Cellulosic ethanol: From revolutionary consolidated bioprocessing idea to proof of concept

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Map of Western Cape
Cape of Good Hope

Arial view of Stellenbosch
Stellenbosch - Jewel of the Cape

Panoramic view of Stellenbosch

“Wine country”
Central campus - “The Red Plane”
1. Next generation technologies for cellulose conversion

2. What is Consolidated Bioprocessing?

3. Recent advances towards realizing CBP

4. Rolling out cellulosic ethanol in southern Africa
Next generation technologies for cellulose conversion
Technologies for Ethanol Production

Ethanol production from sugar

Sugarcane
Sugarbeet
Sweet sorghum

Sugar
Storage tank

Crashing
Sugar extraction

Yeast
Alcohol recovery

Fermentation ➔ Distillation & dehydration

Storage tank

Spent yeast

Fuel blending
Technologies for Ethanol Production

Ethanol production from cellulosics

Pre-treatment ➔ Saccharification ➔ Fermentation ➔ Distillation & dehydration

Agric Res
Woody
Material
Grasses

Chipping
Grinding

Water

Mixing tank

Steam explosion
~200°C

Cellulases

Yeast

Alcohol recovery

Storage tank

Fuel blending

Spent material
Pretreatment for Ethanol Production

Pretreatment - produces an enzyme accessible substrate

Lignin

Cellulose

Amorphous Region

Crystalline Region

Hemicellulose

Ladisch, 2006
**Enzymes required for CBP**

<table>
<thead>
<tr>
<th>Component</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucan</td>
<td>41.6</td>
</tr>
<tr>
<td>Xylan</td>
<td>15.9</td>
</tr>
<tr>
<td>Galactan</td>
<td>0.7</td>
</tr>
<tr>
<td>Mannan</td>
<td>2.2</td>
</tr>
<tr>
<td>Arabinan</td>
<td>0.8</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>5</td>
</tr>
<tr>
<td>Extractives</td>
<td>1.4</td>
</tr>
<tr>
<td>Lignin</td>
<td>25.6</td>
</tr>
<tr>
<td>Ash</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>93.7</strong></td>
</tr>
</tbody>
</table>

**Cellulose**
- Cellbiohydrolases
  - Endo-glucanase
  - β-glucosidase
- Endo-xylanase
- β-xylosidase
- Acetyl xylan esterase
- α-glucuronidase
- α-arabinofuransidase
- Endo-mannanase
- β-mannosidase
- etc.

**Hemicellulose**
- Partially hydrolyzed during feedstock pretreatment
- Xylose
- Manose
- Arabinose
- Galactose
Enzyme system development

**T. reesei secretome**

- CBHs are the major constituent of the *T. reesei* cellulase system
- Second most important species are the EGs
- Broad diversity of enzymes contributes to highly active system
Largest Component of Recalcitrance Barrier: Cost of Cellulase


![Graph showing estimated cellulase cost over years](image-url)
Technologies for Cellulose Conversion

Biomass Processes for EtOH production

Biologically-Mediated Event

Enzyme Hydrolysis Processing Strategy
(Each box represents a bioreactory - not to scale)

Cellulase Production
Lignocellulose Hydrolysis
Hexose Fermentation
Pentose Fermentation

SHF: Separate Hydrolysis & Fermentation
SSF: Simultaneous Saccharification & Fermentation
SSCF: Simultaneous Saccharification & Co-Fermentation
CBP: Consolidated Bioprocessing
Consolidated BioProcessing (CBP)
Consolidated BioProcessing (CBP)

Fundamentals of Microbial Cellulose Utilization

Microbial Cellulose Utilization: Fundamentals and Biotechnology
Lee R. Lynd,1* Paul J. Weimer,2 Willem H. van Zyl,3 and Isak S. Pretorius4

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Consolidated Bioprocessing (CBP)

Consolidated bioprocessing: update (2)

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Consolidated Bioprocessing for Bioethanol Production
Using *Saccharomyces cerevisiae*

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Consolidated BioProcessing (CBP)

Consolidated bioprocessing: update (3)

DOI 10.1007/s00253-010-2660-x

MINI-REVIEW

Engineering cellulolytic ability into bioprocessing organisms

Daniel C. la Grange • Riaan den Haan • Willem H. van Zyl

Applied Microbiology and Biotechnology 87: 1195-1208 (2010)
Technologies for Cellulose Conversion

Consolidated BioProcessing (CBP)
Technologies for Ethanol Production

Ethanol production from cellulosics

- Agric Res Woody Material Grasses
- Water
- Chipping Grinding
- Steam explosion ~200°C
- Mixing tank
- Cooling & conditioning
- Cellulases
- Yeast
- Alcohol recovery
- Pre-treatment ➔ Saccharification ➔ Fermentation ➔ Distillation & dehydration
- Storage tank
- Fuel blending
- Spent material
Technologies for Ethanol Production
Ethanol production from cellulosics

Agric Res
Woody
Material
Grasses

Chipping
Grinding

Water

mixing
tank

Steam
explosion
~200°C

Cooling &
conditioning

Cellulolytic Yeast

Pre-treatment ➔ Saccharification & Fermentation ➔ Distillation & dehydration

Alcohol
recovery

Storage
tank

Spent
material

Fuel
blending

Fuel
recovery

Distillation & dehydration

Saccharification & Fermentation

Technologies for Ethanol Production
Ethanol production from cellulosics
Expression of cellobiohydrolases in yeast

CBH1 cellobiohydrolase production by yeast

Expression of cellobiohydrolases in yeast

Cellobiohydrolase production by yeast

• CBH1 requirements calculated based on ratio of CBH1 to other cellulase components in T. reesei cellulase mixtures to allow growth rate of 0.02 hr⁻¹

2.6% of t.c.p.

Mascoma Corporation
Technical facilities, Lebanon, NH, USA
(www.mascoma.com)
Leading Investment, Unprecedented Focus on CBP

Technical Focus: Overcoming the biomass recalcitrance barrier and enabling the emergence of a cellulosic biofuels industry via pioneering CBP technology integrated with advanced pretreatment

Partners in Mascoma’s CBP Organism Development Effort

- VTT, Finland
- Dartmouth College, USA
- University of Stellenbosch, ZA
- BioEnergy Science Center, USA
- Department of Energy, USA

Three Platforms

1. *T. saccharolyticum*, thermophilic bacterium able to use non-glucose sugars
2. *C. thermocellum*, thermophilic cellulolytic bacterium
3. Yeast engineered to utilize cellulose and ferment glucose and xylose

Multiple chances to succeed near-term & long-term
Screen CBH1 for high level expression

Enzyme activity:
- 48 hour Avicel hydrolysis
- Best enzyme x13 greater than starting point

Enzyme Production:
- 94 mg/L CBH1
- ± 2.5% of Total cell protein in minimal medium
Screen CBH1 for high level expression

Cellulose binding domain

Attachment of CBD

- Best CBM ± 2x improvement in activity
- ± 2x decrease in protein level
Screen CBH2 for high level expression

Enzyme activity:
- 48 hour Avicel hydrolysis
- Best enzyme x2 greater than starting point

Enzyme Production:
- 140 mg/L CBH2
- ± 5% of Total cell protein in minimal medium
- x3 higher than starting point
Combinations of CBHs

Best combination x2.5 greater than single enzyme
Mascoma Cellulolytic Yeast

Cellulase expression in Mascoma Yeast (robust C₅/C₆ fermenting)

Protein Expression (mg/g DCW)

- CBH2 expressed (Reinikainen et al., 1992)
- March 2007
- March 2008
- Oct. 2008
- Dec. 2008

- Improved CBH2; ~1500X ↑
- Improved CBH1; ~400X ↑

Improved host and culture conditions; ~2500X ↑
Enzyme Reduction on Paper Sludge

Mascoma CBP technology on 18% w/w paper sludge

Appearance at 120 hrs.

**Ethanol concentration, g/L**

- CBP + 1 mg Xylanase
- Background + Cellulase + BGL + Xylanase
- Background + BGL + Xylanase

**Time (hours)**

0  50  100

0  10  20  30  40  50  60
Enzyme Reduction on Hardwood

Mascoma CBP Strain (robust C5/C6 fermenting yeast) + 22% w/w unwashed Pretreated Hardwood

Equivalent performance with 2.5-fold less added enzyme

Further reduction likely
Rome, NY Pilot & Demonstration Plant

January 2008

November 2008
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Merja Penttilä
Rolling out cellulosic ethanol in southern Africa
Biomass Technologies will lead the production of next generation cellulosic ethanol in Africa... and drive a sustainable bioenergy future for the continent.

www.sbmt.co.za
SBMT holds the exclusive rights to Mascoma technology for Southern Africa.

- **Mascoma**: Exclusive license (>30 patents, process designs)
- **SBMT**: Value addition to process
- **SU**: Commercialization
- **Africa demonstration**: Map showing the geographical locations and connections.
Costs vs timeline for technology development

1. Mobile conversion unit
   - R5-10 M

2. Demonstration Plant
   - R75-150 M

3. Paper Sludge? Woodchips commercial
   - ~R200-500 M

4. Integrated 1st/2nd generation (sugar cane)
   - >R1 B
Thank you