Development of lignocellulosic biomass adapted hemicellulases pretreatment

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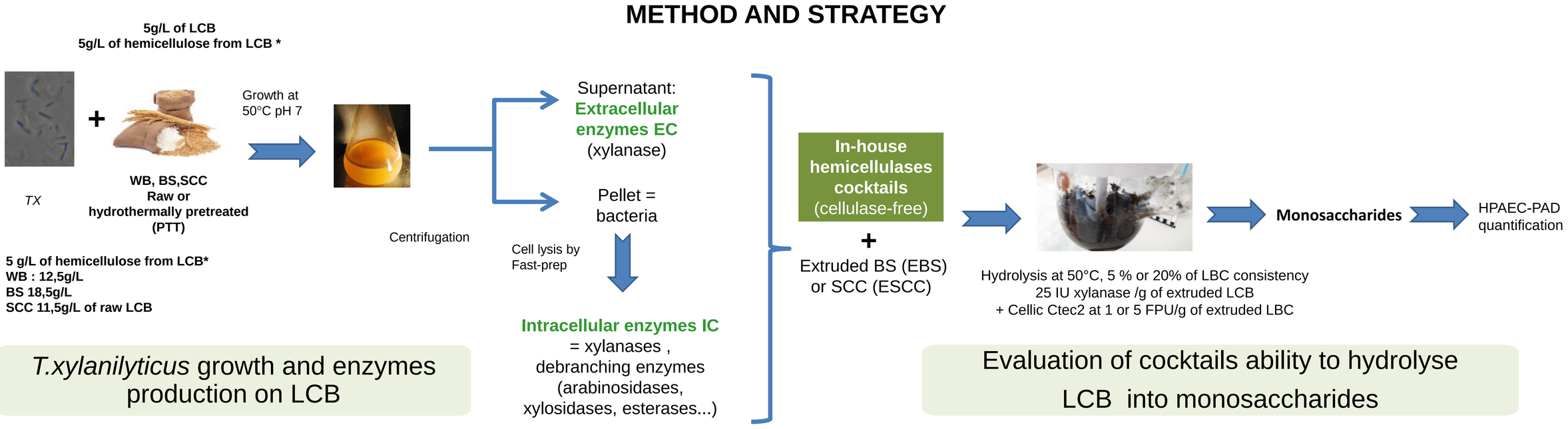
INTRODUCTION

The BABET-REAL5 project aims to develop an integrated process for the production of bioethanol at small industrial scale from various biomasses such as barley Straw (BS) and sweet corn cob (SCC). The enzymatic transformation of these biomasses is known to be difficult and requires a mechanical and/or chemical pretreatment to facilitate the access of enzymes to their substrates. One challenge to be raised by the project consists to combine a pretreatment by extrusion of the lignocellulosic biomass (LCB) and the hydrolysis step to favor the liberation of sugars (hexoses and pentoses). *Thermobacillus xylanilyticus (TX)* is a thermophilic and hemicellulolytic bacterium able to produce enzymatic cocktails during its growth on various LCB. The enzymes produced are endoxylanases (mainly secreted) and other enzymes that are cytoplasmic (debranching activities like acetyl-esterases (AE), arabinosidases (ABF) and xylosidases (Xylo)). Our studies aim to produce and characterize hemicellulases cocktails obtained by cultivating *TX* onto BS and SCC used in the Babetreal project and onto wheat bran (WB). Evaluation of hemicellulases cocktails were investigated onto raw or extruded LCB for the liberation of monosaccharides (glucose and pentoses) in combination with a cellulase cocktail (Cellic Ctec 2).

METHOD AND STRATEGY



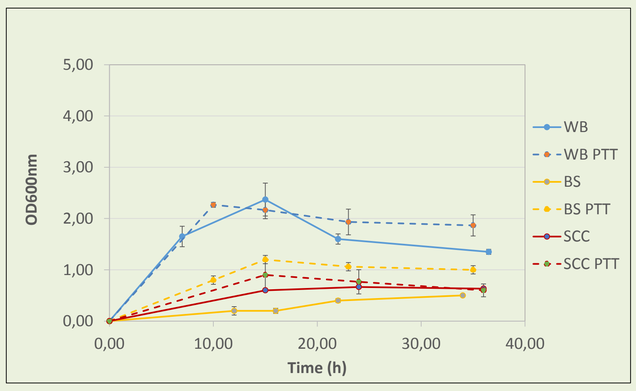
RESULTS

1. Study of the growth of *Thermobacillus xylanilyticus* on different LCB

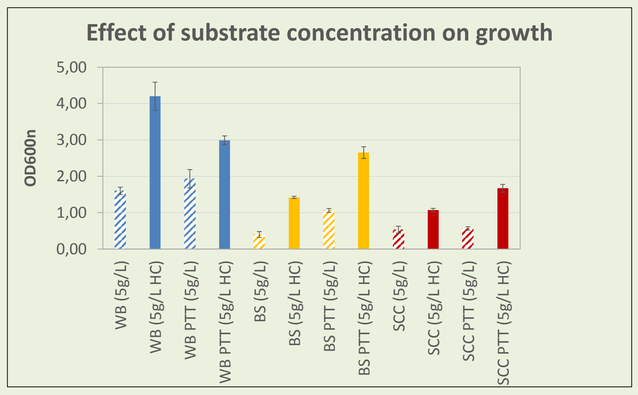
Global overview of *TX* growth and enzymes production

TX growths

Growth course on various LCB



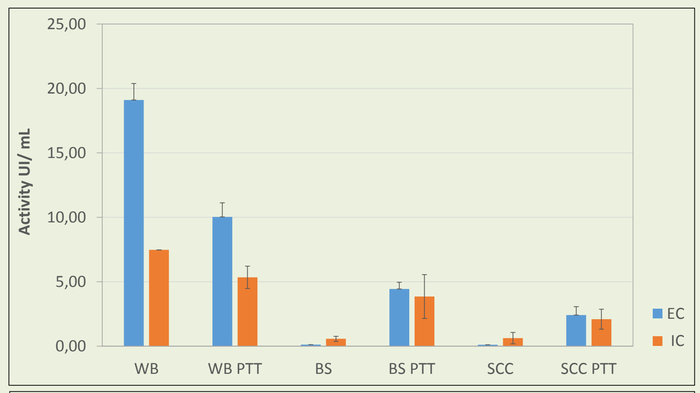
Effect of LBC concentration



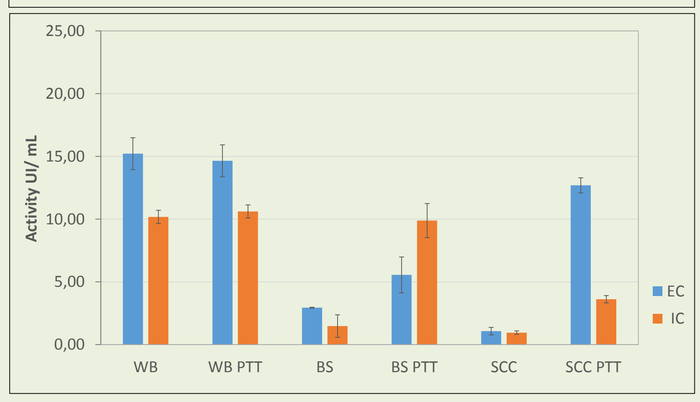
- The growth of TX depends on the LCB used and their concentrations
- Increase of hemicellulose content allows better growth : between 1,5x and 3,6x increase observed with 5g/L hemicellulose
- Hydrothermal pretreatment (PTT) allows better growth mainly with BS and SCC

Xylanase activities

5g/L of LBC



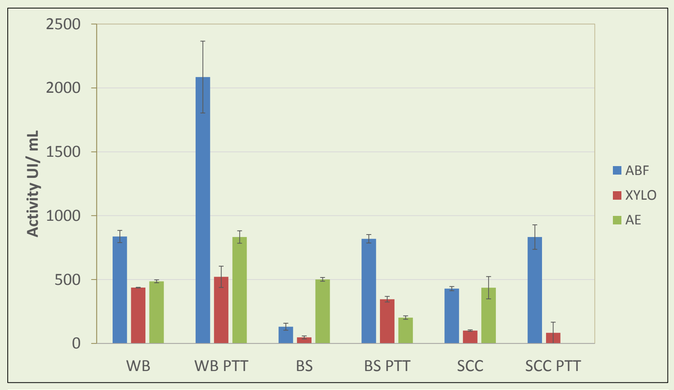
5g/L of hemicellulose



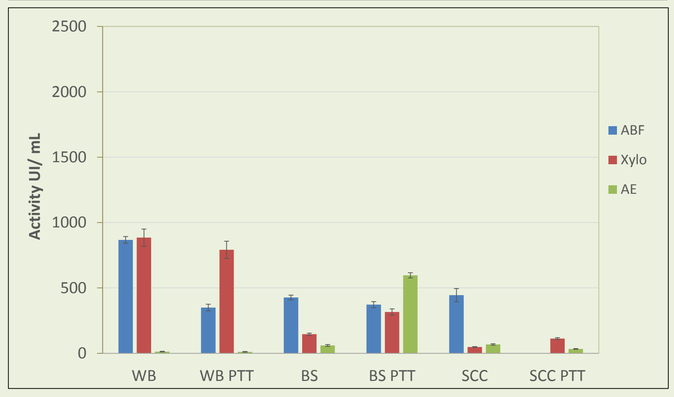
- The main hemicellulasic activity produced is xylanase. Globally, the extracellular (EC) xylanase activity is higher than intracellular (IC) activity.
- Low xylanase activities produced on raw BS and SCC compared to WB
- PTT associated with increase of LBC loading (5 g/L hemicellulose) improves the xylanase production (except with WB)

Debranching activities

5g/L of LBC



5g/L of hemicellulose



- Debranching enzymes are produced on all LCB
- Higher amount of enzymes obtained with PTT LCB (except of esterase activities with BS and SCC)
- No significant effect of increase of LBC loading (5 g/L hemicellulose) on debranching activities production

2. Hydrolysis of LCB with in-house hemicellulases cocktails

Hydrolysis of raw and extruded BS with BS cocktails

Hydrolysis of raw and extruded SCC with SCC cocktails

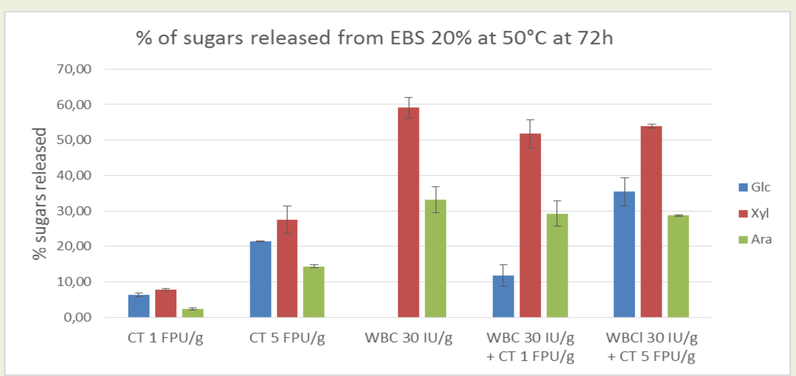
LCB 5%; TX-hemicellulases loading 25 IU/g of substrate

	BS			EBS		
	Time (h)			Time (h)		
Sugar Yields (%)	T0	T8	T24	T0	T8	T24
Xyl (mean±SD)	0.12±0.09	5.59±0.23	12.04±1,.3	0.49±0.21	9.43±0.13	25.68±0.70
Ara (mean±SD)	0.21±0.03	2.98±0.15	6.58±0.69	0.47±0.20	6.75±0.66	16.12±1.65
Glc (mean±SD)	0.67±0.17	2.11±0.10	3.41±0.36	nd	0.10±0.01	Nd

	SCC			ESCC		
	Time (h)			Time (h)		
Sugar Yields (%)	T0	T8	T24	T0	T8	T24
Xyl (mean±SD)	1.07±0.13	3.10±0.21	5.43±0.27	0.48±0.09	8.06±1.81	17.66±2.38
Ara (mean±SD)	0.40±0.07	3.35±0.25	7.22±0.09	0.33±0.035	9.12±2.51	22.88±1.65
Glc (mean±SD)	1.86±0.10	2.48±0.15	3.68±0.11	0.21±0.01	0.37±0.12	1.31±0.35

- In-house hemicellulases cocktails are efficient for the hydrolyses of hemicelluloses from BS and SCC.
- The extrusion pretreatment increases by 2-fold to 3-fold the arabinose and xylose release.
- Glucose liberation is <4% of Glc content whatever the cocktail tested; Combination with cellulases (Celic Ctec 2) is under progress.

Hydrolysis of extruded BS with WB hemicellulase cocktail at high LCB loading and combination with cellulases



20%; TX-hemicellulases loading 30 IU/g of LCB + Cellic Ctec 2 at 1 or 5 FPU/g of EBS

Study of WB hemicellulase cocktail was investigated onto EBS at high consistency.

- The complementation of Cellic Ctec2 with WBC allowed increasing the sugar released from EBS.
- Compared to Cellic CTec2 alone at 1 FPU/g, the complementation with WBC led to a 1.8-fold, 6.6-fold and 12.7-fold higher glucose, xylose and arabinose liberation respectively.
- The same trend was obtained with complementation of Cellic Ctec2 at 5 FPU/g with WBC: glucose, xylose and arabinose released were increased by 1.6-fold, 2.5-fold and 2-fold respectively compared to Cellic CTec2 alone.

CONCLUSION AND FUTURES

*Thermobacillus xylanilyticus is able to g*rowth and produce hemicellulases cocktails on various LCB substrates. The pretreatment of recalcitrant LCB and the increase of C available (higher hemicelluloses loading) provide better conditions for growth of the bacterium.
Dissimilar enzymes production according to the LCB used is observed : identification of different enzymes within the cocktails is needed to implement effective LCB hydrolysis.
The in-house hemicellulases cocktails are able to hydrolysate xylans from various LCB. Due to its higher enzyme concentration, WB cocktail allowed working at high substrate loading, thus improving the monomerization yields of sugars. For the other cocktails improvement of enzyme production is needed.

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