Optimization of alkali pretreatment of wheat straw to be used as substrate for biofuels production

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ABSTRACT

Alkali pretreatment of wheat straw was optimized by response surface methodology to maximize yields of fermentable sugars in subsequent enzymatic hydrolysis and to remove maximum lignin in order to improve rheological attributes of the media. The effects of pretreatment conditions on biomass properties were studied using the Expert Designer software. Concentration of sodium hydroxide and temperature were the factors most affecting pretreatment efficiency. At the optimum (80°C, 39 min, 0.18 g NaOH and 0.06 g lime per g of raw biomass), 93.1 ± 1.0% conversion of cellulose to glucose after enzymatic hydrolysis and 80.3 ± 1.2% yield of monosaccharides (glucose plus xylose and arabinose) from cellulose and hemicellulose of wheat straw were achieved.

Keywords: alkali hydrolysis; agrowaste; response surface methodology; enzymatic hydrolysis; bioethanol

Lignocellulose is the most abundant biomass with an estimated annual production of 10^{11} t containing potential energy of about 2×10^{21} J (Binder and Raines 2010), which makes it a promising feedstock for biotechnological production of biofuels. It is composed of cellulose, hemicellulose and lignin, which are tightly bound together by hydrogen and covalent bonds. Due to its complex structure, lignocellulosic biomass cannot be directly utilized by most bioethanol producers and must be pretreated in several consecutive steps to achieve fermentable sugars. Firstly, pretreatments have to be applied to decompose the complex matrix of biomass prior to enzymatic hydrolysis of cellulose into glucose. In the next paragraphs only alkaline biomass pretreatment, which was used in our work will be discussed; detailed information on other pretreatment methods is reviewed in many articles (Alvira et al. 2010, Menon and Rao 2012).

Alkaline biomass pretreatment is normally performed at a lower temperature and pressure than other pretreatment methods (Farid et al. 2010) and is suitable for processing of agricultural residues such as wheat straw (Sánchez and Cardona 2008). During alkaline pretreatment, ester bonds, which cross-link lignin and xylan, are degraded by a similar mechanism as in soda or kraft processing (Sun and Cheng 2002). In this process, glycosidic linkages in the lignocellulosic cell wall matrix are broken down, resulting in alteration of the structure of lignin to polymeric lignin-like compounds (Bobleter 1994), reduction of the lignin-hemicellulose complex, cellulose swelling, and the partial decrystallization of cellulose (Cheng et al. 2010). Alkali also neutralizes the organic acids and phenols normally formed during this process, less sugar is degraded and fewer inhibitors are formed compared to acid pretreatment. Sodium hydroxide (NaOH), lime ($Ca(OH)_2$) and ammonia (NH_4OH) are frequently used as chemical agents in this process. The type of catalyst together with pH, highly influence monosaccharide yield and lignin removal from wheat straw (Pedersen et al. 2010). Lime can be combined with sodium hydroxide to reduce the cost of pretreatment, stabilize pH and prevent sugar loss due to linkage of the calcium

ion with functional groups (carboxyl-, methoxyl-, and hydroxyl- groups) in the biomass (Torre et al. 1992, Mielenz 2009, Xu and Cheng 2011).

In our work, combination of lime and NaOH was applied to wheat straw to achieve the highest total sugars and lignin removal by using response surface methodology (RSM). RSM is an effective optimization tool which combines mathematical and statistical techniques and it is used for empirical model building and process optimization. It can be well applied when a final response (output variable) is influenced by several independent variables and their interactions.

MATERIAL AND METHODS

Experimental design. RSM was used to find the optimum conditions for pretreatment of wheat straw. Central composite factorial design with four independent variables: (a) pretreatment time (t); (b) temperature (T); (c) concentration of sodium hydroxide (C_S) and (d) concentration of lime (C_L) was applied to maximize lignin removal (L_r), glucose yield (Y_{G_E/G_S}) from cellulose and yield of total sugars (glucose, xylose and arabinose) (Y_{F_E/F_S}) from cellulose and hemicellulose of wheat straw. Each factor was involved at five coded levels (–2, –1, 0, +1, +2) calculated according to following equation:

$$x_i = (X_i - X_0)/\Delta X_i \qquad (1)$$

Where: x_i – dimensionless and X_i – actual value of the independent variable i. X_0 – actual value of the independent variable i at the centre point. ΔX_i – step change of X_i corresponding to a unit variation of the dimensionless value. The experimental design created by Design-Expert 8.0 software (Stat-Ease, Inc, Minneapolis, USA) resulted in 30 experimental trials (16 trials for factorial design, 8 trials for axial points and 6 trials for replication of the central points) as shown in Table 1.

Mechanical treatment of wheat straw. Air dried wheat straw was milled using a knife mill Grindomix GM 200 (Retch, Haan, Germany) with sieve diameter of 2 mm, and was stored in sealed plastic bags at room temperature.

Alkali pretreatment of wheat straw. 20 g of milled wheat straw with 200 mL of NaOH and $Ca(OH)_2$ solutions (as specified in Table 2) was placed into stainless steel reactor with inner height 30 cm and diameter 4.8 cm equipped with a thermocouple, electrical heating and air cooling. To achieve non-oxidative condition the reactor was flushed with nitrogen. After pretreatment, the reactor was cooled by air to room temperature and solid and liquid phases were separated by centrifugation at 5471 × g for 5 min. The solid phase was washed two times with 200 mL of deionized water, dried and used for enzymatic hydrolysis.

Determination of composition of wheat straw before and after pretreatment. Carbohydrate, lignin and ash content analysed according to the NREL laboratory analytical procedure (Sluiter et al. 2008) are shown in Table 3.

Enzymatic hydrolysis. Enzymatic hydrolysis of pretreated samples (5% w/w) was performed in 5 mmol sodium-citrate buffer (pH 5) at 45°C (orbital shaker, rotation 120/min) using cellulase complex NS22086 (Novozymes A/S, Bagsvaerd, Denmark). Enzyme dosing was 35 FPU/g of original dry matter. Concentration of released sugars was analysed by HPLC. The glucose yield (Y_{G_E/G_S}) was calculated according to Eq. (2):

$$Y_{G_E/G_S} = \frac{G_E}{G_S \times C_F} \times 100 \ (\%) \qquad (2)$$

Where: G_S – content of cellulose in one gram of air dried wheat straw and G_E – amount of glucose released per gram of wheat straw after enzymatic hydrolysis. C_F – correction factor (1.11 for glucan and 1.14 for xylan and arabinan). The yield of total sugar (Y_{F_E/F_S}) was calculated according to Eq. (3):

$$Y_{F_E/F_S} = \frac{F_E}{F_S \times C_F} \times 100 \ (\%) \qquad (3)$$

Where: F_S – cellulose plus hemicellulose content in one gram of dry wheat straw; F_E – amount of total sugar (glucose, xylose and arabinose) released per gram of wheat

Table 1. Coded levels of factors for variables used for optimization

Level of factors	–2	–1	0	1	2
Temperature (°C)	80	100	120	140	160
Pretreatment time (min)	10	35	60	85	110
NaOH concentration (g/g) wheat straw	0	0.05	0.1	0.15	0.2
Lime concentration (g/g) wheat straw	0	0.025	0.05	0.075	0.1

Table 2. Experimental design showing actual values of variables and their influence on lignin removal, glucose yield from cellulose and total sugars yield from cellulose and hemicellulose of wheat straw

Temperature (°C)	Time (min)	NaOH	Ca(OH)$_2$	Solid recovery	Lignin content	Lignin removal	Glucose yield	Total sugars yield
		(g/g)		(g/100 g DW)			(%)	
100	35	0.05	0.025	86.7	24.3	8.3	76.6*	84.7*
140	35	0.05	0.025	83.6	23.1	12.8	70.6	72.6
100	85	0.05	0.025	78.9	21.5	18.9	51.1	56.2
140	85	0.05	0.025	79.3	20.6	22.2	72.8	75
100	35	0.15	0.025	70.0	17.3	34.8	77.7	79.3
140	35	0.15	0.025	74.1	18.3	30.9	72.6	72.9
100	85	0.15	0.025	70.1	17.2	34.9	73.3	73.8
140	85	0.15	0.025	71.1	17.8	32.7	68.3	70.7
100	35	0.05	0.075	81.4	21.2	19.9	55.8	61.3
140	35	0.05	0.075	84.5	22.2	16.1	66.2	69
100	85	0.05	0.075	85.9	21.6	18.3	47.3	52.2
140	85	0.05	0.075	82.4	22	16.8	64.7	66.6
100	35	0.15	0.075	64.3	13.2	50.1	76.2	76.6
140	35	0.15	0.075	61.7	12.2	53.9	68.7	68.4
100	85	0.15	0.075	68.9	16.7	37*	71.8	72.3
140	85	0.15	0.075	58.3	10.7	59.7	74.6	72.4
80	60	0.1	0.05	69.7	16.2	38.6	72.3	73.6
160	60	0.1	0.05	67.8	15.6	41.1	81.9	79.4
120	10	0.1	0.05	78.8	19.8	25	69.4	70.6
120	110	0.1	0.05	67.9	14.7	44.4	79.9*	80.3*
120	60	0	0.05	84.8	23.1	12.7	39.7	43.7
120	60	0.2	0.05	53.2	10.3	61	87.7*	84.4*
120	60	0.1	0	73.7	16.9	36.1*	72.1	74.8
120	60	0.1	0.1	71.9	16.8	36.3	68.9	69.2
120**	60	0.1	0.05	72.2 ± 2.7	16.78 ± 0.89	36.6 ± 3.4	76.6 ± 2.9	76.3 ± 2.9

DW – dry weight; *values were excluded from model fitting since their normal probability plot indicates residuals do not follow normal distribution; **the mean value together with standard deviation of 6 trials for replication of the central point

straw after enzymatic hydrolysis. Lignin removal (L$_r$) was calculated according to Eq. (4):

$$L_r = \frac{L_S - L_P}{L_S} \times 100 \ (\%) \tag{4}$$

Where: L$_s$ – lignin content in wheat straw (g/100 g DW) and L$_p$ – lignin content in wheat straw after pretreatment (g/100 g DW).

Statistical analysis and response surface evaluation. Design-Expert 8.0 software was used for

model fitting and statistical data analysis, the proposed model was (5):

$$Y_i = \beta_0 + \sum_{i=1}^{4} \beta_i x_i + \sum_{i=1}^{4} \beta_{ii} x_i^2 + \\ + \sum_{i=1}^{3} \sum_{j=i+1}^{4} \beta_{ij} x_i x_j + \varepsilon \tag{5}$$

Where: Y_i – predicted response; x_i – coded value for each independent variable as listed in Table 1; β_i, β_{ii} and β_{ij} –

linear, quadratic, and interaction regression coefficients respectively, β_0 – intercept coefficient and ϵ – random errors. The individual effect of each variable and the effects of their interaction were determined at statistical significance ($\alpha = 0.05$) by the *F* test.

RESULTS AND DISCUSSION

Effect of pretreatment conditions on lignin removal. Since release of lignin from wheat straw would have a positive effect on enzymatic cellulose digestibility (Yang and Wyman 2004), it was used as one criterion to evaluate the effectiveness of pretreatment. After alkaline pretreatment performed under different conditions, percentage of lignin removal varied from 8% to 61% (Table 2). The influence of analysed factors on lignin removal (L_r) is expressed in Eq. (6):

$$L_r = 35.84 + (3.15 \times t) + (13.35 \times C_S) + \\ + (6.70 \times C_L) + (5.07 \times C_S \times C_S) - (4.22 \times C_L^2) \quad (6)$$

The model shows that only three factors; time of pretreatment, concentration of sodium hydroxide and lime have a significant effect on lignin removal ($P \leq 0.05$) and fits the data with $R^2 = 0.9417$ and adjusted $R^2 = 0.9285$. According to equation (6) the interaction between sodium hydroxide and lime has positive contributions to lignin removal (Figure 1) as also published by Pedersen et al. (2010, 2011). However, clear correlation between delignification and the yield of fermentable sugars was not found (Table 2). This was caused by enzyme surplus used in this experiment, which offset the potential adsorption of enzyme into lignin (Paulová et al. 2012). As published (Öhgren et al. 2007, Pedersen et al. 2011) the correlation between delignification and the yield of fermentable sugars is linearly positive only if delignification is above 60%.

Table 3. Chemical composition of wheat straw (dry weight)

Component	Content (%)
Cellulose	36
Hemicellulose	16.7
Acid soluble lignin	1.7
Acid insoluble lignin	26.5
Ash	6.9

Effect of pretreatment conditions on glucose release in subsequent enzymatic hydrolysis. As shown in Table 2, the yield of glucose released by enzymatic hydrolysis of pretreated wheat straw varied from 47% to 88% depending on the pretreatment condition. Based on the measured data (Table 2), Eq. (7) expressing the significant effects of variables and their interactions on glucose yield after enzyme hydrolysis was derived.

$$Y_{G_{E/G_S}} = 76.76 + (2.83 \times T) - (1.26 \times t) + (6.16 \times C_S) - \\ - (1.00 \times C_L) + (1.59 \times T \times t) - (4.90 \times T \times C_S) - \\ - (2.41 \times t^2) - (6.11 \times C_S^2) - (1.53 \times C_L^2) \quad (7)$$

All variables were expressed in code value as listed in Table 1. The reduced quadratic model is significant ($P \leq 0.0001$) and fits the data with $R^2 = 0.9584$ and adjusted $R^2 = 0.9364$. In the design space, glucose yield is positively influenced mainly by increasing concentration of NaOH (Eq. 7). Maximum glucose yield could be obtained if biomass was pretreated for 60 min at 120°C with 0.13 g of sodium hydroxide and 0.05 g of lime per gram of biomass (Figure 2a,b). Interactive effects of time and temperature (Figure 2a) on the amount of glucose released were not as distinct as that of NaOH and temperature (Figure 2b).

Effect of pretreatment conditions on yield of total sugars released by enzyme hydrolysis. Since 15% of pentoses (mainly D-xylose and L-arabinose) can be found in alkali pretreated wheat straw, it would be economically viable to convert
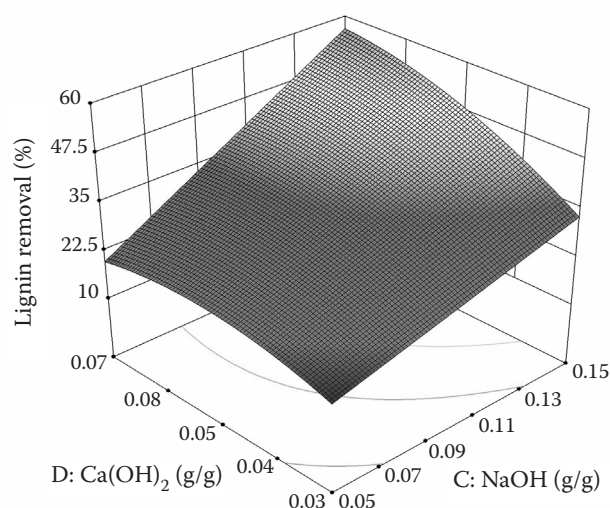

Figure 1. Interaction effect of two independent variables (sodium hydroxide and lime) on lignin removal at 120°C and 60 min pretreatment
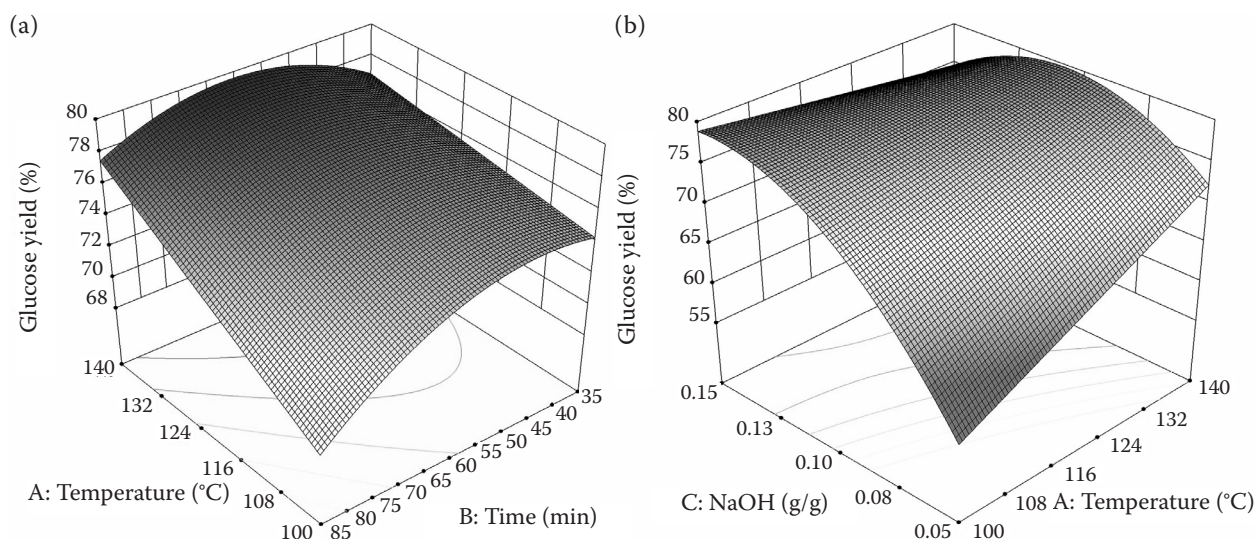
(a)

(b)

Figure 2. Response surfaces representing the interaction effect of two variables on glucose yield: (a) temperature and time when concentration of sodium hydroxide (C_S) and concentration of lime (C_L) are constant at 0.1 and 0.05 g/g dry biomass respectively; (b) C_S and temperature when time and C_L are constant at 60 min and 0.05 g/g dry biomass, respectively

them into ethanol. Taking into account the possibility of using recombinant strains, which are able to utilize both pentose and hexose sugars (Dellomonaco et al. 2010), the yield of total sugars (glucose, xylose and arabinose) released in pretreatment and subsequent enzymatic hydrolysis was taken as criterion of optimization. The model expressing the influence of variables on release of glucose, xylose and arabinose from cellulose and hemicellulose of wheat straw is represented in Eq. (8) (all variables were expressed in code value as listed in Table 1).

$$Y_{F_{E/F_S}} = 75.67 + (1.88 \times T) - (1.31 \times t) + (4.59 \times C_S) - (1.45 \times C_L) + (1.67 \times T \times t) - (4.30 \times T \times C_S) - (1.65 \times t^2) - (5.43 \times C_S^2) \quad (8)$$

The reduced quadratic model is significant ($P < 0.0001$) and fits the data with $R^2 = 0.9388$ and adjusted $F = 0.9116$.

Temperature of 120°C, 60 min of pretreatment time, 0.05 g/g lime and 0.15 g/g NaOH was found as an optimum (Figure 3). Although a higher concentration of $Ca(OH)_2$ had a positive effect on delignification (positive coefficient of C_L in Eq. (4),
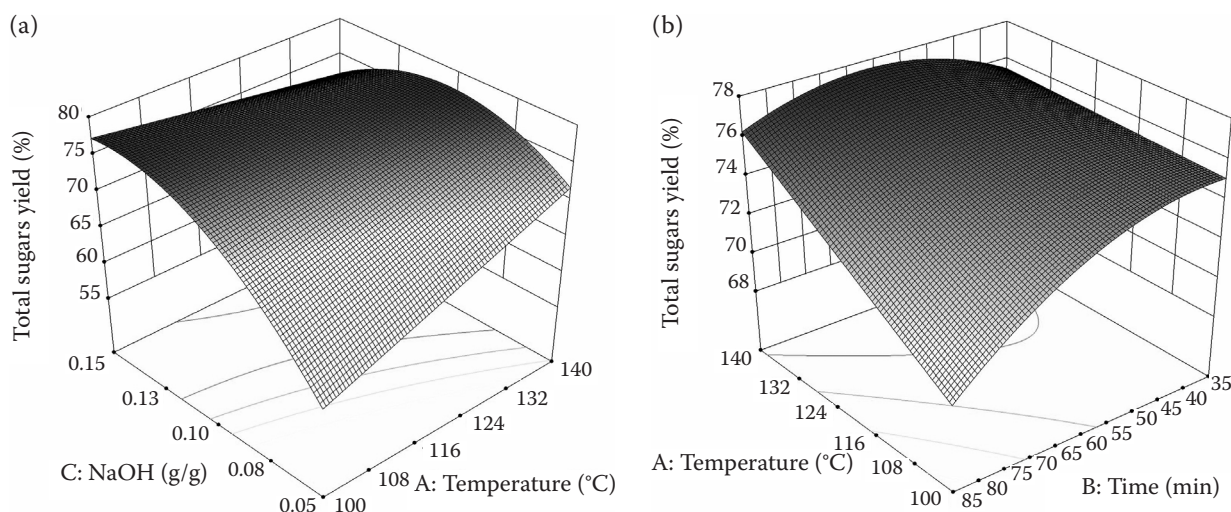
(a)

(b)

Figure 3. Response surfaces representing the interaction effect of two variables on total sugars yield: (a) temperature and time of pretreatment when concentration of sodium hydroxide and concentration of lime (C_L) are constant at 0.1 and 0.05 g/g dry biomass respectively; (b) sodium hydroxide loading and temperature when time and C_L are constant at 60 min and 0.05 g/g dry biomass, respectively

it can cause a reduction in total sugar yields (negative coefficient of C_L in Eq. (6) as reported also by Wang and Cheng (2011) and Xu and Cheng (2011).

Optimization. Finally all three criteria were combined to find the optimum conditions for pretreatment using numerical optimization module provided by Design-Expert 8.0 software.

According to the program prediction, 61% delignification, 82% glucose yield from cellulose and 81% yield of total sugars (glucose, xylose and arabinose) from cellulose plus hemicellulose should be achieved if pretreatment is performed at 80°C for 39 min with 0.18 g of sodium hydroxide and 0.06 g of lime per gram of biomass. In the validation experiment performed in triplicate at these condition 62.5 ± 1.7% of lignin was removed, which is well comparable to the predicted value. Glucose yield from cellulose and total sugars (glucose plus xylose and arabinose) yield from cellulose plus hemicellulose in the enzymatic hydrolysis following the pretreatment were 93.1 ± 1.0% and 80.3 ± 1.2%, respectively. The glucose yield achieved in experiment was 13% higher, while the measured total sugar yield was approaching the value predicted by model.

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