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Dry pretreatment of lignocellulose with extremely low steam and water usage for bioethanol production

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ABSTRACT

Two rarely noticed but important parameters of the dilute sulfuric acid pretreatment of lignocellulose biomass, the feedstock filling ratio to the pretreatment reactor and the solids/liquid presoaking ratio, were extensively studied. The effects of the two parameters on the steam consumption, waste water generation, and pretreatment efficiency were investigated. At the full filling ratio and high solids/liquid presoaking ratio, this "dry" pretreatment method provided at least the following advantages: (1) the steam consumption was significantly reduced; (2) no aqueous acid containing waste water was generated; (3) high solids content of the pretreated materials were obtained and the consequent saccharification and fermentation was carried out at high solids loading easily. This method was applied to various lignocellulose feedstocks successfully and provided a practical means to produce ethanol economically feasible.

1. Introduction

Pretreatment is a requisite step in overcoming biorecalcitrance of lignocellulose for production of bioethanol and is considered as the core of the lignocellulose bioprocessing because its outcome gives significant impacts on the overall conversion scheme such as size reduction, hydrolysis, fermentation, product recovery, residue processing, and co-product potentials (Yang and Wyman, 2008). Different pretreatment methods have been investigated including chemical, physical, thermal, and biological approaches, but only those employ chemicals currently offer the high yields and low costs vital to economic success (Wyman et al., 2005; Zhu and Pan, 2010).

Dilute sulfuric acid pretreatment was one of the leading technologies with potentials of commercial application in the future biofuel industry (Torget et al., 1990, 1991, 1992; Wooley et al., 1999; Aden et al., 2002; Saha et al., 2005; Lloyd and Wyman, 2005; Wyman et al., 2005). This method has been successfully applied to different lignocellulosic biomass for bioethanol production (Torget et al., 1990, 1991, 1992; Ewanick et al., 2007; Linde et al., 2008; Guo et al., 2008; Cara et al., 2008; Ferreira et al., 2010; Kreuger et al., 2010; Sipos et al., 2010). The major technical barriers of the method to the cost reduction may include: (1) the considerable fresh water was used (the dilute acid solution used was about 6–10-folds greater than the solids weight) and the large amount of acid containing waste water was generated, (2) the considerable energy cost (hot steam or electricity) to elevate the pretreatment reaction system to the temperature close to 200 °C, and (3) the considerable amount of various toxins, including organic acids (acetic acid, formic acid, levulinic acid, and ferulic acid), furan derivatives (furfural and 5-hydromethylfurfural (HMF)), and phenol derivatives (vanillin, guaiacol, and 4-hydroxybenzaldehyde), corrosion products, such as metal ions, were generated. The reduction of the fresh water and steam used, the waste water generated, and the toxins produced in the pretreatment would significantly contribute to the overall cost reduction of bioethanol production from lignocellulose material.

In the previous studies on dilute sulfuric acid pretreatment, efforts had been devoted on the optimization of operation parameters such as pretreating temperature, time, and sulfuric acid concentration to improve the yield of glucose and reduce the concentration of toxins (Wyman et al., 2005; Galbe and Zacchi, 2007; Yang and Wyman, 2008; Sipos et al., 2010). In this study, two rarely noticed but important parameters for eliminating the fresh water and steam usage, the feedstock filling ratio to the pretreatment reactor and the presoaking solids (dry feedstock)/liquid (dilute sulfuric acid solution) ratio, were extensively studied. The results show that the full feedstock filling and the high solids/liquid presoaking ratio in the pretreatment resulted in the significant reduction on steam consumption and waste water generation, while the pretreatment efficiency remained at a satisfactory level. After the pretreatment, the inhibitor substances produced were degraded using the biological detoxification method developed in our lab without adding any water to the process

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(Zhang et al., 2010b). The biodetoxified pretreated feedstock was sent for the simultaneous saccharification and ethanol fermentation at the high solids loading. The resulting fermentation broth with high ethanol titer further leads to the reduction of energy consumption in the final distillation step. The method was applied to various lignocellulose feedstocks and the similar results were obtained. The purpose of this dry pretreatment study was to significantly reduce the energy cost and the waste water generation in the dilute acid pretreatment of lignocellulose, and the aim was achieved with extremely low hot steam and water usage by simply regulating two pretreatment operation parameters, the feedstock filling ratio and the presoaking solids/liquid ratio. The method provided a simple and practical option of energy saving and waste water reduction for bioethanol production using lignocellulose feedstock.

2. Methods

2.1. Raw materials

Corn stover (CS) was grown in Jilin, China, and harvested in fall, 2007. The other two CS were from Shandong and Henan, China, harvested in fall 2008. Wheat straw was from Shandong and Henan, China, and harvested in fall 2008. Rice straw was from Jiangxi, China and harvested in fall 2008. Cotton stalk was grown in Hubei, China, and harvested in 2008. Rape straw was from Henan, China, and harvested in 2008. After collection, the materials were milled coarsely using a beater pulverizer (SF-300, Ketai Milling Equipment, Shanghai, China) and screened through a mesh with the circle diameter of 5 mm. The milled raw materials were washed to remove the field dirt, stones and metals, then dried at 105 °C until the weight was constant and stored in sealed plastic bags for use.

The sand used in the mock-up experiment was the common yellow sand used for house-building with the average diameter of 0.3–1.7 mm. It was dried and mixed with water at a solids/liquid ratio of 10 before the mock-up pretreatment experiment. The apparent density of the presoaked sand was about 1120 kg/m^3. The full filling of the pretreatment reactor required approximately 10 kg of the presoaked sand.

2.2. Pretreatment reactor

The self-made pretreatment reactor was a stainless cylinder with the working volume of 10 l (180 mm in diameter and 400 mm in height). The reactor was carefully sealed by an asbestos textile for insulation. The hot steam was generated by a high pressure steam generator (DZFZ4.5C, Zhengyuan Electromechanics, Shanghai, China) and jetted into the pretreatment reactor from the bottom at 3.0 MPa. The steam was dispersed upward uniformly through a steam distributor at the bottom. A stainless mesh made cylinder container was used to take the presoaked feedstock and installed into the pretreatment reactor tightly. After the pretreatment, the mesh container with the pretreated materials was removed from the reactor. Two thermocouples were placed at different locations of the pretreatment reactor: one was vertically placed in the center of the reactor at the 1/3 of the height from the bottom, the other was placed between the outside surface of the reactor and the asbestos insulation, thus the temperature difference between inside and outside of the reactor could be determined.

The dried lignocellulose materials were presoaked using the dilute sulfuric acid solution at the presoaking ratio of the solid (the dry materials) to the liquid (the sulfuric acid solution) of 2.0–0.5 (w/w) by slowly poured the dilute sulfuric acid solution onto the dry materials in a plastic container then roughly stirred. The mixed material was sealed into a plastic bag and stayed for 18 h at the ambient temperature. The apparent density of the presoaked feedstock was 151.3 kg/m^3 (at the solids/liquid presoaking ratio of 2.0) and 1200 g of the presoaked feedstock occupied the whole space of the pretreatment reactor.

2.3. Pretreatment operation

The pretreatment was carried out at the following procedure: first, the presoaked feedstock was filled into a stainless mesh cylinder and then the cylinder was packed into the pretreatment reactor. Then the reactor was sealed and the steam was jetted into the reactor to start the pretreatment. When the inner temperature reached 100 °C, the purge valve was opened briefly to release the inert air. When the temperature reached the required value, the pretreatment residue time was counted. The temperature was controlled within ±2 °C from the target temperature. When the pretreatment was finished, the two upward valves were completely opened and the pressure was released quickly to the atmospheric pressure. The mesh cylinder containing the pretreated material was removed from the reactor, then the pretreated materials were poured into another stainless container for quenching the pretreated material in the cold water bath. The material was sealed into a plastic bag, sealed, and stored at 4 °C until use. The interval between the two operations was kept as short as possible to keep the reactor temperature high for lessening the possible heat loss. In the first operation, the reactor was pre-heated to the required temperature without the feedstock addition using steam jetting.

The mock-up pretreatment using the sand as the feedstock was carried out in the pretreatment reactor and the same procedure was followed at the condition of 190 °C for 3 min.

2.4. Pretreatment assay

The "severity factor" for measuring the pretreatment intensity was not applied because no aqueous water was generated after the pretreatment in the dry pretreatment operation. The intensity was assayed by the individual sugar yields and inhibitor formations.

The cellulase enzyme used was Accellerase 1000 from Genencor International (Rochester, NY, USA). The filter paper activity and the cellobiase activity were determined to be 65.8 FPU/ml and 152.0 IU/ml, respectively (Zhang et al., 2010a). The enzymatic hydrolysis for the pretreatment efficiency assay was carried out according to the protocol of the National Renewable Energy Laboratory (NREL) (Brown and Torget, 1996). One gram (dry base) of the pretreated material, without washing, was poured into 10 ml of 0.1 M citrate buffer (pH 4.8) in a 100 ml flask. Deionized water was used to dilute the suspension to the solid concentration to 5% (w/w) and again the pH was adjusted to 4.8. 0.06 ml of tetracycline (10 mg/ml in 70% ethanol, Sigma–Aldrich, America) and 0.08 ml of cycloheximide (10 mg/ml in deionized water, Amersco, America), and 15 FPU/g DM of Accellerase 1000 cellulase were added. The flask was incubated at 50 °C for 3 days in a water incubator at the constant rotation rate of 180 rpm. Each data were duplicated and averaged.

2.5. Simultaneous saccharification and ethanol fermentation at high solids loading

The simultaneous saccharification and ethanol fermentation (SSF) at the solids loading of 30% (w/w) was operated in a 5 l helical stirring bioreactor (Zhang et al., 2010a). *Saccharomyces cerevisiae* DQ1 was used for ethanol fermentation after the adaptation procedure as described in detail in Zhang et al. (2010a). The pretreated materials were biologically detoxified to remove the inhibitor substances using the solid state culture of *Amorphotheca resinae* ZN1 (Zhang et al., 2010b).

The inhibitors in the pretreated or detoxified CS were tested as follows: adding 45 ml deionized water to 5 g CS (the moisture content measured precisely), then shaking at 150 rpm, 30 °C for about 2 h. And then the mixture was centrifuged at 10,000 rpm for 5 min, and the supernatants were used for HPLC analysis.

In the prehydrolysis stage, the Accellerase 1000 cellulase enzyme was fed into the tank at the dosage of 15.0 FPU/g DM. Then the total CS feedstock after detoxification was fed into the bioreactor within 12 h for prehydrolysis at 50 °C, 150 rpm. The SSF started by reducing the temperature to 37 °C and inoculating the *S. cerevisiae* DQ1 seeds into the hydrolysate. The SSF operation continued for 60 h and the samples were withdrawn at regular intervals and centrifuged at 10,000 rpm for 5 min. The supernatant was stored frozen until analysis on high-performance liquid chromatography (HPLC). The pH was maintained at pH 5.0 during the hydrolysis and SSF stages by addition of 5 M NaOH solution or 1 M H_2SO_4 solution.

2.6. Cellulose and hemicellulose measurement

The contents of cellulose and hemicellulose of the raw lignocellulose materials and the pretreated material were determined by a two-step H_2SO_4 hydrolysis method according to NREL (Sluiter et al., 2008a) with minor modification. One-hundred milligrams of thoroughly washed corn stover was added to 1 ml of 72% (w/w) H_2SO_4, and the mixture was stirred by using a glass rod until the sample was completely mixed with the acid solution. After incubation in a water bath at 30 °C for 1 h, the mixture was diluted by adding 28 ml of distilled water, and the diluted mixture was hydrolyzed at 121 °C for an hour. The mixture was neutralized with $CaCO_3$ powder and then centrifuged at 10,000 rpm for 5 min. The supernatant was used for HPLC analysis and the glucose and xylose concentrations were obtained in the supernatant. Also, glucose and xylose were used as controls for compensating the loss of the monomers due to its degradation during this process.

2.7. Analysis of sugars, ethanol and inhibitors on HPLC

Glucose, ethanol, and lignocellulose degradation compounds, such as furfural, 5-hydroxymethylfurfural (HMF), acetic acid, formic acid, levulinic acid were analyzed using high-performance liquid chromatography (LC-20AD, refractive index detector RID-10A, Shimadzu, Japan) with a Bio-rad Aminex HPX-87H column at the column temperature 65 °C. The mobile phase was 5 mM H_2SO_4 at the rate of 0.6 ml/min. All samples were centrifuged to remove the cell mass and other water insoluble substances, and then filtered through a 0.22 μm filter before analysis.

2.8. Calculation

The sulfuric acid percentage was calculated by dividing the sulfuric acid weight to the dry feedstock weight. The steam used in the pretreatment was calculated by subtracting the presoaked material weight before the pretreatment from the pretreated material after the pretreatment. The total water used in the pretreatment was calculated by subtracting the dry material weight from the pretreated material weight. The oligomer concentrations in the pretreated CS were analyzed using the procedure discussed by Sluiter et al. (2008b). The ethanol yields were calculated using the method listed in Zhang et al. (2010a). The glucose yields were calculated using the method listed in Zhang et al. (2010b).

3. Results and discussion

3.1. Full feedstock filling ratio of the pretreatment reactor reduced the steam consumption

The feedstock filling ratio by lignocellulose feedstock material into the pretreatment reactor is rarely noticed because of its shrinking property during the pretreatment. However, the reduction of the steam consumption by the full filling ratio of CS to the reactor was found to be very significant. Fig. 1 shows that the steam consumption and the total waste water generation (the presoaked sulfuric acid solution plus the steam condensation water) per 100 g of the dry CS decreased significantly with the increasing feedstock filling ratio in the wide range from 12.5% to 100% (v/v). The decrease of the steam consumption resulted in the increase of the solids content of the pretreated CS material from 27.1% to 50.1% (w/w).

To eliminate the reason of the heat dissipation of the pretreatment reactor, a mock-up pretreatment experiment was carried out under the feedstock filling ratio from 25% to 100% (v/v) using the sand as the feedstock at the same pretreatment condition. Table 1 shows that the weight of the presoaked sand did not increase at each filling ratio, indicating the heat dissipation did not cause the detected steam condensation and could be neglected.

Then the pretreatment at the two feedstock filling ratios (the half filling with 400 g of dry CS and the full with 800 g) were further investigated under the different operation conditions. The steam consumption, the waste water generation, and the solids content of the pretreated CS were shown in Fig. 2. The pretreatment efficiency assay results were shown in Table 2. Fig. 2 shows that similar to the result in Fig. 1, the steam consumption and the waste water generation per 100 g of dry CS significantly decreased with the increasing filling ratio at each pretreatment condition, and led to the increase of the solids content in the pretreated CS materials: a 50% (w/w) value was reached for each pretreatment condition at the full filling ratio, comparing to less 40% (w/w) at the half filling ratio. Table 2 shows that the cellulose content of the pretreated CS was not changed significantly at the two filling ratios; the hemicellulose content in the half filling ratio was smaller than that at the full filling ratio. The hydrolysis yields of glucose and xylose of the pretreated CS (without washing) changed with the pretreatment temperature, time, and sulfuric acid concentration, other than with the feedstock filling ratio.

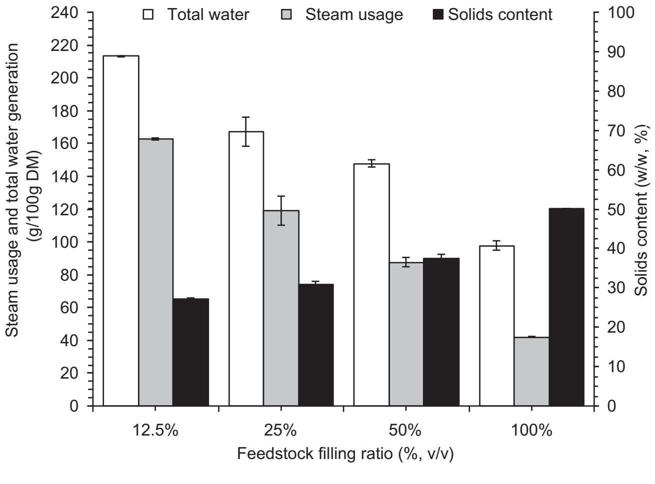

Fig. 1. Effect of the feedstock filling ratio on the steam consumption and waste water generation at the same pretreatment condition. The feedstock was CS with the solids/liquid presoaking ration of 2.0. The pretreatments were performed at 2.5% (w/w) dilute sulfuric acid, 190 °C for 3 min.

Table 1
Steam and water consumption in the mock-up pretreatment using sand as the feedstock.

Feedstock filling ratio(%, v/v)	Sand before pretreatment(kg)	Sand after pretreatment(kg)
25	2.60 ± 0.05	2.62 ± 0.04
50	5.47 ± 0.0	5.45 ± 0.01
100	10.01 ± 0.0	9.82 ± 0.30

The conditions for the mock-up pretreatment were at 190 °C for 3 min at the pressure of 1.2 MPa. The sand before mock-up experiment was presoaked with water at a solids/liquid presoaking ratio of 10.0 (w/w).

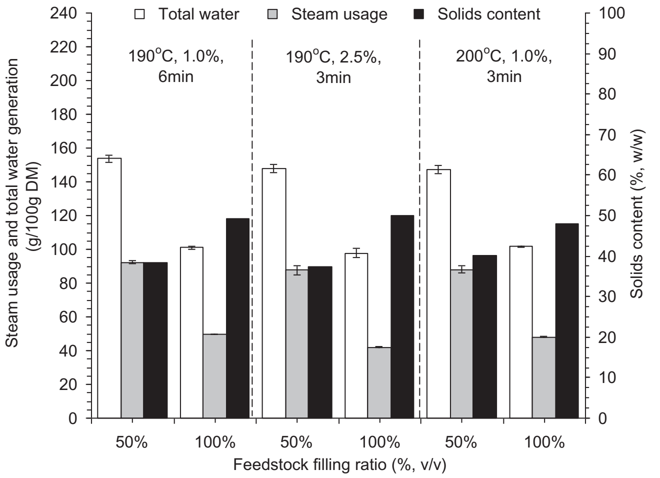

Fig. 2. Effect of the feedstock filling ratio on the steam consumption and waste water generation at the different pretreatment conditions. The pretreatment conditions on the figure legend indicate temperature, sulfuric acid concentration, and time.

The concentrations of glucose, xylose, the oligo-glucose and xylose, and the inhibitor substances (acetic acid, furfural, and HMF) in the pretreatment liquor did not show a significant change with different filling ratio, only slightly smaller at the full filling ratio than that at the half filling ratio. The results show that the full feedstock filling ratio of CS in the reactor significantly reduced the steam consumption in the dilute acid steam pretreatment, while maintaining the same pretreatment efficiency.

The feedstock filling ratio experiment gave an interesting result: the steam usage was almost independent of the feedstock material weight in the pretreatment reactor. The full feedstock filling of the reactor by CS led to the half reduction of the steam consumption, comparing to that with the half filling ratio at different pretreatment conditions. The phenomenon seems hard to understand since the feedstock weight at the full filling case were doubled and the energy to elevate and maintain the pretreatment operation should be also doubled at least, compared to that at the half filling case. The mock-up experiments using sand as feedstock verified that the reason was not from the heat dissipation during the pretreatment. Thus, the reason might relate to the unique property of the lignocellulose materials to absorb the water or steam. Generally, the lignocellulose materials such as CS easily and quickly absorb water above 90% of the total weight. When the CS feedstock filling was not full and there was free space left in the pretreatment reactor, the steam would occupy the free space and in constant contact with the CS materials inside. The unique water absorption property of CS materials resulted in the quick steam removal from the free space to the solid CS feedstock. The absorption phenomenon led to the drop of the partial pressure of steam in the free space, and then the more steam was required to compensate the pressure drop in the free space. As the result, the steam absorption inside the reactor and the steam compensation from the steam generator took place continuously until the pretreatment ends due to the existence of the free space in the pretreatment reactor. On the other hand, if the reactor space was fully filled with the presoaked CS, the steam was only used to heat the CS feedstock (CS solid and the presoaked liquid) to the required temperature and sustained for a certain period of time, but no free space for steam to occupy. As soon as the temperature was reached, no further steam was required unless the compensation of the heat loss of the reactor. As the conclusion of the feedstock filling ratio experiment, the pretreatment reactor should be always full of the lignocellulose feedstock and free space should be definitely avoided.

3.2. High solids/liquid presoaking ratio reduced the waste water generation and steam consumption

The dry CS material was presoaked by the dilute sulfuric acid solution before the pretreatment. The increase of the solids (the dry CS)/liquid (the diluted acid solution) ratio certainly reduced the fresh water use, but might result in the sharp increase of the steam consumption and the decrease of the pretreatment efficiency because of the use of the relative drier CS material during the pretreatment. Fig. 3 and Table 3 show the detailed data of the steam consumption and pretreatment efficiency at different solids/liquid presoaking ratio, respectively, in the range of the solids/liquid presoaking ratio from 0.5 to 2.0.

Fig. 3 indicates that, contrary to the estimation, the steam consumption per 100 g dry CS at the first pretreatment condition (200 °C, 1.0%, 3 min) remained almost unchanged with the increasing solids/liquid presoaking ratio; and at the second condition (190 °C, 2.5%, 3 min), the steam consumption even decreased with the increasing solids/liquid presoaking ratio. The result thus led to

Table 2
Effect of the feedstock filling ratio on the pretreatment efficiency.

Parameters	Filling ratio (%)	Cellulose (%, w/w)	Hemicellulose (%, w/w)	Glucose yield (%)	Xylose yield (%)	Composition of pretreatment liquor						
						Glucose	Xylose	Acetate	HMF	Furfural	O-Cel	O-Xyl
						(g/100 g DM)						
190 °C; 1.0%; 6 min	50	33.32	6.75	60.4	57.0	0.46	2.74	0.71	0.07	0.18	2.08	11.6
	100	32.38	8.54	62.0	58.2	0.41	1.76	0.58	0.11	0.17	2.21	9.91
190 °C; 2.5%; 3 min	50	36.62	3.19	89.3	54.3	1.67	8.17	1.37	0.33	0.66	0.87	3.21
	100	33.72	3.70	85.1	51.5	1.58	8.02	1.20	0.21	0.90	1.57	4.29
200 °C; 1.0%; 3 min	50	35.37	5.48	73.9	60.8	0.49	3.54	0.88	0.11	0.36	2.33	10.8
	100	32.73	7.44	72.3	58.4	0.44	2.27	0.67	0.06	0.18	1.74	7.44

Data in the parameters column indicate the temperature, sulfuric acid concentration, and time; the solids/liquid presoaking ratio was 2.0; the calculation of the cellulose and hemicellulose was based on the dry solids matter (DM). The enzymatic hydrolysis conditions were: 5.0% (w/w) solids loading (without washing), pH 4.8 adjusted with 100 mM citrate acid buffer (pH 4.8), 15.0 FPU/g DM, 50 °C, 150 rpm in a water-bath shaking incubator. All the tests were performed twice and averaged.

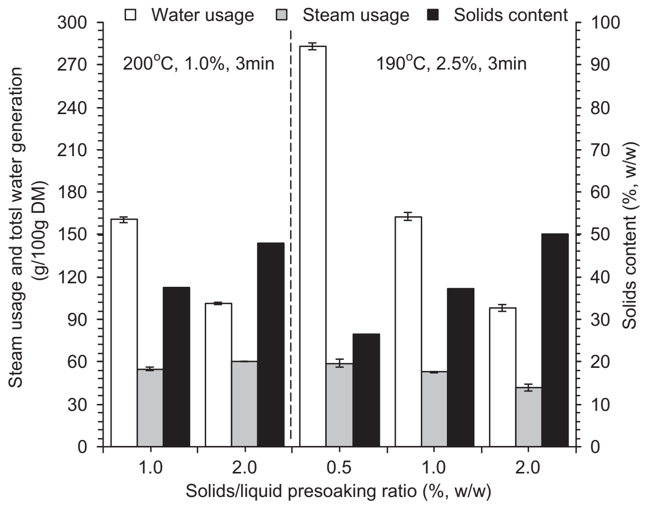

Fig. 3. Effect of the solids/liquid presoaking ratio on the steam consumption and waste water generation at the different pretreatment conditions. The conditions were listed in Table 3.

the significant increase of the solids content of the pretreated CS because the fresh water usage in the presoaking step was reduced at the high solids/liquid ratio. Table 3 indicates that the glucose yield of the pretreated CS approximately maintained the similar values; the xylose yield constantly decreased with the increasing solids/liquid presoaking ratio. The results were not in a perfect agreement with those recently reported by Ferreira et al. (2010), mainly because of the different operation parameters (relative low biomass/liquid ratio from 1.5 to 3.0 g/10 ml, low temperature, and the longer residence time) and the different substrate nature. The concentration of the inhibitor substances in the pretreatment liquor per 100 g of dry CS increased with the increasing solids/liquid presoaking ratio at various pretreatment conditions. Glucose concentration in the pretreatment liquor increased moderately with the increasing solids/liquid presoaking ratio, while the xylose concentration sharply decreased with the increasing solids/liquid presoaking ratio. For the sugar oligomers, both glucose and xylose oligomers decreased with the increasing ratio. The results indicate that the lignocellulose degradation at different solids/liquid presoaking ratio affected the degradation of the components differently. The degradation of cellulose to glucose was not significantly affected by the solids/liquid presoaking ratio, but the degradation of hemicellulose to xylose was affected significantly. On the other hand, the degradation of both glucose and xylose to the secondary products (acetic acid, furfural, and HMF) was accelerated, although it was not significant, with the increasing solids/liquid presoaking ratio.

The solids/liquid presoaking ratio experiment reflected the other side of the unique water absorption capacity of lignocellulose. Usually, the presoaking by the dilute acid solution was intensive (3–10-folds of the water to the solids), even rinsed completely in the dilute acid solution (Yang and Wyman, 2008; Lynd et al., 2008). One consideration of the high dilute acid solution use was to intensify the pretreatment efficiency by sufficient mixing between the solid feedstock and liquid acid solution. The other consideration was to avoid the high steam consumption by the dry feedstock at the high solids/liquid presoaking ratio because of the strong water absorption property of lignocellulose. However, our experiment showed that the steam consumption remained almost unchanged at the high or low solids/liquid presoaking ratio, and even reduced with the increasing solids/liquid ratio at certain pretreatment conditions. The results also indicated that the powerful water absorption capacity of lignocellulose materials was not strictly related to the initial water content of the materials in a certain range. Even with different initial solids/liquid presoaking ratios, the steam consumption for maintaining the pretreatment condition was not affected by the ratio.

The possible reason for the unusual constant steam consumption is still related to the feedstock filling ratio in the reactor. It was found at each pretreatment operation that when the presoaked CS was filled into the reactor at the full filling ratio, the presoaked CS shrunk quickly after the hot steam was jetted onto the loosely packed CS material. The shrinking leaved a free space approximately 20% to 30% of the total reactor space on the top of the reactor, depending on the presoaked ratio of the feedstock CS. The free space in the reactor played the same role to draw the hot steam onto the CS as in the not fully filled case by the CS feedstock discussed in Section 3.1. The free space in the pretreatment reactor was a determinant factor for the steam consumption. The contribution of different solids/liquid presoaking ratio on the steam consumption was less important comparing to that of the filling ratio (either at the beginning of the operation, or during the operation). One consideration to the further reduction of steam consumption might be the tight packing of the CS feedstocks in the reactor by extra pressing to reduce the free space formation, or even completely remove the free space during the pretreatment. However, too tight packing might cause difficulties on the mass and heat transfer inside the feedstock, thus a balance between the reduced steam consumption and the satisfied pretreatment efficiency. The further experiment is on the way and the result might give an optimal answer.

Furthermore, the positive results of present dilute acid pretreatment at higher solids/liquid presoaking ratio of 2 will greatly advance other pretreatment technologies, such as the sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL) and steam explosion, etc., especially in terms of the energy efficiency and the waste streams (Zhu and Pan, 2010).

Table 3
Effect of the solids/liquid presoaking ratio on the pretreatment efficiency.

Parameters	Solids/liquid ratio	Glucose yield (%)	Xylose yield (%)	Composition of pretreatment liquor						
				Glucose	Xylose	Acetate	HMF	Furfural	O-Cel	O-Xyl
				(g/100 g DM)						
200 °C; 1.0%; 3 min	1.0	75.54	65.75	0.36	2.17	0.44	0.05	0.07	2.4	12.8
	2.0	72.34	58.45	0.44	2.14	0.67	0.06	0.18	2.2	11.7
190 °C; 2.5%; 3 min	0.5	82.74	68.35	2.20	19.3	1.84	0.31	0.54	4.2	13.6
	1.0	83.07	59.42	1.29	8.99	0.86	0.24	0.52	2.0	5.4
	2.0	84.50	49.74	1.57	8.13	1.20	0.37	0.82	1.5	4.4

Data in the parameters column indicate the temperature, sulfuric acid concentration, and time; the feedstock filling ratio to the pretreatment reactor was 100% (v/v). The enzymatic hydrolysis conditions for different pretreated CS were: 5.0% (w/w) solids loading (without washing), pH 4.8 adjusted with 100 mM citrate acid buffer (pH 4.8), 15.0 FPU/g DM, 50 °C, 150 rpm in a water-bath shaking incubator. All the tests were performed twice and averaged.

Besides, the very corrosive environment of dilute acid in the high temperature mandates exotic and expensive construction materials of the pretreatment reactor (Hinman et al., 1992). Although no direct measurement of erosion in the present pretreatment bioreactor, the shining appearance of the reactor inner wall after one year use was clearly different from the previous reactor used in our lab with strong erosive spots even after several months use. The major difference between the two reactors was the existence of the aqueous dilute acid solution within the reactor: the present one had no liquid phase, while the old one had.

As the conclusion of the solids/liquid presoaking experiment, the high solids/liquid presoaking ratio significantly reduced the fresh water usage as well as the steam consumption, while the pretreatment efficiency kept at the satisfactory level. Since the pretreated CS contained only 50% water, no aqueous sulfuric acid containing water was generated during the pretreatment.

3.3. Optimization of operation parameters under full filling ratio and high solids/liquid presoaking ratio

The pretreatment operation was carried out at various operation parameters under the full reactor filling ratio and high solids/liquid presoaking ratio for searching an optimal pretreatment efficiency (high monosaccharide yield and less inhibitor formation) and the results were shown in Table 4 and Fig. 4. Table 4 shows that the steam consumption increased with the increasing pretreatment time and temperature, but was almost constant with the increasing sulfuric acid concentration. The decrease of cellulose content was within ±1–2% before and after the pretreatment (34.1%) among all operation conditions tested, indicating that the pretreatment parameter changes did not give a strong effect on the cellulose content. On the other hand, the greater degradation of hemicellulose was observed and the hemicellulose hydrolysis yield increased with the increasing sulfuric acid concentration significantly. The degradation product concentrations in the pretreatment liquor (glucose, xylose, acetic acid, furfural, and HMF) increased with the increasing pretreatment temperature, time, and the sulfuric acid concentration, while the oligomers concentrations of glucose and xylose decreased. The results indicate that the pretreatment severity increased with the increasing pretreatment intensity such as the increased temperature, time, and sulfuric acid concentration.

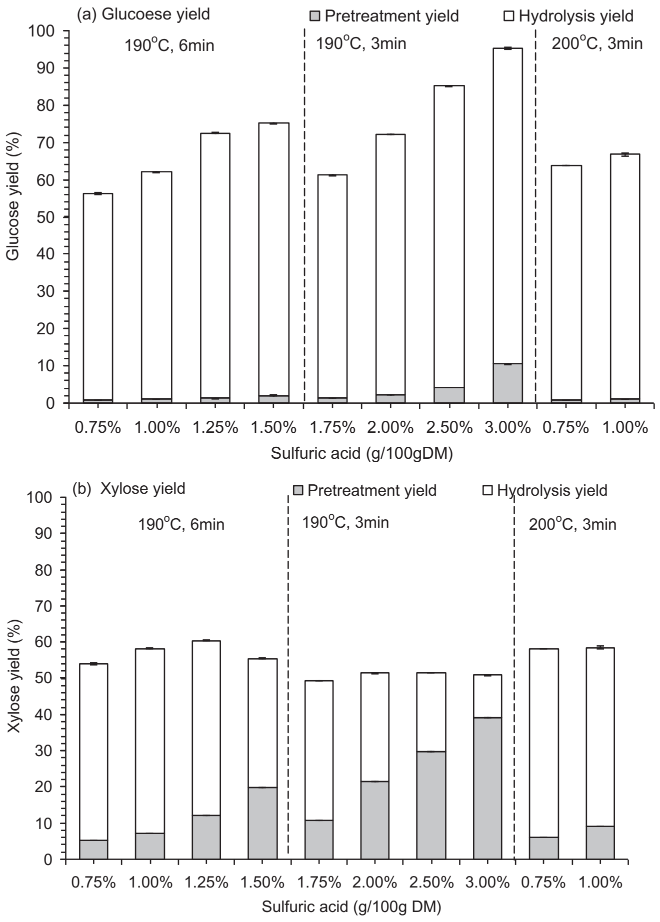

Fig. 4. Glucose and xylose yields of CS in the pretreatment and hydrolysis steps. The conditions were listed in Table 4.

Fig. 4 shows that the glucose yield was mainly from the enzymatic hydrolysis step, while the pretreatment yield took a small percentage. The glucose yield increased with the increasing sulfuric acid concentration significantly in the experimental range from 0.75% to 3.0% (w/w), but the effect of the pretreatment time and temperature experienced a complicated mechanism, thus the glucose yield did not just simply increase with the increasing temperature and time. For xylose yield, Fig. 4 indicates that the xylose yields were kept in a relative low value (less than 60%) at different conditions. Different from the glucose yield, the xylose yield in the pretreatment step took a considerable percentage, especially at the high sulfuric acid concentration conditions. The xylose yield in the pretreatment step increased with the sulfuric acid concentration,

Table 4

Optimization of pretreatment parameters on the steam consumption and waste water generation.

Parameters	Consumption		Composition of pretreated CS			Composition of pretreatment liquor						
	Total Water	Steam	Solids content	Cellulose content	Hemi-cellulose	Glucose	Xylose	Acetate	HMF	Furfural	O-Cel	O-Xyl
	(g/100 g DM)		(%, w/w)			(g/100 g DM)						
190 °C; 0.75%; 6 min	107.3	54.1	46.8	32.36	9.08	0.29	1.30	0.54	0.07	0.12	2.21	10.41
190 °C; 1.00%; 6 min	101.0	49.9	49.2	32.38	8.54	0.41	1.76	0.58	0.11	0.17	2.21	9.91
190 °C; 1.25%; 6 min	100.6	47.7	48.4	34.54	6.84	0.49	3.03	0.70	0.11	0.25	2.12	9.07
190 °C; 1.50%; 6 min	103.2	49.8	47.6	31.34	4.74	0.79	4.94	1.00	0.23	0.56	1.90	6.34
190 °C; 1.75%; 3 min	98.9	44.7	48.3	32.01	7.80	0.51	2.94	0.49	0.12	0.22	2.21	9.91
190 °C; 2.00%; 3 min	95.4	44.0	50.5	33.39	5.56	0.88	5.88	0.77	0.25	0.50	2.27	7.55
190 °C; 2.50%; 3 min	97.8	41.9	50.1	33.72	3.70	1.58	8.02	1.20	0.21	0.90	1.57	4.29
190 °C; 3.00%; 3 min	97.3	44.0	49.0	33.89	1.91	3.96	10.71	2.36	0.41	1.12	0.97	1.88
200 °C; 0.75%; 3 min	109.4	55.9	46.2	32.50	8.11	0.30	1.54	0.67	0.05	0.15	1.74	7.18
200 °C; 1.00%; 3 min	101.5	47.9	47.9	32.73	7.44	0.44	2.27	0.67	0.06	0.18	1.74	7.44

Data in the parameters column indicate the temperature, sulfuric acid concentration, and time; the feedstock was filling into the pretreatment reactor fully 100% (v/v) and the solids/liquid presoaking ratio was 2.0; the calculation of the cellulose and hemicellulose was based on the dry solids matter (DM). The enzymatic hydrolysis conditions for different pretreated CS were: 5.0% (w/w) solids loading (without washing), pH 4.8 adjusted with 100 mM citrate acid buffer (pH 4.8), 15.0 FPU/g DM, 50 °C, 150 rpm in a water-bath shaking incubator. All the tests were performed twice and averaged.

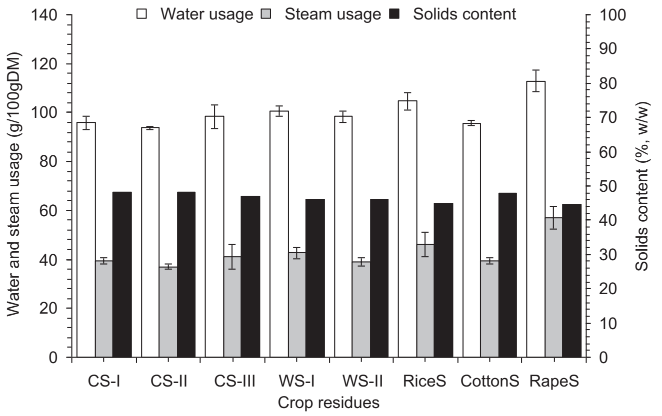

Fig. 5. Steam consumption and waster water generation of the different pretreated feedstocks during the pretreatment. The abbreviation of the feedstocks and the pretreatment conditions were listed in Table 5.

but similar to the glucose yield, the effects of the pretreatment temperature and time on the xylose yields were complicate.

A relative optimal pretreatment parameter set was recommended for the further pretreatment test: 190 °C for 3 min using the 2.5% (w/w) sulfuric acid concentration, at the full feedstock filling ratio and the solids/liquid presoaking ratio of 2.0. The glucose and xylose yields for the CS were 85.2% and 50.9% (without washing the pretreated CS), respectively; the solids content of the pretreated CS was 50.1% (w/w); the inhibitors concentrations were 1.20, 0.90, 0.21 g/100 g DM for acetic acid, furfural, and HMF, respectively; and the cellulose and hemicellulose contents after the pretreatment were 33.9% and 3.7% (w/w), respectively.

3.4. Application to various lignocellulose materials and the consequent SSF performance

The dry pretreatment method with extremely low steam usage and waste water generation was applied to different lignocellulose materials for testing its flexibility. Three kinds of corn stover, two wheat straw, one rice straw, one cotton straw, and one rape straw collected from different sites and years were applied at the optimal pretreatment condition of 190 °C, 3 min, 2.5% (w/w) sulfuric acid, at the full feedstock filling ratio and the solids/liquid presoaking ratio of 2.0.

Fig. 5 shows that the steam consumption and waste water generation were essentially the same in the error range, with the exception for rape straw in which the steam consumption increased by 10–15%. The solids content of these pretreated materials were approximately 50% (w/w). Table 5 shows that the cellulose contents before and after the pretreatment changed little during the pretreatment, while the hemicellulose reduced to approximately 1/5–1/10 of the original content. The degradation products in the pretreatment liquor were approximately constant among the same lignocellulose feedstock, even collected from different sites and years, while the results for the different biomass showed clear differences. The two non-food crop residues, cotton straw and rape straw, were different from the other food crop residues by its low level of degradation products. The glucose yield was similar for the same feedstock, but significantly different among the different biomass. The wheat straw and rice straw showed the optimal glucose yields, while the two non-food crop residues, cotton straw and rape straw, showed the worst glucose yields.

The pretreated materials were biodetoxified and used as the feedstocks for the simultaneous saccharification and fermentation (SSF). Fig. 6 shows the simultaneous saccharification and ethanol fermentation at 30% (w/w) solids loading using five different lignocellulose feedstocks. The three food crop residues, corn stover, rice straw, and wheat straw, showed better fermentation performances in glucose consumption rate, the ethanol production rate, the final ethanol titer and the ethanol yield. On the other hand, the two non-food crop residues, cotton stalk and rape straw, showed the worse performances in glucose consumption rate, the ethanol production rate, the final ethanol titer and the ethanol yield. This phenomenon was in accordance with the enzymatic hydrolysis results shown in Table 5, perhaps resulting from the feedstock pretreatment intensity diversified between the food and non-food crop residues.

Conclusively, the present pretreatment method with extremely low steam and water usage provided at least the following advantages: (1) the hot steam was significantly reduced – only half of the waste water generation was from steam condensation – thus the energy consumption was sharply reduced correspondingly; (2) no aqueous acid containing water was released during the pretreatment operation, which significantly reduced the burden of the large amount of waste water treatment; (3) half of the pretreated materials was the dry solids matter, thus the pretreatment was a "dry" to "dry" process: feeding with the dry lignocellulose feedstock and resulting with the dry pretreated product without

Table 5
Compositions of the feedstock and the pretreatment liquors of the different lignocellulose materials.

Feedstocks	Cellulose (%, w/w)		Hemicellulose (%, w/w)		Glucose yield	Xylose yield	Composition of pretreatment liquor						
							Glucose	Xylose	Acetate	HMF	Furfural	O-Cel	O-Xyl
	Before	After	Before	After	(%)	(%)	(g/100 g DM)						
CS-I	34.1	33.7	24.6	3.7	85.1	51.5	1.58	8.02	1.20	0.21	0.90	1.57	4.29
CS-II	35.4	37.4	23.5	5.2	74.7	60.8	1.55	8.50	1.46	0.32	0.38	0.76	5.23
CS-III	36.3	36.3	21.5	4.7	72.9	64.5	1.82	8.35	1.48	0.35	0.37	0.65	4.13
WS-I	33.4	33.0	26.5	4.3	94.6	65.3	1.09	12.5	1.26	0.23	0.33	1.01	5.56
WS-II	31.7	32.7	23.2	3.5	95.7	73.2	1.86	13.3	2.01	0.47	0.40	0.88	4.19
RiceS	35.4	36.5	22.8	4.7	99.5	67.3	0.93	8.08	0.65	0.12	0.19	1.31	5.34
CottonS	38.2	31.0	18.8	4.5	41.5	57.5	0.52	4.15	1.04	0.07	0.10	0.48	6.08
RapeS	33.9	33.0	19.9	7.1	43.3	62.8	0.65	4.58	0.83	0.08	0.15	0.69	8.89

The calculation of the cellulose and hemicellulose was based on the dry solids matter (DM). CS-I, CS-II and CS-III: Corn stover from Jilin, Shandong, and Henan, China, respectively; WS-I and WS- II: wheat straw from Shandong and Henan, China, respectively; RiceS: rice straw from Jiangxi, China; CottonS: cotton stalk from Hubei, China; RapeS: rape straw from Henan, China. The pretreatment was carried out at the full feedstock filling ratio and the solids/liquid presoaking ratio of 2.0. The enzymatic hydrolysis were carried out at 5.0% (w/w) solids loading (without washing), pH 4.8 adjusted with 100 mM citrate acid buffer (pH 4.8), 15.0 FPU/g DM, 50 °C, 150 rpm in a water-bath shaking incubator. All the tests were performed twice and averaged.

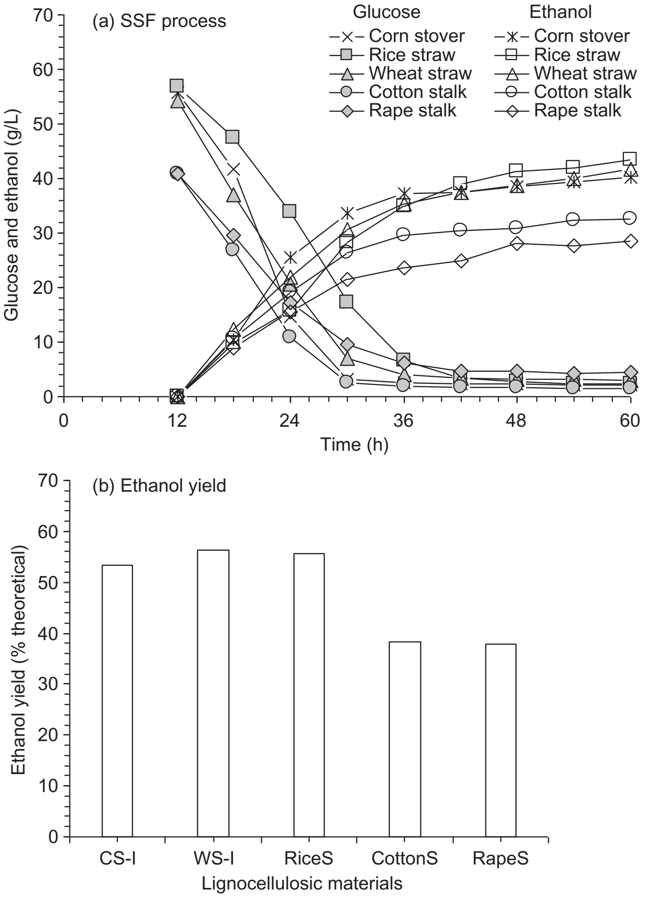

Fig. 6. Simultaneous saccharification and ethanol fermentation of different pretreated lignocellulose feedstocks at the high solids loading. (a) SSF process; (b) ethanol yield.

any waster water generation; (4) the dry pretreated product made the consequent saccharification and fermentation could be carried out at the high solids loading (30%, w/w) and low water usage. The resulting fermentation broth with high ethanol titer further leads to the reduction of energy consumption in the final distillation step. The method was applied to various lignocellulose feedstocks and the similar results were obtained.

Lynd et al. (2008) proposed two scenarios for lignocellulose bioprocessing for ethanol production: Scenario 1 set a target of 7% (w/w) ethanol titer in the fermentation broth using dilute acid pretreatment as the process start. The present dry pretreatment method may provide a practical means to reach this goal with significant potential of the energy saving and waste water reduction.

4. Conclusions

The full feedstock filling ratio and the high solids/liquid presoaking ratio significant reduced the steam consumption and generated no aqueous acid containing waste water during this “dry” dilute acid pretreatment, while maintained the satisfactory pretreatment efficiency. The strong water absorption capacity of lignocellulose materials played a unique role in the reduction of steam consumption and waste water generation. The method was applied to various lignocellulose materials, and the high ethanol titer in the fermentation broth was obtained in the simultaneous saccharification and fermentation at the high solids loading. This method provided a practical means to produce ethanol economically feasible.

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