

Supplementation of fungal cocktails with recombinant hydrolases as a strategy to overcome bagasse hydrolysis limitations

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Biomass conversion by enzymatic hydrolysis still represents a bottleneck for the production of second generation ethanol from sugarcane bagasse and straw. Two major factors can be highlighted: the biomass recalcitrance, even after the pretreatment, and the biocatalysts efficiency. In this work, the study of enzymatic cocktail supplementation using recombinant hydrolases was performed in order to evaluate the effect on sugars release from sugarcane bagasse. Three pretreated bagasse, defined as BED (steam exploded and delignified), BEX (steam exploded) and HID (hydrothermal) were used as substrate in hydrolysis experiments. *Penicillium echinulatum* cocktail provided the core hydrolytic enzymes which was supplemented with different combinations of two endoxylanases (xynA and xynC) and one endoglucanase, all from *Bacillus subtilis*, and produced in *E. coli*. Different ratios between total activity and xylanase activity were studied and the best result obtained was at 100 UI/FPU. At this condition, a synergic effect was observed and final glucose and xylose release increased by 33% and 200%, respectively, using BEX as substrate. Delignified bagasse hydrolysis was poorly affected by the supplementation, indicating that there is a strong commitment between pretreatment and the cocktail formulation to optimize the hydrolysis enzymatic reaction. Experiments using hydrothermal pretreated bagasse are currently being done in order to confirm this observation. By this way, we expect to contribute to overcome bagasse hydrolysis limitations, establishing efficient enzymatic cocktails for each kind of pretreated bagasse, also improving the current knowledge on the role of specific enzymes on the hydrolysis of this biomass.

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