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The role of hemicellulose in nanofibrillated cellulose networks

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Cellulose nanofibrils show remarkable properties with applications in several fields of materials science, such as for composites, hydrogels, aerogels, foams, and coatings. Cellulose nanofibrils are typically produced by mechanical and enzymatic processing leading to fibrils having a width in the nanometer range and very high aspect ratios. The formation of percolating networks and interactions between fibrils lead to useful properties in for example gel formation and composites. In this work we studied how the residual xylan that is found in cellulose nanofibrils that have been produced from hardwood pulp affects these properties. We used enzymatic hydrolysis to specifically remove xylan and studied rheological properties, morphological features, and properties of paper-like films of cellulose nanofibrils. We found that removal of xylan enhances the formation of fibril networks, resulting in both stiffer gels and stronger films. However xylan also stabilizes the fibrils against flocculation. Also the history of processing of the preparations affects the results significantly.

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Introduction

New developments for processing cellulose into fibrils with widths in the nanometer range have widely expanded its potential for use in materials science. As a polymer chain, cellulose is a homopolymer formed by the repeating unit of β-(1-4)-D-glucopyranose. In natural materials such as wood the cellulose polymer is packed into fibrils with a diameter of 3–5 nm. These fibrils are referred to as elemental fibrils and they are further packed together with hemicellulose to form what is known as microfibrils with a diameter of ~10 to 20 nm.[1] The microfibrils are packed further into larger fibrils finally forming macroscopic wood cellulose fibres.[2,3] The processing methods for the production of native fibrillar nanocellulose materials are based on disintegration of fibres mechanically and biochemically into high aspect ratio nanosized fibrils.[4] The resulting fibrils are 3–20 nm in diameter and microns long and referred to as nanofibrillated cellulose (NFC) or microfibrillated cellulose (MFC). The high aspect ratio and the excellent mechanical properties of the fibrils make NFC special as a material.[1,5] It is also possible to obtain high strength NFC fibrils by other means such as TEMPO-oxidation which are reviewed elsewhere.[6] The stiffness of individual cellulose I crystals is very high, measured to be between 134 and 143 GPa and the strength in the GPa range.[7,8]

NFC has been shown to form excellent stiff gels as aqueous dispersions at low solid content,[4] flexible and tough aerogels,[9] composite materials,[10,11] foams,[12] stabilizing emulsions and dispersions,[13–15] biomedical applications[16] with applications for different types of materials even for oil recovery.[17] Many of these applications rely on the stiffness and the high aspect ratio of the fibrils in NFC materials which leads to percolating networks that are further influenced by fibril entanglement and interactions between fibrils.[9,18]

Depending on the origin and the processing history, there can be up to 30% of hemicellulose present in NFC.[19] In softwood NFC, the main hemicellulose is galactoglucomannan and in hardwood NFC it is glucuronoxylan.[20] It is known that the fibrillation of pulp into microfibrillated cellulose is aided by the presence of hemicellulose, which is most likely due to the negative charges on hemicellulose that lead to charge repulsion between the fibrils.[21] The drying of pulp has a negative effect on the fibrillation process.[21] This is most likely due to irreversible coalescence of fibrils together[22–24] which could be driven by the formation of strong hydrogen bonding and contamination of the fibril surface during the drying process making the cellulose surface passive, *i.e.* less reactive.[25] It has been shown that the more hemicellulose is present in the material the less drying affects the fibrillation and other properties of pulp.[21]

A full understanding is still lacking of how the hemicellulose is located in the fibril structure and how it affects the NFC material properties in solution or in the dried state. It is

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expected that at least part of the hemicellulose is on the surface of the fibrils.[26,27] Previous studies on total enzymatic hydrolysis of cellulosic material, also NFC, have shown that hemicellulases especially xylanases are crucial for the efficient hydrolytic solubilisation of cellulosic materials.[28] Xylanases are enzymes that specifically hydrolyse xylans to xylose and small xylo-oligomers leaving cellulose intact.[29] The results suggest that hemicelluloses are associated in the fibril structure in such a way that they prevent cellulases to freely access the cellulose.[28] Other studies on hard wood pulp materials have shown that only some 35% of xylan can be solubilized from the materials by using xylanases demonstrating that not all the xylan in pulp is accessible for the enzyme.[30] The rest of the hemicellulose is most probably located within the fine structure of fibrils in between elemental fibrils. Therefore it is unclear how much hemicellulose can be solubilized from fibrillated material such as NFC where still elemental fibrils are closely associated and protecting at least some fraction from hydrolysis.

The adsorption of different hemicelluloses has been studied on a variety of cellulosic materials.[31–33] There are certain structural features, such as a critical length of the hemicellulose back-bone and the substitution degree that determine the affinity of hemicellulose on the cellulose surface.[31,32] For example, it has been shown that the shortest xyloglucan that can adsorb onto Avicel cellulose must be at least 12 sugar units long.[32] The critical length for xylan adsorption onto bacterial cellulose has been shown to be 15 xylose units.[31] It has also been shown that the more substituents the hemicellulose has the less it will adsorb onto the cellulose surface.[31] The two major xylanases from the filamentous fungi *Trichoderma reesei* hydrolyse xylan to xylose and 2–7 unit xylo-oligomers and are thus expected to release the hemicellulose from the cellulose surface.[34]

In this work we were interested in understanding how the percolating networks in NFC dispersions are affected by the presence of hemicellulose and how hemicellulose may affect interactions between cellulose fibrils and thereby nodes of interactions between fibrils that affect the rheological properties. Hemicellulose (in our case xylan) was partially hydrolysed by an enzymatic treatment and the effect of xylan hydrolysis on fibril interactions in the NFC dispersion was studied by rheological measurements and microscopy. More information on the organisation of the fibrils was obtained by studying the performance of dry films prepared from the NFC dispersions.

Experimental

Preparation of nanofibrillated cellulose

NFC was prepared by mechanical disintegration of bleached birch kraft pulp by ten passes through a M7115 Fluidizer (Microfluidics Corp.), essentially according to previous reports.[4] The solid content of the prepared water dispersion was ~1.9%.

Total hydrolysis of NFC and the analysis of sugar content

A total hydrolysis of the NFC suspension (dry weight 1.9%) was performed to determine its total xylan content.[35] An enzyme mixture containing four different commercial enzyme products was used (Econase (cellulase, Roehm Enzyme Finland) 20 mL, Ecopulp X-200 (xylanase, Roehm Enzyme Finland) 50 mL, Gamanase (mannanase, Novo) 100 mL and Novozyme 188 (β-glucosidase, Novo) 50 mL). The mixture was then diluted with 200 mL of 50 mM sodium acetate buffer pH 5 and desalted using a Biogel P-6 gel (Bio-Rad) column using the same buffer. The total activity of the mixture was 29.8 FPU (filter paper units) mL^{-1}. The load of enzyme used for total hydrolysis of NFC was 50 FPU g^{-1} and the reaction was allowed to proceed for 48 h at 40 °C (stirring 250 rpm), after which an additional 10 FPU g^{-1} amount of enzyme cocktail was added and the reaction continued for another 18 h to ensure total hydrolysis of the sample. The mixture was cleared by centrifugation (4000 rpm, 10 min, Eppendorf), supernatant was boiled for 5 min to inactivate enzymes and centrifuged again. Monosaccharides were determined by chromatography (DIONEX ISC-5000, CarboPac PA20) from the second supernatant. The dry weight of the first pellet from the total hydrolysis reaction was also determined.

NFC sample preparation

A stock solution of NFC was prepared as described previously.[15] For experiments, an aliquot of the stock solution was diluted with 50 mM sodium acetate pH 5 to a concentration of 4.55 g L^{-1} while mixing with a magnetic stirrer (<1000 rpm). Mixing was continued for about 30 seconds after which the solution was sonicated with an ultrasonifier (Vibra-Cell VXC 750, Sonics and Materials Inc., U.S.A.) using a standard tip (20%, 5 min). After sonication the solution was again mixed with the magnetic stirrer for about ~30 s. After this pre-treatment, xylan was hydrolysed using purified pI9 xylanase from *T. reesei*.[34] The total volume of the reactions was 3.52 mL with a final concentration of 0.4% NFC and an enzyme dosage of 3 mg g^{-1}. The samples were incubated at 45 °C for 24 h. Part of the samples were mixed with a magnetic stirrer (800 rpm) and part were allowed to react without stirring. Control samples were treated identically except that buffer was added instead of enzyme.

Analysis of xylan released from NFC

Samples were first clarified by centrifugation (20 000 rpm, 20 min). The clarified samples were then subjected to further enzymatic hydrolysis to break down possible oligosaccharide products into constituent monosaccharides. The following enzyme mixture was used: endoglucanase (24 nk at mL^{-1}), xylanase (187 nk at mL^{-1}), mannanase (18 nk at mL^{-1}), β-xylosidase (1 nk at mL^{-1}), β-mannosidase (18 nk at mL^{-1}), β-glucosidase (4 nk at mL^{-1}), α-galactosidase (11 nk at mL^{-1}), arabinosidase (6 nk at mL^{-1}), and α-glucuronidase (9 nk at mL^{-1}). A 1 : 4 ratio of enzyme to sample was used and sodium acetate buffer pH 5 (100 mM) was used for dilutions. Incubation was continued for 24 h at 45 °C. Finally the samples were boiled for 5 min to inactivate the enzymes and then centrifuged. The monosaccharide composition was analysed from the supernatant by chromatography as described above.

Rheological characterization of NFC suspensions

Xylanase treated and control samples were characterized for their rheological properties using cylinder and cup geometry (CC15, gap between the cup wall and bob 0.5 mm), with a stress controlled rheometer (StressTech, Reologica Instruments Ab, Sweden). Viscoelastic properties of the NFC dispersions were measured by oscillatory stress sweep tests (0.01–1 Pa) under a constant frequency of 0.1 Hz and by frequency sweep tests (0.01–10 Hz) at a constant stress amplitude of 0.03 Pa. Three samples were analysed for each treatment. The error of the measurements was calculated from the set of samples and it represents the accuracy of the sample preparation, *i.e.* how reproducible the sample preparation was. After the xylanase treatment the samples were allowed to equilibrate to room temperature for 3 h prior to rheological measurements. 3 mL of each sample was pipetted into the measuring cup and the bob was lowered to the measuring position. The sample was allowed to relax for 5 min before the measurement was started.

Preparation and characterization of dried NFC films

Xylanase treated NFC and control samples were used to make thin, paper-like films. The films were prepared passing NFC dispersion (1 mL, 4 g L^{-1}) through a membrane (Durapore GVWP, 0.22 μm, Millipore, US) in a vacuum filtering device whereby the NFC deposited on the membrane as a film. The deposited NFC film was peeled off from the supporting membrane and gentle pressure was applied on it using a 300 g load for 10 min. Films were dried overnight at 65 °C, and then allowed to stabilize at 50% humidity at 21 °C before tensile testing under ambient conditions. For the tensile testing, a mini tensile tester (Deben, UK) was used. A 20 N load cell was used with a nominal strain rate of 0.5 mm min^{-1}. At least 4 duplicates were made from each sample. The films were cut into strips of 2 mm × 20 mm and had a thickness of about 7–10 μm. A micrometer slide calliper was used to determine the widths of the samples. The thickness was measured with a scanning electron microscope (JEOL JSM-7500F FEG, Japan) using an acceleration voltage of 1–15 kV depending on the sample. Samples were sputtered with palladium to enhance imaging conditions and prevent the charging of the samples. For the thickness measurements, films were aligned perpendicular to the electron beam. At least 8 measures were made from different places of the film cross-section.

Cryogenic transmission electron microscopy (Cryo-TEM) imaging of NFC fibrils

Cryo-TEM was used to analyse the morphology of the NFC fibrils in solution. NFC dispersions were diluted 1 : 10 and vitrified by purging in a liquid propane–ethane mixture on c-flat grids using a vitrobot (FEI, U.S.A.). Imaging was performed with a JEOL JEM-3200FSC Cryo-TEM 300 kV.

Atomic force microscopy (AFM)

NFC samples were diluted 1 : 400 with water and a 30 μL aliquot was spread on freshly cleaved mica (Electron Microscopy Sciences). The samples were allowed to dry for 24 h, attached on steel supports with double-sided tape (Scotch) and imaged using a NanoScope IIIa Multimode AFM instrument (E-scanner, Digital Instruments/Veeco) with an NSCI5/AlBS cantilever (μMASCH, USA). All images were recorded in tapping mode in air with scan rates of 0.8–1 Hz (free amplitude was about 0.65 V). The damping ratio was around 0.7–0.85. Images were flattened to remove possible tilts in the image data. Otherwise no further processing of the images was done.

Dynamic vapour sorption (DVS) measurement of NFC films

Dynamic water vapour sorption of NFC films was done with a DVS-1 instrument (Surface Measurement Systems, UK) to investigate if the amount of xylan within the films has an effect on the water sorption capability of NFC. NFC films were dried overnight at 60 °C in a desiccator containing phosphorus pentoxide before the measurement. The measurement had five 16 hour steps at 0%, 25%, 50%, 75% and 90% relative humidity. The amount of water vapour content was measured during the whole experiment.

Results

Total xylan content and release of xylan from NFC

Table 1 summarises the sugar content results from total enzymatic hydrolysis of NFC analysed by chromatographic methods. In addition to solubilised sugars there were 1.7% of non-soluble materials such as lignin.

We conclude that ~27% of the NFC sample consisted of xylan (xylose, methylglucuronic acid and xylotrioses), ~71% of cellulose (glucose), ~0.3% of glucomannan (2 : 1 mannose to glucose[20]) and 1.7% of insoluble materials. The hydrolysis with only pI9 xylanase for 24 hours at 45 °C (with stirring and without) showed that only part of the total xylan was accessible to the enzyme releasing 32–36% of the total xylan as soluble saccharides. There were no significant differences between samples that had been stirred or not. The release of xylan during the hydrolysis corresponded to approximately a 10% reduction of total mass. Experiments showed that increasing enzyme load or hydrolysis time did not increase the release of xylan.

Effect of xylan removal and continuous mixing on NFC dispersion rheology

Stress sweep measurements were performed on xylanase-treated samples that had been mixed or not mixed during the

Table 1 The amount of mono- and oligosaccharides released from NFC by total enzymatic hydrolysis

Mono-/oligosaccharide[a]	Glu	Xyl	Man	Me-gluA	Xyl_3
Amount solubilised (%)	71	26.2	0.2	0.6	0.3

[a] Glu = glucose, Xyl = xylose, Man = mannose, Me-gluA = methylglucuronic acid, and Xyl_3 = branched xylotrioses (hexenuronic acid xylotriose and 2-*O*-(4-*O*-methyl-3-L-idopyranosyluronic acid)-xylotriose).

hydrolysis. The elastic modulus (G'), viscous modulus (G'') and phase angle of a typical measurement (NFC) are presented in Fig. 1A. The average G' and G'' values in the linear viscoelastic region and the critical stress at the onset of non-linear behaviour were determined from the curves (Fig. 1B). From Fig. 1B it is seen that G' values are an order of magnitude higher than G'' values and thus the systems are all predominantly elastic. Xylan hydrolysis had a clear effect on the elastic modulus, but the effect was dependent on the treatment procedure. Letting the xylan hydrolysis proceed without mixing resulted in a higher G' than the corresponding control sample without xylanase. Mixing with a magnetic stirrer during the hydrolysis on the other hand led to a significant decrease in G'. This lowering of G' was more pronounced when xylan had been removed. The critical stress values present the onset of elastic-to-viscous transition and clearly show that this phenomenon occurs at lower stress values for samples that are mixed compared to those that are not mixed. From the samples that are mixed the one treated with xylanase stands a significantly lower stress value than the one not treated with xylanase. Xylan hydrolysis increases the critical stress value compared to native NFC.

No significant differences were observed in frequency dependent behaviour of the different NFC samples. The modulus of all NFC solutions rose as a function of frequency with an equal slope (data not shown).

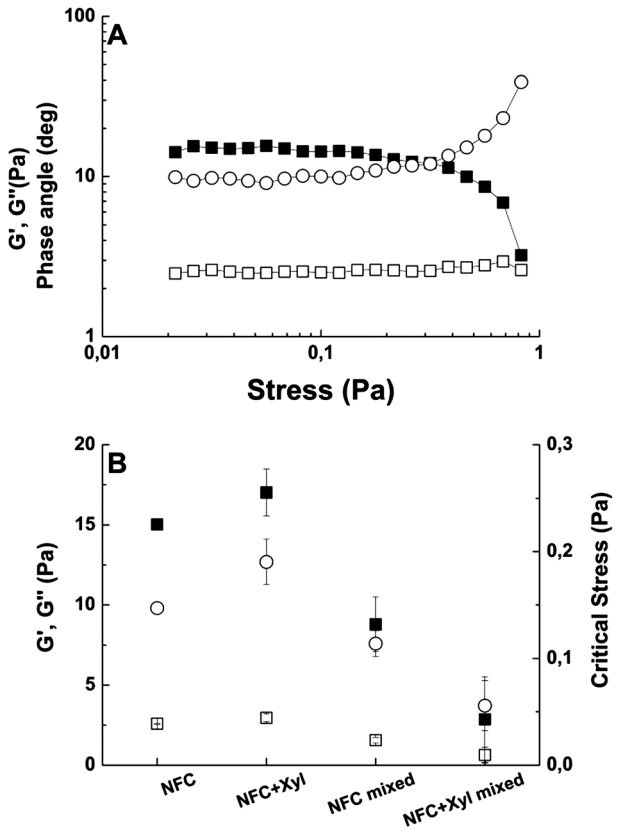

Fig. 1 Effects of xylanase treatment and continuous mixing on rheological properties of NFC dispersion. (A) A typical stress sweep test for NFC dispersion showing the behaviour of elastic and viscous moduli (G', G'') and phase angle. (B) Elastic moduli (G'), viscous moduli (G'') and the critical stress values of the different samples. In graph (A) ■: G', □: G'', and ○: phase angle. In graph (B) ■: G', □: G'', and ○: critical stress.

Effect of xylan removal and continuous mixing on NFC fibril morphology

Cryo-TEM and AFM images were taken for samples (Fig. 2) that had been treated with xylanase with and without mixing and controls. No differences between treatments were observed compared to non-treated controls. Xylanase treatment or continuous mixing does not seem to have an effect on the morphology of the fibrils; no shortening of the fibrils or significant differences in bundling can be seen in any of the samples. The fibril diameters, *i.e.* heights, from the AFM images are very similar for all samples; NFC 6.8 ± 4.0 nm, mixed NFC 9.1 ± 4.2 nm, xylanase treated NFC 9.0 ± 4.3 nm and xylanase treated mixed NFC 9.6 ± 3.4 nm. The heights and deviations were calculated from heights obtained from the cross-section of 20 fibrils in different images.

Preparation and characterization of thin paper-like films

Films of NFC were made using samples that had been treated with xylanase and controls without xylanase. Each set included samples that had been mixed or not mixed during the treatment. Although the film preparation followed an identical procedure, the different NFC treatments resulted in different film thicknesses and thus different film densities. The film thicknesses and densities are summarised in Table 2.

Typical stress–strain curves for differently processed NFC film samples are shown in Fig. 3. The mechanical properties of

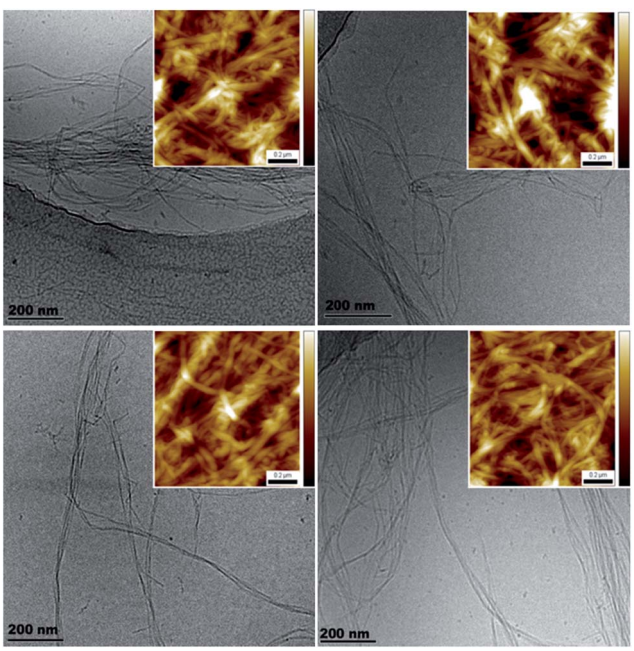

Fig. 2 Cryo-TEM images of NFC suspensions: top left, NFC; top right, NFC mixed; bottom left, NFC treated with xylanase; and bottom right, NFC treated with xylanase and mixed. Insets: AFM height images of the corresponding samples spread and dried on freshly cleaved MICA. Scan size 1 × 1 μm and height 60 nm.

Table 2 Thickness and average density of differently treated NFC films

	NFC	NFC–Xyl[a]	NFC mixed[b]	NFC–Xyl mixed[c]
h (μm)	12.2 ± 1.45	8.2 ± 0.90	8.5 ± 0.84	9.4 ± 0.83
ρ (mg mm^{-3})	1.2	1.6	1.8	1.4

[a] Xylanase treated NFC. [b] Continuously mixed NFC. [c] Continuously mixed NFC that was treated with xylanase.

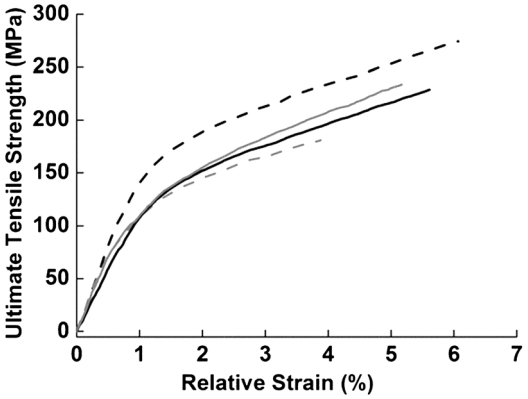

Fig. 3 Typical stress–strain curves for the differently processed NFC films. NFC (solid black line), continuously mixed NFC (dashed black line), NFC treated with xylanase (solid grey line), and continuously mixed NFC treated with xylanase (dashed grey line).

Table 3 Mechanical properties of differently treated NFC films

	Ultimate tensile strength (MPa)	Young's modulus (GPa)	Strain-to-failure (%)	Yield strength (MPa)
NFC	199 ± 35	10.0 ± 3.1	5.24 ± 0.6	113 ± 22
NFC–Xyl[a]	243 ± 38	13.1 ± 1.9	4.81 ± 0.3	126 ± 24
NFC mixed[b]	252 ± 17	14.6 ± 1.3	5.55 ± 0.4	149 ± 6.3
NFC–Xyl mixed[c]	166 ± 34	11.1 ± 1.3	3.80 ± 0.6	100 ± 26

[a] Xylanase treated NFC. [b] Continuously mixed NFC. [c] Continuously mixed NFC that was treated with xylanase.

the four NFC films are summarised in Table 3. Treatments showed in general significant effects on the mechanical properties. The measured parameters (stiffness (Young's modulus), yield strength, ultimate tensile strength, and strain to failure) show dependence on both mixing and xylanase treatment of the samples. A general trend is that in mixed samples the xylanase treatment weakened the mechanical properties and in non-mixed samples the xylanase treatment improved mechanical properties. However for the values for strain to failure, the xylanase treatment generally led to lower values.

Dynamic water sorption measurement of NFC films

Water uptake was used as a way of identifying differences between xylanase treated and non-treated NFC films. Control samples and samples with xylanase treatment either with or without mixing showed very similar water uptake profiles

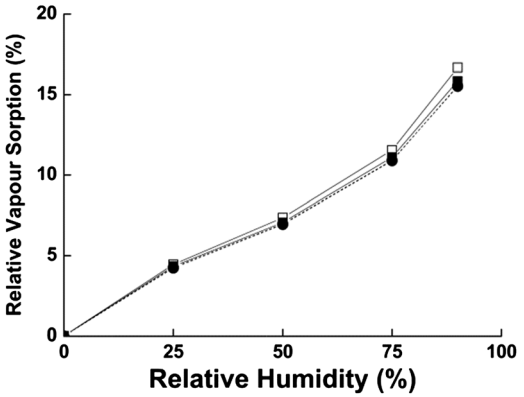

Fig. 4 Relative water vapour uptake by paper-like films made of differently treated NFC. –□– NFC, –○– xylanase treated NFC, –■– continuously mixed NFC and –●– continuously mixed xylanase treated NFC. Treating NFC with xylanase with or without mixing did not significantly change moisture uptake compared to controls without xylan treatment.

(Fig. 4). In total a 16% mass gain was reached at 90% relative humidity in all films.

Discussion

NFC prepared from hard and softwood has been widely used for developing new innovative materials and has proven to be very promising for many applications. In this work we addressed the question of how the hemicellulose content of the NFC can affect its properties. We used a type of NFC preparation that has been widely used in other studies.[9,11,13,17,36–54]

Initially we found that 27% of the NFC preparation consisted of xylan, the major hemicellulose in hardwood. Treating the NFC with a purified xylanase (pI 9 variant from *T. reesei*) released 32–35% of xylan. Extending hydrolysis time or enzyme dosage did not release more xylan indicating that the remaining xylan was not accessible. Xylan is naturally found between elementary microfibrils of cellulose in the plant cell walls. Most likely the xylan that was hydrolyzed and removed in the current experiments was located easily accessible on the surface of the small bundles of microfibrils that form the NFC. The xylan that was not hydrolysed by the enzyme was most likely in between the microfibrils of the NFC. Interestingly, the hydrolysis of xylan did not result in any notable change in the dimensions or shape of the NFC as analyzed by both Cryo-TEM and AFM. It is therefore difficult to estimate exactly how the xylan is associated with the cellulose, if it was thinly and evenly spread out along the fibrils or only as loosely associated. The water sorption behaviour was performed to detect possible changes in the cellulose fibril structure during xylan hydrolysis. Previously it has been shown that the water sorption capability of cellulosic material is dictated by the crystallinity of the materials below RH 75% and by structural features such as porosity above RH 75%.[55] In our work we saw no differences in water sorption behaviour of the different samples suggesting that the xylanase has not altered the cellulose structure.

Rheological measurements showed significant differences between xylanase treated and non-treated NFC samples.

However, it was found already in preliminary experiments that the viscoelastic properties of NFC dispersions are very much dependent on their shearing history even in the absence of xylanase, which is probably related to shear-induced changes in the floc structure of the dispersions.[56] In our work, we also observed a marked difference in G' between the mixed and non-mixed NFC dispersions without xylanase (Fig. 1B), although they had identical chemical composition. This highlighted the necessity of developing a pre-treatment protocol involving initial break-up of aggregates and networks by sonication and mixing prior to enzymatic treatment. This pre-treatment protocol served very well to achieve a reproducible base-level to which different treatments could be performed and reduced significantly the variation between experiments.

The enzymatic hydrolysis of xylan led to a clear increase in the elastic modulus of the sample when the sample was not mixed (Fig. 1B). Continuous mixing lowered the modulus of the non-treated samples but the samples that were both mixed and treated with xylanase resulted in the lowest modulus values overall. Interpretation of the results is difficult because apparently several different effects are occurring simultaneously. The initial pre-treatment of the samples involving sonication is expected to open up bundles of fibrils and increase fibril entanglement and therefore interaction nodes between fibrils. We also note that this non-enzyme treated, non-mixed sample gave high modulus values. Treating the sample with xylanase, but not mixing increased the modulus further. We interpret this change as an increase of interactions between fibrils resulting in a stronger percolating network. On the other hand, the continuous mixing of samples without the addition of xylanase significantly decreased the modulus. The mechanism behind this is most likely related to less effective percolation due to the formation of flocs as a result of the shear forces during mixing.[18] The bundling might cause the loss of percolation by the loss of interaction nodes throughout the gel and result in local concentrated interactions of fibrils in the gel. In the work by Vian *et al.*[22] it was shown that the presence of hemicellulose did not affect the bundling of fibrils during dehydration of the system yet it prevented the irreversible flocculation of the fibrils. It was also shown that reducing the amount of hemicellulose in the system caused bundling of the fibrils all the more extensively the more hemicellulose was extracted and the flocs grew larger when larger amounts of hemicellulose were removed. This suggests that there might be a similar mechanism of bundling caused by shear forces with and without the removal of hemicellulose, yet the exact structure of the flocs in solution conditions remains unclear. Sonication of samples that were not treated by xylanase led again to increased viscosity, most likely due to/consistent with the opening up of flocculated structures. This finding is consistent with the findings of Vian *et al.*[22] that fibrils with hemicellulose do not flocculate irreversibly and can be dispersed again. The xylanase treatment of samples that were mixed led to a still larger decrease in modulus. The mechanism that leads to the decrease of modulus in the mixed samples was therefore apparently enhanced by xylanase treatment. This is contradictory to the effect of xylanase without mixing. It could be possible that the increase in interactions brought about by xylanase treatment is favourable to a certain extent (as when the dispersion is at rest), but that a further increase in interactions during mixing results in such a high level of flocculation that the network structure is hampered.

The elastic-to-viscous transition of the gels as studied by increasing the applied stress supports these conclusions (Fig. 1B, Critical stress). This transition occurs at the lowest stress values for the mixed and xylanase treated samples. The critical stress value then increased in the order, mixed and non-treated, non-mixed and non-treated, with the strongest gels for the xylanase treated and non-mixed. The results follow the logic that mixing induces aggregation of fibrils that leads to weaker gels. Xylan removal enhances this effect, but if samples were not mixed, the xylan removal made the gelling network stronger.

The effect of xylanase treatment for making paper-like films was seen in two ways. Firstly, the thickness of the films was reduced as expected by the loss of mass due to hydrolysis. However also mixing reduced the thickness apparently by allowing a denser packing of the fibrils. In line with this it has previously been shown with pulp materials that the density is increased and that the pore size is decreased when xylan is enzymatically removed indicating that the packing of the fibres is more efficient.[30] There was also a clear effect on the mechanical properties of the films by the treatment with xylanase. When NFC samples were not mixed during the enzymatic reaction, the films became stiffer and stronger as a result of the treatment. This is in line with the rheology data where gel-stiffening occurred by the same treatment. However, continuous mixing of NFC also resulted in stronger and stiffer films without enzymatic treatment. Since the mixed/non-mixed samples without enzymatic treatment were chemically identical there must be other factors leading to increased properties. As can be seen from the measured thicknesses, also the thickness of the mixed samples was lower, which indicates better packaging of fibrils in the films and thus increased interactions between fibrils. The denser packaging allowed by the aggregation of the fibrils then seems to lead to better properties. On the other hand, as the further weakening of the gel properties in the rheological experiments indicated, the enzymatic treatment further weakened interactions between fibrils in mixed samples and thus led to weaker films. Thus the effect of xylanase treatment was in the same way opposite in both film formation and for gel formation depending on how the fibrils were mechanically treated. However, in all cases the values for strain-to-failure of the samples were lower for xylanase treated NFC. This shows that the stiffness and strain values are not necessarily directly dependent and that xylan plays a role in the extension of fibril networks on the region of the plastic deformation. Interactions between fibrils that are mediated by xylan then can allow more creep and extension, while the interactions in fibrils where xylan was removed were stiffer but less flexible. It appears that softening of the interface between NFC fibrils due to the xylan layer is beneficial and allows movement of the fibrils under tensile stress. Although it is difficult to distinguish between the fine effects of dispersion quality and the strength of the interaction between the fibrils, the film properties show consistent results and reveal different aspects of the fibril

properties. It has also been shown previously for pulp materials that the tensile strength is decreased and that the fibre stiffness is increased when hemicelluloses are removed.[30]

The formation of interacting nodes in percolating networks has been seen as a major explanation of the properties of NFC. In this work we addressed the question of how hemicellulose (in our case xylan), a major component of typical NFC preparations, affects its properties. The results support a major role of fibril networks in NFC properties, but show that xylan as an additional component clearly affects fibril interactions and therefore the details of how NFC performs. Investigating the role of interactions within NFC networks is greatly affected by the history of the preparation and in which form the fibrils are present, *i.e.* if they are open or aggregated. Microscopy imaging did not reveal such differences and they were only seen by detailed rheological measurements. The results show that xylan in this way plays a key role in fibril stability in aqueous surroundings preventing for example fibril coalescence during mechanical mixing. In some cases the mechanical properties can be enhanced by xylan removal, but as stability may be decreased a better control of processing conditions may be required when xylan is present.

Conclusions

Only some 35% of the xylan present in the NFC network can be removed by or is accessible to xylanase. The presence of xylan affects the NFC network properties clearly but effects are not unambiguous and the comparison of the effects to gel properties and film properties is somewhat difficult to correlate. The biggest effect of xylan still seems to be on the association behaviour of the NFC fibrils. The xylan that is easily removed by enzyme hydrolysis and affects the stability of NFC seems to be on the surface of the fibrils and the rest of the xylan not affecting the association of the fibrils is within the fibril structure. Despite the presence of xylan NFC is very sensitive to processing history. Mixing for example induces changes in the hydrogel structure that affect the mechanical properties.

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