Biorefinery development of lignocellulosic biomass for lignin extraction and succinic acid production via fermentation

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Keywords: Succinic Acid, Lignin, Lignocellulosic biomass, lignin particles

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Introduction

In this study, an integrated biorefinery has been developed utilising lignocellulosic biomass as by-product stream for the sustainable production of two value-added products, lignin and succinic acid. Three different pretreatment technologies were tested in this work, to evaluate their effect in: (1) lignin isolation and (2) its characteristics, (3) enzymatic hydrolysis of the carbohydrate-rich solids and (4) bio-based succinic acid production. Based on that, two novel technologies, deep eutectic solvents (DES) and non-thermal plasma, have been evaluated while alkaline pretreatment as a conventional method was also used. The fractions rich in lignin derived from these pretreatment methods were also characterised to investigate the potential of these streams to be used as additives in packaging materials, adhesives, resins or cosmetics.

Materials & methods

In this study, different technologies were evaluated for the pretreatment of lignocellulosic biomass in order to enhance the enzymatic hydrolysis yield of the remaining biomass.

Method 1: Alkaline delignification with NaOH was performed using 1.19% (w/v) NaOH at 70°C for 30 min (Filippi et al., 2022). After pretreatment, the alkali lignin (AL) was obtained with precipitation of the liquid fraction with HCl.

Method 2: In this method DES was used, which was produced with choline chloride (ChCl) and lactic acid (LA) (molar ratio 1:10) at 120°C for 1 h. After pretreatment, lignin was separated from the liquid fraction with filtration and the solvent was recycled and reused for the next pretreatment cycle as reported by Filippi et al. (2023). The lignins obtained by this process were referred to as DES-L1 after the first cycle and DES-L2 after the second pretreatment cycle.

Method 3: GS solids were treated with non-thermal dielectric barrier discharge air plasma (using a 20% duty cycle, with a voltage of 200 V, frequency of 20 Hz and 60 min of duration) and hydrolysed using commercial enzyme preparation. After enzymatic hydrolysis, the solid fraction was separated from the sugar-rich liquid and pretreated with ChCl: LA (molar ratio 1:2) for 6 h at 120°C to obtain lignin particles (DES-L6) (Lou et al., 2019).

The dissolution of the resulting lignins in different solvents (acetone, ethanol, methanol, DMSO, THF and ethyl acetate) was tested. Detection of carboxyl content in lignins was carried out through ^{31}P NMR while FT-IR and SEM analysis was also performed.

The enzymatic hydrolysis of the lignocellulosic material used after each pretreatment method was carried out using 20 FPU cellulase, 80 U β-glucosidase and 10 U xylanase per g pretreated biomass. All hydrolysis experiments were conducted at 50^{o}C for 48 h under continuous stirring. The hydrolysate produced was evaluated as the sole carbon source for the production of bio-based succinic acid (SA) using the bacterial strain *Actinobacillus succinogenes* in batch fermentation mode. The fermentation medium was supplemented with 5 g/L yeast extract and minerals while the fermentation conditions were based on Filippi et al. (2022). The lignocellulosic content was determined based on NREL method (Sluiter et al., 2012).

Results & discussion

To assess the findings of this study, mass balances were conducted for the three pretreatment processes, focusing on lignin isolation and succinic acid production, using 1,000 kg of lignocellulosic biomass as the raw material for each case. In the case of alkali pretreatment (Method 1), the result was the production of 343.4 g of AL and 78.9 g of succinic acid. For Method 2, the lignin fraction obtained after the initial pretreatment cycle was 453.4 g, with the succinic acid concentration reaching 103.2 g. When employing non-thermal plasma (Method 3), maximum concentrations of 690 g for lignin and 169.9 g for succinic acid were achieved. The fermentation yield in this case was 0.68 g of succinic acid per g of total solids, with a productivity equal to 0.67 g/(L·h).

Regarding the characterization of generated lignin particles, all samples demonstrated maximum solubility in DMSO, with no dissolution observed in acetone. SEM analysis suggests that lignin particles produced with DES exhibit a distinctive shape on the micrometer scale.

Conclusions

Non-thermal plasma treatment gave the most promising results in terms of SA production and lignin isolation. Also, the utilisation of DES treatment results in dispersed lignin particles predominantly within micro dimensions with a specific shape. As a result, incorporating DES and non-thermal plasma as innovative, eco-friendly pretreatment technologies, as opposed to conventional alkali pretreatment, proves to be a highly efficient method capable of both replacing and enhancing the production and morphology of the resulting lignin particles.

References

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Acknowledgements

The project is supported by the Circular Bio-based Europe Joint Undertaking and its members. Funded by the European Union (LUCRA project). Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or CBE JU. Neither the European Union nor the CBE JU can be held responsible for them. This work was also co-funded by UK Research and Innovation (UKRI) under the UK government's Horizon Europe funding guarantee grant number 10082169.