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PRODUCTION OF CELLULOSE NANOFIBERS BY ENZYMATIC HYDROLYSIS IN A STIRRED TANK REACTOR

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ABSTRACT

The biorefinery concept involves the use of renewable biomass such as lignocellulosic residues for the production of biofuels together with other chemicals and bioproducts. Among these bioproducts, nanocellulose can significantly contribute to the economic viability of the overall process. However, the development of a bioprocess for large-scale production of nanocellulose remains a challenge. In this work, it was studied the estimation of scale-up parameters for the production of nanocellulose via the enzymatic hydrolysis using eucalyptus cellulose pulp as feedstock. The enzymatic hydrolysis reactions were carried out in a stirred tank reactor (5L) using 10% w/v of solid loading and enzyme loadings of 5 and 10 mg/g of cellulose. The impeller used was the up and down-pumping Elephants Ears at the rotation of 470 rpm. The system used allowed the determination of power consumption and cellulose conversion for different process conditions. The residual solids of the hydrolysis presented nanocelulose characteristics, as shown by FEG-SEM and XRD analysis. In conclusion, our results show that nanocellulose can be produced in a stirred tank reactor using the enzymatic route.

1. INTRODUCTION

The biorefineries concept integrates lignocellulosic biomass conversion processes to obtain biofuels and bioproducts with high-added value, which may contribute to reduce dependence on fossil fuels and products currently obtained from non-renewable sources (Garcia et al., 2016). Nanocellulose have attracted interest in many applications, like biomedicine, packaging and mechanical reinforcement, due their optical and mechanical properties (Lin & Dufresne, 2014). However, the sustainable production of nanocellulose at large-scale remains a challenge. The present study evaluates the production of nanocellulose in a stirred tank reactor using the enzymatic route. For that, it was carried out the estimation of scale-up parameters for the production of nanocellulose using eucalyptus cellulose pulp as feedstock. The nanocellulose produced was characterized by FEG-SEM and XRD analysis.

2. Materials and methods

2.1. Enzymatic Hydrolysis

The eucalyptus kraft pulp (by the Suzano Pulp and Paper Company - São Paulo, Brazil) was grinded to a particle size smaller than 2 mm. Cellulose pulp was then hydrolyzed in a citrate buffer 0.1 M, pH 5.0 and 50 °C, 470 rpm at 48h with the commercial enzymatic complex Cellic CTec3 (Novozymes - Bagsvaerd, Denmark) (206 FPU/g, 122 mg protein/g). Cellulose conversion were calculated by value of glucose released. Glucose concentration was monitored using an enzimatic kit GOOD-PAD (Labtest®) by fabricator protocol.

2.2 Experimental apparatus and Power Consumption analysis

Experimental apparatus used was previously described by (Correa et al., 2016) and consist in a bench-stirred tank reactor with a 5L (New Brunswick Scientific, USA). The dimensions of reactor were: tank diameter (D_T) = 0.160 m; tank height (H_T) = 0.370 m; liquid height (H_L) = 0.210 and four baffles. The Elephant Ear impellers with diameter (Di) = 0.080 m were employed with one in down-pumping mode (EEDP) and other in up-pumping (EEUP). Temperature was controlled by a thermostatic bath. The digital dynamometer (FG 6005SD, Lutron) was used by determine power consumption based on the force measured. The power consumption was determined by the Equation 1

$$P=T.\omega \qquad (1)$$

2.3 Cellulose nanofiber characterization

The solid material was evaluated by X-ray diffraction (XRD) in a Shimadzu 6000, with radiation CuKa (λ = 1.54 Å) angle 2θ = 5-40º at 2º/min. The cristallinity index (CI) was measure by (Segal et al., 1959). Morphological analysis were were evaluated by FEG-SEM (JEOL Model JSM-607 1F) microscope operated at 2.0 kV and 1.0 nm resolution.

3. Results and discussion

3.1. Enzymatic hydrolysis and power consumption determination

Enzymatic hydrolysis experiments were carried out for 48 h at solids loading (SL) of 10% and enzyme loading (EL) of 5 and 10 mg/g cellulose. Cellulose conversion was 49.6% for the EL 5 and 71.3% for the EL 10, indicating that higher enzyme loadings are required for higher conversion values. Power consumption during enzymatic hydrolysis was evaluated for the different conditions (Figure 1). A significant reduction in power consumption was observed along the reaction time, reducing from 16.8 to 3.7 W when using SL 10%, EL 5 mg/g, and from 15.4 to 3.9 W when using SL 10%, EL 10 mg/g after 48 h. The enzyme performance contributed to a reduction of power consumption by reducing the viscosity of the slurry through physically disrupting the structure of the fibrous cellulosic, thus requiring less energy for rotation of the impellers. In the initial hours, the higher power consumption observed was due the high solid content which originated a stagnant region, in which material stayed located close to the wall to reactor, affecting diffusion process, leading to limitation of cellulose conversion. This phenomena was minimized during the course of the enzymatic reaction, as also previously reported by Corrêa and co-workers (2016).

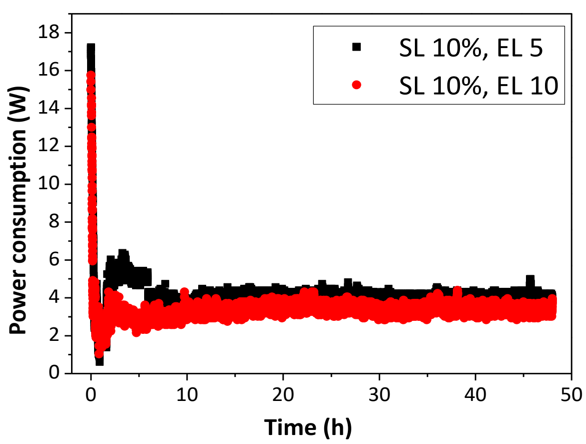

Figure 1 - Power consumption during enzymatic hydrolysis

3.2 Cellulose nanofiber (CNF)

Figure 2 show the FEG-SEM of the structures obtained after enzymatic hydrolysis for the different conditions tested. The cellulose nanofiber obtained present a rodlike structure, evidencing the nanocellulose predominance in the solid material. The reduction of amorphous cellulose and increase of crystalline cellulose was evidenced by the crystallinity index (CI) analysis. In the raw material, the CI was 72% and it increased to 82% for the condition using SL 10%, EL 5 and 83% to SL 10%, EL 10. A similar behavior was reported by Camargo, *et. al.* (2016) that observed a solid material rich in crystalline cellulose after enzymatic hydrolysis of sugarcane bagasse.

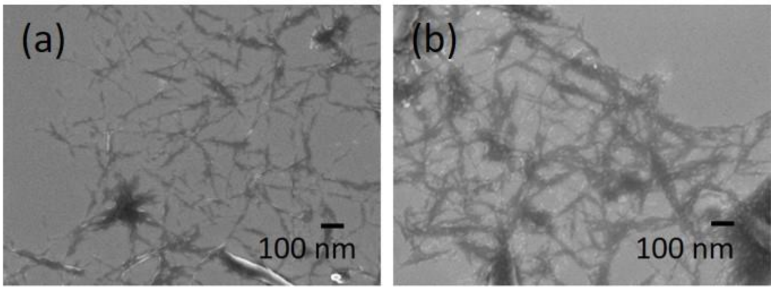

Figure 2 - Cellulose nanofiber produced in stirred tank reactor (a) SL 10%, EL 5 (b) SL 10%, EL 10

4. Conclusion

The power consumption during enzymatic hydrolysis decreased along the cellulose conversion reaction. The degradation of amorphous cellulose was verified by the presence of the crystalline domain in the cellulose nanofiber. This work encourage further studies towards the development of scale-up protocols for nanocellulose production via enzymatic hydrolysis in stirred tank reactors.

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