Production of bioethanol from lignocellulosic biomasses – recent developments at VTT

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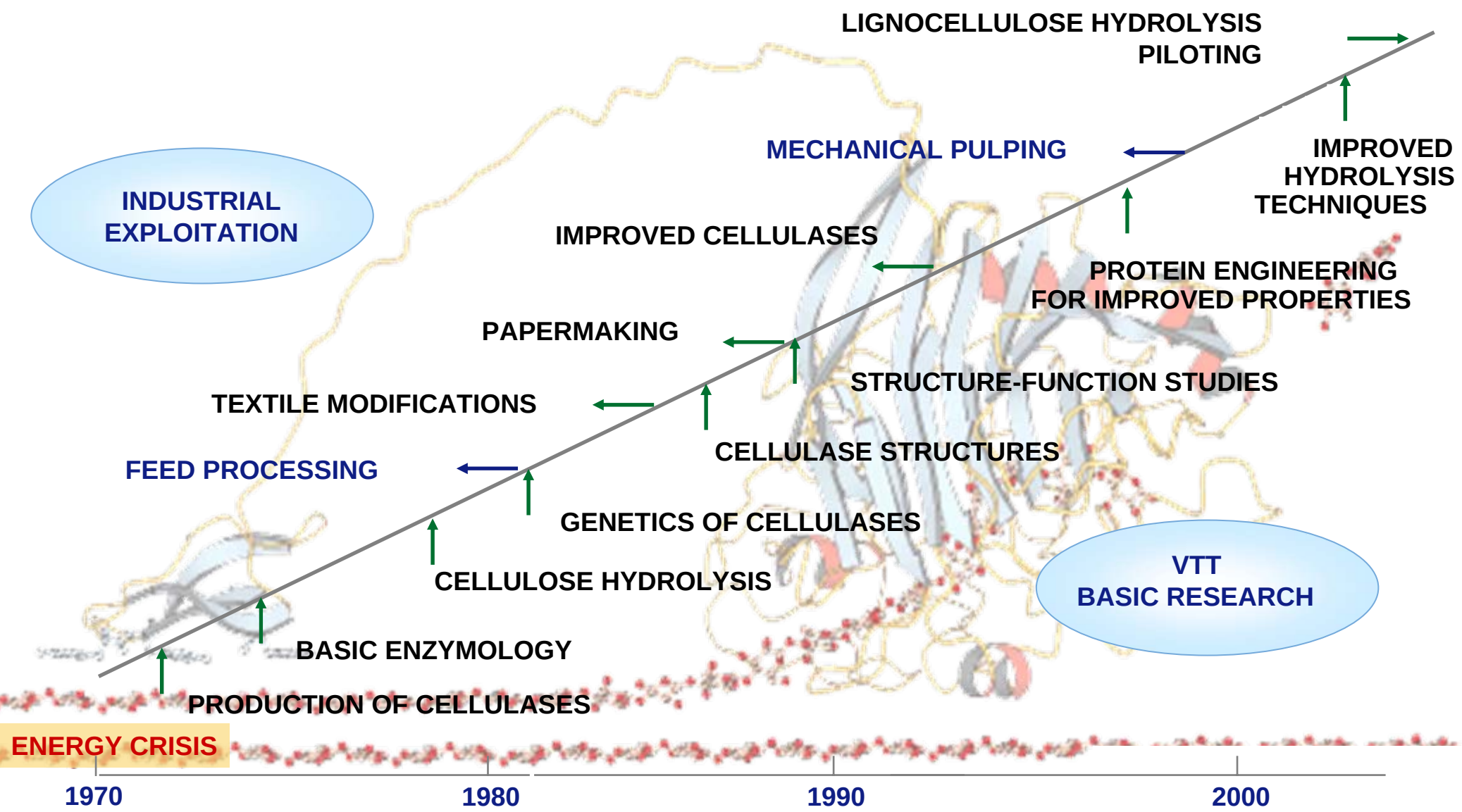
CELLULASE RESEARCH AT VTT



OUTLINE

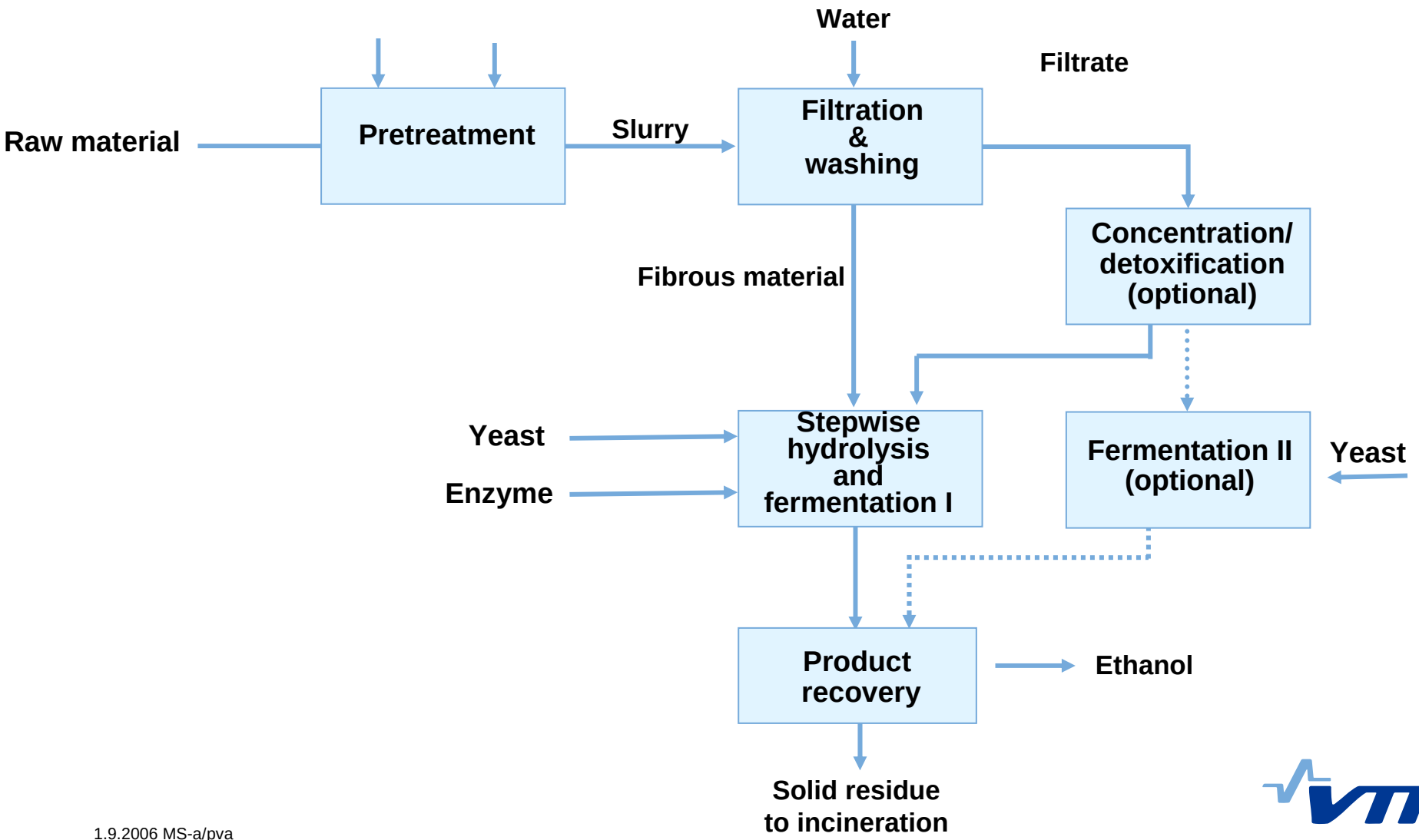
1. **Process for inhibitor-rich feedstocks**
2. **Research within the TIME project**
   - Novel thermophilic enzymes
   - Pre-hydrolysis process concept
   - Xylose-to-ethanol fermenting yeasts
3. **Research within the AGROETA project**
   - Energy crop reed canary grass vs. barley straw
4. **Decentralized bioethanol production (not lignocellulose)**
5. **Conclusions**

NOVEL PROCESS CONCEPTS

Aims: High final ethanol concentration, fast, high yield and low costs

1. High initial dry matter content
2. Concentration / detoxification of the hemicellulose fraction (optional)
3. Minimization of effects of inhibitors by phased addition
4. Thermophilic hydrolysis steps (several options)
5. Improved enzyme compositions

VTT PROCESS CONCEPT FOR INHIBITOR-RICH FEEDSTOCKS



TIME PROJECT

- “Technological improvement for ethanol production from lignocellulose”
- FP5-financed EU project (finished)
- Raw materials: corn stover, willow and spruce
- Pre-treatment: steam explosion or wet oxidation
- **VTT role in regard to enzymatic hydrolysis**
  - Novel thermostable hydrolytic enzymes
  - High-temperature pre-hydrolysis concept
- **VTT role in regard to fermentation**
  - Yeasts that ferment xylose to ethanol

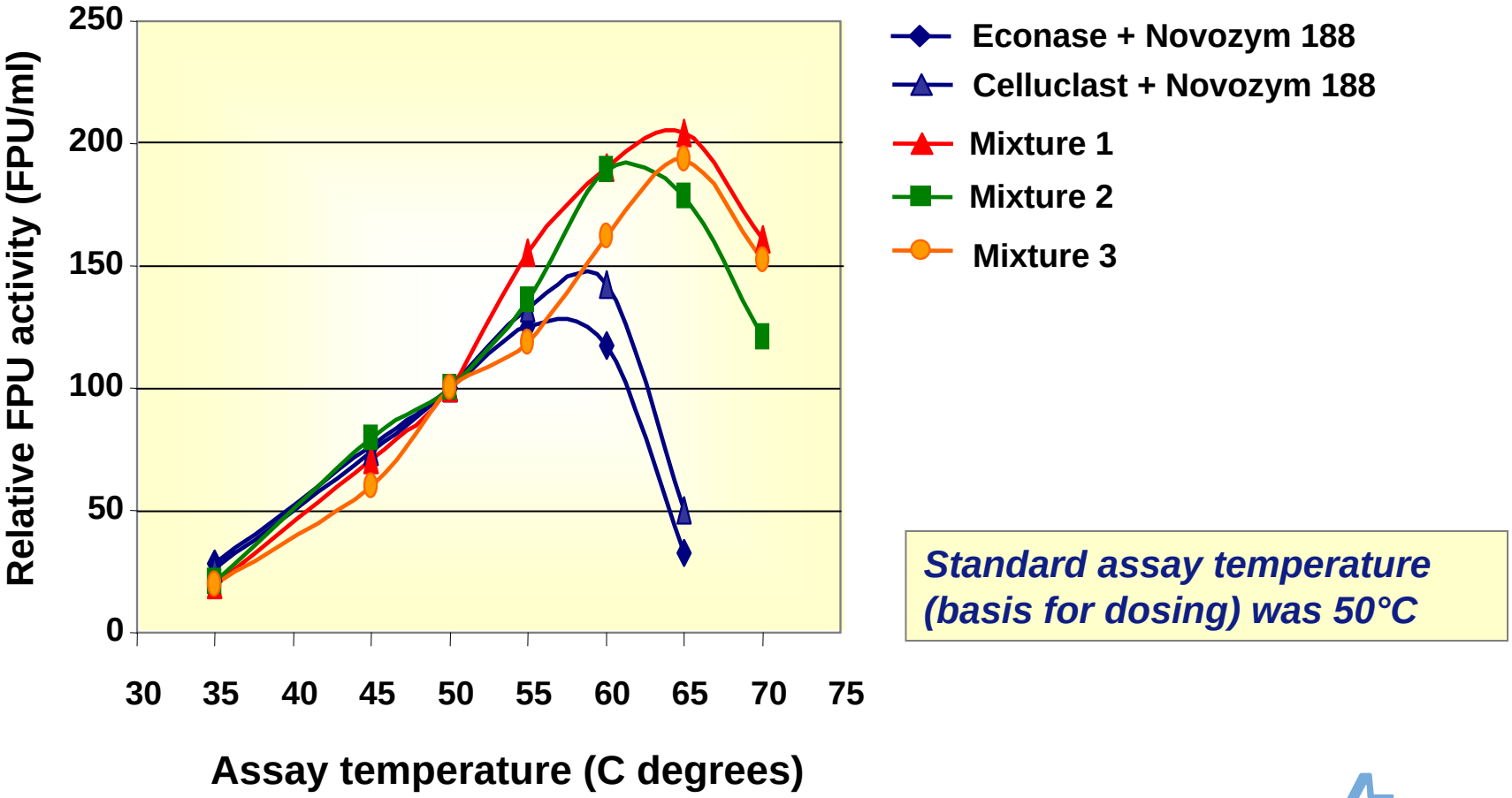
POTENTIAL ADVANTAGES OF THERMOSTABLE ENZYMES IN LIGNOCELLULOSE HYDROLYSIS

1. **Higher specific activity, i.e. decreased enzyme loading**
2. **Higher stability, i.e. extended life-time, reuse of enzymes**
3. **Allow more flexibility in the process configuration**
4. **Allow process with improved integration in terms of heat recovery and recycling of process streams**
5. **Allow increased dry matter content due to lower viscosity at high temperature**
6. **Smaller risk of contamination**

STRATEGY FOR DEVELOPING THERMOPHILIC ENZYME MIXTURES

1. **Screening and characterization of potential thermophilic proteins (CBH's, EGs, betaglucosidases & 1 XYL), activity profiles**
2. **Cloning of the most potential candidates (*T. reesei*)**
3. **Overproduction of candidate proteins (*T. reesei*)**
4. **Purification and characterization of candidate enzymes**
5. **Evaluation of the most interesting proteins and mixtures thereof**

FPU ACTIVITY OF THE NOVEL THERMOPHILIC ENZYMES (COMPOSED OF CBH-CBD, EG, β-Glu and XYL)

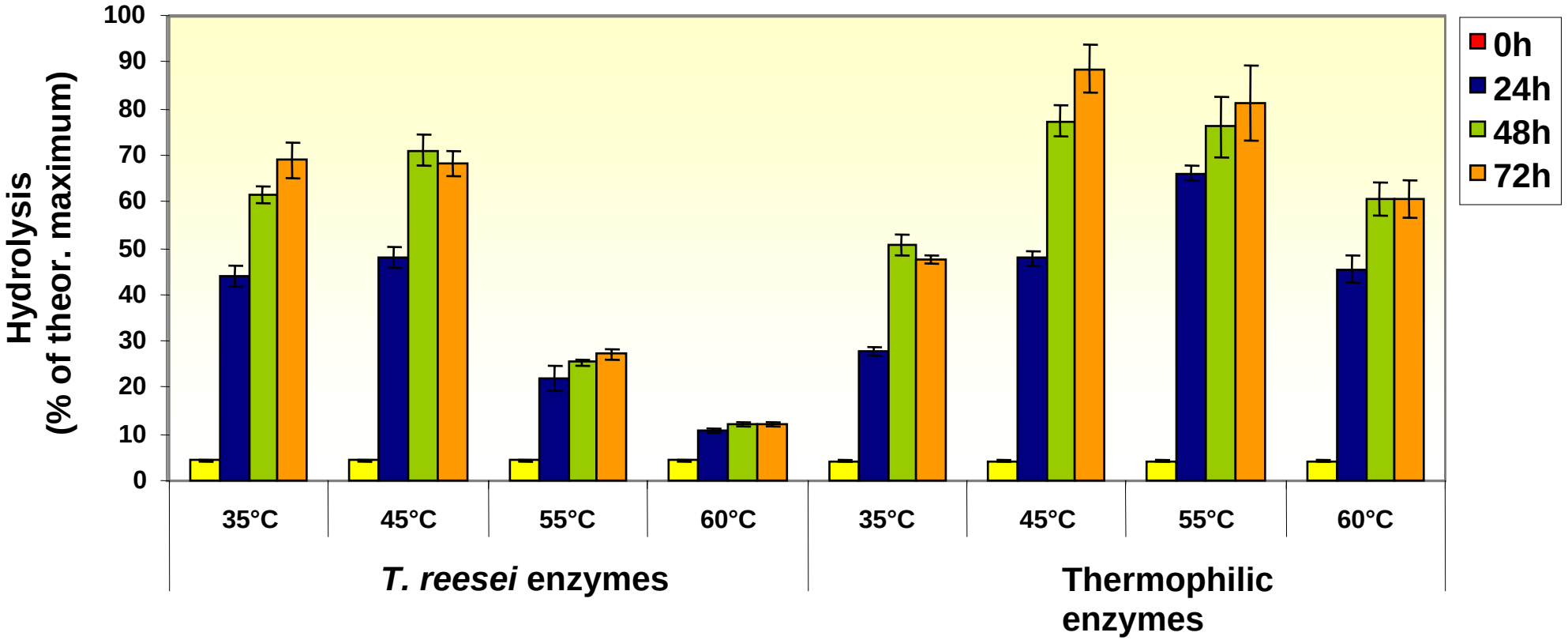


Standard assay temperature (basis for dosing) was 50°C

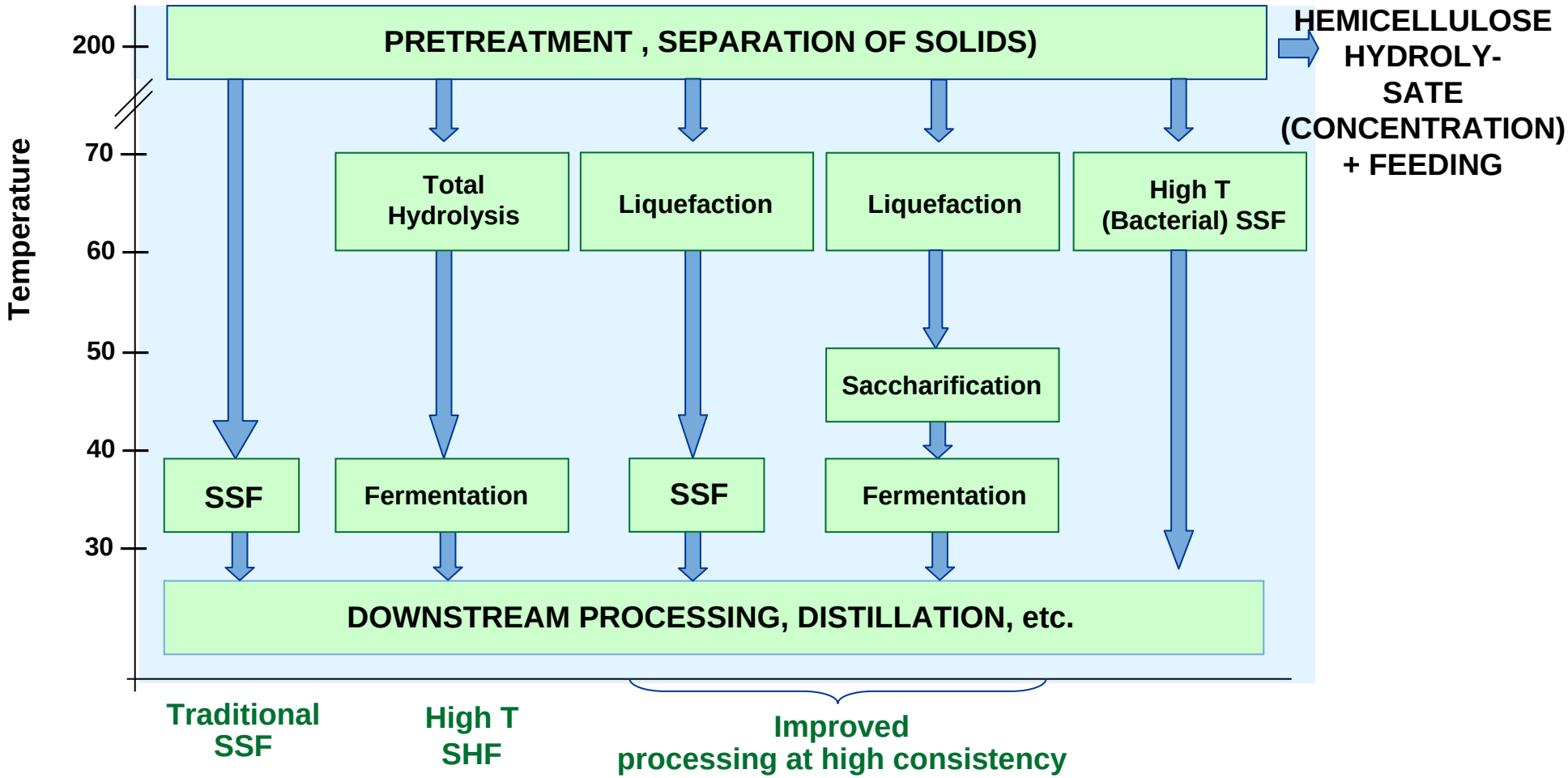
HYDROLYSIS OF SPRUCE WITH THE DESIGNED THERMOPHILIC ENZYME MIXTURE

Thermophilic enzymes (CBH, EG, β-Glu, XYL): 9.8 FPU/g cellulose

State-of-art enzymes (Celluclast + Novozym 188): 11.5 FPU/g cellulose



PROCESS CONCEPTS FOR THERMOPHILIC CELLULASES: HIGH CONSISTENCY BIOETHANOL PRODUCTION



YEAST DEVELOPMENT AT VTT

1. **Hydrolysate tolerant *Saccharomyces* strains and yeasts producing ethanol from xylose (XR/XDH and XI pathways)**
2. **Sugar transport and effect of XK over-expression**
3. **Analysis and modification of pentose phosphate pathway reactions**
4. **Redox engineering**
5. **Genome-wide and flux analyses (MFA) of pentose fermentation**
6. **Mutagenesis and chemostat screens for strain improvement**
7. **Cloning of the fungal arabinose utilisation pathway**

TIME project

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AGROETA PROJECT

- “Bioethanol production from local agricultural residues”
- TEKES-financed project (on-going)
- Raw materials: reed canary grass, barley straw and oat husk
- Effect of harvest time (collaborator)
- Pre-treatment: steam explosion (collaborator)
- **VTT & enzymatic hydrolysis**
  - Comparison of hydrolysis efficiency
- **VTT & fermentation**
  - Ethanol production utilizing engineered yeast
- **VTT & process feasibility**

BARLEY STRAW VS. REED CANARY GRASS

- **Reed canary grass**
  - Two fields (1990 and 2003)
  - Biomass yield 6 t/ha
  - An energy crop
- **Barley straw**
  - Biomass yield 2 t/ha
  - Food production waste
- **Effect of harvest time**
  - **On composition**
  - **On hydrolysis**
  - **On fermentation**
- **Harvest logistics**

BARLEY STRAW VS. REED CANARY GRASS 2

- Composition alike (of dry mass)
  - Straw: glucose 49%/xylose 24%
  - Grass: glucose 49%/xylose 19%
- After steam explosion similar dry mass contents and sugar compositions (fibre fraction)
- Released sugars after hydrolysis of fibre fraction with state-of-the-art enzymes
  - Straw: glucose 64%/xylose 11%
  - Grass: glucose 71%/xylose 6%
- Both feedstocks have been hydrolyzed and fermented without a separation step with promising results
- The VTT yeast converts xylose to ethanol, but also some xylitol was formed

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DECENTRALIZED BIOETHANOL PRODUCTION

- **Joint venture between VTT and a Finnish filling station company -> St1 Biofuels Oy**
- **Raw material: Food industry waste products (whey, dough, etc.) -> not lignocellulose!**
- **Combined, continuous fermentation & evaporation process results in a 20-40% ethanol solution**
- **Centralized distillation**
- **Currently piloted**
- **Contact: antti.pasanen@st1.fi**

CONCLUSIONS

- New process concept improves the performance of the process with feedstocks containing high amounts of inhibitors
- Novel thermophilic enzymes identified enabling increased hydrolysis temperatures (by 10-15 °C compared to the performance of state-of-the-art enzymes)
- Only four thermophilic enzymes are needed to top the current enzymes
- Short liquefaction step in combination with thermostability enable high consistency hydrolysis (up to 20%)
- Yeast strain development on several levels
- Reed canary grass – a interesting (local) energy crop
- Decentralized production being evaluated