Making Cost Effective Cellulosic Biofuel: Achieving High-Level Expression of Exo-Glucanase Cellulases in Plants

Simon Warner – EPSO Biofuels workshop

May 28th & 29th, 2008

Our purpose

BRINGING PLANT POTENTIAL TO LIFE

Syngenta: Global R&D Capabilities

JEALOTT'S HILL, UK

Chemical Discovery
Weed Control
Formulation
Bioscience
Environmental Science

SBC, Beijing

Biotech R&D

GREENSBORO, NC

Formulation
Environmental Science

GOA, INDIA

Chemistry

RESEARCH TRIANGLE, NC

Biotechnology R&D

Other sites:

Marker-assisted breeding support

Global field station network

BASEL/STEIN, SWITZERLAND

Fungicides, Insecticides & Professional Products

BRISBANE, AUSTRALIA

Syngenta Centre for Sugarcane Biofuels Development

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Syngenta Biotechnology Inc. (SBI)

Biotech R&D Center

- Crop Transformation
- Enzyme Biochemistry & Protein Design
- Plant Protein Expression
- Plant Analysis & Immunology
- Patents and Licensing
- Regulatory Affairs
- Bioinformatics & Scientific Computing

History

- 1st to Market with GM event - Bt corn
- Other GM crops on the market

For Syngenta, the path to efficient and sustainable renewable fuels will encompass three horizons

Today

Maximizing yield and decreasing pressure on key feedstocks

- Best genetics
- Optimized starch
- Protecting yield
- Facilitate crop production and availability

3 - 5 years

Innovating within the existing industry to maximize efficiency

- Tailored crops
- Corn Amylase
- Tropical Sugar Beet
- Processing changes

5+ years

Help make sustainable cellulosic conversion a reality

- Plant-expressed enzymes
  - Self-processing biomass crops
  - Plant made additive enzymes
- Improved feedstocks
- Biomass conversion process around key feedstocks

Syngenta Technology Development

Tropical Sugar Beet – a global opportunity

TSB span:
30 N to 30 S.

Strengths

- **Water –33% to -50% vs. sugar cane**
- **Fit to saline/alkaline soils**
- **Cost competitive with sugarcane**
- **Increased farmer income**
- **Provides an additional cash crop**

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Expression of the amylase enzyme in corn

Benefits

- Greater ethanol output per bushel
- Increased plant through-put
- Reduced energy and water use
- Processing flexibility
- Reduced chemical usage

Cane Biomass = Boiler fuel and Cogen or more liquid fuel?

Total Sugar 153 kg	2.55 GJ
Bagasse 50% mositure 275 kg	2.3 GJ
Straw 15% moisture 165 kg	2.15 GJ

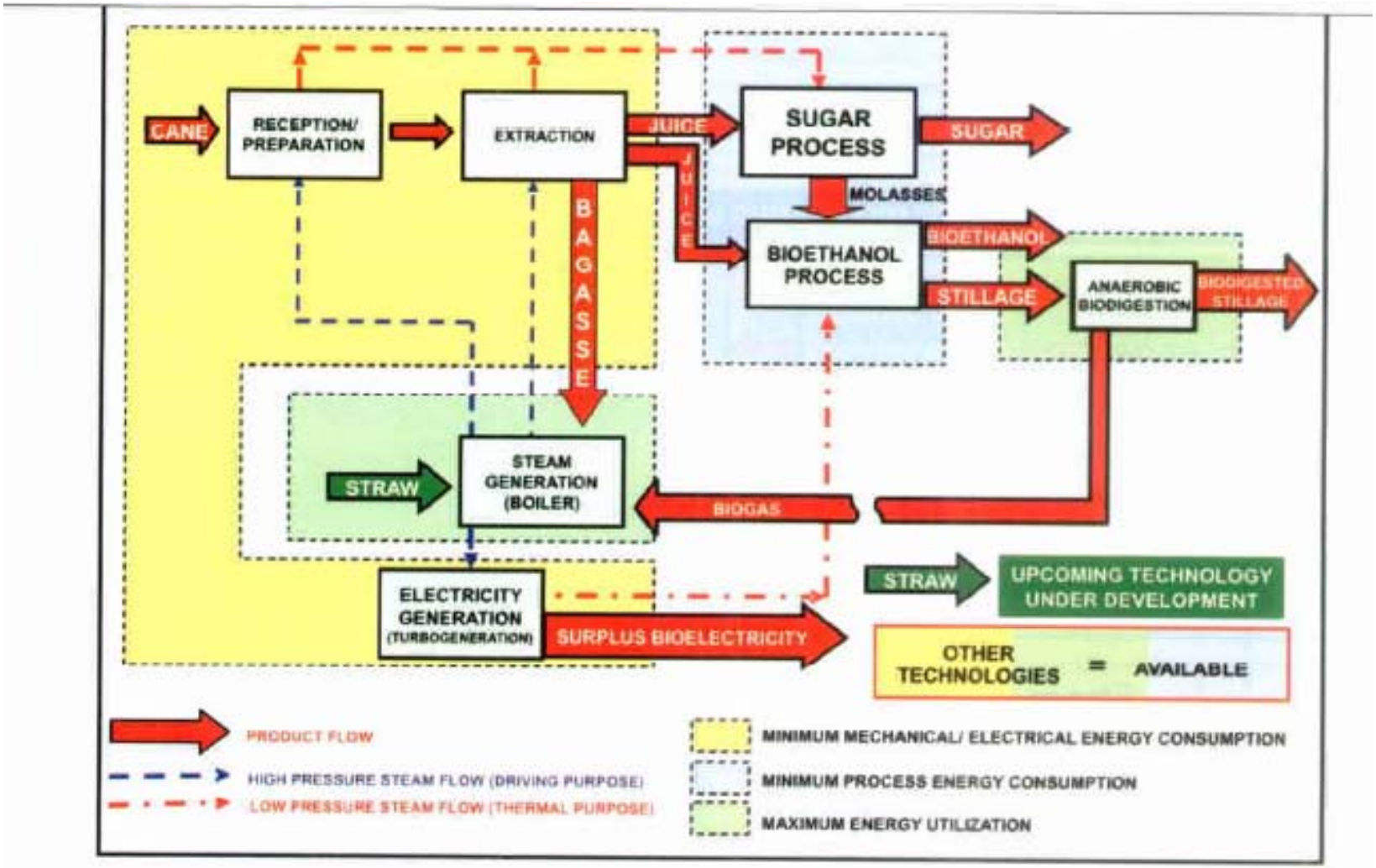
1 tonne of Cane in the field 7.25 GJ

Barrel of Oil 5.74 GJ

Tonne of cane = 1.2 Barrels of oil

After **Cogeneration in Brazilian sugar and bioethanol mills: past, present and challenges.** Oliverio, Jose Luiz; Ribeiro, Jose Eduardo. International Sugar Journal (2006), 108(1291)

Current Co-Gen process is cheap and efficient



After **Cogeneration in Brazilian sugar and bioethanol mills: past, present and challenges.** Oliverio, Jose Luiz; Ribeiro, Jose Eduardo. International Sugar Journal (2006), 108(1291)

Cane Biomass = Boiler fuel and Cogen or more liquid fuel?

	Cellulosic Ethanol	Electricity
Total Sugar 153 kg	98 L	0 KWh
Bagasse 50% mositure 275 kg	60 L	109 kWh
Straw 50% moisture 165 kg	39 L	65 kWh
	99 L	174 KWh
1 tonne of Cane in the field	**US$ 46 / tonne**	**US$ 17 / tonne**

Converting everything possible into ethanol – 0.8 barrels of oil equivalent

Cogeneration in Brazilian sugar and bioethanol mills: past, present and challenges. Oliverio, Jose Luiz; Ribeiro, Jose Eduardo. International Sugar Journal (2006), 108(1291)

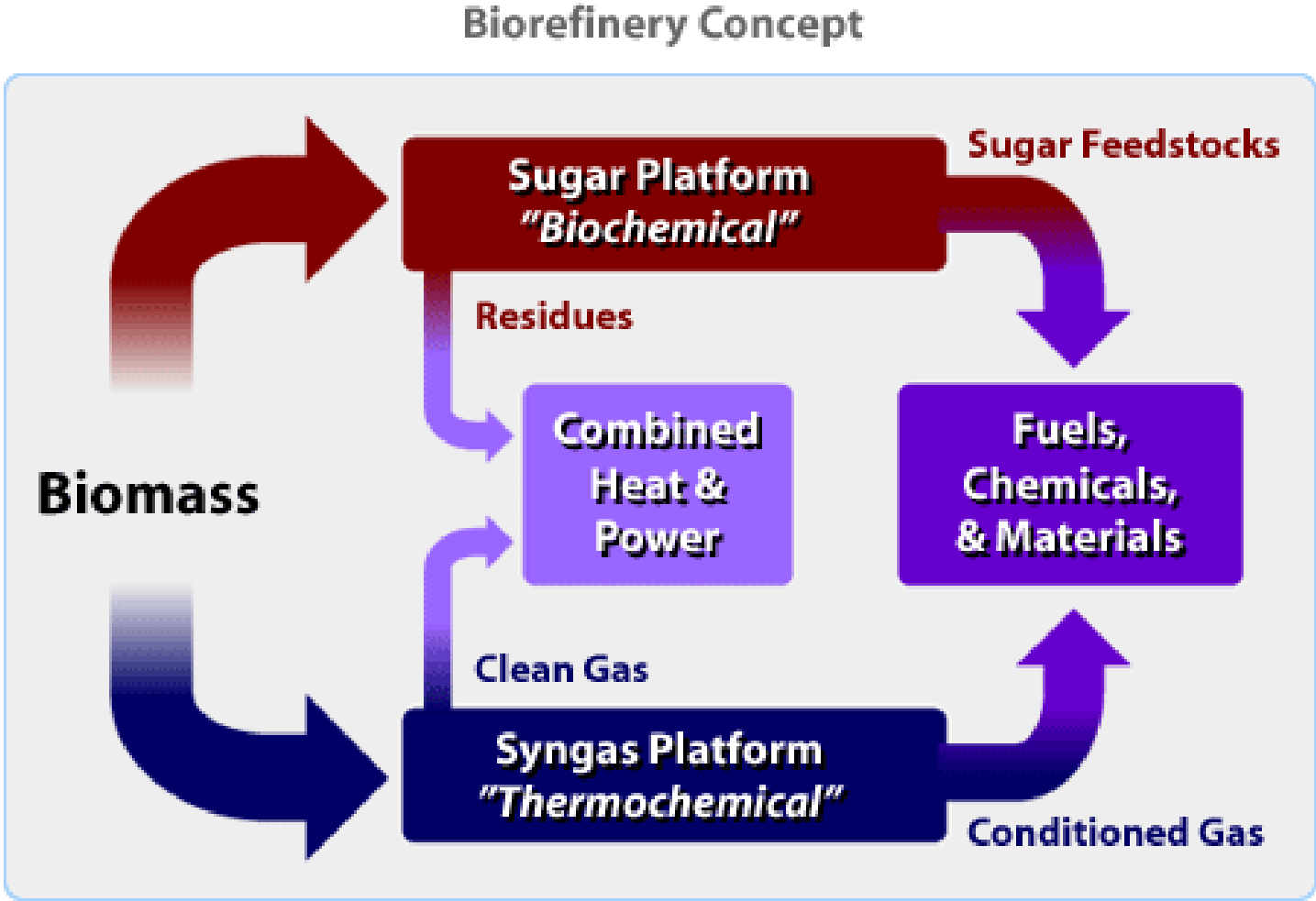
Cost comparisons more electricity vs more ethanol?

- Mill's have mandates for production set by the government
- They want to make money, not sugar, alcohol and electricty
- If a cellulosic ethanol process existed today in Brazil the value of a tonne of bagasse when converted to electricity is similar to that if converted to ethanol
- The capital investment is not the same and currently highly favors co-gen'.
- Market volatility in co-gen' – Hydroelectric program.
- If there is a driver for liquid transport fuels – biochemical and thermochemical conversion may be economic in Brazil
  - **Todays US prices; Ethanol is $31.18/GJ and Electricity is $17.36/GJ**

What about Corn and other residues?

- First step – incremental value in corn seed fiber (change in DDGS)
- Second step – corn stover collection to power existing ethanol plants
  - We have to add value or its expensive ($165 per tonne colllection cost)
  - Max ethanol value today assuming ca 60 gallon production $162
- Keep inventing…..

NREL Thermochemical Conversion



Convert the best and burn the rest

Addressing key challenges to reduce cellulosic ethanol cost

	Source optimum feedstock	Pretreatment (break up fiber)	Hydrolysis (Make sugars)	Fermentation (Sugars to fuel)
Costs (USD/Gal.)*	$0.15–1.15**	$0.35 – 0.60	$0.75–1.00	$0.40–0.75
		Total: $1.8 – $3.0+		
Key Challenges	• High cost of management and logistics	• High cost of capital equipment	• Low enzyme activity and expression	• Non-robust C5 sugar fermenting micro-organisms
Syngenta Focus	• **Feedstock productivity and management**		• **Unique, evolved enzymes with low loading requirements** • **Plant expressed enzymes**	

Collaborations to integrate process and reduce to practice

Focused improvements will enable $1.25 - $1.50 USD/Gal. cellulosic ethanol

Source: Syngenta analysis
*Estimated costs in 2007, ** High variability due to management and logistics for certain feedstocks

Syngenta – Reducing the Concepts to Practice

- We have expressed multiple cellulases in plants
  - Exo Cellulases, Endo Glucanases, Beta Glucosidases and Hemi cellulases
- Progress towards commercially relevant expression levels
- Early technology – but will provide the lowest cost performance enzymes for cellulosic biomass conversion

Syngenta plant-expression capability for two concepts

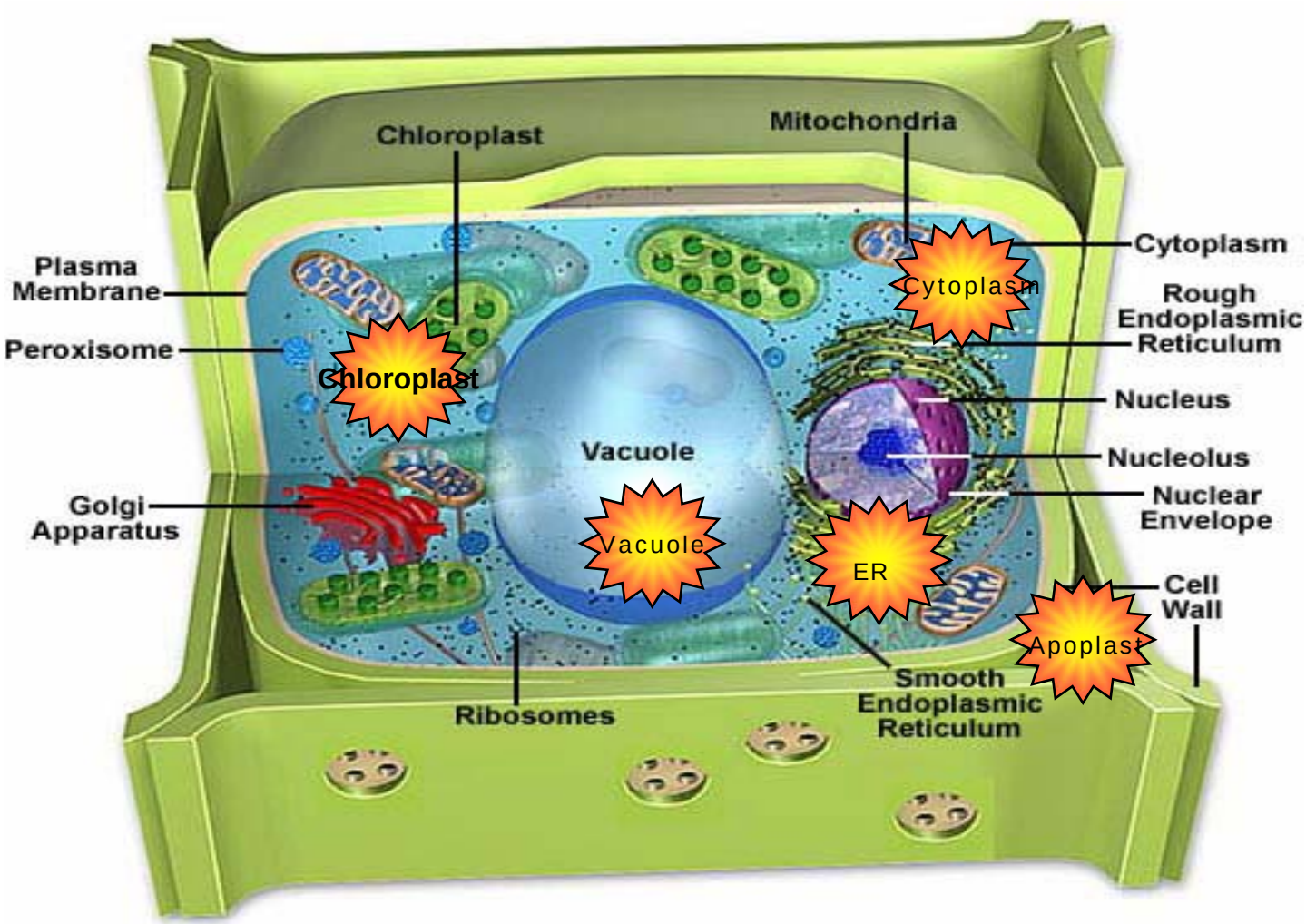
Concept	Feedstock Expression	Plant-Expressed Additive
	 	
Description	- Enzymes expressed in the biomass that is to be processed (e.g. Corn, cane & beet)	- Enzymes expressed in an “additive” protein crop (e.g. canola, soy)
Advantages	- “Self-processing” feedstock - Lowest cost enzyme production - Large amounts may be made	- Flexible for different feedstocks - Can be added after pretreatment - Storage and transport possible
Challenges	- Process design around feedstock to maximize performance	- Additional logistics necessary - Carries some additional processing cost

Delivering plant enzyme expression concepts

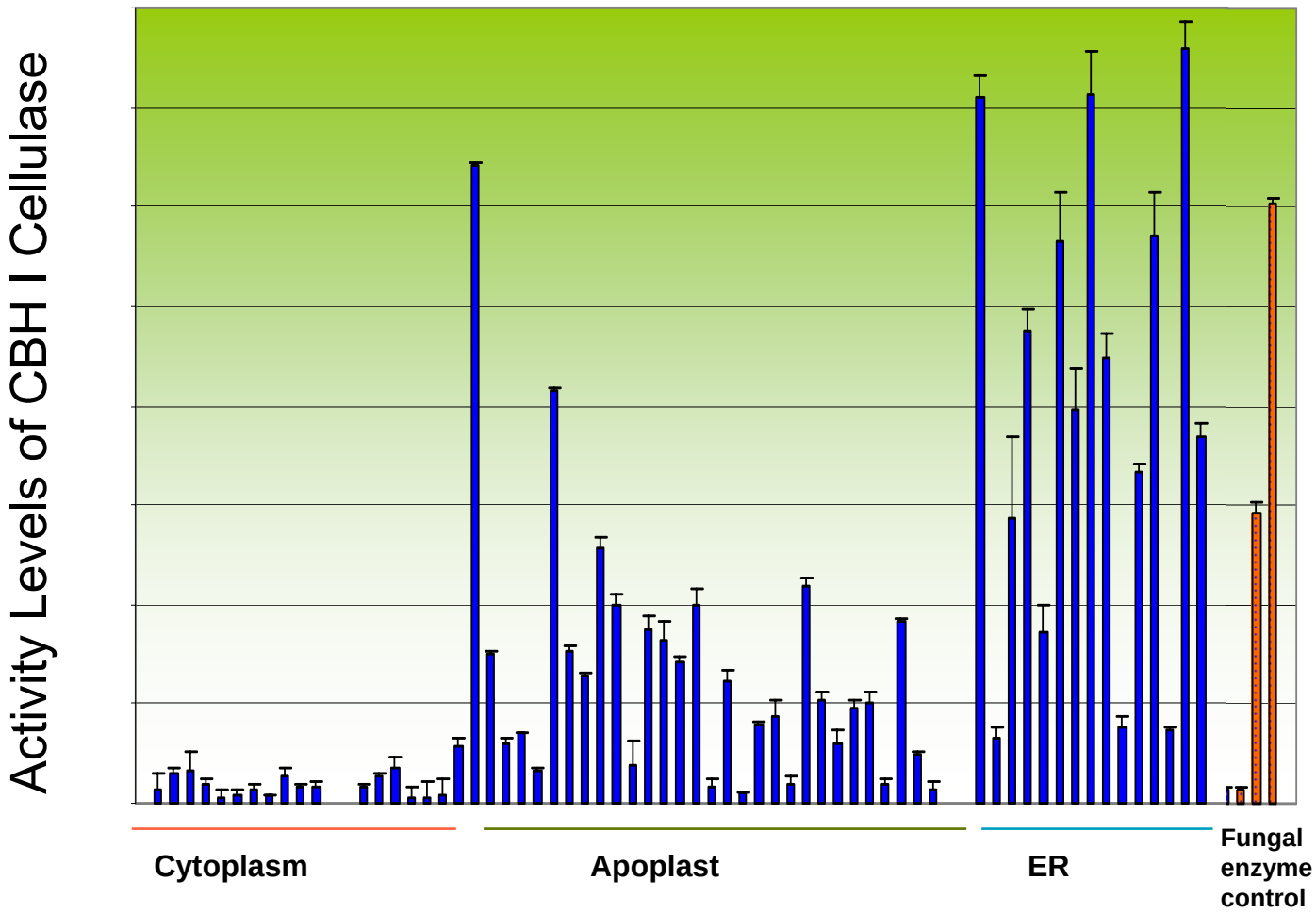
- Expression in certain tissues or cell type

Delivering the plant enzyme expression concepts

- Subcellular targeting – important for cellulase expression



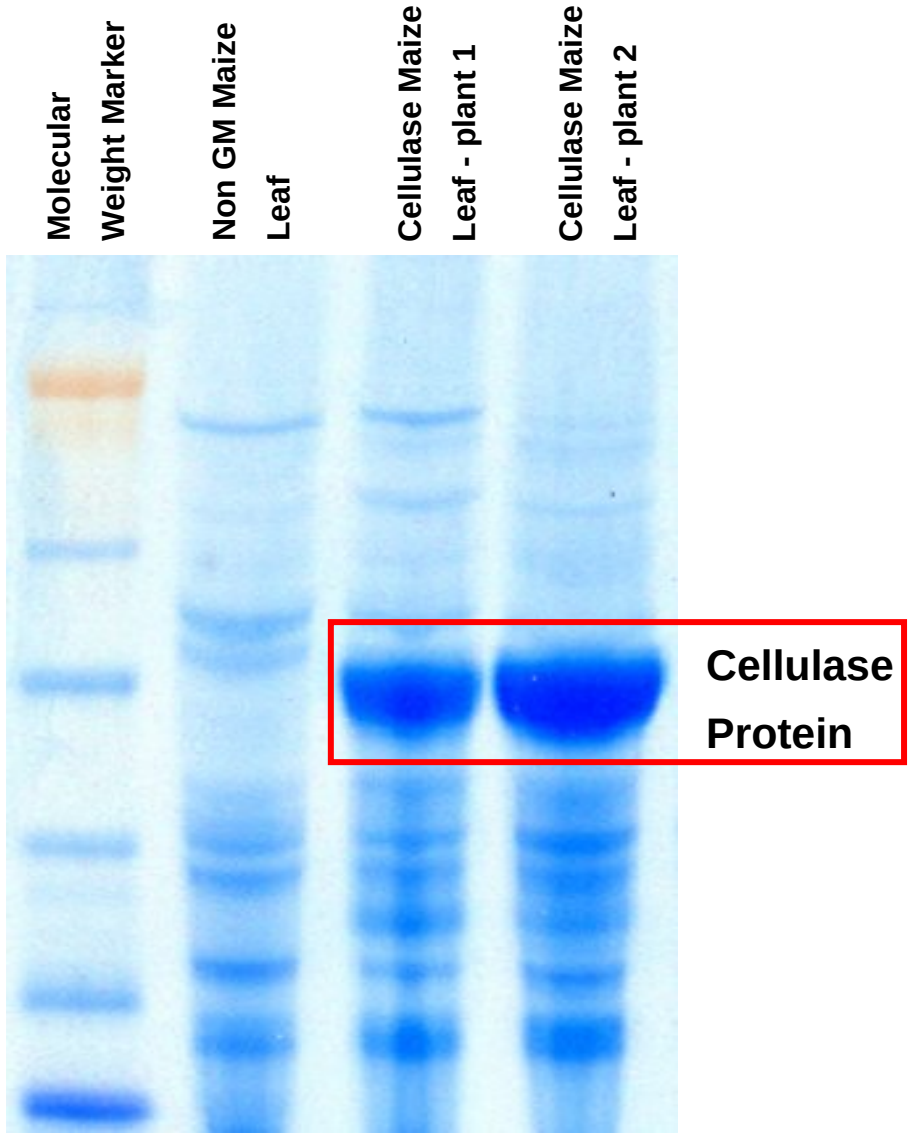
Accumulation of active CBH1 cellulase in grain is dependent on sub cellular targeting



Each bar represents an independent event

High Level Cellulase Expression in Corn Leaf

- Protein Gel analysis of GM corn leaf
- Picture shows relative abundance of cellulase protein
- Plants look perfectly normal and set seed



Cellulase from collaboration with VERENIUM THE NATURE OF ENERGY

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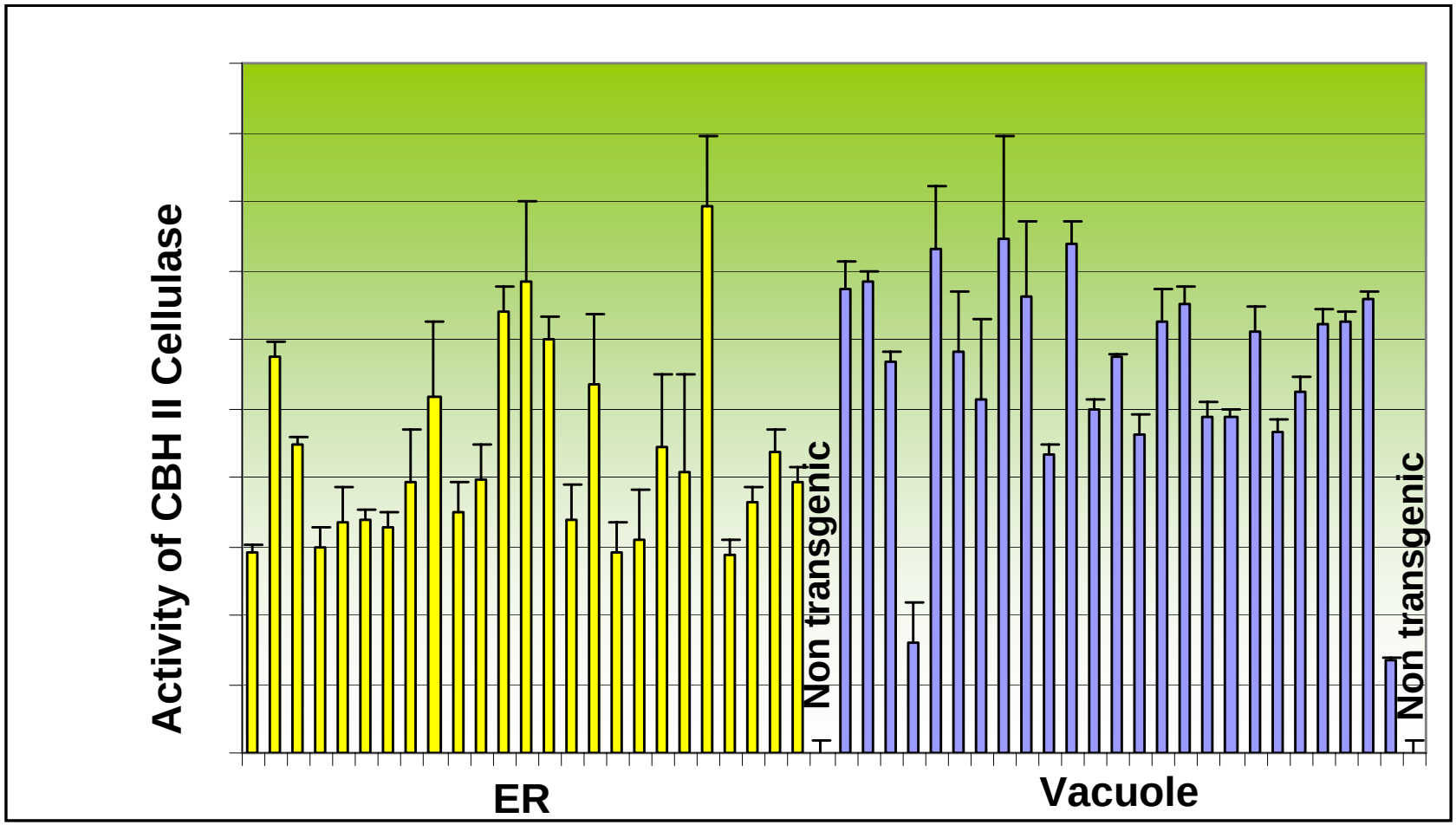
CBH II cellulase expression in maize leaf



CBHII from collaboration with VERENIUM THE NATURE OF ENERGY™

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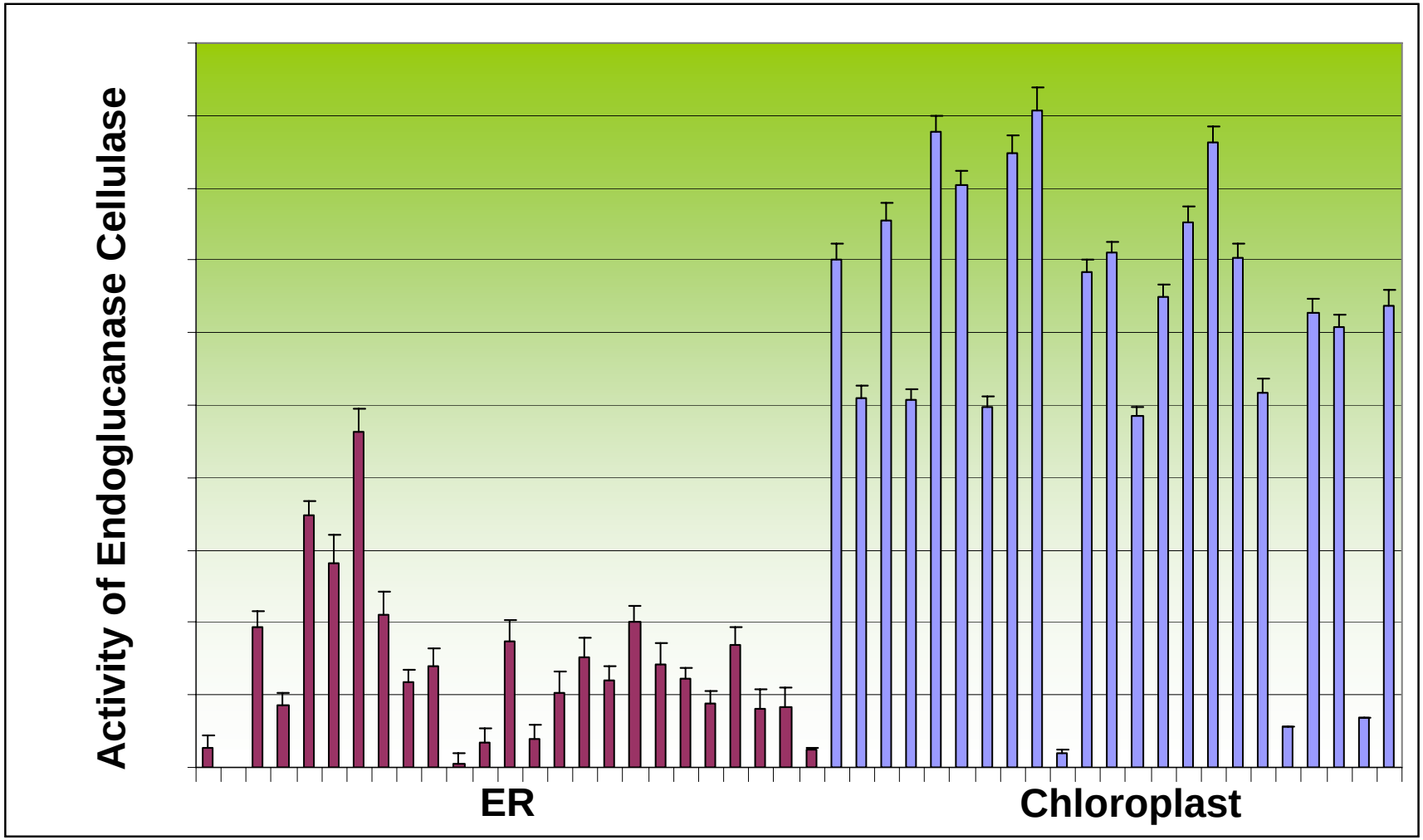
Endoglucanase cellulase activity in maize leaf



EG from collaboration with VERENIUM THE NATURE OF ENERGY

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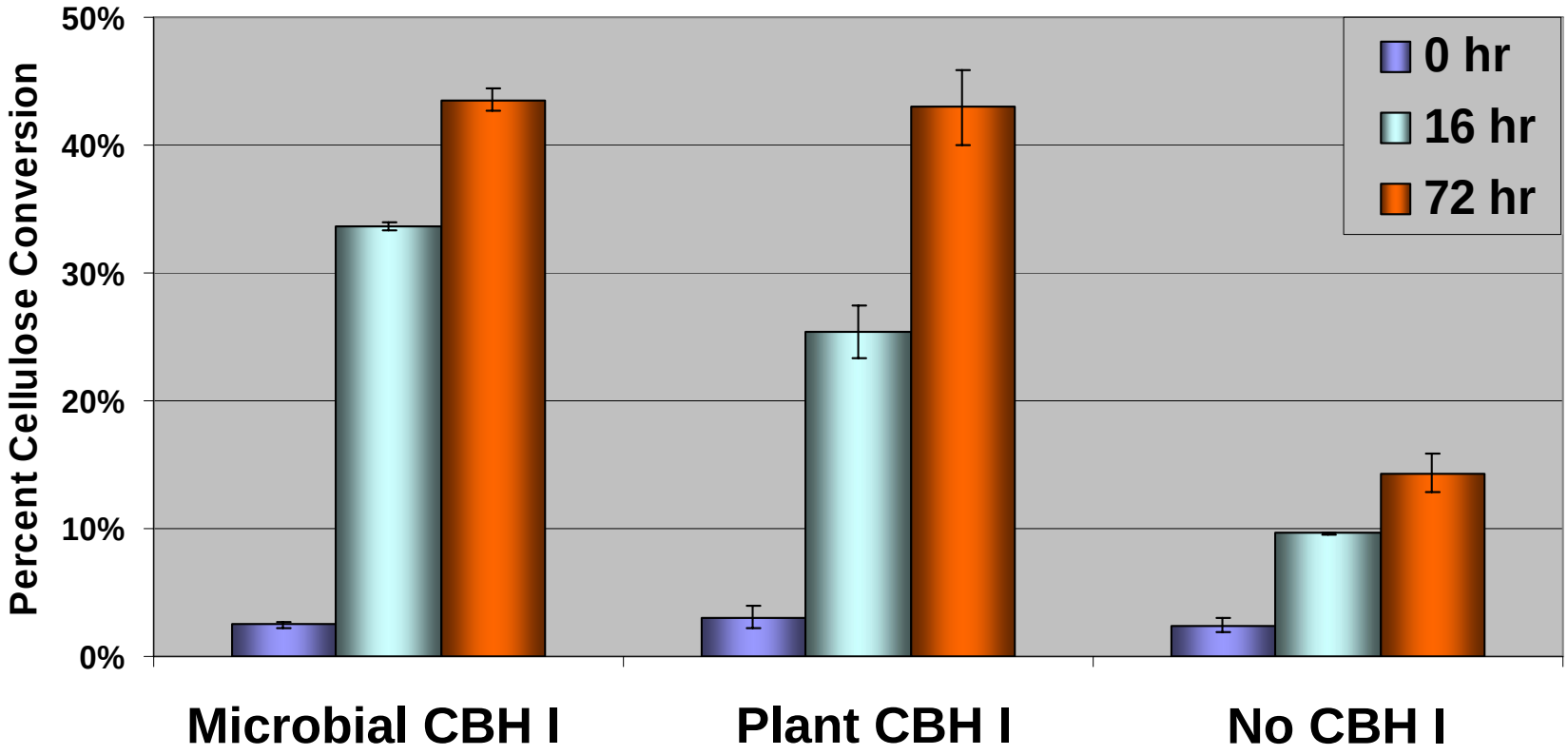
Plant produced cellulases perform like microbial cellulases in cocktails



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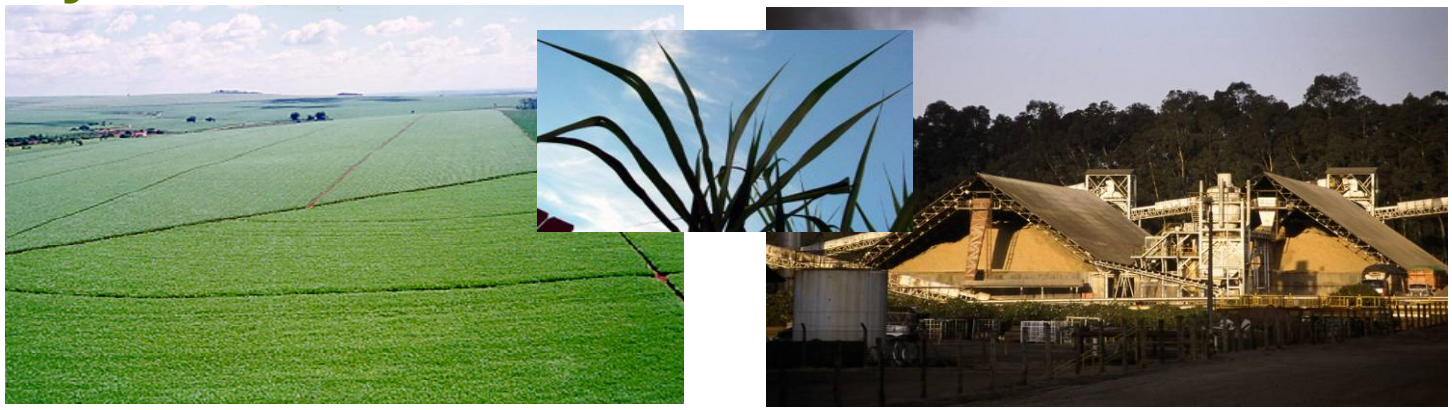
How will the world deliver enzyme cocktails?

Concept	Feedstock	Plant Additive	Microbial
			
Enzyme cost today $/gal EtOH	$0.05 - $ 0.25	$1 -10	$0.75 -$1.0
Enzyme cost at target $/gal EtOH	Approx $0	$0.01 - $0.1	Approx $0.13
Capital need	No	Existing processor	Fermentation
Running Costs	Zero	Low	High
25+ M/Tonne/yr Enzyme capacity	Yes	Not certain	Capex Cost $

Target assumes DOE enzyme cost reductions are met

Summary



Syngenta is uniquely positioned: why?

- Innovating in plant expressed enzymes
  - Reducing the cost of cellulosic biomass conversion
  - US Patent no. 7,361,806 issued (April 22, 2008), Plant expressed endo-cellulases
- Tailoring Energy crops to meet the world future needs
- Proven record of taking GM crops to market

Thank You:

Science

Stacy Miles

Sergio Arellano

Bruce Link

Jason Nichols

Paul Oeller

Myoung Kim

Chris Batie

Business

Mark Bidwell

Tim Kroenke

Rod Coleman

Jeff Miano

Karen Bruce

Ian Jepson

Terry Stone

Tom Bregger

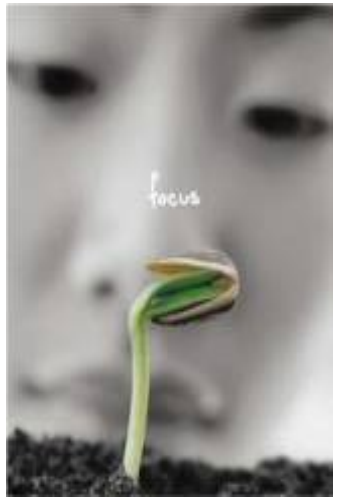
Martha Dunn

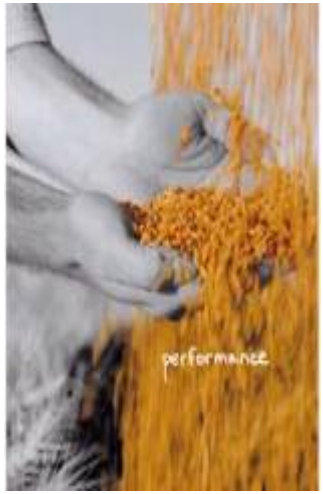
Dan Tangeman

John Steffens

David Patton

Neal Briggi





Thank you

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