Integration of cellulases recycling with 2nd generation bioethanol production from waste paper residues

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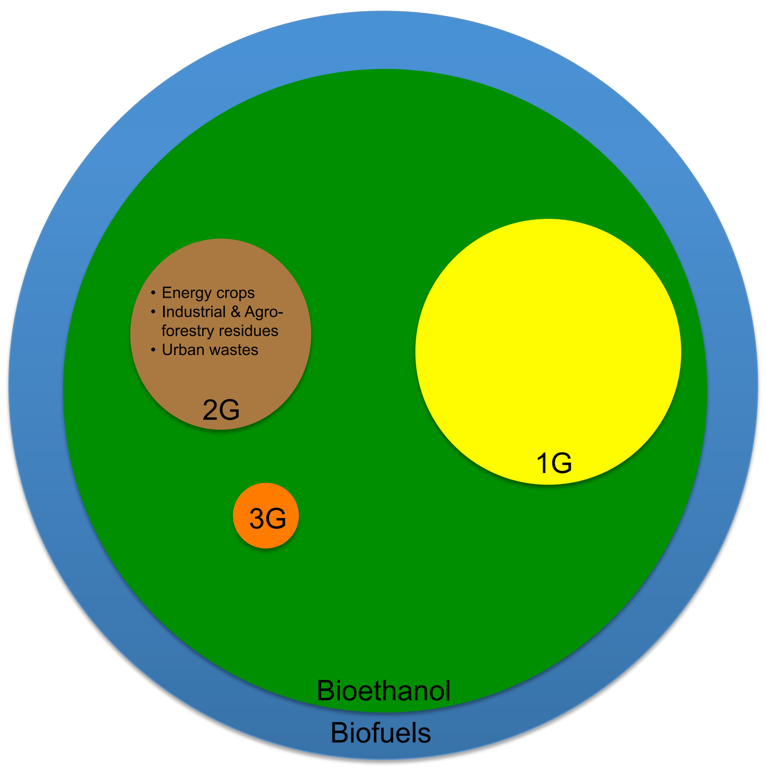
Abstract

Nowadays, the economic feasibility of second-generation bioethanol production depends on two important factors: raw material and the cost of enzymes. The utilization of urban and industrial residues may represent a significant improvement on this process, as it refers in some cases to a material with a negative cost and with no soil competition with food-crops. The intensive utilization of paper and paper-based materials originates high amounts of different paper residues, which present a considerable fraction of carbohydrates, therefore arising as an interesting material to be hydrolysed. Reporting to other determinant of the process economics, the high cost of cellulases demands immediate improvements on their efficiency, where enzyme recycling comes as a promising strategy to achieve that. Few works have been conducted regarding this concept, therefore, different factors still need to be studied and optimized. The need for more thermostable enzymes has been recently identified as a vital feature for enzyme recycling by one of the supervisors of this project, therefore, special attention will be given to this factor on this project.

This PhD project will explore the strategy of cellulases recycling applied more specifically to the fermentation of paper-based residues. Aiming the optimization of the overall process, and considering some of the limitations already identified for this type of substrates, the proper way to integrate saccharification and fermentation processes will also be pursued.

State of the art

Agricultural and forestry residues, as well as wastes, could considerably reduce the land required for 2G biofuel production. Also, since they are by-products, their cost is inferior comparatively to 1G biofuels. For some residues, disposal costs have to be paid; these costs can be avoided – with a positive effect on overall production costs – if the residues are used for biofuel production.

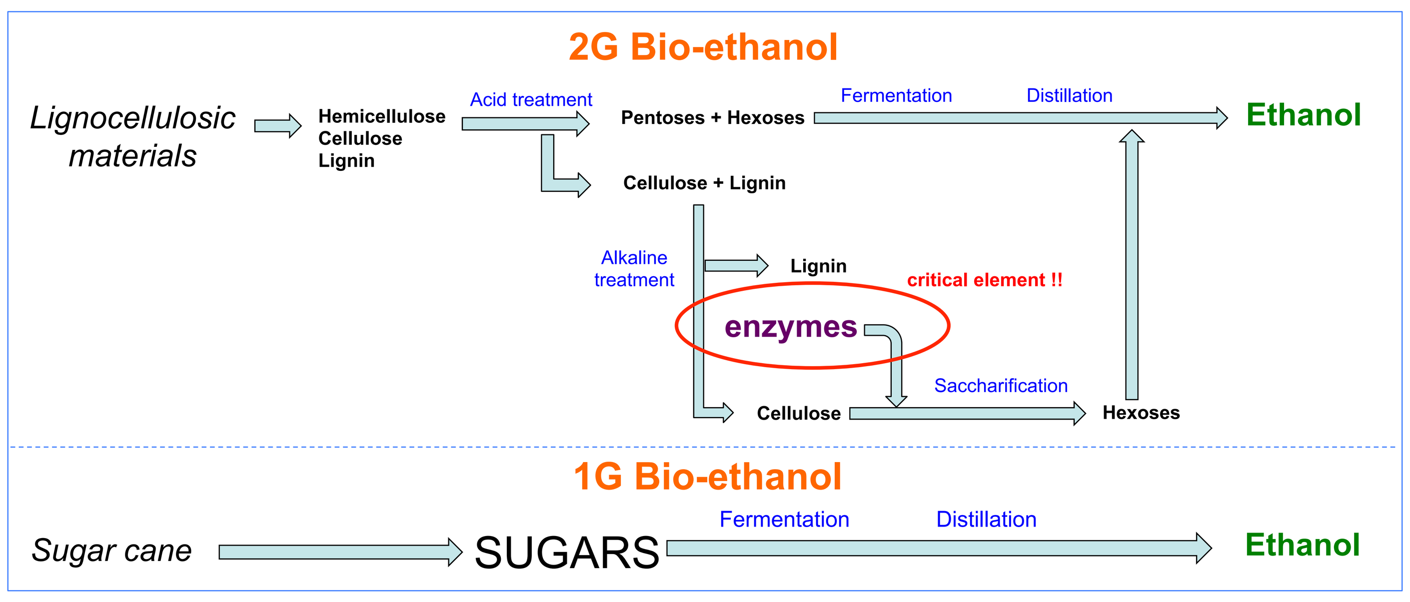


Lignocellulosic materials usually requires pre-treatments to facilitate the access of cellulose from cellulases. Beyond the costs associated to the energy consumption and the utilization of chemicals, these pre-treatments can originate different types of inhibitors (*e.g.* acetic acid, furfural, etc.) that may affect either the enzymes or, posteriorly, the cells in the fermentation process. Paper-based residues, on the other hand, already present a considerable level of processing, therefore becoming an easily digestible material [1]. Additionally, they are available in high quantities and present a significant carbohydrates content: 47.2 % cellulose for newspaper; 58.6 % for office paper and 52.6 % for cardboard [2].

The possible application of paper-derived residues for bioethanol production has been intensively studied in the last years with several works already published on the subject [1; 2; 3; 4]. The amount of sugars released are however small, caused by a low initial solids concentration [5], leading to the production of low ethanol titers, considerably affecting the process economics.

Sludges from paper recycling facilities have not received yet a proper attention as substrate for 2G biofuels. These are composed mostly by poor-quality non-recyclable paper fibers together with some chemicals, such as ink particles [6]. Nevertheless, similarly to the paper residues, their cellulose content is considerably high. Marques *et al.* [6] reported a cellulose content of 34.1 % and Lark *et al.* [7] reported a value of 50 %. As a residue that requires to be disposed off, this is a material with a negative cost for the paper industries, therefore with no cost for biorefineries.

Substrates of 2G-bioethano, usually requires the action of cellulases in order to convert cellulose to fermentable sugars. This step corresponds to one of the biggest costs in lignocellulosics fermentations since those enzymes usually present a high cost. Cellulase recycling showed in the last years that can be a promising way to overtake this limitation. As a relatively new concept, the effect of many factors on the process still remain unclear. One of the determinants of recycling seems to be the temperature, as was observed by Rodrigues *et al.* [8]. The authors verified that alkaline elution was efficient in the recovery of enzyme after hydrolysis and fermentation, and that the efficiency of this recovery increased with the hydrolysis of cellulose. Also, after testing different temperatures, they observed that by exposing the enzymes to high temperatures for large periods of time, their activity decreased. More recently, Rodrigues *et al.* [9] observed that enzymes significantly loose its activity for a temperature of 50ºC, independently of the initial concentration of enzyme. However, the decrease on cellulose hydrolysis over consecutive rounds of hydrolysis was higher when low concentrations of enzymes were used. Other works already observed the importance of this factor, such as Lindedam *et al.* [10] and Tu *et al.* [11].



Fermentation stage also faces important challenges. The development of improved microorganisms more tolerant to toxics and temperature variations, as well as able to ferment pentoses should be pursued. Additionally, a suitable process design to integrate saccharification and fermentation must be developed.

Work plan

Phase 1

Evaluation of different paper residues

- Are the cellulases able to act on these substrates ?
- What is the toxicity of the substrates to the fermentation organism ?
- Are these substrates suitable for enzyme recycling?
- How to further improve enzyme's accessibility to the substrate?

Phase 2

Screening for cellulase systems

- Which enzymes present the best saccharification kinetics?
- Which enzymes more efficiently cope with substrate's toxicity?
- Which enzymes are more suitable for enzyme recycling ?

Phase 3

Optimization of cellulase recycling

- Which is the best process design to conduct cellulase recycling ?
- Among other factors, what are the best enzyme concentration and operation temperature to conduct enzyme recycling ?

Phase 4

Screening of fermentation microorganisms

- Which microorganisms present high fermentation performances on these specific substrates ?
- Which are the most suitable microorganisms to an efficient integration of saccharification and fermentation processes ?

Phase 5

Improved process design

- Considering both the application of enzyme recycling and the limitations commonly found in the hydrolysis of paper residues, what is the best process design to efficiently integrate saccharification and fermentation processes ?

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