



# Update on Compositional Analysis/Aqueous and AFEX Pretreatments

Nathan S. Mosier, Venkatesh Balan, Richard Hendrickson, Miroslav Sedlak,, Bryan Bal, Ming-Woei Lau, John Schumm, Bruce Dale and Michael R. Ladisch

Purdue University and Michigan State



Midwest Consortium for Biobased  
Products & Bioenergy





# Acknowledgements

**Material in this work supported by US Dept of Energy**

DE-FG36-04GO14220

**For gifts of enzymes**

Genencor (Enzymes)

**Liquid and solid fiber streams from dry mill**

Big River Resources

**Industrial Cooperators**

Aventine

Dow AgroSciences

Big River Resources

Genencor

Countrymark Coop (170,000 Agr. Producers; Indiana based refiner and supplier of wholesale fuels)

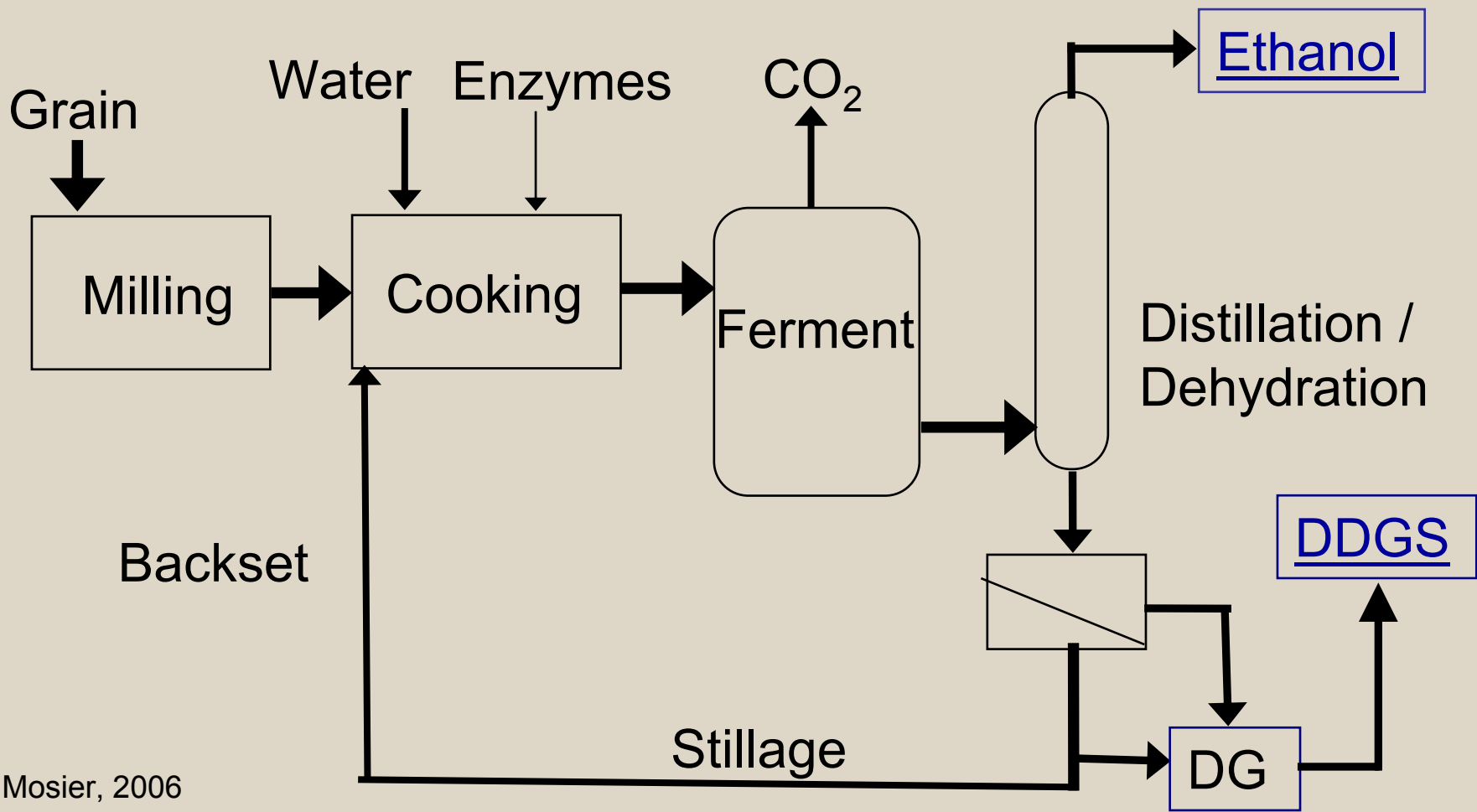


**Midwest Consortium for Biobased  
Products & Bioenergy**





# Dry Mill (Grind) Process



Mosier, 2006



Midwest Consortium for Biobased Products & Bioenergy





# DDGS Composition

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

Based on analysis at U. Illinois, USDA NCAUR, Purdue

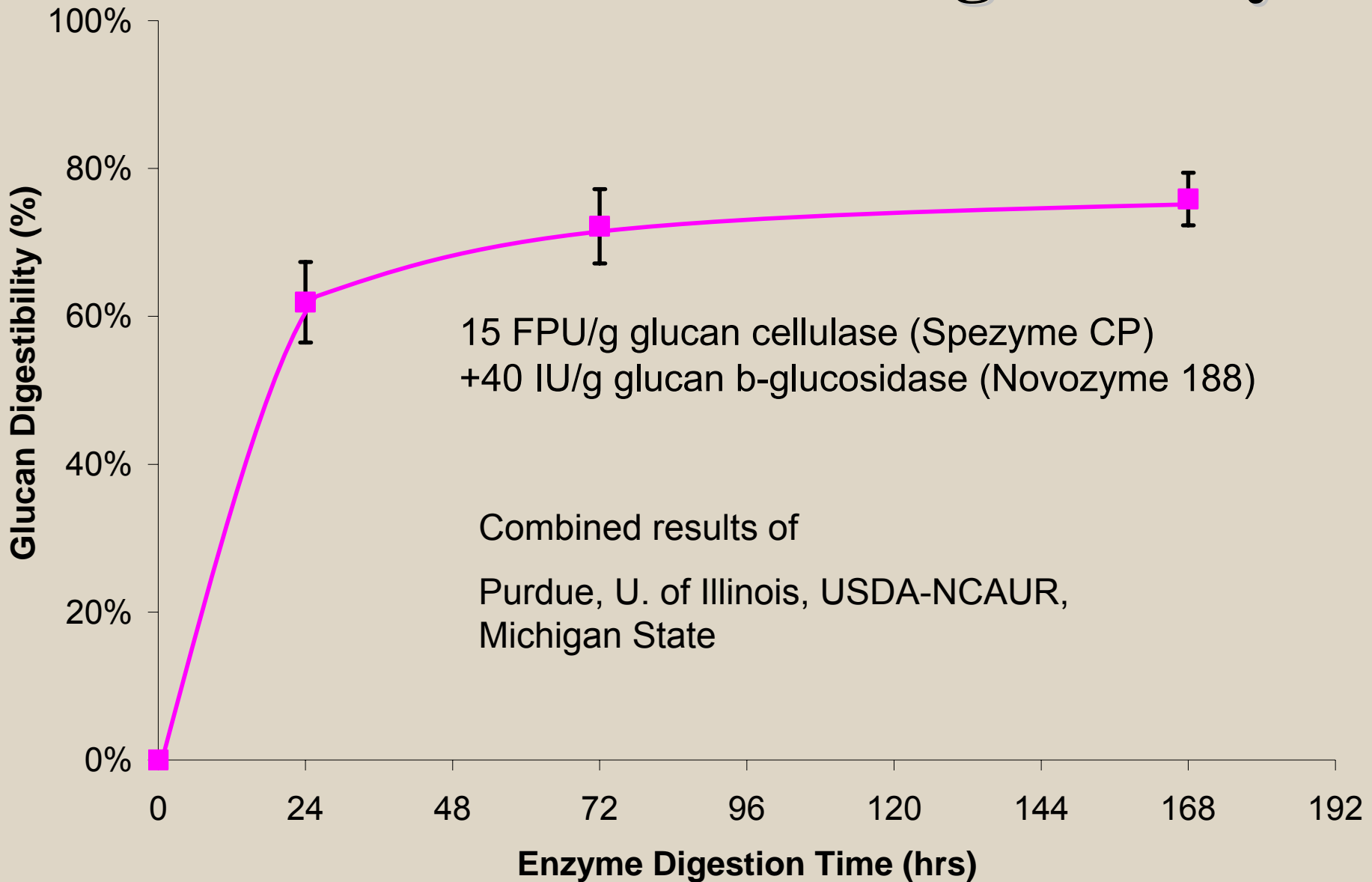


Midwest Consortium for Biobased  
Products & Bioenergy





# DDGS: Cellulose Digestibility



# Cellulose Digestibility of Untreated DDGS

Much slower than starch

Much faster than other lignocellulose (corn stover)

at enzyme loadings of 15 FPU / g cellulose for  
Spezyme with 40 IU / g Novozyme 188 ( $\beta$ -glucosidase)

63% of cellulose converted to glucose in 24 hrs

76% converted in 168 hrs

Based on combined results of Purdue, U. of Illinois, USDA-NCAUR, Michigan State



**Midwest Consortium for Biobased  
Products & Bioenergy**



# Pretreatment

Improves reactivity of plant polysaccharides  
(cellulose and hemicellulose)

Reduces time of hydrolysis (rate)

Increases yield

Reduces enzyme demand (loading)

DDGS used, as received (no grinding)



**Midwest Consortium for Biobased  
Products & Bioenergy**



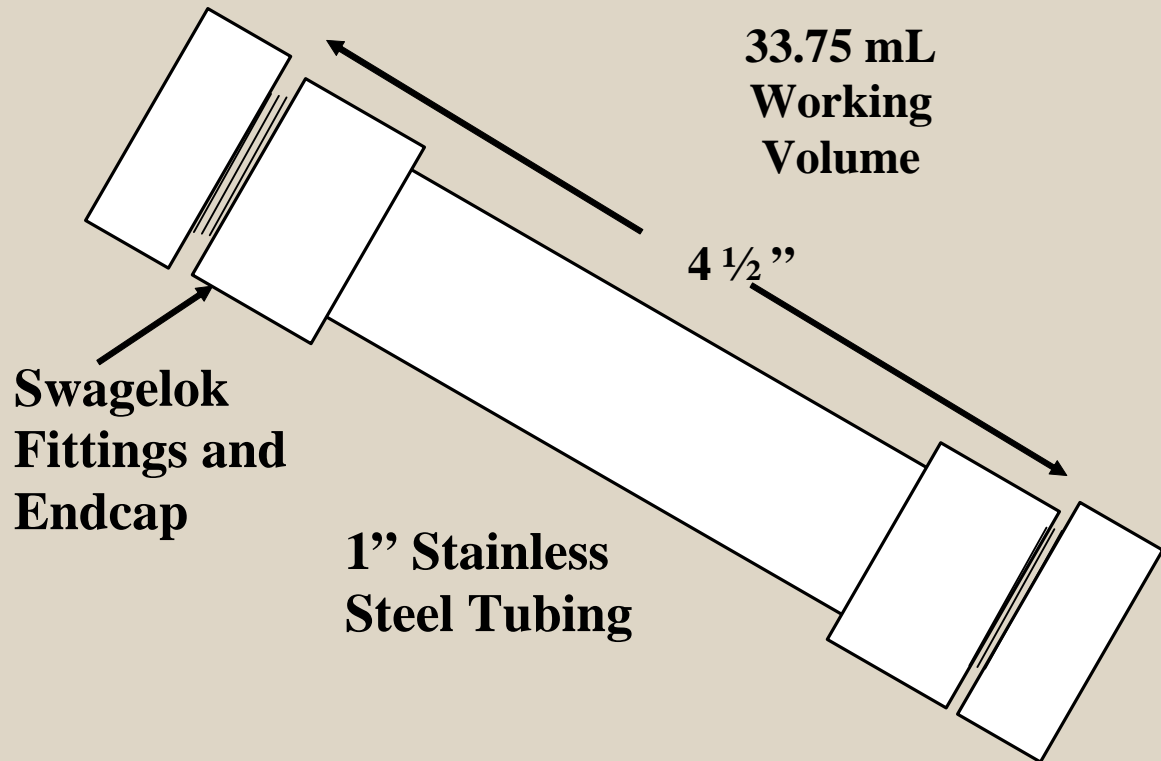
# Controlled pH Liquid Hot Water Pretreatment

Conditions 160 C, 20 min

pH control through buffer capacity of liquid

Minimal fermentation inhibitors, no wash stream

Minimize hydrolysis to monosaccharides thereby minimizing degradation



Midwest Consortium for Biobased Products & Bioenergy



# Pretreated DG – Effect of Solids Loading



Midwest Consortium for Biobased  
Products & Bioenergy



# Pretreated DG – Effect of Enzymatic Hydrolysis (1.5 hrs)

Liquefaction  
by enzyme  
hydrolysis



No  
additional  
liquid  
added

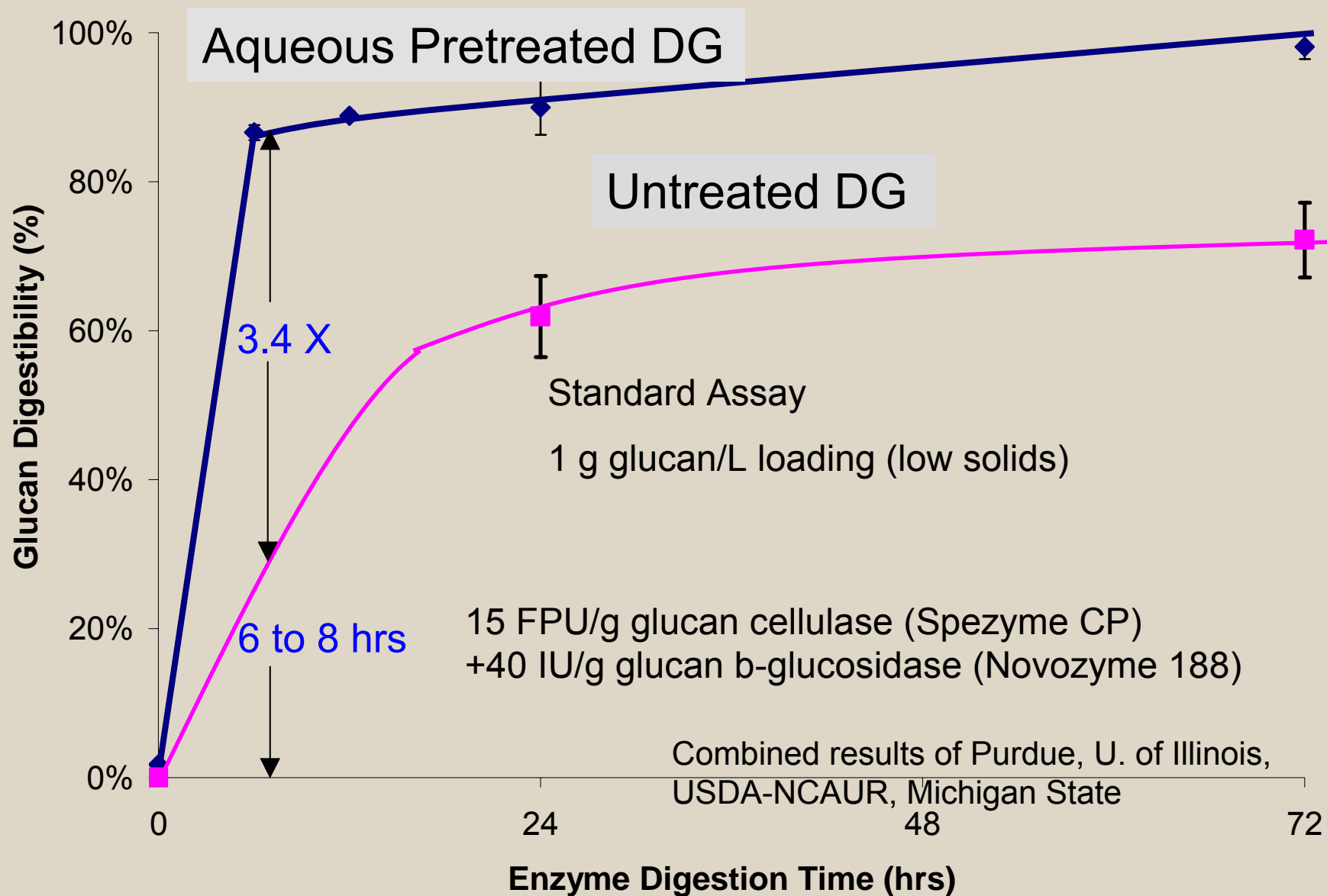


Midwest Consortium for Biobased  
Products & Bioenergy





# Cellulose Digestibility





# Comparison

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

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

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



Midwest Consortium for Biobased  
Products & Bioenergy



# AFEX: Pretreatment and Hydrolysis

AFEX = Ammonia Fiber Explosion

	Temp	NH3	72h hydrolysis yield, 16.6 FPU cellulase / g
11.5% MC	70°C	0.8:1 kg/kg dry	108%
60% MC	80°C	0.6:1 kg/kg dry	103%

Milder than other types of biomass

Very low xylose yields (<10% after 168h)

65% of dry matter solubilized

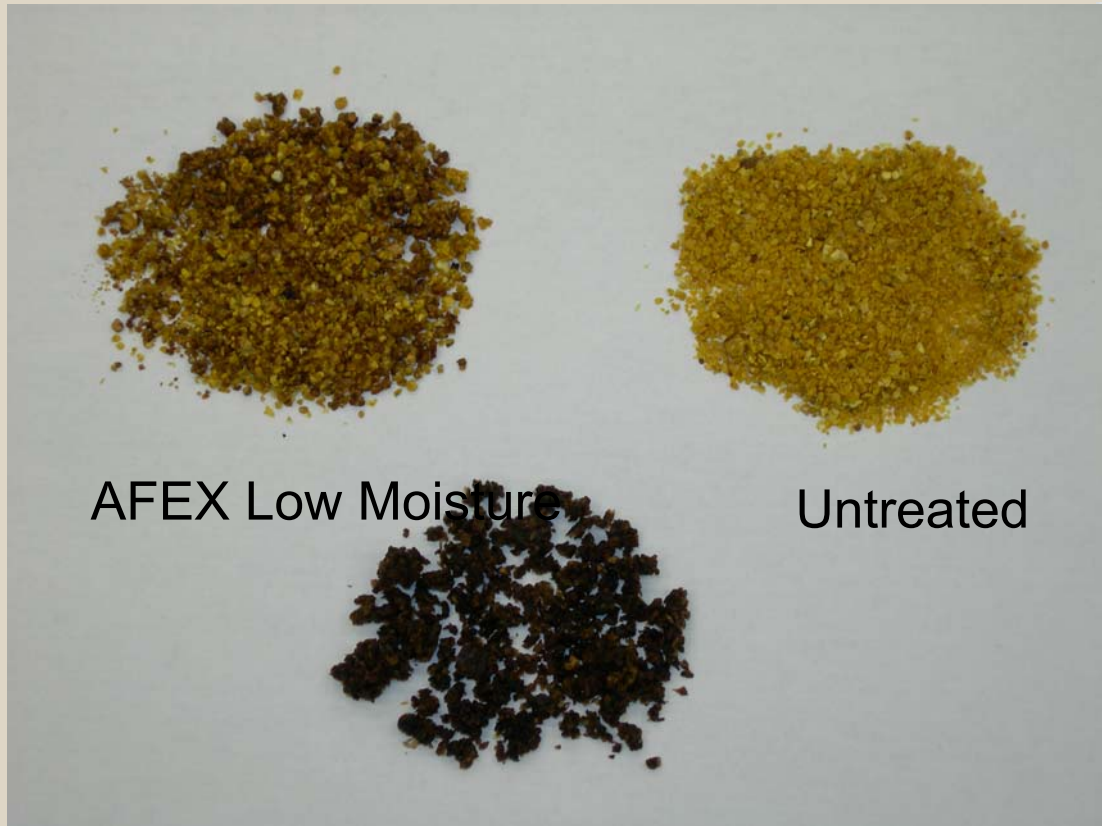
Dale, Balan, Bals



Midwest Consortium for Biobased  
Products & Bioenergy



# AFEX vs Untreated



AFEX Low Moisture

Untreated

AFEX High Moisture



AFEX

Untreated

Dale, Balan, Bals



Midwest Consortium for Biobased  
Products & Bioenergy



# AFEX: Ammonia Loading Effect

## Pretreatment

11.5% moisture

70°C, 5 min

## Hydrolysis

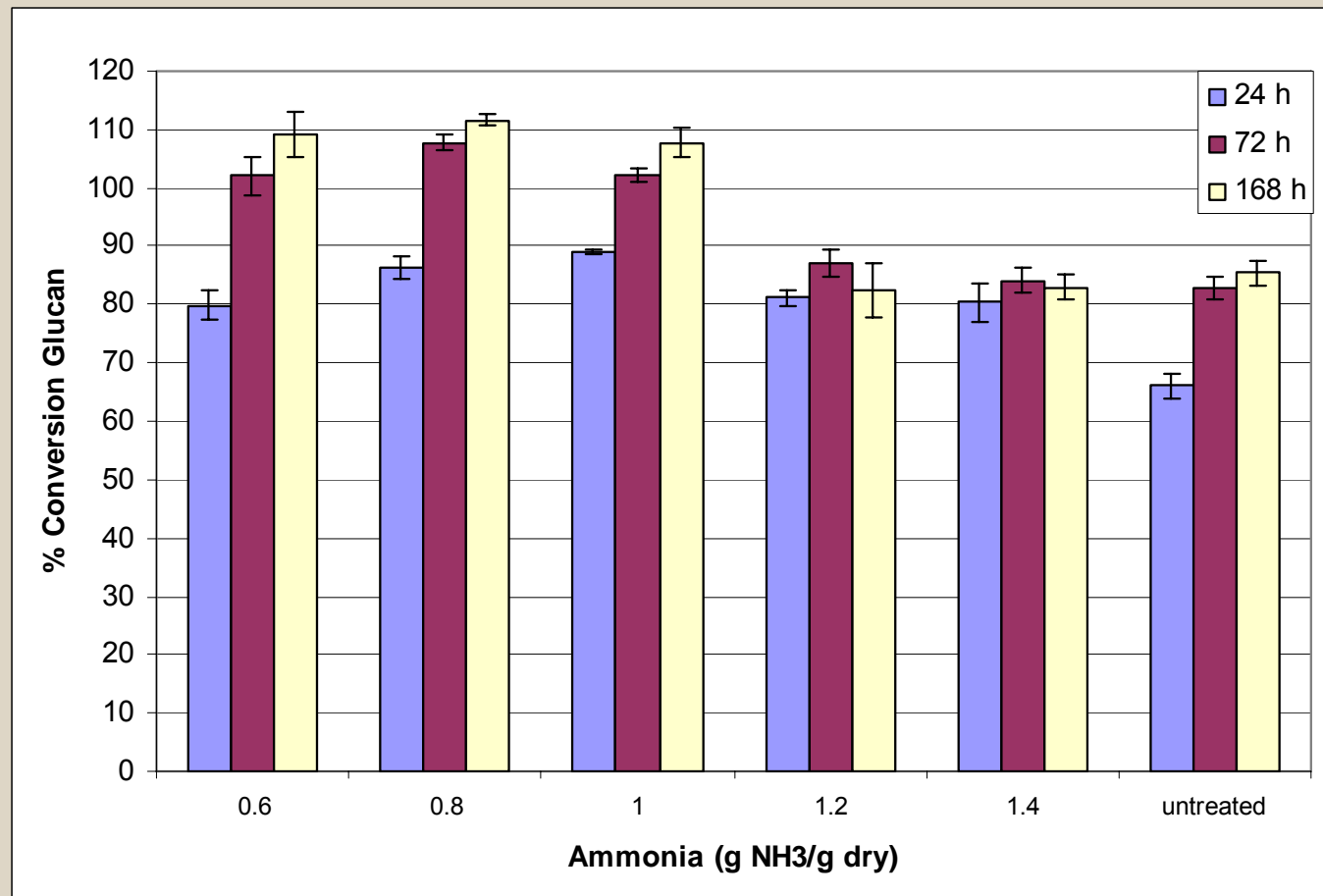
16.6 FPU

cellulase / g

cellulose gives

90% conversion in

24 hours



Midwest Consortium for Biobased  
Products & Bioenergy



# Ammonia Effect

## Constants

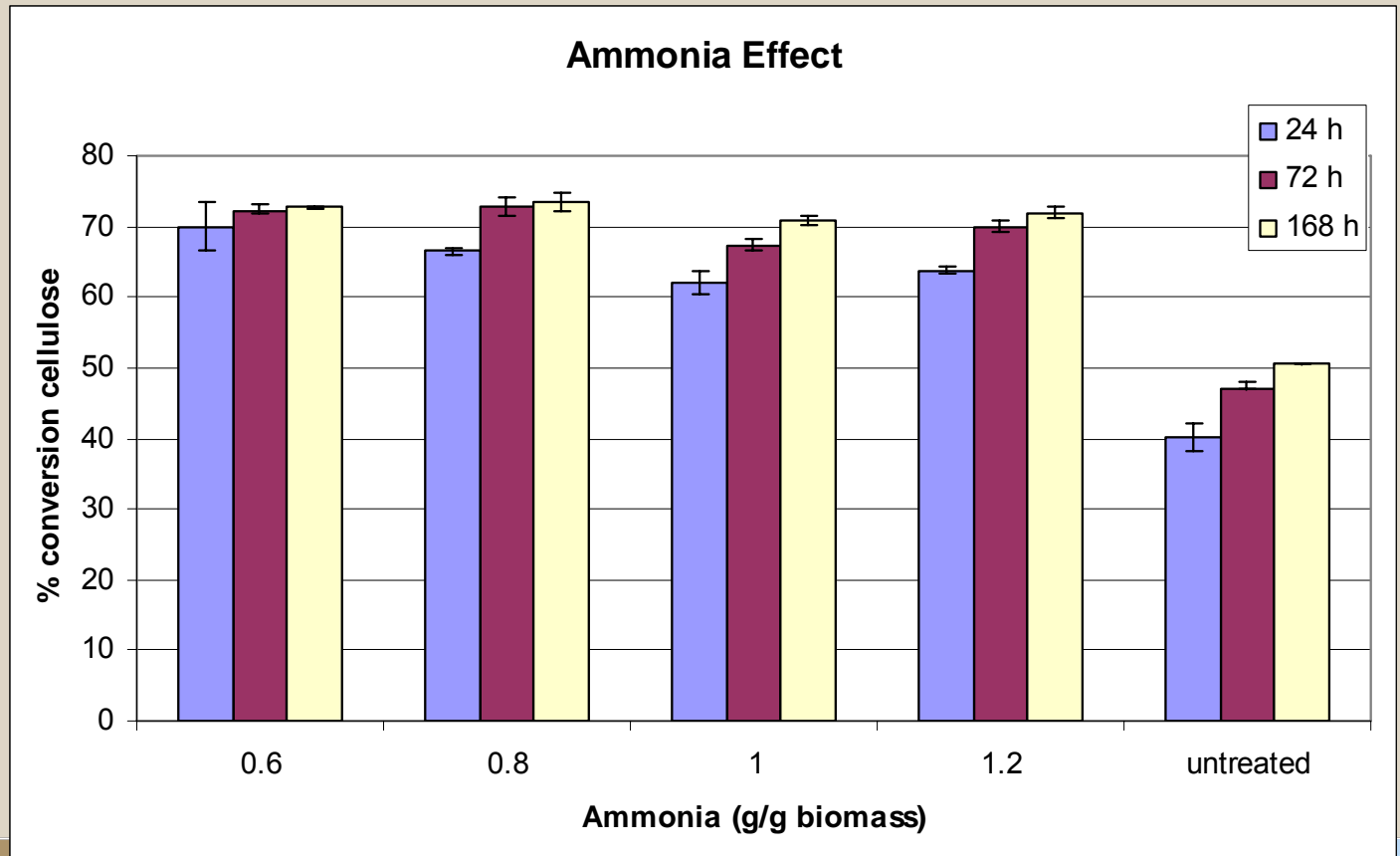
MC: 150% dwb

80 C

5 minute  
residence time

Spezyme CP –  
15 FPU

Assumed 21.0%  
Cellulose



Midwest Consortium for Biobased  
Products & Bioenergy



# Temperature Effect

## Constants

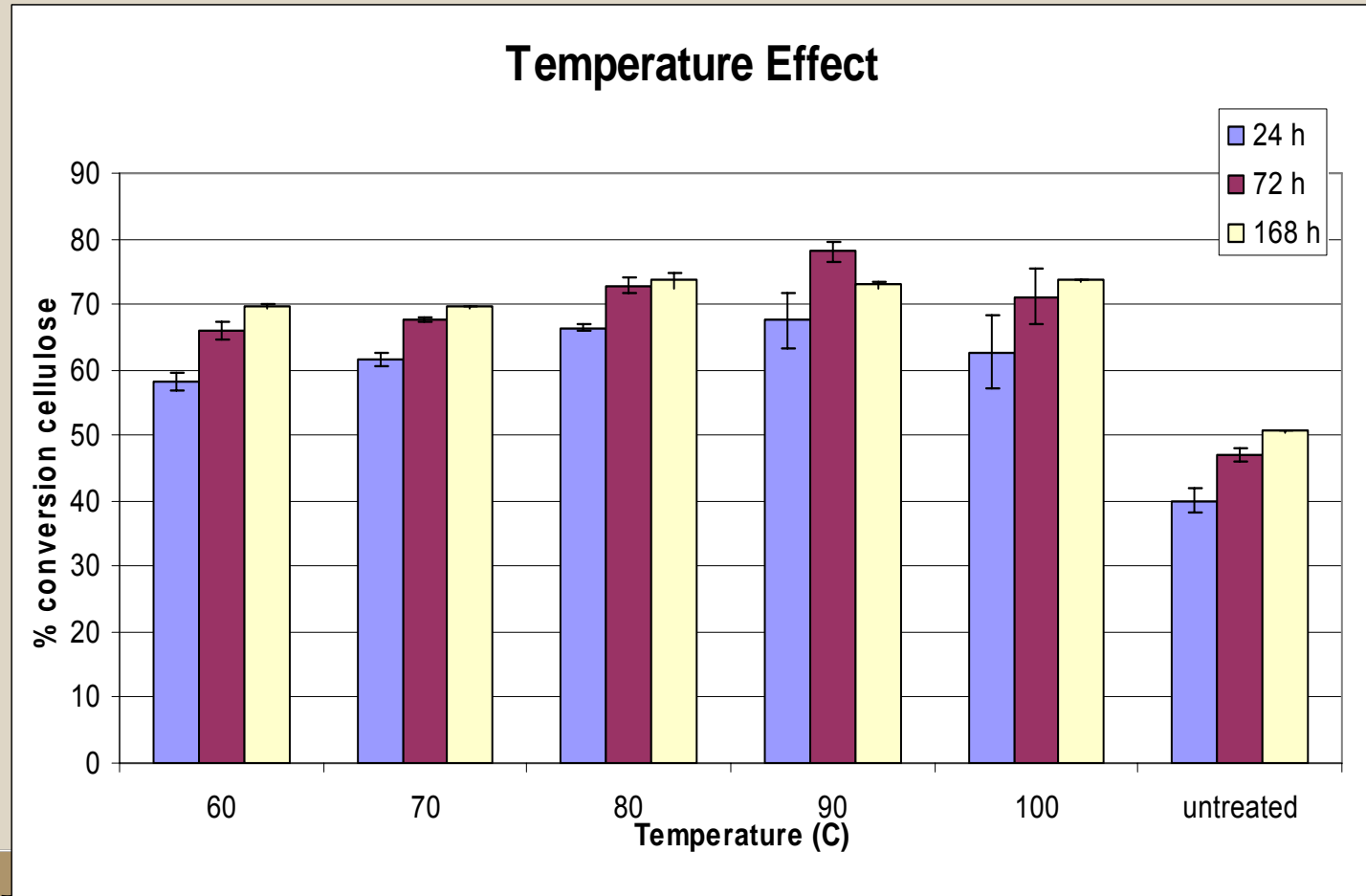
MC: 150% dwb

0.8:1 NH<sub>3</sub>

5 minute  
residence time

Spezyme CP –  
15 FPU

Assumed 21.0%  
Cellulose



Midwest Consortium for Biobased  
Products & Bioenergy



# Summary of Results

Biomass	Treatment	% Cellulose	% yield	g sugar per Kg biomass
DDGS	untreated	16.0%	83%	146
DDGS	13%, 70C, 0.8:1 NH <sub>3</sub>	16.0%	108%	190
DDGS	150%, 80C, 0.6:1 NH <sub>3</sub>	16.0%	103%	181
WC	untreated	21.0%	47%	109
WC	150%, 90C, 0.8:1 NH <sub>3</sub>	21.0%	78%	180

Moisture content too high? Errors in % glucan numbers? Wet cake is more difficult to hydrolyze?



**Midwest Consortium for Biobased  
Products & Bioenergy**



# Amino Acid Profile

Amino Acid	DDGS	Hydrolyzed DDGS	
	(%)	(%)	(g/100g DDGS)
Arginine	1.05%	0.72%	0.30
Histidine	0.71%	0.01%	0.00
Isoleucine	0.99%	0.73%	0.30
Leucine	3.99%	10.85%	4.45
Lysine	0.78%	2.31%	0.95
Methionine	0.50%	0.15%	0.06
Cystine	0.07%	0.02%	0.01
Phenylalanine	1.60%	1.42%	0.58
Threonine	1.04%	0.94%	0.39
Valine	1.48%	1.82%	0.75



Midwest Consortium for Biobased  
Products & Bioenergy





# Corn Co-products Contain Cellulose

	<b>Corn Fiber<sup>1</sup></b>	<b>DDGS</b>	<b>Corn Stover<sup>2</sup></b>
<b>Cellulose</b>	14%	16%	34%
<b>Hemicellulose</b>	24%	13%	29%
<b>Starch</b>	28%	5%	Negligible
<b>Protein</b>	12%	26%	2.3
<b>Lignin</b>	8%	2%	11.0

<sup>1</sup> Mosier *et al.* Appl. Biochem. Biotech. 125(2):77-98 (2005).

<sup>2</sup> Pioneer 33A14, harvested 2002, analyzed by NREL (CAFI 2 project)



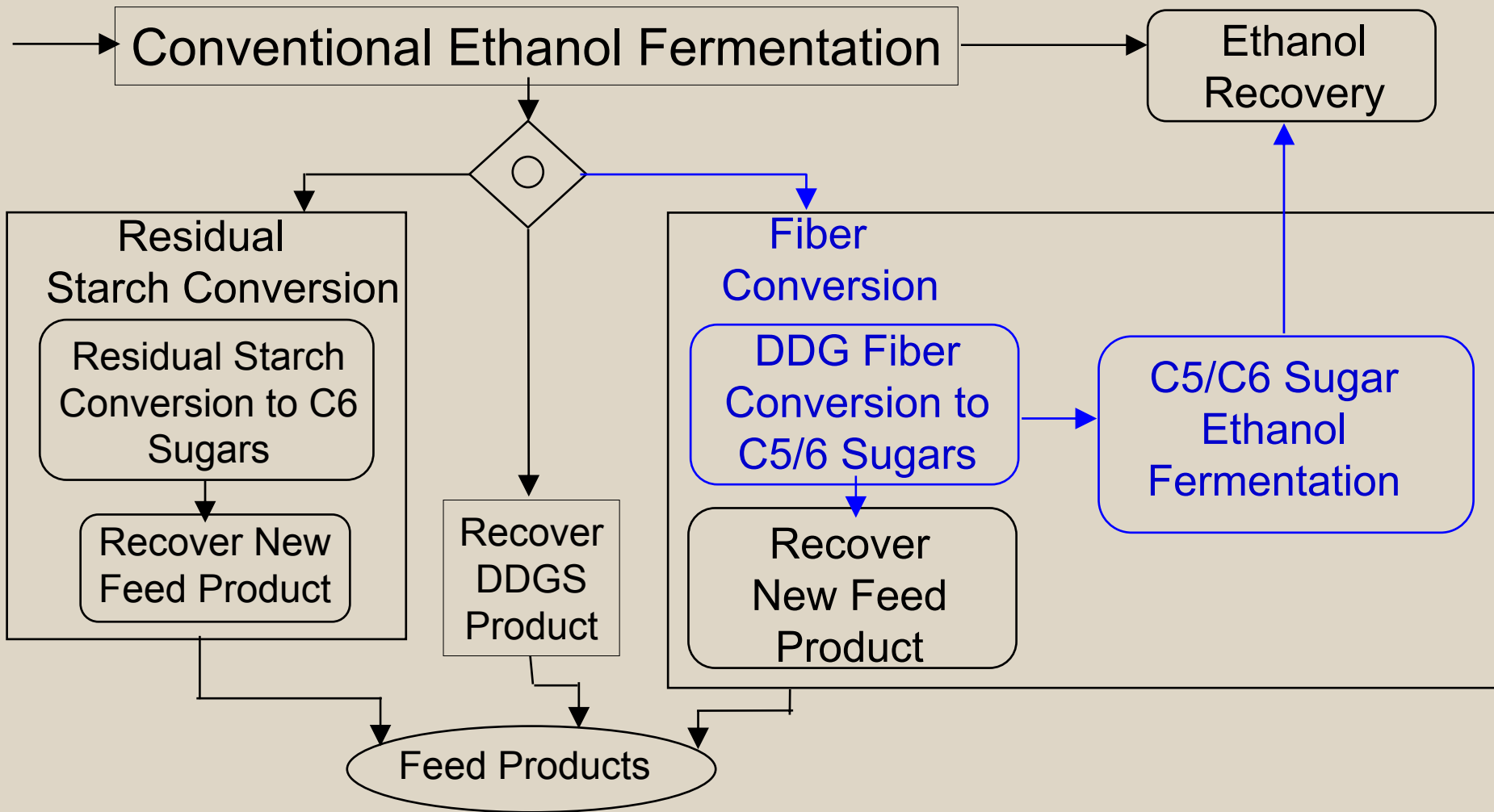
**Midwest Consortium for Biobased  
Products & Bioenergy**





*This paper:*

# Mixed Sugars to Ethanol

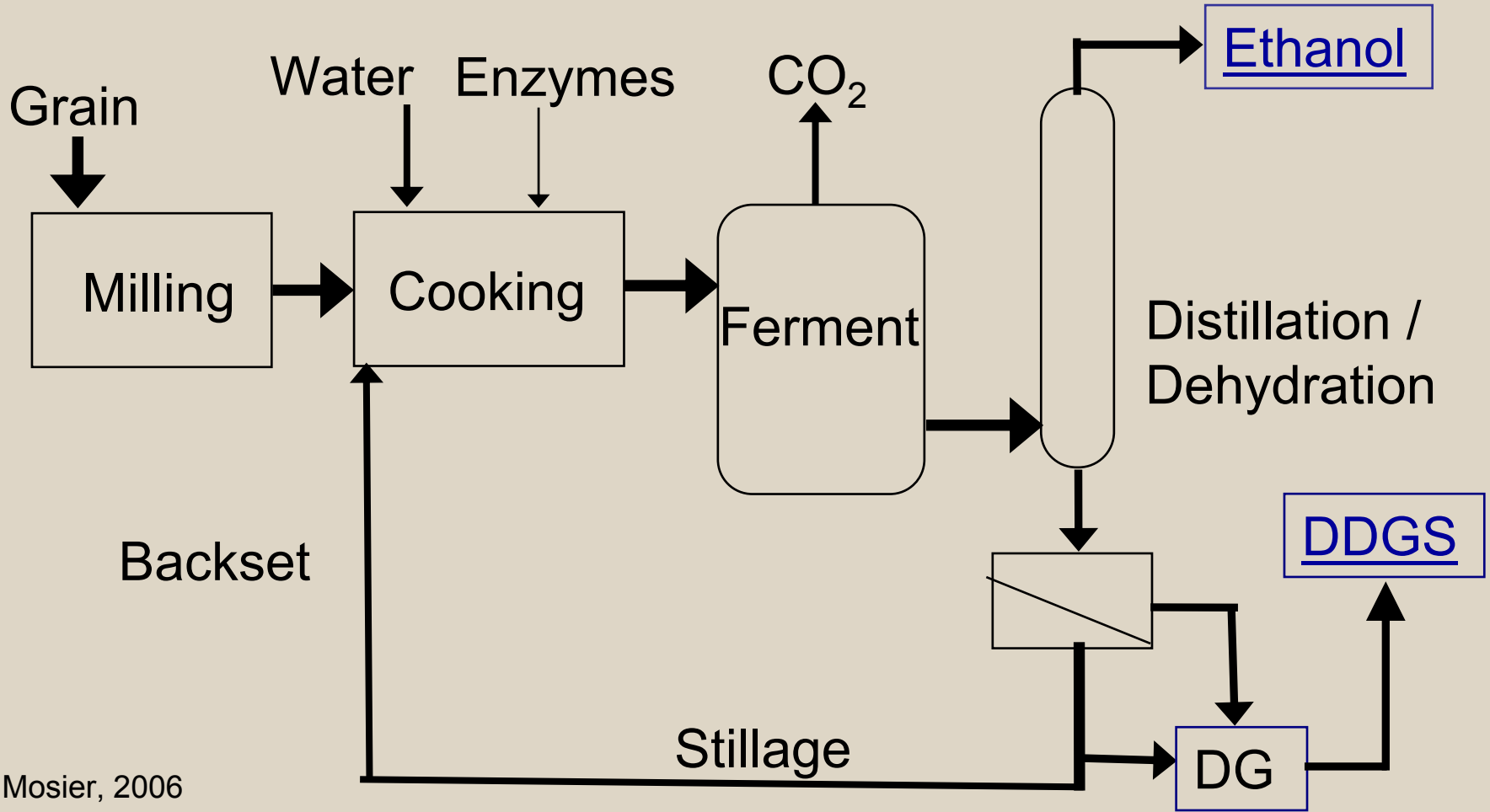


**Midwest Consortium for Biobased Products & Bioenergy**





# Dry Mill (Grind) Process



Mosier, 2006



Midwest Consortium for Biobased  
Products & Bioenergy





# DDGS Composition

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

Based on analysis at U. Illinois, USDA NCAUR, Purdue

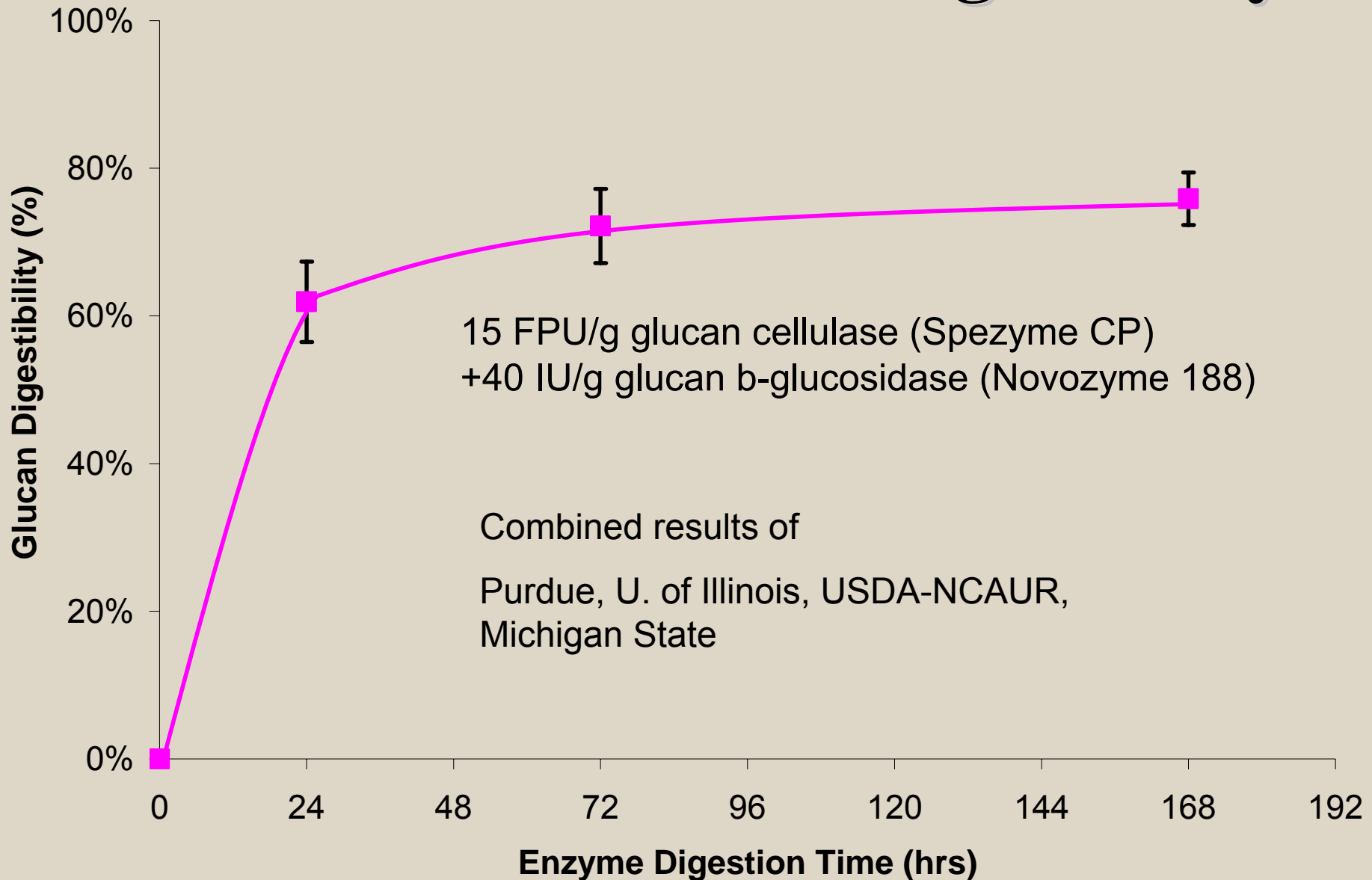


Midwest Consortium for Biobased  
Products & Bioenergy





# DDGS: Cellulose Digestibility



# Cellulose Digestibility of Untreated DDGS

Much slower than starch

Much faster than other lignocellulose (corn stover)

at enzyme loadings of 15 FPU / g cellulose for Spezyme with 40 IU / g Novozyme 188 ( $\beta$ -glucosidase)

63% of cellulose converted to glucose in 24 hrs

76% converted in 168 hrs

Based on combined results of Purdue, U. of Illinois, USDA-NCAUR, Michigan State



**Midwest Consortium for Biobased  
Products & Bioenergy**



# Pretreatment

Improves reactivity of plant polysaccharides  
(cellulose and hemicellulose)

Reduces time of hydrolysis (rate)

Increases yield

Reduces enzyme demand (loading)

DDGS used, as received (no grinding)



**Midwest Consortium for Biobased  
Products & Bioenergy**



# AFEX: Pretreatment and Hydrolysis

AFEX = Ammonia Fiber Explosion

	Temp	NH3	72h hydrolysis yield, 16.6 FPU cellulase / g
11.5% MC	70°C	0.8:1 kg/kg dry	108%
60% MC	80°C	0.6:1 kg/kg dry	103%

Milder than other types of biomass

Very low xylose yields (<10% after 168h)

65% of dry matter solubilized

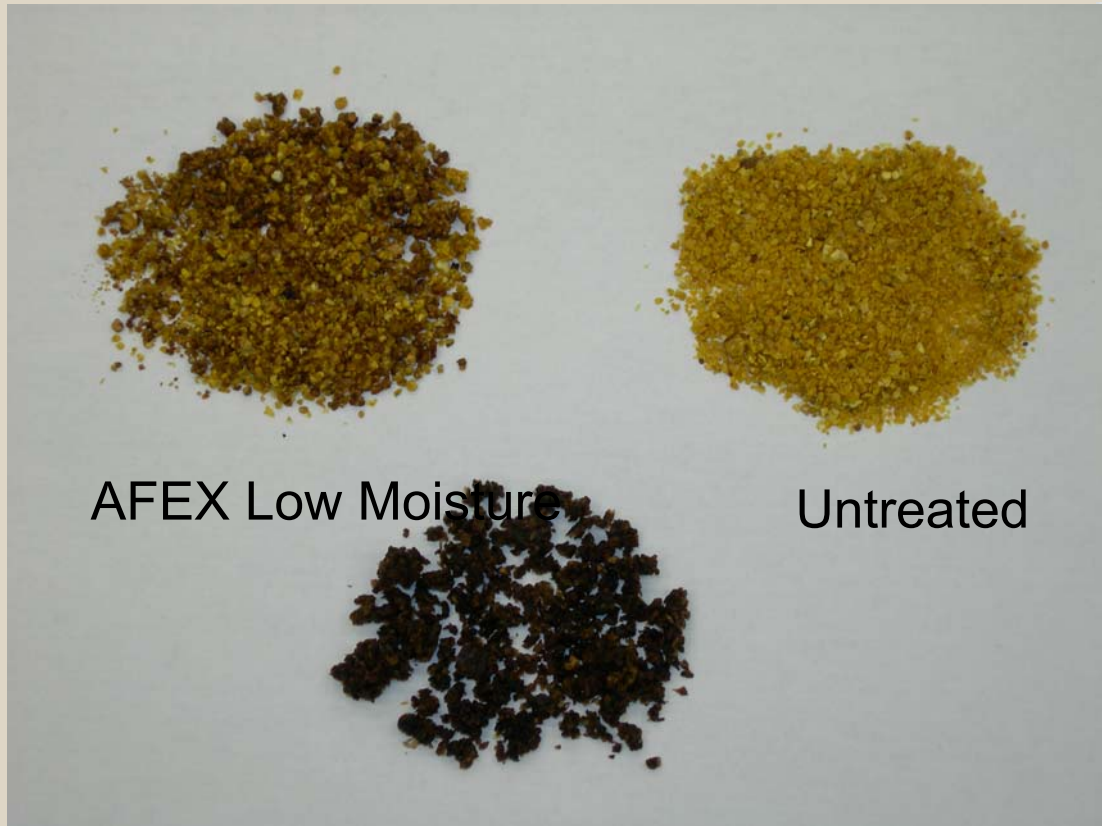
Dale, Balan, Bals



Midwest Consortium for Biobased  
Products & Bioenergy



# AFEX vs Untreated



AFEX Low Moisture

Untreated

AFEX High Moisture



AFEX

Untreated

Dale, Balan, Bals



Midwest Consortium for Biobased  
Products & Bioenergy



# AFEX: Ammonia Loading Effect

## Pretreatment

11.5% moisture

70°C, 5 min

## Hydrolysis

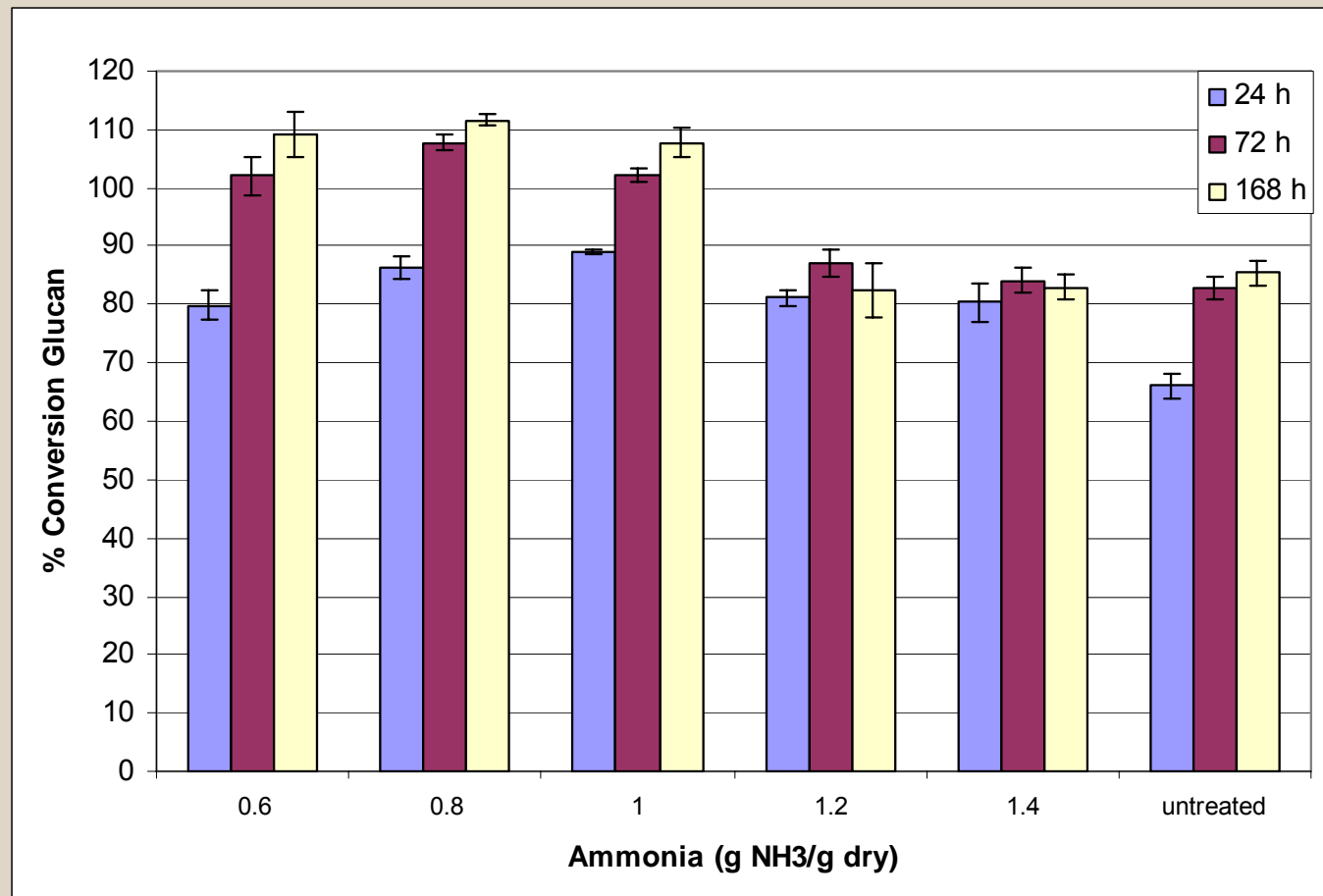
16.6 FPU

cellulase / g

cellulose gives

90% conversion in

24 hours



Midwest Consortium for Biobased  
Products & Bioenergy





# Comparison

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

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

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



Midwest Consortium for Biobased  
Products & Bioenergy





# Sugar Yields: 24 hr Hydrolysis with Cellulase

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

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

% yield includes polysaccharides from both DG and stillage

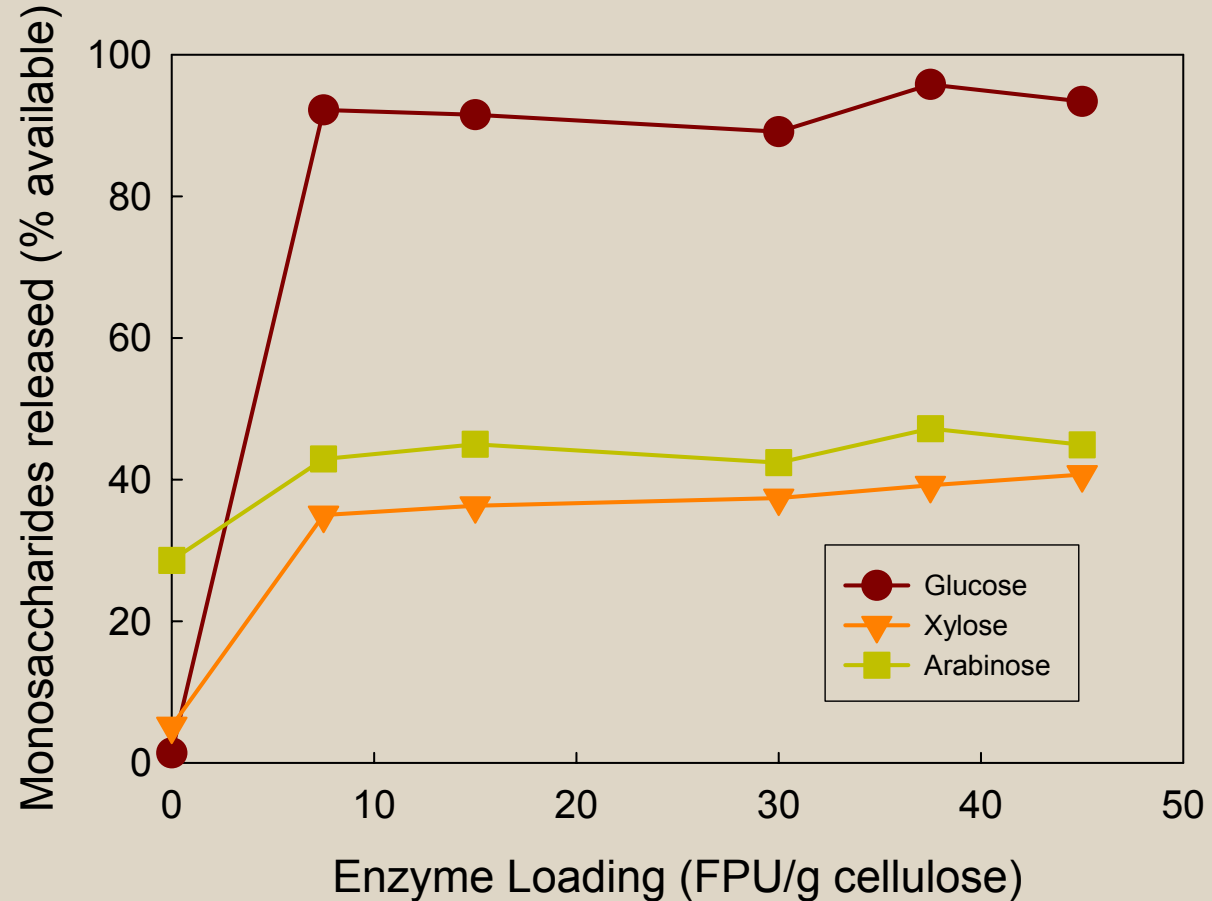


Midwest Consortium for Biobased Products & Bioenergy



# Effectiveness of using a cellulase preparation on hot-water treated DDGS

GC220 Cellulase  
+ Novo188  
(40 U/g cellulose)



Midwest Consortium for Biobased  
Products & Bioenergy



# Phenolic acids in corn fiber

	% w/w of Cell Wall
<i>p</i> -Coumaric acid	0.3
Ferulic acid	2.9
Dehydrodiferulic acids	2.5
5-5' dimer <sup>a</sup>	23
8-O-4' dimer <sup>a</sup>	35
8-8' dimer <sup>a</sup>	11

<sup>a</sup> Values are expressed as % of total dimers.

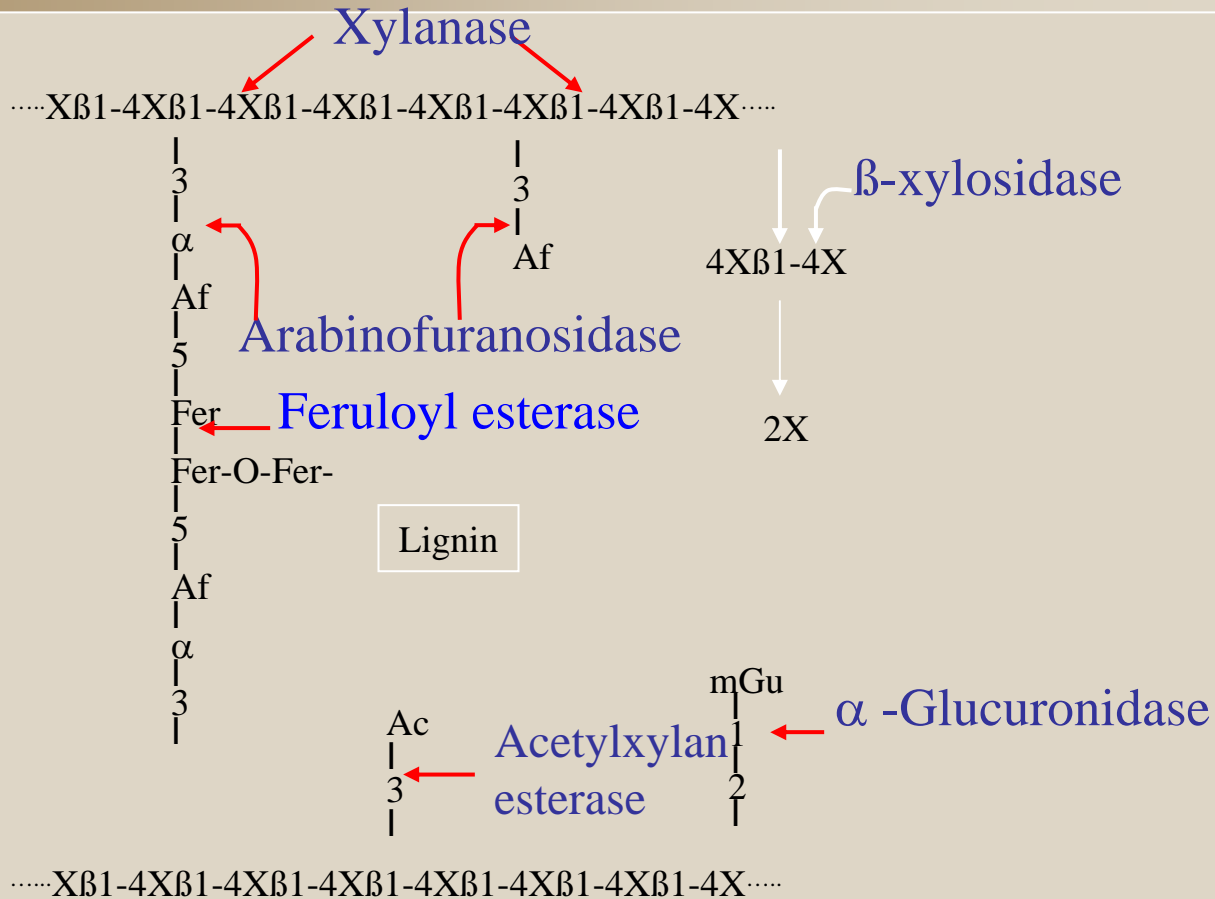
Saulnier and Thibault, 1999



Midwest Consortium for Biobased  
Products & Bioenergy



# Complex Mixture of Enzymes Needed to Degrade Arabinoxylan



Selinger et al., 1996



**Midwest Consortium for Biobased  
Products & Bioenergy**



# Effect of xylanase

<u>Xylanase</u>	<u>Hemicellulase</u>	<u>Glucose</u>	<u>Xylose</u>	<u>Arabinose</u>
(Yields as % available)				
none	none	92.0%	37.9%	47.0%
GC260	Multifect A40	90.2%	34.8%	46.5%
GC260	Optimash BG	95.5%	40.1%	47.8%
GC260	Optimash XL	92.4%	38.0%	47.7%
Multifect XL	Multifect A40	92.3%	35.9%	47.0%
Multifect XL	Optimash BG	95.8%	40.4%	45.8%
Multifect XL	Optimash XL	95.1%	35.7%	43.8%
Multifect Xylanase	Multifect A40	99.0%	43.9%	48.3%
Multifect Xylanase	Optimash BG	96.8%	42.4%	48.8%
Multifect Xylanase	Optimash XL	97.5%	43.0%	47.3%

**Conclusion: Multifect Xylanase combinations were most effective.**

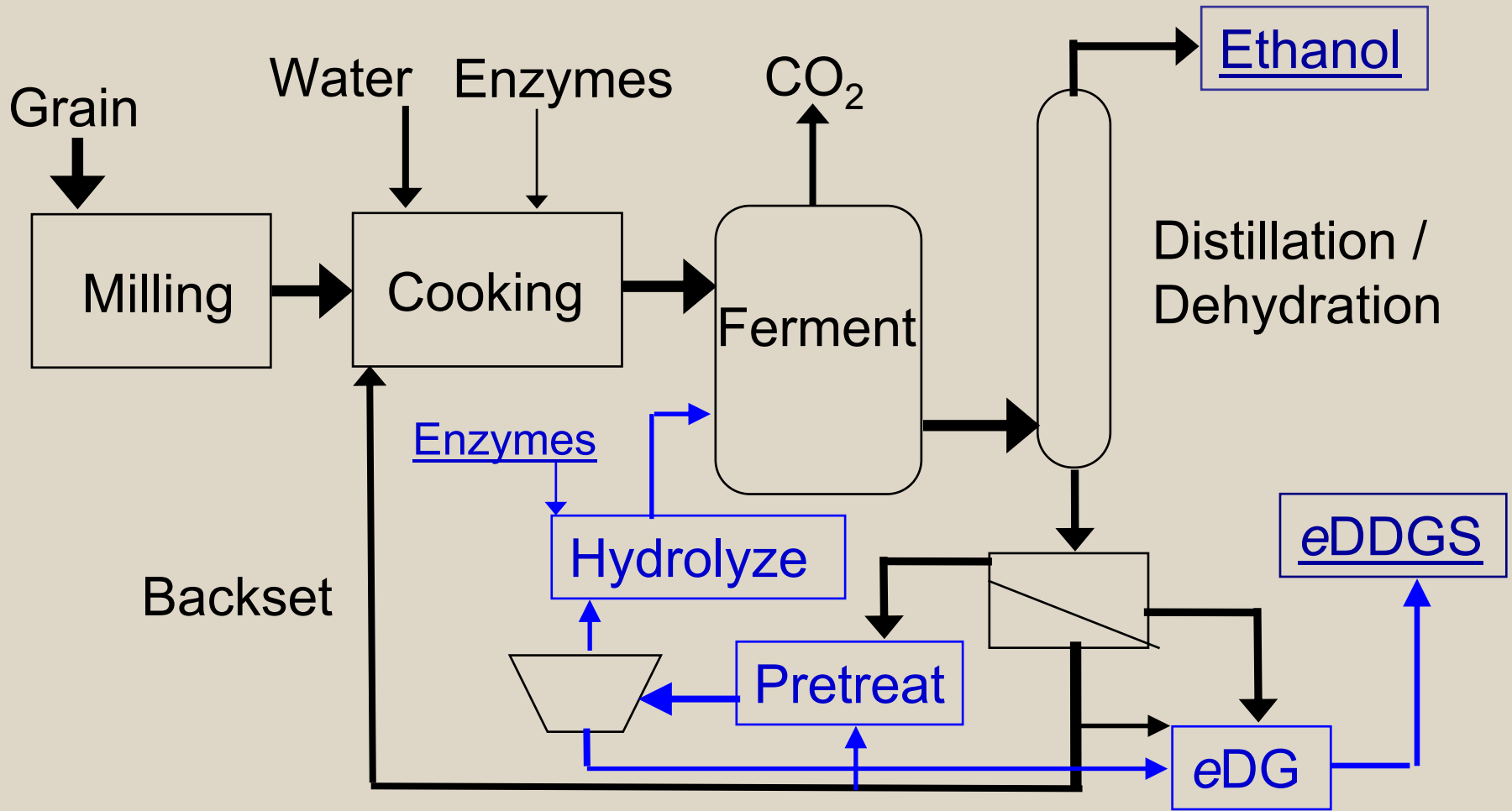


Midwest Consortium for Biobased  
Products & Bioenergy





# Dry Mill (Dry Grind) Process with Cellulose Conversion



Midwest Consortium for Biobased Products & Bioenergy





# Conclusions

Distillers Grain is more easily digested than other lignocellulosic biomass

Liquid hot water and AFEX pretreatments increase reactivity

Hemicellulose hydrolysis requires new enzyme formulations

Unconditioned sugar streams fermentable to ethanol by yeast

Cellulose conversion fundamentals apply to DDGS



**Midwest Consortium for Biobased  
Products & Bioenergy**

