Development of Improved Enzymes for the Bio-refinery

esEGP5
Norwich, UK
April 2, 2008

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Genencor, a Danisco Division
Palo Alto, California, USA

Agenda

- Enzyme Technology for Fuel Ethanol from Starch
- Transition to Cellulosic Ethanol
- The Challenges of Cellulosic Ethanol and Genencor's Role
- Integrating Technology
- Conclusions

Genencor Division at a Glance

GENENCOR®
A Danisco Division

Develops, produces & sells enzymes for industrial applications

- Founded 1982
- May 2005: Danisco-Genencor Division
- 9 Bioproducts manufacturing sites (Europe, Americas, China)
- 4 R&D sites (Europe, USA, China)
- Approximately 1300 Employees Worldwide
- Proven innovator ~4000+ owned and licensed patents and patent applications
- Making cellulases since 1985
- First recipient of 2000 US Department of Energy subcontract for cellulase cost reduction
- Winning proposal for 2008 US DOE subcontract for cellulase cost reduction

The BioRefinery

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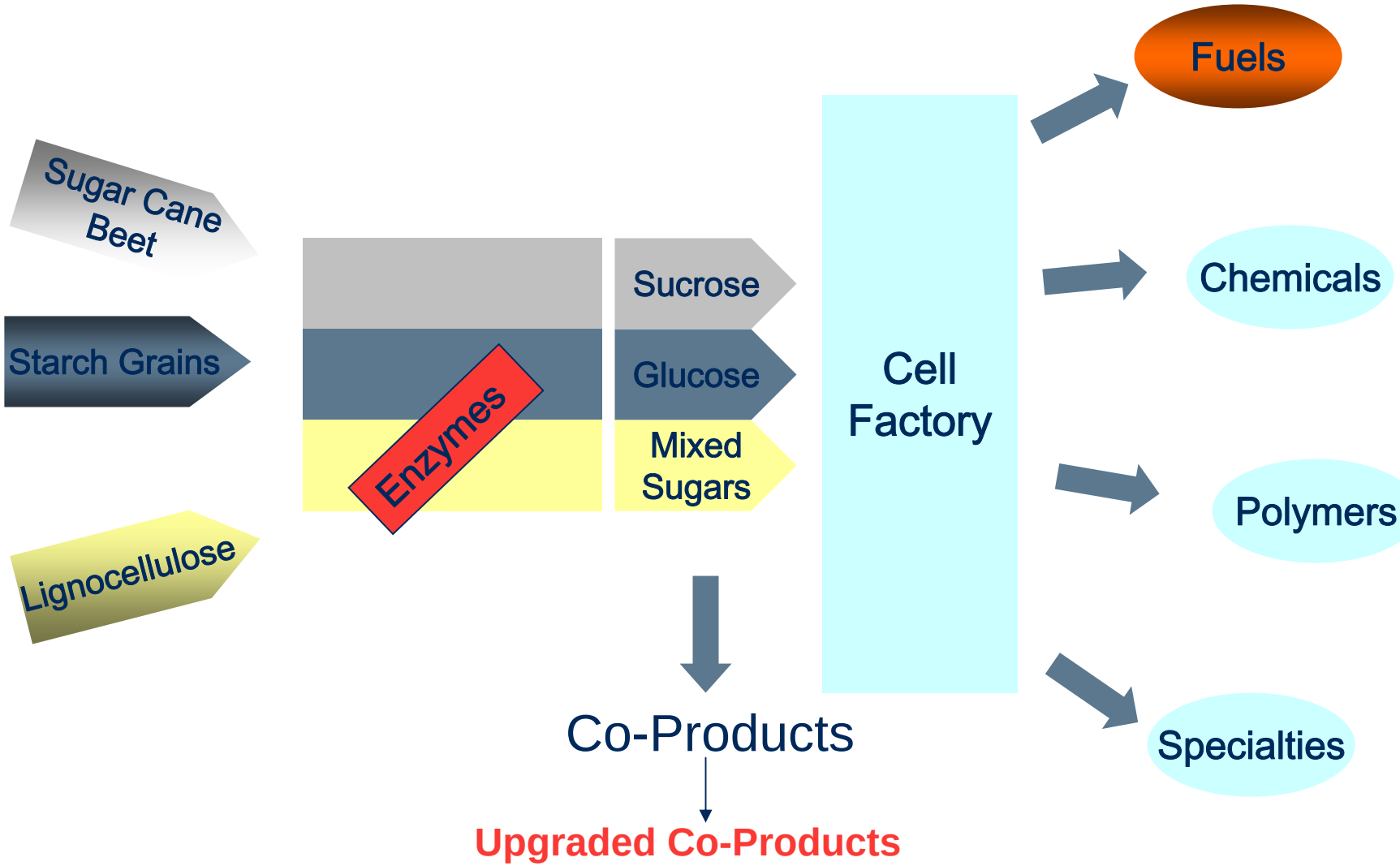

Corn-to-Ethanol Biorefinery US 2007

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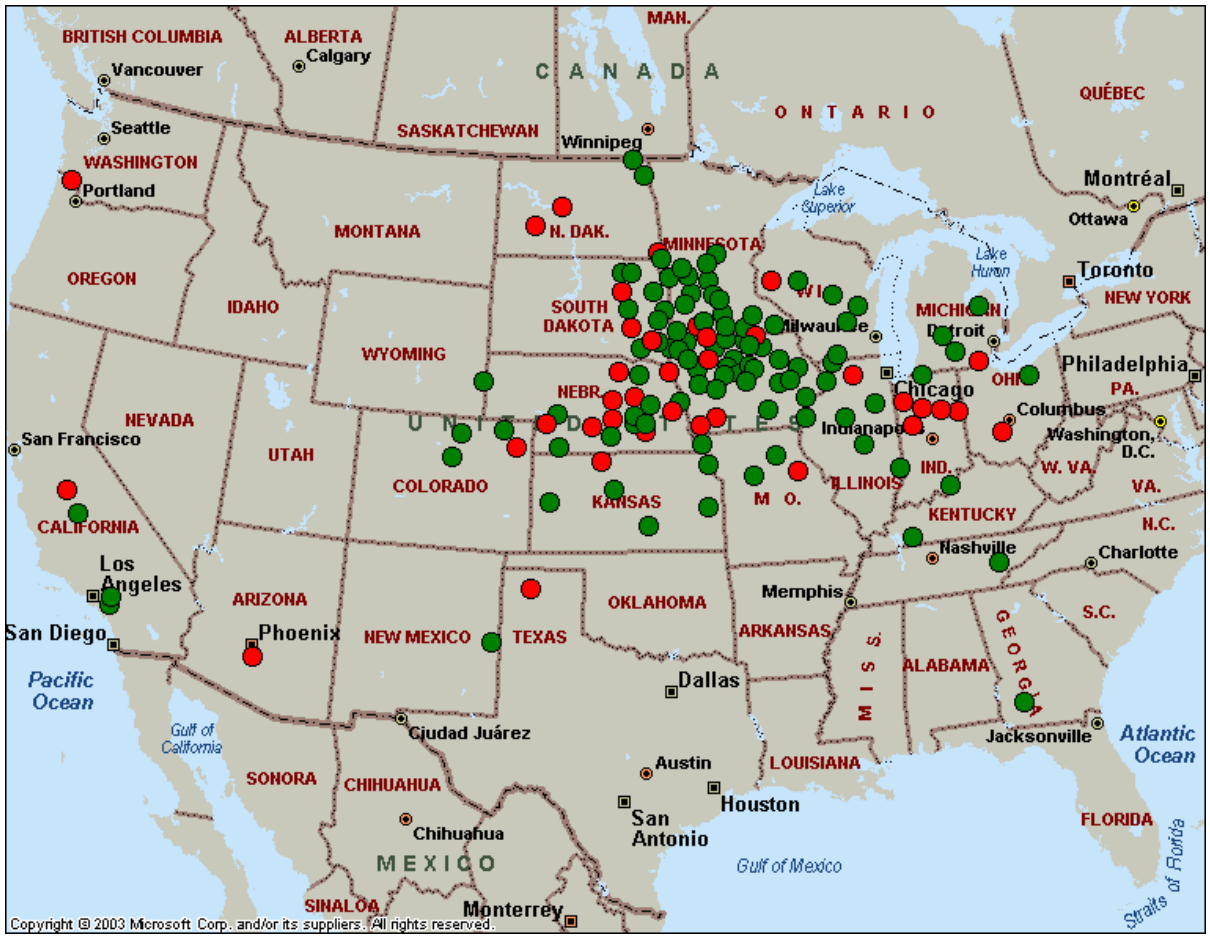

Source: John Urbanchuk and Renewable Fuels Association

● = operating, ● = under construction

136 Operating Plants with 6.9 Billion Gallon Capacity

13 Plants in expansion with 1.0 Billion Gallon Capacity

88 Plants under Construction will Provide another 6.6 Billion Gallons

Total Capacity When Completed = 14.5 Bil. Gal.

Hundreds of other plants in Planning Stage!

Conventional Ethanol Production from Starch

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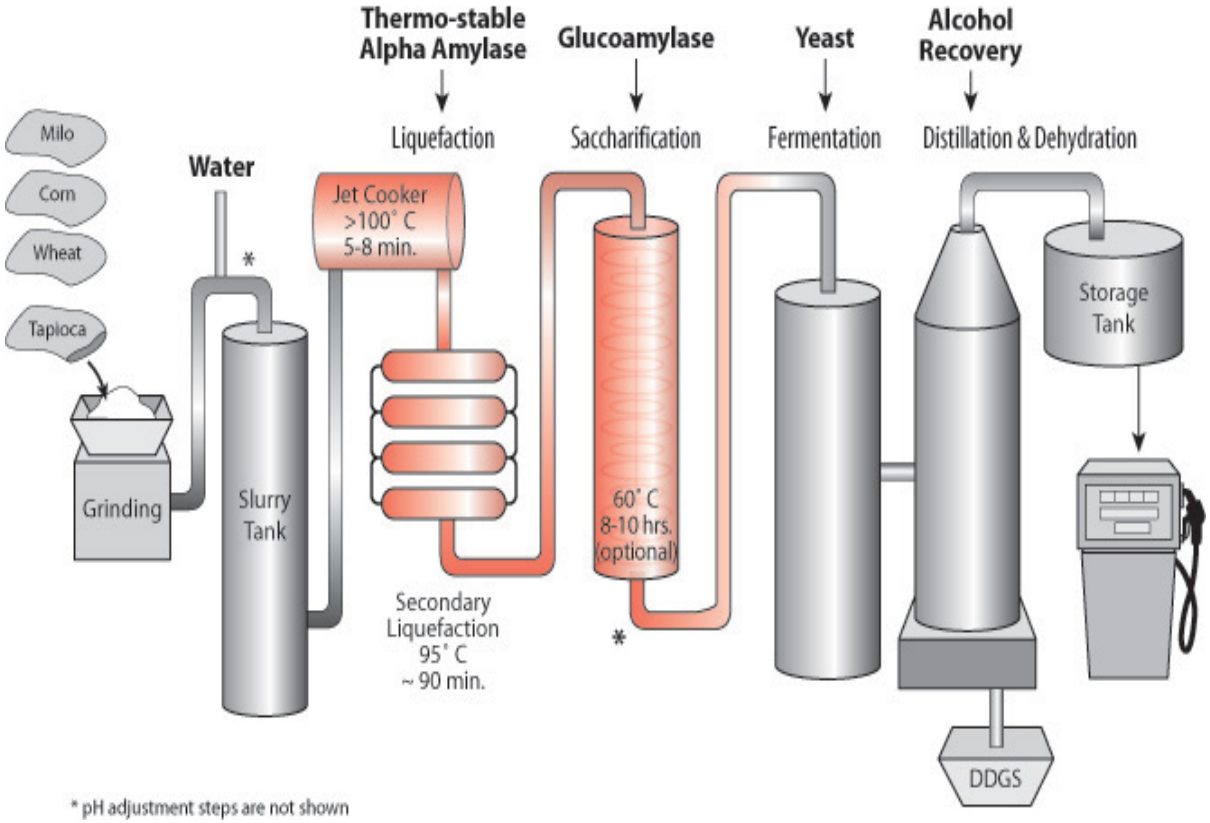

Need: Better Enzymes and Processes for the Grain Processing Industry

Granular Starch Hydrolyzing Enzyme (GSHE): Glucoamylase and Alpha Amylase

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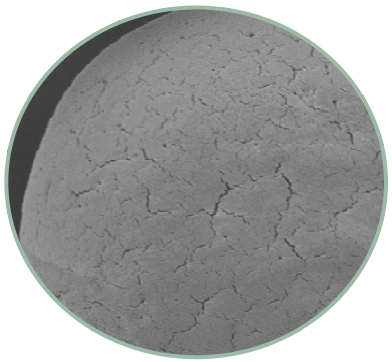
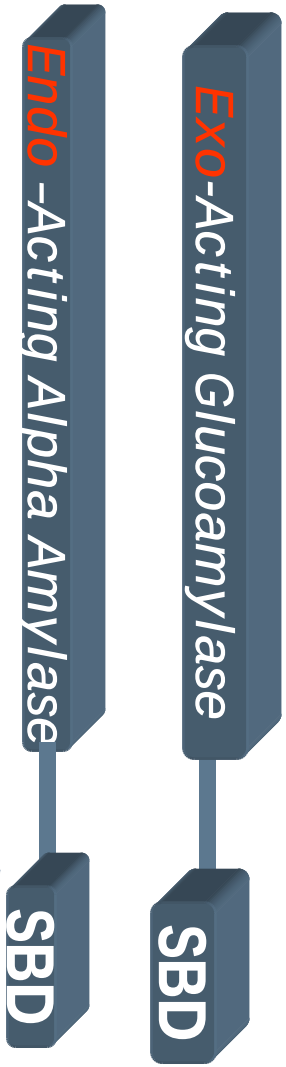
STARGEN 001 product

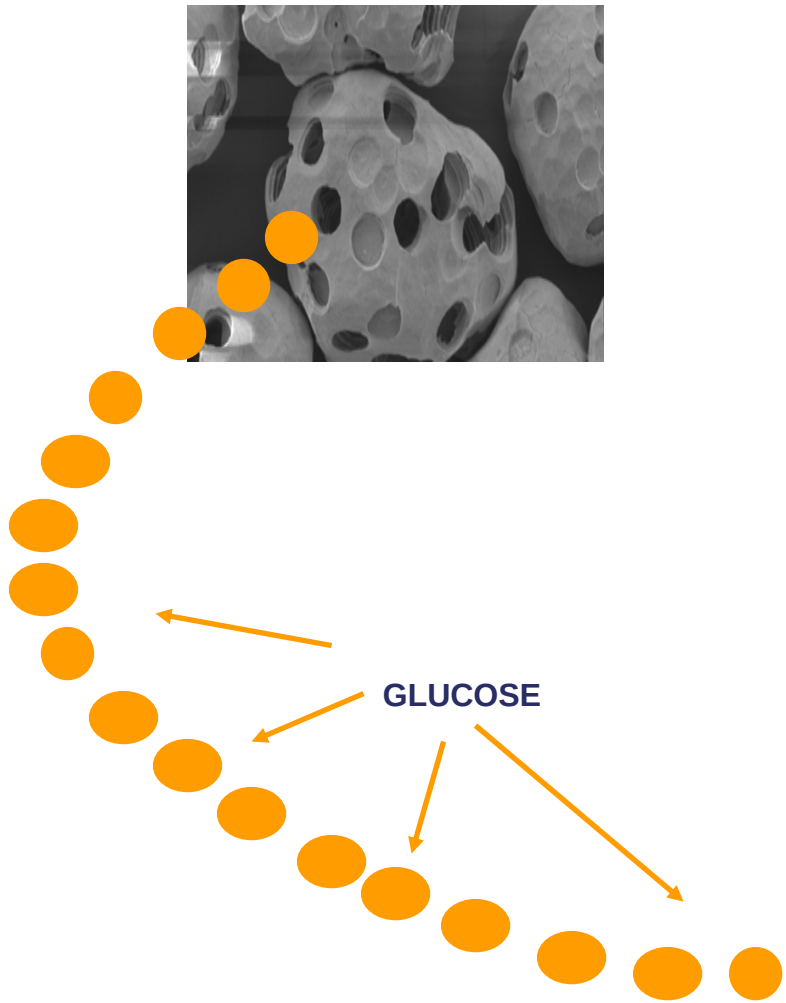
Granular Starch



Aspergillus kawachi **alpha-amylase**
Expressed in *Trichoderma reesei*

Glucoamylase from *Aspergillus niger*





GSHE: Low Energy Starch-Ethanol Process

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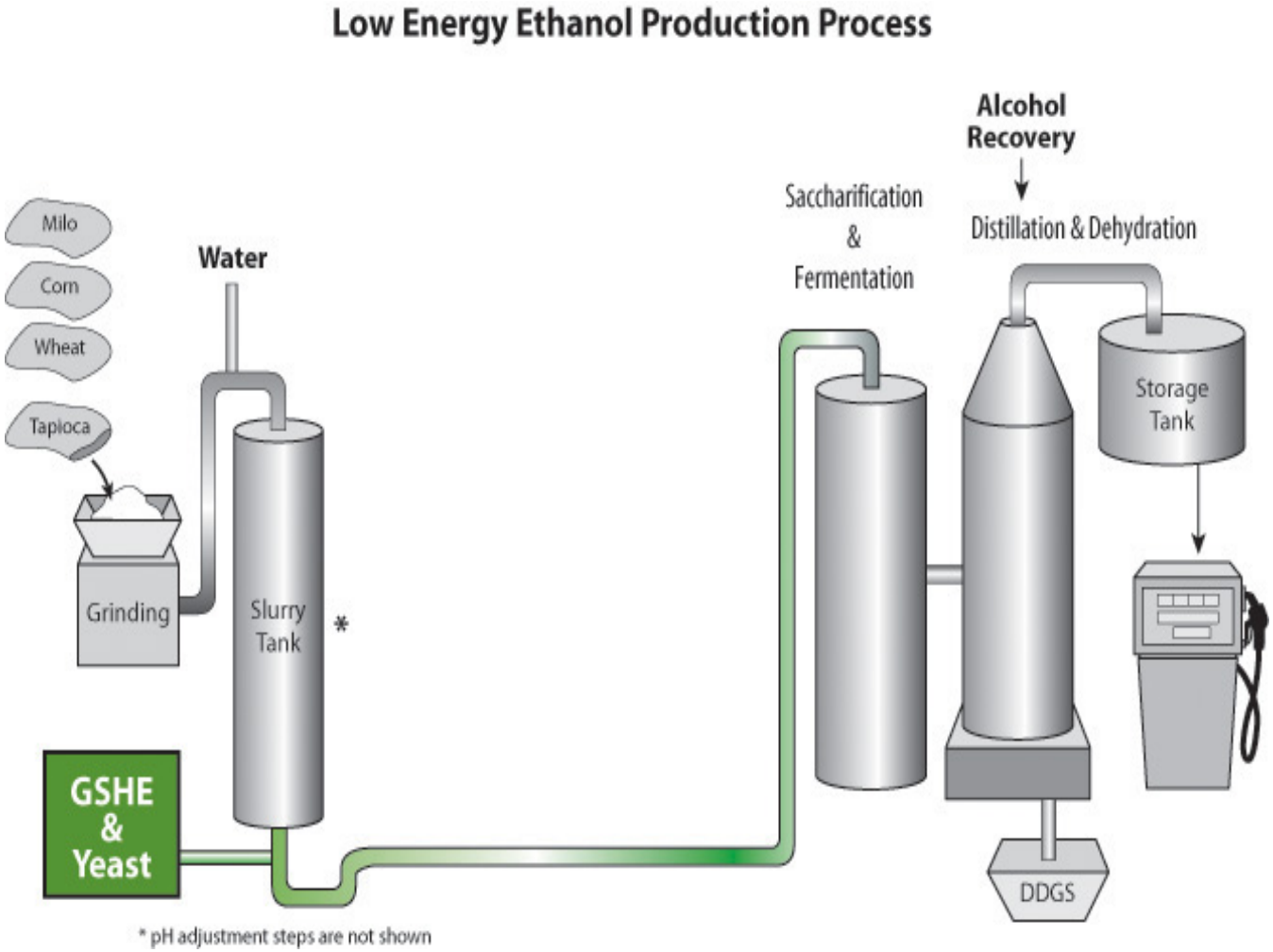
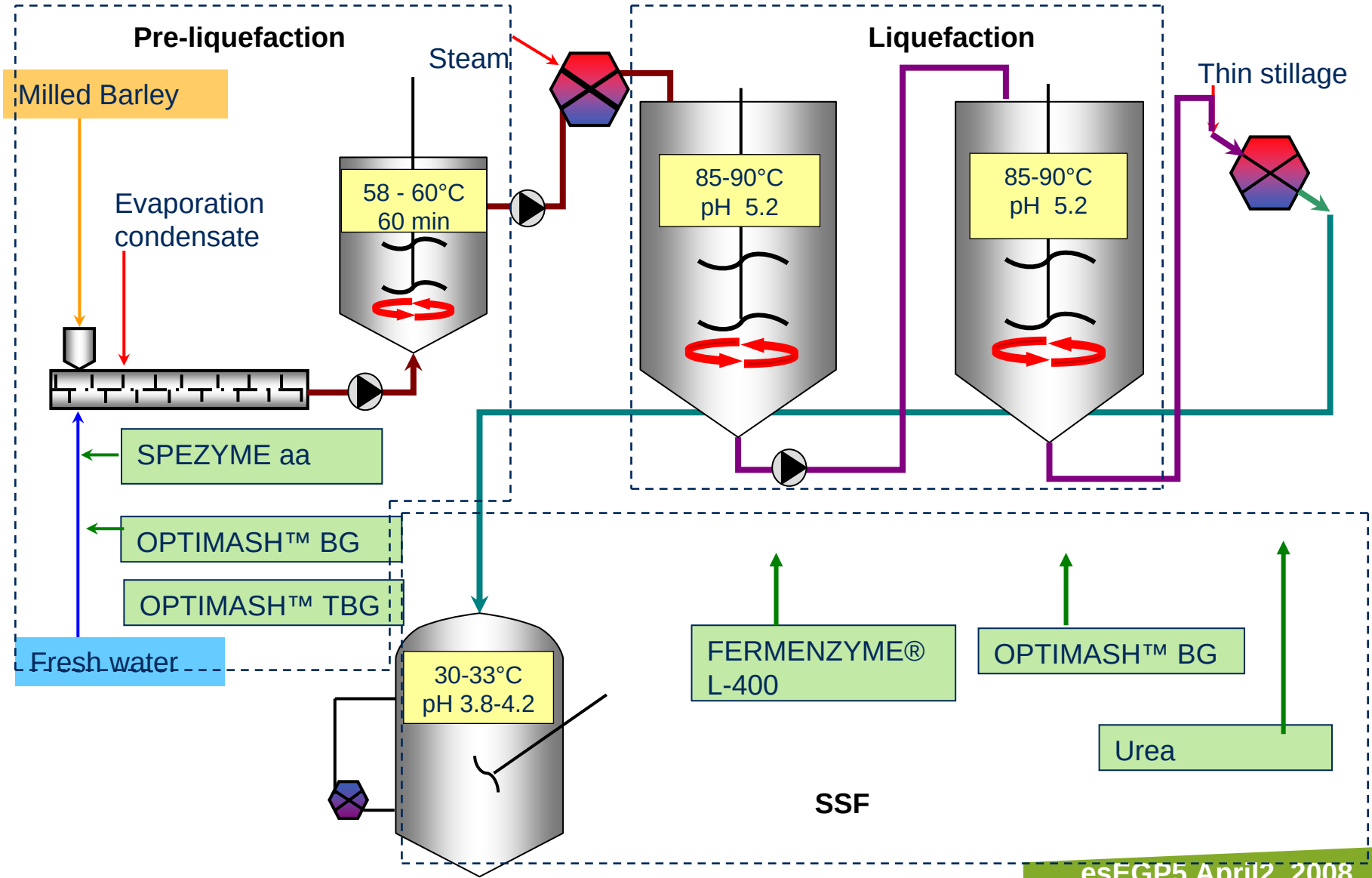


Barley Process: Conventional



Barley Biorefinery: STARGEN™ Process

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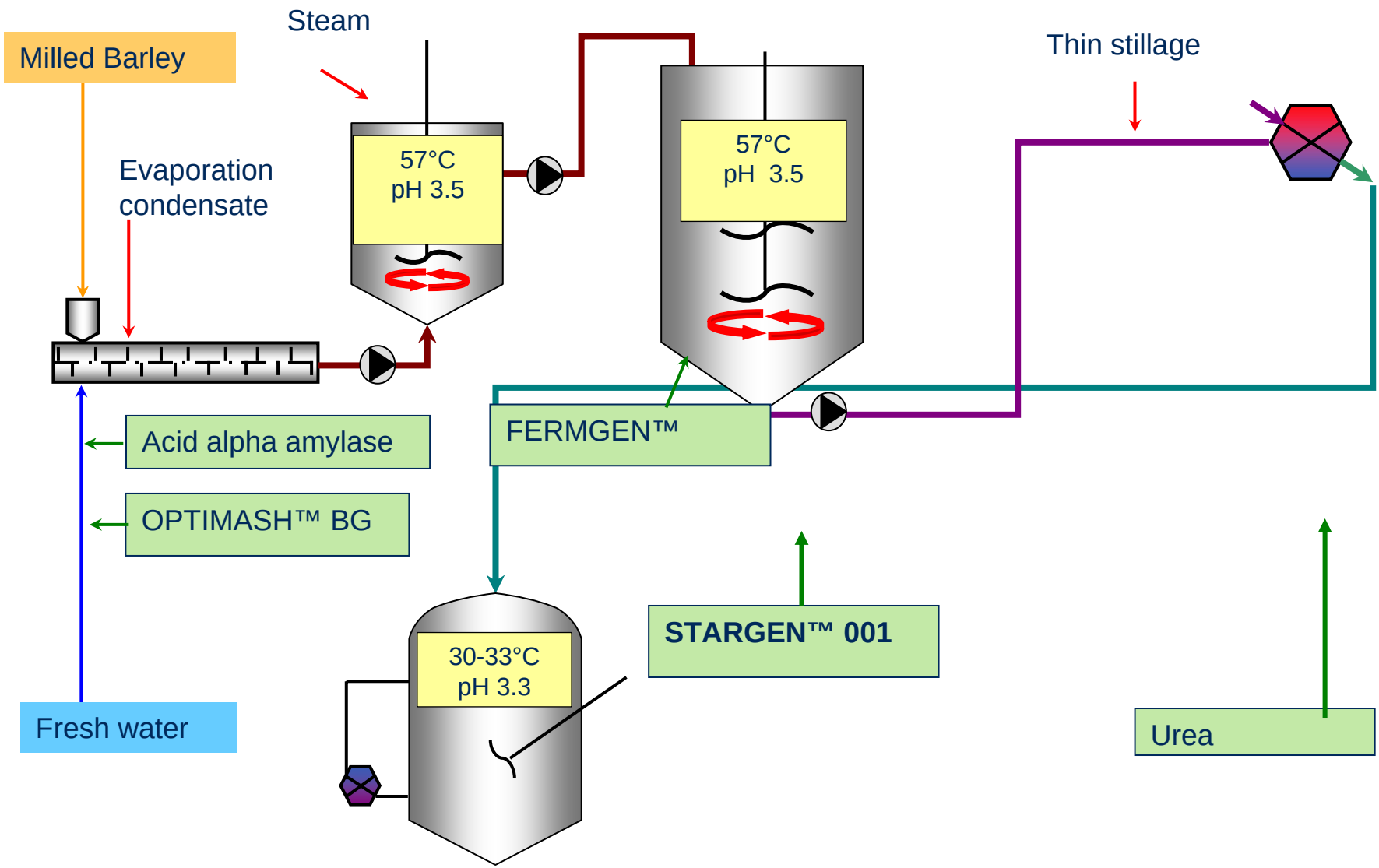

Comparison of Barley Ethanol Processes

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	Conventional	STARGEN™
Ethanol Yield (Gal/bu)*	2.36	2.60

*** Thoroughbred hulled barley with 15% moisture and 53 lb/bu**

Genencor Enzymes for Fuel Ethanol Production from Starch

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Enzyme	Purpose	Product name
Liquefying enzymes	Thermostable alpha-amylases for liquefaction of starch at high temperatures	**SPEZYME® XTRA** **SPEZYME® FRED** **MAXALIQ® ONE**
Saccharifying enzymes	Glucoamylases and blends for hydrolysis of starch to glucose	**G-ZYME ® 480** **FERMENZYME ® L-400**
Protein hydrolyzing enzymes	Proteases for improved fermentation efficiency	**FERMGEN ™**
Viscosity reduction enzymes	Multi-component enzymes for wheat, barley & rye	**OPTIMASH ™ (EUR) (wheat, rye and barley)**
Granular starch hydrolyzing enzymes	Alpha-amylase and glucoamylase blend for processing of uncooked starch	**STARGEN ™ 001 (US)** **STARGEN ™ 002 (EUR)**

Biofuel Opportunity in US Gasoline Replacement

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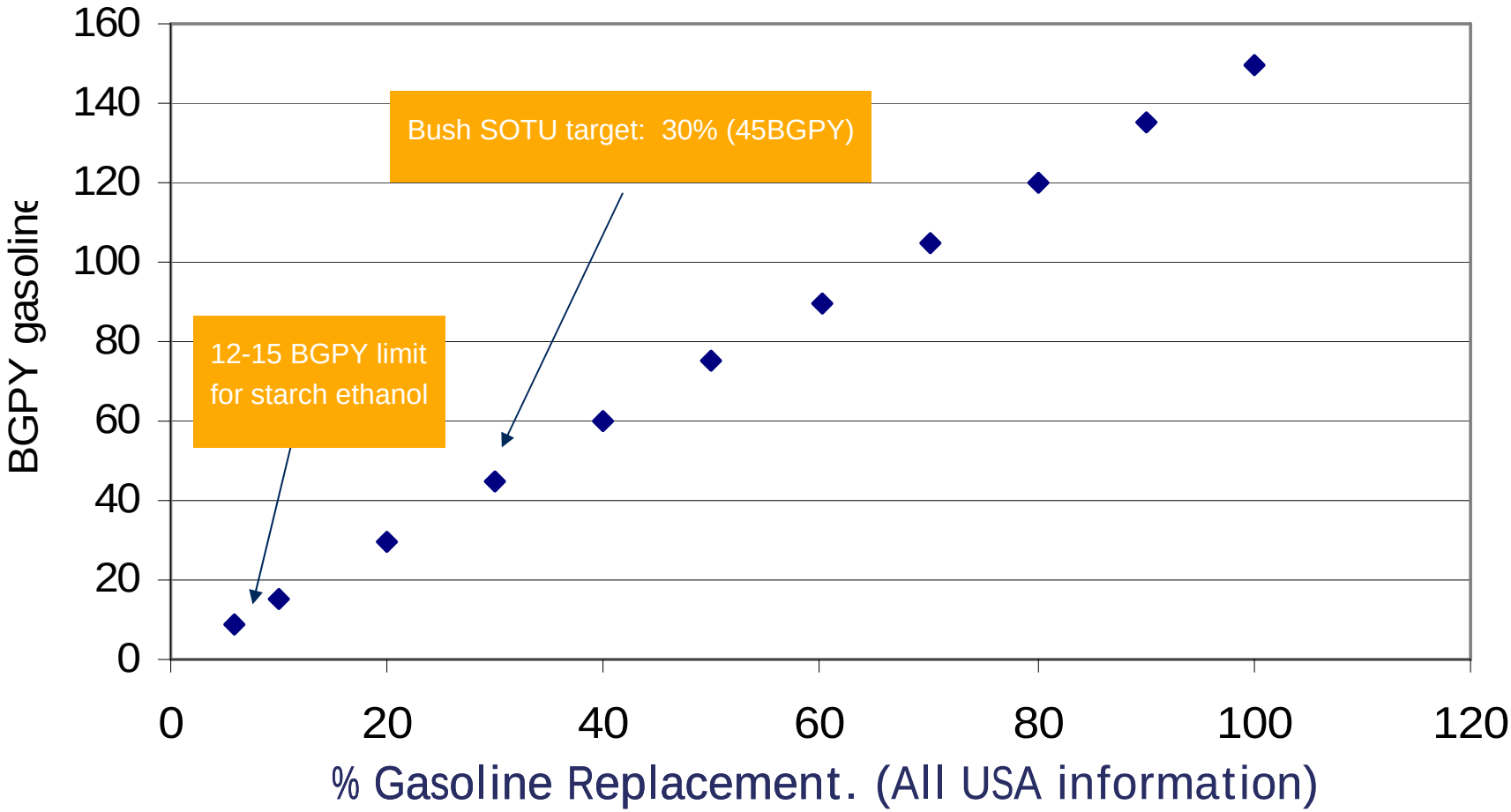

US Sources of Cellulosic Biomass

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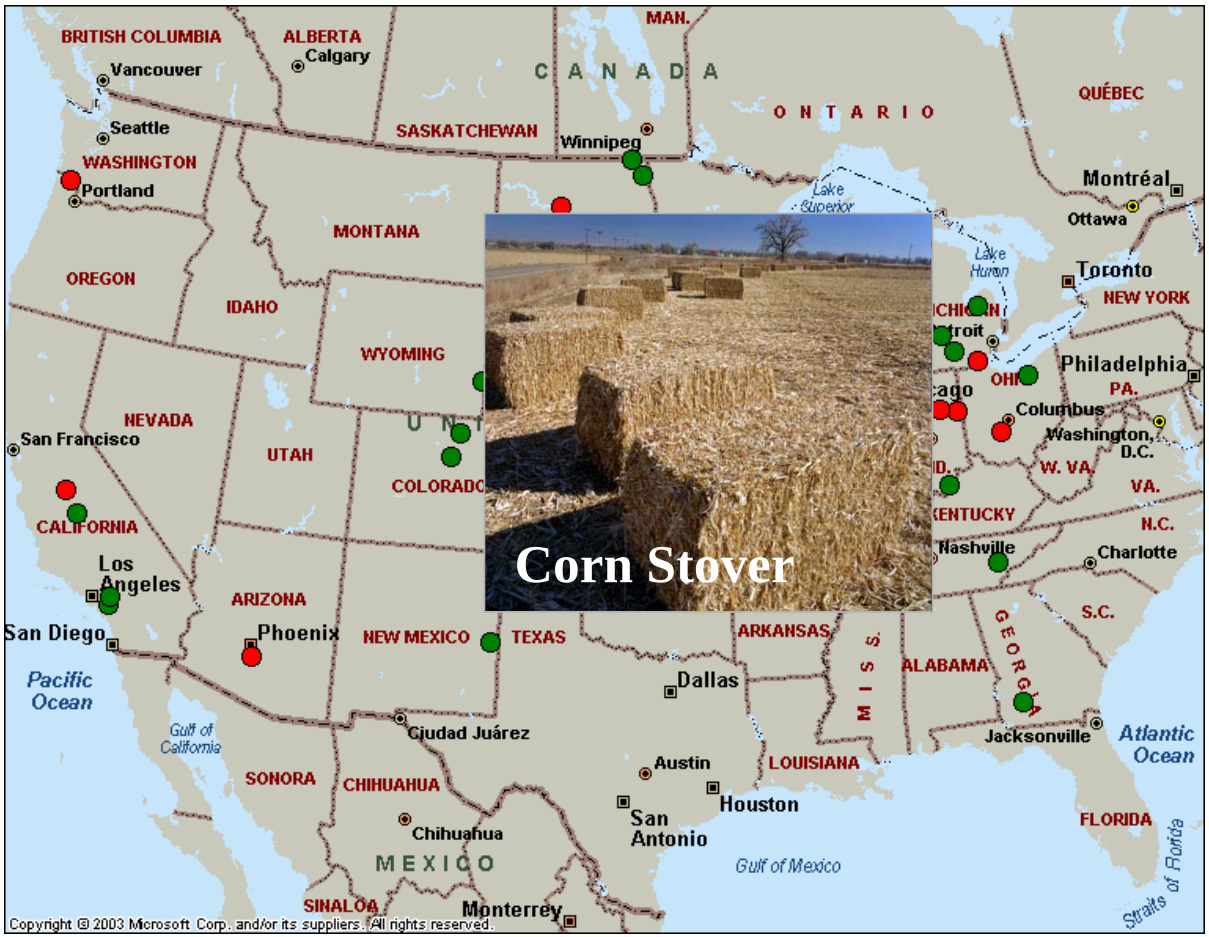

US Sources of Cellulosic Biomass

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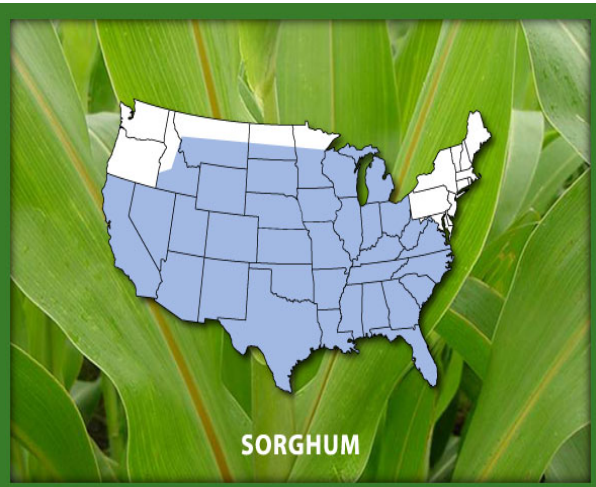

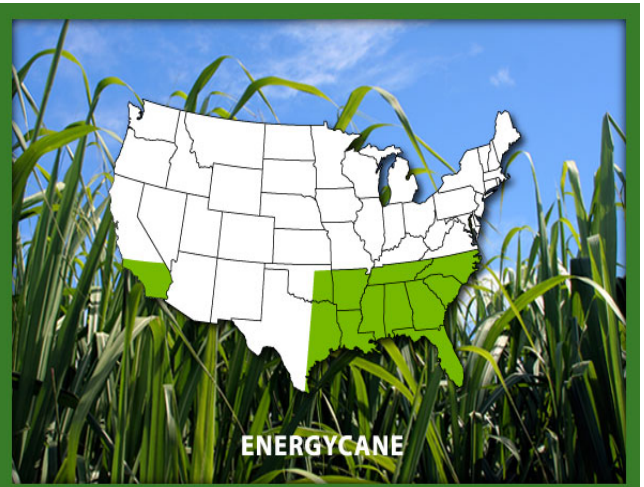

Source: http://www.ceres.net

Cellulosic ethanol : Enzyme challenges

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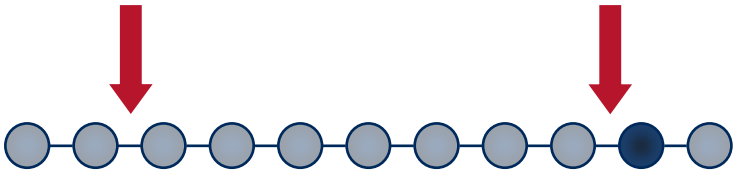
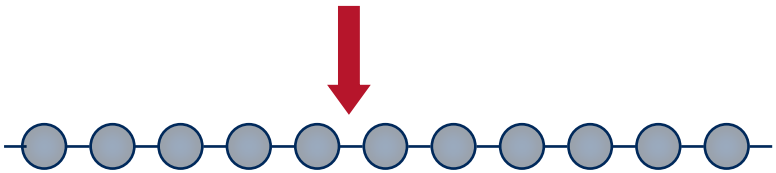
- Biomass much more recalcitrant to hydrolysis
- Complex, synergistic mix of cellulases & hemicellulases needed
- End product inhibition, enzyme stability, non-productive binding…..
- Enzyme dosing is much higher than for starch

Cellulose: Hydrolyzing the β-1,4 linkage

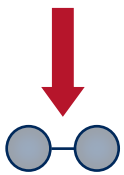
Cellobiohydrolase



Endoglucanase



β-Glucosidase



- Cellulose digestion requires at least 3 types of activities working synergistically:
  - Exo-1,4-β-glucanase (cellobiohydrolase): processive, attacks reducing (CBH I) or non-reducing (CBH II) end of cellulose chain, releasing cellobiose units.
  - Endo-1,4-β-glucanase: non-processive, attacks along chain; reduce dp and viscosity, creating more chain ends for the exo's.
  - Beta-glucosidase (β-glu): converts soluble oligosaccharides to glucose, relieving product inhibition.

Plus hemi-cellulases etc etc.

Advanced Cellulase Development Programs

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Project Structure and Funding

- U.S. Department of Energy (DOE) funded >$15 million over a 4-year period beginning in June 2000
- Sub-contract through the National Renewable Energy Laboratory (NREL)
- Genencor contributed ~$5 million

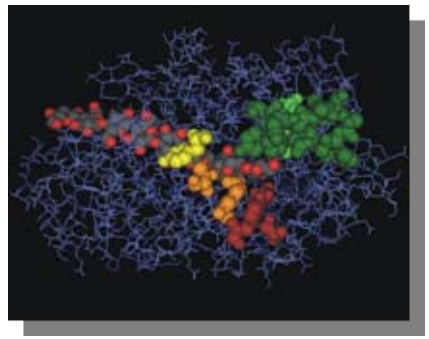
Goal: Improved Low Cost Cellulase for Biomass Conversion to Ethanol

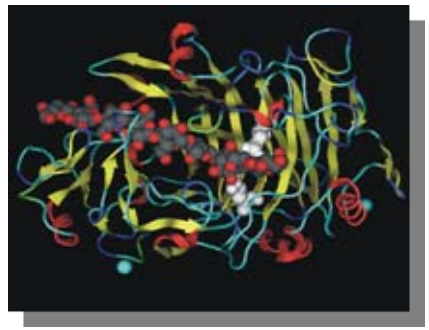
- Original program targeted a 10-fold cellulase cost reduction
- Program achieved a 30-fold reduction per gallon on NREL model substrate

Awards

- R&D Magazine, Top 100 Innovations, 2004

2008: Genencor proposal chosen for new DOE Enzyme Improvement subcontract





How Will the Market Emerge?

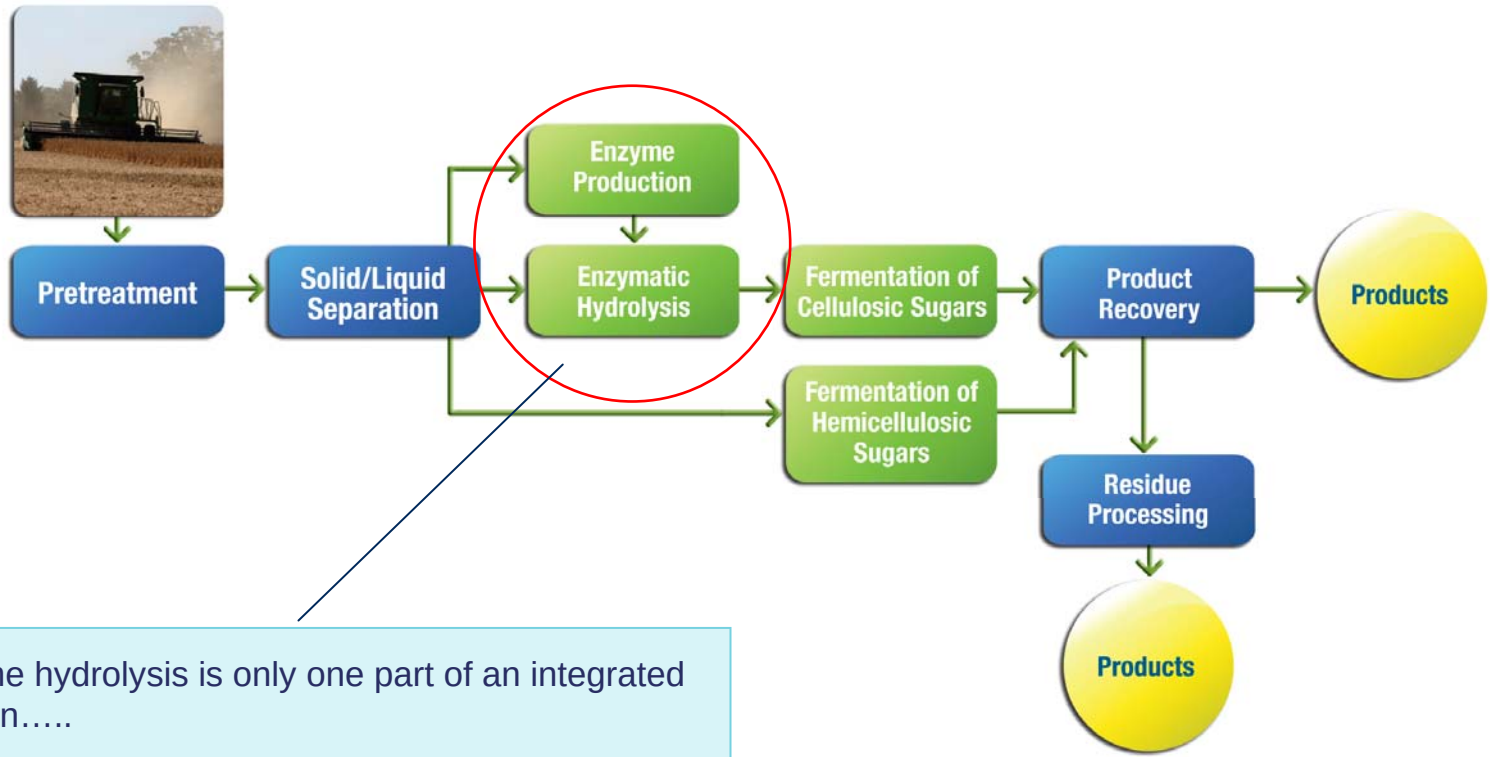
- 2008 to 2010 – pilot plants at 0.5 to ~ 2 MGPY
- 2009 to 2012 – demonstration plants at 2 to 10 MGPY
- 2009 to 2014 – first commercial plants deployed @ 20 to 50 MPGY

2007 Unmet Need:

Process developers & engineers need an enzyme solution that is representative of final, optimal enzyme preparations so they can design their systems.

System Level Solution

Figure 1: Production of ethanol from cellulosic biomass.



Enzyme hydrolysis is only one part of an integrated solution…..

ACCELLERASE™ 1000 at a Glance

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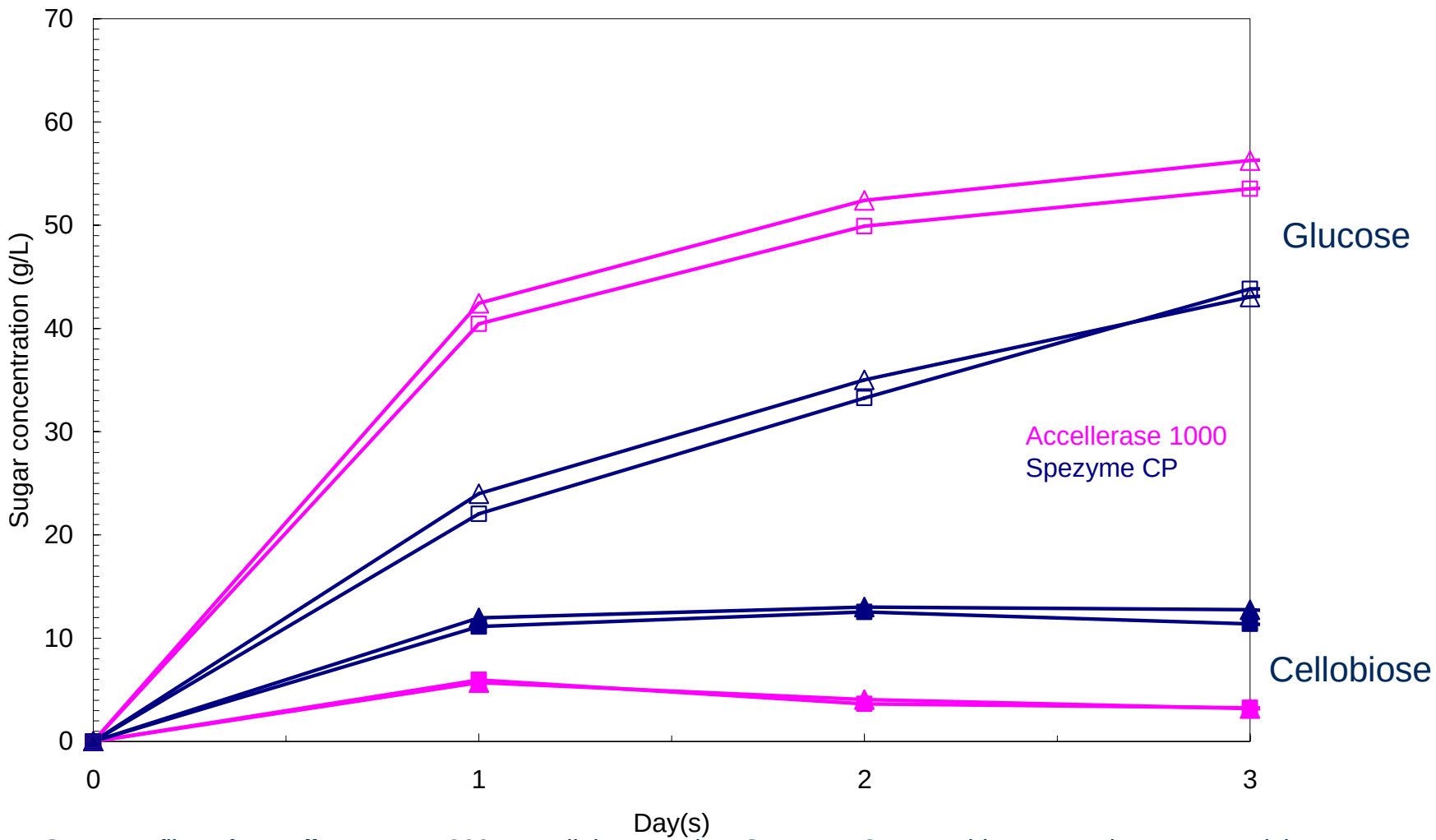
- First True Biomass Targeted Enzyme Launched on October 15, 2007
  - High β-glucosidase activity to minimize residual cellobiose potentially increasing saccharification rate, speeding ethanol fermentation, and improving yield
  - Unclarified product with remaining nutrients from enzyme production available to the yeast in addition to the hydrolysis sugars after saccharification
  - Minimal formulation ensures no chemical interference with saccharification carbohydrate analysis or subsequent yeast fermentation
- Delivers Significantly Enhanced Performance Over Previous Product

accellerase™
FUELING THE BIOBASED ECONOMY

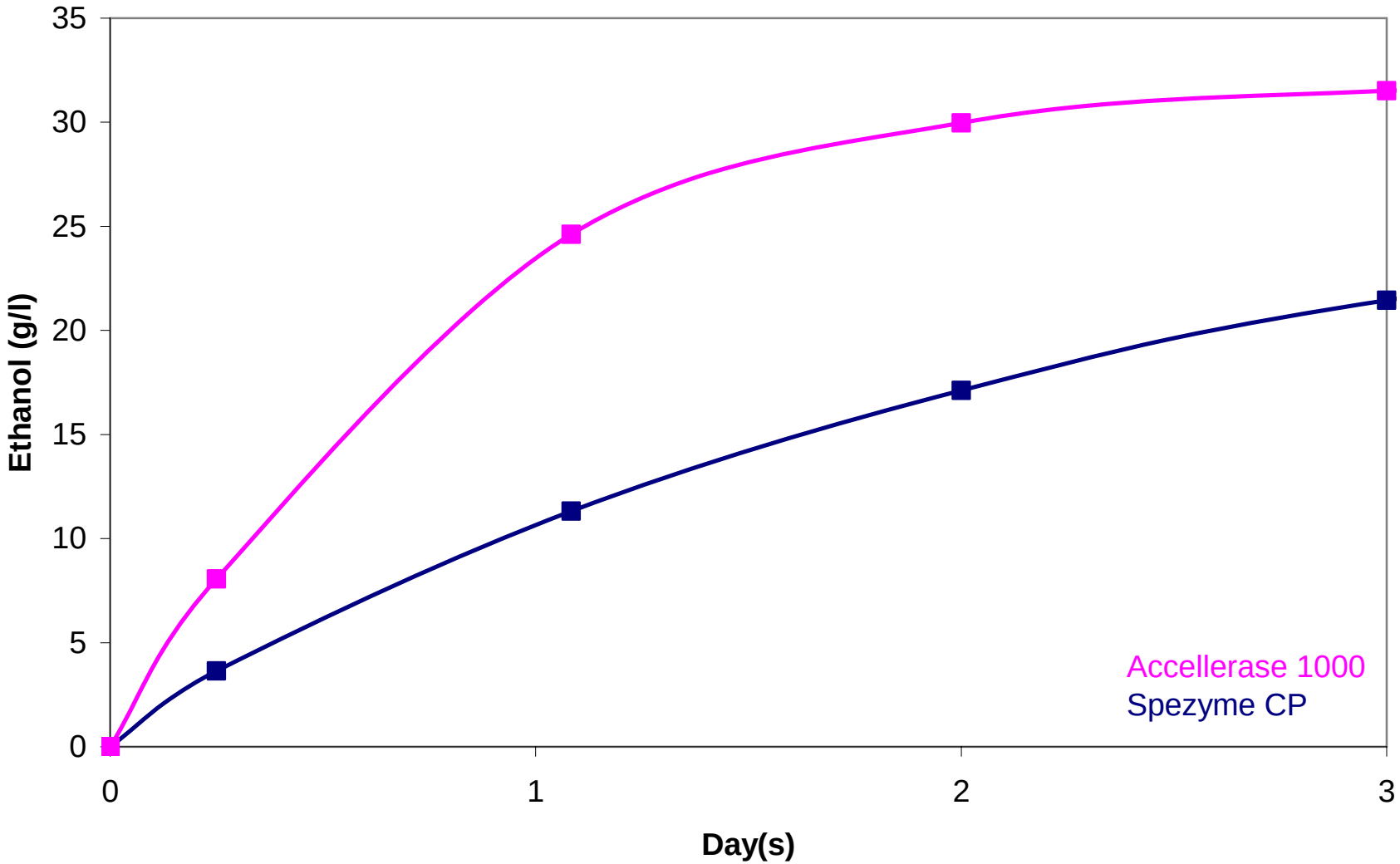
www.genencor.com/cellulosicethanol

Significantly Lower Cellobiose Levels During Hydrolysis



Sugar profiles of **AccelleraseTM 1000** vs cellulase product Spezyme CP on acid pretreated corn stover (Δ) and sugar cane bagasse (□), 7% cellulose loading, 50° C, and pH 5.0.

Enhanced SSF Performance: Ethanol Yield



Ethanol profiles of **AccelleraseTM 1000** vs cellulase product Spezyme CP in SSF on acid-pretreated sugar cane bagasse, 7% cellulose loading, 37˚ C, and pH 5.0.

The BioRefinery

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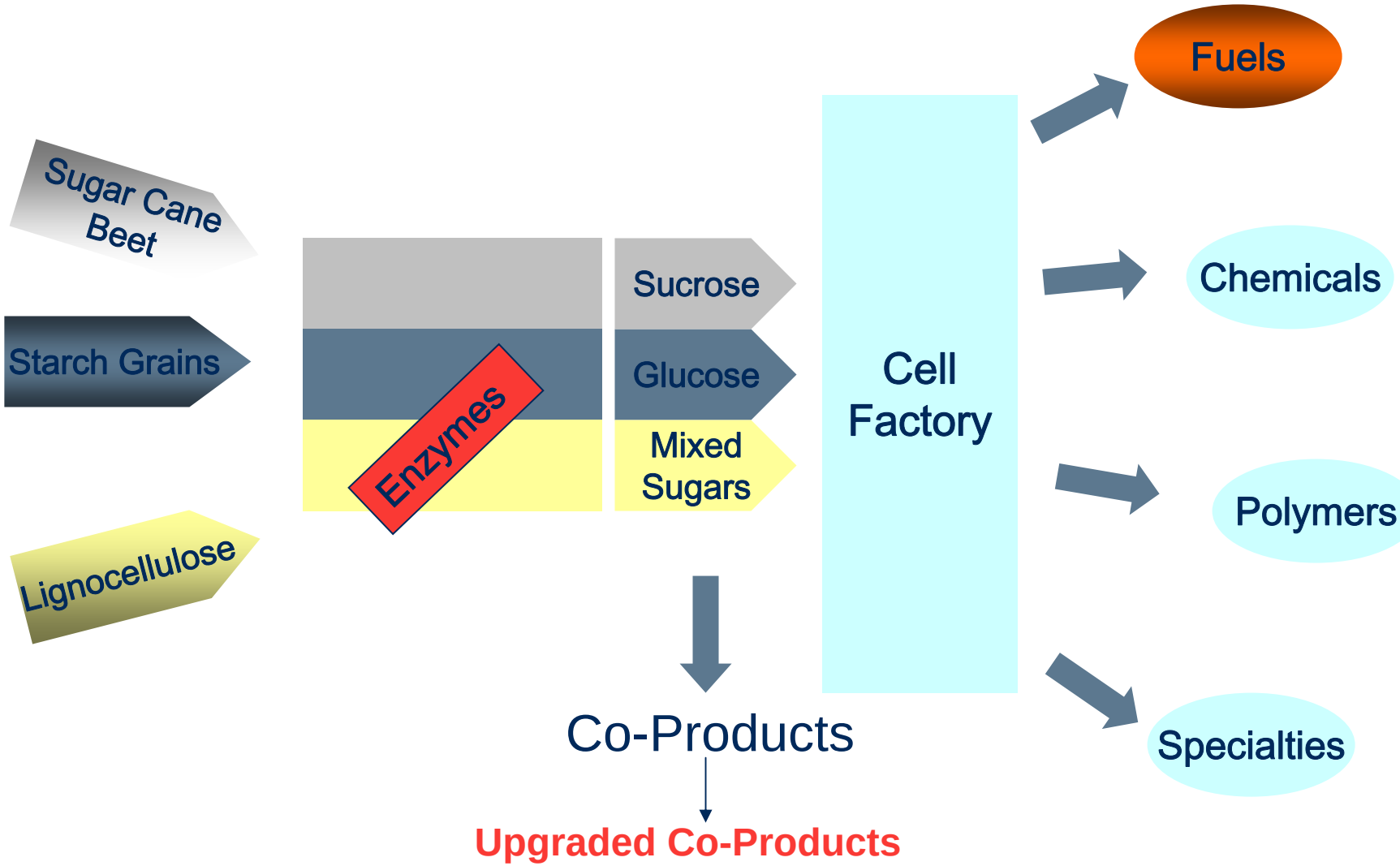

Integrating Starch & Cellulosic Biomass Technologies

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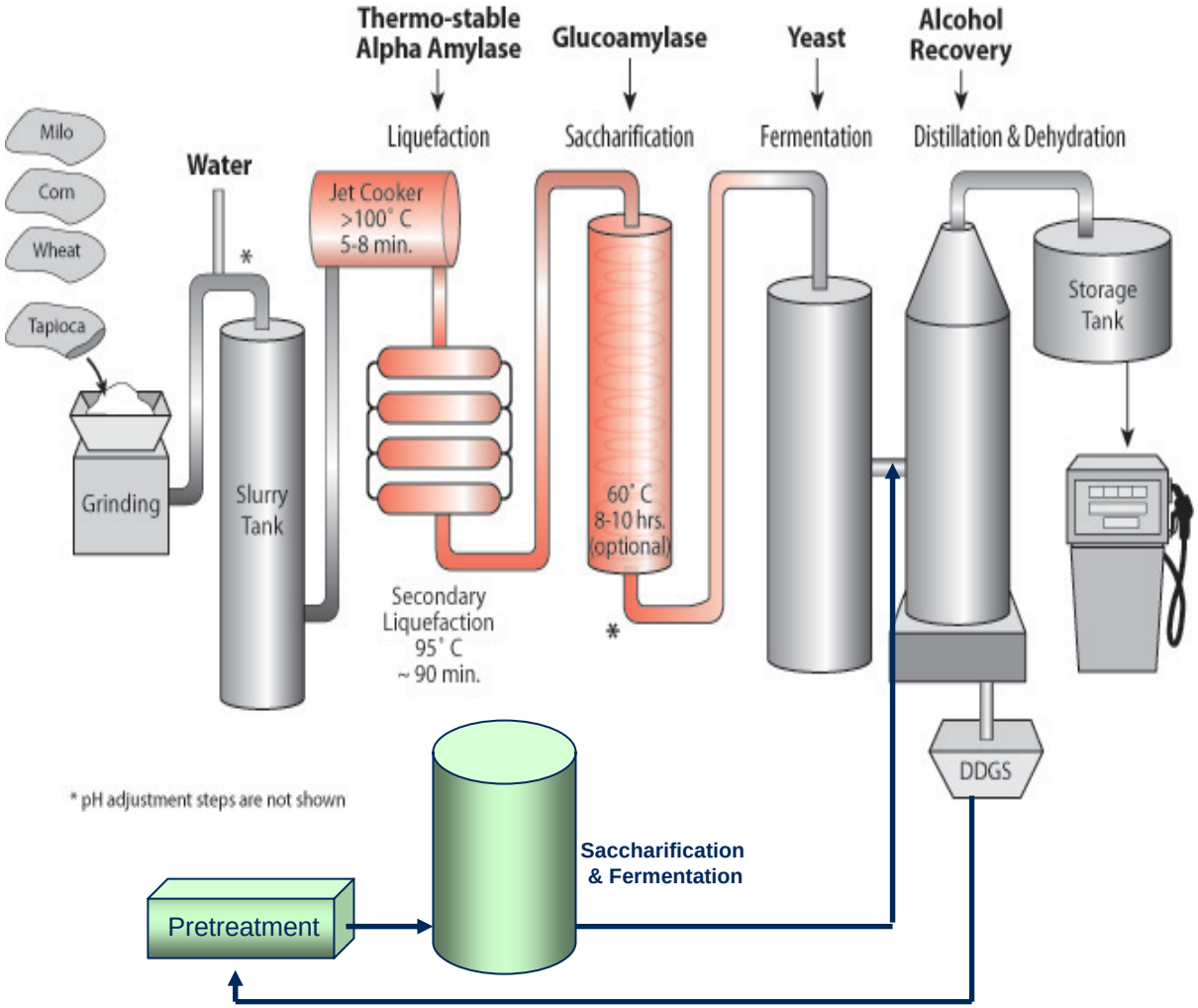
Mike Ladisch: MidWest Consortium / Purdue University



Conclusions

- Starch process is an enzymatic process, and starch processing technology is rapidly evolving.
- Cellulosic biomass processing is much more complicated, best solution will be through integrated processes.
- Cellulosic conversion will enhance starch processing economics, and cellulosic & starch technologies & processes could very well merge in synergistic ways.

Acknowledgements

Genencor

R&D

Biomass Applications Group

Grain Applications Group

Biorefinery Business Development