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Hydrolyzability of xylan after adsorption on cellulose: Exploration of xylan limitation on enzymatic hydrolysis of cellulose

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ABSTRACT

During pretreatment of lignocellulosic materials, the dissolved xylan would re-adsorb on cellulose, and then inhibits the cellulose hydrolysis by cellulases. However, the hydrolyzability of xylan adsorbed on cellulose is not clear. In this work, the adsorption behavior of xylans on celluloses and the hydrolysis of adsorbed xylan by xylanase (XYL) were investigated. The results indicated that the adsorption of beechwood xylan (BWX) and oat spelt xylan (OSX) on Avicel was conformed to Langmuir-type adsorption isotherm. Higher ion strength increased the adsorption of BWX on Avicel, but not that of OSX. Both BWX and OSX adsorbed on Avicel and corn stover after dilute acid pretreatment (CS-DA) could be hydrolyzed by XYL. Compared to OSX, BWX adsorbed on cellulosic materials could be more easily hydrolyzed by XYL. Thus, supplementation of XYL could hydrolyze the xylan adsorbed on cellulose and potentially improved hydrolysis efficiency of lignocelluloses.

1. Introduction

Lignocellulosic biomass has been recognized as a potential sustainable source of mixed sugars for the production of biofuels and biochemicals (Himmel et al., 2007). Highly efficient conversion of carbohydrates in biomass into fermentable sugars still represents a bottleneck (Kumar & Wyman, 2009; Wyman, 2007). There are some key limiting factors during enzymatic degradation of cellulose, e.g. substrate recalcitrance, hydrolysate limiting, hemicellulose and lignin hindrance, etc. It has been reported that xylan, hemicellulose in hardwoods and annual plants, is closely associated to the surface of the stiff cellulose crystallite forming the microfibril network in plant cell walls (Dammström, Salmén, & Gatenholm, 2009; Ding & Himmel, 2006; Himmel et al., 2007). The xylan backbone has an affinity to the cellulose surface and adsorb irreversibly on cellulosic surfaces, and this character plays an important role in biosynthetic processes in plants and papermaking industrial processes (Köhnke, Östlund, & Brelid, 2011).

Adsorption of xylan on cellulose is dependent on the structure and concentration of xylan, pH of the solution, time and temperature, and the nature of the cellulose fibers (Meller, 1965). Adsorption of xylan on cellulosic fibers has been shown strongly influenced by temperature and the rate of adsorption increases with an increase in temperature under alkaline conditions (Yllner, Östberg, & Stockman, 1957; Eriksson, Samuelson, & Viale, 1963; Köhnke, 2010). The adsorption of xylan on cellulose is found to be affected by the fine structure of xylan, e.g. type of substituent, molecular weight, and degree of substitution (Köhnke, 2010; Kabel, van den Borne, Vincken, Voragen, & Schols, 2007). It has been confirmed that degradation of xylan side groups increases xylan aggregation and thus the driving force for xylan assembly on cellulose surfaces (Linder, Bergman, Bodin, & Gatenholm, 2003). Increasing adsorption of xylan on cellulose is with the decreasing of the degree of substitution, especially with low content of 4-*O*-methyl glucuronic acid substituent in xylan (Linder et al., 2003). The unsubstituted xylose backbone has an increased tendency to self-associate (Kabel, van den Borne et al., 2007; Köhnke, Pujolras, Roubroeks, & Gatenholm, 2008; Eronen, Österberg, Heikkinen, Tenkanen, & Laine, 2011), which reduces solubility but also increases the amount of xylan adsorbed onto aggregated structures (Eronen et al., 2011; Linder et al., 2003).

It has been reported that such irreversible adsorption of xylans on cellulose inhibits the enzymatic degradation of cellulose (Köhnke, 2010; Taylor & Haigler, 1993) and the existence of xylans is one of the main barriers in the enzymatic hydrolysis of

Abbreviations: Ac, *O*-acetyl; BWX, beechwood xylan; CEL, mixture of Celluclast 1.5 L and Novozyme 188; CS-DA, corn stover after dilute acid pretreatment; DM, dry matter; ESI–MS, electrospray ionization-mass spectrometry; FPU, filter paper unit; MeGlcA, 4-*O*-methyl-α-D-glucuronic; OSX, oat spelt xylan; X_2, xylobiose; X_3, xylotriose; X_4, xylotetraose; XOS, xylo-oligosaccharide; XYL, xylanase.

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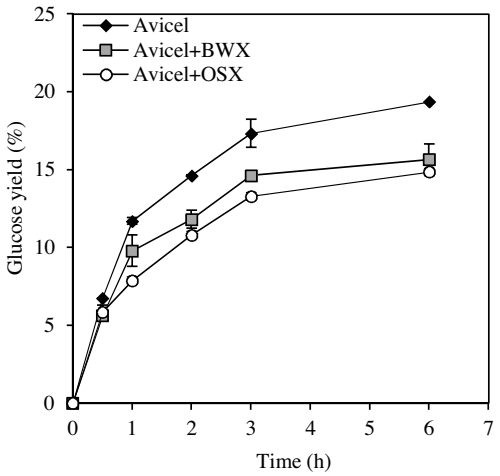

Fig. 1. Hydrolysis of Avicel (20 mg/mL) with or without adsorption of BWX (8.28 mg/mL) and OSX (8.28 mg/mL) by CEL for 0.5, 1, 2, 3, and 6 h at pH 5.0 and 50 °C. CEL contained Celluclast 1.5 L (10 FPU/g DM) and Novozyme 188 (500 nkat/g DM). The error bars indicate standard errors.

different pretreated lignocellulosic materials (Yang & Wyman, 2004; Öhgren, Bura, Saddler, & Zacchi, 2007). It has been reported that isolated xylan can inhibit the enzymatic hydrolysis of Avicel and nanocellulose by individual cellulases, which might be due to the adsorption of xylan covering cellulose and/or the binding of xylans to active sites of cellulases (Zhang, Tang, & Viikari, 2012). However, the inhibition of xylan to cellulose hydrolyzation can be overcome in the hydrolysis of xylan-containing lignocellulosic materials by the addition of xylanase (Öhgren et al., 2007; Selig, Knoshaug, Adney, Himmel, & Decker, 2008; Kumar & Wyman, 2009; Bura, Chandra, & Saddler, 2009), which increases the accessibility of cellulases to cellulose. It has been reported that the removal of xylan prior to hydrolysis increases the pore volume of the substrates and the surface areas accessible for enzymes (Penttilä et al., 2013).

For efficient enzymatic hydrolysis of lignocelluloses, pretreatment was a prerequisite. However, it has been confirmed that a large part of the dissolved xylan would re-adsorb or precipitate on cellulose during pretreatment, and then inhibits the cellulose hydrolysis by cellulases (Kumar & Wyman, 2009; Kabel, Bos, Zeevalking, Voragen, & Schols, 2007). However, to the best of our knowledge, the hydrolyzability of xylan adsorbed on cellulose is not clear. In this work, the adsorption of xylans from beechwood (BWX) and oat spelt (OSX) on celluloses and the hydrolyzabilities of xylans adsorbed on celluloses by xylanase were investigated. The main objective of this work was to understand the role of xylanase in the hydrolysis of xylans adsorbed on cellulose that occurred during the pretreatment of lignocelluloses, which would investigate the xylan limitation on enzymatic hydrolysis of cellulose further.

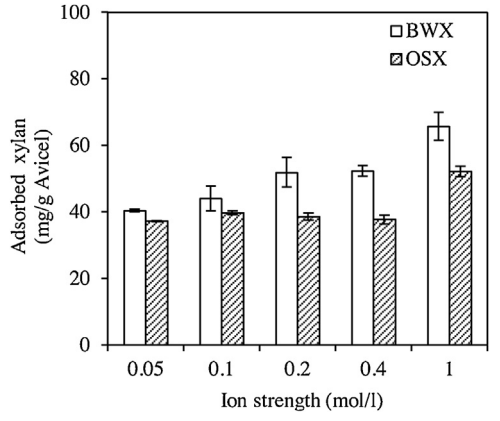

Fig. 2. Effect of ionic strength on the adsorption of BWX and OSX (5 mg/mL) on Avicel (50 mg/mL) at 50 °C for 6 h. The error bars indicate standard errors.

2. Materials and methods

2.1. Materials

Beechwood xylan (BWX) and microcrystalline cellulose (Avicel PH-101) in ~50 μm particle size were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Oat spelt xylan (OSX) was supplied by Shanghai Hualan Chemical Technology Co., Ltd. (Shanghai, China). Corn stover was collected from a local farm in Yangling, Shaanxi Province, China. It was milled to give a size less than 0.3 mm and was soaked in 1% H_2SO_4 (w/w) in a 500 mL screw-capped bottle at a solid-to-liquid ratio of 1:10 and incubated at 121 °C for 1 h. After pretreatment, the mixture slurries were filtered by sand core funnel, and the solids (CS-DA) were washed with distilled water until the filtrate pH was neutral. Chemical composition of CS-DA was determined using Laboratory Analytical Procedures established by National Renewable Energy Laboratory (Sluiter et al., 2008). The chemical composition (% of dry matter (DM)) of CS-DA contained 51.9% cellulose, 8.3% xylan, and 28.9% lignin. All other chemicals used were analytical grade reagents.

Oat spelt xylan (8 g) was suspended in 80 mL of 50 mM sodium citrate buffer (pH 5.0) and stirred for 5 h at room temperature, and then the soluble fraction was recovered by centrifugation for 10 min at 10,000*g* (Ryan et al., 2003). After that, the supernatant was lyophilized and used as soluble OSX for following experiments.

Celluclast 1.5 L, containing major cellulolytic activities, with a filter paper activity of 74.7 FPU/mL and Novozyme 188 with a β-glucosidase activity of 8451 nkat/mL were obtained from Novozymes A/S (Bagsvaerd, Denmark). The *Thermomyces lanuginosus* xylanase, Pentopan Mono BG (Sigma Chemical Co., USA), was used as xylanase preparation (XYL).

2.2. Adsorption of xylan on avicel

To investigate the effect of ion strength on xylan adsorption on Avicel, the incubation of Avicel (50 mg/mL) with BWX and OSX (5 mg/mL) were performed in the same system as described previously. The buffer with different ion strengths (I) was 50 mM sodium citrate buffer (pH 5.0) adjusted with NaCl to I = 0.05, 0.1, 0.2, 0.4, and 1 mol/L, and the ionic strength was calculated by ionic strength formula:

$$I = \frac{I}{2}\sum_{B} m_B z_B^2$$

In this formula, m_B (mol/L) is the molar concentration of each ion; z_B is the valence number of each ion.

The adsorption isotherm experiments of xylan on Avicel and CS-DA were implemented in 50 mM sodium citrate buffer (pH 5.0) with 2 mL working volume. 5% Avicel and CS-DA was incubated with 0.5, 1, 2.5, 10, and 15 mg/mL BWX and OSX for 6 h at 50 °C on an orbital shaker with 200 rpm. After adsorption, samples were immediately centrifuged at 10,000*g* for 10 min, and then the supernatants were collected for further total polysaccharides analysis. The amount of absorbed xylan was calculated from the difference between the total polysaccharides in the supernatants before and after incubation. The solid residues after adsorption before or after washing (washed by 50 mM sodium citrate buffer for 3 times) were hydrolyzed by XYL for 6 h as described later. The total carbohydrates released from Avicel or CS by xylanase was used as control. The total carbohydrates in hydrolysates were determined to