Available online at www.sciencedirect.com
ScienceDirect
Carbohydrate Polymers 69 (2007) 94–105
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Structural differences of xylans affect their interaction with cellulose

Mirjam A. Kabel, Hein van den Borne, Jean-Paul Vincken, Alphons G.J. Voragen, Henk A. Schols *

Wageningen University, Department of Agrotechnology and Food Sciences, Laboratory of Food Chemistry, Bomenweg 2, 6703 HD, Wageningen, The Netherlands

Received 7 June 2006; received in revised form 1 September 2006; accepted 8 September 2006
Available online 20 October 2006

Abstract

The affinity of xylan to cellulose is an important aspect of many industrial processes, e.g. production of cellulose, paper making and bio-ethanol production. However, little is known about the adsorption of structurally different xylans to cellulose. Therefore, the adsorption of various xylans to bacterial cellulose (BC) was studied. Also, the relationship between xylan size and adsorption was analysed. BC was used as cellulosic material, because of its high specific surface area and homogeneous structure.

In general, unsubstituted linear xylan parts favoured adsorption to BC. Xylan affinity for BC was also related to xylan *size*. The presence of arabinosyl and *O*-acetyl substituents to xylan decreased the adsorption of xylan to BC considerably. Removing substituents resulted in higher amounts of adsorbed material. Most likely, increasing the number of unsubstituted xylosyl residues induced the formation of xylan–xylan interactions, which contributed to adsorption to BC.

Schematic models are proposed showing the adsorption of structurally different xylans to BC.

Keywords: Xylan; Bacterial cellulose; Adsorption; Affinity; Structural characteristics

1. Introduction

The interactions between cellulose and hemicelluloses play an important role in biosynthetic processes in plants as well as in industrial processes. Well studied is the adsorption of xyloglucan, which is the most abundant hemicellulose in dicotyledonous plants, to cellulose microfibrils (Chambat, Karmous, Costes, Picard, & Joseleau, 2005; Levy, Maclachlan, & Staehelin, 1997; Vincken, De Keizer, Beldman, & Voragen, 1995) and the consequences thereof for cell growth (Cosgrove, 1997; Kerr & Fry, 2003; Levy et al., 1997; Pauly, Albersheim, Darvill, & York, 1999). Xylan, which is the most abundant hemicellulose in monocotyledonous plants and hard wood, is also reported to interact with cellulose. Compared to xyloglucan, less is known about its critical backbone length required for interaction with cellulose, as well as about the influence of xylan substituents on this interaction.

Xylans from different sources can differ highly in structural complexity. Xylan as present in the cell walls of monocots (grasses and cereals) consist of linear chains of β-D-(1,4)-linked D-xylopyranosyl residues, which can be substituted with α-L-arabinofuranosyl at the 2-*O* and/or 3-*O*-position(s), and α-D-glucuronopyranosyl or its 4-*O*-methyl derivative at the 2-*O*-position (Brillouet, Joseleau, Utille, & Lelievre, 1982; Carpita, 1996; Shibuya & Iwasaki, 1985). Furthermore, small amounts of ester-linked coumaric and ferulic acid can be linked to (some of) the arabinosyl residues (Ishii, 1997; Saulnier, Vigouroux, & Thibault, 1995; Wende & Fry, 1997). In contrast to xylans from grasses and cereals, xylan from hardwoods is a highly acetylated 4-*O*-methyl-α-D-glucuronoxylan, essentially without arabinosyl substitution. On average, every tenth xylosyl residue carries an α-4-*O*-methylglucuronyl residue at *O*-2

* Corresponding author. Tel.: +31 317 482239; fax: +31 317 484893.
E-mail address: henk.schols@wur.nl (H.A. Schols).

0144-8617/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2006.09.006

(Evtuguin, Tomas, Silva, & Neto, 2003; Puls & Poutanen, 1989; Shatalov, Evtuguin, & Neto, 1999).

Only few publications mention the impact of the different xylan substituents on the assembly of the xylan-cellulose network in the plant cell wall (Carpita et al., 2001; Hendriksson & Gatenholm, 2001; Iwata, Indrarti, & Azuma, 1998; Kerr & Fry, 2003; Linder, Bergman, Bodin, & Gatenholm, 2003a; Linder, Roubroeks, & Gatenholm, 2003b; Reis & Vian, 2004), but it is difficult to derive the key-factors governing the xylan–cellulose interactions. Other publications describe the role of xylans in industrial processes. For example, in the pulping process of wood for paper manufacturing, the presence of xylan is desirable, because the xylan–cellulose interactions result in paper with good tensile properties (Schonberg, Oksanen, Suurnakki, Kettunen, & Buchert, 2001). However, in the same process, xylans can hinder the removal of lignin essential for paper bleaching (Subramaniyan & Prema, 2000). Also in processes dealing with the enzymatic conversion of cellulose from biomass to fermentable glucose, aiming at the production of bio-ethanol, the interaction of xylans with cellulose needs to be reckoned with (Lynd, 1996).

In the present research the adsorption of xylan to bacterial cellulose (BC) is studied. The effect on adsorption of different substituents of xylans purified from various sources is studied. Also, the critical backbone length needed for adsorption is determined. Finally, a schematic overview explaining xylan–cellulose interactions is proposed.

2. Experimental

2.1. Materials used

2.1.1. Xylans and corresponding fractions

Wheat arabinoxylan (WAX) of medium viscosity and linear oat spelt xylan (OS xylan) were purchased from Megazyme and Koch-Light, respectively. Acetylated xylan was extracted from *Eucalyptus* wood (*Euc* xylan). *Euc* wood alcohol insoluble solids (AIS; 10 g), which was prepared as described previously (Kabel et al., 2002), was delignified in 500 mL of 10% peracetic acid kept for 30 min at 85 °C under continuous stirring. The material was cooled rapidly and diluted twice with distilled water. The delignified wood AIS was collected on a glass filter G3, washed with a mixture of water and acetone (1:1), and air-dried. The isolation of the acetylated xylan from the delignified wood was performed as described by Evtuguin et al. (2003) with this modification that pure acetylated xylans were obtained by dialysis and freeze drying.

OS xylan and WAX were partially degraded by endoxylanase III (1.6 U/g substrate; glycosylhydrolase family 11) purified from *Aspergillus awamori* (Kormelink, Searle-van Leeuwen, Wood, & Voragen, 1993d) in 50 mM NaOAc, pH 5. The enzyme was inactivated by boiling the solutions for 5 min. The degraded xylan solution was reduced in volume under vacuum and subjected to preparative scale size-exclusion chromatography. Hereto, an Akta explorer system (Amersham Pharmacia) equipped with a column (100 × 2.6 cm i.d.) of BioGel P-6 material (200–400 mesh, BioRad) thermostated at 60 °C was used. Elution was performed at 0.5 mL/min with distilled water (60 °C), and the eluent was monitored by a Shodex RI-72 detector. Fractions of 2.6 mL were assayed for the total sugar and uronic acid content. Additionally, the masses of oligomers present in each fraction were analysed by using MALDI-TOF MS. Appropriate fractions were combined and freeze dried. Finally, OS xylan resulted in the fractions OS I, OS II, OS III, OS IV and OS V; WAX resulted in the fractions WAX I, WAX II, WAX III, WAX IV and WAX V of which in this research only fraction WAX II was used.

2.1.2. Cellulose

To obtain bacterial cellulose (BC) a strain of *Acetobacter pasteurianus* NCIMB 8034 (Aberdeem, Scotland) was pre-grown on agar (Oxoid) plates containing Yeast Extract Dextrose medium, pH 5.71. This YED medium was composed of 10 g/L yeast extract (Oxoid), 20 g/L Bacto-peptone (Difco) and 20 g/L glucose. Single colonies grown on the agar-plates were added to YED medium (30 mL) in 0.1 L flasks with a wide base and incubated at 30 °C in a New Brunswick Scientific Innova 4000 incubator shaker (140 rpm) for 48 h. Next, 0.01 L of the grown suspension was again added to YED medium (100 mL) in 0.25 L flasks with a wide base and incubated for 10 days at 30 °C without shaking. The BC was collected on a glass filter G3 and washed extensively with pure water. The BC was suspended in 4% NaOH and stirred overnight at 4 °C, and filtered; this procedure was repeated five times. The BC was then added to 2.5 M HCl, and kept in 2.5 M HCl for 1 h at 30 °C, and collected again on a glass filter and washed thoroughly with pure water. Finally, BC was suspended in water and homogenized (3 mg/mL based on dry weight). The BC was composed for 81%w/w of sugars (Glc 90% mol, Ara 2% mol, Xyl 2% mol, Man 4% mol and uronic acids 2% mol). Avicel Cellulose, Type SF, was obtained from Serva.

2.2. Adsorption experiments

Tamarind seed xyloglucan and fraction $[xg]_5$ were obtained as described by Vincken et al. (1995). Experiments were performed in a total volume of 1 mL for adsorption of xyloglucan and fraction $[xg]_5$ and of 1.5 mL for xylan and xylan fractions.

Adsorption of xyloglucan and $[xg]_5$ with varying concentrations (25–1000 μg) was performed by treating 2.5 mg of Avicel cellulose or 1 mg of BC in 100 mM NaOAc, pH 5.5, at 40 °C and head-over-tail mixing for 16 h.

All xylans and xylan fractions used were dissolved first in 50 mM NaOAC, pH 5, and centrifuged. The supernatants were used for adsorption experiments. Adsorption isotherms of the adsorption of the four xylans and of the OS fractions to BC were obtained by treating 3 mg of BC in 50 mM NaOAC, pH 5, with varying concentrations of

xylan or xylan fraction (25–1000 μg) for 16 h at 40 °C and head-over-tail mixing.

About 400 μg of WAX treated with arabinose releasing enzymes or 25–900 μg *Euc* xylan treated with an *O*-acetyl releasing enzyme was adsorbed to 0.5 mg of BC in 50 mM NaOAc, pH 5 (16 h, 40 °C, head-over-tail).

All samples were centrifuged (3 min, 14000*g*) prior to analysis. The amount of unbound material in the supernatants was quantified by the determination of the total sugar content. In all experiments, adsorption was calculated from the difference of total sugar in solution with and without addition of BC.

2.3. Enzymatic release of arabinosyl-residues from WAX

Both wheat arabinoxylan (WAX) and fraction WAX II (obtained as described above) were subjected to enzymatic hydrolysis in a concentration of 570 μg of substrate/mL of 50 mM NaOAc buffer, pH 5. Degradation experiments were performed with a purified arabinofuranohydrolase-*d*3 (AXHd3) from *Bifidobacterium adolescentis* (Megazyme) and 4 μl of enzyme (200 U/mg) was added per mg of substrate. Similarly, WAX and WAX II were subjected to enzymatic hydrolysis by using an in our laboratory purified arabinofuranosidase B (arafur B; 4 μl/mg of substrate; 294 U/mg) from *Aspergillus niger* (Kormelink, Gruppen, & Voragen, 1993b). WAX and WAX II were also subjected to enzymatic hydrolysis by using both AXHd3 and arafurB. Enzyme reactions were stopped by boiling samples for 5 min. Samples were centrifuged (5 min, 10000*g*) and supernatants were used for adsorption experiments.

2.4. Enzymatic removal of O-acetyl groups from Euc xylan

Euc xylan was subjected to enzymatic hydrolysis in a concentration of 2.5 mg of substrate/mL of 20 mM piperazine buffer, pH 5. Deacetylation experiments were performed with a purified acetyl xylan esterase purified from *Aspergillus niger* (Kormelink, Lefebvre, Strozyk, & Voragen, 1993c). About 10 μg of enzyme was added per 2.5 mg of substrate. Enzyme activity was stopped by boiling samples for 5 min. The supernatants collected after centrifugation (5 min, 10000*g*) were used for adsorption experiments.

2.5. Total sugar content

The total neutral sugar content was determined colorimetrically with an automated orcinol/H_2SO_4 assay (Tollier & Robin, 1979), using an autoanalyser (Skalar Analytical BV). Xylose was used to make a calibration curve (0–200 μg/mL).

2.6. Uronic acid content

The uronic acid content was determined (UA) by an automated *m*-hydroxydiphenyl assay (Blumenkrantz & Asboe-Hansen, 1973; Thibault, 1979) using an autoanalyser (Skalar Analytical BV).

2.7. Neutral sugar composition

The neutral sugar composition was determined by gas chromatography according to Englyst and Cummings (1984), using inositol as an internal standard. The samples were treated with 72% w/w H_2SO_4 (1 h, 30 °C), followed by hydrolysis with 1 M H_2SO_4 for 3 h at 100 °C, and the constituent sugars released were analysed as their alditol acetates by using GC.

2.8. O-Acetyl content

The degree of acetylation was determined on a Thermo Separation Products system HPLC, using an Aminex HPX column (Voragen, Schols, & Pilnik, 1986). The amount of *O*-acetyl groups was corrected for the free acetic acid in the sample.

2.9. HPSEC

Xylan and xylan fractions were dissolved in pure water (4 mg/mL) and subjected (20 μl) to HPSEC. High-performance size-exclusion chromatography was performed on a Thermo Separation Products HPLC system equipped with a membrane solvent-degasser, three TSKgel columns (7.8 mm ID × 30 cm per column) in series (G4000, G3000, G2500; Tosohaas), in combination with a PWX-guard column (Tosohaas). Elution took place at 30 °C with 0.2 M sodium nitrate at 0.8 mL/min. The eluate was monitored using a refractive index (RI) detector (Shodex RI-71). Calibration was performed using pullulans (Polymer Labs).

2.10. HPAEC (pH 12)

High-performance anion-exchange chromatography was performed on a Thermo Separation Products system equipped with a Dionex CarboPac PA-20 column (3 mm ID × 150 mm) in combination with a Dionex CarboPac PA guard column (3 mm × 25 mm) and PAD-detection (Dionex) (Lee, 1996). Enzymatic released arabinose from WAX was analysed by using HPAEC performing an isocratic elution (0.5 mL/min) during 20 min with a solution of 18 mM NaOH. To analyse cellobiose (enzymatic released from BC) a gradient of 0–550 mM sodium acetate in 100 mM NaOH during 25 min was applied. Each elution was followed by a washing and equilibration step.

2.11. MALDI-TOF mass spectrometry

MALDI-TOF (Matrix-Assisted Laser Desorption/Ionisation Time-Of-Flight) mass spectrometry was performed using an Ultraflex instrument (Bruker Daltonic's) equipped with a nitrogen laser of 337-nm. After a delayed extraction time of 200 ns, the ions were accelerated to a kinetic energy

of 12,000 V. Hereafter, the ions were detected using the reflector mode, and selected for positive ions. The lowest laser power required to obtain good spectra was used and at least 100 spectra were collected. The mass spectrometer was calibrated with a mixture of maltodextrins (mass range 365–2309).

Matrix solution was prepared by dissolving 9 mg of 2,5-dihydroxybenzoic acid (Bruker Daltonic's) in 1 mL of an acetonitrile–water-mixture (300 μl:700 μl). The samples were mixed with a matrix solution (1 μL of sample in 1 μL of matrix), after desalting the samples with resin (AG 50W-X8 Resin; Biorad). Prepared solutions (1 μL) were put on a MALDI-TOF-plate (Bruker Daltonic's) and allowed to dry under a constant stream of air.

2.12. NMR spectroscopy

Samples were exchanged in D_2O (99.9 atom% D, Cambridge Isotope Laboratories) with intermediate freeze drying, dissolved in 600 μL 99.96% D_2O (Cambridge Isotope Laboratories) and inserted in 5 mm NMR-tubes. NMR spectra were recorded at a probe temperature of 25 °C on a Bruker AMX-500 spectrometer located at the Wageningen NMR Centre. ^{1}H chemical shifts are expressed in ppm relative to internal acetone (δ 2.225). 1D ^{1}H NMR spectra were recorded at 500.13 MHz using 64 scans of 8192 data points and a sweep width of 3000 Hz.

2.13. Dynamic light scattering

Dynamic light scattering (DLS) was performed as described previously (Van der Burgh, De Keizer, & Cohen Stuart, 2003). An ALV light scattering instrument was used equipped with a 200 mW argon ion laser at 90 °C, tuned at a wavelength of 514.5 nm. Temperature was controlled by a Haake C35 thermostat, providing cell accuracy (0.1 °C) and kept at 30 °C. To analyze the measured autocorrelation functions, the method of cumulants was used (Stock & Ray, 1985). Xylan solutions of 1–2 mg/ml were applied to DLS.

3. Results

3.1. Purification and fractionation of oat spelt xylan, wheat xylan and Eucalyptus wood xylan

To study the affinity for cellulose of a variety of xylan structures, four xylans were used from three different sources. Oat spelt xylan was used as a linear xylan; a highly arabinosyl substituted xylan was obtained from wheat; a xylan substituted with 4-*O*-methylglucuronic acid residues and *O*-acetyl groups was purified from *Eucalyptus* wood. The latter xylan was studied after an alkali treatment to remove all *O*-acetyl groups as well. The sugar composition of these xylans (Table 1) corresponded to the detailed structural characteristics as described in the introduction for cereal xylans (wheat and oat spelt) and for hardwood xylan (*Euc* xylan).

In addition to xylan *structure*, this research also focussed on xylan *size* in relation to cellulose affinity. Therefore, the pure xylans were subjected to enzymatic degradation by using a purified and well characterized endo-xylanase III (Kormelink, Gruppen, Vietor, & Voragen, 1993a). The partially degraded xylan was fractionated by using size exclusion chromatography and different populations were obtained. Fig. 1 presents the HPSEC elution patterns of oat spelt xylan and the fractions prepared. A clear shift from high to lower molecular weight is present from OS I to OS V, respectively. However, in OS I, II and III still some larger xylans, having a Mw higher than 34 kDa (based on pullulans), were present. Additionally, OS IV contained xylans having a Mw of 1.5–35 kDa, and showed quite some overlap with xylans present in OS V. These results clearly present the difficulty to obtain higher amounts of homogeneous xylan fractions, which is the consequence of working with highly heterogeneous starting xylan materials. Nevertheless, the fractions obtained were expected to be sufficiently pure to study the relationship between xylan size and cellulose affinity. Similarly, four wheat arabinoxylan fractions were prepared. The molecular weight distributions as analysed by

Table 1
Sugar composition of xylan (-fractions) from oat spelt (OS), wheat (WAX) and *Eucalyptus* wood (*Euc*)

	% w/w								mol/100 mol Xyl		
	Sugars (total)[a]	Ara	Xyl	Man	Gal	Glc	UA	Ac	Ara (DS_{Ara})	UA (DS_{GlcA})	Ac (DS_{Ac})
OS xylan	89	7	77	0	1	1	3	0	9	3	0
OS I	76	9	53	0	3	3	8	0	16	11	0
OS II	75	8	54	0	3	2	8	0	14	11	0
OS III	70	7	52	0	2	2	7	0	13	10	0
OS IV	66	7	53	0	1	1	4	0	12	6	0
OS V	51	6	44	0	0	0	1	0	14	2	0
WAX	94	34	59	0	0	0	1	0	57	2	0
WAX II	91	40	48	0	1	0	2	0	83	3	0
Euc AIS	61	0	9	1	1	40	6	4	2	46	87
Euc xylan	61	0	36	1	1	1	10	12	0	20	70

[a] Including uronic acids (UA) and acetyl-esters (Ac).

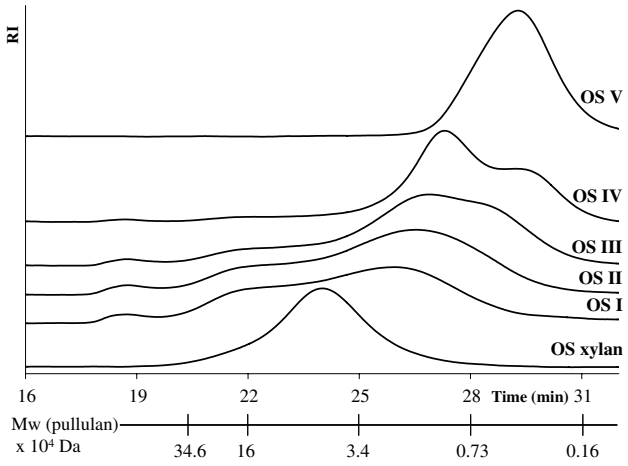

Fig. 1. HPSEC elution profiles of oat spelt (OS) xylan and corresponding fractions obtained after xylanase treatment and size exclusion chromatography.

using HPSEC of the WAX and fraction WAX II used in this research are presented in Fig. 2. WAX II showed to be quite heterogeneous, although the size of the predominant xylans was much lower compared to WAX. Both WAX and WAX II were used in our experiments without further fractionation. Fig. 2 also shows the molecular weight distribution of *Euc* xylan.

3.2. Solubility aspects

Besides the structural details of the xylans, also the solubility aspects of xylan are of importance for xylan adsorption. In general, xylans having few substituents tend to self-associate, forming aggregates with a low solubility (Andrewartha, Phillips, & Stone, 1979). Such a decrease in solubility was observed during enzymatic hydrolysis of both *O*-acetyl and arabinosyl residues from *Euc* xylan and WAX, respectively, by studying the behaviour of the xylans with dynamic light scattering (DLS). As an example, the DLS-graph for the *Euc* xylan subjected to enzymatic removal of *O*-acetyl groups is presented in Fig. 3. At about 270 min of enzyme treatment the hydrodynamic radius decreased. This most likely meant that, by the removal of *O*-acetyl, first the xylan contracts itself by the interaction of unsubstituted parts with other unsubstituted xylan parts within the same molecule. After this decrease in hydrodynamic radius an increase was observed. This was related with the interaction of unsubstituted xylans with other unsubstituted xylans forming aggregated structures, which, finally, resulted in the formation of insoluble aggregates. Furthermore, the solution became opaque ($t > 270$ min) and a pellet was observed after centrifugation of samples taken after 270 min. The latter resulted in the enormous increase in light intensity observed at the moment that the radius started to increase ($t > 270$ min) as well. Similar results were seen in DLS of WAX with addition of arabinosyl hydrolysing enzymes. At a DS_{Ara} of about 40, the light intensity increased enormously and some of the xylan material became insoluble.

These observed tendencies showing self-association of xylans should be kept in mind studying the adsorption of xylan to BC.

3.3. Choice of cellulose needed for adsorption experiments

To study the affinity of xylans to cellulose, the choice of cellulose should be considered first, since parameters representing cellulose characteristics, like surface area, pores and crystallinity, will influence adsorption (Vincken et al., 1995; Zhang & Lynd, 2004). Therefore, adsorption isotherms of two celluloses, Avicel cellulose and bacterial cellulose (BC), were analysed by using the well-characterised xyloglucan fraction $[xg]_5$, containing various xyloglucan-oligomers with the same backbone length, but with differences in side-chains (Vincken et al., 1995), as well as by using a polymeric xyloglucan.

From Fig. 4, it can be concluded that Avicel cellulose and BC showed a different adsorption for both the polymeric xyloglucan and the xyloglucan fraction $[xg]_5$. Avicel cellulose adsorbed much lower amounts of xyloglucan than BC. This difference is most likely due to a relatively small external surface area of Avicel cellulose (20 m^2/g) compared to BC (200 m^2/g) (Zhang & Lynd, 2004). Nevertheless, for both Avicel and BC was seen that the higher the

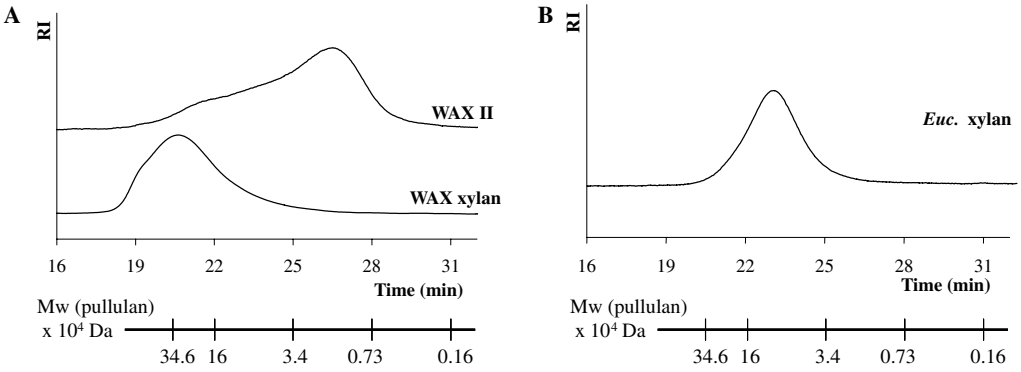

Fig. 2. HPSEC elution profiles of wheat (WAX) xylan and fraction WAX II (A), and of *Eucalyptus* wood (*Euc*) xylan (B).

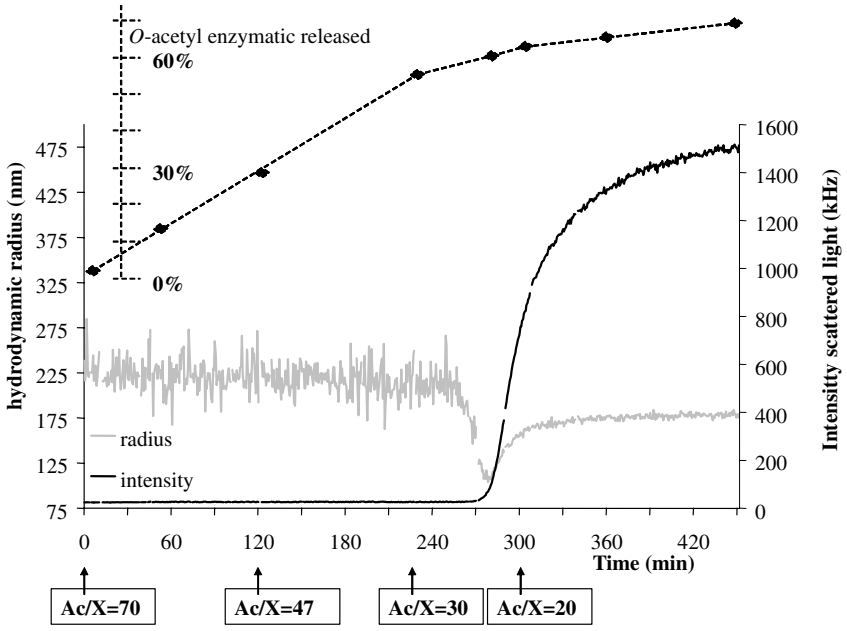

Fig. 3. Dynamic light scattering of *Euc* xylan hydrolysed with AXE. Both hydrodynamic radius (nm) and light intensity (kHz) are analysed in time. The number of *O*-acetyl groups per 100 xylosyl residues in time is presented under the *x*-axis.

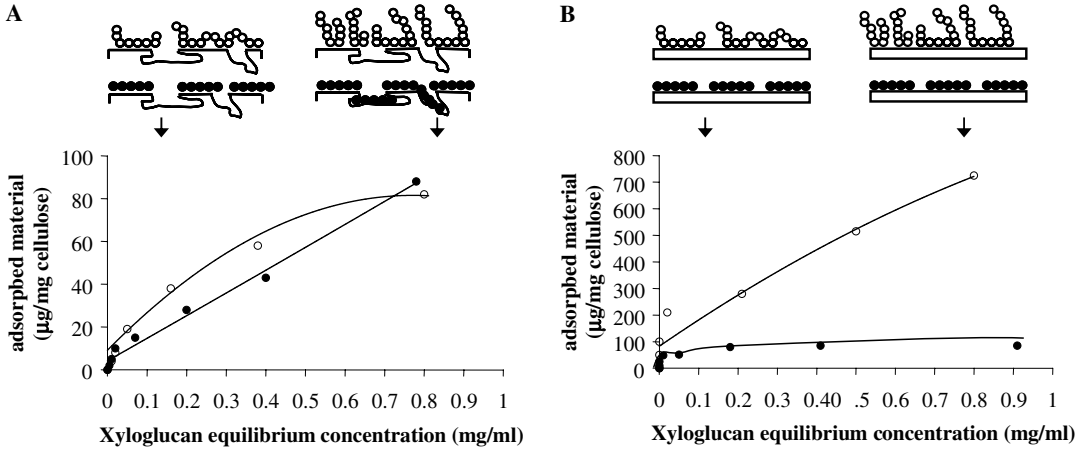

Fig. 4. Adsorption isotherms of oligomeric ($[xg]_5$) and polymeric xyloglucan to Avicel (A) and BC (B), where ●, oligomeric xyloglucan and ○, polymeric xyloglucan.

amount of xyloglucan still in solution after adsorption, which is named "equilibrium concentration" (Fig. 4), the higher amount of xyloglucan adsorbed onto the cellulose surface. The results were compared with the previously reported model for polymeric xyloglucan adsorption of Vincken et al. (1995). At a low xyloglucan equilibrium concentration, xyloglucan can interact several times with the cellulose, resulting in an almost linearly adsorbed polymer train (Fig. 4). At higher xyloglucan concentration, a new adsorption-equilibrium will result in more xyloglucan adsorbing to cellulose by the formation of loops and tails, which are associated to only a few positions in the chains to cellulose (Fig. 4). Our results of the xyloglucan adsorption to both Avicel cellulose and BC fit well with the theory of Vincken et al. (1995).

Also, a different adsorption was shown for Avicel cellulose compared to BC for the adsorption of the xyloglucan-fraction $[xg]_5$. The adsorption isotherm of $[xg]_5$ to BC reached a plateau value at relatively low $[xg]_5$-equilibrium concentrations indicating that a maximum in adsorption was reached, while for Avicel no plateau value was reached. Again according to the theory of Vincken et al. (1995), the $[xg]_5$-fragments only can adsorb to cellulose as linear trains, because they are too small to form loops and tails. Therefore, it was expected that a maximum in adsorption was reached already at low equilibrium concentrations as was the case for BC adsorption. The increase in adsorption to Avicel at higher concentrations of $[xg]_5$, could be related to the presence of small pores in Avicel cellulose (Vincken et al., 1995). In Avicel cellulose, besides

adsorption to the cellulose surface, $[xg]_5$-fragments are expected to occupy small pores present in Avicel cellulose. Additionally, the heterogeneity of $[xg]_5$ also contributed to an increase in adsorption at higher concentrations. More $[xg]_5$-fragments having a relatively low amount of side-chains were present at high concentrations, which might more easily enter the pores in Avicel cellulose compared to $[xg]_5$-fragments having a higher amount of side-chains. Xyloglucan *polymers* can not enter these small pores in Avicel.

The xylan fractions prepared in this research are even more polydisperse compared to the $[xg]_5$-fragments. Based on this heterogeneity together with the shown heterogeneity of the cellulose surface of Avicel cellulose, it was concluded that BC was best to use in the adsorption experiments performed in the present study.

3.4. Effect of xylan structure on adsorption to BC

To rule out insolubility aspects, only soluble xylan and xylan fractions were used in adsorption experiments. Adsorption was calculated from the difference of xylan in solution, determined as total sugar, with and without addition of BC.

The relationship between structural characteristics of xylans and the adsorption onto BC was studied by determining the adsorption isotherms of the four purified xylans: WAX, OS xylan, *Euc* xylan with *O*-acetyl groups, and *Euc* xylan without *O*-acetyl. The results are shown in Fig. 5. The four adsorption isotherms all showed a different course, which could be divided in two parts: a first steep increase of the adsorption isotherm (Fig. 5), and an increase in the adsorption isotherm after the first steep part. Only for WAX a maximum value of adsorption was reached.

WAX showed a maximum in adsorption at the lowest concentration of xylan still in solution after adsorption, which is named "equilibrium concentration" (Fig. 5), while oat spelt xylan never reached a maximum in our experiment and adsorbed in higher amounts to BC. The presence

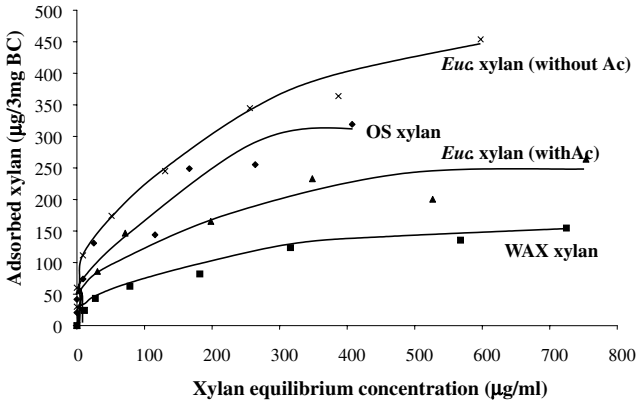

Fig. 5. Adsorption isotherms of four structurally different xylans adsorbed to bacterial cellulose.

of high amount of arabinosyl side chains in WAX, and thus low amount of unsubstituted linear parts, most likely resulted in a low adsorption to BC. The much lower amount of arabinosyl substituents in OS xylan, and thus more unsubstituted linear parts, resulted in a higher amount of adsorbed material. These differences in adsorption by the presence of arabinosyl side groups were also seen in the cell walls of maize coleoptile tissue. The maize xylans having a low degree of arabinosyl substitution and interlaced with β-glucans are detected tightly associated around cellulose microfibrils, but more highly arabinosyl substituted xylans are found in spaces between fibrils and, therefore, are suggested to interconnect the microfibrils (Carpita et al., 2001).

The *Euc* xylan from which all *O*-acetyl groups were removed adsorbed the most to BC compared to the other three xylans tested. This observation seemed slightly in contradiction with the in the above text mentioned hindrance of adsorption by the presence of side-groups, since the DS with 4-*O*-methylglucuronic acid groups (Degree of Substitutions per 100 xylosyl residues; $DS_{GlcAme} = 20\%$) of *Euc* xylan is still relatively high. However, in previous research is proposed that part of the xylan from *Eucalyptus* wood contained a blockwise distribution of 4-*O*-methylglucuronic acid substituents over the xylan backbone (Kabel et al., 2002), leaving the major part of the xylan without substitutes. Therefore, it is suggested that mainly xylan parts without substitutions interacted directly with BC. Similar results were shown by Linder et al. (2003a). Their observation was that the non-branched part of xylan from birch wood had a high affinity for the BC surface, while the 4-*O*-methylglucuronic acid substituted xylan part had not.

The highly acetylated xylan from *Eucalyptus* wood, also containing 4-*O*-methylglucuronic side groups, adsorbed slightly better to BC when compared to WAX, but less than OS xylan (Fig. 4). Apparently, the high degree of *O*-acetyl substituents in *Euc* xylan ($DS_{Ac} = 70\%$) hindered adsorption, but less than a lower degree of arabinosyl substitution in WAX ($DS_{Ara} = 57\%$) did. Both arabinosyl and *O*-acetyl substituents can be linked to *O*-2 and *O*-3 of the xylosyl residues in the backbone. However, the relatively small *O*-acetyl substituents seem to influence adsorption to BC less than the relatively large arabinosyl substituents. From our results it was difficult to conclude if the presence of 4-*O*-methylglucuronic acids ($DS_{GlcAme} = 20\%$) in the highly acetylated *Euc* xylan had an effect on adsorption to BC.

3.5. Effect of xylan size on adsorption to BC

The enzymatically prepared fractions of oat spelt xylan (OS I, II, III, IV and V) were used to study the effect of xylan size on adsorption to BC. The adsorption isotherms of OS xylan and fractions are presented in Fig. 6. From this figure differences are visible mainly at higher xylan equilibrium concentrations. Nevertheless, a distinctly higher

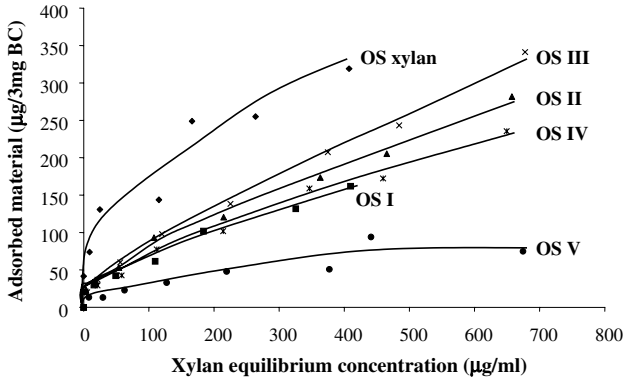

Fig. 6. Adsorption isotherms of OS I, II, III, IV and V adsorbed to bacterial cellulose.

adsorption was seen for OS xylan than for OS I–IV, and OS V adsorbed the least to BC. Therefore, it was concluded that size is an important parameter affecting adsorption and that smaller molecules tend to adsorb less to the cellulose surface then the larger ones. Following, the minimal DP needed for adsorption was studied in more detail. Hereto, OS V was analysed by using MALDI-TOF mass spectrometry, before and after adsorption to BC. In Fig. 7 the *y*-axes represented the peak height observed in the mass spectra calculated as a percentage of the sum of the peak heights per spectrum. Fig. 7 shows clearly that adsorption to BC was preferred over solution for oligomers in OS V having a backbone-length of at least 15 unsubstituted xylosyl residues. This is rather comparable with the minimal backbone length of xyloglucan oligomers of about 12 residues needed for adsorption (Vincken et al., 1995). The results of the adsorption of OS V to BC leave open the question whether these minimal 15 unsubstituted xylosyl residues are contiguous or not.

3.6. Effect of arabinosyl substitution on adsorption to BC

The effect of the number of arabinosyl substituents and their distribution over the xylan backbone was studied in relation to adsorption to BC. It was already mentioned that the low adsorption of WAX compared to OS xylan to BC was most likely caused by the high amounts of arabinosyl residues in WAX. However, besides the total number of arabinosyl residues present, their distribution over the xylan-backbone was expected to influence adsorption to BC as well. To study this effect, first, xylans having different arabinosyl distribution patterns were prepared from WAX and WAX II. Hereto, WAX and WAX II were treated with the arabinosyl releasing enzymes arafur B, AXHd3, and a combination of these two as described in detail in the experimental part. Arafur B is able to split off arabinosyl residues from singly substituted xylosyl residues (Kormelink et al., 1993b), which results in an increase of *unsubstituted* xylosyl residues, and maintaining the amount of *doubly* substituted xylosyl residues. AXHd3 hydrolyses only *O*-3 linked arabinosyl residues from doubly substituted xylosyl residues (Van Laere et al., 1999) resulting in a decrease of arabinosyl residues, but maintaining the same amount of substituted xylosyl residues. The combination of these two enzymes will result in a relatively lowly substituted xylan.

The adsorption of WAX and WAX II treated with these enzymes was compared (Fig. 8). This figure showed that the action of arafur B or AXHd3 alone had almost no effect on the adsorbed amount of WAX or WAX II to BC, although a distinct decrease in the DS_{Ara} was observed. This suggested that no clear difference in adsorption was observed for xylans containing doubly substituted xylosyl residues compared to xylans having a similar arabinosyl residue substitution of (mainly) singly substituted xylosyl residues. However, the combined hydrolysis of arafur B and AXHd3 resulted both in a decreased DS_{Ara} and a remarkable increase in adsorbed amount to BC. Apparently, adsorption depended primarily on the length of unsubstituted xylan backbone. The latter conclusion also partly explained the low adsorption of WAX II to BC, since WAX II mainly contained highly substituted xylan. This was substantiated by calculating the average

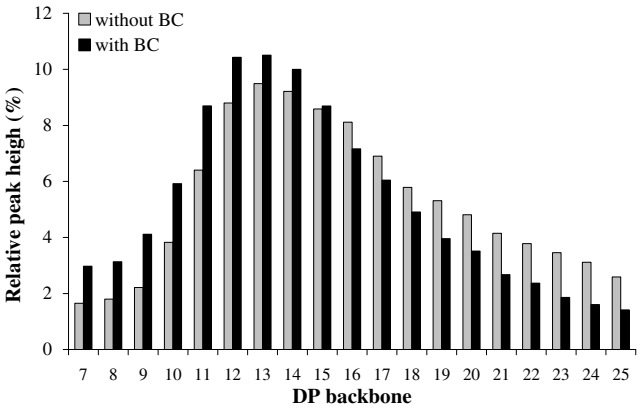

Fig. 7. Relative peak height (%) of MALDI-TOF mass spectra from pool OSV before and after adsorption to BC. The *x*-axis represents the xylan backbone-length, assuming that 85% mol of the oligomer consists of xylose.

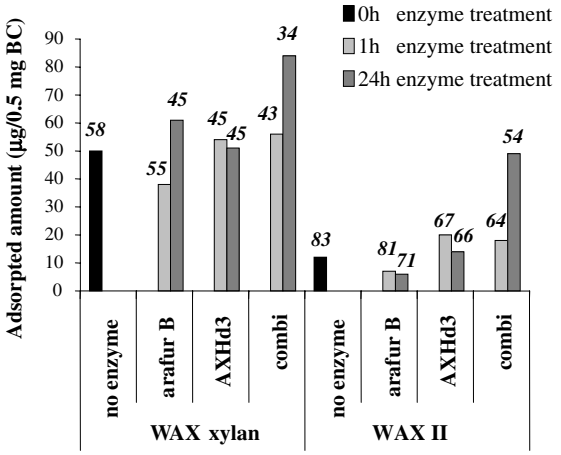

Fig. 8. Adsorption of enzymatic treated (0, 1 and 24 h) WAX and WAX II to BC. The values in italic above each bar represent the number of arabinosyl substitutes per 100 xylosyl residues.

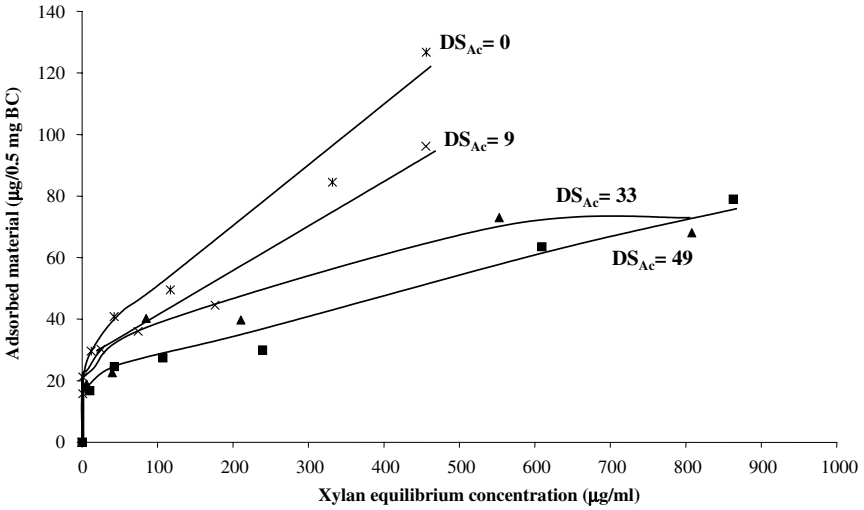

Fig. 9. Adsorption isotherms of xylans with a different degree of *O*-acetyl groups adsorbed to BC. DS_{Ac} = number of *O*-acetyl groups per 100 xylosyl residues.

number of unsubstituted xylosyl residues. MALDI-TOF mass results (not shown) showed that WAX II contained oligomers having a DP of 30–70. Using the average DP of 51 in combination with the average DS_{Ara} of 83 (Table 1), it was calculated that on average oligomers having a backbone of 28 xylosyl residues and 23 arabinosyl side-chains were present in WAX II. NMR-data (not shown) revealed that in WAX II 16% of the arabinosyl residues present was substituted at *O*-2 of a xylosyl residue, 38% at *O*-3 and 46% was substituted at both *O*-2 and *O*-3 of the same xylosyl residue. Thus, WAX II contained oligomers with on average only 10 unsubstituted xylosyl residues, while at least 15 xylosyl residues were needed for adsorption (see text above).

3.7. Effect of O-acetyl substitution on adsorption to BC

The effect of the number of *O*-acetyl groups attached to *Euc* xylan on the adsorption to BC was also studied. The adsorption isotherms of *Euc* xylan treated with a pure xylan acetyl esterase, which hydrolyses *O*-acetyl groups from the xylan backbone, are presented in Fig. 9. In this figure, also the DS_{Ac} is shown. The less *O*-acetyl groups were attached to the xylan backbone the higher was the adsorbed amount to BC. Again, the increase in number of unsubstituted xylosyl residues in the backbone is increasing BC affinity strongly.

4. Discussion

The results obtained in this research, concerning adsorption of various xylan structures to BC, were summarized in a model represented in Fig. 10. This model was partly based on the model described in Fig. 4 for xyloglucan adsorption (Vincken et al., 1995).

In this research, OS xylan was used as a linear xylan, which was observed to adsorb well to BC compared to the other xylans tested (Fig. 5). At low xylan concentrations, the OS xylan most likely will extend itself over the BC surface, driven by the unsubstituted regions in the xylan. This high affinity of the xylan for BC is represented by the steep first part of the adsorption isotherm of OS xylan (Fig. 5). At higher xylan concentrations, it is suggested that a new adsorption-equilibrium will be established, with progressively less junction zones between unsubstituted xylan and cellulose, and more material in loops and tails (Fig. 10). This can be seen as a direct result of the polydispersity of the polysaccharide populations used (Vincken et al., 1995). Arabinosylated and glucuronylated xylosyl residues are proposed to be part of the loops and tails. The increase in adsorbed xylan in loops and tails is predominantly represented by the increase in the adsorption isotherm, which directly follows on the first steep part (Fig. 5). Furthermore, since no plateau value was reached and full surface area coverage was expected, it was assumed that xylan–xylan interactions occurred as well. Unsubstituted linear parts were expected to induce interactions (↔ in Fig. 10) with unsubstituted linear parts present in the loops of adsorbed xylans, resulting in relatively high amounts of adsorbed material. Such xylan–xylan interactions could be related with the observation of Linder et al. (2003a, 2003b), who suggested that xylans adsorb to BC as soluble xylan aggregates.

High concentrations of OS V were used for adsorption and analysed by using MALDI-TOF MS (Fig. 7). Here from it was concluded that at least 15 unsubstituted xylosyl residues were needed for adsorption. It should be noted that unsubstituted xylo-oligosaccharides with a smaller backbone length may have the possibility to adsorb to BC, but that under the conditions used there are sufficient oligomers having a minimal backbone length of 15 xylosyl residues which adsorb preferentially. Adsorption of xylan polymers at high xylan equilibrium concentrations might also require this minimum of 15 xylosyl residues, contigu-

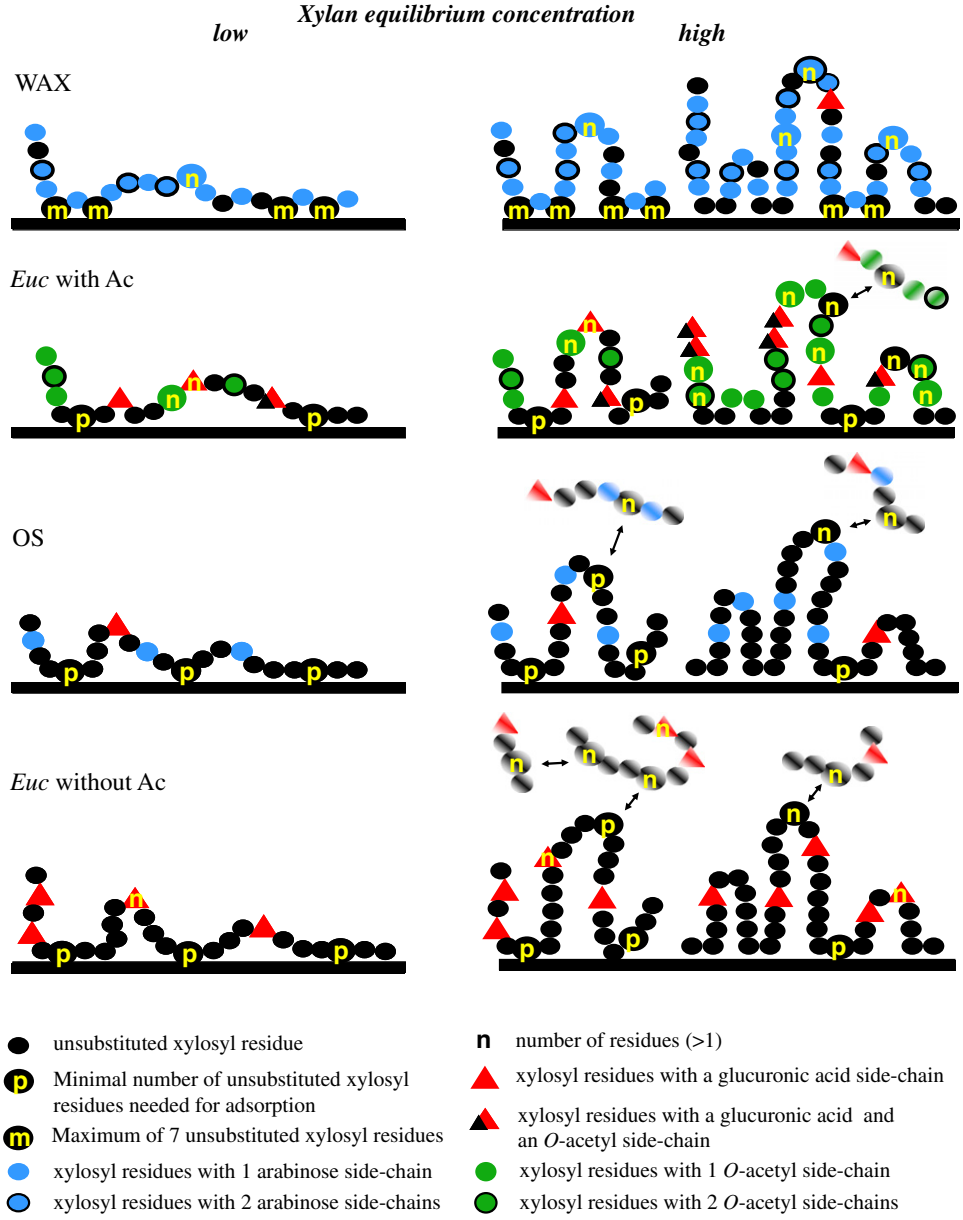

Fig. 10. Model representing the interaction of structurally different xylans with BC. The dark colored beads belong to adsorbed xylans and the shaded colored beads belong to xylans forming associations (↔) with the adsorbed xylans.

ous or not, but this could not be concluded explicitly from our research.

Similar considerations as for OS xylan were applicable to adsorption of *Euc* xylan without *O*-acetyl (Fig. 10). A first steep part of the adsorption isotherm (Fig. 5) was related with a high affinity of the *Euc* xylan for cellulose at low xylan concentrations, possibly by many junction zones between unsubstituted xylan and cellulose. At higher xylan concentration, as predominantly presented by the increase in the adsorption isotherm, the increase in larger xylans within the polydispers polysaccharide population adsorbing in loops and tails was expected to occur as for OS xylan (Fig. 5). However, *Euc* xylan adsorbed in even higher amounts to BC than OS xylan (Fig. 5). In general, more material might adsorb to BC, being part of adsorbed loops and tails, when the xylan polymers are larger. As the molecular weight distributions of *Euc* and OS xylan are almost similar (Figs. 1 and 2), this seemed unlikely. The higher amount of xylan adsorbed to BC is most likely related with the distribution of the glucuronic acid groups over the xylan backbone. Considering a blockwise distribution (Kabel et al., 2002), it is suggested that compared to OS xylan, larger unsubstituted regions were present in *Euc* xylan without *O*-acetyl, which could lead to more xylan–xylan assembly (↔ in Fig. 10). This was also shown by a more pronounced increase in the last part of the adsorption isotherm comparable to OS xylan (Fig. 5).

Of the four xylans tested WAX adsorbed in the lowest amounts to BC. The main part of WAX remained in solution as loose and large molecules containing arabinosyl side groups hindering adsorption. Also, Dervilly-Pinel, Thibault, and Saulnier (2001) showed that arabinoxylans having a DS_{Ara} of 37–130 behave as semi-flexible random-coils. Still, at low WAX equilibrium concentrations, a high affinity of unsubstituted xylosyl residues for BC, as indicated by the first steep part of the adsorption isotherm (Fig. 5), might be expected, resulting in many xylan–cellulose junction zones. Additionally, the structural model of Gruppen, Kormelink, and Voragen (1993), covering both water soluble and insoluble wheat arabinoxylans, suggests that in these arabinoxylans less dense branched regions are present, containing up to seven contiguous unsubstituted xylosyl residues, alternated with highly arabinosylated xylan regions. This implies that also in the WAX used in our research less than 15 contiguous unsubstituted xylosyl residues could be sufficient for adsorption.

At high WAX equilibrium concentrations, the least junction zones with cellulose as needed for adsorption are expected comparable to OS xylan, with arabinosylated xylosyl residues being part of loops and tails (Fig. 10). Furthermore, the adsorption isotherm of WAX reached a maximum in adsorption at a relatively low WAX concentration (Fig. 5). This suggested the absence of xylan–xylan interactions. Such a self-association is not expected to occur between highly arabinosylated xylans, which was reported previously (Andrewartha et al., 1979).

Following the model for adsorption of WAX to BC (Fig. 10), it is quite clear that, by using the AXHd3 enzyme, a reduction of the doubly arabinosyl substituted xylosyl residues to singly substituted ones did not alter the arabinosyl distribution over the xylan backbone, and therefore hardly influenced adsorption at high xylan equilibrium concentration. However, removal of all arabinosyl sidechains from one xylosyl residue, by using a combination of enzymes, resulted in more unsubstituted xylosyl residues (Fig. 10), which resulted in higher amounts of adsorbed material (Fig. 8). This higher amount of material adsorbed was expected to result from the formation of xylan–xylan interactions between unsubstituted xylosyl residues, as shown for e.g. OS in Fig. 10 (↔ in Fig. 10).

Euc xylan with *O*-acetyl groups is proposed to contain lowly substituted parts, which have high affinity for BC as shown in Fig. 10, but the main part will remain in solution comparable to WAX. Again, at low xylan equilibrium concentrations the xylan is expected to adsorb to BC forming many junction zones of unsubstituted xylan with cellulose (Fig. 10), as indicated by the first steep part of the adsorption isotherm (Fig. 5). At high xylan equilibrium concentration the xylan is expected to interact with BC forming loops and tails, with the *O*-acetyl and the (4-*O*-methyl-)glucuronyl substituents in the loops and tails. Furthermore, comparable to arabinosyl side chains, the presence of *O*-acetyl groups was expected to prevent self-association of the xylans (Fig. 10). However, some self-association of unsubstituted linear xylan parts (↔ in Fig. 10) was assumed, because the adsorption isotherm of *Euc* xylan with *O*-acetyl groups still increased unlike WAX (Fig. 5). The possibility that more *Euc* xylan adsorbed to BC by forming larger loops or tails compared to WAX was excluded, because the molecular weight of *Euc* xylan was much lower than the molecular weight of WAX (Fig. 2). Finally, the removal of *O*-acetyl groups by using a pure xylan acetyl esterase resulted in an increase in adsorbed xylan (Fig. 9). This higher amount of adsorbed xylan was expected to result from the formation of xylan–xylan interactions between unsubstituted xylosyl residues, as described for WAX after removal of arabinosyl substituents.

Acknowledgements

This project is supported with a grant of the Dutch Programme EET (Economy, Ecology, Technology) a joint initiative of the Ministries of Economic Affairs, Education, Culture and Sciences and of Housing, Spatial Planning and the Environment. The programme is run by the EET Programme Office, SenterNovem. The authors thank Ben van den Broek for his contribution in the preparation of BC and for the gift of AXHd3. Also, the authors thank Bram Sperber for his help with the DLS experiments and the discussions about polymer behavior.

References

Andrewartha, K. A., Phillips, D. R., & Stone, B. A. (1979). Solution properties of wheat-flour arabinoxylan and enzymically modified arabinoxylans. *Carbohydrate Research, 77*, 191–204.

Blumenkrantz, N., & Asboe-Hansen, G. (1973). New method for quantitative determination of uronic acids. *Analytical Biochemistry, 54*, 484–489.

Brillouet, J. M., Joseleau, J. P., Utille, J. P., & Lelievre, D. (1982). Isolation, purification and characterization of a complex heteroxylan from industrial wheat bran. *Journal of agricultural and food chemistry, 30*(3), 488–494.

Carpita, N. C. (1996). Structure and biogenesis of the cell walls of grasses. *Annual Review of Plant Physiology. Plant Molecular Biology, 47*, 445–476.

Carpita, N. C., Defernez, M., Findlay, K., Wells, B., Shoue, D. A., Catchpole, G., et al. (2001). Cell wall architecture of the elongating maize coleoptile. *Plant Physiology, 127*, 551–565.

Chambat, G., Karmous, M., Costes, M., Picard, M., & Joseleau, J. P. (2005). Variation of xyloglucan substitution pattern affects the sorption on celluloses with different degrees of crystallinity. *Cellulose, 12*, 117–125.

Cosgrove, D. J. (1997). Assembly and enlargement of the primary cell wall in plants. *Annual Review of Cell and Developmental Biology, 13*, 171–201.

Dervilly-Pinel, G., Thibault, J. F., & Saulnier, L. (2001). Experimental evidence for a semi-flexible conformation for arabinoxylans. *Carbohydrate Research, 330*, 365–372.

Englyst, H. N., & Cummings, J. H. (1984). Simplified method for the measurement of total non-starch polysaccharides by gas–liquid chromatography of constituent sugars as alditol acetates. *Analyst, 109*, 937–942.

Evtuguin, D. V., Tomas, J. L., Silva, A. M. S., & Neto, C. P. (2003). Characterization of an acetylated heteroxylan from Eucalyptus globulus Labill. *Carbohydrate Research, 338*, 597–604.

Gruppen, H., Kormelink, F. J. M., & Voragen, A. G. J. (1993). Water-unextractable cell wall material from wheat flour. III. A structural model for arabinoxylan. *Journal of Cereal Science, 18*, 111–128.

Hendriksson, A., & Gatenholm, P. (2001). Controlled assembly of glucuronoxylans onto cellulose fibres. *Holzforschung, 55*, 494–502.

Ishii, T. (1997). Structure and functions of feroylated polysaccharides. *Plant Science, 127*, 111–127.

Iwata, T., Indrarti, L., & Azuma, J.-I. (1998). Affinity of hemicellulose for cellulose produced by *Acetobacter xylinum*. *Cellulose, 5*, 215–228.

Kabel, M. A., Carvalheiro, F., Garrote, G., Avgerinos, E., Koukios, E., Parajó, J. C., et al. (2002). Hydrothermally treated xylan rich by-products yield different classes of xylo-oligosaccharides. *Carbohydrate Polymers, 50*(1), 47–56.

Kerr, E. M., & Fry, S. C. (2003). Pre-formed xyloglucans and xylans increase in molecular weight in three distinct compartments of a maize cell-suspension culture. *Planta, 217*, 327–339.

Kormelink, F. J. M., Gruppen, H., Vietor, R. J., & Voragen, A. G. J. (1993a). Mode of action of the xylan-degrading enzymes from *Aspergillus awamori* on alkali-extractable cereal arabinoxylans. *Carbohydrate Research, 249*, 355–367.

Kormelink, F. J. M., Gruppen, H., & Voragen, A. G. J. (1993b). Mode of action of (1,4)-β-D-arabinoxylan arabinofuranohydrolase (Axh) and alpha-L-arabinofuranosidases on alkali-extractable wheat-flour arabinoxylan. *Carbohydrate Research, 249*(2), 345–353.

Kormelink, F. J. M., Lefebvre, B., Strozyk, F., & Voragen, A. G. J. (1993c). Purification and characterization of an acetyl xylan esterase from *Aspergillus niger*. *Journal of Biotechnology, 27*, 267–282.

Kormelink, F. J. M., Searle-van Leeuwen, M. J. F., Wood, T. M., & Voragen, A. G. J. (1993d). Purification and characterization of three endo-(1,4)-beta-xylanases and one beta-xylosidase from *Aspergillus awamori*. *Journal of Biotechnology, 27*, 249–265.

Lee, Y. C. (1996). Carbohydrate analysis with high-performance anion-exchange chromatography. *Journal of Chromatography A, 720*, 137–149.

Levy, S., Maclachlan, G., & Staehelin, L. A. (1997). Xyloglucan sidechains modulate binding to cellulose during *in vitro* assays as predicted by conformational dynamics simulations. *Plant Journal, 11*(3), 373–386.

Linder, A., Bergman, R., Bodin, A., & Gatenholm, P. (2003a). Mechanism of assembly of xylan onto cellulose surfaces. *Langmuir, 19*, 5072–5077.

Linder, A., Roubroeks, J. P., & Gatenholm, P. (2003b). Effect of ozonation on assembly of xylans. *Holzforschung, 57*, 496–502.

Lynd, L. R. (1996). Overview and evaluation of fuel ethanol from cellulosic biomass: technology, economics, the environment, and policy. *Annual Reviews Energy Environment, 21*, 403–465.

Pauly, M., Albersheim, P., Darvill, A. G., & York, W. S. (1999). Molecular domains of the cellulose/xyloglucan network in the cell walls of higher plants. *The Plant Journal, 20*(6), 629–639.

Puls, J., & Poutanen, K. (1989). Mechanisms of enzymatic hydrolysis of hemicelluloses (xylans) and procedures for determination of the enzyme activities involved. In M. P. Coughlan (Ed.), *Enzyme systems for lignocellulose degradation*. Elsevier Applied Science.

Reis, D., & Vian, B. (2004). Helicoidal patterns in secondary cell walls and possible role of xylans in their construction. *Comptes Rendus Biologies, 327*, 785–790.

Saulnier, L., Vigouroux, J., & Thibault, J. F. (1995). Isolation and partial characterization of feruloylated oligosaccharides from maize bran. *Carbohydrate Research, 272*, 241–253.

Schonberg, C., Oksanen, T., Suurnakki, A., Kettunen, H., & Buchert, J. (2001). The importance of xylan for the strength properties of Spruce kraft pulp fibres. *Holzforschung, 55*, 639–644.

Shatalov, A. A., Evtuguin, D. V., & Neto, C. P. (1999). (2-*O*-α-D-galactopyranosyl-4-*O*-methyl-α-D-glucurono)-D-xylan from *Eucalyptus globulus labill*. *Carbohydrate Research, 320*, 93–99.

Shibuya, N., & Iwasaki, T. (1985). Structural features of rice bran hemicellulose. *Phytochemistry, 24*(2), 285–289.

Stock, R. H., & Ray, W. H. (1985). Interpretation of photon correlation data: a comparison of analysis methods. *Journal of Polymer Science, Polymer Physics Edition, 23*, 1147–1393.

Subramaniyan, S., & Prema, P. (2000). Cellulase-free xylanases from *Bacillus* and other microorganisms. *FEMS Microbiology Letters, 183*, 1–7.

Thibault, J. F. (1979). An automated method for the determination of pectic substances. Automatisation du dosage des substances pectiques par la methode au meta-hydroxydiphenyl. *Lebensmittelen Wissenschaft Technologie, 12*(5), 247–251.

Tollier, M., & Robin, J. (1979). Adaptation de la methode a l'orcinol-sulfurique au dosage automatique des glucides neutreux totaux: conditions d'application aux extraits d'origine vegetale. *Annales de Technologie Agricole, 28*, 1–15.

Van der Burgh, S., De Keizer, A., & Cohen Stuart, M. A. (2003). Complex coacervation core micelles. Colloidal stability and aggregation mechanism. *Langmuir, 20*, 1073–1084.

Van Laere, K. M. J., Voragen, C. H. L., Kroef, T., Broek, L. A. M. v. d., Beldman, G., & Voragen, A. G. J. (1999). Purification and mode of action of two different arabinoxylan arabinofuranohydrolases from *Bifidobacterium adolescentis* DSM 20083. *Applied Microbiology and Biotechnology, 51*, 606–613.

Vincken, J.-P., De Keizer, A., Beldman, G., & Voragen, A. G. J. (1995). Fractionation of xyloglucan fragments and their interaction with cellulose. *Plant Physiology, 108*, 1579–1585.

Voragen, A. G. J., Schols, H. A., & Pilnik, W. (1986). Determination of the degree of methylation and acetylaton of pectins by h.p.l.c. *Food Hydrocolloids, 1*(1), 65–70.

Wende, G., & Fry, S. C. (1997). Digestion by fungal glycanases of arabinoxylans with different feruloylated side-chains. *Phytochemistry, 45*(6), 1123–1129.

Zhang, Y.-H. P., & Lynd, L. R. (2004). Toward an aggregated understanding of enzymatic hydrolysis of cellulose: noncomplexed cellulase systems. *Biotechnology and Bioengineering, 88*(7), 797–824.