Industrial Crops & Products 113 (2018) 348–357

Contents lists available at ScienceDirect

Industrial Crops & Products

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

Comprehensive evaluation of combining hydrothermal pretreatment (autohydrolysis) with enzymatic hydrolysis for efficient release of monosaccharides and ferulic acid from corn bran

Kankan Jiang[a,b], Lulu Li[a], Liangkun Long[a], Shaojun Ding[a,*]

[a] The Co-Innovation Center of Efficient Processing and Utilization of Forest Resources, Jiangsu Key Lab for the Chemistry & Utilization of Agricultural and Forest Biomass, College of Chemical Engineering, Nanjing Forestry University, Nanjing, 210037, Jiangsu, China
[b] Jiangsu Key Laboratory for Bioresources of Saline Soils, Yancheng Teachers University, Yancheng, 224051, Jiangsu, China

ARTICLE INFO

Keywords:
Corn bran
Autohydrolysis
Enzymatic hydrolysis
Monosaccharide
Ferulic acid

ABSTRACT

The combination of hydrothermal pretreatment (autohydrolysis) and enzymatic hydrolysis was comprehensively evaluated for the efficient release of monosaccharides and ferulic acid from corn bran. Arabinan was depolymerized and solubilized more easily during autohydrolysis compared to xylan, esterified ferulic acid, and the acetyl group. Also, the enzymatic xylose yield showed strong linear correlation with arabinan, ferulic acid, and acetic acid content in autohydrolysis residues while correlations between enzymatic glucose yield and hemicellulose contents were separated into two stages with different slopes. The addition of a few debranching enzymes to commercial cellulase and xylanase only slightly enhanced enzymatic hydrolysis of autohydrolysis residues, whereas an enzyme blend from *Aspergillus oryzae* and *Eupenicillium parvum* showed a significant synergistic effect. Desirable combined hydrolysis yields of glucose (72.26%), xylose (75.87%), arabinose (76.95%), and ferulic acid (74.13%) were obtained after autohydrolysis at 165 °C for 40 min and subsequent hydrolysis by an equal mixture blend produced by *A. oryzae* and *E. parvum* at an enzyme loading dosage of 14.1 mg protein/g dry destarched corn bran.

1. Introduction

Corn is one of the three most important food and industrial crops in the world. China is one of the world's two largest producers of corn with approximately 218 × 10^6 t of corn grown in 2016, of which more than 10% is allocated for the manufacture of food products (Liu and Guo, 2013). With a cautious estimation, 5% (by weight) of the corn is separated as corn bran during the process of obtaining corn starch. The recovered corn bran has low value and is often used for animal feed alone or in combination with corn germ cake or meal (Rose et al., 2010). Corn bran is mainly composed of arabinoxylan, comprising up to 56% of the biomass dry matter. Furthermore, it contains approximately 20% cellulose and has a high content of ferulic acid (approximately 3%) (Rose et al., 2010). Therefore, corn bran can serve as a low-cost organic source of sugars and natural ferulic acid production.

One of the primary requirements in the utilization of corn bran is to achieve an efficient decomposition of corn bran into monosaccharides and ferulic acid. However, the complete enzymatic hydrolysis of corn bran is still a challenge because of its native recalcitrant properties (Appeldoorn et al., 2010; Faulds and Williamson, 1995). The recalcitrance could be attributed to a combination of several factors. First, corn bran has an exceptional rigid and tight exterior that leaves it virtually impenetrable to enzymes. Second, up to 70% of the xylopyranosyl moieties in arabinoxylan are heavily substituted with various components, such as α-L-arabinofuranosyl, D-galactopyranosyl, D-glucuronyl, acetyl residues, and ferulic acid (Nghiem et al., 2011; Saha 2003). This heterogeneous arabinoxylan is crosslinked through covalent linkages between arabinofuranosyl residues and ferulic acid, which makes it more difficult for even the correct enzymes to catalyze complete hydrolysis (Dodd and Cann, 2010). In addition, corn bran contains lignin (10–14%) and structural proteins (5%). It has been suggested that the lignin and structural proteins also participate in intermolecular interactions with arabinoxylan through diferulate cross-linking, giving rise to a highly complex network of heterogeneous molecules.

Therefore, in order to enhance the susceptibility and substantially release the monosaccharides and ferulic acid from corn bran, pretreatment before enzymatic hydrolysis seems indispensable at present. Furthermore, a full complement of cellulolytic and hemicellulolytic enzymes would be required to cooperatively act in order to completely release the monosaccharides and ferulic acid from pretreated corn bran.

* Corresponding author.
E-mail address: dshaojun@njfu.edu.cn (S. Ding).

https://doi.org/10.1016/j.indcrop.2018.01.047
Received 19 September 2017; Received in revised form 16 December 2017; Accepted 19 January 2018

To date, the reported corn bran pretreatment methods include hydrothermal pretreatment, acidic or alkaline pretreatment, and steam explosion technology (Agger et al., 2011; Bonnin et al., 2002; Dien et al., 2006; Grohmann and Bothast, 1997; Saulnier et al., 2001; Zhao et al., 2014). Among all of the pretreatment methods, hydrothermal pretreatment (autohydrolysis) is relatively simple, environmentally friendly, and cost effective (Batalha et al., 2015; Cara et al., 2012). However, this pretreatment method also causes side effects, such as the production of degraded compounds, including furfural and acetic acid, especially at extreme processing conditions (Baêta et al., 2015; Zhang et al., 2013a,b). A few experiments with autohydrolysis pretreatment and enzymatic hydrolysis of corn bran have been reported (Bonnin et al., 2002; Dien et al., 2006), but details on the solubilization and degradation of hemicellulose and the chemical composition changes in solid residues with various autohydrolysis pretreatment severity have not been well understood. The effect of autohydrolysis pretreatment severity on subsequent enzymatic hydrolysis of autohydrolysis products (the solid and liquid fractions) has not been comprehensively elucidated. Additionally, the commercial cellulase/hemicellulases were ineffective for enzymatic saccharification of corn bran because of the lack or insufficiency of some key enzyme constituents (Agger et al., 2010). The proper pretreatment conditions and a more efficient enzyme cocktail are still needed to be designed in the pursuit of full component valorization of corn bran, particularly with respect to glucose, xylose, arabinose, and ferulic acid.

In this paper, the combination of hydrothermal pretreatment and enzymatic hydrolysis was thoroughly investigated with the aim of maximizing monosaccharide and ferulic acid release from corn bran. To this end, the first step of this work was to hydrothermally treat corn bran to formulate a solubilization model of hemicelluloses of corn bran based on the combined severity (CS) factor. Second, crude enzyme preparations from *Eupenicillium parvum* 4–14 and *Aspergillus oryzae* were blended for the synergetic hydrolysis of autohydrolyzed corn bran for the efficient release of monosaccharides and ferulic acid. The objective of this work was to complete the information about the influence of the autohydrolysis and enzymatic hydrolysis on the efficient decomposition of corn bran into monosaccharides and ferulic acid.

2. Materials and methods

2.1. Materials

Corn bran was collected from Nanyang, Henan Province, China. Destarched corn bran was prepared by treating raw material with amylase and papain according to the method used by Rose and Inglett (2010) with modifications. Amylase and papain were purchased from Imperial Jade Bio-Technology Co., Ltd. (Ningxia, China). The commercial enzymes of cellulase, β-glucosidase, and xylanase were purchased from Sigma Chemicals (St. Louis, MO, USA). Arabinofuranosidase and acetyl xylan esterase were purchased from Megazyme (Bray, Ireland). Ferulic acid esterase from *Myceliopthora thermophila* ATCC 42464 was recombinantly expressed in *Pichia pastoris* strain X33, which was kindly provided by Prof. Christakopoulos at Luleå University of Technology in Sweden (Antonopoulou et al., 2017). Crude enzymes were produced by fungal strains using modified Mandels' medium with corn bran as the carbon source under solid state fermentation following the method used by Long et al. (2016). All of the other chemicals used in the study were analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). *Eupenicillium parvum* 4–14 was isolated from soil (Nanjing, China) and deposited in the China Center for Type Culture Collection (CCTCC) (Long et al., 2016). *Aspergillus oryzae* 2011 was obtained from the China Center of Industrial Culture Collection (CICC). *Thielavia heterothallica* D-76003, *Trichoderma reesei* D-86271 (Rut C-30), and *Thermomyces lanuginosus* D-96488 were obtained from the VTT Culture Collection (VTTCC, Finland). *Myceliopthora thermophila* ATCC 42464 was supplied by the American Type Culture Collection (ATCC, USA).

2.2. Autohydrolysis processing

Autohydrolysis was conducted in duplicate in a stainless steel batch reactor (model YRG2-10 × 1.25 L, ZhengJie Technology and Development Co., Ltd., Nanjing, China). The combined severity factor (CS) was used to characterize the pretreatment intensity by coupling the reaction conditions of time and temperature into one single variable. The CS factor was calculated by the following equation (Overend and Chornet, 1987):

$$CS = \log\{t \cdot \exp[(T_H - T_R)/14.75]\},$$

where t is reaction time (min); T_H is the target temperature (°C); T_R is a reference temperature (most often 100 °C); and the value of 14.75 is present as an empirical parameter related to the activation energy (pseudo first-order kinetics).

Destarched corn bran (30 g) and ultrapure water (300 mL) were mixed in reactors and then immersed in an oil bath. The autohydrolysis was conducted at different temperatures (155 °C, 165 °C, and 175 °C) for different times (10, 20, 30, and 40 min, excluding heating and cooling periods) based on a preliminary experiment. The rotation speed of the reactors during autohydrolysis was approximately 10 rpm. The

Table 1
The content of carbohydrates, esterified ferulic acid, and acetyl group in destarched corn bran and autohydrolysis residues.

Destarched corn bran or autohydrolysis residue	CS[a]	Cellulose (%)	Xylan (%)	Arabinan (%)	Acetic acid (%)	Ferulic acid (%)	Acid insoluble lignin (%)	Ara.[b]/Xyl.[c]	Ace.[d]/Xyl.
Destarched corn bran	–	22.50	30.50	16.62	4.20	2.06	9.06	0.54	0.14
155 °C, 10 min	6.03	24.38	29.43	15.95	3.25	1.90	9.80	0.54	0.11
155 °C, 20 min	6.72	25.85	29.74	14.41	3.17	1.85	9.83	0.49	0.11
155 °C, 30 min	7.13	27.42	28.76	12.87	3.16	1.82	11.17	0.45	0.11
155 °C, 40 min	7.42	29.66	27.93	11.00	3.11	1.51	12.73	0.39	0.11
165 °C, 10 min	6.71	25.64	29.30	14.33	3.33	1.77	10.27	0.49	0.11
165 °C, 20 min	7.40	29.58	27.74	11.78	3.06	1.59	12.70	0.43	0.11
165 °C, 30 min	7.81	36.63	24.08	8.00	2.60	1.06	15.87	0.33	0.11
165 °C, 40 min	8.10	43.61	19.52	6.13	2.14	0.71	17.97	0.31	0.11
175 °C, 10 min	7.39	37.51	22.22	7.26	2.59	0.97	15.03	0.33	0.12
175 °C, 20 min	8.08	51.18	12.08	2.56	1.48	0.28	21.37	0.21	0.12
175 °C, 30 min	8.49	54.31	9.24	1.44	1.12	0.16	20.87	0.16	0.12
175 °C, 40 min	8.77	53.95	8.97	1.27	1.01	0.15	23.33	0.14	0.11

[a] CS, combined severity.
[b] Ara., arabinan.
[c] Xyl., xylan.
[d] Ace., acetic acid.