Industrial Crops and Products 102 (2017) 51–57

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Optimization of ethylenediamine pretreatment and enzymatic hydrolysis to produce fermentable sugars from corn stover

Lei Qin[a,b,1], Xia Li[a,b,1], Jia-Qing Zhu[a,b], Wen-Chao Li[a,b], Hui Xu[a,b], Qi-Man Guan[a,b], Man-Tong Zhang[a,b], Bing-Zhi Li[a,b,*], Ying-Jin Yuan[a,b]

[a] Key Laboratory of Systems Bioengineering (Ministry of Education), School of Chemical Engineering and Technology, Tianjin University, Weijin Road 92, Nankai District, Tianjin 300072, PR China
[b] SynBio Research Platform, Collaborative Innovation Center of Chemical Science and Engineering (Tianjin), Tianjin University, Weijin Road 92, Nankai District, Tianjin 300072, PR China

ARTICLE INFO

Article history:
Received 14 November 2016
Received in revised form 13 March 2017
Accepted 17 March 2017

Keywords:
Lignocellulosic biomass
Corn stover
Ethylenediamine pretreatment
Enzymatic hydrolysis

ABSTRACT

Ethylenediamine (EDA) pretreatment is an effective pretreatment technology to improve enzymatic digestibility of corn stover for the production of fermentable sugars. In this study, key pretreatment parameters were identified and optimized to improve enzymatic digestibility of corn stover. We found that agitation and biomass stack height during pretreatment had significant impacts on enzymatic digestibility. Response surface experiment showed that optimal condition to achieve maximum total sugar enzymatic yield was 150 °C and 80 mL EDA/100 g corn stover. Under this condition, glucose yield was greater than 90% in enzymatic hydrolysis at 1% glucan loading. Optimized temperature to minimize residual EDA in pretreated corn stover was 200 °C. Two-stage pretreatment was carried out to maximize both sugar yields and EDA removal, in which glucose and xylose enzymatic yields reached 92% and 70% respectively at 1% glucan loading, and EDA residue reduced to 27 g/kg corn stover. With the optimal enzyme loadings (both enzymes of Ctec2 and Htec2 were loaded at 30 mg protein/g glucan), glucose and xylose yields at 6% glucan loading reached 81% and 58%, respectively.

1. Introduction

Lignocellulosic biomass, such as corn stover, has been recognized as a potential sustainable resource. The amount of sustainably collected corn stover is estimated to be 80–100 million dry tons annually on the earth (Kadam and Mcmillan, 2003). Corn stover can be converted into fermentable sugars and thus various fermentation products through biochemical process, including pretreatment, enzymatic hydrolysis and fermentation (Himmel et al., 2007; Hansen et al., 2014). Pretreatment is a key step to enhance enzymatic digestibility of corn stover via breaking down recalcitrant cell wall structure and increasing accessibility of cellulose. The choice of pretreatment method has significant impact on overall conversion, biorefinery costs and most other processing decisions (Yang and Wyman, 2008).

Several pretreatment methods have been applied to improve the enzymatic hydrolysis of lignocellulose, such as dilute acid, steam explosion, alkaline, and ionic liquid pretreatment (Kataria et al., 2016; Liu et al., 2016; Haykir and Bakir, 2013; Sundaram and Muthukumarappan, 2016). Among these methods, ammonia-based pretreatment, such as ammonia fiber expansion (AFEX) pretreatment, was one of the leading technologies with potential of commercial application in the biorefinery industry (Li et al., 2010; da Costa Sousa et al., 2016). The major barrier of this method against commercialization is the considerable operating pressure (Sendich et al., 2008), that gives rise to elevated facility costs and security risks. As an alternative method, ethylenediamine (EDA) pretreatment can be operated at ambient pressure and provides biomass of high enzymatic digestibility. Enzymatic conversion of cellulose after EDA pretreatment was higher than 80% without optimization (Qin et al., 2015). During EDA pretreatment, part of crystalline cellulose transforms into amorphous cellulose, and lignin is degraded partially and relocated on the surface of biomass with ether bonds in lignin-hemicellulose complex cleaved (Qin

* Corresponding author at: Key Laboratory of Systems Bioengineering (Ministry of Education), School of Chemical Engineering and Technology, Tianjin University, Weijin Road 92, Nankai District, Tianjin 300072, PR China.
E-mail addresses: qinlei@tju.edu.cn (L. Qin), lixia01@tju.edu.cn (X. Li), zjq0519qing@163.com (J.-Q. Zhu), liwenchao@tju.edu.cn (W.-C. Li), xuhui0723@tju.edu.cn (H. Xu), guanqiman@126.com (Q.-M. Guan), mantong1021@163.com (M.-T. Zhang), bzli@tju.edu.cn (B.-Z. Li), yjyuan@tju.edu.cn (Y.-J. Yuan).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.indcrop.2017.03.026
0926-6690/

et al., 2015). In comparison to other pretreatments, such as dilute acid pretreatment, EDA pretreatment has several potential advantages: it can be implemented without water addition (a dry-to-dry process), that reduces energy consumption during pretreatment (Pan et al., 2016) and avoids solid-liquid separation after pretreatment; it was carried out at ambient pressure, that reduces investment and operation cost; it preserves hemicellulose and does not produce furans which inhibit fermentation.

EDA is evaporated simultaneously with pretreatment reaction so that EDA can be recycled. To reduce refinery cost, EDA should be removed from biomass completely. Previous study suggested that temperature and EDA loading in pretreatment affected EDA removal and enzymatic digestibility of biomass (Qin et al., 2015). In fact, other factors during pretreatment may potentially affect enzymatic digestibility and EDA removal, such as residence time, moisture loading, biomass filling ratio and agitation (Murnen et al., 2007; Zhang et al., 2011; He et al., 2014). In order to remove the EDA residue in pretreated biomass and improve biomass enzymatic digestibility, crucial factors and optimal conditions in pretreatment and enzymatic hydrolysis should be investigated.

In this study, we investigated the effect of several parameters on enzymatic digestibility and EDA removal in EDA pretreatment. The optimal pretreatment condition and optimal enzyme loadings were studied for achieving maximum sugar yields. Two-step pretreatment process was designed based on the understandings about EDA pretreatment to obtain both high sugar yields and EDA removal.

2. Materials and Methods

2.1. Materials

Corn stover used in this study was collected from the suburb of Tianjin, China, and then was milled and screened. The fractions between 20 and 80 meshes were collected and then air dried at room temperature. Air-dried CS had an average moisture content of 5%. EDA (≥99%) was purchased from Yuanli Co. (Tianjin, CN). The commercial enzymes used in this study are Cellic Ctec2 (182 mg protein/mL) and Cellic Htec2 (198 mg protein/mL), which were gifted by Novozymes (Beijing, CN).

2.2. Pretreatment

CS was filled into 1-L beaker and mixed with the specific loadings of EDA (40–80 mL/100 g CS). No water was added unless otherwise specified. The beaker covered with a piece of aluminum foil, was put into an electric oven (with a fume hood at the air outlet) set at designated temperature. Timing started when the beaker was put into the oven. After a period of residence time (including heating-up period), aluminum foil was taken off to evaporate EDA. During the evaporation process, the mixture was periodically taken out of the oven and stirred manually. Pretreated CS was then cooled down at room temperature, preserved in hermetic bags and subjected to composition analysis and enzymatic hydrolysis. Without otherwise specified, default pretreatment conditions were: temperature 140 °C, EDA loading 80% (v/w), moisture loading 5% (w/w), residence time 20 min, drying time 40 min, biomass stack height 5 cm, agitating every 10 min, and ambient pressure.

2.3. Enzymatic hydrolysis

Enzymatic hydrolysis was conducted with 20 mL reaction volume in 100-mL Erlenmeyer flasks at 50 °C and 200 rpm. 50 mM citrate buffer (pH 4.8) with 40 mg/L tetracycline was used in enzymatic hydrolysis. Without otherwise specified, default hydrolysis conditions were: 1% glucan loading, 20 and 10 mg protein/g glucan of Cellic Ctec2 and Htec2, respectively, and hydrolysate samples withdrawn at 72 h. After enzymatic hydrolysis, samples were centrifuged at 12,000 rpm for 10 min to separate hydrolysates from solid residues. The hydrolysates were frozen at −20 °C for subsequent analysis.

Table 1
Central composite design for the optimization of EDA pretreatment.

Parameters	Coded factor levels				
	−1.414	−1	0	1	1.414
Temperature (°C)	123.8	130	145	160	166.2
EDA loading (mL/100 g CS)	31.7	40	60	80	88.3

Table 2
Central composite design for the optimization of enzyme loading in enzymatic hydrolysis.

Parameters	Coded factor levels				
	−1.414	−1	0	1	1.414
Cellic Ctec2 (mg protein/g glucan)	5.9	10	20	30	34.1
Cellic Htec2 (mg protein/g glucan)	5.9	10	20	30	34.1

2.4. Analytical methods

Composition of CS (dry basis), including glucan, xylan, acid-insoluble lignin (AIL) and acid-soluble lignin (ASL), was determined by two-step acid hydrolysis method following the Laboratory Analytical Procedure (LAP) of the National Renewable Energy Laboratory (Sluiter et al., 2008). Glucose and xylose were analyzed by HPLC with an Aminex HPX-87H organic acid column (Bio-rad, Hercules, CA) and a refractive index detector. Mobile phase was 5 mM sulfuric acid in deionized water filtered through 0.22 μm filter. Operating condition for the HPLC column was 65 °C with mobile phase flow rate of 0.6 mL/min. ASL was estimated by UV spectrophotometer at 210 nm according to LAP method. The composition of raw CS material is: 40.2% glucan, 26.8% xylan, 21.3% AIL and 3.0% ASL.

2.5. Response surface methodology

Response Surface Methodology was used to optimize the pretreatment condition and enzyme loading by software Design-Expert 8.0 (Stat-Ease Inc., US) using rotatable central composite statistical designs ($\alpha = 1.414$), as shown in Tables 1 and 2. Center values and intervals were decided according to previous experiences. The central design point was conducted in quintuplicate to give a total of 13 experiments (Additional file 1: Tables S1 and S3).

3. Results and discussion

3.1. The underlying factors in pretreatment

To improve enzymatic digestibility of pretreated CS, we investigated several key factors in EDA pretreatment process. Besides temperature and EDA loading as previous report (Qin et al., 2015), effects of feedstock stack height, agitation, vacuum degree, feedstock moisture content and residence time were taken into account. Agitation played a crucial role in pretreatment. Pretreatment without the intermittent agitation in beaker resulted in the burnt and carbonized biomass (Additional file 1: Fig. S1), unless the stack height was less than 3 cm. This is probably because the reaction between biomass and EDA was exothermic, in addition that EDA was difficult to be evaporated out of the beaker without any agitation due to its high relative vapor density (2.07) compared with air, that caused temperature in biomass exceeding the ignition point of biomass (about 260 °C) (Liu et al., 2015). We found