CoER : Biofuels

Department of Microbiology • Faculty of Natural Sciences

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

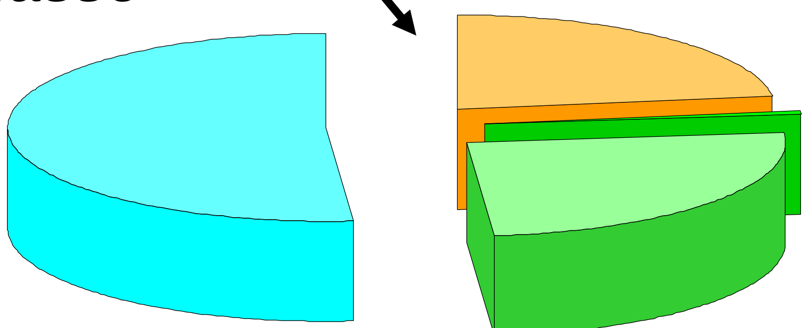
Technologies for Cellulose Conversion

Lignocellulose composition

Sugarcane bagasse

Non-fermentable sugars
high energy aromatics

Lignin
28%



Hexoses (fermentable)

Cellulose
46%

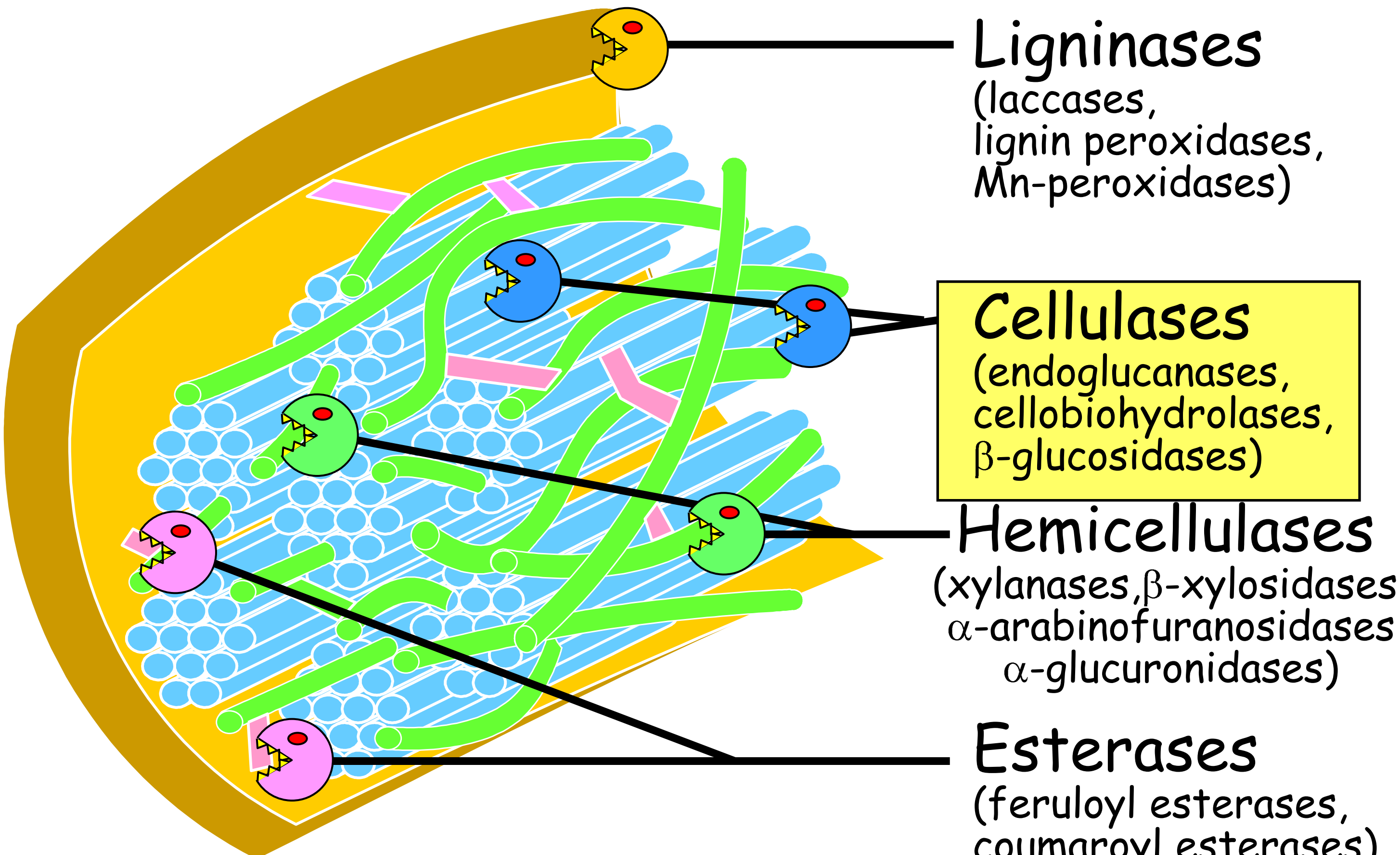
Arabinan
2%

Pentoses (fermentable)

Xylan
25%

Technologies for Cellulose Conversion

Enzymatic hydrolysis of biomass

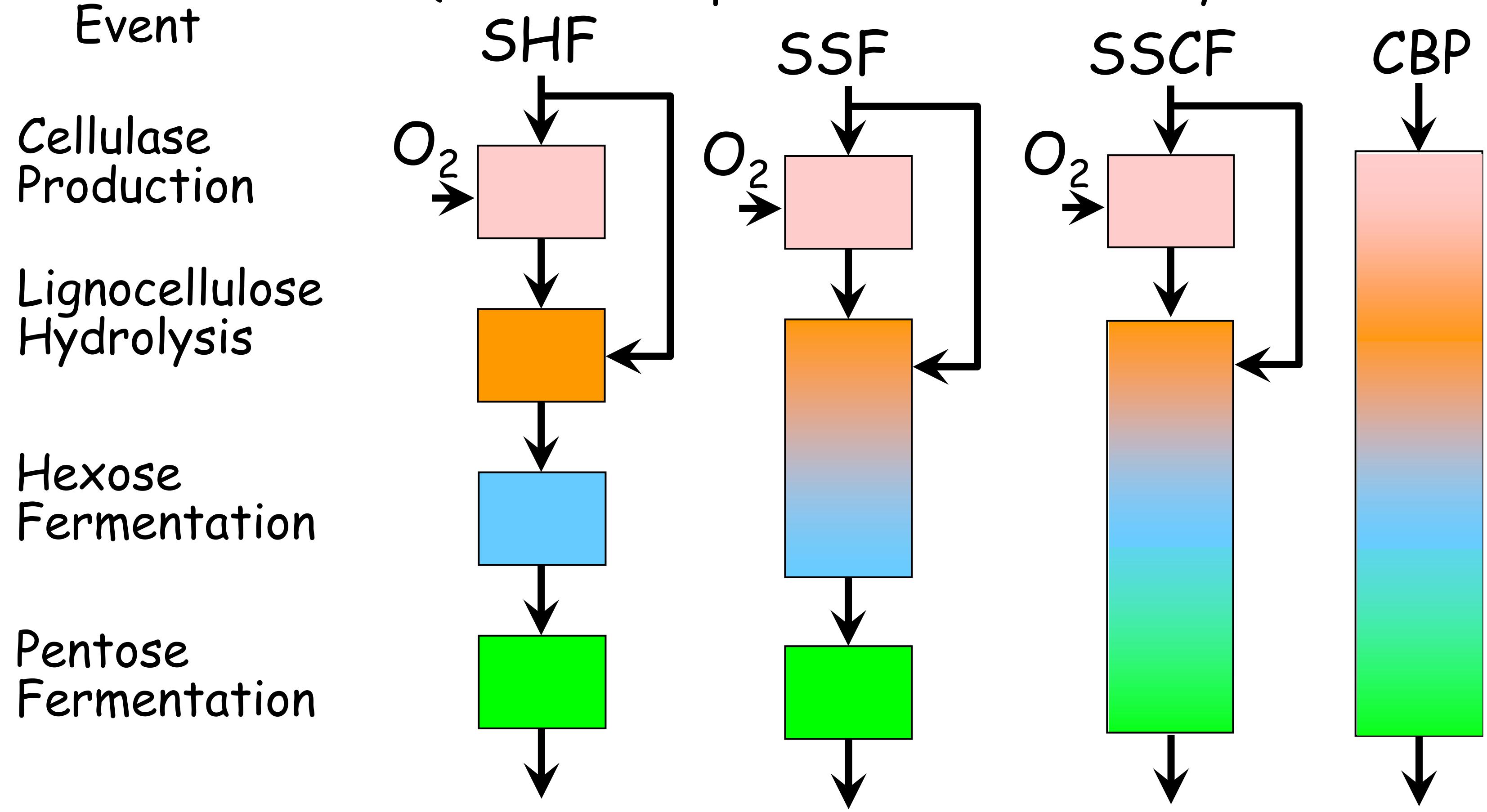


Technologies for Cellulose Conversion

Biomass Processes for EtOH production

Biologically-Mediated Event

Enzyme Hydrolysis Processing Strategy[1]
(Each box represents a bioreactory - not to scale)



SHF: Separate Hydrolysis & Fermentation
SSF: Simultaneous Saccharification & Fermentation
SSCF: Simultaneous Saccharification & Co-Fermenation
CBP: Consolidated Bioprocessing

Consolidated BioProcessing (CBP)

Consolidated BioProcessing (CBP)

Fundamentals of Microbial Cellulose Utilization

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

Microbial Cellulose Utilization: Fundamentals and Biotechnology

Lee R. Lynd,[1*] Paul J. Weimer,[2] Willem H. van Zyl,[3] and Isak S. Pretorius[4]

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

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

Consolidated bioprocessing : update

Full text provided by www.sciencedirect.com
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Consolidated bioprocessing of cellulosic biomass: an update

Lee R Lynd[1,2], Willem H van Zyl[2], John E McBride[1] and Mark Laser[1]

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

Consolidated BioProcessing (CBP)

Consolidated bioprocessing : update (2)

Adv Biochem Engin/Biotechnol (2007) 108: 205–235
DOI 10.1007/10_2007_061

Published online: 21 April 2007

Consolidated Bioprocessing for Bioethanol Production Using *Saccharomyces cerevisiae*

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Advances in Biochemical Engineering / Biotechnology (2007)

Consolidated BioProcessing (CBP)

Consolidated bioprocessing : update (3)

Appl Microbiol Biotechnol
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

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Applied Microbiology & Biotechnology (2010) [corrected proofs]

Consolidated BioProcessing (CBP)

Consolidated bioprocessing: update (3)

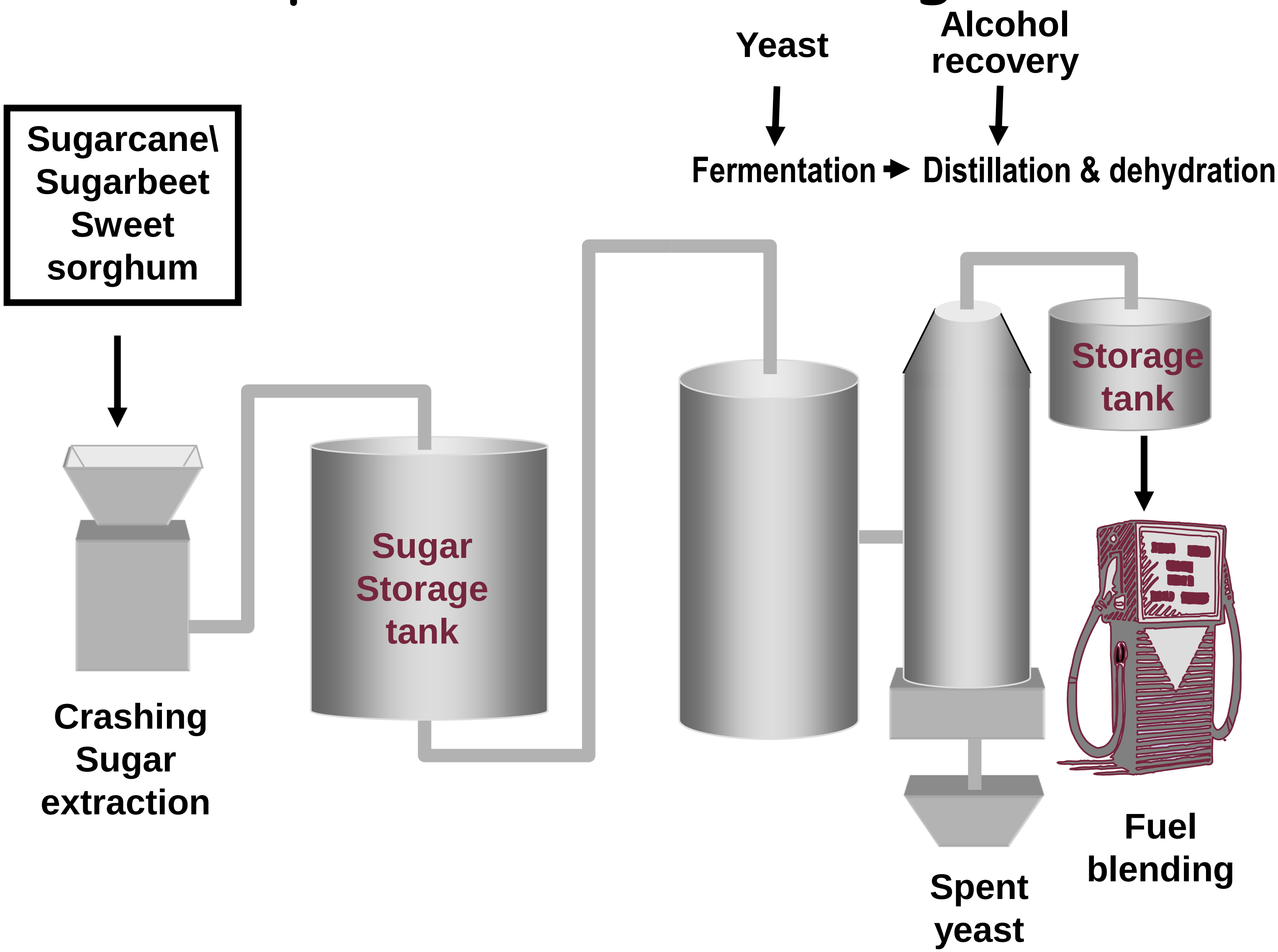
Good activity	green
Low activity	yellow
Very low activity	orange
No activity	red

	S. cerevisiae		*K. marxianus*		*P. stipitis*		*H. polymorpha*		*E. coli*		*K. oxytoca*		*Z. mobilis*		*C. acetobutylicum*		Lactic acid bacteria		*T. saccharolyticum*	
	WT	GE	WT	WT	GE	WT	WT	GE	WT	GE	WT	GE	WT	GE	WT	GE	WT	GE	WT	GE
Breakdown crystalline cellulose	red	orange	red	red	red	red	red	red	red	red	red	red	red	red	red	red	red	red	red	red
Breakdown amorphous cellulose	red	green	red	orange	red	yellow	red	yellow	red	orange	red	orange	red	yellow	yellow	orange	yellow	orange	red	red
Breakdown hemicellulose	red	yellow	red	orange	orange	yellow	red	yellow	red	orange	red	orange	red	NA	orange	orange	yellow	yellow	red	red
Utilize cellobiose	red	green	red	green	green	green	green	green	red	orange	green	green	red	orange	green	green	green	green	green	green
Utilize xylobiose	red	green	green	green	green	green	red	green	red	orange	green	green	red	orange	green	green	NA	NA	green	green
Grow on glucose	green	green	green	green	green	green	green	green	green	green	green	green	green	green	green	green	green	green	green	green
Grow on xylose	red	yellow	green	green	green	green	green	green	green	green	green	green	red	orange	green	green	green	green	green	green
Ferment glucose to ethanol	green E	green E	yellow E	yellow E	orange E	orange E	yellow E	yellow E	red	green E	red	green E	green E	green E	orange B	orange B	yellow L	yellow L	red	green E
Ferment xylose to ethanol	red	orange E	orange E	orange E	green E	green E	yellow E	yellow E	red	orange E	red	green E	red	orange E	orange B	orange B	yellow L	yellow L	red	green E
Resistant to hydrolysate inhibitors	yellow	green	yellow	yellow	orange	orange	orange	orange	red	yellow	red	orange	orange	orange	NA	NA	NA	NA	NA	NA
GRAS status	green	green	green	green	green	green	green	green	red	red	red	red	green	green	NA	NA	green	green	NA	NA
Low pH	green	green	green	green	green	green	green	green	orange	orange	orange	orange	orange	orange	green	green	green	green	NA	NA
High temperature	red	yellow	green	green	red	red	green	green	red	red	red	red	red	red	yellow	yellow	yellow	yellow	green	green

Applied Microbiology & Biotechnology (2010) [corrected proofs]

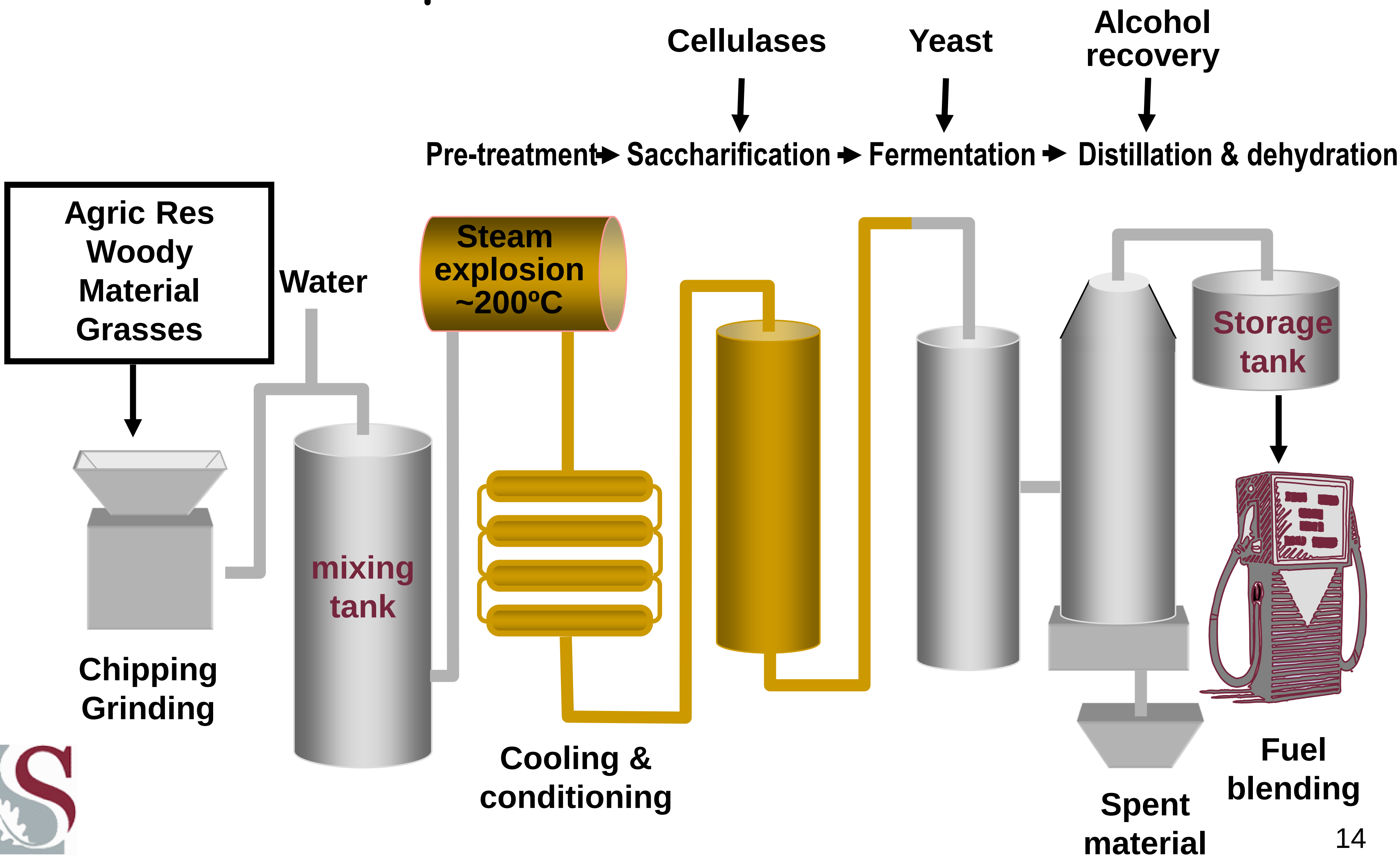
Technologies for Ethanol Production

Ethanol production from sugar

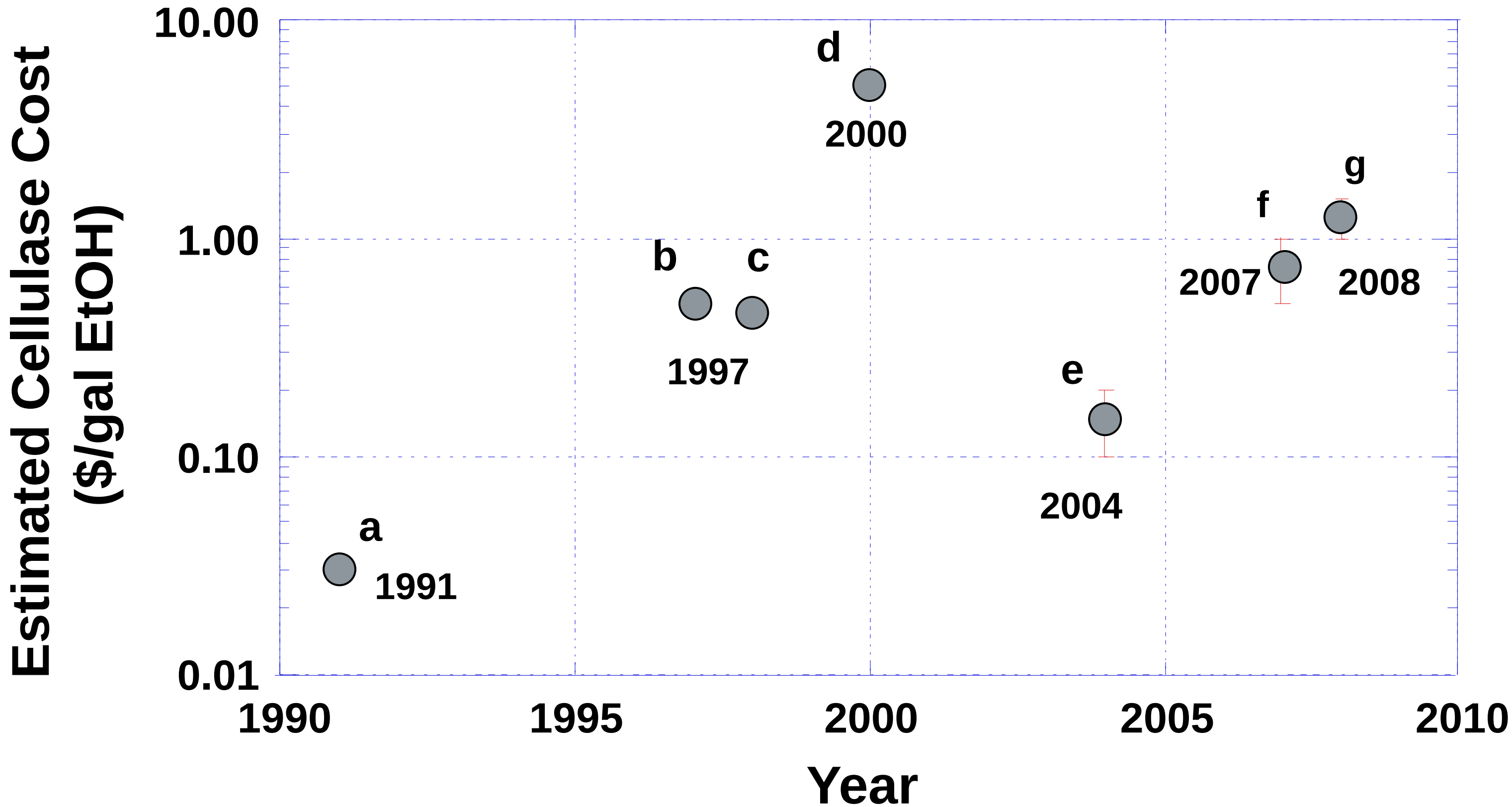


Technologies for Ethanol Production

Ethanol production from cellulosics

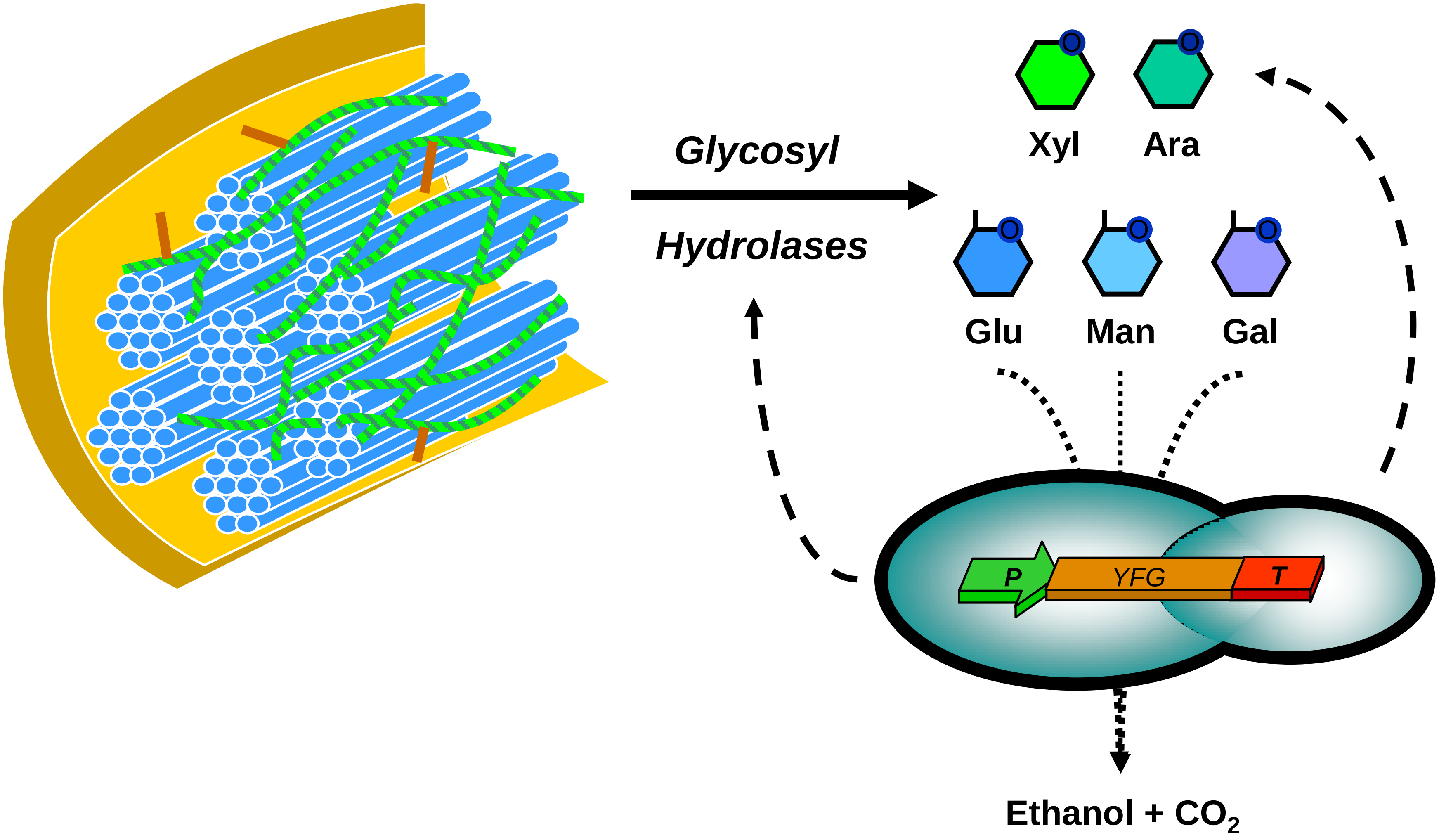


Largest Component of Recalcitrance Barrier: Cost of Cellulase



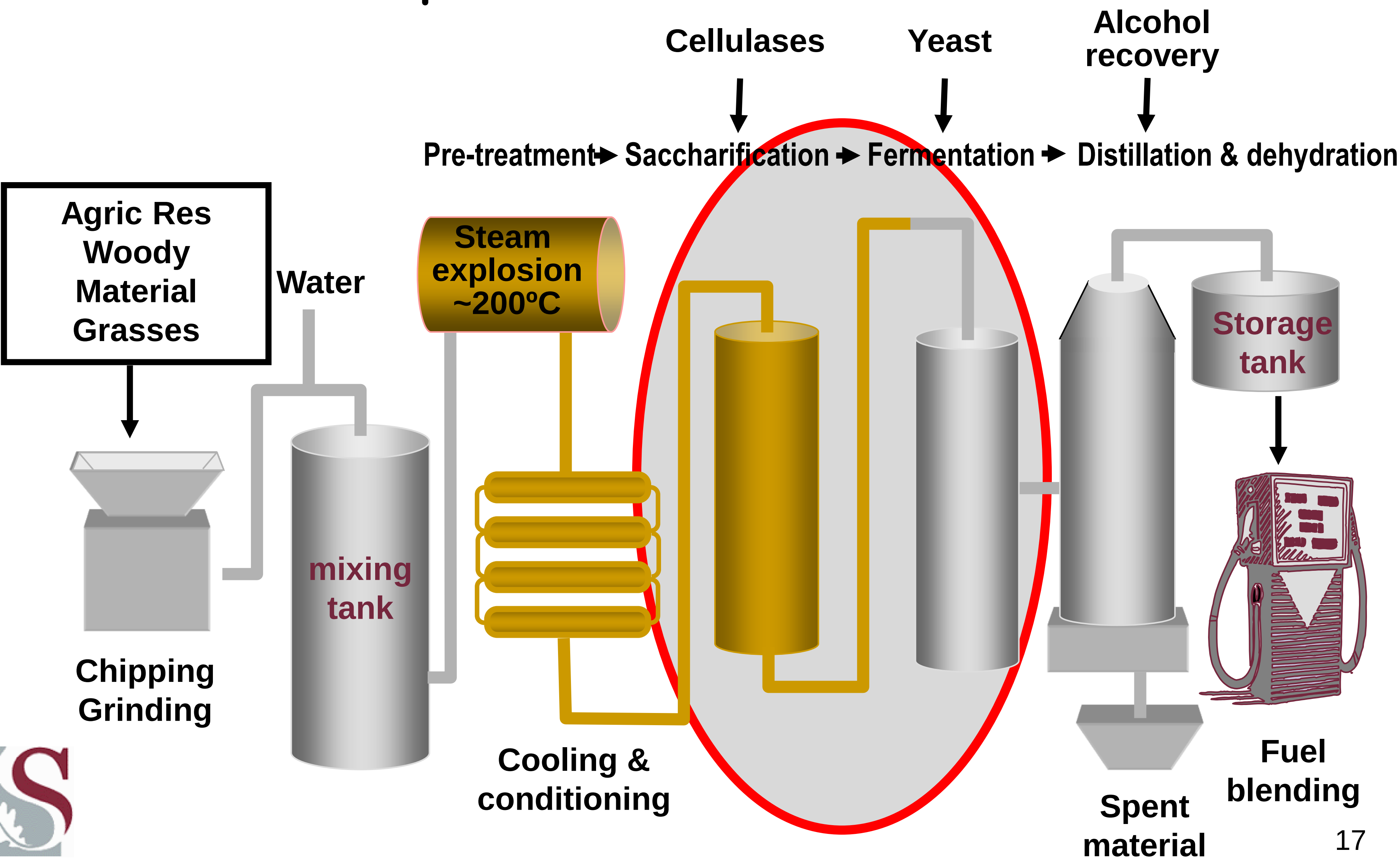
a) Hinman et al. 1991. Appl. Biotechnol. Bioeng. 34/35:639-657.
b) Hettenhaus & Glassner, 1997 (http://www.ceassist.com/assessment.htm).
c) NREL, 1998. Bioethanol from the corn industry. DOE/GO-1009-577.
d) Schell, 2004. ASM Natl Meeting; McMillan, 2004. DOE/NASULGS Biomass & Solar Energy Workshops.
e) Genencor & Novozyme, 2004. Press releases (e.g. http://www.genencor.com/wt/groc/pr 109831360).
f) Petiot, Novozymes, Platts Cellulosic Ethanol & Second Generation Biofuels, 2007.
g) Sheridan (Novozymes) Nature Biotech, 2008.

Consolidated BioProcessing (CBP)



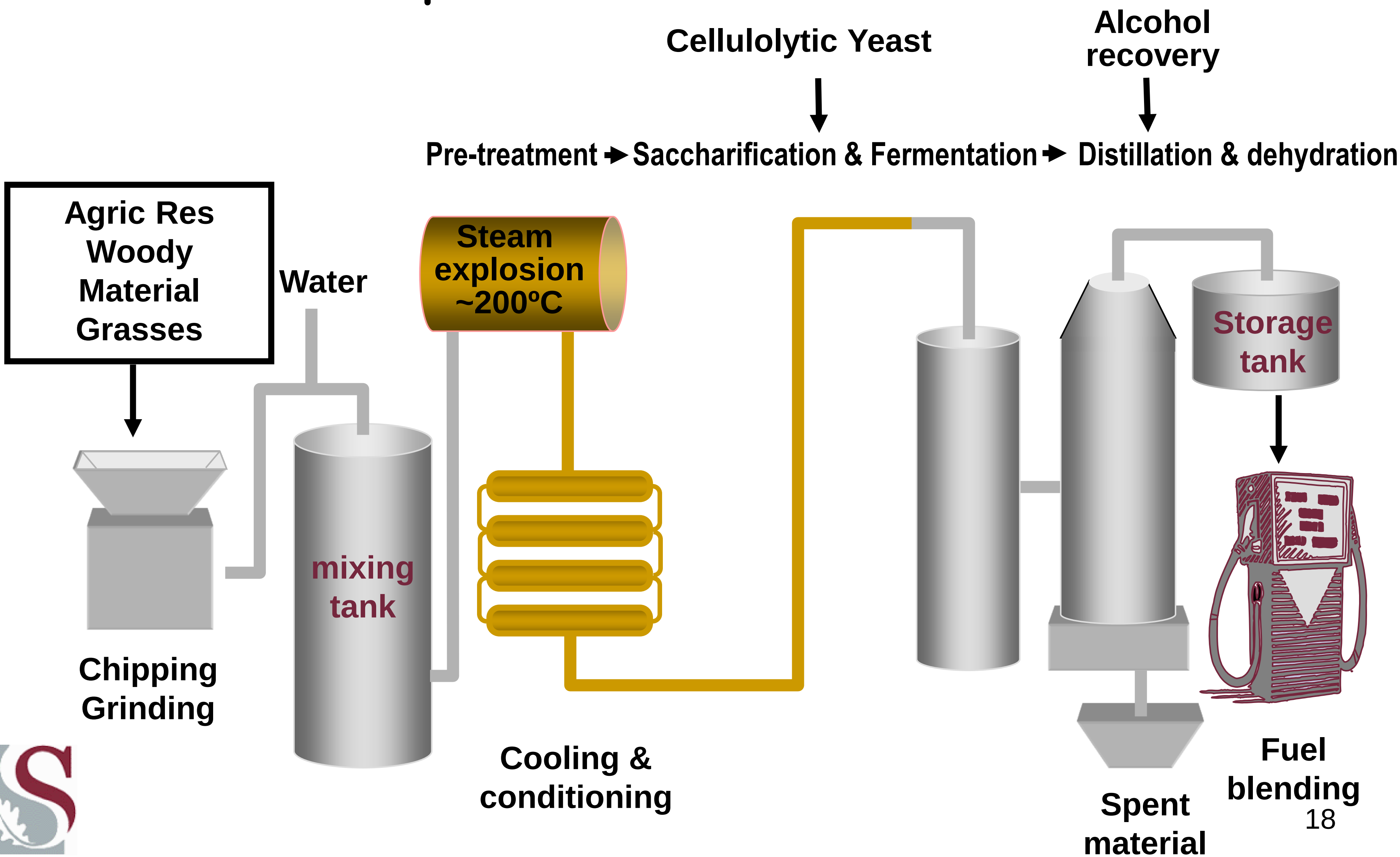
Technologies for Ethanol Production

Ethanol production from cellulosics



Technologies for Ethanol Production

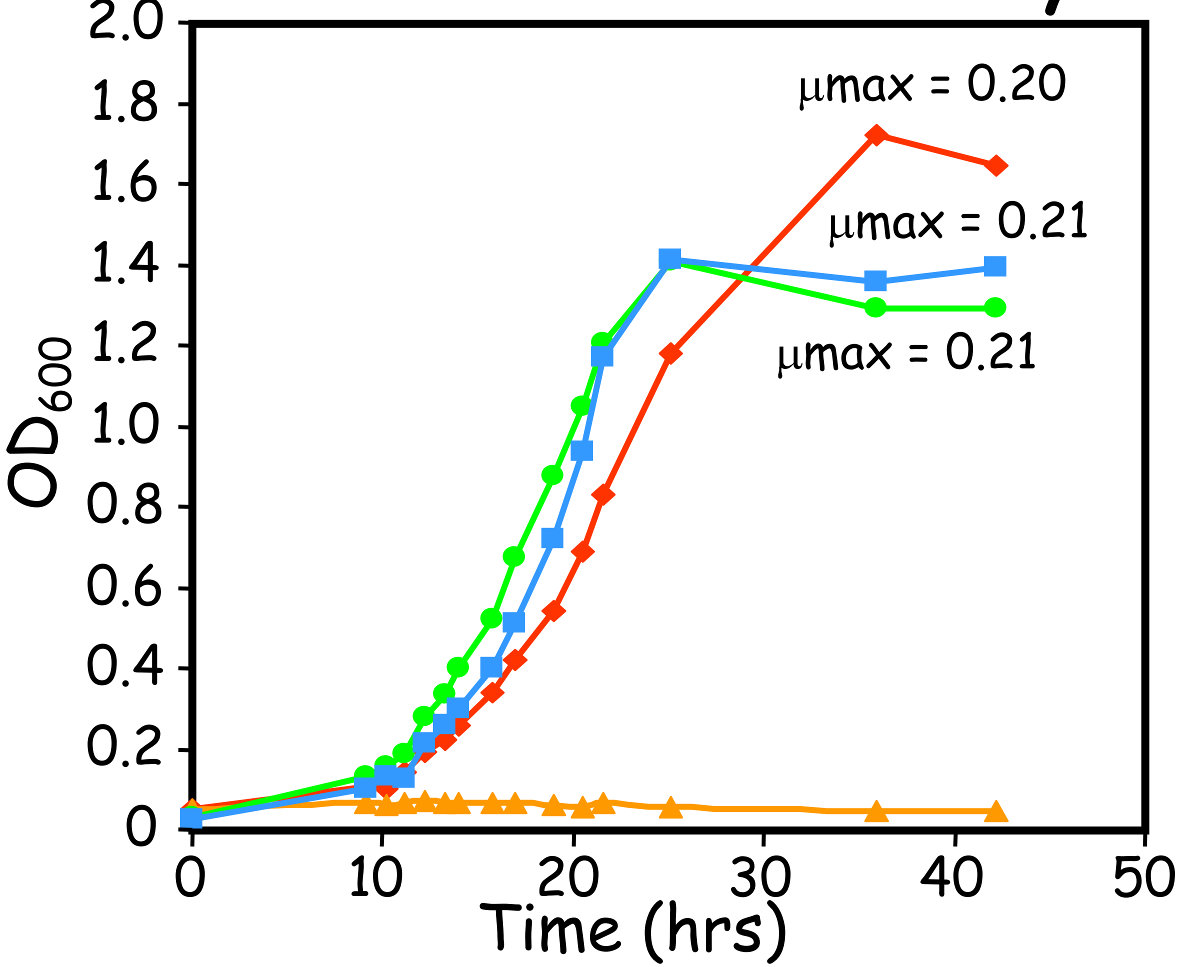
Ethanol production from cellulosics



Conversion of amorphous cellulose to yeast biomass

Cellobiose utilization in yeast

Cellobiose utilization by *S. cerevisiae*



ΣySF1 = *S. cerevisiae* expressing *S.fibuligera* β-glucosidase (BGL1) gene from 2μ plasmid.

- Σ host - glucose
- Σ host - cellobiose
- ΣySF1 - glucose
- ΣySF1 - cellobiose

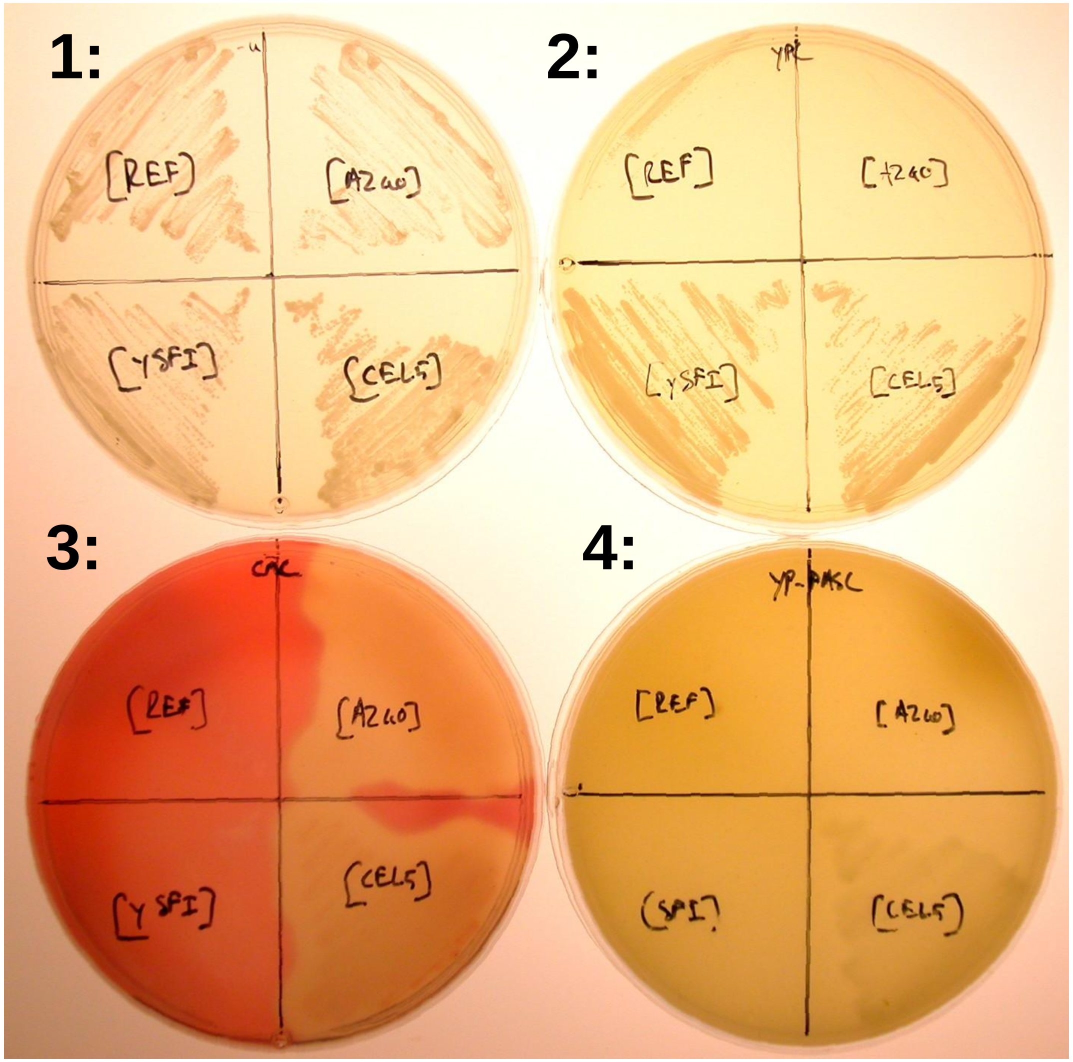
SC media;
5g/L cellobiose; 30°C / pH adjusted to 6.0 (KOH)

Van Rooyen, R., B. Hahn-Hägerdal, D.C. La Grange, W.H. Van Zyl. 2005. Construction and characterization of cellobiose-growing and fermenting *Saccharomyces cerevisiae* strains. *J. Biotechnol* **20:** 284 – 295.

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

SC Glucose

SC cellobiose



SC CMC

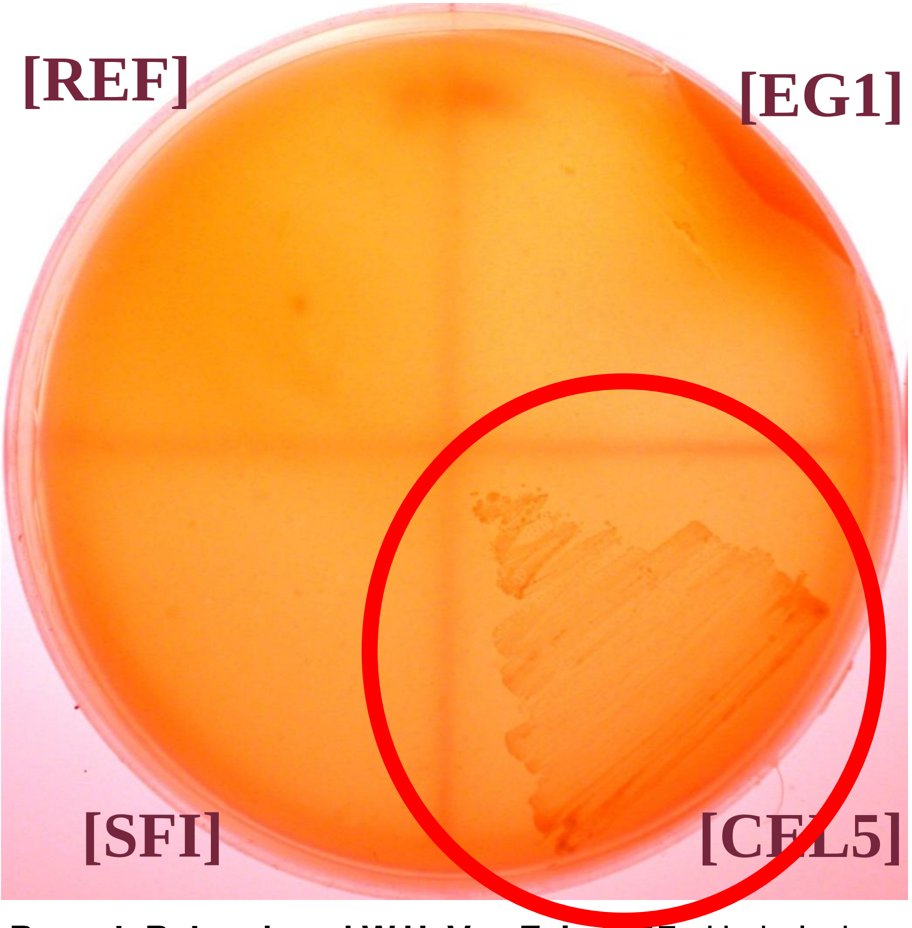
YPD PASC

[REF] = Y294 [yEP352]::*fur1;* [EG1] = Y294 [*EG1*]::*fur1*

[SFI] = Y294 [*BGL1*]::*fur1;* [CEL5] = Y294 [*EG1*+*BGL1*]::*fur1*

Conversion of amorphous cellulose

Growth on amorphous cellulose (PASC)



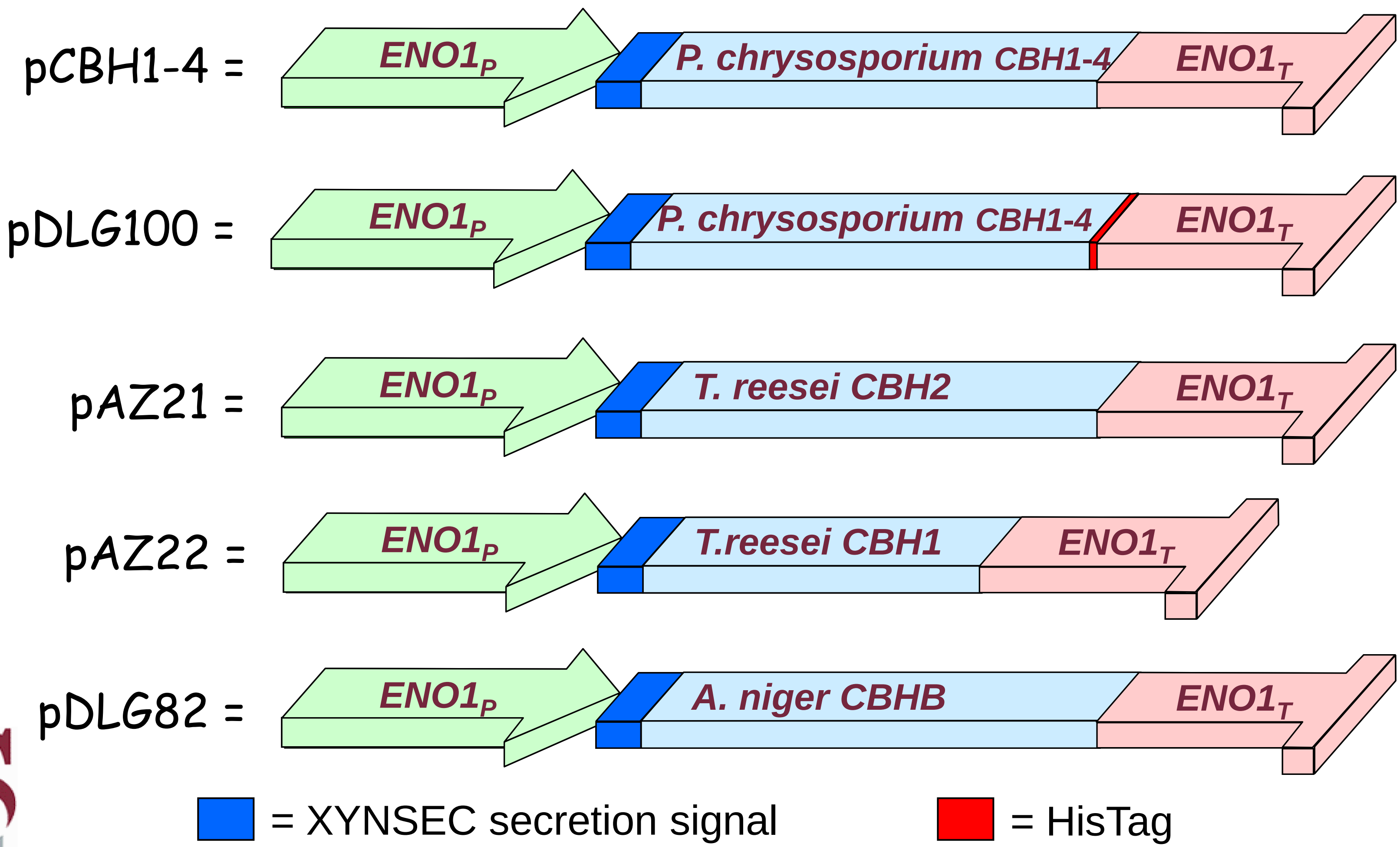
Den Haan, R., S.H. Rose, L.R. Lynd, and W.H. Van Zyl. 2007. Hydrolysis and fermentation of amorphous cellulose by recombinant *Saccharomyces cerevisiae*. *Met. Eng.* **9:** 87–94.

Functional expression of cellobiohydrolases in yeast

Expression of cellobiohydrolases in yeast

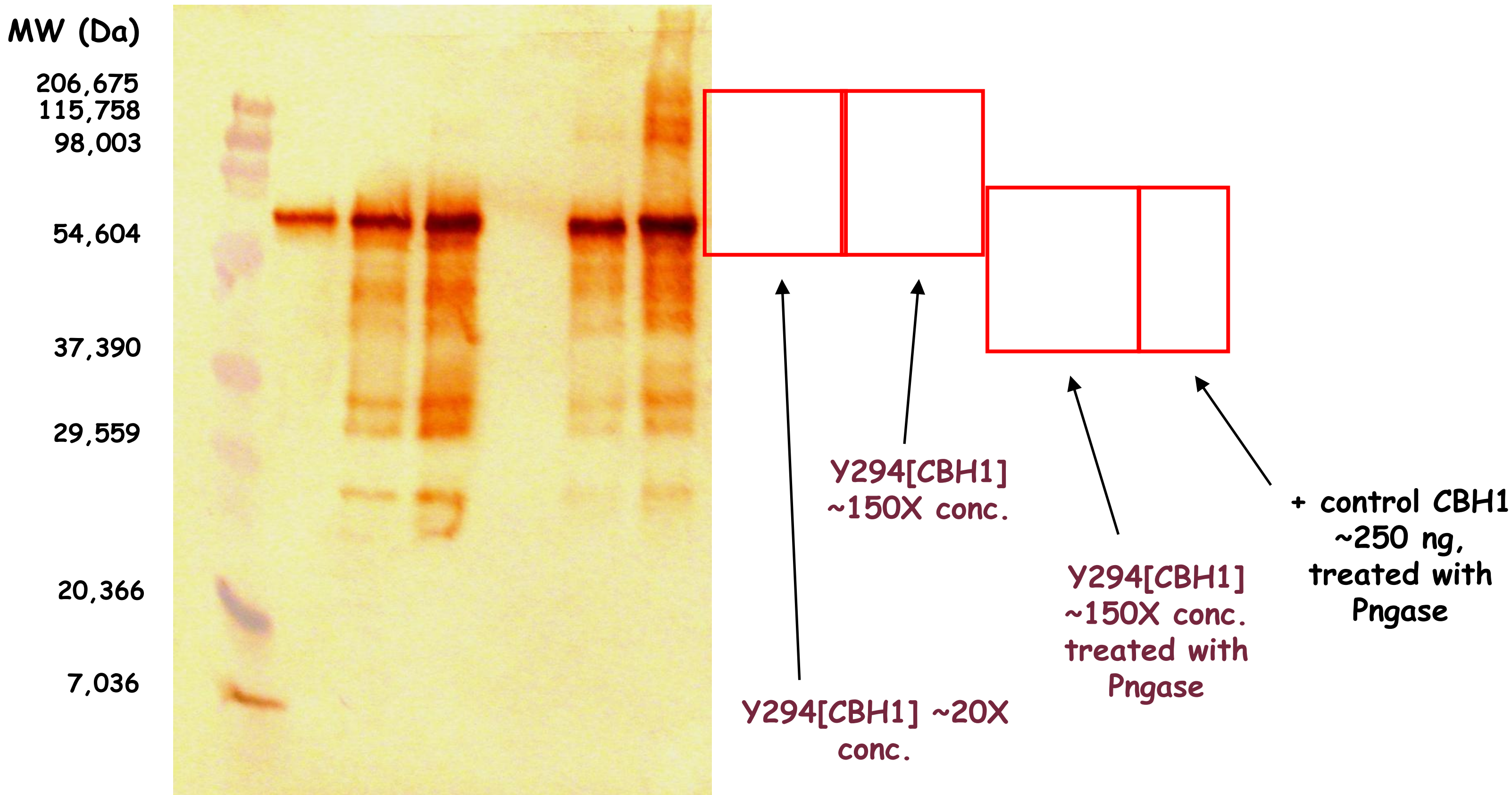
CBH expression in *S. cerevisiae*

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



Expression of cellobiohydrolases in yeast

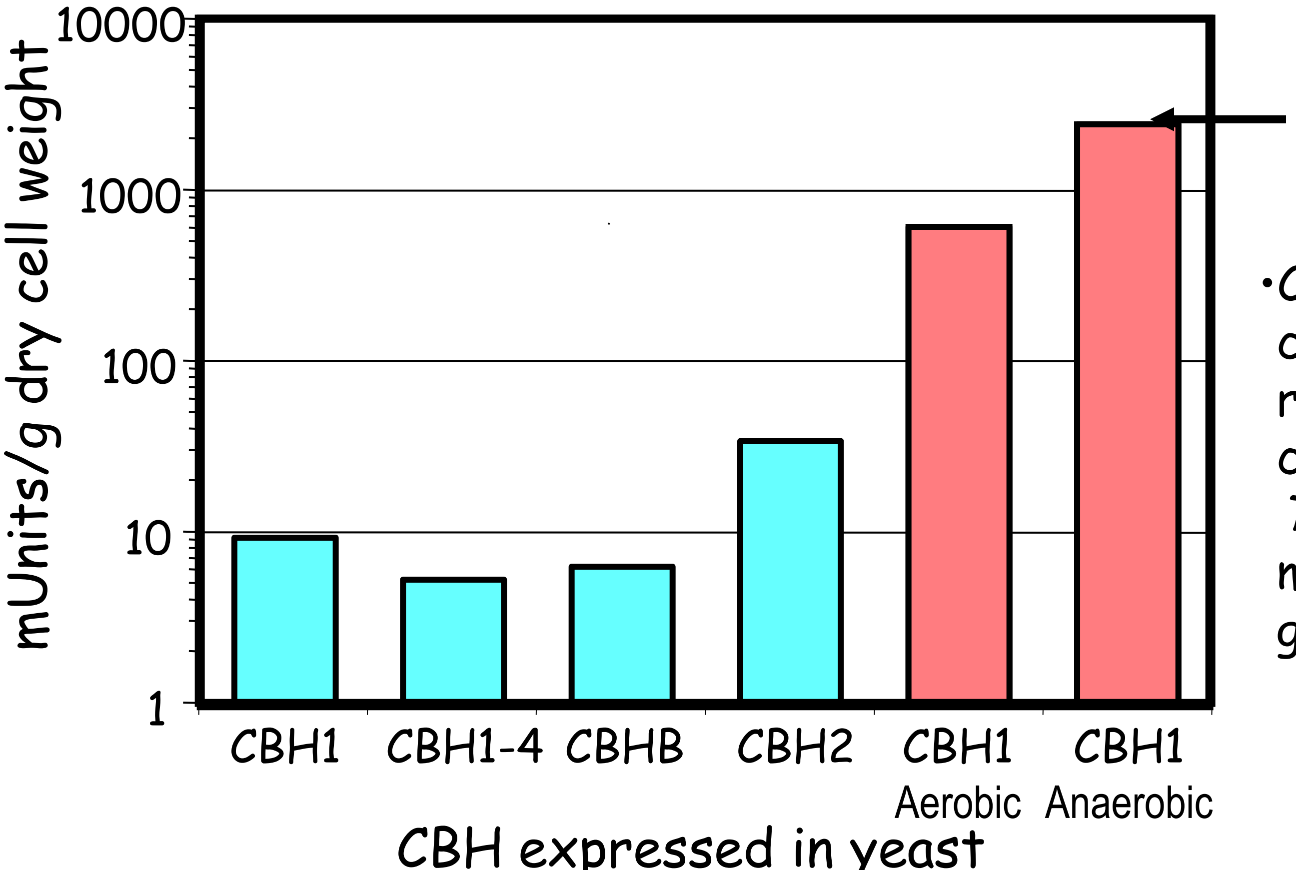
CBH1 cellobiohydrolase production by yeast



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



- 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}

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.

Recent advances towards realizing CBP

MASCOMA

Mascoma Corporation
Technical facilities, Lebanon, NH, USA
(www.mascoma.com)

MASCOMA

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

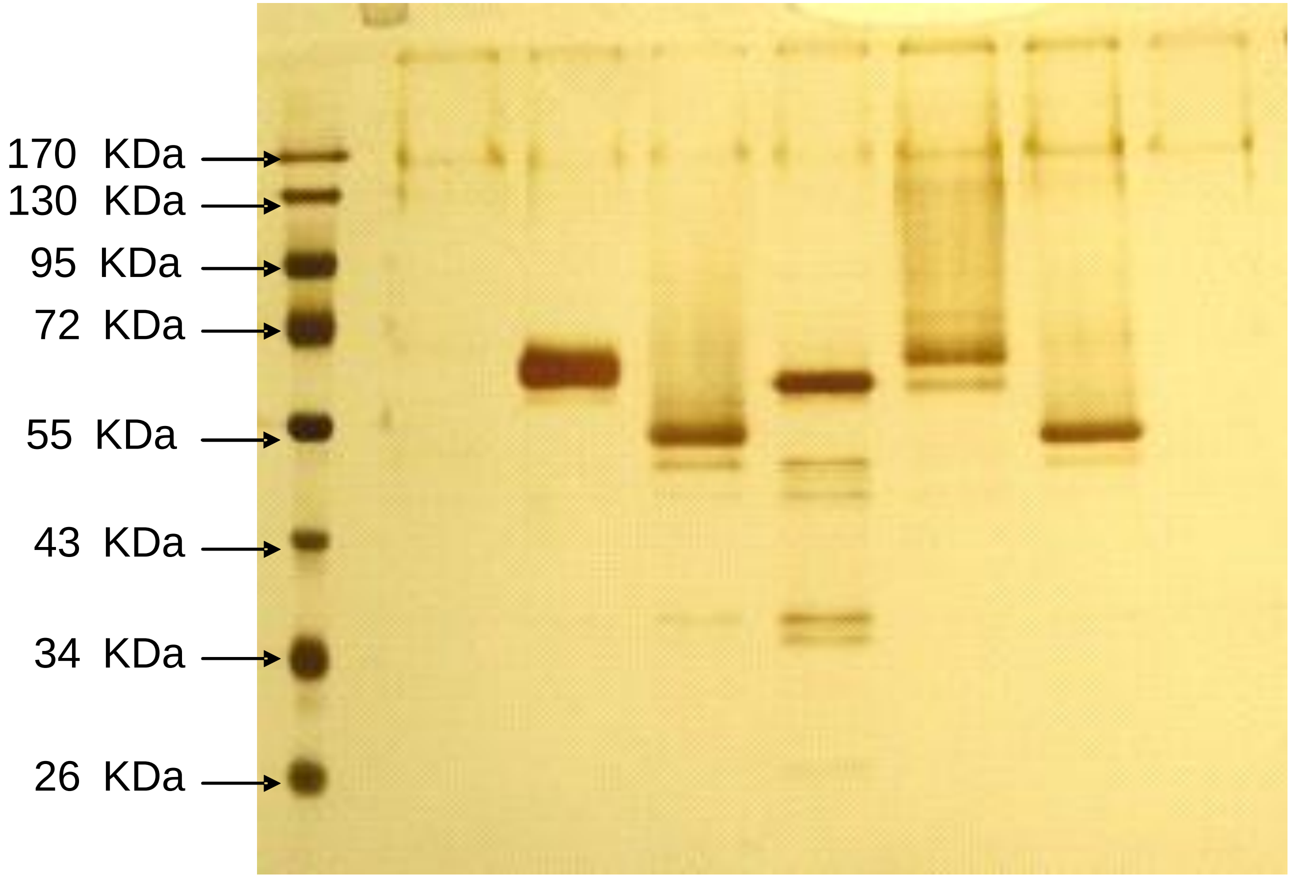
Three Platforms

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

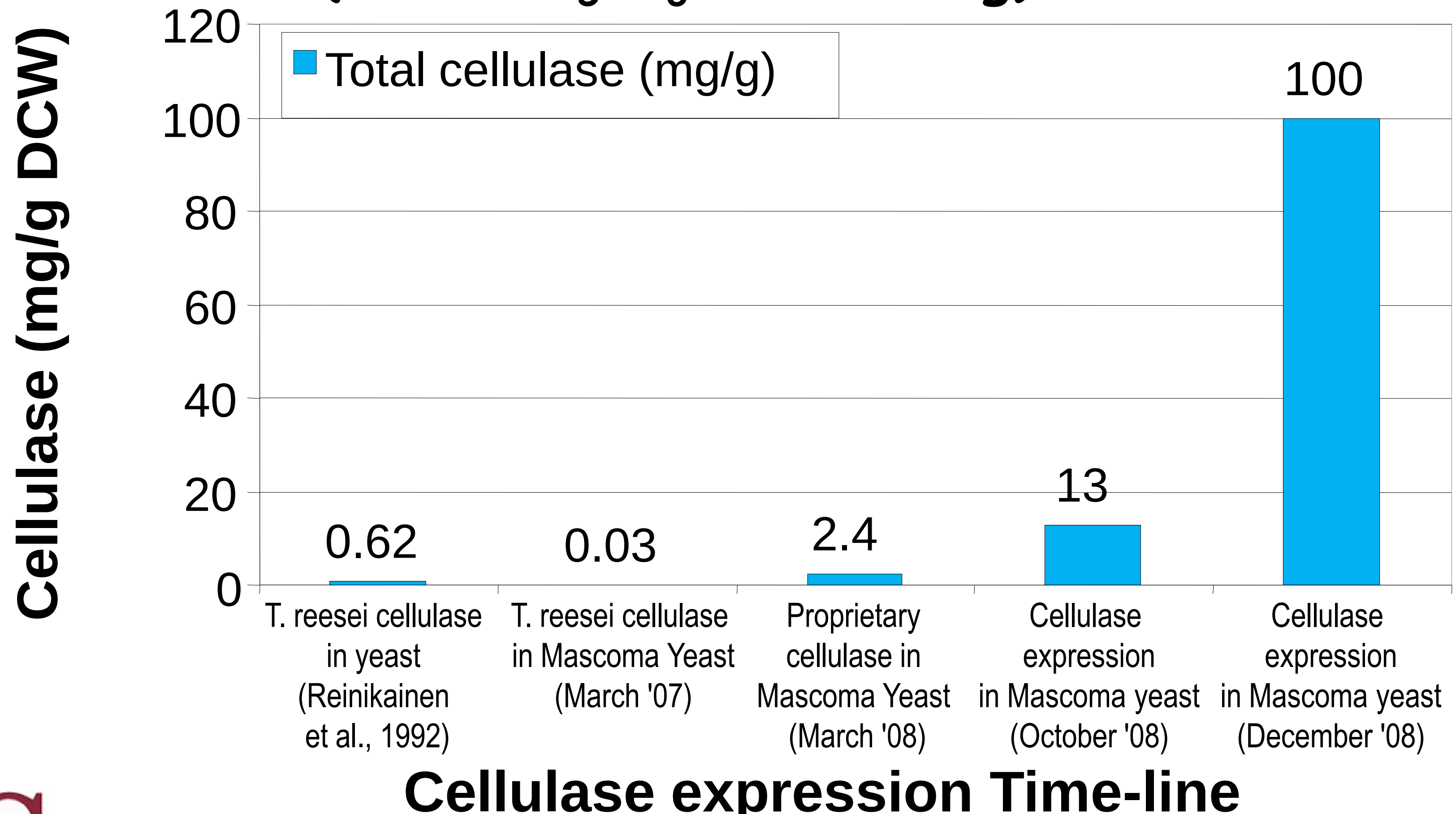
Subsequent expression of cellobiohydrolases!

12% SDS-PAGE, silver staining



Mascoma Cellulolytic Yeast

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

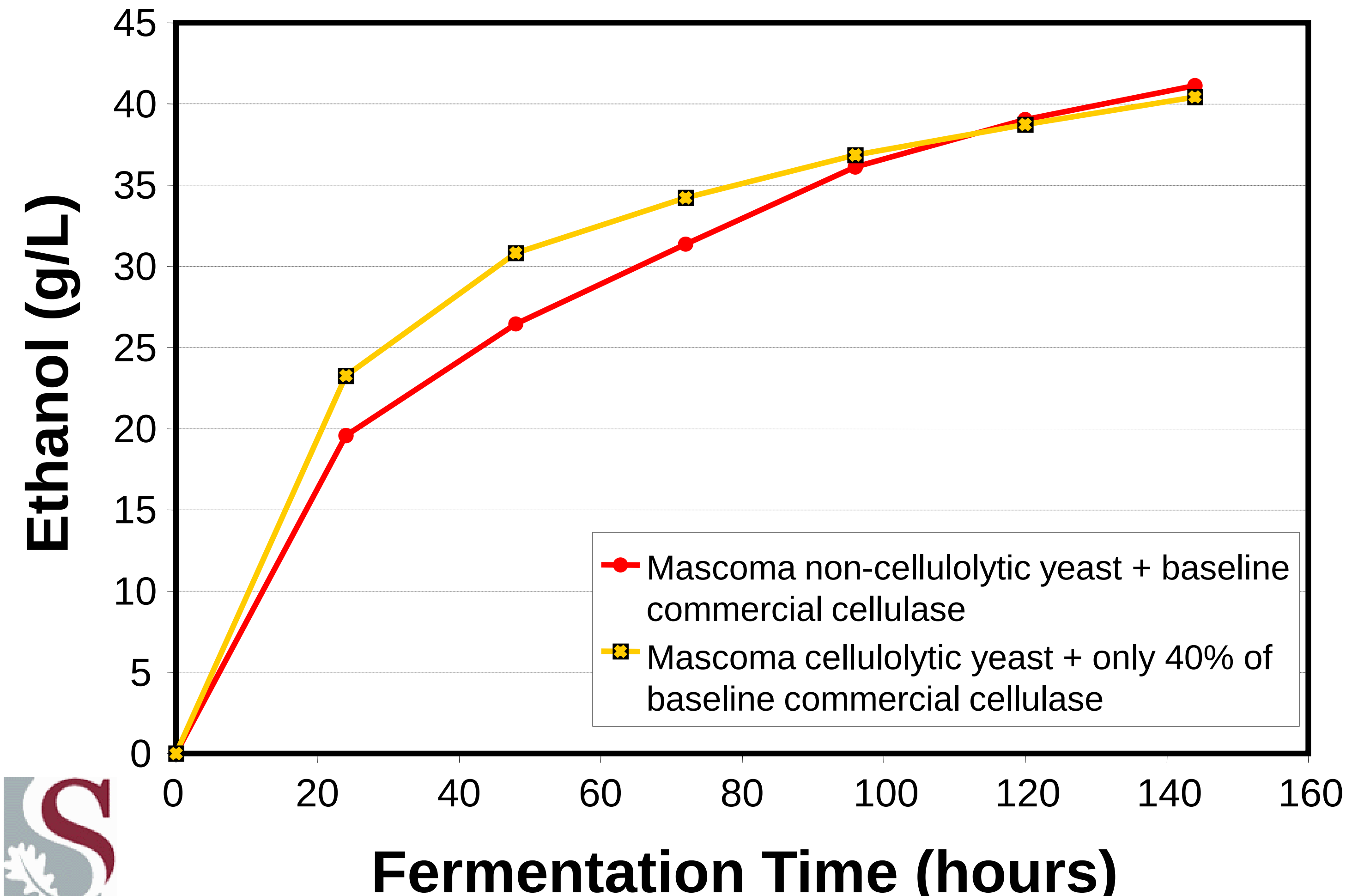


Mar, 2007 to Dec, 2008: >3,000-fold improvement in expression levels

Enzyme Reduction on Hardwood

MASCOMA

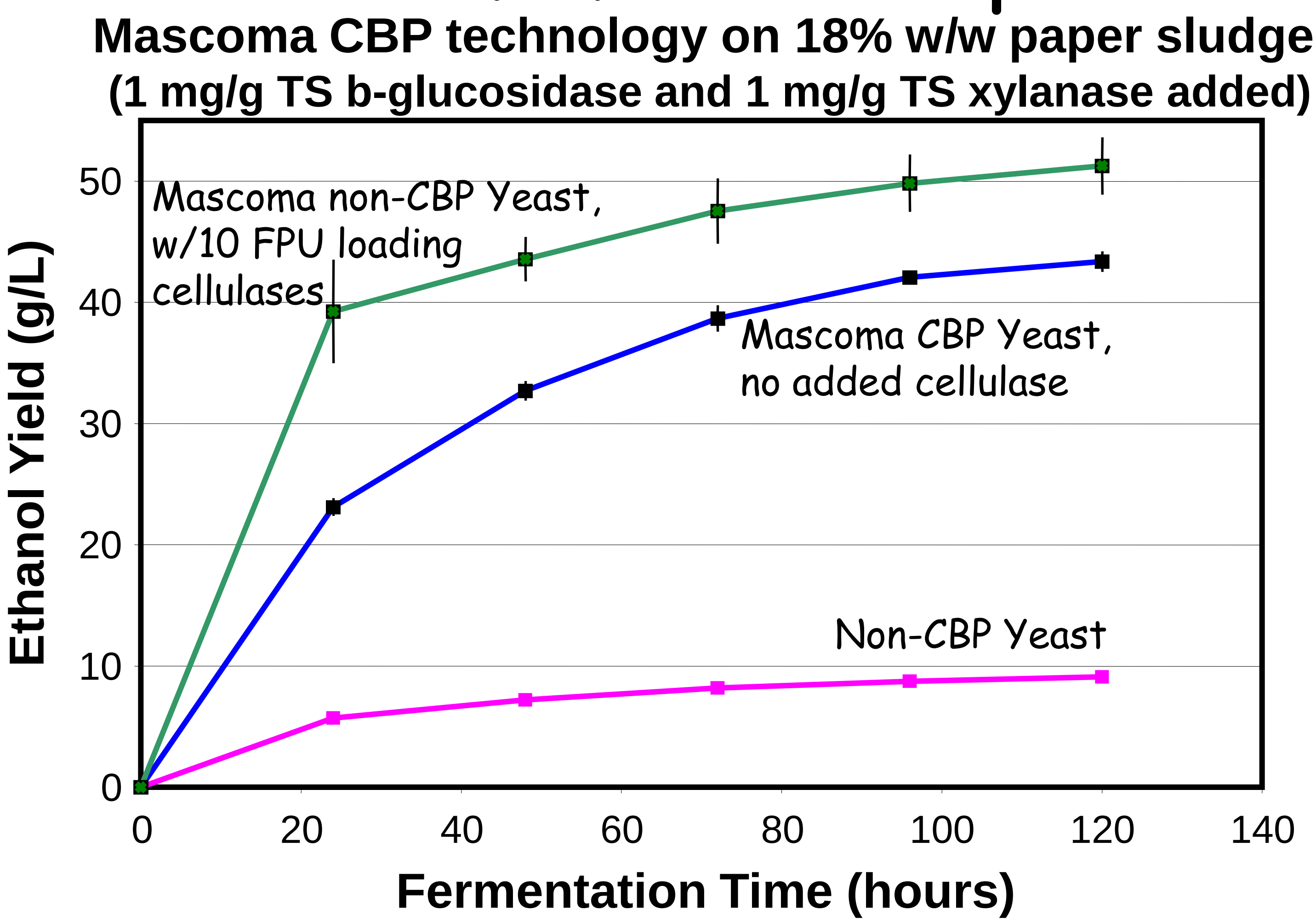
Mascoma CBP Strain (robust C5/C6 fermenting yeast) + 22% w/w unwashed Pretreated Hardwood + Commercial cellulase



Equivalent performance with 2.5-fold less added enzyme

Further reduction likely

Conversion of Paper Sludge to Ethanol: Proof of CBP Concept



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

Rome, NY Pilot & Demonstration Plant

January 2008

November 2008

Acknowledgements

- Stellenbosch University, South Africa

  Danie la Grange | Riaan den Haan
  Shaunita Rose | Ronél van Rooyen

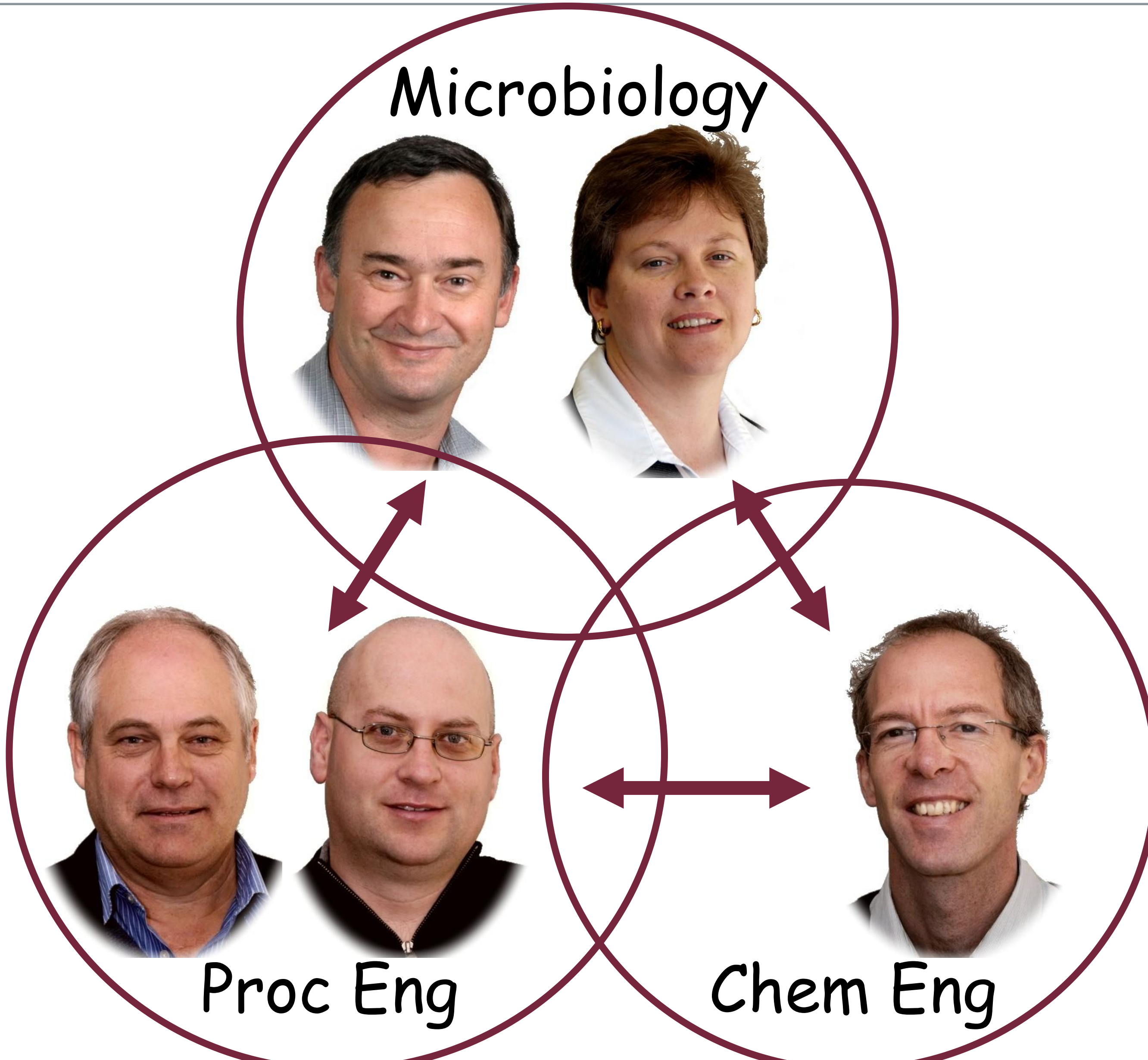
- Dartmouth College, USA

  John McBride | Lee Lynd

- VTT, Finland

  Marja Ilmen | Merja Penttilä

Chair of Energy Research : Biofuels (members)



Technologies for Cellulose Conversion

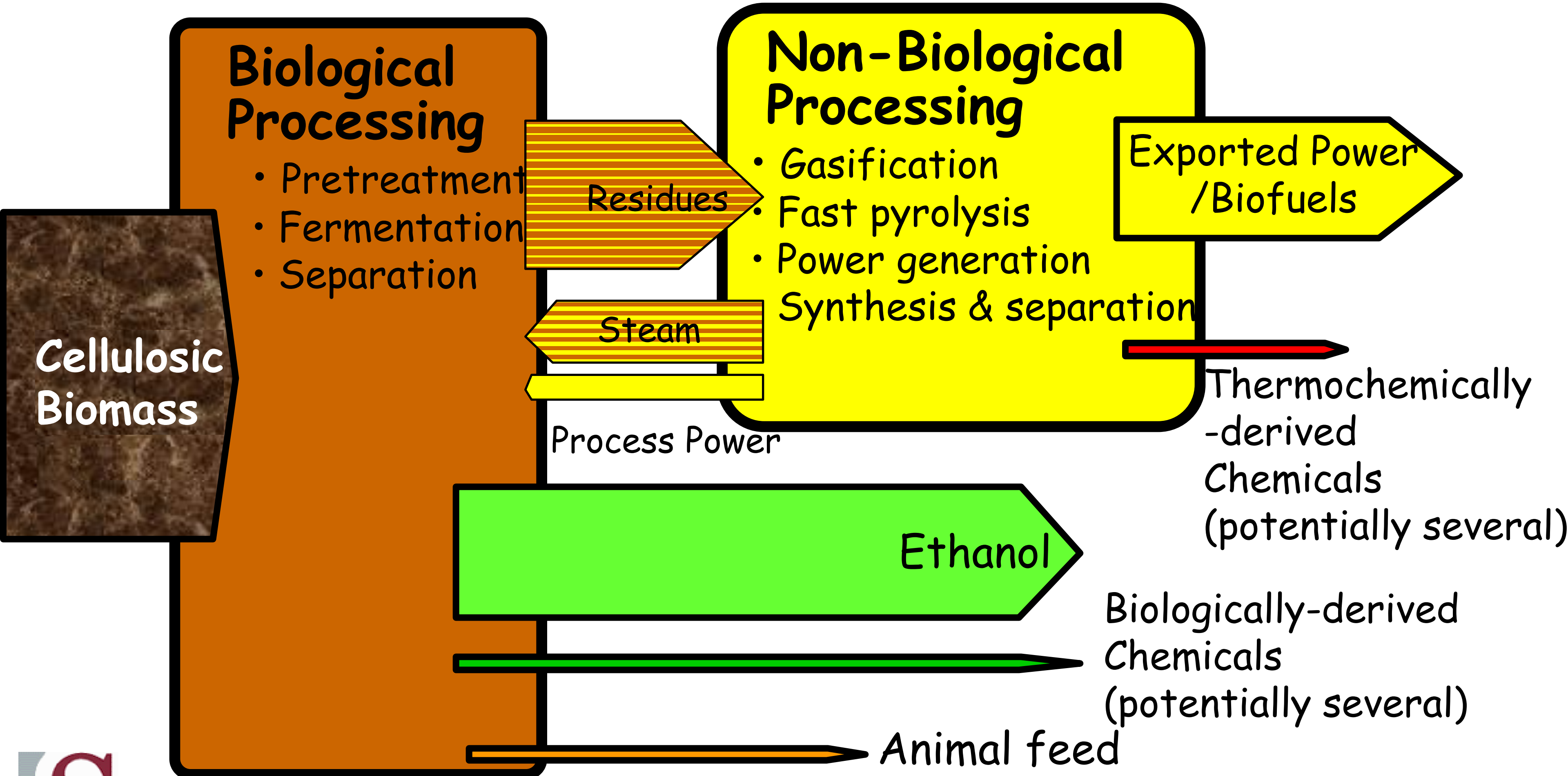
Cellulosics biofuels production value chain:

Primary Biomass Production → Biomass Transport → Biomass Primary Processing → **Biomass Conversion to Biofuels and By-products** → Product Distribution & Marketing

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

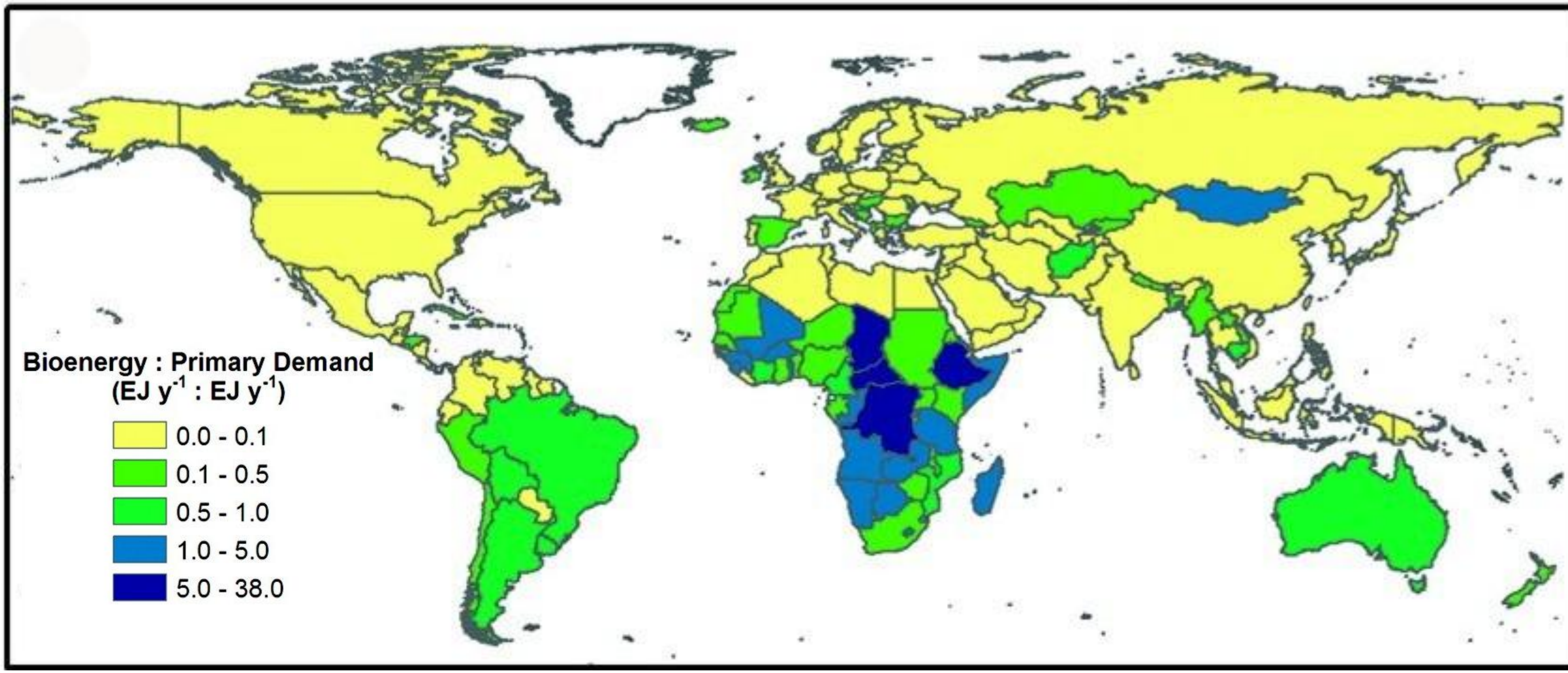
Technologies for Cellulose Conversion

Biomass Biorefinery Concept



Lynd et al. 2003. Plant Biomass Conversion to Fuels and Commodity Chemicals in South Africa: A Third Chapter? South African Journal of Science **99:** 499 – 507.

Biomass potential of Africa at large



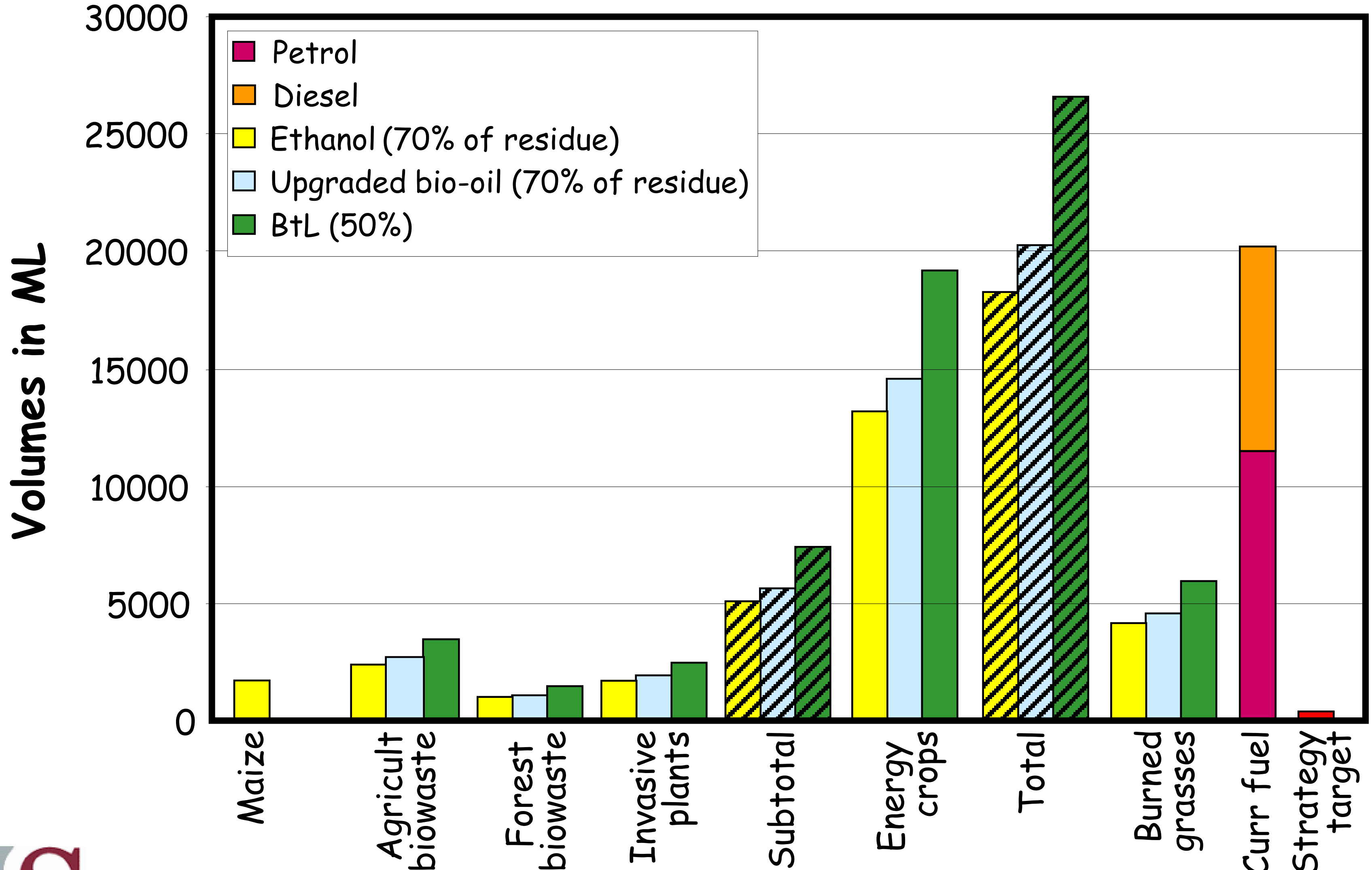
Ratio of the energy content of the biomass on abandoned agriculture lands relative to the current primary energy demand at the country level. The energy content of biomass is assumed to be 20 kJ g^{-1}. Source: Campbell et al. (2008)

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 (Marrison and Larson, 1996)	**67 Mt/a**	**(1 171 PJ/a)**
3. Invasive plant species	**8.7 Mt**	**(151 PJ)**
Total, annual basis	**93 Mt/a**	**(1 622 PJ/a)**

Lynd et al. 2003. Plant Biomass Conversion to Fuels and Commodity Chemicals in South Africa: A Third Chapter? South African Journal of Science **99:** 499 – 507.

South Africa's potential:
Biofuels production



Maize to Ethanol = 430 L/ton

Biomass to upgraded bio-oils = 310 L/ton

Biomass to ethanol = 280 L/ton

Biomass to liquid (BtL) = 570 L/ton

Thank you!