Combined Severity during Pretreatment Chemical and Temperature on the Saccharification of Wheat Straw using Acids and Alkalis of Differing Strength

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Acids and alkalis are considered important catalysts in biomass pretreatment, which is essential to overcome the recalcitrance of lignocellulose for sugar release. In this study, the effects of various chemicals and temperatures on the pretreatment and subsequent enzymatic hydrolysis of wheat straw were investigated. The conversions of glucan and xylan during pretreatment and enzymatic hydrolysis were examined. The temperature and different ions in pretreatment govern the dissociation constant and hydrogen ion concentration. Due to higher dissociation at higher temperature, weak acids and weak alkalis can produce high glucose yields, similar to strong acids and alkalis. The concept of modified combined severity for weak acid pretreatment was explored. The pH value and real combined severity of weak acids at reaction temperatures were estimated according to xylan recovery during pretreatment. Glucose yield in enzymatic hydrolysis is mainly decided by xylan recovery for acidic pretreatment and by total content of xylan and acid-insoluble-lignin in solids for alkaline pretreatment.

Keywords: Wheat straw; Pretreatment; pH; Temperature; Enzymatic hydrolysis; Combined severity

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INTRODUCTION

Lignocellulosic biomass, due to its low cost and sustainability as a resource, has been considered one of the most promising feedstocks for the production of fuels, especially ethanol. The technology for the conversion of lignocellulosic biomass to ethanol has been under development for decades (Zhong *et al*. 2010). Wheat straw is an abundant by-product from wheat production and is a suitable type of lignocellulosic raw material for use as a feedstock. To make the conversion process efficient and economical, a variety of parameters and techniques need to be investigated, such as the storage (Liu *et al*. 2013a,b), pretreatment of wheat straw, enzymatic hydrolysis, and fermentation (Talebnia *et al.* 2010). Biomass pretreatment has been considered as one of the most crucial but expensive steps in the lignocellulose conversion process (Mosier *et al.* 2005). The goal of pretreatment is to increase the accessibility of cellulose and to release fermentable sugars as much as possible (Han *et al*. 2007).

The current hydrothermal pretreatment methods can be basically classified into two clusters: acidic pretreatment and alkaline pretreatment (Pedersen and Meyer 2010).

In acidic pretreatment, hydrogen ions mainly catalyze the hydrolysis of glycosidic bonds and monosaccharide dehydration, resulting in hemicellulose degradation and furan production (Hsu *et al*. 2010; Wei *et al*. 2012). Kabel *et al.* (2007) studied the combined severity of sulfuric acid pretreatment of wheat straw relative to the sugar release. Higher severity not only reduced more xylan, but also produced more furfural when using sulfuric acid. Some organic acids have been suggested as alternatives for sulfuric acid in the pretreatment, because they produce lower amounts of degradation products (such as furfural) and have less negative effect on subsequent fermentation (Lee *et al*. 2011; Qin *et al*. 2012). A previous study by the authors showed that the different acids influence the degradation of xylan due to the dissociation constant (K_d) of each acid (Qin *et al*. 2012). As known, the K_a value of weak acids is changed as temperature changes. In alkaline pretreatment, hydroxyl ions break the ester and ether bonds in lignin and the lignin-carbohydrate complex (Gupta and Lee 2010; Pedersen and Meyer 2010), allowing lignin and hemicellulose fragments to dissolve into the liquid fraction. Previous work suggests that a higher temperature during ammonia pretreatment can lead to higher enzymatic conversion of pretreated solid (Qin *et al*. 2013). Similarly, the dissociation constant of many alkali sources also changes as the temperature changes.

A variety of parameters have been optimized for different pretreatment methods and technologies, and it has been observed that temperature and pH play the most important roles in influencing sugar yield among several pretreatment conditions (Mosier *et al*. 2005; Pedersen and Meyer 2010; Li *et al*. 2010). It is believed that accessibility of cellulose decides the enzymatic yield of biomass, which involves crystallinity, degree of polymerization, hemicellulose content, lignin content, specific surface area, pore size, and particle size (Zhao *et al.* 2012). Out of this list, hemicellulose and lignin content have been found to play leading roles relative to other parameters (Adani *et al*. 2010). How the combined effect of changed temperatures and changed ionic concentrations in pretreatment affect the content of hemicellulose and lignin, and the saccharification during pretreatment and enzymatic hydrolysis needs to be studied. In addition, many comparative studies have focused on the specific efficient pretreatment method or several pretreatments with each representative condition (Hsu *et al.* 2010; Kumar *et al.* 2009), which are not global comparison. A systematic study of the effects and the interactions of different chemicals and temperatures on pretreatment will give more information about the minor chemical species present and may aid in the improvement of lignocellulosic conversion processes.

EXPERIMENTAL

Materials

Wheat straw was obtained from the suburb of Liaocheng, Shandong, China. The wheat straw was air-dried until the moisture content was no more than 10% based on the total weight. The knife-milled wheat straw was screened using 20 and 80 mesh screens to collect particles within the size range of 0.2 to 0.9 mm. These were stored in sealed polyethylene plastic bags at room temperature.

Accellerase 1500 was a gift from Genecor (Suzhou, China). Novozyme 188 was purchased from Sigma-Aldrich (St. Louis, MO). The cellulase activity of Accellerase 1500 was 77 FPU (filter paper units)/mL; the β-glucosidase activity of Novozyme 188 was 250 *p*NPGU (p-nitrophenyl-β-D-galactopyranoside units)/mL.

Pretreatment

Five acids (sulfuric, oxalic, tartaric, citric, and acetic acids) and five alkalis (sodium carbonate, calcium hydroxide, sodium hydroxide, ammonia, and methylamine) were used in the pretreatment of wheat straw. All chemical concentrations were 90 mM based on the total reaction volume by molecular weight, except for the ammonia and methylamine concentrations, which were each 15% (w/v). The pH of each solution was listed in Table 1. At first, 15 g of milled wheat straw (dry matter) was mixed with the chemical solution to reach a final weight of 150 g. The mixture was then transferred to a 316 stainless steel reactor and heated in an oil bath as previously described (Qin *et al*. 2012). The reactor was plunged into the preheated oil at the desired temperature. After 30 min, the reactor was cooled to room temperature by quenching in ice water. The pretreated slurry was pressed through filter cloth to separate the free liquid from the solid. Then, the liquid stream was collected for component analysis. The solid fraction was washed with ~750 mL of deionized water and dried at room temperature. The stream used to wash the solid fraction was also separated by filtration and collected for characterization.

Table 1. Solution pH (20 °C)

Solution	pH at 20 °C
sulfuric acid (90 mM)	1.2
oxalic acid (90 mM)	1.6
tartaric acid (90 mM)	2.6
citric acid (90 mM)	2.8
acetic acid (90 mM)	4.1
water	6.0
sodium carbonate (90 mM)	11.2
calcium hydroxide (90 mM)	12.6
sodium hydroxide (90 mM)	12.7
ammonia (15%,w/w)	13.0
methylamine (15%, w/w)	13.5

Enzymatic Hydrolysis

The substrates were hydrolyzed at a glucan loading of 1% (w/v) in a 50 mM citrate buffer solution (pH=4.8) at a cellulase loading of 15 FPU/g glucan and at a *β*-glucosidase loading of 64 *p*NPGU/g glucan in 100-mL Erlenmeyer flasks. Sodium azide (0.04%) was added to the mixture to inhibit microbial contamination during hydrolysis. Flasks were shaken in an incubator at 200 rpm for 168 h at 50 °C. At the end of the reaction, the liquid fractions were collected for sugar analysis.

Analytical Methods

The National Renewable Energy Laboratory (NREL) laboratory analysis protocol (LAP) was followed to determine the composition of the biomass. The glucose, xylose, and furfural concentrations were analyzed *via* high performance liquid chromatography (HPLC) using an Aminex 87H column (Bio-Rad, Hercules, CA).

The composition of the untreated wheat straw used in this study was as follows: glucan, 37.1%; xylan, 19.8%; arabinan, 2.9%; acid-insoluble lignin, 12.8%; acetyl, 2.5%; ash, 5.9%; water extractives, 15.1%; and ethanol extractives, 2.4%.

Glucan/xylan/AIL (acid-insoluble lignin) recovery was defined as:

Glucan/xylan/AIL recovery = (glucan/xylan/AIL determined in pretreated solids) / (glucan/xylan/AIL in untreated biomass) (1)

Glucose/xylose yield for acidic pretreatment was defined as:

Glucose yield = (glucose in enzymatic hydrolysate + glucose in pretreated liquid) ×0.9 / (glucan in untreated biomass) (2)

Xylose yield = (xylose in enzymatic hydrolysate + xylose in pretreated liquid) ×0.88 / (xylan in untreated biomass) (3)

Glucose/xylose yield for alkaline pretreatment was defined as:

Glucose yield = (glucose in enzymatic hydrolysate) ×0.9 / (glucan in raw biomass) (4)

Xylose yield = (xylose in enzymatic hydrolysate) ×0.88 / (xylan in raw biomass) (5)

RESULTS AND DISCUSSION

Compositions of Pretreated Solids

The compositions of the pretreated wheat straw following different chemical pretreatments are listed in Table 2. Compared with those of the untreated wheat straw, following each acidic pretreatment, the glucan and AIL contents of the pretreated solids noticeably increased, while after each alkali pretreatment, the glucan and xylan contents of the pretreated solids increased. Meanwhile, as temperature increased, the contents of xylan decreased and AIL increased. It was noticeable that the changed trend of glucan content as temperature increased was not clear due to the varied removal of xylan and AIL.

The pattern was more obvious by considering the glucan, xylan, and AILS recovery of pretreated solids rather than their compositions, as shown in Fig. 1. The glucan recovery was above 80% and decreased slightly as the temperature increased under most conditions, except for a significant decrease when the pretreatment involved sulfuric acid at 180 to 200 °C (Fig. 1A). The loss of glucan varied from 4% to 13% after alkali pretreatment and from 2% to 70% after acid pretreatment. The results implied that acid pretreatment degraded glucan more than alkali pretreatment at the same temperature. Xylan recovery was noticeably reduced as the temperature increased in pretreatment with any chemical (Fig. 1B). Xylan recovery varied from 50% to 92% after alkali pretreatment and from 0% to 90% after acid pretreatment at different temperatures. Xylan was almost completely removed after pretreatment with sulfuric and oxalic acid at temperatures above 180 °C. Acidic pretreatment reduced the xylan recovery significantly in comparison to alkali pretreatment at the same temperature.

Table 2. Composition of Pretreated Solids *

Catalysts	Temperature (°C)	Glucan	Xylan	AIL
Sulfuric acid	140	55.1 ± 1.1%	9.6 ± 0.4%	27.1 ± 1.3%
	160	55.9 ± 0.6%	6.6 ± 0.6%	29.7 ± 1.6%
	180	55.0 ± 1.2%	0.8 ± 0.1%	34.5 ± 1.1%
	200	30.3 ± 0.8%	0.0 ± 0.0%	58.1 ± 1.8%
Oxalic acid	140	48.7 ± 0.8%	15.5 ± 0.7%	22.7 ± 0.8%
	160	54.2 ± 0.3%	7.0 ± 0.5%	29.0 ± 0.9%
	180	56.6 ± 1.0%	3.5 ± 0.5%	29.6 ± 1.3%
	200	54.6 ± 0.7%	1.4 ± 0.1%	33.9 ± 1.2%
Tartaric acid	140	43.5 ± 1.3%	21.4 ± 0.8%	20.4 ± 1.0%
	160	51.1 ± 0.9%	13.9 ± 1.2%	23.5 ± 0.7%
	180	57.4 ± 1.0%	9.5 ± 0.4%	28.8 ± 1.6%
	200	55.5 ± 1.2%	3.2 ± 0.3%	34.3 ± 1.9%
Citric acid	140	44.4 ± 0.8%	19.4 ± 0.2%	21.3 ± 1.1%
	160	51.5 ± 1.4%	13.5 ± 0.9%	23.9 ± 1.3%
	180	58.6 ± 1.4%	7.7 ± 0.4%	28.6 ± 0.9%
	200	54.5 ± 1.0%	2.2 ± 0.3%	35.6 ± 1.4%
Acetic acid	140	40.0 ± 0.8%	21.1 ± 0.5%	18.9 ± 0.7%
	160	43.3 ± 0.7%	18.4 ± 0.4%	19.0 ± 1.0%
	180	51.7 ± 0.9%	12.8 ± 0.5%	23.8 ± 0.5%
	200	57.4 ± 0.5%	2.5 ± 0.2%	30.1 ± 1.2%
Water	140	58.0 ± 1.0%	29.3 ± 0.6%	19.7 ± 0.9%
	160	50.3 ± 0.5%	25.1 ± 0.8%	17.8 ± 1.1%
	180	46.5 ± 0.7%	20.7 ± 0.6%	21.1 ± 0.8%
	200	56.3 ± 1.5%	4.7 ± 0.5%	30.2 ± 2.0%
Sodium carbonate	140	57.4 ± 1.1%	29.0 ± 1.2%	12.8 ± 0.8%
	160	57.6 ± 0.9%	29.3 ± 0.9%	12.0 ± 0.6%
	180	45.1 ± 1.1%	24.7± 0.6%	19.0 ± 0.3%
	200	52.0 ± 1.3%	16.0± 0.7%	20.0 ± 0.7%
Calcium hydroxide	140	56.7 ± 0.6%	28.0 ± 0.4%	13.8 ± 0.8%
	160	52.2 ± 0.5%	24.7 ± 0.4%	13.8 ± 1.0%
	180	45.3 ± 1.0%	21.0 ± 0.7%	20.2 ± 1.5%
	200	49.0 ± 1.2%	13.9 ± 1.0%	22.5 ± 1.1%
Sodium hydroxide	140	57.4 ± 0.7%	25.0 ± 0.5%	2.7 ± 0.3%
	160	65.5 ± 1.0%	27.8 ± 0.9%	2.9 ± 0.5%
	180	47.8 ± 0.8%	21.1 ± 0.6%	8.2 ± 0.8%
	200	51.8 ± 1.1%	18.6 ± 1.1%	9.2 ± 0.5%
Ammonia	140	57.7 ± 0.2%	27.6 ± 0.3%	3.9 ± 0.7%
	160	65.6 ± 1.5%	24.5 ± 1.2%	3.9 ± 0.6%
	180	60.3 ± 0.8%	22.2 ± 0.7%	6.5 ± 0.2%
	200	61.4 ± 1.2%	20.2 ± 0.7%	5.8 ± 0.7%
Methylamine	140	57.4 ± 0.4%	26.3 ± 0.3%	3.3 ± 0.2%
	160	71.1 ± 1.4%	24.3 ± 0.9%	3.9 ± 0.4%
	180	61.0 ± 1.0%	20.4 ± 0.7%	6.0 ± 0.5%
	200	63.0 ± 1.1%	20.0 ± 0.6%	6.0 ± 0.6%

* All data in this table are mean values of duplicate experiments.

AIL recovery was generally increased as temperature increased (Fig. 1C). AIL recovery was above 100% under acidic conditions and was even higher when the temperature reached 200 °C. This high AIL recovery was attributed to the formation of lignin-like compounds (“pseudo-lignin”) under the acidic conditions from re-condensation reactions, especially those between carbohydrates or carbohydrate degradation products and other components from the water extractives (Sannigrahi *et al*. 2011). However, less than 50% AIL recovery was observed for pretreatments with sodium hydroxide, ammonia, and methylamine, a trend that increased as temperature increased. Sodium carbonate and calcium hydroxide resulted in much less AIL removal than did the other alkalis.

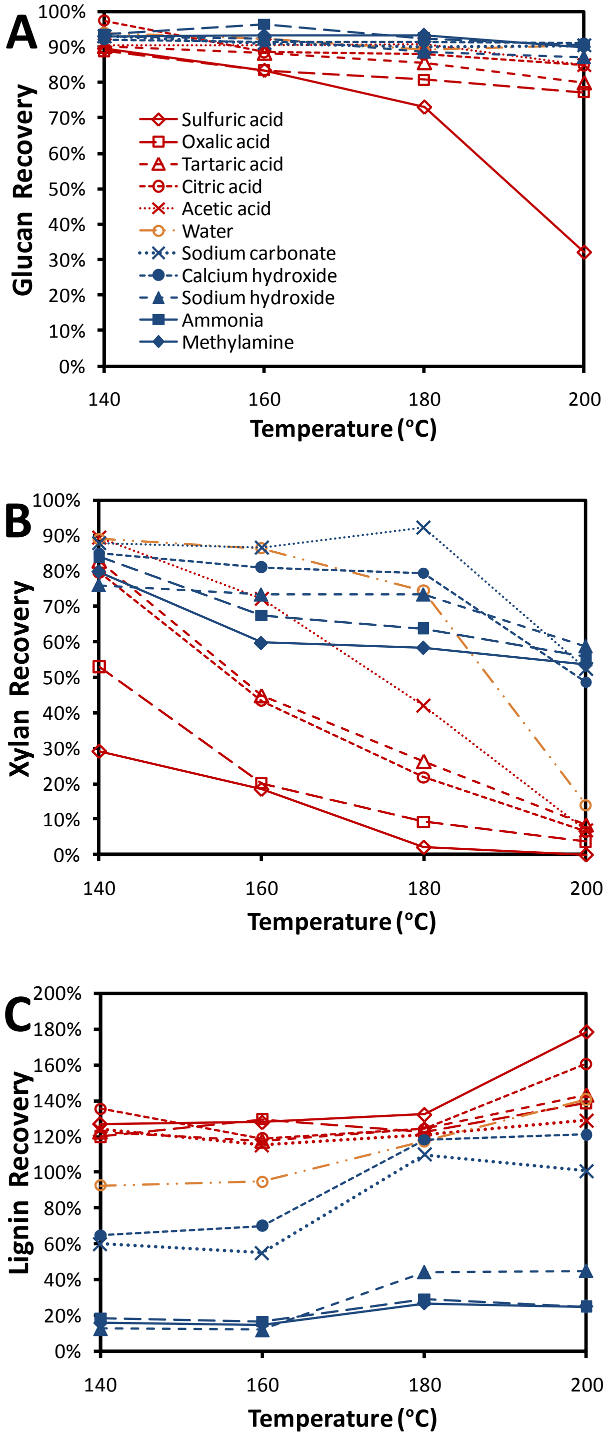

Fig. 1. Glucan recovery (A), xylan recovery (B), and AIL recovery (C) after pretreatments with different chemicals and temperatures. Values are the means of duplicate experiments (standard error＜10%).

Components in Pretreated Liquid Fraction

The liquid fraction generated from acidic pretreatment contains considerable amounts of fermentable sugars while also containing other compounds such as HMF (hydroxymethylfurfural) and furfural (Fig. 2). It was found that sulfuric acid produced more furfural and HMF than weak acids, in agreement with previous reports (Qin *et al*. 2012). Furfural, which is a by-product of the dehydration of xylose, has been considered one of the main inhibitors in the following fermentation (Li and Yuan 2010). The main inhibitors produced in pretreatment are furans and acids (Ding *et al*. 2012; Wang *et al*. 2013), and the yields of these inhibitors were much lower in alkaline pretreatment than in acidic pretreatment (Chundawat *et al*. 2010).

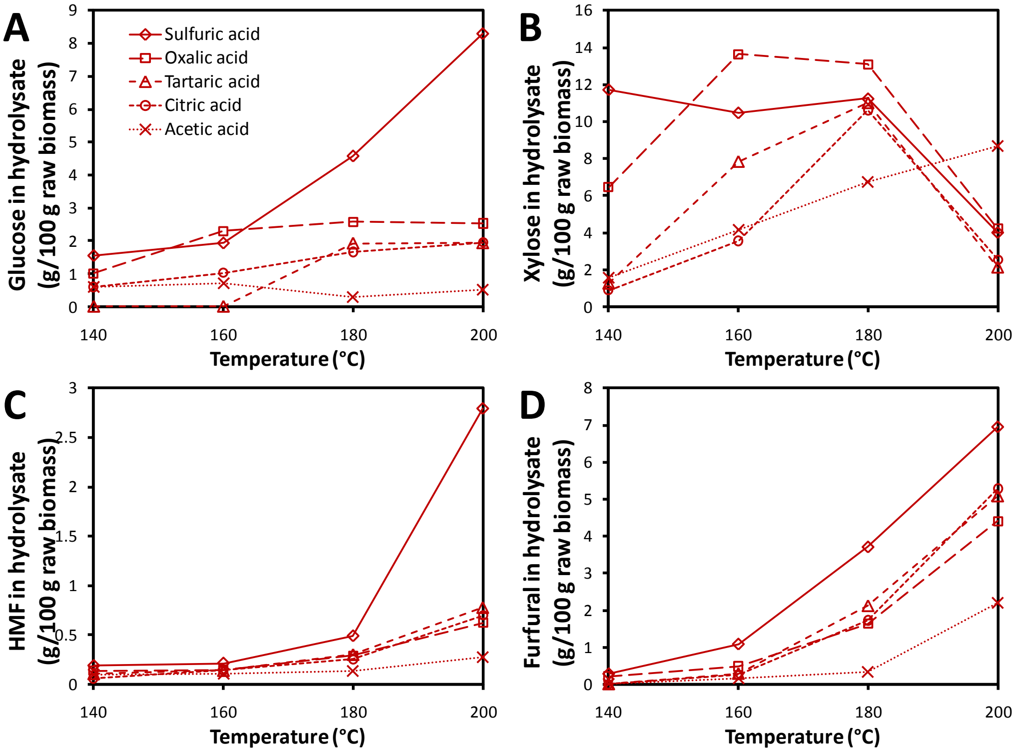

Fig. 2. Glucose (A), xylose (B), HMF (C), and furfural (D) amounts determined in pretreated liquid with different chemicals and at different temperatures. Values are the means of duplicate experiments (standard error＜10%).

Sugar Yield after Pretreatment and Enzymatic Hydrolysis

The pretreated solids were hydrolyzed at a glucan loading of 1% for 168 h with cellulase and β-glucosidase loadings of 15 FPU and 64 *p*NPGU /g glucan, respectively. The total sugars derived from pretreatment and the enzymatic hydrolysis process were used to calculate the total glucose and xylose yields in accordance with Eqs. 2 through 5. As shown in Fig. 2, high temperatures generally resulted in high glucose yields, except for the sulfuric acid pretreatment at 200 °C. High glucose yields (above 85%) were achieved for solids pretreated with oxalic acid, tartaric acid, citric acid, and liquid hot water at 200 °C, and sodium hydroxide, ammonia, and methylamine above 160 °C (Fig. 2A). At each temperature, the glucose yield was increased as the acidity or alkalinity increased. However, this trend did not apply to the pretreatment at 200 °C. The optimal conditions for xylose yield were alternative to glucose yield (Fig. 2B). High xylose yields were achieved after pretreatment with sulfuric acid at 140 °C, oxalic acid at 160 °C, and tartaric and citric acids at 180 °C. For alkaline-pretreated solids, the xylose yield was less affected by temperature. Sodium hydroxide and ammonia resulted in higher xylose yields than did the other alkalis.

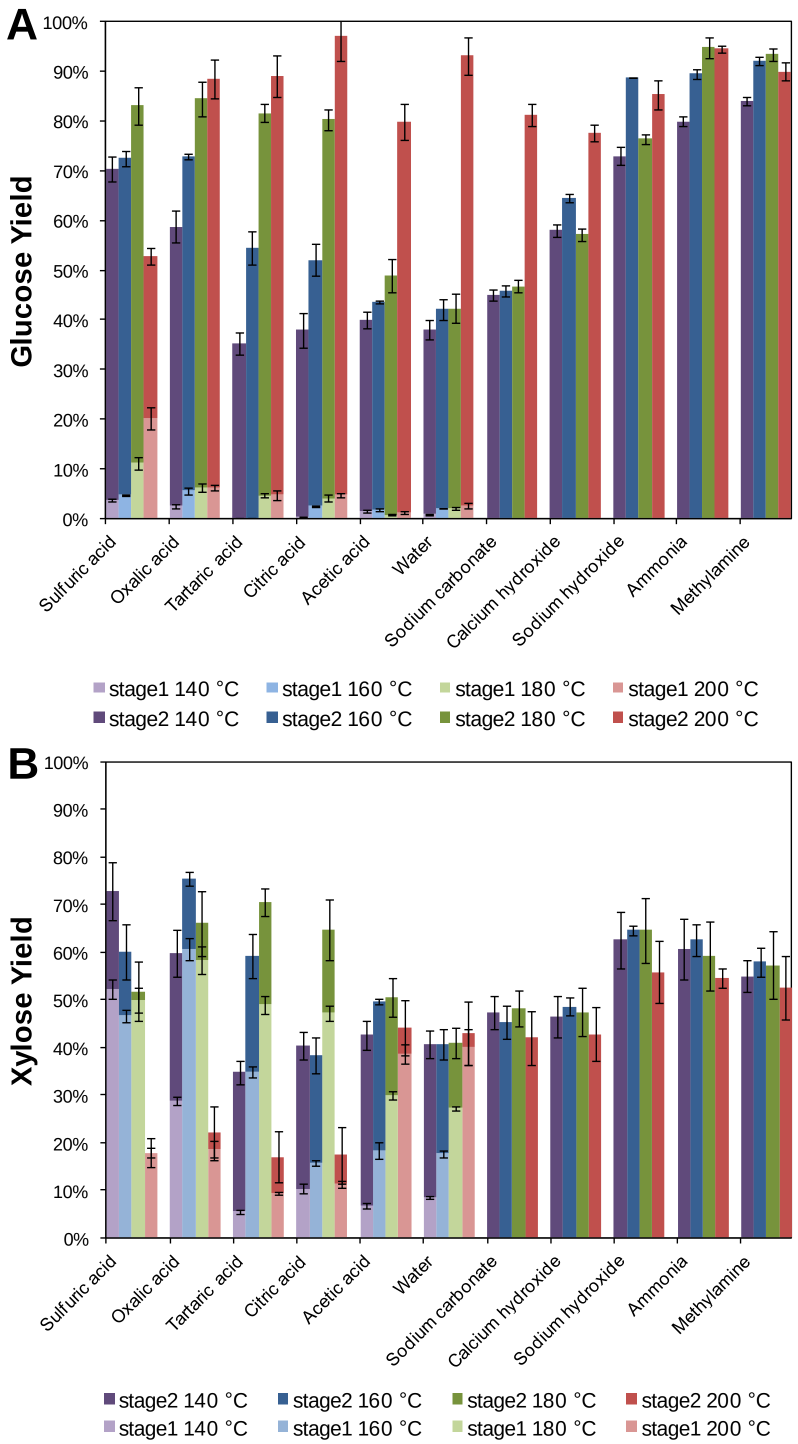

Fig. 3. Glucose yield (A) and xylose yield (B). Values are the means of duplicate experiments. Stage1 stands for pretreatment process in which sugar yield is calculated based on raw material. Stage2 stands for enzymatic hydrolysis process in which sugar yield is calculated based on raw material.

Combined Severity for Weak Acid Pretreatment

The net effect of temperature and chemical on the biomass can be attributed to the effect on the ion concentrations, especially the hydrogen ion and hydroxyl ion concentrations (pH and pOH), as well as their subsequent effects on the lignocellulose. When the chemicals are changed, the dissociation constant changes, leading to the different pH values. However, when temperature increases, the dissociation constant of specific ionic compounds increase, leading to the hydrogen ion and hydroxyl ion concentrations to increase in weak acid and weak alkali solutions. In addition, the dissociation constant of water in these solutions also increases, resulting in a decrease in the pH of the alkaline solution. For example, the pH of 90 mM sulfuric acid is 1.2 at 20 °C, and it remains constant with increasing temperature. The pH of weak acid solutions decrease in varying degrees and approach the values associated with strong acids as temperature is increased. The dissociation degree of dicarboxylic acids (tartaric acid and oxalic acid) and triprotic acid (citric acid) are enhanced as temperature is increased. For alkalis, pH values were decreased (*e.g.* pH of sodium hydroxide solution decreases from 12.7 to 11.9 as temperatures were raised from 20 °C to 50 °C). Calcium hydroxide is a special case because its solubility dramatically decreases as temperature increases, implying that it is not suitable for use at high temperatures.

Moreover, the effect of temperature itself cannot be overlooked. High temperature means that more of the molecules will get close to the activation energy, resulting in the transformation of the cellulose crystal form (Horii *et al*. 1987). High temperature combined with high hydrogen ion concentration (high combined severity) can even break down the cellulose and hemicellulose structures, as observed in the case of sulfuric acid pretreatment at 200 °C. It was found that weak acids and water pretreatment with high temperature led to higher glucose yield than sulfuric acid (Fig. 3), which reflected the advance of combination of weak acid and high temperature. High temperature also reduces the chemical dosage to achieve high saccharification yield.

As temperature was increased, xylan recovery was significantly decreased for both sulfuric acid and weak acids. Xylan recovery was decreased to a greater extent in the case of weak acids due to the increased hydrogen concentration. Xylan recovery was slightly decreased for alkalis due to the greater dissociation of water. Hydroxyl ion concentration also affected xylan removal by hydrolyzing the bonds between lignin and carbohydrate. It was noticeable that increasing temperature was no help for the removal of lignin. On the contrary, higher temperature increased the apparent amount of “lignin” in pretreated solids (Fig. 1C). In brief, the acidity and temperature of pretreatment determined the loss of xylan and recovery of AIL in any pretreated solid. As a result, it is assumed that xylan recovery has a relationship with combined severity of pretreatment (Silverstein *et al*. 2007), *i.e.* a relationship that involves a combination of pretreatment temperature, time, and pH value.

Overend and Chornet (1987) initially defined this severity parameter to relate temperature and time for steam explosion pretreatment based on the assumption that the effects of pretreatment follow first-order kinetics and obey the Arrhenius equation. Using this relationship they defined a reaction ordinate (R_0, min),

$$R_0 = t \times \exp\left[(T_H - T_R) / 14.75\right] \qquad (6)$$

where t is the residence time (min), T_H is the reaction temperature (°C), T_R is the base temperature (100 °C), and 14.75 is the conventional energy of activation assuming the overall reaction is hydrolytic and the overall conversion is first order. The logarithm of the reaction ordinate defines the severity during steam explosion pretreatment such that severity is equal to log R_0.

A modified severity parameter was later developed by Chum *et al*. (1990) for use with sulfuric acid pretreatment,

$$\text{Combined severity (CS)} = \log M_0 = \log R_0 - pH \tag{7}$$

It can be shown that Eqs. 7 and 8 are not suitable for weak acids, because the pH value under such high temperature is very hard to be determined. If the pH at room temperature in Table 1 is used to calculate CS (apparent combined severity, CS_a), then the relationship between xylan recovery during pretreatment and CS_a can be depicted as shown in Fig. 4. The parameter CS_a was found to have no comparability among different acids due to the drift of pH value, which was also found in previous work (Qin *et al*. 2012). As weak acids are finding increasing study in lignocellulose pretreatment (Lee *et al*. 2011; Scordia *et al*. 2011), the need for discussion of its pretreatment severity is increasing.

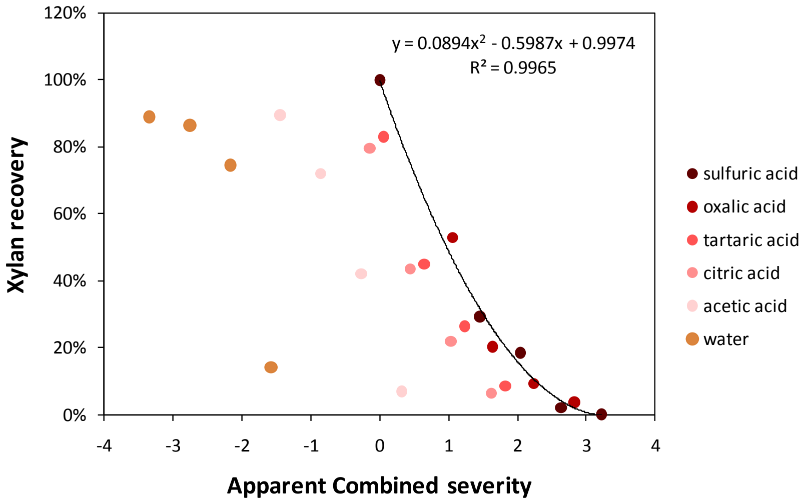

Fig. 4. Relationship between xylan recovery during acid pretreatment and apparent combined severity

In an attempt to improve upon the model based on room temperature pH values, the next step was to explore the real CS for weak acids. To estimate the real pH value at reaction temperature, a modified combined severity (CS_m) is substituted for CS_a based on the following assumption: 1, the real combined severity should be a function of xylan recovery in spite of the type of acid; 2, the pH value of sulfuric acid is kept constant; and 3, the real pH at the reaction temperature of weak acid should decrease, following the relationship {pH (20 °C) – δ} ($\delta \geqslant 0$).

When the proposed model was employed, a quadratic functional relationship between xylan recovery of sulfuric pretreatment and combined severity (Fig. 4) was found to have a good fit to the data points (R^2=0.9965). According to the first assumption, CS_m should be transformed to make every data point for all pretreatments conform to the regression of sulfuric acid. By adjusting the value of δ, CS_m of each acid and each temperature was achieved. Figure 5 shows the relation between xylan recovery and CS_m

of different acid pretreatments. Table 3 shows the estimated pH values. The estimated pH values were in the range of 1.6 to 3, which are much lower than that at 20 °C.

This method is practicable because the assumption of xylan degradation during acid pretreatment depending on hydrogen ion concentration is reasonable. Parameter CS includes all the factors influencing xylan degradation (temperature, time, and hydrogen ion concentration). Nevertheless, this method has difficulty in estimating the reaction pH of alkaline pretreatment, because the degradation reactions of xylan and lignin both are related to the hydrogen ion and hydroxyl ion concentrations.

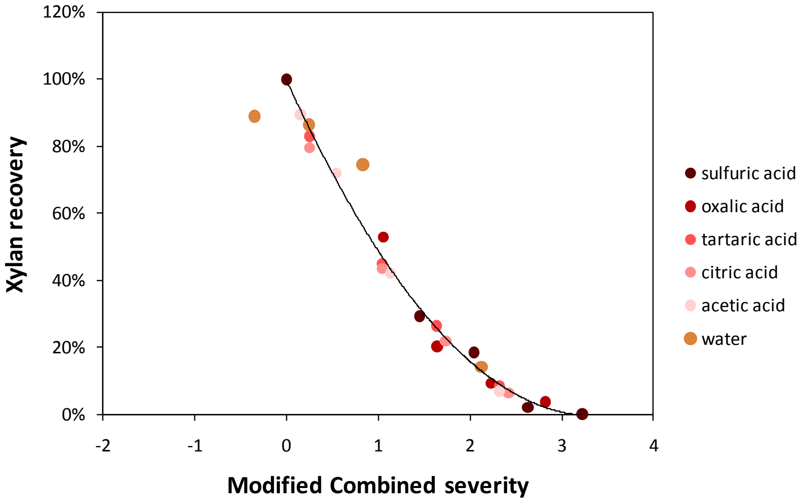

Fig. 5. Relationship between xylan recovery during acid pretreatment and modified combined severity.

Table 3. Estimated pH Values of Various Acids at Reaction Temperatures.

Solutions	140 °C	160 °C	180 °C	200 °C
oxalic acid (90 mM)	1.6	1.6	1.6	1.6
tartaric acid (90 mM)	2.4	2.2	2.2	2.1
citric acid (90 mM)	2.4	2.2	2.1	2.0
acetic acid (90 mM)	2.7	2.7	2.7	2.1
water	3	3	3	2.3

Relationship between Glucose Yield and Solid Composition

It is believed that hemicellulose and lignin had general and significant effects on the enzymatic hydrolysis of lignocellulosic biomass (Zhao *et al*. 2012), although biomass after some particular pretreatments can achieve high enzymatic yield by increasing cellulose surface area but not by removing hemicellulose or lignin (Rollin *et al*. 2011). Among those parameters associated with pretreatment, xylan recovery was best-related to glucose yield for acid pretreatments (Fig.6, R^2=0.95), and total amount of xylan and AIL in pretreated solids was best-related to glucose yield for alkaline pretreatments (Fig.7, R^2=0.82). These relationships reflect the different main governing parameters for the enzymatic hydrolysis of acid *vs*. alkali pretreated solids.

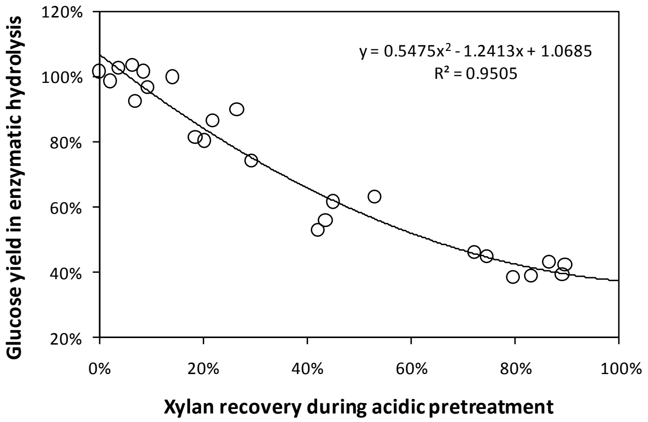

Fig. 6. Relationship between glucose yield in enzymatic hydrolysis process and xylan recovery during acidic pretreatment

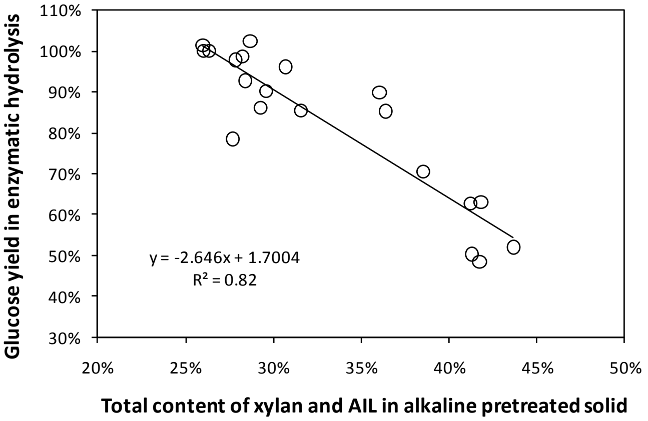

Fig. 7. Relationship between glucose yield in enzymatic hydrolysis process and the total amount of xylan and AIL in alkaline pretreated solids

As a result, temperature and different ions control the enzymatic yield indirectly by means of changing the hydrogen ion concentration and successively affect the xylan and lignin contents. The results of this study showed that a series of weak acids and alkalis, such as citric acid and methylamine, could also be appropriate for achieving a high sugar yield. However, from an economic point of view, one pretreatment using sulfuric acid, hot water, or lime (calcium hydroxide) is still the best. If the chemical agent used in pretreatment could be efficiently recovered, then weak acids and weak alkalis could have great potential for development in this type of application.

CONCLUSIONS

1. Chemical and temperature together govern the hydrogen ion concentration. The combined severity calculated using the pH measured at room temperature for a weak acid is defective in reflecting the severity of pretreatment. Therefore, a modified combined severity, as demonstrated in this work, needs to be applied.

2. The pH value and real combined severity of weak acids at reaction temperatures were estimated according to xylan recovery during pretreatment. The estimated pH values of weak acids were in the range of 1.6 to 3.
3. Glucose yield in enzymatic hydrolysis is mainly affected by xylan recovery for acidic pretreatment and by total content of xylan and AIL in solids for alkaline pretreatment.

ACKNOWLEDGMENTS

This work was financially supported by the National Natural Science Foundation of China (Major International Joint Research Project: 21020102040) and the International Joint Research Project of Tianjin (11ZCGHHZ00500).

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Article submitted: August 12, 2013; Peer review completed: September 11, 2013; Revised version received: September 23, 2013; Second revised version received: October 22, 2013; Accepted: October 25, 2013; Published: November 4, 2013.