MASCOMA
ADVANCED BIOCONVERSION PRODUCTS

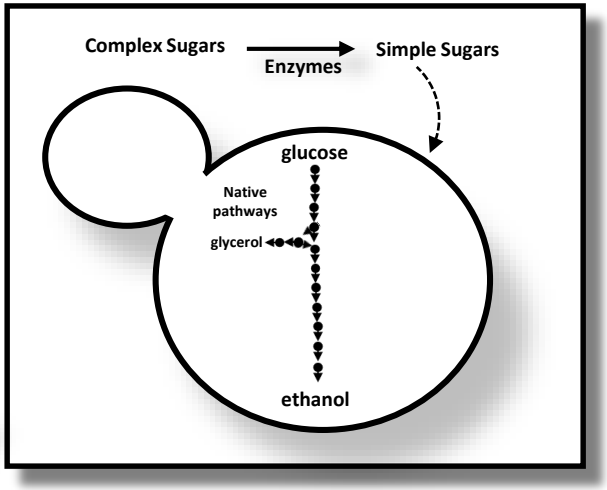
LALLEMAND

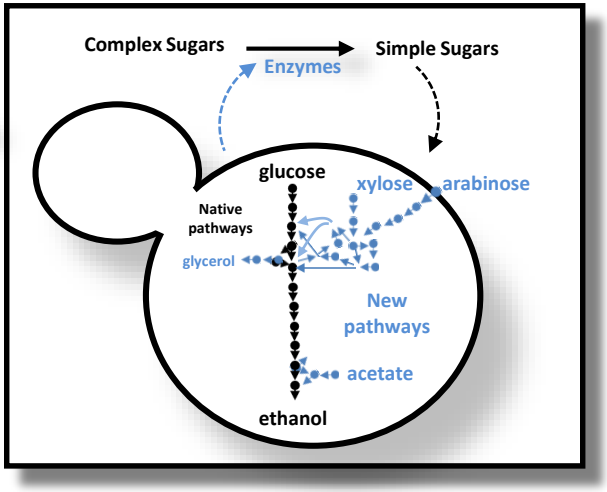
Advanced Yeast Biocatalysts for 2nd Generation Ethanol Production

BIO Pacific Rim; December 8, 2014

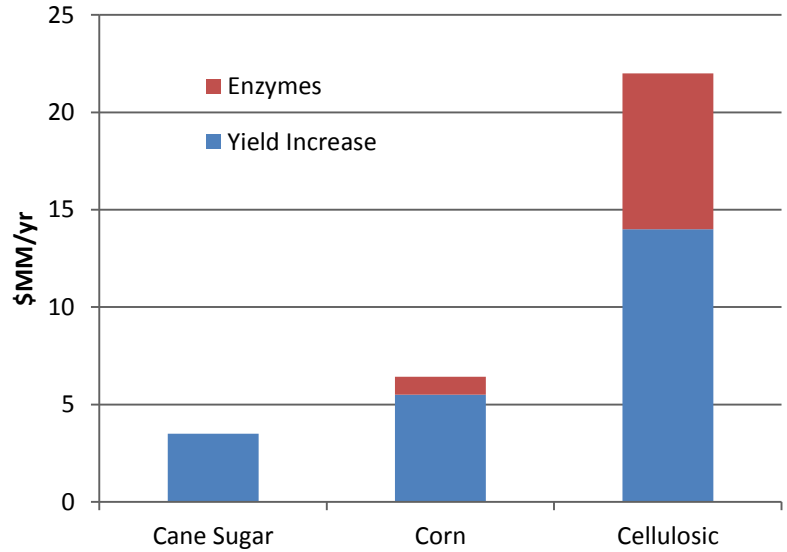
John McBride, Ph.D.

Value of Advanced Yeasts





Potential value created by bioengineered Yeast:



Mill Size (MGY): 40 80 20

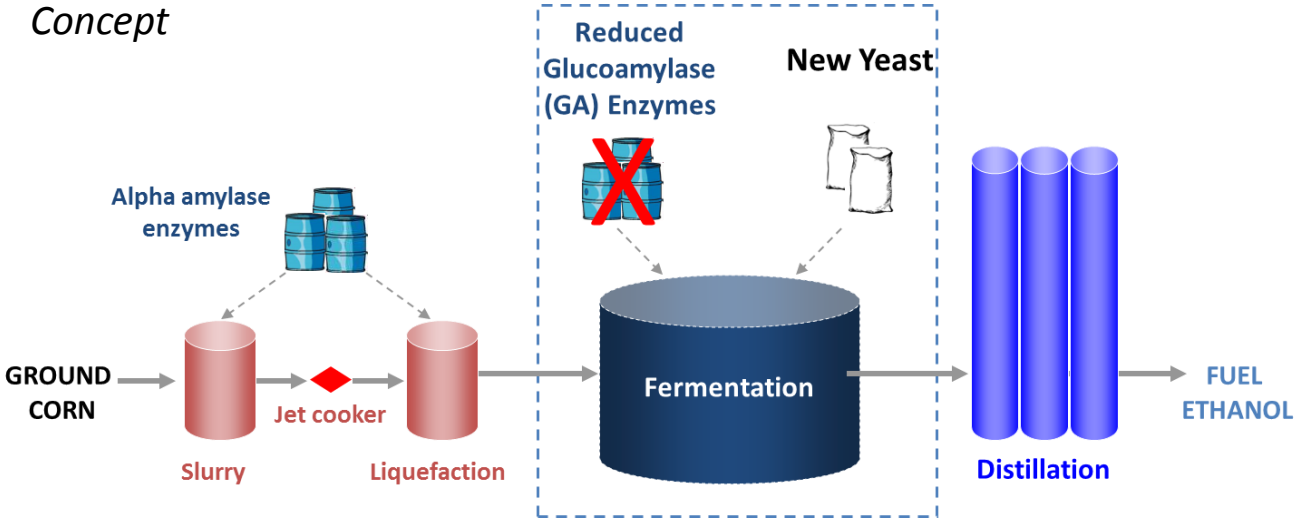
Assumptions:

- $1.75/gal EtOH selling price
- $12/Kg enzyme selling price

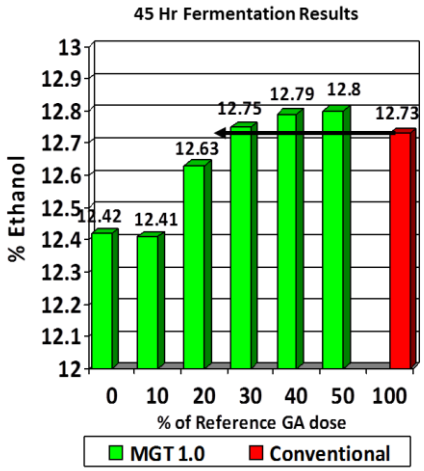
- 2 products being used at commercial scale
- >3 Billion gallons of EtOH made with Mascoma strains to date

Application in Grain-Based Fuel Ethanol Production: Transferm

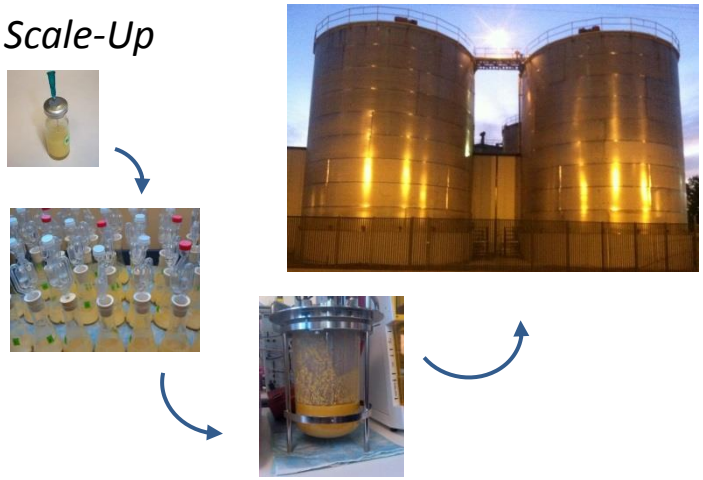
Concept



Lab Scale Demonstration

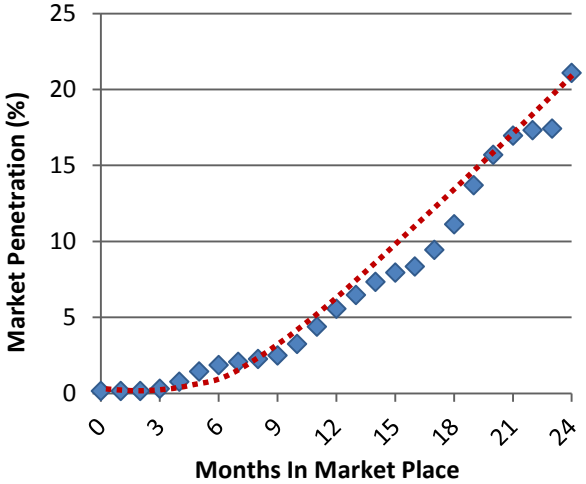


Scale-Up



Rapid Commercial Adoption

Liquid Yeast dosing & Storage

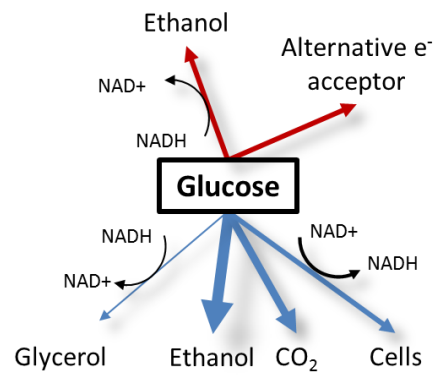


MASCOMA

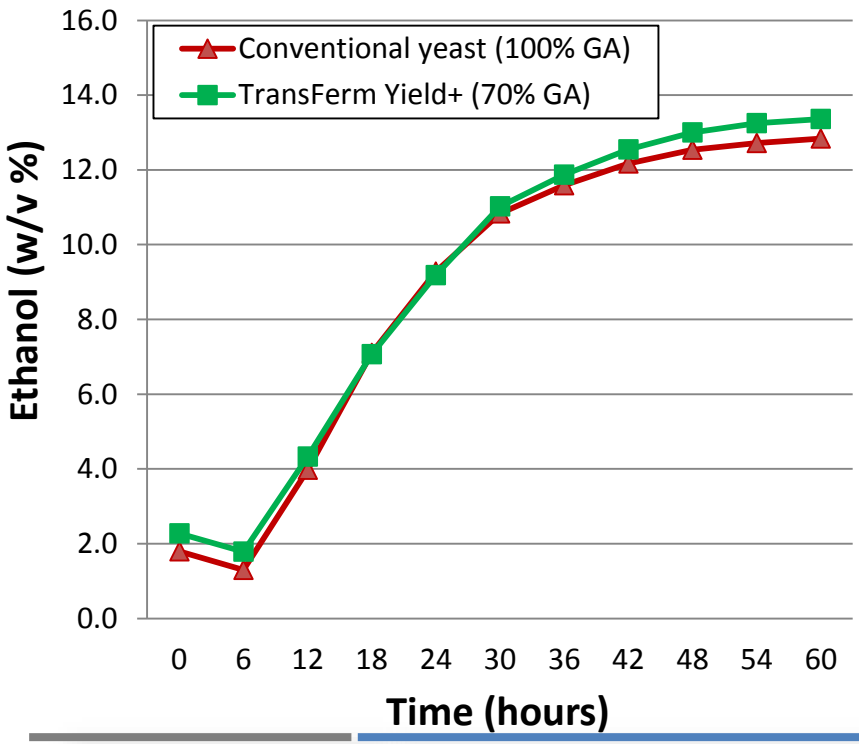
TransFerm® Yield+, Increased Yields and GA for Corn Ethanol

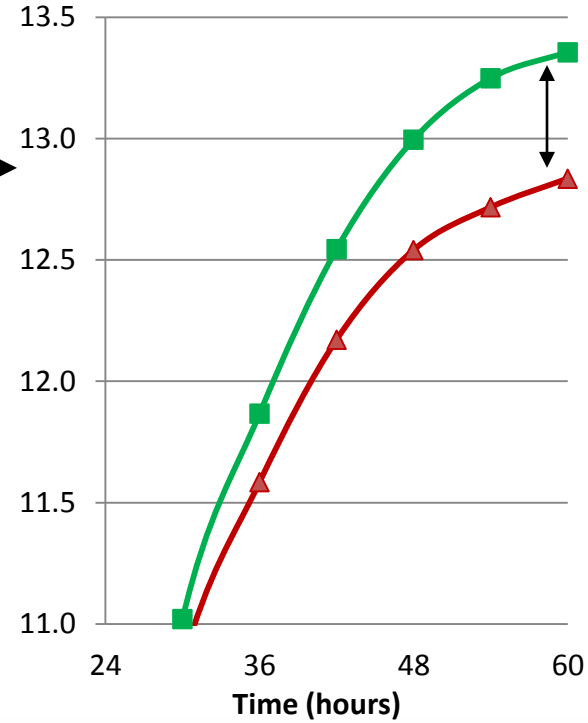
TransFerm Yield+

- Balances the redox during anaerobic growth with a new metabolic pathway
- Makes sufficient glycerol to balance osmotic stress



Pilot Scale Data:



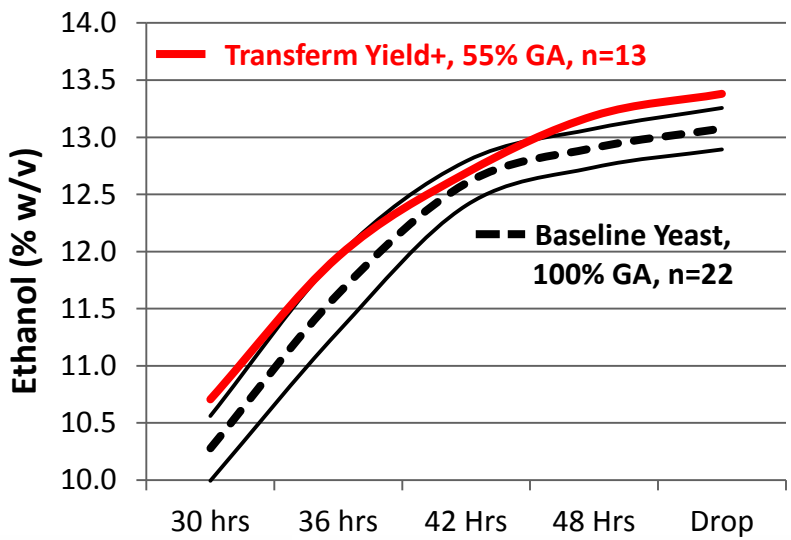


- 4.5% Yield increase
- Same Kinetics

Note: *pilot data based on n=3 for conventional yeast; n=5 for TransFerm Yield+*

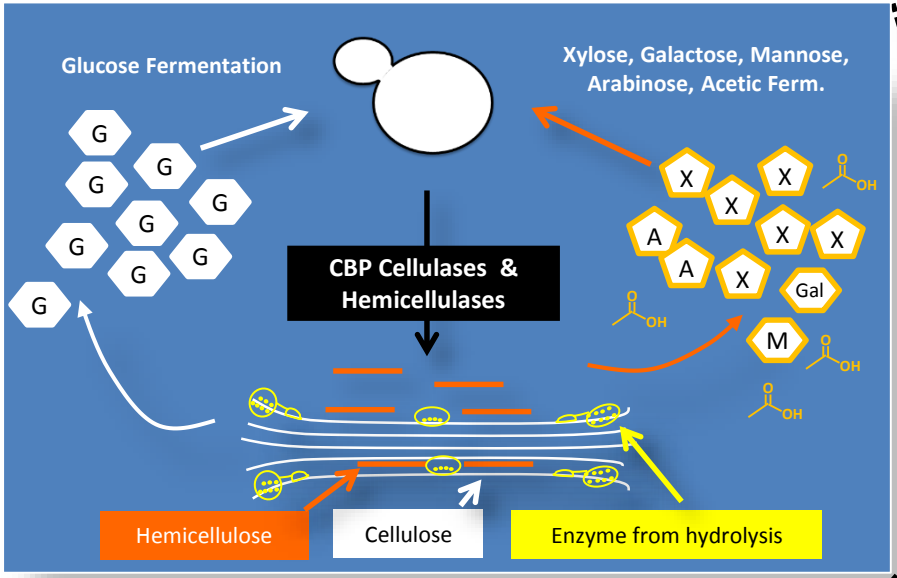
TransFerm® Yield+ Commercial Scale Results

Customer	Plant Scale (MGPY)	Design Type	N=	Ethanol Yield Increase Achieved	Glycerol Reduction	Enzyme Reduction	Estimated Total Value per Year (MPY)
1	110	ICM	13	2.9%	21%	45%	$6.3
2	125	ICM	10	1.6%	40%	30%	$3.9
3	115	ICM	29	2.0%	37%	25%	$3.6
4	60	ICM	53	2.0%	30%	40%	$2.1
5	35	Delta T	88	1.7%	26%	28%	$0.9

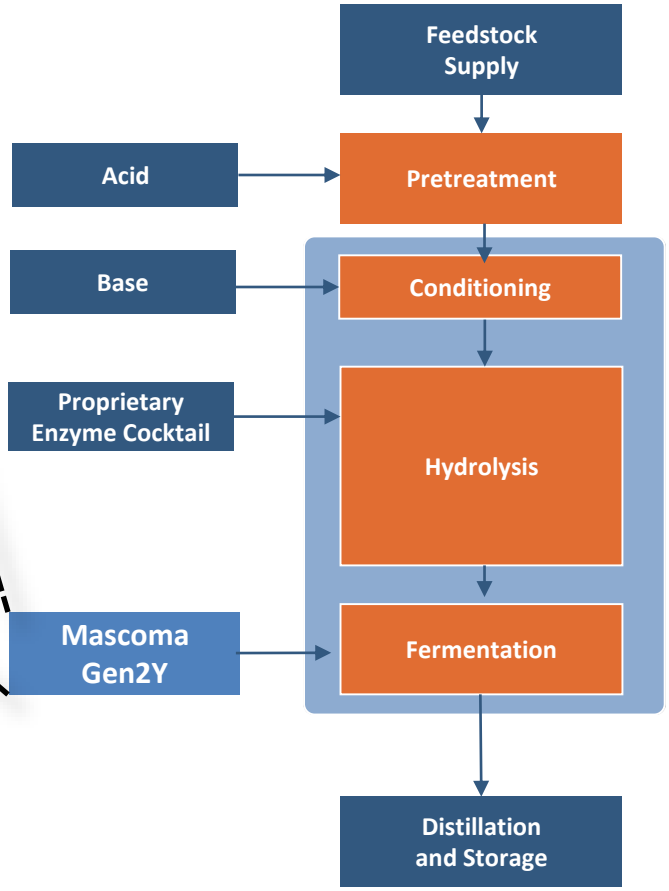


- Robust commercial scale results, kinetics
- Average value of $0.04/gal delivered
- >$500M value creation for the U.S. ethanol industry from this product

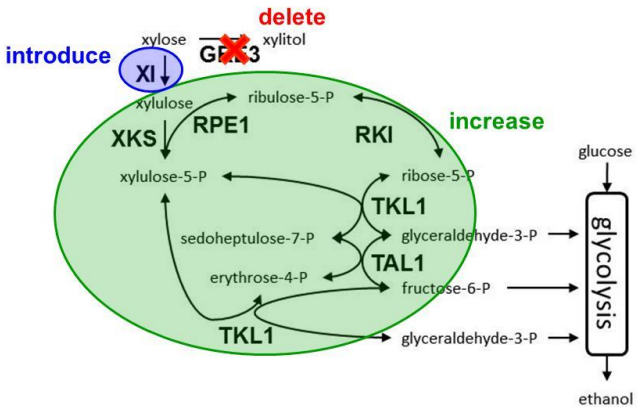
Gen2 Yeast: Drop-in Value



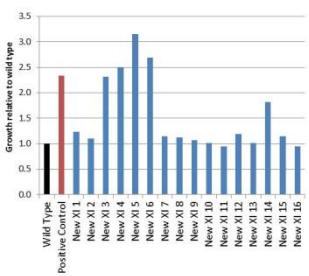
Technology for Bagasse Sugar Fermentation	Yield +
Xylose Fermentation	33%
Glycerol Reduction	5%
Acetate Conversion to Ethanol	5%
Arabinose Utilization	5%
Cellulase and Hemicellulase Secretion	>10%

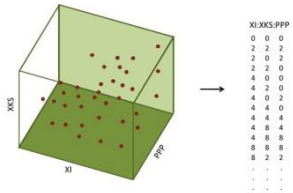


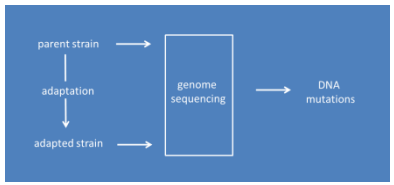
Xylose Fermentation



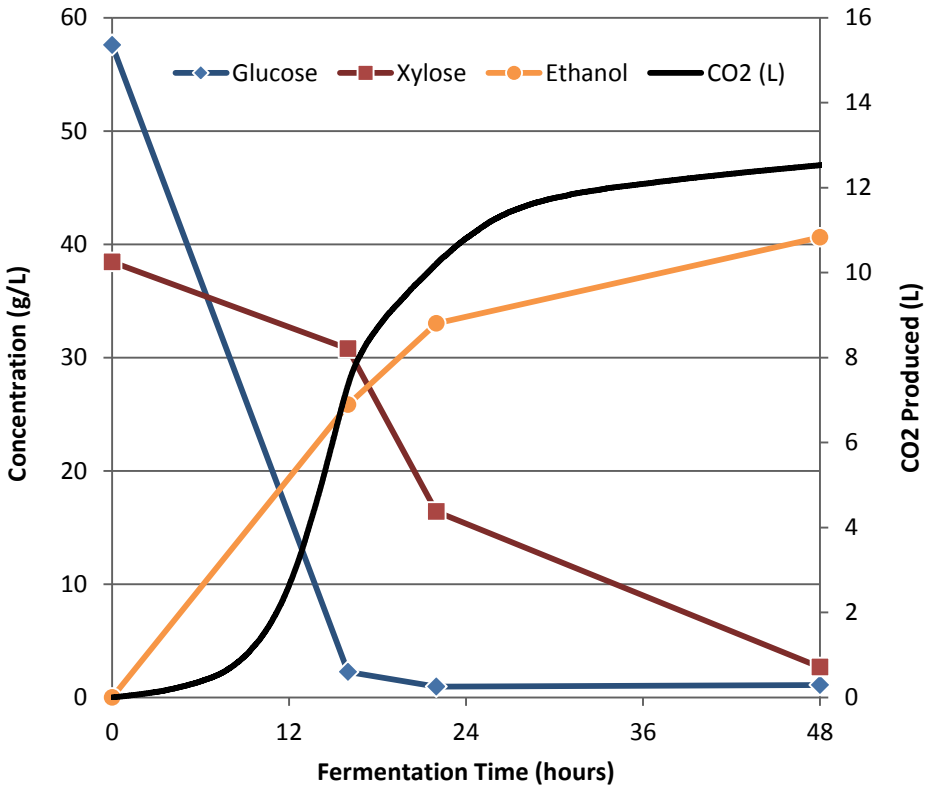
A. Isolation of unique xylose isomerases
B. Optimization of core genes
C. Identification of new mutations







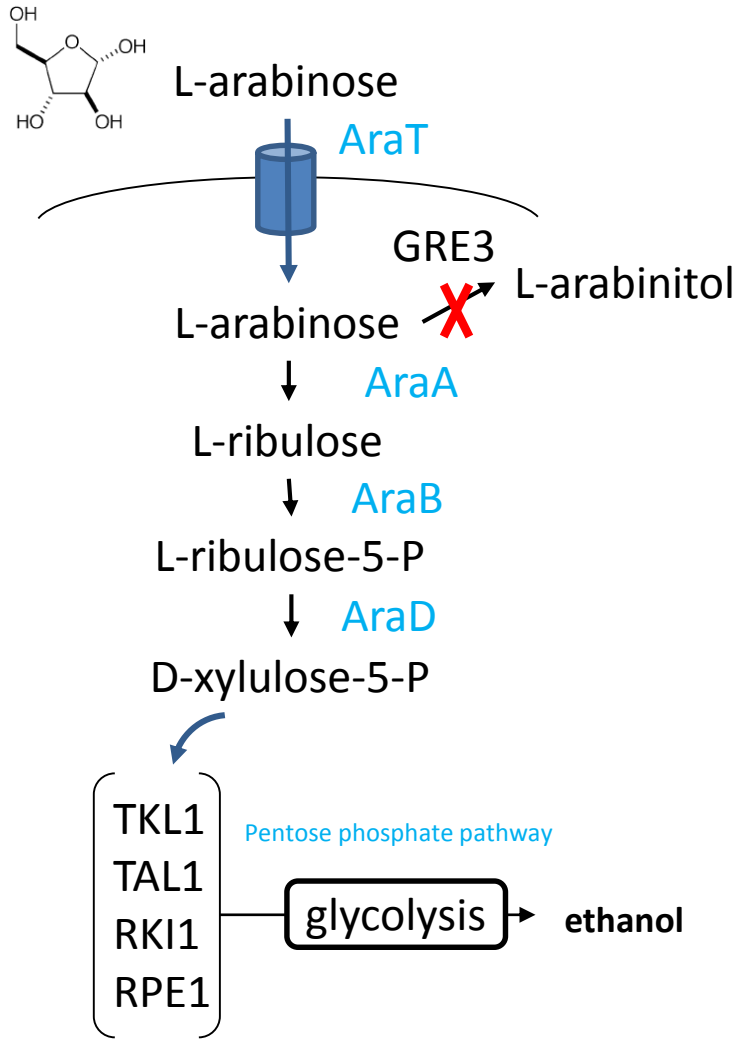
Commercial corn stover hydrolyzate fermentation

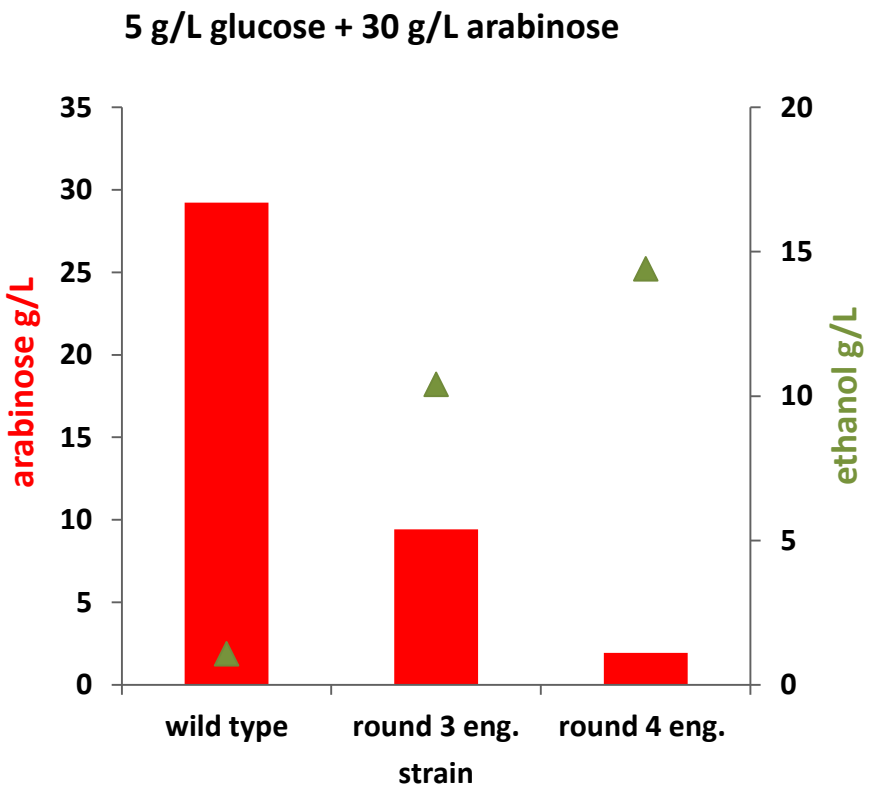


- Finishes fermentation in ~30 hours from small inoculum
- 0.45 g/g ethanol yield
- **Directly Engineered, no adaptation**

MASCOMA

Arabinose Fermentation



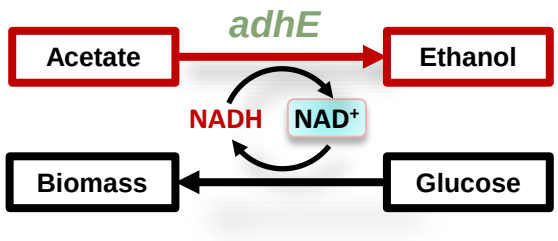


- 93% arabinose utilized
- 0.45 g/g yield on added arabinose
- **Directly engineered, no adaptation**

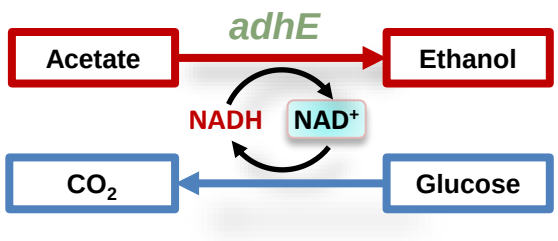
Acetate Fermentation

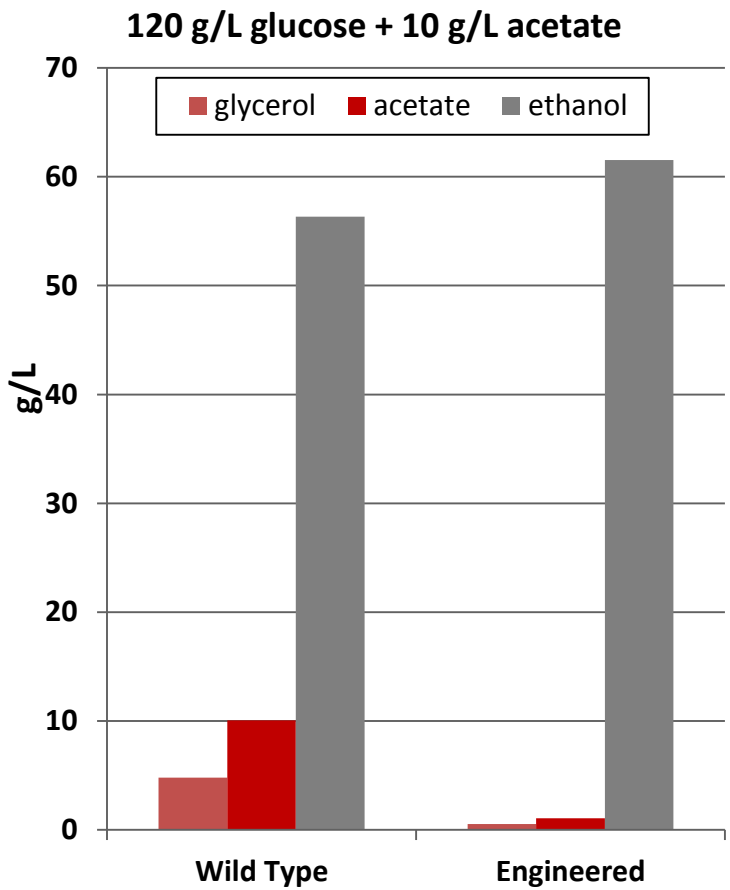
Novel Pathway for Acetic Acid Fermentation

~10% via Glycerol reduction



~90% via new pathway





- 9 g/L acetic acid consumed
- 9.3% yield increase
- Yield on glucose is 0.513 g/g

Yeast Secreted Hemicellulase Enzymes

Direct Fermentation of Hardwood Xylo-oligomers from 2-stage Pretreatment with no Enzyme:

Unique Enzyme Combinations:

Screened activities

xylanase

xylosidase

acetyl xylan esterase

glucuronyl esterase

acetyl esterase

endoglucanases

α-galactosidase

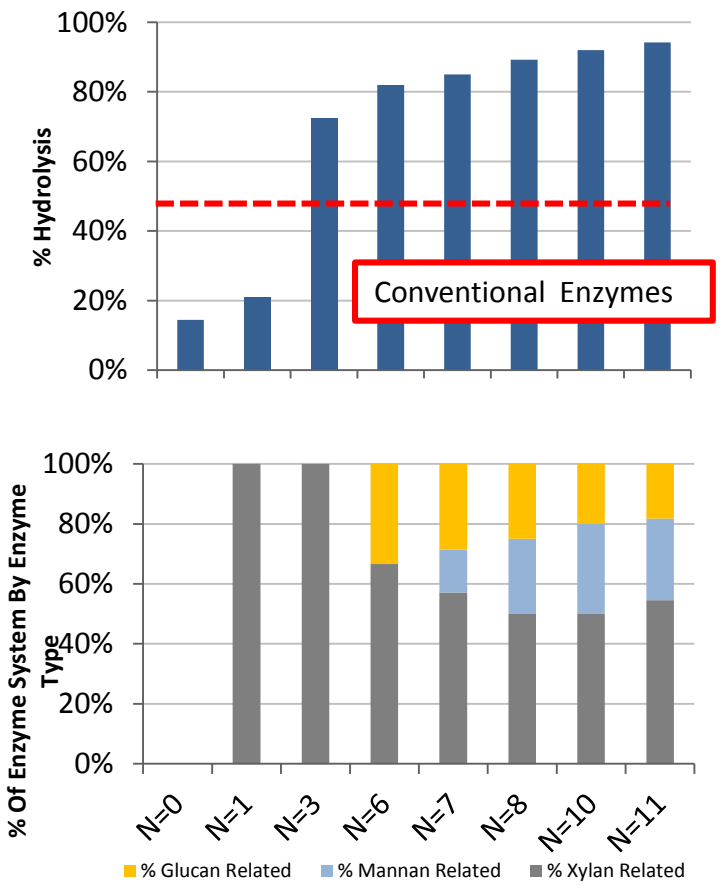
β-glucosidase

α-glucuronidase

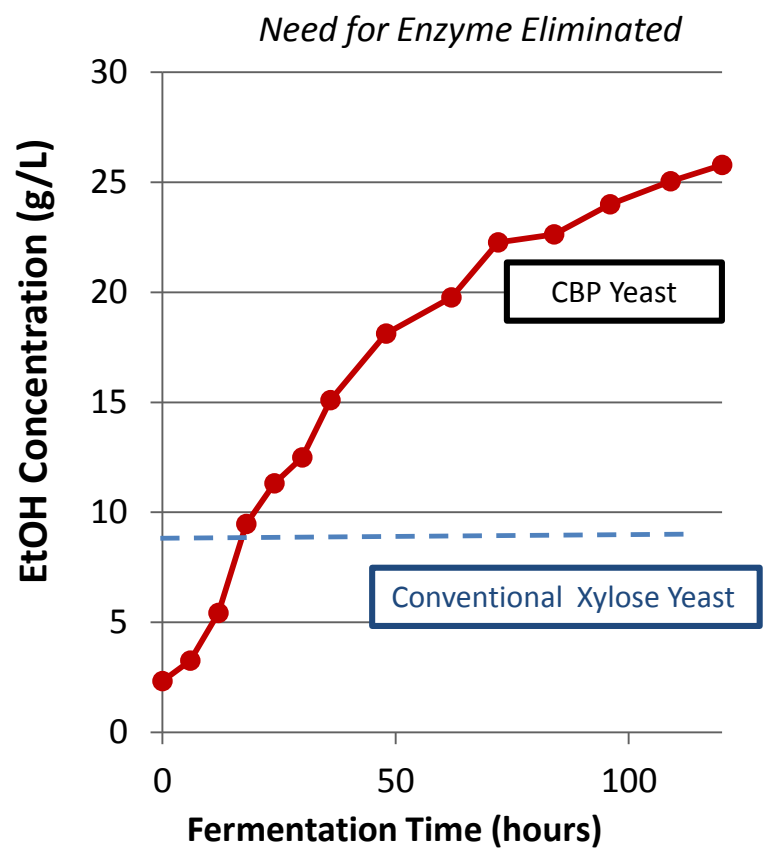
β-mannanase

β-mannosidase

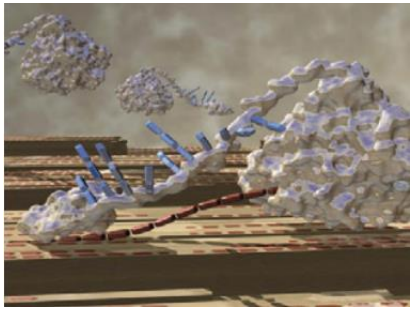
others



Applied in Pilot Scale Fermentations:



Cellulase Secretion



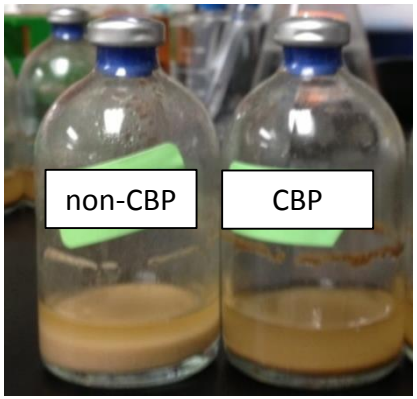
Source: NREL

Enzymatic Activities Expressed in Yeast		
cellobiohydrolase I	*CBH I chimeras**	*cellobiohydrolase II*
endoglucanases	*arabinofuranosidase*	*feruloyl esterase*
swollenin	*α-expansin*	*β-expansin*

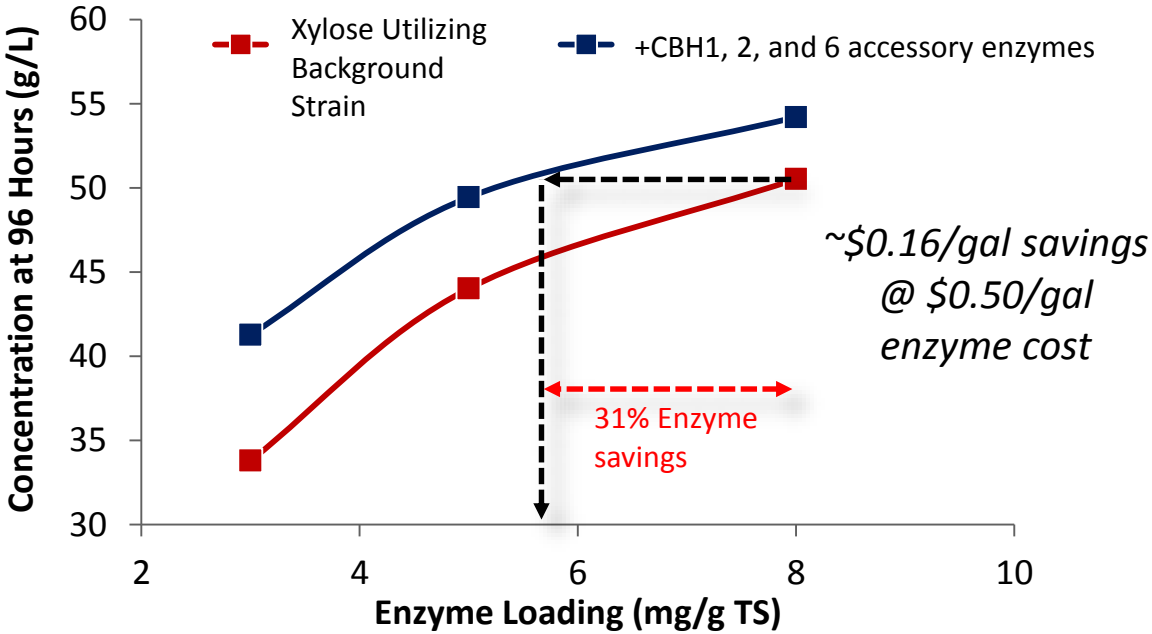
* See Ilmen *et al*. "High Level Secretion of Cellobiohydrolases by *Saccharomyces cerevisiae*." *Biotechnol. Biofuels* 4, no. 1: 30.

Highest Reported CBH1 and CBH2 Expression by 10X

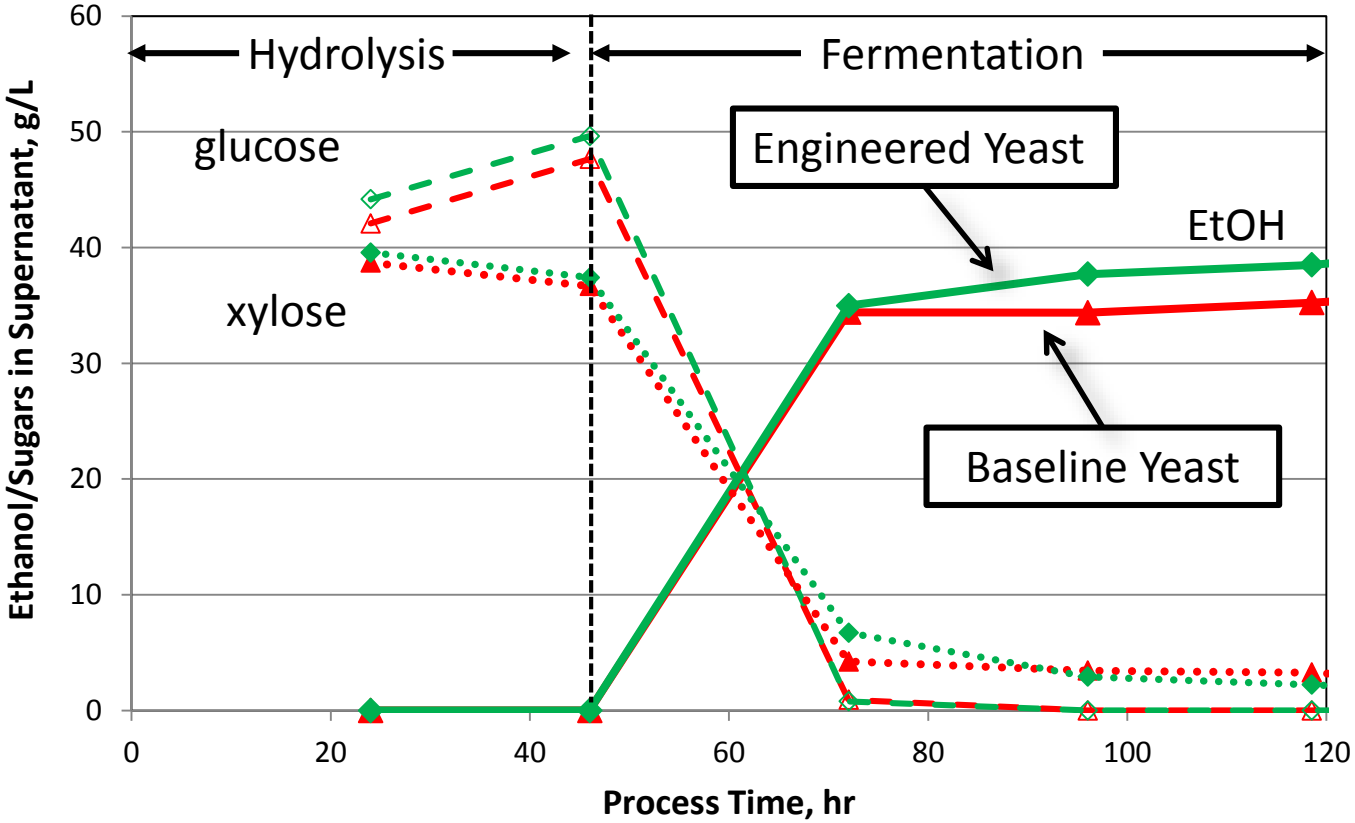
Impact of secreting cellulases:



Hydrolysis of cellulose



Hemicellulase Secretion in Corn Stover HHF Process

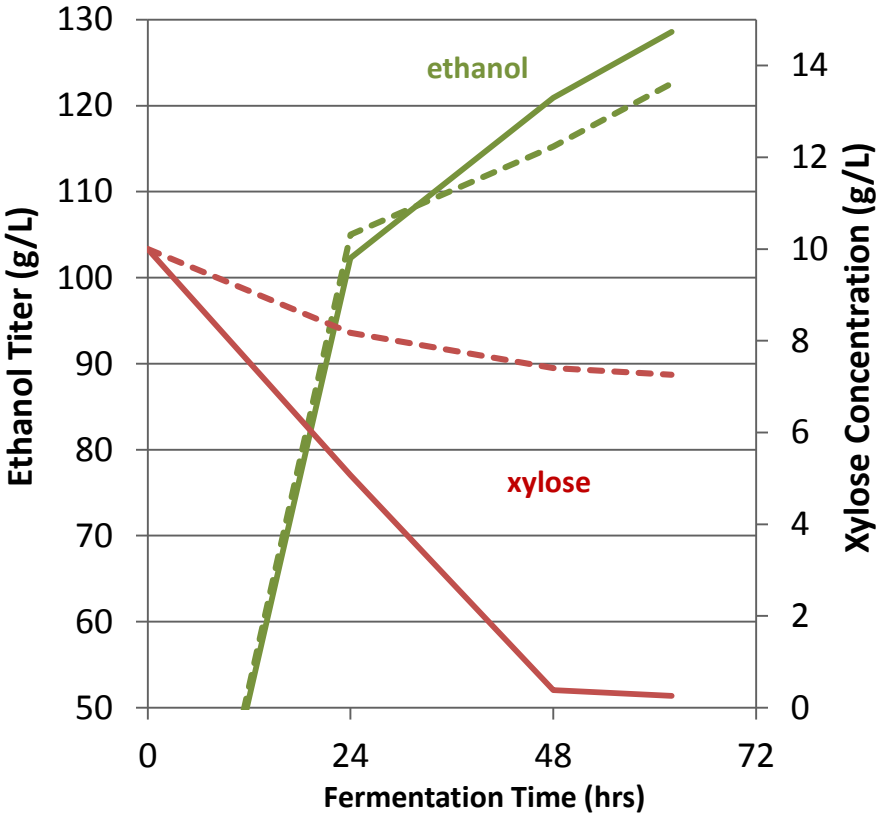


10% Yield improvement by hemicellulase enzyme expression

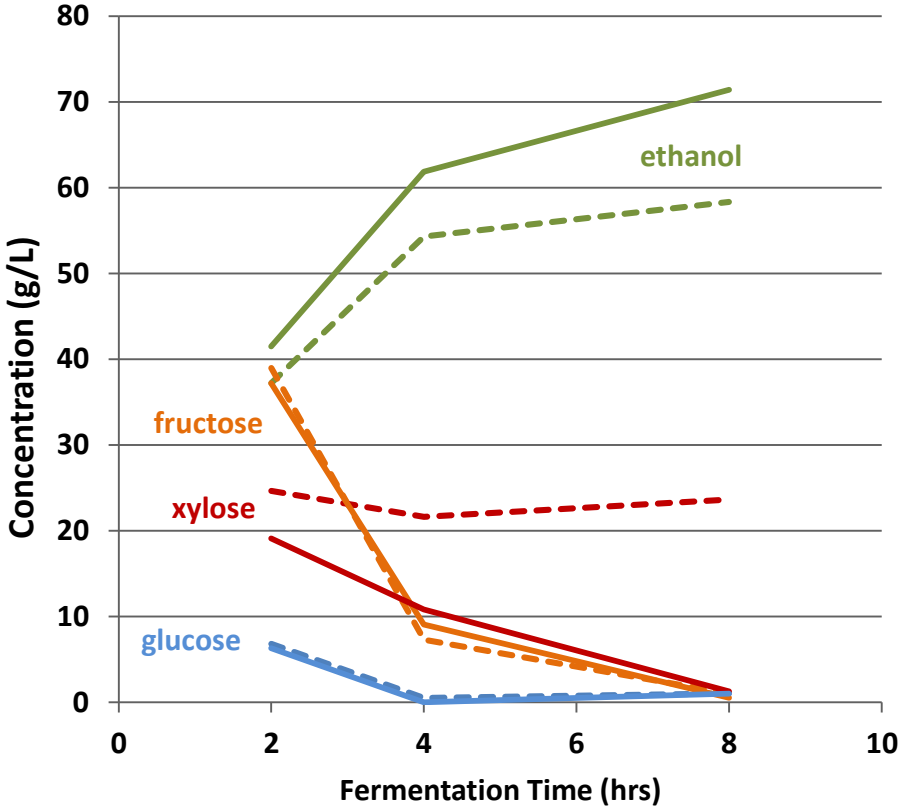
@20MM gal scale, Value = +2 MM gal x $3/gal = $6M/yr = $0.30/gal

Co-Fermentation to Extend 1G Capital

Corn Mash and Xylose:



Molasses and Xylose:



Dash = wild type

Solid = C5 strain

Summary

- Proven ability to deploy GM Yeasts for commercial fuel production
- Suite of technologies developed for 2G ethanol production (xylose, glycerol, acetic acid, enzyme secretion)
- Yield increases of 25% and cost savings of ≥$0.30/gal above xylose utilizing strains
- Novel process configurations to extend 1G fermentation capital are now possible