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Effect of acetylation/deacetylation on enzymatic hydrolysis of corn stalk

Wei Jiang [1], Huadong Peng [1], Hongqiang Li, Jian Xu [*]

National Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100190, China

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ABSTRACT

Effect of degree of acetylation (DA) within corn stalk (CS) on the enzymatic hydrolysis was investigated. By acetylation and deacetylation treatment, CS with different DA from 0.34% to 11.20% was prepared. The analysis of chemical composition by two-step acid hydrolysis and characteristic functional groups via FTIR implied that the structure of the three major components (cellulose, hemicellulose and lignin) in the treated CS was not changed noticeably. The correlation between enzymatic yield and DA was navigated well by the nonlinear curve fit with Adj. R-Square greater than 0.90. The DA was proved to be a crucial barrier to the digestibility of CS. The efficiency of the enzymatic hydrolysis was negatively correlated with DA value.

1. Introduction

Although a rapid progress has been made on enzymatic hydrolysis of lignocelluloses, mechanisms correlating the deconstruction of plant cell wall matrix with its components and structures are not yet fully clarified. The objective of understanding the effect of the key structural features within biomass on the hydrolysis process of polysaccharides is beneficial to reducing the treatment severity in various kinds of physical, chemical and biological pretreatments, which are often used to damage the natural recalcitrance of lignocelluloses [1,2]. Some structural features significantly affecting the enzymatic hydrolysis efficiency have been identified, such as degree of acetylation (DA), lignin content, crystalline index, specific surface area, degree of polymerization, etc [3,4].

As one of the most important structural features of biomass, DA has attracted a lot of attention since 1990. By investigating the effect of DA on the enzymatic digestibility of acetylated oatspelts xylans with DA from 0.26 to 1.67 mol acetyl groups per mole of anhydro-xylose units, Mitchell et al. [5] found that the enzymatic digestibility was dramatically affected by DA. Rivard et al. [6] prepared the cellulose, starch, xylan samples with various DA and observed significant reductions in anaerobic biodegradability and enzymatic hydrolysis at substitution levels between 1.2 and 1.7. Kong et al. [7] deacetylated wood by various alkali metal hydroxide solutions at different alkali/wood ratios. It was found that the sugar yield in enzymatic hydrolysis was directly associated with acetyl groups, but not the swelling feature. Chang and Holtzapple [8] reported that acetyl groups were less important than lignin content and crystallinity index in biomass bioconversion. Later in 2006, Kim and Holtzapple [3] found

* *Corresponding author*. Tel./fax: +86 10 8254 4852.
E-mail addresses: jxu@home.ipe.ac.cn, super_xujian@yahoo.com (J. Xu).
[1] Both authors contributed equally to this work.
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Table 1 – Scheme of deacetylation/acetylation process on CS.

Sample	Solid to liquid ratio	Acetic anhydride (mL)	NaOH (mL)	Water (mL)	Time (h)	Temperature (°C)
A1	1: 20	200	–	0	0.5	90
A2	1: 20	200	–	0	2	90
A3	1: 20	200	–	0	4	90
A4	1: 20	200	–	0	8	90
Raw	–	–	–	–	–	–
D1	1: 20	–	4	196	24	25
D2	1: 20	–	16	184	24	25
D3	1: 20	–	48	152	24	25
D4	1: 20	–	80	120	24	25

A1~A4: acetylation with acetic anhydride; D1~D4: deacetylation with 1% NaOH.

that acetyl groups could be removed much easier than lignin. Selig et al. [1] observed that the acetyl groups and xylan removal can enhance not only the initial hydrolysis rates of xylan and glucan, but also the overall conversion extent.

Although progresses obtained from the above studies could improve the understanding of the effect of acetyl groups on the bioconversion, more detailed mechanism on how the acetyl groups affected the whole enzymatic hydrolysis process still needs to be further investigated.

In the present work, not only deacetylation was carried out on CS as other studies did, but also acetylation was included to prepare samples with different gradients of DA in order to investigate the relationship between acetylation degree and efficiency of biomass enzymolysis. Analysis of chemical composition and characteristic functional groups were used to reflect variation of major structures of CS in the deacetylation/acetylation process. Enzymatic hydrolysis was employed to testify that whether DA was crucial to the bioconversion process or not.

2. Material and methods

2.1. Feedstock preparation

Maize (*Zea mays* L., 1# Jiyuan) was grown from May to October, 2011 at Yanqing County (115°97′E Longitude, 40°45′N Latitude) of Beijing, China. After maize ear was harvested, corn stalk (CS) was collected and air-dried, which was then milled to ≤ 2 mm with a grinder (RT-34S, Beijing Kun Jie Yu Cheng Machinery Co., Ltd.). To avoid the effect of monosaccharides remained in the CS on the analysis, the milled CS was washed thoroughly with tap water. It was then dried at 45 °C overnight and sealed into plastic bags for the following experiments.

2.2. Acetylation/deacetylation process

In both acetylation and deacetylation process, 10 g dried CS was used. As shown in Table 1, acetylation was carried out as follows: Dried CS was mixed with the acetic anhydride as the set solid to liquid ratio (SLR) of 1: 20, 150 rpm at 90 °C for 0.5 h, 2 h, 4 h, 8 h.

Deacetylation process: Dried CS was mixed with different amount of NaOH as the set SLR of 1: 20, 150 rpm at 25 °C for 24 h. The final NaOH concentration was 0.005, 0.02, 0.06, 0.1 mol L^{-1}, respectively.

The treated CS from both processes was then washed with tap water until the eluant was neutral. It was then dried at 45 °C before being milled to 1 mm for the following experiments. The acetylated CS was labeled as A1, A2, A3, A4, respectively, based on different treatment time. The deacetylated CS was named as D1, D2, D3, D4, according to the NaOH concentration employed.

2.3. Enzymatic hydrolysis

The detailed procedure of enzymatic hydrolysis was the same as in our previous study [9]. The acetylated/deacetylated CS of 2 g (Dry Matter) was employed in the enzymatic hydrolysis. Cellulase purchased from the Zesheng Bioengineering Technology Co., Ltd. was the same as used in Li and Xu's study [10]. The filter paper activity (FPA) and β-glucanase activity were 46.65 ± 0.85 IU FPA cm^{-3} and 4.97 ± 0.14 IU cm^{-3}, respectively. The enzymatic hydrolysis yield was calculated according to Eq. (1).

$$Y_E(\%) = C_G \times V_E \times (9/10)/M_C \times 100 \quad (1)$$

Y_E: Enzymatic yield of the theoretical, %;
C_G: Concentration of glucose, $g\ L^{-1}$;
V_E: Volume of the enzymatic reaction mixture, L;
M_C: Mass of cellulose in treated or raw CS, g;
(9/10): Conversion coefficient of glucose to cellulose.

2.4. Analytical methods

2.4.1. Chemical composition

The chemical composition of treated/raw CS was analyzed according to the procedure from the National Renewable Energy Laboratory (NREL) [11]. The structural polysaccharides of CS were broke down into sugar monomers by a two-step sulfuric acid hydrolysis process. The concentration of sugar monomers, acetic acid were quantified by HPLC (Agilent 1260 Infinity, USA) via a refractive detector. A Hi-Plex H column (300 × 7.7 mm) was used at 65 °C with H_2SO_4 of 0.005 mol L^{-1} as the eluant at a flow rate of 0.6 $cm^3\ min^{-1}$. The Klason lignin was determined gravimetrically by subtracting the ash content from the solid residue obtained from acid hydrolysis. The content of acetyl groups was regarded as one component of the CS which was calculated based on Eq. (2).

$$DA(\%) = M_A/(M_P \text{ or } M_R) \times 100 \quad (2)$$