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ORIGINAL PAPER

Effect of xylanases on refining process and kraft pulp properties

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Abstract Results of this study demonstrate that enzymatic pretreatment of pulps enables energy savings in the refining process. Pretreatments of NBSK pulp with 3 different commercial xylanases resulted in a faster increase in the pulp freeness that reduced energy input for refining. The partial xylan removal by these enzymes affected the properties of cellulosic pulp and paper. The tensile properties of paper were improved by pretreatment with a pure xylanase while the additional activity of cellulases in the other two tested enzymes negatively influenced the tear resistance. Only the pure xylanase improved the pulp and paper properties. The results of this study provide evidence that the purity of xylanases used in papermaking is of great importance and may decide of paper quality and production costs.

Keywords Refining · Cellulose · Enzymes · Xylanases

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Introduction

Modern technologies of paper production must ensure the high paper quality and be sustainable, cost-effective and energy-efficient (Znidarsic-Plazl et al. 2009). Paper quality depends on the quality of cellulosic pulp and one of most important unit operations, deciding of the physical properties of paper is pulp refining. Refining modifies the surface of fibres and causes their shortening (Przybysz 1997; Biermann 1996). Mechanical treatment increases the flexibility of fibres and fines fraction content. Because of the growing external fibrillation, the specific area of fibres is increased that in turn enhances the bonding forces between fibres. Internal fibrillation reduces the stiffness of fibres that allows to form the dense structure of paper sheet. Fibrillation and shortening of fibres caused by the action of refiners blades cause the appearance of fines. During dewatering of paper web, the fines fill spaces between the entangled fibres. In consequence, the density and smoothness of paper increase while the air permeability decreases (Przybysz 1997). Pulp refining is the key operation in papermaking and simultaneously one of the most energy-consuming steps, consuming up to 15–18% of total electric energy used for paper production (Cui et al. 2015). Therefore, some options that reduce the energy input were proposed, e.g., steam explosion or pulp freezing and thawing (Przybysz and Przybysz 2013). However, none of them was implemented in industry, mainly because of high investment costs (Cui et al. 2015). A promising, environmentally-friendly approach, enabling energy savings involves enzymes that have been increasingly applied in papermaking since 1986. Since 1995 the number of publications related to these enzymes has increased each year and over 300 papers were published in years 2006–2010. Additionally, 25 patents related to the application of enzymes in papermaking have been published each year since 2001. The enzymes that are used in papermaking, such as cellulases, lipases, xylanases and laccases, have to be sufficiently stable and active under technological conditions (elevated temperature and pH different from neutral). Usually, cellulases were used for fibres modification while xylanases were applied for pulp bleaching (Demuner et al. 2011).

In 2000, it was found that enzyme-assisted refining may consume even twice less energy (Dickson et al. 2000). According to Bajpai et al. (2006), the treatment of pulp with a mixture of cellulases and hemicellulases resulted in a reduction of the energy requirement for refining of 25 kWh/ton pulp. Also the results of our previous study showed that pulp pretreatment with xylanases significantly reduced the energy consumption (Przybysz Buzała et al. 2016). However, there must be a balance between the energy savings caused by the use of enzymes and the price of the latter to avoid an increase in the overall costs of paper production. Furthermore, the enzymes have to be active and stable at approximately 50 °C for around 60 min. This time may be shorter if enzyme concentration is higher but it leads to the higher process costs. These enzymes may be easily inactivated by pH change or at elevated temperature (100 °C), e.g. in the drying section of paper machine. To reduce the costs of enzymatic pretreatment, the active enzymes contained in white water from paper mill may be reused. The price of enzyme preparations depends on their purity. Purification of enzymes is usually conducted using sophisticated techniques, including liquid chromatography, and therefore pure enzymes are more expensive than crude enzyme preparations. However, pure enzymes, deprived of additional activities, outperform crude enzyme preparations that usually show many different enzymatic activities, in terms of treatment outcomes.

The objective of this work was to compare the influence of three different xylanases, characterized by different cellulolytic activity (only one of them did not exhibit this activity) on a cellulosic pulp, which was then used for paper production. The authors attempted to determine the impact of changes in cellulose and hemicelluloses contents in the pulp and dimensional characteristics of fibres, caused by enzymatic pretreatment, on the consumption of energy for pulp refining as well as pulp and paper properties.

Materials and methods

Pulp and its characterization

A bleached pine kraft pulp was kindly donated by one of Polish paper mills. Typically, this pulp is used for production of high quality graphic papers. The pulp had a form of air-dried sheets. The parameters of the unbeaten pulp were as follows:

- Dry matter content—96.16%,
- Water retention value (WRV)—96.87% (according to the ISO 23714:2014 standard),
- Weighted average fibre length—2030 µm (according to the ISO 16065-2:2014 standard),
- Polymerization degree—1250 (according to the ISO 5351:2012 standard).

Enzymes and chemicals

The following three commercial xylanases were used for pulp pretreatment:

- Xylanase 1: an endo-1,4-β-D-xylanase (EC 3.2.1.8) from *Neocallimastix patriciarum*, from Megazyme company
- Xylanase 2: an endo-1,4-β-D-xylanase (EC 3.2.1.8) from *Trichoderma longibrachiatum*, from Creative Enzymes company
- Xylanase 3: an endo-1,4-β-D-xylanase (EC 3.2.1.8) from *Thermomyces lanuginosus.* from Sigma-Aldrich

All other chemicals were analytical grade and procured from either Sigma-Aldrich (USA) or POCh (Poland).

Activities of cellulases and xylanases were assayed by the 3,5-dinitrosalicylic acid (DNS) method (Miller 1959) at pH 5.0 and 50 °C for 0.5% carboxymethylcellulose (CMC) and 0.5% birch xylan, respectively (reaction time of 5 min). Activities of both the glycosidases were expressed as micromoles of reducing sugars released from the polysaccharide substrates in 1 min (U). The filter paper activity was determined at pH 5.0 and 50 °C according to Adney and Baker (2008) and expressed in FPU.

Enzymatic treatments of pulps

Samples of the pulp (22.5 g dry weight) were soaked in water for 24 h before the treatment with enzymes. Then these samples were disintegrated in a laboratory propeller pulp disintegrator (type SHPD28D from Danex, Poland) according to the standard ISO 5263-1:2006. Pulp consistency was set to 2.25% (total volume of each sample was 1 dm^3). Then samples of pulp (pH of approximately 7) were treated with the three listed above enzyme preparations, used separately. After addition of the enzyme preparation to the pulp, the mixture was incubated in a water bath (at 50 °C) with shaking (60 rpm) for 60 min. Then the enzymes were inactivated by 10 min incubation of the enzyme-treated pulp in a boiling water bath and the pulp was cooled and filtered through a mesh sieve no. 200. The filtrates were analyzed for glucose concentration using a commercial GOD-POD enzymatic analytical kit (Biomaxima, Poland), to determine the extent of enzymatic pulp degradation. Changes in the chemical composition of pulps, it means the decrease in hemicellulose contents caused by the treatment with the three mentioned above commercial xylanases were determined by means of their enzymatic hydrolysis, using the preparation NS-22086 from Novozymes A/S (Denmark) and HPLC analysis of the hydrolysates obtained (Buzała et al. 2015). Sugar profiles of these hydrolysates were compared with the sugar profile of the hydrolysate of the reference pulp which was not treated with any of these xylanases. Mono- and disaccharide contents in the hydrolysates of the pulps were determined by HPLC using an Ultimata 3000 Dionex liquid chromatograph equipped with a Rezex RPM-Monosaccharide Pb^{2+} column (8 µm, 7.8 × 300 mm) and a Shodex-RI-10 refractive index detector. The temperatures of the column and the RI detector were set at 80 and 40 °C, respectively. The volume of injected samples of hydrolysates, which were filtered through a nylon syringe filter (0.45 mm) before HPLC analysis, was 10 µl. The rate of flow of HPLC grade water (Sigma), which was the mobile phase, was 0.6 ml/min.

Pulp refining and freeness measurements

After inactivation of the enzymes, the pulps were refined using a PFI laboratory mill at 3.4 kG, according to the standard ISO 5264-2:2011. In preliminary tests, in which optimum doses of the three enzyme preparations were determined, the pulp samples were refined for 30 s (720 revolutions). In all other experiments, the process was accomplished when the pulp freeness value was 30°SR (its duration was measured with precision of 1 s to calculate the number of mill revolutions). Then, samples of the refined pulp were transferred from the mill to a mixer.

Pulp quality analysis

Analysis of chemical composition of untreated and enzyme pre-treated pulps included quantification of extractives, lignin, cellulose, hemicelluloses and ash. The content of lignin was determined by a gravimetric method in compliance with the TAPPI T222 standard after the removal of extractives according to the TAPPI T204 standard. The content of holocellulose was determined according to the TAPPI T249 standard. Cellulose was quantified as alpha-cellulose, according to the TAPPI T203 standard. The content of hemicelluloses was calculated as the difference between the holocellulose and cellulose contents. Ash content was determined by gravimetric method in compliance with TAPPI T211 standard. All these assays were performed in triplicate for each pulp.

The refined pulp samples were placed in the mixer and the concentration of their suspensions was adjusted to 0.25%. The thoroughly mixed pulp samples were analyzed for the: freeness (according to the standard ISO 5267-1:2002), dimensional properties of fibres such as weighted average fibre length, fibre width etc. (according to standards ISO 16065:2014) using a Morfi Compact Black Edition device (Techpap, France) and WRV (ISO 23714:2014) using a

MPW 352 centrifuge (MPW, Poland), and then they were used to produce handsheets of paper.

Paper production and properties

Handsheets of paper were produced with the use of a standard laboratory Rapid-Köthen class sheet former, according to the standard ISO 5269-2:2007.

The following paper properties were measured after conditioning of the handsheets in standard conditions, in compliance with ISO 187:1990 (at air relative humidity φ of 50% and temperature of 23 °C):

Grammage—according to ISO 536:2012,
Bulk density—according to ISO 534:2012,
Tensile index—according to ISO 1924-2:2010,
Tear index—according to ISO 1974:2012,
TEA index—according to ISO 1924-2:2010.

Only the handsheets of grammage equal to 75 ± 1 g/m^2 were used for evaluation of paper properties.

Results

Enzymatic activities

The applied enzymatic preparations showed activities of both cellulases and xylanases (Table 1) with an exception of the *N. patriciarum* xylanase, which degraded neither CMC nor filter paper. The xylanolytic activity of this enzyme was very high (4590 U/µl). The *T. longibrachiatum* xylanase (736.8 U/g) was characterized by the high activity of cellulases (133 U/g for CMC and 256 FPU/g). The xylanolytic activity of *T. lanuginosus* enzyme was very high (7460 U/g for birch xylan). This preparation exhibited also the cellulolytic activity (38 U/g, 122 FPU/g).

The results of preliminary tests that were carried out to determine the optimal doses of the enzymatic preparations are presented in Table 2. In preliminary tests, the pulps were refined in the PFI mill for 30 s (720 revolutions). The optimum dose of each of the three enzyme preparations corresponded to the maximum tear resistance of paper produced from the enzymatically pretreated pulps.

These doses were as follows:

- *N. patriciarum* xylanase—0. 033 µl/1 g d.w.,
- *T. longibrachiatum* xylanase—0.5 mg/1 g d.w.,
- *T. lanuginosus* xylanase—2.5 mg/1 g d.w.

One of the products of pulp treatment by the three enzyme preparations was glucose. Its concentrations in the filtrates of enzyme-treated pulp were relatively low, of 0.010, 0.023 and 0.061 mg/ml for the *N. patriciarum*, *T. longibrachiatum* and *T. lanuginosus* xylanase preparation, respectively.

The impact of enzymatic pretreatment on chemical composition of the bleached cellulosic pulp

The data presented in Table 3 demonstrate that pretreatment of the bleached pine pulp with xylanases caused only slight changes in the ratio of cellulose to hemicelluloses while the contents of lignin, extractives and ash was maintained below 0.05% d.w. The content of hemicelluloses was decreased from around 3.5% d.w. in the original pulp to around 3.0, 2.0 and 2.8% d.w. after digestion with the xylanases from *N. patriciarum*, *T. longibrachiatum* and *T. lanuginosus*, respectively. The partial hemicellulose removal brought about a small increase (below 1% d.w.) in the cellulose content in the pulp. To elucidate which fractions of hemicelluloses were digested by the three xylanases, samples of the pretreated pulp were saccharified using the preparation NS-22086 from

Table 1 Activities of cellulases and xylanases (at 50 °C and pH 5.0) of the three tested preparations of xylanases

Enzymes	Xylanase from *Neocallimastix patriciarum*		Xylanase from *Trichoderma longibrachiatum*		Xylanase from *Thermomyces lanuginosus*	
	U/µl	FPU/µl	U/g	FPU/g	U/g	FPU/g
Cellulases	0	0	133	256	38	122
Xylanases	4590	–	736.8	–	7460	–

Table 2 The dependence of tear resistance and breaking length of paper on the dose of xylanase preparation

Enzyme	Dose	Duration of pretreatment (min)	Freeness (°SR)	Tear resistance (mN)	Breaking length (m)
Control	n/a	n/a	14	895	4700
N. patriciarum xylanase	0.0165	60	13	840	4500
(µl/1 g s.m.)	0.033	60	13	920	5000
	0.066	60	13	900	5000
	0.264	60	13	890	4950
T. longibrachiatum xylanase	0.5	60	13	620	5200
(mg/1 g s.m.)	1	60	14	600	5350
	2.5	60	14	540	5600
	5	60	15	520	5850
T. lanuginosus xylanase	2.5	60	14	910	5000
(mg/1 g s.m.)	5	60	14	860	5200
	10	60	13	800	5150
	20	60	13	770	4950

Table 3 The effect of enzymatic pretreatment on chemical composition of the bleached cellulosic pulp

Enzyme	Cellulose % d.w.	Hemicellulose	Lignin	Extractives	Ash
Control	96.45 ± 0.22	3.49	0.011 ± 0.006	0.030 ± 0.011	0.020 ± 0.011
N. patriciarum xylanase	96.93 ± 0.36	3.02	0.012 ± 0.006	0.022 ± 0.006	0.022 ± 0.008
T. longibrachiatum xylanase	97.94 ± 0.24	2.01	0.005 ± 0.001	0.029 ± 0.003	0.026 ± 0.009
T. lanuginosus xylanase	97.11 ± 0.19	2.84	0.008 ± 0.004	0.018 ± 0.008	0.024 ± 0.007

Novozymes A/S (Denmark), which digests cellulose and hemicelluloses as was described by Buzała et al. (2015). The comparison of sugar profiles of enzymatic hydrolysates of the pulp samples, which were pretreated by the xylanases with the profile of hydrolysate of the reference, not pretreated pulp showed that the pretreatment caused a partial xylan removal, which was reflected by the decrease in xylose concentration in the hydrolysates (Table 4). The weakest impact on the xylose concentration had the treatment with *N. patriciarum* xylanase (a decrease from 1.34 to 1.13% w/w) and the strongest—with the *T. longibrachiatum* xylanase (a decrease to 0.73% w/w). The treatment of the bleached pine pulp with the xylanases caused that also the concentration of rhamnose in the hydrolysates was slightly reduced (from 0.07% w/w to even 0.03% w/w in case of the treatment with the *T. longibrachiatum* xylanase) like the concentration of arabinose, which was reduced from around 2% w/w in the hydrolysate of the not pretreated pulp to around 1.25%

Table 4 The effect of enzymatic pretreatment on sugar profile of enzymatic pulp hydrolysates

Enzyme	Glucose % w/w	Cellobiose	Xylose	Rhamnose	Arabinose
Control	83.41	13.04	1.34	0.07	2.08
N. patriciarum xylanase	83.41	12.82	1.13	0.05	1.84
T. longibrachiatum xylanase	84.81	13.13	0.73	0.03	1.25
T. lanuginosus xylanase	84.73	12.38	0.99	0.04	1.81

w/w in the hydrolysate of pulp pretreated with the *T. longibrachiatum* xylanase. These results demonstrate that pretreatment with the latter xylanase preparation caused the most advanced degradation of hemicelluloses while the *N. patriciarum* xylanase had the weakest impact on these heteropolysaccharides.

The impact of enzymatic and mechanical treatments on fibre morphology

Pulp freeness is the relatively easily measurable parameter, characterizing refining level and negatively correlated with the pulp dewatering ability, which decreases with the increase in freeness. Because the yield of paper production is diminished when dewatering ability is too low, in industrial conditions freeness values should not be higher than 30°SR. Therefore, the pulps were analyzed before and after refining to 30°SR, which corresponded to the highest paper strength (Przybysz 2011). To determine the effect of enzymatic pulp pretreatment on energy consumption for refining, each time the number of the PFI mill revolutions that were necessary to achieve the freeness of 30°SR was calculated (Table 5).

Pretreatment of pulp with the xylanases significantly reduced the number of the PFI mill revolutions that were necessary to achieve the freeness of 30°SR. The decrease in this number was the smallest (22.2%) for the *N. patriciarum* xylanase and the greatest (41.9%) for the *T. longibrachiatum* xylanase. Also the values of WRV of the enzyme-treated pulp were lower, both before and after refining. It was caused by the partial removal of hemicelluloses from the pulp. The latter polysaccharides are more prone to swelling than cellulose. The greatest decrease in WRV, by 12.26% before refining and by 56.93% after refining, was observed after pretreatment with the *T. longibrachiatum* xylanase. The decrease in WRV was the lowest after pretreatment with *T. lanuginosus* xylanase, of 0.43 and 21.47%, respectively. The decrease in pulp yield ranged from 0.012 to 0.069 g per kilogram of pretreated pulp.

The effect of enzymatic pretreatment on the size of fibers and the content of fines was also determined. These measurements were performed by the fiber image optical analysis using a Morfi Compact Black Edition apparatus, according to ISO 18065:2014. The results are shown in Table 6.

Pretreatments with xylanases from *N. patriciarum* and *T. lanuginosus* caused that after refining the mean weighted fibre length was decreased to 1875 and 1881 µm, respectively (the fibres were around 100 µm longer than the fibres of the reference pulp). The shortest fibres were observed after pretreatment with *T. longibrachiatum* xylanase (their length after refining was only 1380 µm). Enzymatic pulp pretreatment caused also changes in the diameter of fibres. The largest increase in the diameter was observed after pretreatment with the *T. longibrachiatum* xylanase (to nearly 32 µm), while the diameter of fibres of the reference pulp was increased by only 0.3 µm. Noteworthy, the *T. lanuginosus* xylanase outperformed the other two tested xylanases because it caused the smallest decrease in fibre length and the smallest rise in the fines content after pulp refining.

Table 5 The effect of enzymatic pretreatment on pulp characteristics

Sample	PFI revolutions	Reduction in PFI revolutions (%)	Freeness (°SR)	WRV (%)
Unbeaten				
Control	0	–	13	96.87 ± 0.67
N. patriciarum	0	–	11	88.46 ± 0.43
T. longibrachiatum	0	–	11	84.61 ± 0.55
T. lanuginosus	0	–	11	96.45 ± 0.87
Refined to 30°SR				
Control	5850	0.0	30	226.34 ± 1.21
N. patriciarum	4550	22.2	30	182.69 ± 1.08
T. longibrachiatum	3400	41.9	30	169.41 ± 1.89
T. lanuginosus	4050	30.8	30	204.87 ± 1.68

Table 6 The effect of enzymatic pretreatment on fibres dimensional characteristics

Sample	Mean fibre arithmetic length (μm)	Mean weighted fibre length (μm)	Mean fibre width (μm)	Mean fibre coarseness (mg/m)	Kinked fibre content (%)	Mean fibre curl index (%)	Macro fibrillation index (%)	Fine content (% in length)
Unbeaten								
Control	1311	2030	28.3	0.1487	46.9	11.8	0.289	13.8
	(12)	(14)	(0.2)	(0.0056)	(2.1)	(0.1)	(0.059)	(1.8)
N. patriciarum	1302	1995	28.3	0.1380	56.4	15.2	0.249	21.9
	(14)	(12)	(0.1)	(0.0084)	(1.6)	(0.3)	(0.034)	(1.8)
T. longibrachiatum	1308	1874	28.9	0.1692	60.0	16.3	0.326	19.0
	(11)	(13)	(0.1)	(0.0046)	(2.9)	(0.3)	(0.038)	(1.0)
T. lanuginosus	1307	1917	28.9	0.1568	58.9	15.5	0.312	19.9
	(8)	(12)	(0.1)	(0.0078)	(2.8)	(0.5)	(0.038)	(1.4)
Refined to 30°SR								
Control	1102	1782	28.6	0.1512	28.1	8.8	0.923	28.9
	(12)	(9)	(0.1)	(0.0024)	(1.9)	(0.2)	(0.042)	(1.4)
N. patriciarum	1156	1875	30.4	0.1645	36.6	10.3	0.825	26.8
	(10)	(11)	(0.1)	(0.0058)	(1.5)	(0.3)	(0.065)	(1.3)
T. longibrachiatum	885	1380	31.9	0.1745	32.6	9.0	0.935	31.5
	(10)	(8)	(0.1)	(0.0072)	(1.6)	(0.2)	(0.047)	(1.2)
T. lanuginosus	1157	1881	30.2	0.1939	33.3	9.6	0.721	23.3
	(8)	(9)	(0.1)	(0.0064)	(1.8)	(0.1)	(0.071)	(1.6)

The effect of enzyme treatments on physical properties of handsheets

The strength properties of fibers were significantly changed by the pretreatment with xylanases. The physical properties of paper handsheets were correlated with the dimensional characteristics of the fibers (Table 7) and the best results were observed after pulp pretreatment with *T. lanuginosus* xylanase, which caused a rise in tensile index and TEA index (from 22.8 before refining to 96.4 after this process, and from 0.32 to 2.29, respectively). Thus these indices increased by 18 and 17%, respectively while the tear index was reduced by 10%. Also the pretreatment of pulp with *N. patriciarum* xylanase positively affected paper properties (the tensile index and TEA index were 13 and 11% higher, respectively, while the tear index was not reduced). The pretreatment with *T. longibrachiatum* xylanase did not increase the values of tensile and TEA indices and significantly reduced the value of tear index because of the significant reduction of the length of fibres.

Discussion

Many authors have investigated the effect of cellulases on refining process as well as kraft pulp and paper properties (Cadena Eith et al. 2010; Gil et al. 2009; Lecourt et al. 2010; Singh et al. 2014). The results obtained by Cadena Eith et al. (2010) revealed a substantial increase in strength-related properties of paper. The recombinant endoglucanase, which contained the catalytic domain, increased the WRV and drainage resistance by 5 and 25%, respectively. Gil et al. (2009) observed that the pretreatment with cellulases caused the increase of SR and fibre hydratation of a bleached *Eucalyptus globulus* pulp The enzymes cleaved the polysaccharide chains on the fibre surface without liberating them from the surface. They observed that the small changes in the hemicelluloses content did not significantly affect pulp properties during refining despite the higher macrofibrillation and breaking length, and lower tear index. On the other hand, Lecourt et al. (2010) observed that fibres were more hydrated, swollen, fibrillated and cut, but the latter phenomenon negatively affected tear

Table 7 The effect of enzymatic pretreatment on laboratory handsheets characteristics

Sample	Bulk (cm^3/g)	Tensile index (Nm/g)	TEA index (J/g)	Tear index (mN m^2/g)
Unbeaten				
Control	1.89	17.8	0.26	5.4
		(1.8)	(0.06)	(0.2)
N. patriciarum	1.86	19.8	0.21	8.0
		(1.4)	(0.08)	(0.3)
T. longibrachiatum	1.88	20.2	0.28	7.3
		(1.6)	(0.09)	(0.3)
T. lanuginosus	1.93	22.8	0.32	6.3
		(1.9)	(0.07)	(0.2)
Refined to 30°SR				
Control	1.37	79.2	1.96	6.8
		(2.1)	(0.17)	(0.3)
N. patriciarum	1.30	89.5	2.18	6.7
		(2.9)	(0.12)	(0.5)
T. longibrachiatum	1.27	79.2	1.80	5.1
		(1.9)	(0.15)	(0.1)
T. lanuginosus	1.43	96.4	2.29	6.1
		(2.4)	(0.06)	(0.2)

index. According to Singh et al. (2014), the treatment of pulp with endoglucanase improved mechanical properties of paper sheets. We also investigated the effect of cellulases and xylanases on refining process and kraft pulp properties (Przybysz Buzała et al. 2016). Partial cellulose degradation by the two preparations of cellulases increased the contents of fines and reduced the weighted average fiber length that caused deterioration of paper strength properties. The xylanase partially removed xylan, associated with cellulose fibers, and slightly loosened their structure and reduced the weighted average fiber length.

Enzymatic pulp treatment with xylanases has been increasingly used in papermaking, mainly for pulp bleaching (Nair et al. 2010), production of dissolving grade pulp (Ibarra et al. 2009; Hakala et al. 2013), separation of hemicelluloses for further conversion (Huang et al. 2009) or to improve refining (Chen et al. 2012).

The assessment of the impact of xylanases requires characterization of both the pulp and paper properties (Cui et al. 2015). The application of xylanases to reduce the energy input for pulp refining was reported by Chen et al. (2012), Dickson et al. (2000) and Bhardwaj et al. (1996). The results reported by Chen et al. (2012) who used xylanases to pretreat pulps obtained from various poplar varieties suggested that the strength paper properties that are characterized by the tear, burst and tensile indices were not changed. Bhardwaj et al. (1996) observed the increased tensile index and tear index of enzyme-treated mixed pulp (60% waste corrugated kraft cutting and 40% softwood). However, these results indicate that enzyme-modified fibres show strong beatability due to the cell wall loosening action of the xylanases and the decrease in the energy demand may be gained in the paper making process.

Chen et al. (2012) noticed that the length of fibres after refining was longer when the pulp was pretreated with xylanases while neither the diameter of fibres nor fines content were significantly affected. The results obtained by Chen et al. (2012) suggest that the number of PFI mill revolutions that are necessary to obtain the given value of freeness may be reduced by 5-9% when cellulosic pulp is pretreated with xylanases while the results of this work provide evidence that this number is reduced by 22.2–41.9%. Furthermore, in contrast to the results reported by Chen et al. (2012), the results of this study demonstrate that pulp pretreatment with preparations of xylanases that are either free of cellulases or display the low cellulolytic activity enables to improve the static strength properties (tensile index) and maintain the dynamic strength properties (tear index). This finding is consistent with the results of Dickson et al. (2000). However, pulp pretreatment with preparations of xylanases that display the relatively high cellulolytic activity negatively affected the dynamic strength properties that was correlated with a significant decrease in the mean weighted fibre length, which was greater than in the studies of Chen et al. (2012) and Saukkonen et al. (2014), and nearly the same as reported by Derkowska (2015) and Borkowski (2015). Also Znidarsic-Plazl et al. (2009) observed the improvement of paper strength properties while Saukkonen et al. (2014) reported only slight differences in paper properties (changes in the indices by only several %). This study showed that these differences (an increase in tensile index and a decrease in tear index) reach even 20%.

Also Bhaduri et al. (1995) and Blomstedt et al. (2010) reported that pulp and paper properties were very slightly influenced by enzymatic pulp pretreatment. Interestingly, Christiemin et al. (2014) found that the addition of xyloglucan to cellulosic pulp improved the strength paper properties. This finding suggests that partial removal of hemicelluloses from pulps leads to worse paper strength properties. Also Han et al. (2012) reported that supplementation of cellulosic pulp with birch xylan improved the strength properties but the changes were only around 5–7% with an exception of the burst index which was improved by 10–12%. This study showed that the smallest decrease in the hemicellulose content (from around 3.5–3.0% d.w.) in the bleached pine pulp, which was caused by the pure xylanase from *N. patriciarum*, resulted in the most acceptable pulp and paper properties. Partial digestion of hemicelluloses contained in the bleached pine pulp by this enzyme caused a slight decrease in the content of xylose (by around 16% w/w), rhamnose (by around 28.5% w/w) and arabinose (by less than 12% w/w) in the pulp.

Conclusions

Pretreatment of the bleached kraft pulp with xylanases reduced the number of PFI mill revolutions that were necessary to achieve the freeness of 30°SR and significantly affected paper properties. Pretreatment with the *N. patriciarum* xylanase, which caused the smallest decrease in the content of hemicelluloses (by around 0.5% d.w.) in the pulp, improved the static paper strength properties by 13% while the dynamic properties were not significantly changed. The more advanced degradation of hemicelluloses by the *T. longibrachiatum* and *T. lanuginosus* xylanases, which showed cellulolytic activity, negatively affected the dynamic strength properties of paper.

Pretreatments with the *N. patriciarum* and *T. lanuginosus* xylanases caused that the decrease in the mean weighted fibre length and the increase in fines content were relatively small while the *T. longibrachiatum* xylanase, which was characterized by the relatively high cellulolytic activity, reduced the mean fibre length by almost 400 µm, and enhanced formation of fines (their content increased to 32% while in the reference to 28%).

Reassuming, the best pulp and paper properties were obtained using the xylanases from *N. patriciarum* and *T. lanuginosus*, which caused the least changes in the structure of hemicelluloses and cellulose fibres. Pulp pretreatment with these xylanases enabled to reduce duration of refining and increased the static strength properties of paper by above 10%.

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References

Adney B, Baker J (2008) Measurements of cellulase activities, NREL/TP-510-42628

Bajpai P, Mishra SP, Mishra OP, Kumar S, Bajpai PK (2006) Use of enzymes for reduction in refining energy—laboratory studies. Tappi J 5:25–32

Bhaduri SK, Ghosh IN, Deb Sarkar NL (1995) Ramie hemicellulose as beater additive in paper making from jute-stick kraft pulp. Ind Crop Prod 4:79–84

Bhardwaj NK, Bajpai P, Bajpai Pramod K (1996) Use of enzymes in modification of fibres for improved beatability. J Biotechnol 51:21–26

Biermann CJ (1996) Handbook of pulping and papermaking, 2nd edn. Academic Press An Imprint of Elsevier, San Diego, pp 137–149. ISBN 978-0-12-097362-0

Blomstedt M, Asikainen J, Lahdeniemi A, Ylonen T, Paltakari J, Hakala TK (2010) Effect of xylanase treatment on dewatering properties of birch kraft pulp. BioResources 5:1164–1177

Borkowski J (2015) Xylanase-assisted pulp refining process. Bachelor Thesis, Lodz University of Technology

Buzała K, Przybysz P, Rosicka-Kaczmarek J, Kalinowska H (2015) Production of glucose-rich enzymatic hydrolysates from cellulosic pulps. Cellulose 22:663–674

Cadena Eith M, Chriac Iulia A, Pastor Javier FI, Diaz P, Vidal T, Torres Antonio L (2010) Use of cellulases and recombinant cellulose binding domains for refining TCF kraft pulp. Biotechnol Prog 26(4):960–967

Chen JC, Yang GH, Kong FG, Lucia LA, Liu Y (2012) Influence of xylanase pretreatment on refining energy and brightness of P-RC APMP pulp of Italian black poplar branches. Cellul Chem Technol 46:283–290

Christiemin M, Henriksson G, Lindstrom ME, Brumer H, Teeri TT, Lindstrom T, Laine J (2014) The effects of xyloglucan on the properties of paper made from bleached kraft pulp. Nord Pulp Pap Res J 21:3677–3690

Cui L, Meddeb-Mouelhi F, Laframboise F, Beauregard M (2015) Effect of commercial cellulases and refining on kraft pulp properties: correlations between treatment impacts and enzymatic activity components. Carbohyd Polym 115:193–199

Demuner BJ, JrN Pereira, Antunes AMS (2011) Technology prospecting on enzymes for the pulp and paper industry. J Technol Manag Innov 6:148–157

Derkowska M (2015) Enzymatic support for pulp refining process. Master Thesis, Lodz University of Technology

Dickson AR, Wong KKY, Mansfield SD (2000) Response of xylanase-treated kraft pulp to Escher-Wyss and PFI refining. Tappi J 83:64–74

Gil N, Amaral NE, Costa AP, Duarte AP (2009) Use of enzymes to improve the refining of a bleached *Eucalyptus globulus* kraft pulp. Biochem Eng J 46:89–95

Hakala TK, Liitia T, Suurnakki A (2013) Enzyme- aided alkaline extraction of oligosaccharides and polymeric xylan from hardwood kraft pulp. Carbohyd Polym 93:102–108

Han W, Zhao C, Elder T, Chen K, Yang R, Kim D, Yunqiao P, Hsieh J, Ragauskas AJ (2012) Study on the modification of bleached eucalyptus kraft pulp using birch xylan. Carbohyd Polym 88:719–725

Huang HJ, Lin W, Ramaswamy S, Tschirner U (2009) Process modeling of comprehensive integrated forest biorefinery: an integrated approach. Appl Biochem Biotechnol 154:26–37

Ibarra D, Kopcke V, Ek M (2009) Exploring enzymatic treatments for the production of dissolving grade pulp from different wood and non-wood paper grade pulps. Holzforschung 63:721–730

Lecourt M, Meyer V, Sigoillot J-C, Petit-Conil M (2010) Energy reduction of refining by cellulases. Hlozforschung 64:441–446

Miller GL (1959) Use of dinitrosalicylic reagent for determination of reducing sugar. Anal Chem 31:426–428

Nair SG, Sindhu R, Shashidhar Shankar (2010) Enzymatic bleaching of kraft pulp by xylanase from *Aspergillus sydowii* SBS 45. Indian J Microbiol 50(3):332–338

Przybysz K (1997) Pulp and paper technology part 2: paper technology, 2nd edn. WSiP, Warsaw, pp 51–77

Przybysz K (2011) Paper technology part I: papermaking pulps. Wist, Lodz

Przybysz Buzała K, Przybysz P, Kalinowska H, Derkowska M (2016) Effect of cellulases and xylanases on refining process and kraft pulp properties. PLoS ONE 11:8

Przybysz P, Przybysz K (2013) An overview of pulp refining concepts. Part I. Development of construction and operation of pulp refining installations and units. Pol Pap Rev 69:383–389

Saukkonen E, Lyytikainen Geydt P, Backfolk K (2014) Surface selective removal of xylan from refined never-dried birch kraft pulp. Cellulose 21:3677–3690

Singh R, Bhardwaj NK, Choudry B (2014) An experimental study of the effect of enzyme-assisted refining on energy consumption and paper properties for mixed hardwood pulp. Appita J 67:226–231

Znidarsic-Plazl P, Rutar V, Ravnjak D (2009) The effect of enzymatic treatments of pulps on fiber and paper properties. Chem Biochem Eng 23:497–506