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# 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


**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 |

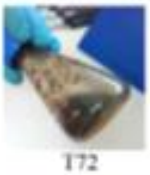
Filtrate

### 3- Hydrolysis

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

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


T0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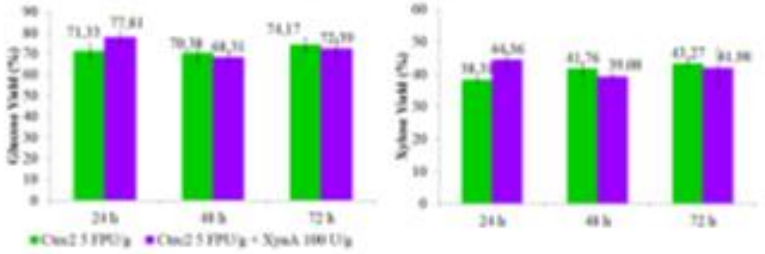
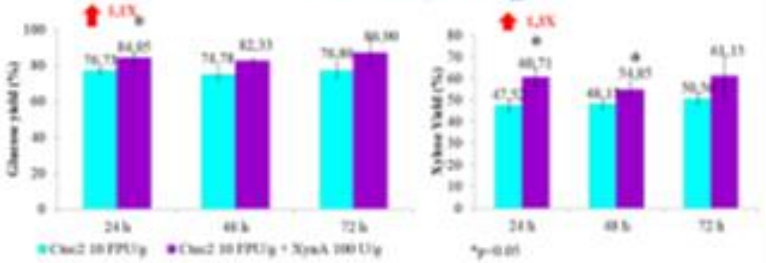
### 4-Conversion yields to monomeric fermentable sugars (vs theoretical amount)

Cellic Ctec2 (5 FPU/g)




Cellic Ctec2 (10 FPU/g)




## 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) (80° C, flow 0.6 ml/min), with a RI detector at 35°C.

**-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.**

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