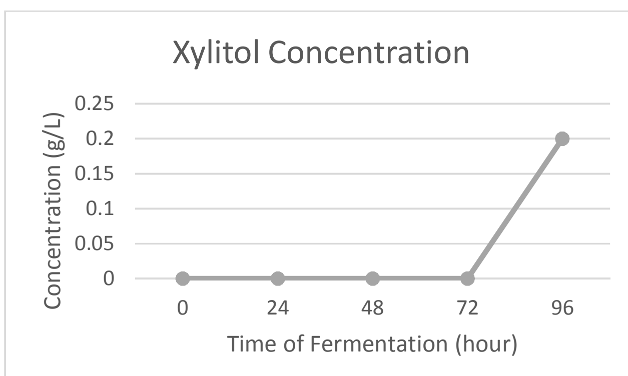
Glucose was used by *D. hansenii* to form biomass and other metabolites compound. Because there were different metabolic pathways between xylose and glucose utilization, xylitol was not converted by yeast if glucose was used as carbon sources. Table 1 shows that glucose utilization value was higher than xylose utilization. It occurred because the ratio of xylose-glucose concentration was very low.

The best ratio of xylose-glucose concentration in hydrolysate as the best raw material for xylitol was 1:4 [2]. If xylose content was high but glucose content was also high then the glucose content in hydrolysate will be fermented into ethanol, therefore will inhibit fermentation process and reduce yeast ability to convert xylose into xylitol. The acetic acid resulted from acid hydrolysis could produce inhibitor compound such as acetic acid and furfural which could change the morphology of yeast cell and caused the death of cell during xylitol fermentation [8].

Based on Fig 4, the concentration of xylose and glucose were decreasing during the fermentation process. It occurred because xylose and glucose were the substrate of yeast for metabolism process. The formation of xylitol occurred at the 96th hour fermentation time. The concentration of xylitol obtained was 0.2 g/L or 0.101 g/g towards xylose. This result was very low. It happened because of the lack of fermentation time operated. It is shown in Fig 4. that until the time fermentation finished, xylitol concentration hadn't decreased yet. It indicates that the conversion process of xylose to xylitol started to occur at the 96th hour fermentation time.



(a)



(b)

Fig. 4. Concentration of xylose, glucose (a) and Xylitol (b) per 24 hours

Fermentation process has a different rate of metabolism in converting xylose to xylitol [6]. Otherwise, there are factors that can obstruct fermentation process in xylitol production such as high concentration of glucose, low concentration of dissolved oxygen (can convert sugar to become ethanol), an inhibitor compound such as acetic acid and furfural (can damage cell morphology which causes cell death) [4].

4. Conclusion

Mathematical models were obtained in order to predict the concentration of xylose, the ratio of xylose-glucose concentration, acetic acid concentration in the hydrolysate and to optimize the process. The optimum condition of acid hydrolysis in multi-objective responses was obtained at 12.54 minutes hydrolysis time and 3.19% usage of sulfuric acid concentration. The optimum of xylose concentration, the ratio of xylose-glucose concentration, and acetic acid concentration found in the hydrolysate was 8.89 g/L; 1.06; and 1.27 g/L, respectively. Xylitol concentration obtained after fermentation process from acid hydrolysate at 15 minutes hydrolysis time and 4% H_2SO_4 concentration usage was 0.2 g/L or 0.101 g/g tion time.

Acknowledgment
The authors are grateful to funding sources. This research was funded by Universitas Padjadjaran and Directorate of Higher Education, Indonesian Ministry of National Education (DIKTI).

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