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Development and characterization of bacterial cellulose produced by cashew tree residues as alternative carbon source

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ABSTRACT

Bacterial cellulose (BC) has been extensively exploited for applications in materials science, biomedical and technological fields. The BC production demands culture media rich in carbon sources. Agro-forestry residues constitute an interesting source of nutrients for microorganism, but they are frequently wasted. For cashew crop, exudate is periodically extracted from the tree trunks to increase the production of cashew nut, the most valuable product from cashew trees that produces about 700 g of exudate/year, which remains wasted. Here, we associated the nutritional properties of residues from cashew tree with the need of carbon sources for BC, in attempt to valorize the residue and to decrease the costs of BC production. The carbon source from Hestrin Schramm culture medium was totally or partially replaced by cashew tree residues and the BC production was evaluated. The produced BC membrane in static medium was characterized by FTIR, SEM and TGA and the kinetics of production was determined, suggesting the cashew tree residues as a potential carbon source for BC production.

1. Introduction

The use of cellulose in paper making is one of its most noble and known applications (Klemm et al., 2005). The exceptional physical chemical properties, availability and renewability of cellulose have been widening its ranges of applications in the last decades. Encouraged by the decrease in paper demand, cellulose has been used to develop new materials and composites, including the multifunctional and/or nanostructured ones (Carreira et al., 2011; Joye and McClements 2016; Wang et al., 2016).

Cellulose is a homopolymer of D-glucopyranose residues linked by β-(1 → 4) glycosidic linkages (Esa et al., 2014), synthetized by plants and other organisms, such as bacteria, fungi and animals (Trovatti 2013; Garcia et al., 2016). The most representative producers within the bacteria kingdom belong to *Gluconacetobacter*, *Acetobacter and Komagataeibacter genera* (De Salvi et al., 2014; Shoda and Sugano 2005; Machado et al., 2016). The cellulose produced by these microorganisms, known as bacterial cellulose (BC) or biocellulose, is obtained as a gel-like three-dimensional mat formed by entangled nanofibrils of cellulose. BC is produced in high pure state and it has unique mechanical properties related to the intrinsic properties of cellulose macromolecules, associated to the morphology of the membranes woven by the bacteria, which represents a crucial point for its application in the development of composites, in which the nanofibers are used for reinforcement (Tabarsa et al., 2017). Despite the promising properties of BC, its application remains limited because of the high costs of production, including the culture media. In attempt to overcome this disadvantage, some strategies have been proposed, such as the genetic improvement of the cellulose producing microorganisms and the formulation of low cost culture media (Cacicedo et al., 2016).

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The conventional nutrient source for BC production in academic studies is Hestrin and Schramm (HS) culture medium, composed by glucose, yeast extract, peptone and mineral salts (Hestrin and Schramm 1954). Many modifications on the composition of HS medium have been proposed, such as the use of mono- and disaccharides like fructose, sucrose, maltose, cellobiose, xylose, galactose and alditols, which have been successfully used as carbon sources (Ishihara et al., 2002; Keshk and Sameshima 2005). Industrial wastes or by-products from agro-forest and food industry have been also used for BC production, for instance, Konjac powder (Hong and Qiu 2008), beet molasses (Keshk et al., 2006), sugar-cane molasses (Tyagi and Suresh 2016), corn steep liquor (Noro et al., 2004), as source of carbon, nitrogen and other nutrients.

Regarding the utilization of residues/by-products, Brazil is an essentially an agroforest country, in which cashew crop and processing represent an important branch of the food chain. The main products in cashew industry are the cashew nut and cashew juice, and the main by-products are cashew pulp and the exudate. Exudate is periodically extracted from the tree trunks to increase the production of cashew nut, the most valuable product from cashew trees. Each tree of the specie *Anacardium occidentale* produces about 700 g of exudate/year, which remain wasted (Costa et al., 1996). The cashew tree exudate (CTE) is rich in mono- and oligosaccharides, arabinogalactan-protein and mineral salts (Menestrina et al., 1998; Pereira-Netto et al., 2007; Rodrigues et al., 1993; Silva et al., 2010). CTE is constituted of *ca.* 70% of a branched heteropolysaccharide, the called cashew gum (CG). Its main polymeric chain is formed by D-galactopyranose units linked by β-(1 → 4) glycosidic linkages (Rodrigues et al., 1993). Arabinose, ramnose, glucose, and glucuronic acid are other sugars that can integrate the branched chemical structure of CG. The annual production of CTE and CG is at least 68.000 and 48.000 tons/year, respectively (Rodrigues et al., 1993). These large amounts of wasted by-products show the economic importance in adding value to these residues.

These residues have been extensively used in pharmaceutical industry (as excipient binder), in biotechnology industry and food industry (in beverages, as thickening agent, gelling agent and colloidal stabilizer) (Kumar et al., 2012). However, it is the first time that cashew crop residues are exploited as a source of nutrients for BC production.

Considering that BC has shown tremendous potential as an effective biopolymer in various fields, the use of CTE and CG as a source of nutrients for its production is a good opportunity to reuse the wastes, adding value to these by-products which can decrease the costs of BC production. Thus, the hypotheses of this research is to use CTE and CG as a source of nutrients for BC production. For such, the carbon source from HS medium was replaced (totally or partially) by CTE or CG. BC production was determined and BC was characterized to check if it kept its intrinsic properties.

2. Materials and methods

2.1. Materials

Anhydrous D-glucopyranose and ethanol, citric acid, KH_2PO_4, Na_2HPO_4, $MgSO_4{\cdot}7H_2O$, all P.A. grade were purchased from Synth®. Bacteriologic peptone, agar and yeast extract were purchased from Merck. CTE was collected at Center of Agrarian Sciences – CCA at Federal University of Piauí (UFPI), Teresina, Piauí state, Brazil, and used as such, or after purification steps (gum polysaccharide, CG).

2.2. CTE purification – isolation of CG

CG isolation was carried out using the methodology described by Rodrigues et al., 1993, with some modifications. Shortly, an aqueous solution of CTE (10%, w/v) was prepared and stirred for 12 h. The solution was then neutralized with 1 M NaOH solution and filtered. CG was subsequently precipitated by adding ethanol (4× in volume) to the CTE aqueous solution. Precipitated CG was separated by filtration and successively washed with ethanol and acetone, generating a white powder, which was oven dried (30 °C) for 24 h and stored at room temperature.

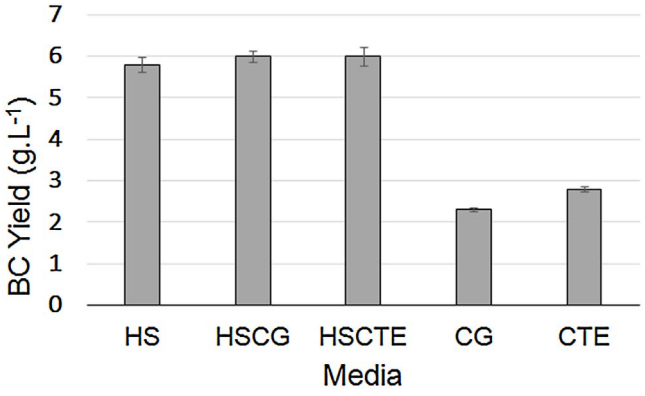

Fig. 1. BC production from HS, HSCG, HSCTE, CG and CTE at 7th days time point.

2.3. Microorganism maintenance and pre-inoculum

Komagataeibacter rhaeticus previously isolated by Santos et al. (2014) was maintained in HS solid culture medium at 4–8 °C. One single colony from the solid medium was used to inoculated into 50 mL of modified HS liquid medium (50 g L^{-1} glucose, 4 g L^{-1} of yeast extract, 0.73 g L^{-1} of $MgSO_4{\cdot}7H_2O$, 2 g L^{-1} KH_2PO_4, 20 g L^{-1} ethanol and distilled water, 1000 mL, pH 6.5) and incubated at 28 °C for 24 h in static condition. The liquid medium was then homogenized and used as the pre-inoculum.

2.4. BC production and culture media

The production of the BC membranes was carried out by cultivation of 5 mL from the previously prepared *K. rhaeticus* suspension in 45 mL of liquid culture media in a 250 mL Erlenmeyer flask, at 28 °C for 168 h (7 days), without agitation. HS was used as the standard culture medium. The media prepared using cashew residues as alternative carbon sources were formulated by replacing total or partial glucose by CTE or CG and labeled HSCTE (25 g L^{-1} glucose plus 25 g L^{-1} of CTE), HSCG (25 g L^{-1} glucose plus 25 g L^{-1} of CG), CG (50 g L^{-1} of CG) and CTE (50 g L^{-1} of CTE).

2.5. Production kinetics, purification and drying the BC membranes

The samples for BC production kinetics study were carried out at 0, 48, 72, 96, 120, 144 and 168 h time points using the best culture medium (HSCTE) based on BC production. It was not possible to evaluate the BC production at 24 h because only a thin and no homogeneous membrane was obtained. The BC membranes were exhaustively washed with distillated water in order to remove the impurities from the culture medium. Then, they were treated with an aqueous solution of 0.1 M NaOH at 80 °C for 45 min and washed with distillated water until neutral pH. The purified BC membranes were dried at 38 °C for 48 h and weighted.

2.6. Field emission gun scanning electron microscopy (FEG-SEM)

SEM experiments were carried out using samples previously coated with evaporated carbon. The images were obtained using the JEOL T-300 microscope operating at 2 kV.