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Acetylation of bacterial cellulose catalyzed by citric acid: Use of reaction conditions for tailoring the esterification extent

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ABSTRACT

Bacterial cellulose (BC) nanoribbons were partially acetylated by a simple direct solvent-free route catalyzed by citric acid. The assay of reaction conditions within chosen intervals (i.e. esterification time (0.5–7 h), catalyst content (0.08–1.01 mmol/mmol AGU), and temperature (90–140 °C)), illustrated the flexibility of the methodology proposed, with reaction variables which can be conveniently manipulated to acetylate BC to the required degree of substitution (DS) within the 0.20–0.73 interval. Within this DS interval, characterization results indicated a surface-only process in which acetylated bacterial cellulose with tunable DS, preserved fibrous structure and increased hydrophobicity could be easily obtained. The feasibility of reusing the catalyst/excess acylant in view of potential scale-up was also illustrated.

1. Introduction

Cellulose fibers are known to display a hierarchical organization consisting of micrometric-in-length microfibrils composed of amorphous and crystalline domains, with diameter in the ≈2–20 nm range. Individualization of cellulose microfibrils from plant sources requires intensive mechanical treatment, in many cases aided by chemical or enzymatic pretreatments of the lignocellulosic raw materials. Besides, concentrated solutions of strong acids are commonly used to yield the so-called cellulose nanocrystals by selective removal of amorphous domains.

On the other hand, bacterial cellulose (BC) is a quite green alternative for the isolation of pure lignin/hemicellulose-free-cellulose nanoribbons with low energy demand, and no need of harsh chemicals other than diluted aqueous alkali solutions used to remove bacterial cells. Besides chemical purity, bacterial cellulose is also distinguished by its high polymerization degree, high crystallinity, high mechanical strength, and well-separated nano- and microfibrils which create an extensive surface area, and confer BC a high liquid loading capacity.

The mentioned characteristics make BC a remarkably versatile biomaterial with applications in paper products, electronics, acoustic membranes, reinforcement of composite materials, membrane filters, hydraulic fracturing fluids, edible food packaging films, and –due to its unique nanostructure and properties-, in numerous medical and tissue-engineered applications (tissue-engineered constructs, wound healing devices, etc) (Foresti, Cerrutti & Vazquez, 2015). In the widely studied use of BC as nanofiller/reinforcement, however, the OH-rich structure and high hydrophilicity of BC promotes aggregation and hampers its use in most common plastic composites. Chemical modification of cellulose aimed at reducing the number of hydroxyl interactions and increasing its compatibility with several matrices is therefore of great interest. Particularly, the possibility of conferring a hydrophobic character only to the surface of cellulose microfibrils, while keeping the integrity of their crystalline core unchanged, is a challenge that in the last years has triggered much research (Missoum, Belgacem, & Bras, 2013).

To achieve this aim, in the last decade a number of protocols devoted to the esterification (reaction in which hydroxyl

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groups are replaced by less hydrophilic ester groups) of never-dried BC pellicles, homogenized BC suspensions, and freeze-dried BC suspensions and pellicles, have been proposed. Among them, methodologies catalyzed by perchloric acid (Gonçalves et al., 2015, 2016; Ifuku et al., 2007; Kim, Nishiyama, & Kuga, 2002; Yamamoto, Horii, & Hirai, 2006), sulphuric acid (Cunha, Zhou, Larsson, & Berglund 2014; Tomé et al., 2011a, 2011b), *p*-toluenesulfonyl chloride (Blaker, Lee, Walters, Drouet, & Bismarck, 2014; Lee & Bismarck, 2012; Lee, Blaker, & Bismarck, 2009; Lee et al., 2011), iodine (Hu, Chen, Xu, & Wang, 2011), pyridine (Agustin, Nakatsubo, & Yano, 2016; Tomé et al., 2010); and α-hydroxy acids such as tartaric, lactic and citric acid (Ávila Ramírez, Gómez Hoyos, Arroyo, Cerrutti, & Foresti, 2016; Ávila Ramírez, Juan Suriano, Cerrutti, & Foresti, 2014) have been reported. Esterification of BC in palmitoyl chloride vapor (Berlioz, Molina-Boisseau, Nishiyama, & Heux, 2009) and in DMAc/LiCl (De Marco Lima, Sierakowski, Faria-Tischer, & Tischer, 2011) has also been proposed.

In reference to the esterification of BC catalyzed by α-hydroxy acids of natural origin, our previous contributions explored the esterification of BC suspensions with acetic and propionic acid catalyzed by L-tartaric acid, as well as the acetylation of BC with acetic anhydride catalyzed by L-tartaric, lactic and citric acids. The acylant was always added in great excess to provide sufficient liquid medium to disperse BC (no extra solvent was incorporated), and the extent of esterification was tuned by adjusting the esterification interval. From the results obtained in the mentioned contributions, it came clear that the α-hydroxy acid with highest activity towards BC acetylation was citric acid. The higher reactivity of acetic anhydride when compared with the corresponding acid was also evidenced (Ávila Ramírez et al., 2014, 2016). So, in the current contribution, the esterification of BC with acetic anhydride using citric acid as catalyst is further explored, but the focus of research is now shifted to the effect of reaction conditions (not only time but also temperature and catalyst content) on the DS conferred to BC. Given the novelty and potentiality of the route, the aim of this study is to gain knowledge on significant variables that would allow tailoring the acetylation degree conferred to BC. The possibility of reusing the catalyst/excess acylant is also of interest for the potential scale up of the route. Characterization in terms of chemical structure, morphology, crystallinity, thermal decomposition and wettability of acetylated BC samples as a function of DS is also provided.

2. Experimental

2.1. Materials

BC production was carried out in static culture using the bacterial strain *Gluconacetobacter xylinus,* NRRL B-42. BC culture medium was formulated using anhydrous dextrose (Biopack), meat peptone (Britania, Laboratorios Britania S.A.), yeast extract (Britania, Laboratorios Britania S.A.), disodium phosphate (Anedra), citric acid (Merck), glycerol (Sintorgan) and corn steep liquor (Ingredion). Acetic anhydride (Merck), citric acid (Merck), hydrochloric acid (Anedra) and sodium hydroxide (Biopack) were all reagent grade chemicals.

2.2. Production of BC

Inocula of *Gluconacetobacter xylinus* NRRL B-42 were cultured in 100 mL Erlenmeyers flasks containing 20 mL of Hestrin and Schramm (HS) medium (Hestrin & Schramm, 1954**)** and incubated under orbital agitation (200 rpm) for 48 h at 28 °C. For BC production, 1% (v/v) inocula were transferred to 10 L steel trays with 5.0 L of fermentation medium containing 4.0% w/v glycerol and 8.0% corn steep liquor, and statically incubated at 28 °C during 14 days. After that time the pellicles were harvested, thoroughly rinsed with distilled water to remove the culture medium, homogenized in a blender in KOH solution (5% w/v) for 5 min, left in alkali at room temperature for 14 h to eliminate the bacterial cells, and finally rinsed with distilled water till neutralization.

2.3. Esterification of BC catalyzed by citric acid

Homogenized BC (0.5 g dry weight, 3.087 mmol AGU (anhydroglucose units)) was solvent exchanged from water through acetic acid into acetic anhydride prior to reaction. Solvent exchange steps implied contacting the BC with 20 mL of acetic acid or acetic anhydride (twice each) for 10 min with stirring followed by vacuum filtration. BC was then contacted with citric acid (0.08–1.01 mmol/mmol AGU) and 50 mL (i.e. 0.53 mol) of acetic anhydride in a 100 mL glass flask equipped with a reflux condenser. The mixture was then heated to the chosen temperature (90–140 °C) under continuous magnetic agitation in a thermostatized oil bath, and acetylation was alternatively run for different reaction intervals (0.5–7 h). Reaction and reaction conditions intervals are schematized in Scheme 1.

Once the chosen reaction time interval was over, the solid product was separated by vacuum filtration and thoroughly washed with distilled water. The filtrate (acetic anhydride in excess + citric acid) was recovered for reuse assays. Reactions were performed in duplicate, being in all cases the error lower than 3%.

The extent of esterification conferred to BC was determined by heterogeneous saponification and back titration with HCl, as detailed elsewhere (Ávila Ramírez et al., 2014**)**. The acyl content and the degree of substitution achieved were then calculated as stated in Eqs. (1) and (2), respectively:

$$\text{Acyl}(\%) = [(V_B - V_S) \times N_{HCl} \times 4,3]/W \quad (1)$$

$$DS = (162 \times \text{Acyl}\%)/[4300 - (42 \times \text{Acyl}\%)] \quad (2)$$

where V_B (mL) is the volume of HCl required for blank titration, V_S (mL) is the volume of HCl required to titrate the sample, N_{HCl} is the normality of the HCl solution, and W (g) is the mass of sample used.

2.4. Characterization of esterified BC

2.4.1. Solid-state CP/MAS ^{13}C nuclear magnetic resonance spectroscopy (CP/MAS ^{13}C NMR)

High-resolution ^{13}C solid-state spectra of grinded samples were collected using the ramp $\{1H\} \rightarrow \{13C\}$ CP/MAS pulse sequence (cross-polarization and magic angle spinning) with proton decoupling. Spectra were collected at room temperature in a Bruker Avance II-300 spectrometer equipped with a 4-mm MAS probe. The operating frequency for protons and carbons was 300.13 and 75.46 MHz, respectively. Glycine was used as an external reference for the ^{13}C spectra and to set the Hartmann-Hahn matching condition in the cross-polarization experiments. According to the sample, the recycling time varied from 5 to 6 s. The contact time during CP was 2 ms. The SPINAL64 sequence (small phase incremental alternation with 64 steps) was used for heteronuclear decoupling during acquisition with a proton field H1H satisfying $\omega 1H/2\pi = YHH1H = 62$ kHz. The spinning rate for all the samples was 10 kHz.

2.4.2. Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectra of native and acetylated grinded BC samples were acquired on an IR Affinity-1 Shimadzu Fourier Transform Infrared Spectrophotometer in absorbance mode. Carefully dried (12.5 mg, 110 °C, 1 h) samples were mixed with previously dried KBr (130 °C, overnight) in the ratio 1:20 and