XIII SEMINÁRIO BRASILEIRO DE TECNOLOGIA ENZIMÁTICA

ENZITEC 2018 >>>FLORIPA

AT5-1268376

Addition of a bacterial GH10XynA xylanase to a commercial enzymatic cocktail improves bioconversion of extruded sweet corn cob at high solids consistency

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Enzyme-mediated hydrolysis of lignocellulose, to release soluble and fermentable sugars, is a key step for lignocellulosic based biofuels. In order to develop an alternative solution for the production of second generation ethanol based on smaller industrial scale, our **objective** was to optimize the enzymatic hydrolysis step to achieve high sugar conversion (glucose and xylose). Our experimental design was based on the following **hypothesis**: Addition of hemicellulases to Cellic Ctec2® (Novozymes) will improve the sugar conversion yields in PH-SSCF (pre-hydrolysis and simultaneous saccharification and co-fermentation) conditions.

Experimental design

Biomass → Pre-treatment (Extrusion) → Pre-hydrolysis (50°C, 24 h) → SSCF (30°C, 72 h) → ETHANOL

Cellic C-Tec2 + In-house xylanases

BABET REAL5 PROJECT
https://www.babet-real5.eu/

RESULTS

1-Selected Xylanase

Recombinant GH10-XynA ((EC 3.2.1.8), from *Paenibacillus* sp. A59 (Ghio et al., Bioenergy Research 2018)
Xylanase Activity: 100 - 160 IU/mg, optimal pH range: 5.5 to 8; optimal temperature range 45°C to 60°C.
Thermal stability: More than 60% activity after 24 h at 50°C.

2-Biomass and Pre-treatment

Sweet Corn Cobs (SCC)
Optimal Extrusion: **NaOH/SCC ratio of 5.9%, T° 130°C**

Alkali extrusion

SCC (milled)

Extruded SCC (eSCC):
42.1% cellulose
26.6% hemicellulose
6.4% lignin

Alkali (NaOH) **Acid (H_2SO_4)**

M1	M2	M3	M4	M5	M6	M7	M8	M9	M10
NC	80°C	130°C	130°C	40°C	NC	50°C	50°C	50°C	50°C

Filtrate

3- Hydrolysis

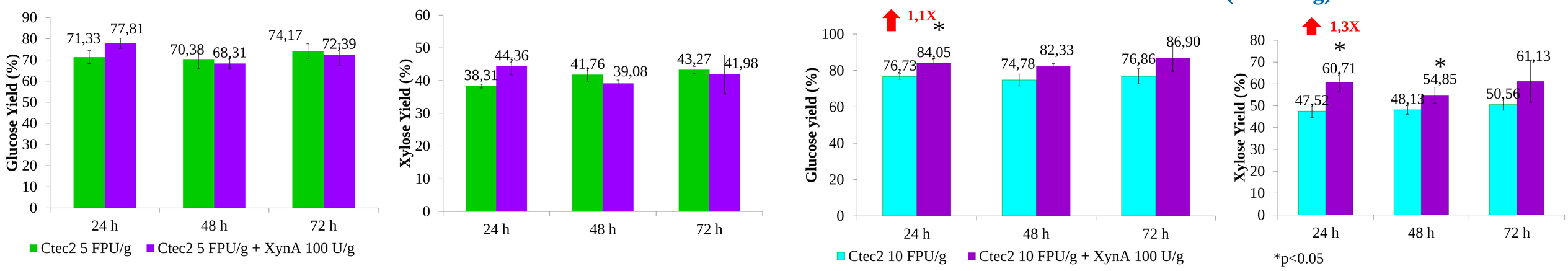
eSCC + GH10XynA (100 IU/g_{dw})
Cellic C-Tec2: 5 or 10 FPU/g_{dw}

Pre-hydrolysis: 50°C, pH: 5.5, 24 h, followed by incubation at SCCF conditions: 30°C, pH: 5.5, 48 h

T0

T72

4-Conversion yields to monomeric fermentable sugars (vs theoretical amount)



METHODS

-Biomass and pre-treatment: Sweet corn cob (SCC) was pre-treated by a continuous alkaline thermo-mechano-chemical process (NaOH/SCC ratio of 6.1%, T° 137°C), in an extruder at LCA, INP, Toulouse (France).

-Hydrolysis: Experiments were carried out with 20% solids load (5g of dry weight biomass/ 25 g total) using CellicCtec2 (Novozymes), at 5 or 10 FPU/g, supplemented or not with GH10-XynA (100 IU/g), based on the conditions for pre-hydrolysis and simultaneous saccharification and co-fermentation (PH-SSCF): pH 5.5, 24h at 50°C, followed by 48h at 30°C. Samples were taken at 24, 48 and 72 hs. There were three replicates for each condition.

-HPLC analysis: Glucose and xylose were quantified by HPLC (Agilent 1100), using a Rezex RPM-Monosaccharide column (Phenomenex) (80º C, flow 0.6 ml/min), with a RI detector at 35ºC.

-Statistical annalysis. One way ANOVA ($p<0,05$) followed by Tukey´s multiple comparison test, using Graphpad 5 software.

CONCLUSIONS

- **Addition of GH10XynA to CCtec2 resulted in an increased conversion of extruded sweet corn cob to xylose and also glucose.**
- **The cocktail CCtec2+GH10XynA will be assayed on other hemicellulose rich biomasses.**

Acknowledgments

This work was funded by BABETREAL5, Cofunded by the EU within the Horizon 2020 programme. Grant Agreement n°654365, INTA PNAIyAV 1130034 and MINCYT PICT2014-0791 projects. We thank Novozymes for gently supplying Cellic Ctec2®