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EVALUATING THE EFFECTS OF ENZYMATIC PRETREATMENT ON EUCALYPTUS PULP FOR THE EXTRACTION OF CELLULOSE NANOFIBERS BY BALL MILLING

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1. INTRODUCTION

Cellulose nanofibers (CNF) is usually produced from diluted cellulosic suspensions by mechanical fibrillation using a refiner, high-pressure homogenizer or microfluidizer, which operate under high shear force, resulting in the cleavage of cellulose fibers and fibrillation. However, high energy consumptions are required, which becomes an economic issue that limits its implementation. To overcome this, mechanical fibrillation can be combined with chemical or enzymatic pretreatment (Bauli et al., 2019). Even though the TEMPO chemical pretreatment ((2,2,6,6-tetramethylpiperidine-1-oxyl) oxidation and carboxymethylation) is considered one of the most effective, the use of hazardous reagents and chemical recovery limits its application at an industrial scale. Enzymatic pretreatment represents an environmentally friendly alternative for CNF production due to high enzyme specificity and less harmful reaction conditions. The use of enzymes catalyzes the hydrolysis of cellulose fibers and makes fiber fibrillation by mechanical pretreatment easier. During enzymatic hydrolysis highly ordered regions of microfibers are delaminated and disrupted, causing the increase of external surface area and porosity as enzyme further opened up the cellulosic structure (Michelin et al. 2020). Studies on enzymatic production of CNF have been sparse in the literature compared to acid hydrolysis methods. Due to this, it is not clear which parameters have more influence on the action of the enzyme and thus on the extraction yield and properties of the extracted CNF. The aim of this work is to evaluate the effect of an enzyme cocktail under different pretreatment conditions on bleached eucalyptus Kraft pulp (BEKP) for the extraction of CNF by ball milling.

2. MATERIAL AND METHODS

BEKP was provided by a commercial local pulp mill composed of 77% glucan, 18% xylan, and 0,7% lignin. Cellic CTec3 and Cellic HTec preparations were used to study the effect of enzymatic pretreatment conditions (solid concentration and reaction time) on BEKP processing. The conditions evaluated were: 4%-4 h; 4%-8 h; 10%-4 h; 10%-8 h; 16%-4 h; and 16%-8 h. The reaction was conducted at 50°C, pH 4.8, and 150 rpm. Liquid fractions after enzymatic pretreatment were evaluated for sugar release by HPLC. The solid yield (SY), degree of polymerization (DP), water retention value (WRV), and acid groups content were determined for the solid residues (enzyme-pretreated pulp) following reported methods by Cebreiros et al. (2021). Solid residues were ball milled for CNF extraction. CNF morphology was evaluated by Transmission Electron Microscopy (TEM), and fiber size fragmentation was monitored by dynamic light scattering (Malvern Zetasizer Nano-ZS).

3. RESULTS AND DISCUSSION

Enzymatic hydrolysis resulted, for all the conditions evaluated, in a significant DP reduction (>50%), a greater exposure of cellulose chains that enhanced water retention and increased external surface area (Table 1). The lowest solid loading (4%) condition was the most efficient regarding cellulose and xylan hydrolysis for a short reaction time (4 h). However, by extending the reaction time to 8 h, solids

loading of 4% and 8% achieved comparable results. High solid loading is attractive since it allows to recover the released sugars at high concentrations (>30 g/L), which can be converted to biofuels or high value-added chemicals. This contributes to an integral exploitation of cellulosic feedstocks in a biorefinery concept.

Table 1. *Cellulose and xylan conversion yields, and characterization of solid residues and liquid fractions.*

Sample	CC (%)	XC (%)	Solid fraction				Liquid fraction	
			SY (%)	DP	WRV	AA (mmol/kg)	Glucose (g/L)	Xylose (g/L)
BEKP	-	-	-	1196	2.7	45	-	-
4%-4h	43	68	52	332	4.1	65	14.6	5.4
4%-8h	53	80	42	288	4.3	77	18.1	6.3
10%-4h	30	55	65	345	3.5	59	26.1	10.6
10%-8h	56	82	39	258	4.0	75	40.2	13.6
16%-4h	22	39	75	524	3.5	49	29.8	12.1
16%-8h	35	59	60	458	3.6	66	46.5	17.8

CC: cellulose conversion; XC: xylan conversion; SY: solid yield; DP: degree of polymerization; WRV: water retention value; AA: weak acid groups.

Differences on the size distribution of CNF samples indicates that both solid loading and reaction time conditions used for enzymatic hydrolysis influenced fiber fragmentation and/or fibrillation by ball milling through fiber structure alterations. The enzymatic cocktail makes the cellulose fibers more swollen and accessible by the breakage of the cellulose network. It can be observed that fiber surface changes occurring during enzymatic hydrolysis at 4% and 10% solid loadings enhanced fiber size reduction by ball milling (Fig. 1a). Extending hydrolysis time from 4 h to 8 h provided higher fiber fragmentation at 10% solid loading, which favored the extraction of CNF.

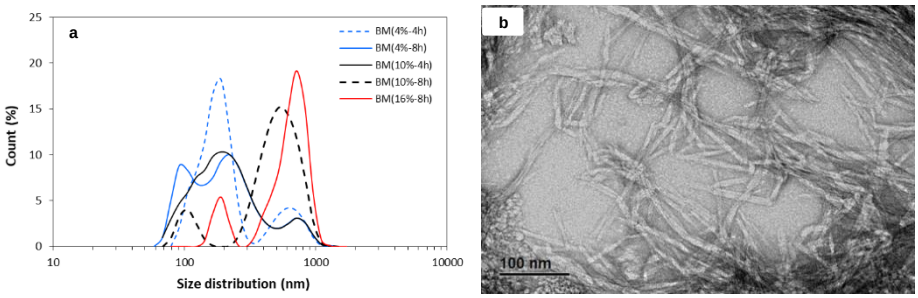

Figure 1. *CNF extraction by ball milling (BM): (a) Particle size distribution of CNF samples; (b) TEM of CNF.*

4. REFERENCES

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