


Integration of Pretreatment in Ethanol Dry Mills

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Acknowledgements

Material in this work supported by US Dept of Energy

DE-FG36-04GO14220

For gifts of enzymes

Genencor (Enzymes)

Distillers Grains and Stillage

Big River Resources

Interactions and Inputs

Bruce Dale, Michigan State

Mike Cotta, Bruce Dien, USDA NCAUR

Linda Liu, LORRE Purdue University

Whole Corn Grain Composition

Dry Matter Composition*	Glucan (total) (% dry matter)	64.4 – 82.3%
	Cellulose (% dry matter)	3.3 – 4.3%
	Starch (% dry matter)	61 – 78%
	Xylan (% dry matter)	5.8 – 6.6%
	Protein (% dry matter)	6 – 12%
	Oil (% dry matter)	3.1 – 5.7

* Watson, Stanley A. “Structure and Composition,” in Corn: Chemistry and Technology (1987).

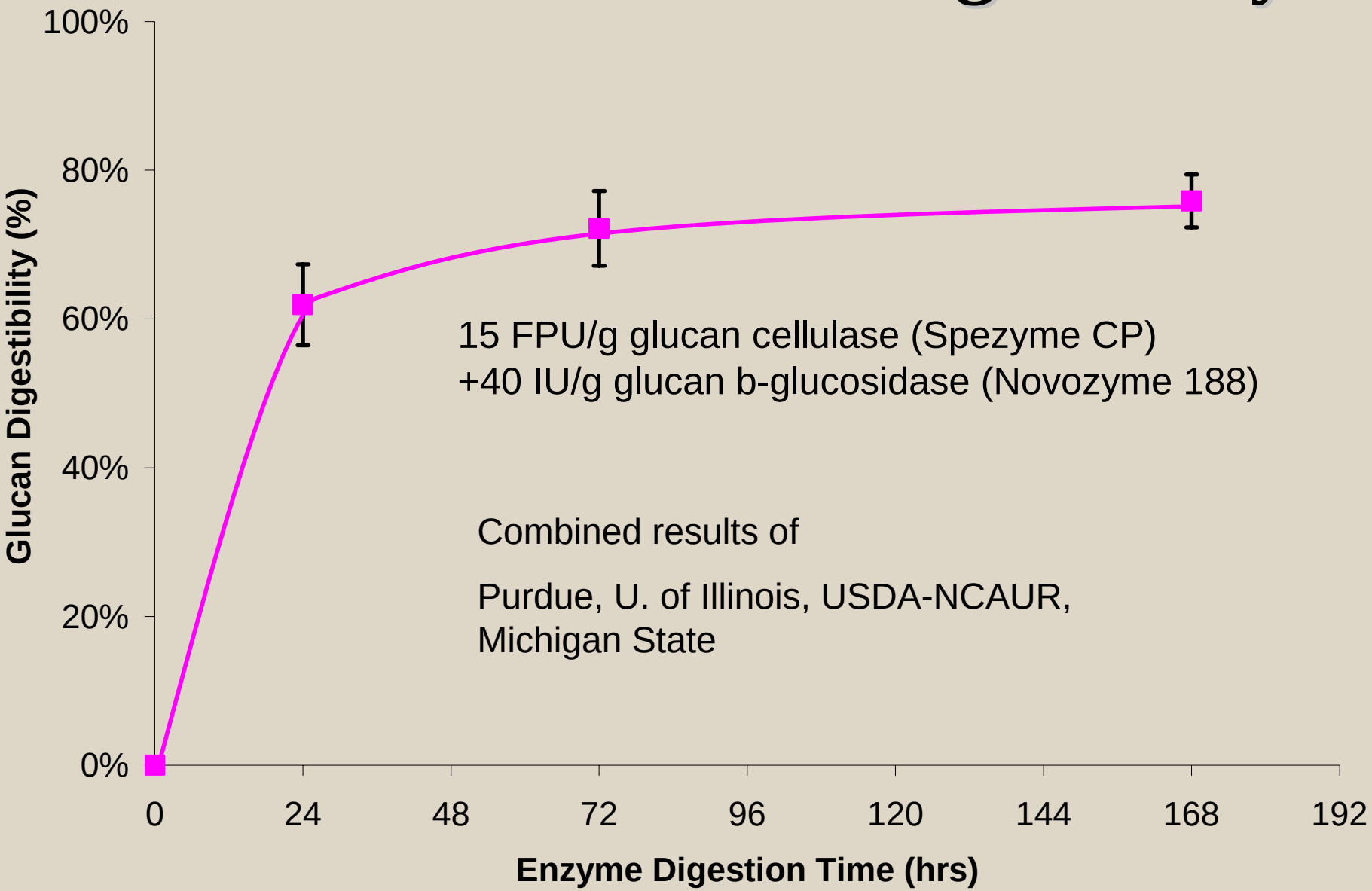
DDGS Composition

		Average (95% confidence intervals)
Gross Matter Composition	Moisture Content (% total)	11.2 ± 0.6%
	Dry Matter Content (% total)	88.8 ± 0.6%
Dry Matter Composition	Glucan (total) (% dry matter)	20.9 ± 7.1%
	Cellulose (% dry matter)	16.0 ± 6.6%
	Starch (% dry matter)	5.2 ± 1.0%
	Xylan (% dry matter)	8.2 ± 3.3%
	Arabinan (% dry matter)	5.3 ± 0.7%
	Protein (% dry matter)	26 - 34%
	Oil (% dry matter)	10 - 13%

Integration of Cellulosic Ethanol in Dry Grind Process

- Cellulose present in DG (DDGS)
  - Not utilized in dry grind process
- Cellulose has low value as animal feed
- Cellulose potential increase to ethanol yield (gal/bu) >5%
- Cellulose + Hemicellulose potential increase to yield >10%

DDGS: Cellulose Digestibility



Cellulose Digestibility of DDGS

- 63% of cellulose converted to glucose in 24 hrs
- 76% of converted in 168 hrs (7 days)
- Much slower than starch
- Much faster than other lignocellulose (corn stover)

Pretreatment Needed

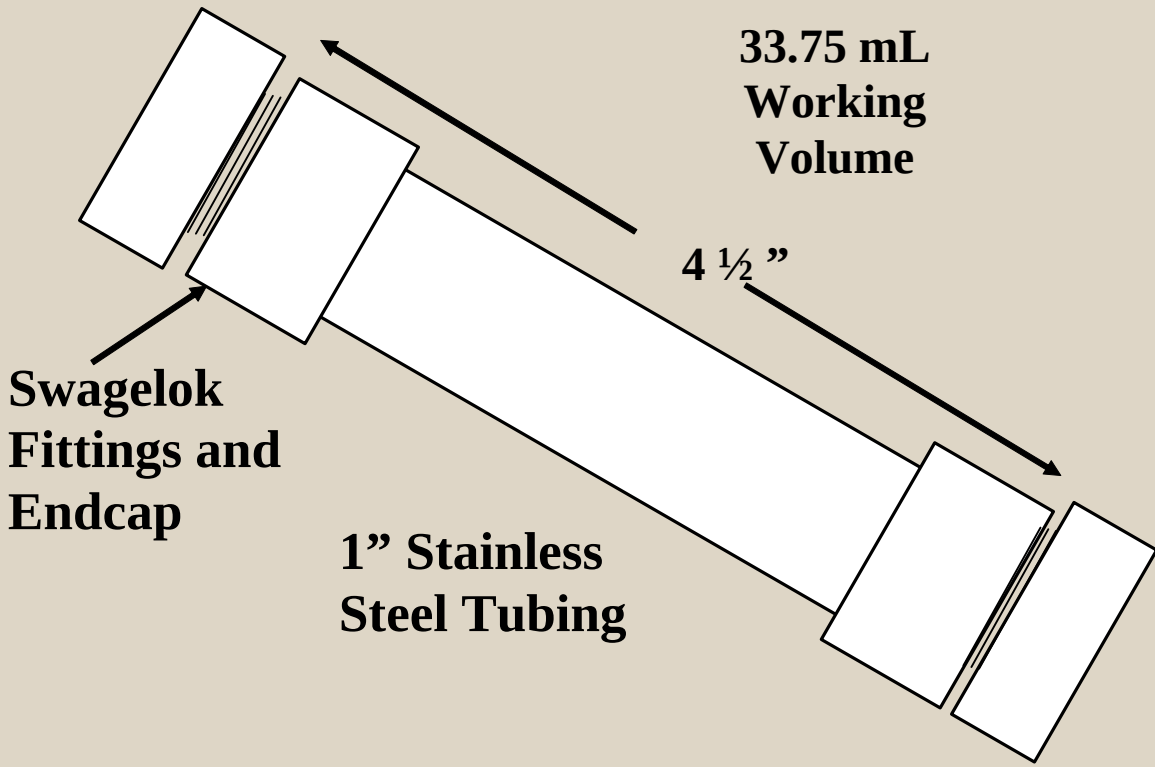
- Improve reactivity of plant polysaccharides (cellulose and hemicellulose)
- Reduce time of hydrolysis (rate)
- Increase yield
- Reduce enzyme demand (loading)

Controlled pH Aqueous Pretreatment

- Cook DDGS in liquid water at high temperatures and pressures
- Thermohydrolysis of hemicellulose
  - Maximize solubilization to hemicellulose oligosaccharides
  - Minimize hydrolysis to monomeric sugars (xylose)
  - Minimize sugar degradation
- Improves reactivity of cellulose

Controlled pH Liquid Hot Water Pretreatment

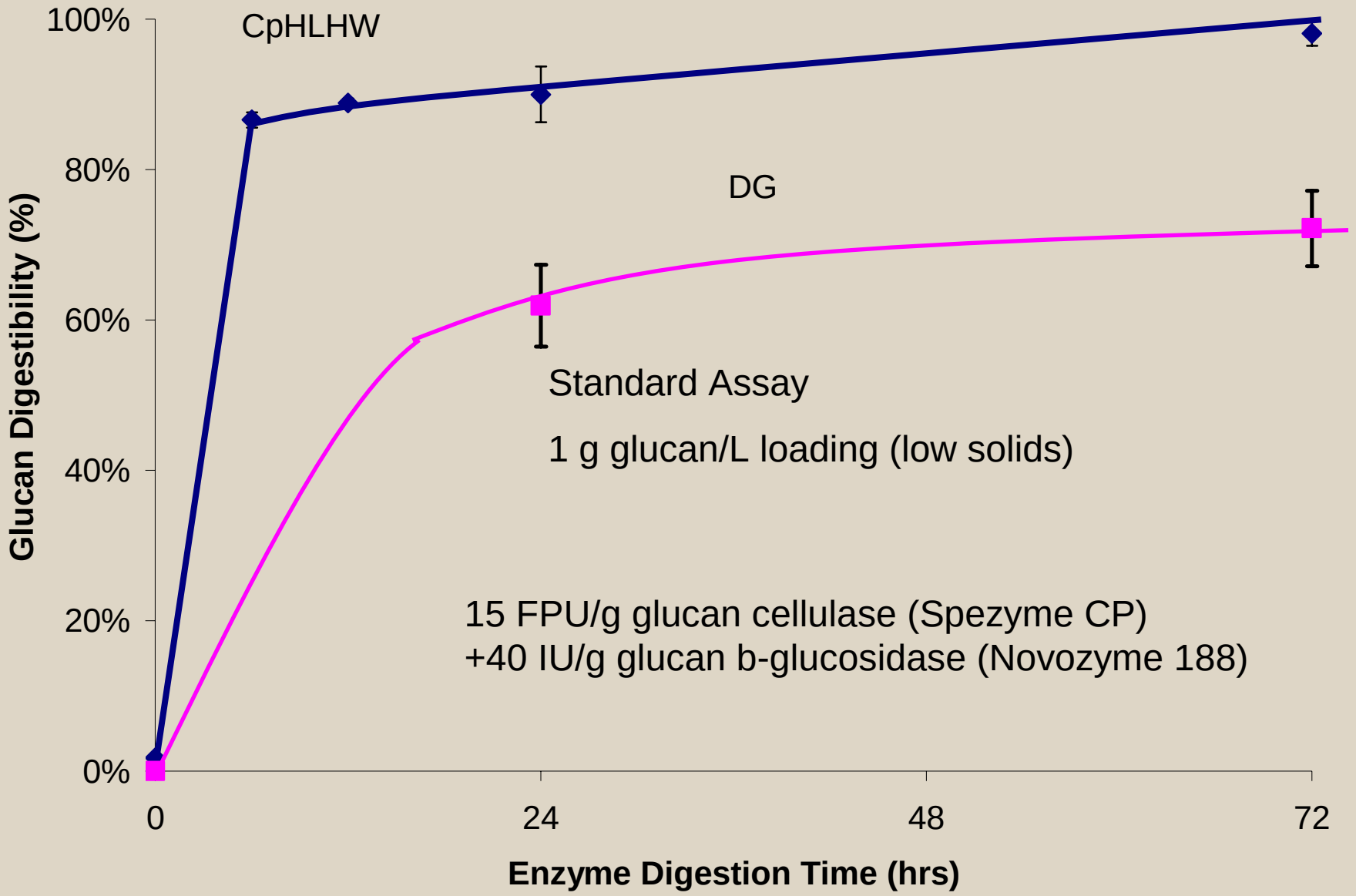
- pH control through buffer capacity of liquid
- Minimal fermentation inhibitors, no wash stream
- Minimize hydrolysis to monosaccharides thereby minimizing degradation



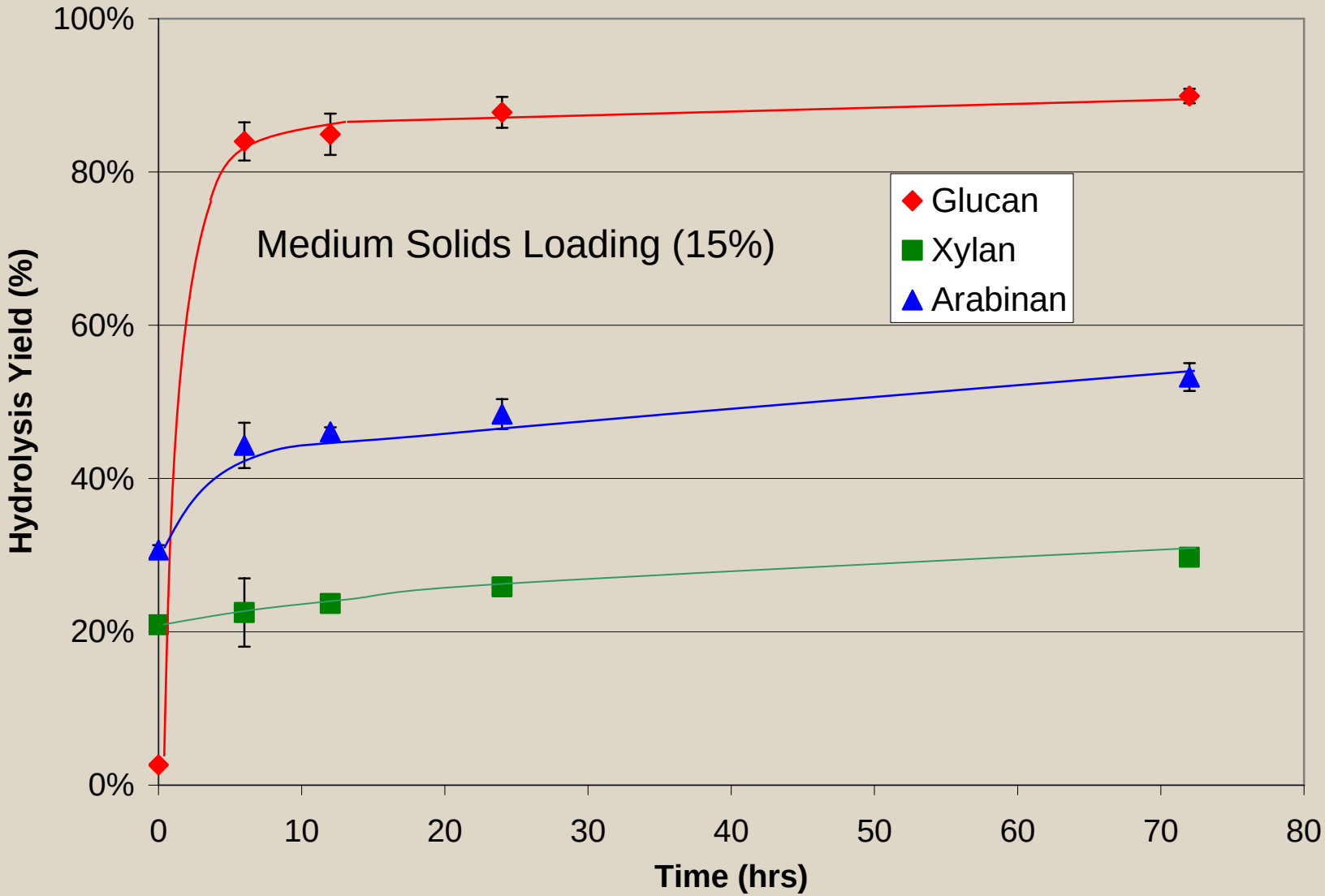
Pretreatment of Distillers Grain

- Wet Cake
- 15 – 30% Wet Cake Slurry in Light Stillage
- 160 C
- 20 minutes

Cellulose Digestibility



Combined Glucose, Xylose, and Arabinose Yields



Pretreated DG – Effect of Solids Loading

Pretreated DG – Effect of Enzymatic Hydrolysis (1.5 hrs)

Liquefaction by enzyme hydrolysis

No additional liquid added

Effect of Solids Loading on Pretreatment and Hydrolysis

	15% DG Solids (% mass dissolved)	20% DG Solids (% mass dissolved)	30% DG Solids (% mass dissolved)
Pretreatment 160° C, 20 Min	29.8%	29.7%	30.0%
Pretreatment + Enzyme Hydrolysis	45.2%	45.1%	47.8%


Sugar Yields: 24 hr Hydrolysis with Cellulase

24 Hours of Hydrolysis by 15 FPU/g glucan in DG Cellulase + 40 IU/g glucan β-glucosidase

Sugar (g/L)	15% Solids	20% Solids	30% Solids
Glucose (g/L)	26.0	32.8	42.2
(% yield)	68%	71%	73%
Xylose (g/L)	4.5	5.8	2.8
(% yield)	19%	19%	19%

% yield includes polysaccharides from both DG and stillage


Total Phosphorus Results

	Total P (% dry matter)
Ground Corn	0.42%
DG	0.49%
Stillage	0.195%
DDGS	1.05%
DG Pretreated Solids	0.00%
DG Pretreated Liquid	0.28%


Fermentation

- DG + Stillage Pretreated
- Cellulase hydrolysis (50 C) for 24 hrs
- Inoculation Cell Mass – 9.5 g/L (400 KU)
- 30 C, 200 rpm
- Fermentation stopped after 48 hrs



Fermentation: 30% DG + Stillage

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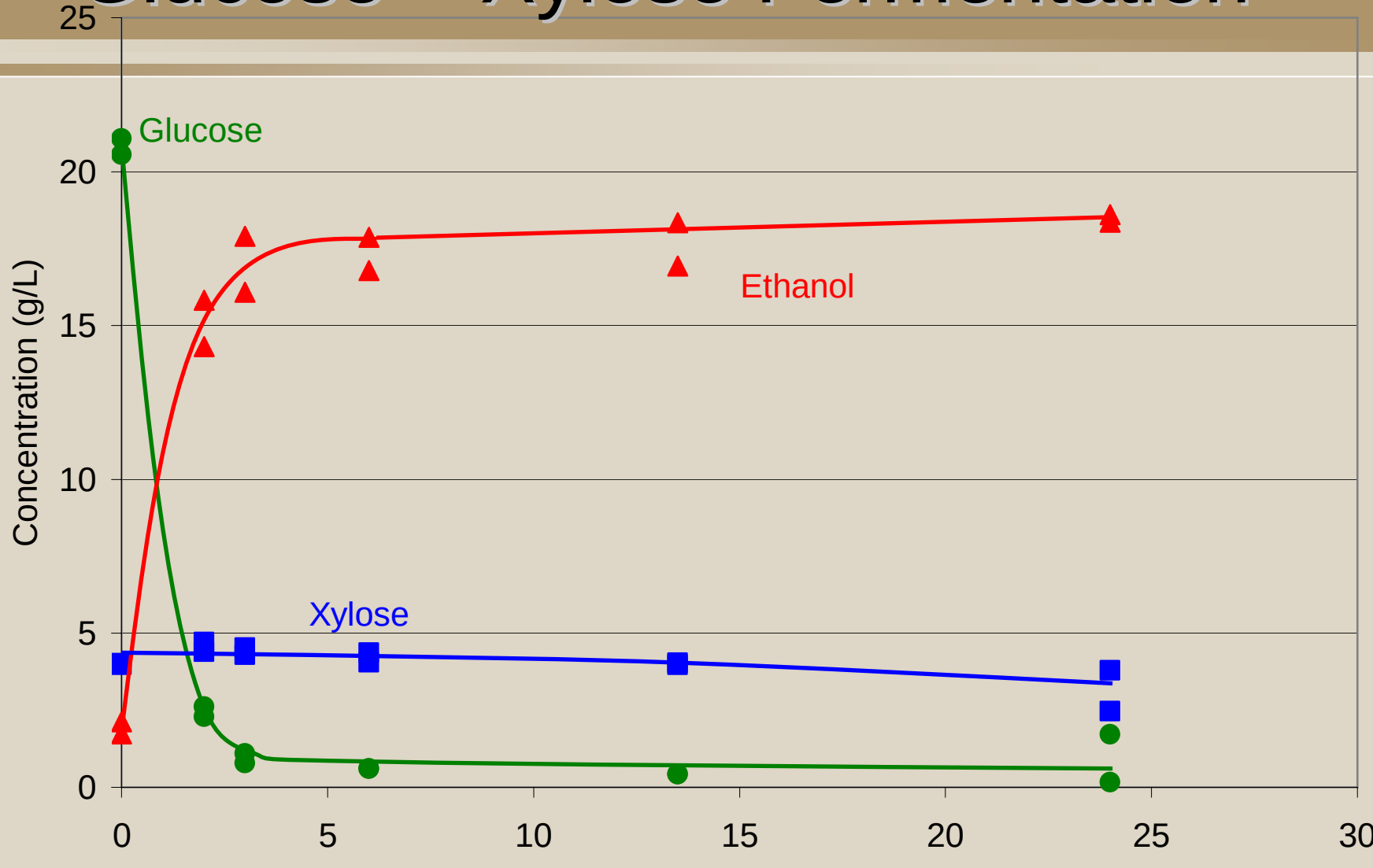
Utilization of Xylose

- Yeast unable to ferment xylose
- Genetic engineering

- Enzymes not optimized for hemicellulose hydrolysis
- Genetic engineering!

Glucose + Xylose Fermentation



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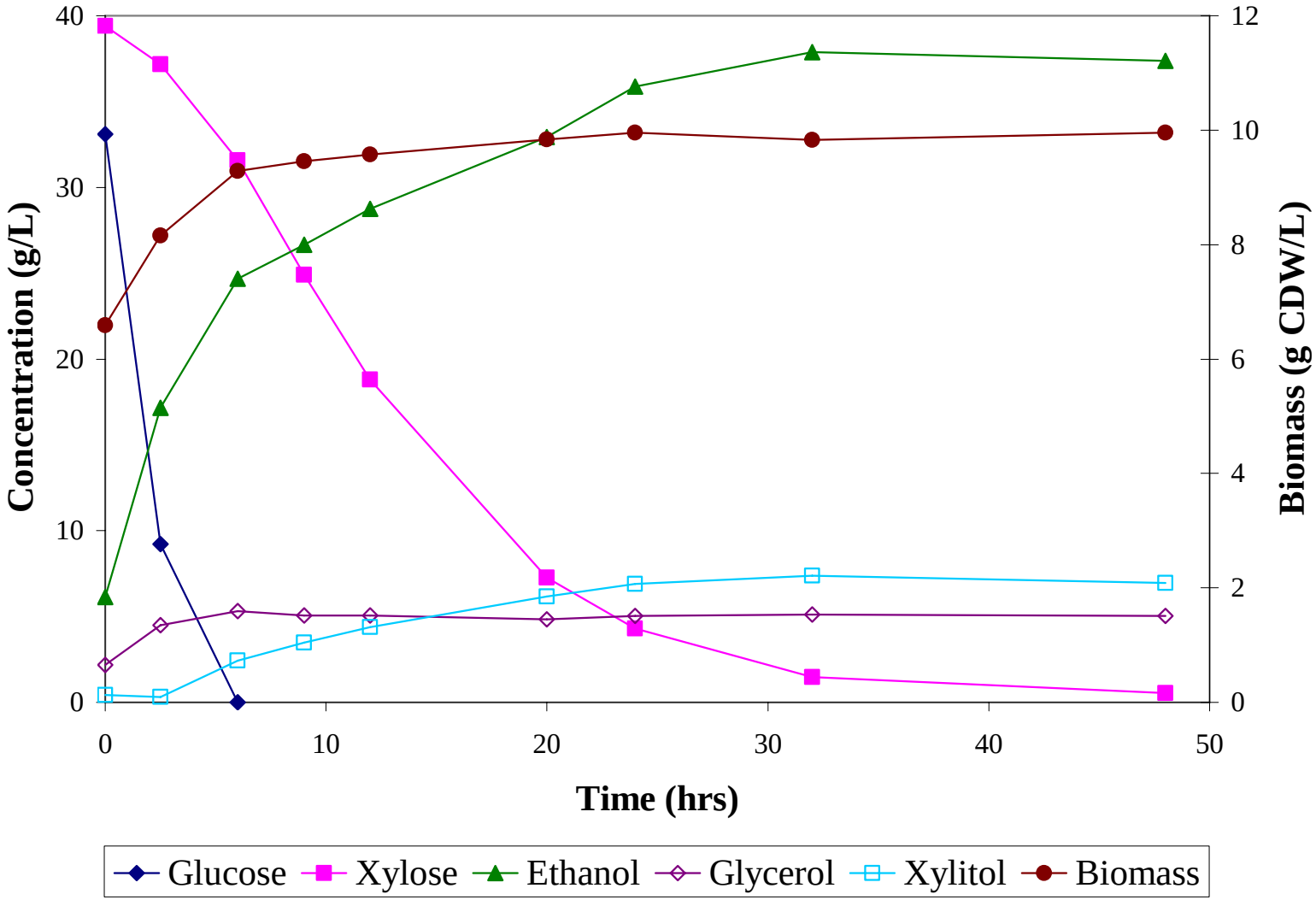
Control Fermentation



Xylan hydrolyzing enzymes (ENZ)

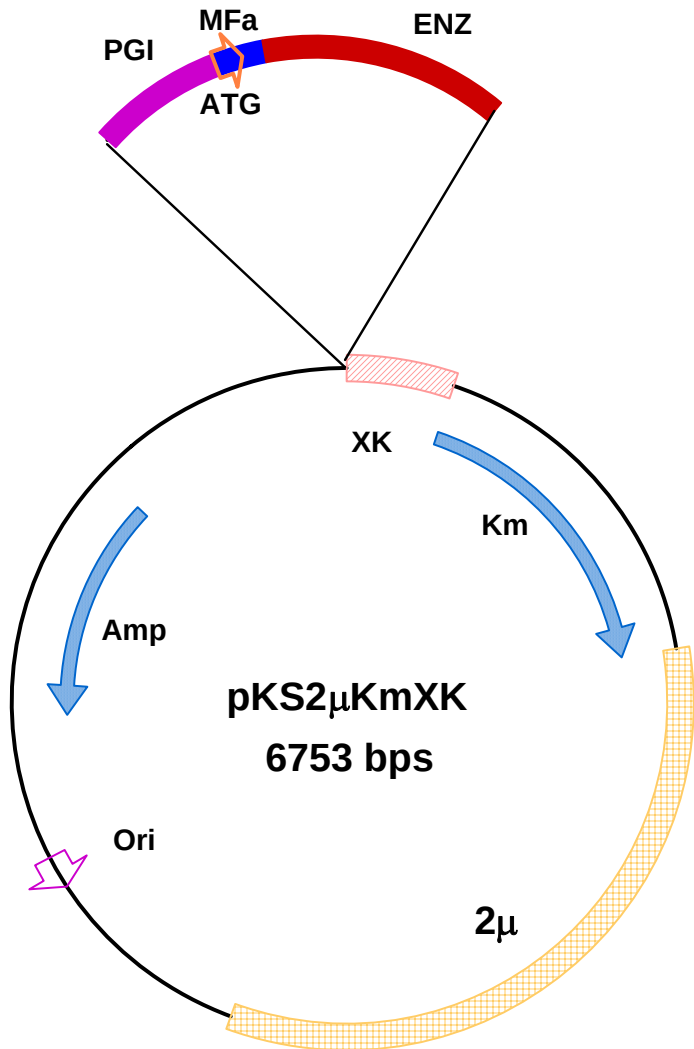
α - Glucuronidase - Y15405 (*A. tubingensis*)

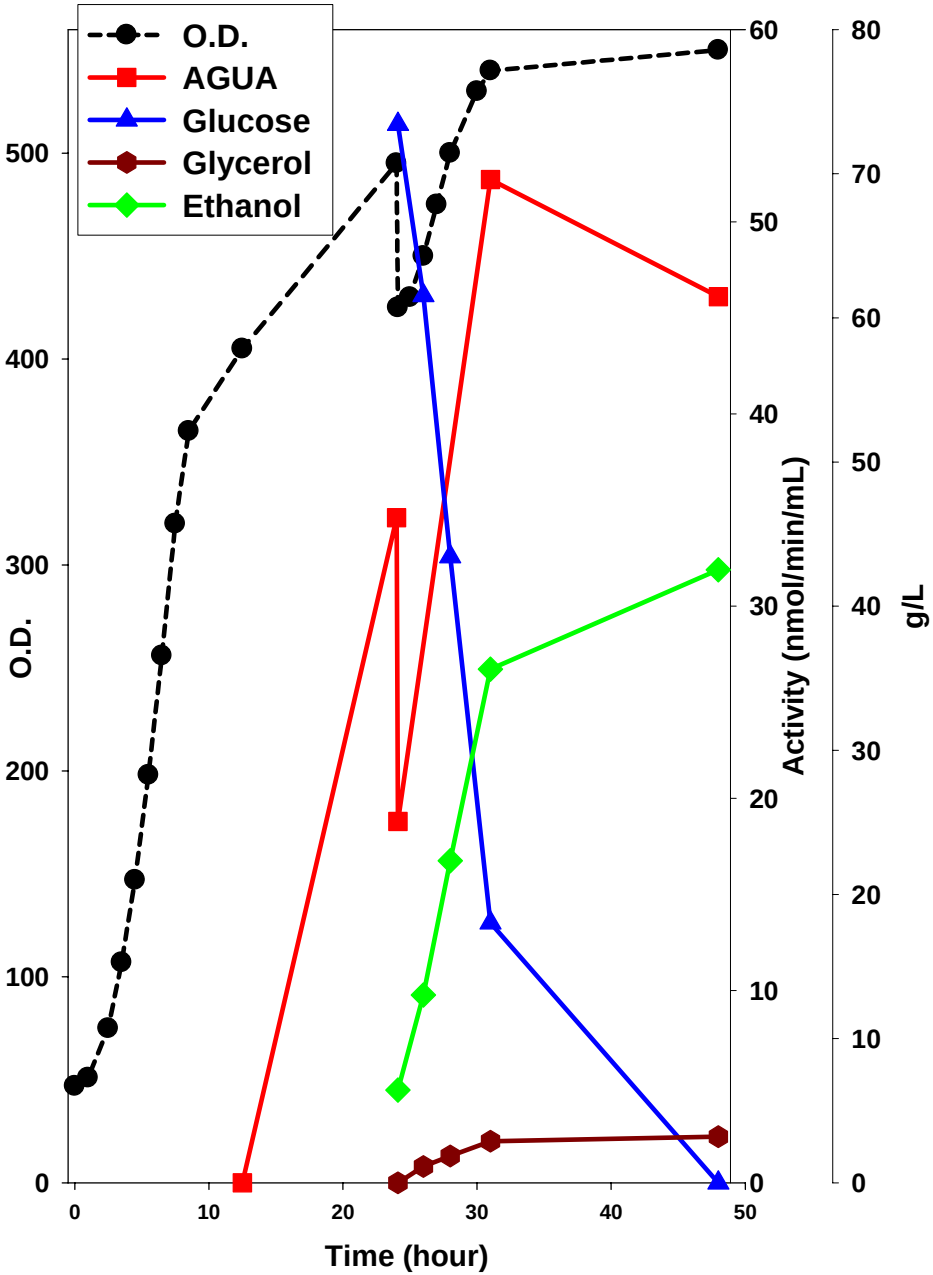
β - Xylosidase - Y13568 (*A. nidulans*)

β - Xylosidase/ arabinosidase - AF040720 (*Selenomonas ruminantium*)

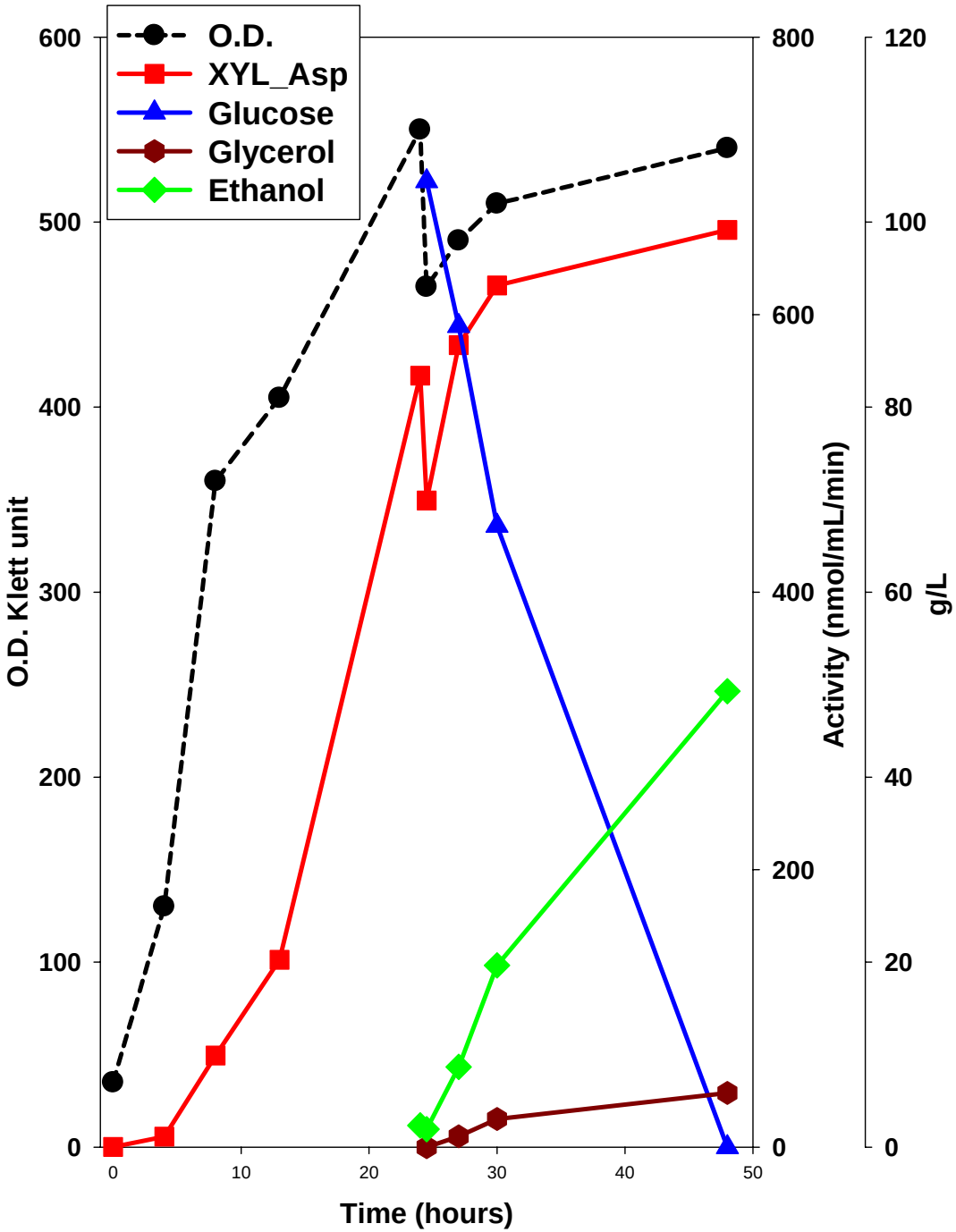
Combination of OE-PCR and PCR

PGI-MFa-ENZ

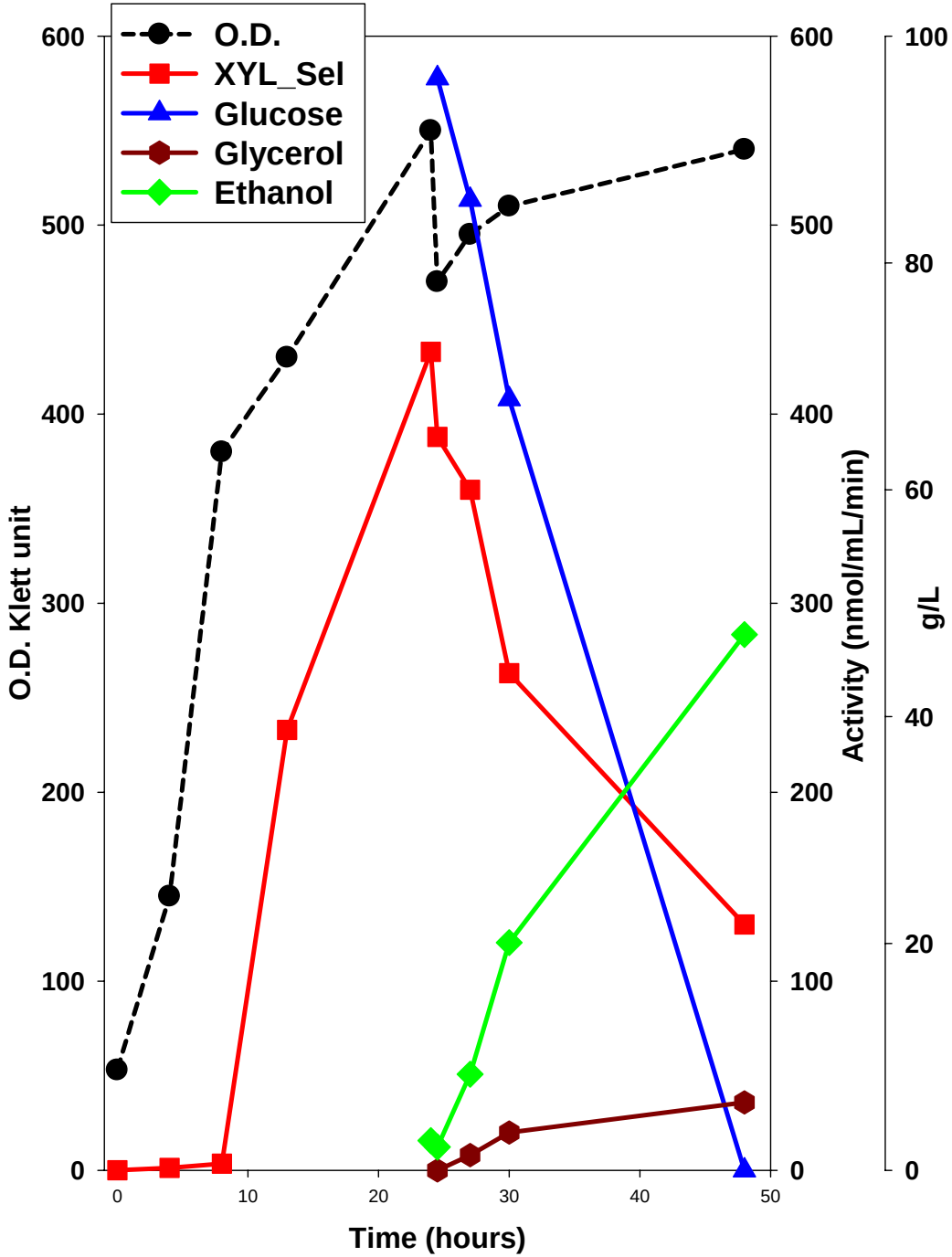




Production of *A. tubingensis* α- glucuronidase by *S. cerevisiae* during growth and fermentation



Production of *A. nidulans* β-xylosidase by *S. cerevisiae* during growth and fermentation



Production of *S. ruminantium* β- xylosidase/ arabinosidase by *S. cerevisiae* during growth and fermentation (Gene supplied by NCAUR)

Summary

- Distillers Grain is more easily digested than other lignocellulosic biomass
- Liquid hot water pretreated (controlled pH) increases reactivity
  - Rate
  - Yield
- Sugar stream fermented to ethanol at high yield