Improvement of the enzymatic hydrolysis of olive stones by two-stage pretreatment of acid hydrolysis and organosolv

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Lignocellulosic wastes are the most common and quantitatively relevant wastes produced by agro-food industries. Their chemical composition makes them particularly suitable to be upgraded in co-located/associated biorefineries. Among many examples, the wastes derived from the olive oil sector are particularly relevant for Mediterranean countries. Specifically, Olive Stones (OS) are generated in the olive oil and olive pomace mills in large quantities, but notwithstanding their interesting chemical composition, the high recalcitrance of this material, as compared to other lignocellulosic residues, is still hampering its integral valorisation within the biorefinery concept. This work aims to develop an effective pretreatment strategy, based in a two-step approach to overcome this recalcitrance.

In order to selectively fractionate the macromolecular components, different pretreatment methods can be applied. The dilute acid pretreatment is one of the most widely used and it is considered an efficient method for the solubilisation and upgrade of the hemicellulosic fraction. On the other hand, organosolv fractionation may enable the selective delignification. When used in conjugation, these methods may allow a significant increase of cellulose content of the processed solids and of its digestibility.

The OS used in this work present a content of 21% cellulose, 28% hemicellulose (including hemicellulosic sugars, 25%, and acetyl groups, 3%), 33% lignin and 4% extractives. In order to selectively hydrolyse the hemicellulosic fraction an acid pretreatment with sulphuric acid in autoclave was carried out under the optimal conditions reported by Padilla-Rascón *et al* (2020), e.g. 128 °C, a 1/3 solid/liquid ratio and a sulphuric acid concentration of 10.5 g/100 g biomass. The solid fraction obtained in the acid pretreatment, after washing and drying, was used in the second fractionation stage, consisting of an organosolv pretreatment carried out with ethanol:water (50:50) in a Parr reactor. The experimental conditions were 1/6 solid/liquid ratio at 190 ºC, the reaction time varied from 0 min to 150 min. All solids obtained in both stages were chemically characterised and subjected to enzymatic hydrolysis for solubilisation of the cellulosic fraction. Saccharification experiments were carried out with Cellic® CTec2 enzyme in a buffer solution (pH 5) at a concentration of 15 FPU/g substrate and 5% solid ratio w/v was used and kept in agitation for 72 h at 50 °C.

The liquid fractions obtained in all steps were analysed for sugars, furans (5-hydroximethylfurfural and furfural) and aliphatic acids (acetic, formic and levulinic acid) in an HPLC, with an ICSep ICE-COREGEL 87H3 column operating at 65 ºC with 5 mM sulfuric acid as mobile phase (0.6 mL/min). The solids obtained in the acid and organosolv pretreatments were characterised for their content in structural carbohydrates, lignin, and ash, according to NREL methodology (Sluiter et al., 2012).

In the first acidic stage, a xylose-rich liquor with a concentration of 62 g/L xylose (78.9% recovery) was achieved. Despite the efficient hydrolysis of hemicellulose the enzymatic hydrolysis yield for the solid fraction from the first acidic stage was only 2%, clearly indicating the need for a subsequent pretreatment.

The solid obtained after the second stage (organosolv pretreatment), retained almost all of the initially available cellulose, and in contrast to the solids obtained in the first stage they exhibited a remarkable increase of the enzymatic hydrolysis yield, exceeding 80% of the potential glucose in the OS.

The results obtained showed that this two-stage experimental methodology had a deep impact on the recovery of products from OS. The first acid stage was efficient for obtaining a liquor rich in xylose, appropriate for conversion into furfural or xylitol. The subsequent organosolv stage allowed an important removal of lignin and its recovery in the liquid phase from which it was selectively precipitated with water and then characterized by Capillary Zone Electrophoresis that enabled the identification of relevant phenolic compounds. These results, in particular the efficient overall saccharification of OS that enabled the recovery of 41g of sugars per 100 g of OS, equivalent to 81.8% total sugars, reported for the first time in this work, together with the lignin-derived products recovered from organosolv liquors, provide a promising approach for the effective valorization of OS in a biorefinery framework.

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