Appl Biochem Biotechnol (2013) 169:2430–2441
DOI 10.1007/s12010-013-0154-x

Maximum Production of Fermentable Sugars from Barley Straw Using Optimized Soaking in Aqueous Ammonia (SAA) Pretreatment

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Received: 31 August 2012 / Accepted: 18 February 2013 /
Published online: 2 March 2013

Abstract Soaking in aqueous ammonia (SAA) pretreatment was investigated to improve enzymatic digestibility and consequently to increase total fermentable sugar production from barley straw. Various effects of pretreatment process parameters, such as reaction temperature, reaction time, solid:liquid ratio, and ammonia concentration, were evaluated, and the optimum conditions for two of the most important factors, reaction temperature and time were determined using response surface methodology. Optimized reaction conditions were 77.6 °C treatment temperature, 12.1 h. treatment time, 15 wt.% ammonia concentration, and 1:8 solid-to-liquid ratio, which gave a sugar recovery yield of 71.5 % (percent of theoretical sugar recovered from the untreated barley straw) with enzyme loading of 15 FPU/g-glucan. In the optimization of the SAA pretreatment process, ammonia concentration, reaction temperature, and reaction time were determined to be the most significant factors correlated to subsequent enzyme digestibility. Based on tested conditions exhibiting high sugar recovery yields of >60 %, it appeared that reaction temperature affected total fermentable sugar production more significantly than reaction time.

Keywords Fermentable sugars · Sugar recovery yield · Lignocellulosic biomass · Ammonia pretreatment

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Introduction

It has recently been suggested that power, fuels, and other chemicals could be produced from various renewable resources, particularly lignocellulosic materials, such as agricultural residues, woody biomass, and dedicated energy crops. The US Department of Energy (US-DOE) defined a biorefinery as a processing plant where biomass feedstocks are converted and extracted into a spectrum of these types of valuable products [1]. One of the major proposed products of future biomass biorefineries is the fuel ethanol, which is currently the most widely used liquid biofuel alternative to fossil fuels [2].

Lignocellulosic biomass primarily consists of carbohydrates (cellulose and hemicellulose) and lignin, which represent about 50–70 % and 15–30 % of the dry weight of plants, respectively. Cellulose and hemicellulose can be enzymatically hydrolyzed to monomeric sugars and then fermented to produce ethanol or chemically converted to other products [3–7]. Bioconversion of lignocellulosic material, which involves enzymatic saccharification followed by microbial fermentation, offers many advantages over chemical conversion such as mild processing conditions, minimal by-product formation, and lower energy requirements. However, lignocellulosic biomass is only partially digestible in its native form, i.e., less than 10–20 % can be hydrolyzed by enzymes into fermentable sugars. Major difficulties in the conversion of biomass include chemical and physical barriers such as heterogeneous composition, recalcitrant nature, crystalline structure of cellulose, and others [8–10]. To overcome these barriers and thus improve enzymatic hydrolysis rate and yield, several pretreatment methods have been developed. The primary goal of pretreatment is to make the fibers (cellulose and hemicellulose) more amenable to enzymatic action [11, 12]. In the past, pretreatment research has generally been focused on developing an efficient method for maximizing fermentable sugar production. However, the biggest challenge today is to advance the pretreatment technology to the point of becoming economically viable for production of fuels and chemicals at lower costs compared to petroleum refineries.

One of the methods developed for pretreatment of lignocellulosic material in our group is soaking in aqueous ammonia (SAA), which uses mild reaction conditions (30–80 °C, 1–2 atm) and does not require detoxification of the sugar stream, thus providing a simple process with low energy requirements [13, 14]. In addition, the ammonia used for pretreatment can be evaporated, recovered, and recycled. Whereas most other chemical pretreatment methods hydrolyze a significant amount of hemicellulose and generate hydrolysates containing a mixture of lignin and sugars, the SAA method preserves most of the hemicellulose (~80 %) and almost all of the cellulose (~95 %) in the solid residue, which then are available for enzymatic hydrolysis and fermentation [13, 14]. The SAA process has been demonstrated as a pretreatment method suitable for agricultural residues [15, 16].

This study first focused on evaluating the various effects of parameters such as solid-to-liquid ratio, reaction temperature, reaction time, and ammonia concentration on compositional changes and enzymatic digestibility of SAA-treated barley straw. Then, the optimum conditions for temperature and time for SAA pretreatment of barley straw, which would result in highest yields of fermentable sugars in the subsequent enzymatic hydrolysis, were determined using response surface methodology (RSM). In order to find the optimal pretreatment conditions and to predict the highest sugar recovery yields, optimum ammonia concentration was first determined. Then, the effect of the two most important remaining factors, i.e., reaction time and temperature, were determined based on results of RSM results.

Materials and Methods

Materials

Straw of the Thoroughbred barley variety was obtained from the Foundation Seed Farm of the Virginia Crop Improvement Association (Mt. Holly, VA) in September 2010. The straw was stored in a low-humidity room at ambient temperature (18–24 °C) and relative humidity below 25 % until used. It was ground in a Wiley mill and screened, and the fractions between 10 and 35 mesh (0.5 and 2.0 mm) were collected and air-dried at room temperature (25 °C). The initial composition of barley straw was 41.0 wt.% glucan, 22.4 wt.% xylan, 1.2 wt.% galactan, 3.0 wt.% arabinan, 21.3 wt.% lignin (acid insoluble+acid soluble), and 4.1 wt.% ash.

Cellulase GC 220 was provided by DuPont Industrial Biosciences (formerly Genencor International, Rochester, NY). The average activity of cellulase (GC-220) was 45 filter paper units (FPU)/ml. The β-glucosidase enzyme, Novozyme 188 (Novo Inc., Lot #11 K1088), was purchased from Sigma-Aldrich (St. Louis, MO). Activity of Novozyme 188 was 750 cellobiase units (CBU)/ml.

Ammonium hydroxide (Fisher Cat # A669C) was used for the pretreatment. All other chemicals used were of reagent grade and purchased from various suppliers.

Methods

SAA Pretreatment of Barley Straw

Barley straw was treated with 5–15 wt.% aqueous ammonia in screw-capped bottles at 25–80 °C for 3–96 h. Solid-to-liquid ratios ranging from 1:3 to 1:10 were used. After pretreatment, the solids were separated from the liquid by filtration under vacuum, washed with deionized (DI) water until the pH reached 7.0, and subjected to enzymatic digestibility tests. Acid soluble and insoluble lignin and carbohydrate contents were determined by the NREL Chemical Analysis and Testing Standard Procedure [17].

Enzymatic Digestibility Tests

The enzymatic digestibilities of SAA-treated barley straw solids were determined in duplicate according to the NREL Chemical Analysis and Testing Standard Procedure NREL/TP-510-42629 [17]. The conditions of the enzymatic digestibility test were pH 4.8 (0.05 M sodium citrate buffer) in an incubator shaker agitated at 150 rpm and maintained at 50 °C.

For the enzymatic digestibility tests of the pretreated solid residue, 15 FPU of GC-220/g-glucan supplemented with 30 CBU of β-glucosidase (Novozyme 188)/g-glucan were used. The initial glucan concentration was 1 % (*w*/*v*) based on 100 ml of total liquid and solid. The solid residue samples used in the digestibility tests were wet samples collected after SAA pretreatment. Avicel was taken through the same procedure as a reference. Two different enzymatic digestibilities for glucan and xylan were measured with SAA-treated solid. The 250-ml screw-capped Erlenmeyer flasks containing the enzyme hydrolysis preparations were placed in an incubator shaker (New Brunswick Scientific, Edison, NJ). Samples were taken periodically (6, 12, 24, 48, 72, and 96 h) and analyzed for glucose and xylose contents using HPLC.

The glucan and xylan digestibilities were calculated as follows:

$$Glucan\ digestibility = \frac{Total\ release\ glucose(g) \times 0.9}{Initial\ glucan\ loading(g)} \times 100 \quad (1)$$

(0.9 is the conversion factors of glucose to equivalent glucan.)

$$Xylan\ digestibility = \frac{Total\ release\ xylose(g) \times 0.88}{Initial\ xylan\ loading(g)} \times 100 \quad (2)$$

(0.88 is the conversion factors of xylose to equivalent xylan.)

Response Surface Methodology

Response surface methodology (RSM) was used to optimize the reaction time and temperature of SAA pretreatment conditions. The series of experiments designed and conducted are shown in Table 1. The two factors, reaction time and reaction temperature, were chosen based on results of preliminary tests on digestibility of pretreated barley straw. The pretreatment was performed with solid-to-liquid ratio of 1:8 at 30–90 °C for 3–21 h. The optimal reaction conditions for maximizing glucan and xylan digestibility with minimal sugar loss during the pretreatment were determined. To achieve high enzymatic digestibility after SAA pretreatment, pretreatment conditions were optimized by RSM based on the 2^2 factorial central composite design (CCD). The matrix corresponding to the CCD is presented in Table 2. Fifteen experiments were carried out with two variables, and each variable varied at three levels (α=1.50) for sugar recovery yields. The sugar recovery yield (Y_S) was calculated according to the following equation:

$$\text{Sugar recovery yields, } Y_s[\%] = \frac{\text{Glucan remaining[g]after SAA} \times \text{Glucan digestibility[\%]} + \text{Xylan remaining[g]after SAA} \times \text{Xylan digestibility[\%]}}{(\text{Glucan} + \text{Xylan})\text{[g]in untreated barley straw}} \times 100 \quad (3)$$

The quadratic polynomial model was fitted for the sugar recovery yield (Y) using the following equation:

$$Y = \alpha_0 + \alpha_1 A + \alpha_2 B + \alpha_{11} A^2 + \alpha_{22} B^2 + \alpha_{12} AB \quad (4)$$

Where A and B represent coded levels of the independent variables; α_0 is intercept term; α_1 and α_2 are linear terms; α_{11} and α_{22} are quadratic terms; and α_{12} is an interaction term. The statistical analysis of the data was performed using Design Expert software (version 7.1.1, Stat-Ease, Inc., Minneapolis, USA).

Table 1 Independent variables and their levels in the experimental design

Independent variable	Symbols	Unit	Code levels		
			−1	0	1
Reaction temperature	A	°C	40	60	80
Reaction time	B	h	6	12	18

Table 2 Experimental design of the central composite design

Run	Variables	
	(A) Reaction temperature [°C]	(B) Reaction time. [hours]
1	−1	−1
2	−1	−1
3	1	−1
4	1	−1
5	−1	1
6	−1	1
7	1	1
8	1	1
9	−1.5	0
10	1.5	0
11	0	−1.5
12	0	1.5
13	0	0
14	0	0
15	0	0

Pretreatment conditions: solid: liquid=1:8, 15 wt.% ammonia concentration

Analytical Methods

Treated and untreated barley straw were analyzed for carbohydrates (sugars) and lignin [17]. Each sample was analyzed in duplicate. The conditions of the first hydrolysis were 1:10 of solid-to-liquid ratio using 72 wt.% sulfuric acid and 30 °C for 1 h. The secondary hydrolysis was performed using 4 wt.% sulfuric acid at 121 °C for 1 h.

Carbohydrates were determined by HPLC with a Bio-Rad Aminex HPX-87P column (Bio-Rad Laboratories, Hercules, CA) and a refractive index detector (Varian 356-LC, Varian, Inc., CA) operated at 60 °C. The solvents were DI water at a flow rate of 0.6 ml/min.

For acid insoluble lignin analysis, the autoclaved hydrolysis solution was vacuum filtered, and the recovered solid sample was dried and weighed. The dried samples were then combusted in a furnace at 575±25°C for 16 h to determine the ash content. The difference of the two weights was taken as the acid insoluble lignin. The absorbance of the hydrolysis liquor in the aliquot obtained from the vacuum filter sample at 320 nm on a UV–visible spectrophotometer was used to determine the acid soluble lignin [17].

Results and Discussion

Effects of Ammonia Concentrations

The results of the experiments performed to study the effects of ammonia concentration in SAA of barley straw are summarized in Table 3. Three different ammonia concentrations (0, 5, and 15 wt.%) were used while maintaining reaction temperature at 60 °C and reaction time at 24 h. As Table 3 shows, there were no significant differences in glucan and xylan compositions of the residual solids after SAA pretreatment over the entire range of ammonia

Table 3 Effect of ammonia concentration on solid composition and enzymatic digestibility after SAA pretreatment

NH_3 concentration	S.R.[a]	Lignin[b]	Solid		Enzymatic digestibility[c]		Sugar recovery yield, Y_S
			Glucan	Xylan	Glucan	Xylan	
[wt.%]	[%]	[%]	[%]	[%]	[%]	[%]	[%]
Untreated	100	21.3 (±1.8)	41.0 (±1.5)	22.4 (±1.2)	11.0 (±1.0)	7.5 (±0.9)	–
0	91.8 (±2.0)	18.8 (±0.3)	38.0 (±1.5)	21.1 (±2.1)	27.9 (±1.5)	13.2 (±0.7)	19.9
5	80.7 (±2.3)	9.5 (±1.9)	37.2 (±1.7)	17.5 (±1.1)	69.6 (±2.5)	61.8 (±3.1)	57.9
15	72.4 (±2.5)	8.8 (±2.0)	37.5 (±1.2)	17.4 (±0.4)	83.4 (±2.3)	69.2 (±1.7)	68.3

Data in the table are based on the oven-dry untreated biomass. Values are expressed as mean and standard deviation; other pretreatment conditions: 60 °C reaction temperature, solid:liquid=1:10 and 24 h reaction time

[a] S.R. stands for solid remaining (wt.%) after reaction

[b] Acid soluble lignin+acid insoluble lignin

[c] Digestibility at 72 h

concentrations. Relative to the starting amount of untreated oven-dry biomass, the glucan and xylan contents of the pretreated solids, due to loss of overall mass, were both lower than those in the untreated material (~37 wt.% compared to ~41 wt.% for glucan and ~17 wt.% compared to ~22 wt.% for xylan). Most importantly, both carbohydrate fractions were well preserved during the pretreatment (>90 % of glucan and >78 % of xylan). Surprisingly, increasing of ammonia concentration from 5 to 15 wt.% showed little additional effect on delignification. However, SAA pretreatment with 15 % ammonia significantly enhanced enzymatic digestibility of treated solids as compared to SAA treatment with 0 and 5 wt.% ammonia (Table 3). When ammonia concentration was increased from 5 to 15 wt.%, the 72-h digestibility increased from 69.6 % to 83.4 % and from 61.8 % to 69.2 % for glucan and xylan, respectively. Because one of the primary objectives of the pretreatment is to improve yields of fermentable sugars by enzymatic hydrolysis, it was decided to use 15 wt.% ammonia in the subsequent experiments.

Effects of Reaction Temperature

The effects of reaction temperature were also investigated. The recovery of polysaccharides in the SAA-treated solid and 72-h enzymatic digestibility are shown in Fig. 1. Four different reaction temperatures (25 °C, 40 °C, 60 °C, and 80 °C) were applied while maintaining other conditions (15 wt.% ammonia concentration, solid:liquid ratio at 1:10, and reaction time of 24 h.) The data indicated that the xylan and lignin remaining in the solid fraction decreased as reaction temperature increased. Solubilization of xylan was increased from 13.0 % to 26.8 %, and lignin removal increased from 42.7 % to 60.1 % when temperature was increased from 25 °C to 80 °C. The glucan contents in the treated solid did not seem to be significantly affected by the temperatures studied. All temperatures tested resulted in a high recovery of glucan in the solid residue.

Pretreatment temperature did not show significant effects on digestibility of both glucan and xylan until it was increased above 40 °C. Digestibility of glucan increased from 68.0 % to 83.4 % when pretreatment temperature was increased from 40 °C to 60 °C. The corresponding increase in xylan digestibility was from 58.6 % to 69.8 %.

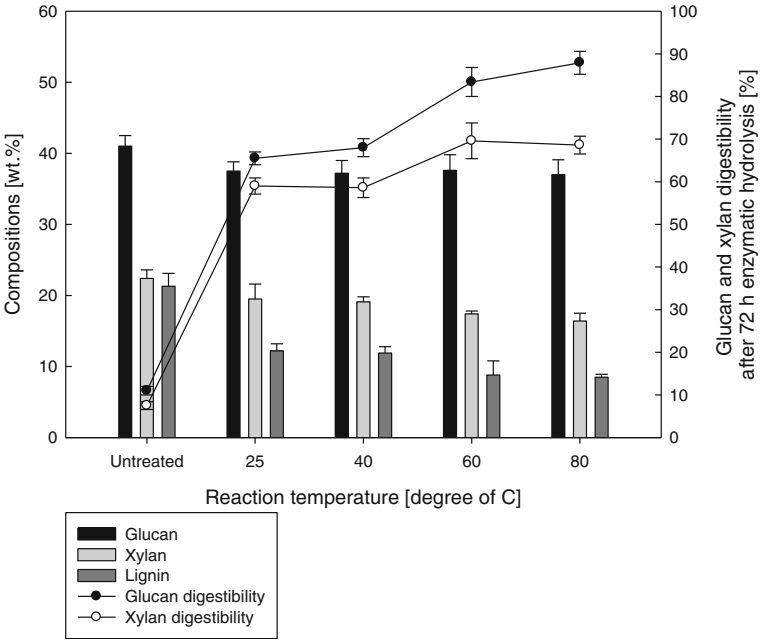

Fig. 1 Effect of reaction time on recovery of glucan, xylan, and lignin in the solid residue and its enzymatic digestibility after SAA pretreatment

Increase of pretreatment temperature above 60 °C resulted in further increase in glucan digestibility but did not have any effect on xylan digestibility.

Effects of Reaction Time

To study the effects of reaction time, reaction times of 3, 12, and 24 h were applied while maintaining the other conditions (reaction temperature at 60 °C, ammonia concentration at

Table 4 Effect of reaction time on the compositions and enzymatic digestibility in SAA-treated barley straw

Reaction time	S.R.[a]	Lignin[b]	Solid		Enzymatic digestibility[c]		Sugar recovery yield, Y_S
			Glucan	Xylan	Glucan	Xylan	
[h]	[%]	[%]	[%]	[%]	[%]	[%]	[%]
Untreated	100	21.3 (±1.8)	41.0 (±1.5)	22.4 (±1.2)	11.0 (±1.0)	7.5 (±0.9)	-
3	79.7 (±1.7)	11.1 (±0.7)	38.3 (±1.1)	19.8 (±1.0)	65.9 (±2.4)	56.5 (±2.7)	57.5
12	75.5 (±1.0)	9.5 (±0.9)	38.0 (±1.1)	18.2 (±0.5)	80.4 (±1.2)	70.8 (±1.5)	68.5
24	72.4 (±2.5)	8.8 (±2.0)	37.5 (±1.2)	17.4 (±0.4)	83.4 (±2.3)	69.2 (±1.7)	68.3

Data in the table are based on the oven-dry untreated biomass. Values are expressed as mean and standard deviation; other pretreatment conditions: 15 wt.% of ammonia concentration, 60 °C reaction temperature; solid:liquid=1:10

[a] S.R. stands for solid remaining (wt.%) after reaction

[b] Acid soluble lignin+acid insoluble lignin

[c] Digestibility at 72 h

15 wt.%, and the solid:liquid ratio at 1:10). The compositions of the pretreated solids relative to the untreated barley straw (percent recoveries of lignin, glucan, and xylan) and their enzymatic digestibility are presented in Table 4. The results showed that lignin removal was quite significant even with shortest reaction time of 3 h (47.9 % removal). Further delignification, however, was less significant when reaction time was increased to 12 and 24 h. Lignin removal was 55.4 % and 58.7 % in these two cases, respectively. Extending reaction time past 3 h did not have significant effects on glucan and xylan contents of the pretreated materials. When reaction time was increased from 3 to 24 h, the glucan content was virtually unchanged whereas the xylan content was reduced only slightly from19.8 % to 17.4 %, which resulted in an increase of xylan solubilization from 11.6 % to 22.3 % of the xylan in the untreated material. Digestibility of both glucan and xylan increased, however, when reaction time was increased from 3 to 12 h (from 65.9 % to 80.4 % for glucan and from 56.5 % to 70.8 % for xylan.) Increasing the reaction time to 24 h did not improve xylan digestibility and improved glucan digestibility only slightly. The net result was significant improvement of total fermentable sugar yield (sugar recovery yield, Y_S) when reaction time was increased from 3 to 12 h (from 57.5 % to 68.5 %), but further increase in reaction time did not result in further improvement of total fermentable sugar yield.

Effects of Solid:Liquid Ratio

The effects of solid:liquid ratios of 1:3, 1:6, and 1:10 were studied while maintaining the other conditions at 60 °C reaction temperature, 15 wt.% ammonia, and 24 h reaction time. The compositions and enzymatic digestibility results of the pretreated barley straw are shown in Table 5. The results indicated that decreases of the solid:liquid ratio did not change the glucan and xylan contents but increased the delignification and enzyme digestibility of the pretreated materials. The increases of glucan digestibility as the results of lower solid: liquid ratios were more significant than those observed for xylan digestibility. When the solid:liquid ratio was decreased from 1:3 to 1:10 glucan digestibility increased from 73.2 %

Table 5 Effect of solid:liquid ratio on the compositions and enzymatic digestibility in SAA-treated barley straw

Solid:liquid	S.R.[a]	Lignin[b]	Solid		Enzymatic digestibility[c]		Sugar recovery yield, Y_S
			Glucan	Xylan	Glucan	Xylan	
[−]	[%]	[%]	[%]	[%]	[%]	[%]	[%]
Untreated	100	21.3 (±1.8)	41.0 (±1.5)	22.4 (±1.2)	11.0 (±1.0)	7.5 (±0.9)	–
1:3	75.7 (±1.0)	11.5 (±0.5)	37.0 (±0.8)	17.6 (±0.2)	73.2 (±1.0)	65.3 (±2.5)	60.8
1:6	73.6 (±1.1)	9.5 (±0.3)	37.2 (±1.1)	17.5 (±0.6)	76.2 (±1.6)	66.1 (±1.7)	63.0
1:10	72.4 (±2.5)	8.8 (±2.0)	37.5 (±1.2)	17.4 (±0.4)	83.4 (±2.3)	69.2 (±1.7)	68.3

Data in the table are based on the oven-dry untreated biomass. Values are expressed as mean and standard deviation; other pretreatment conditions: 15 wt.% of ammonia concentration, 60 °C reaction temperature and 24 h reaction time

[a] S.R. stands for solid remaining (wt.%) after reaction

[b] Acid soluble lignin+acid insoluble lignin

[c] Digestibility at 72 h

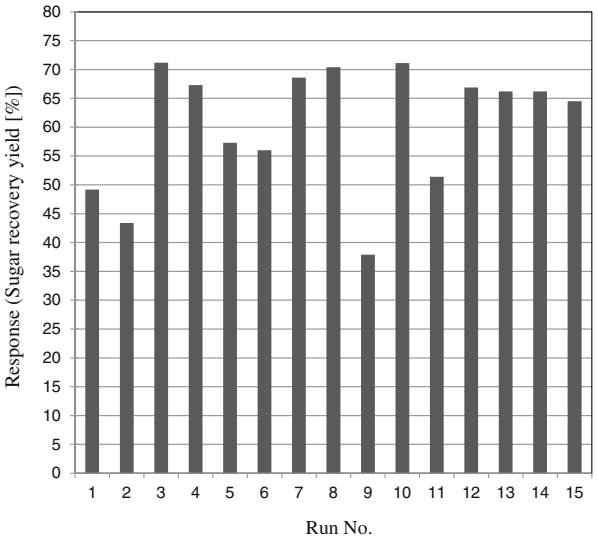

Fig. 2 Experimental results of the central composite design. Note. Variables (*A*: Reaction temperature; *B*: Reaction time) are shown in Table 2; Pretreatment conditions: solid:liquid = 1:8, 15 wt.% ammonia concentration

to 83.4 %, whereas xylan digestibility only increased from 65.3 % to 69.2 %. The total fermentable sugar yield (Y_S) increased accordingly from 60.8 % to 68.3 %.

RSM Study

A CCD set up with the Design Expert software was employed to investigate the simultaneous effects of reaction temperature and reaction time on sugar recovery yield. The performances of various combinations of pretreatment conditions are summarized in Fig. 2. The polynomial equation, describing the sugar recovery yield (Y_S) as a simultaneous

Table 6 ANOVA analysis for responses *Y* [sugar recovery yield (%)]

Source	Sum of squares	Degree of freedom	Mean squares	*F* values	*Prob* [a] > *F*
For *Y*					
Model	1,542.96	5	308.59	33.88	<0.0001
A	1,208.35	1	1,208.35	132.68	<0.0001
B	158.06	1	158.06	17.36	0.0024
AB	51.00	1	51.00	5.60	0.0421
A^2	118.07	1	118.07	12.96	0.0057
B^2	36.04	1	36.04	3.96	0.0779
Residual	81.96	9	9.11		
Lack of fit	53.15	3	17.72	3.69	0.0815
Pure error	28.82	6	4.80		

[a] Probability values (*P* values)

function of reaction temperature and reaction time of SAA pretreatment, is shown below:

$$Y_S = 65.91 + 9.83A + 3.56B - 2.52AB - 4.12A^2 - 2.28B^2 \quad (5)$$

The RSM was used to optimize the SAA pretreatment conditions. Two factors, reaction time and temperature, were chosen and the values of these two process parameters used in the RSM experiments are previously shown in Table 1. The RSM pretreatment experiments were performed with solid-to-liquid ratio of 1:8 and 15 wt.% ammonia concentration. The optimal conditions were found to maximize glucan and xylan digestibility with the minimal sugar loss during the pretreatment. The sugar recovery yield (Y_S) was calculated and used because it is a combined result of the two factors, carbohydrate remaining and enzymatic digestibility (see Eq. (1)–(3)). To achieve high sugar recovery and saccharification yield after SAA pretreatment, i.e., sugar recovery yield (Y_S), pretreatment conditions were optimized by RSM based on the 2^2 factorial central composite designs. The matrix corresponding to the CCD is previously presented in Table 2. Fifteen experiments were carried out with two variables, and each variable varied at three levels (α=1.50) for sugar recovery yield. The highest sugar recovery yield (71.2 % with Run no. 3 in Fig. 2) was observed at 80 °C reaction temperature, 6 h reaction time, 15 wt.% ammonia concentration with 1:8 of solid:liquid ratio. High sugar recovery yields over 70 % were also observed from run no. 8 (treated at 80 °C for 18 h.) and run no. 10 (treated at 90 °C for 12 h.). Compared to the wide range of reaction times (6–18 h.) that resulted in high sugar recovery yield (Y_S above 70 %), reaction temperature range for high sugar recovery yield was relatively narrow (80–90 °C). Judging from these results, the reaction temperature was more critical than reaction time.

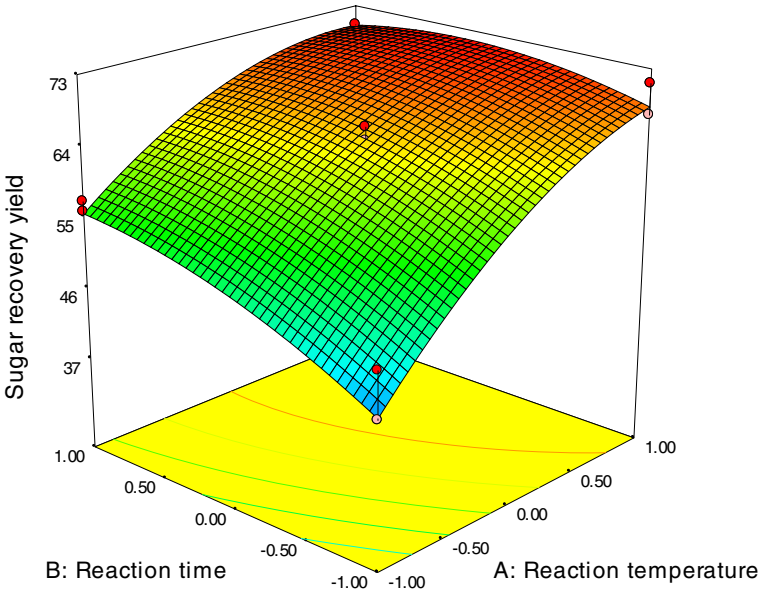

Fig. 3 Response surface curve showing combined effect of reaction temperature and reaction time on sugar recovery by SAA pretreatment of barley straw

The summary of the analysis of variance (ANOVA) is also presented in Table 6. The values of *Prob>F* for *A*, *B*, *AB*, and A^2 less than 0.05 indicated that these coefficients in the model in Eq. (5) significantly affected sugar recovery yield, and coefficient of B^2 also influenced sugar recovery yield in the range used in this study. The model's *F* value of 33.88for *Y* and the value of *Prob>F* (<0.0001) for the overall model showed that the overall model was adequate. The determination coefficient (R^2=0.9496) indicated that 94.96 % of the variability in the response could be explained by the model.

The 3-D response surface using Eq. (5) for sugar recovery yield is shown in Fig. 3. The optimization of reaction temperature and reaction time of SAA treatments to achieve maximum sugar recovery yield was carried out in the range of experimental runs using the Design Expert software. The maximum sugar recovery yield and optimal conditions were also determined as 71.5 % with 77.6 °C reaction temperature, 12.1 h reaction time, 15 wt.% NH_4OH, and 1:8 solid-to-liquid ratio.

Conclusion

Various effects of processing parameters in SAA pretreatment of barley straw were evaluated and their effects on total sugar recovery yield were calculated. It was found that the sugar recovery yield (Y_S) generally increased at increasing ammonia concentration, temperature, and decreased with solid:liquid ratio. Compared to the reaction temperature, reaction time appeared to be less critical in improving fermentable sugar production. The sugar solutions obtained by enzyme hydrolysis of the SAA pretreated biomass can be used for production of ethanol and various value-added co-products in which both digestibility and sugar recovery after pretreatment are important.

Acknowledgments This work was supported by the New Growth Technology Strategy Department grant funded by POSCO (2012A076).

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