Construction of cellulolytic Saccharomyces cerevisiae strains for consolidated bioprocessing

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7. SANERI Senior Chair of Energy Research: Biofuels
Next generation technologies for cellulose conversion
Technologies for Cellulose Conversion

Lignocellulose composition

Sugarcane bagasse

- Lignin: 28%
- Xylan: 25%
- Cellulose: 46%
- Arabinan: 2%
- Pentoses (fermentable)
- Hexoses (fermentable)

Non-fermentable sugars
high energy aromatics

Lignin
28%

Arabinan
2%

Pentoses
(fermentable)
Enzymatic hydrolysis of biomass

**Ligninases**
(laccases, lignin peroxidases, Mn-peroxidases)

**Cellulases**
(endoglucanases, cellobiohydrolases, β-glucosidases)

**Hemicellulases**
(xylanases, β-xylosidases, α-arabinofuranosidases, α-glucuronidases)

**Esterases**
(feruloyl esterases, coumaroyl esterases)
Technologies for Cellulose Conversion

Biomass Processes for EtOH production

Biologically-Mediated Event

Enzyme Hydrolysis Processing Strategy
(Each box represents a bioreactor - 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)
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

Chemical and Biochemical Engineering, Thayer School of Engineering and Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire 03755; USDA Agricultural Research Service, U.S. Dairy Forage Research Center and Department of Bacteriology, Madison, Wisconsin, 53706; and Department of Microbiology and Institute for Wine Biotechnology,⁴ University of Stellenbosch, Stellenbosch 7600, South Africa

Consolidated Bioprocessing (CBP)

Consolidated bioprocessing: update

Consolidated bioprocessing of cellulosic biomass: an update
Lee R Lynd¹,², Willem H van Zyl², John E McBride¹ and Mark Laser¹

Current Opinion in Biotechnology 16:577-583 (2005)
Consolidated BioProcessing (CBP)

Consolidated bioprocessing: update (2)

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Consolidated Bioprocessing for Bioethanol Production Using *Saccharomyces cerevisiae*

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Advances in Biochemical Engineering / Biotechnology (2007)
MINI-REVIEW

Engineering cellulolytic ability into bioprocessing organisms

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## Consolidated BioProcessing (CBP)
### Consolidated bioprocessing: update (3)

<table>
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<th>Activity</th>
<th>S. cerevisiae</th>
<th>K. marxianus</th>
<th>P. stipitis</th>
<th>H. polymorpha</th>
<th>E. coli</th>
<th>K. oxytoca</th>
<th>Z. mobilis</th>
<th>C. acetobutylicum</th>
<th>Lactic acid bacteria</th>
<th>T. saccharolyticum</th>
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### Breakdown Capacities
- **Breakdown crystalline cellulose**
- **Breakdown amorphous cellulose**
- **Breakdown hemicellulose**
- **Utilize cellobiose**
- **Utilize xylobiose**
- **Grow on glucose**
- **Grow on xylose**
- **Ferment glucose to ethanol**
- **Ferment xylose to ethanol**
- **Resistant to hydrolysate inhibitors**
- **GRAS status**
- **Low pH**
- **High temperature**

**Notes:**
- WT: Wild Type
- GE: Genetic Engineering
Technologies for Ethanol Production

Ethanol production from sugar

- Sugarcane
- Sugarbeet
- Sweet sorghum

Crashing Sugar extraction → Sugar Storage tank → Yeast Alcohol recovery → Fermentation → Distillation & dehydration → Storage tank → Spent yeast → Fuel blending
Technologies for Ethanol Production
Ethanol production from cellulosics

- Agric Res
- Woody Material
- Grasses

- Chipping
- Grinding

- Mixing tank

- Water

- Steam explosion ~200ºC

- Cooling & conditioning

- Pre-treatment ➔ Saccharification ➔ Fermentation ➔ Distillation & dehydration

- Cellulases
- Yeast
- Alcohol recovery

- Storage tank

- Spent material
- Fuel blending
Largest Component of Recalcitrance Barrier: Cost of Cellulase

Consolidated BioProcessing (CBP)

Glycosyl Hydrolases

Ethanol + CO₂
Technologies for Ethanol Production

Ethanol production from cellulosics

Agric Res
Woody
Material
Grasses

Water

Chipping
Grinding

mixing
tank

Steam explosion
~200°C

Celluloses

Yeast

Alcohol
recovery

Pre-treatment ➔ Saccharification ➔ Fermentation ➔ Distillation & dehydration

Cooling & conditioning

Storage tank

Fuel blending

Spent material
Technologies for Ethanol Production

Ethanol production from cellulosics

Pre-treatment ➔ Saccharification & Fermentation ➔ Distillation & dehydration

Agric Res
Woody Material
Grasses

Water

Steam explosion
~200°C

Cellulolytic Yeast

Alcohol recovery

Chipping
 Grinding

mixing tank

Cooling & conditioning

Storage tank

Fuel blending

Spent material
Conversion of amorphous cellulose to yeast biomass
Cellobiose utilization in yeast

Cellobiose utilization by *S. cerevisiae*

\[
\Sigma ySF1 = S. cerevisiae \text{ expressing } S. fibuligera \beta\text{-glucosidase (BGL1) gene from 2µ plasmid.}
\]

\[
\Sigma \text{ host - glucose}
\]

\[
\Sigma \text{ host - cellobiose}
\]

\[
\Sigma ySF1 - glucose
\]

\[
\Sigma ySF1 - cellobiose
\]

*SC media:* 5g/L cellobiose; 30°C / pH adjusted to 6.0 (KOH)

Co-expression of endoglucanase & β-glucosidase in *S. cerevisiae*

\[
\begin{align*}
[\text{REF}] &= Y294 [\text{yEP352}]:\text{fur1}; \\
[\text{SFI}] &= Y294 [\text{BGL1}]:\text{fur1}; \\
[\text{EG1}] &= Y294 [\text{EG1}]:\text{fur1}; \\
[\text{CEL5}] &= Y294 [\text{EG1+BGL1}]:\text{fur1}.
\end{align*}
\]
Conversion of amorphous cellulose

Growth on amorphous cellulose (PASC)

Functional expression of cellobiohydrolases in yeast
Expression of cellobiohydrolases in yeast

CBH expression in *S. cerevisiae*

Functional CBH expression: a long-standing but elusive goal!

- **pCBH1-4** = ENO1<sub>p</sub> \(\rightarrow\) P. chrysosporium CBH1-4 \(\rightarrow\) ENO1<sub>T</sub>
- **pDLG100** = ENO1<sub>p</sub> \(\rightarrow\) P. chrysosporium CBH1-4 \(\rightarrow\) ENO1<sub>T</sub>
- **pAZ21** = ENO1<sub>p</sub> \(\rightarrow\) T. reesei CBH2 \(\rightarrow\) ENO1<sub>T</sub>
- **pAZ22** = ENO1<sub>p</sub> \(\rightarrow\) T. reesei CBH1 \(\rightarrow\) ENO1<sub>T</sub>
- **pDLG82** = ENO1<sub>p</sub> \(\rightarrow\) A. niger CBHB \(\rightarrow\) ENO1<sub>T</sub>

= XYNSEC secretion signal

= HisTag
Expression of cellobiohydrolases in yeast

CBH1 cellobiohydrolase production by yeast

MW (Da)
206,675
115,758
98,003
54,604
37,390
29,559
20,366
7,036

Y294[CBH1] ~150X conc. treated with Pngase

Y294[CBH1] ~20X conc.

+ control CBH1 ~250 ng, treated with Pngase

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.

Recent advances towards realizing CBP
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
- Dartmouth College
- University of Stellenbosch
- BioEnergy Science Center
- Department of Energy

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*
Subsequent expression of cellobiohydrolases!

12% SDS-PAGE, silver staining
Mascoma Cellulolytic Yeast

Cellulase expression in Mascoma Yeast (robust $C_5/C_6$ fermenting) vs Time

- **T. reesei cellulase in yeast** (Reinikainen et al., 1992): 0.62 mg/g DCW
- **T. reesei cellulase in Mascoma Yeast (March '07)**: 0.03 mg/g DCW
- **Proprietary cellulase in Mascoma Yeast (March '08)**: 2.4 mg/g DCW
- **Cellulase expression in Mascoma yeast (October '08)**: 13 mg/g DCW
- **Cellulase expression in Mascoma yeast (December '08)**: 100 mg/g DCW

Mar, 2007 to Dec, 2008: >3,000-fold improvement in expression levels
Enzyme Reduction on Hardwood

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

Equivalent performance with 2.5-fold less added enzyme

Further reduction likely
Conversion of Paper Sludge to Ethanol: Proof of CBP Concept

Mascoma CBP technology on 18% w/w paper sludge
(1 mg/g TS b-glucosidase and 1 mg/g TS xylanase added)

- Mascoma CBP Yeast, no added cellulase
- Mascoma non-CBP Yeast, w/10 FPU loading cellulases
- Non-CBP Yeast

85% cellulose conversion with production of recoverable ethanol with no added cellulase!
Acknowledgements

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Dartmouth College, USA
John McBride         Lee Lynd

VTT, Finland
Marja Ilmen          Merja Penttilä
Chair of Energy Research : Biofuels (members)
Cellulosics biofuels production value chain:

1. The CoER: Biofuels positions itself in the conversion technologies, but acknowledges the importance of establishing the whole value chain.

2. These includes both biochemical and thermochemical processes and integration of the processes if applicable.
Technologies for Cellulose Conversion

Biomass Biorefinery Concept

Biological Processing
- Pretreatment
- Fermentation
- Separation

Non-Biological Processing
- Gasification
- Fast pyrolysis
- Power generation
- Synthesis & separation

Exported Power / Biofuels

Thermochemically-derived Chemicals (potentially several)

Biologically-derived Chemicals (potentially several)

Ethanol

Animal feed

Residues

Steam

Process Power

Cellulosic Biomass

Biomass potential of Africa at large

Ratio of the energy content of the biomass on abandoned agriculture lands relative to the current primary energy demand at the country level. The energy content of biomass is assumed to be 20 kJ g\(^{-1}\). Source: Campbell et al. (2008)
South Africa's potential: Renewable biomass available

1. Residues
   Agricultural
   - Maize stover: 6.7 Mt/a (118 PJ/a)
   - Sugar cane bagasse: 3.3 Mt/a (58 PJ/a)
   - Wheat straw: 1.6 Mt/a (28 PJ/a)
   - Sunflower stalks: 0.6 Mt/a (11 PJ/a)
   **Agricultural subtotal**: 12.3 Mt/a (214 PJ/a)

   Forest industry
   - Left in forest: 4.0 Mt/a (69 PJ/a)
   - Saw mill residue: 0.9 Mt/a (16 PJ/a)
   - Paper & board mill sludge: 0.1 Mt/a (2 PJ/a)
   **Forest industry subtotal**: 5.0 Mt/a (87 PJ/a)

2. Energy crops
   - From 10% of available land: 67 Mt/a (1 171 PJ/a)
   (Marrison and Larson, 1996)

3. Invasive plant species
   **Total, annual basis**: 93 Mt/a (1 622 PJ/a)

South Africa’s potential: Biofuels production

Maize to Ethanol = 430 L/ton
Biomass to ethanol = 280 L/ton
Biomass to upgraded bio-oils = 310 L/ton
Biomass to liquid (BtL) = 570 L/ton
Thank you!