Characterization of the Detailed Relationships of the Key Variables in the Process of the Alkaline Sulfite Pretreatment of Corn Stover by Multivariate Analysis

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In biomass pretreatment processes, both the properties of feedstock and process parameters play important roles in the yield of downstream enzymatic hydrolysis. More importantly, like many other industrial processes, the pretreatment system is multivariate and the variables in the system are inter-related to different extents, which means that studying the relationships of the key variables is of critical importance for the improvement of downstream enzymatic saccharification yield. In this work, two multivariate analysis methods of the Principal Component Analysis (PCA) and Partial Least Square (PLS) were employed to characterize the detailed relationships of the key process variables of alkaline sulfite pretreatment of corn stover. The results showed that the total alkali charge is positively correlated with the sugar content in pretreated biomass, lignin removal efficiency, and final sugar yield; pretreatment temperature has negative impact on the recovery of polysaccharides; and total alkali charge is more influential than other pretreatment process variables (such as Na_2SO_3/NaOH and temperature) under the conditions studied.

Keywords: Multivariate analysis; Alkaline sulfite pretreatment; Corn stover; Enzymatic hydrolysis

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INTRODUCTION

The increasing energy demand, depletion of petroleum sources, and concern of global climate change have motivated the development of renewable and sustainable energy and chemicals by the exploitation and utilization of lignocellulosic biomass (Mosier *et al.* 2005; Agbor *et al.* 2011). However, the enzymatic conversion of carbohydrates in lignocelluloses to fermentable sugars is difficult, as lignocellulosic biomass has evolved a complex structure and chemical compositions to protect the structural sugars from outside attack (Ding *et al*. 2012; Leu and Zhu 2013). Therefore, pretreatment is required to disrupt the natural recalcitrance of lignocellulosic biomass for effective enzymatic hydrolysis.

Up to now, many biological, physical, chemical, and physicochemical pretreatment methods have been investigated on various feedstocks and are still under development with varying levels of success, including dilute acid/alkali pretreatment, autohydrolysis, organosolv process, ammonia fiber explosion (AFEX), the Sulfite

Pretreatment to Overcome Recalcitrance of Lignocellulose (SPORL) method, and hydrotropic pretreatment (Mosier *et al.* 2005; Zhu and Zhuang 2012; Mou *et al.* 2013a). It has been known that the effect of pretreatment is mainly dependent upon the properties of raw material (*e.g.* the particle size of feedstock) and the process conditions of pretreatment (*e.g.* the chemical charge) (Jin *et al.* 2013). For instance, severe pretreatment conditions (*e.g.* high temperatures) are generally required for woody biomass due to its more compact structure and higher lignin content, compared to herbaceous biomass; appropriately increasing pretreatment temperature may lead to better removal of lignin/hemicellulose, thus enhancing the downstream enzymatic digestibility (Zhu and Zhuang 2012).

However, the pretreatment system is multivariate. The compositional and structural features of raw material are closely associated, and many pretreatment process variables are more or less inter-related (Mosier *et al.* 2005). The variability of these variables accounts for the variation of digestibility between different sources of lignocellulosic biomass. Thus, the interactions between the variables in pretreatment system are as important as the variables themselves. Studying the interactions between the variables is of crucial importance for better understanding the interaction mechanism among the variables, optimization of process conditions, and the decrease of production cost, particularly for large scale application.

The multivariate analysis methods (Principal Component Analysis (PCA) and Partial Least Square (PLS) analysis) have been widely used to analyze or monitor the complex operations of biorefinery-alike pulp and papermaking, such as the rapid and nondestructive assessment of wood properties (Evans *et al.* 1995; Xu *et al.* 2011), the measurement of wood chip quality (Ding *et al.* 2005, 2009), the troubleshooting of the TMP process (Browne *et al.* 2004), process control (Strand *et al.* 2001; Bendwell 2002), and quality prediction (Phung and Nguyen 2003). Recently, multivariate analysis methods have been used to evaluate pretreatment effects (such as enzymatic digestibility and final sugar yield) based on the quantitative analysis of NIR spectra of pretreated biomass (Baum *et al.* 2012), and the developed models can predict the monosaccharides yield after enzymatic hydrolysis (Krasznai *et al.* 2012).

In this study, to characterize the detailed relationships among key process variables of alkaline sulfite pretreatment (NaOH + Na_2SO_3) of corn stover on enzymatic saccharification, 28 pretreatment experiments at lab scale were conducted under different conditions by varying total alkali charge, liquid to solid ratio, temperature, cooking duration at the maximum temperature, and Na_2SO_3/NaOH ratio. After analysis of chemical composition of pretreated biomass and enzymatic hydrolysis sugar yield, PCA was performed on the entire dataset to establish the correlations between the pretreatment process variables, the properties of pretreated biomass (glucan content, lignin content, *etc.*), and the final effect of pretreatment (total sugar yields, *etc.*). In addition, quantitative characterization of the effect of the properties of pretreated biomass on enzymatic hydrolysis was investigated by PLS method.

EXPERIMENTAL

Materials

Corn stover was harvested in the fall of 2012 from Qingdao, Shandong Province, China, and was cut into pieces having 3 to 5 cm length. After being air-dried, the corn stover was milled and screened to obtain the particles in the size range of 0.425 to 8 mm. The screened corn stover was stored in a plastic bag before component analysis and pretreatment. The raw corn stover contains 31.22% glucan, 17.66% xylan, 1.91% arabinan, 14.20% acid insoluble lignin, 0.85% acid soluble lignin, 22.61% extractives (hot water soluble plus ethanol soluble substances), and 6.89% ash.

Commercial enzymes, Celluclast 1.5L (cellulase) and Novozyme 188 (β-glucosidase), were purchased from Sigma-Aldrich China Inc. The activities of cellulase and β-glucosidase were 121 FPU/mL and 741 IU/mL, respectively, as tested following IUPAC standard methods (Ghose, 1987). Sodium hydroxide and sodium sulfite were obtained from Sinopharm Chemical Reagent Co. Ltd. All enzymes and chemicals were used as received.

Methods

Alkaline sulfite pretreatment

The alkaline sulfite pretreatment of corn stover was carried out in a reactor (PL1-00, Xianyang TEST Equipment Co., Ltd. China). For each test, 50 g screened corn stover (bone dry) was used, and the pretreatment was performed under different conditions as designed (Table I and Table II in appendix). During pretreatment, the reactor was rotated at 1 rpm. After pretreatment, the reactor tubes were cooled immediately to room temperature with tap water, and the samples were transferred in a Nylon bag (300 meshes) and then rinsed with tap water to neutralize pH. Finally, the washed samples were completely transferred to a pre-weighed plastic bag, and stored at 4 ºC for the next steps of analysis.

Enzymatic hydrolysis

To evaluate the effectiveness of pretreatment under different conditions, enzymatic hydrolysis of pretreated corn stover was conducted using the National Renewable Energy Laboratory (NREL) method. Briefly, the pretreated corn stover was enzymatically hydrolyzated with a substrate consistency of 2% (w/v). A mixture of cellulase (20 FPU/g-substrate) and β-glucosidase (5 IU/g-substrate) was added together with 0.05 M sodium citrate buffer (pH 4.8) and hydrolysis took place at 50 °C for 48 h in serum bottle (25 mL) placed in an incubator shaker at 90 rpm. To each bottle, 200 μL of a 2% sodium azide solution was added to prevent the growth of organisms during hydrolysis. Upon completion, the supernatant was filtered through a 0.22 μm membrane to be ready for further analysis.

Composition analysis

The component analysis of untreated and pretreated corn stover was conducted according to the NREL analytical procedures. Acid and enzymatic hydrolyzates (0.22 μm filtered) were analyzed by a high performance liquid chromatography (HPLC) system (Model 1200, Agilent, USA) equipped with a refractive index detector and Bio-Rad Aminex HPX-87H column (300 × 7.8 mm). The column was run at 55 ºC with 5 μM H_2SO_4 (0.5 mL/min) as a mobile phase. All of the pretreatments, enzymatic hydrolysis, and component analysis were carried out in duplicate, and the average for each test was reported and detailed calculations were shown in the appendix.

Multivariate analysis

In this work, 18 key variables in alkaline sulfite pretreatment system were studied at lab scale, including total alkali charge (Alkali, based on the oven dried corn stover and calculated as NaOH), liquid to solid ratio (L/S ratio), pretreatment temperature (T), holding time at maximum temperature (H time), and the ratio of Na_2SO_3 to NaOH (Na_2SO_3/NaOH); chemical composition of pretreated corn stover including glucan content in pretreated corn stover (Glucan), xylan content in pretreated corn stover (Xylan), extractives content in pretreated corn stover (Extractive), and lignin content in pretreated corn stover (Lignin); the variables regarding pretreatment effectiveness including solid yield after pretreatment (Solid Yield), delignification rate (D-lignin), recovery rate of glucan remaining in pretreated corn stover (R-glucan), recovery rate of xylan remaining in pretreated corn stover (R-xylan), and the variables regarding enzymatic hydrolysis effect of enzymatic hydrolysis percentage of glucan (E-glucan), enzymatic hydrolysis percentage of xylan (E-xylan), final glucan yield after pretreatment and enzymatic hydrolysis (Y-glucan), final xylan yield after pretreatment and enzymatic hydrolysis (Y-xylan), and final total sugar yield after pretreatment and enzymatic hydrolysis (Y-total sugar). The final dataset including all variables with units and ranges, and the detailed calculations are provided in the appendix.

Among these available variables selected, there are some obvious interrelations. For instance, Solid Yield is inter-related with R-glucan, R-xylan, and D-lignin. Y-total sugar is strongly associated with Y-glucan and Y-xylan. But more detailed relationships of the key variables in alkaline sulfite pretreatment can be revealed by multivariate analysis. The first step of the analysis was to perform the PCA on the entire dataset, to obtain an overall picture. PCA is the most common method used to reduce a large number of original variables to a smaller number of principle components (latent vectors) that explain the maximum amount of variance in the dataset. The first principal component (p1) is in the direction of greatest variability in the dataset, while the second one (p2) is in the orthogonal direction to p1. Each successive component explains less variance than its predecessors. In the second step, the PLS analysis was performed by using pretreatment effectiveness variables as Y set. Pretreatment process parameters and chemical composition variables of pretreated biomass were set as the X set. In contrast to PCA, PLS is a supervised method, which models the relationship between the two matrices (X and Y). The PLS analysis was performed to establish an orthogonal space structure and to relate these new vectors to the real variables (Lanouette *et al.* 2004). Two important results of PLS analysis were the variable importance plot (VIP) and coefficient plot for each Y variable. VIP plots summarized the influence of all X variables on all Y variables simultaneously. As the sum of squares of all VIP values is equal to the number of variables in the model, the average VIP would be 1, thus any VIP value bigger than 1 is above average and thus more important, while anything lower than 1 is less important. For coefficient plots, the regression coefficients were presented for the quantitative relationships between the X variables and the given Y variable. In this study, both PCA and PLS were carried out by using the SIMCA (Soft Independent Modelling of Class Analogy) software package (SIMCA-P 13.0.3 by Umetrics). The detailed procedure regarding how to carry out PCA and PLS in SIMCA together with some training dataset can be downloaded at the official web of Umetrics (http://www.umetrics.com/downloads/other-downloads).

RESULTS AND DISCUSSION

PCA Analysis

The PCA results suggested two principal components with a combined R^2 of 0.722 and Q^2 of 0.505. The R^2 value is the percent of variation of the entire dataset for all of variables (X with PCA and Y with PLS), explained by the model. A large R^2 (close to 1) is a necessary qualification for a good model, but it is not sufficient. The Q^2 value is an estimate of the predictive ability of the model (X with PCA and Y with PLS), and it is calculated by cross validation. The value of Q^2 indicates how well the model predicts the new data. A large Q^2 ($Q^2 > 0.5$) indicates a good predictive capability. Thus, the PCA model can explain about 72% variations of all variables.

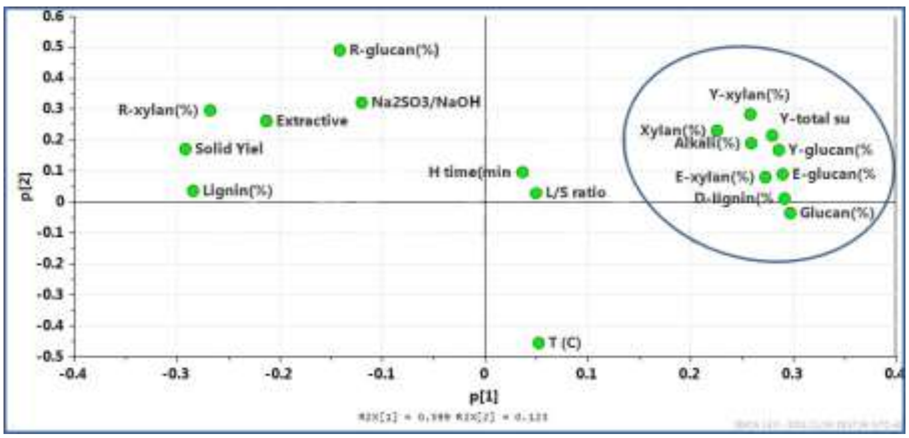

Fig. 1. Loading scatter plot for the PCA model

Figure 1 shows the loading scatter plot for each of the two principal components in the PCA model. In loading plots, p1 *vs.* p2 displays how the variables relate to each other. The further the variable is from the origin, the higher its influence will be. Variables near to each other are positively correlated, whereas variables opposite to each other are negatively correlated. As shown in Fig. 1, variables of Y-total sugar, Y-glucan, Y-xylan, E-glucan, E-xylan, Gluan, Xylan, Alkali, and D-lignin are grouped together, which means that they are strongly correlated, and the total alkali charge has big impact on delignification (D-lignin) under the pretreatment conditions employed, hence improving enzymatic digestibility, as lignin is one of main barriers of enzyme hydrolysis (Mosier *et al.* 2005; Mou *et al.* 2013b). Alkali has some negative impact on R-xylan and R-glucan, because the increase of Alkali may result in more degradation of carbohydrates, particularly for hemicellulose (*e.g.* xylan). In p2, T (pretreatment temperature) is influential and negatively related to R-glucan and R-xylan. This is because a higher temperature can result in more degradation of carbohydrates (Yu *et al.* 2013). Figure 1 also shows that Na_2SO_3/NaOH is positively correlated with R-glucan and Extractive, but it has less impact than T. In addition, factors of H time and L/S ratio are close to 0, which means these two factors have no clear relationship with other variables under the conditions studied and they are not well explained by the PCA model.

Analysis for the Overall PLS Model

When the pretreatment process parameters were set as X variables, and the variables of chemical composition of pretreated corn stover, Solid Yield, R-glucan, R-xylan, and D-lignin were set as Y variables, two principal components of PLS model 1 yielded a cumulated R^2 of 0.416 for X variables (R^2X(cum)), R^2 of 0.715 for Y variables (R^2Y(cum)), and Q^2 of 0.506 for prediction (Q^2(cum)). In other words, in the PLS model 1, these two principal components accounted for about 72% of the variation in the properties of pretreated corn stover, and about 51% of the predictive ability of the variation in the effects of alkaline sulfite pretreatment.

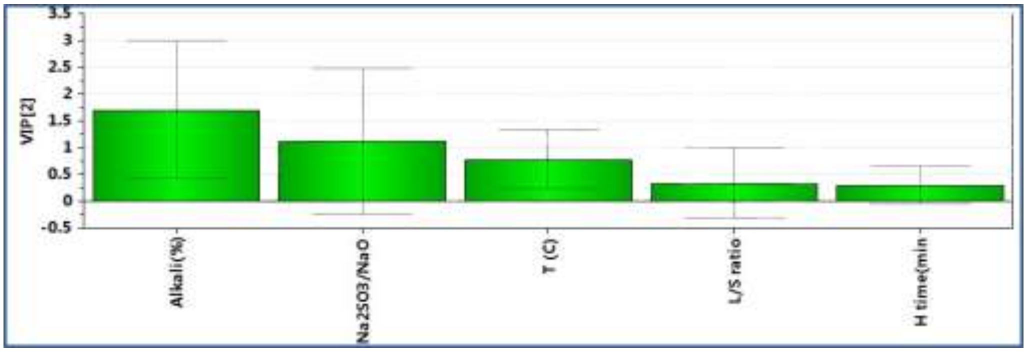

Fig. 2. Variable importance plot for PLS model 1 (X: pretreatment process parameters; Y: chemical composition variables of pretreated corn stover, Solid Yield, R-glucan, R-xylan, D-lignin)

Figure 2 is the variable importance plot (VIP), which presents the impact of all X variables on all Y variables simultaneously. It can be seen that the two most important variables were Alkali and Na_2SO_3/NaOH. Hence, the total alkali charge and the ratio of Na_2SO_3 to NaOH at the same total alkali charge played a more important role with respect to the properties of pretreated corn stover. More detailed information can be found in Fig. 3, which shows that p1 was dominated by Alkali. Alkali was positively related with Glucan, Xylan, and D-lignin, but negatively related to R-glucan, R-xylan, Solid Yield, Lignin, and Extractive. Therefore, under the conditions employed in this study, the higher the total alkali charge, the higher the glucan and xylan content could be obtained in the pretreated corn stover, and the more lignin and extractives could be removed. Stronger alkali can promote lignin removal, but it can also result in higher degradation of carbohydrates, thus lowering the solid yield (Li *et al.* 2012). For example, as shown in Table I in the appendix, for the sample No. 1 with Alkali of 6%, after pretreatment, the Glucan, Xylan, R-glucan, and R-xylan were 43.13%, 23.75%, 99.73%, and 97.08%, respectively. However, for the sample No. 4 with Alkali of 12%, after pretreatment, the Glucan, Xylan, R-glucan, and R-xylan were 55.74%, 25.1%, 98.64%, and 78.53%, respectively. T dominates in p2, and it is negatively correlated to R-glucan and R-xylan. This is because higher temperature can degrade more sugars (Sixta 2006; Yu *et al.* 2013). Hence, to retain more carbohydrates after pretreatment, the pretreatment temperature needs to be suitably decreased. It's better to be not higher than 140 ºC, as the random hydrolysis of carbohydrates will take place when the temperature is over 140 ºC under the alkaline conditions (Sixta 2006). In addition, Na_2SO_3/NaOH is positively related with R-glucan and R-xylan. This may be due to the fact that Na_2SO_3 can stabilize carbohydrates by oxidizing the reducing end groups (Sixta 2006). On the other hand, increasing the Na_2SO_3/NaOH at a given total alkali charge may lower the dosage of NaOH, thereby reducing the degradation of carbohydrates with the relatively mild alkaline condition, and decreasing delignification as well. Thus, Na_2SO_3/NaOH is somewhat negatively related to D-lignin in this study, despite the fact that the sulfite

(SO_3^{2-}) in pretreatment liquor can lead to sulfonation of lignin, enhancing its hydrophilicity (Zhu *et al.* 2009). The increase of hydrophilicity can make lignin easier to be washed out during washing process after pretreatment. Therefore, in this work, Alkali was a more influential factor than Na_2SO_3/NaOH, and the total alkali charge could be appropriately increased (may be no less than 11%) to achieve a satisfied pretreatment effect.

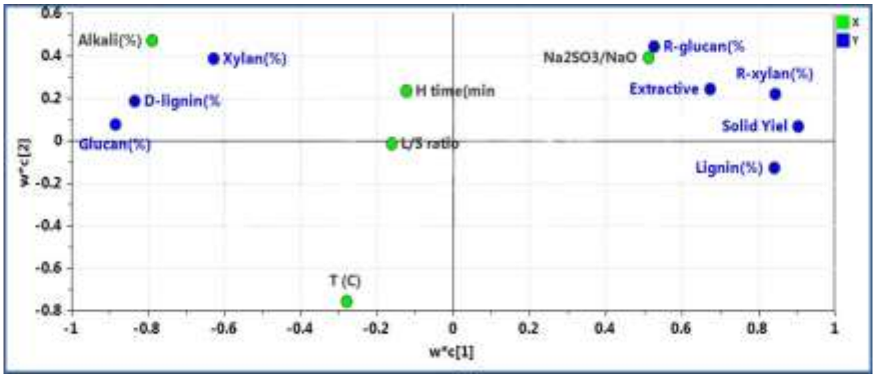

Fig. 3. Loading scatter plot for PLS model 1 (X: pretreatment process parameters; Y: chemical composition variables of pretreated corn stover, Solid Yield, R-glucan, R-xylan, D-lignin)

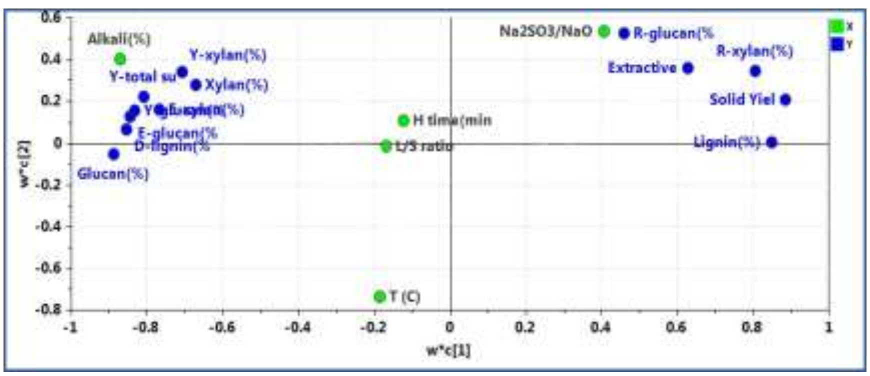

Fig. 4. Loading scatter plot for PLS model 2 (X: pretreatment process parameters; Y: chemical composition variables of pretreated corn stover, Solid Yield, R-glucan, R-xylan, D-lignin, E-glucan, E-xylan, Y-glucan, Y-xylan, Y-total sugar)

When the variables of E-glucan, E-xylan, Y-glucan, Y-xylan, and Y-total sugar were added in PLS model 1 and also set as Y variables, a new two principal components PLS model 2 could be obtained with R^2X(cum) of 0.412, R^2Y(cum) of 0.717, and Q^2(cum) of 0.466. The PLS model 2 has similar model structure with the PLS model 1, as shown in Fig. 4, but its performance is weaker. This may be due to the lack of some important information, which needs to be included in the model analysis in future as well. For instance, properties of raw material (*e.g.* particle size of corn stover) may affect both the process of treatment (*e.g.* energy consumption) and qualities of end product (Lanouette *et al.* 2004; Li *et al.* 2011).

To investigate the impact of pretreatment process variables and the properties of pretreated corn stover on enzymatic hydrolysis yield, the PLS model 3 was proposed by setting the pretreatment process variables (Alkali, T, H time, Na_2SO_3/NaOH, L/S ratio), the properties of pretreated corn stover (Glucan, Xylan, Extractive, Lignin), and pretreatment effect variables (Solid Yield, R-glucan, R-xylan, D-lignin) as X variables, while the factors of E-glucan, E-xylan, Y-glucan, Y-xylan, and Y-total sugar were set as Y variables. Two principal components can be obtained for the PLS model 3 with R^2X(cum) of 0.667, R^2Y(cum) of 0.787, and Q^2(cum) of 0.589. The corresponding VIP plot is exhibited in Fig. 5. As can be seen, the three most important variables were Alkali, D-lignin, and Glucan. The influence of other process parameters on enzymatic hydrolysis yield (such as Na_2SO_3/NaOH and T) was not as significant as total alkali charge. This is in good agreement with the results shown in Figs. 2, 3, and 4.

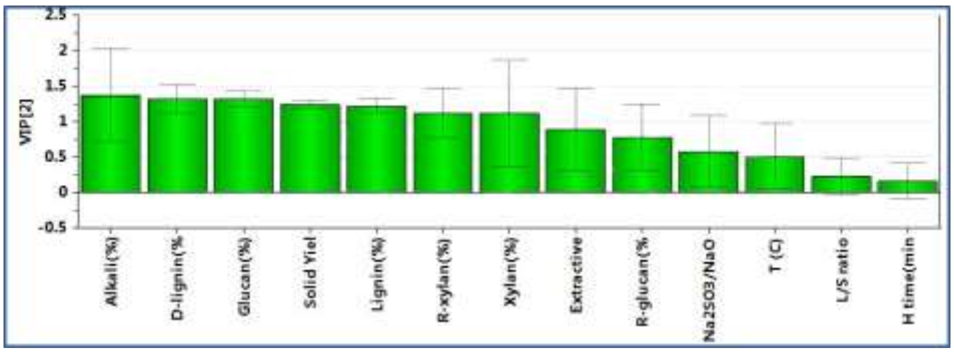

Fig. 5. Variable importance plot for PLS model 3 (X: pretreatment process parameters, chemical composition variables of pretreated corn stover, Solid Yield, R-glucan, R-xylan, D-lignin; Y: E-glucan, E-xylan, Y-glucan, Y-xylan, Y-total sugar)

Quantitative Analysis of the Impact of the Properties of Pretreated Corn Stover on Enzymatic Saccharification

In order to quantify the impact of the properties of pretreated corn stover and the pretreatment variables on the enzymatic hydrolysis yield, Glucan, Xylan, Lignin, Extractive, Solid Yield, R-glucan, R-xylan, and D-lignin were set as X variables, and the factors of E-glucan, E-xylan, Y-glucan, Y-xylan, and Y-total sugar were set as Y variables (pretreatment process parameters were not included). In this case, the PLS model 4 with two principal components was proposed, and it was able to account for about 89% of the variation of the properties of pretreated corn stover and the pretreatment effect (R^2X(cum)), 75% of the variation of the enzymatic hydrolysis effects (Y^2X(cum)) under the same hydrolysis conditions, as well as about 60% of the predictive ability (Q^2 (cum)) of the variation in the effects of enzymatic hydrolysis. Figure 6 is the corresponding VIP plot for the PLS model 4, which shows that D-lignin, Glucan, and Solid Yield (mainly inter-related to R-glucan, R-xylan, and D-lignin) played a more important role on enzymatic saccharification compared to other variables (*e.g.* Extractive). Usually, to achieve a high fermentable sugar yield in enzymatic hydrolysis, lignin should be removed sufficiently, while the loss of polysaccharides should be minimized as long as their degrees of polymerization (particularly for xylan) need to be reduced to the appropriate extent during the pretreatment process (Mosier *et al.* 2005; Leu and Zhu 2013).

The effects of all X variables on E-glucan and E-xylan are shown in Fig. 7(a) and Fig. 7(b), respectively. E-glucan was positively correlated with D-lignin, Glucan and Xylan, but negatively correlated with Lignin, Solid Yield, and R-xylan. Many studies have reported a strong positive relationship between the percentage of lignin removal and the yield of enzymatic saccharification (Agbor *et al.* 2011; Gu *et al.* 2012; Ding *et al.* 2012).

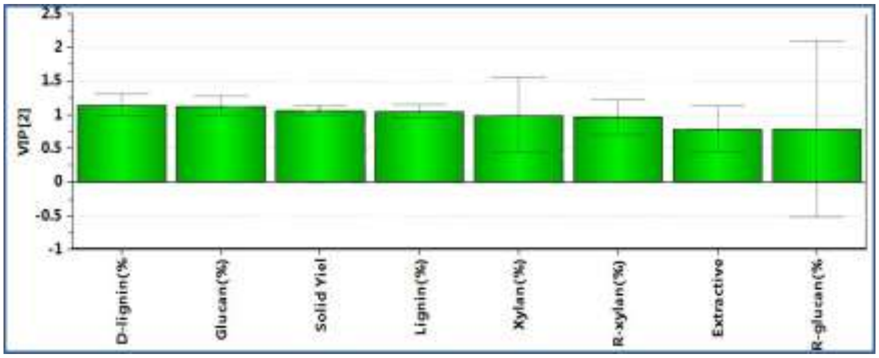

Fig. 6. Variable importance plot for PLS model 4 (X: Glucan, Xylan, Lignin, Extractive, Solid Yield, R-glucan, R-xylan, D-lignin; Y: E-glucan, E-xylan, Y-glucan, Y-xylan, Y-total sugar)

Solid Yield has some negative impact on E-glucan, which may be due to the insufficient lignin removal and low de-polymerization of polysaccharides (Leu and Zhu 2013). In addition, Extractive also has a slight negative impact on both E-glucan (Fig. 7(a)) and E-xylan (Fig. 7 (b)). But most of extractives can be removed after the alkaline sulfite pretreatment based on the data listed in the appendix (Table I). Appropriately increasing Alkali and T can enhance the removal of extractives, as both Alkali and T are negatively correlated with Extractives, as presented in Figs. 3 and 4.

For the final sugar yields (Y-glucan, Y-xylan, and Y-total sugar), the corresponding coefficient plots are given in Figs. 7(c), (d), and (e), respectively. Again, both the percentage of lignin removal (D-lignin) and the recovery rate of carbohydrates (particularly for R-glucan) play an important role on enzymatic saccharification.

Based on the analyses above, in order to achieve a satisfactory effect of the alkaline sulfite pretreatment for ameliorating enzymatic saccharification, Alkali can be properly increased (no less than 11%), while T should be suitably decreased (not higher than 140 ºC). As for the Na_2SO_3/NaOH, it should be appropriately adjusted. At a given total alkali charge, the addition of Na_2SO_3 in alkaline pretreatment can improve not only the recovery of carbohydrates, but also can promote lignin removal, thus enhancing the enzymatic hydrolysis yield (Franco *et al.* 2012; Li *et al.* 2012). According to the data listed in the appendix (Table I), it can be seen that, for the sample No. 22, at the total alkali charge of 12% with the Na_2SO_3/NaOH of 1.2, about 78% of final total sugar yields can be obtained, and this is about 14% higher than the sample No. 28 obtained from the pretreatment without addition of Na_2SO_3. Similar results were found in a previous study (Li *et al.* 2012). In addition, more intensive studies are underway by including more parameters (such as the properties of raw material) and more samples with reasonable experiment design.

On the other hand, it should be noted that multivariate analysis can also be used for other pretreatment systems. The biomass pretreatment process which is in common with many other industrial processes (*e.g.* pulp and papermaking), has high requirements for process stability (Lanouette *et al.* 2004; Li *et al.* 2011). Outcomes from pretreatment process are determined by two groups of variables: 1) those from feedstocks (*e.g.* species), and 2) those from pretreatment operation (*e.g.* chemical charge).

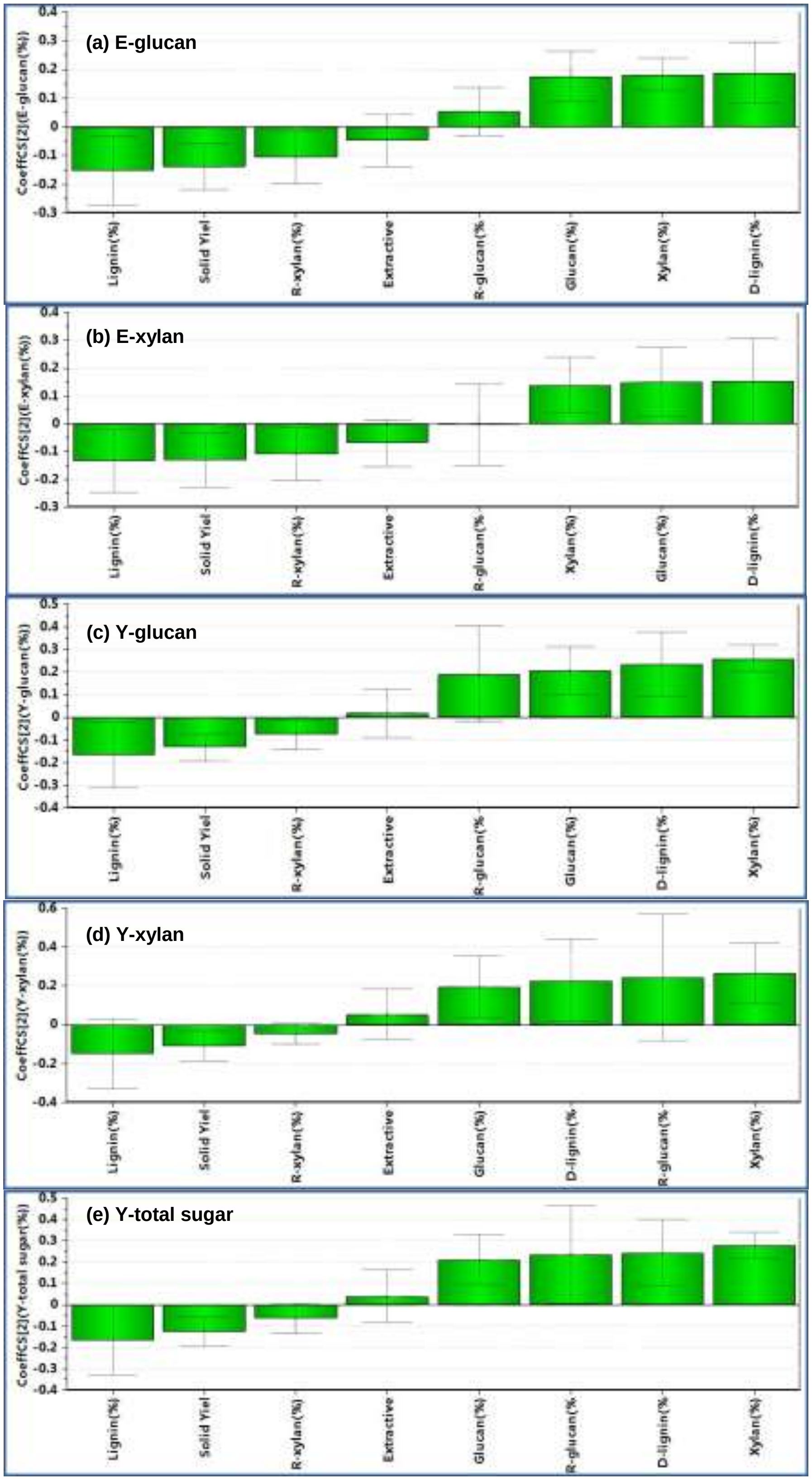

Fig. 7. PLS coefficient plots for E-glucan (a), E-xylan (b), Y-glucan (c), Y-xylan (d), and Y-total sugar (e)

By monitoring the properties of feedstock with online meters (developed based on multivariate analysis) (Ding *et al.* 2005, 2009), the operators can adjust the key process variables (*e.g.* energy consumption) in advance to minimize the process variations, thus getting the end products with uniform quality. Also, the process operators can quickly find solutions for troubleshooting on the basis of the model developed by multivariate analysis for a given process system (Browne *et al.* 2004). Therefore, multivariate analysis would be a useful approach to improve and sustain the pretreatment process in future commercial production.

CONCLUSIONS

1. The detailed relationships of key process variables of alkaline sulfite pretreatment of corn stover were characterized by the Principal Component Analysis (PCA) and Partial Least Square (PLS) methods. These two multivariate analysis methods give a good overview of the correlation of the variables in the pretreatment system and can be a useful tool to optimize the pretreatment conditions for the enhancement of enzymatic hydrolysis yield.
2. In this work, pretreatment temperature was negatively correlated with the yield of carbohydrates, while the Na_2SO_3/NaOH ratio was positively correlated with the yield of carbohydrates. Total alkali charge had a strongly positive impact on the percentage of delignification and final sugar yields, and it was more influential than the pretreatment temperature and the Na_2SO_3/NaOH ratio.
3. Based on the results from PCA and PLS methods, by suitably increasing the total alkali charge (no less than 11%), decreasing the pretreatment temperature (not higher than 140 ºC), and appropriately adjusting the Na_2SO_3/NaOH ratio at a given total alkali charge, satisfactory pretreatment effects (*e.g.* final sugar yields) could be achieved for the system of alkaline sulfite pretreatment.

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Appendix: Table I. Experimental Dataset

ID	Alkali (%)	L/S ratio	T (ºC)	H time (min)	Na_2SO_3/NaOH	Solid Yield (%)	Glucan (%)	Xylan (%)	Extra-ctive (%)	Lignin (%)	D-lignin (%)	R-glucan (%)	R-xylan (%)	E-glucan (%)	E-xylan (%)	Y-glucan (%)	Y-xylan (%)	Y-total sugar (%)
1	6	6	140	20	1	72.19	43.13	23.75	7.94	16.56	20.55	99.73	97.08	40.73	31.43	40.62	30.51	36.97
2	8	6	140	20	1	64.07	47.98	23.28	6.63	11.92	49.28	98.47	84.46	63.32	55.97	62.35	47.27	56.9
3	10	6	140	20	1	59.12	52.98	24	6.08	8.24	67.62	100.3	80.36	77.14	68.24	77.4	54.83	69.25
4	12	6	140	20	1	55.25	55.74	25.1	5.32	4.36	84.01	98.64	78.53	86.81	73.56	85.63	57.76	75.56
5	14	6	140	20	1	53.16	57.83	25.92	4.75	3.94	87.66	98.47	78.02	89.87	75.36	88.49	58.8	77.77
6	12	12	140	20	1	54.56	57.58	25.58	3.5	4.55	83.52	98.18	77.14	84.79	73.05	83.24	56.36	73.53
7	12	10	140	20	1	55.25	55.74	25.1	3.93	4.36	84.01	98.64	78.53	86.81	73.56	85.63	57.76	75.56
8	12	8	140	20	1	54.27	56.8	25.73	4.01	5.82	79.03	96.32	77.16	87.62	75.59	84.4	58.33	74.98
9	12	6	145	20	1	53.32	57.5	26.11	5.09	4.25	85.04	95.79	76.93	87.39	74.87	87.72	58.6	76.28
10	12	4	140	20	1	61.1	50.74	24.83	9.07	11.46	53.48	96.88	83.83	80.8	66.3	78.27	55.58	70.07
11	12	6	120	20	1	59.04	52.96	25.04	6.81	5.05	80.19	99.95	83.71	73.62	65.84	73.73	55.11	67.01
12	12	6	130	20	1	56.74	54.32	25.37	6.71	4.67	83.03	95.24	78.64	81.21	70.18	77.34	55.19	69.34
13	12	6	136	20	1	55.62	57.5	26.11	5.64	4.25	84.04	96.79	78.93	86.39	72.87	83.72	57.6	74.28
14	12	6	150	20	1	50.95	57.43	25.2	5.17	3.35	88.64	93.72	72.7	84.86	71.68	79.53	52.11	69.63
15	12	6	160	20	1	48.47	58.16	24.49	4.13	2.59	91.66	90.3	67.22	85.85	72.13	77.51	48.48	67.02
16	12	6	140	10	1	53.68	57.06	26.07	5.46	5.13	81.69	98.11	79.24	84.04	69.64	82.45	55.19	72.6
17	11	6	140	20	1	55.74	57.22	26.06	5.82	4.72	82.68	99.24	81.51	84.46	69.77	85.51	56.87	75.16
18	12	6	140	30	1	53.32	57.5	26.11	5.12	4.25	85.04	95.79	76.93	87.39	74.87	83.72	57.6	74.28
19	12	6	140	40	1	53.64	57.43	26.05	5.08	4.23	84.92	98.67	79.12	84.93	70.28	83.8	55.61	73.62
20	12	6	140	50	1	52.62	57.69	26.17	5.13	3.55	87.6	97.23	77.98	86.1	70.92	83.72	55.3	73.45
21	12	6	140	20	2	63.36	51.35	24.92	9.87	8.25	65.26	100.2	89.41	67.52	50.87	71.37	46.48	64.37
22	12	6	140	20	1.2	55.39	56.52	25.39	5.96	3.30	87.87	100.3	79.63	79.88	64.02	89.1	58.98	78.58
23	12	6	140	20	0.5	53.02	57.92	26.16	5.82	2.12	92.52	98.36	78.54	82.98	66.3	88.62	58.07	78.94
24	12	6	140	20	0.25	51.08	58.63	26.08	5.67	1.12	96.20	95.93	75.43	83.31	66.56	86.92	57.21	77.81
25	12	6	140	20	0.167	51.05	57.89	25.27	5.23	1.12	96.21	94.66	73.05	83.53	68.56	87.07	57.08	76.59
26	12	6	140	20	0.125	50.32	58.71	25.79	4.19	1.58	94.73	94.63	73.49	83.55	67.78	88.07	57.81	75.49
27	12	6	140	20	0.1	50.52	57.89	25.35	3.78	2.61	91.24	93.68	72.52	83.01	68.25	85.75	55.49	73.54
28	12	6	140	20	0	51.38	59.01	25.47	1.98	3.41	3.20	79.20	97.12	74.1	79.86	67.75	77.55	68.67

Notes: Alkali: total alkali charge (based on the oven-dried corn stover and calculated as NaOH); L/S ratio: liquid to solid ratio; T: the maximum pretreatment temperature; H time: holding time of pretreatment at the Max. temperature; Na_2SO_3/NaOH: the ratio of Na_2SO_3 to NaOH; Solid Yield: solid yield after pretreatment; Glucan: glucan content in pretreated corn stover; Xylan: xylan content in pretreated corn stover; Extractive: extractives content in pretreated corn stover; Lignin: lignin content in pretreated corn stover; D-lignin: delignification rate; R-glucan: recovery rate of glucan in pretreated corn stover; R-xylan: recovery rate of xylan in pretreated corn stover; E-glucan: enzymatic hydrolysis percentage of glucan; E-xylan: enzymatic hydrolysis percentage of xylan; Y-glucan: glucan yield after pretreatment and enzymatic hydrolysis; Y-xylan: xylan yield after pretreatment and enzymatic hydrolysis; Y-total sugar: final total sugar yields after pretreatment and enzymatic hydrolysis.

Calculations:

Solid Yield (%) = (Pretreated biomass (g) / Original biomass (g)) × 100%

R-glucan (%) = (Solid Yield × $C_{\text{glucan of pretreated biomass}}$) / $C_{\text{glucan of original biomass}}$ × 100%

R-xylan (%) = (Solid Yield × $C_{\text{xylan of pretreated biomass}}$) / $C_{\text{xylan of original biomass}}$ × 100%

D-lignin (%) = 1 – (Solid Yield × $C_{\text{lignin of pretreated biomass}}$) / $C_{\text{lignin of original biomass}}$ × 100%

E-glucan (%) = ($M_{\text{glucose in hydrolyzate}}$ × 0.9 / $M_{\text{glucan in pretreated biomass}}$) × 100%

E-xylan (%) = ($M_{\text{xylose in hydrolyzate}}$ × 0.88 / $M_{\text{xylan in pretreated biomass}}$) × 100%

Y-glucan (%) = ($M_{\text{glucose in hydrolyzate}}$ × 0.9 / $M_{\text{glucan in original biomass}}$) × 100%

Y-xylan (%) = ($M_{\text{xylose in hydrolyzate}}$ × 0.88 / $M_{\text{xylan in original biomass}}$) × 100%

Y-total sugar (%) = (($M_{\text{glucose in hydrolyzate}}$ × 0.9 + $M_{\text{xylose in hydrolyzate}}$ × 0.88) / $M_{\text{glucan plus xylan in original biomass}}$)) × 100%

Where, *C* is the content of the corresponding component in biomass (wt.%), and *M* is the mass of sugar (g).

Table II. Variables in Alkali Sulfite Pretreatment

Pretreatment parameters		Component content in pretreated corn stover		Pretreatment effectiveness variables		Enzymatic hydrolysis effect variables	
Variables	**Range**	**Variables**	**Range**	**Variables**	**Range**	**Variables**	**Range**
Alkali	6~14%	Glucan	43.13~59.01%	Solid Yield	48.47~72.19%	E-glucan	40.73~89.87%
L/S ratio	4~12	Xylan	23.28~26.17%	D-lignin	3.2~96.21%	E-xylan	31.43~79.86%
T	120~160ºC	Extractive	1.98~9.87%	R-glucan	79.2~100.3%	Y-glucan	40.62~89.1%
H time	10~50 min	Lignin	1.12~16.56%	R-xylan	67.22~97.12%	Y-xylan	30.51~77.55%
$Na_2SO_3/NaOH$	0~2	-	-	-	-	Y-total sugar	36.97~78.94%