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In situ enzyme aided adsorption of soluble xylan biopolymers onto cellulosic material

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ABSTRACT

The functional properties of cellulose fibers can be modified by adsorption of xylan biopolymers. The adsorption is improved when the degree of biopolymers substitution with arabinose and 4-O-methyl-glucuronic acid (MeGlcA) side groups, is reduced. α-L-Arabinofuranosidase (AbfB) and α-D-glucuronidase (AguA) enzymes were applied for side group removal, to increase adsorption of xylan from sugarcane (*Saccharum officinarum* L) bagasse (BH), bamboo (*Bambusa balcooa*) (BM), *Pinus patula* (PP) and *Eucalyptus grandis* (EH) onto cotton lint. The AguA treatment increased the adsorption of all xylans by up to 334%, whereas, the AbfB increased the adsorption of the BM and PP by 31% and 44%, respectively. A combination of AguA and AbfB treatment increased the adsorption, but to a lesser extent than achieved with AguA treatment. This indicated that the removal of the glucuronic acid side groups provided the most significant increase in xylan adsorption to cellulose, in particular through enzymatic treatment.

1. Introduction

Xylan is a biomaterial with many potential applications in food, medical, pharmaceutical and packaging industries as coating, additive, emulsifying and entrapment agents that have not yet been fully explored. The biopolymer is a major hemicellulose present in cell walls of higher plants, existing in close association with cellulose, encrusted by lignin (Söjström, 1993). The xylan–cellulose association occurs even outside the cell wall matrix, thus presenting a variety of opportunities to improve and introduce new functional properties in cellulosic materials. Previous reports have demonstrated the phenomena on various cellulosic materials including never dried and dried pulp fibers (Köhnke, Pujolras, Roubroeks, & Gatenholm, 2008), bleached and unbleached pulp from softwood Kraft pulp (Ramírez, Puls, Zúñiga, & Saake, 2008), chemi-thermomechanical pulps (CTMP) (Henriksson & Gatenholm, 2002), plant cotton fibers (Henriksson & Gatenholm, 2001), cotton whiskers (Saxena & Ragauskas, 2009), recycled fibers (Arndt & Zelm, 2008) and bacterial cellulose (Kabel, van den Borne, Vincken, Voragen, & Schols, 2007). The xylan that adsorbed onto these cellulosic surfaces was either in unmodified or modified form (nano- and micro-hydrogels), which inherently could swell to different degrees under varying solution conditions (Henriksson & Gatenholm, 2001; Linder, Roubroeks, & Gatenholm, 2003; Numan & Bhosle, 2006). The presence of xylan on the fiber surface enhances the strength of cellulose fibre joints and crossings.

The incorporation of hydrogels onto cellulose fiber surfaces allows application as implantation matrices in biosensors, packaging, sanitary and textile products, as well as the slow release of bioactive substances or other compounds (Kishida and Ikada, 2002; Lagaron et al., 2005; Silva et al., 2007; Daus and Heinze, 2009; Chimphango, van Zyl, & Görgens, 2012a). Furthermore, xylan hydrogel coatings enabled development of novel packaging material with restricted gas permeability named xylophane (Gröndahl, Eriksson, & Gatenholm, 2004; Gatenholm, Grondahl, Dammstrom, & Eriksson, 2008) and xylan–cellulosic composites with a reduction of 362% in specific water transmission (Saxena & Ragauskas, 2009). Both the formation of the hydrogels and adsorption onto cellulose are enhanced when xylan has a lower degree of substitution (DS), while preserving a higher degree of polymerization (DP). These trends have been observed towards end of delignification in conventional Kraft pulping, where less substituted xylan irreversibly adsorbed onto the pulp fibers under high alkalinity [pH 12–14] and high temperatures [165–170 °C] (Söjström, 1993). The xylan adsorption of cellulose is influenced for example by xylan surface charge, purity, concentration, temperature and duration of the reactions (Henriksson & Gatenholm, 2001). The xylan adsorbed at

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Table 1
Xylan structural properties and chemical composition.

Xylan			Content (% substrate dry weight)					
	Mw (Da)[a]	Poly-dispersity	Arabi-nose	MeGlcA[b]	Xylose	DS[c] arabinose	DS[d] MeGlcA	Lignin (Klason)
Sugarcane (*Saccharum officinarum* L) Bagasse (BH)	20,100	3.70	17.4	8.5	71.0	1:4	1:8	28
giant bamboo (*Bambusa balcooa*) (BM)	17,224	3.26	10.5	11.2	79.5	1:7.6	1:7	32
Eucalyptus (*Eucalyptus grandis*) (EH)	73,736	1.32	0.3	12.8	82.1	1:274	1:6	31
Pine (*Pinus patula*) (PP)	55,170	1.46	15.5	11.5	61.3	1:4	1:5	55

[a] Molecular weight.
[b] Methyl glucuronic acid.
[c] Degree of polymerization.
[d] Degree of substitution.

the surfaces of cellulosic material either through co-crystallization with cellulose (Henriksson & Gatenholm, 2001) or through hydrogen bonding (Atalla & Isogai, 2005).

The xylans from wood, grass, and cereals consist of a 1,4-β-linked D-xylose backbone chain substituted with L-arabinose or 4-*O*-methylglucuronic acid (MeGlcA), or both (Timell, 1967; Wilkie, 1979). The type of substitution, DS and the substitution pattern control the solubility properties of the xylan (Ebringerova & Heinze, 2000), hence, the ability to either adsorb onto cellulosic products or form hydrogels would rely, in part, on manipulation of these parameters. The DS and substitution patterns are dependent on the xylan type, isolation and modification methods. For example, xylans with MeGlcA groups can develop stronger bonds between adjacent cellulose fibres through coulomb forces (Miletzky et al., 2015a,b) than xylans without these groups. Higher molecular weight (*Mw*) xylan fractions have a greater affinity towards cellulose that improves adsorption (Kabel et al., 2007).

Process conditions in conventional modification and adsorption methods will typically degrade polymeric components or require multistage processing. For example, Kraft pulping conditions used xylan adsorption onto the cellulosic fibers resulted in some polymer degradation (Gabrielii, Gatenholm, Glasser, Jain, & Kenne, 2000; Lindblad, Ranucci, & Albertsson, 2001; Henriksson & Gatenholm, 2002; Linder et al., 2003; Lindblad & Albertsson, 2005; Köhnke et al., 2008). Alternative debranching methods involving oxalic acid could remove 6% of the arabinose side groups from oatspelt xylan (Sternemalm, Höije, & Gatenholm, 2008), although simultaneously reducing *Mw* from 305 kDa to 169 kDa. Furthermore, xylan hydrogels produced through esterification using carboxylic acid with *N*,*N*′-carbonyldiimidazole (CDI) that is sulphated with SO_3 and DMF (Daus and Heinze, 2009) may contain residual toxic substances, necessitating detoxification.

Enzymatic methods of xylan modification offer improved selectivity, thus, reducing chances of polymer degradation. In addition, the hydrogels produced are non-toxic because the enzymes are produced from microbial systems with generally regarded safe (GRAS) status (Chimphango, Rose, van Zyl, & Görgens, 2012b). Application of α-L-arabinofuranosidase (AbfB) (EC3.2.1.55) and α-D-glucuronidase (AguA) (EC 3.2.1.131-) enzymes, capable of removing side groups from polymeric xylans in the absence of endoxylanase, is a preferred method of xylan modification (Chimphango et al., 2012b).

The present study assessed the effect of *in situ* removal of side chains from xylans by recombinant AbfB and AguA enzymes on the efficiency of modified xylan adsorption onto cotton lint. The xylans were extracted from sugarcane (*Saccharum officinarum L)* bagasse (BH), bamboo (*Bambusa balcooa*) (BM), *Pinus patula* (PP) and *Eucalyptus grandis* (EH) using mild-alkaline methods (Chimphango et al., 2012a). The efficiency of xylan adsorption was determined as the difference between the amount of xylan adsorbed onto cellulose fibers without modification and with *in situ enzymatic* modification. The degree of adsorption was defined as the xylose content of the cotton lint expressed as a percentage of the initial amount of soluble xylan present in the adsorption mixture.

2. Materials and methods

2.1. Materials

Xylan was extracted from *S. officinarum* L (sugarcane) bagasse (BH), *Bambusidae balcooa* [giant bamboo (BM)], *E. grandis*, [Eucalyptus (EH)], and *P. patula* (PP) with mild alkaline methods (Chimphango et al., 2012a). The structural and chemical properties of the extracted xylans are presented in Table 1, from which 1% w/v solutions were prepared according to de Wet, Matthew, Storbeck, Van Zyl, and Prior (2008). Non-absorbent cotton lint (Grade 1, Cotton King) was used as the cellulosic material.

Crude α-L-arabinofuranosidase (AbfB) with volumetric activity of 18.0 nkat mL^{-1} (IU mL^{-1}) on *p*-nitrophenyl arabinofuranoside (*p*-NPA) was produced using recombinant *Aspergillus niger* D15 (Chimphango et al., 2012b). The 4-*O*-methylglucuronic acid (MeGlcA) side chains were removed by α-D-glucuronidase (AguA) with specific activity of 300 nkat mg^{-1} (approx. 17.9 IU mg^{-1}) that was purified from wild type *Schizophyllum commune* (VTT-D-88362-ATCC 38548, generously donated by Prof. Matti Siika-aho (VTT Biotechnology, Finland)).

2.2. Xylan adsorption onto cellulosic material

2.2.1. Adsorption of glucuronoxylan

Cotton lint (0.2 g) was placed in 15 mL Schott bottles, and autoclaved at 121 °C for 15 min to reduce the risk of microbial contamination. Upon cooling to room temperature, the adsorption of EH, was performed in a reaction mixture of 2.0 mL containing 0.2 g cotton lint, 1.0 mL EH xylan (1% w/v), 0.04 mL AguA (300 nkat mg^{-1}) and 0.86 mL of 0.05 M acetate buffer pH 4.8. The reaction was performed in a water bath for 16 h at 40 °C. Control mixtures consisted of 0.2 g cotton lint and 2 mL acetate buffer. The experimental design is shown in Table 2.

2.2.2. Adsorption of arabinoglucuronoxylan

Cotton lint (0.2 g), autoclaved as specified in Section 2.2.1, was treated in a reaction mixture of 2.0 mL in Schott bottles, containing 1.0 mL xylan (1% w/v) prepared from BH, BM and PP, 0.1 mL AbfB, 0.04 mL AguA and 0.86 mL of 0.05 M acetate buffer pH 4.8. The reactions were performed under similar conditions as indicated in Section 2.2.1. Similarly the control sample was prepared as explained in Section 2.2.1. The experimental design is presented in Table 2.

2.2.3. Determining degree of arabinose and MeGlcA side chain release during in-situ xylan modification

The release of arabinose and MeGlcA side chains in the adsorption mixtures was analysed using a high pH anion exchange