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Short Communication

Lower pressure heating steam is practical for the distributed dry dilute sulfuric acid pretreatment

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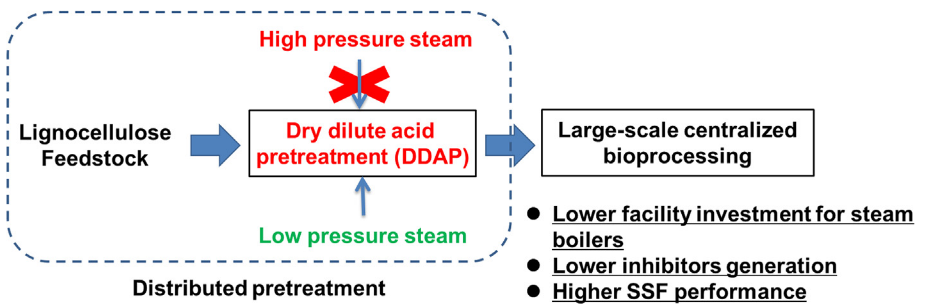
HIGHLIGHTS

- Lower pressure heating steam was practical for dry dilute acid pretreatment.
- Lower inhibitors generated in pretreatment using lower pressure heating steam.
- High SSF performance was obtained for corn stover pretreated by lower pressure steam.
- Lower pressure heating steam caused shorter mixing time during pretreatment.

GRAPHICAL ABSTRACT



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ABSTRACT

Most studies paid more attention to the pretreatment temperature and the resulted pretreatment efficiency, while ignored the heating media and their scalability to an industry scale. This study aimed to use a relative low pressure heating steam easily provided by steam boiler to meet the requirement of distributed dry dilute acid pretreatment. The results showed that the physical properties of the pretreated corn stover were maintained stable using the steam pressure varying from 1.5, 1.7, 1.9 to 2.1 MPa. Enzymatic hydrolysis and high solids loading simultaneous saccharification and fermentation (SSF) results were also satisfying. CFD simulation indicated that the high injection velocity of the low pressure steam resulted in a high steam holdup and made the mixing time of steam and solid corn stover during pretreatment much shorter in comparison with the higher pressure steam. This study provides a design basis for the boiler requirement in distributed pretreatment concept.

1. Introduction

Pretreatment is a prerequisite step to destroy the lignocellulose structures and enhance cellulose hydrolysis efficiency for ensuring extensive lignocellulose biorefining for biofuels and biochemicals production (Yang and Wyman, 2008). A number of pretreatment methods including dilute acid, steam explosion, ammonia fiber expansion, alkaline treatment, and ionic liquid have been developed (Tucker et al., 2003; Brownell et al., 1986; Teymouri et al., 2005; Chang et al., 1998; Lee et al., 2009), and almost all of these pretreatments have a temperature effect, namely the relatively higher temperature used, the more effective pretreatment performance obtained (Mosier et al., 2005).

Most studies only paid their attentions to the pretreatment temperature and the resulted pretreatment efficiency, while ignored the heating media and their scalability to an industry scale. The oil or sand bath or the electrical heating coils are widely used to heat the pretreatment reaction in bench-scale experiments (Wyman et al., 2009), but hot steam is the preferred option in

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industry. In a bench-scale process development unit for ethanol production from lignocellulose proposed by Palmqvist et al., the saturated steam with high pressure of 3.0 MPa and 235 °C was used for heating the pretreatment (Palmqvist et al., 1996). In National Renewable Energy Laboratory (NREL)'s design report, the superheated steam with 1.3 MPa and 268 °C for heating the pretreatment reaction was supplied by the unit "combustor, boiler, and turbogenerator" (Humbird et al., 2011). As for the newly proposed distributed pretreatment concept, which can ensure a secure and reliable feedstock supply, to build a steam boiler with 3.0 MPa pressure is a great challenge for both manufacturing and its made material (Eranki et al., 2011; Zhang et al., 2016). The thermal plant could supply superheated steam to meet the requirement in pretreatment unit, but it is more practical for the centralized bioprocessing plant with respect to the high investment, other than the small decentralized pretreatment centers. Therefore, it is better to employ a low pressure heating steam or look for a suitable and easy-scalable heating method for pretreatment.

The main objective of this study was to explore the effect of different heating steam pressure supplied by boilers on dilute sulfuric acid pretreatment and the following bioprocessing steps. The changes of physical properties, compositions, enzymatic hydrolysis and ethanol fermentability of the corn stover pretreated using the heating steam pressure varying from 1.5, 1.7, 1.9 to 2.1 MPa were tested. Then a simplified CFD model was established to simulate the steam holdup during dry dilute acid pretreatment in the helical ribbon pretreatment reactor to explain the heating steam pressure effect. This study provided a design basis for the boiler requirement in the distributed bioprocessing concept.

2. Materials and methods

2.1. Raw materials

Corn stover was obtained from Dancheng County, Henan Province, China, in fall 2013. Corn stover was water-washed to remove the field dirts, air-dried, and milled using a beater pulverizer to pass through the 10-mm diameter apertures. The milled corn stover was sealed in plastic bags and stored at room temperature until used.

2.2. Strains and enzymes

Saccharomyces cerevisiae DQ1 (CGMCC 2528, China General Microbial Collection Center, Beijing, China) was used for ethanol fermentation. *Amorphotheca resinae* ZN1 (CGMCC 7452, China General Microbial Collection Center, Beijing, China) was used for biodetoxification of dry dilute sulfuric acid pretreated corn stover (He et al., 2016).

The cellulase enzyme Youtell #6 was purchased from Hunan Youtell Biochemical Co., Yueyang, Hunan, China. The filter paper activity of Youtell #6 was 135 FPU/g determined using the NREL protocol LAP-006 (Adney and Baker, 1996), the cellobiase activity was 344 CBU/g, and the protein content was 90 mg per gram of cellulase reagent determined by Bradford method.

2.3. Dry dilute sulfuric acid pretreatment and biodetoxification operations

The pretreatment unit consists of a 20 L pretreatment reactor and a 36 kW electric steam generator. A single helical ribbon stirrer was driven by a motor mounted on top of the reactor through an electromagnetic convertor. Four symmetrical distributed nozzles were designed on the distributor to disperse the steam jetted into the reactor (He et al., 2014). The steam generator was manufactured by Shanghai Huazheng Boiler Manufacture Co, Shanghai, China. It was heated by electricity and generated the saturated steam vapor up to 2.5 MPa and 225 °C. Corn stover was pretreated using dry dilute sulfuric acid pretreatment (DDAP) according to He et al. (2014). Briefly, corn stover and dilute sulfuric acid solution were co-currently fed into the 20 L pretreatment reactor at a solid/liquid ratio of 2:1 (w/w) with sulfuric acid of 2.5 g per 100 g solid lignocellulose and helically stirred at 50 rpm. The saturated heating steam was adjusted to 1.5 MPa (198 °C), 1.7 MPa (204 °C), 1.9 MPa (210 °C) and 2.1 MPa (215 °C), respectively, to heat the mixed corn stover and dilute sulfuric acid to 175 °C for 5 min. The ramping time of the pretreatment using different pressure steam was nearly the same due to the marginal difference in the steam temperature (shown in Table 1). The solids content of the pretreated material was about 50% (w/w) with pH 2.0 and no wastewater was generated.

The pretreated corn stover was detoxified via solid state biodetoxification according to He et al. (2016) before it was hydrolyzed and fermented to ethanol. Briefly, the pretreated corn stover was neutralized with the suspended slurry of 20% (w/w) $Ca(OH)_2$ to pH of 5–6, and then inoculated with *A. resinae* ZN1 spores to start the biodetoxification and lasted for 48 h at 28 °C with sterilized aeration at 1.0 vvm. The solids content of biodetoxified corn stover was still around 50% (w/w). Corn stover composition after biodetoxification showed no obvious change compared with the freshly pretreated corn stover.

2.4. Enzymatic hydrolysis and simultaneous saccharification and fermentation tests

Enzymatic hydrolysis assay of the pretreated corn stover feedstock was carried out according to the protocol of NREL LAP-009 (Brown and Torget, 1996). 0.5 g of the pretreated corn stover (dry base) and 10 mL of deionized water were loaded into a 100 mL flask to prepare the slurry in 0.1 M citrate buffer containing 2.5% (w/w) solids and pH was finely adjusted to 4.8 by adding 5 M NaOH solution. 0.08 mL of cycloheximide (10 mg/mL in deionized water) was added to avoid the microbial contamination. 20 FPU/g DM (dry pretreated corn stover matter) of cellulase was added and the hydrolysis lasted for 72 h at 50 °C and 150 rpm in a water-bath shaking incubator.

SSF was carried out in a 5 L helical ribbon stirrer agitated bioreactor as described in Zhang et al. (2010). Briefly, the pretreated and biodetoxified corn stover was loaded into the bioreactor to reach 25% (w/w) solids content. 15 FPU/g DM of cellulase enzyme was added and the prehydrolysis was carried out for 12 h at 50 °C, then the temperature was reduced to 37 °C and seed cultures of *S. cerevisiae* DQ1 were inoculated into the bioreactor at a 10% ratio (v/v) to start the simultaneous saccharification and fermentation step (SSF). Samples were taken periodically for analysis of ethanol and glucose.

2.5. CFD modeling of pretreatment reactor

A commercial grid-generation tool, ICEM CFD 14.0 (Ansys Inc., Canonsburg, PA, USA) was used to generate the 3D grids of the reactor model for running Fluent 14.0 (Ansys Inc.). The Eulerian-Eulerian two-fluid model was used for the two-phase flow calculations (Xiong et al., 2013, 2015; Aramideh et al., 2015). The corn stover was set as continuous phase and water vapor was set as dispersed phase. The drag force and heat transfer between the phases was simulated by the Schiller-Naumann's model and Ranz-Marshall's model, respectively. The sliding mesh method was used to characterize the impeller rotation. In the simulation, corn stover was considered as incompressible and non-Newtonian fluid and

Table 1
Partial physical properties of the saturated steam with different pressure and the mixing time of steam and corn stover during pretreatment calculated based on CFD model.

Pressure (MPa)	Temperature (°C)	Density (kg/m^3)	Injection velocity (m/s)	Mixing time (s)
1.5	198.327	7.59	1.94	3.6
1.7	204.346	8.57	1.72	4.2
1.9	209.838	9.55	1.54	4.6
2.1	214.898	10.53	1.40	5.6

The temperature and density of the steam at different pressure are the inherent properties of the saturated steam and the injection speed was calculated based on the mass flow rate of the steam. The mixing time was calculated based on the CFD simulations.

the chemical reaction during pretreatment was neglected (Hou et al., 2016).

Velocity inlet with a mass flow of 6 kg/h according to the specification of the boiler and pressure outlet under the atmospheric condition was used in the simulation, while no-slip and adiabatic condition was used for walls. The physical properties of high pressure vapor (temperature and density) in the inlet were shown in Table 1 at different cases. The physical and rheological properties of raw corn stover were referred to our previous study (Hou et al., 2016). In the initial time, the temperature of corn stover was set at 100 °C and the zone above corn stover was assumed to full of water vapor. Transient calculation was used and the time step was set at 0.025 s^{-1}.

2.6. Analysis

Cellulose and hemicellulose contents of the corn stover were analyzed using a two-step sulfuric acid hydrolysis method (Sluiter et al., 2008a). Oligomers of glucan and xylan were measured according to a one-step sulfuric acid hydrolysis method (Sluiter et al., 2008b). Glucose, xylose, ethanol, acetic acid, furfural and HMF were analyzed on HPLC (LC-20AD pump, RI detector RID-10A, Shimadzu, Kyoto, Japan) with Bio-Rad Aminex HPX-87H column at 65 °C and 0.6 mL/min of 5 mM H_2SO_4 as the mobile phase.

3. Results and discussion

3.1. Heating steam pressure did not affect the enzymatic hydrolysis and SSF performance of the pretreated corn stover

Our previous studies showed that the dry dilute sulfuric acid pretreatment at 175 °C (1.1 MPa) for 5 min gave a satisfactory pretreatment performance (He et al., 2014). Based on the temperature and pressure needed for the optimal pretreatment conditions, the heating steam pressure of 1.5 MPa (198 °C), 1.7 MPa (204 °C), 1.9 MPa (210 °C) and 2.1 MPa (215 °C) was chosen for testing its effect on pretreatment performance. The structural components of the pretreated corn stover were shown in Fig. 1. The cellulose content stabilized around 35% and hemicellulose was around 2% in the pretreated corn stover heated by different steam pressure ranging from 1.5 MPa to 2.1 MPa. Soluble components including oligomer glucose, oligomer xylose, and the monomer xylose showed a slight decreasing with the heating steam pressure increasing, while the glucose content was much stable (shown in Table 2). Furfural and HMF increased marginally, while acetic acid was decreased with steam pressure increasing. Roughly, the sugars in the pretreated corn stover were decreasing and the inhibitors exhibited an increasing tendency with the increased heating steam pressure. The possible reason was that a little more time was needed for the higher pressure steam with higher temperature to transfer its heat to solid corn stover, and led to some extent overcook of the local materials.

Enzymatic hydrolysis and SSF at 25% solids loading was also conducted on the corn stover pretreated using different pressure steam. Enzymatic hydrolysis results in Fig. 1 show that glucose yields of the pretreated corn stover were all above 90% (91.8%, 97.9%, 98.9%, and 98.2%, corresponding to the steam pressure of 1.5, 1.7, 1.9, and 2.1 MPa, respectively). SSF at 25% solids loading was conducted in a 5 L helical ribbon agitated bioreactor using the pretreated and biodetoxified corn stover. The results in Fig. 1 show that a small decrease of the ethanol titer and yield were observed with the heating steam pressure increasing (from 50.8, 48.7, 44.7, to 45.6 g/L with the steam pressure of 1.5, 1.7, 1.9, and 2.1 MPa, respectively, corresponding to the ethanol yield of 88.9%, 83.0%, 77.0% and 76.7%). The increasing acetic acid concentration in the SSF system (from 1.4, 1.7, 2.5, to 3.2 g/L with the steam pressure of 1.5, 1.7, 1.9, and 2.1 MPa, respectively) was responsible for the ethanol titer and yield decreasing (During biodetoxification, the fungus *A. resinae* ZN1 firstly degraded furfural and HMF, and then degraded acetic acid (Ran et al., 2014). As for the higher furan inhibitors in corn stover pretreated by higher pressure steam, the relative more acetic acid left after same biodetoxification period). Taken together, lower pressure steam used for heating the pretreatment gave a relative lower inhibitors content in the pretreated material (4.0 ± 0.7 mg/g DM of furfural and 1.7 ± 0.0 mg/g DM of HMF respectively with 1.5 MPa heating steam pressure compared with 4.1 ± 1.0 mg/g DM of furfural and 3.0 ± 0.2 mg/g DM of HMF respectively with 2.1 MPa heating steam pressure) and a slight higher ethanol yield in SSF at high solids loading.

3.2. Computational fluid dynamics simulation of the pretreatment heating by different pressure steam

In order to clarify the effect of different heating steam pressure on the mixing of solid corn stover and hot steam and the heat transfer from hot steam to corn stover and liquid acid during pretreatment, a simplified CFD model was established to simulate the steam holdup (represented by the conservative gas volume fraction) during dry dilute acid pretreatment in the helical ribbon stirrer agitated reactor. Fig. 2 shows the steam holdup in the solid corn stover was decreasing with heating steam pressure increasing, indicating the more sufficient contact between the steam and the corn stover materials heating with lower pressure steam. The mixing time calculated by the CFD simulation model showed an increasing trend (shown in Table 1). The lower pressure steam with higher density has a higher injection velocity at the same mass flow rate, which makes the hot steam contact with more corn stover materials at once and is much helpful for the hot steam distribution and heat dispersion. This is the reason for the shorter mixing time during pretreatment using the lower pressure steam and might be responsible for the lower inhibitors generation.

Dry dilute acid pretreatment has showed its potential as a distributed pretreatment method embodied in the distributed bioprocessing concept (Zhang et al., 2016). Pretreatment temperature, other than the heating steam pressure is a key parameter for the dry dilute sulfuric acid pretreatment, therefore we could obtain a similar glucan conversion at the same pretreatment condition heating by different pressure steam in spite of a little difference in the inhibitors generation. However, lower pressure heating

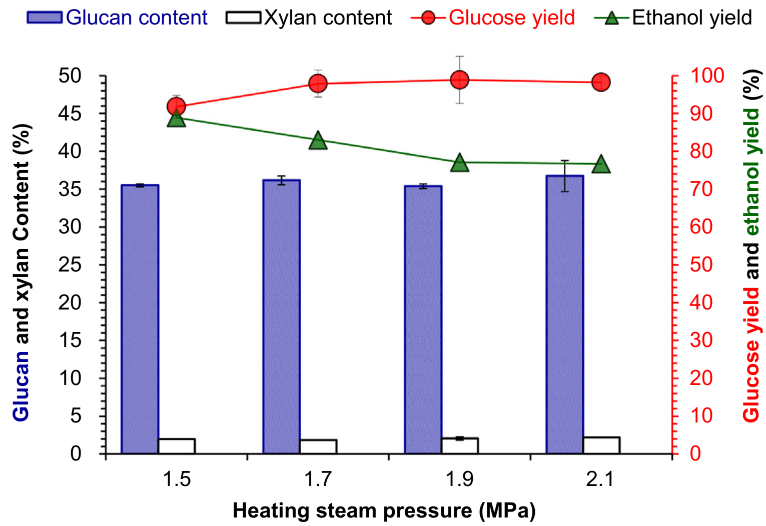
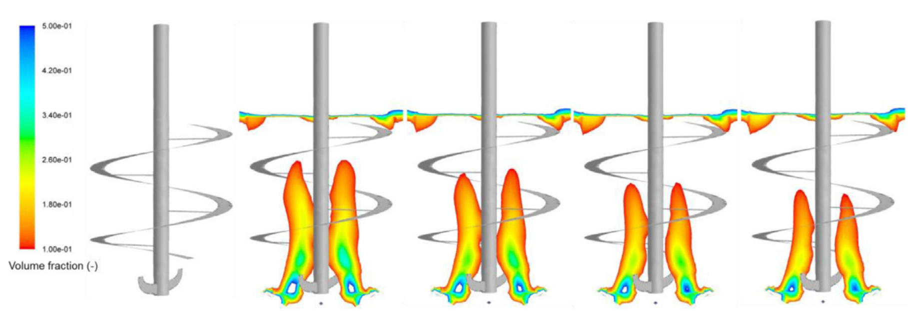


Fig. 1. Changes of structural compositions, glucose and ethanol yield of the corn stover pretreated with different heating steam pressure. Enzymatic hydrolysis conditions: 2.5% (w/w) solids loading, pH 4.8, 20 FPU/g DM, 50 °C, 150 rpm in the shaking water-bath incubator for 72 h; SSF conditions: prehydrolysis with 25% solids loading (w/w) of pretreated and biodetoxified corn stover, cellulase dosage of 15 FPU/g DM, 50 °C, pH 4.8, 150 rpm for 12 h; then SSF with inoculation of *S. cerevisiae* DQ1 at 37 °C, pH 5.0, 150 rpm for another 60 h.

Table 2
Effect of heating steam pressure on the soluble components of the dry dilute sulfuric acid pretreated corn stover.

Heating steam pressure (MPa)	Glucose (mg/g DM)	Xylose (mg/g DM)	Acetic acid (mg/g DM)	Furfural (mg/g DM)	5-HMF (mg/g DM)	O-Glu[a] (mg/g DM)	O-Xyl[b] (mg/g DM)
1.5	21.7 ± 0.3	149.4 ± 1.0	16.1 ± 0.5	4.0 ± 0.7	1.7 ± 0.0	7.6 ± 2.4	25.5 ± 9.0
1.7	23.1 ± 0.0	132.8 ± 0.2	17.1 ± 0.9	4.2 ± 0.1	2.0 ± 0.6	5.3 ± 1.1	20.8 ± 0.0
1.9	22.2 ± 0.3	124.1 ± 1.0	14.8 ± 1.2	4.4 ± 0.1	2.8 ± 0.2	5.6 ± 1.0	17.7 ± 3.6
2.1	21.6 ± 0.1	120.8 ± 0.6	13.7 ± 0.4	4.1 ± 1.0	3.0 ± 0.2	5.9 ± 0.2	18.6 ± 0.5

Corn stover was pretreated at 175 °C for 5 min using 2.5% sulfuric acid at 50 rpm agitation rate. Unit here was defined as micrograms components in per gram dry corn stover.
[a] O-Glu represents the oligomer of glucan.
[b] O-Xyl represents the oligomer of xylan.



(a) Impeller diagram (b) 1.5 MPa steam (c) 1.7 MPa steam (d) 1.9 MPa steam (e) 2.1 MPa steam

Fig. 2. CFD simulation of the steam holdup in the helically agitated pretreatment reactor. (a) Impeller diagram; (b) heating with 1.5 MPa steam; (c) heating with 1.7 MPa steam; (d) heating with 1.9 MPa steam; (e) heating with 2.1 MPa steam. The rheological properties of corn stover in this mixing system were described by power law model. The consistency coefficient Kp (Pa·s^n), the dimensionless power-law index n and the maximum apparent viscosity η_a (Pa·s) at the beginning of pretreatment were 11, 0.188 and 1.08, respectively (Hou et al., 2016).

steam is much more preferred for the small distributed pretreatment centers with respect to the easily-constructing boilers and simple safety precautions compared to the higher pressure steam boilers. In addition, the capital investment for manufacturing 1.5 MPa boilers can be reduced to 80% of that for 2.1 MPa boilers according to the private discussion with Shanghai Huazheng Boiler Manufacture Co, Shanghai, China. This study provided a solid basis for engineering design of the steam boilers used in distributed pretreatment.

4. Conclusion

The effect of different heating steam pressure varying from 1.5, 1.7, 1.9 to 2.1 MPa was tested on the dry dilute acid pretreatment. The results show that both enzymatic hydrolysis and high solids loading SSF of the pretreated corn stover heating by lower pressure steam were satisfying. CFD simulation showed that the high injection velocity of the low pressure steam resulted in a high steam holdup and made the mixing time of steam and solid corn stover during pretreatment much shorter. This study indicated that the relatively low pressure steam was more practical as the heating media for distributed pretreatment.

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