OPTIMIZATION AND COMPARISON OF DILUTE ACID PRETREATMENT OF SELECTED AGRICULTURAL RESIDUES FOR RECOVERY OF XYLOSE

Ozlem Akpinar,[a,*] Okan Levent,[a] Serdal Sabanci,[a] Reyhan Selin Uysal,[a] and Burcu Sapci[a]

Two different agricultural wastes, sunflower stalk and tobacco stalk, were evaluated for the production of xylose, which can be used as a raw material and converted to xylitol, a highly valued product. The objective of the study was to determine the effects of H_2SO_4 concentration, temperature, and reaction time on the production of sugars (xylose, glucose, and arabinose), and on the reaction by-products (furfural and acetic acid) from sunflower stalk and tobacco stalk and to compare the hydrolysis conditions of these wastes. Since both agricultural wastes had different structures, they had different responses to experimental conditions. Response surface methodology (RSM) was used to optimize the hydrolysis process in order to obtain high xylose yield and selectivity. The optimum reaction temperature, reaction time and acid concentration were 120 ^{o}C, 30 min and 4% of acid concentration for sunflower stalk and 133 ^{o}C, 27 min and 4.9% of acid concentration for tobacco stalk.

Keywords: Xylose; Xylitol; Sunflower stalk; Tobacco stalk; Optimization

Contact information: Gaziosmanpasa University, Department of Food Engineering, Taslıciftlik 60100, Tokat, Turkey; Corresponding author: ozlem.akpinar@gop.edu.tr*

INTRODUCTION

Agricultural wastes widely available in Turkey are produced at an annual rate of more than 50 million tons. These wastes can be used as animal feed, but this use has slight economical significance. They are usually left to rot or be burned in the field after harvesting (Agrupis and Maekawa 1999). Nowadays, the conversion of these materials to fuels and chemicals has been receiving enormous attention because they are cheap, renewable, and contain high amount of carbohydrates (cellulose and hemicellulose). Therefore, utilization of agricultural biomass for industrial purposes offer economic and ecological benefits by solving the proper disposal of them, providing additional income for farmers and generating employment.

Since agricultural wastes are mainly composed of hemicellulose, cellulose, and lignin, they can be used as a renewable material for production of valued added products (Silverstein et al. 2007). They contain around 20% of hemicellulose. The main component of the hemicellulosic fraction of these wastes is xylan, a heteropolysaccharide, made of xylose sugar that can be used as source for production of chemicals including food-related products (Saha 2003). One such compound is xylitol, a five carbon sugar alcohol that is a natural sweetener. Xylitol has been extensively used in various food products such as chewing gum, candy, soft drinks, and ice cream (Olinger and Pepper 2001; Rivas et al. 2002).

The biotechnical production of xylitol, employing a hemicellulose fraction of lignocellulosic materials, instead of pure xylose is a more versatile approach to reduce the cost of production (Parajo et al. 1998; Winkelhausen and Kuzmanova 1998). Therefore, the first step is the careful hemicellulose depolymerization to monomeric sugars during production of xylitol. This can be easily accomplished by mild acid treatment due to its amorphous structure, while cellulosic and lignin fractions remain unaltered (Rahman et al. 2007).

Hemicellulose hydrolysis of different lignocellulosic materials using dilute acid pretreatment has been studied by many researchers (Canettieri et al. 2007; Herrara et al. 2003; Liavoga et al. 2007; Rahman et al. 2007; Roberto et al. 1995, 2003). The results showed that the amount of sugar released during pretreatment is dependent on the source of lignocellulosic materials and operating conditions of the experiments, such as temperature reaction time and acid concentration. Although controlled acid hydrolysis pretreatment of lignocellulosic biomass mainly produces xylose from hemicellulose, other by-products such as glucose, acetic acid, and furfural are produced in low amounts. Since lignocellulosic materials contain different amounts of lignin, cellulose, and hemicellulose, the amount of these degradation products depends on the amount of the polymer and structure of hemicellulose in the lignocellulosic biomass. Acid concentration is the most important parameter affecting the sugar yield, while temperature is mainly responsible for degradation of sugars to various by-products such as furfural (Rahman et al., 2007), which strongly affect the microbial metabolism during xylitol production. To overcome this problem, it is necessary to run the hydrolysis reaction under less severe conditions for each lignocellulosic waste to keep the degradation products at low concentration.

Tobacco and sunflower are important crops in the Middle Black Sea region in Turkey. The previous studies showed that xylan from tobacco and sunflower stalk are mainly composed of xylose units (>90%) and that they have a more linear structure than xylan from softwood (Akpinar et al. 2009). As there has been no study on the optimization of dilute acid hydrolysis pretreatment conditions of sunflower and tobacco stalks to produce xylose, the aim of this study was to produce xylose from the agricultural wastes, find the optimum conditions, and to compare xylose production conditions (Bascetincelik et al. 2006).

EXPERIMENTAL

Materials

Agricultural wastes were collected from local farmers in Turkey, air-dried, and milled to obtain particles that were 1 to 5 mm long and 1 mm thick. An Aminex HPX 87H column (dimension: 300x7.8 mm; average particle size: 9 μm) and cation H cartridge were purchased from Bio-Rad Laboratories, CA, USA. All the chemicals were analytical grade and obtained either from Sigma Chemical Company, MO, USA, or Merck KGaA, Germany.

Agricultural Wastes Composition

The stalks were analyzed following standard methods for the determination of moisture, ash, and lignin (ASTM 1993). Moisture and ash were determined gravimetrically by desiccation of the samples at 105 °C and by ignition in an oven at 600 °C, respectively. Klason lignin (acid insoluble lignin) was gravimetrically measured as the insoluble fraction after digestion with 72% sulfuric acid. Acid-soluble lignin was determined by measuring the UV absorption at 205 nm using an extinction coefficient of 1101 $g^{-1}cm^{-1}$ (Canettieri et al. 2007). Uronic acid was determined spectrophotometrically using glucoronic acid as a standard for quantification (Melton and Smith 2001). Protein content of the agricultural waste was measured by the Kjeldahl N method (Protein=6.25xN).

The polysaccharides in the stalk were hydrolyzed according to Browning (1967), and the monosaccharide composition was determined. Ground agricultural waste (300 mg) was mixed with 72% sulfuric acid (3 mL), and the mixture was held at 30 °C for 1 h with stirring. The concentration of acid in the mixture was adjusted to 4.0% by adding water, and the mixture was refluxed for 2 h. The sugars in the aliquot of the hydrolysate were assayed by HPLC on Aminex HPX 87H (300 x 7.8 mm) column at 45 ^{o}C with a flow rate of 0.5 mL/min as described in analytic methods.. The monosaccharide presents in the hydrolysate were converted to percent monosaccharides: D-glucose to glucan, D-xylose to xylan, and D-arabinose to arabinan.

Acid Hyrolysis Pretreatment

Acid pretreatment experiments were performed in a 100 mL stainless-steel pressure batch reactor. The reactor was loaded 2 g of agricultural waste (dry weight) and 20 mL of sulfuric acid solution. The reactions were carried in the range of 86.7 to 153.3 ^{o}C under different sulfuric acid concentrations (0.7 to 7.3% $H_2 SO_4$) and residence times (5 to 55 min). After the reaction was completed, the solid material was separated with filtration and the filtrate was analysed for xylose, glucose, acetic acid and furfural.

Analytical Methods

Hydrolysates from acid pretreated samples were analysed with an HPLC system equipped with a refractive index detector (Perkin Elmer Series 200), and column oven (Perkin Elmer Series 200) on Aminex HPX 87H (300 x 7.8 mm), which was preceded by its complimentary cation H cartridge. Before injection, samples were filtered through a 0.20 μm filter. Aliquots of filtered sample (20 μL) were injected to the HPLC system. Sugars and acetic acid were eluted with 5 mmol/L H_2SO_4, the mobile phase from the column. It was used at 45 ^{o}C and a flow rate of 0.5 mL/min (Canettieri et al. 2007). A complete analysis was carried out in 70 min. A computing integrator determined the start, retention time, and end of the peak, and it integrated the area under each peak as a function of height and width of the peak. Their concentration was quantified using average peak areas compared with mixture of standard (xylose, glucose, arabinose, acetic acid, and furfural) and expressed as g/L sugar.

Experimental Design and Response Surface Methodology (RSM)

A 2^3 rotatable central composite design (CCD) was used in order to fit a second order model and the design consisted of 20 sets of experiments. Experimental range and

levels of independent variables investigated are given in Table 1. The quadratic model was selected for predicting the optimal point and is expressed as,

$$Y= b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 \quad (1)$$

where Y represents response variables (xylose yield and selectivity), b_0 is the interception coefficient, b_1, b_2, and b_3 are the linear terms, b_{11}, b_{22}, and b_{33} are the quadratic terms and X_1, X_2, and X_3 represent the variables studied. Xylose yield ($Y1$) was calculated as a ratio of xylose concentration of hydrolysate to xylose content of stalk that was defined as maximum xylose concentration. Hydrolysis of selectivity (Y_2) was calculated as a ratio of xylose to glucose in the hydrolysate.

Table 1. Experimental Range and Levels of Independent Process Variables

Independent variables	Symbol	Range and Levels				
		-α	-1	0	+1	+α
Temperature (°C)	X_1	86.7	100	120	140	153.3
Reaction time (min)	X_2	5	15	30	45	55
Acid concentratin (%)	X_3	0.7	2	4	6	7.3

The Design Expert v. 7 software (Stat-Ease Inc., Minneapolis) was used for regression and graphical analyses of the data obtained. Fischer's test was used for determination of the type of model equation, while the student's t-test was performed for the determination of statistical significance of regression coefficients.

RESULTS AND DISCUSSION

Composition of Sunflower Stalk and Tobacco stalk

Table 2 presents the composition of tobacco stalk and sunflower stalk. Like most of the non-wood fibers, ash content was markedly higher than that of the wood species (Agrupis and Maekawa 1999). The major component was determined as glucan, which can be used in the production of ethanol, followed by xylan and Klason lignin. The chemical compositions of tobacco stalk and sunflower stalk were similar to each other. Other components (acid soluble lignin and protein) also were determined. The rest of the components (extractives such as hot water, cold water or ethanol extractives) have minor importance for this study, and are reported as "others".

Although these materials are very complex, a detailed knowledge of their composition is necessary in order to calculate the theoretical yield of xylose. The potential maximum concentration of xylose produced in the sunflower stalk and tobacco stalk hydrolysate was 24.9 g/L and 24.2 g/L, respectively.

Table 2. Composition of the Raw Material, Expressed as Weight Percent of Dry Weight

Components	Content (g/100 g tobacco stalk)	Content (g/100 g sunflower stalk)
Glucan	33±3	36±3
Xylan	21±1	22±4
Arabinan	0.77±0.07	0.66±0.05
Acetyl groups	2.8±0.9	2.8±0.8
Uronic acid	8.5±0.3	6.2±0.6
Klason lignin	23±1	26±0
Acid soluble lignin	1.5±0.1	1.3±0.0
Proteins	1.8±0.3	1.1±0.2
Ash	6.4±0.9	3.1±0.3
Others (by diff.)	1.1	0.84

Sugar and By-product Formation

The xylose and glucose concentrations showed a dependence on the experimental operating conditions. The highest xylose concentration was 10.9 g/L in sunflower stalk hydrolysate, achieved at 120 ^{o}C for 30 min with 4% of acid concentration (19th run), and 13.4 g/L in tobacco stalk hydrolysate, achieved at 140 ^{o}C for 15 min with 6% of acid concentration (14th run) (Figs. 1 and 2). Generally, in lignocellulosic biomass, xylan is present as a xylan-lignin complex and becomes resistant to hydrolysis. Therefore, the higher amount of lignin in the sunflower stalk (Table 1) tended to limit the xylan hydrolysis and decreased xylose production.

During the acid pretreatment of agricultural wastes other sugars are released, mainly glucose, which are produced from the cellulosic fraction or some heteropolymers of hemicellulosic fraction. In addition, there is another reaction taking place, the dehydration of xylose to furfural. High concentrations of glucose in the fermentation media adversely affect microbial conversion of xylose to xylitol, and furfural is toxic to yeast (Walther et al., 2001). Therefore, it is necessary to optimize the pretreatment parameters to minimize the levels of glucose and furfural in the hydrolysate. When the operating temperature and the reaction time were 153.3 ^{o}C and 30 min, respectively, with an acid concentration held at 4% sulfuric acid (5th run); glucose and furfural production were maximized in both hydrolysates (Fig. 1A and B). However, glucose levels were higher in the tobacco stalk hyrolysate than in the sunflower stalk hydrolysate at the same reaction conditions. The previous studies showed that sunflower and tobacco stalks xylans were heterogeneous polymers of pentose and hexose sugars and sugar acids. It was found that tobacco stalk xylan had higher glucose content than did the sunflower stalk xylan (Akpinar et al. 2009).

Since xylans of plants are partially acetylated, the concentration of acetic acid coming from the hydrolysis of the acetyl groups also increased with an increase in the time, temperature, and acid concentration. Depending on the pretreatment conditions, 13 to 89% of the acetyl groups of sunflower stalk and 8 to 80% of the acetyl groups of tobacco stalk were converted to acetic acid. Under the same reaction conditions acetic acid levels were higher in sunflower stalk than tobacco stalk (Fig. 1A and B).

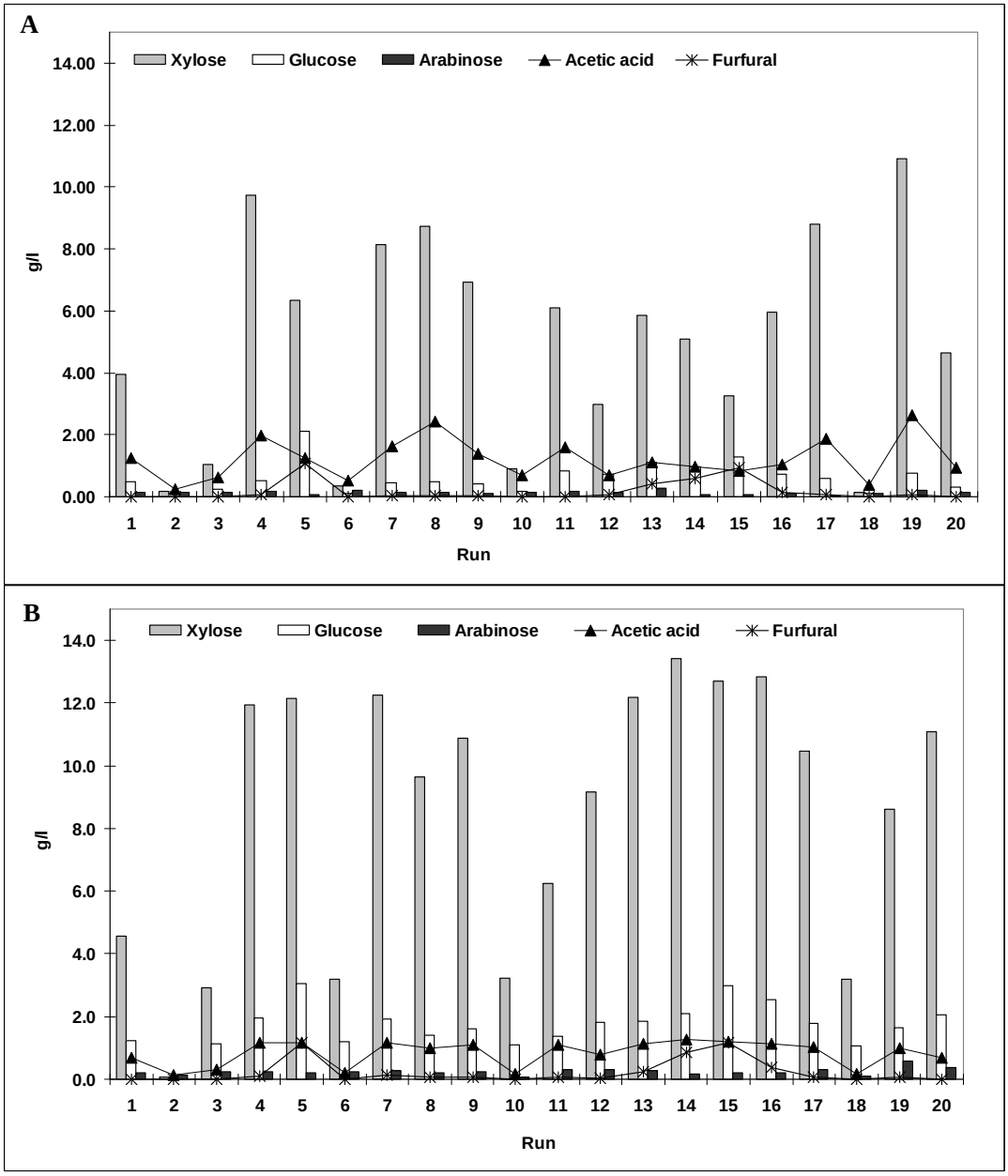

Fig. 1. Formation of xylose, glucose, arabinose, acetic acid and furfural under selected pretreatment conditions from sunflower stalk (A) and tobacco stalk (B)

Statistical Modelling

Although the compositions of tobacco stalk and sunflower stalk are similar to each other to some degree, they are most different with respect to lignin and glucan content. Generally, in lignocellulosic biomass, xylan exists in xylan-lignin complexes and becomes resistant to hydrolysis (Zhu et al. 2006). Therefore these two wastes have to be optimized separately, since they will have different responses to reaction conditions depending on their composition. The design of this research, including the dependent (or response) variables, xylose yield from sunflower stalk (Y_{1S}) and tobacco stalk (Y_{1T}) and

selectivity of pretreatment condition for sunflower stalk (Y_{2S}) and tobacco stalk (Y_{2T}) are given in Table 3. The quadratic models with coded variables are shown in Eq. (2), (3), (4), and (5), where the xylose yield (Y_{1S} and Y_{1T}) and selectivity (Y_{2S} and Y_{2T}) as a function of temperature (X_1), time (X_2), and acid concentration (X_3).

$$Y_{1S} = 35.6 + 6.56X_1 + 0.55X_2 + 3.51X_3 - 8.12X_1^2 - 5.36X_2^2 - 10.2X_3^2 - 1.04X_1X_2 - 2.48X_1X_3 - 1.00X_2X_3 \quad (2)$$

$$Y_{2S} = 16.6 + 0.34X_1 + 1.36X_2 + 0.42X_3 - 4.90X_1^2 - 1.98X_2^2 - 5.13X_3^2 - 0.48X_1X_2 - 1.55X_1X_3 - 0.69X_2X_3 \quad (3)$$

$$Y_{1T} = 53.5 + 12.8X_1 + 0.085X_2 + 4.17X_3 - 10.7X_1^2 + 0.049X_2^2 - 8.11X_3^2 - 8.97X_1X_2 - 8.53X_1X_3 + 1.82X_2X_3 \quad (4)$$

$$Y_{2T} = 13.0 - 2.95X_1 + 0.55X_2 + 0.60X_3 - 2.70X_1^2 - 0.52X_2^2 - 1.03X_3^2 - 1.79X_1X_2 - 2.86X_1X_3 - 5.85X_2X_3 \quad (5)$$

Table 3. Experimental Design and Results Obtained from Acid-Pretreated Sunflower Stalks and Tobacco Stalks

Runs	Variables			Responses			
				Sunflower stalk		Tobacco stalk	
	X_1	X_2	X_3	Y_{1S} (%)	Y_{2S} (g/g)	Y_{1T} (%)	Y_{2T} (g/g)
1	100	45	6	15.8	8.18	18.73	3.68
2	100	15	2	0.730	1.62	0.30	2.93
3	120	30	0.7	4.12	4.10	11.96	2.57
4	120	30	4	38.9	18.2	49.13	6.16
5	153.3	30	4	25.5	3.01	50.08	3.95
6	100	45	2	1.42	3.59	13.06	2.62
7	120	30	4	32.5	17.8	49.93	6.35
8	120	30	4	35.2	17.9	39.55	6.82
9	120	30	4	27.8	17.1	44.48	6.75
10	100	15	6	3.67	4.89	13.22	2.96
11	120	5	4	24.6	7.30	25.61	4.56
12	140	15	2	12.0	4.10	37.68	4.99
13	120	30	7.3	23.5	5.34	50.06	6.55
14	140	15	6	20.5	5.28	55.12	6.44
15	140	45	6	13.1	2.55	52.25	4.26
16	140	45	2	24.0	8.24	52.76	5.08
17	120	30	4	35.1	14.7	43.02	5.85
18	86.7	30	4	0.500	1.38	13.12	2.97
19	120	30	4	43.6	14.4	35.43	5.22
20	120	55	4	18.6	14.4	45.29	5.39

Y1 (xylose yield)= 100 x (X_{yl}/X_{ylmax}); Y_2 (selectivity) = X_{yl}/G_{lc}, X_{yl} = xylose concentration obtained in the hydrolysate X_{ylmax} = maximum xylose concentration based on the xylan concent, G_{lc}=glucose concentration obtained in the hydrolysate

Regression analysis was performed to fit the response function and experimental data. The second order model for xylose yield and selectivity was evaluated by ANOVA, and results are shown in Tables 4 and 5. The ANOVA for xylose yield from sunflower stalk and tobacco stalk obtained the determination coefficients of 0.89 and 0.89, explaining 89% and 89% of the variability in the responses. The selectivity was also evaluated by ANOVA, as presented in Table 4 and 5. The regression for the selectivity of pretreatment condition for sunflower stalk and tobacco stalk was statistically significant at the 95% confidence level. Again the model for selectivity for both of them did not show any lack of fit, and the determination coefficients (R^2) obtained were 0.96 and 0.77, explaining 96% and 77% of the variability in the responses.

Table 4. Analysis of Variance for Xylose Yield and Selectivity for Sunflower Stalk

Source	Sum of squares		Degress of freedom		Mean square		*F*-value		*P*-value	
	Y_{1S}	Y_{2S}	Y_{1S}	Y_{2S}	Y_{1S}	Y_{2S}	Y_{1S}	Y_{2S}	Y_{1S}	Y_{2S}
Model	3025.15	680.03	9	9	336.13	75.56	8.60	25.79	0.0012	<0.0001
Residual	39.98	29.30	10	10	39.10	2.93				
Lack of fit	246.16	14.89	5	5	49.23	2.98	1.70	1.03	0.2873	0.4858
Pure error	144.82	14.40	5	5	28.96	2.88				
Total	3416.14	709.32	19	19						
R^2	0.89	0.96								

Table 5. Analysis of Variance for Xylose Yield and Selectivity for Tobacco Stalk

Source	Sum of squares		Degress of freedom		Mean square		*F*-value		*P*-value	
	Y_{1T}	Y_{2T}	Y_{1T}	Y_{2T}	Y_{1T}	Y_{2T}	Y_{1T}	Y_{2T}	Y_{1T}	Y_{2T}
Model	5099.93	32.23	9	9	566.66	3.58	9.37	3.70	0.0008	0.0267
Residual	640.70	9.68	10	10	60.47	0.97				
Lack of fit	449.78	7.88	5	5	89.96	1.58	2.90	4.40	0.1335	0.0648
Pure error	154.92	1.79	5	5	30.98	0.36				
Total	5704.64	41.90	19	19						
R^2	0.89	0.77								

Figures 2 through 5 show the response surfaces to estimate the xylose yield and selectivity relative to the independent variables temperature (X_1), time (X_2), and acid concentration (X_3). When acid concentration was selected at 4% as the center point, the effect of temperature and time on xylose yield for sunflower stalk and tobacco stalk are shown in Fig. 2. The maximum xylose yield for sunflower stalk (37%) was obtained working at 128 ^{o}C and 30 min reaction time, while the maximum xylose yield for tobacco stalk (56%) was obtained working at 140 ^{o}C and 40 min reaction time. When reaction temperature was selected at 120 ^{o}C as the center point, from Fig. 3, it was interpreted that

the maximum xylose yield for sunflower stalk (36%) was obtained working with 4.3% acid concentration and 31 min reaction time, and the maximum xylose yield for tobacco stalk (44%) was obtained working with 6% acid concentration and 45 min reaction time.

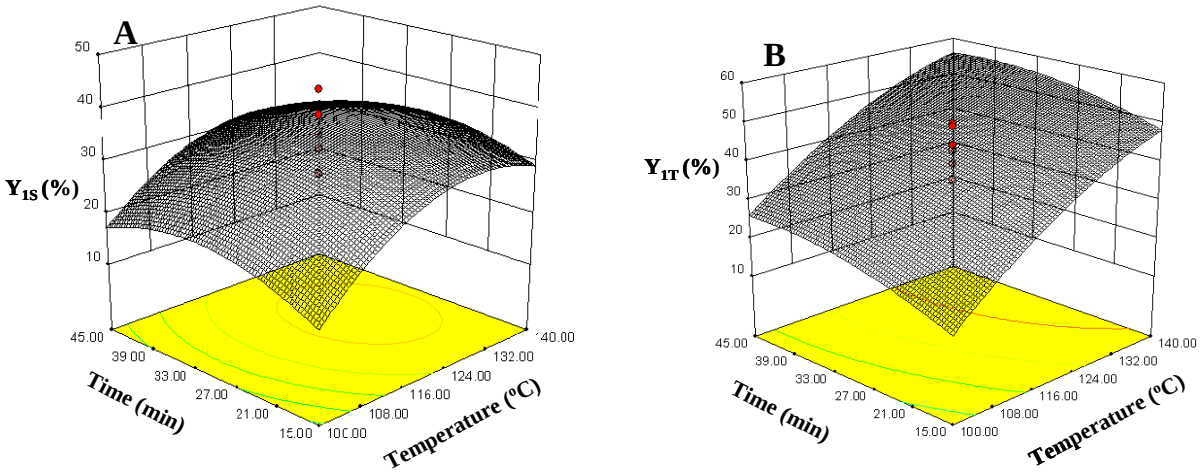

Fig. 2. Effect of reaction temperature and time on xylose yield when acid concentration was selected at 4% as a center point. A: sunflower stalk, B: tobacco stalk

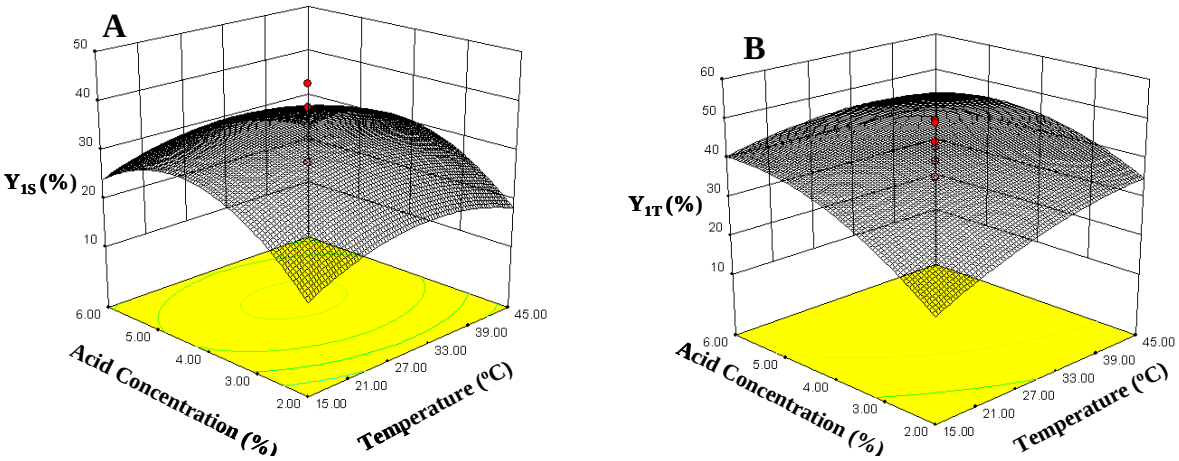

Fig. 3. Effect of H_2SO_4 conentration and reaction time on xylose yield when time was selected at 30 min as a center point. A: sunflower stalk, B: tobacco stalk

Figure 4 shows the effect of temperature and time on selectivity. When acid concentration was selected at 4% as the center point, the maximum selectivity for sunflower stalk (17 gg^{-1}) was obtained at 120 ^{o}C and 35 min of reaction time and the maximum selectivity for tobacco stalk (6.33 gg^{-1}) was obtained at 128 ^{o}C and 27 min of reaction time. When reaction temperature was selected at 120 ^{o}C as the center point, the maximum selectivity for sunflower stalk (17 gg^{-1}) was obtained with 4% acid concentration and 35 min reaction time; maximum selectivity for tobacco stalk (6.32 gg^{-1}) was obtained working with 4.9% acid concentration and 29 min reaction time (Fig. 5).

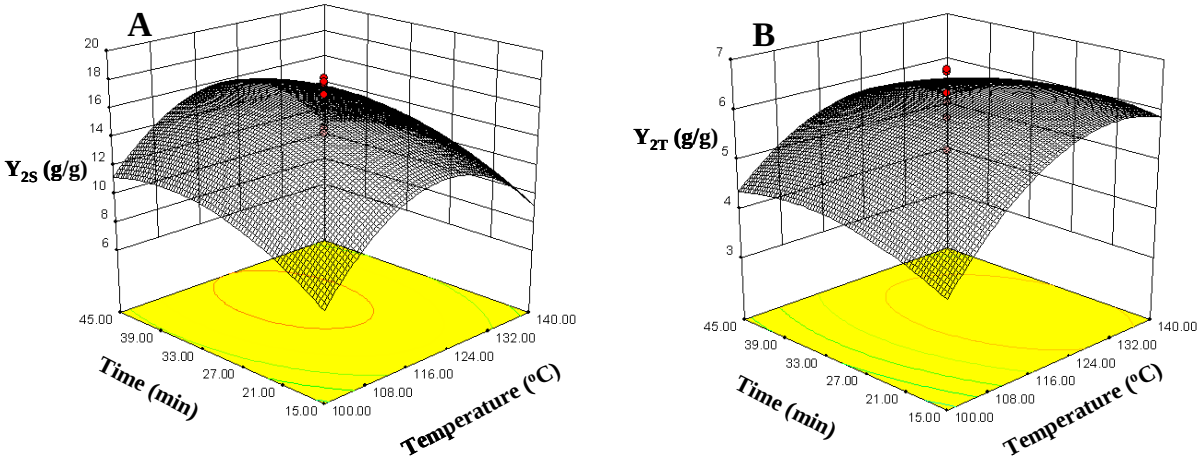

Fig. 4. Effect of reaction temperature and time on selectivity when acid concentration was selected at 4% as a center point. A: sunflower stalk, B: tobacco stalk

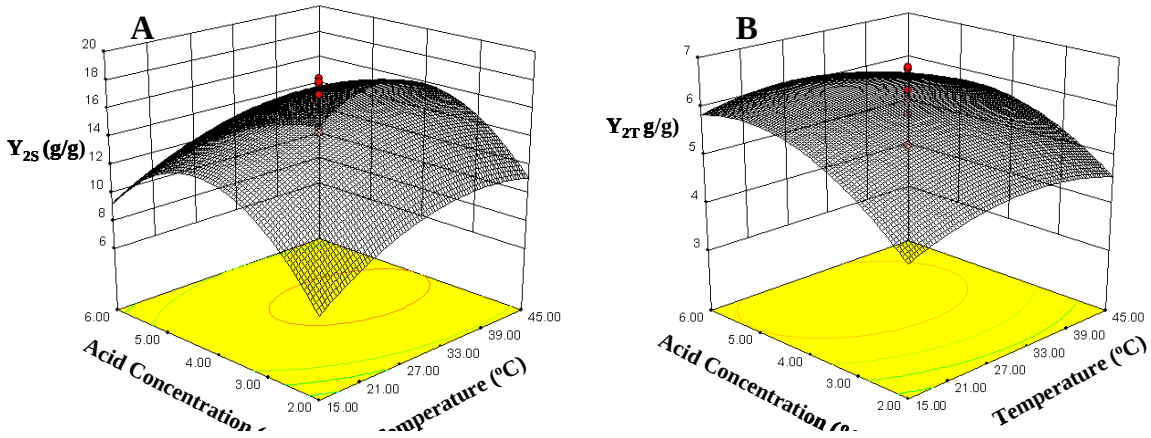

Fig. 5. Effect of H_2SO_4 concentration and reaction time on selectivity when when time was selected at 30 min as a center point. A: sunflower stalk, B: tobacco stalk

When overall xylose yield and selectivity of this study for sunflower stalk and tobacco stalk were compared with each other, it was found that at the same reaction conditions, xylose yield in sunflower stalk was lower than for tobacco stalk due to the higher lignin content of sunflower stalk, while the selectivity values in sunflower stalk were higher than those in tobacco stalk. It was speculated that the lower lignin content in tobacco stalk made cellulose more accessible to acid hydrolysis. In all of the experiments, xylose yield and selectivity remained in between 0.5-39% and 1.4-18 gg^{-1} for sunflower stalk and 0.3-55% and 2.6-6.8 gg^{-1} for tobacco stalk.

Based on the models, numerical optimization was carried out with Design Expert program. The optimal working conditions, based on high level of xylose yield and

selectivity, were chosen using the following criteria: xylose yield >35 and selectivity >15 g/g for sunflower stalk and xylose yield >50 and selectivity >5.5 g/g for sunflower stalk. Ten solutions for both agricultural residues were obtained, as shown in Table 6. As an optimum point for sunflower stalk 4% acid concentration, 120 ^{o}C, and 30 min was selected and the xylose yield and selectivity were predicted as 36% and 17 gg^{-1}, respectively (Table 7). For tobacco stalk 4.9% acid concentration, 133 ^{o}C, and 27 min were selected. Under these conditions xylose yield and selectivity were predicted as 52% and 6.1 gg^{-1}, respectively (Table 7). To confirm these results, hydrolysis runs were conducted in triplicate under these optimized conditions, the xylose yield and selectivity for sundlower stalk were obtained as 36% and 16 gg^{-1}, respectively and for tobacco stalk, were found as 50% and 6.6 gg^{-1}, respectively (Table 7).

Table 6. Solution for Optimimum Conditions for Sunflower Stalk (SS) and Tobacco Stalk (TS)

Solution number	Temperature (^{o}C)	Time (min)	Acid (%)	Desirability
1SS	125.04	24.20	4.55	1
2SS	125.04	35.80	4.55	1
3SS	**120.00**	**30.00**	**4.00**	**1**
4SS	128.41	20.69	4.25	1
5SS	132.05	36.96	3.98	1
6SS	119.17	31.55	3.95	1
7SS	131.73	31.67	4.10	1
8SS	122.57	29.85	3.62	1
9SS	129.78	21.33	4.44	1
10SS	129.49	32.58	4.92	1
1TS	**133**	**27**	**4.9**	**1**
2TS	130	21	5.2	1
3TS	134	36	4.0	1
4TS	135	39	3.9	1
5TS	137	38	3.2	1
6TS	134	21	4.6	1
7TS	133	20	4.8	1
8TS	128	25	5.8	1
9TS	125	40	5.3	1
10TS	126	40	5.4	1

Table 7. Experimental Validity Test for the Optimized Values Predicted by the Statistical Analysis for Sunflower Stalk (SS) and Tobacco Stalk (TS)

Response variables	Observed Response	Predicted Response
Y_1SS	36	36
Y_2SS	16	16.6
Y_1TS	50	52
Y_2TS	6.6	6.1

Roberto et al. (2003) obtained 77% xylose yield and 5 g/g selectivity from rice straw with 1% H_2SO_4, 121 ^oC and 27 min of reaction time. Canettieri et al. (2007) conducted dilute acid pretreatment of *Eucalyptus grandis* residue using 0.65% H_2SO_4, 157 oC and with a reaction time of 20 min and obtained 80 % xylose yield and 8 g/g selectivity. Rahman et al. (2006) studied dilute acid hydrolysis of palm empty fruit bunch fiber at 119 ^oC, 60 min using 2% H_2SO_4. Akpinar et al. (2011) showed that dilute acid pretratment of cotton stalk at 140 ^oC, 15 min using 6% H_2SO_4 yielded 48% xylose with 2.3 g/g selectivity. These results showed that yields of recovered xylose and selectivity for hemicellulose hydrolysis can be different due to the variation in the chemical composition of the biomass, which influences the degree of hemicellulose degradation.

CONCLUSIONS

1. Dilute acid hydrolysis of sunflower stalk and tobacco stalk were carried out under different operating conditions to produce xylose. A 2^3 rotatable central composite design was used in designing experiments, and response surface methodology was used to optimize the hydrolysis conditions.
2. The optimum reaction conditions were found as 4% acid concentration, 120 ^oC and 30 min for sunflower stalk, whereas the corresponding optimum was 4.9% acid concentration, 133 ^oC, and 27 min for tobacco stalk.
3. During the acid pretreatment of agricultural wastes, additional compounds are released, and further reactions take place. Most important are the production of glucose and the dehydration of xylose to furfural. The amounts of glucose and furfural depend on on the temperature, acid concentration, and the reaction time.
4. Under selected hydrolysis conditions, both waste showed promising sources of xylose with high yield which could be used for production of different chemicals, mainly xylitol.

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