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Research paper

Periodic peristalsis enhancing the high solids enzymatic hydrolysis performance of steam exploded corn stover biomass

Zhi-Hua Liu [a, b], Hong-Zhang Chen [a, *]

[a] State Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100190, China
[b] Graduate University of Chinese Academy of Sciences, Beijing 100190, China

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ABSTRACT

Enzymatic hydrolysis beyond 15% solid loading offers many advantages such as increased sugar and ethanol concentrations and decreased capital cost. However, difficult mixing and handling limited its industrialized application. A novel intensification method, periodic peristalsis, had been exploited to improve the high solids enzymatic hydrolysis performance of steam exploded corn stover (SECS). The optimal steam explosion conditions were 200 °C and 8 min, under which glucan and xylan recovery was 94.3% and 64.8%, respectively. Glucan and xylan conversions in periodic peristalsis enzymatic hydrolysis (PPEH) were 28.0–38.5% and 25.0–36.0% higher than those in static state enzymatic hydrolysis with solid loading increasing from 1% to 30%, respectively, while they were 1.0–11.2% and 3.0–9.2% higher than those in incubator shaker enzymatic hydrolysis (ISEH). Glucan and xylan conversion in PPEH at 21% solid loading reached 71.2% and 70.3%, respectively. Periodic peristalsis also facilitated fed-batch enzymatic hydrolysis of which SECS was added completely before transition point. Results presented that PPEH shortened the transition point time from solid state to slurry state, decreased the viscosity of hydrolysis mixture, and reduced the denaturation effect of enzymes compared with ISEH, and hence improve the high solids enzymatic hydrolysis efficiency.

1. Introduction

Biomass refinery has attracted much attention for biofuels production worldwide in response to energy security and environmental concerns [1–3]. Pretreatment and enzymatic hydrolysis are two key unit operations in which biomass is fractionated into fermentable sugars and then used for biochemical conversion processes such as ethanol fermentation [4–6]. Steam explosion (SE) has been recognized as one of the most effective pretreatments [5,7,8]. SE increases the accessible surface area of carbohydrates to enzymes due to the part removal of hemicellulose, the redistribution of lignin, and the separation of fibers, and hence improves the subsequent enzymatic hydrolysis performance.

An important factor in the process economy is the solid loading in the stream entering the hydrolysis step [9–11]. An ethanol concentration of >4% (w/w) is considered to be the benchmark for an efficient distillation in ethanol production from biomass, and thus the corresponding sugar levels of >8% (w/w) are needed in enzymatic hydrolysis. It implies an initial solids loading of >20% (w/w) for most types of biomass [12–14]. The enzymatic hydrolysis operated beyond 15% (w/w) solid loading is generally determined as high solids enzymatic hydrolysis [11,12]. High solids enzymatic hydrolysis offers several advantages including the improved overall productivity, the size reduction of equipment, and the energy usage for heating and cooling [14,15]. Unfortunately, it presents several technical problems. Although sugar and consequently final ethanol concentrations are higher by increasing the solid loading, sugar and ethanol yields decrease due to the mass transfer limitations and the inhomogenous distribution of enzymes and products [12,15]. High solids enzymatic hydrolysis also leads to high initial viscosity, resulting in the difficulty of mixing and handing. Especially the mixing of biomass by shaking in laboratory scale is inadequate and even cannot be conducted at higher solid loading [14,15]. The

Abbreviations: CBU, cellobiase units; DW, dry weight; FPU, filter paper unit; FB-ISEH, fed-batch incubator shaker enzymatic hydrolysis; FB-PPEH, fed-batch periodic peristalsis enzymatic hydrolysis; ISEH, incubator shaker enzymatic hydrolysis; PPEH, periodic peristalsis enzymatic hydrolysis; SEH, static state enzymatic hydrolysis; SE, steam explosion; SECS, steam exploded corn stover; UCS, untreated corn stover.

* Corresponding author. 1 North 2nd Street, Zhongguancun, Haidian District, Beijing 100190, China.
E-mail address: hzchen@ipe.ac.cn (H.-Z. Chen).

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drastically increased viscosity with the increase of solid loading also leads to the increase of power consumption in traditional stirred reactor [16,17]. Thus, an effective mixing method should be exploited to overcome these challenges and improve high solids enzymatic hydrolysis performance.

Fed-batch operating mode has been suggested as a possibility to solve some problems related to high solids enzymatic hydrolysis [14,18]. Fed-batch enzymatic hydrolysis allows time for the solid to liquefy at low solid loading firstly and reduces the products' feedback inhibition effects on enzymes by maintaining a level of free water and facilitating products diffusion. The initial viscosity is low in fed-batch operating mode, so mass diffusion limitations can be minimized or altogether avoided. However, the results of feeding strategy for fed-batch enzymatic hydrolysis are currently still inconclusive [14,18,19]. In order to maintain high sugar conversion and fully exploit the advantages of fed-batch operating mode, how and when to add the solid into the hydrolysis mixture should be evaluated.

Periodic peristalsis was exploited to improve the high solids enzymatic hydrolysis performance of steam exploded corn stover. SE conditions were evaluated and compared. Periodic peristalsis was compared with other intensification methods of static state and incubator shaker especially beyond 15% solid loading. Periodic peristalsis was also used to optimize the feeding strategy and increase the fed-batch enzymatic hydrolysis efficiency. Enzymatic hydrolysis kinetics, enzyme activity, and viscosity transition were analyzed in order to reveal how different intensification methods can be employed to increase the high solids enzymatic hydrolysis efficiency.

2. Materials and methods

2.1. Biomass resource

Corn stover biomass was provided by Chinese Academy of Agricultural Sciences in Beijing, China. Sample was air dried to 5–10% moisture content based on dry weight (DW) for the composition analysis according to the laboratory analysis protocol of National Renewable Energy Laboratory, Colorado, USA (Table 1).

2.2. Steam explosion (SE)

SE was carried out in a 20-L steam explosion reactor connected with a steam generator (Weihai Automatic Control Co. Ltd., Shandong, China). During SE, 1.0 kg sample (DW) was loaded into the steam explosion reactor. Steam supplied by the steam generator was rapidly injected into the steam explosion reactor until holding temperature reached setting values (190, 195, and 200 °C). After 4, 8, or 12 min residence time, sample was exploded into a reception tank. Steam exploded corn stover (SECS) was washed using 15-L water. The solid and liquid fractions were stored at 4 °C for further use.

Pretreatment severity (log R_0) is calculated as described by Overend and Chornet [20]:

$$\log R_0 = t \times \exp[(T - 100)/14.75] \quad (1)$$

where t is the residence time, min; T is the pretreatment temperature, °C.

2.3. Enzymatic hydrolysis

Cellic CTec2 was kindly provided by Novozymes (China) investment Co., Ltd (Beijing, China). Filter paper activity (FPU) of cellulases was 108 FPU/mL, while cellobiase activity of β-glucosidase was 1290 CBU/mL.

A novel intensification method, periodic peristalsis, is developed in this study and the periodic peristalsis enzymatic hydrolysis reactor system is given in Supplementary material A. Periodic peristalsis enzymatic hydrolysis reactor consists of a hydrolysis reactor and four peristalsis arms connecting with several peristalsis balls, which were driven by Motor. Enzymatic hydrolysis of SECS intensified by normal force mode was carried out in periodic peristalsis enzymatic hydrolysis reactor, which was named periodic peristalsis enzymatic hydrolysis (PPEH). PPEH was conducted at 1%–30% solid loading (w/w) with an enzyme loading of 10 FPU/g glucan at 50 °C for 120 h with a low mixing speed of 20 rpm. Samples were adjusted to a pH of 4.8 using a 50 mM citrate buffer.

Enzymatic hydrolysis of SECS intensified by shear force mode was carried out in 250 mL flasks by an incubator shaker at 1%–30% solid loading (w/w) in a 50 mM citrate buffer solution (pH 4.8) by adding an enzyme loading of 10 FPU/g glucan with 100 mL reaction volume, which was named incubator shaker enzymatic hydrolysis (ISEH). ISEH was conducted at 50 °C for 120 h with a high mixing speed of 200 rpm. The procedure of static state enzymatic hydrolysis (SEH) was the same as that of ISEH, but it was conducted without agitation. The feeding strategy of fed-batch enzymatic hydrolysis with different intensification methods was also evaluated (Table 2). As for different feeding strategies, SECS was added at certain time to get a final solid loading of 18%, 21%, 24%, 27% and 30%, respectively, while Cellic CTec2 was added at 0 h enzymatic hydrolysis. Fed-batch enzymatic hydrolysis was conducted with an enzyme loading of 10 FPU/g glucan at 50 °C for 120 h with a mixing speed of 20 rpm for PPEH and 200 rpm for ISEH.

2.4. Enzyme activity assay

Measurement of enzyme activities under different intensification methods was performed. Cellulase activity was determined using Whatman No.1 filter paper strips as substrate while β-glucosidase activity was measured using cellobiose as substrate. The procedure used 1.0 mL of citrate buffer (50 mM, pH 4.8), 0.5 mL

Table 1
Compositions of untreated and steam exploded corn stover biomass.

Experiments	Pretreatment conditions			Composition content (%)					Composition recovery (%)		
	Temperature (°C)	Time (min)	Severity (log R_0)	Glucan	Xylan	Araban	Lignin	Ash	Glucan	Xylan	Lignin
Case 1	–	–	–	36.1 (0.9)	16.5 (0.7)	3.5 (0.5)	17.1 (1.1)	4.2 (0.2)	–	–	–
Case 2	190	8	3.55	48.8 (1.2)	10.4 (1.1)	1.6 (0.6)	27.5 (1.2)	2.9 (0.6)	95.8 (1.6)	73.5 (2.6)	95.9 (3.1)
Case 3	195	8	3.70	49.7 (1.6)	8.1 (0.6)	1.2 (0.4)	30.1 (1.6)	2.5 (0.5)	94.8 (1.5)	70.3 (3.1)	102.9 (2.6)
Case 4	200	4	3.55	49.5 (1.8)	10.7 (1.0)	1.5 (0.8)	26.3 (2.1)	2.7 (0.3)	99.4 (2.1)	71.3 (2.8)	92.6 (3.4)
Case 5	200	8	3.85	53.1 (1.2)	7.1 (0.9)	0.9 (0.2)	31.9 (1.1)	2.3 (0.4)	94.3 (2.3)	64.8 (2.3)	108.2 (2.9)
Case 6	200	12	4.02	52.5 (1.4)	4.7 (0.3)	0.6 (0.6)	35.7 (1.5)	1.8 (0.6)	91.8 (1.9)	56.8 (2.4)	115.5 (2.4)

Note: Case 1 is untreated corn stover (UCS); lignin is acid insoluble lignin (AIL); standard deviations are shown in parentheses.