Carbohydrate Polymers 189 (2018) 39–47

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

A novel enzymatic approach to nanocrystalline cellulose preparation

Facundo Beltramino, M. Blanca Roncero, Teresa Vidal, Cristina Valls*

CELBIOTECH_Paper Engineering Research Group, Universitat Politècnica de Catalunya (UPC. BarcelonaTech), Colom 11, E-08222, Terrassa, Spain

ARTICLE INFO

Keywords:
Nanocrystalline cellulose
Cellulase
Optimization
Yield increase
Fiber length

ABSTRACT

In this work, conditions for an enzymatic pretreatment prior to NCC isolation from cotton linter were assessed. Different cellulase doses and reaction times were studied within an experimental design and NCC were obtained. At optimal enzymatic conditions (20U, 2 h), a total yield greater than 80% was achieved and the necessary enzymatic treatment time was reduced 90%. Different intensities of enzymatic treatments led to proportional decreases in fiber length and viscosity and also were inversely proportional to the amount of released oligosaccharides. These differences within fibers lead to quantitative differences in NCC: increase in acid hydrolysis yield, reduction of NCC surface charge and crystallinity increase. Benefits produced by enzymatic treatments did not have influence over other NCC characteristics such as their sulfur content (≈1%), size (≈200 nm), zeta potential (≈ −50 mV) or degree of polymerization (≈200). Evidence presented in this work would reduce the use of harsh sulfuric acid generating a cleaner stream of profitable oligosaccharides.

1. Introduction

Research in nanocrystalline cellulose (NCC), a material also named cellulose nanocrystals, started some years ago (Rånby, 1951; Revol, Bradford, Giasson, Marchessault, & Gray, 1992) and has generated a huge interest in recent years due to the promising features this material holds (Habibi, Lucia, & Rojas, 2010; Sun et al., 2014; Trache, Hussin, Haafiz, & Thakur, 2017). Typically, it consists on a rigid rod-like monocrystalline cellulose domain with dimensions among 1–100 nm in width and up to several hundred nanometers in length (Lin & Dufresne, 2014). Also, they are produced from cellulose fibers, a very abundant raw material (Zhu et al., 2016). NCC has a high degree of crystal structure, a high aspect ratio (length-to-diameter, up to 300), a large surface area (above 150 $m^2 g^{-1}$), a very high elastic moduli, (estimated to be over 130–150 GPa) and a low thermal expansion coefficient (6 ppm K^{-1}) (Tanaka, Saito, Ishii, & Isogai, 2014). This material finds many potential applications in diverse fields such as an additive for composite materials (Moon, Martini, Nairn, Simonsen, & Youngblood, 2011), optical applications (Lin, Huang, & Dufresne, 2012), or diverse uses in biomedicine (Lin & Dufresne, 2014), to name a few.

Biotechnology has been used for several applications in cellulose industry, such as biobleaching, biorefining, or even pulp quality upgrades (Beltramino, Valls, Vidal, & Roncero, 2015; Beltramino, 2016; Garcia-Ubasart, Torres, Vila, Pastor, & Vidal, 2013; Quintana, Valls, Vidal, & Blanca Roncero, 2013; Valls & Roncero, 2009). Generally, the use of enzymes as a green technology allows reducing the pollution generated by traditional chemical processes, providing a solution for an enormous social concern. Cellulases, enzymes degrading cellulose include three different enzymatic activities (Teixeira et al., 2015). Endoglucanases (E.C. 3.2.1.4) catalyze the hydrolysis of the 1, 4-glycosidic linkages of the amorphous regions of cellulose. In nature, they hydrolyze cellulose in synergy with cellobiohydrolases (E.C. 3.2.1.91), which act upon the reducing and non-reducing ends of cellulose chains. Finally, β-glucosidases (E.C. 3.2.1.21), catalyze the hydrolysis of cellobiose into glucose. Generally, this enzymatic cellulose degrading activity is capable of participating into NCC preparation, fact that is reflected in some examples of authors successfully introducing enzymes (cellulases) into nanocellulose preparation process (Anderson et al., 2014; Teixeira et al., 2015; Zhang, Xue, Zhang, & Zhao, 2012). The first proposal of the concept of using enzymes for producing cellulose nanomaterials was stated by Zhu, Sabo, & Luo, 2011. Furthermore, enzymatic preparation of NCC has been related with an improved quality of final product compared to pure chemical processes (George, Ramana, Bawa, & Siddaramaiah, 2011).

One of the main drawbacks associated with NCC preparation is the low yield presented by the typical acid hydrolysis with sulfuric acid used for its preparation (Chen et al., 2015). Considering this evidence, a previous work from our group demonstrated that a cellulase pretreatment on cotton linters could increase the yield of NCC as well as to influence other characteristics of them (Beltramino, Roncero, Vidal, Torres, & Valls, 2015). Optimizations via factorial designs have been widely used in literature for optimizing enzymatic and chemical treatments for diverse applications (Bondeson, Mathew, & Oksman, 2006; Fillat & Roncero, 2009, 2010; Valls & Roncero, 2009). In a previous study, conditions of sulfuric acid

* Corresponding author.
E-mail addresses: facundo.beltramino@upc.edu (F. Beltramino), blanca.roncero@upc.edu (M. Blanca Roncero), teresa.vidal@upc.edu (T. Vidal), cristina.valls@upc.edu (C. Valls).

https://doi.org/10.1016/j.carbpol.2018.02.015
Received 6 November 2017; Received in revised form 5 February 2018; Accepted 5 February 2018
Available online 08 February 2018
0144-8617/

hydrolysis in order to maximize NCC yield from cellulase-pretreated fibers were optimized using a factorial design (Beltramino, Roncero, Torres, Vidal, & Valls, 2016). Maximal yield was achieved with 25 min of hydrolysis at 47 °C and using 62% wt. H_2SO_4. In the light of the results formerly obtained, this work intended to find the best conditions for obtaining the maximum profit of enzyme action. For this, conditions for the enzymatic pretreatment were optimized before and after obtaining NCC within a 2^2 complete factorial design. The main objective was to maximize the yield of the whole enzymatic and chemical process. We focused into the assessment of quantitative effects of these pretreatments of different intensity and their relations in both cellulose fibers and NCC. The purpose of this study was to find the best conditions for the enzymatic pretreatment providing the highest NCC yield in combination with optimal conditions established in a previously reported work (Beltramino et al., 2016).

2. Materials and methods

2.1. Cellulose source and enzyme

Cotton linters provided by Celsur (Spain) were used as a raw material for experiments. Composition of fibers was: glucans content (cellulose) 97.7% ± 0.3; xylans content 2% ± 0.2; Rhamnans 0.2% ± 0.15; acetyl groups 0.1% ± 0.1. Fibers, as received from provider, were beated in a valley mill for 90 min for reducing average length. Obtained fibers were named as "initial". A commercial cellulase preparation (named "C"), provided by Fungal Bioproducts (Spain) and obtained from *Cerrena* sp. fungus was used for treatments. Previous works demonstrate that it is not a mono-component enzyme (Beltramino, Valls et al., 2015; Beltramino, 2016). Activity as U g^{-1} from enzyme stock was 1700 and was expressed as CMCase units i.e. the amount of enzyme degrading 1 μmol of CMC (carboxymethilcellulose) per minute.

2.2. Enzymatic treatments

Enzymatic treatments were held using cellulase C on an Ahiba Easydye (Datacolor, USA) apparatus having independent 250 mL vessels with agitation consisting on upside-down inversions at 20 oscillations per minute. Treatments were performed at 55 °C, 5% consistency and pH 5 maintained with a 50 mM sodium acetate buffer solution on distilled water. Enzyme dose and reaction time were variables chosen in accordance to an experimental design (Table 1). After reactions a liquor sample was recovered for residual enzymatic activity determination and enzyme was deactivated by heating samples to 105 °C during 15 min. Fibers were then filtered using a filter with pore size N°2 and reaction liquor was passed through fibers 3 times in order to recover fines. No washing was performed after treatments in order to avoid sample loss and samples of reaction liquor were saved for sugar content analysis. A control for enzymatic treatments was also performed on fibers, applying the same conditions as for treatments during 2 h, but with no enzyme addition.

Table 1
Experiences of the statistical plan with their conditions.

Y	X1	X2	Cellulase dose (U g^{-1} odp)	Enzymatic treatment time (h)
Y1	−1	−1	2	2
Y2	1	−1	20	2
Y3	−1	1	2	24
Y4	1	1	20	24
Y5	0	0	11	13
Y6	0	0	11	13
Y7	0	0	11	13
Y8	1	0	20	13
Y9	0	−1	11	2

2.2.1. Experimental design

Enzymatic treatments were applied in accordance to a 2^2 statistical factorial plan involving two levels and two variables plus three repetitions in the central point, which required a total of 7 experiences (Table 1). Variables were: X1(enzyme dose), varied within 2–20 U g^{-1} odp (oven-dried pulp) range and X2 (reaction time) varied within 2–24 h. These independent variables were coded as −1 or +1; both for direct comparison of coefficients and to better understand the effect of each variable on the responses. The results of the three repetitions at the central point and their variance were used in combination with the variance of the saturated model to calculate Snedecor's F-value in order to determine whether the variance was homogeneous or heterogeneous. Since the variance was homogeneous in all cases, a linear model was constructed, its significant terms identified and potential curvature detected. Two additional points were required for solving quadratic terms confounding. Linear multiple regression technique was applied by using an Microsoft Excel spreadsheet to implement the stepwise backward regression method and discard all terms with a probability (*p*-value) less than 0.05.

2.3. Nanocrystalline cellulose preparation

Nanocrystalline cellulose (NCC) was obtained from initial, control and enzymatically pretreated fibers by a controlled sulfuric acid hydrolysis, using the protocol proposed by Dong et al., 1998. Fibers were fluffed prior to hydrolysis, oven dried and cooled in a desiccator. Typically, 1.5 g of sample weighted immediately from desiccator was hydrolyzed with 62% (w/w) sulfuric acid for 25 min at 47 °C with an acid-to-fibers ratio of 10:1 (i.e. 10 mL g^{-1} cellulose), optimal hydrolysis conditions described in a previous work (Beltramino et al., 2016). In all cases, hydrolysis reaction was stopped by diluting the acid with chilled (4 °C) distilled water in a 10-fold basis, and also cooling samples immediately on an ice bath. Samples were then centrifuged at 6000 rpm for 15 m and supernatant was discarded. Samples were re suspended in distilled water and centrifugation step was repeated, discarding supernatant. Samples were then sonicated to disperse them using a Hielscher UP100H ultrasonic processor at 100% amplitude and 0.75 cycles for 20 min on an ice bath to prevent heating which may cause desulfation (Dong et al., 1998). Re suspended samples were then dialyzed against distilled water using a 10 kDa Thermo Fischer dialysis membrane until pH 3. Final samples were filtered through Whatman ashless paper filters, N° 41 (pore size 20–25 μm).

2.4. Samples characterization

2.4.1. Cellulose fibers

Enzymatic treatment yield was calculated by determining the solid residue (treated fibers) after treatments and was indicated as% of recovered fibers mass. Initial and enzymatically treated fibers were characterized in terms of viscosity and fiber length according to ISO 5351:2010, and TAPPI Standard T271, respectively.

Infrared spectra of fibers samples were recorded at room temperature using a Perkin Elmer Spectrum 100 ATR-FTIR spectrophotometer. Fourier transformed infrared spectroscopy (FTIR) spectral analysis was conducted within the wavenumber range of 600–4000 cm^{-1}. A total of 64 scans were run to collect each spectrum at a 1 cm^{-1} resolution. Total crystallinity index (TCI) as proposed by Nelson and O'Connor (Nelson & O'Connor, 1964) was estimated from the ratio between the absorption peaks at 1370 cm^{-1} and 2900 cm^{-1}, respectively.

2.4.2. Enzymatic treatment effluents

Released reducing sugars on enzymatic reaction effluents were analyzed using a 1100 Agilent HPLC instrument (Agilent technologies, USA) furnished with a BIO RAD Aminex HPX-42A ion-exchange column. Residual enzymatic activity on effluents was determined using an adapted version of Somogyi-Nelson method to determine reducing