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Effect of Xylanase-Assisted Treatment of Oxygen-Delignified Eucalypt Kraft Pulp on ECF Bleaching

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Abstract: The effects of treating industrial (laboratory-unwashed) oxygen-delignified eucalypt kraft pulp with a commercial endo-xylanase (X) on ECF bleaching performance were evaluated. Changes in fibre morphology, pulp bleachability and quality, and bleaching effluent parameters were assessed. Although no significant morphological changes were observed, fibres showed some external fibrillation. The X stage reduced both the amount and the integrity of xylans remaining in the fibres, causing their redistribution inside the cell wall. In bleaching, the X treatment allowed the reduction of ClO_2 and NaOH loads by 20 and 10%, respectively. Furthermore, the brightness stability of enzyme-treated bleached pulps was improved, which was assigned to the decrease in the content of hexenuronic acid residues. The X treatment did not affect the cellulosic counterpart of pulp and did not cause a significant impact on the papermaking properties, even when xylan degradation was somewhat excessive. The enzymatic treatment caused a significant increase in the chemical oxygen demand (COD) of the respective effluent. The realistic conditions used provide a better insight into the overall impact of this technology at a pulp mill.

Keywords: cellulose technology; chlorine dioxide savings; ECF biobleaching; eucalypt kraft pulp; green industry; xylanase

1. Introduction

The delignification of wood is currently carried out using various pulping processes, depending on the purpose of the resulting cellulosic pulp. These include, for example: (i) kraft pulping for the production of bleached pulp, suitable for the manufacture of printing and writing (P&W) paper; (ii) acid sulphite pulping for the production of high purity dissolving pulp, required to produce regenerated fibres used in the textile industry [1]; or (iii) organosolv pulping for the recovery and use of lignin and sugars [2,3]. Nowadays, kraft cooking is the most common pulping process used for P&W paper production. However, by the end of cooking, the pulp still possesses a dark colour, needing further processing to acquire the desired brightness. The dark colour is mainly due to residual lignin, which, together with cellulose and hemicelluloses, are the main constituents of lignocellulosic biomass. Although lignin is extensively removed during kraft pulping, residual lignin still represents about 2%–4.5% of pulp weight [4]. It contains chromophoric structures of phenol, catechol and quinone types [5], which are responsible for the dark colour of the pulp. The removal of these chromophores from pulp is achieved by so-called pulp bleaching processes, which enable pulp to achieve ISO brightness values above 88% [6], making it appropriate for the production of high-grade P&W paper. Bleached pulp usually has a lignin content below 1% [7].

In the past, the pulp bleaching process consisted of chlorine-based sequences applying elemental chlorine and hypochlorite [8], which produced organochlorinated species (AOX), some of them toxic [6]. Environmental concerns and legal restrictions pushed the industry

towards "greener" solutions, and, as a result, elemental chlorine-free (ECF) bleaching was introduced and became the dominant bleaching technology today [4,9]. ECF bleaching is based on the use of chlorine dioxide (instead of chlorine) as the main bleaching agent [9]. Considerably fewer AOX are produced [9,10], thus decreasing their load in the mill treatment plant and enabling operational efficiency and environmental benefits [11]. Another technology that has allowed mills to reduce the need for chlorine-based chemicals is oxygen delignification [6,8,9], which is usually applied after pulping and before bleaching [7]. ECF bleaching of oxygen-delignified kraft pulp to produce white P&W paper is nowadays a steady trend and widespread. Hydrogen peroxide is also commonly applied in ECF bleaching sequences, either simply as an adjuvant in alkaline extraction stages (E_P) or in a dedicated bleaching stage (P). The different loadings of chemical reagents, time and temperature applied in the E_P and P stages affect pulp differently [12]. In any case, hydrogen peroxide is used with the intention of further increasing the brightness or brightness stability of the pulp [8].

Although chlorine dioxide and oxygen bleaching technologies have allowed for reducing the environmental impact of bleaching, pulp mills are continuously making efforts to further decrease their emissions [13], not only due to the tendency for the implementation of increasingly restrictive laws concerning the emission of such compounds, as well as growing public awareness, but also in order to comply with internal environmental performance indicators of the mill. Therefore, the search for new ways to achieve those goals has been active for decades. One of those alternative bleaching technologies is xylanase-assisted technology, the commercial application of which started in the early 1990s [14]. It usually takes place at the beginning of the bleaching sequence (as a pre-bleaching stage) [10]. The treatment of pulp with xylanase does not properly bleach the pulp, but it facilitates the removal of chromophore structures related to residual lignin and degraded xylan. In others words, the enzymatic stage can be considered a bleach-boosting step [15] that increases pulp bleachability, thus allowing a lower consumption of chemical bleaching reagents in later steps. In fact, xylanase treatment has been seen to decrease the consumption of bleaching chemicals by 10%–30% in ECF bleaching sequences [14] while allowing lower bleaching costs [16] and decreased emissions of organochlorinated compounds [17].

Xylanase (endo-xylanase) is a hydrolytic enzyme that acts directly on xylan, which is the major hemicellulose found in hardwoods. Endo-xylanase cleaves linkages within the xylan main chain, thereby causing the depolymerization of xylans into smaller fragments [18]. Xylan degradation is linked to the bleach-boosting effect of xylanase in the way that the enzyme preferably removes xylan molecules that precipitate on the surface of the fibres at the end of cooking. These molecules act as a barrier that hampers both the penetration of bleaching agents into the fibres and the diffusion of oxidised lignin fragments outwards [19]. The xylanase treatment can also somewhat act as a direct delignification step in the way that liberated xylans may be linked to lignin fragments in the form of lignin-carbohydrate complexes (LCCs) [20], thus enabling lignin liberation, as well. Lastly, xylanase may also contribute to improving pulp brightness stability by the indirect removal of hexenuronic acids (HexA). HexA groups exist as substituents in xylan chains [21] and are widely considered to be chromogenic structures, thus contributing to the yellowing (brightness reversion) of bleached pulp [22]. Besides, HexA reacts with chlorine dioxide, thereby increasing its consumption during pulp bleaching [9].

For pulp mills, the use of xylanase pre-bleaching is very easy and cheap to implement [14]. Furthermore, due to its high selectivity, it does not cause side reactions, nor does it produce harmful by-products. The mill application of xylanase is also generally reported not to impair pulp strength properties [10,17]. Breakthroughs in genetics and protein engineering have led not only to the development of microorganisms capable of effectively producing high amounts of enzymes but also to the obtention of thermo- and alkali-tolerant enzymes [23]. This has made enzyme production more cost-effective and allowed the production of enzymes that are active under the harsh conditions encountered at the pulp mill (pH 9–10 and temperature 65–80 °C), thus almost eliminating the need for

temperature and pH adjustment before the enzymatic treatment. However, xylanase treatment is associated with a loss of pulp yield and increased chemical oxygen demand (COD) of the effluents. These negative effects originate from the removal of hemicelluloses [24], which have inhibited a more widespread use of this technology. On the other hand, the xylanase pre-bleaching treatment represents a solution, for instance, for mills with limited chlorine dioxide production [8–10].

In this work, the application of a xylanase stage on unwashed oxygen-delignified eucalypt kraft pulp, followed by a $D_0E_PD_1D_2$ bleaching sequence, was studied. The initial goal was the assessment of both morphological and structural modifications of the pulp introduced by the xylanase treatment. Thereafter, this work focused on the bleach-boosting effect of introducing the X stage at the start of an ECF bleaching sequence, namely through the evaluation of chemical savings in the subsequent bleaching sequence (and hence the decrease in the environmental impact of bleaching). The impact on pulp quality and bleaching effluents/filtrates was assessed, as well. The use of pulp as collected at the mill, without further washing, in opposition to what happens in most published works, allowed studying the effect of xylanase in the presence of organic carryover, which makes this study more realistic from an industrial point of view.

2. Materials and Methods

2.1. Raw Materials

Oxygen-delignified eucalypt kraft pulps (with a kappa number of around 10 and produced mainly from *Eucalyptus globulus*) were collected at a Portuguese pulp mill employing an $OD_0E_PD_1D_2$ bleaching sequence and were used in the lab trials without further washing. A commercially available endo-1,4-β-xylanase product, Xylio Pre, supplied by Novozymes, Bagsværd, Denmark, was used.

2.2. Xylanase Activity

Xylanase activity determination was based on the hydrolysis of a model xylan substrate (oat spelts xylan) by xylanase, which releases xylan fragments in the form of reducing sugars. These were afterwards quantified using the DNS (3,5-dinitrosalicylic acid) colorimetric method. This quantification method is based on the oxidation of carbonyl groups of the reducing sugars to carboxyls, with simultaneous DNS reduction to 3-amino-5-nitrosalicylic acid, shifting the colour of the solution from yellow to brownish red. The diluted xylanase solution was incubated with xylan substrate solution and Britton–Robinson buffer (pH 9) at 70 °C for 60 min. Afterwards, DNS solution was added, and the mixture was boiled for 15 min. Samples were then filtered using nylon syringe filters with a 0.45 μm pore diameter before their absorbance was measured in a Cary 100 UV-Vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) at a 540 nm wavelength. Xylanase activity is expressed in U/g and is defined as the amount of enzyme which produces 1 μmol of xylose equivalents (sugars with reducing power equivalent to that of xylose) in one minute. This methodology was adapted from the previously described methodology by Ghose and Bisaria [25].

2.3. Xylanase Treatment

Xylanase (X) pre-bleaching treatments were performed at 10% consistency in polyethylene bags placed in a water bath at 70 °C; pulp pH (9) was not adjusted, in order to mimic the industrial conditions. Two combinations of duration and xylanase dose were tested: (i) 30 min, 75 g/t odp (oven-dried pulp), and (ii) 60 min, 25 g/t odp. Control treatments (c) employed the exact same conditions as their respective X treatments but without enzyme addition. At the end of the treatment, a pulp washing step was performed by vacuum filtration and using a volume of distilled water 15 × the odp weight. The filtrates from the treatments were collected (before washing) and stored for further characterisation.

2.4. Fibre Testing

Pulp morphological properties (average fibre length, width, shape and coarseness, as well as fines content) were evaluated employing an L&W Fibre Tester Plus equipment. The analyses were performed using 100 mL of pulp suspensions at 0.1% consistency.

2.5. Scanning Electron Microscopy (SEM)

Sampling for SEM analysis began by suspending pulp in 2 mL of distilled water at 1% consistency, followed by homogenisation. Afterwards, the sample was centrifuged at $3000 \times g$ rpm for 5 min, and after discarding the supernatant, it was left to dry at room temperature. Air-dried samples were attached to a support with carbon tape and then subjected to analysis in a Hitachi TM4000plus scanning electron microscope at 15 kV of accelerating voltage (Tokyo, Japan).

2.6. Enzymatic Peeling

Enzymatic peeling consisted in subjecting pre-treated pulps (following X or c) to an enzymatic hydrolysis treatment with a different commercial xylanase (X2753, a recombinant endo-xylanase expressed in *Aspergillus oryzae*, purchased from Sigma-Aldrich, St. Louis, MO, USA) under highly favourable conditions for hydrolysis to occur (0.5% consistency, pH 5, and 40 °C for 210 min). Then, 0.5 mL aliquots were collected from the reaction medium in 30 min intervals and immediately mixed with 1 mL DNS. After boiling the mixture for 15 min and dilution with 10 mL of ultra-pure water, samples were filtered with the help of nylon syringe filters (0.45 μm pore diameter), and their absorbance was measured at 540 nm wavelength using a Cary 100 UV-Vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA).

2.7. Bleaching Sequences

A complete $D_0E_PD_1D_2$ bleaching sequence was employed following each enzymatic (and control) treatment to assess the impact on pulp bleachability (D–chlorine dioxide, ClO_2; E_P–alkaline extraction with sodium hydroxide, NaOH, reinforced with hydrogen peroxide, H_2O_2). Depending on the pulp kappa number, total chemical doses applied varied between 35 and 40 kg/t odp of ClO_2, 10 and 14 kg/t odp of NaOH, and 2 and 4 kg/t odp of H_2O_2. In the xylanase-assisted sequences ($XD_0E_PD_1D_2$), ClO_2 and NaOH doses in the D_0 and E_P stages were reduced by 20 and 10%, respectively, in comparison with the control sequences ($cD_0E_PD_1D_2$). The temperature and duration of all chemical stages were unchanged between control and enzyme-assisted sequences. All stages were conducted at 10% consistency in polyethylene bags placed in a water bath at the desired temperature. At the end of each stage, a pulp washing step was performed by vacuum filtration using a volume of distilled water $15 \times$ the odp weight. Analogously to the filtrate from the X stage, the filtrates from the chemical stages were collected and stored for later characterization.

2.8. Pulp Characterization

The bleach-boosting effect of the X stage was assessed in terms of ISO brightness gain, as well as brightness stability (PC Number) improvement. ISO brightness determination was performed according to ISO 2470-1 in hand sheets prepared according to ISO 3688. Accelerated dry aging of hand sheets was simulated by conditioning them in the dark at 105 °C for 60 min. This aging treatment was followed by another brightness reading, following equilibration to the appropriate temperature (23 ± 1 °C) and relative humidity (50 ± 2%) conditions. ISO brightness was read using an ABB L&W Elrepho 071 spectrophotometer (Zürich, Switzerland). The PC number was calculated using Equation (1) [26].

$$\text{PC Number} = 100 * \left(\frac{\left(1 - \frac{\text{brightness after reversion (\%)}}{100}\right)^2}{\left(2 * \frac{\text{brightness after reversion (\%)}}{100}\right)} - \frac{\left(1 - \frac{\text{initial brightness (\%)}}{100}\right)^2}{\left(2 * \frac{\text{initial brightness (\%)}}{100}\right)} \right) \tag{1}$$

The determination of pentosan content consisted of the distillation of the furfural produced during the hydrolysis of pulp with hydrochloric acid, followed by absorbance analysis at 630 nm wavelength in a Cary 100 UV-Vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA), as described in TAPPI Standard T 223 cm-01.

Hexenuronic acid quantification was based on the acid hydrolysis of pulp with sodium formate (pH 3.5) for 6 h at 105 °C in a nitrogen atmosphere, followed by absorbance analysis of the hydrolysate filtrate at 245 and 480 nm wavelengths in a Cary 100 UV-Vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). The used methodology was adapted from the one described by Vuorinen et al. [27].

The method for intrinsic viscosity determination relates to the complete dissolution of cellulosic chains in a cupriethylenediamine (CED) solution, which does not affect chain length. Viscosity is determined by measuring the flow time of the solution in a viscosimeter under standard conditions and with a very low fibre concentration. Intrinsic viscosity determination was performed according to ISO 5351.

2.9. Papermaking Quality

Papermaking properties were evaluated on isotropic hand sheets of approximately 60 g/m² grammage, produced according to ISO 5269-1, after refining pulps at 2000 revolutions in a PFI refiner (L&W MKV, Hamjern, Norway), following the indications in ISO 5264-2. Evaluated properties included: drainability (ISO 5267-1), bulk (ISO 534), burst index (ISO 2758), tensile index (ISO 1924), tear index (ISO 1974), internal bond strength (TAPPI Standard UM 403), opacity and light scattering coefficient (ISO 2469), Klemm capillary rise (ISO 8787), Gurley air resistance (ISO 5636-5), Bendtsen roughness (ISO 5636-3), and water retention value (adapted from ISO 23714). Handsheet grammage was determined as indicated in ISO 536.

2.10. Chemical Oxygen Demand

Chemical oxygen demand (COD) determination relies on the oxidation of almost all organic compounds, either solubilised or suspended in a liquid sample. Filtrate samples were initially oxidised by digestion with sulfuric acid and potassium dichromate in the presence of silver sulfate and mercury sulfate (in reaction tubes Hach Lange LCK 514, Düsseldorf, Germany) at 170 °C for 15 min in a Hach Lanhat HT 200 S digital thermostate. The amount of dichromate spent in the oxidation of the sample was calculated after absorbance analysis of the formed Cr (III) at 600 nm wavelength in a Hach Lange DR 2800 spectrophotometer. COD measurement was performed according to ISO 15705.

2.11. Adsorbable Organic Halides

The determination of the adsorbable organic halides (AOX) in the bleaching filtrates was based on the adsorption of the organic constituents of a previously acidified sample onto activated charcoal. The charcoal was afterwards incinerated in an oxygen-rich current, and the formed hydrochloric acid was quantified by a microcoulometric titration. Analyses were performed in an Analytik Jena multi X 2500 analyser (Jena, Germany) under the following conditions: oven temperature: 1050 °C; combustion time: 300 s; oxygen flow: 100 mL/min. This methodology was adopted from ISO 9562. AOX content in fully bleached pulp samples was determined through the quantification of AOX removable by pulp washing, as described in SCAN-CM 44:97.

3. Results

3.1. Impact of the X Treatment on Pulp Morphology and Structure

The X treatments of oxygen-delignified eucalypt kraft pulp were performed with 75 and 25 g/t odp of xylanase for 30 and 60 min, respectively. For each X treatment, a control assay was carried out for comparison reasons. No pH adjustment was made, so the pH applied in the X treatments was the original value (pH 9). Temperature (70 °C) and consistency (10%) in these assays were chosen according to conventional mill operating conditions.

Pulp analysis in fibre tester equipment allowed the observation of morphological changes in fibres. The most important parameters evaluated were average length, width, shape, and coarseness of the fibres, as well as fines content. The higher the shape value, the less deformed the fibres. Coarseness refers to fibre wall thickness (a measure of fibre weight per length unit). Fines are defined as fibres with less than 0.2 mm length. The results are presented in Table 1. Average length, width, and shape seem to show a very slight decrease tendency after all treatments (X and control) with respect to the initial pulp. However, no significant differences were observed between enzyme-treated and control pulps. Additionally, both average coarseness and fines content do not appear to suffer any significant change after the treatments. These data, therefore, suggest that xylanase has not caused a visible effect on fibre morphology. Nevertheless, temperature and pH conditions themselves slightly modify average fibre length, width and shape; this is, however, unimportant for this work since these are the conditions that pulp would normally be subjected to at the mill.

Table 1. Average fibre morphology properties after X and control treatments of oxygen-delignified eucalypt kraft pulp.

	Length (mm)	Width (μm)	Shape (%)	Coarseness (μg/m)	Fines Content (%)
Initial pulp	0.767 ± 0.002	18.5 ± 0.0	90.3 ± 0.0	62.2 ± 0.5	22.9 ± 0.1
Control 30 min	0.763 ± 0.002	18.4 ± 0.0	89.4 ± 0.0	60.6 ± 1.1	23.3 ± 0.4
X 30 min, 75 g/t odp	0.761 ± 0.002	18.3 ± 0.0	89.4 ± 0.0	63.9 ± 1.1	23.8 ± 0.7
Control 60 min	0.760 ± 0.002	18.3 ± 0.0	89.5 ± 0.1	63.0 ± 0.6	23.7 ± 0.2
X 60 min, 25 g/t odp	0.766 ± 0.002	18.3 ± 0.0	89.3 ± 0.1	62.4 ± 1.3	23.0 ± 0.8

Despite finding no apparent changes in fibre morphology, enzyme-treated and control pulps revealed some differences, as observed by SEM analysis (Figure 1). No major differences can be identified, although there seems to exist a slight fibrillation in xylanase-treated fibres (Figure 1c,d), identified by some peeling on their surface. External fibre peeling is indeed often reported as an effect of pulp treatment with xylanase and relates to surface xylan degradation [28,29]. Xylan is an important constituent of fibre walls, meaning its degradation and removal may lead to fibre wall weakening and external peeling. These morphological changes then lead to increased permeability to bleaching agents and lignin fragments [30]. However, these alterations are very modest, and therefore, overall fibre morphology remained essentially unaffected, as corroborated by the data in Table 1.

The enzymatic peeling technique was conducted with the intent of identifying modifications produced by the X stage inside the cellulosic layers of the fibres, namely concerning xylan presence, distribution, and integrity. Enzymatic peeling assays were performed following the enzymatic or the control stage and consisted of evaluating the release of reducing sugars throughout the reaction of the pre-treated fibres with another xylanase. Figure 2 shows both the cumulative and fractional (in 30 min intervals) release of reducing sugars throughout the enzymatic peeling treatments. Beginning with the cumulative release analysis (Figure 2a), it is noted that a higher reducing sugar release from control pulps took place. This may be ascribed to some degree of xylan removal occurring in the X stages, which would mean a lower abundance of this hemicellulose in xylanase-treated pulps, which would, in turn, translate into a lower amount of degradation products released.

Regarding the fractional reducing sugars release (Figure 2b), which corresponds to the amount of xylan degradation products formed in 30 min intervals, each release "peak" would indicate the ease of xylanase access to a xylan layer within the fibre cell walls. Thus, the order and intensity of these "peaks" would correspond to the allocation and abundance of the xylan layers reached by the xylanase, respectively. Therefore, the shorter the time for the appearance of the "peak", the closer the allocation of xylan to the fibre surface. At the same time, the larger the "peak", the greater the abundance of xylan in a layer. Based on these basic considerations, the results obtained suggest that the X stages led to the removal of xylan from the outer and inner layers of the cell wall, with the former being predominant. This is in line with the commonly reported preferential elimination of xylan from the fibre surface after hydrolysis with xylanase. The plausible explanation for this observation is the easier xylan access from the surface, where its concentration is commonly high in hardwood kraft pulps [31]. The inner layers of the fibre are less accessible due to the well-known structural organisation of the secondary cell wall. As the enzymatic hydrolysis proceeds, the concentration of xylan in the outer layers decreases while still maintaining considerable amounts in the inner layers. The redistribution of xylan abundance in the inner layers of the cell wall after enzymatic treatment is also noteworthy, especially at longer treatment times. This can presumably be explained by the migration of xylan between the inner layers after its partial depolymerization in the enzymatic treatment. It is therefore suggested that, compared to the control treatments, the X stage not only reduces the amount of xylan remaining in the fibres but also depolymerizes xylan, leading to its redistribution inside the cell wall. Thus, it can also be concluded that enzymatic peeling is an interesting tool to evaluate the mechanisms of enzymatic action inside cellulosic fibres during the X stage.
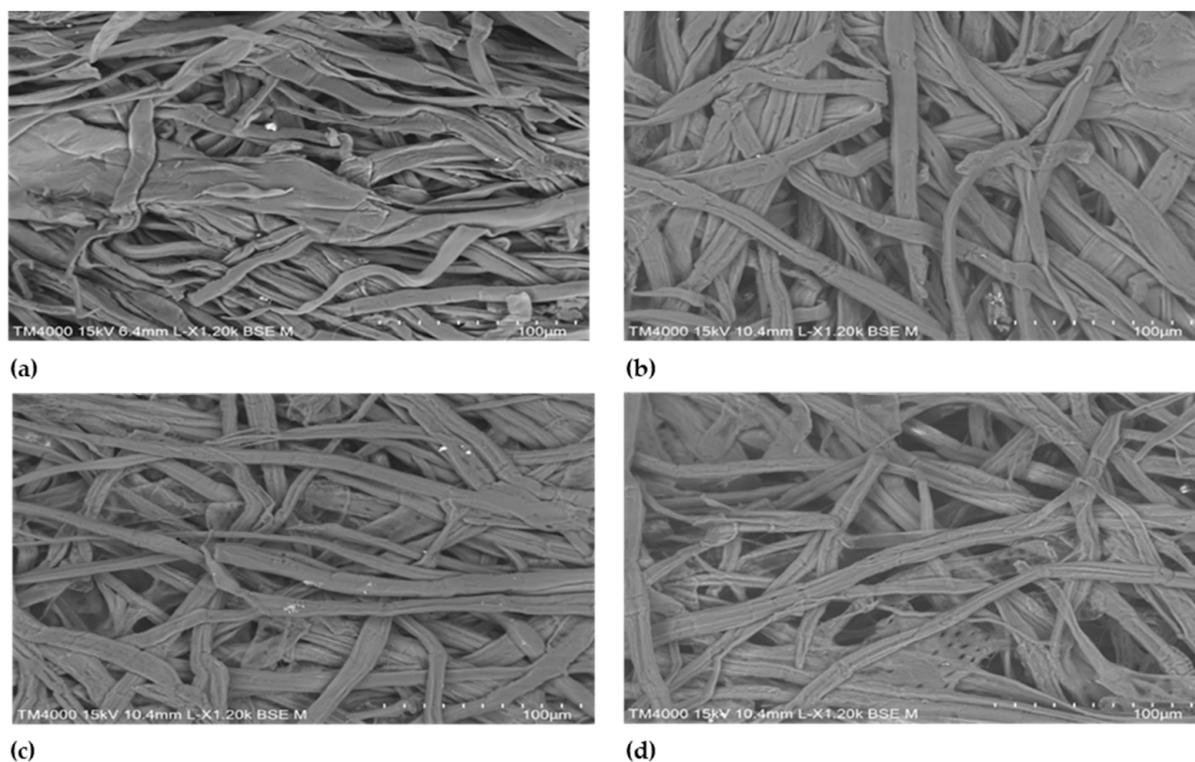

Figure 1. SEM images of pulp fibres following treatment of oxygen-delignified eucalypt kraft pulp with: (**a**) control 30 min; (**b**) control 60 min; (**c**) X 30 min, 75 g/t odp; (**d**) X 60 min, 25 g/t odp.
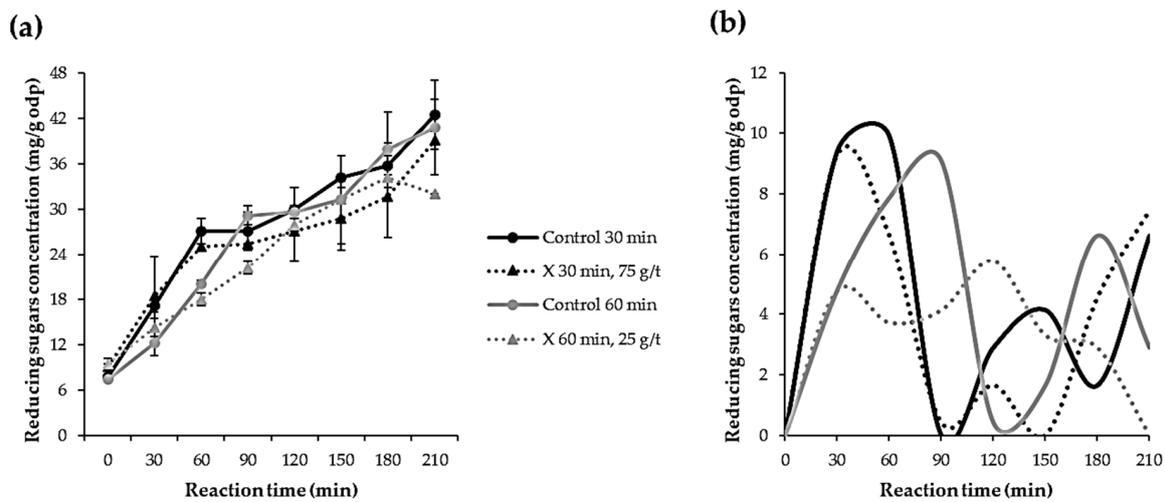
(a)

(b)

Figure 2. Cumulative (**a**) and fractional (**b**) reducing sugar release during the enzymatic peeling treatments.

3.2. Savings of Chemicals in $XD_0E_PD_1D_2$ Bleaching Sequences

Figure 3 shows both the brightness and brightness stability (given by the PC number) obtained for the pulp after each bleaching sequence tested. Since the final pulp brightness obtained after the complete bleaching of enzyme-treated pulps was approximately the same as that obtained for the respective control pulps, it can be concluded that the pre-treatment of oxygen-delignified pulp with an X treatment of either 30 min and 75 g/t odp xylanase dose, or 60 min and 25 g/t odp, allowed the reduction of ClO_2 dose in D_0 by 20%, and NaOH dose in E_P by 10%. Despite the harsh conditions used in this study, from the xylanase activity point of view (pH 9), the results are in agreement with other studies that focused on the use of xylanase preceding the ECF bleaching of eucalypt kraft pulp. For instance, a bacterial xylanase that was used to treat oxygen-delignified *Eucalyptus globulus* kraft pulp (at 45 °C and pH 7, for 2 h) in a XDE_PD bleaching sequence caused a decrease in over 30% chlorine dioxide consumption [32]. In another work, ClO_2 savings in an $OXDE_{OP}D$ bleaching sequence were as high as 40% when commercial xylanases were applied (at 55 °C and pH 8, for 2 h) on oxygen-delignified *Eucalyptus globulus* kraft pulp [33], as well.
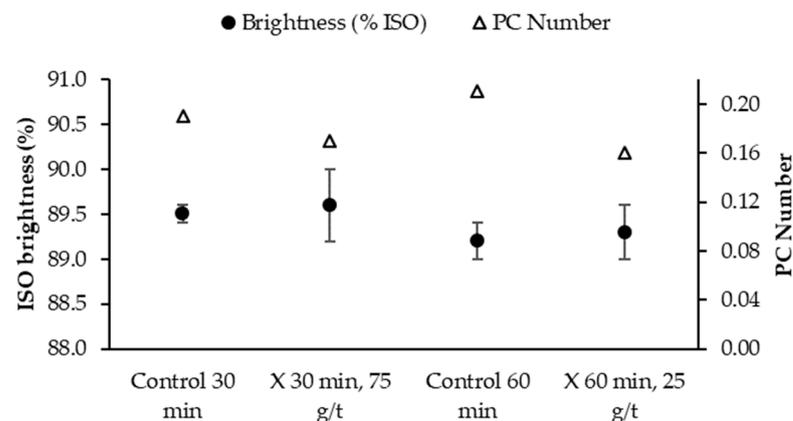

Figure 3. Effect of the bleaching sequences on pulp brightness and brightness stability (PC number).

Additionally, both enzymatic treatments improved brightness stability (lowered PC number), especially the longer one (PC number decreased from 0.21 to 0.16), meaning that X duration might positively affect brightness stability. A reduction in the PC number caused by the xylanase treatment is also commonly reported [34–36].

3.3. Impact of the X Pre-Bleaching Treatment on Pulp Quality

Because the primary target for xylanase is xylan, the removal of this hemicellulose is expected to occur during the X stage. The control bleaching sequence ($cD_0E_PD_1D_2$) reflects the changes in xylan content that normally take place during the conventional industrial sequence. Accordingly, the variations in xylan content found between the xylanase-aided sequence and the control sequence by the end of bleaching should be an indication of the additional removal of those structures. In *Eucalyptus* wood (and pulp), almost the total amount of pentosans is represented by xylans. Being the main hemicellulose in *Eucalyptus*, it is believed that xylan plays an important role in promoting greater refining efficiency, as well as in developing satisfactory papermaking quality. Thus, quantification of the pentosans content in bleached pulp was performed in order to evaluate the extent of xylan removal caused by the treatment with xylanase and thus predict an effect on the papermaking quality of pulp. In addition to pentosans, the quantification of hexenuronic acids (HexA) was carried out in bleached pulp, as well. HexA residues are formed during kraft pulping and result from the elimination of a methoxyl group from 4-*O*-methyl-glucuronic acid residues [37], which are naturally present in *Eucalyptus* xylan as substituents of the main backbone [4]. As previously mentioned, HexA are thought to be chromogenic structures, thereby contributing to brightness loss over time. Therefore, the removal of xylan fragments containing HexA groups by xylanase action is expected to contribute to pulp brightness stability. Pentosans and HexA contents were also determined in the control bleached pulps for comparison purposes.

The results presented in Figure 4 show that both pentosan and HexA contents drop as a result of either enzymatic pre-treatment. The drop in pentosan content is more noticeable after the shorter enzymatic treatment, in which a higher enzyme dose was applied (1.5% drop vs. 0.8% in the longer treatment). This suggests that the xylanase load had a higher impact on xylan degradation than the duration of the treatment. However, the effect on pulp bleachability by both treatments was the same, as well as the chemical savings achieved. This suggests that, above a certain degree of xylan degradation, no additional bleaching gains can be achieved. In other words, limited xylan hydrolysis is enough to boost subsequent chemical bleaching. Similar observations have been previously reported regarding the leachability of lignin from xylanase-treated birch kraft pulp [15]. Thus, xylan degradation caused by the shorter X stage may have been excessive and is, therefore, more likely to affect pulp quality.
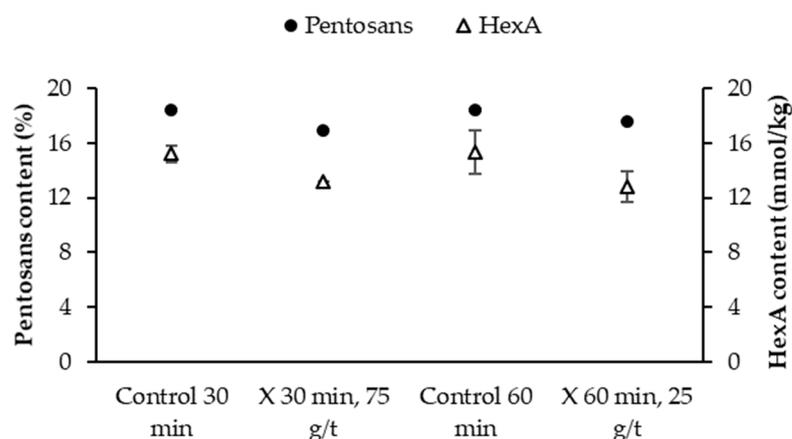

Figure 4. Effect of the bleaching sequences on pentosans and hexenuronic acid (HexA) contents.

HexA content in the original pulp was 58.0 mmol/kg. The HexA content of enzyme-treated pulps was lower than that of untreated pulps as a result of their removal (Figure 4). As aforementioned, some of the xylan fragments that are released from the pulp by xylanase may contain HexA groups, thus explaining their lower amount in enzyme-treated pulps. Unlike pentosans, the HexA fraction shows changes during the chemical bleaching se-

quence because of its oxidation by ClO_2 during the D stages, which leads to HexA removal from the pulp. However, since in the enzyme-assisted bleaching sequences, a lower ClO_2 was applied, there should be less removal of HexA resulting from the action of ClO_2 alone. Thus, the drop in the HexA content of enzyme-treated pulps can only be attributed to the xylanase treatment. Indeed, the removal of HexA from the pulp by xylanase action is often reported [29,34,38]. The data for HexA content is also in accordance with the brightness stability results presented in Figure 3: a lower HexA content corresponds to a lower PC number, which means a higher brightness stability. The pulp subjected to the longer enzymatic pre-treatment is the one that showed the greatest brightness stability improvement, and it is also the one for which the drop in HexA content seems more significant. This consolidates the idea of a direct relationship between HexA content and brightness stability.

The intrinsic viscosity of pulp is commonly used as an indicator of the extent of cellulose degradation through its degree of polymerisation (DP). The lower the drop of cellulose DP in pulp, the higher the pulp viscosity value. Cellulose degradation is an undesired phenomenon because it deteriorates pulp strength properties [16]. In the $D_0E_PD_1D_2$ bleaching sequence, cellulose degradation is quite insignificant. The exception is the E_P stage, when hydrogen peroxide (P) is added, which leads to a small degradation of cellulose, as seen by the slight pulp viscosity drop following that stage (data not shown). Nonetheless, hydrogen peroxide was added in the same amount in all assays. Intrinsic viscosity was determined for all fully bleached pulps, and the respective values are presented in Table 2. The determined intrinsic viscosity values confirm that no significant modification of cellulose occurs as a result of enzyme addition following $OXD_0E_PD_1D_2$. This was quite expected since the commercial enzyme preparation used in this study was almost pure xylanase, with no cellulase activity, meaning no cellulose degradation took place. Indeed, viscosity is often observed not to be affected by the xylanase treatment [29,39–42]. However, a slight tendency towards increasing viscosity was observed in the enzyme-treated pulps. This can be explained by the fact that the removal of the comparatively short xylan chains increases the average DP of the polysaccharides in the pulp, thus increasing the intrinsic viscosity of the pulp.

Table 2. Intrinsic viscosity of bleached pulp after complete bleaching of enzyme-treated and untreated oxygen-delignified eucalypt kraft pulp.

	Intrinsic Viscosity (dm^3/kg)
Control 30 min	931 ± 10
X 30 min, 75 g/t odp	937 ± 0
Control 60 min	919 ± 8
X 60 min, 25 g/t odp	925 ± 13

Since hemicelluloses increase paper strength through the increase in free hydroxyl groups on the surface of the fibres, which strengthen the hydrogen bonds between fibres, their removal by xylanase can lead to decreased paper strength [34]. The papermaking properties of each enzyme-treated and untreated pulp were therefore assessed following a refining treatment. Before hand sheet formation, the drainability of the refined pulp was evaluated. Afterwards, hand sheets were subjected to the analysis of the main quality parameters, which included strength (burst, tensile, tear and internal bond strengths), optical (opacity and light scattering coefficient), and structural (capillary rise, air permeability, roughness and water retention value) properties.

Table 3 shows that the X pre-bleaching stages seem to cause a slight drainability reduction in refined pulp (from 26 to 25 °SR), which may be attributed to xylan removal [43]. Hemicelluloses, namely xylans, are highly hydrophilic and therefore capable of absorbing water efficiently, thus promoting fibre swelling during refining and favouring this operation [44]. Since enzyme-treated pulps possess a lower xylan content, it is likely that less fibre swelling occurred during refining, leading to lower drainability. It had been observed

that pentosan content decreased more significantly during the shorter X treatment, with higher enzymatic load (Figure 4), though this was not translated into a greater decrease in drainability. Decreases in drainability resulting from xylan removal have been previously reported elsewhere [43,45].

Table 3. Papermaking properties of refined bleached pulp after complete bleaching of enzyme-treated and untreated oxygen-delignified eucalypt kraft pulp.

	Control 30 min	X 30 min, 75 g/t	Control 60 min	X 60 min, 25 g/t
Drainability (°SR)	26 ± 0.7	25 ± 0.4	26 ± 0.7	25 ± 0.7
Bulk (cm^3/g)	1.37 ± 0.01	1.34 ± 0.01	1.36 ± 0.01	1.36 ± 0.01
Burst index (kPa.m^2/g)	5.20 ± 0.21	5.15 ± 0.10	5.31 ± 0.14	5.10 ± 0.12
Tensile index (kN.m/kg)	71.8 ± 2.9	73.1 ± 2.5	74.2 ± 2.5	73.6 ± 2.8
Tear index (mN.m^2/g)	8.89 ± 0.49	9.91 ± 0.69	9.47 ± 0.64	8.68 ± 0.39
Internal bond strength (J/m^2)	394 ± 26	405 ± 36	402 ± 34	393 ± 15
Light scattering coefficient (m^2/kg)	28.2 ± 0.4	28.0 ± 0.2	28.3 ± 0.3	28.0 ± 0.3
Opacity (%)	70.2 ± 0.3	69.8 ± 0.2	69.8 ± 0.2	69.6 ± 0.3
Klemm capillary rise (mm)	4.4 ± 0.4	4.3 ± 0.2	4.2 ± 0.2	4.6 ± 0.3
Gurley air resistance (s/100 mL)	9.2 ± 1.2	9.6 ± 1.0	9.8 ± 0.9	8.6 ± 0.9
Bendtsen roughness (mL/min)	91 ± 8	91 ± 6	88 ± 4	92 ± 4
Water retention value (%)	117 ± 5	124 ± 5	123 ± 1	128 ± 5

The bulk was found to decrease slightly when the greater enzymatic load was applied in the enzymatic treatment. This was unexpected because hemicelluloses usually contribute to bulk reduction [46]. However, bulk decrease following the treatment of pulp with xylanase has been previously reported, as well [37]. The tear index showed a different behaviour between both enzymatic treatments, being increased after the higher enzymatic load and shorter treatment duration and decreased following the lower load and longer treatment. Tear strength depends mainly on individual fibre strength, though it is also significantly affected by fibre bonding [47]. The modest tear index decrease observed in pulp treated with the lower xylanase load may be explained by the reduction in xylan content, which has been reported to improve tear strength in *Eucalyptus globulus* bleached kraft pulp [48]. However, the increase in the tear index that was observed in pulp treated with the high xylanase load had also been previously observed [43,45]. The reason for the increase in tear strength has been suggested to be due to the higher external fibrillation often caused by the xylanase treatment [49], which is, to some extent, seen in Figure 1. Although water retention value (WRV) is favoured by fibre swelling and is therefore favoured by a higher hemicellulose content [46], a slight tendency for increased WRV is observable in the enzyme-treated pulps. On the opposite, a decrease in WRV has been reported in other works as a result of xylanase's action [37,45]. The tendency for increased WRV could be explained by the external fibrillation that the xylanase treatment caused on the fibres (Figure 1), which is suggested to cause increased fibre swelling and could thereby overcome the loss of hemicelluloses. The light scattering coefficient was slightly decreased in enzyme-treated pulps, which is in accordance with previous data reported by Sousa et al. [43]. Overall, the papermaking properties of xylanase-treated pulps are similar to those of control pulps, in agreement with results published in previous studies [29,33]. On the other hand, either impairments [34] or improvements [50] in papermaking quality have also been reported by other researchers. Apparently, the origin of the enzymes and the conditions of their application play an important role in the papermaking properties of final bleached pulp.

3.4. Impact of the X Pre-Bleaching Treatment on Bleaching Filtrates

The bleaching effluents were analysed with respect to their COD and AOX levels (Figure 5). COD is an indicator of the total amount of organic species (dissolved or suspended) in the filtrate, while AOX gives the concentration of organochlorinated species that originate from the application of chlorine-based bleaching chemicals, which are considered environmentally hazardous. COD was expected to rise in the filtrates of the X treatments as a result of the release of xylan fragments from the pulp. The COD value of the filtrate/liquor from the initial pulp was 1.9 g/L, which means that the control treatments caused some release of organic material from the pulp (0.6 g/L by the 30 min control and 0.7 by the 60 min control). A great COD increase was indeed observed in the filtrates from both X treatments compared to the control ones, especially from the high-load treatment (1.9 g/L increase). This was expected, considering the higher pentosan content drop (Figure 4). The 60 min X treatment increased the COD by 1.4 g/L. COD increases in the filtrate from the X treatment are generally reported [38,45]. An increase in COD could have a positive effect from the point of view of the mill since a higher COD presumes a higher potential for energy recovery through the concentration and burning of the filtrate in a recovery boiler. On the other hand, it could overload an already overcharged energy recovery system and cause serious operational issues. Increased COD in X treatments is also a clear indicator of pulp yield loss, which means decreased mill productivity. The impact on COD of the filtrates from the following bleaching stages does not seem significant, though it tends to be higher in the D_0 and E_P filtrates from the bleaching sequences containing the enzymatic stage. This might be a result of the increased extractability of lignin degradation products and/or of the lower ClO_2 load applied in the D_0 stage since less material might have been oxidised by this agent in that case.
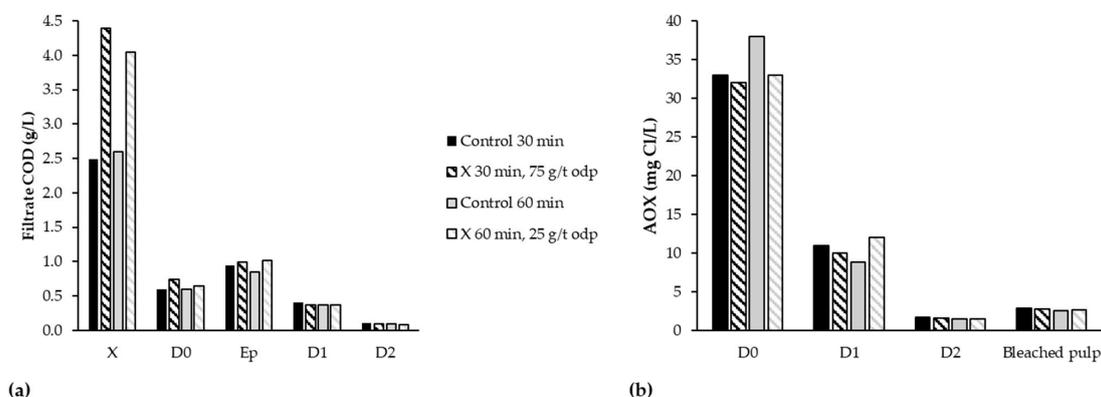

Figure 5. COD (**a**) and AOX (**b**) values observed in the filtrates from bleaching of enzyme-treated and untreated oxygen-delignified eucalypt kraft pulp. AOX was also determined in fully bleached pulps.

The decrease in AOX formation in the D stages has been reported to be proportional to the reduction in ClO_2 load [9,17]. This means that a decrease of around 20% in AOX should be observed in the D_0 filtrate when the enzymatic treatment was applied since the ClO_2 load reduction was 20%. In practice, only an insignificant AOX decrease was detected in the D_0 filtrate after the X treatment, and even the reduction observed for the shorter enzymatic stage stood far from the aforementioned 20% decrease (13%). Moreover, AOX slightly increased in the D_1 filtrate following the 30 min X stage, although no change in ClO_2 load was introduced. AOX was also quantified on fully bleached pulp in order to understand if some of its amounts could have been retained in the pulp, which would explain the lack of visible differences in the filtrates. However, the AOX value in bleached pulp was approximately the same in all pulps. Since AOX formation during a D stage is much dependent on pH [8,11], these observations could be explained by the fact that neither initial nor final pH were controlled in the D stages, which could lead to different AOX formation.

The results suggest that, at a mill in which neither temperature nor pH adjustment before the enzymatic pre-treatment is an option, the choice of enzyme load to be added to the pulp should be made according to the time available to perform such a treatment. Thus, a shorter treatment time would require applying a higher xylanase dose, which would cause a higher pulp yield loss and higher filtrate COD; this could be problematic, both operationally and economically. In case a longer treatment time is possible, the xylanase load could be lowered, meaning the aforementioned issues could be attenuated. Furthermore, brightness stability seems to be favoured by the longer X treatment with a lower enzymatic load. Moreover, the longer treatment allows a decrease in costs directly associated with xylanase. Therefore, the rule of thumb should be to always apply the lowest effective xylanase load.

4. Conclusions

The application of both low-duration high-enzyme dose (30 min, 75 g/t odp) and high-duration low-enzyme dose (60 min, 25 g/t odp) X stages employing a commercial endo-xylanase on oxygen-delignified eucalypt kraft pulp did not cause significant fibre morphology changes, although slight fibrillation was observed in both situations. Nevertheless, cell wall structure was modified by the enzyme action, as xylan molecules were removed from the outer layers, and xylan depolymerisation led to their redistribution inside the cell walls. Although different amounts of xylan were released from pulp, both X treatments revealed the same effect on pulp bleachability in terms of chemical savings. This suggests that xylan degradation and removal from pulp increased the efficiency of chemical bleaching, thereby allowing the reduction of chemical consumption. At the same time, above a certain degree of xylan degradation, no further gain in pulp bleachability is obtained. Thus, the shorter enzymatic treatment required a higher xylanase dose to achieve the same chemical savings as the longer treatment and caused the greatest yield loss, as seen by the pentosans content values. Moreover, the longer X treatment allowed better brightness stability as a result of a greater HexA removal. Therefore, regarding brightness stability, treatment duration seems important. Despite considerable xylan removal (especially by the high enzymatic load treatment), neither X treatment significantly affected pulp strength properties, which shows the potential of xylanase biobleaching technology to reduce the use of chemicals in ECF bleaching without decreasing pulp quality. Despite the fact that AOX reduction in the D_0 filtrate was lower than expected, the xylanase pre-treatment of oxygen-delignified eucalypt kraft pulp was still able to decrease the environmental impact of bleaching.

The conclusions from this study could be of special interest from the point of view of the pulp mill since it was conducted on unwashed pulp, i.e., the xylanase stage was applied on pulp as collected at the mill, without any washing step or additives application in between, opposite to what happens in most published works. This means that the effect of organic carryover was taken under consideration, which makes all conclusions more realistic from an industrial point of view.

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