Optimization of Xylose Production from Sugarcane Trash by Microwave-Maleic Acid Hydrolysis

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(Received: December 02, 2019; Accepted: May 06, 2020)

Abstract

Sugarcane trash contains significant amount of xylan that could be hydrolysed to xylose. The xylose could be further fermented to produce xylitol, a sugar alcohol that has low calories and does not cause caries of teeth. In this study we optimized the production of xylose from sugarcane trash by microwave-assisted maleic acid hydrolysis using response surface methodology (RSM). The factors optimized were acid concentration, time, and temperature. The xylose yield based on the weight of initial biomass was determined and it served as a response variable. Results show that acid concentration and interaction between time and temperature had significant effect on xylose yield. The quadratic regression model generated from the optimization was fit and can be used to predict the xylose yield after hydrolysis with various combinations of acid concentration, time, and temperature. The optimum condition for xylose production from sugarcane trash was using maleic acid of 1.52%, and heating at 176 °C for 6.8 min. At this condition the yield of xylose was 24.3% per initial biomass or 0.243 g/ g biomass.

Keywords: maleic acid; microwave heating; response surface methodology; sugarcane trash, xylose

How to Cite This Article: Hermiati, E., Oktaviani, M., Ermawar, R.A., Laksana, R.P.B., Kholida, L.N., Thontowi, A., Mardiana, S., and Watanabe, T., (2020), Optimization of Xylose Production from Sugarcane Trash by Microwave-Maleic Acid Hydrolysis, Reaktor, 20(2), 81-88, http://doi.org/10.14710/reaktor.20.02.81-88.

INTRODUCTION

Sugarcane trash (ST) is biomass left in the field when the sugarcane is harvested. It consists of leaves and tops of sugarcane plant. The amount of the biomass is approximately 15-30% of the total above ground biomass at harvest. Some farmers used ST for
cattle or goat feed, but only in a small amount. Some biomass is left on the field, and the majority of the biomass is usually burnt at harvest. Thus, it creates environmental problem, especially in the sugarcane plantation. Some studies have been conducted in order to better utilize the ST, either for bioenergy (Jenjarayakosohn et al., 2014; Smithers, 2014) or for producing compost (Suma and Savitha, 2015) and for growing mushrooms (Nadkarni, 1997). ST contains significant amount of xylan that could be hydrolyzed to xylose, and further fermented to xylitol.

There are different methods of biomass fractionation using thermochemical process. The use of acid in the fractionation of lignocellulosic material was aimed at hydrolyzing of hemicellulose fraction from the biomass by randomly cleaves the glycosidic bonds between adjacent xylose units. Microwave heating has been proved more efficient than conventional heating, because the heating process using microwave is direct, uniform, and faster (Tsubaki et al., 2013, Ohashi and Watanabe, 2018). The use of organic acids, such as maleic or oxalic acid, in the fractionation of lignocellulosic biomass has been reported by Kootstra et al., 2009; Jung et al., 2014, 2015; Barisik et al., 2016; Kundu and Lee, 2015; Solihat et al., 2017; Fatriasari et al., 2018; Anita et al., 2019). However, they mostly focused on the effects of the pretreatment on the enzyme susceptibility of the solid residues resulted from pretreatment. On the other hand, reports on the production of xylose from the biomass were mostly used inorganic acids, such as sulfuric, hydrochloric, and nitric acids and combined with conventional heating (Ahmed et al., 2001, de Paiva et al., 2009, Rahman et al., 2007, Rocha et al., 2014, Ji et al., 2017, Krishania et al., 2018). The use of microwave and organic for producing xylooligosaccharides from beechwood xylan and corncob hemicellulose with xylose as by product has been reported by Lin et al. (2017). This report concluded that oxalic acid was the best organic acid for the production of xylooligosaccharides. Lee and Jeffries (2011) reported the effects of conventional heating with organic and inorganic acid on the sugar monomers produced in the hydrolysate and from the saccharification and fermentation of the solid residues of corncob. They found that organic acids, such as maleic acid and organic acid could produce more monomer sugars in the hydrolysates and more ethanol from the solid residuals. Studies on the use of microwave heating combined with maleic acid for hydrolysis of hemicellulose in ST to produce xylose, including its optimum hydrolysis conditions, have not been reported. Therefore, in this study we optimized the conditions for hemicellulose hydrolysis from ST to produce xylose by combining microwave heating with maleic acid.

**Materials and Methods**

**Materials**

The ST was obtained from PT PG Rajawali II plantation in Subang, West Java in 2017. The material was chopped, dried, ground, and sieves into particles of sizes 40-60 mesh (250-420 µm), then it was put in a sealed plastic bag and stored in a plastic container. Maleic acid was analytical grade and was used without further purification.

**Design of Experiment**

\[
Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3
\]  

(1)

The factors that were optimized were acid concentration, temperature, and heating time. The response was xylose yield obtained in the hydrolysate. The optimization of those factors was conducted using rotatable central composite design (CCD) (Cochran and Cox 1992) with 20 experimental points (2^3 factorial points, 6 star points, and 6 center points). With three factors to be optimized, the distance of each star point from the center in CCD, or we call it α value, becomes [2^{1/4}] or 1.682. The experiments at those points were conducted in duplicates. The ranges of acid concentration (0.5-2%), temperature (170-200 °C), and heating time (2-8 min) for the optimization were selected based on our previous experiments on microwave-maleic acid pretreatment of some biomass, such as sugarcane bagasse, oil palm empty fruit bunch, and sugarcane trash (Sari et al., 2016, Solihat et al., 2017, Fatriasari et al., 2018, Anita et al., 2019). The quadratic second order polynomial model was fitted to the data by multiple regression analysis as follows.

Where Y represents the response variable (xylose yield, %); X_1 (heating time, min), X_2 (temperature, °C), and X_3 (acid concentration, %) represent the independent variables; b_0 represents the interception coefficient; and b_{11}, b_{22}, b_{33}, b_{12}, b_{13}, and b_{23} represent the regression coefficients, respectively. The data obtained were analyzed using the response surface methodology (RSM) with Design Expert® 12 software. The analysis of variance (ANOVA) was used to analyze the adequacy of the model.

**Hydrolysis of Xylose**

The hydrolysis of xylose from ST was carried out in a microwave digester (Milestone START D) with maleic acid catalyst. The picture of the equipment is shown in Fig 1. Fig 1A shows the complete system of the microwave digester used in this research, while Fig 1B shows the rotor with the teflon vessels inside it, and Fig 1C shows the safety system of the reaction vessels in the rotor. As much as three grams of ST was put in a teflon tube, and added with maleic acid with solid liquid ratio of 1:10. The tube was degassed at -20 bar vacuum for 5 min. The heating process in the microwave digester was...
Figure 1. START D microwave digester: Complete microwave system (A), rotor and vessels (B), and safety system of a vessel within the rotor (C)

performed at 50% magnetic stirring with 12 min pre-heating and about 10 min of cooling. After the process was completed the hydrolysate was separated from the insoluble fraction by filtration. The hydrolysate was kept in a plastic tube and stored in a refrigerator for further analysis.

Analysis of Xylose in The Hydrolysates

The hydrolysate was analyzed for its xylose concentration using high performance liquid chromatography (HPLC) (Ultra Fast Liquid Chromatography/UFLC, Shimadzu, Japan) equipped with a Coregel 87H3 column from Transgenomic, USA and refractive index detector (RID). The analysis was run for 40 min with oven temperature of 80 °C, and 0.05 mM H_2SO_4 as an eluent with a flow rate of 0.6 mL/min. The xylose yield was reported based on the weight of initial biomass.

RESULTS AND DISCUSSION

Model Fitting

The xylose yields obtained from the optimization experiment of 40 runs are presented in Table 1. The analysis of variance of the data (Table 2) shows that the model generated from the experiment was significant (p value <0.05). The table also shows that acid concentration and the interaction between heating time and temperature had significant effects on xylose yield from the hydrolysates. The lack of fit F-value of 0.97 with p value of 0.4572 implies the lack of fit is not significant relative to the pure error (p value >0.10), which means that the model was good and fit. It can be used for predicting the xylose yield that could be obtained from the hydrolysis process using microwave heating with maleic acid catalyst. The coefficient of determinant (R^2) was high enough (0.7138). Considering the significant model, the insignificant lack of fit, and the high R^2, it can be suggested that the quadratic regression equation model generated in this experiment (Eq. 2 in coded form or Eq. 3 in actual form) was fit and can be used to predict the xylose yield of microwave-assisted maleic acid hydrolysis of ST.

\[
Y = 22.67 + 0.50 X_1 + 0.45 X_2 + 2.72 X_3
- 0.47 X_1^2 - 1.10 X_2^2 - 2.59 X_3^2 - \\
2.18 X_1 X_2 - 0.58 X_1 X_3 - 1.02 X_2 X_3 \tag{2}
\]

\[
Y = -672.08 + 27.53 X_1 + 6.07 X_2 + \\
88.36 X_3 - 0.14 X_1^2 - 0.01 X_2^2 - \\
12.81 X_1^3 - 0.13 X_1 X_2 - 0.72 X_1 X_3 - \\
0.25 X_2 X_3 \tag{3}
\]

Analysis of Response Surface

The results of response surface analysis in contour plots and surface plots are shown in Fig 2A, 2B, and 2C. Fig 2A shows that at shorter heating time the yield of xylose is increased with increase of temperature, while at longer heating time was vice versa, the yield was decreased with increase of temperature. Similarly, at lower temperature, the xylose yield was increased with increase of heating time, while at higher temperature the xylose yield was decreased with increase of heating time. These mean that the hydrolysis should use either low temperature and long heating time or high temperature and short heating time. Fig 2B shows that xylose yield was increased greatly with increase of acid concentration up to 1.5%, then the yield was slightly decrease when acid concentration was higher than 1.75%. On the other hand, the xylose yield was increased only slightly with increase of heating time. Fig 1C shows that at low temperature the xylose yield was greatly increased up to acid concentration of 1.5%, then it was slightly decreased when acid concentration was higher than 1.75%. At high temperature the xylose yield was greatly increased up to acid concentration of 1.25%, then it was decreased when acid concentration was higher than 1.5%. These suggest that in order to get high xylose yield, the acid concentration should not be higher than 1.5%. Fig 2C also shows that at low acid concentration the yield of xylose was steadily increased with increase of temperature; however, at high acid concentration it was only slightly increased up to about 175 °C, then it was slightly decreased when the temperature was higher than 190 °C.

The severity during the hydrolysis of hemicellulose or xylan in biomass to xylose involves three main factors, namely temperature, duration of heating, and acidity. Data in Table 1 and Fig 2a-2c show that the xylose yields generally increased due to increase of either temperature, duration of heating or acid concentration, but only until certain temperature, time and acid concentration. The xylose yield decreased when the temperature, duration of heating and acid concentration were further increased.
Table 1. Design of experiment and xylose yields from microwave-assisted maleic acid hydrolysis of sugarcane trash

| Run | $X_1$ | $X_2$ | $X_3$ | Time (min) | Temperature (°C) | Acid Concentration (%) | Xylose Yield (%)$^d$ |
|-----|-------|-------|-------|------------|------------------|------------------------|----------------------|
| 1   | 1     | 1     | 1     | 6.8        | 194              | 1.7                    | 19.24                |
| 2   | 1     | 1     | 1     | 6.8        | 194              | 1.7                    | 19.14                |
| 3   | 1     | 1     | -1    | 6.8        | 194              | 0.8                    | 15.40                |
| 4   | 1     | 1     | -1    | 6.8        | 194              | 0.8                    | 16.75                |
| 5   | 1     | -1    | 1     | 6.8        | 176              | 1.7                    | 27.02                |
| 6   | 1     | -1    | 1     | 6.8        | 176              | 1.7                    | 23.75                |
| 7   | 1     | -1    | -1    | 6.8        | 176              | 0.8                    | 22.13                |
| 8   | 1     | -1    | -1    | 6.8        | 176              | 0.8                    | 15.80                |
| 9   | -1    | 1     | 1     | 3.2        | 194              | 1.7                    | 21.62                |
| 10  | -1    | 1     | 1     | 3.2        | 194              | 1.7                    | 24.12                |
| 11  | -1    | 1     | -1    | 3.2        | 194              | 0.8                    | 19.22                |
| 12  | -1    | 1     | -1    | 3.2        | 194              | 0.8                    | 17.21                |
| 13  | -1    | -1    | 1     | 3.2        | 176              | 1.7                    | 21.64                |
| 14  | -1    | -1    | 1     | 3.2        | 176              | 1.7                    | 20.61                |
| 15  | -1    | -1    | -1    | 3.2        | 176              | 0.8                    | 13.46                |
| 16  | -1    | -1    | -1    | 3.2        | 176              | 0.8                    | 9.74                 |
| 17  | +α    | 0     | 0     | 8          | 185              | 1.25                   | 21.86                |
| 18  | +α    | 0     | 0     | 8          | 185              | 1.25                   | 19.53                |
| 19  | 0     | +α    | 0     | 5          | 200              | 1.25                   | 19.75                |
| 20  | 0     | +α    | 0     | 5          | 200              | 1.25                   | 21.60                |
| 21  | 0     | 0     | +α    | 5          | 185              | 2                      | 20.54                |
| 22  | 0     | 0     | +α    | 5          | 185              | 2                      | 16.26                |
| 23  | -α    | 0     | 0     | 2          | 185              | 1.25                   | 19.52                |
| 24  | -α    | 0     | 0     | 2          | 185              | 1.25                   | 20.69                |
| 25  | 0     | -α    | 0     | 5          | 170              | 1.25                   | 19.98                |
| 26  | 0     | -α    | 0     | 5          | 170              | 1.25                   | 13.12                |
| 27  | 0     | 0     | -α    | 5          | 185              | 0.5                    | 10.93                |
| 28  | 0     | 0     | -α    | 5          | 185              | 0.5                    | 9.84                 |
| 29  | 0     | 0     | 0     | 5          | 185              | 1.25                   | 21.33                |
| 30  | 0     | 0     | 0     | 5          | 185              | 1.25                   | 22.76                |
| 31  | 0     | 0     | 0     | 5          | 185              | 1.25                   | 27.03                |
| 32  | 0     | 0     | 0     | 5          | 185              | 1.25                   | 19.35                |
| 33  | 0     | 0     | 0     | 5          | 185              | 1.25                   | 25.39                |
| 34  | 0     | 0     | 0     | 5          | 185              | 1.25                   | 19.47                |
| 35  | 0     | 0     | 0     | 5          | 185              | 1.25                   | 20.04                |
| 36  | 0     | 0     | 0     | 5          | 185              | 1.25                   | 22.65                |
| 37  | 0     | 0     | 0     | 5          | 185              | 1.25                   | 27.41                |
| 38  | 0     | 0     | 0     | 5          | 185              | 1.25                   | 21.22                |
| 39  | 0     | 0     | 0     | 5          | 185              | 1.25                   | 26.13                |
| 40  | 0     | 0     | 0     | 5          | 185              | 1.25                   | 19.96                |

$^a$ $X_1$ = time; $^b$ $X_2$ = temperature; $^c$ $X_3$ = acid concentration; $^d$ = based on the weight of initial biomass
Figure 2. Contour plots obtained from the optimization of xylose hydrolysis from sugarcane trash, which show the effects of heating time and temperature (A), the effects of heating time and acid concentration (B), the effects of temperature and acid concentration (C), and xylose yield obtained from one of optimum conditions of xylose hydrolysis (D).
As we know, the xylan in the hemicellulose was hydrolyzed to xylose by heat treatment with acid. However, the xylose was degraded to furfural when the biomass exposed to the more severe treatment, such as due to increase of temperature, duration of heating, and acid concentration. Yemis and Mazza (2011) reported that the increase of temperature, heating time and acidity enhanced the conversion of xylose and xylan to furfural. Therefore, the xylose yield could be decreased when more severe conditions were applied. These phenomena were also reported by other researchers who applied acid hydrolysis of biomass either using conventional heating (Rahman et al., 2007, de Paiva et al., 2009, Zhang et al., 2015, Ji et al., 2017) or microwave heating (Yan et al., 2018). Data in Fig. 2a-2c and Table 2 show that acid concentration had significant and greater effect than did temperature or duration of heating alone on the xylose yield. This is in agreement with the results of study on optimization of xylose production from sugarcane bagasse by sulfuric acid with conventional heating, that showed the greater effect of sulfuric acid concentration than the temperature (de Paiva et al., 2009).

Optimum condition of hydrolysis for producing xylose was using maleic acid of 1.52% with heating temperature of 176 °C for 6.8 min (Fig 2D). At this condition the xylose yield reached 24.3% based on weight of initial biomass or 0.243 g/ g ST. With that yield the xylose concentration in the hydrolysate was approximately 22.3 g/L. The xylose yield obtained in this study is similar with that reported by de Paiva et al (2009) who obtained 266.73 mg/ g sugarcane bagasse, but higher than that obtained from wheat straw (0.185 g/ g wheat straw) reported by Ji et al (2017) or from corn cob (3.575 kg / 22 kg corn cob equal to 16.25%) reported by Zhang et al (2015).

**CONCLUSIONS**

Xylose could be produced from ST through hydrolysis using microwave heating and organic acid catalyst, such as maleic acid. At optimum hydrolysis conditions (maleic acid 1.52%, 6.8 min, 176 °C) the xylose yield could reach 24.3% or 0.243 g / g ST. By using microwave heating the hydrolysis of hemicellulose to xylose was fast, it could be completed in less than 10 minutes. However, further study is needed to hydrolyze the rest of the xylooligomers that might be present in the hydrolysate, so that the xylose yield and xylose concentration in the hydrolysate could increase.

**ACKNOWLEDGEMENT**

The authors would like to thank Japan Science and Technology Agency and Ministry of Science, Technology and Higher Education of the Republic of Indonesia for funding this research through JASTIPNet Project "Development of Integrated Process for Conversion of Sugarcane Trash to Bioethanol and Value-added Chemicals" 2016-2018 and Insinas Project "Pengembangan Teknologi Proses Pembuatan Biosurfaktan dan Xilitol sebagai Ko-Produk pada Produksi Bioetanol Generasi 2” 2019, respectively.
AUTHOR CONTRIBUTIONS
All authors contributed equally to this work

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