Enzyme technology for cellulosic ethanol production

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Business from technology

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Introduction – Bioethanol

Globally

- Demand and markets for ethanol increasing rapidly; USA and Brazil leading the development
- Ethanol from cellulose is necessary for improved GHG balance
- Oil prize increasing rapidly
- Countries are pursuing for energy self-sufficiency

In Europe

- EU Biofuel directive 2003: 5.75% of transport fuels substituted with biofuels by 2010; EU Comission strategy 2007: binding minimum 10 % of transport fuels substituted with biofuels by 2020
- Variable local raw materials for cellulosic ethanol

Markets

- Estimated global biofuel production 45 billion litres/year
- Production will possibly double from this by 2011
- In the range of at least 100 new plants (mainly 1st generation) would start within 5 years in USA

Key topics with biofuels production

- **First generation biofuels**
  - Raw materials - sustainability - conversion efficiency
  - Energy balance, CO_2/GHG emissions - side stream utilization
  - Integration with second generation fuel production
- **Second generation (e.g. from cellulose) biofuels**
  - Improved conversion technologies for various raw materials
  - Reduction of production costs - integrated process concepts
  - LCA, CO_2/GHG - energy balances
  - Product quality

Source: Prof. L. Viikari

Raw Materials

Major raw materials for bioethanol production

- **1st generation raw materials**
  - Sugar cane juice (sucrose)
  - Corn starch (glucose)
  - Wheat and barley starch (glucose)
  - Sugar beet juice (sucrose)

- **2nd generation raw materials**
  - Sugar cane bagasse
  - Sugar cane trash
  - Corn stover
  - Wheat and barley straw
  - Sugar beet pulp
  - Energy crops
  - Forest industry side streams
  - Municipal waste streams

Typical raw material composition (% of DM) and theoretical maximum ethanol yield (L/ton DM)

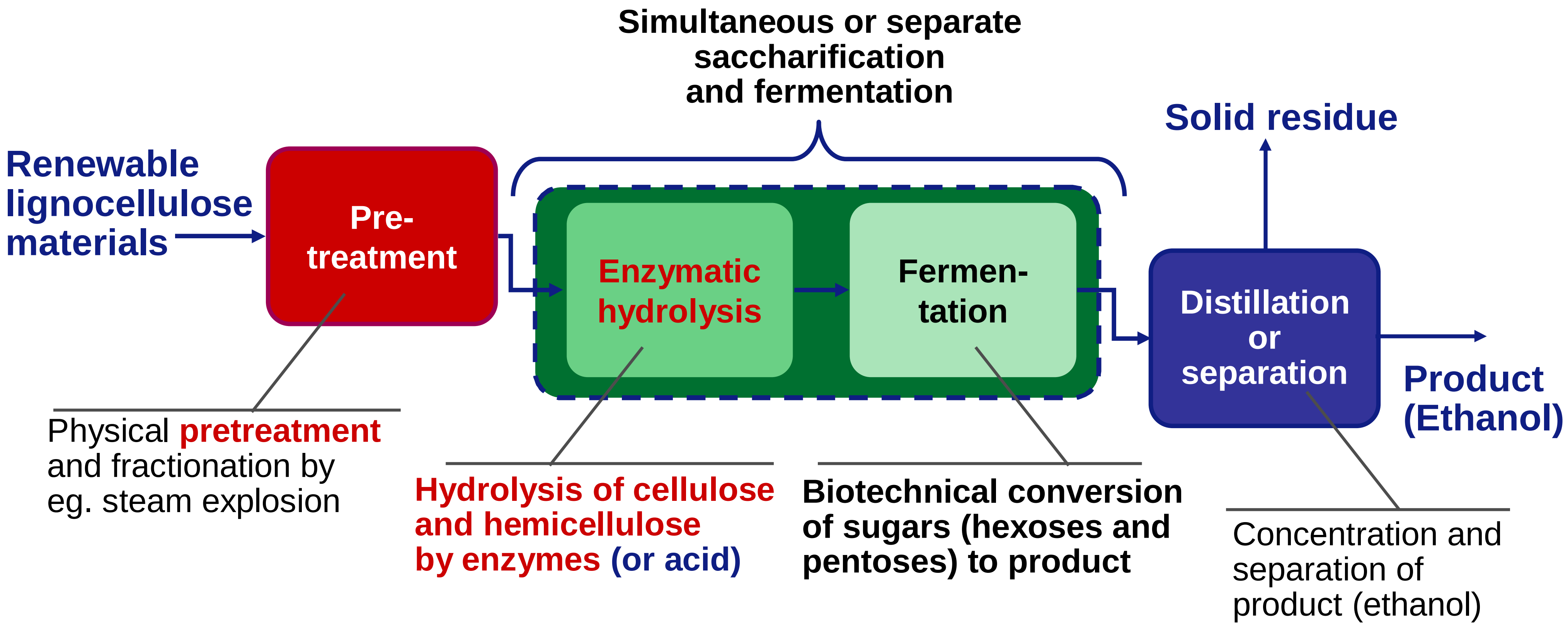
	Softwood (spruce	Hardwood (willow)	Corn stover	Sugarcane bagasse
Glucan	45	42	37	43
Mannan	14	3	-	-
Galactan	2	2	3	0.4
Xylan	7	15	22	24
Arabinan	1	2	5	2
Lignin	**28**	**25**	**23**	**22**
Other components	4	11	10	8
Ethanol from C6	420	330	280	300
Ethanol from C5	57	120	200	190

Data from Lund University

Processes

Process concepts

Major steps of typical ethanol process (2nd generation)



Drivers and targets for enzyme development

Major bottlenecks of the present conversion technologies

1. **Resistant structure of (ligno)cellulose**: efficient pretreatment technologies are needed
2. **Efficiency of the enzymatic hydrolysis of cellulose is too low**
3. Fermentation of pentoses is restricted on real substrates
4. **Too low yield** and concentration of product (ethanol)
5. High energy demand in the production process

Factors affecting the efficiency and economics of enzymatic hydrolysis

- Composition and accessibility of substrate (cellulose, hemicellulose, lignin), improved by pre-treatment
- Properties of enzymes: specific activity, stability, end-product inhibition, unproductive binding, role of CBD's etc.
- Composition of enzyme mixtures (cellulase mixtures for optimal synergy), role of additional enzymes (hemicellulases, lignin modifying etc.)
- Hydrolysis technologies: separate/simultaneous/stepwise, temperature, mixing (affecting e.g. diffusion), recycling of enzymes
- Enzyme prize; efficient production systems

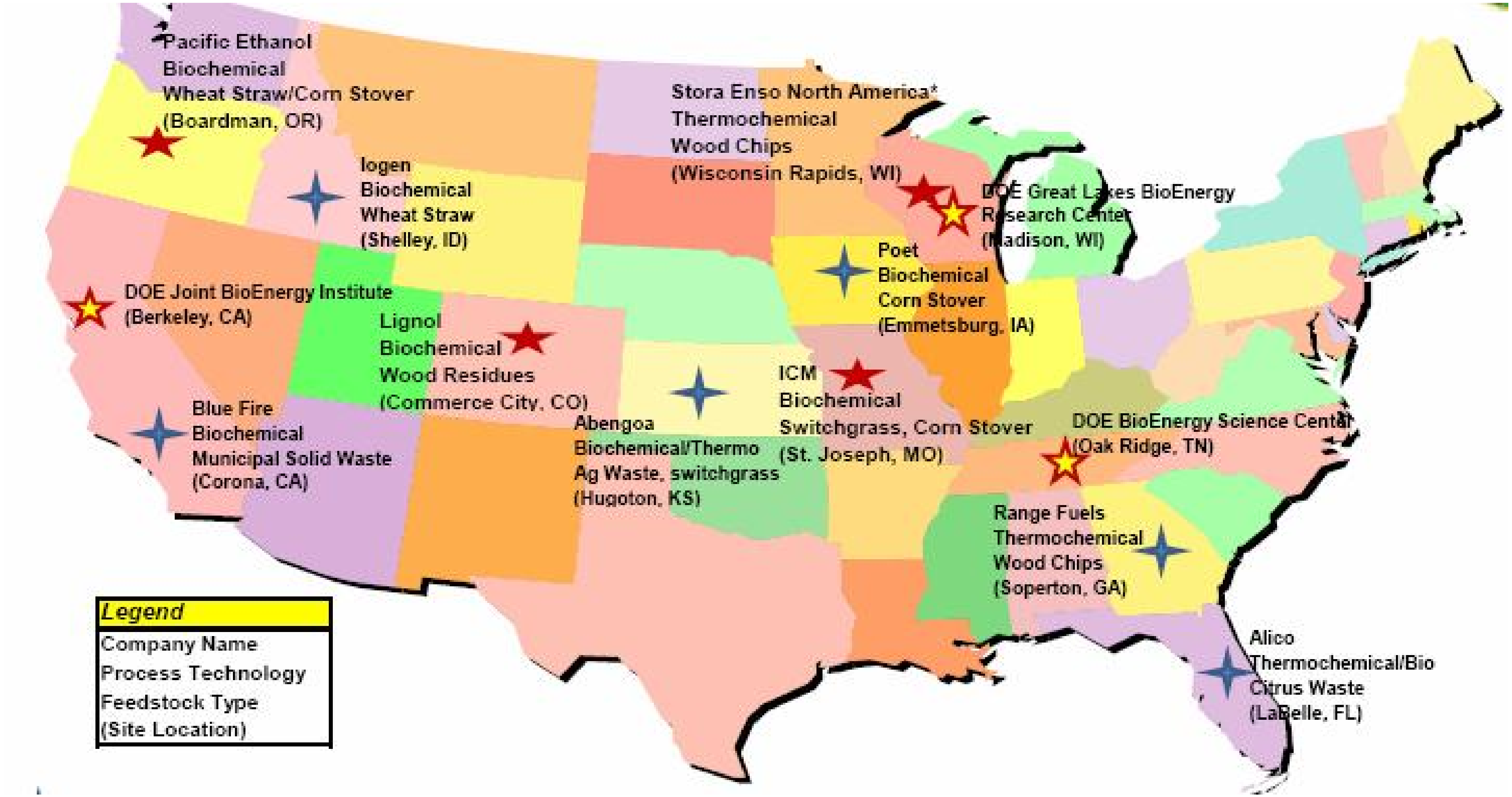
Feasibility and costs

Production costs of cellulose ethanol

- In several moderate estimations, production costs of 2nd generation ethanol are ca. 0.4-0.5 €/L. But "Iogen ready to sell by 0.25 €/L"
- Cost structure (European estimate; TIME project, 2004; by courtesy of Per Sassner, Lund University):
  - *Production costs:* 0.45 – 0.6 €/L, depending on raw material and process
  - *Raw materials*: total ca. 40 %
  - *Chemicals (incl. enzymes)*: 20 - 25 %
  - *Residue (energy)*: income 20 - 25 % (i.e. -20 - -25 %)
  - *Utilities and other costs:* ca. 15 %
  - *Capital costs:* 40 - 45 %

Ongoing activities

USA: Major DOE biofuels project locations



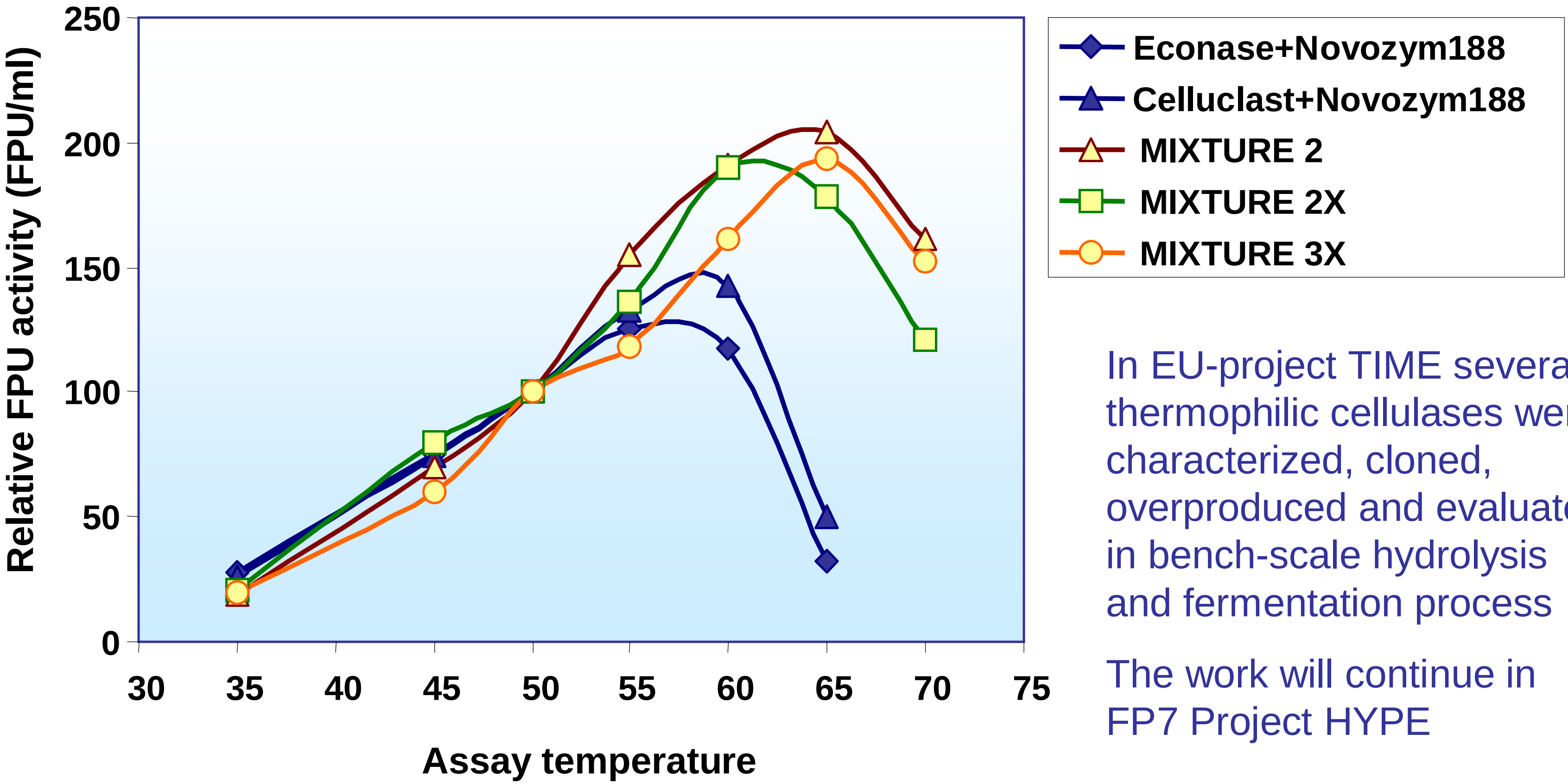
Six Commercial-Scale Biorefinery Projects; DOE will invest up to $385 million

Four Small-Scale Biorefinery Projects; DOE will invest up to $114 million (first round)

Three Bio-Energy Centers; DOE will invest up to $405 million

* Acquired by NewPage Corporation

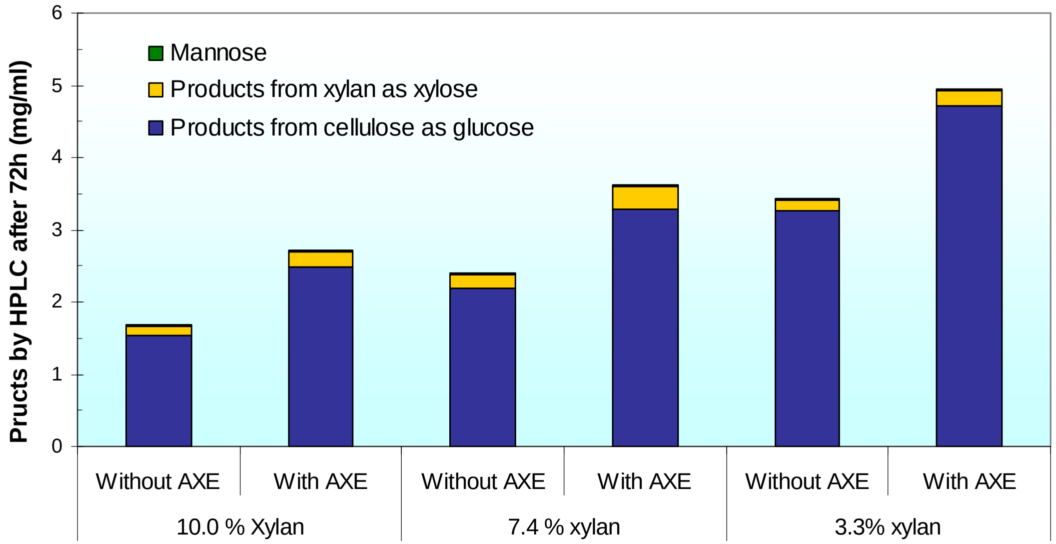
Example 1: Development of more thermophilic enzymes

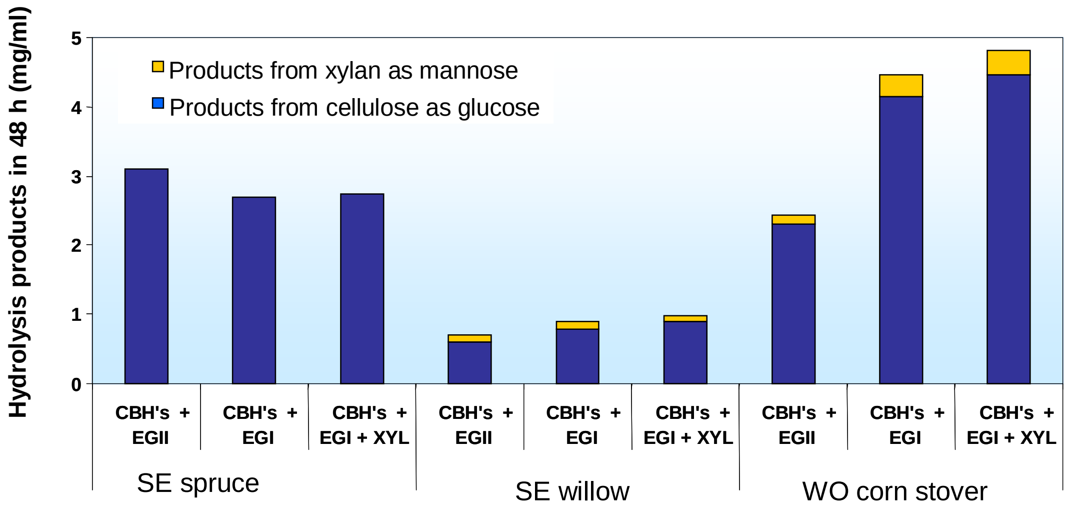


In EU-project TIME several thermophilic cellulases were characterized, cloned, overproduced and evaluated in bench-scale hydrolysis and fermentation process

The work will continue in FP7 Project HYPE

Example 2: Development of helper enzymes





Hemicellulases and other helper enzymes have major role in the efficient hydrolysis of technical agro or forest-based materials

These enzymes have been studied and their role has been evaluated in several projects, including TIME and EU-project NILE

Example 3: Development of more efficient enzymes

Comparison of the Michaelis-Menten, cellobiose inhibition constants and the temperature optimum of the four cellobiohydrolases

Enzyme	On CNPLac					On MULac			
	k_{cat} (min^{-1})	K_m (μM)	k_{cat}/K_m ($min^{-1}M^{-1}$)	K_i (Glc_2) (μM)	Type of inhibition	T_{opt} (°C)	k_{cat} (min^{-1})	K_m (μM)	k_{cat}/K_m ($min^{-1}M^{-1}$)
Ct **Cel7A**	19 ±1	2000 ±200	$9.5\ 10^3$	39 ±14	comp.	65	69 ±3.2	221 ±35	$3.1\ 10^5$
Ta **Cel7A**	1.7 ±0.1	990 ±70	$1.7\ 10^3$	107 ±14	comp.	65	19.5 ±0.8	268 ±38	$7.3\ 10^4$
At **Cel7A**	2.8 ±0.1	2100 ±150	$1.3\ 10^3$	141 ±25	comp.	60	11.3 ±0.7	220 ±44	$7.3\ 10^4$
Tr **Cel7A**	2.6 ±0.05	520 ±30	$5.0\ 10^3$	19± 4	comp.	60	28.7 ±1.0	287 ±31	$1.0\ 10^5$

Ref. Voutilainen et al., Biotechnol. Bioeng, in press.

Catalytic efficiency of the key cellulase enzymes, cellobiohydrolases is of extreme importance. New and more efficient CBH enzymes have been found characterized in our earlier projects.

This work will be continued in our on-going projects (e.g. EU-project DISCO, co-ordinated by VTT).

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TIME project