Optimization of Alkaline Pretreatment for Enzymatic Saccharification of Poppy Stalks

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Response surface methodology (RSM) was employed to optimize dilute alkaline pretreatment conditions for the maximum glucose yield of poppy stalk, with respect to NaOH concentration (1.0 to 3.5%, w/v), pretreatment temperature (50 to 100 °C), and pretreatment time (10 to 110 min). Recovery of glucan ranged from 61.02% to 99.14%, based on the initial glucan in the raw material. The highest lignin removal (43.43%) was obtained at the pretreatment conditions of 90 °C and 3.50% NaOH for 90 min. The optimum pretreatment conditions for maximum glucose yield after enzymatic hydrolysis were found to be 2.40% w/v NaOH, 70 min, and 80 °C. Under these conditions, experimental glucose and xylose yields were 499.35 mg/g glucan and 498.66 mg xylose/g xylan, respectively.

Keywords: Poppy stalks; Alkaline pretreatment; Enzymatic saccharification; Optimization

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INTRODUCTION

Lignocellulosic materials are natural renewable resources that can be converted into useful materials and energy. Utilization of lignocellulosic materials for these purposes requires effective pretreatment to disrupt the structure of the lignocellulose structure to increase accessible surface area and porosity (Kumar and Wyman 2009b). Although, a range of chemical, physical, and biological processes have been configured to release constituent sugars from lignocellulose, these processes suffer several shortcomings, such as high energy consumption, low conversion efficiency, and technological impasses (Sun and Cheng 2002). Alkaline treatment processes are generally very effective in the pretreatment of agricultural residues (Mosier *et al.* 2005). The major effect of alkaline pretreatments is the delignification of lignocellulosic biomass, thus enhancing the reactivity of the remaining carbohydrates (Taherzadeh and Karimi 2008). Sodium hydroxide pretreatment on a variety of lignocellulosic materials has been reported to increase conversion of biomass by increasing surface or cellulose content, decreasing the degree of polymerization, and reducing lignin content (Gupta and Lee 2010; Hendriks and Zeeman 2009; Xu *et al.* 2010)

Poppy (*Papaver somniferum* L.) is an important industrial crop, which has been grown since almost ancient times, for pharmaceutical and food applications in Turkey. While alkaloids from poppy capsules and straw are widely used in the legal pharmaceutical industry, its seeds are used for producing edible oil. The major legal opium poppy growing areas in the world today are in government-regulated opium farms in Turkey and India. In Turkey, although it was grown extensively without any

limitations prior to 1975, poppy has been cultivated only in limited areas under strictly controlled conditions by the Turkish Government. The area under opium poppy cultivation in Turkey is reported to be 55,000 hectares for the year 2011 (Turkish Statistical Institute 2011). Assuming that an average of 50% of whole plants are fibrous waste residue (poppy stalk, it does not contain alkaloid) (Laughlin 1978), the total poppy stalk yield from opium poppy cultivation can be estimated to be more than 100,000 ton/year. Poppy stalks do not have any suitable end use, and are generally burnt in the fields or utilized as boiler fuels. Therefore, poppy stalks, as lignocellulosic biomass, afford a renewable and low-cost raw material for the production of fermentable sugars.

The objectives of this study were to optimize dilute alkaline pretreatment of poppy stalks, using a response surface method with respect to the maximum yield of glucose following enzymatic hydrolysis, and to investigate the effect of pretreatment conditions on the lignin removal, glucan, and xylan recovery.

EXPERIMENTAL

Materials

Papaver somniferum stalks used in this study were kindly provided by Afyon Alkaloids Plant (belongs to Turkish Grain Board), Bolvadin, Turkey, where opium alkaloid derivatives from the crushed poppy capsules produced for legal medicinal uses such as analgesic and antitussive. Afyon Alkaloids Plant, with the largest capacity of alkaloid production of the world, utilizes traditional poppy crop to meet legal alkaloid requirement of domestic and international market. Production in this plant is implemented according to the needs and expectations of the customers, and in consistency to BP, USP, EU, INT. PH pharmacopeias (www.tmo.gov.tr/Upload/Document/poppy.pdf).

Raw material was air dried to 10% moisture content. The dried materials were ground by a grinder and screened with a sieve shaker to obtain particle sizes between 0.224 and 0.850 mm. Samples were stored in plastic bags at 4 °C for future use. Celluclast 1.5 L was purchased from Sigma Aldrich (St. Louis, USA). Aminex HPX 87P column was purchased from Bio-Rad Laboratories (California, USA). All chemicals used were of standard analytical grade.

Methods

Pretreatment and enzymatic hydrolysis of poppy stalks

Pretreatment experiments were performed in a water bath. Dilute sodium hydroxide (1.0-3.5 w/v%) was used for pretreatment of ground poppy stalk at a solid to liquid ratio of 1:20.

Enzymatic hydrolyses were carried out in stoppered conical flasks (50 mL). The pH was adjusted to 4.8 with acetate buffer, and cellulase (30 FPU/g dry biomass, Celluclast 1.5 L) was added to the pretreated substrate in a total working volume of 20 mL. The hydrolysis reactions were carried out at 50 °C in an incubator for 72 h with shaking at 150 rpm. The reactions were stopped in a boiling water bath for 15 min and hydrolysates were clarified by centrifuging at 5000 rpm for 5 min. The supernatants were analyzed for glucose and xylose using HPLC.

Analytical Methods

The chemical composition of raw and pretreated poppy stalk were determined according to the National Renewable Energy Laboratory (NREL) (Sluiter *et al.* 2008a) methods. First, 0.3 g of solid stalk was hydrolyzed by 3 mL of 72% (w/w) H_2SO_4 at 30 °C for 60 min, and then the reaction mixture was diluted to 4% (w/w) and autoclaved at 121 °C for 60 min. Lignin was determined by solid residue, and glucan and xylan amounts were determined from the filtrate using high-performance liquid chromatography (Agilent 1100, Germany). The HPLC system was equipped with a Bio-Rad Aminex HPX-87P (USA) column (300 mm × 7.8 mm) and a refractive index detector. The analytical column was operated at 80 °C with 0.2-µm filtered HPLC grade water as the mobile phase. The mobile phase flow rate was 0.6 mL/min.

Extractive (Sluiter *et al.* 2008b) and ash (Sluiter *et al.* 2008c) analysis was conducted according to NREL procedures. Enzyme activity of Celluclast 1.5L® was determined by NREL protocols and reported as filter paper units (FPU) (Adney and Baker 2008). One unit of FPU is defined as the amount of enzyme required to liberate 1 µmol of glucose from Whatman No. 1 filter paper per min at 50 °C.

Lignin removal (Eq. 1), glucan recovery (Eq. 2), and glucose recovery (Eq. 3) were calculated as follows;

$$Lignin\ removal\ (\%) = \left(100 - \frac{\text{Amount Lignin in pretreated solid}}{\text{Amount of lignin in initial solid}}\right) x100 \quad (1)$$

$$Glucan\ Recovery\ (\%) = \frac{\text{Amount of glucan in pretreated solid}}{\text{Amount of glucan in initial solid}} x100 \quad (2)$$

$$Glucose\ Recovery\ (mg/g) = \frac{\text{Amount of glucose producedx0.9}}{\text{Amount of glucan in unpretreated solid}} x100 \quad (3)$$

Experimental Design for Response Surface Methodology

Response surface methodology was employed to optimize the alkaline pretreatment conditions. The studied parameters and their ranges were as follows: temperature (X_1) from 50 to 100 °C, NaOH concentration (X_2) from 1.0 to 3.5% (w/v), and pretreatment time (X_3) from 10 to 110 min. The experimental design was based on the central composite design (CCD) using a total of 20 experimental settings with 8 factorial points, 6 axial points (α=1.682), and 6 center points. A generalized second-order polynomial equation was assumed for predicting the Y variable. The model proposed for the response of Y (Eq. 4) was as follows,

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_0 X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum \sum_{i<j=1}^{3} \beta_{ii} X_i X_j \quad (4)$$

where β_0, β_i, β_{ii} and β_{ij} are the regression coefficients for intercept, linear, quadratic, and interaction terms, respectively.

The model was built based on the variables with confidence levels of 95%. The coefficients of the response surface equation were estimated using Minitab 16 (UK) software. The significance of the model and regression coefficient was tested by the analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Composition of Untreated Poppy Stalks

The composition of unpretreated poppy stalks used in this study is presented in Table 1. Glucan was the main component in the raw biomass, followed by hemicellulose and lignin, respectively. The holocellulose content was lower than those found from published data as in Cengiz *et al*. (2010). This difference might be due to several factors, such as location of cultivation, seasonal conditions, and analytical procedures. The results indicate that poppy stalk is rich in carbohydrates and a potential source for fermentable sugars.

Table 1. Composition of Untreated Poppy Stalk

Component	Percentage (%)*
Holocellulose	44.81 ± 2.72
Glucan	24.36 ± 1.29
Xylan	7.41 ± 0.45
Mannan	5.33 ± 0.19
Arabinan	4.73 ± 0.56
Galactan	2.98 ± 0.22
Lignin	19.79 ± 0.95
Extractives	14.21 ± 0.55
Ash	9.01 ± 0.37
Other	13.69
*calculated on a dry-weight basis (DM)	

Effect of Pretreatment Parameters on the Solid Recovery, Lignin Removal, Glucan, and Xylan Recovery

The combinations of the variables randomized by the Minitab software, along with observed values for solid recovery, lignin removal, and glucan and xylan recovery, are given in Table 2. The ANOVA fittings of quadratic models, in an unreduced state for non-significant terms for lignin removal and glucan and xylan recovery are shown in Table 3.

The insoluble solid recovery decreased significantly as the severity of pretreatment conditions increased and ranged from 71.86% (50 °C, 2.25% NaOH, 60 min) to 45.50% (90 °C, 3% NaOH, 90 min), based on the initial biomass weight. The weight loss was mostly due to the removal of lignin and degradation of xylan (Karunanithy and Muthukumarappan 2011). Similar to the results found by other authors for different crop residues pretreated under similar conditions. Kim and Han (2012) reported that solid losses in rice straw pretreatment increased with increasing alkaline concentration from 1% to 4% at 60 ^{o}C. Solid losses for sorghum bicolor straw are reported ranging from 30% (w/w) under mild pretreatment conditions (1% NaOH; 60 min; 60 ^{o}C) up to 63% when pretreated in 2% NaOH for 90 min at 121 ^{o}C (McIntosh and Vancov 2010). The same authors also showed that solubilisation of hemicellulose in the pretreatment of wheat straw at 121 ^{o}C for 30 min increased from 20% to 33% as NaOH concentration increased from 0.75% to 2.0% (McIntosh and Vancov 2011).

The highest experimental lignin removal (43.43%) was obtained when pretreatment conditions of 90 °C and 3.5% NaOH for 90 min were used, which also resulted in the lowest insoluble solid (45.50%) recovery. A similar lignin removal degree has been reported for other agricultural residues, such as rice straw (Kim and Han 2012), wheat straw (Kumar *et al.* 2009), and barley straw (Duque *et al.* 2013). The factor with the largest effect on the lignin removal (Table 3) was the linear terms of temperature and alkaline concentration ($p < 0.05$), followed by the quadratic terms of temperature and time ($p < 0.05$) and the interactions between alkaline concentration and time ($p < 0.05$). As expected, lignin removal increased with temperature and alkaline concentration (Fig. 1A). However, alkaline concentration did not show positive effects on the delignification at lower pretreatment times, while the temperature effect remained significant ($p < 0.05$).

Table 2. Experimental Data of Solid Recovery, Lignin Removal, Glucan, and Xylan Recoveries

T (°C)	NaOH (%)	Time (min)	Solid recovery (%DM)	Lignin removal (%)	Glucan recovery (%)	Xylan recovery (%)
75	1.00	60	61.66	30.22	62.89	80.17
75	2.25	60	63.17	32.10	92.00	63.50
60	3.00	30	61.39	31.15	97.73	70.34
50	2.25	60	71.86	27.56	98.12	72.08
90	3.00	90	45.50	43.43	92.61	53.79
75	2.25	60	64.32	31.64	96.38	70.32
75	2.25	60	61.60	34.12	95.21	64.72
75	2.25	60	63.79	30.00	89.61	63.38
60	1.50	90	67.95	22.04	77.12	59.57
60	3.00	90	61.21	34.00	97.48	56.54
75	3.50	60	56.71	36.44	99.14	56.58
75	2.25	10	66.74	28.57	89.34	35.83
90	1.50	90	50.47	31.69	89.37	65.17
75	2.25	110	52.98	30.07	95.76	38.58
100	2.25	60	46.42	42.83	98.61	69.56
60	1.50	30	64.63	27.01	61.02	54.56
90	1.50	30	55.50	35.29	84.46	68.33
75	2.25	60	62.00	34.50	96.44	70.28
90	3.00	30	49.17	36.52	85.75	73.66
75	2.25	60	64.00	33.09	95.76	60.63

Glucan content ranged from 23.00% to 51.75% in the pretreated solid. The maximum glucan recovery (99.14%) was found for the treatment conditions of 75 °C, 3.50% NaOH, and 60 min (Table 2). Although all parameters exhibited positive effects on the glucan recovery, the most effective variables were NaOH concentration and pretreatment time. These variables had a linear effect, while temperature and NaOH concentration showed an interaction effect (Table 3). The rate of glucan recovery

decreased at higher temperatures and alkaline concentrations due to a decrease in solids recovery (Fig. 1B).

Table 3. ANOVA for the Response Surfaces Quadratic Model for Lignin Removal and Glucan and Xylan Recoveries

Term	Lignin removal (%)		Glucan recovery (%)		Xylan recovery (%)	
	Estimate	*p*-Value	Estimate	*p*-Value	Estimate	*p*-Value
Constant	32.58	0.000	94.39	0.000	65.33	0.000
X1: T (°C)	4.31	0.000	1.44	0.270	1.15	0.543
X2: NaOH (%. w/v)	2.85	0.000	8.96	0.000	-2.42	0.215
X3: Time (min)	0.23	0.615	2.81	0.046	-1.99	0.301
X1*X1	0.10	0.042	0.46	0.710	2.81	0.145
X2*X2	0.23	0.597	-5.67	0.001	1.94	0.300
X3*X3	-1.19	0.020	-1.59	0.214	-9.08	0.000
X1*X2	-0.47	0.437	-6.57	0.002	-2.35	0.348
X1*X3	0.60	0.318	-0.51	0.759	-1.78	0.473
X2 X3	2.22	0.003	-1.80	0.291	-4.44	0.092
Lack-of-Fit	0.554		0.064		0.056	
R-Sq	94.43		91.02		80.33	
R-Sq-Adj	89.42		82.93		62.63	

Xylan content varied between 3.98% and 11.11% in the pretreated stalks. Xylan recovery (35.83% to 80.17% of the initial xylan in the raw material) was lower compared to glucan recovery because of the solubility of xylan in alkaline (Egües *et al.* 2012). Similar results have been reported in previous studies (Karagöz *et al.* 2012; McIntosh and Vancov 2011). For xylan recovery, pretreatment time showed a quadratic effect, and no significant linear or interaction effects of the variables were observed.

Effect of Pretreatment Parameters on Glucose and Xylose Recovery

Experimental and statistically predicted data for the recovery of glucose and xylose for various treatment combinations are presented in Table 4. Application of Central Composite Design on the pretreatment process generated the following second order polynomial equations in terms of actual factors for recovery of glucose and the xylose:

$$Y_{\text{glucose (mg/g glucan)}} = -418.54 + 15.02\,X_1 + 128.12\,X_2 + 4.76\,X_3 - 0.11\,X_1X_1 - 36.94\,X_2X_2 - 0.0289\,X_3X_3 + 0.95\,X_1X_2 + 0.003\,X_1X_3 - 0.43\,X_2X_3$$

$$Y_{\text{xylose (mg/g xylan)}} = 855.55 - 8.35\,X_1 - 233.13\,X_2 + 13.21\,X_3 + 0.03\,X_1X_1 - 1.32\,X_2X_2 - 0.013\,X_3X_3 - 4.78\,X_1X_2 - 0.073\,X_1X_3 - 3.32\,X_2X_3$$

Analysis of variances (ANOVA) of the models for the recovery of glucose and xylose are given in Table 6. The predicted models were significant ($p < 0.05$) for both glucose and xylose recovery. The coefficients of determination (R^2) and lack of fit values were 90.59% (R^2-adjusted: 82.13%) and 0.059 for glucose, and 79.61% (R^2-adjusted: 63.08%) and 0.266 for xylose recovery, respectively. Although temperature and time had

strong linear effect on the glucose, quadratic effects were significant for all parameters, and no interaction effect was observed. For xylose recovery, only alkaline concentration showed significant linear effect, and interaction effect was observed between alkaline concentration and hydrolysis time.

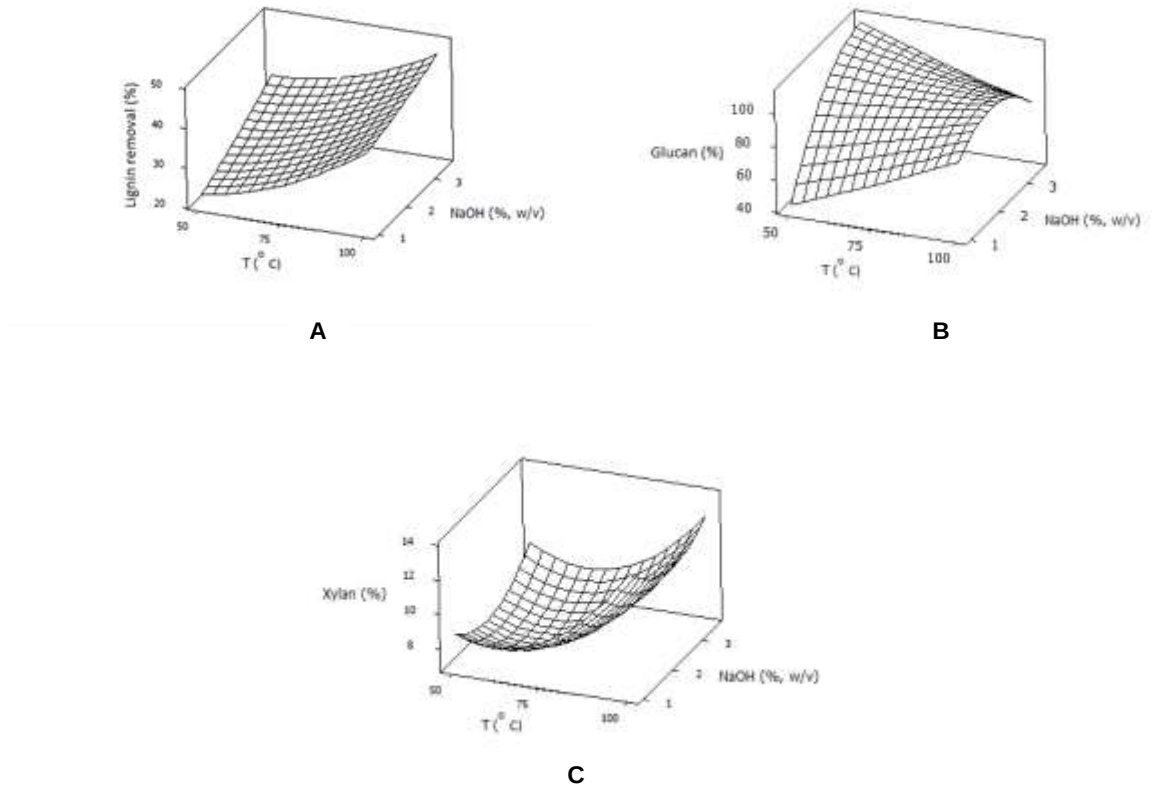

Fig. 1. Response surface plots for the effect of temperature (°C) and NaOH (%, w/v) concentration on the lignin removal (%) (A), glucan recovery (%) (B), and xylan recovery (%) (C)

The effects of both temperature and NaOH% on the glucose yield at constant time of center point value (60 min) are shown in Fig. 2. The glucose yield increased with the increase of temperature and alkaline concentration. However, further increase of these two variables resulted in a decrease of glucose yield. Similar phenomena were also observed for the effect of other parameters. In other words, lower or higher pretreatment values both had negative effects on glucose yield. Experimental maximum glucose recovery of 489.11 mg glucose/g glucan was achieved at the center point condition of 75 °C, 2.25% NaOH, and 60 min. The recovery of xylose increased with increasing temperature and hydrolysis time at lower alkaline concentrations, however, at higher alkaline concentrations, it decreased as temperature and hydrolysis time increased. This phenomenon is explained with the alkaline hydrolysis mechanism which is based on the saponification of intermolecular ester bonds crosslinking xylan hemicelluloses (Sun and Cheng 2002).

Optimization of Parameters to Maximize Glucose Recovery

The predicted optimum process variables to yield the maximum amounts of glucose were established by the response optimizer of Minitab 16 software. The optimum pretreatment conditions were 2.40% w/v alkaline concentration, 70 min hydrolysis time, and a temperature of 80 °C. At the optimum conditions, predicted maximum glucose yield was 524.86 mg/g glucan. To validate the result of the predicted value, experiments were conducted at the optimal conditions, displaying the glucose yield of 499.35 mg/g glucan. Contrary to maximum glucose recovery, at these conditions, a significantly ($p < 0.05$) lower xylose recovery (498.66 mg xylose/ g xylan) was obtained. Under the optimal condition lignin removal, glucan and xylan recovery were about 99%, 79%, and 36% of their experimental maximum, respectively.

Table 4. Experimental(1) and Predicted(2) Data of Glucose and Xylose Recoveries

T (°C)	NaOH (%, w/v)	Time (min)	Glucose yield (mg/g glucan)[1]	Glucose yield (mg/g glucan)[2]	Xylose yield (mg/g xylan)[1]	Xylose yield (mg/g xylan)[2]
75	1.00	60	421.48	425.83	754.77	743.38
75	2.25	60	509.43	494.03	665.79	645.24
60	3.00	30	399.21	397.61	578.31	567.30
50	2.25	60	384.68	398.93	517.88	600.17
90	3.00	90	457.75	461.39	491.22	527.52
75	2.25	60	492.46	494.03	605.04	645.24
75	2.25	60	485.28	494.03	626.83	645.24
75	2.25	60	470.29	494.03	655.96	645.24
60	1.50	90	455.78	436.18	795.94	785.14
60	3.00	90	407.05	406.73	487.36	409.06
75	3.50	60	458.09	444.69	516.61	542.89
75	2.25	10	411.51	394.45	686.31	675.17
90	1.50	90	440.07	448.06	687.94	688.42
75	2.25	110	438.03	446.04	524.09	550.12
100	2.25	60	474.34	451.04	795.82	728.42
60	1.50	30	385.77	388.52	691.66	644.83
90	1.50	30	389.10	395.83	611.12	678.89
75	2.25	60	485.62	494.03	581.93	645.24
90	3.00	30	421.69	447.69	816.25	816.53
75	2.25	60	491.56	494.03	738.46	645.24

Table 5. ANOVA for the Response Surfaces Quadratic Model for Glucose and Xylose

Term	Glucose recovery (mg/g initial glucan)	Xylose recovery (mg/g initial xylan)
	p-Value	*p*-Value
X1: T (°C)	0.008	0.056
X2: NaOH (%. w/v)	0.262	0.007
X3: Time (min)	0.009	0.062
X1*X1	0.000	0.703
X2*X2	0.001	0.966
X3*X3	0.000	0.518
X1*X2	0.113	0.042
X1*X3	0.856	0.187
X2 X3	0.149	0.009
Lack-of-Fit	0.059	0.266
R-Sq (%)	90.59	79.61
R-Sq-Adj (%)	82.13	61.26

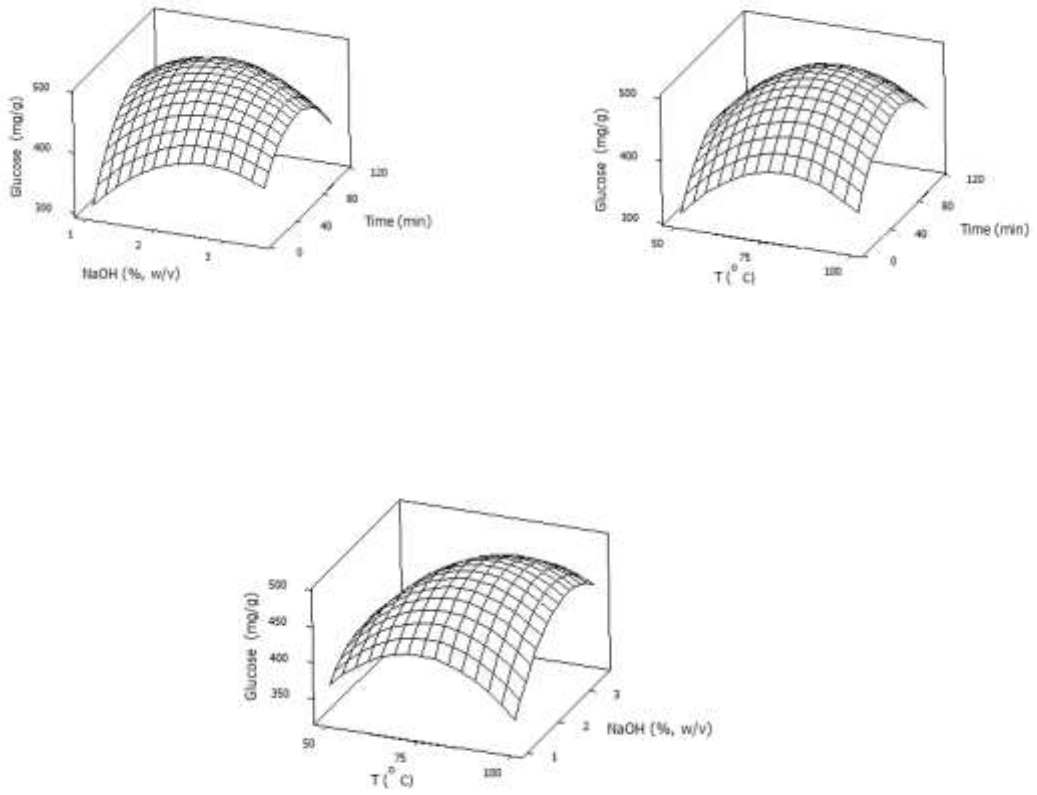

Fig. 2. Response surface plots for the interactive effects of temperature (°C), NaOH (%, w/v), and pretreatment time (min) on the glucose recovery (mg/g initial glucan)

CONCLUSIONS

1. In response to dilute alkaline (NaOH) pretreatment, glucan recovery and lignin removal were improved by increasing the loading amount of NaOH and temperature.
2. The optimum dilute alkaline pretreatment obtained for maximizing glucose recovery were as follows: 2.40% (w/v) NaOH, 80 °C pretreatment temperature, and 70 min of pretreatment time, which produced 499.35 mg/g glucan.
3. The experimental value was well within the estimated value of the model obtained by RSM.
4. The results of this study showed that poppy stalks with their high sugar content can be considered an important feedstock for ethanol production.

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REFERENCES CITED

Adney, B., and Baker, J. (2008). "Measurement of cellulase activities," Technical Report, NREL/TP-510-42628.

Cengiz, M., Dincturk, O. D., and Sahin, H. T. (2010). "Fractional extraction and structural characterization of opium poppy and cotton stalks hemicelluloses," *Pharmacogn. Mag*. 6(24), 315-319.

Duque, A., Manzanares, P., Ballesteros, I., Negro, M. J., Oliva, J. M., Saez, F., and Ballesteros, M. (2013). "Optimization of integrated alkaline–extrusion pretreatment of barley straw for sugar production by enzymatic hydrolysis," *Process Biochem.* 48(5-6), 775-781.

Egües, I., Sanchez, C., Mondragon, I., and Labidi, J. (2012). "Effect of alkaline and autohydrolysis processes on the purity of obtained hemicelluloses from corn stalks," *Bioresour. Technol.* 103(1), 239-248.

Gupta, R., and Lee, Y. Y. (2010). "Pretreatment of corn stover and hybrid poplar by sodium hydroxide and hydrogen peroxide," *Biotechnol. Prog.* 26(4), 1180-1186.

Hendriks, A., and Zeeman, G. (2009). "Pretreatments to enhance the digestibility of lignocellulosic biomass," *Bioresour. Technol.* 100(1), 10-18.

Karagöz, P., Rocha, I. V., Özkan, M., and Angelidaki, I. (2012). "Alkaline peroxide pretreatment of rapeseed straw for enhancing bioethanol production by same vessel saccharification and co-fermentation," *Bioresour. Technol.* 104, 349-357.

Karunanithy, C., and Muthukumarappan, K. (2011). "Optimization of alkali soaking and extrusion pretreatment of prairie cord grass," *Biochem. Eng. J.* 54(2), 71-82.

Kim, I., and Han, J.-I. (2012). "Optimization of alkaline pretreatment conditions for enhancing glucose yield of rice straw by response surface methodology," *Biomass Bioenerg*. 46, 210-217.

Kumar, P., Barrett, D. M., Delwiche, M. J., and Stroeve, P. (2009). "Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production," *Ind. Eng. Chem. Res.* 48(8), 3713-3729.

Kumar, R., and Wyman, C. E. (2009). "Access of cellulase to cellulose and lignin for poplar solids produced by leading pretreatment technologies," *Biotechnol. Prog.* 25(3), 807-819.

Laughlin, J. C. (1978). "The effect of band placed nitrogen and phosphorus fertilizer on the yield of poppies (*Papaver somniferum* L.) grown in Krasnozem soil," *Acta Hort.* 73, 165-169.

McIntosh, S., and Vancov, T. (2010). "Enhanced enzyme saccharification of *Sorghum bicolor* straw using dilute alkali pretreatment," *Bioresour. Technol.* 101(17), 6718-6727.

McIntosh, S., and Vancov, T. (2011). "Optimization of dilute alkaline pretreatment for enzymatic saccharification of wheat straw," *Biomass Bioenerg.* 35(7), 3094-3103.

Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y. Y., Holtzapple, M., and Ladisch, M. (2005). "Features of promising technologies for pre-treatment of lignocellulosic biomass," *Bioresour. Technol*. 96, 673-686

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., and Crocker, D. (2008a). "Determination of structural carbohydrates and lignin in biomass," Technical Report NREL/TP-510-42618 Revised April 2008.

Sluiter, A., Ruiz, R., Scarlata, C., Sluiter, J., and Templeton, D. (2008b). "Determination of extractives in biomass," Technical Report, NREL/TP-510-42619.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., and Templeton, D. (2008c). "Determination of ash in biomass," Technical Report, NREL/TP-510-42622.

Sun, Y., and Cheng, J. (2002). "Hydrolysis of lignocellulosic materials for ethanol production: A review," *Bioresour. Technol.* 83, 1-11

Taherzadeh, M. J., and Karimi, K. (2008). "Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: A review," *Int. J. Mol. Sci.* 9(9), 1621-1651.

www.tmo.gov.tr/Upload/Document/poppy.pdf. Poppy and Alkaloid Affairs Department/ Ankara/Turkey

Turkish Statistical Institute (TUIK) (2011). *The Summary of Agricultural Statistics*.

Xu, J. L., Cheng, J. J., Sharma-Shivappa, R. R., and Burns, J. C. (2010). "Sodium hydroxide pretreatment of switchgrass for ethanol production," *Energ. Fuel.* 24, 2113-2119.

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