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Assessment and regression analysis on instant catapult steam explosion pretreatment of corn stover

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HIGHLIGHTS

- Instant catapult steam explosion (ICSE) was used to pretreat corn stover.
- The response surface methodology was applied to optimize the process parameters.
- Structural and morphological changes of the pretreated biomass were characterized.
- The ICSE provided a novel strategy for biomass pretreatment with less inhibitors produced.

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ABSTRACT

Instant catapult steam explosion (ICSE) offers enormous physical force on lignocellulosic biomass due to its extremely short depressure duration. In this article, the response surface methodology was applied to optimize the effect of working parameters including pressure, maintaining time and mass loading on the crystallinity index and glucose yield of the pretreated corn stover. It was found that the pressure was of essential importance, which determined the physical force that led to the morphological changes without significant chemical reactions, and on the other hand the maintaining time mainly contributed to the thermo-chemical reactions. Furthermore, the pretreated biomass was assessed by scanning electron microscope, X-ray diffraction and Fourier transform infrared spectra to understand mechanisms underlying the ICSE pretreatment.

1. Introduction

Although fuel ethanol is now being used as an alternative to petroleum-derived transportation fuels (Bai et al., 2008), many controversies have been raised with its current production from sugar- and starch-based feedstocks, which are major sources of food supply. Lignocellulosic biomass, particularly agricultural residues such as corn stover and wheat or rice straw, has garnered worldwide attention as sustainable feedstock for fuel ethanol production, the so-called cellulosic ethanol (Zhao et al., 2012). But unfortunately, many challenges are still on the way for the commercial production of cellulosic ethanol, and the pretreatment of lignocellulosic biomass to render its recalcitrance for efficient enzymatic hydrolysis of the cellulose component is one of them (Himmel et al., 2007).

Among various technologies that have been developed for biomass pretreatment, steam explosion is one of the most commonly employed physico-chemical process, in which lignocellulose is subjected to steam pressure at a period of time for thermo-chemical reactions to occur and hydrolyze hemicelluloses. When the pressure is released, pressure force further destroys the complex composed mainly of cellulose and lignin, making the cellulose component more accessible for cellulase attack (Hendriks and Zeeman, 2009). However, for conventional steam explosion, pressure release is not quick enough since valves with diameters much smaller than that of pressurized vessels are employed, which significantly compromise the de-crystallization effect of the pressure force on biomass, and a longer time is thus required for the pretreatment process with more toxic byproducts produced (Alvira et al., 2010; Zhang et al., 2013).

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In order to address these problems, an innovative explosion pretreatment named instant catapult steam explosion (ICSE) device was developed, in which a piston with the same diameter as the pressured vessel was employed and the duration for pressure release was significantly shortened for more powerful effect on the pretreated biomass. Compared to the time of seconds, even minutes for the de-pressurization process with conventional steam explosion, the ICSE system completed pressure release within 0.1 s (Yu et al., 2012), which consequently exerted much higher explosion power density on the pretreated biomass to de-crystallize its structure more effectively. As a result, much shorter time was required for biomass pretreatment, and toxic byproducts released during the thermo-chemical reactions would be reduced significantly.

In this work, the ICSE pretreatment of corn stover was evaluated by the response surface methodology (RSM) based on the Box–Behnken design. The optimal operation parameters including pressure, maintaining time and mass loading were identified, taking crystallinity index (CrI) of the pretreated corn stover or glucose yield after enzymatic hydrolysis as the response value. Furthermore, the structural and morphological changes of the pretreated corn stover were characterized with scanning electron microscopy, X-ray diffraction and Fourier transform infrared spectra for more understanding of the mechanisms underlying the pretreatment process.

2. Methods

2.1. Feedstock

Corn stover was donated by Jilin Chemical Industry Company Ltd., CNPC, one of the major companies dedicated to the development of cellulosic ethanol in China. The feedstock was collected from the local farmland, dried and comminuted through a 4 mesh screen with sieve size of 4.75 mm (Humbird et al., 2011).

2.2. ICSE pretreatment

A device with catapult explosion structure (QBS-80 SE, Hebi Gentle Bioenergy Co. Ltd., China) was used. The corn stover at the mass loading of 20, 40 and 60 g was fed into the 400 ml chamber, making the uploading per unit volume at 50, 100 and 150 g/L, respectively. The pressure was controlled at 1.5, 2.5 and 3.5 MPa, and after the maintaining time of 10, 50 and 90 s for each of the pressure and uploading, the piston driving device was triggered to release pressure, resulting in an intense explosion within 0.1 s (Yu et al., 2012).

2.3. Enzymatic hydrolysis

All ICSE-pretreated corn stover was collected without washing, and hydrolyzed by cellulases (GENENCOR Accellerase 1500) with a loading of 30 FPU/g substrate. The reaction was conducted at 50 °C for 72 h in the acetate buffer solution of pH 4.8 (Hsu et al., 2010). Then, the samples were centrifuged at 3000 × g for 10 min and filtered through a 0.45 μm syringe filter. The concentrations of glucose and inhibitors including acetic acid, furfural and 5-HMF in the supernatant were determined by HPLC (Waters 410, Waters, MA, USA) with the column (Bio-red Aminex HPX-87H, 300 mm × 7.8 mm, Hercules CA) and Waters 410 refractive detector. A flow rate of 0.4 ml/min was applied with 10 mmol/L H_2SO_4 as the mobile phase.

The glucose yield was calculated by the following equation:

$$\text{Glucose yield} = \text{glucose in the enzymatic hydrolysate}/(\text{cellulose in the feedstock} \times 1.11) \quad (1)$$

All experiments were carried out in duplicate.

2.4. Chemical composition analysis

The chemical composition in the corn stover, including cellulose, hemicelluloses, acid soluble lignin (ASL) and acid-insoluble lignin (AIL), were analyzed by the method from the NREL laboratory analytical procedures (Sluiter et al., 2011). This method contained a two-step acid hydrolyzed process: Firstly, the corn stover was hydrolyzed by 72% (w) H_2SO_4 at 30 °C for 1 h; Then, the reaction mixture was diluted by deionized water and further hydrolyzed with 4% (w) H_2SO_4 in autoclave at 121 °C for 1 h; Finally, the reaction mixture was filtered, and the solid residue was washed and dried to determine the AIL, while the filtrate was collected to analyze the chemical composition of cellulose, hemicelluloses and ASL.

2.5. Fourier transform infrared (FTIR) analysis

The FTIR spectrum of the samples was recorded by the FTIR spectrometer (EQUINOX55, BRUKER, Germany) between 500 and 4000 cm^{-1} at 2 cm^{-1} nominal resolution and 25 °C.

2.6. X-ray diffraction (XRD) analysis

The XRD diffractogram of the samples was obtained by the X-ray diffractometer (D/MAX-2400, RIGAKU, Japan). The lignocellulosic biomass was scanned in the range of 10–80° (2θ) with a step size of 0.02° and step time of 1 s at 40 kV and 100 mA under 25 °C. The crystallinity index (CrI) of corn stover is defined by:

$$\mathrm{CrI} = (I_{002} - I_{\mathrm{am}})/I_{002} \times 100\% \quad (2)$$

where I_{002} is the maximum intensity of crystalline portion at $2\theta = 22.6°$, and I_{am} is the intensity attributed to the amorphous portion at $2\theta = 18.7°$ (Xu et al., 2013).

2.7. Scanning electron microscopy (SEM) analysis

The SEM (Quanta 450, FEI, USA) was used to observe the morphology of corn stover. The solid samples with the high vacuum gold jetting were fixed on the aluminum sample stubs. Images were acquired with a 20 kV acceleration voltage.

2.8. Regression analysis

The Box–Behnken design was chosen to investigate the effect of the pressure, maintaining time and mass loading on the performance of the ICSE pretreatment by the software Design-export 8.0 (Statease, USA, MN). An analysis of variance (ANOVA) was employed to evaluate the fitness of the model.

Glucose yield and CrI as response variables (Y) were fitted in the quadratic polynomial equation:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i<j}^{k} \beta_{ij} x_i x_j + \sum_{i=1}^{k} \beta_{ii} x_i^2 \quad (3)$$

where x_i and x_j are independent variables: pressure (x_1), maintaining time (x_2) and mass loading (x_3), and β_0, β_i, β_{ij} and β_{ii} are intercept effect, linear effect, linear-by-linear interaction and quadratic effect, respectively (Yoon et al., 2012).

3. Results and discussion

3.1. Response surface analysis for CrI and glucose yield

Based on the Box–Behnken design of RSM, 17 experimental runs were performed for the ICSE pretreatment, and CrI and glucose yield are recorded in Table 1. As can be seen, CrI and glucose yield were affected by pressure, maintaining time and mass loading, which were correlated by Eq. (3). The results of ANOVA are given in Table 2. Since glucose yield was analyzed after the enzymatic hydrolysis of the pretreated biomass, its experimental error was accumulated, resulting in higher p-value and lower R^2.

Acetic acid and 5-HMF were detected, but another inhibitor furfuran was not detectable. The RSM analysis was also applied to evaluate the production of acetic acid and 5-HMF, but very high p-values and low R^2 made the model not significant, indicating their production could not be correlated with the pretreatment conditions by Eq. (3). Without doubt, much less acetic and 5-HMF produced during the ICSE pretreatment would benefit microbial fermentations thereafter.

ANOVA indicated that pressure played a dominant role in the ICSE pretreatment, since the p-value as low as 0.0003 was observed for both CrI and glucose yield. On the other hand, the impact of mass loading was not significant since much higher p-values of 0.2328 and 0.7218 were obtained for CrI and glucose yield. Similar results were also observed in the simulation (Fig. 1), in which pressure was the most significant factor affecting both CrI and glucose yield. When the pressure increased from 1.5 to 2.5 MPa, CrI decrease rendered glucose yield improvement. However, when the pressure further increased to 3.5 MPa, no significant impact on CrI was observed, but glucose yield was still improved, suggesting that CrI was not correlated with glucose yield under the high pressure condition for the ICSE pretreatment, as that reported previously in steam explosion (Zhu et al., 2008). More efficient removal of hemicelluloses might be the reason for the improvement of glucose yield due to their role in blocking cellulases from attacking the cellulose component. When corn stover was maintained at high temperature associated with the high pressure, thermo-chemical reactions occurred, since hot water condensed onto the surface of the feedstock as a weak acid attacked hemicelluloses to generate porous structure (Kim et al., 2014), making the explosion thereafter more effective to expose the cellulose component for enzymatic hydrolysis, which would be further discussed in Section 3.2.

The conditions for the ICSE pretreatment were optimized by the RSM model with glucose yield or CrI as the responding value (Table 3). The highest glucose yield of 97.75% was predicted at 3.5 MPa with maintaining time of 90 s, mass loading of 146 g/L and CrI of 34.05%, while the lowest CrI of 32.17% was predicted at 3.45 MPa with maintaining time of 10 s, mass loading of 50 g/L and glucose yield of 75.52%. The reason for this phenomenon was due to the much shorter maintaining time of 10 s, compared to that of 90 s for more efficient degradation of hemicelluloses to improve glucose yield significantly, indicating that the ICSE process destroyed the biomass recalcitrance more effectively, even without enough degradation of hemicelluloses. When the pressure was fixed at 2.5 MPa and 1.5 MPa, the highest glucose yield was predicted to be 79.80% and 54.71%, with CrI of 32.77% and 37.55%, respectively. These optimization results are in accordance with the experimental results in Table 1 and simulation analysis in Fig. 2. Therefore, glucose yield instead of CrI should be used for process optimization with the ICSE system.

3.2. Structural and morphological changes after the ICSE pretreatment

Since pressure was the most important factor for the ICSE pretreatment, maintaining time and mass loading were fixed at 10 s and 150 g/L, respectively, to explore the structural and morphological changes of corn stover under different pressure conditions. The SEM images clearly indicated that the surface of the raw corn stover was compact and smooth presenting recalcitrance to enzymatic deconstruction (Himmel et al., 2007), but the surface cracked at 1.5 MPa, and broke up at 2.5 MPa. As the pressure was increased to 3.5 MPa, the corn stover was split into fine debris, and became more porous. The XRD analysis gave similar results, and CrI decreased from 49.72% with the untreated corn stover to 43.78%, 34.91% and 32.17% for the pretreated samples by the ICSE process at 1.5, 2.5 and 3.5 MPa.

Chemical changes of the corn stover associated with the ICSE pretreatment were evaluated by FTIR. There were no significant difference in the FTIR spectra among the raw feedstock and samples pretreated at 1.5, 2.5 and 3.5 MPa, indicating that the pretreatment, to a large extent, was a physical process. As an exception, absorbance at 1104 cm^{-1} representing the association of cellulose and hemicellulose (Labbe et al., 2005) increased under the high pressure condition, due to the degradation of hemicelluloses under the pretreatment condition (Alvira et al., 2010), which was supported by the component analysis in Fig. 2. The removal of hemi-

Table 1
Experimental designs and results of the pretreatment of corn stover by the ICSE process.

Run	x_1 (MPa)	x_2 (s)	x_3 (g/L)	CrI (%)	Glucose yield (%)	5-HMF (mg/g)	Acetate (mg/g)
1	1.5	10	100	45.25	47.52	2.00	0.00
2	3.5	10	100	35.38	83.63	2.25	5.75
3	1.5	90	100	38.95	43.16	1.50	0.00
4	3.5	90	100	32.52	93.26	2.25	2.00
5	1.5	50	50	41.64	50.26	1.00	22.00
6	3.5	50	50	32.38	85.62	1.50	7.00
7	1.5	50	150	42.99	50.62	1.33	0.00
8	3.5	50	150	38.49	81.72	1.17	3.67
9	2.5	10	50	35.56	54.92	2.00	0.00
10	2.5	90	50	35.66	77.77	1.50	3.00
11	2.5	10	150	37.43	56.99	1.67	0.67
12	2.5	90	150	32.13	87.80	2.33	13.17
13	2.5	50	100	34.78	68.25	2.25	15.50
14	2.5	50	100	36.98	75.11	1.25	13.50
15	2.5	50	100	35.83	73.12	2.25	0.00
16	2.5	50	100	35.89	76.90	2.00	0.00
17	2.5	50	100	34.38	65.20	2.50	3.50

Table 2
ANOVA of the quadratic model for the CrI and glucose yield.

Factor	DF*	CrI (%)			Glucose yield (%)		
		$\beta_0/\beta_i/\beta_{ij}/\beta_{ii}$	*F*-Value	*p*-Value	$\beta_0/\beta_i/\beta_{ij}/\beta_{ii}$	*F*-Value	*p*-Value
		70.1588			−5.3973		
x_1	1	−22.54	45.52	0.0003	34.70	43.68	0.0003
x_2	1	0.0075	10.41	0.0145	−0.0557	6.51	0.038
x_3	1	−0.0301	1.71	0.2328	0.112	0.14	0.7218
x_1x_2	1	0.0214	1.19	0.3118	0.0875	0.73	0.42
x_1x_3	1	0.0238	2.28	0.1748	−0.0213	0.07	0.8019
x_2x_3	1	−0.0007	2.95	0.1298	0.001	0.24	0.6407
x_1^2	1	3.068	15.99	0.0052	−3.5718	0.81	0.3993
x_2^2	1	−0.0004	0.64	0.4491	−0.0008	0.1	0.7619
x_3^2	1	0.0001	0.1	0.766	−0.0004	0.08	0.792
Model	9		8.96	0.0043		5.82	0.015
R^2		0.920			0.882		

* DF: degree of freedom.

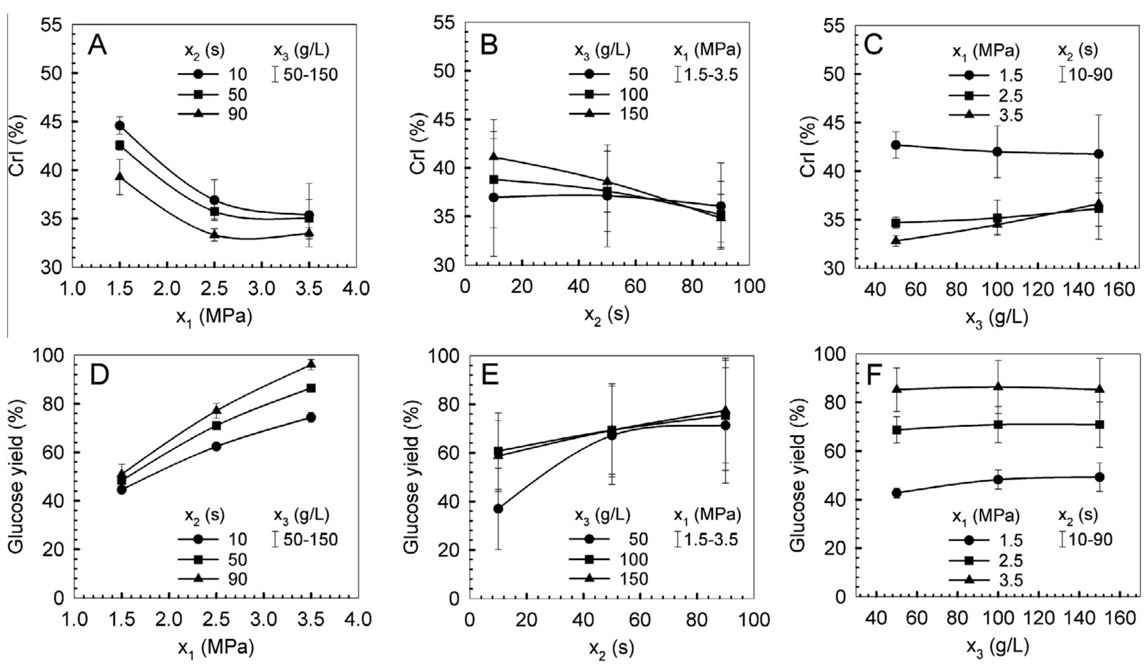

Fig. 1. Simulation of the impact of the ICSE pretreatment conditions (pressure x_1, maintaining time x_2 and mass loading x_3) on CrI (A–C) and glucose yield (D–F). The solid legends represent the averages predicted with the factor illustrated by the bars.

Table 3
Optimization of the ICSE pretreatment conditions based on maximal glucose yield or minimal CrI.

	x_1 (MPa)	x_2 (s)	x_3 (g/L)	Glucose yield (%)	CrI (%)
a	3.50	90	146	**97.75**	34.05
b	3.45	10	50	75.52	**32.17**
c	2.50	90	150	**79.80**	32.77
d	1.50	90	150	**54.71**	37.55

a: based on maximal glucose yield; b: based on minimal CrI; c and d: maximal glucose yield predicted when pressure was fixed at 2.5 and 1.5 MPa, respectively. All the response values are highlighted.

celluloses improved the accessibility of cellulases to the surface of the cellulose component.

3.3. Overall assessment on the ICSE pretreatment

The severity factor log R_0 can be used to evaluate the pretreatment of steam explosion, which reflects the combined impact of

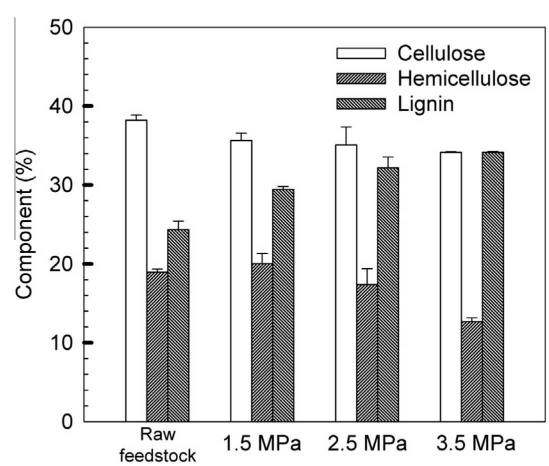

Fig. 2. Component analysis of the raw feedstock and samples pretreated by the ICSE process at 1.5, 2.5 and 3.5 MPa with maintaining time of 50 s and mass loading of 100 g/L.

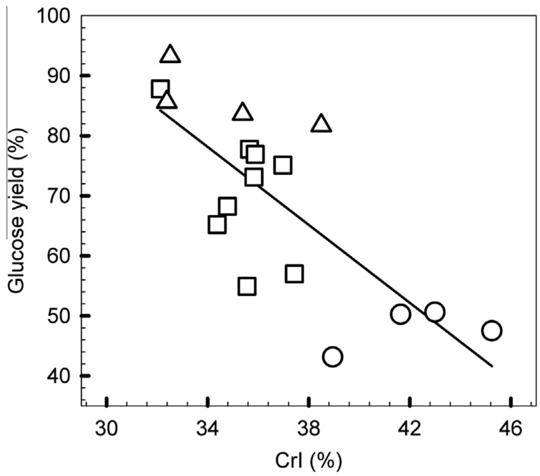

Fig. 3. Correlation of CrI and glucose yield for the ICSE pretreatment under conditions employed in Table 1 (○1.5 MPa, □2.5 MPa, Δ3.5 MPa).

temperature and duration (Vivekanand et al., 2013), but this parameter alone is not suitable for evaluating the ICSE pretreatment, since the maintaining time is much shorter and the pressure release is extremely quick. In fact, the ICSE process can be divided into two stages: the maintaining process at high temperature that fits the severity factor theory, and the pressure release that can be explained by the explosion power density (Yu et al., 2012).

Fig. 3 correlates CrI and glucose yield for the ICSE pretreatment. Roughly, a negative correlation is observed, since cellulases were able to easily access the loose and porous cellulose substrate. High pressure treatment provided powerful explosion force for the biomass and resulted in lower CrI and higher glucose yield. In addition, high temperature associated with the high pressure facilitated the degradation of hemicelluloses as discussed previously. Therefore, for the ICSE process, high pressure is preferred for more effective pretreatment. Unfortunately, employing high pressure is energy-intensive, and in the meantime requires more capital investment for pressurized vessels. Among all setting pressures in this work, 3.5 MPa was enough because glucose yield almost reached 100%. If combined with other pretreatment strategies with acid, alkali or solvents, the ICSE pretreatment would perform well under moderate pressure conditions.

Maintaining time contributed to the destruction of lignocellulosic compact structure by removing most hemicelluloses (Zhang et al., 2012), which was significantly reduced with the ICSE pretreatment, and consequently improved productivity of the system. Biomass loading did not significantly influence the ICSE process in this work, since all biomass in the small chamber was homogenized by the explosion force, indicating that biomass could be pretreated at high mass loading that depends on its density (~150 g/L in this work) to fully explore the capacity of the ICSE system. When it is scaled up, maintaining time would be extended properly to render the mass transfer process for hemicelluloses degradation, but high mass loading would not be compromised significantly for the ICSE process, taking into account of much smaller vessel volume and high explosion power density compared with traditional steam explosion systems.

The traditional steam explosion pretreatment generates many by-products such as acetic acid, furfural and 5-HMF, which are extremely toxic to microbial fermentations (Hendriks and Zeeman, 2009). Thanks to much shorter time for maintenance and quick pressure release, less inhibitory by-products were produced with the ICSE pretreatment. For example, no furfural was detected in this work, both acetic acid and 5-HMF were reduced significantly (Table 1), which inevitably benefits the production of biofuels such as ethanol and bio-based chemicals through microbial fermentations.

4. Conclusions

The ICSE was an effective strategy for corn stover pretreatment. Two processes occurred sequentially: a thermo-chemical process degrading hemicelluloses during the maintenance at high temperature and a physical process destroying the biomass structure by rapid depressurization. Pressure that provides explosion energy played a dominant role in the ICSE process, while mass loading had no significant impact on either CrI or glucose yield, making it possible for the ICSE system to be operated at high solid uploading to improve productivity.

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