Xylanase-Induced Liberation of Negatively Charged Species and Their Effect on Colloidal Interactions and the Retention of Bleached Kraft Pulp Fibers

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The ability and specificity of various monocomponent endo-1-4-β-xylanases to release negatively charged species from never-dried, bleached, birch kraft pulp was studied. The effects of dissolution of these xylan-based components on pulp filtrate properties and the subsequent chemical retention were determined. The results revealed that the amount of charged species released depended on the xylanase and that the ratio of charged species released to dissolved xylan is not linear. Chemical retention tests showed that high levels of dissolved xylan interfere with the fixation of colloidal species, which was confirmed by removing the dissolved hemicelluloses. The roles of residual hemicellulose and the properties of modified fibers on chemical retention and the level of internal sizing are discussed.

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INTRODUCTION

During recent decades, a strong interest in using enzymes more within the forest products industry has emerged, with a large number of research papers and patent applications in this field (Demuner *et al.* 2011). At the moment, enzymes are successfully being used in several operations in industrial kraft pulp fiber lines. The most well-established enzyme applications include the use of xylanases in kraft pulp pre-bleaching and the use of both cellulases and xylanases to reduce the energy required for refining (Bajpai 1999; Kenealy and Jeffries 2003; Dhiman *et al.* 2008). Some potential future applications include the selective modification of bleached kraft pulps for papermaking purposes (Blomstedt *et al.* 2010); separating potential value-added side streams, such as xylo-oligosaccharides (Hakala *et al.* 2013; Metsämuuronen *et al.* 2013), from kraft pulp fiber lines with the aid of xylanases; and the use of endoglucanases to facilitate the fibrillation of cellulose during the production of nanoscale cellulose fibrils (Pääkkö *et al.* 2007).

The properties of xylanase-modified kraft pulps have been investigated by several groups (Oksanen *et al.* 1997; Roncero *et al.* 2005; Moss and Pere 2006; Blomstedt *et al.* 2010; Saukkonen *et al.* 2014). In general, xylanase treatments are considered highly selective and primarily affect surface xylan. Improved dewatering properties, increased brightness, decreased swelling, and slightly reduced beatability, with minor changes in the physical properties of the paper, have been reported for xylanase-treated pulps.

Although the effects of xylanase treatments on the basic properties of kraft pulps are quite well understood, the xylanase-induced effects on the furnish and papermaking colloidal chemistry have been explored less. However, since it is known that xylanases act

primarily on the surface of fibers, and because the surface charge of the fiber and the accessible surface are especially important for colloidal interactions and wet end chemistry, it is expected that xylanase treatment would change the retention behavior of the kraft pulp fiber suspension. In both softwood and hardwood, the fiber charge originates primarily from 4-O-methyl-α-D-glucopyranosyluronic acids bound to the xylan backbone (Sjöström 1989), and any modification of the xylan fraction therefore influences the adsorption of polyelectrolytes and colloidal interactions.

Only a few studies of the effects of xylanase treatments on fiber surface charge and the electrokinetic properties of bleached pulps can be found in the literature. Horvath and Lindström (2007) reported a slightly reduced fiber charge in fines-free, xylanase-treated, bleached softwood kraft pulp. Recently, it was reported that the treatment of birch kraft pulp with xylanase reduced the negative zeta potential charge of the fibers (Saukkonen *et al.* 2014). Moreover, Du *et al.* (2012) reported that the xylanase treatment of bleached softwood kraft pulp causes the zeta potential to increase and the cationic demand of the pulp to decrease, but it was not clear whether the cationic demand was determined in the pulp filtrates prior to or after washing. In addition, in studies by Saukkonen *et al.* (2014) and Du *et al.* (2012), the experimental conditions were not properly adjusted for electrokinetic determinations (*i.e.*, the pH and electrolyte concentration), making it impossible to draw clear conclusions regarding the effects of xylanase treatment on the pulp's charge characteristics and the colloidal behavior.

There is a lack of understanding of the effect of the xylanase treatment of never-dried kraft pulp fibers on the colloidal interactions, and particularly on the adsorption and retention behavior of modified kraft pulps. In the present study, the ability of three different xylanases to liberate anionic charged species from the fiber surface was determined. The resulting changes in zeta potential of the fibers, the amount of dissolved species present, and the cationic demands of the filtrates were determined in the absence and in the presence of various wet-end additives, and the subsequent effects on chemical retention were monitored.

EXPERIMENTAL

Comparison of Xylanases

Three commercial xylanase enzymes were used: an endo-1,4-β-xylanase produced by *Bacillus sp.* (Pulpzyme HC, (PZ)); an endo-1,4-β-xylanase produced by *Aspergillus oryzae*, carrying the gene-encoding family 10 xylanase from *A. aculeatus* (Shearzyme 500L (SZ)); and a thermo-stable endo-1,4-β-xylanase from *Thermomyces lanuginosus*, produced by genetically modified *A. oryzae* (Novozym 51024 (NZ)).

The xylanase activities and endoglucanase side activities of the different enzyme products were determined against xylan from beech wood (Sigma) and hydroxyethyl cellulose (HEC), respectively. The xylanase activity assay was carried out at 50 °C in 100-mM citrate-phosphate buffer at pH 7, while the endoglucanase activity assay was carried out at 50 °C in 50-mM Na-citrate buffer at pH 5. The reducing sugars from the hydrolyzates were determined using the DNS method (Miller *et al.* 1959). The determined and declared enzyme activities are given in Table 1.

Never-dried, bleached birch kraft pulp was obtained from a local pulp mill. Prior to xylanase treatment, the pulp was refined to °SR 24.5 using a laboratory Hollander beater (Oy Lorentzen and Wettre Ab, Sweden). The enzyme treatments were done in a water bath

with pulp consistency 1.58 wt.%, pH 7, temperature 50 °C, and 0.01 M NaCl. Gentle mixing was applied during the enzymatic hydrolysis. After the set reaction time (1 h) had elapsed, the filtrate was separated from the pulp *via* Büchner filtration. To deactivate the enzymes, the filtrate was heated to 90 to 95 °C for 10 min and the pulp cake was washed three times using near-boiling water. The samples were then cooled and stored in a cold room until further use.

Table 1. Xylanase and Endoglucanase Activities of the Endo-1-4-β-Xylanases

Xylanase	Declared Activity (FXU/g)	Xylanase Activity (nkat/mL)	Endoglucanase Activity (nkat/mL)
PZ	360	116315	5
SZ	650	37650	17
NZ	650	142426	4

Xylanase activity was determined against xylan from beech wood and endoglucanase activity against hydroxyethylcellulose (hec). FXU = fungal xylanase unit, nkat = nanokatal.

The filtrate sample was analyzed for the dissolved carbohydrate residues after acid hydrolysis using the HPLC method described by Metsämuuronen *et al.* (2013). The cationic demand of the filtrate samples was determined using a Mütek PCD 02 particle charge detector (BTG, Switzerland) with 0.001 N poly-DADMAC as titrant. The pulp's zeta potential was determined at 0.6 wt.% consistency in a solution of 0.01 M NaCl electrolyte using streaming potential equipment (Mütek SZP 06; BTG, Switzerland). The water retention value (WRV) and Schopper-Riegler (°SR) number were determined according to the ISO 23714 (2014) and SCAN-C 19:65 (1965) standards, respectively. The fiber dimensions and pulp fines contents were determined using an optical fiber analyzer (L&W FiberTester; Lorentzen & Wettre, Sweden).

Up-Scaled Xylanase Treatment

Of the three tested xylanases, the hydrolytic efficiency of PZ was selected to be determined in a pilot-scale environment. This enzyme was able to dissolve more negatively charged species from the fiber wall than the other two xylanases. The pulp raw material used was industrial, never-dried, bleached birch kraft pulp, the same material used in the previous section of this work.

The enzyme treatments were done in 5-m^3 pilot-scale vessels with pulp consistency 3.5 wt.%, temperature 50 °C, pH 7, and a treatment time of 90 min. The applied enzyme dose was 2 kg/t or 10 kg/t, corresponding to 217 or 1087 nkat/g of O.D. pulp, respectively. The enzyme treatments were done either after refining or together with refining. In the first case, the pulp was first slightly "over-refined" to °SR 21 using a pilot-scale disc refiner before the enzyme treatment. After the completed hydrolysis, the pulp temperature was raised to over 90 °C and maintained for approximately 30 min to deactivate the enzyme. In the second case, the pulp was refined during the enzyme treatment; refining was initiated after 60 min of hydrolysis and the hydrolysis was allowed to continue during refining. After 90 min, the deactivation stage was initiated. In addition, one pulp batch was prepared without the enzyme deactivation step. A reference pulp was refined to °SR 16.5 and was also subjected to the deactivation stage. All pulps were refined at 50 °C. A fraction of the pulps were washed in a Büchner funnel immediately after sampling to remove dissolved material.

The filtrate and pulp properties from the up-scaled xylanase treatment were determined as described in the previous section. In addition, total organic carbon (TOC) in pulp filtrates was determined using a TOC analyzer (Shimadzu TOC-5050A; Shimadzu Corp., Japan).

Retention Chemical Performance

A moving belt former (MBF) equipped with a commercial triple-layer SSB forming fabric was used to investigate the retention behavior of the unwashed and washed pulp samples. The pulp sheets were prepared according to a typical fine paper furnish, including PCC filler, AKD, cationic starch, a retention polymer, and bentonite. The doses, dosing points, and delay times for the chemical additives are given in Table 2.

Table 2. Chemical Additives and Dosing Points in the MBF Trials

Additive	Trade Name	Added Amount (%)	Dosing Point	Delay Time (s)
Cationic starch (wet-end)	Raisamyl 50021	0.40	Thick stock	90
Alkyl ketene dimer (AKD)	EKA DR28HF	0.15	Thin stock	60
Precipitated calcium carbonate (PCC)	Optical HB	29	Thin stock	45
Cationic starch (retention)	Raisamyl 50021	0.15	To MBF	30
Cationic polyacrylamide (C-PAM)	Percol 47	0.015	To MBF	15
Bentonite	Hydrocol SH	0.15	To MBF	4

Delay time is the time prior to sheet formation (*i.e.*, when the vacuum was switched on in the MBF). The added amount (%) indicates the dry weight of the additive with respect to the dry weight of the formed paper product when assuming 100 % first pass retention.

The formed sheets were drum-dried at 90 °C for 3 h, and their dry weight and ash content (at 525 °C) were determined. These data were used to calculate the total and filler retention during the sheet forming process. In addition, the $Cobb_{60}$ sizing degree of the sheets was determined according to ISO 535 (1991) after the sheets had been stored for 12 d. During storage, the sheets were protected from light. No additional curing was performed prior to the Cobb testing.

RESULTS AND DISCUSSION

Comparison of Xylanases

In the first part of this study, the abilities of the three xylanase enzymes to hydrolyse and dissolve xylo-oligosaccharides from bleached birch kraft pulp were compared, as shown in Fig. 1. The tests were done at various enzyme levels but with the treatment time and other conditions remaining constant. In the present study, the dissolved oligosaccharides were quantified as xylose after acid hydrolysis. However, it is known from previous work that the endoxylanase from *Bacillus* sp. (PZ) produces primarily xylotriose, while the main degradation products for the endoxylanases from *A. aculeatus*

(SZ) and *Thermomyces lanuginosus* (NZ) are xylobiose and xylose, respectively (Metsämuuronen *et al.* 2013).

For all three xylanases, there was a relatively low concentration limit beyond which the further release of xylose did not occur. This shows that accessible xylan at the surface can be removed at roughly the same enzyme concentration, but that the selectivity and efficiency of different enzymes vary significantly. In particular, NZ released significantly lower amounts of xylose, although it exhibited the highest enzymatic activity toward xylan from beech wood.

In all pulp filtrates, xylose was the only monosaccharide detected after acid hydrolysis, confirming the high selectivity of the xylanases applied, which is in agreement with previously published results concerning specificity in the treatment of bleached kraft pulps with monocomponent xylanases (Metsämuuronen *et al.* 2013). In a recent paper, it was also demonstrated that the treatment of refined, never-dried pulp with an endoxylanase resulted in rapid, highly selective hydrolysis of xylan from the outermost layers of the fiber wall (Saukkonen *et al.* 2014). The high selectivity of a purified xylanase preparation was also reported by Hakala *et al.* (2013) for a consistent pulp raw material. The common features of xylanases, including their high selectivity and poor accessibility within the fiber wall, have been exploited to study the presence of carbohydrate-lignin linkages in kraft pulps (Tenkanen *et al.* 1999; Karlsson *et al.* 2001) and the location of charged groups in the fiber wall (Laine *et al.* 1996), among other things.
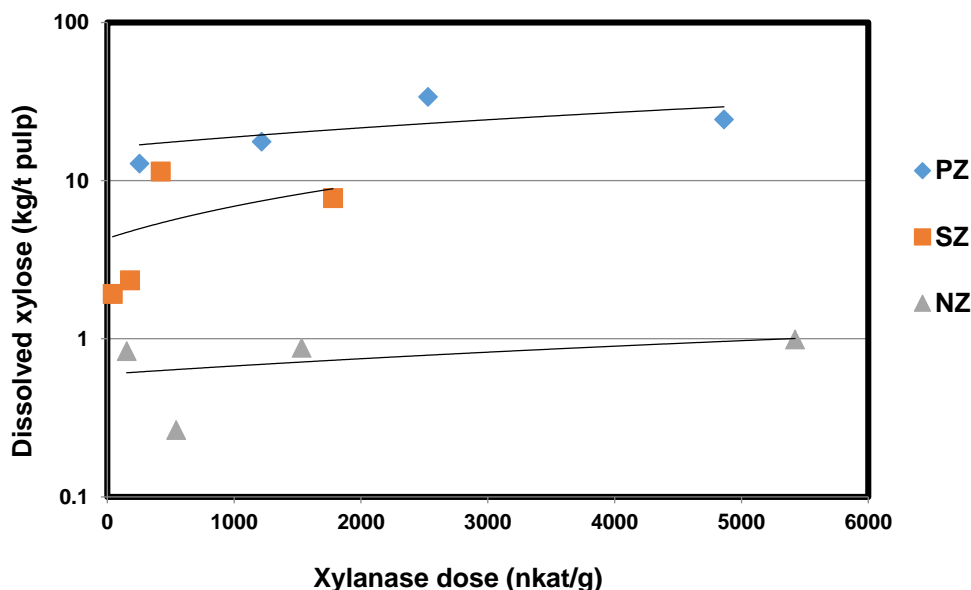

Fig. 1. The effect of xylanase treatment on the liberation of xylose from bleached kraft pulp fiber wall as determined from pulp filtrates after acid hydrolysis. The lines are included to illustrate the differences between the xylanases.

According to the literature, the productivity of xylanases depends on the substrate pore structure and the molecular size of the xylanase (Maiti and Whitmire 1997). Törrönen *et al.* (1994) estimated that an endo-1-4-β-xylanase from *T. reesei* has a molecular weight of approximately 20 kDa and a diameter of 3 to 4 nm. Because most of the pores in bleached birch kraft pulp have been reported to have diameters greater than 10 nm, most should be accessible for enzyme action. However, because the hydrolytic action of

xylanases is, in practice, limited primarily to fibril surfaces (Teleman *et al*. 2001), it seems that no suitable xylan substrate may be present in these accessible pores, or that the penetration of xylanases into the pores is restricted (*e.g.*, by electrostatic repulsion).

Figure 2 shows the anionic charge, determined as the cationic demand of the pulp filtrates, plotted against the released xylose. The xylanases yielded hydrolyzed xylan residues with significantly different characteristics. The plot shows a strong correlation between the amount of released xylose and the cationic demand of the filtrate. The highest amount of negatively charged species was released during enzymatic hydrolysis of the sample treated with PZ, exhibiting increasing cationic demand with increasing amounts of degraded xylan. SZ, on the other hand, exhibited an initial increase in the cationic demand, after which the demand remained constant, although the amount of released xylose increased significantly. NZ seems incapable of liberating anionic material from the fiber wall because the cationic demand remained the same as that of the reference sample, despite the fact that some xylan degradation did occur. An attempt is made to explain this behavior in the following discussion.
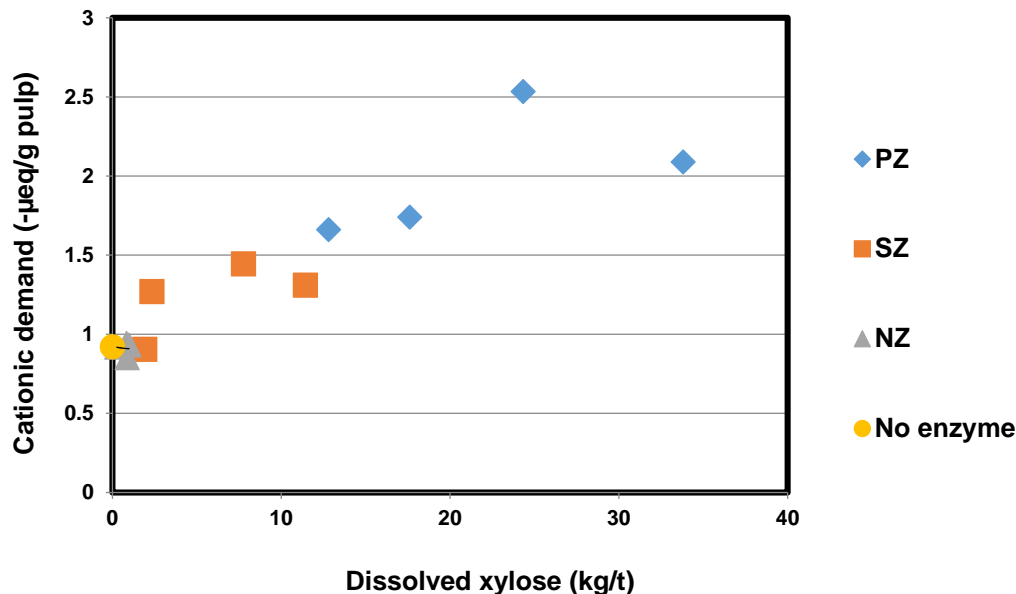

Fig. 2. The effect of xylan-based residues on the cationic demand of pulp filtrates. The pH of the filtrates fell slightly, from approximately 7 to approximately 6, for the samples treated with PZ. For the other two enzyme preparations, the pH reduction was negligible. The filtrates contained 0.01 M NaCl as background salt.

Figure 2 shows that the three enzymes exhibited different hydrolysis efficiencies. For SZ, there was an initial increase in the cationic demand that then levelled off; no further increase was observed, even though the amount of dissolved xylose increased. In the case of PZ, on the other hand, the cationic demand increased with increasing amount of dissolved xylose. The detected difference can probably be attributed to the physical characteristics of the enzymes; SZ contains family 10 endoxylanases, which typically have higher molar masses than family 11 endoxylanases such as PZ and NZ. Thus, because of its larger molecular size, the hydrolytic action of SZ on the fiber surfaces can be expected to be more limited than PZ or NZ. Because the outermost layers of bleached kraft fibers have a relatively low uronic acid content (Dahlman *et al.* 2003), the cationic demand of the

filtrate remains at a certain level, even though the amount of released xylose increases. However, SZ appears able to hydrolyze the lightly substituted xylan at the fiber surface, as indicated by the slight initial increase in cationic demand. Of the tested xylanases, PZ seems to have the highest accessibility toward substrate xylan, exhibiting the highest productivity of xylose and the largest amount of anionic species released.

In contrast to SZ and PZ, NZ seems to have been very inefficient toward birch kraft xylan, in spite of the fact that its activity assayed against beechwood xylan was significantly higher than that of the other xylanases. It is suggested that this xylanase type is likely incapable of hydrolyzing a xylan substrate having a low frequency of side groups. The xylan from beechwood contains five to seven acetyl groups linked in the C-2 or C-3 positions and one 4-O-methyl glucuronic acid residue per 10 xylose units (Fengel and Wegener 1989). The inefficiency of NZ toward bleached hardwood pulps has also been reported by Žnidaršič-Plazl *et al.* (2009), who related its inefficiency to its lower declared activity than that of some other xylanases. However, in light of the present results and activity data, it seems that the presence of side groups on the xylan substrate is more likely the key factor determining the hydrolytic efficiency of NZ.

For selected pulp samples, the °SR value, WRV, zeta potential, and fiber dimensions were also determined, as shown in Table 3. An exact comparison of the action of different enzymes is somewhat difficult because the applied enzyme dosages were not initially fixed to similar nkat/g levels using a correct substrate. Nevertheless, the data presented in Table 3 show that the xylanase treatment reduced drainage resistance and water retention value, while the fiber length and width were only marginally reduced. In addition, the electrostatic charge of the fibers was reduced with a slight change in the zeta potential, which further confirms that negatively charged xylan is removed from the surface.

Table 3. Effect of Low and High Xylanase Dosages on Pulp Properties

Xylanase	Xylanase Dose (nkat/g)	°SR	WRV (g/g)	Zeta potential (mV)	Length (mm)*	Width (µm)*	Fines (%)*
No xylanase	0	24.5	1.99	-26.0	0.924	21.5	3.0
PZ (low)	252	22.5	1.98	-24.0	0.916	21.4	3.2
PZ (high)	2524	21.5	1.96	-22.4	0.896	21.3	3.1
SZ (low)	45	22.0	1.91	-25.7	0.899	21.3	3.0
SZ (high)	453	21.0	1.90	-22.4	0.897	21.3	2.9
NZ (low)	185	24.0	1.96	-26.0	0.900	21.2	3.0
NZ (high)	1859	23.0	1.90	-23.2	0.895	21.3	3.0
* length-weighted properties							

Prior to analysis, the pulp samples were washed with hot deionized water to remove any dissolved material from the suspension. The zeta potential was measured at pH 7 with 0.01 M NaCl as background salt, yielding a sample conductivity of 1.2 mS/cm.

The observed improvement in pulp dewatering properties is in agreement with the previously published data of Blomstedt *et al.* (2010), who suggested that, in addition to the partial removal of water-binding xylan from the fiber surface, the enzyme had an effect on the macropore structure of the fiber wall, reducing the amount of water carried in the fiber wall. In general, cellulosic fibers are known to exhibit swelling behavior typical of a

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polyelectrolyte gel. Because xylan is the main source of ionic groups in bleached kraft pulps, its presence in the fiber cell wall can be expected to significantly contribute to fiber swelling or the water-binding properties of the fibers (Sjöström 1989; Wågberg and Annergren 1997).

According to Table 3, the zeta potential of the pulp samples was changed from an initial -26.0 to -22.4 mV with higher doses of PZ and SZ. In addition, the zeta potential of the sample with a high NZ dose also had lower anionicity, despite the fact that there was no increase in the cationic demand of the corresponding pulp filtrate. Thus, this result may indicate that NZ is irreversibly adsorbed onto the fiber surface. Xylanases may carry a positive or a negative charge at a given pH, depending on their amino acid sequence. Typically, xylanases belonging to family 10, such as SZ, are characterized by high M_w and low isoelectric point (pI), while family 11 xylanases, such as PZ and NZ, are characterized by low M_w and high pI (Pastor *et al.* 2007). According to Tenkanen *et al.* (1995), there is electrostatic interaction between the xylanase and the substrate xylan when the pH is below the pI of the xylanase. Accordingly, the enzyme should be totally bound to the xylan substrate at a pH below its pI and primarily unbound at a pH above its pI (Tenkanen *et al.* 1995). Thus, if NZ has a pI higher than 7, it might in principle be possible for it to be irreversibly adsorbed onto the fiber. In combination with the observation that NZ does not seem to have the capability to hydrolyze a slightly surface-substituted xylan, this could explain why the pulp's zeta potential is shifted in the "less negative" direction even though no anionic species were present in the pulp filtrate. In the case of both PZ and SZ, it seems that the zeta potential is reduced mostly as a result of the removal of anionic xylan moieties from the fiber surface.

Properties of Pulps from Up-Scaled Enzyme Treatment in the Absence and Presence of Additives

Table 4 shows selected properties of the pulps after up-scaled xylanase treatment in the absence of and in the presence of wet-end additives. Interestingly, without any additives, the washed pulp samples (B) exhibited higher drainage resistance, determined as the °SR number, than the unwashed pulps (A). The target °SR level for unwashed pulp samples was 16.5; the °SR number of the pulp refined to °SR 21 was reduced to 16.5 with the treatment of the pulp with 10 kg/t PZ after refining. Because the °SR values of all the washed pulp samples were higher than those of the unwashed samples, the presence of dissolved xylan moieties in the liquid phase reduces the drainage resistance of the pulps. This can probably be ascribed to the ability of the dissolved anionic xylo-oligosaccharides to act as weak electrolytes. In general, while polyelectrolytes such as polymeric xylan on the fiber surface increase pulp swelling, the small-sized electrolytes present in the external solution are known to reduce pulp swelling by screening charges, increasing the dewatering rate (Lindström 1992).

The water retention values of the washed pulp samples show that mild xylanase treatment (2 kg/t) after refining slightly increased the pulp's WRV from 1.88 to 1.93 g/g (Table 4), but the WRV decreased under harsher treatment conditions (*i.e.*, 10 kg/t during refining). The most significant reduction, from 1.88 to 1.38 g/g, occurred in the pulp treated with 10 kg/t xylanase during refining, without enzyme deactivation. This sample also suffered a reduction in fiber length, which was not observed in any other samples. However, the decrease in fiber length did not result in the expected increase in the amount of fines, so it may be that the prolonged action of xylanase in this sample especially targeted the fines fraction, causing some to disappear. The fines typically contain larger amounts of

charged groups than long fibers and are known to significantly contribute to swelling. The fines content in all enzyme-treated pulps was slightly lower than that of the reference.

According to Table 4, the zeta potential changed from -26.5 to -20.9 mV when the refined pulp was treated with 10 kg/t xylanase. This change in zeta potential agrees well with the results given in the previous section of this paper, confirming that charged groups were released from the surface. When the refining was performed during the xylanase treatment, the zeta potential was reduced even more significantly, which is related to the ability of simultaneous refining to continuously remove the dissolved and degraded xylan residues from the fiber surface and to expose new substrate area for xylanase action. The more efficient removal of xylan-based material from the fiber surface also increased the cationic demand and TOC of the pulp filtrates, both of which indicate that the highest levels of dissolved material were obtained from the sample prepared with 10 kg/t xylanase during refining and without enzyme deactivation.

Table 4. Properties of Xylanase-Treated Pulps and Corresponding Papermaking Stocks Including All Wet-End Additives

	Reference (no enzyme treatment)		2 kg/t after refining		10 kg/t after refining		10 kg/t during refining		10 kg/t during refining, no deactivation	
Sample type	A	B	A	B	A	B	A	B	A	B
Properties of pulps treated with PZ										
°SR	16.5	19.0	17.5	21.0	16.5	19.5	15.0	16.5	17.0	20.0
WRV (g/g)	-	1.88	-	1.93	-	1.90	-	1.83	-	1.38
Fiber length (mm)	0.944	-	0.941	-	0.949	-	0.946	-	0.931	-
Fiber width (µm)	20.7	-	21.1	-	20.9	-	20.7	-	20.8	-
Fines (%)	2.6	-	2.4	-	2.5	-	2.2	-	2.1	-
Zeta potential (mV)	-26.5	-	-24.4	-	-20.9	-	-20.7	-	-19.5	-
pH	7.0	-	7.0	-	6.5	-	6.9	-	6.7	-
Cationic demand (µeq/g pulp)	4.09	-	4.34	-	4.60	-	5.24	-	6.15	-
TOC (mg/g pulp)*	11.5	-	23.1	-	27.7	-	35.2	-	44.3	-
Properties of colloid systems (pulp + all additives included)										
pH	8.5	8.5	8.5	8.6	8.2	8.3	8.3	8.6	8.4	8.5
Conductivity (µs/cm)*	169	157	169	152	169	153	177	151	152	151
Cationic demand (µeq/L)**	19	12	18	5.5	18	6.1	16	1.9	13	1.8
Zeta potential (mV)	-43.8	-19.3	-45.5	-20.8	-56.3	-20.1	-35.3	-7.4	-16.5	-44.0

*determined from filtrate samples, represents dissolved charged species
** determined from the fraction that passes 200 mesh wire, represents dissolved and colloidal fraction

Zeta potential of pulps was determined at pH 7 in 0.01 M NaCl. A = unwashed pulp sample or pulp filtrate, B = washed pulp sample. The xylanase treatments were carried out on a pilot scale.

If the reference sample, without any contribution of enzyme on TOC value, is excluded, the TOC values were excellently linearly correlated ($R^2 > 0.99$) with the cationic demands of the filtrates, which further confirms that the action of PZ is very selective toward xylan substrates containing anionic charged species.

The cationic demands of the pulp filtrates given in Table 4 (expressed as µeq/g pulp to exclude the effect of pulp consistency) are significantly higher than the values shown in Fig. 2, even though the same enzyme and pulp raw material were used. The lowest cationic demand was around 4 µeq/g for the reference pulp, whereas the harshest xylanase treatment gave a value higher than 6 µeq/g. The surface charge of the bleached hardwood kraft pulps typically falls between 10 and 27 µeq/g, and with respect to these values, the values obtained seem reasonable (Laine and Stenius 1997). The fact that greater amounts of charged species were dissolved in up-scaled xylanase treatments than in the laboratory scale-treatments can probably be ascribed to differences in the treatment conditions. In the up-scaled treatment, the pulp consistency was higher and the deactivation stage (if any) had to be implemented in a different way than in the laboratory, causing the hydrolysis reaction to continue after the set treatment time because it took some time to heat the pulp from 50 °C to over 90 °C. According to the supplier, the PZ enzyme is active within the temperature range of 50 to 80 °C.

Effect of Dissolved Xylan Residues on Retention Chemical Performance and Internal Sizing

To investigate the effect of enzymatically degraded xylan residues on the performance of retention chemicals, laboratory sheets were prepared from (A) unwashed and (B) washed xylanase-treated pulp samples in the presence of wet-end additives typically used in fine paper production. A moving belt former (MBF) was used for sheet preparation because it better simulates the wet-end and fiber retention behavior than a conventional sheet mold because of its pulsating vacuum dewatering, higher pulp consistency, and the presence of substantially greater shear forces in the mixing of the additives (Strengell *et al.* 2004; Blomstedt *et al.* 2010). Because of the relatively harsh sheet forming conditions, the formation of a MBF sheet is typically quite poor and the MBF sheets were therefore only used for retention and water absorption (determined as the $Cobb_{60}$ value) measurements; no basic paper properties were determined using the filled sheets.

Figures 3 and 4 show the total retention and filler retention, respectively, based on the dry weights and the ash contents of the MBF sheets. Similar trends were observed for both total and filler retention, which was expected since the total retention is governed primarily by the fiber and fines retention and filler retention; the contribution of other papermaking additives on the total retention is minor because of their low dosages.

According to the present results, the removal of dissolved substances from pulps by washing has a different effect on the retention behavior depending on the initial amount of negatively charged species present in the pulp filtrate. For the reference sample and the sample treated with 2 kg/t xylanase after refining, which had cationic demands of 4.09 and 4.34 µeq/g, respectively, washing decreased or had no effect on the retention levels. However, when the cationic demand of the filtrates was significantly increased to 4.60 µeq/g or more after treatment with higher xylanase doses and with a different application strategy, the washing of the pulps was found to make a positive contribution to the retention level, which confirms that there was an interaction between the dissolved xylan residues and cationic retention additives.
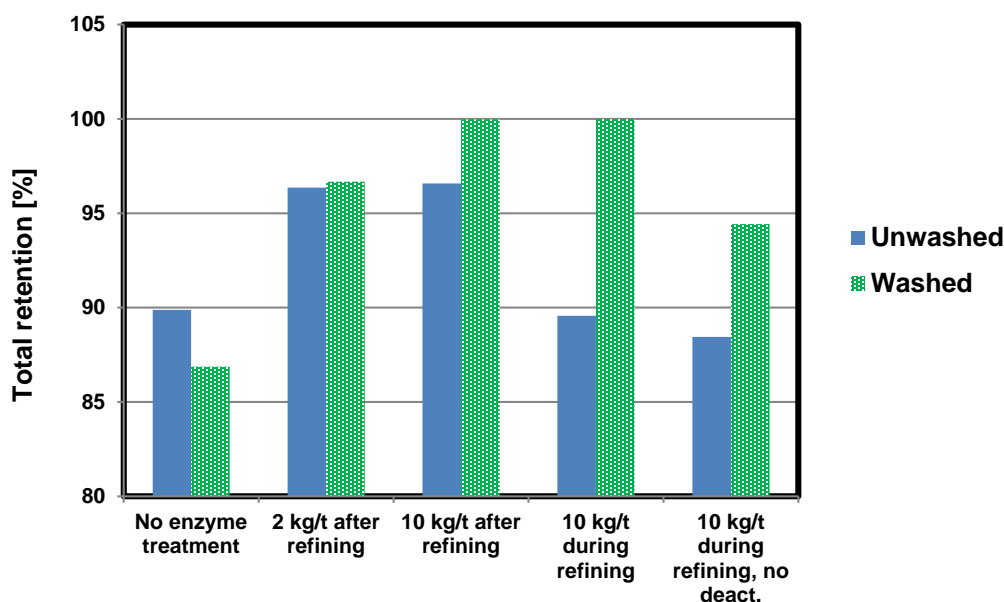
Fig. 3. The effect of xylanase treatment on total retention. Unwashed = pulp samples used as such, containing all the xylan-based material dissolved during the xylanase treatment. Washed = pulp samples washed prior to use, containing no significant amounts of dissolved substances
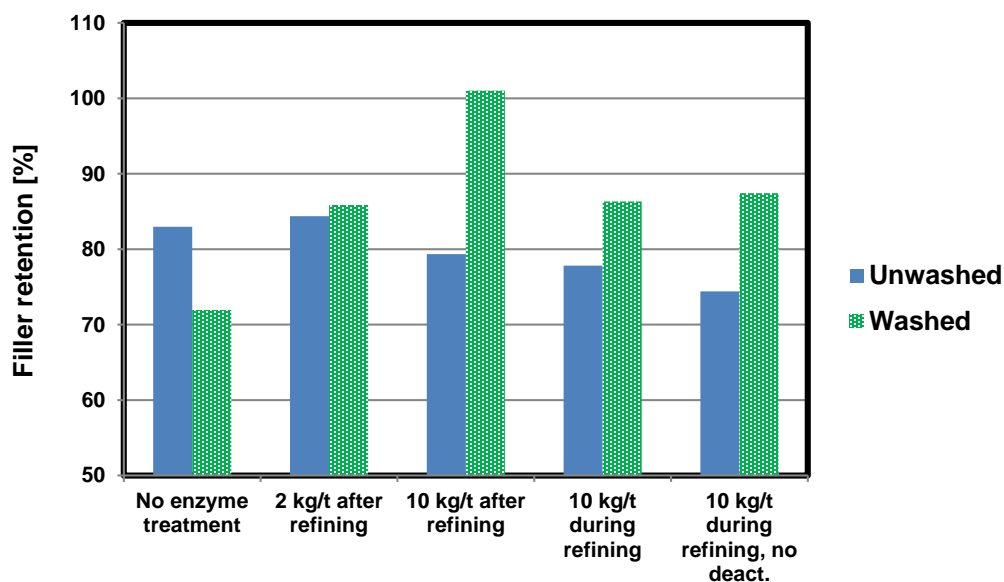

Fig. 4. The effect of xylanase treatment on filler retention. Unwashed = pulp samples used as such, containing all the xylan-based material dissolved during the xylanase treatment. Washed = pulp samples washed prior to use, containing no significant amounts of dissolved substances

In the case of the samples with the highest cationic demands, the retention could be expected to be lower if the pulp samples were used in the unwashed form. It is well known that dissolved anionic substances may consume cationic wet end additives such as starch, retention polymer, and sizing agent (Lindström 1992). However, because the opposite effect was observed for the reference sample, which initially contained a small amount of negatively charged species, the effect of pulp washing on the total and filler retention may

depend on the initial ratio of cationic demand to pulp zeta potential. If this ratio is low (*i.e.*, a low cationic demand of the filtrate with a high zeta potential), pulp washing has a negative effect on the total retention, and *vice versa*.

A comparison of the retention levels of the unwashed samples shows that the highest retention levels were obtained with pulps treated with xylanase after refining. The present results indicate that the cationic retention additives are able to bind the dissolved substances, to a certain extent, without losing their functionality. Below this level, the dissolved substances are retained in the paper web, causing the total retention to be higher. However, when this limit is exceeded, the retention drops because the retention additives lose some of their functionality because of the neutralization of their cationic sites by anionic dissolved material. In addition, pulps that were xylanase-treated after refining exhibited substantially higher retention values than the reference pulp, regardless of whether the pulps had been washed or not.

Interestingly, the retention levels of the washed pulp samples show that with xylanase-treated pulps, higher retention was always obtained than with the reference pulp without enzyme treatment. This supports the earlier hypothesis that the action of xylanases especially targets pulp fines. The lower charge of the fines fraction has been shown to be beneficial for filler retention in the presence of a cationic retention polymer (Lyytikäinen *et al.* 2011). In addition, it is possible that there are differences in the conformation with which the retention polymers are adsorbed onto the fibers with different surface charges. According to Wågberg and Ödberg (1989), a more extended conformation of oppositely charged polymer molecules, which is believed to be beneficial for bridging flocculation, can be expected when there is increased competition for the fiber surface charges. The xylanase-treated fibers are expected to have lower surface charge content than the reference pulp, as indicated by the lower zeta potential of the pulps.

Figure 5 shows the effect of xylanase treatment on the water absorption capacity of the MBF sheets, determined as the $Cobb_{60}$ value.
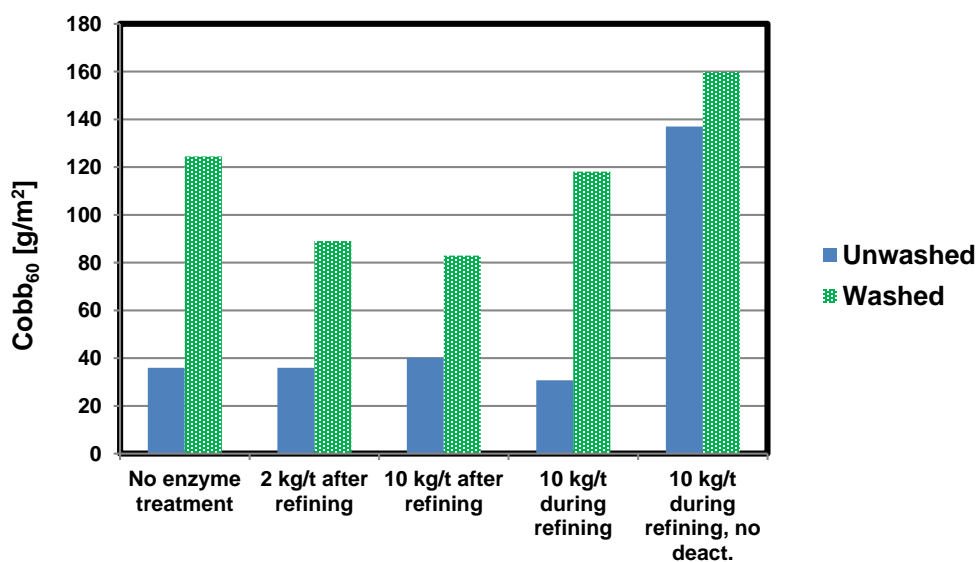

Fig. 5. The effect of xylanase treatment on sizing efficiency, determined as the $Cobb_{60}$ value. Unwashed = pulp samples used as such, containing all the xylan-based material dissolved during the xylanase treatment. Washed = pulp samples washed prior to use, containing no significant amounts of dissolved substances

In general, the $Cobb_{60}$ value cannot be used to directly indicate AKD retention, but rather provides information regarding the performance of AKD in the system. Interestingly, lower $Cobb_{60}$ levels were obtained for the paper samples prepared in the presence of dissolved xylan residues, despite the fact that both the total and filler retention were poorer in this case. Typically, AKD retention is expected to be high when the filler retention is high because the cationic AKD particles are often adsorbed onto the filler and fines surfaces because of their higher specific surface area.

By comparing the $Cobb_{60}$ values with the cationic demands determined for the papermaking stock filtrates (Table 4), it can be seen that notably lower $Cobb_{60}$ levels were obtained with cationic demands of 16 µeq/L or more than with cationic demands of 13 µeq/L or less. Furthermore, the cationic AKD dispersion was dosed into the thin papermaking stock at the point where cationic wet-end starch (4 kg/t) was already applied to the pulp. As it is known that the anionic species present in process water may neutralize the cationic sites of papermaking additives, it can be assumed that with higher levels of dissolved anionic species, there should be fewer cationic sites left in the starch to adsorb onto the negative sites present on fiber surfaces, thus leaving a greater amount of negative sites on the fibers available for interaction with cationic AKD particles after starch adsorption. The lower or negligible amount of anionic charges present on the fiber surfaces, together with the fact that AKD was introduced into the thin stock, lowers the probability of AKD colliding with a potential retention site, causing the initial attachment of AKD particles to be very slow (Champ and Ettl 2004). In the present study, where the chemical dosing points were located quite near to the MBF headbox (*i.e.*, the delay times prior to sheet formation were short), it is suggested that for the enzyme-treated, washed pulp samples with low cationic demand, the amount of cationic wet-end starch dosed prior to the AKD was too high, leaving only a few possible adsorption sites for AKD free.

In general, any AKD that is not adsorbed onto the fiber surfaces at the filler dosing point is likely to be adsorbed onto the surface of the filler that, in this study, was dosed 15 s after the AKD. According to Novak and Rende (1993), the size located on the filler surface is not able to chemically bond, so that any adsorbed size becomes hydrolyzed with time. Therefore, for the washed pulp samples having a greater probability of AKD adsorbing onto the filler surfaces because of the lack of free adsorption sites on the fibers after starch adsorption, better sizing responses could probably have been obtained by reducing the amount of wet-end starch adsorption or by increasing the delay time between AKD and filler addition to leave more time for AKD particles to attach to anionic sites on the fibers. This likely also applies to the unwashed sample "10 kg/t during ref., no deact.," which for some reason had a much lower cationic demand in the presence of wet-end additives than any other unwashed sample.

CONCLUSIONS

1. The ability of the commercial xylanase enzymes to dissolve xylan from bleached birch kraft pulp varies significantly, and the ratio of dissolved anionic species to dissolved xylan was different for the three xylanases studied.

2. The degradation of xylan can be intensified if enzymatic treatment is implemented simultaneously with refining.

3. Xylanase treatment increases the cationic demand of the pulp filtrate and reduces the anionic zeta potential of the fibers. Especially with harsher enzyme treatment conditions, the removal of large amounts of dissolved substances by washing is necessary to maximize the total and filler retention in subsequent papermaking processes.

4. In the retention system studied, the efficiency of AKD as an internal sizing agent was significantly greater in the presence of dissolved xylan residues. This effect is related to the changed wet-end charge balance, requiring a modified dosing strategy for cationic wet-end additives.

ACKNOWLEDGMENTS

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