Effect of commercial xylanases applied at extreme conditions in a eucalyptus pulp mill

URSULA FILLAT, M. BLANCA RONCERO, ALEXANDRE BASSA, AND VERA MARIA SACÓN

ABSTRACT: In this study, we examined the effect of treating eucalyptus pulp with various commercial xylanases to identify the most effective enzyme for use under the industrial bleaching conditions used at the Jacareí mill of the Brazilian firm Fibria, which include a high pH and temperature. Based on the results, the use of two of the nine enzymes studied reduced the kappa number by 1.5 units, increased brightness by 2.5% ISO, and decreased hexenuronic acids (HexA) content by more than 10 µmol/g relative to a control treatment in the absence of enzyme. The most marked changes in brightness were observed on application of an oxidative D stage to enzyme-treated pulp samples. Finally, the chemical oxygen demand (COD), total organic carbon (TOC), color, and turbidity of the effluents obtained at the end of the processes involving the enzymes were all higher than in the control process.

Application: The use of xylanases can raise productivity in mills with limited ability to produce chlorine dioxide, can potentially reduce bleaching reagents cost, and can increase the flexibility of the bleaching sequence.

Current interest in the use of biotechnology principles in pulp and paper production processes has arisen from the high potential of biological treatments for meeting environmental restrictions. The use of enzymes – laccases [1-4] and xylanases [5-10] – in the bleaching section of pulping processes has aroused much interest. Xylanases have facilitated the industrial implementation of biobleaching treatments [11-13], saved bleaching reagents, and reduced the presence of halogen-containing compounds in the effluents [14-18].

Various xylanases act differently on xylan chains [8,18-21] and differ in their ability to reduce the kappa number and chromophoric groups of pulp; however, the exact mechanisms of action of xylanases are still poorly understood. Factors influencing the efficiency of a xylanase treatment are related to the characteristics of the particular enzyme (type, activity, optimum pH and temperature, and penetration ability), the properties of the pulp (lignin content, bleaching sequence used, substrate composition, and accessibility), and miscellaneous variables (pulp consistency, salt concentrations, degree of enzyme dispersion, and reaction time) [19,22]. Kraft cooking and oxygen delignification of pulp occur at an alkaline pH and a high temperature. This has led enzyme producers to develop formulations capable of withstanding these conditions to facilitate their industrial use [22,23].

In this study, preliminary tests were carried out to identify the most efficient commercial enzyme at the temperature used in the storage tower of the B fiber line in Fibria's Jacareí unit (located in the São Paulo state of Brazil) and a pH closer to that in the tower. This previously was deemed the most suitable point in the bleaching sequence for using the enzyme. Biobleaching tests with xylanases were used to characterize the pulp obtained after the enzyme (X) stage, and the XD and XDE sequences (where D denotes bleaching with chlorine dioxide and E alkaline extraction), in terms of kappa number, brightness, viscosity, and hexenuronic acids (HexA) content. The pH, chemical oxygen demand (COD), color, and turbidity of the effluents from the X stage also were calculated.

MATERIALS AND METHODS

Raw material

The raw material used was a mixture of pulp from *Eucalyptus saligna* and *Eucalyptus grandis* supplied by Fibria. Samples were collected at a preset point in the B bleaching stage, where the pulp had been delignified with oxygen and allowed to stand in the storage tower before acid washing. Before treatment with the xylanases, the pulp was washed with distilled water at 10% consistency. **Table I** shows the properties of the pulp and its filtrate.

Commercial enzymes

For this study, we used nine commercial enzymes from four U.S. companies. EDT (Enzymatic Deinking Technologies; Nor-

Initial Pulp	
Kappa number	11.5
Brightness (%ISO)	55.3
Viscosity (mL/g)	1080
HexA (µmol/g)	61± 2
Pulp Filtrate	
COD ($kgO_2 \cdot t^{-1}$)	30.8
pH	11.3

I. Properties of pulp and filtrate.

cross, GA, USA) supplied five of the enzymes, which we coded enzA, enzB, enzC, enzD, and enzE. Verenium (San Diego, CA, USA) supplied enzL; Buckman (Memphis, TN, USA) supplied enzB11 and enzB14; and Genencor (DuPont Genencor Sciences; Palo Alto, CA) supplied enzG. For commercial confidentiality reasons, no further information about their characteristics can be disclosed.

Enzyme treatment (X stage)

Xylanase treatments were performed on 70 g of pulp. The xylanase dose used was 0.3 kg/a.d. ton, equivalent to 0.03% o.d. pulp. Tests were performed by placing the pulp samples at pH 9.5 in polyethylene bags that were immersed in a thermostatically temperature controlled water bath at 80ºC for 2 h at 10% consistency. After the treatment was completed, the bags were cooled, the pulp filtered, the liquor collected for subsequent characterization, and the pulp washed with distilled water and centrifuged. The control treatment was performed under the same conditions, but without xylanase.

Chlorine dioxide treatment (D stage)

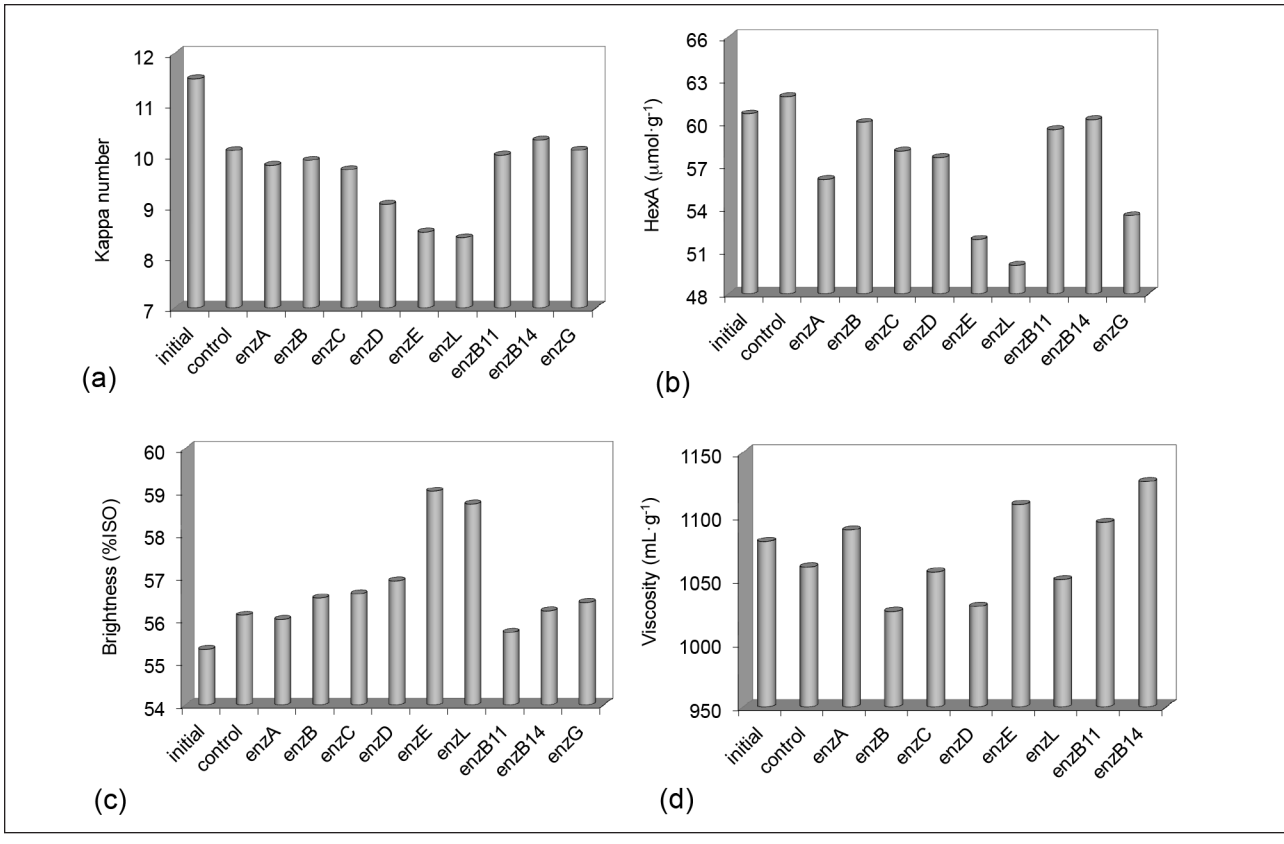
We treated 20-g samples of pulp at 10% consistency at pH 3.8, using a chlorine dioxide dose of 19.8 kg active Cl_2/o.d. ton. Samples were placed in polyethylene bags that were immersed in a thermostatically temperature controlled bath at 60ºC for 90 min. We then filtered the pulp and saved the liquor for subsequent determination of residual chlorine dioxide and pH. Finally, the pulp was washed with distilled water and centrifuged.

Alkaline extraction (E stage)

Alkaline extraction was performed on 10 g of pulp at 10% consistency placed in polyethylene bags that were immersed in a thermostatically temperature controlled bath at 75ºC for 1 h. The NaOH dose was 1.2% o.d. pulp and the treatment pH always higher than 11.5. After the treatment was completed, the pulp was filtered, and the liquor collected and stored frozen for subsequent characterization. Finally, the pulp was washed with distilled water and centrifuged.

Pulp and effluent properties

Pulp samples obtained after the X stage and the XDE sequence were characterized in terms of brightness, kappa number, and viscosity in accordance with their respective standards (ISO 2470:1999 "Paper, board and pulps - Measurement of diffuse blue reflectance factor [ISO brightness]," ISO 302:2004 "Pulps - Determination of Kappa number," SCAN-CM 15:88 "Viscosity in cupri-ethylenediamin [CED] solution") and for HexA (TAPPI T 282 pm-07 "Hexeneuronic acid content of chemical pulp"). We analyzed effluents from the X stage for pH, COD, color, total organic carbon (TOC), and turbidity in accordance with their respective standards (SMEWW 5220 D "Chemical



1. (a) Kappa number, (b) hexenuronic acids (HexA) content, (c) brightness, and (d) viscosity of the pulp samples after the X stage.

oxygen demand, closed reflux, colorimetric method," CETESB NT-07/L5.117 "Determinação de cor em águas," SMEWW 5310 "Total organic carbon," and ASTM D6698 "Standard test method for on-line measurement of turbidity below 5 ntu in water"). Effluents from D also were analyzed for pH and residual chlorine dioxide.

RESULTS AND DISCUSSION

Application point

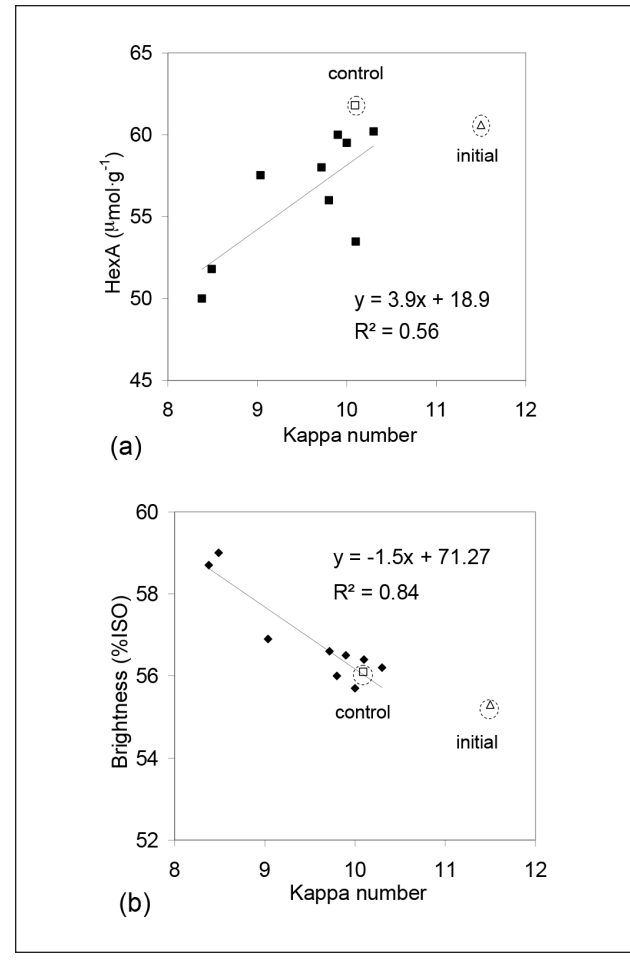
The Jacareí mill has been using an OAZDP bleaching sequence. For the experiments, the first decision was to determine the most suitable point in the industrial bleaching sequence for insertion of an X stage. Xylanase treatment usually occurs immediately before or after oxygen delignification [24], usually in the raw pulp storage tower [23,25]. In the B bleaching line of the Jacareí unit of Fibria, pulp from the O (oxygen predelignification) stage is subject to acid washing (an A stage) before ozonation (a Z stage). A prebleached pulp storage tower is used between the oxygen predelignification and acid washing towers that was identified as the optimum point for implementation of a xylanase treatment. The conditions in the storage tank were 10% pulp consistency, pH >9.5, and 80ºC.

Effect of X stage on pulp and effluent properties

Pulp properties

Figure 1 shows the kappa number, HexA content, and brightness obtained with the enzymes compared with initial and control samples. The control treatment (heating at 80ºC for 2 h) reduced the kappa number by 1.5 units. The results provided by the enzymes enzA, enzB, enzC, enzB11, enzB14, and enzG differed little from those of the control treatment. On the other hand, enzymes enzD, enzE, and enzL reduced the kappa number by an additional 1 unit to 1.7 units (to 8.5) after the X stage (Fig. 1a). A similar reduction was accomplished in studies with different xylanases [16,26,27], albeit at lower pH and temperature levels.

Some 4-O-methylglucuronic acids present in xylans are converted into the corresponding unsaturated HexA through elimination of methanol during alkaline cooking of wood. HexA increase the kappa number, consume bleaching reagents, retain metal ions, favor pulp yellowing, and facilitate the formation of oxalic acid [27]. Although all enzymes reduced the HexA content somewhat, only enzE and enzL decreased it to about 50 µmol/g (about 17%) (Fig. 1b). As found in previous studies [9,28], enzyme treatments reduce HexA contents and consumption of bleaching reagents in subsequent stages as a result. Some studies have revealed a relationship of the reductions in HexA contents and kappa number; thus, a reduction of 10 µmol/g in HexA seemingly corresponds to one of 0.86-1.05 units in kappa number [29]. **Figure 2** shows the kappa number related to HexA content. If the kappa number decreases, the HexA content also decreases. However, other parameters need to be considered, because the correlation is not so good. In fact, other authors [30-32] have pointed at

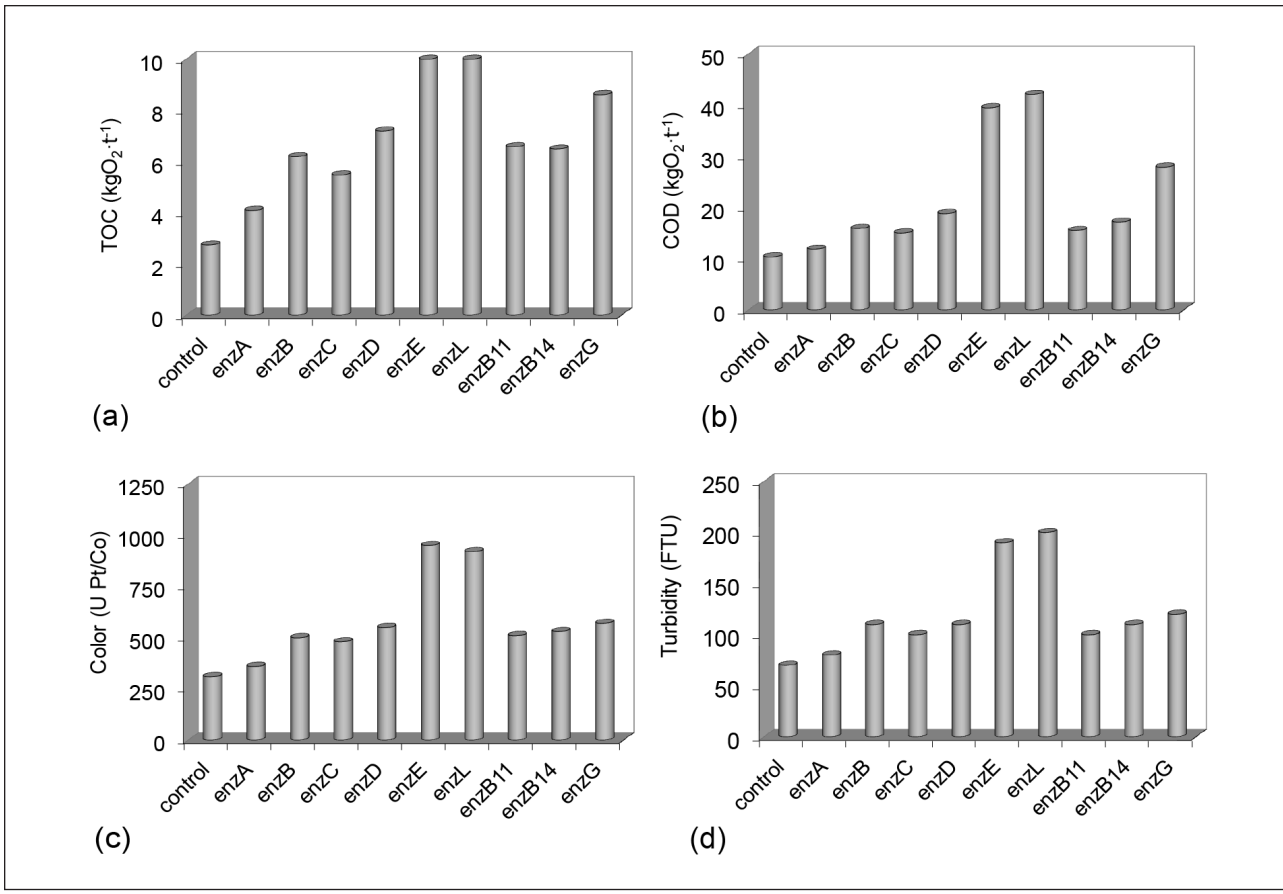


2. Relationship of (a) HexA content and (b) pulp brightness to the kappa number.

other compounds, such as carbohydrate degradation products or even lignin products removed during the enzymatic treatment. Therefore, some yield loss is expected [33].

The control treatment raised pulp brightness by 1% ISO (to 56.1% ISO) with respect to the initial pulp (Fig. 1c). Using enzymes enzA, enzB, enzC, enzD, enzB11, enzB14, or enzG in the X stage resulted in no substantial increase in brightness relative to the control treatment. On the other hand, using enzE or enzL raised brightness by 2.5% ISO (to 59% ISO) relative to the control pulp (Fig. 1c). The brightness increase was correlated with the reduction in kappa number (**Fig. 3**). The viscosity was about 1050 mL·g-1 and the xylanases had virtually no effect on it (Fig. 1d).

Xylanase does not react directly with lignin in pulp. Xylans are hemicellulose components falling between lignin and the cellulose matrix. Because hemicelluloses can bind some lignin [34], if a xylanase degrades xylans, it can cause lignin-cellulose complexes to dissociate, reducing the lignin content. Kraft cooking causes xylose and xylan macromolecules to form colored species containing double bonds; xylanases also can remove these chromophoric compounds [21,30]. Previous studies related the removal of chromophores, hydropho-



3. (a) TOC, (b) COD, (c) color, and (d) turbidity of the effluents from the X stage. Color and turbidity directly measured in the pulp filtrate at 10% o.d. pulp consistency.

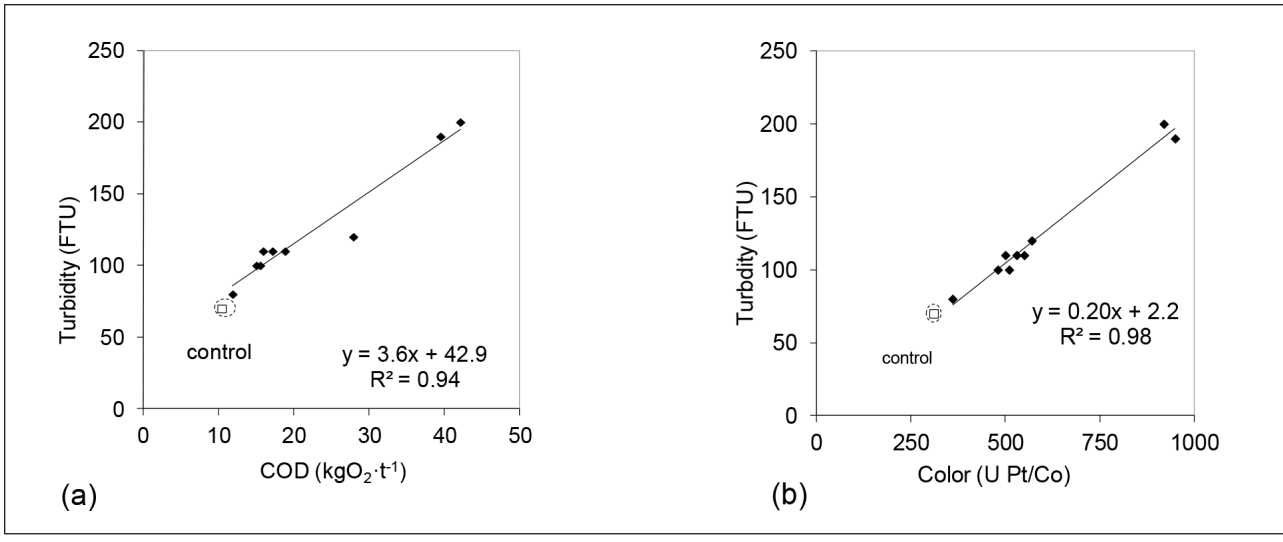
bic compounds, and reduced sugars present in the effluents from an X stage to the reduction in kappa number [26,35,36].

Effluent properties

Figure 3 shows that the COD, TOC, color, and turbidity of the final filtrate in X stage all were higher than in the control treatment. Xylanases enzA, enzB, enzC, enzD, enzB11, and enzB14 produced a slight increase in COD (from 1.4 kg O_2/ton to 8.4 kg O_2/ton), TOC (from 1.4 kg C/ton to 4.5 kg C/ton), color (from 360 U PtCo to 550 U PtCo), and turbidity (from 80 FTU to 110 FTU) in the X stage effluents. EnzG caused a more marked increase in COD (17.5 kg O_2/ton) and TOC (5.9 kg C/ton). However, the strongest effects were those of enzE and enzL, which increased COD and TOC in the filtrates by more than 29 kg O_2/ton and 13 kg C/ton, respectively, and led to color and turbidity levels above 950 U PtCo and 190 FTU, respectively. Previous studies also have reported a COD increase resulting from xylanase treatment [15,18].

The enzymes enzE and enzL, which were those providing the smallest kappa numbers and highest brightness (Figs. 1a and 1c), were also those resulting in the highest COD, TOC, color, and turbidity values for the effluents (Fig. 3). The increase in COD, TOC, color, and turbidity fit straight lines with coefficients of determination from 0.93 to 0.98 (**Fig. 4**). Some authors [15] have ascribed the increased COD of the effluents from an X stage to the hydrolysis of xylans (i.e., the presence of dissolved sugars), which reduces pulp yield at an early bleaching stage. Other authors [26,36] have found increased concentrations of hydrophobic and phenolic compounds in the effluents. We found that the enzymes enzE, enzL, and enzG lowered the pH of the initial filtrate, 9.5, by one unit (to about 8.5); however, the other enzymes caused no substantial changes in pH. The decreased pH obtained with these three enzymes might have resulted from their reducing the acid content of the pulp (HexA groups) through hydrolysis of hemicelluloses and dissolution in the liquor of the acids thus formed.

Although the X stage effluents increased COD, TOC, color, and turbidity values, previous studies revealed sequences involving xylanases that reduced the overall effect of COD [37] and resulted in better biodegradability of the effluents [38,39], partly because they reduced the need for bleaching agents in subsequent stages. In addition, the effluent from the X stage contains no chlorinated compounds, so it can be recycled together with the effluents from the oxygen delignification stage and sent to the recovery section [15], or, by virtue of its increased biodegradability, it can be treated at the effluent processing plant.



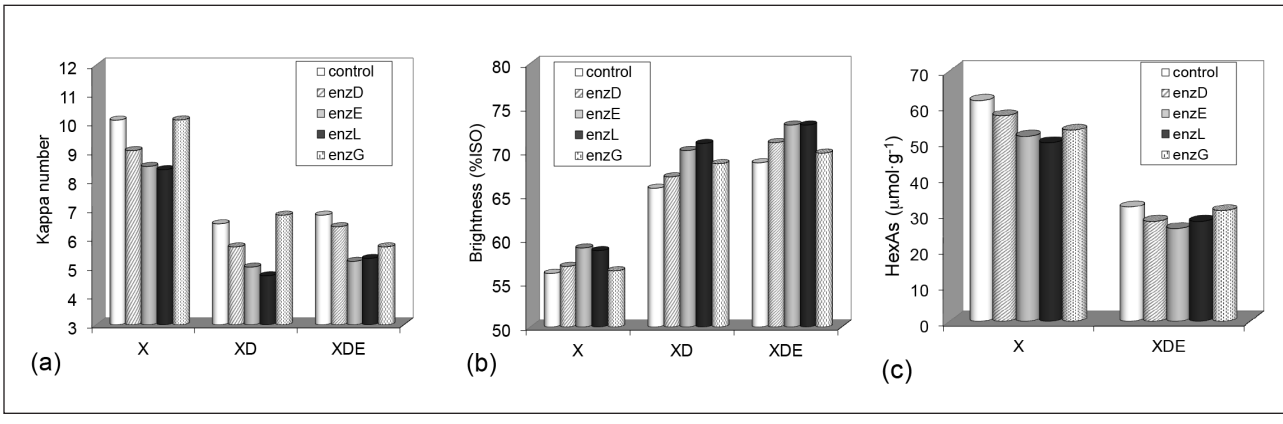
4. Relationship between effluent properties. Color and turbidity directly measured in the pulp filtrate at 10% o.d. pulp consistency.

Final bleaching: XD and XDE sequences

Enzymes enzE and enzL exhibited the strongest influence on pulp properties after the X stage. Samples were subjected to D and E bleaching stages, and the resulting pulp characterized (**Fig. 5**). Using a D stage after the X stage reduced the kappa number by 3.5 units (to 6.5) in the control treatment. Alkaline extraction (an E stage) after D resulted in no substantial change in kappa number with respect to D. The enzD and enzG treatments had virtually no effect on the kappa number (Fig. 5a); by contrast, the enzE and enzL treatments reduced it by more than 1.5 units with respect to the control treatment. This difference is similar to that between the control treatment and both enzyme treatments after the X stage (Fig. 1a).

DE sequences reduced the HexA content of the pulp by about 30 µmol/g as a result of the chlorine dioxide stage instead of alkaline extraction stage. Some authors [40] suggest that hypochlorous acid (HOCl), an oxychlorine intermediate formed during ClO_2 bleaching, is the primary agent involved in HexA. They found that some of the initial ClO_2 reactions with lignin generate HOCl, which seems to react faster with HexA than with lignin. After xylanase treatments, the HexA content was lower than the control in all cases (Fig. 5c). By effect of the differences in HexA content after X, the control and enzD-treated pulp samples required more chlorine dioxide to remove HexA than did the enzE and enzL treated samples; this had an obvious effect on pulp properties after the XD and XDE sequences.

The D stage raised pulp brightness by nearly 10% ISO (to 65.8% ISO) (**Fig. 5b**) in the control treatment. The enzD and enzG treatments increased brightness after the XD sequence by 1.3% and 2.8% ISO, respectively. The enzE and enzL treatments resulted in the greatest differences, however, raising brightness by 4.3% and 5.1% ISO, respectively (to 70.1% and 70.9% ISO, respectively). These differences are greater than those between the control and enzyme treatments after the X stage (Fig. 1c). Therefore, the X stage alters the pulp in such a way that it raises its brightness after the subsequent oxidative D stage. The E stage in the DE and XDE sequences increased pulp brightness by 1.2% to 1.3% ISO with respect to D. The control treatment raised brightness by 2.9% ISO (to 68.7% ISO). The enzD and enzG treatments increased brightness after the XDE sequence by 2% and 1% ISO, respectively.



5. (a) Kappa number, (b) brightness, and (c) HexA content of the pulp after the X stage, and the XD and XDE sequences.

Finally, enzE and enzL afforded the highest brightness levels (70.1% and 70.9% ISO, respectively, which were the result of increases of 4.3% and 5.1% ISO, respectively). These differences are similar to those between the control and the enzE and enzL treatments after the X stage. Therefore, the E stage fails to expand the brightness difference between the control treatment and these two enzyme treatments.

Some authors [22,27] have suggested that xylanases open up pores in fiber cell walls; this alters fiber crystallinity, thereby facilitating access by bleaching reagents and decreasing diffusional resistance in degraded lignin fragments. This effect, together with the differences in kappa number and HexA content after the X stage, helps the oxidative D stage expand the brightness differences between the control and enzyme treatments.

Pulp viscosity after the XDE sequence was about 950 mg/L; therefore, the D and E stages reduced viscosity by about 100 mg/L. The xylanase treatments resulted in no substantial difference in pulp viscosity after XDE with respect to the control treatment. The initial pH in the D stage ranged from 3.6 to 4.2, depending on the treatment used. The final pH was always lower: 3.0 to 3.4. The other enzymes produced no substantial changes in pH.

CONCLUSIONS

Experimental evidence suggests that, in terms of pulp properties, xylanases enzE and enzL were the best enzymes tested in Fibria pulp at high pH and temperature. Their use reduced the kappa number after the X and the HexA content with respect to the control treatment in the absence of enzymes. The reduction in kappa number was correlated with that in HexA content through hydrolysis of xylans.

The test with enzG did not alter significantly the kappa number nor pulp brightness, but did decrease HexA content. This enzyme is more selective in reducing HexA than were enzE and enzL; not all xylanases have the same behavior.

Although the X stage by itself increased pulp brightness, the greatest brightness changes were observed after the oxidative D stage, in the enzyme-treated pulp samples. The xylanase treatments improved the properties of pulp without reducing its viscosity.

The COD, TOC, color, and turbidity values of the final filtrate were higher in all enzyme treatments than in the control treatment. The most marked increase was obtained with enzE and enzL.

These results are promising for industrial application of enzymes in settings such as the Jacareí unit of Fibria. Introducing enzymes in the current process will require no special alteration or investment in new equipment. By virtue of the potential savings in bleaching reagents, the use of xylanases can raise productivity in plants with a limited ability to produce chlorine dioxide, which is the case with the Jacareí unit, and it can increase the flexibility of the bleaching sequence. **TJ**

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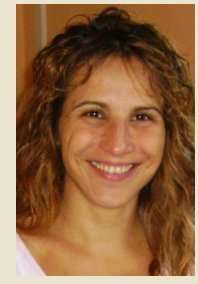
ABOUT THE AUTHORS

This study was a collaboration between an industrial firm, which has an interest in studying xylanase application in the mill, and a research center. The study differs from previous research in that conditions where enzymes were applied (high temperature and pH) were not the usual conditions used in this kind of study.

The most difficult aspect of this research was to select the best treatment conditions. To do that, we conducted some preliminary experiments. We found good results with some of the tested enzymes at extreme conditions.

The findings in this study might help mills interested in industrial xylanase application. The next step in our research is to optimize the enzymatic treatment conditions.

Fillat



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