



Enzyme Digestibility of Distillers' Grains for Additional Ethanol Production

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For gifts of enzymes

Genencor (Enzymes)

Pretreated liquid and solid fiber streams

Big River Resources

Interactions and Inputs

Bruce Dale, Michigan State

Mike Cotta, Bruce Dien, USDA NCAUR

Linda Liu, John Schumm, LORRE Purdue University



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DDGS Composition

Gross Matter Composition		Average (95% confidence intervals)
	Moisture Content (% total)	
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)	26.4%
	Total Dry Matter Mass Closure	92.7%

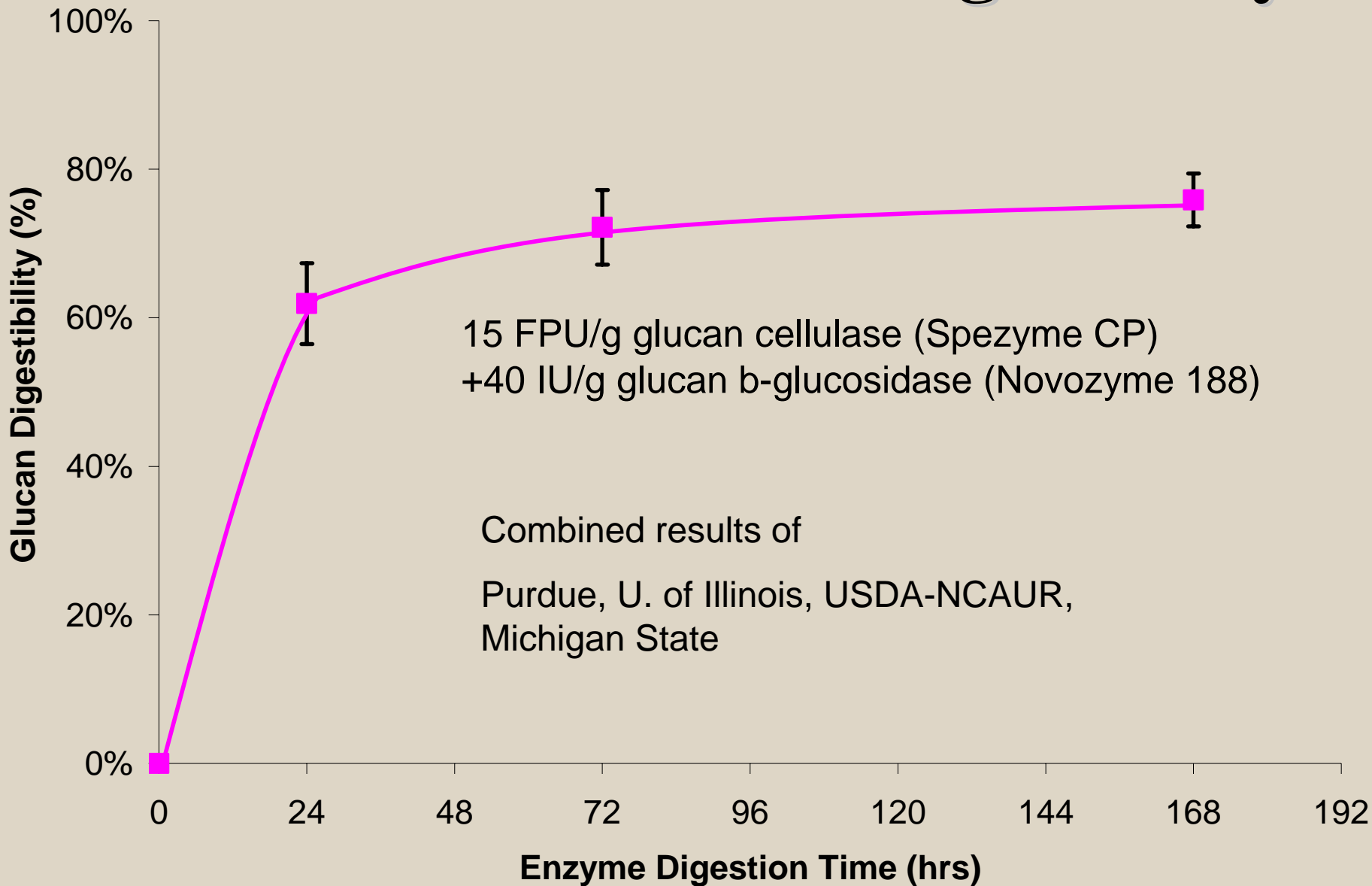


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DDGS: Cellulose Digestibility



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)



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Pretreatment Needed

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



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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

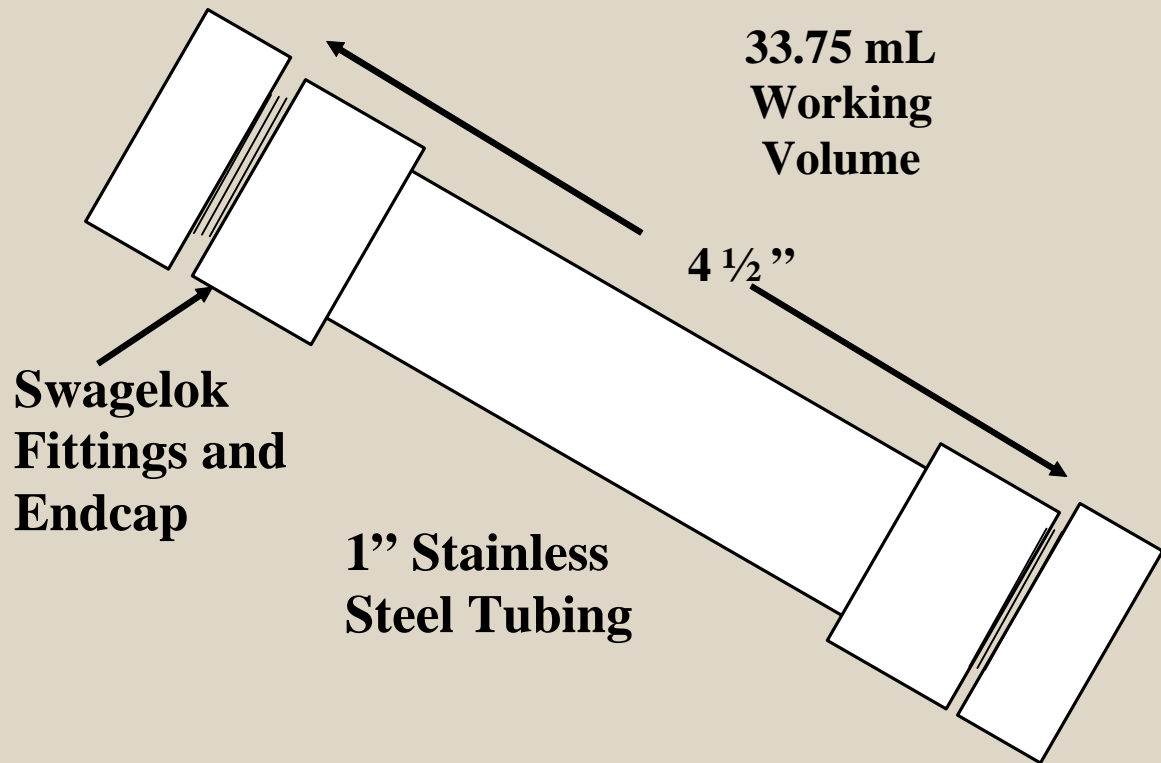


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Controlled pH Liquid Hot Water Pretreatment

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



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Preliminary Pretreatment

- DDGS as received (no further grinding)
- 15 % Solids Slurry in Water
- 160 C
- 20 minutes

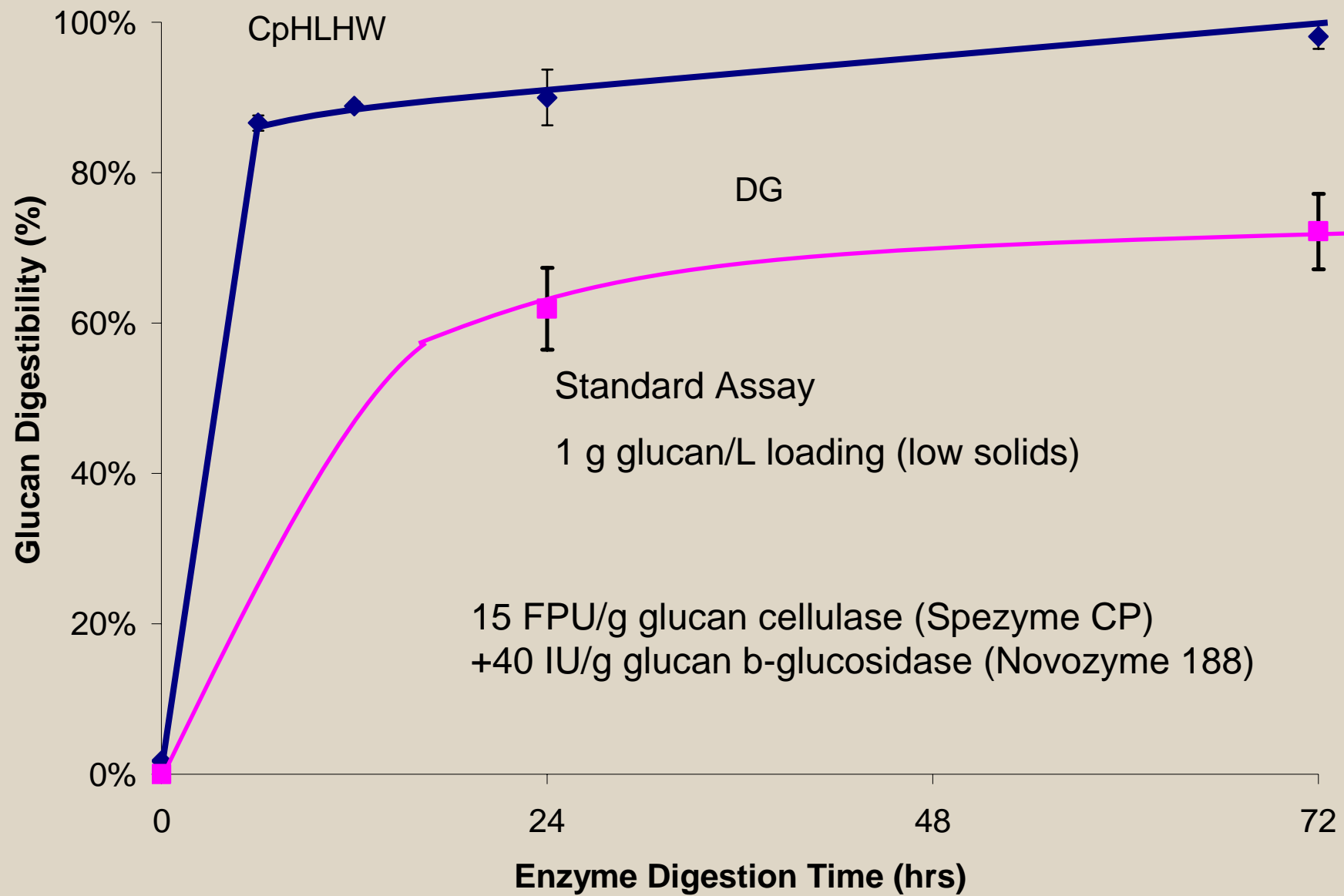


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Cellulose Digestibility





Comparison

Enzyme Digestibility of DDGS (%) Measured at U. Illinois

Time (h)	Untreated DDGS	AFEX treated DDGS	Hot Water treated DDGS
0	2.3	2.4	1.7
6	-	64.3 ± 1.1	78.3 ± 1.1
12	-	75.6 ± 0.7	88.4 ± 1.1
24	62.7 ± 0.5	89.6 ± 1.6	95.8 ± 1.0
48	-	97.8 ± 0.9	99.6 ± 0.8
72	71.5 ± 1.4	102.6 ± 2.7	99.1 ± 1.9
168	76.2 ± 1.1	-	-

Need to optimize with respect to hemicellulose hydrolysis, enzyme composition, sugar concentration

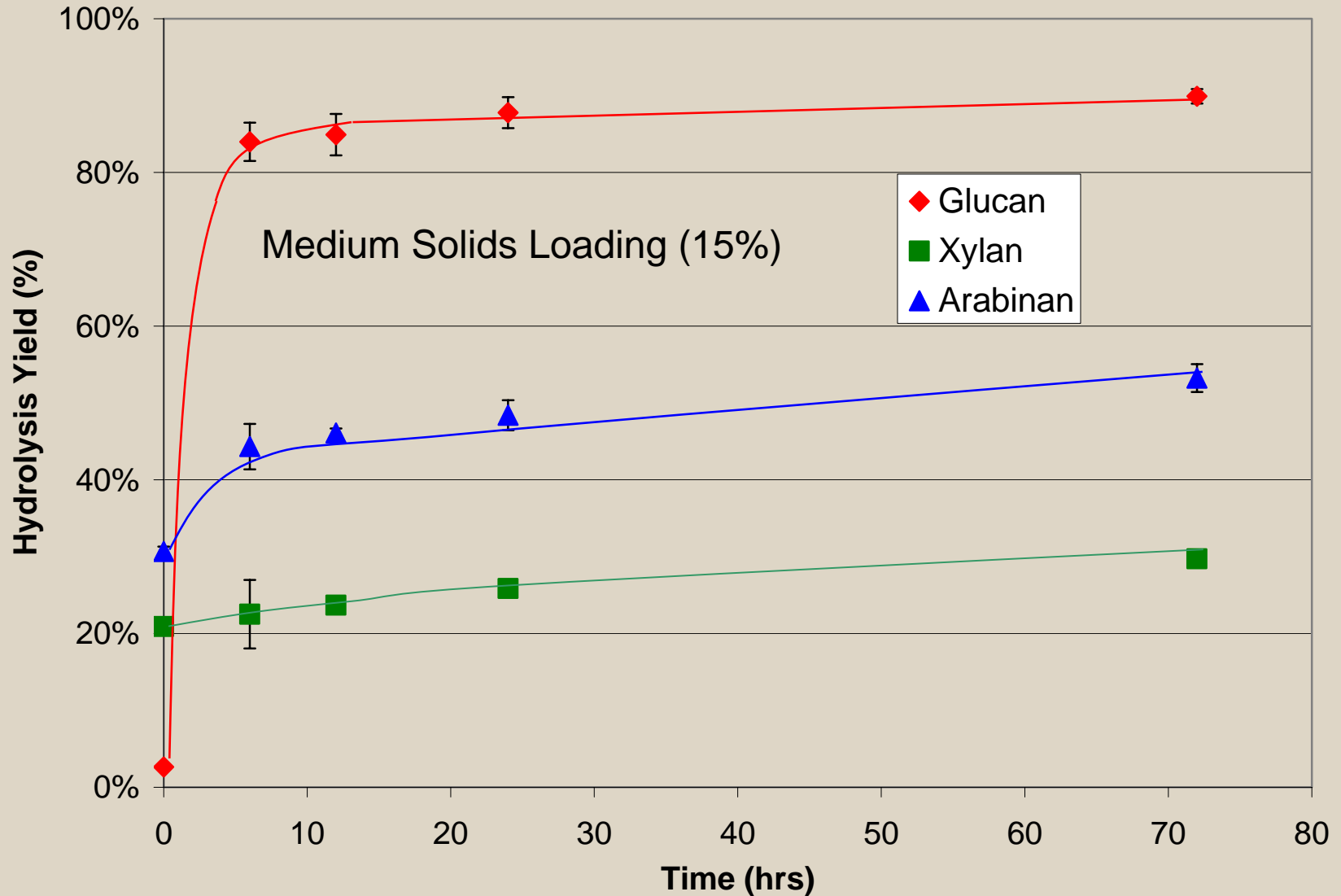


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Combined Glucose, Xylose, and Arabinose Yields



Pretreatment of Wet DG

- Wet DG as received (no further grinding)
- 15 - 30% Solids Slurry in Light Stillage
- 160 C
- 20 minutes



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DG – Before Pretreatment



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DG – Compositional Analysis

Gross Matter Composition	Average	
	Moisture Content (% total)	64.7%
	Dry Matter Content (% total)	35.5%
Dry Matter Carbohydrate Composition	Glucan (total) (% dry matter)	21%
	Xylan (% dry matter)	15.4%
	Arabinan (% dry matter)	11.6%



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Stillage – Compositional Analysis

	Average Concentration (g/L)
Glucose	0.2 ± 0.003
Glucan (oligosaccharide)	12.6 ± 0.5
Xylose	0.3 ± 0.003
Xylan (oligosaccharide)	2.1 ± 0.1
Arabinose	0.3 ± 0.5
Arabinan	0.7 ± 0.007



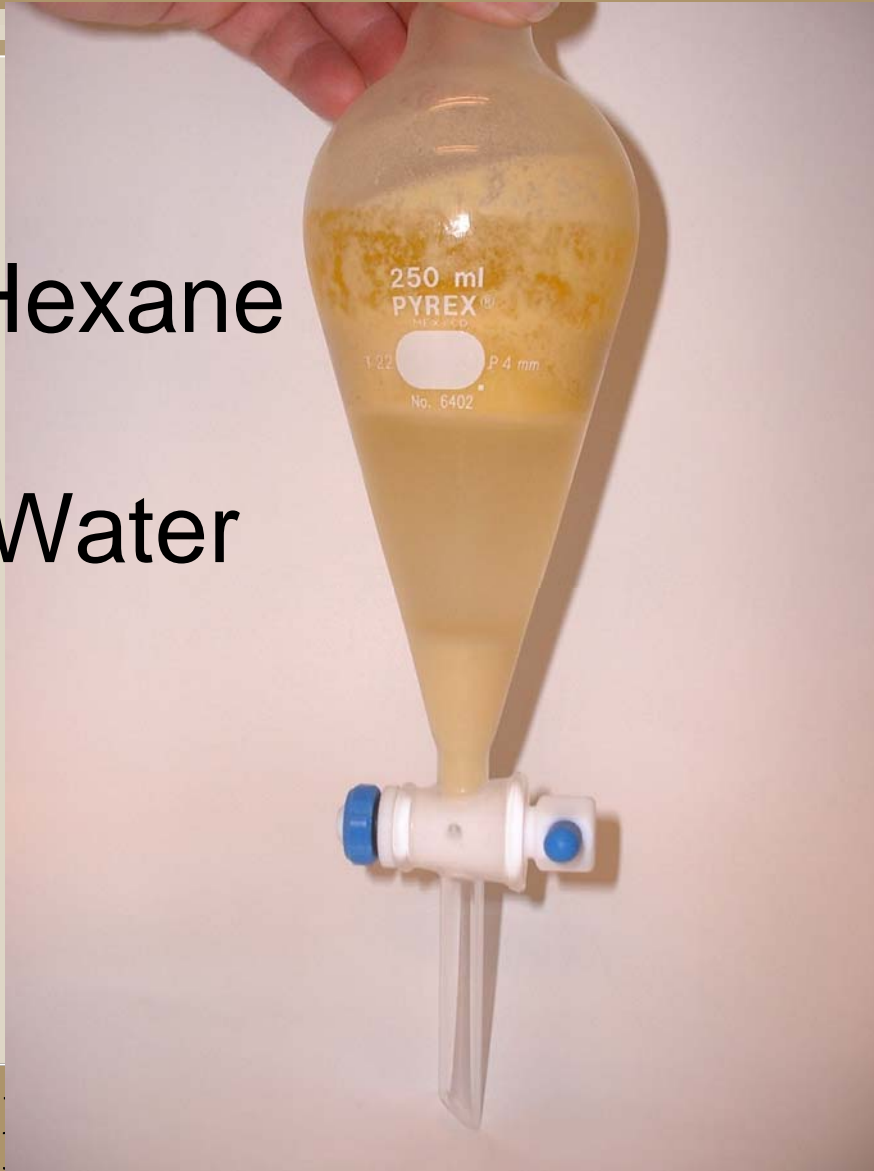
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Stillage – Oil and Protein

Hexane

Water



100 mL
stillage +

100 mL
hexane



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Pretreated DG – Effect of Solids Loading



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Pretreated DG – Effect of Enzymatic Hydrolysis (1.5 hrs)

Liquefaction
by enzyme
hydrolysis



No
additional
liquid
added



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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



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Fermentation

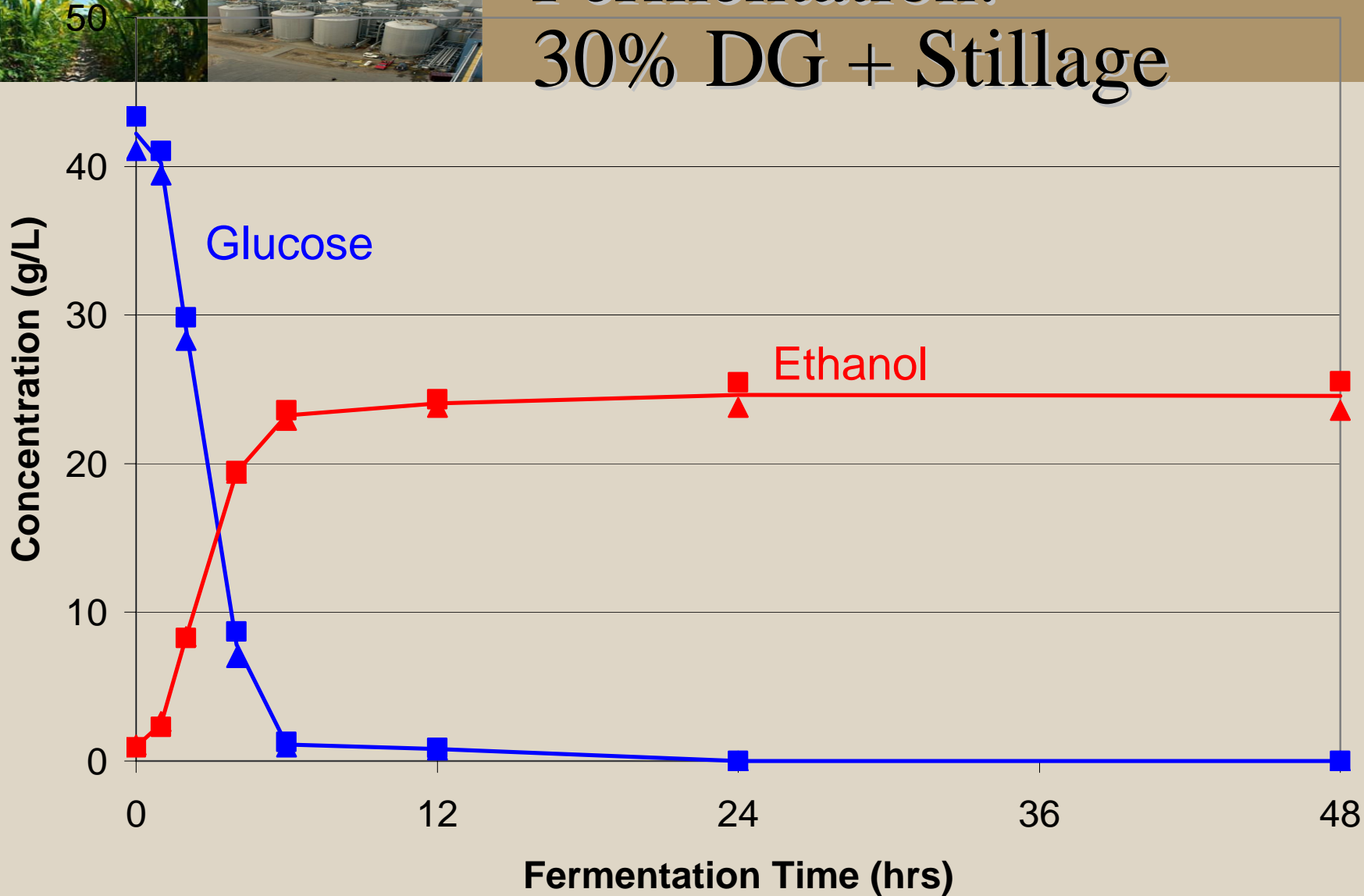
- DG + Stillage Pretreated
- Cellulase hydrolysis (50 C) for 24 hrs
- Inoculation Cell Mass – 9.5 g/L (400 KU)
- 30 C, 200 rpm
- Fermentation stopped after 48 hrs



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Fermentation: 30% DG + Stillage



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Summary

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



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