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Study of xylan and cellulose interactions monitored with solid-state NMR and QCM-D

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Abstract: Development of physical properties of bleached eucalyptus kraft pulp is typically based on the refining process. However, many studies have reported that xylan deposition is a viable alternative. As the mechanisms of xylan and cellulose interactions are not clear, the main goal of this study was to achieve a better understanding of these interactions. Considering that a sample of pulp enriched with xylan is a very complex matrix, a model system was developed. Cellulosic thin films were prepared by spincoating and the Langmuir-Schaefer (LS) method from trimethylsilylcellulose (TMSC). Their interactions with xylan were analyzed using the quartz crystal microbalance with dissipation (QCM-D) monitoring technique. The topological changes on cellulose were studied using atomic force microscopy (AFM). For the ^{13}C solid-state nuclear magnetic resonance (NMR) studies, samples were prepared using commercial microcrystalline cellulose (MCC) and xylan. The xylan was extracted from bleached birch kraft pulp using a cold caustic extraction (CCE) method. The QCM-D monitoring showed deposition only with higher concentrations of xylan solution ($1\text{ mg}\cdot\text{l}^{-1}$) for the LS method. The AFM images showed that xylan deposits as agglomerates on the cellulose surface, and the NMR experiments showed that there are interactions for the more ordered region of the cellulose fiber and for the less-ordered region.

Keywords: AFM, cellulose, deposition, NMR, QCM-D, xylan

Introduction

Cellulose is the most abundant organic compound in the planet and the main structural component of plant cells. It is a linear polysaccharide formed by β -D-glucopyranose units linked by β -1,4-glycosidic covalent bonds (Sjöström 1981). Native cellulose is formed by two crystalline allomorphs, $\text{I}\alpha$ and $\text{I}\beta$, demonstrated by cross-polarization magic angle spinning (CPMAS) ^{13}C nuclear magnetic resonance (NMR) techniques. Those two allomorphs are different regarding the crystalline structure, hydrogen bonds and molecular conformation (Atalla and Vanderhart 1984).

Hemicelluloses, along with cellulose and pectin, are polysaccharides that form the cell wall of higher plants (Sjöström 1981). Hemicelluloses can be found both in the primary and secondary walls and, in a small amount, in the middle lamella. Hemicelluloses are branched heteropolymers constituted by different monosaccharides in pyranose or furanose forms in different proportions (Timell 1967; Srndovic 2011).

Hemicelluloses are amorphous polymers, without the tendency to form crystalline regions in their native forms on the fiber walls, providing flexibility (Atalla et al. 1993).

It has recently been proposed that xylan might be able to form hydrogen bonds with the hydrophilic surfaces of cellulose through folding as a twofold helical screw (Bromley et al. 2013; Busse-Wicher et al. 2014, 2016a,b) while it adapts a threefold helical screw xylan in solution (Simmons et al. 2016).

Xylans are hemicelluloses easily removed by alkali solutions, due to their amorphous structure and high amount of acid groups typically present as side groups in xylans (Aurell 1965).

The main sources to xylan extraction in a kraft pulp mill are wood chips, cooking liquors and bleached pulp (Axelsson et al. 1962; Simonson 1963; Sjöström and Enström 1967; Janzon et al. 2006; Dahlman et al. 2008; Krogerus and Fuhrmann 2009).

An increase in the amount of hemicelluloses in the fiber wall can be achieved in two different ways: improving the retention of hemicelluloses in the fibers during the delignification process (Kleppe 1970) and conducting

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a deposition process of dissolved hemicellulose polymers onto the fiber (Yllner and Enström 1956; Aurell 1965; Dahlman et al. 2003).

Earlier studies of xylan sorption onto cellulose surfaces have reported first-order kinetics. This indicates a physical process, with Van der Waals or hydrogen bonds between the sorbed xylan and the cellulose substrate (Clayton and Phelps 1965; Hansson 1970; Mora et al. 1986; Mitikka-Eklund, 1996; Danielsson and Lindström 2005; Ribe et al. 2009). Others studies have reported the adsorption of xylan on the cellulose fibers as a relatively slow process, probably due to the molecular diffusion in the porous fiber wall (Clayton and Phelps 1965; Hansson 1970).

Hydrogen bonds have been demonstrated to be involved in the xylan retention during sorption-desorption experiments with hydrogen bond disruptor reagents (Mora et al. 1986). However, the hydrogen bond formation between xylan and cellulose has been questioned. Previous studies have affirmed that if hydrogen bond formation does occur, the xylan layer on the cellulose surface should be flat, according to the models for free energy between flat surfaces (Derjaguin 1934), and not swollen with water as observed in experiments using techniques such as quartz crystal microbalance with dissipation (QCM-D). They suggest, then, that the driving force is a combination of entropy increasing, associated with the release of solvent molecules during the polymer adsorption, and the weak Van der Waals attraction, rather than formation of hydrogen bonds as has been cited before (Mora et al. 1986; Tammelin et al. 2009). Despite that, hydrogen bonds may be important for dried systems (Paananen et al. 2004).

Other studies suggested that xylan exists both as single molecules in aqueous solution and as aggregates in the colloidal size. The aggregate formation is provided by the interaction of unsubstituted regions of the xylan chain and by the hydrophobic interaction due to lignin residues covalently bonded to xylan. Consequently, the adsorption on the cellulose surface happens through single molecules and aggregates. This mechanism is, probably, more relevant for systems in which the xylan solubility is lower (Mora et al. 1986; Linder et al. 2003).

Xylan adsorption is considered as irreversible and only small amounts are removed by dilution (Paananen et al. 2004) or water washing (Eriksson et al. 1963). Exposure to alkaline conditions under high temperatures increases the desorption rate (Hansson and Hartler 1969). It is easy to understand as the solubility of hemicelluloses is higher under alkaline conditions (Miao et al. 2014; Li et al. 2015).

However, it has been reported that adsorbed xylan from cold caustic extraction (CCE) liquors is kept in the cellulose surface even after the delignification process or bleaching sequences (Dahlman et al. 2003; Soares 2009).

The interest of the pulp and paper industry over the xylan content of cellulose pulps is based on the carboxylic groups which are introduced in the cellulose fibers by xylan (Kleppe 1970). Fibers with high xylan content get swollen easily, exposing a higher superficial area, thereby increasing the active sites for fiber reactions (Eriksson and Sjöström 1968; Mobarak et al. 1973). It has been reported that adsorbed xylan on pulp increases tensile properties of the paper (Sihtola and Blomberg 1975; Schönberg et al. 2001) and decreases the energy consumption during the refining process (Aurell 1965; Mobarak et al. 1973; Vaaler et al. 2002).

NMR has been used as an extensive physical method to determine the structure of organic compounds. Developments in high-resolution solid-state NMR of organic materials over the last 30 years have been deeply established (Dybowski and Bai 2006). The materials that are investigated can be natural, synthetic and even biological systems, and ^{13}C is undoubtedly the most feasible nucleus that can be studied with high-resolution NMR in the solid phase. It can be easily observed and the interpretation of ^{13}C spectra is almost a direct reading of the carbon skeleton of molecules (Ghisalberti and Godfrey 1998; Lambert et al. 2000; Alesiani et al. 2005; Pournou 2008; Dupree et al. 2015; Santonia et al. 2015).

The structures of cellulose in wood and pulp have been extensively studied employing CPMAS ^{13}C NMR spectroscopy (Van der Hart and Atalla 1984; Newman and Hemmingson 1990; Newman 1992; Newman et al. 1993; Lindgren et al. 1995; Larsson et al. 1997; Newman 1998; Wickholm et al. 1998; Larsson et al. 1999; Hult et al. 2000; Maunu et al. 2000). Other advanced techniques such as atomic force microscopy (AFM) and QCM-D monitoring have been used as support for a better knowledge of xylan and cellulose interactions (Binnig et al. 1986; Ducker et al. 1991; Fatisson et al. 2009; Thio et al. 2011; Chen et al. 2016; Mocchiuttia et al. 2016; Korolkov et al. 2019).

As there is a great interest in the xylan content from pulp and paper producers, knowledge of the mechanisms of xylan adsorption on cellulose surface is desirable. Therefore, the main goal of this work was to study the interactions of xylan and cellulose using techniques such as ^{13}C solid-state NMR, AFM and QCM-D associated with nanofilm samples to achieve a purer model than the industrial pulp samples.

Materials and methods

Xylan samples: Xylan has been extracted from commercial bleached birch kraft pulp using 1 M NaOH solution at room temperature followed by ultra- and diafiltration as described earlier (Laine et al. 2015) and as shown in Figure 1.

Saturated solutions/dispersions of xylan were prepared, at different pH, from a xylan slurry of dry matter content (DMC) by adding deionized water and 1 M NaOH solution and regulating the pH several times during stirring for 48 h at room temperature. After centrifugation, the supernatants were the samples used for the adsorption trials, according to Figure 2.

The carbohydrate content of the saturated xylan solutions and the DMC were determined (Table 1).

Preparation of cellulose samples with adsorbed xylan: Microcrystalline cellulose (Sigmacell Type 50 S-5504, Sigma-Aldrich Oy, Espoo, Finland) (MCC) was selected as the cellulose model. Xylan isolated from bleached birch kraft pulp was added to the cellulose suspension at selected pH values, 13 (represents a high pH during pulp processing) and 10 (where xylan precipitation in practice may take place at the final phase of pulping). Adsorption experiments were performed by mixing soluble/dispersed xylan and MCC at defined conditions.

The DMCs were 95.1% for MCC and 9.18% for the original xylan dispersion.

The soluble part of the xylan dispersion was isolated by regulating the pH of 1.2 l of xylan dispersion to pH 13 and pH 10, respectively, and a final xylan content of 2.5%wt. After stirring at room

temperature for 48 h and pH adjustment as necessary, the dispersion/solution was centrifuged at 4750 rpm (5250 g) for 1 h at room temperature using an Allegra X-15R (Beckman Coulter, Inc., Brea, CA, USA) centrifuge and the supernatant was collected. The resulting samples were saturated xylan solution at pH 13 (Xyl sol pH 13) and saturated xylan solution at pH 10 (Xyl sol pH 10).

Ten grams of MCC (as bone dry) and 990 g of Xyl sol pH 13 or Xyl sol pH 10 were stirred at room temperature for 7 days. The pH was recorded several times during this period.

After the adsorption, both of the samples were divided into two equal parts for further analysis.

- A. Half of the sample was centrifuged resulting in the solid sample MCC + Xyl pH 13 unwashed and the supernatant (Sup Xyl pH 13). The same procedure was used for the samples at pH 10. The solid sample was air-dried except a small portion (20% of the original sample).
- B. The other half was centrifuged and redispersed into 990 ml pH 13 water and centrifuged. This was repeated once more resulting in MCC + Xyl pH 13 washed. The same procedure was performed at pH 10. The solid sample was air-dried except for a small portion (20% of the original sample).

Carbohydrate analysis of MCC, freeze-dried xylan and the four solid MCC + Xyl samples were performed after prehydrolysis with 72% (w/w) sulfuric acid for 60 min at 30°C and autoclaving at 4% (w/w) sulfuric acid concentration for 60 min at 120°C. The resulting mono-saccharides were determined by high-performance anion-exchange chromatography (HPAEC) with pulse amperometric detection (Dionex ICS 3000 equipped with CarboPac PA1 column or Dionex ICS 5000 equipped with CarboPac PA20 column; Dionex, Sunnyvale, CA, USA) according to the NREL method (National Renewable Energy Laboratory). The carbohydrate analysis of Xyl sol pH 13 and Xyl sol

Figure 1: Schematic flow to obtain xylan dispersion according to Laine et al. 2015.

Table 1: Xylan and dry matter content of the xylan solutions/ dispersions for the adsorption trial.

Sample	Xylan content (g · l ⁻¹)	Dry matter content (wt.-%)
Xyl sol pH 13	9.22	2.20 ^a
Xyl sol pH 10	11.19	1.27

^aThe sample contains significant amounts of sodium hydroxide from the pH regulation.

Figure 2: Schematic flow to obtain saturated xylan dispersion samples.

pH 10 was performed after neutralization using the same protocol but without prehydrolysis.

The carbohydrate composition, including neutral and acidic sugars, was determined according to Sundberg et al. (1996) with two parallel determinations.

Solid-state NMR experiments: The ^{13}C CPMAS NMR measurements were performed using an Agilent DD2 600 NMR spectrometer (Agilent Technologies, Santa Clara, CA, USA) with magnetic flux density of 14.1 T, equipped with a 3.2-mm T3 MAS NMR probe operating in a double resonance mode. The MAS rate in experiments was set to 10 kHz. For the CPMAS spectra of the reference materials, 8000 scans were accumulated using a 1.3-ms contact time and a 6.0-s delay between successive scans. The relaxation time constants $T_{1\rho}(\text{H})$ for pure xylan and MCC were determined with a delayed contact pulse sequence to be 9.2 ms (xylan) and 18.5 ms (MCC). The clear difference in $T_{1\rho}(\text{H})$ values indicates that the proton spin relaxation-based spectral edition (PSRE) for ordered MCC and amorphous xylan components can be carried out. The delayed contact pulse sequence was used also for the PSRE experiments with a duration for the spinlock pulse set as 8.0 ms for the partially relaxed spectra. Four thousand scans were collected for both relaxed and non-relaxed spectra. Protons were decoupled during acquisition using SPINAL-64 proton decoupling with a field strength of 80 kHz. Ninety-degree pulse durations and Hartmann-Hahn match for cross polarization were calibrated using α -glycine. The spectra were processed using the TopSpin 3.5 software (Bruker Scientific Instruments, Billerica, MA, USA).

Adsorption of xylan on cellulose analyzed using QCM-D and AFM:

Adsorption and desorption of xylan on cellulose was investigated using the E4 QCM-D instrument (Q-sense AB, Gothenburg, Sweden). QCM-D allows the simultaneous monitoring of changes in frequency and dissipation at the solid/liquid interface at the fundamental frequency of 5 MHz and its six overtones. These changes are further translated to mass changes and changes in viscoelastic properties taking place during the adsorption/desorption process. The interpretation is described in detail in Rodahl et al. (1995). Briefly, when the adsorbed mass is evenly distributed, rigidly attached and fully elastic, the areal mass can be calculated according to the Sauerbrey equation (Sauerbrey 1959) where Δm is the adsorbed mass per unit surface, $\Delta f = f - f_0$ is the frequency change, n is the overtone number ($n = 1, 3, 5, 7, 9, 11, 13$) and C is the sensitivity constant of the device. In the present case, $C = 0.177 \text{ mg} \cdot \text{m}^{-2} \cdot \text{Hz}^{-1}$ as reported by the supplier (Q-sense AB).

$$\Delta m = -\frac{C\Delta f}{n}$$

If the material on the QCM-D sensor surface is not fully elastic, unevenly distributed or relatively thick, frictional losses occur that lead to a damping of the oscillation with the decay rate of amplitude that depends on the viscoelastic properties of the material. This is monitored by following the changes in dissipation energy, D , which is defined as follows:

$$D = \frac{E_{\text{dissipation}}}{2\pi E_{\text{storage}}}$$

where $E_{\text{dissipation}}$ is the total dissipated energy during one oscillating cycle and E_{storage} is the total energy stored in the oscillation.

Cellulose-coated thin films were deposited on gold QCM-D crystals by spincoating or by the Langmuir-Schaefer (LS) method using trimethylsilylcellulose (TMSC) (Kontturi et al. 2003; Tammelin et al.

Table 2: Sample set points (all sample points are at least duplicated).

Samples	Xylan concentration (mg · ml ⁻¹)
r1. pH 13 NaOH solution with 1 mM NaCl (reference)	0
r2. pH 10 NaOH solution with 1 mM NaCl (reference)	0
a1. pH 13 NaOH solution with 1 mM NaCl	0.01
a2. pH 13 NaOH solution with 1 mM NaCl	0.1
a3. pH 13 NaOH solution with 1 mM NaCl	1
b1. pH 10 NaOH solution with 1 mM NaCl	0.01
b2. pH 10 NaOH solution with 1 mM NaCl	0.1
b3. pH 10 NaOH solution with 1 mM NaCl	1

2006). Hexamethyl disilazane, dimethylacetamid (DMA) and lithium chloride were purchased from Sigma-Aldrich, Finland. All other chemicals were analytic grade. LS surfaces were prepared on gold QCM-D sensors with polystyrene coating. Polystyrene (0.1 w-%, Mw 280 000 g · mol⁻¹) in toluene was first spincoated on the sensor (4000 rpm, 30 s) and dried in an oven (60°C for 10 min). TMSC was dissolved in chloroform (0.4 mg · ml⁻¹), and the 30 TMSC layers were deposited on the sensor using the LS method.

TMSC surfaces were regenerated to cellulose via desilylation in 10% HCl vapor in vacuum for 5 min (Schaub et al. 1993).

TMSC was prepared from MCC powder (Fluka) by first dissolving in dimethylacetamide (DMAC)/LiCl followed by silylation with hexamethyl silazane (Cooper et al. 1981; Greber and Paschinger 1981). Successful silylation was evidenced by ^1H NMR in chloroform.

Prior to the adsorption studies, the cellulose surfaces were swollen in water overnight. The QCM-D sensors with cellulose thin films were placed in the QCM-D measurement cells, and the surfaces were allowed to stabilize in the appropriate buffer solution until a stable baseline was attained. Xylan dispersions were allowed to be adsorbed on the cellulose surfaces in the selected conditions which are shown in Table 2. After the measurement, the sample surfaces were rinsed with the given buffer solutions and dried by using nitrogen gas.

AFM microscopy: Changes in the topography of the cellulose surfaces before and after the xylan adsorption were monitored using AFM [ANASYS AFM + ® (ANASYS Instruments Inc., Santa Barbara, CA USA)]. The AFM characterization was carried out in dry conditions directly after the QCM-D measurement. The images were taken in the tapping mode in air using aluminum-coated n-type silicon cantilevers (HQ:NSC15/Al BS, Micromasch, Tallinn, Estonia) with typical probe radius of 8 nm, force constant of 40 N · m⁻¹ and nominal resonance frequencies between 265 and 410 kHz. The images were not processed in any way except for flattening.

Results and discussion

Preparation and characterization of the samples

The xylan content was 9.22 and 11.19 g · l⁻¹ for Xyl sol pH 13 and Xyl sol pH 10, respectively. This could not be

calculated directly from the DMC and pH because the NaOH addition was not literally the amount necessary for adjusting aqueous solutions due to the buffering capacity of the xylan. It is interesting that more xylan remained dissolved or dispersed at pH 10 compared to pH 13. This phenomenon could not be studied in more detail in this work.

Adsorption experiments were performed using 10 g MCC (as bone dry) with 990 g of the Xyl sol pH13 and Xyl sol pH10, respectively. This means that approximately equal amounts of xylan and MCC were present in the experiments (xylan content of 9.13 g at pH 13 and 11.08 g at pH 10). The pH was not adjusted after addition of xylan dispersions/solutions and mixing at room temperature for 7 days. For the experiment with Xyl sol pH 13, the final pH was 10.71 while for that with Xyl sol pH 10, the final pH was 8.60. Most probably, also the MCC was buffering the pH together with the xylan. After 7 days, the samples were separated into two equal parts and centrifuged.

The solids from one-half were collected as such as “unwashed sample”, and the wet yield and DMC were determined. The solids of the other half were washed with pH-adjusted water and centrifuged again and this was repeated one more time. After that, the solids were collected (“washed sample”). Again, the wet yield and DMC were determined. A part of all samples was dried for carbohydrate analysis and NMR analysis, and the rest was stored wet.

The list of obtained samples with the yields and carbohydrate contents are given in Table 3. The xylan content was high in the MCC samples after adsorption at pH 13 accounting for even 60% of the unwashed sample and 28% of the washed samples. This indicates a strong adsorption tendency that was only partly reversible during washing. During the adsorption trial, the pH decreased, which either enhanced the adsorption or was a consequence of it. At pH 10, the xylan content was moderate with 7.6% in the unwashed sample and 2.5% in the washed sample, respectively.

The ratio of xylose to 4-methyl glucuronic acid was calculated based on the analysis data for the xylan sample

used for fractionation as well as for the xylan dispersed at pH 10. The ratios were 19 and 22, respectively. As the difference was only minor, uronic acid substitution is not expected to have a significant effect on solubility or dispersibility under these conditions.

Adsorption of xylan on cellulose analyzed by NMR

The solid-state NMR spectra for the reference materials, pure MCC and xylan are shown in Figure 3.

Each carbon atom in cellulose and xylan produces distinctive signals to the solid-state NMR spectrum according to their chemical environment in the molecule and to the level of molecular ordering. Due to similarity in the chemical structure of MCC and xylan, most of their solid-state ¹³C NMR signals overlap. However, the signals from xylan carbon X1 at 100.46 ppm and C4 carbon in ordered cellulose from crystalline interiors of MCC at 87.19 ppm

Figure 3: ¹³C CPMAS NMR spectra of MCC and amorphous xylan reference samples.

Table 3: Yield of MCC samples with adsorbed xylan and polysaccharide contents.

Sample	Wet yield (g)	DMC (%)	Dry yield (g)	Cellulose (g)	Xylan (g)	Mannan (g)
MCC + Xyl pH 13 unwashed	115.7 ^a	14.95	17.30	6.7	10.4	0.1
MCC + Xyl pH 13 washed	34.0	8.89	3.02	2.14	0.85	0.04
MCC + Xyl pH 10 unwashed	19.27 ^b	30.92	5.96	5.46	0.45	0.09
MCC + Xyl pH 10 washed	14.76	27.54	4.06	3.89	0.10	0.06

^a50.8% of the wet sample; ^b51.5% of the wet sample.
DMC, Dry matter content; MCC, microcrystalline cellulose.

Figure 4: Solid-state ^{13}C NMR spectra of MCC + xylan samples prepared at different pH before and after the sample washing step. The arrow on the right-hand side figure marks the xylan X1 signal.

appear in the spectral regions with only partial overlap. These signals were chosen for spectral editing purposes to distinguish the ordered and less-ordered components into subspectra, as described later. The cellulose C4 signal at 82.2 ppm is known to originate from somewhat less ordered fibril surfaces.

The NMR spectra for MCC samples with adsorbed xylan, before and after the washing step, are shown in Figure 4. It can be seen that in MCC + Xyl pH 10, there are no visible signals from xylan present. In contrast to this, there is a strong signal from xylan X1 observed close to the cellulose C1 in MCC + Xyl pH 13. The washing step decreases the intensity of the X1 signal, but it can still be observed in the spectrum as a shoulder on the cellulose C1 signal.

Figure 5 shows the result of PSRE in the case of MCC + Xyl pH 13 unwashed. The xylan subspectrum correlates well with the reference xylan spectrum. This suggests that xylan is precipitated mainly in an unordered form. There is also a clear xylan signal left in the MCC

subspectrum at 100.4 ppm, representing a fraction closely associated with the ordered cellulose fibrils.

In the PSRE spectra of MCC + Xyl pH 13 washed (Figure 6), the remaining xylan does not contribute to the MCC subspectrum, which is assumed to represent the crystalline cellulose from the interiors of the crystallites. Xylan is observed in the subspectrum of less-ordered material, together with some cellulose signals, which most likely originate from the somewhat less ordered cellulose on the fibril surfaces, which apparently show similar relaxation behavior with xylan.

In the case of MCC + Xyl pH 10 unwashed (Figure 7), the only effect of PSRE appears to be a separation of cellulose signals into highly crystalline and less-ordered parts, the latter presumably originating from the fibril surfaces. The same result is obtained from the MCC + Xyl pH 10 washed (spectra not shown here).

According to CPMAS ^{13}C spectra, the amount of xylan in MCC + Xyl pH 10 is below the detection limit of the experiment. In the MCC + Xyl pH 13, most of the xylan is in amorphous form, and not closely associated with

Figure 5: PSRE spectra of MCC + Xyl pH 13 unwashed. (a) Original spectrum. (b) MCC sub spectrum. (c) Xylan subspectrum, together with the overlaid xylan reference spectrum (in purple).

Figure 6: PSRE spectra of MCC + Xyl pH 13 washed. (a) Original spectrum. (b) MCC sub spectrum. (c) Xylan subspectrum.

Figure 7: PSRE spectra of MCC + Xyl pH 10 unwashed. (a) Original spectrum. (b) MCC subspectrum. (c) Xylan or less-ordered MCC subspectrum.

cellulose. However, part of the xylan that could not be removed by washing is closely associated with MCC and follows into the subspectrum of cellulose at fibril surfaces. Even though the fibril surfaces are less ordered than cellulose in the interiors of cellulose crystallites, it is more ordered compared to the amorphous isolated xylan, suggesting that the xylan associated with the fibril surfaces might also have an ordered conformation.

Adsorption of xylan on cellulose analyzed by QCM-D and AFM

The adsorption behavior of xylan on cellulose at high pH was investigated using surface-sensitive methods (QCM-D and AFM) to reveal the interactions taking place directly at the cellulose surface. At highly alkaline conditions (pH 10 and pH 13), it is essential to follow and ensure the durability of the cellulose thin films which are deposited on the QCM-D sensor surfaces. Based on the preliminary tests, it was found that only highly crystalline cellulose surfaces prepared via the LS method were able to tolerate high alkaline conditions. More amorphous ultrathin films of cellulose produced via spincoating instantaneously dissolved when contacted with alkaline buffer solutions (results not shown). Furthermore, the most reliable adsorption experiments were produced at pH 10 (LS-cellulose surfaces), and are therefore presented in the following.

Prior to the xylan adsorption experiments, the QCM-D sensors with cellulose thin films were first allowed to swell and stabilize overnight in milliQ water. Subsequently, the cellulose thin films were contacted with buffer solution (1 mM NaCl at pH 10) until no changes in frequency and dissipation were detected, indicating that no changes due to swelling and/or cellulose dissolution occur (see Figure 8). As shown by Figure 8, the plateau level was

Figure 8: Changes in frequency (orange curve) and dissipation (blue curve) as a function of time recorded for LS-cellulose ultrathin films when stabilized and contacted with buffer solution of pH 10 and 1 mM NaCl. ($f_0 = 5 \text{ MHz}$ $n/3$).

attained after 150 min in contact with the buffer. A minor positive change in frequency indicates a corresponding loss of cellulose from the sensor surface. Such a small change in frequency ($<4 \text{ Hz}$ corresponds to a mass change of $<0.7 \text{ mg} \cdot \text{m}^{-2}$ calculated using the Sauerbrey equation) can be considered as a negligible mass loss and does not interfere in the following xylan adsorption experiments.

Figure 9 shows the adsorption isotherm of dissolved xylan on the cellulose surface using three concentration levels of xylan ($0.01 \text{ mg} \cdot \text{ml}^{-1}$, $0.1 \text{ mg} \cdot \text{ml}^{-1}$ and $1 \text{ mg} \cdot \text{ml}^{-1}$) at pH 10. Again, the changes in frequency and dissipation as a function of time were followed in order to estimate the mass change due to xylan adsorption and to evaluate the physical properties of the formed xylan layers. Figure 9 clearly illustrates that significant xylan adsorption takes place only at the highest solution concentration of $1 \text{ mg} \cdot \text{ml}^{-1}$, showing that xylan concentration has an important role in the deposition kinetics. Simultaneously, the dissipation change at the end of the adsorption was relatively high ($\sim 6.5 \times 10^{-6}$), although the frequency change is as low as -11 Hz . This suggests that the formed xylan layer is not uniformly attached on the cellulose surface.

Figure 9: Adsorption isotherm of xylan from solutions containing $0.01 \text{ mg} \cdot \text{ml}^{-1}$, $0.1 \text{ mg} \cdot \text{ml}^{-1}$ and $1 \text{ mg} \cdot \text{ml}^{-1}$ of xylan in pH 10 and 1 mM NaCl. Δf and ΔD vs. time for $n = 3$.

Evenly distributed thin and rigidly attached polymer layer normally generates a dissipation change lower than 1×10^{-6} with similar levels of frequency change. These findings are fully consistent with our previous results and are also supported by the AFM imaging (see Figure 10). After adsorbing xylan on cellulose, clear changes could be seen in both AFM height and phase contrast images (see Figure 10). Spherical xylan particles (Figure 10b and d) with the diameters varying between 0.01 and 0.1 μm were seen. The height image of the reference cellulose surface is shown in Figure 10a. Our system here behaved exactly in the same way as discussed earlier (Paananen et al. 2004; Tammelin et al. 2009). The main driving force for xylan to adhere to the cellulose surface seems to be the poor solubility of xylan in aqueous solutions. High xylan concentration in solution restricts the solubility of the xylan chains and favors the formation of agglomerates. Attachment on the cellulose surface begins only when the

concentration is relatively high, probably close to the limit of precipitation. In solution, polymer-polymer contacts dominate over polymer-solvent contacts, which promotes adsorption as xylan chains tend to escape from poor solvents. Chains either form agglomerates, get adsorbed on surfaces or even precipitate to avoid polymer-solvent contacts. Here, any precipitation was observed and care was taken to utilize freshly prepared xylan dispersions in all QCM-D experiments.

Conclusions

Adsorption of xylan from saturated solutions on MCC particles was successful, when starting from pH 13. A significant amount of xylan was strongly adsorbed and could not be removed by washing. Only minor irreversible enrichment of xylan was detected when the starting pH

Figure 10: AFM images were taken for a better understanding of xylan and cellulose interaction and xylan behavior after during the deposition trials.

AFM height images of the cellulose surface before (a) and after (c) xylan adsorption. AFM phase image of the cellulose surface after xylan adsorption with two magnifications (b) and (d).

was 10. The pH dropped during the 7-day adsorption time, indicating a buffering effect by the cellulose.

The xylan-adsorbed MCC samples before and after washing were analyzed by solid-state NMR. The amount of xylan in the MCC + Xyl pH 10 samples was below the detection limit of the experiment. In the MCC + Xyl pH 13 sample, the xylan was closely associated with MCC as it could not be completely separated from the MCC subspectrum. However, the washing step removed the xylan signal from the MCC subspectrum, while the remaining xylan signals were observed in the xylan subspectrum together with less-ordered cellulose signals, which are known to originate from the accessible fibril surfaces. This may indicate that the xylan fraction that remains after the washing step is closely associated with the surface of MCC. Therefore, through the use of NMR techniques, two distinct regions of xylan adsorption on cellulose fibers were possible to be seen. It suggests that there may be different adsorption mechanisms for each region, probably related to the size of the xylan fragment.

QCM-D experiments showed that xylan was irreversibly deposited on the cellulose surface, when the xylan concentration was high enough ($1 \text{ mg} \cdot \text{ml}^{-1}$). The AFM image of the formed surface indicated that xylan had deposited as oval particles with diameters around $0.01\text{--}0.1 \mu\text{m}$, while most of the cellulose surface seems to be uncovered.

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