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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.

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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.

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