

Recent breakthroughs in enzymes for biomass hydrolysis



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- ➔ Background on Genencor
- ➔ Accellerase[®] Enzyme Product Line
- ➔ Recent Developments – Accellerase[®] 1500
- ➔ Challenges Ahead
- ➔ Summary

Genencor in brief: a world leader in industrial biotechnology



- Pioneer in biotech - history traced to 1982, when Genentech, Corning Glass Works joined to form Genencor
- Over 250 commercial enzyme products
- ~3500+ owned and licensed patents and applications
- Parent company Danisco - more than USD \$3.5 billion in revenues and 9,700 employees
- Global sales and manufacturing

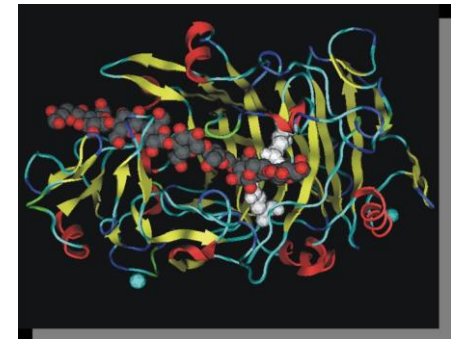


Genencor's longstanding leadership in cellulosic biofuels



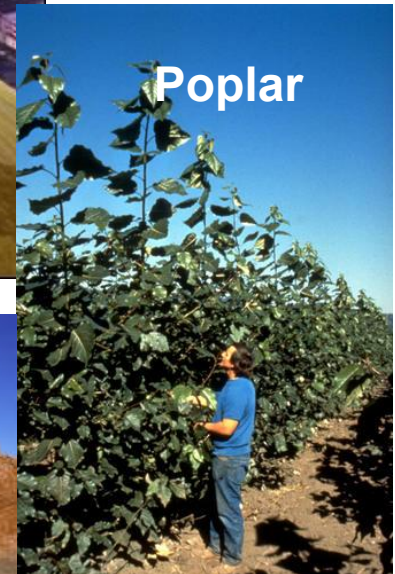
25+ years of investment and R&D in biofuels

- **Pre 2000** – Genencor builds the leading production systems for cellulase enzymes and patents key genes required for economic biomass degradation
- **2004** - Genencor completes 4-year contract with NREL, achieves 30-fold reduction in cost to convert biomass to fermentable sugars
- **2007** - Launched first commercially available enzyme for cellulosic ethanol -Accellerase®
- **February 2008** – DOE awarded Genencor 2nd round of funding for cellulosic ethanol enzyme improvement
- **May 2008** – Genencor and DuPont enter 50/50 joint venture: DuPont Danisco Cellulosic Ethanol LLC (DDCE)
- **February 2009** – Launch Accellerase® 1500



Extensive feedstock / pretreatment experience

- Genencor is engaged in leading academic / industrial consortia and customers pushing the boundaries of developing cellulosic technology
- **CAFI 1 / 2 / 3**
Corn-stover / Poplar / Switchgrass pretreatment comparison by leading academics
AFEX, Dilute Acid, Hot Water, Lime, Sulfur Dioxide, Aqueous Ammonia, etc...
- **Midwest Consortium**
DDGS/Wet Cake, Grain Fiber conversion
- **French Pulp & Paper consortium**
Recycled fibers, softwood and hardwood pulp
- **Wheat Straw, Sugarcane Bagasse and More!**



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Genencor's two-pronged strategy



Genencor has embraced a two-pronged complementary strategy to serve the biorefinery market:

1. Create a global Accellerase® enzyme business to enable cellulosic ethanol and other biochemical production using fermentable sugars from cellulose
2. Provide an integrated solution through our new Joint Venture with Dupont to produce cellulosic ethanol: **DDCE**



Pretreatment



Enzymatic hydrolysis



Fermentation to ethanol





- **Accellerase[®] 1000** was the first commercial enzyme product specifically designed for biomass hydrolysis.
- First generation representative product for process developers & engineers
- This week we announced the next step and significant improvement in the Accellerase[®] Product Line:

Accellerase[®] 1500!



Now we launch Accellerase[®] 1500



- Accellerase[®] 1500 designed to replace Accellerase[®] 1000
- Strengthens Genencor's Leadership in Biomass Hydrolysis Enzymes
 - Complex of many important activities necessary for biomass hydrolysis.
 - Unclarified product.
 - Lower cost in operation than Accellerase[®] 1000.
 - Even higher β -glucosidase activity drives more complete conversion to glucose.
 - Improved formulation
- Delivers Significantly Enhanced Performance

GENENCOR[®]
A Danisco Division www.genencor.com

Introducing
ACCELLERASE[®] 1500
Together we can fuel the future with biomass.

Genencor[®] and you—
a partnership of possibilities

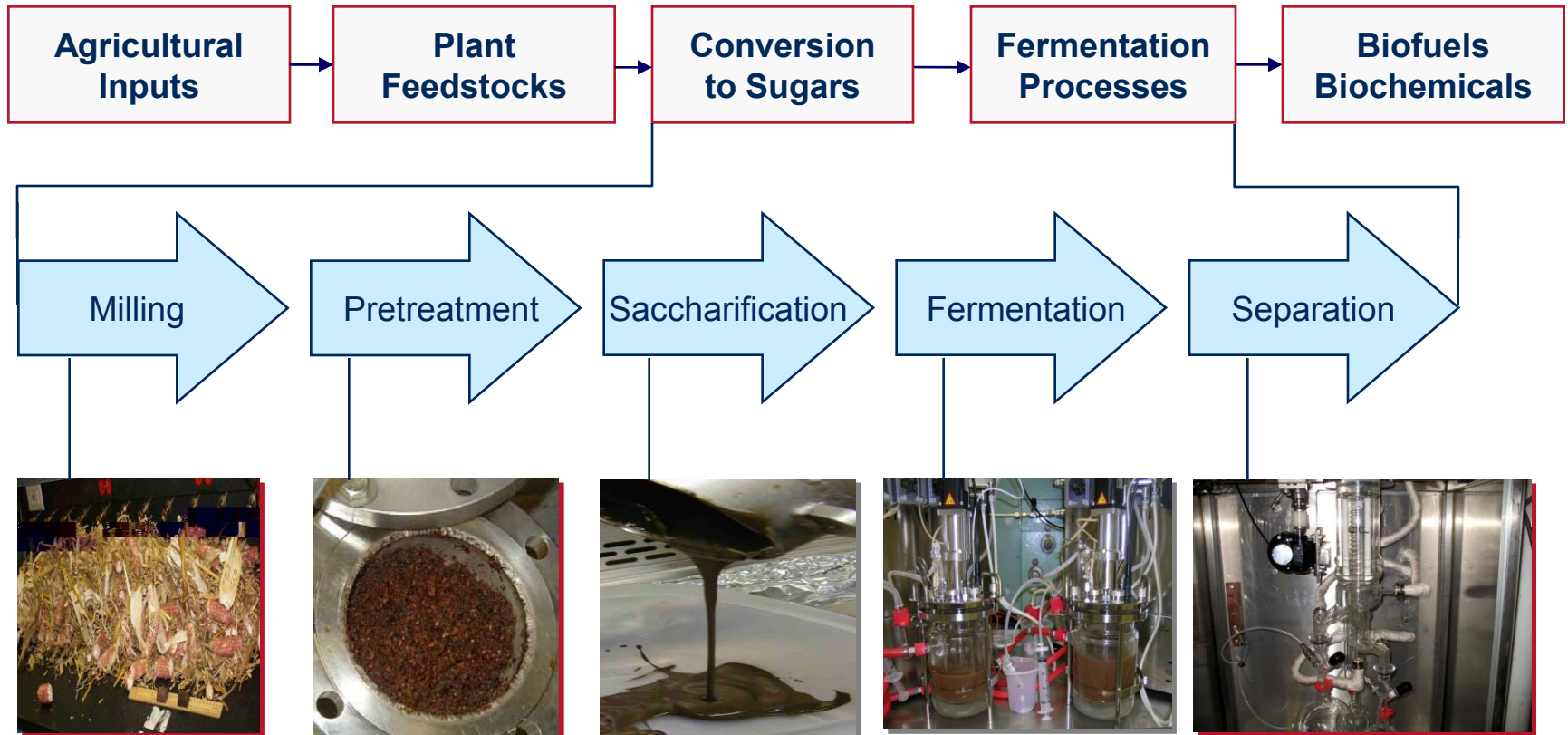
ACCELLERASE[®] 1500 is the next big step toward cost-effective production of fuel ethanol and other biochemicals from lignocellulosic biomass.

- Lower cost in operation
- Improved formulation
- Higher beta-glucosidase activity
- Higher ethanol yield potential
- More robust operation
- Effective for a variety of cellulosic substrates

accellerase[®]
FUELING THE BIOBASED ECONOMY

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Required operations for cellulosic ethanol process



Process Development: Different Process Configurations (1)

Separate Hydrolysis and Fermentation (SHF)

SHF

Enzymatic
Hydrolysis



Fermentation

T=50 °C
pH=5.0

T=32-38 °C
pH=4.0-5.0

Simultaneous Saccharification and Fermentation (SSF)

SSF

Enzymatic
Hydrolysis

Fermentation

T=32-38 °C
pH=4.0-5.0

Process Development: Different Process Configurations (2)

Hybrid Saccharification and Fermentation (HSF)

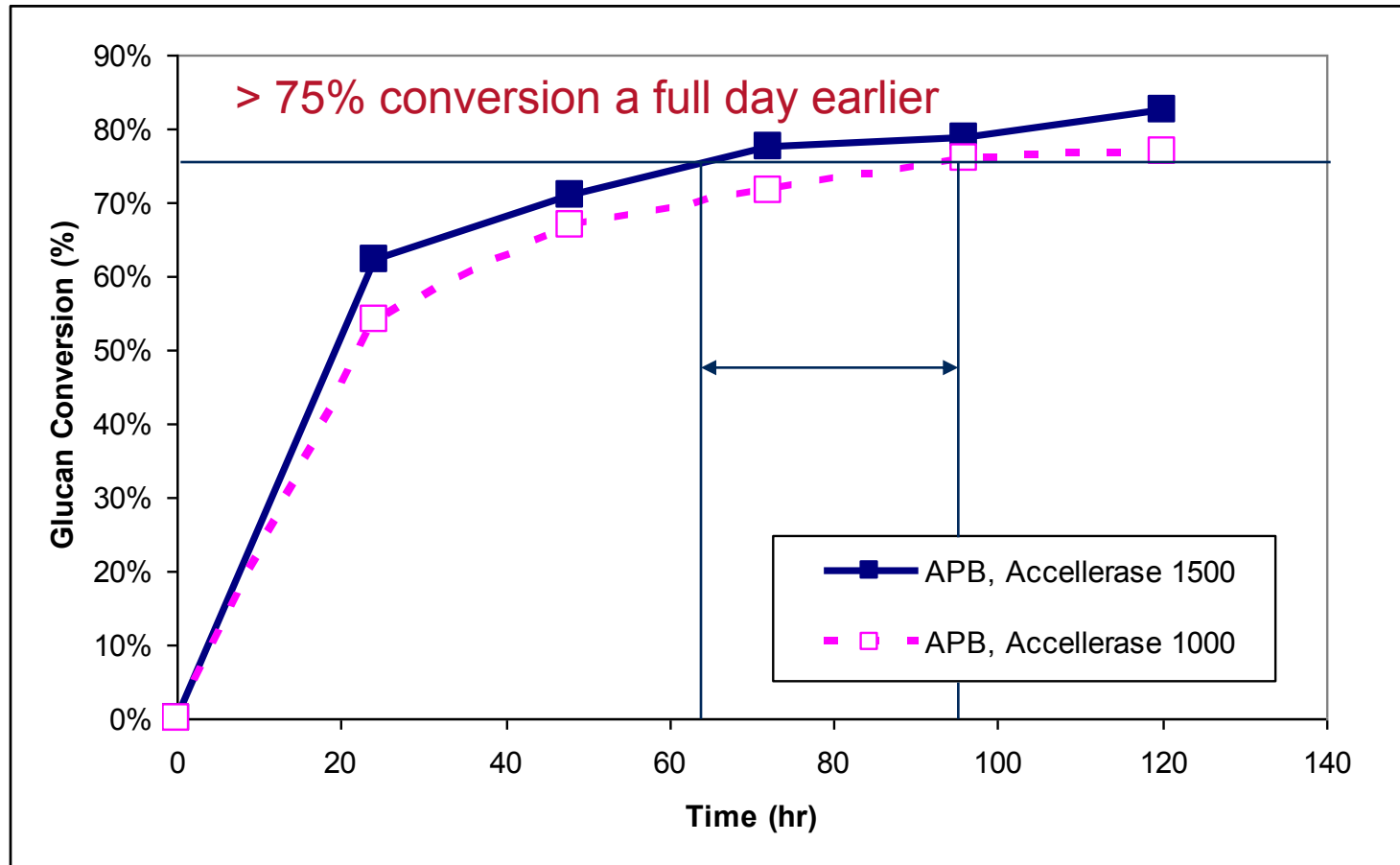
HSF

Enzymatic
Hydrolysis

Fermentation

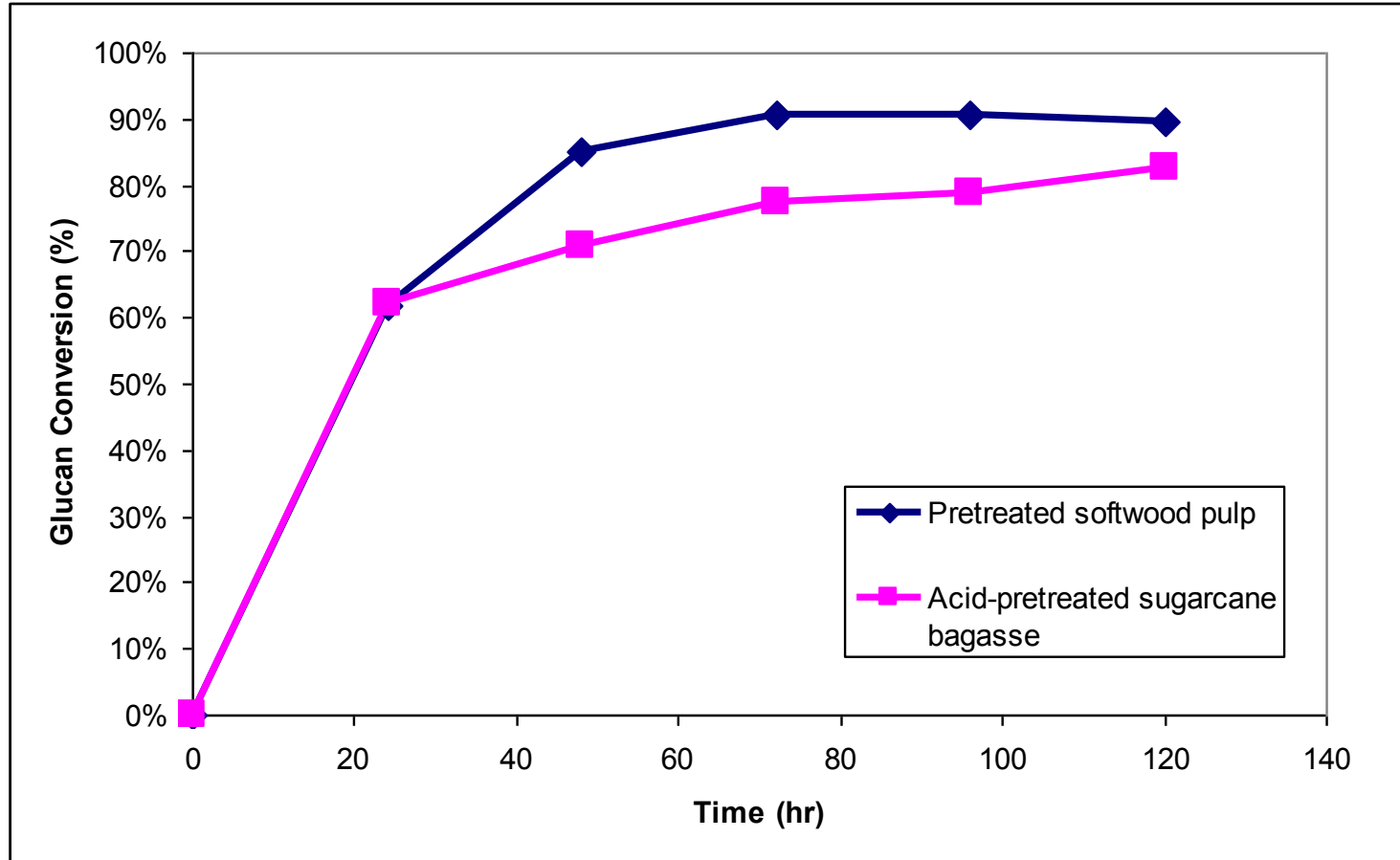
T=50 °C for certain time, then
T=32-38 °C, pH=4.0-5.0

Accellerase[®] 1500 vs Accellerase[®] 1000 Saccharification



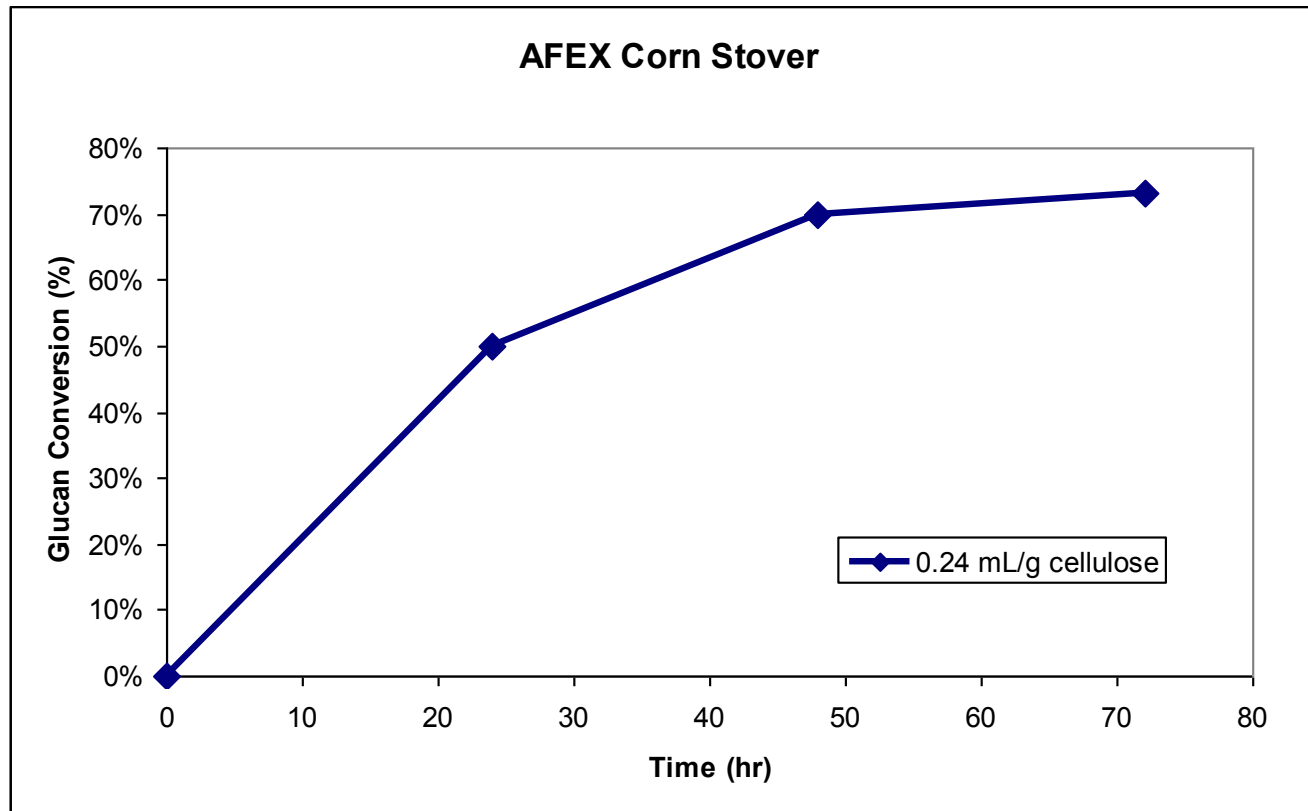
% Glucan conversion of and a washed acid-pretreated sugarcane bagasse (APB) using **ACCELLERASE[®] 1500** and **ACCELLERASE[®] 1000** at 0.24 mL/g cellulose, 7% cellulose loading, 50°C, and pH 5.0.

Accellerase[®] 1500 performance on a variety of feedstocks



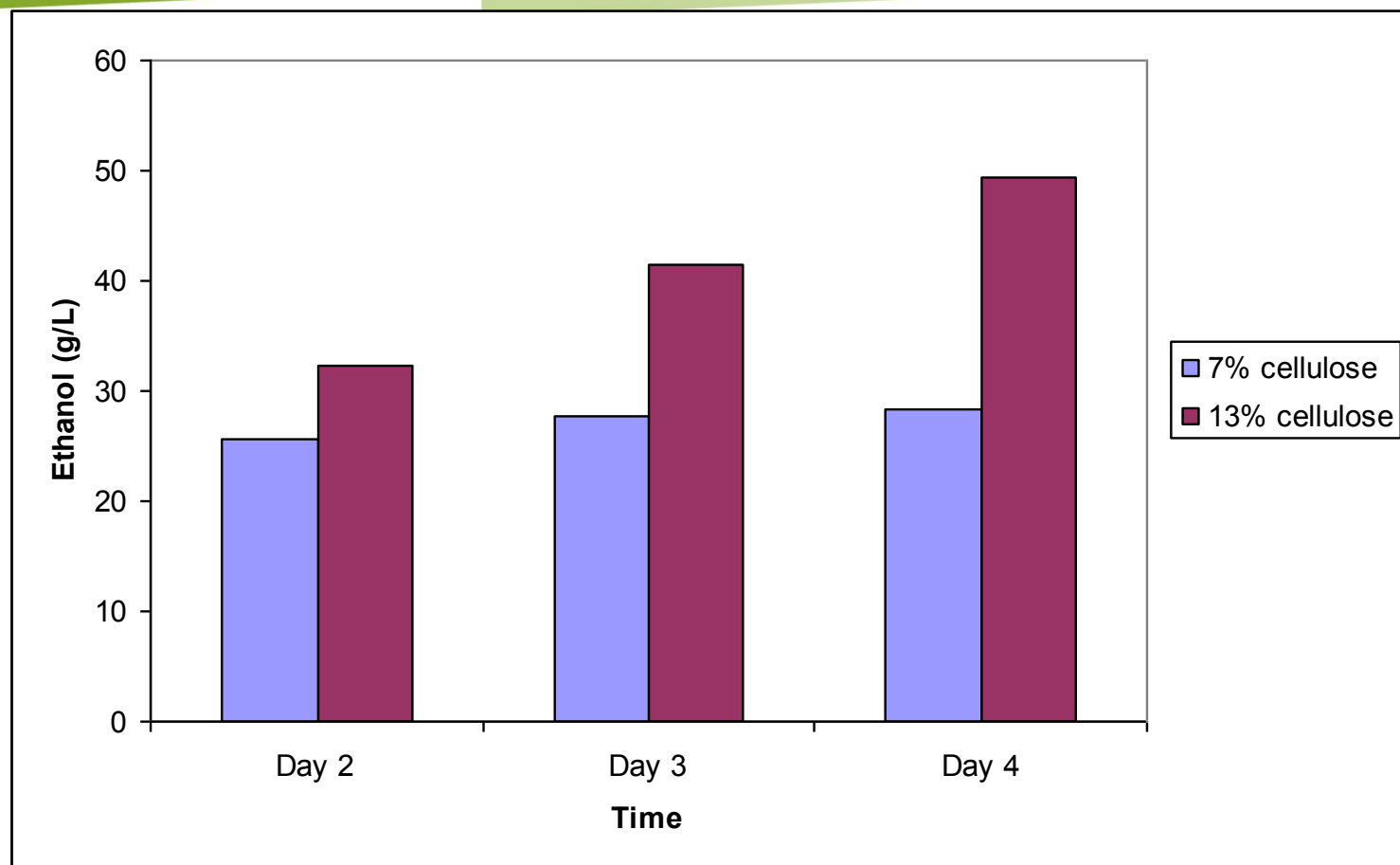
% Glucan conversion of a pretreated softwood pulp**, and a washed acid-pretreated sugarcane bagasse (APB) using **ACCELLERASE[®] 1500** at 0.24 mL/g cellulose, 7% cellulose loading, 50°C, and pH 5.0.

Accellerase[®] 1500 performance on a variety of Feedstocks



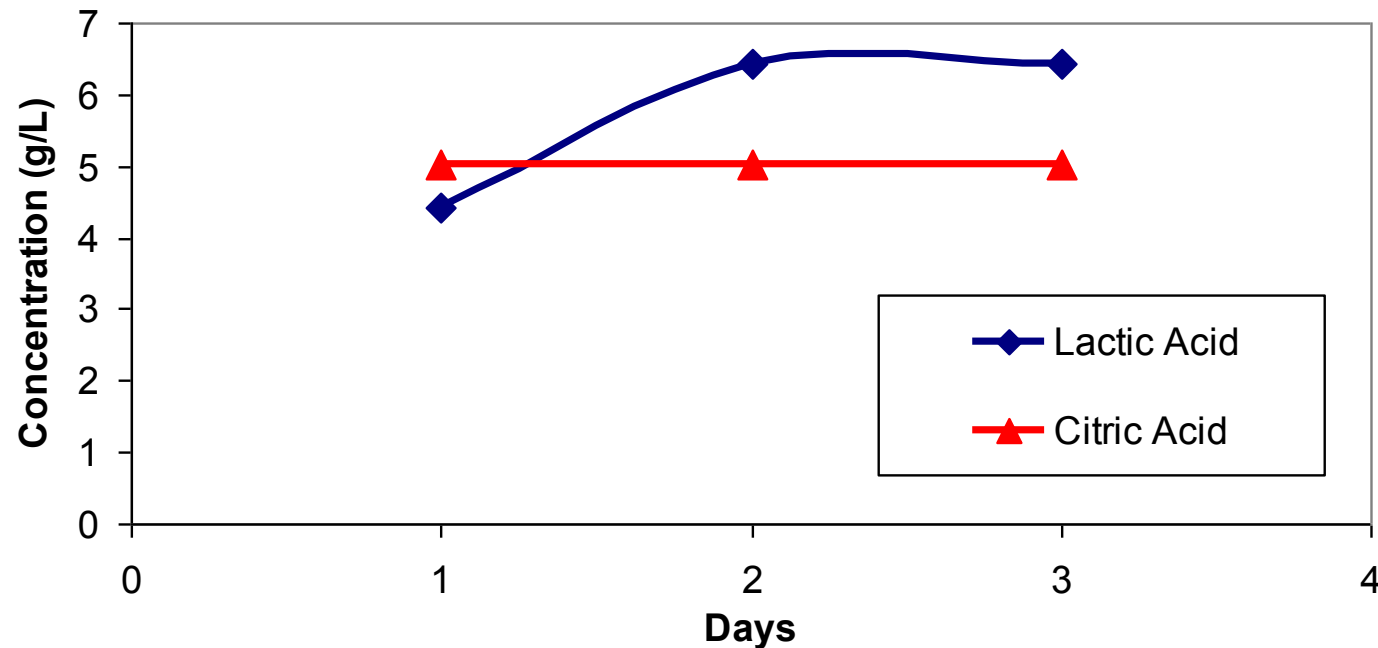
% Glucan conversion of a ammonia fiber-expansion (AFEX) corn stover using **ACCELLERASE[®] 1500** at 0.24 mL/g cellulose, 2% cellulose loading, 50°C, and pH 5.0.

Accellerase® 1500 performance in SSF mid to high solids loading



Simultaneous saccharification and fermentation of washed acid-pretreated sugarcane bagasse using **ACCELLERASE**[®] 1500 at 0.24 mL/g cellulose, 7% and 13% cellulose loading, pH 5.0, and 38°C with THERMOSACC[®] DRY yeast (Milwaukee, WI).

Lactic Acid Production from *L. rhamnosus* and Citric Acid Production from *A. niger* on 7% Glucan Loading of Washed Acid Pretrated Sugarcane Bagasse (APB) Hydrolysate Produced from Accellerase 1500



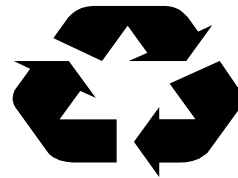
Lab scale experiments show other biochemicals can be made from sugars produced from biomass using Accellerase® 1500!!

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Process economics impacted by ALL steps

- Genencor is investing in delivering next generation enzymes, but all parts of the process count to deliver improved overall economics.
- Capital and Operations Cost
 - Feedstock Choice and Cost
 - Pretreatment Choice and Cost
 - Conversion of sugars to final product
 - For ethanol production: Ethanologen Performance
 - For biochemicals: Efficiency of pathways to final product.
 - Water and Energy Recycle
 - Waste Disposal
- Process integration key to success!



Pretreatment	Substrate	% Glucan	% Xylan	% Lignin (K+AS)	Dose for 80% Glucan Conversion in 3 days @ 7% Cellulose (mgTP Accellerase/g cellulose)
Kraft	Paper Pulp*	81.9	8	3.1	
Kraft	Paper Pulp*	74.6	20.7	2.6	
Kraft	Paper Pulp	77.3	21.5	0.7	
Kraft	Paper Pulp*	82.2	9.9	3.5	
Kraft	Paper Pulp*	74.3	21.1	2.7	
Kraft	Paper Pulp*	77.8	9.4	3.1	
Dilute Acid (NREL)†	Stover	55.6	3	30	
Dilute Acid (NREL)†	Bagasse	56.9	3	30	
Kraft	Paper Pulp	74	5.5		
Autocatalytic	Bagasse	54.4	3.2	30.3	
Autocatalytic	Bagasse	54.2	3.2	26.7	
Autocatalytic	Bagasse	52.9	5.3	29	
Steam Expansion	Hardwood	54.4	6.5	34	
AFEX	Stover	31.7	19.1		
Autocatalytic	Bagasse	55	5	25	
Steam/SO2	Spruce	46.5	4.1	32.1	
Autocatalytic	Bagasse	59.7	4.1	26	
Autocatalytic	Bagasse	59.4	1.6	25.5	
Two stage acid/alkali	Rice Straw	60.5	15.7	8.6	
Autocatalytic	Bagasse	64.2	3	26	
Autocatalytic	Bagasse	65.9	2.4	20.1	
Steam Expansion	Stover	40.6	11.7	31.4	
Steam Expansion	Stover	43.1	5.2	36.3	
Steam Expansion	Hardwood	47.9	15.2	29.7	
Steam Expansion	Bagasse	49.4	13.9	27.5	
AFEX	Bagasse	43.1	22.6		
Kraft	Recycle Pulp	51.4	8.3	19	
Steam Expansion	Bagasse	40.6	22.6	25.5	
Steam Expansion	Bagasse	41.4	21.1	27.6	

Note: data for Accellerase® 1000, mg total protein / g cellulose

Future improvements in enzyme efficacy driven by active RnD today!

- Reduce product inhibition of enzymes
- Increase ethanol tolerance of enzymes
- Increase thermo tolerance of enzyme suite
- Increase hemicellulase activity
- Reduce ineffective binding/ interaction with substrate
- Increase specific activity of enzymes

= Improved Process Economics

Summary



- Genencor has been at the forefront of cellulase and hemicellulase enzyme improvement for years and continues to actively innovate.
- ACCELLERASE® 1500 is our new commercially available biomass enzyme complex developed specifically for second generation biorefineries
- Government support for technology development and pilot plant and larger projects are critical for this emerging industry.
- Process integration at pilot / demo scale will continue to improve the current state of cellulosic ethanol and biochemical economics and environmental impact.



Acknowledgements: Genencor Biomass Team



Biomass Applications Group

Biomass R&D Teams

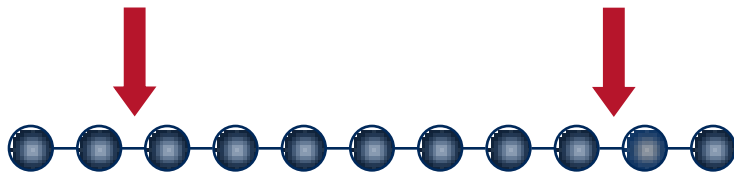
Manufacturing Plants

Biorefinery Business Group

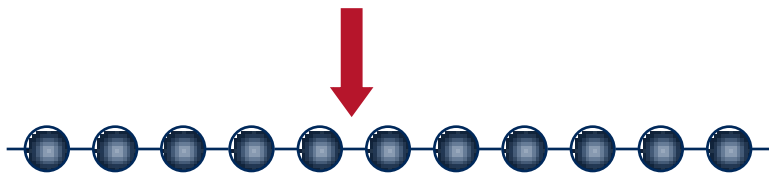
**** Some research on pulp and paper feedstocks funded by
Agence Nationale de la Recherche, France (ANR-05-BIOE-
007)**

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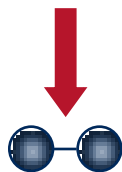
Cellobiohydrolase



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.
- Beta-glucosidase (**β -glu**): converts soluble oligosaccharides to glucose, relieving product inhibition.

Existing and planned US cellulose biorefineries



Source: The Advanced Biofuels & Climate Change Information Center, Feb 2009

<http://biofuelsandclimate.wordpress.com/about/>

Red: In Operation, **Green:** Commercial Scale, **Blue:** Pilot or Pre-commercial Scale