Utilisation of ethylene glycol- based deep eutectic solvent for enhancing cellulose enzymatic digestibility of vine shoot biomass

A. Duque, I. Higueras, R. Iglesias, P.Manzanares

Biofuels Unit. Renewable Energies Division. CIEMAT.

Avda. Complutense 40, 28040 Madrid-Spain.

Keywords: lignocellulosic biomass, deep eutectic solvents, enzymatic hydrolysis

Presenting author email: aleta.duque@ciemat.es

Vineyards are a relevant cultivation in Europe, occupying 3.2 million hectares of land (or 2.0% of the utilized agricultural area) in the European Union in 2020 (Eurostat, 2021). Spain (with 0.9 million hectares), France and Italy are the major producers within the EU. Grape crop generates a big amount of residual biomass from the pruning of vines, the vine shoots (VS), which is currently underutilised. At present, VS are usually left on the land as soil conditioner, or directly burnt in the field (Bisaglia and Romano, 2018). In contrast to these traditional methods, biomass refining for the production of bioproducts and bioenergy offers an opportunity for the valorisation of these residues and their integration into a circular economy.

Lignocellulosic biomass, such as VS, is mainly composed of cellulose, hemicellulose and lignin, forming a very compact and recalcitrant structure. For this reason, a previous step known as pretreatment is usually needed to facilitate the access to the carbohydrates in the biomass. Among the variety of pretreatment methods, deep eutectic solvents (DES) have attracted a growing interest as a "green" method for the fractionation of biomass due to their superior solvent capacity (Ab Rasid, 2021). Deep Eutectic Solvents (DES) are eutectic mixtures of hydrogen donors and acceptors with an atypically low melting point. They share some physico-chemical characteristics with ionic liquids, but are considered to be less expensive, easier to prepare and more environmentally friendly than those solvents (Hansen, 2021).

In the present work, VS are pretreated with a DES formed by choline chloride and ethylene glycol (ChlCl:EG) to enhance the enzymatic susceptibility of the biomass to hydrolytic enzymes, with the final aim to maximise monomeric sugar production. A factorial 2^3 experimental design was proposed to test the effect of temperature, solids load, and particle size on the DES pretreatment. The conditions chosen for the experimentation are summarised in Table 1.

Table 1. Experimental design factors and corresponding levels.

Factor	Units	Low level	High level
Temperature	ºC	120	150
Solids load	% (w/w)	5	10
Particle size	mm	0.5	2

Vine shoots were obtained from VanMander S. L (Santa Margalida, Barcelona, Spain), milled at 2 mm at CEDER-CIEMAT (Soria, Spain) and stored in plastic bags until use. DES was synthesized by mixing choline chloride and ethylene glycol (reagent grade, Sigma-Aldrich Chemie GmbH, Germany) at a molar ratio of 1:2 at 60ºC and 100 rpm until complete dissolution.

The pretreatment of VS with ChlCl:EG was carried out in pressure glass tubes at 5 or 10% w/w solids. The tubes were incubated at the chosen temperature for 17h in an oven. After this time, the content of the tubes was filtrated under vacuum and the first filtrate was collected. Water was added as antisolvent and the filtrate was left in the refrigerator overnight. The pretreated solid was then washed thoroughly with hot water to eliminate the DES from the pulp. The washing water was also collected and saved refrigerated for further processing. The washed solid was dried in an oven at 40ºC. A sample of this solid was used for compositional analysis and other fraction was submitted to enzymatic saccharification to determine its sugar release potential. Saccharification experiments were carried out in 100 ml flasks at 5% w/w solids, with the addition of 15 FPU/g substrate of a commercial enzymatic cocktail (SAE0020, Sigma-Aldrich, Co.). The flasks were incubated at 50ºC and 150 rpm for 72h and a sample was taken at the end of the hydrolysis to determine by HPLC the monomeric sugars concentration. After the resting time, the first and second filtrate were centrifuged at 9,0000 rpm for 15 min. The supernatant was removed and the pellet was washed twice with distilled water, then dried in a freeze drier and collected.

The results presented in Figure 1 show that 120ºC are not enough to provoke a strong reaction of the ChlCl:EG with VS. In fact, the carbohydrate conversions at this temperature were very similar to that of the raw

material (10% glucan conversion, and 2% xylan conversion). However, at 150ºC, the pretreated substrates showed a better saccharification efficiency that reached almost 50% and 60% for glucan and xylan, respectively.

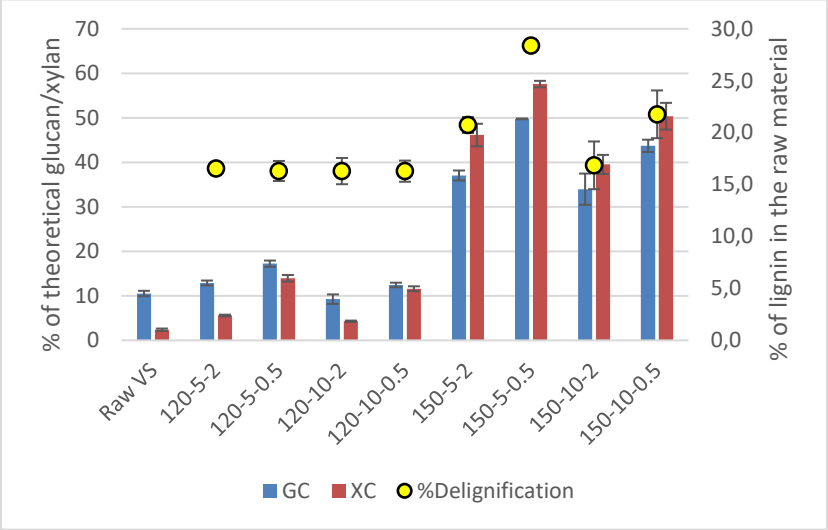

Figure 1. Glucan and xylan conversion (in % of theoretical sugars in the pretreated material) for untreated vine shoots and pretreated materials, and degree of delignification (in %) for pretreated materials. Experiments are named as X-Y-Z, where X is the temperature (ºC), Y is the solids load (% w/w), and Z is the particle size (mm).

Both the solids load and the particle size had also a significant effect on the glucan and xylan conversions, although their effect was smaller than of the temperature. Thus, increasing the solids load, or the particle size of the biomass, led to a certain decrease of the saccharification efficiency. Taking as a reference the best condition tested (150ºC, 5% solids load and 0.5 mm particle size), the glucan conversion was reduced by 25% when the particle size increased to 2 mm, and it experienced 12% decrease when the solids load increased to 10%w/w.

One of the causes for the improvement of the saccharification efficiency using the pretreatment with ChlCl:EG can be the dissolution of lignin achieved by this solvent, as proved by the delignification degree presented in Figure 1. Removal of lignin with ChlCl:EG was not as high as with other choline chloride-based DES (Su, 2021), but in the best conditions, the delignification percentage could reach almost 30% of the lignin in the raw material.

ChlCl:EG is an interesting DES, especially for having a moderate pH between 4-4.5 (Skulcova, 2018). This makes ChlCl:EG less prone to cause corrosion problems than other acidic DES and it also simplifies the washing step, as less water is needed to reach a suitable pH for the saccharification step. Overall, the results obtained in this work, showed that there is a temperature threshold that limits the efficacy of the pretreatment with this DES. Moreover, the study revealed important information about the effects of solids load and particle size that would be very relevant for the further improvement of the pretreatment. In this respect, it would be essential to test the process in a reactor able to work at high solids load, and that considers the particle size reduction.

Acknowledgements

Authors acknowledge MCIN/AEI/ (10.13039/501100011033) for funding this research work through PID2020-112594RB-C32 project.

References

Ab Rasid, N.S., Shamjuddin, A., Abdul Rahman, A.Z., Saidina Amin, N.A. (2021) J Cleaner Prod, 321, 129038.

Bisaglia, C., Romano, E. (2018) Biomass Bioenergy, 115, 136-142.

European Commission, Eurostat (2021).

Hansen, B.B., Spittle, S., Chen, B., Poe, D., Zhang, Y., Klein, J.M, Horton, A. *et al.* (2021) Chem Rev, 121 (3), 1232-1285.

Skulcova, A., Russ, A., Jablonsky, M., and Sima, J. (2018). BioRes, 13(3), 5042-5051.

Su, Y., Huang, C., Lai, C., Yong, Q. (2021). Bioresour Technol, 321, 124471.