Grape crop waste biomass as feedstock for a lignocellulosic biorefinery: effect of Deep Eutectic Solvent on biomass fractionation

A. Duque, C. Alvarez, J.M. Oliva, R. Iglesias, P. Manzanares
Keywords: vine shoot, enzymatic hydrolysis, choline chloride, delignification
Advanced Biofuels and Bioproducts Unit, Renewable Energies Department, CIEMAT, Madrid, 28040, Spain
Presenting author email: aleta.duque@ciemat.es

Introduction

Lignocellulosic biorefineries, through the production of bioenergy and bioproducts from lignocellulosic biomass (LB) can significantly contribute to decrease the oil dependence in transport and chemical sectors, while making a more sustainable and efficient use of biomass resources. This strategy is particularly important in the context of the use of residual biomass from some widely grown agricultural crops, as is the case of the grapevine. Grape crop is a very important economic activity that generates yearly large amounts of agricultural residues, particularly vine shoots (VS). VS biomass is generated during elimination of dry VS operations (pruning), resulting in an average yield of 2 to 4 tons of these residues for each hectare of cultivation (Nabais *et al.*, 2010). According to FAOSTAT statistics (FAOSTAT, 2022), close to 6.9 million hectares were cultivated in the world in 2020, with close to 45% of the total area being located in Europe. Currently, VS biomass has a limited use as a fuel in power generation industries and mainly in domestic applications. The most common practice is to leave the VS in the field as organic fertilizer, or to burn them, with the associated negative environmental impacts. Thus, a new valorization scheme of VS biomass as feedstock in a biorefinery can be an interesting approach in the future.

To undertake VS valorization in a biorefinery scheme, a first step is the pretreatment, which is required to break down the LB structure of the material and facilitate the recovery and use of main lignocellulose components, i.e., carbohydrates (cellulose and hemicellulose) and lignin, in the subsequent conversion process stages. Among the different pretreatment technologies reported in the literature to successfully fractionate LB, the use of deep eutectic solvents (DES) has attracted great attention in the last decades as an environmentally-friendly technique to be applied in LB processing. DES are mixtures of two different constituents (one acts as hydrogen bond donor while the other is hydrogen bond acceptor) at certain ratios. The key feature of DES is that the melting point of the mixture is much lower than its components due to the strong hydrogen-bonding interaction between them. DES have been claimed to be an interesting alternative to the use of ionic liquids (ILs), due to they are easy for preparation, low cost, low toxicity, and high biodegradability. In this work, a DES mixture consisting of choline chloride (ChCl) and lactic acid has been tested as fractionation method for VS biomass and the results have been evaluated in terms of lignin removal and enzymatic digestibility of the pretreated material. For comparison purposes, the same pretreatment has been applied to cereal straw biomass.

Materials and Methods

VS biomass was kindly supplied by VanMander S. L (Santa Margalida, Barcelona, Spain) and wheat straw (WS), used as reference for an herbaceous biomass, was a material previously provided by WIP Renewable Energies (Munich, Germany), in the context of another research project. Both biomasses were milled at 2 mm. Choline chloride (≥ 98%) and lactic acid (≥85%) were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany).

Compositional analysis was carried out through the analytical methods described by Sluiter et al. (2012). DES solution was prepared mixing choline chloride and lactic acid in a molar ratio 1:5. The mixture was incubated for 30 min at 60ºC in an orbital shaker at 100 rpm, until the colour is clear and no traces of solids are left in the solution. For pretreatment experiments, 1.25 g dry biomass were mixed with 25 ml of the DES solution or distilled water (in the case of control experiments) were added. For the experiments at low temperature, Erlenmeyer flasks were incubated in an orbital shaker at 60 ºC and 100 rpm for 14 h. In the case of high temperature experiments, the reaction took place in pressure tubes placed inside an oven at 120ºC for 6 h.

After the pretreatment, the reaction mixture was filtrated under vacuum by 0.45µm filters. The first filtrate was collected and distilled water was added (4 times the volume of filtrate) to precipitate the extracted lignin. This liquid was left overnight in the refrigerator and next day it was centrifuged in Falcon tubes at 9,000 rpm for 15 min to recover the precipitated material. The resulting pellet was washed twice with distilled water and centrifuged again between washes. The tubes were then dried in a vacuum oven at 40 ºC and the weight was recorded. When possible, a sample of the pellet was taken to perform compositional analysis.

After collecting the first filtrate, the solid residue was washed thoroughly with hot water until pH of the filtrate was neutral and dried in an oven at 40 ºC. A portion of the dried residue was taken for compositional analysis, while the rest was submitted to saccharification to evaluate the amount of sugars that could be produced.

Enzymatic hydrolysis (EH) was performed in 50 ml Erlenmeyer flasks at 5 % solids, adding 15 FPU/ g substrate of a cellulolytic cocktail (SAE0020, Sigma-Aldrich, Co.). The flasks were incubated in an orbital shaker

at 50 ºC and 150 rpm. Sugars were measured by high performance liquid chromatography as explained in a previous work (Duque *et al*., 2020).

Results

The composition analysis of vine shoot shows the following results (in % dry weight basis): glucan, 35.5; hemicellulose, 18.4; acid insoluble lignin, 25.4, extractives, 8.3 and ash, 3.6. On the other hand, wheat straw biomass contained 33.7 glucan, 22.7 hemicellulose, 14.8 acid insoluble lignin, 10.8 extractives and 6.8 ash.

The mass balances after pretreatment show that the amount of solubilised material in the pretreatment carried out at 120ºC was 12.2% of the raw material for the vine shoot and 7.8% for wheat straw. Nevertheless, at 60ºC, the solubilisation is negligible and would likely correspond to fine biomass particles, as it can be deducted by comparison with the control samples, whose values of solids recovery in the filtrate are very similar. The pretreatment with DES seems to be quite selective and it almost does not solubilise glucan or xylan. In contrast, the lignin content in the solid residue of the biomasses pretreated at 120ºC was noticeably lower, resulting in a delignification of about 30% for WS and almost 50% for VS.

Another important variable to evaluate the success of the pretreatment with DES was the sugar production potential from the solid residues submitted to EH. The results of these experiments (in % of the maximum theoretical that could be achieved if all glucan and xylan in pretreated substrates are fully hydrolysed) are presented in Figure 1.

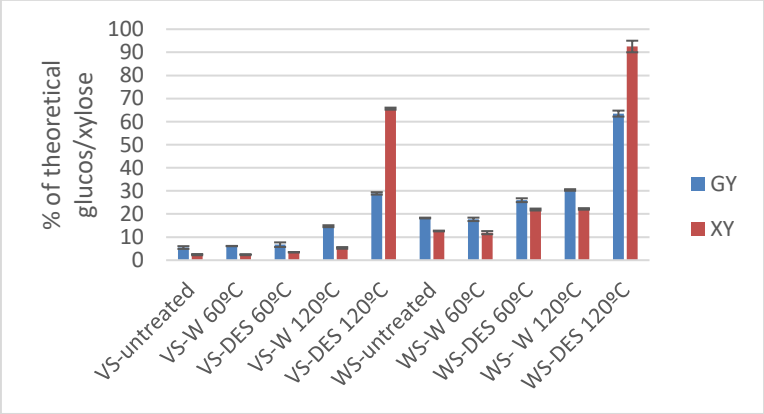

Figure 1. EH yield of the pretreated biomass with respect to glucan or xylan content in the pretreated material.

As it can be seen in Figure 1, experiments at 60ºC do not have any effect in the case of VS, while only a small improvement of the hydrolysis yield with respect to the raw material is observed for DES-pretreated WS at this temperature. However, the pretreatment at 120ºC is undoubtedly more effective: glucan hydrolysis yield reaches almost 30% in the case of VS, whereas more than 60% of the glucan is hydrolysed in the pretreated WS at 120ºC. The effects are even higher for the xylan in the pretreated biomasses. Thus, xylan hydrolysability increases to 65% and over 90% for VS and WS, respectively. Nevertheless, these results are highly conditioned by the actual amount of xylan left in the solid residue.

Summarizing from the results shown above, it can be concluded that, in general, vine shoot proved to be more difficult to pretreat and convert into fermentable sugars than wheat straw. Also DES pretreatment at low temperature and long times was not very effective in fractionating the biomass or increasing its enzymatic accessibility. On the contrary, higher temperatures and shorter times resulted in the delignification of the biomass up to almost 50%, in the case of VS. Moreover, the enzymatic digestibility of the pretreated biomasses was increased by 3-5 fold for glucan and 7-27 fold for xylan, for VS and WS biomass, respectively.

Acknowledgements

Grant PID2020-112594RB-C32, funded by MCIN/AEI/ 10.13039/501100011033.

References

Duque, A.; Domènech, P.; Älvarez, C., Ballesteros, M.; Manzanares, P. (2020) Renewable Energy 158, 263-270

FAOSTAT (2022). http://faostat.fao.org (last accessed 18.02.22).

Nabais J.M.V., Laginhas C., Carrott P.J.M., Carrott M.M.L.R. (2010) Journal of Analytical and Applied Pyrolysis 87(1) 8-13.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Crocker, D. (2012) Determination of Structural Carbohydrates and Lignin in Biomass, Golden, CO, USA.