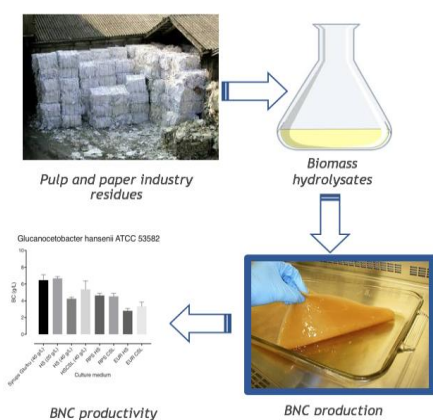


Bacterial cellulose production through hydrolysates produced with cellulosic residues

F. Garrett^{*}, A. Rodrigues, F. Dourado, M. Gama³

¹CEB - Centro de Engenharia Biológica, departamento de engenharia biológica, Universidade do Minho, Gualtar, Braga, Portugal.

*francisco.agss@gmail.com



In the paper industry, significant fraction of fibers that cannot be re-utilized are wasted by the paper companies, which raise economic and environmental concerns. An increasing demand of bacterial cellulose (BC) has been noticed in the last years. In order to ally the recycling of lignocellulosic residues and the production of bacterial cellulose, Recycled paper sludge (RPS) and rejected fibers (EUR) were enzymatically hydrolyzed to obtain sugar hydrolysates, which were used for BC production. Exploratory assay (different strains and nitrogen sources) was performed with RPS and EUR hydrolysates. RPS hydrolysate showed to be an interesting alternative carbon source for *G. hansenii* (5 g/L of BC) and EUR hydrolysate showed potential as carbon source for *G. xylinum* (4-5 g/L of BC). Overall, the results suggest that RPS and EUR residues have potential to be alternatives of carbon source for BC production, after a further optimization of the BC production and the enzymatic hydrolysis.

In the paper industry, significant fraction of fibers that cannot be re-utilized are wasted by the paper companies, which raise economic and environmental concerns [1]. Additionally, aggressive methods to retrieve the cellulose are used for papermaking, such as acid hydrolysis. Therefore, alternative materials were being studied in the last decade[2,3].

An increasing demand of bacterial cellulose (BC) has been noticed in the last years. Bacterial cellulose (BC) is a known polymer produced by Acetobacteraceae family, which consist of a wide group of strictly aerobic, gram negative bacteria[4]. Among several genera of the Acetobacteraceae family, acetobacter and komagataebacter genus are known have high yields of BC production. The interest in producing bacterial cellulose is due to the advantages that this product offers among the unique characteristics such as high porosity, high water retention capacity, low density, biocompatibility, non-toxicity and biodegradability, which promotes a variety of applications in the food industry as food additive, in paper industry as coating material and in the biomedical industry as regenerative material[2]. However, the production of BC still presents challenges, as high production costs[5]. Therefore, the use of low-value waste is an alternative that may lower the cost of producing BC and at same time, take advantage of using by-products of the papermaking industry[6]. In order to ally, the recycling of lignocellulosic residues and the production of bacterial cellulose, the production of bacterial cellulose through lignocellulosic residues has been studied. Recycled paper sludge from RENOVA and rejected fibers from EUROPAC were kindly supplied for this study. Recycled paper sludge (RPS) is a residue originated from the paper recycling process, more specifically, from the treatment of the liquid effluents generated in that process. It is mostly composed of small fibers with approximately 40% of carbohydrates that cannot be incorporated on recycled paper [7]. Rejected fibers (EUR) is a residue originated from the paper pulp production.

Therefore, the goal is to obtain interesting yields of BC from this lignocellulosic residues, which could be difficult due to contaminants present in the residues and the availability of the cellulose in the residue.

To achieve the goals established above, RPS and EUR residues were hydrolyzed enzymatically (with Cellic Ctec 2;

Novozymes) in order to obtain the hydrolysate for bacterial cellulose production [7]. Along with enzymatic hydrolysis, acid hydrolysis were also performed in order characterize each residue in terms of cellulose and hemicellulose present in the residues [4]. The concentration of reducing sugars retrieved through the saccharification process were 54 g/L (yield recovery sugars of 69,58 %) and 24 g/L (yield recovery sugars of 42,30%) for RPS and EUR respectively.

Table 1: Characterization of RPS and EUR

Sample	RPS	EUR
glucans (%)*	30	30
Xylans (%)*	16,11	17,8
Reducing sugars (g/L)**	54,24	24,29
yield recovery sugars (%)**	69,58	42,30

Ac Acid hydrolysis characterization

E Enzymatic hydrolysis results

After hydrolysate production, an assay of BC production through static fermentation for 9 days at 30 °C was performed with the goal of optimizing the yield of BC by testing two different strains (*Glucanocetobacter hansenii* ATCC 53582 and *Glucanocetobacter xylinus* ATCC 700178) and nitrogen sources (combination of yeast extract/peptone and corn steep liquor (CSL)) on the hydrolysates previously prepared [8]. For the strain *G. hansenii*, the highest yield (6 g/L) was obtained with the standard medium (Hestrin & Schramm medium). However, RPS hydrolysate showed to be an interesting alternative carbon source for *G. hansenii* since the yield of BC with RPS was around 5 g/L. For the *G. xylinum* strain, EUR hydrolysate (4-5 g/L) is shown as an interesting alternative to HS medium (2-3g/L). Overall, the results suggest that RPS and EUR residues have potential to be alternatives of carbon source for BC production, after a further optimization of the BC production and the enzymatic hydrolysis.

Acknowledgements

The authors wish to thank Daniel Gomes for technical help. This work received financial support from FCT via project ID520405.ID1956.

References

- [1] C. Sánchez, *Biotechnology Advances*, 27 (2009) 185-194.
- [2] A.F. Jozala et al., *Applied microbiology and biotechnology*, 100 (2016) 2063-2072.
- [3] A.F. Jozala, et al. *Applied Microbiology and Biotechnology*, 99 (2015) 1181-1190.
- [4] M. Gama, F. Dourado, S. Bielecki, *Bacterial nanocellulose: From biotechnology to bio-economy*, 2016, Elsevier.
- [5] F. Esa, S.M. Tasirin, N.A. Rahman, *Agriculture and Agricultural Science Procedia*, 2(2014) 113-119.
- [6] P. Carreira et al., *Bioresource technology*, 102 (2011) 7354-7360.
- [7] S. Marques et al., *Biological upgrading of wastes from the pulp and paper industry*. 2009.
- [8] H.-I. Jung, et al., *Applied biochemistry and biotechnology*, 162 (2010) 486-497.