Lignocellulosic enzymes

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US-EC Task Force on Biotechnology Research
Biotechnology for the Development of Sustainable Bio-energy
OUTLINE OF THE PRESENTATION

1. Background: from first to second generation fuels
2. Approaches to improve bioethanol production processes
3. Potential of thermostable enzymes in cellulose hydrolysis
4. Other enzymes
5. Conclusions
GREENHOUSE GAS REDUCTIONS

Figure 2. Range of estimated Greenhouse Gas Reductions from Biofuels

Source: IEA, 2006a

Doornbosch & Steenblick, OECD, 2007
First and second generation biofuels

Raw materials

1. Generation:
   - Sugars \( C_6H_{12}O_6, C_{12}H_{22}O_{11} \)
   - Starch \( (C_6H_{10}O_5)_n \)
   - Sugar-cane
   - Sugar
   - Corn
   - Wheat

2. Generation:
   - Fatty acids \( (C_{18}H_{34}O_2) \)
   - Rapeseed
   - Palm oil
   - Jatropha
   - Algae

Processes

1. Generation
   - Bioethanol \( C_2H_5OH \)
     - \( \geq 5 \% \) gasoline
     - \( \sim 0.5 \) €/litre

2. Generation
   - Methylester-diesel
     - \( \geq 5 \% \) diesel-mix
     - \( \sim 0.7 \) €/litre

Products

1. Generation
   - Synthetic biodiesel \( C_nH_{2n+2} \)

2. Generation:
   - Bioethanol, butanol etc..
Lignocellulose as raw material

Because of the resistant structure of cellulose and natural composite structures of lignocellulosics, efficient pretreatment technologies are needed prior to the enzymatic hydrolysis.

Ref. Wyman, 1994
THE CHALLENGING RAW MATERIAL

Diameter of each tracheid is approximately 30 µm (left), wood cell wall layers S1-S3: secondary cell wall layers, P: primary wall, M.L. middle lamella (middle) and lignin-carbohydrate complex of the secondary cell wall (right)

Adapted from Kirk and Cullen (1998).
GENERAL OUTLINE OF THE LIGNOCELLULOSE-TO-BIOETHANOL PROCESS

Pre-treatment

Hydrolysis

Fermentation

Distillation or separation

Simultaneous or separate saccharification and fermentation

Renewable lignocellulosic materials

Physical deconstruction and fractionation by refining, steam explosion or other methods

Hydrolysis of cellulose and hemicellulose by acid or enzymes

Fermentation of sugars (hexoses and pentoses) to ethanol by yeast or bacteria

Solid residue

Fuels: Ethanol...

Concentration and separation of product

Courtesy of K. Reczey
IMPROVEMENT OF THE ENZYMATIC HYDROLYSIS OF LIGNOCELLULOSE

Composition and accessibility of substrate
n Feedstock improvement (long term)
 n Pretreatment and fractionation of cellulose, hemicellulose and lignin (short term)

Properties of cellulases
n Specific activity
 n End-product inhibition
 n Stability

Composition and production of enzyme mixtures
n Optimal cellulase mixtures
 n Optimal accessory enzymes
 n Efficient production of necessary components

Hydrolysis technologies
 n Separate/simultaneous, recycling of enzymes etc.
Main enzymes in lignocellulose hydrolysis

- **Cellulases**
  - Endo-β-1.4-glucanases, cellobiohydrolases, β-glucosidases
  - Fungal cellulases *e.g.* *Trichoderma, Humicola, Acremonium*
  - Bacterial cellulases *e.g.* *Clostridium thermocellum*

- **Hemicellulases**
  - Backbone degrading enzymes
  - Enzymes removing the side groups
  - β-xylosidases

- **Lignin modifying enzymes?**
  - Laccases, peroxidases
  - Enzymes hydrolyzing lignin-carbohydrate complexes?

- **Other helper enzymes/proteins?**
  - Swollenin
<table>
<thead>
<tr>
<th>POTENTIAL ADVANTAGES OF THERMOSTABLE ENZYMES IN LIGNOCELLULOSE HYDROLYSIS</th>
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<tbody>
<tr>
<td>• Higher specific activity, <em>i.e.</em> decreased enzyme loading</td>
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<tr>
<td>• Higher stability; <em>i.e.</em> extended life-time, reuse of enzymes</td>
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<td>• Allow more flexibility for process configuration</td>
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<tr>
<td>• Allow process with improved integration in terms of heat recovery and recycling of process streams</td>
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<tr>
<td>• When expressed in plants, allow more flexible processing</td>
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<td>• Allow increased dry matter content due to lower viscosity at high temperature</td>
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BIOETHANOL PRODUCTION CONCEPTS
with various options in relation to process temperature

Viikari et al. (2007) Advances in Biochemical Engineering Biotechnology 108, 121-145
Thermostable enzymes

Methylumbelliferyl lactoside (MULac) used as a substrate

Results:
- \( T_{\text{opt}} \geq 65 \, ^{\circ}\text{C} \) for \( Ct \) Cel7A and \( Ta \) Cel7A, and \( \geq 60 \, ^{\circ}\text{C} \) for \( At \) Cel7A and \( \sim 60 \, ^{\circ}\text{C} \) for \( Tr \) Cel7A
- \( Ct \) Cel7A clearly the most active cellobiohydrolase on soluble substrate (already at lower temperatures).

Hydrolysis of microcrystalline cellulose at 70 °C

2-module versions of the cellobiohydrolases

**Results:**
- *Ta Cell7A + Tr CBM* the most efficient enzyme

The time-course of Avicel hydrolysis was followed for 24 hours by measuring soluble reducing sugars.

Kinetic constants and cellobiose inhibition, soluble model substrate, 22°C

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>$k_{cat}$ (min$^{-1}$)</th>
<th>$K_m$ (μM)</th>
<th>$k_{cat}/K_m$ (min$^{-1}$M$^{-1}$)</th>
<th>$K_i$(Glc$_2$) (μM)</th>
<th>Type of inhibition</th>
</tr>
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<tbody>
<tr>
<td>Ct Cel7A</td>
<td>19 ±1</td>
<td>2000 ±200</td>
<td>9.5 x 10$^3$</td>
<td>39 ±14</td>
<td>comp.</td>
</tr>
<tr>
<td>Ta Cel7A</td>
<td>1.7 ±0.1</td>
<td>990 ±70</td>
<td>1.7 x 10$^3$</td>
<td>107 ±14</td>
<td>comp.</td>
</tr>
<tr>
<td>At Cel7A</td>
<td>2.8 ±0.1</td>
<td>2100 ±150</td>
<td>1.3 x 10$^3$</td>
<td>141 ±25</td>
<td>comp.</td>
</tr>
<tr>
<td>Tr Cel7A</td>
<td>2.6 ±0.05</td>
<td>520 ±30</td>
<td>5.0 x 10$^3$</td>
<td>19 ±4</td>
<td>comp.</td>
</tr>
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</table>

HYDROLYSIS OF STEAM PRETREATED SPRUCE

- Thermostable enzymes (CBH, EG, β-Glu, XYL): 9.8 FPU/g cellulose
- Reference enzymes (Celluclast + Novozym 188): 11.5 FPU/g

Viikari et al. (2007) Advances in Biochemical Engineering Biotechnology 108, 121-145
HEMICELLULOSES AND HEMICELLULULASES

Hemicellulases are essential components in efficient LC enzyme mixtures

The need for accessory enzymes depends on the substrate & pretreatment used
CONCLUSIONS: IMPROVED LIGNOCELLULOSE ENZYMES

**Feed stock improvement**
- Improved raw materials & pretreatments for better hydrolyzability
- Modified carbohydrate/lignin structures
- Expression of LC enzymes in plants

**Cellulases & other enzymes**
- Short & long term challenges for enzyme development
- Thermostability a generally useful parameter
- Integrated hydrolysis technologies
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