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Exploring the effects of treatments with carbohydrases to obtain a high-cellulose content pulp from a non-wood alkaline pulp

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ABSTRACT

In this work, treatments with a xylanase (X) and carbohydrases mixture (Cx) were applied on a TCF bleached sisal pulp in order to obtain high-cellulose content fibers applicable on a wide range of uses. A limit of ≈12% w/w final content in hemicelluloses was found regardless of the enzymatic treatment assessed. An extraction with 4% and 9% w/v NaOH was performed for further hemicelluloses removal. We found that NaOH dose could be strongly reduced if combined with Cx or Cx + X treatments. Also, if necessary, a stronger reduction could be obtained with 9% w/v NaOH, which was found to be boosted in a 14% if performed after a treatment with Cx. An end-product with a low content in xylans (≈2.9% w/w) and in HexA (5.8 μmol/odp) was obtained. Pulp Fock solubility was also increased (≈30%) by enzymatic treatments. HPLC analysis of effluents provided useful information of enzymatic catalytic mechanisms.

1. Introduction

There is a growing interest in society to move toward a bio-based economy where a bigger part of our daily products can be provided by agriculture (Ibarra, Kopcke, Larsson, Jaaskelainen, & Ek, 2010). In this direction, making agriculture not just a food provider, but a producer of other raw materials, by taking advantage of non-food residues generated upon food cropping or cultivating other fiber-providing species, can be a good way to move toward this new economic model. Fibers quality improvement from paper grade to high cellulose content has attracted plenty of interest in recent years (Li, Zhang, Duan, Liu, & Ni, 2015). Traditionally, pulps with low hemicelluloses content have been obtained through acid sulphite or pre-hydrolysis Kraft process (Li et al., 2015). On these processes, hemicelluloses that are present on pulp suffer a greater attack than during alkaline processes such as Kraft or NaOH-AQ, reducing their presence on final product. However, pulps obtained through these processes have some drawbacks related to quality of final product or the pollution they generate. Also, these pulping processes imply higher costs than alkaline ones in terms of chemical consumption, production rate, inventories and storage space (Barlow & Hillman, 2006). For these reasons, several methods have been studied in order to carry out the selective elimination of hemicelluloses from alkaline pulps (Bajpai & Bajpai, 2001; Jackson, Heitmann, & Joyce, 1998; Kopcke, Ibarra, & Ek, 2008). These methods include nitren, cuen and alkaline extraction. Besides them, enzymatic hydrolysis of different components of lignocellulose has attracted special attention because of its potential as a "green" process. It is well known that biomass availability to enzymes is hindered by diverse factors (Zhu, O'Dwyer, Chang, Granda, & Holtzapple, 2008). Because of this, several methods have been studied for enhancing enzymatic biomass conversion, including physical, chemical, biological or thermophysical pretreatments (Maache-Rezzoug, Pierre, Nouviaire, Maugard, & Rezzoug, 2011; Pierre, Maache-Rezzoug, Sannier, Rezzoug, & Maugard, 2011). Among enzymes, xylanases have been traditionally used in pulp and paper industry for pulp bleaching (Fillat, Roncero, & Vidal, 2011; Valls & Roncero, 2009). In this work, however, they are applied on bleached pulps with the purpose of removing hemicelluloses. Other enzymes, such as endoglucanases (cellulases), have been mainly used by authors for fibers biorefining (Garcia-Ubasart, Torres, Vila, Javier Pastor, & Vidal, 2013), biomass saccharification (Pierre, Sannier, et al., 2011; Pihlajaniemi, Sipponen, Sipponen, Pastinen, & Laakso, 2014; Zhang, Tang, & Viikari, 2012) or increasing cellulose reactivity (Kopcke et al., 2008; Miao et al., 2014; Pierre, Sannier, et al., 2011).

Among the formerly stated fiber-providing species, sisal constitutes a raw material with a great potential for several applications. These fibers have traditionally been used to manufacture natural ropes, cordage and sacking. Regarding their potential as a raw material for pulp and paper industry, sisal fibers present some

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positive features including a high tear resistance, alpha cellulose content, porosity, bulk, absorbency and folding endurance, making it excellent for a variety of specialty papers (Aracri & Vidal, 2012). In addition, as sisal fibers have better physical properties than softwood kraft fibers, they become a good raw material for reinforcing fiber in paper with high recycled content, or for reducing basis weight while maintaining product quality (Maddern & French, 1995). This study focuses on the possibility of using a bleached non-wood pulp, from sisal (*Agave sisalana*), to obtain a high cellulose-content pulp by means of enzymatic treatments, combined with alkaline extractions. A bleached sisal pulp was used and different enzymatic treatments with new carbohydrases were applied in order to modify this pulp. Although similar works have been published in literature (Henriksson, Christiernin, & Agnemo, 2005; Ibarra, Kopcke, & Ek, 2009; Wang et al., 2014), this study introduced new enzymes, a xylanase and a carbohydrases mixture (containing endoglucanase and xylanase activities, not still commercially available) together with a NaOH extraction. Enzymes also permitted a reduction in the use of NaOH. Furthermore, possibilities of enzymatic treatments were studied by assessing different ways of applications including a newly focused evaluation of xylanolytic treatments. Also, a comprehensive study of enzymatic effects on fibers was performed for better understanding the effects of these new catalysts.

2. Materials and methods

2.1. Pulp

A totally chlorine free (TCF) bleached pulp from sisal (*A. sisalana*) was used as a raw material. Pulp was provided by Celesa (Spain), and was obtained by an alkaline NaOH-AQ process. Pulp initial parameters were: Content in hemicelluloses (xylans, % w/w) = 16.1 ± 0.3; kappa number (KN) = 4.7 ± 0.2; ISO brightness (%) = 82.1 ± 0.3; viscosity (mL/g) = 616 ± 41; HexA content (μmol/g odp) = 45.1 ± 1.5; Fock solubility 13.2 ± 0.2%.

2.2. Enzymes

A xylanase (X) and a carbohydrases mixture (Cx) were used for treatments, both provided by Fungal Bioproducts (Spain) and obtained from *Cerrena* sp. fungus. Carbohydrases mixture (Cx) had both Carboxymethylcellulase (CMCase) and xylanase activities. Activities as U/g dried enzyme powder were: 11000 U/g for the xylanase (X), 1700 U/g and 680 U/g for the cellulase and xylanase activity on the mixture (Cx), respectively. Enzymatic activity was determined at application conditions (50 °C and pH 7 for X; and 55 °C and pH 5 for Cx). An activity unit (U) is defined as the amount of enzyme capable of converting 1 μmol of substrate per minute. Enzymatic activity was determined using Spiro method to quantify released reducing sugars (Spiro, 1966) after a microscale enzymatic reaction carried out for 15 min. Substrate was carboxymethyl cellulose (CMC) or birchwood xylan for measurement of cellulolytic and xylanolytic activity, respectively. Prior to treatments, sugar presence on enzymatic preparations was analyzed using the same method as for effluents (described below). None of the studied oligosaccharides was found on these preparations.

2.3. Enzymatic treatments

Enzymatic treatments were held using X enzyme (Fig. 1A) and combined treatments using Cx and X enzymes (Fig. 1B). Letter "K" in a sample name indicates a control (*e.g.*, KX indicate control for X treatments, and KCx for Cx). Control samples were prepared using the same conditions as in enzymatic treatments, but with no enzyme addition. After treatments, an aliquot of effluents was saved for analysis. Enzymatic reactions were then stopped washing pulps with decalcified water three times and one time with deionized water.

1 Treatments with xylanase (X) (Fig. 1A) were applied in plastic bags on a thermostatic bath with a 10 U/g oven-dried pulp (odp) dose at 50 °C, 10% consistency, pH 7 (adjusted with 50 mM Tris–HCl buffer) and manual agitation every 10 min according to two different procedures:
   1.1 Direct (X/KX): Reaction was carried out up to 5 h, and samples were collected after each hour for characterization (X_1, X_2, X_3, X_4 and X_5).
   1.2 Stepwise addition (Xs/KXs): 2 U/g odp xylanase were added 1 h periods which were immediately followed by washing with deionized water (Xs_1, Xs_2, Xs_3, Xs_4 and Xs_5). At the end of treatment (5 h) a final dose of 10 U/g odp was applied, equal to that used on "direct" treatment.
2 Treatments with carbohydrases mixture (Cx) and xylanase (X) (Fig. 1B):

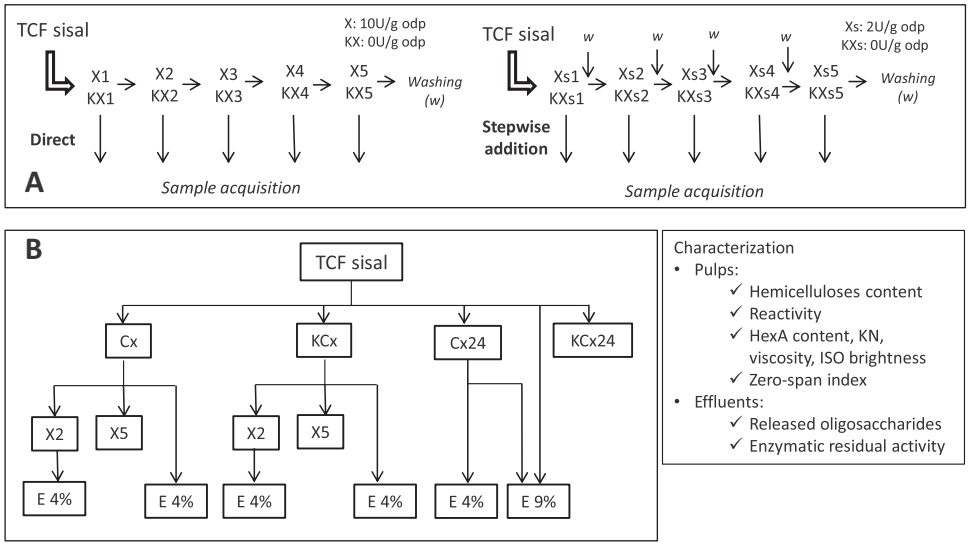

Fig. 1. Work scheme of the present study.