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Microwave-assisted acid hydrolysis to produce xylooligosaccharides from sugarcane bagasse hemicelluloses

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ABSTRACT

Hemicelluloses from sugarcane bagasse were subjected to microwave-assisted acid hydrolysis at mild temperature to produce xylooligosaccharides (XOS). The hydrolysis was performed with dilute H_2SO_4 at 90 °C and the influence of acid concentration (0.1–0.3 M) and reaction time (20–40 min) on the XOS production was ascertained with response surface methodology based on central composite design. The fitted models of XOS and xylose yields were in good agreement with the experimental results. Compared to hydrolysis time, acid concentration was a more significant coefficient in the production of XOS. A well-defined degree of polymerisation of XOS and the monomer in the hydrolysates were quantified. No sugar-degraded byproduct was detected. The maximum XOS yield of 290.2 mg g^{-1} was achieved by hydrolysis with 0.24 M H_2SO_4 for 31 min. The results indicated that the yields of xylose and the byproducts can be controlled by the acid concentration and reaction time in microwave-assisted acid hydrolysis.

1. Introduction

Currently, there has been increasing interest in the production of high-value products from ligocellulosic biomass. Particular emphasis has been placed on xylooligosaccharides (XOS), which are sugar oligomers made up of xylose residues through β-(1 → 4)-linkages where the number of xylose residues involved in their formation varies from 2 to 7 (Caparrós, Garrote, Ariza, Díaz, & López, 2007). This is mainly due to their remarkable potential for utilisation in many fields including pharmaceuticals, feed formulations and agricultural purposes (Vázquez, Alonso, Domínguez, & Parajó, 2000). Additionally, XOS possess a variety of excellent physiological properties such as lowering cholesterol, improving bowel function, calcium absorption and lipid metabolism, and reducing the risk of colon cancer, since they are not metabolised by human digestive system but promote the growth of beneficial intestinal bacteria such as *Bifidobacterium* and *Lactobacillus* (Chapla, Pandit, & Shah, 2012; Moure, Gullón, Domínguez, & Parajó, 2006; Vázquez et al., 2000). Thus, XOS have also been found numerous applications in food-related fields.

XOS are usually produced from xylan rich lignocellulosic materials by breaking down some ether bonds in the xylan backbone to give compounds with a lower degree of polymerisation (Vázquez et al., 2000). In recent years, several methods are being explored to generate XOS. The technologies used are classified into physical, chemical, and biochemical methods, among which autohydrolysis, enzymatic and acid hydrolysis have been studied extensively. Autohydrolysis involves the deacetylation of xylans to produce acetic acid, which hydrolyses the hemicelluloses from lignocellulosic materials. This process eliminates the use of corrosive chemicals for the extraction and hydrolysis of xylan. However, special equipment operated at high temperatures and pressures are employed (Wang, Sun, Cao, Tian, & Wang, 2009). In addition, extensive purification processes are needed because of the production of a variety of undesirable products such as degraded lignin, monosaccharides, as well as their degraded products. Enzymatic hydrolysis does not produce undesirable byproducts, but the time is relatively longer as compared to autohydrolysis. It also requires special conditions for storage and handling of the enzymes (Samanta, Jayapal, Kolte, Senani, Sridhar, Suresh, et al., 2012). In addition, the complete enzymatic hydrolysis of xylan depends on different enzymes acting in synergy since some of them prefer to randomly cleave unsubstituted xylan, but some prefer more highly branched xylan (de Menezes, Silva, Pavarina, Guímaro Dias, Guímaro Dias, Grossman, et al., 2009). Acid hydrolysis randomly cleaves the glycosidic bonds between adjacent xylose units and

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usually large amounts of undesirable degraded by-products are generated. With respect to the heating process, acid hydrolysis is usually conducted through convection or conduction, which has low process efficiency.

Microwave irradiation is an attractive technology with advantages of lower energy requirements, uniform and selective heating, the ability to start and stop instantaneously, as well as low equipment size and waste (Delazar, Nahar, Hamedeyazdan, & Sarker, 2012; Gabriel, Gabriel, Grant, Grant, Halstead, & Mingos, 1998; Warrand & Janssen, 2007). These benefits lead to its wide applications such as food processing, wood drying, natural products extract, and lignocellulosic biomass treatment. When biomass is subjected to microwave irradiation, it is assumed that the induced hot spots in the biomass disrupt the structure and thus improve the disintegration of the material (Janker-Obermeier, Sieber, Faulstich, & Schieder, 2012). Recently, reports on microwave-assisted acid hydrolysis for the production of xylose or furfural have been already available (Kumar & Wyman, 2008; Rose & Inglett, 2010; Yemis & Mazza, 2011), but less attention has been paid on the treatment of hemicelluloses to produce oligosaccharides.

The aim of the present study was to apply microwave-assisted acid hydrolysis to produce XOS from sugarcane bagasse, an industrial crop with an annual production of up to 100 million tons (Zhao, Wang, Lin, & Guo, 2012). The effects of sulfuric acid concentration and hydrolysis time on the hydrolysis were assessed in terms of the composition and degree of polymerisation (DP) of hydrolysates. In order to maximise efficiency in the production of XOS, the optimum microwave-assisted hydrolysis conditions were identified by response surface methodology (RSM).

2. Experimental

2.1. Raw material

Sugarcane bagasse used in this work was collected from a sugar factory in Guangzhou, China. It was dried in an oven at 55 °C for 16 h, ground using a laboratory mill, and screened to obtain a fraction with average mesh size of 20–80. The composition of the sugarcane bagasse was cellulose 43.6%, hemicelluloses 33.5%, lignin 18.1%, ash 2.3%, and wax 0.8% on a dry weight basis.

2.2. Preparation of hemicellulosic fraction from sugarcane bagasse

Wax and other extracts of sugarcane bagasse were firstly removed by refluxing in a Soxhlet apparatus with ethanol/toluene (1:2, v/v) for 8 h. Then the powder obtained was delignified with sodium chlorite in acidic solution (pH 3.8–4.0, adjusted by acetic acid) at 75 °C for 2 h. Subsequently, the delignified material was extracted with 10% KOH, with a solid to liquor ratio of 1:20 (g ml^{-1}) at 30 °C for 10 h. The liquid fraction was collected, neutralised to pH 5.5–6.0 with acetic acid, concentrated, and precipitated by the addition of three equivalent volumes of 95% ethanol. The resulting precipitate was dissolved in distilled water after separating by filtration, dialysed (molecular weight cut-off 3500 g mol^{-1}) against water, and then freeze-dried.

2.3. Chemical characterisation of isolated hemicellulosic fraction

The composition of xylan-rich hemicelluloses was determined by high-performance anion-exchange chromatography (HPAEC) ICS3000 gradient using a Carbopac™ PA-20 column (4 × 250 mm, Dionex). The monosaccharides and uronic acids were separated in 18 mM NaOH (carbonate free and purged with nitrogen), with post-column addition of 0.3 M NaOH at a rate of 0.5 ml min^{-1}. Run time was 45 min, followed by a 10 min elution with 0.2 M NaOH to wash the column and then a 15 min elution with 18 mM NaOH was carried out to re-equilibrate the column. Calibration was performed with a standard solution of L-arabinose, D-galactose, D-glucose, D-xylose, D-mannose, galacturonic acid, and glucuronic acid (Sigma–Aldrich, China). The molecular weights of the precipitated xylan-rich fraction were determined by gel permeation chromatograph (GPC) on a PL aquagel-OH 50 column calibrated with PL pullulan polysaccharide standards. The eluent was 0.02 M NaCl in 0.005 M sodium phosphate buffer (pH 7.5). A flow rate of 0.5 ml min^{-1} was maintained. FT-IR spectrum was measured using a Nicolet iN 10 FT-IR Microscope (Thermo Nicolet Corporation, Madison, WI) equipped with a liquid nitrogen cooled MCT detector. The spectrum was measured in the range of 4000–750 cm^{-1} at a resolution of 8 cm^{-1}.

2.4. Microwave-assisted hydrolysis of hemicellulosic fraction

Microwave irradiation was performed by using a microwave oven (Beijing Xiang-Hu Science and Technology Development Reagent Co., Ltd.) equipped with a magnetic stirrer and a temperature probe. Microwave intensity and irradiation time were set by a control panel. According to the experimental design, the xylan-rich hemicelluloses were suspended in dilute sulfuric acid solution with a solid to liquor ratio of 1:25 (g ml^{-1}) in each run. A microwave irradiation power of 700 W was applied to heat the suspension to 90 °C and held for the desired time with refluxing. After irradiation, the reactant was immediately cooled in an ice bath to room temperature and centrifuged to separate solubilised fraction for analysis. Acid concentration and hydrolysis time for the experimental design are given in Table 1. A total of 11 runs were carried out and the experimental runs were randomised to minimise the effect of unexpected variability in the observed responses.

2.5. Experimental design and data analysis

Preliminary experiments with microwave-assisted hydrolysis of sugarcane bagasse hemicelluloses indicated that sulphuric acid concentration and hydrolysis time had significant effects on the yield of xylooligosaccharides. The hydrolysis parameters were optimised using response surface methodology. Central composite design (CCD) for 2 factors and three replicates at the center point was employed. Sulfuric acid concentration (U_1) and hydrolysis time (U_2) were chosen for independent variables. The range of the two independent variables and the center point values are presented in Table 1 based on the preliminary experiments. The yields of XOS and xylose were determined as the response variable (Y). The variables were coded according to the equation:

$$X_i = (U_i - U_{i,0})/\Delta U_i, \quad (1)$$

where x_i is the coded value of the variable U_i, $U_{i,0}$ is the value of U_i at the center point, and ΔU_i is the step change.

The relationship of the independent variables and response was calculated by the quadratic polynomial equation:

$$Y = A_0 + \sum A_i U_i + \sum A_{ii} U_i^2 + \sum \sum A_{ij} U_i U_j, \quad (2)$$

where U_i and U_j are independent variables, which influence the response variable Y, A_0 is the intercept, A_i is the linear coefficient, on the response Y; A_{ii} is the quadratic coefficient and A_{ij} estimates the interaction effect between variables i and j on the response Y. The statistical software Design-Expert (Version 8.0.6) was used for the regression analysis of the experimental data and the response surface plots. Analysis of variance (ANOVA) was used to estimate the statistical parameters.

Table 1
Independent variables of the central composite design and the experimental results.

Run	Variables		Coded levels		Responses	
	U_1 (M)	U_2 (min)	x_1	x_2	XOS yield (mg g^{-1})	Xylose yield (mg g^{-1})
1	0.1	20	−1	−1	72.3	8.7
2	0.1	30	−1	0	138.3	19.9
3	0.1	40	−1	1	160.8	28.0
4	0.2	20	0	−1	204.7	42.6
5	0.2	30	0	0	290.1	85.5
6	0.2	30	0	0	277.9	99.9
7	0.2	30	0	0	280.5	97.6
8	0.2	40	0	1	262.2	100.2
9	0.3	20	1	−1	248.9	101.2
10	0.3	30	1	0	255.1	116.9
11	0.3	40	1	1	232.5	172.8

2.6. Hydrolysate analysis by HPAEC

Released xylooligosaccharides were quantified by HPAEC Dionex ICS 3000 using a Carbopac™ PA-100 column (4 × 250 mm, Dionex) in combination with a PA-100 guard column (4 × 50 mm, Dionex). The column temperature was 30 °C and the flow rate of the gradient elution was 0.4 ml min^{-1}. XOS was separated with 0–80 mM NaAc gradient in a 100 mM NaOH isocratic (carbonate free and purged with nitrogen) for 15 min, followed by a 80–300 mM NaAc gradient in 100 mM NaOH for 10 min, then a 10 min elution with 100 mM NaOH was used to re-equilibrate the column before the next injection. The concentration of the oligosaccharides was quantified using peak area and compared with those for xylobiose (X2), xylotriose (X3), xylotetraose (X4), xylopentaose (X5), and xylohexose (X6), purchased from Megazyme (Ireland). XOS yield (w/w) was calculated as (X2 + X3 + X4 + X5 + X6)/xylan weight. The free monosaccharides in the hydrolysates were determined by HPAEC as described above. The quantitative analysis of the degradation products in hydrolysate was performed on an high performance liquid chromatography (HPLC, Agilent 1200 series, Agilent Technologies, USA) equipped with a refractive index detector (Yang, Wang, Xu, Sun, & Lu, 2012). The separation was performed on an aminex column (HPX-87H ion exclusion column with a length of 300 mm and an inner diameter of 7.8 mm, Bio-Rad Laboratories, USA) at 50 °C with 5 mM sulfuric acid at a flow rate of 0.6 ml min^{-1}. Before measurements, all the samples were filtered through 0.22 μm syringe filter.

3. Results and discussion

3.1. Chemical composition of hemicellulosic preparation

Sugarcane bagasse is made up of structural and non-structural components. The non-structural components of sugarcane bagasse were removed and the dewaxed feedstock was then subjected to delignification and alkaline extraction to obtain hemicellulosic fraction. The fraction obtained was chemically characterised, since an accurate determination of the starting material is of vital importance in the XOS production. The structure and purity of the XOS were strongly affected by the chemical composition of the hemicelluloses utilised (Parajó, Garrote, Cruz, & Dominguez, 2004). Compositional analysis indicated that xylose was the dominant component of the isolated hemicellulosic fraction, comprising 83.8% of the total neutral sugars. Arabinose (11.3%) appeared to be the secondary major sugar and galactose (0.5%), glucose (3.1%) and uronic acid (1.4%) were detected in minor amounts. The results showed that the fraction obtained was mainly composed of xylan, which was beneficial for XOS production. The molecular weights and the polydispersity of the obtained hemicellulose preparation were also determined. The weight-average molecular weight was 76,880 g mol^{-1} and the polydispersity was 3.12, which indicated the relatively homogeneous structure of the isolated hemicellulose preparation. The structure of the hemicelluloses was determined by FT-IR (Fig. 1) since it has been proven to be a easy and quick tool for studying the structure and property of polysaccharides (Kacurakova & Mathlouthi, 1996). The absorbances at 3386, 2908, 1643, 1458, 1331, 1241, 1160, 1030, 975 and 899 cm^{-1} in the spectrum are associated with typical hemicelluloses. The presence of arabinosyl side chains is documented by the two low-intensity shoulders at 1160 and 975 cm^{-1}. The intensive band at 1030 cm^{-1} is due to C—O, C—C stretching and the glycosidic (C—O—C) contributions, indicating a dominant xylan in the isolated hemicelluloses, in good agreement with the compositional analysis aforementioned. The sharp band at 899 cm^{-1} is indicative of the β-configuration for the glycosidic linkages between xylopyranose units in the main xylan chains. Data from analytical investigations indicated that the isolated hemicelluloses were potentially applicable for XOS production. Although the branched structure causes the XOS to have an enhanced variety of structures, some substitute unit, especially arabinose linked to the xylan backbone, is susceptible to hydrolysis and gives furfural and other undesired reaction byproducts during the production (Parajó et al., 2004). Therefore, the efficient and proper production method and operation conditions should be chosen ideally.

3.2. Effects of microwave-assisted acid hydrolysis of hemicelluloses

Acid hydrolysis leads to partial degradation of hemicelluloses into soluble lower molecular weight polymers, oligosaccharides and monosaccharides. The proportion of the soluble compounds depends on the operation conditions. Temperature, acid concentration, and reaction time are the most crucial parameters in the hydrolysis of hemicelluloses, since they affect hydrolysis rate and selectivity. In the present study, a mild temperature of 90 °C was utilised as this was the maximum temperature measured after the longest microwave irradiation in atmospheric pressure (Warrand & Janssen, 2007). In fact, both the mild temperature and the way of applying the heat were important in the study. According to the preliminary experiment, the dilute acid concentrations from 0.1 to 0.3 M and reaction time 20–40 min were selected for hydrolysis treatment.

After the microwave-assisted acid hydrolysis of sugarcane bagasse hemicelluloses was completed, the hydrolysis products in the solution of each sample were analysed. Although the DP larger than X6 could be seen, the amount of each sample was reported up to DP 6, due to the lack of X7 of the standard (Gullon, Gonzalez-Munoz, & Parajo, 2011). The relative contents of X2, X3, X4, X5, and X6 in XOS are shown in Fig. 2. When the acid concentration increased from 0.1 to 0.3 M with 20 min reaction time, the

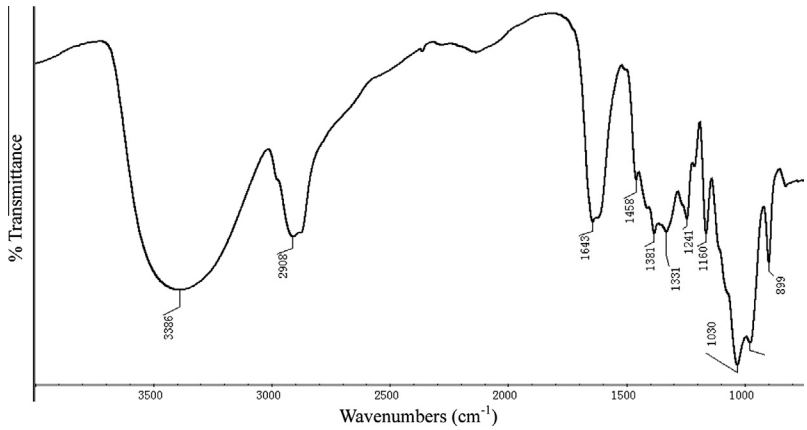

Fig. 1. FT-IR spectrum of hemicellulosic fraction from sugarcane bagasse.

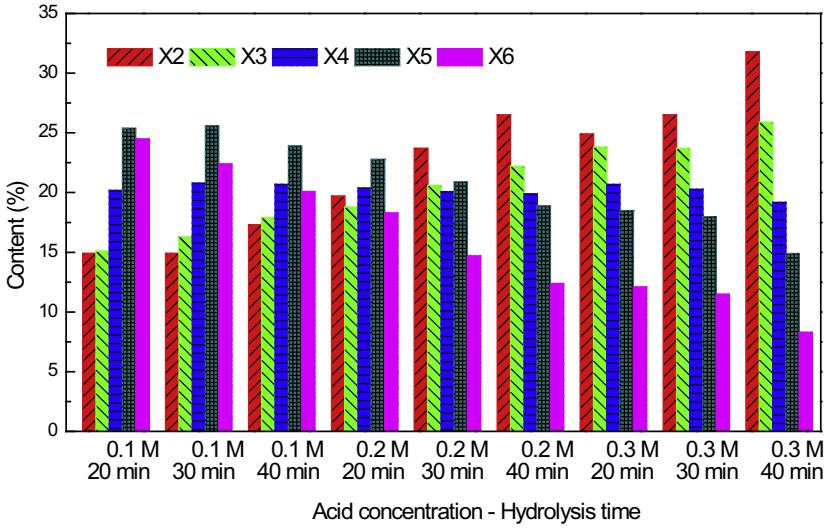

Fig. 2. The relative contents of xylobiose (X2), xylotriose (X3), xylotetraose (X4), xylopentaose (X5), and xylohexose (X6) in XOS produced from sugarcane bagasse hemicelluloses with different acid concentrations and times.

content of X6 decreased drastically from 24.5% to 12.1%; the content of X5 decreased from 25.4% to 18.5%, but the contents of X2 and X3 increased from 14.9% and 15.1% to 24.9% and 23.8%, respectively. This indicated that xylopentose and xylohexaose continued to be hydrolysed into smaller oligosaccharides after release with the increased acid concentration. As similar observation was also obtained with the hydrolysis times of 30 and 40 min. The effect of different reaction times on XOS production can also be observed in the figure. When the reaction time increased from 20 to 40 min at an acid concentration of 0.1 M, the contents of X2 and X3 slightly increased from 14.9% and 15.1% to 17.3% and 17.9%, accompanied by a decrease in the contents X5 and X6 from 25.4% and 24.5% to 23.9% and 20.1%, respectively. The results were paralleled with the acid concentrations of 0.2 and 0.3 M. In addition, higher level of X2 and X3 concentrations and lower amounts of X5 and X6 were observed with the higher acid concentrations. However, the content of X4 in all of the hydrolysates remained at a constant level. It was concluded that the distribution of DP for the products was dependent on both acid concentration and hydrolysis time under the conditions used.

Since the DP decreased with the increasing acid concentration and hydrolysis time, the formation of monomers in the hydrolysate was unavoidable. Usually, the treatment with concentrated acid and/or high temperature causes the degradation of carbohydrates to form byproducts such as furfural and hydroxymethylfurfural (HMF), reducing XOS purity (Otieno & Ahring, 2012). In the present study, no side products were detected by HPLC under the conditions used. This is probably a consequence of the mild hydrolysis conditions at a low temperature of 90 °C. This can be supported by Neureiter, Danner, Thomasser, Saidi, and Braun (2002) who concluded that temperature has an important impact on the formation of sugar degradation products.

3.3. Fitting models

From the above results, it is clear that acid concentration and hydrolysis time are the major factors that influence the resulting products. When the acid concentration increases to a certain value, the degradation of sugars takes place. It is accepted that the oligosaccharide formation is more prominent in dilute acids (Maki-Arvela, Salmi, Holmbom, Willfor, & Murzin, 2011). However, if the acid concentration is too low, the process becomes economically unfavourable. Thus, acid concentration and hydrolysis time should be optimised to achieve high XOS yield. Response surface methodology is an effective statistical procedure using a minimum set of experiments to determine the coefficients of a mathematical model and the optimum conditions (Yemis & Mazza, 2012). Values of the independent process variables were studied and the

responses obtained from 11 different combinations of microwave-assisted reaction conditions are shown in Table 1. The yields of XOS and xylose were in the range of 72.3–290.1 mg g^{-1} and 8.7–172.8 mg g^{-1}, respectively. The fit summary report produced by Design-Expert recommends the quadratic and linear models for XOS and xylose yields, respectively. The following regression equations represent the description of XOS and xylose yields from the experimental responses:

$$Y_{XOS} = -642.37632 + 4181.4605U_1 + 27.14816U_2 - 26.225U_1U_2 - 6965.52632U_1^2 - 0.32905U_2^2$$

$$Y_{xylose} = -106.2924242 + 557.1666667U_1 + 2.475U_2$$

The correlation measure for testing good-of-fit of the regression equation is the determination coefficient R^2, which is defined as the ratio of the explained variation to the total variation and demonstrates the agreement between the observed and predicted results (Nath & Chattopadhyay, 2007). It is suggested that at least 0.80 is a good fit for a model (Yemis & Mazza, 2012). In the present study, R^2 for XOS yield was found to be 0.982 and 0.915 for the xylose, respectively. The high values of R^2 of the models indicated a close agreement between the experimental results and the theoretical values predicted by the model. This similarity can also be verified by the high correlations between the observed and predicted values in Fig. 3.

In order to obtain the best-fit model, analysis of variance (ANOVA) was considered to determinate the adequacy of the models. Regression coefficients and analysis of variance for XOS and xylose yields are presented in Table 2. The probability value (*P*-value) was used to check the significance of the coefficient, which indicates the interaction between each independent variable. *P*-values less than 0.05 for the regression model are statistically significant and reversed to the not significant model term (Oomah & Mazza, 2008). ANOVA analysis showed that the *P*-values were 0.1385 and 0.1644 for the two models respectively, indicating the lack of fit was not significant and further demonstrated the two models were of good fit. The analysis of XOS yield showed that the *P*-values of U_1, U_2, U_1U_2, U_1^2, and U_2^2 were <0.0001, 0.0103, 0.0106, 0.0004, and 0.01078, respectively, indicating the independent variables U_1, U_2, and the quadratic terms of U_1U_2, U_1^2, and U_2^2 had significant effects on XOS yield. This also demonstrated that acid concentration and reaction time had significant effects on the production of XOS. *P*-value is also an indication of the order of significance for process variables affecting the yields, where smaller *P*-values indicate higher significance of the corresponding coefficient (Muralidhar, Chirumamila, Marchant, & Nigam, 2001). Compared to hydrolysis time, the effect of acid concentration was higher on XOS yield, since a smaller *P*-value of acid concentration indicated a more significant coefficient. As for xylose, both U_1 and U_2 had significant effects on xylose yield as indicated by the low *P*-values of <0.0001 and 0.0054, respectively. Obviously, the effect of acid concentration was more significant than that of hydrolysis time on xylose yield.

3.4. Optimisation of process variables

Two-dimensional contour plot and three-dimensional response surface are the graphical representations of the regression equation, which provide a method to evaluate the interactions between variables and predict the optimum values for each variable at maximum value (Qiao, Hu, Gan, Sun, Ye, & Zeng, 2009). In the present study, two-dimensional contour plots and three-dimensional response surface plots were generated using Design-Expert as presented in Fig. 4.

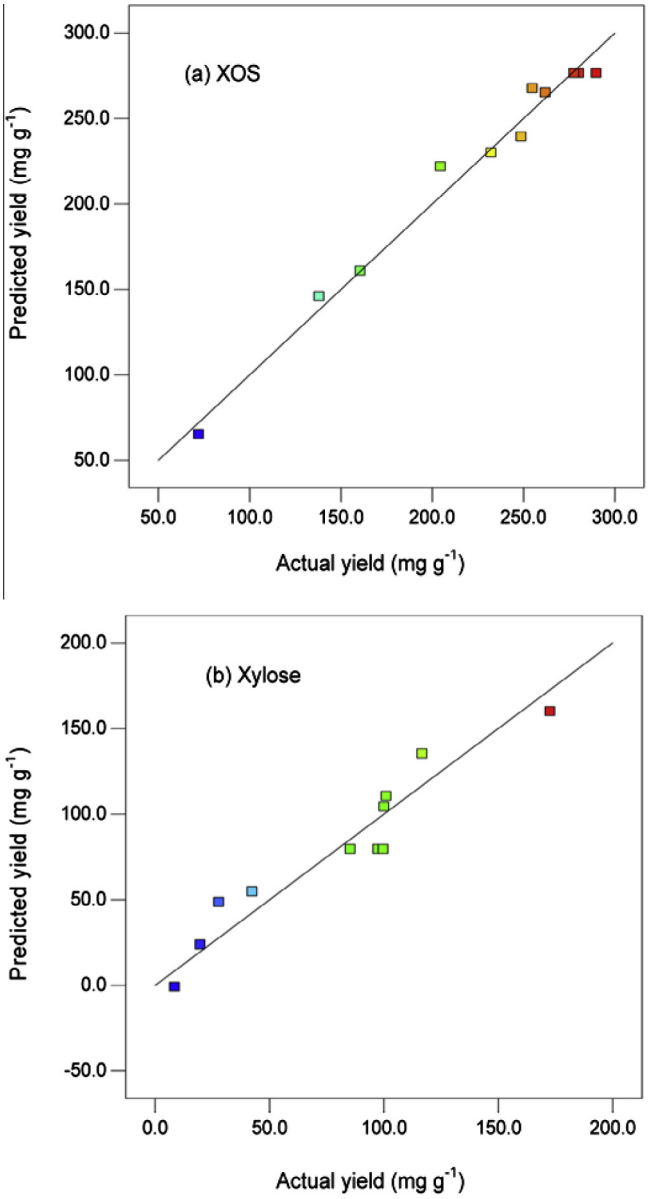

Fig. 3. Actual vs. predicated yields of XOS and xylose.

The linear effect of acid concentration on XOS yield was observed only in the range of 0.1–0.24 M, and further increases in acid concentration resulted in a decrease in XOS yield. Similarly, an increase in XOS yield was observed with the increasing time from 20 to 30 min and then XOS yield decreased as the time increased. The yield of XOS increased as acid concentration increased from 0.1 to 0.24 M, then went down as acid concentration further increased, which indicated that a sulfuric acid concentration of 0.24 M was required to achieve maximum. Likewise, hydrolysis time increased from 20 to 30 min led to an increase in XOS yield and then a decrease with the further increasing time. The results showed that these two variables substantially affected the production of XOS. The experiments performed at both higher acid concentration and longer hydrolysis time led to a decrease in the yield of XOS. This was in accordance with the increase of xylose yield discussed below. The red zone in Fig. 4 is the optimal condition for the production of XOS. The hydrolytic depolymerisation of the xylan-rich hemicelluloses proceeded most efficiently at 0.24 M sulfuric acid and a hydrolysis time of 31 min, with a predicted

Table 2
Analysis of variance of the models for XOS and xylose yields.

Source	Sum of squares	Degrees of freedom	Mean square	*F*-value	*P*-value
XOS (mg g^{-1})					
Model	47285.86	5	9457.171	54.16213	0.0002
U_1	22216.34	1	22216.34	127.2351	<0.0001
U_2	2799.36	1	2799.36	16.0322	0.0103
U_1U_2	2751.003	1	2751.003	15.75526	0.0106
U_1^2	12291.37	1	12291.37	70.39385	0.0004
U_2^2	2742.983	1	2742.983	15.70933	0.0107
Residue	873.0428	5	174.6086		
Lack of fit	790.4561	3	263.4854	6.380821	0.1385
Pure error	82.58667	2	41.29333		
Corrected total	48158.9	10			
Xylose (mg g^{-1})					
Model	22301.46	2	11150.73	43.30648	<0.0001
U_1	18626.08	1	18626.08	72.33878	<0.0001
U_2	3675.375	1	3675.375	14.27418	0.0054
Residual	2059.872	8	257.4841		
Lack of fit	1940.186	6	323.3643	5.403514	0.1644
Pure error	119.6867	2	59.84333		
Corrected total	24361.33	10			
Model	22301.46	2	11150.73	43.30648	<0.0001

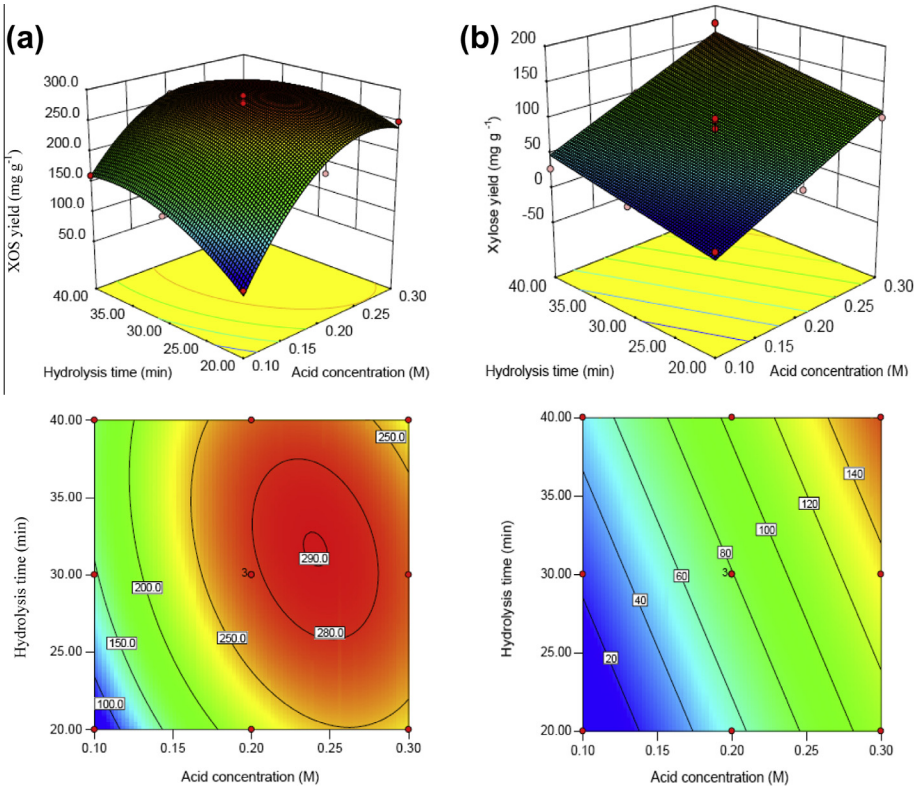

Fig. 4. Response surface and contour plot of acid concentration vs. hydrolysis time on the XOS (a) and xylose (b) yields.

optimum value of 290.2 mg g^{-1}. The XOS yield in the present study was higher than the results reported in the hydrolysis of tobacco stalk xylan in 0.25 M sulfuric acid for 30 min heated by boiling water bath (140 mg g^{-1}), as well as the hydrolysis by xylanase (114 mg g^{-1}) for 24 h (Akpinar, Erdogan, Bakir, & Yilmaz, 2010).

As shown in Fig. 3, xylose yield in the hydrolysis process increased with the increasing of acid concentration and hydrolysis time. The surface analysis showed no interaction between the two variables, and xylose yield linearly increased as a function of the variables under the conditions used. In general, the concentration of 0.1 M sulfuric acid resulted in the lowest level of xylose, followed by 0.2 M, and then 0.3 M. The content of xylose also increased with the increasing reaction time. The phenomenon was also consistent with the distribution of the DP. The XOS with higher DP were hydrolysed into both XOS with lower DP and xylose with the increase in acid concentration and hydrolysis time. Under the optimum conditions for the production of XOS aforementioned, a xylose yield of 104.8 mg g^{-1} was achieved. This indicated that a certain amount of xylose was obtained when maximum XOS was produced during the microwave-assisted acid hydrolysis process. Above all, the phenomena indicated that the monosaccharide and byproduct can be controlled by acid concentration and reaction

time in the microwave-assisted hydrolysis process at mild temperature.

4. Conclusions

In this study, the effect of microwave-assisted acid hydrolysis of sugarcane bagasse on the production of XOS was investigated. The statistical analysis showed that acid concentration was more significant as compared to the hydrolysis time in the production of XOS. Response surface analysis indicated that the microwave-assisted acid hydrolysis performed with 0.24 M H_2SO_4 for 31 min, produced a maximum XOS yield of 290.2 mg g^{-1}. Xylose yield increased with an increasing acid concentration and residence time and no sugar-degraded products were formed during the process. Thus, microwave-assisted acid hydrolysis at mild temperature provides an efficient technique to produce XOS with great application potential.

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