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# Effect of commercial cellulases and refining on kraft pulp properties: Correlations between treatment impacts and enzymatic activity components

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ABSTRACT

The importance of enzymes as biotechnological catalysts for paper industry is now recognized. In this study, five cellulase formulations were used for fibre modification. The number of PFI revolutions decreased by about 50% while achieving the same freeness value (decrease in CSF by 200 mL) with the enzymatic pretreatment. The physical properties of handsheets were modified after enzymatic pretreatment followed by PFI refining. A slight decrease in tear strength was observed with enzymes C1 and C4 at pH 7 while the most decrease in tear was observed after C2, C3, C5 treatments. C1 and C4 which had xylanase activity improved paper properties, while other enzymes had a negative impact. Therefore, the intricate balance between cellulolytic and hemicellulolytic activity is the key to optimizing biorefining and paper properties. It was also observed that C1 impact was pH dependent, which supports the importance of pH in developing an enzymatic strategy for refining energy reduction.



## 1. Introduction

Paper production is an energy-intensive process and as a consequence improving energy efficiency is a matter of high priority for the paper industry (Znidarsic-Plazl, Rutar, & Ravnjak, 2009). Physical properties of paper are another priority, and in papermaking, the high energy consuming process of refining (or beating) is normally used to improve bonding and develop optimum strength properties of the final paper products (Banavath, Bhardwaj, & Ray, 2011; Chen, Wan, Zhang, Ma, & Wang, 2012). Pulp refining, defined as the repeated passage of pulp through zones of compression and shearing, is carried out to a greater or lesser degree in all paper and board mills. This mechanical treatment of cellulosic fibres results in structural changes including fibre shortening and internal/external fibrillation, to name a few (Sain, Fortier, & Lampron, 2002). The primary fines produced by delaminating the outer layers of fibre during refining tend to be slender and flexible, which improves bonding property in a sheet of paper (Sigoillot et al., 2001; Torres, Negro, Fuente, & Blanco, 2012).

Today, because of ever-growing energy costs and environmental regulations, paper producers need to reduce energy consumption associated with refining, which represents 15–18% of the total electrical energy required to produce paper (Bajpai, Mishra, Mishra, Kumar, & Bajpai, 2006). Different solutions were developed, such as adapting plate pattern to modify fibre treatment or varying pulp consistency. Other methods consisted in increasing intensity through refiner or redesigning refining strategy (Lecourt, Sigoillot, & Petit-Conil, 2010; Seth, 1999). Nevertheless, these methods require important investments. Thus, simpler methods with lower implementation costs are preferred. Ideally, they should request limited changes in process or plant configuration, and interact minimally with wet end chemistry. Enzymes-assisted refining (biorefining) comply with such restrictions, and was shown to offer an environmentally friendly means for improving the strength properties of pulp (Pelletier et al., 2013; Spiridon, Duarte, & Belgacem, 2001). The pre-treatment of pulp with commercial cellulase prior to refining can achieve more fibrillation of fibres, which enhances the inter-fibre bonding and increases the tensile strength of softwood kraft pulp (Liu et al., 2012). Some studies have reported that by pre-treating pulp with cellulase enzymes can result in a reduction in refining energy for the same target drainage index

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(García et al., 2002; Kim, Jo, & Lee, 2006). It should be noted, however, that the cellulase treatment can cause loss of fibre intrinsic strength and usually affects the tear strength in a negative way (Gil, Gil, Amaral, Costa, & Duarte, 2009).

Published results vary in response to the various enzymatic activities present in the cellulase preparations (contaminants such as other carbohydrate-modifying enzymes are often present in commercial preparations) (Garcia-Ubasart, Torres, Vila, Pastor, & Vidal, 2013; Oksanen, Pere, Paavilainen, Buchert, & Viikari, 2000). Bajpai et al. (2006) conducted laboratory and process-scale studies with mixtures of cellulase and hemicellulase enzymes for reducing the refining energy requirement of different types of pulps which included virgin pulp and recycled pulp fibres. Gil et al. (2009) used cellulases and beta-glucanases to treat bleached *Eucalyptus globulus* kraft pulp. They showed that such enzymatic treatment could improve pulp drainability, by up to 80%, using the same level of refining energy. In another study, it was shown that the tear strength of paper can decrease after enzymatic treatment depending on the mixture used (Lee, Ibrahim, & Omar, 2013). Further, the exact type of cellulase present (endoglucanase variants) and other factors such as pH (which can affect pulp polymer properties, Taipale, Österberg, Nykänen, Ruokolainen, & Laine, 2010), can affect biorefining (Oksanen, Pere, Bouchert, & Viikari, 1997). To our knowledge, the impact of pH on enzymes themselves has not been reported so far for biorefining.

The aim of this study was to decrease refining energy requirements of kraft pulp with minimal impact on drainability while promoting paper strength properties, using commercial cellulase treatments followed by laboratory PFI refining. For this, five different commercial cellulase formulations were used for enzymatic treatment prior to mechanical refining. The impact of enzymatic treatment and refining on fibre morphology and pulp drainability in kraft pulp were studied. Subsequently, the physical properties of conventional handsheets prepared from enzyme-treated and untreated pulps were determined. Analysis of the various enzymatic activities detected in the commercial cellulase formulations allows shedding light on the importance of non-cellulolytic enzyme components in determining pulp and paper properties.

## 2. Materials and methods

### 2.1. Pulp characterization

The kraft pulp was provided by Tolko paper plant in Manitoba (Canada). The kraft pulping was performed using softwood. The chemical compositions of the pulp were measured according to Van Soest method (Van Soest, Robertson, & Lewis, 1991). Prior to experiments, the pulp was filtered and then air dried to about 6% moisture before enzymatic treatment.

### 2.2. Enzymes and chemicals

Carboxymethyl cellulose (CMC), birchwood xylan and *p*-nitrophenyl β-glucopyranoside (pNPG) were purchased from Sigma. Five different commercial cellulase preparations named C1, C2, C3, C4 and C5, used in this study were produced by various suppliers.

### 2.3. Detection and quantification of enzymatic activities

Cellulase (CMCase), xylanase, and β-glucosidase (i.e. cellobiose) activities were tested using CMC, xylan from birch wood, and pNPG as substrates, respectively. The activities of cellulase and xylanase were assayed quantitatively using the dinitrosalicylic acid method (DNS) which measures the reducing sugars generated by enzymatic hydrolysis (absorption readings at 540 nm) (Miller, 1959). One international enzyme unit (IU) was defined as the amount of enzyme necessary to release 1 μmol of reducing sugar per min under our assay conditions. β-Glucosidase activity was determined by measuring the amount of *p*-nitrophenol released from pNPG used as colorimetric substrate by absorbance measurements at 540 nm (Dashtban, Maki, Leung, Mao, & Qin, 2010). All enzymatic activities were measured at 50 °C and pH 7 unless specified otherwise.

### 2.4. Enzymatic treatments of pulp

The dried pulp was presoaked overnight into the corresponding white water (provided by the pulp supplier) at 5% consistency and disintegrated for 5 min (disintegrator from Labtech Instruments) before enzymatic treatment. Treatments of kraft pulp with the various commercial cellulase preparations were carried out in a 4-L Erlenmeyer flask at 2% pulp consistency, at pH 7 and 50 °C for 1 h with continuous mechanical agitation (unless specified otherwise). Solution pH was adjusted to 7 for enzymatic treatment using diluted $H_2SO_4$. Enzyme solution was added to a final concentration of 0.2 g enzymes per kg of oven dry pulp. The enzymatic digestions were stopped by incubating the pulp 15 min on ice followed by vacuum filtration. The control pulps were treated under similar conditions except for the addition of the enzyme preparations which was omitted. For each experiment, two trials were carried out, and ten handsheets were prepared and analyzed for each trial. Filtrates was boiled for 15 min and kept frozen prior to sugar analysis.

### 2.5. Sugar analysis of filtrates after enzymatic treatment

Carbohydrate (mono- and disaccharides) content in the filtrate after enzymatic treatment was determined by ion chromatography. An ICS-5000 ion chromatography system (Dionex, Sunny-vale, CA, USA) was used in this study. The detection was carried out by an electrochemical detection cell using a combined pH-Ag/AgCl reference electrode. Analyses of standards and filtrates samples were conducted at 40 °C with isocratic elution (NaOH 1 mM at 1 mL/min) using a Dionex PA100 (50 mm × 4 mm) guard and analytical Dionex CarboPac SA10 (250 mm × 4 mm) column. For all analyses, 10 μL was injected. The analysis was performed at 40 °C with the flow rate set at 1.0 mL/min. The Dionex Chromeleon software was used for data processing.

### 2.6. Pulp refining and freeness measurement

The refining of untreated and enzyme-treated pulp fibres (adjusted to 10% consistency) was conducted following the Tappi standard method T 248 sp-00 using a laboratory PFI mill at different number of revolutions. The Canadian Standard Freeness (CSF) was measured according to Tappi method T 227 om-99.

### 2.7. Fibre quality analysis

Fibre characterization of the pulp samples (0.0001% consistency) was carried out with a HiRes LDA02-090 Fibre Quality Analyzer (FQA) (Optest Equipment Inc, Canada). The distributions of fibre fines and fibre mean length, as well as mean kink and curl indexes, were calculated on the basis of 5000 fibres. Fibre fines were defined as the fibre portion with fibre length between 70 and 200 μm and the fibre length was determined over a 70–10,000 μm range.

### 2.8. Physical properties measurement

Handsheets with basis weight of $60 \pm 2\,g\,m^{-2}$ were prepared using a standard handsheet former according to Tappi method T 205 sp-02. Prior to testing, the handsheets were conditioned for 24 h at 23 °C and 50% of relative humidity according to Tappi standard method T 402 sp-03. The physical strength properties such as tensile, burst, tear, and internal bond strength were determined according to Tappi standard test method T 494 om-01, T 403 om-02, T 414 om-98, and T 569 pm-00, respectively.

### 2.9. Statistical methodology

All tests were carried out independently in duplicates. The procedures and statistical methodology for the different tests have been performed following the corresponding standard methods. The mean and standard deviation of the data sets were calculated using the Average and Stdev functions of Microsoft® Office Excel 2012.

## 3. Results

### 3.1. Effect of commercial cellulase treatments and refining on drainage property of kraft pulp

The kraft pulp studied was derived from softwood, and it contained $80.36 \pm 0.05\%$ cellulose, $9.8 \pm 0.6\%$ hemicellulose, and $6.53 \pm 0.22\%$ lignin. The CSF values of enzymatically treated pulps and control pulps measured after different PFI refining intensities are shown in Table 1. As expected, CSF decreased as PFI revolutions increased, indicating a reduction in drainage rate due to fibrillation. The target drop in CSF (200 mL) was achieved after 6000 revolutions on a PFI mill, in the absence of enzymes, regardless of pH. No significant change in CSF between the untreated and the enzymatic-treated pulps was observed (0 revs, Table 1). Adding refining with PFI mill to enzyme treated pulp lead to a decrease in CSF from 20% to 40% (as PFI revolutions increased) compared to unrefined pulp (Table 1). Most enzymatic treatments led to the target reduction in CSF (200 mL) after 4500 revolutions only (except for C5). This suggests that the pulp treated with any cellulase preparation tested here would require less refining energy in order to reach similar drop in freeness. The results show that with enzyme C1 at pH 5 the impact on drainage would be maximal, with a drop in CSF of 298 mL compared to the 117 mL afforded by mechanical refining alone (at 3000 revs). Lower freeness levels may be the consequence of various modifications of fibres, such as fibrillation or increased content in fines, due to fibre cutting (Kim et al., 2006).

### 3.2. Impact of enzymatic and mechanical treatments on fibre morphology

In the absence of mechanical refining, the mean fibre length of pulp was barely affected by any cellulase treatment used in this study (0 revolutions, see Fig. 1a). Mechanical refining alone had nearly no impact on fibre length either, but when combined with enzyme pre-treatment, enzymes C2, C3 and C5 at pH 7, and C1 at pH 5 promoted a decrease in fibre length by 17–32% (up to 0.7 mm, Fig. 1 panel a). While pulp treatment with enzyme C1 at pH 5 followed by PFI refining had a destructive action on the fibre length, the impact of the same enzyme at pH 7 was found to be much less pronounced. A very limited (if any) fibre cutting effect was observed with cellulase samples C1 and C4 at pH 7 compared to untreated pulp. At 3000 revs, fibre length decreased 3.08% and 27.31% for C1 pH 7 and C1 pH 5 treatment, respectively. At 4500 revs, fibre length after C1 pH 7 treatment is the same as control, while it decreased 29.73% for C1 pH 5 treatment.

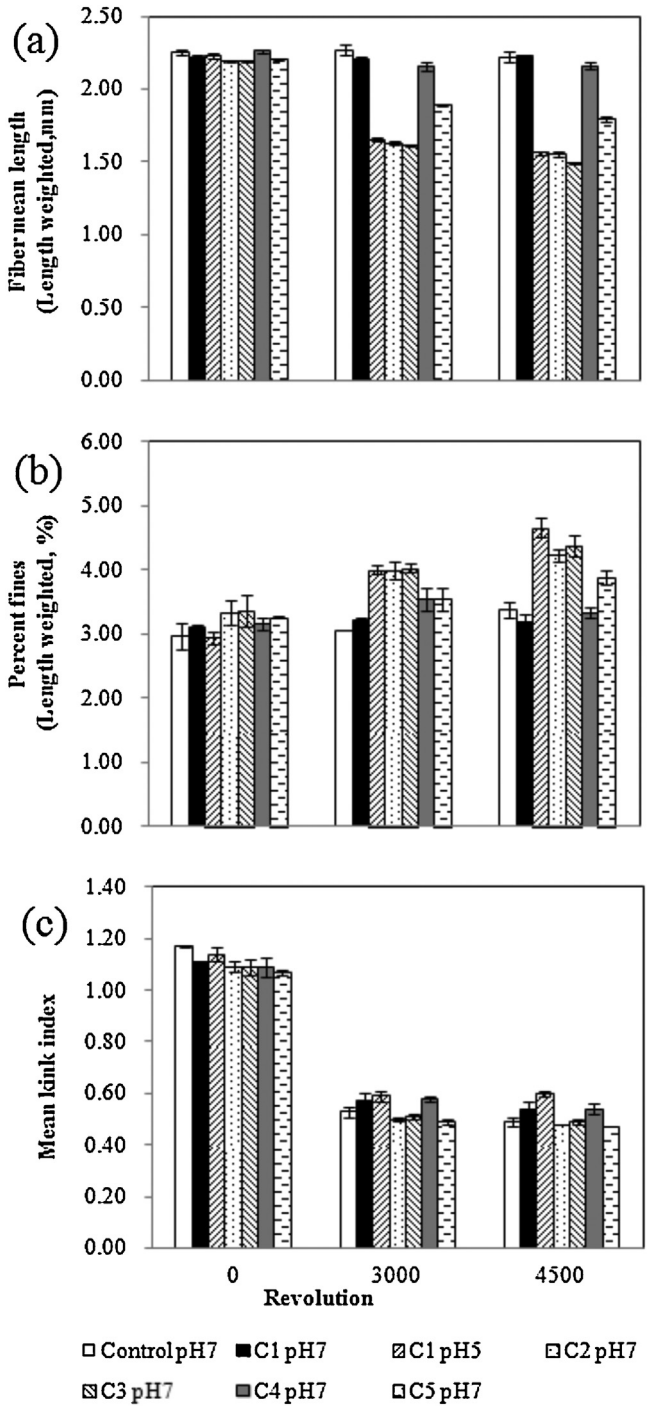


**Fig. 1.** Effect of cellulase treatment and PFI revolution on fibre length (a), percent fines (b) and kink index (c) at different pH values. Control pH 5 is not shown because it was equal to Control pH 7 regarding all properties measured.

The percent of fines generated after PFI refining was analyzed by FQA. After 3000 revolutions on the PFI refiner, all enzymes induced an increased in the percent of fines (from 5% to 30% compared to untreated pulp). After further mechanical treatment (4500 revs), enzymes C2, C3, C6 at pH 7 and C1 at pH 5 generated more fines (about 40%) than for untreated pulp (Fig. 1b). Commercial preparations C1 and C4 led to no additional production of fines compared to pulp without enzyme treatment. As expected, the reduction of fibre length observed in Fig. 1a was accompanied by a consequent impact on fines percentage for all enzyme treatments (panel b).

**Table 1**
Effect of PFI revolution on CSF (mL).

| Enzymes | PFI revolution | | | | |
|---|---|---|---|---|---|
| | 0 | 3000 | 4500 | 6000 | 0 vs 3000 + E[a] |
| Control (pH 7) | 710 ± 1.41 | 581 ± 5.66 | 558 ± 10.61 | 508 ± 2.12 | |
| Control (pH 5) | 692 ± 2.12 | 575 ± 2.83 | 516 ± 7.07 | nd | |
| C1 (pH 7) | 684 ± 0.00 | 499 ± 1.41 | 413 ± 0.71 | nd | 211 |
| C1 (pH 5) | 701 ± 12.73 | 403 ± 9.19 | 288 ± 18.38 | nd | 289 |
| C2 | 718 ± 3.54 | 525 ± 11.31 | 471 ± 2.12 | nd | 185 |
| C3 | 708 ± 0.00 | 514 ± 2.83 | 441 ± 7.78 | nd | 196 |
| C4 | 691 ± 0.71 | 505 ± 5.66 | 430 ± 7.07 | nd | 205 |
| C5 | 707 ± 7.78 | 567 ± 7.07 | 495 ± 11.31 | nd | 143 |

nd: not done.
[a] 0 vs 3000 + E compares the CSF measured after enzymatic treatment and refining (3000 revs) with the CSF observed for the untreated pulp (no enzymes, 0 revs) at the same pH. C2–C5 treatments were carried out at pH 7.

The mean kink index of enzymatically treated and PFI refined samples are reported in Fig. 1c. After PFI refining, the mean kink index dropped by 60% on average. Impact of pH on C1 was not significant. The kink index was barely affected by the enzymatic treatments compared to control, but it was slightly preserved with C1 (at either pH) and C4 treatments after refining with 3000 and 4500 revolutions.

### 3.3. Effect of enzyme treatments on physical properties of handsheet

The results in Fig. 2a–c indicate that the burst, tensile and internal bond strength increased with the increase in PFI refining revolutions. Further, there were no significant differences detected between mechanical treatment at 3000 and 4500 revolutions for handsheet physical properties (no enzymes). Enzyme preparations C1 (pH 7) and C4 led to the best handsheet strength properties. The tensile index, burst index and internal bond strength were improved by 7.47%, 2.76%, and 38.36% respectively after treatment with C1 at 3000 PFI revolutions, compared to control.

Treatment of pulp with enzyme samples C2 and C3 had a more pronounced, negative effect on burst and tensile strength compared to control under the same conditions (Fig. 2a and b). This is probably due reduction of fibre length (as indicated in Fig. 1a). Cellulases C1 and C4 treatment at pH 7 lead to a slight increase in tensile strength (by 7.47% and 3.97% respectively) at 3000 PFI revolutions. This may be explained by modifications to the fibre structure, such as increased flexibility and looseness after enzymatic treatment, which would promote the removal of the primary cell wall during the PFI refining. This would allow them to conform to the shape of neighbouring fibres as discussed by Clark, Allison, and Kibblewhite (1997) and as indicated by preservation of kink index with C1 and C4 demonstrated above. In this study, because the fibre length of pulps which were treated by C1 and C4 was only moderately affected, (see Fig. 1a), their burst index were similar to control values.

Cellulase C1 and C4 pulp treatments followed by 3000 PFI revolutions resulted in high paper internal bond strength compared to control pulps (38% and 40% higher than control sample respectively). These enzyme preparations had a higher impact than the other 3 enzymes (Fig. 2c). This enhancement is probably due to a higher flexibility (kink index shown here), higher collapsibility and higher fibrillation of the fibres (Ramos, Filho, Deschamps, & Saddler, 1999). External fibrillation also causes delamination of the surface layers, which contributes to the fibre–fibre bonds (Fardim & Duran, 2003).

The enzyme impact on tear index was exceptional: at variance with most properties measured in this study, enzymes had a deleterious impact on tear without any mechanical. The loss in tear index was minimal however for C1 (pH 7) and C4 (less than 10% see Fig. 2d). The lower tear index of pulp treated with enzyme may be due to cellulose degradation and reduction of fibre length (Pala, Lemos, Mota, & Gama, 2001), in accordance with fibre length measurements (Fig. 1a). Strey, Wesley-Smith, and Wolfaardt (2009) suggested that endoglucanase in cellulase preparations probably damages fibrils and that this damage becomes apparent after mechanical treatment.

### 3.4. Enzymatic activities

In order to better understand the impact of the various enzymatic treatments used here, an investigation of the actual enzymatic activities afforded by the commercial cellulase preparations was performed. Using model compounds, it was found that the preparations were very different (Table 2). The preparation that led to the largest reduction in CSF was C1 at pH 5, which had the most important xylanase and CMCase activities. Then, when considering C1 and C4 at pH 7, which provided a substantial preservation of strength properties after refining, it was found that the common features of these preparations were xylanase and cellobiase activities. Enzymes C2 and C3 that led to the worst results for handsheet properties did not show any activity other than CMCase.

Fig. 3 shows the amount of selected carbohydrates which were liberated by enzymatic hydrolysis of pulp fibres (i.e. pulp is the substrate for this experiment, not model compounds, Table 2). For the control sample, there was only small amount of hemicellulose-derived sugar in the filtrate after 1 h incubation (0.61 mg/L). The amount of carbohydrates liberated ranged from 6.27 to 51.92 mg/L when enzymes were used. Enzyme C1 (pH 5) liberated the largest amount of cellobiose (24.09 mg/L) and hemicellulose-derived sugars (23.79 mg/L). These findings confirm the activities detected in Table 2. Enzyme preparation C4 released similar amount of cellobiose, but less hemicellulose sugars than C1 (pH 5). The enzyme treatments (C2, C3 and C5) produced comparable amount of cellobiose (between 3.85 and 8.10 mg/L) and a smaller quantity of hemicelluloses sugars. C1 (pH 5) and C4 also produced more glucose than the other four enzyme treatments. For C4 this result confirms the detected β-glucosidase activity shown above (measured using a *p*-nitrophenol derivative). Enzymes C2, C3, and C5 did not liberate much hemicellulose above the control level, confirming the low xylanase activity reported in Table 2. Enzymes C1 (pH 7) and C4 hydrolysed about 2.64 and 7.50 mg/L of hemicellulose-derived sugars, respectively. Although the experimental conditions used for measuring enzymatic activities were very different (model compounds for Table 2 vs pulp-derived sugars for Fig. 3), the results clearly indicate the presence of xylanase in C1 and C4 preparations. Analyses of released sugars also indicate the presence of cellulolytic activities in such preparations, in agreement with detection of CMCase activity.

**Table 2**
Enzyme activities determined at 50 °C and pH 7 for all enzyme samples and at pH 5 and 50 °C for C1 sample enzyme.

| | C1 (pH 7) | C1 (pH 5) | C2 | C3 | C4 | C5 |
|---|---|---|---|---|---|---|
| Endoglucanase (IU/mg) | ND | 26 ± 1.4 | 14.8 ± 0.6 | 10.6 ± 0.5 | 24.7 ± 0.6 | 15.7 ± 0.6 |
| Xylanase (IU/mg) | 55.6 ± 1.1 | 122 ± 0.5 | ND | ND | 57.4 ± 4.9 | ND |
| β-Glucosidase (IU/mg) | 15 ± 1.1 | ND | ND | ND | 168 ± 20 | 21 ± 3.3 |

ND: not detected.

**Table 3**
Impact of enzyme preparations on pulp and handsheet properties (after 3000 revolutions on PFI mill).

| | C1 (pH 7) | C1 (pH 5) | C2 | C3 | C4 | C5 |
|---|---|---|---|---|---|---|
| CSF | ++ | + | ++ | ++ | ++ | +++ |
| Fibre mean length | +++ | + | + | + | +++ | ++ |
| Percent fines | + | +++ | +++ | +++ | ++ | ++ |
| Kink index | ++ | ++ | + | + | ++ | + |
| Burst | +++ | ++ | + | + | +++ | ++ |
| Tensile | +++ | ++ | + | + | +++ | ++ |
| Tear | +++ | ++ | + | + | +++ | ++ |
| Internal bond strength | ++ | +++ | + | + | ++ | + |

Number of + indicates relative value intensity measured for the indicated properties.

## 4. Discussion

Despite compelling evidence of enzymatic biorefining as potentially efficient technology for optimizing paper production, it remains that very few (if any) of the research performed to date has identified the "holy grail" of refining: an inexpensive, environmentally safe way to save energy while preserving or improving paper properties. As a matter of fact, enzymatic improvement of refining efficiency achieved at laboratory scale (sometimes spectacular) has been mostly correlated with decline in paper properties (García et al., 2002; Gil et al., 2009; Kim et al., 2006; Liu et al., 2012; Oksanen et al., 2000). Further, even if one had solved this quest in a research environment, it remains that the availability of relevant enzymes at industrial scale is not straightforward. It is recognized that commercial enzymes too often reflect the needs of the enzyme producers and not specifically the needs of end users (Rosgaard et al., 2007). The availability of enzymes coming from a very limited (easy to grow and manage) number of organisms (*Aspergillus*, *Trichoderma*, etc.) is an obvious consequence of suppliers' cost reduction strategy. Availability of the appropriate of better adapted enzymes in Nature (biodiversity) is certain, but not much effort has been deployed to use it at customers' advantage so far (Saleem et al., 2010; Shanmughapriya, Kiran, Selvin, Thomas, & Rani, 2010). One way to circumvent this limitation is to explore the various "cellulase" formulations commercially available, understand their impact, and eventually develop optimal cocktails for one's particular needs.

The objective of this study was to compare and understand the impact of five commercial enzyme samples, shedding light on how to use such enzymes optimally for biorefining. Table 2 compares the various enzymatic activities that were detected in the 5 enzyme samples selected for this study. Clearly, these "cellulase" preparations are not homogenous preparations. Most contain significant cellulase activity (measured here by the production of cellobiose and CMCase assay), believed to be the spearhead of biorefining (Ko et al., 2010; Zhang, Tuomainen, Siika-aho, & Viikari, 2011), along with various activities as hemicellulase and β-glucosidase (cellobiase).

Comparison of enzymatic activity profiles (Table 2 and Fig. 3) with the summary of the various impacts detected presented in Table 3 reveal a number of correlations: (1) samples C2 and C3 had no xylanase activity (they produced very little amounts of hemicellulose-derived sugars too), which is interesting, considering they were the worst preparations for biorefining in this study. This observation is emphasized in Table 3, where the pulp and paper properties are compiled for all tests: C2 and C3 would be the worst choice for the pulp studied here, for most parameters studied. Severe attack by C2 and C3 reduced fibre length and produced the most important quantity of fines. But its impact on drainage was not as important compared to xylanase-containing treatments. No correlation between fines percent and drainage was observed for most enzymes used here, except for C1 pH 5 where a large proportion of fines resulted into a large drop in CSF. (2) The freeness was minimal for samples treated with enzymes showing xylanase activity. Impact on drainage did not correlate with paper properties: C1 at pH 5 led to the maximal reduction in drainage, and resulted into loss of tear strength and fibre length. Enzymatic treatments by C1 (pH 7) and C4 protected fibres length, enhancing the interweaving of fibres and bonding between them, while reducing significantly drainage compared with C2, C3 and C5. It should be emphasized that non-CMCase cellulolytic activity was detected by sugar analysis for C1 at pH 7 (Fig. 3). Thus, one cannot conclude that xylanase activity alone is sufficient for explaining the impacts observed. The ability of xylanase to affect drainage or help refining was reported by Oksanen et al. (2000), Dickson, Wong, and Mansfield (2000) and Garcia-Ubasart et al. (2013) to name a few. The possible contamination by any cellulolytic activities in the enzymatic products used in such studies should be addressed before concluding that xylanase alone can decrease drainage. It was claimed that CMCase activity was correlated with modification of drainage (Ko et al., 2010; Torres et al., 2012) but in our study, C1 (pH 7) had no CMCase activity but led to a reduction in CSF. (3) The protection of fibre length with minimal production of fines was correlated with β-glucosidase activity, but not with the absence of CMCase. Considering that β-glucosidase is not expected to provide protection to fibres, that the removal of cellobiose should enhance cellulase activity, such an observation is somewhat puzzling. These results could be explained by the different properties of cellulolytic enzymes in C1, C4 and C5 compared to the ones found in C2 and C3. Enzyme preparation C1 at pH 5 showed no CMCase activity, but it did produce cellobiose from pulp (Fig. 3). Enzymes such as exoglucanases that remove small sugars at either end of cellulose are much less effective at weakening fibres compared to endoglucanases that can break cellulose anywhere in the fibres (Kibblewhite & Wong, 1999). Our enzymatic assays do not allow for the specific identification of endohydrolytic activities, and do not confirm nor than oppose this hypothesis. But it has been claimed that endoglucanase activity was responsible for loss in strength after refining (Clark et al., 1997; Oksanen et al., 1997, 2000; Strey et al., 2009). (4) The kink index and the related internal bond strength were correlated with freeness, and with the xylanase content of the preparations. Increased flexibility of fibres after xylanase treatment was observed by Dickson et al. (2000) too. (5) Application pH may have a deleterious impact on a "cellulase" treatment: enzyme preparation C1 responded to pH change from 7 to 5 by a change in the various enzymatic activities tested here. Its ability to generate small sugars from pulp was much more important, and the CMCase and xylanase activities were shifted upward (they were the most important activities detected among all preparations used). This resulted into the maximal impact on drainage observed here, and improvement of kink and inter fibre bond. However, this was accompanied by an important reduction fibre length and tear index. Clearly, this shift in enzymatic activities will lead

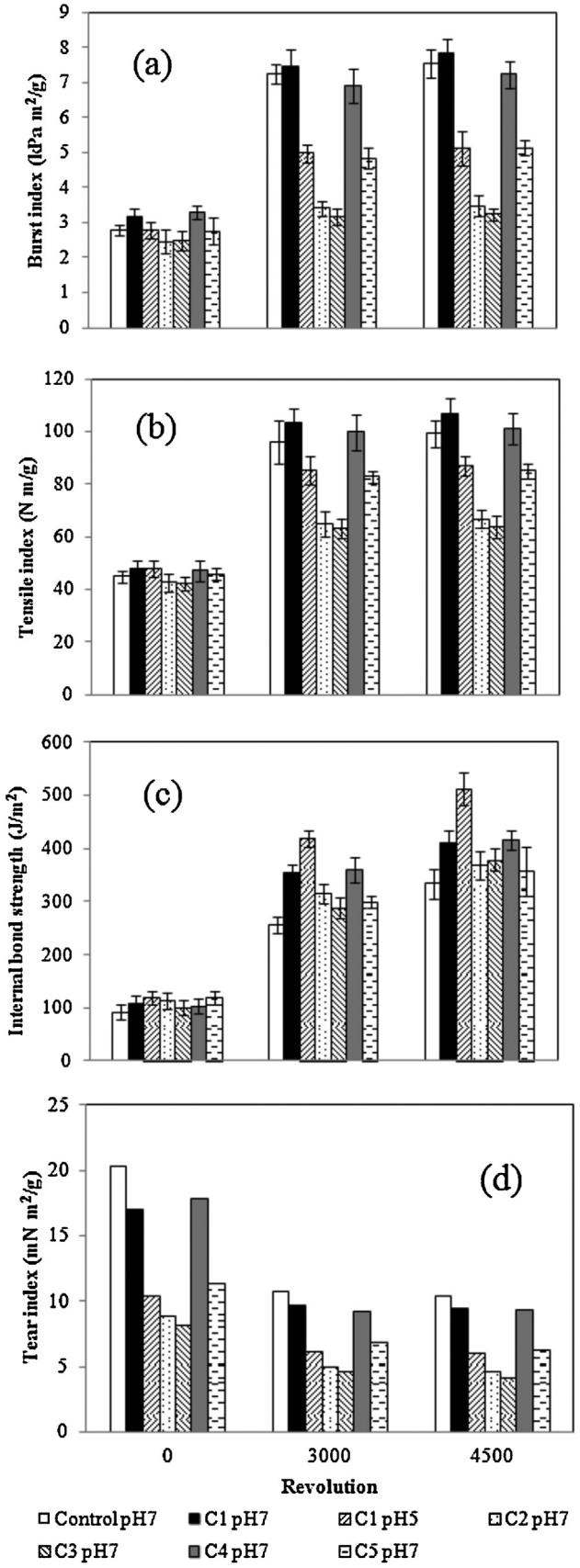


**Fig. 2.** Effect of cellulase treatment and PFI revolution on handsheets burst index (a), tensile index (b), internal bond strength (c) and tear index (d) at different pH values.

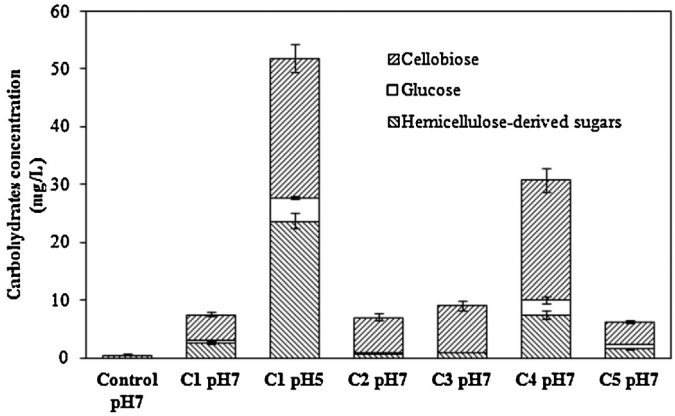


**Fig. 3.** Carbohydrates concentration in the filtrate after enzymatic treatment.

to consequences for pulp and paper properties, regardless of the impact on drainage afforded at either pH value. It was inferred that pH would have an impact on hemicelluloses (they can be ionized) but this impact was not detected here (Taipale et al., 2010). All controls led to similar pulp and handsheet regardless of pH used in this work. (6) Enzymatic treatments in the absence of mechanical refining had little impact on paper properties, except for tear index, which was decreased by all enzymes without any mechanical treatment. Enzyme preparations with xylanase activity had the least deleterious impact on tear index.

Refining has been shown to remove fibers' outer layer, allow for swelling, decrease length and improve flexibility. Enzymatic (cellulase) refining appears to have similar impact: it would remove the primary wall and S1 layer (rich in cellulose). Accordingly, S2 layer becomes fibrillated, and its surface and specific volume are increased (Singh & Bhardwaj, 2010; Zhang et al., 2011). The pretreatment of pulp with commercial cellulase prior to refining can achieve more fibrillation of fibres, which enhances the inter-fibre bonding and increases the tensile strength of softwood kraft pulp (Liu et al., 2012). It has been argued that xylanase too can modify fibres, and induce separation of the secondary wall, increase surface area by fibrillation and reduce pore volume (Lei, Lin, & Li, 2008; Pala, Mota, & Gama, 2007; Singh & Bhardwaj, 2010).

## 5. Conclusions

Our study clearly demonstrates the impact of xylanase and/or cellulase on refining and on pulp and paper properties, and is in line with several reports on biorefining (García et al., 2002; Gil et al., 2009; Kim et al., 2006; Liu et al., 2012; Oksanen et al., 2000). Despite these findings, the problem is far from being solved regarding biorefining at the industrial level. This study clearly demonstrates that fines content or CSF are poor indicators of pulp and paper properties. Correlations between enzymatic activities found in the various commercial samples with their impact on pulp and paper properties indicate that an intricate balance between cellulolytic and hemicellulolytic activities (and levels of activity) is the key to optimal biorefining. The impact of pulp composition, in particular its pH, on the various enzyme products used has to be investigated before this intricate balance can be achieved.

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