# Evaluation of different acid hydrolysis in sugarcane bagasse for cellulose-rich extract production



Ricardo Freixo, Francisca Bastos, Alessandra Ribeiro, Carla Pereira\*, Eduardo Costa, &

Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal \*oramos@porto.ucp.pt; cpereira@porto.ucp.pt

PORTO

# Introduction/Resume

Sugarcane bagasse, the major by-product of the sugarcane industry and a very promising renewable and low-cost raw material, can be used to produce valuable materials such as cellulose or cellulose-based materials. This lignocellulosic biomass constituted mainly by cellulose, hemicellulose and lignin, 42, 28 and 20%, respectively, can be cleaved by enzymatic or acid/alkaline hydrolysis, thus releasing compounds such as glucose, xylose, furfural or xylo-oligosaccharides (Kim and Day, 2011). One of the major barriers to lignocellulosic biomass valorization is its recalcitrance to hydrolysis caused by the hierarchy structure of the plant cell wall (i.e. the strong, rigid and complex microfibril structure of the cellulose-lignin-hemicellulose matrix) (Wyman, Cai et al. 2017). Therefore, to address this and facilitate the access to isolated compounds as celulose for further production of high-value products, different treatments have been tested to obtain fractions rich in cellulose

# **Objectives**

The aim of this work was to obtain a fraction rich in cellulose from sugarcane bagasse through cost-effective process. For this purpose, the impact of different acid hydrolysis treatments were evaluated in the remotion of lignin and hemicellulose in order to produce solid fractions rich in cellulose. Thus, the structural composition of solid fractions rich in cellulose obtained by 4 different acid hydrolysis treatments were characterized in terms of level of cellulose, hemicellulose, Klason lignin and acid soluble lignin by NREL procedure (Sluiter et al., 2008). Also, the functional groups and crystallinity were assessed by Fourier-Transform Infrared spectroscopy (FT-IR) and Powder X-Ray Diffraction (PXRD) analysis, respectively.

### Material and Methods

The sugarcane bagasse was dried and sieved in a blade mill and the fraction greater than 315 μm was selected. After this, the biomass was subjected to four different acid treatments, HA1, HA2, HA3 and HA4 according to Guerra-Rodríguez et al. (2012), Laopaiboon, Thani et al. (2010) and Kumar, Dheeran et al. (2015), respectively. The procedures were summarized

Sulfuric acid solution prepared at 2% (w/v) was added to bagasse at a 12:1 ratio (w/w). Then, the solution was heated in a water bath at 100 °C for 30 min. The solid fraction obtained after filtration was washed with water until neutrality and then dried at 55 °C overnight in an oven.

Hydrochloric acid solution prepared at 0.5% (v/v) and preheated, was added to bagasse at a 15:1 (v/w) ratio. Then, the solution was heated in a water bath at 100 °C for 4h under agitation. The solid fraction obtained after filtration was washed with water until neutrality, and then dried at 55 °C overnight in an oven.

# Acid hydrolysis 3 (HA3)

Sodium hydroxide solution prepared at 5% (w/v) using a 10 M NaOH solution, was added to bagasse at a ratio of 1:20 (v/w). Then, the solution was heated in a water bath at 80 °C for 60 min under agitation. The solid fraction obtained after filtration was washed with water until neutrality and dried at 50 °C until constant weight. Afterwards, hydrochloric acid solution prepared at 0.5% (v/v) and preheated, was added to the solid fraction previously obtained at a 15:1 (v/w) ratio. The solid fraction obtained after filtration was washed with water until neutrality, and then dried at 55 °C overnight in an oven.

### Acid hydrolysis 4 (HA4)

Sulfuric acid solution prepared at 8% (v/v) was added to bagasse at a ratio of 8:1 (w/w), Then, the solution was heated in autoclave at 100 °C for 90 min. The solid fraction was washed with water until neutrality, and then dried at 55 °C overnight in an oven. Afterwards, a sulfuric acid solution prepared at 40% (v/v) was added to the solid fraction previously obtained, at a ratio of 1:4 ratio (w/w). The solution was heated in a water bath at 80 °C for 90 min under agitation, while the solid fraction was washed with water until neutrality, and then dried at 55 °C overnight in an oven.

#### Chemical characterization

The solid fractions obtained from acid treatments were characterized in terms of lignocellulosic composition by National Renewable Energy Laboratory (NREL) following the analytical procedures for standard biomass analysis (Sluiter et al., 2008). The structural characterization was assessed by FT-IR and PXRD analysis. The FT-IR spectra were recorded using the Frontier™ MIR/FIR spectrometer from PerkinElmer in a scanning range of 550-4000 cm $^{-1}$  for 16 scans at a spectral resolution of 4 cm $^{-1}$ , while the PXRD analyses were performed on Rigaku MiniFlex 600 diffractometer with Cu k $\alpha$  radiation, with a voltage of 40 kV and a current of 15 mA (3°≤ 2θ ≥ 60°; step of 0.01 and speed rate of 3.0°/min).

# Results

The data obtained from NREL, photos from the solid fraction, FT-IR and XRD characterization for solid fractions rich in cellulose obtained from different acid treatments (HA1-HA4) were presented in Figure 1, 2, 3 respectively and Table 1.

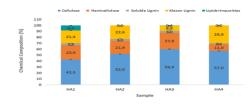


Figure 1. Chemical composition of solid fractions rich in cellulose affected by the different acid treatments









Figure 2. Visual aspect of the solid fractions rich in cellulose affected by the different acid treatments (HA1-

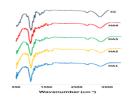


Figure 3. FT-IR spectra of solid fractions rich in cellulose affected by the different acid treatments (HA1-HA4) applied in comparison with the data obtained for a commercial cellulose from Sigma-Aldrich (CC).

Table 1. Crystallinity index of solid fractions rich in cellulose by the different treatments (HA1-HA4)

Treatment	Crystallinity index (%)
HA 1	57.0
HA 2	59.4
HA 3	69.3
HA 4	65.9

### Conclusions

The treatment described as acid hydrolysis 3 (HA3), comprising two steps hydrolysis i.e., an alkaline hydrolysis with sodium hydroxide followed by an acid hydrolysis with hydrochloric acid, showed to be the most promising one in lignin removal, producing a solid fraction rich in cellulose (i.e., 59.9%) (Fig. 1) exhibiting the highest crystallinity (i.e., 69.3%) (Tab. 1) and whitter color (Fig. 2) compared to the other treatments. On other hand, the treatment 4 (HA4) allowed a greater removal of hemicellulose, but the lowest lignin removal proved by the darker color exhibited by the solid fraction (Fig. 2).

#### Acknowledgements

This research was funded by Amyris Bio Products Portugal Unipessoal Lda and Escola Superior de Biotecnología — Universidade Católica Portuguesa through Alchemy project- Capturing high value from industrial fermentation bio products (POCI-01-0247-FEDER-027578). We would also like to thank the scientific collaboration under the Fundação para a Ciência e a Tecnologia (FCT) project UIDB/50016/2020.







References
Guerra-Rodríguez, E., Portilla-Rivera, O., Jarquín-Enríquez, L., Ramírez de León, J., & Vázquez, M. (2012). Acid hydrolysis of wheat straw: A kinetic study. Biomass and Bioenergy, 36, 346-355. doi: 10.1016/j.biombioe.2011.11.005
Kumar, S., Dheeran, P., Singh, S. P., Mishra, I. M., & Adhikari, D. K. (2015). Kinetic studies of two-stage sulphuric acid hydrolysis of sugarcane bagasse. Renewable Energy, 83, 850-858. doi: https://doi.org/10.1016/j.renene.2015.05.033
Kim, M. & Day, D. F. (2011). Composition of sugar cane, energy cane, and sweet sorghum suitable for ethanol production at Louisiana sugar mills. Journal of Industrial Microbiology and Biotechnology, 38, 803-807.
Laopaiboon, P., Thani, A., Leelavatcharamas, V., & Laopaiboon, L. (2010). Acid hydrolysis of sugarcane bagasse for lactic acid production. Bioresource Technology, 101(3), 1036-1043. doi: https://doi.org/10.1016/j.biortech.2009.08.091
Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Suiter, J., Templeton, D., & Crocker, D. (2008). Determination of Structural Carbothydrates and Lignin in Biomass—NREL/TP-510-42618. Laboratory Analytical Procedure (LAP).
Wyman, C. E., Cai, C. M., & Kumar, R. (2017). Bioethanol from Lignocellulosic Biomass. In R. A. Meyers (Ed.), Encyclopedia of Sustainability Science and Technology (pp. 1-27). New York, NY: Springer New York.