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O-09 - ENHANCING THE ENZYMATIC SACCHARIFICATION OF WHOLE SLURRY FROM AUTOHYDROLYZED EUCALYPTUS GLOBULUS WOOD BY SUPPLEMENTATION WITH A RECOMBINANT CARBOHYDRATE-BINDING MODULE

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Background

Lignocellulosic biomass has a recognised potential as a sustainable platform for the production of biofuels and other biochemicals. However, lignin residues and other inhibitory compounds resulting from lignocellulosics pretreatment affect the digestibility of resulting whole slurries. The addition of synergistic proteins that can cooperate with cellulases is an emerging strategy for enhanced lignocellulosics hydrolysis. Carbohydrate-binding modules (CBMs) have been shown to improve the enzymatic hydrolysis of pure cellulose models but their effect on the enzymatic hydrolysis of lignocellulosic materials has not yet been evaluated. Thus, in this work, the potential synergistic effect of a family 3 CBM on the enzymatic saccharification of a pretreated lignocellulosic biomass was studied for the first time.

Method

The CBM3 from the *Clostridium thermocellum* scaffolding protein (CipA) was recombinantly produced in *Escherichia coli*. Different dosages of CBM3 (30-1.5 mg/g_{solids}) were incubated simultaneously with the enzymatic cocktail Cellic CTec2 (15 FPU/g_{solids}) in the whole slurry from autohydrolysed *Eucalyptus globulus* wood (EGW), containing 5% (w/v) solids and 73% (w/v) hydrolysate. Enzymatic hydrolyses were conducted at pH 4.85, 50°C and 150 rpm. Glucose yields were determined over time by HPLC. BSA effect was also evaluated using the same loads as CBM3. Additionally, the binding of CBM3 to solids and lignin of pretreated EGW was studied by fluorescence microscopy.

Results & Conclusions

CBM3 effect was found to be dose-dependent. When added at the higher dosage (30 mg/g_{solids}), CBM3 led to an increase in glucose yield from 75 to a maximum of 89% (14% increase), alleviating significantly the inhibitory effect of EGW hydrolysate on the enzymatic saccharification. BSA, which has a well-documented additive effect, led to a slightly lower glucose yield increment (11%). CBM3 was able to bind to EGW solids but not to the isolated lignin. Therefore, the CBM3 effect is not related with the typical lignin-blocking mechanism attributed to other proteins, such as BSA. CBM3 is a valid and inexpensive additive that may be included in enzymatic cocktails for improved whole slurry saccharification, thus contributing to more efficient biomass conversion bioprocesses.

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Keywords: recombinant CBM3, whole slurry, enzyme inhibition, lignin, enhanced saccharification