



**CoER : Biofuels**

Department of Microbiology • Faculty of Natural Sciences

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

## **Emile van Zyl**

*Department Microbiology  
University of Stellenbosch*





# Western Cape



Map of Western Cape





# Cape of Good Hope



*Arial view of Stellenbosch*



*arial view of the city and harbor*

Water Front

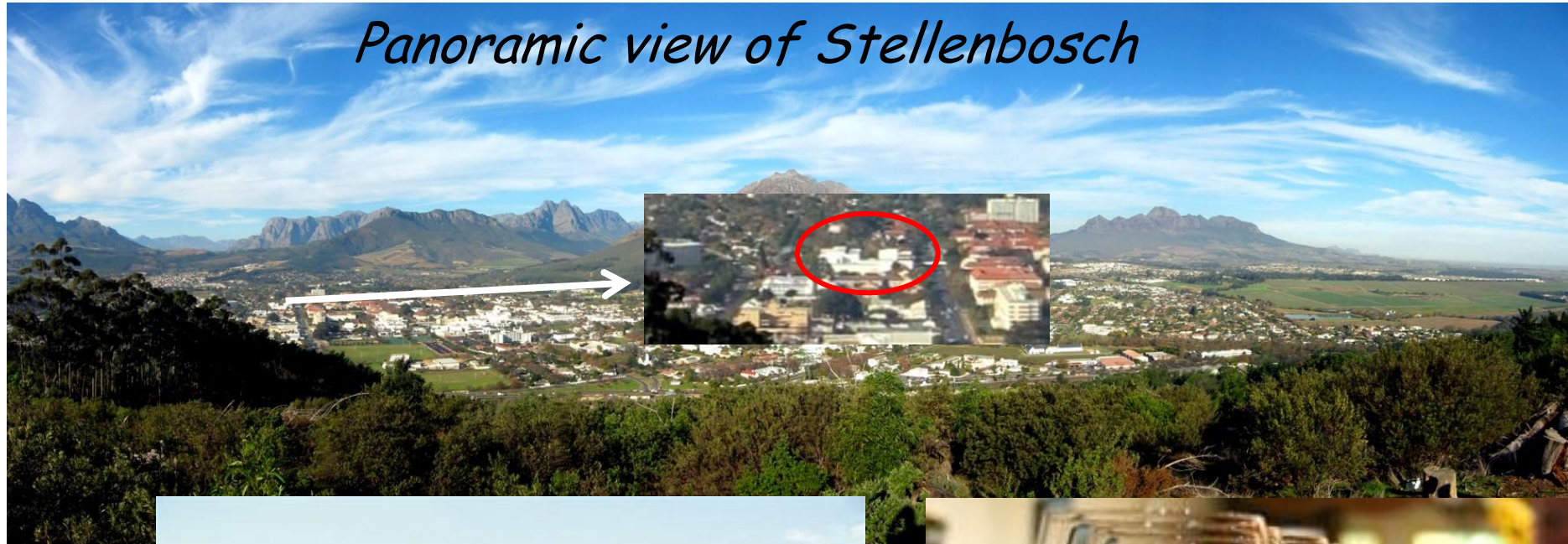




# Stellenbosch- Jewel of the Cape



*Panoramic view of Stellenbosch*



"Wine country"





Central campus - "The Red Plane"



Dept. Microbiology





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





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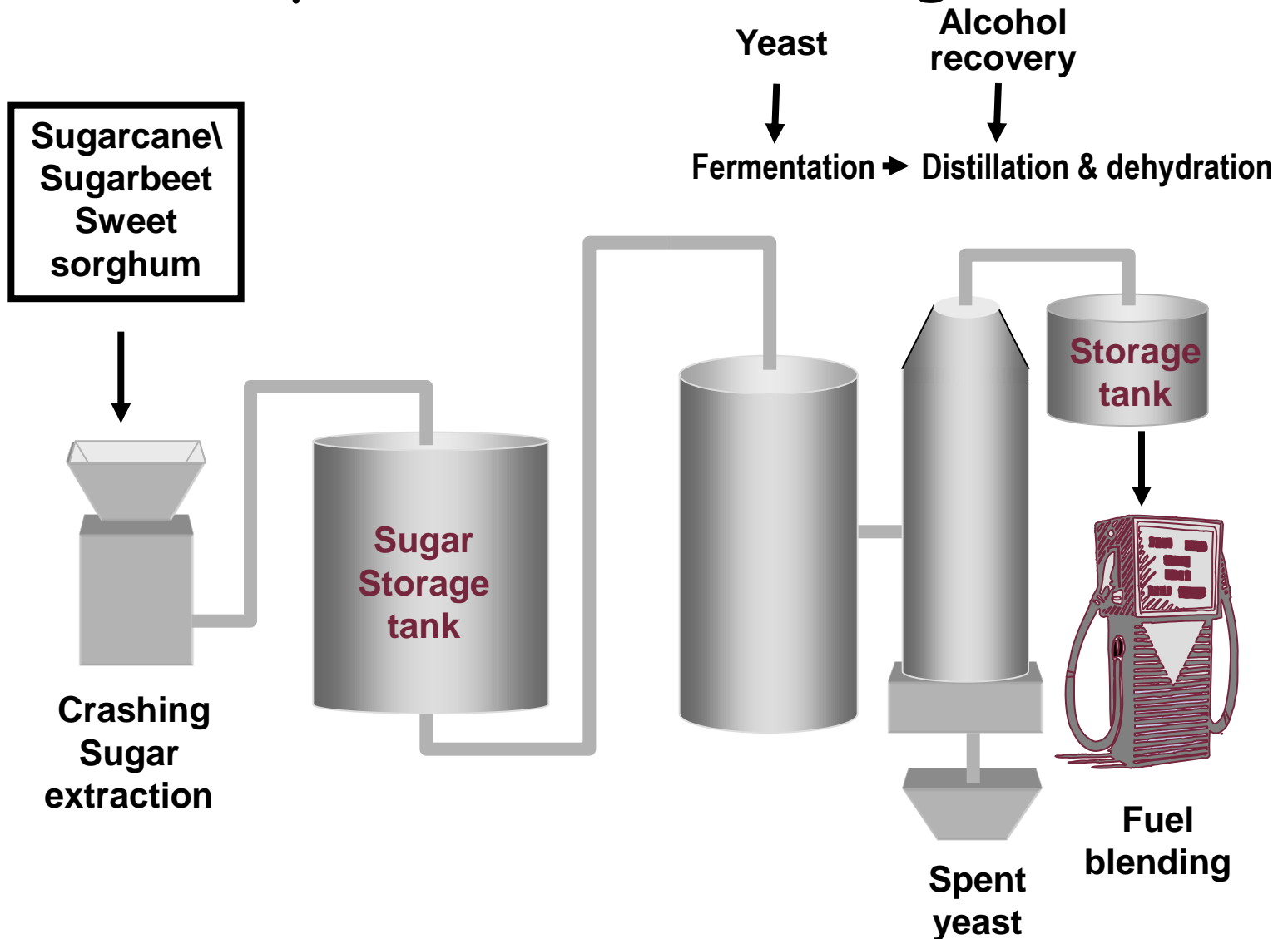
# Next generation technologies for cellulose conversion





# Technologies for Ethanol Production

## Ethanol production from sugar

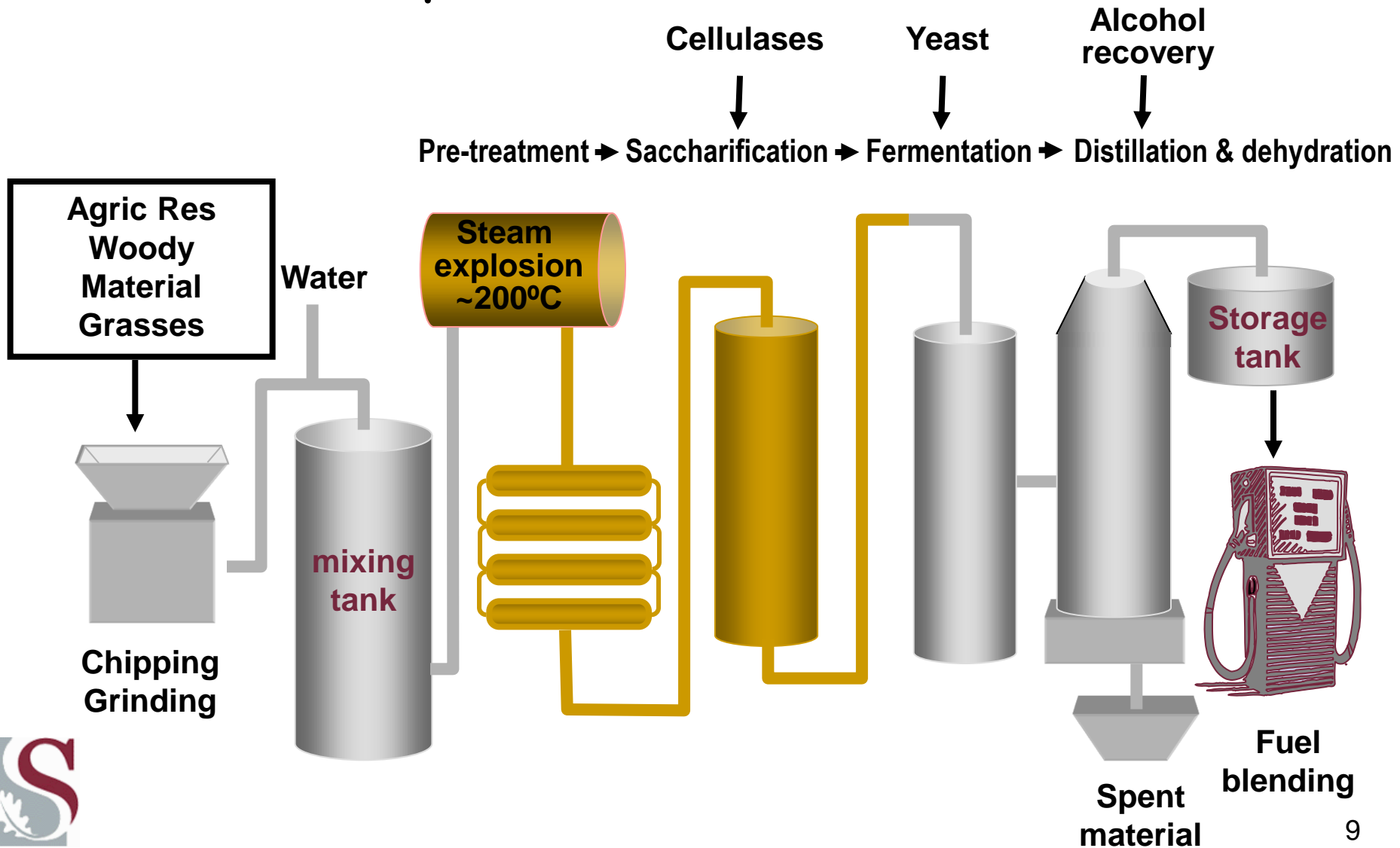






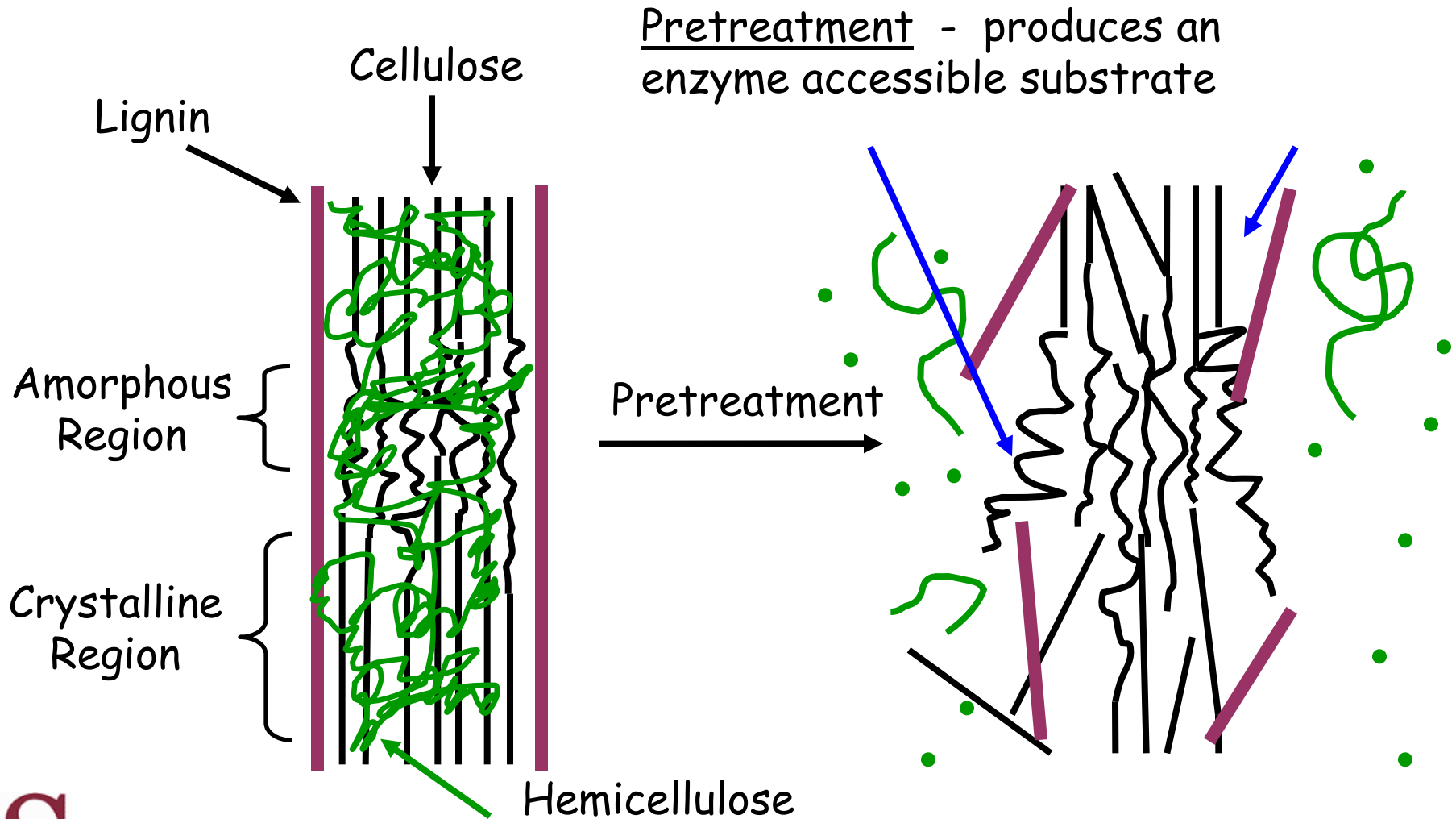
# Technologies for Ethanol Production

## Ethanol production from cellulosics





# Pretreatment for Ethanol Production





# Enzymes required for CBP



Component	
Glucan	41.6
Xylan	15.9
Galactan	0.7
Mannan	2.2
Arabinan	0.8
Acetic acid	5
Extractives	1.4
Lignin	25.6
Ash	0.5
Total	93.7



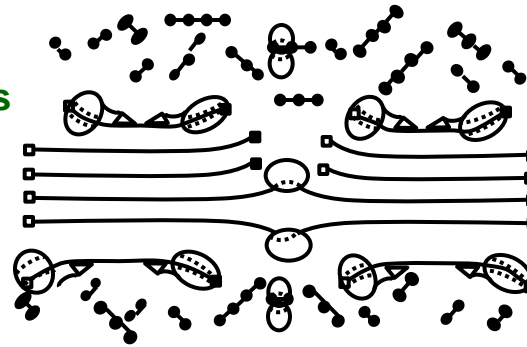
## Cellobiohydrolases

Endo-glucanase  
β-glucosidase

Endo-xylanase  
β-xylosidase  
Acetyl xylan esterase  
α-glucuronidase  
α-arabinofuransidase

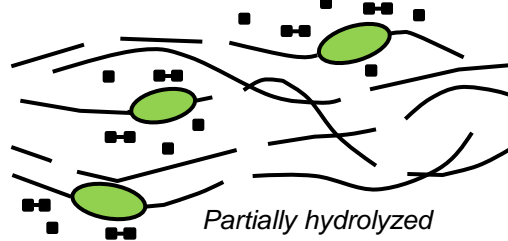
Endo-mannanase  
β-manosidase  
etc.

## Cellulose



→ **Glucose**

## Hemicellulose



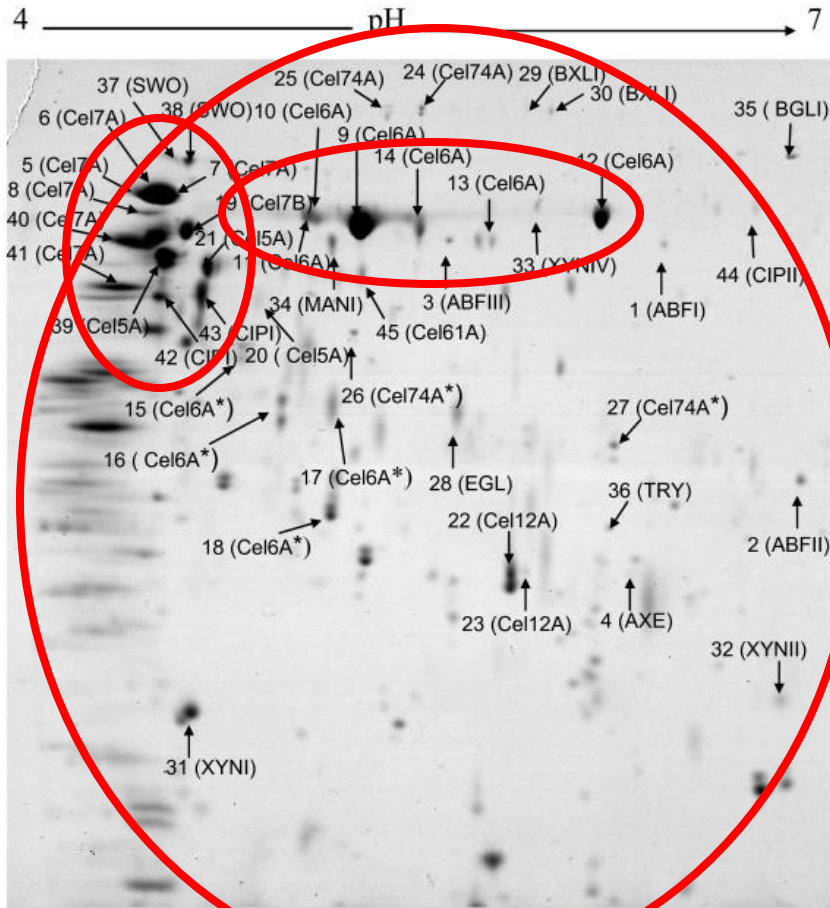
*Partially hydrolyzed during feedstock pretreatment*

→ **Xylose**  
→ **Manose**  
→ **Arabinose**  
→ **Galactose**





# Enzyme system development



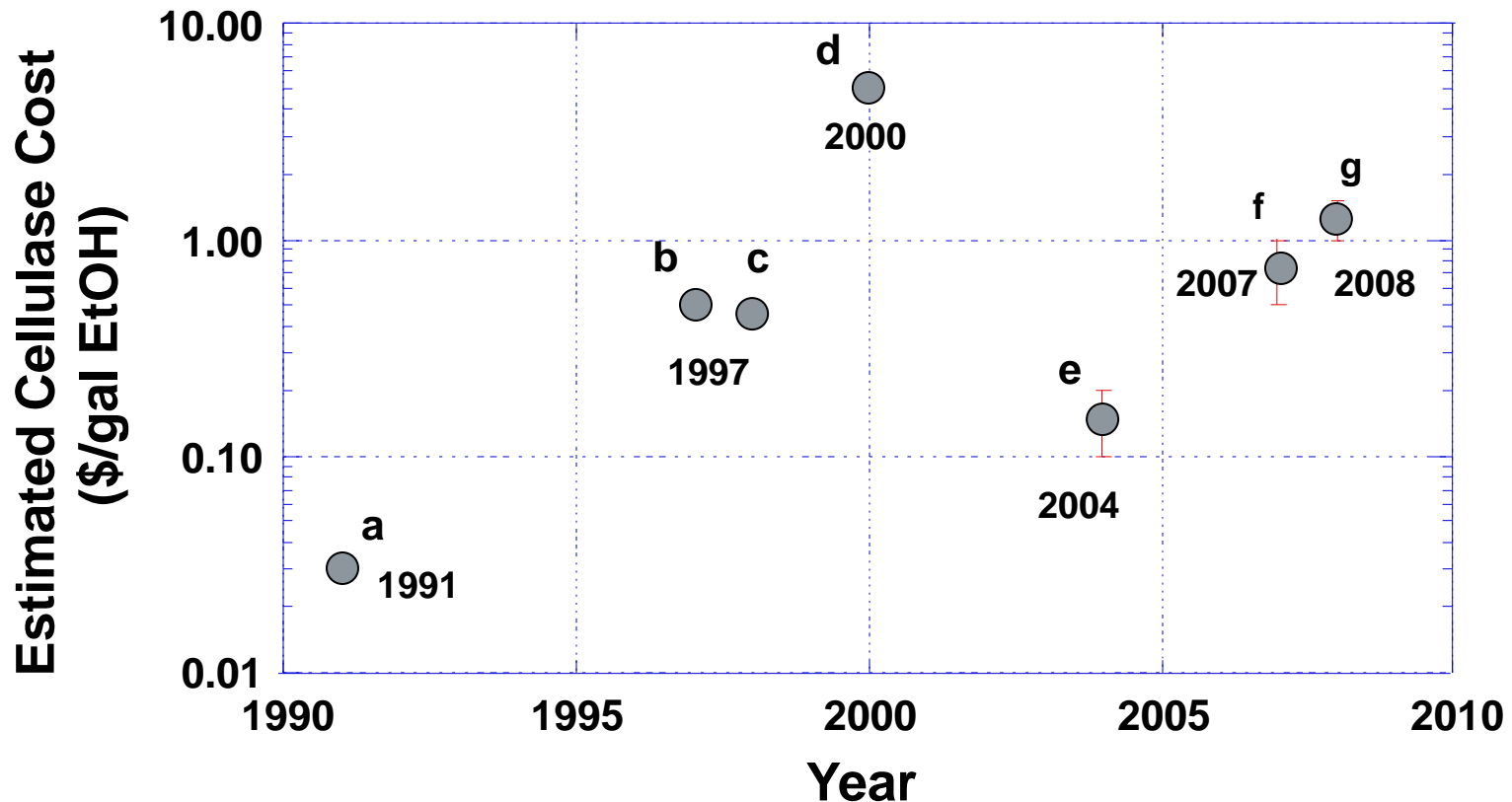
## *T. reesei* secretome

- 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



- a) Hinman et al. 1991. Appl. Biotechnol. Bioeng. 34/35:639-657.
- b) Hettenhaus & Glassner, 1997 (<http://www.ceassist.com/assessment.htm>).
- c) NREL, 1998. Bioethanol from the corn industry. DOE/GO-1009-577.
- d) Schell, 2004. ASM Natl Meeting; McMillan, 2004. DOE/NASULGS Biomass & Solar Energy Workshops.
- e) Genencor & Novozyme, 2004. Press releases (e.g. [http://www.genencor.com/wt/groc/pr\\_109831360](http://www.genencor.com/wt/groc/pr_109831360)).
- f) Petiot, Novozymes, Platts Cellulosic Ethanol & Second Generation Biofuels, 2007.
- g) Sheridan (Novozymes) Nature Biotech, 2008.





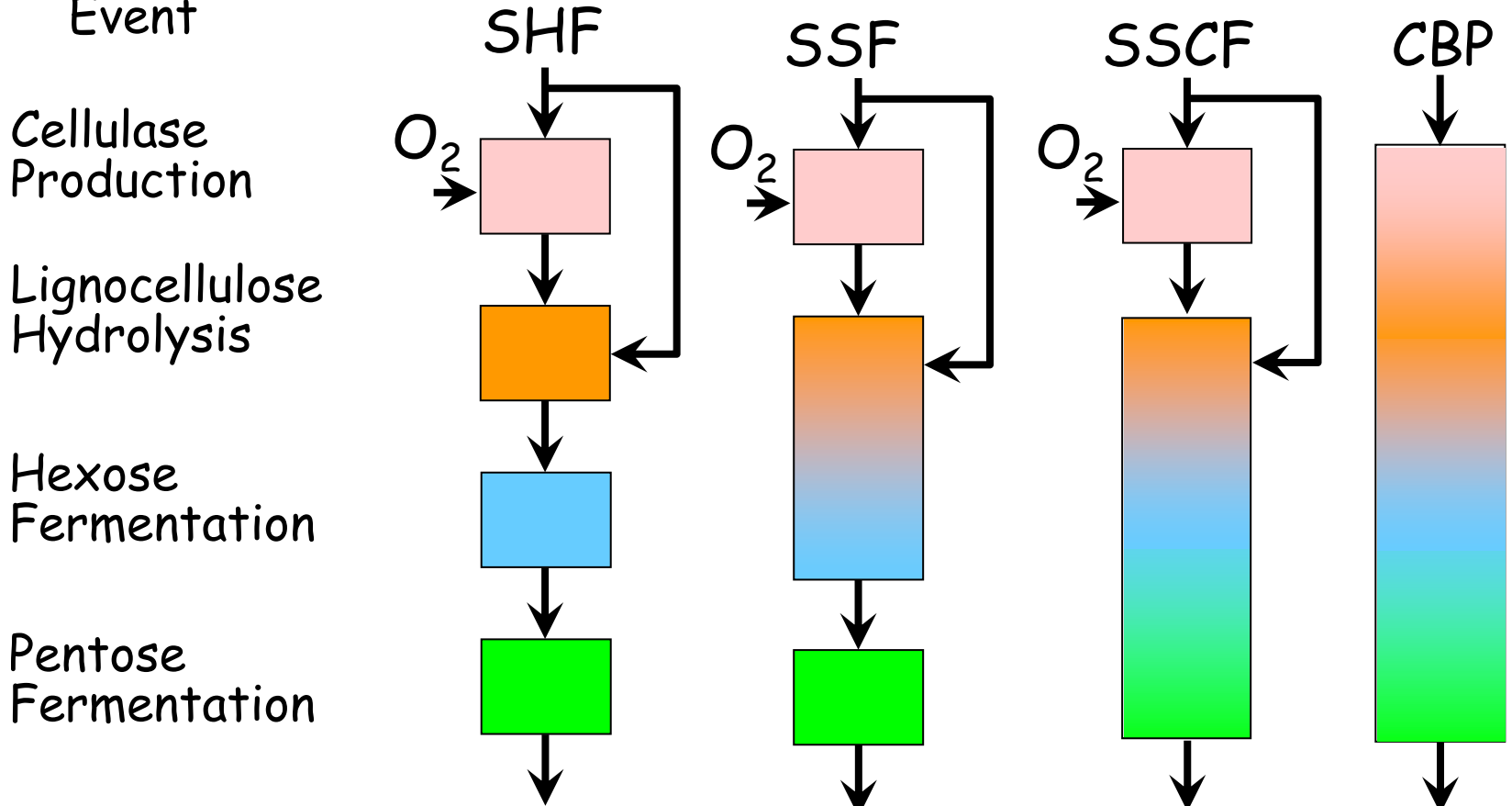
# Technologies for Cellulose Conversion



## Biomass Processes for EtOH production

Biologically-Mediated Event

Enzyme Hydrolysis Processing Strategy<sup>1</sup>  
(Each box represents a bioreactor - not to scale)



SHF: Separate Hydrolysis & Fermentation

SSCF: Simultaneous Saccharification & Co-Fermentation

SSF: Simultaneous Saccharification & Fermentation

CBP: Consolidated Bioprocessing





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# Consolidated BioProcessing (CBP)





# Consolidated BioProcessing (CBP)



## Fundamentals of Microbial Cellulose Utilization

MICROBIOLOGY AND MOLECULAR BIOLOGY REVIEWS, Sept. 2002, p. 506–577  
1092-2172/02/\$04.00+0 DOI: 10.1128/MIMBR.66.3.506–577.2002  
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Vol. 66, No. 3

### Microbial Cellulose Utilization: Fundamentals and Biotechnology

Lee R. Lynd,<sup>1\*</sup> Paul J. Weimer,<sup>2</sup> Willem H. van Zyl,<sup>3</sup> and Isak S. Pretorius<sup>4</sup>

*Chemical and Biochemical Engineering, Thayer School of Engineering and Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire 03755<sup>1</sup>; USDA Agricultural Research Service, U.S. Dairy Forage Research Center and Department of Bacteriology, Madison, Wisconsin, 53706<sup>2</sup>; and Department of Microbiology<sup>3</sup> and Institute for Wine Biotechnology,<sup>4</sup> University of Stellenbosch, Stellenbosch 7600, South Africa*

*Microbiology and Molecular Biology Reviews* **66**: 506-577 (2002)







# Consolidated BioProcessing (CBP)



## Consolidated bioprocessing : update (2)

Adv Biochem Engin/Biotechnol (2007) 108: 205–235  
DOI 10.1007/10\_2007\_061  
© Springer-Verlag Berlin Heidelberg  
Published online: 21 April 2007

### **Consolidated Bioprocessing for Bioethanol Production Using *Saccharomyces cerevisiae***

Willem H. van Zyl<sup>1</sup> (✉) · Lee R. Lynd<sup>2</sup> · Riaan den Haan<sup>1</sup> · John E. McBride<sup>2</sup>

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[whvz@sun.ac.za](mailto:whvz@sun.ac.za)

<sup>2</sup>Thayer School of Engineering, Dartmouth College, 8000 Cummings Hall,  
Hanover, NH 03755-8000, USA

*Advances in Biochemical Engineering/Biotechnology 108: 205 -235 (2007)*





# Consolidated BioProcessing (CBP)



## Consolidated bioprocessing : update (3)

Appl Microbiol Biotechnol (2010) 87:1195–1208  
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

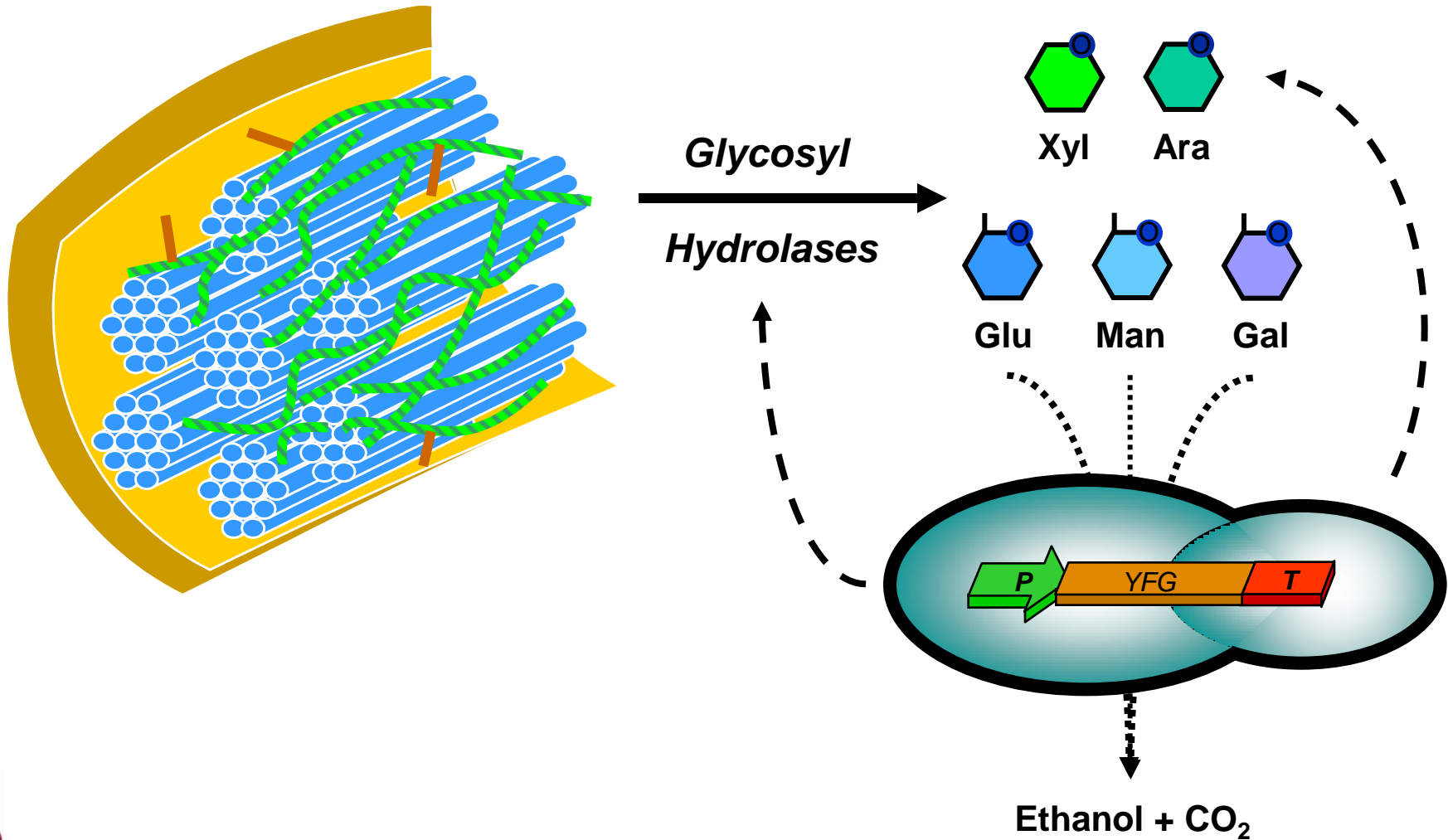
*Applied Microbiology and Biotechnology* **87**: 1195-1208 (2010)





# Technologies for Cellulose Conversion

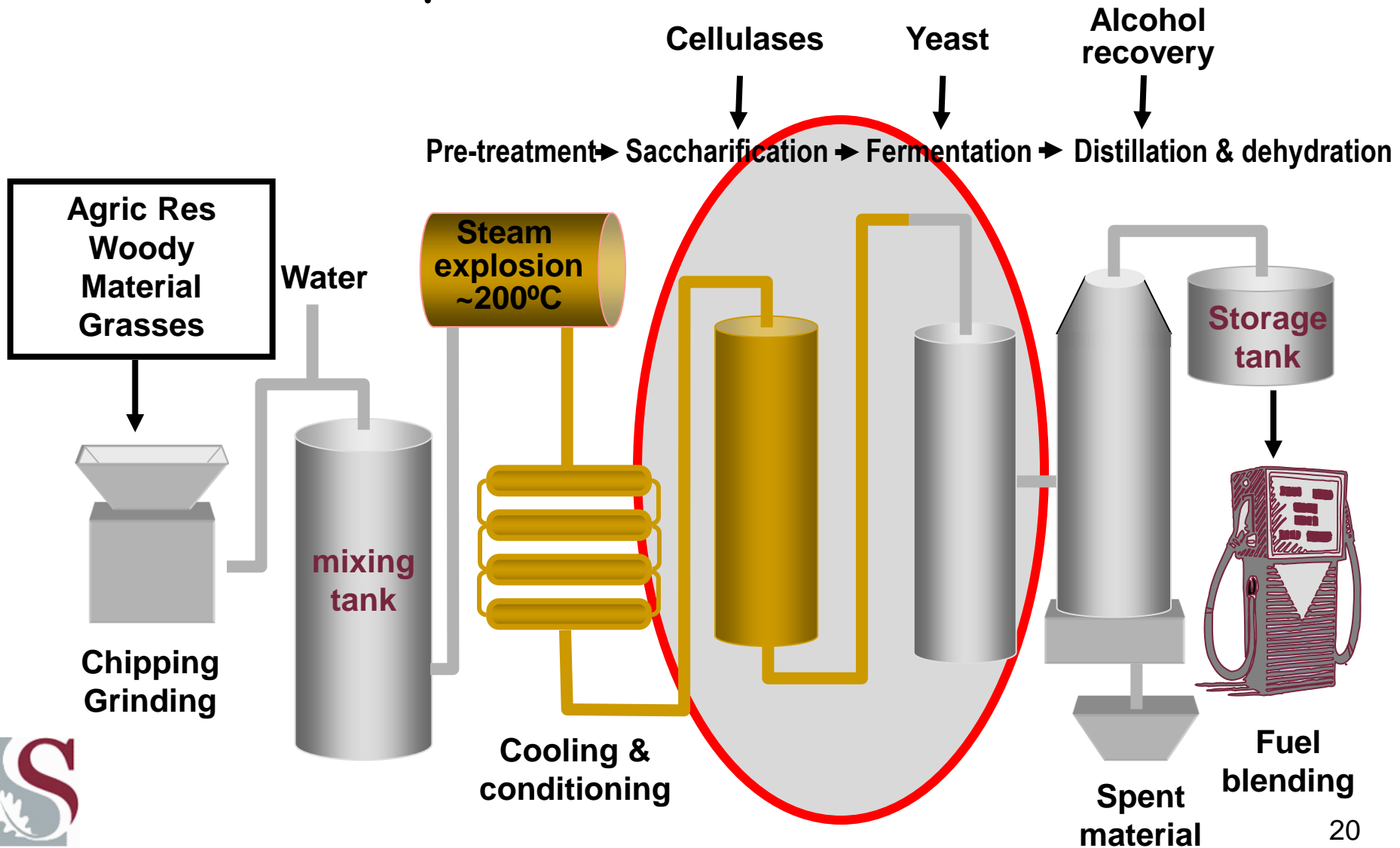
## Consolidated BioProcessing (CBP)





# Technologies for Ethanol Production

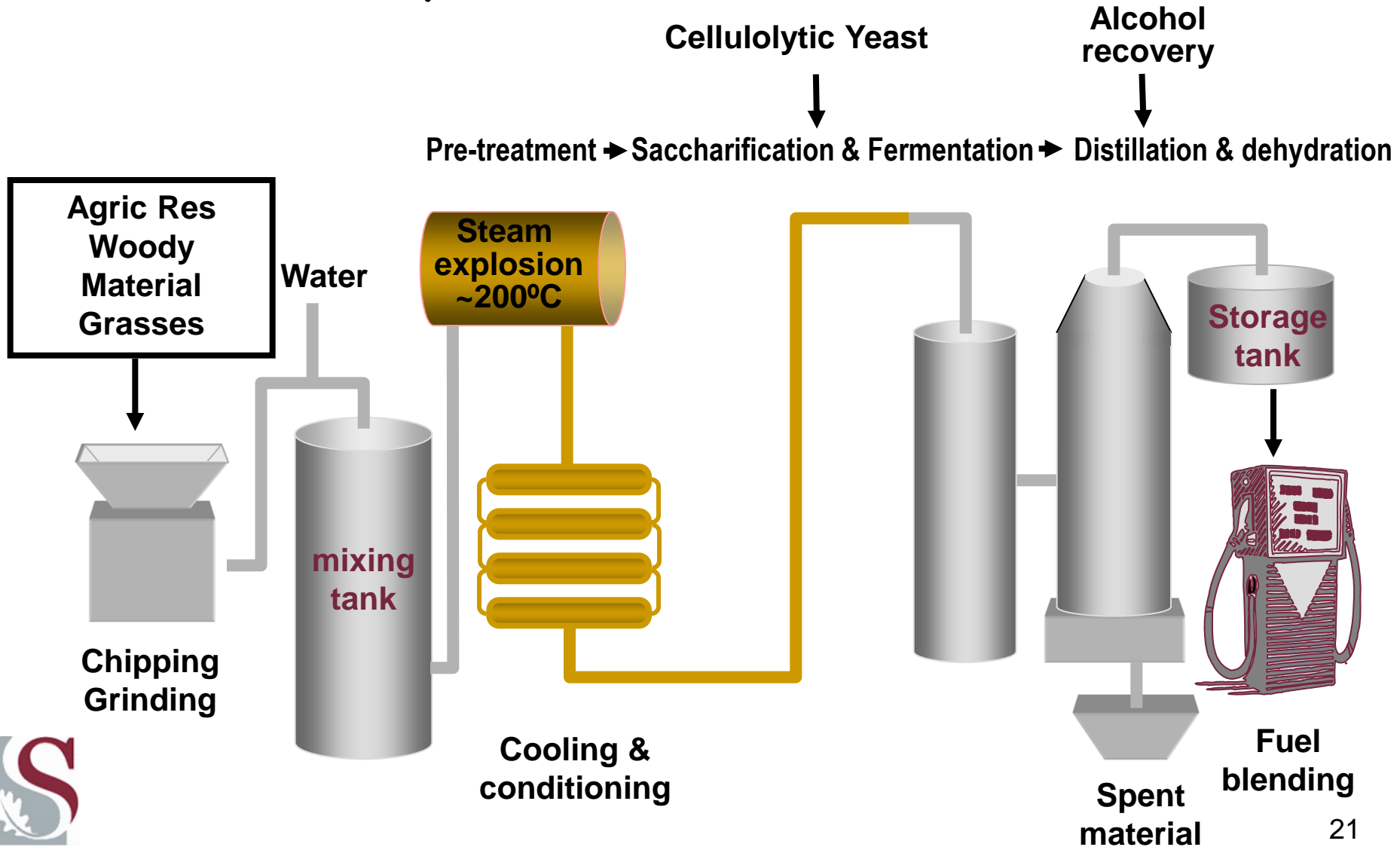
## Ethanol production from cellulosics





# Technologies for Ethanol Production

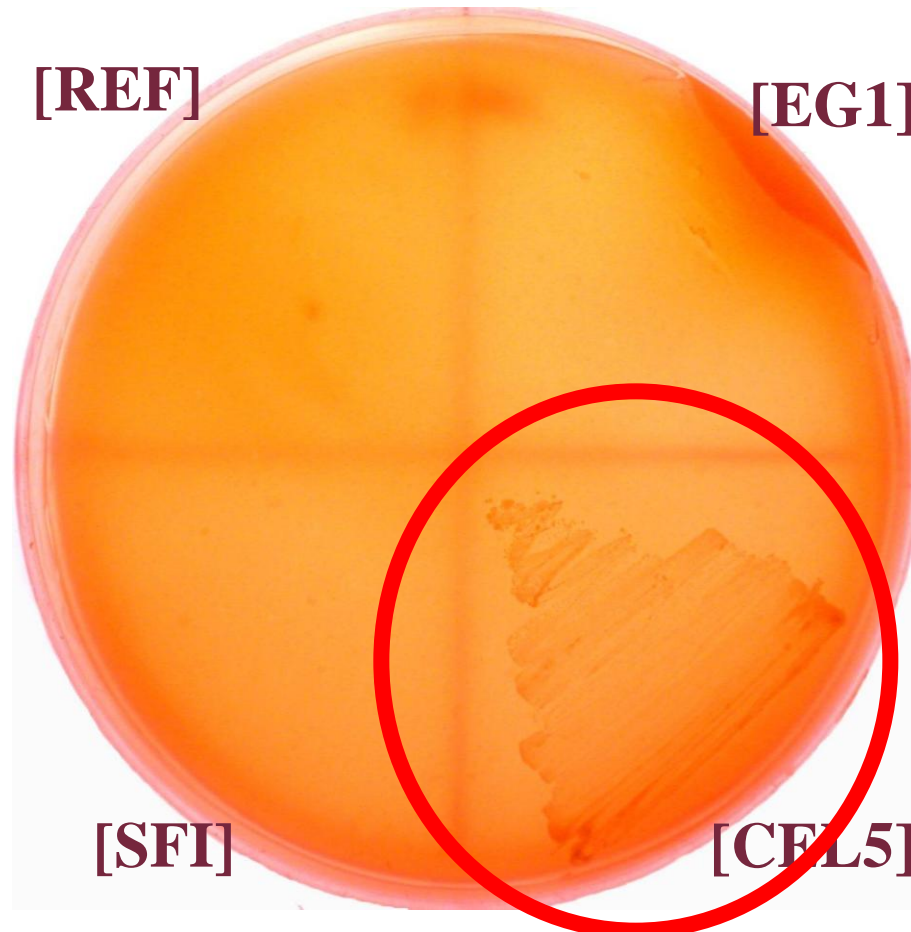
## Ethanol production from cellulosics





# Technologies for Cellulose Conversion

## Growth on amorphous cellulose (PASC)



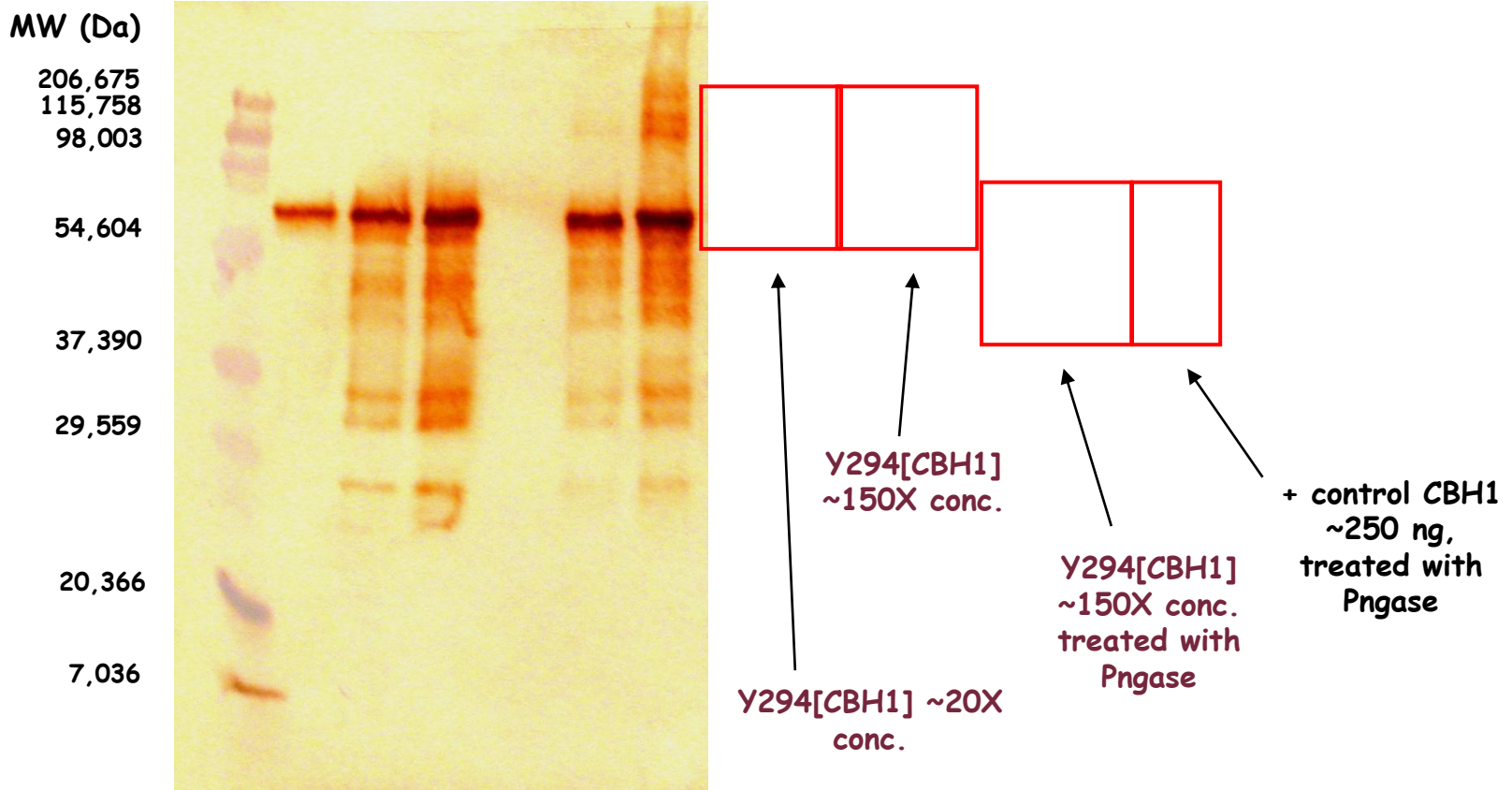
Den Haan, R., S.H. Rose, L.R. Lynd, and W.H. Van Zyl. 2007. Hydrolysis and fermentation of amorphous cellulose by recombinant *Saccharomyces cerevisiae*. *Met. Eng.* **9**: 87–94.



# Expression of cellobiohydrolases in yeast



## CBH1 cellobiohydrolase production by yeast

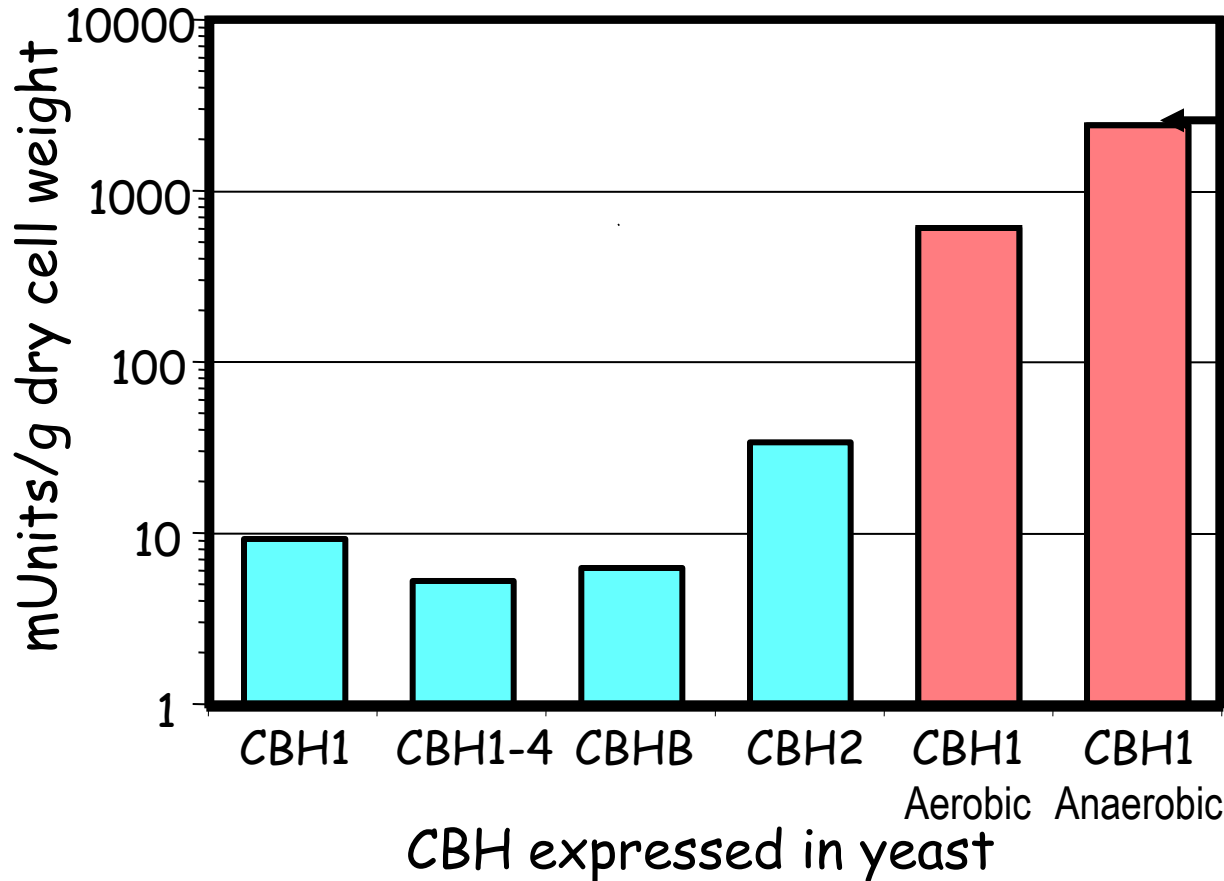


Den Haan, R., J.E. McBride, D.C. La Grange, L.R. Lynd, and W.H. Van Zyl. 2007. Functional expression of cellobiohydrolases in *Saccharomyces cerevisiae* towards one-step conversion of cellulose to ethanol. *Enzyme Microb. Technol.* **40**: 1291–1299.



# Expression of cellobiohydrolases in yeast

## Cellobiohydrolase production by yeast



2.6% of t.c.p.

- 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<sup>-1</sup>



Den Haan, R., J.E. McBride, D.C. La Grange, L.R. Lynd, and W.H. Van Zyl. 2007. Functional expression of cellobiohydrolases in *Saccharomyces cerevisiae* towards one-step conversion of cellulose to ethanol. *Enzyme Microb. Technol.* **40**: 1291–1299.





Mascoma Corporation  
Technical facilities, Lebanon, NH, USA  
([www.mascoma.com](http://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

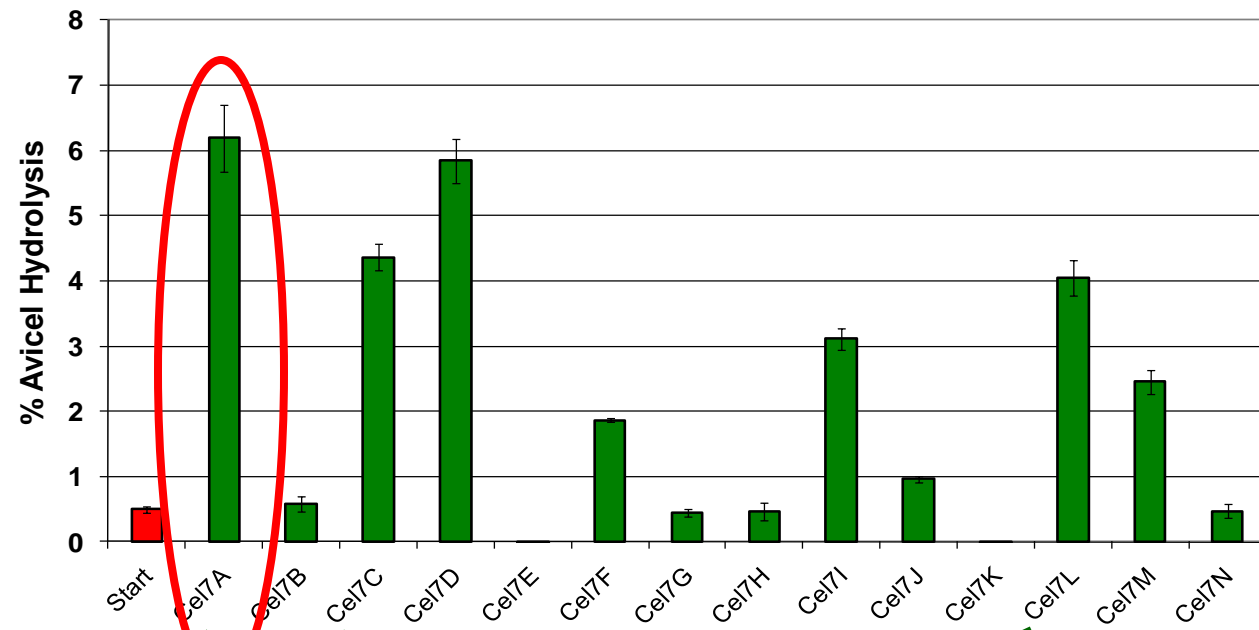
## Three Platforms

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



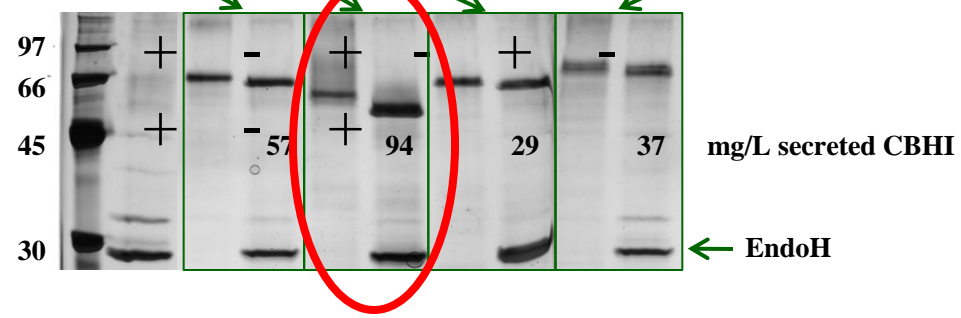
*Multiple chances to succeed near-term & long-term*

MASCOMA **Screen CBH1 for high level expression**



**Enzyme activity:**

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



**Enzyme Production:**

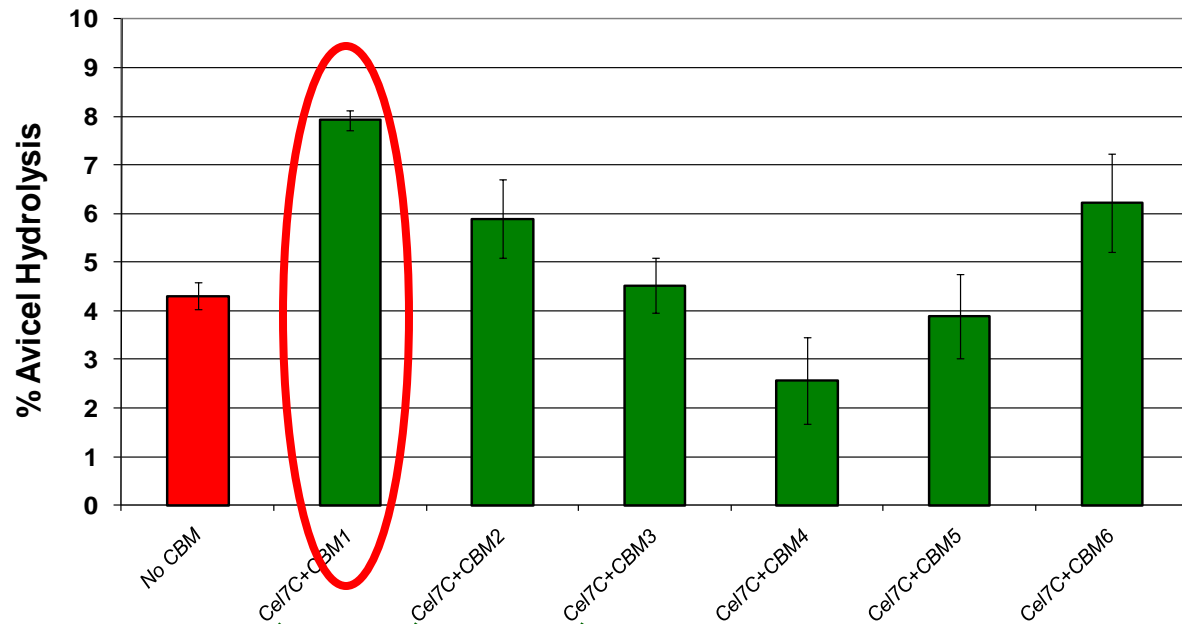
- 94 mg/L CBH1
- $\pm 2.5\%$  of Total cell protein in minimal medium





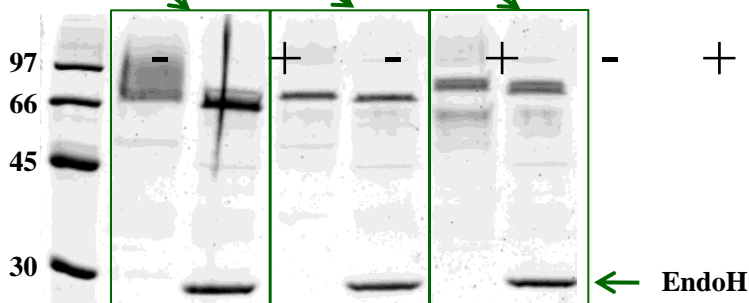
# Screen CBH1 for high level expression

## Cellulose binding domain

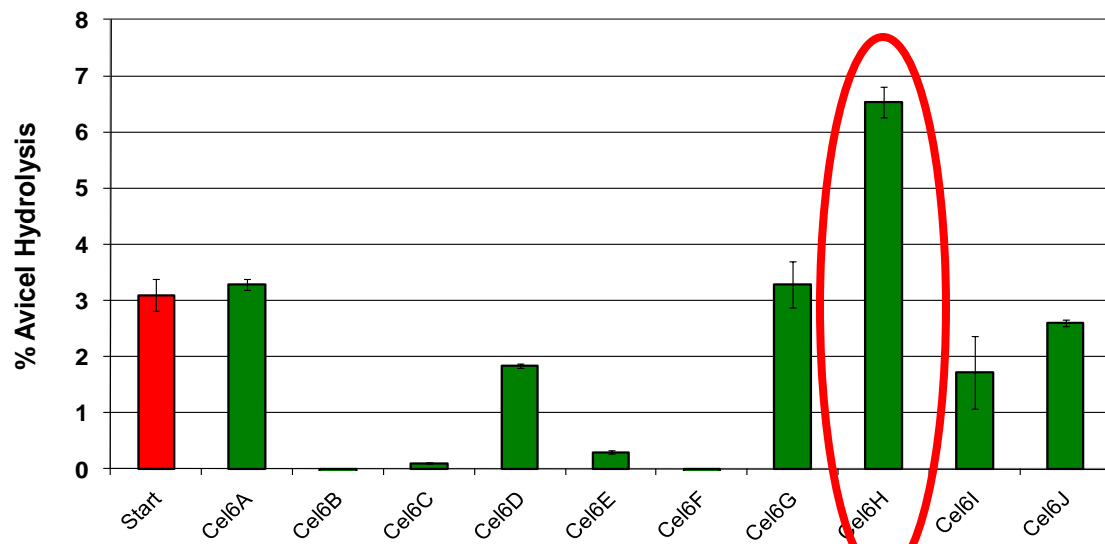


### Attachment of CBD

- Best CBM  $\pm$  2x improvement in activity
- $\pm$  2x decrease in protein level

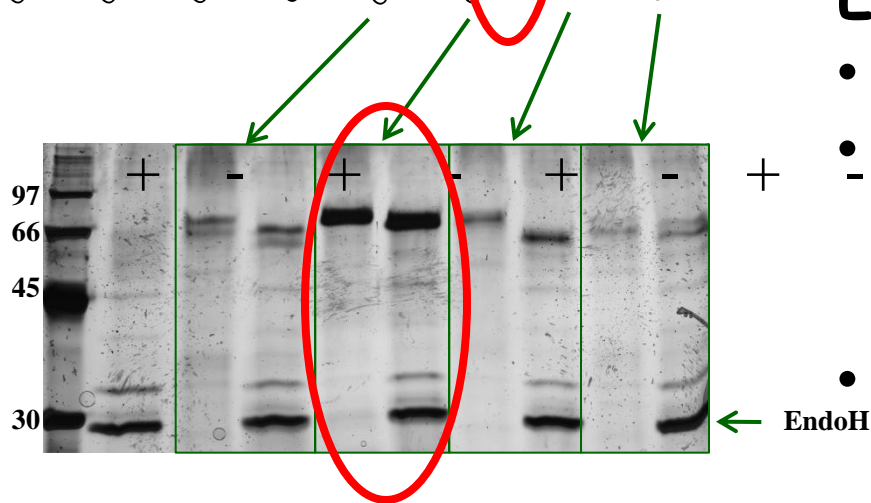


# MASCOMA Screen CBH2 for high level expression



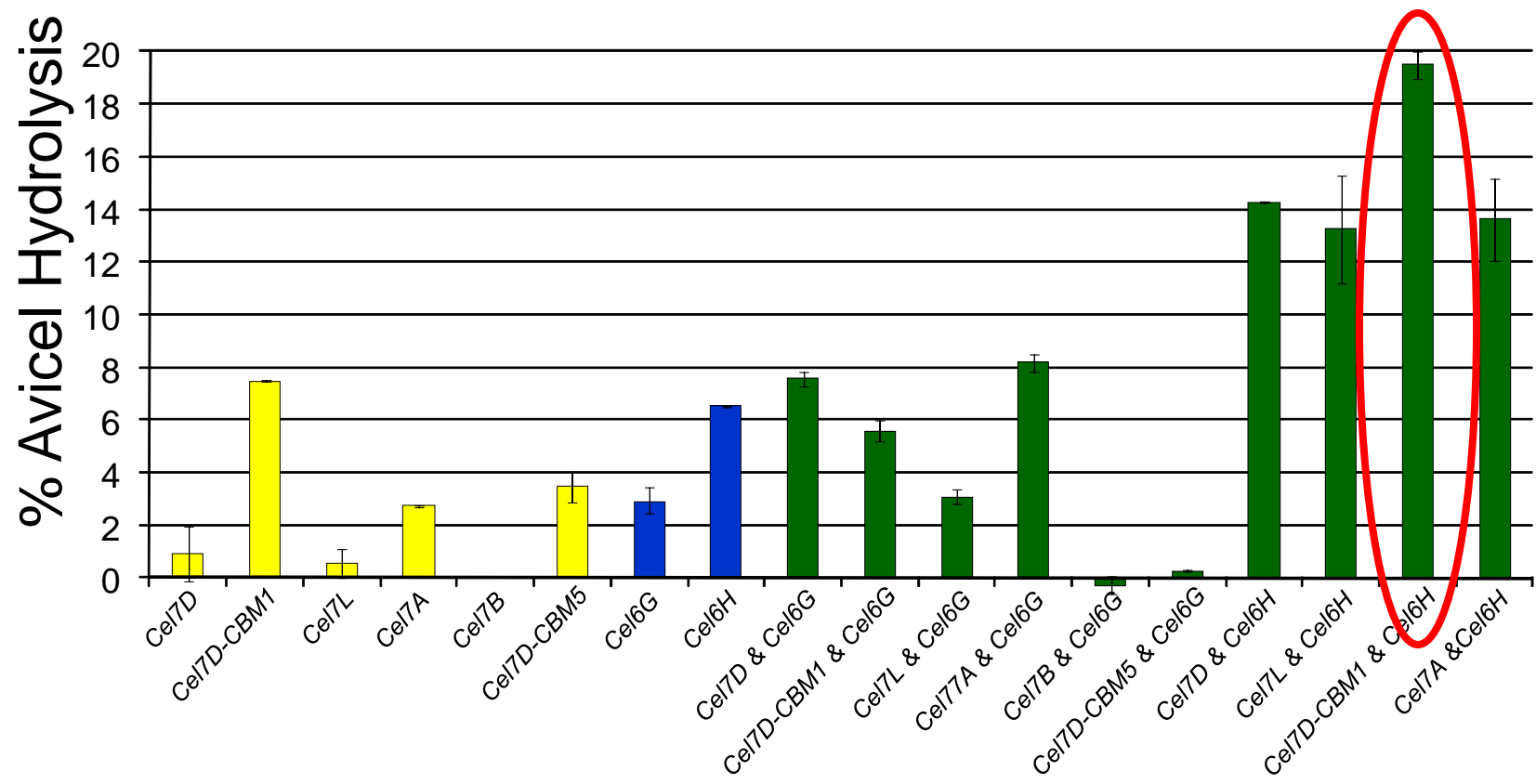
Enzyme activity:

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



Enzyme Production:

- 140 mg/L CBH2
- $\pm$  5% of Total cell protein in minimal medium
- x3 higher than starting point

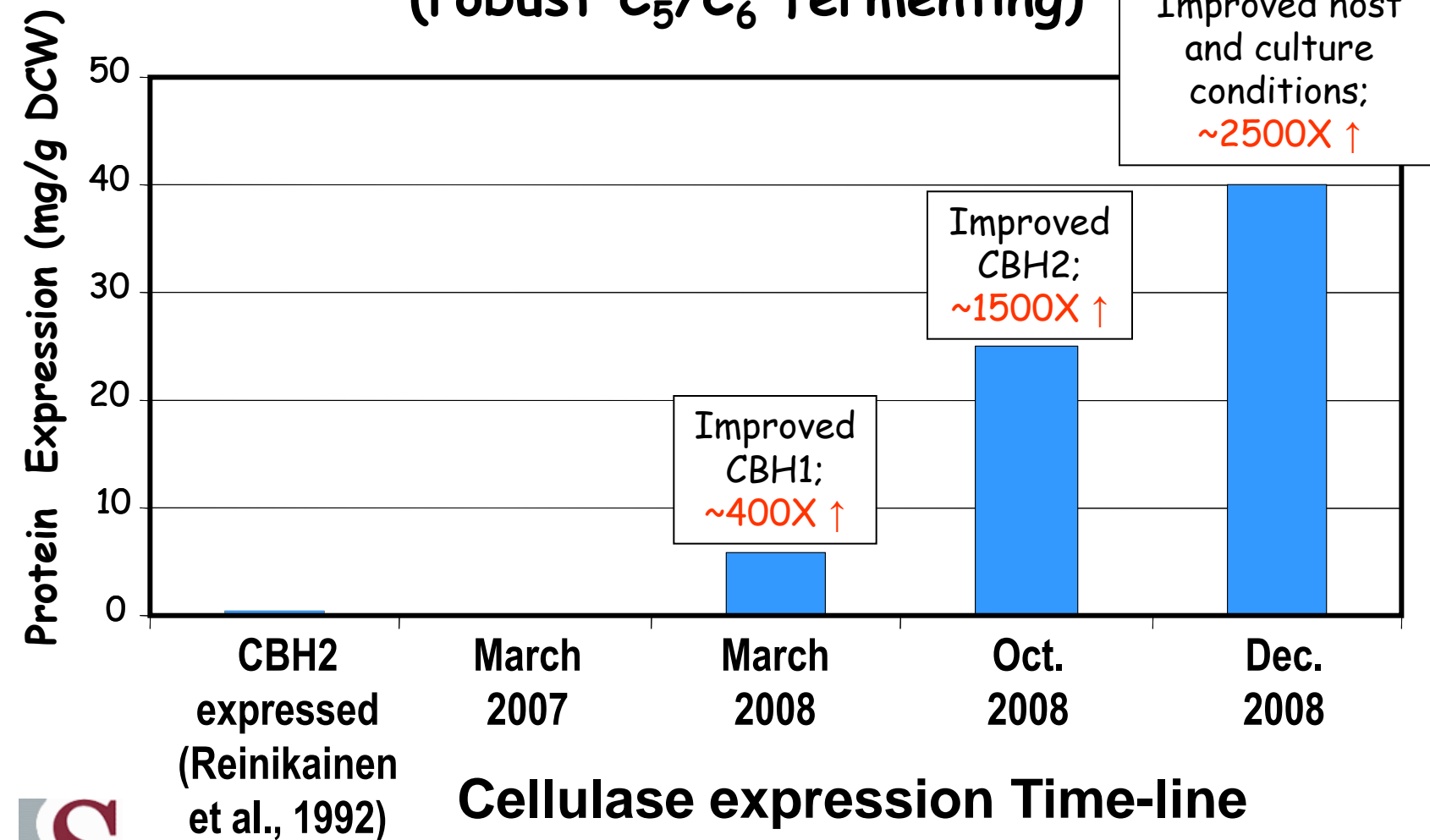


**Best combination x2.5 greater than single enzyme**



# Mascoma Cellulolytic Yeast

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



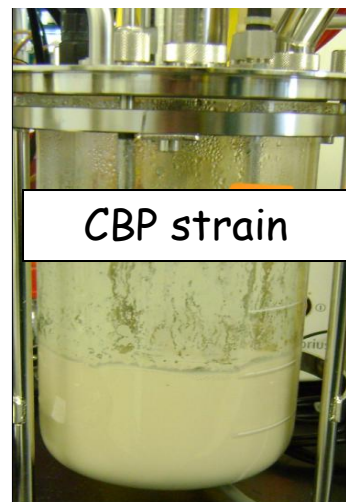
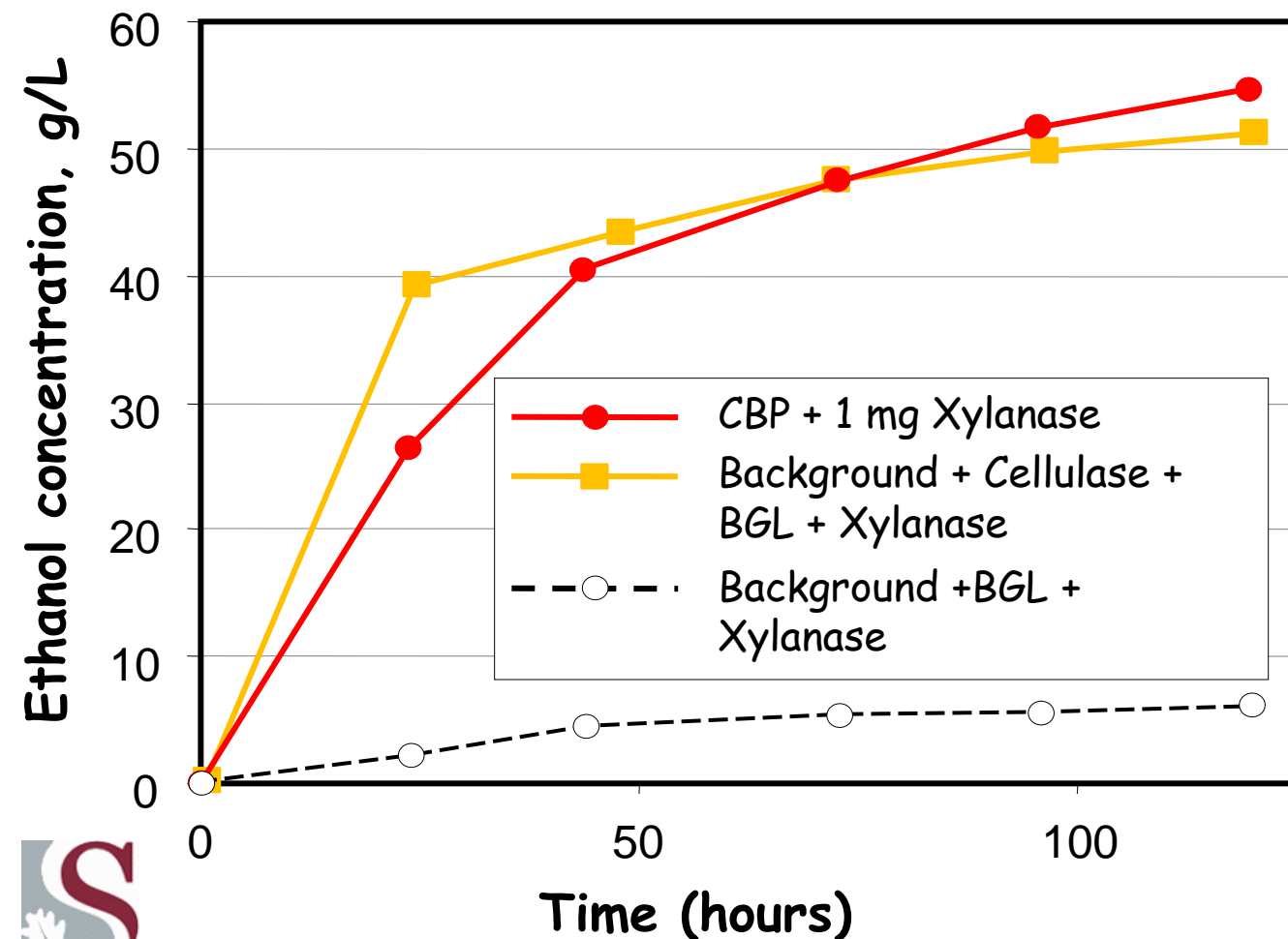
**Cellulase expression Time-line**



# Enzyme Reduction on Paper Sludge

Mascoma CBP technology on 18% w/w paper sludge

Appearance at 120 hrs.

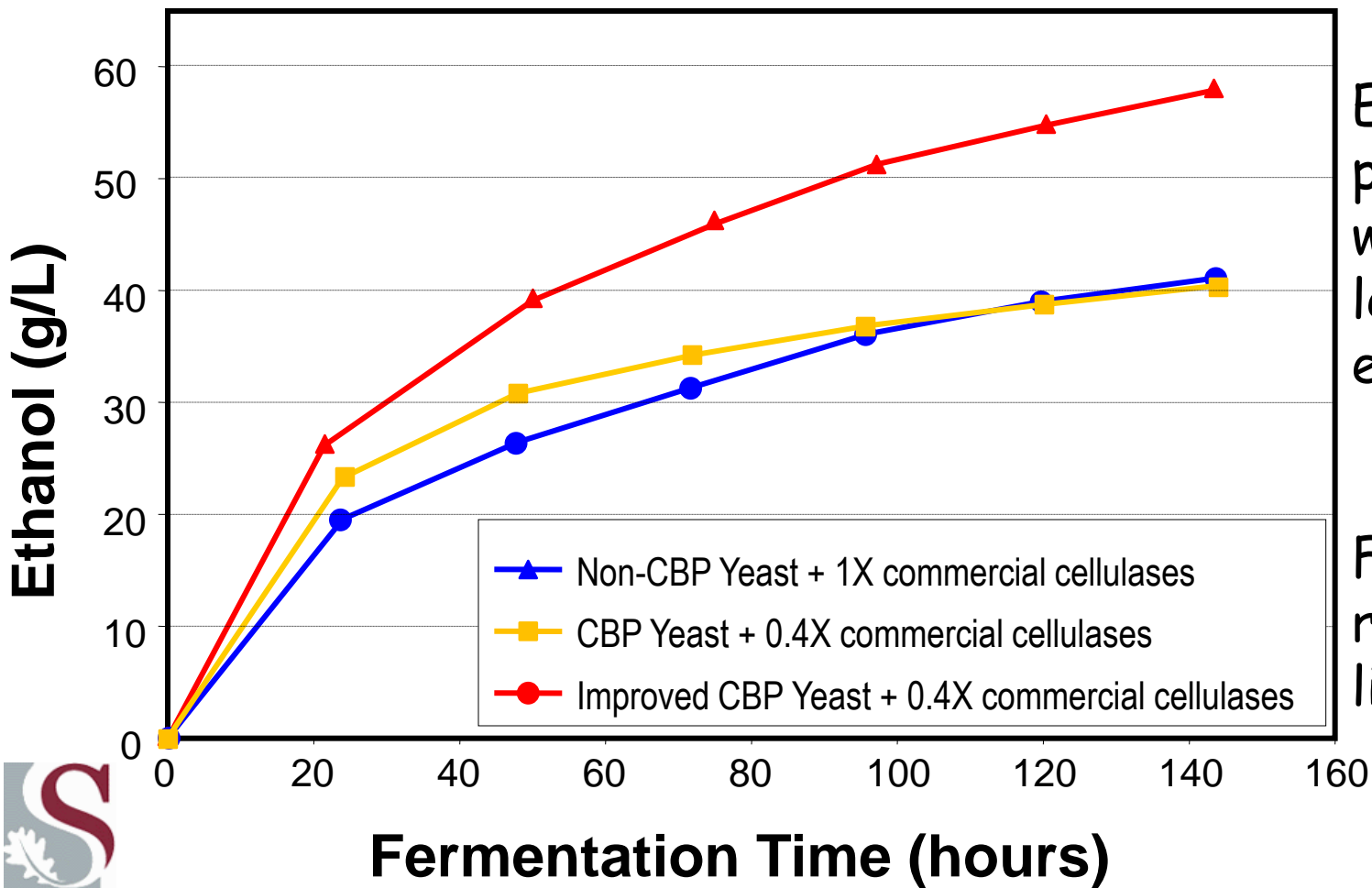




# Enzyme Reduction on Hardwood



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





January 2008



November 2008





# Acknowledgements



Danie la Grange  
Shaunita Rose  
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Naomi Thorngren

Deidre Willies

Vineet Rajgarhia



Marja Ilmen

Merja Penttilä





# Rolling out cellulosic ethanol in southern Africa





will lead the production of  
next generation cellulosic  
ethanol in Africa

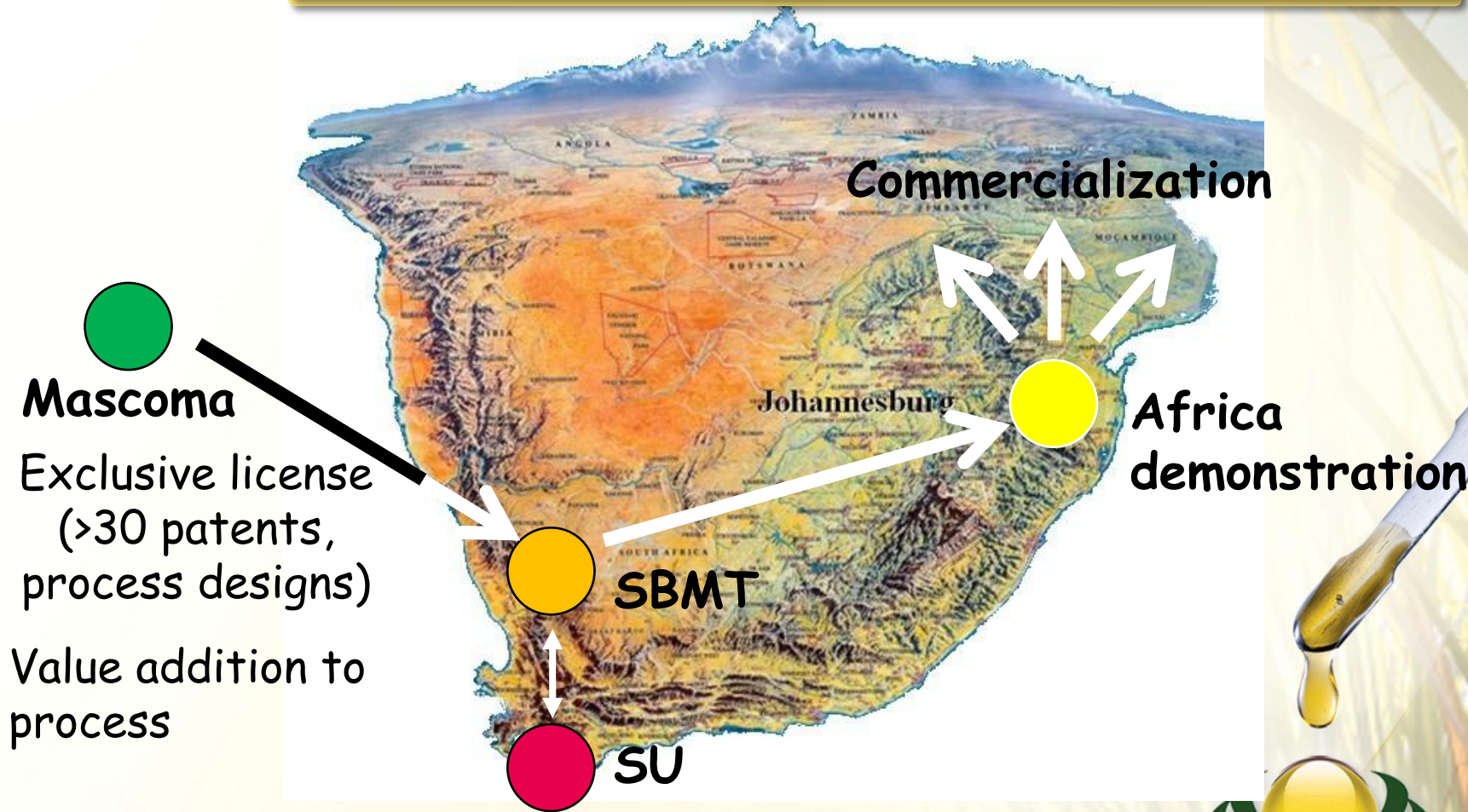


... and drive a sustainable bioenergy future for the continent.

[www.sbmt.co.za](http://www.sbmt.co.za)

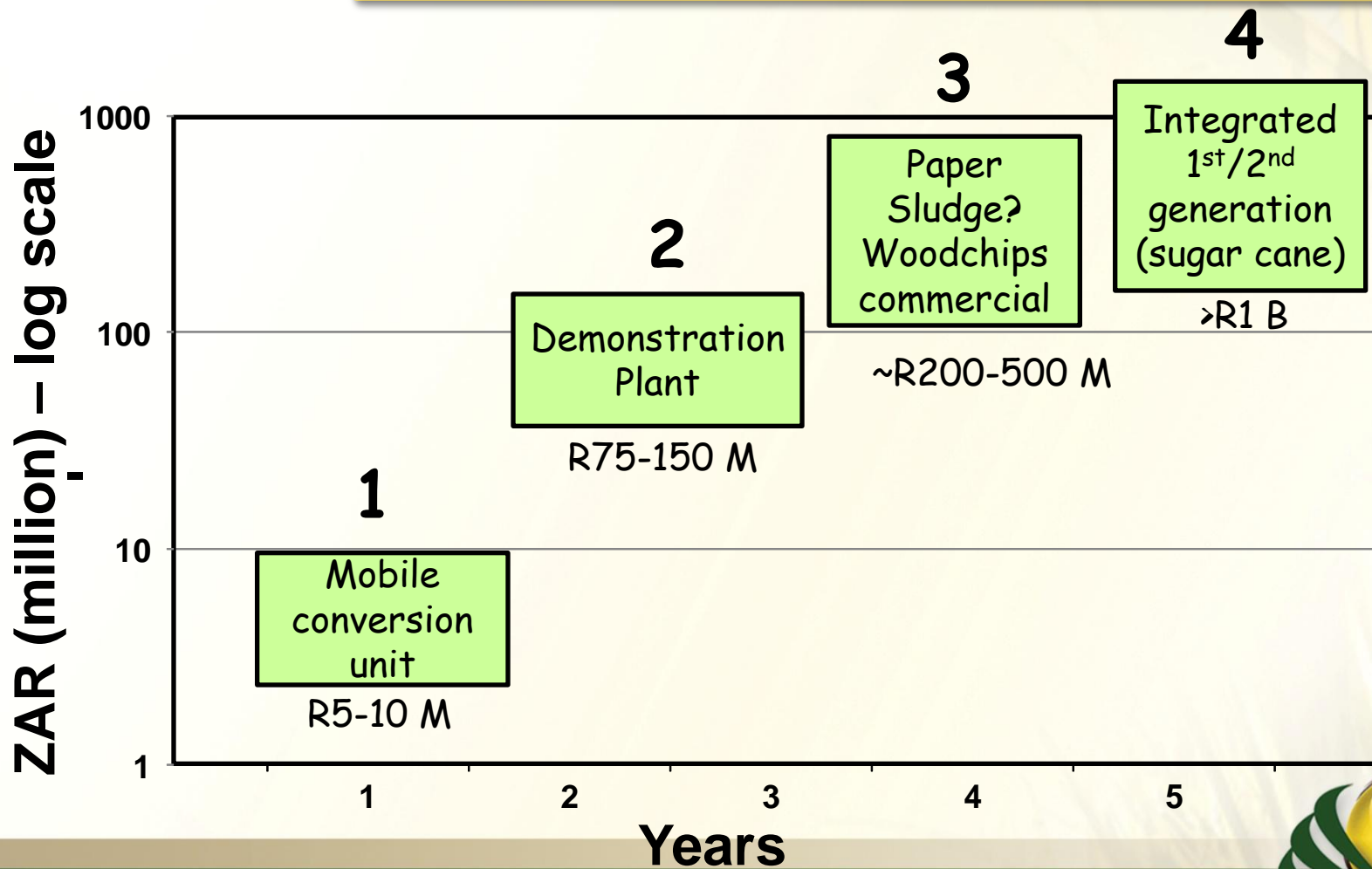


# SBMT business model



SBMT holds the exclusive rights to Mascoma technology for Southern Africa

# Costs vs timeline for technology development





# CoER : Biofuels

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# Thank you

