EFFECTS OF THE ENDOXYLANASE TREATMENT ON FIBER CHARACTERISTICS, BRIGHTNESS STABILITY AND STRENGTH PROPERTIES OF BLEACHED WHEAT STRAW PULP

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The pretreatment of soda-anthraquinone (AQ) wheat straw pulp with purified endo-(1,4)-β-xylanase was studied. Different xylanase dosages (0, 2, 20 and 100 IUg^{-1}) were applied to the pulp before an ECF (Elemental Chlorine Free) bleaching sequence. The fiber characteristics, brightness stability and strength properties of the obtained pulps were determined. The results showed that the fiber characteristics were not significantly affected by the enzyme pretreatment. On the other hand, the xylanase pretreatment lowered the strength properties of the pulp. A slight improvement in lignin removal was observed, thus improving pulp bleachability, the results agreeing with those obtained on wood pulps. Furthermore, higher paper brightness stability was obtained by the xylanase pretreatment, explained by the removal of the metal ions from the pulp samples.

Keywords: wheat straw, endoxylanase, paper ageing, brightness stability, strength properties

INTRODUCTION

Environmental and population growth pressures are contributing to long-term changes in forest land management practices and to a decreased wood harvesting for both forest products industries and pulp and paper manufacture. At the same time, cereal grain production generates tremendous quantities of straw. Therefore, for strong environmental and economic reasons, straw is recommended as an alternative raw material for the production of paper products for both developed and developing countries. Agri-based fibers are of particular importance in countries with high demand for pulp and paper and limited forest resources.[1,2]

Wheat straw is one of the most important agricultural residues that can be utilized as a fiber source.

Conventional chlorine-based bleaching is known for its serious environmental impact, such as the formation of toxic and/or mutagenic organic chlorinated compounds, while the increased amount of chloride and AOX (adsorbable organic halogen) in bleach plant effluents[3] is of special interest in developing environmentally friendly processes including biotechnological methods, which can reduce or totally eliminate the use of chlorine in the process. Biobleaching with xylanase, which has already proven its potential as an environmentally friendly bleaching method,[4] has been applied in many mills for pre-bleaching wood pulps.

Xylan is one of the major hemicelluloses present in the plant cell walls. The backbone of xylan contains only 1,4-linked β-D-xylopyranosyl units. During the biological degradation of xylan in the cell wall, the endoxylanases degrade the xylan backbone into oligosaccharides, which are further degraded by several enzymes, including acetyl esterase, α-glucuronidase and β-xylosidase.[5] Enzymatic degradation is a complex process, involving several hemicellulolytic enzymes (isoenzymes).[6] Because of the key role played in the catalysis of xylan hydrolysis, one of the industrial applications of endoxylanases is in pre-bleaching of chemical pulps in the pulp and paper industry.[7]

During the production of bleached chemical pulps, brightness and its stability values represent important quality parameters. The tendency of brightness loss in fully bleached pulps on storage or exposure to heat, the so-called thermal-induced yellowing, represents a serious problem[8] and is influenced by several parameters, including metal ions and the carbonyl and carboxyl group content.[9,10]

The objective of the paper was to evaluate the effect of pre-bleaching with purified endoxylanase and to determine the influence of its application on the fiber characteristics, brightness stability and strength properties of elemental chlorine free (ECF) bleached wheat straw soda AQ pulp.

MATERIALS AND METHOD

Soda AQ pulping of wheat straw (*Tiriticum aestivum L.*) was carried out in a stainless steel reactor. The cooking chemicals contained 12% active alkali charge and 0.1% antraquinone (AQ); the ratio of cooking liquor to straw was of 6:1. The cooking temperature was ramped to 160 °C within 40 min and maintained throughout the 60 min of cooking.

The commercial endoxylanase enzyme used for bleaching was Cat X2753 from Sigma (purified endo-(1,4)-β-xylanase from *Thermomyces lanuginosus* with 2500 IU/g expressed in *Aspergillus oryzae* microorganism, synonym – Pentopan Mono BG from Novozyme). The enzyme activities were determined by the dinitrosalicylic acid (DNS) method.[11] A diluted enzyme solution (30 µL) was incubated in 300 µL of 1% (w/v) birch wood xylan (Sigma) solution (100 mmol L^{-1} acetate buffer with 0.4% Tween 20, pH 5.0) at 40 °C, for 20 min. One international unit (IU) of xylanase activity was defined as the amount of enzyme catalyzing the release of 1 mmol of xylose equivalent per minute.

Three different enzyme dosages – 2, 20 and 100 IU g^{-1} – were applied to the wheat straw soda AQ pulps. The enzyme pretreatment was carried out at 5% pulp consistency, 50 mmol L^{-1} sodium acetate buffer, pH 5.0, 50 °C, for 90 min. After the pretreatment, the pulp samples were washed in water. The control trial was performed under exactly the same conditions, yet without xylanase addition.

Further bleaching of the control and enzyme pretreated pulps was done in polyethylene bags placed in a water bath. The initial amount of straw pulp for each bleaching was of 50 g (o.d.). The conditions for each bleaching stage of the elemental chlorine free (ECF) bleaching process are shown in Table 1.

The lignin-derived compounds (LDCs) present in the filtrates after the bleaching sequences were measured spectrophotometrically at 280 nm. The carbohydrates loss, measured as the amount of reducing sugars (RS) released from pulp, was determined spectrophotometrically at 540 nm, according to the DNS method.[12]

Table 1
ECF bleaching conditions of wheat straw soda AQ pulps

Conditions	Bleaching sequence		
	D_1	E_p	D_2
Pulp consistency (%, w/w)	10	10	10
Temperature (°C)	75	75	75
Reaction time (min)	60	180	180
Active chlorine (% on pulp, w/w)	2.0	-	1.0
Magnesium sulphate (% on pulp, w/w)	-	0.5	-
Sodium hydroxide (% on pulp, w/w)	-	1.0	0.1
Hydrogen peroxide (% on pulp, w/w)	-	0.5	-

D, chlorine dioxide; E_p, peroxide-reinforced alkaline extraction

The Kappa number, viscosity and metal ion contents of the pulp samples were determined according to TAPPI T 236 cm-85 TAPPI T 230 om-94 and TAPPI T 266 om-94, respectively, while fiber characteristics were measured on a Fiber Quality Analyzer (FQA). Fiber swelling, measured as the water retention value (WRV), was determined[13] experimentally by the centrifugation technique at a centrifugal force of 900·g, for 30 min.

Paper sheets of fully bleached pulps, weighing ~60 g/m^2, were prepared according to Tappi T 272 om-92, and accelerated thermal ageing was conducted according to the ISO method 5630-3:1996 (70 °C and 65% relative humidity). Pulp samples of 92% solid content were sealed in double polyethylene bags placed in a water bath for 1 to 15 days.

Brightness measurements of the paper sheets were made on a Technibrite Micro TB-1C, by the ISO test method (ISO 2470). All brightness values reported, representing an average of ten separate measurements, had a standard deviation ±0.5. The post-color (PC) number,[14] adopted for the evaluation of brightness stability, uses of the Kubelka-Munk relationship (Eq. 1) to convert brightness loss into a parameter related to chromophore concentration, and is calculated according to Eq. 2 (where k and s are the absorption and scattering coefficients of the paper sheets, respectively, and R_∞ is brightness). The PC number is an indicator of the new chromophores generated in the paper sheets. A higher PC number indicates the greater number of chromophore bodies generated during ageing:[15,16]

$$\frac{k}{s} = \frac{(1-R_\infty)^2}{2R_\infty} \quad (1)$$

$$\text{PC number} = 100 \times \left[\left(\frac{k}{s}\right)_{\text{after}} - \left(\frac{k}{s}\right)_{\text{before}} \right] \quad (2)$$

The breaking length and stretch (TAPPI T 404 cm-92), the burst (TAPPI T 403-om-91) and tear index (TAPPI T 414-om-88) of the pulp samples were determined according to standard methods.

RESULTS AND DISCUSSION

Fiber characteristics

The kappa numbers of the control and xylanase-pretreated pulps, prior to DEpD bleaching, were virtually the same, remaining unchanged at 9.5, which indicated that xylanase would mainly act on the xylan, while lignin removal is limited. Figure 1 shows the relationship between lignin removal and the loss of carbohydrates during the enzymatic treatment. The increase in the enzyme charge causes a corresponding increase in the amount of reducing sugars (RS) accumulated in the filtrates. The amount of RS released increased by 81% at an enzyme application of 100 IU g^{-1}, in comparison with that at 20 IU g^{-1}. However, the effect of the xylanase pretreatment on the amount of lignin-derived compounds (LDCs) removed was much lower, the xylanase pretreatment with 100 IU g^{-1} producing only a 14% increase in the LDC, compared to that at 20 IU/g. Clarke *et al.*[17] reported similar results for softwood kraft pulp. Xylan can be a source of new chromophores, as suggested by Beyer *et al.*[18] The xylanase dosage is a critical parameter in releasing the chromophoric material and in reducing the sugars from wheat straw soda-AQ pulp.

Due to the increased loss in carbohydrates, a higher xylanase dosage in the enzymatic pretreatment stage increased the overall mass loss (1.4, 4.8 and 17.8 %, respectively, at 2, 20 and 100 IU g^{-1} xylanase dosage – Fig. 2). A similar loss was reported by Beyer *et al.*,[18] who studied the pretreatment of birch and pine pulps with xylanase. Xylan removal from the pulp, due to the xylanase pretreatment, increased pulp viscosity, as shown in Figure 2. Yang and Ericsson[19] reported earlier that a higher viscosity was obtained after xylanase pretreatment, caused by the selective removal of xylan, which has a lower degree of polymerization (DP) than the other carbohydrates present in pulp. When present in pulp, the low-DP xylan can be expected to give a lower pulp viscosity.[20-23] Figure 2 also shows that the WRV drops sharply with enzyme application, which can be attributed to the removal of the low DP xylan from the fiber surface; with the addition of more enzyme, the WRV increases steadily because of the formation of new hydrophilic end groups from the endoxylanase pretreatment.

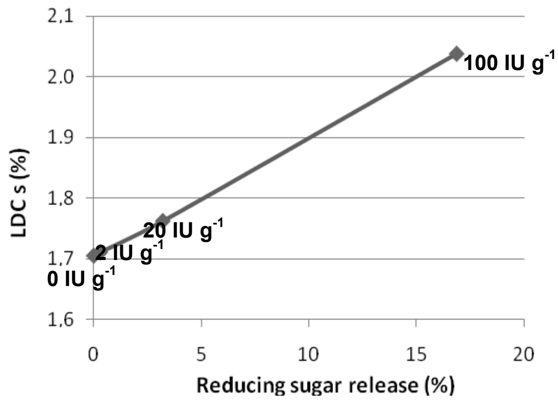

Figure 1: Lignin-derived compounds (LDCs) and reducing sugars (RS) released during enzyme pretreatment

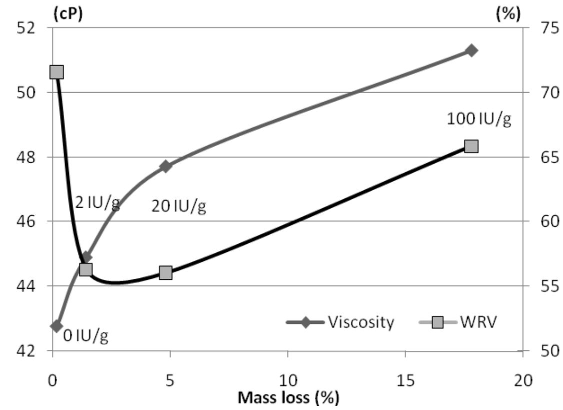

Figure 2: Effect of the mass loss, due to enzyme pretreatment, on pulp viscosity (cP) and WRV (%) (xylanase dosage: 0, 2, 20 and 100 IU g^{-1}, respectively)

Table 2
Fiber properties after xylanase pretreatment

Enzyme dosage (IU g^{-1} pulp)	Coarseness (mg mm^{-1})	Fiber length (mm)	Curl index (%)	Kink index (%)	Fines (%)
0	0.088±0.064	0.423±0.12	0.080±0.072	1.54±0.95	43.09±3.1
2	0.088±0.065	0.433±0.18	0.081±0.054	1.62±0.87	42.61±2.8
20	0.081±0.047	0.434±0.11	0.084±0.048	1.64±0.85	41.52±2.4
100	0.080±0.030	0.413±0.16	0.098±0.026	1.86±0.49	44.40±2.2

Table 2 shows the effect of enzyme pretreatment on the fiber characteristics. No significant changes were noticed in coarseness and fiber length after the xylanase treatment. Similar results were previously reported by Yang *et al*.[24] and Prasad *et al*.[25] However, the curl and kink indexes of the fibers increased with the enzyme dosage, which may have contributed to the loss in paper strength.

Bleaching and brightness stability

The endoxylanase pretreatment can lead to a higher final brightness of the ECF-bleached pulp. At a charge of 2 IU g^{-1}, the brightness increase was of 0.2 points for the fully bleached pulp (Fig. 3); at 20 IU g^{-1}, brightness increased by another 0.4 points; at 100 IU g^{-1}, the brightness increase was of 2.5 points. Previous results showed that the amount of RS released by xylanase did not correlate well with the bleaching abilities of the pulp samples.[26,27]

Brightness stability is one of the most important parameters of the fully bleached chemical pulps. Accelerated thermal ageing was performed for determining the effect of the xylanase pretreatment on the brightness loss of the paper sheets. The effects of the thermal ageing time on the brightness stability of the pulp samples, illustrated in Figure 4, show that almost half of the total brightness loss occurred in the first 3 days of the thermal ageing process. The most significant brightness loss was observed for the control (20.9%), while the brightness loss was of 20.4% at 2 IU g^{-1} xylanase after 15 days. However, the lowest brightness loss was obtained at a 100 IU g^{-1} enzyme dosage (16.8%), indicating that the xylanase pretreatment decreased the brightness loss of the bleached pulp. Table 3 shows the obvious correlation between xylanase dosage and PC number. The PC numbers of the paper sheets were 6.0, 5.7, 5.2 and 3.7, respectively for 0, 2, 20 and 100 IU g^{-1} xylanase dosages. Similar results were obtained by Yang *et al.*[24]

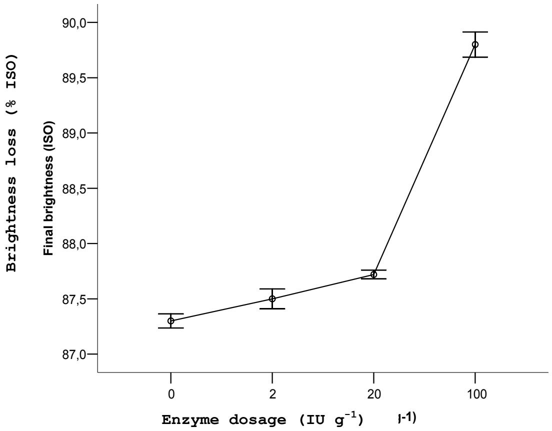

Figure 3: Effect of xylanase pretreatment on the final brightness of the DEpD bleached wheat straw pulps

Most likely, the improved brightness stability was due to the fact that the xylanase pretreatment enhanced the removal of transition metal ions, as shown in Table 3. For a 100 IU g^{-1} xylanase dosage, the removal of iron, manganese, magnesium and calcium was 52.4, 9.3, 56.03 and 33.3%, respectively.

Bleached chemical pulps contain negligible amounts of lignin. Usually, the kappa numbers of the fully bleached ECF (elemental chlorine free) and TCF (total chlorine free) pulps were[28] of about 0.8 units. In bleached pulps, the presence of transition metal ions, such as iron, manganese and copper, could cause a significant brightness loss.[18,28,29-35] A possible explanation for the lower pulp brightness, when metal ions were present in the pulp samples, was the formation of colored metallic complexes with carboxy, oxo-carboxy and, possibly, HexA groups in the carbohydrates. In a systematic study on the formation of colored metallic complexes with extractive model compounds, such as quercetin, Ni *et al.*,[36] showed that the metal ions could also enhance brightness reversion during photo-yellowing of mechanical pulps.[37]

Alternatively, Sevastyanova[8] hypothesized that the metal ions might act as Lewis acids and accelerate the hydrolysis of polysaccharides and their further oxidation during thermal yellowing, therefore enhancing brightness reversion.

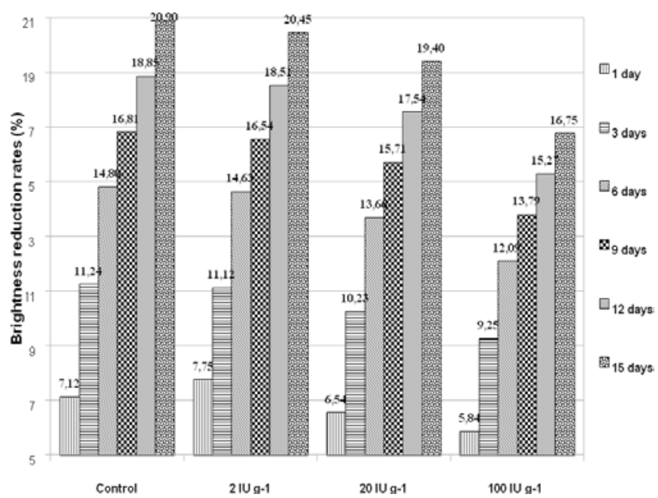

Figure 4: Effect of xylanase pretreatment on brightness loss during accelerated ageing

Strength properties

The physical properties of the fully bleached wheat straw pulp samples were determined both before and after ageing (15 days). The results are presented in Figures 5 and 6. It was evident that the increase in the enzyme dosage decreased the strength properties of the wheat straw soda-AQ pulp. The decreased strength may have been due to the removal of hemicellulose, therefore decreasing the fiber bonding capacity. Prasad *et al.*[25] and Mansfield *et al.*[38] reported similar results, namely that bagasse mechanical pulps showed a decrease in their strength properties by the enzyme pretreatment. Increasing the xylanase dosage resulted in a slightly lower bulk of the paper sheets (Fig. 5). The mass loss (Fig. 2) and increase of kink and curl indexes (Table 2) may be partially responsible for these results.

In addition, Figures 5 and 6 show that the ageing process led to a further decrease in the strength properties (a drop from 4.7 to 9.4%), caused by hydrolytic, oxidative and thermal degradation of cellulose.[39] Similar results have been provided in the literature – for example, Zou *et al*.[40,41] reported a significant strength loss during accelerated paper ageing, attributed to polysaccharide degradation during the process.

Table 3
Metal ion contents and PC number after 15 days of ageing

Enzyme concentration	PC	Metal ion contents (ppm)				
(IU g^{-1} pulp)	Number	Fe	Cu	Mn	Mg	Ca
0	6.0	16.85	u.d.	4.85	381.4	882
2	5.7	12.58	u.d.	4.50	367.2	676
20	5.2	15.60	u.d.	4.75	334.1	317
100	3.7	8.83	u.d.	0.45	213.7	294

PC Number – post-color number, u.d. – undetectable

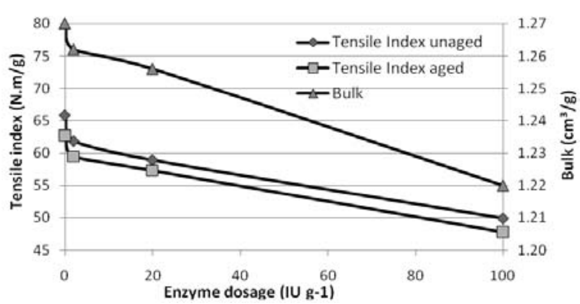

Figure 5: Effect of xylanase pretreatment on bulk and tensile index before and after ageing

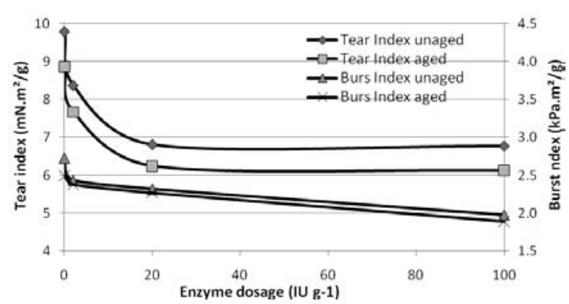

Figure 6: Effect of xylanase pretreatment on tear and burst indexes before and after ageing

CONCLUSIONS

The effect of the pretreatment of soda-AQ wheat straw pulp with purified endo-(1,4)-β-xylanase, prior to an ECF (Elemental Chlorine Free) bleaching sequence, was studied. While the fiber characteristics were not significantly changed, the enzyme pretreatment can facilitate the subsequent bleaching process, a higher final pulp brightness resulting after the same ECF sequence. The boosting effect of the xylanase pretreatment on straw pulp agrees well with that obtained on wood pulp, as reported in literature. Also, the xylanase pretreatment led to an increased brightness stability of the fully bleached pulp, due to the enhanced removal of metal ions from the pulp fibers. On the other hand, at high enzyme dosage, the xylanase pretreatment can induce more carbohydrate degradation, and thus a higher strength loss. At the same time, accelerated thermal ageing decreased the strength properties of the pulp.

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