



Developing Enzyme Systems for Converting Pretreated DDGS to Fermentable Sugars

Mike Cotta
Bruce Dien
Xin-Liang Li
Eduardo Ximenes

National Center for Agricultural Utilization
Research
Peoria, IL



Midwest Consortium for Biobased
Products & Bioenergy





Acknowledgments

Ms. Patricia O'Bryan

Mr. Loren Iten

Dr. Mohammed Moniruzzaman

Dr. Ladisch's and Dr. Dale's Groups

Genencor International for enzymes and discussion

Biocatalysts Limited for additional enzymes

DOE for Funding



**Midwest Consortium for Biobased
Products & Bioenergy**



Why Hemicellulases?

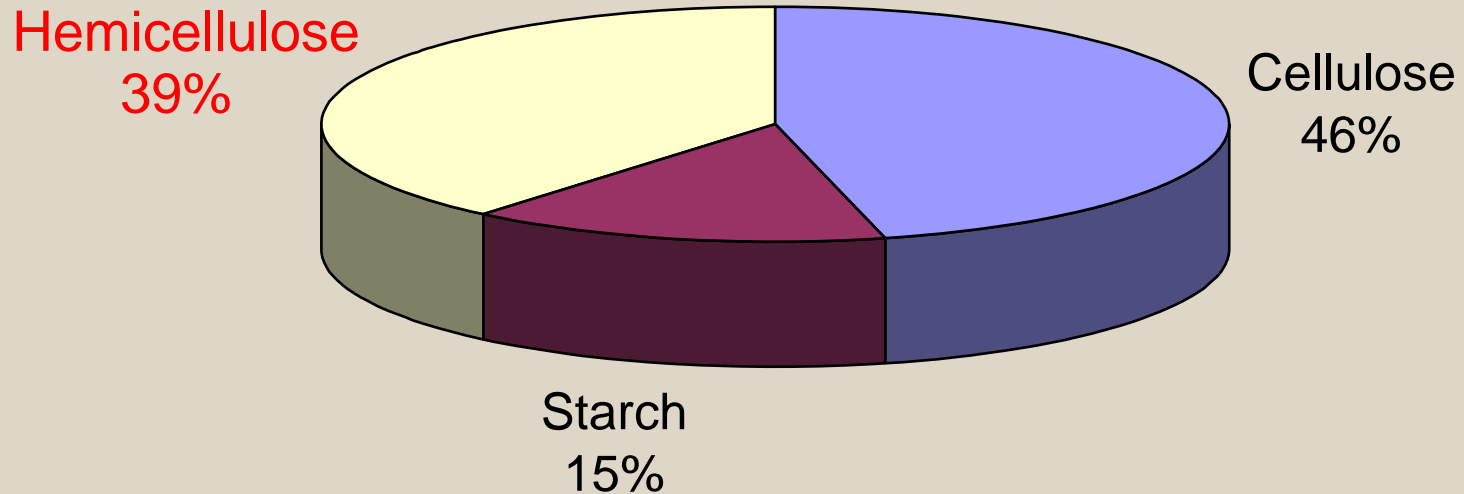
- Hemicellulases are needed to convert AFEX & LHW pretreated xylan into fermentable monosaccharides.
- Hemicellulases may be beneficial auxiliary enzymes for cellulases in some cases.



Midwest Consortium for Biobased
Products & Bioenergy



Pentose sugars are significant



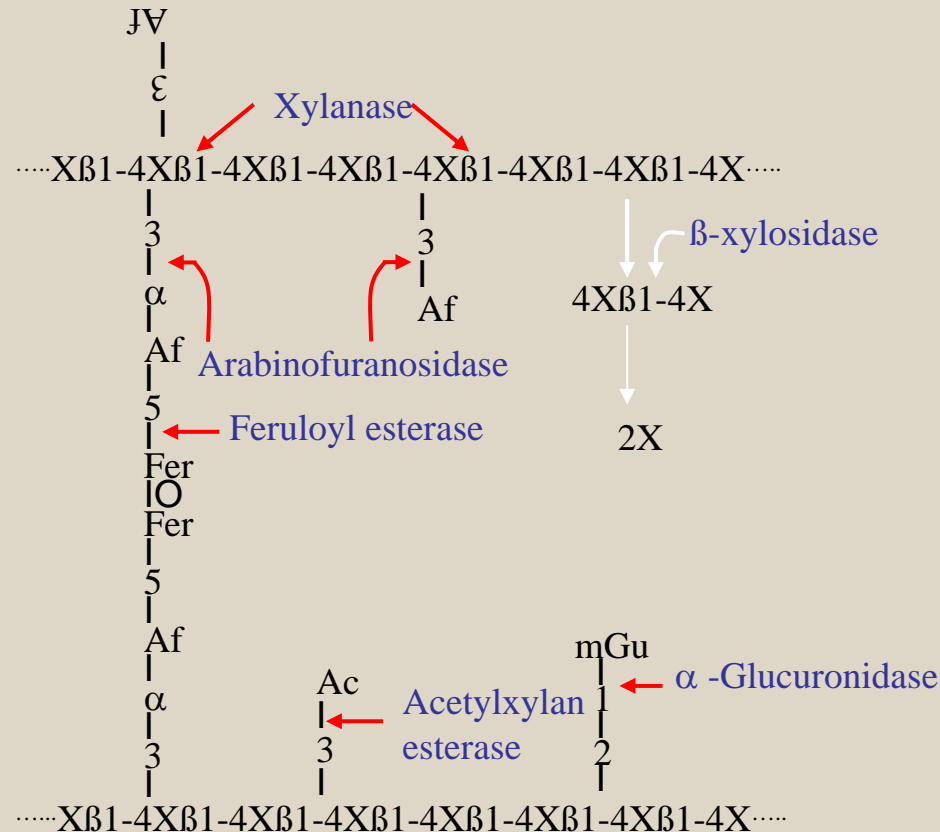
Relative Amounts of Carbohydrates



**Midwest Consortium for Biobased
Products & Bioenergy**



Complex Mixture of Enzymes Needed to Degrade Arabinoxylan



Selinger et al., 1996

Midwest Consortium for Biobased
Products & Bioenergy



Spectra of Activities in Genencor Enzyme Prep.s (pH 4.8, 50°C)

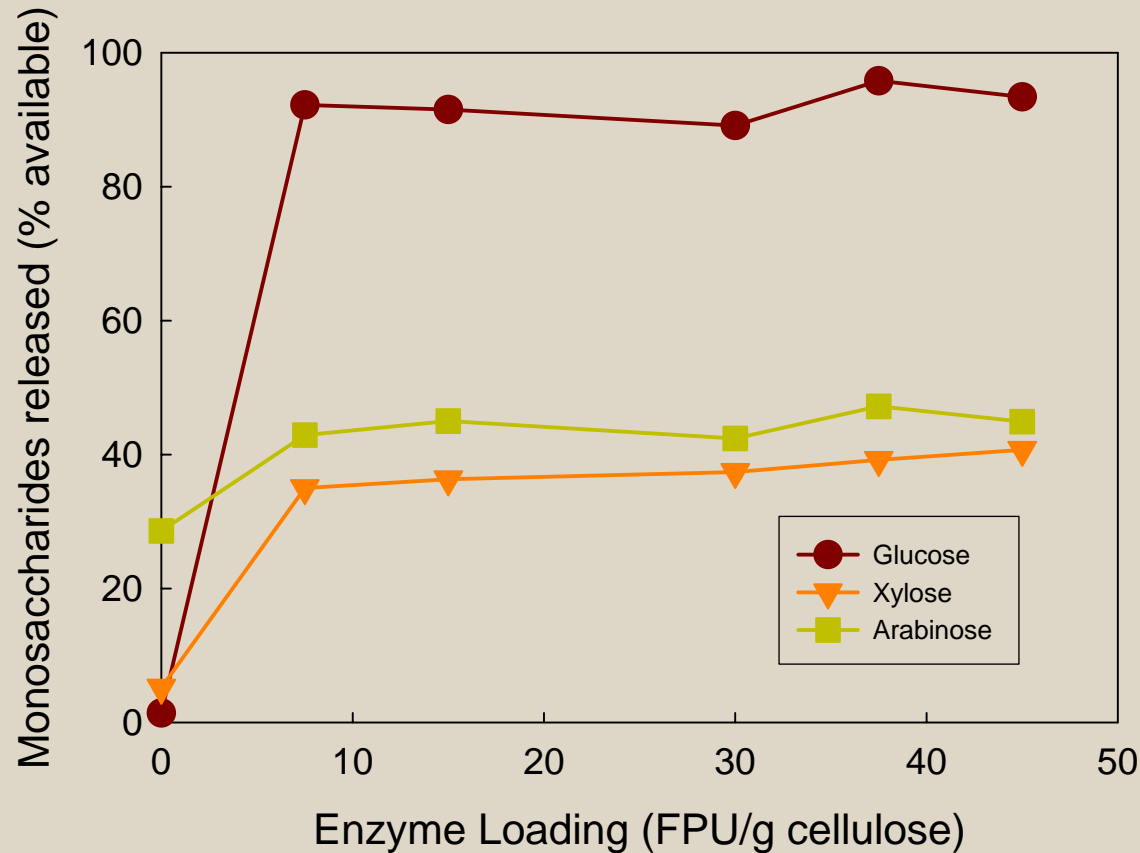
Activity	GC220	Spezyme CP	Novo 188	Multifect	Multifect
				Xylanase	Pectinase
	Activity IU/ml				
Cellulase (FPU)	92.8	58.2	8.5	0.77	4.18
β -glucosidase	99.7	128	665	35.9	345.8
Xylanase (OSX)	2782	2622	123	25,203	1664
α -arabino -furanosidase	3.06	22.6	29.3	9.44	1862
β -xylosidase	23.3	7.3	16.6	22.6	186.2
α -galactosidase	3.9	0.39	116	2.39	31.9
feruloyl esterase	0.0	0.0	0.6	0.0	9.67
p-coumaroyl esterase	?	?	?	1.3	21.7



Midwest Consortium for Biobased
Products & Bioenergy



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



Just adding GC220 Cellulase & Novo188 (40 U/g cellulose)

Midwest Consortium for Biobased
Products & Bioenergy



Digestion Assay

- Pretreatment: AFEX or hot water (HW)
- Substrate: DDGS
- Solids: 6%w/w
- Cellulase: 15 FPU + 40 U β -glu per g cellulose
- Reaction Conditions: pH 4.8; 50°C
- Rxn Duration: 72 hr
- No. of Rxn: (analytical) duplicates
- Analysis: Glu, Ara, Xyl/Gal by HPLC

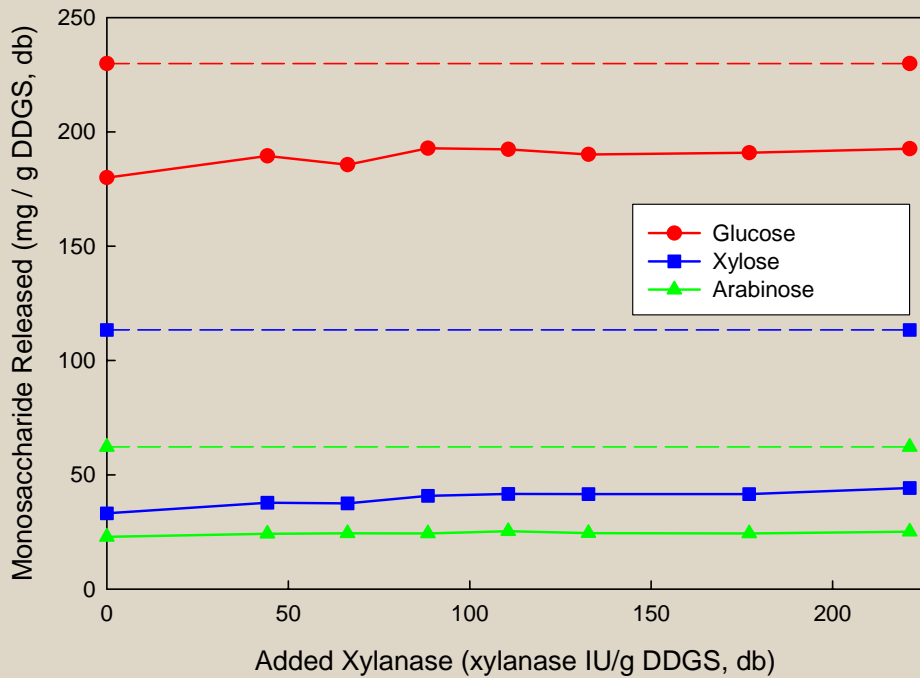


Midwest Consortium for Biobased
Products & Bioenergy

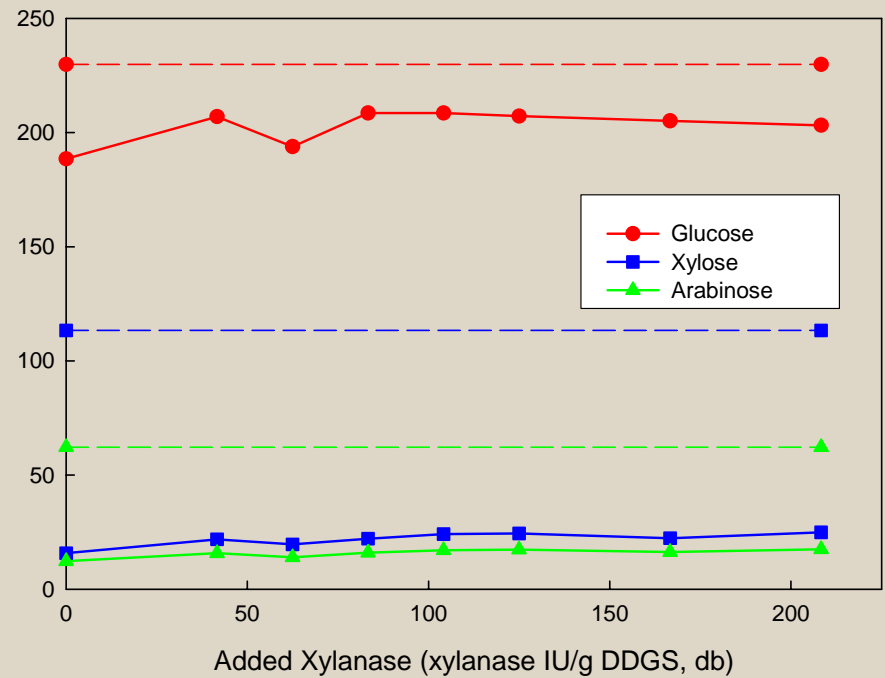


I. + *Xylanase*

LHW



AFEX



Midwest Consortium for Biobased
Products & Bioenergy



Commercial Xylanase Applied to Pretreated LHW DDGS

GC260

Multifect A40

Multifect XL

Multifect Xylanase

Optimash BG

Optimash XL

Result: None had any appreciable effect on monosaccharide yields

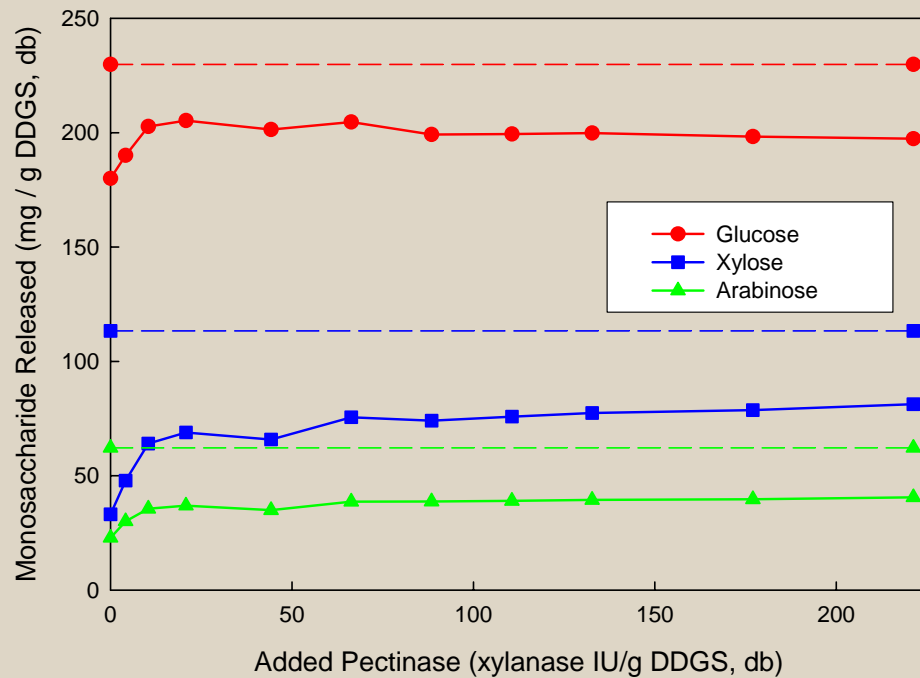


**Midwest Consortium for Biobased
Products & Bioenergy**

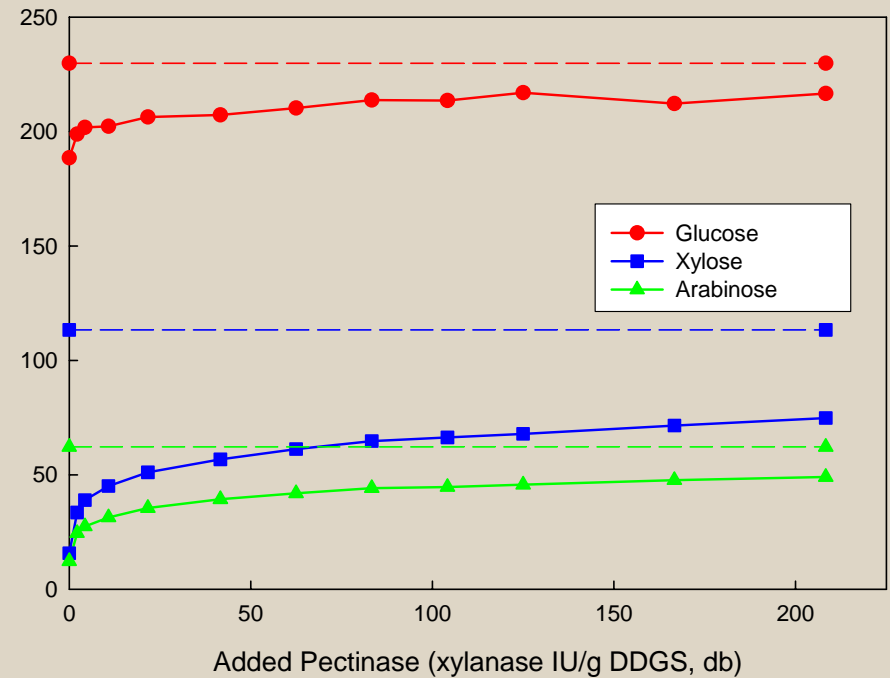


II. + *Pectinase*

LHW



AFEX



Midwest Consortium for Biobased
Products & Bioenergy



Commercial Source of FAE (Depol 740)

<u>Activity</u>	<u>U/ml</u>
feruloyl esterase	15.6
p-coumaroyl esterase	13.8

Note: Rxn conditions: pH 4.8, 50°C

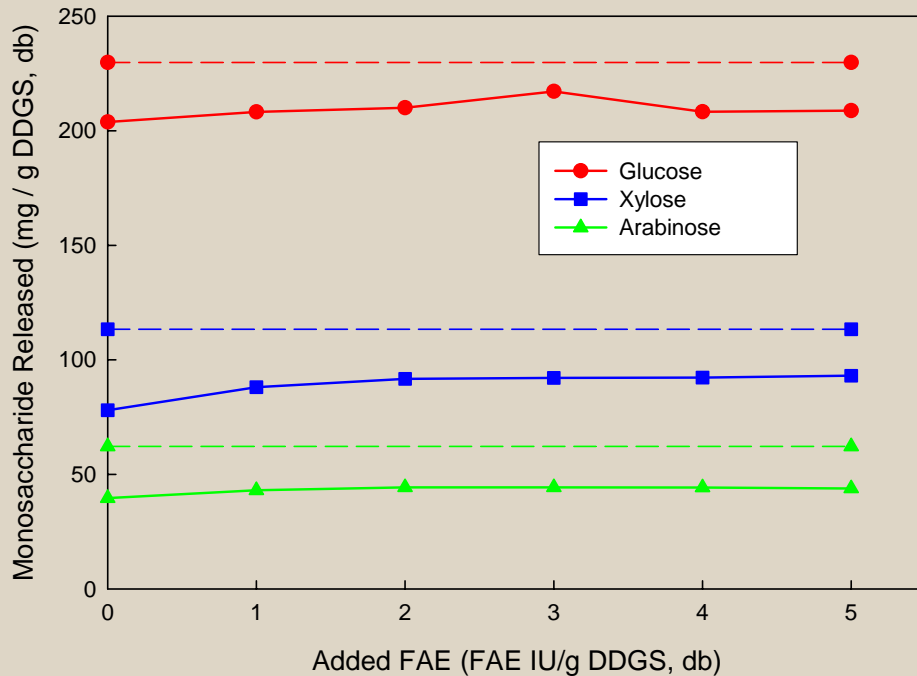


Midwest Consortium for Biobased
Products & Bioenergy

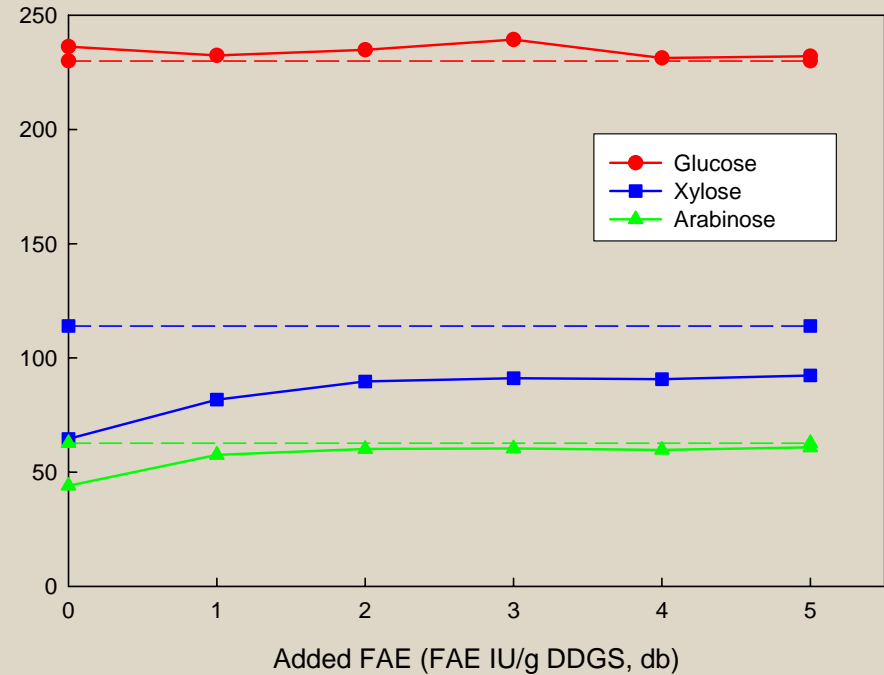


III. + *Pectinase* + *FAE*

LHW



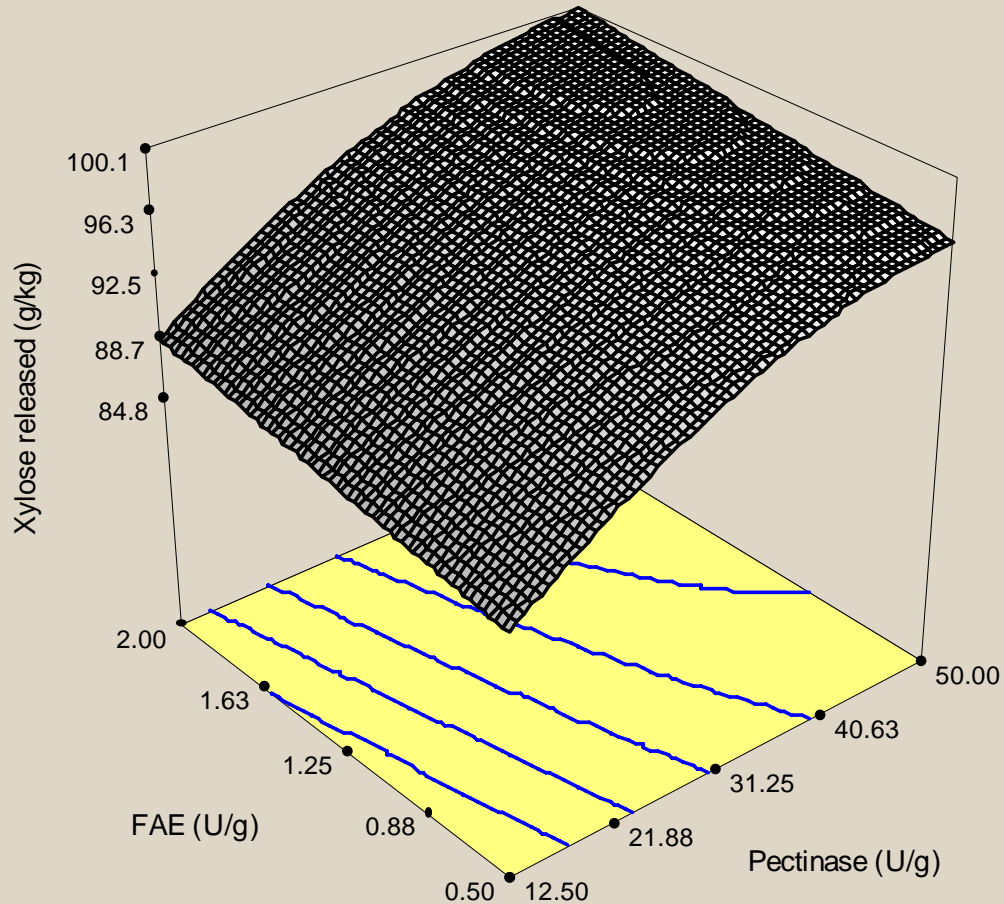
AFEX



Midwest Consortium for Biobased
Products & Bioenergy



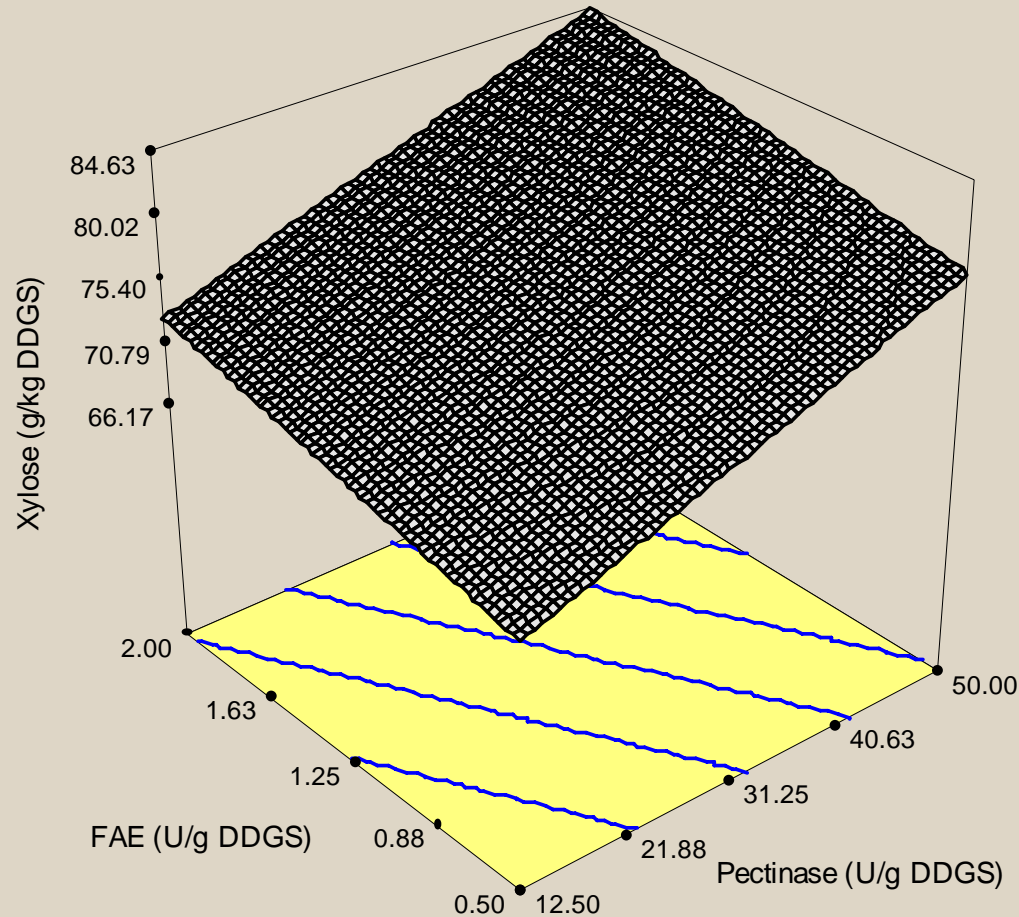
LHW Response Surface



**Midwest Consortium for Biobased
Products & Bioenergy**



AFEX Response Surface



Midwest Consortium for Biobased
Products & Bioenergy



Summary of LHW Results

Multifect Xylanase	Multifect Pectinase FE	DEPOL 740	<u>Glucose</u>	<u>Xylose</u>	<u>Arabinose</u>
			Yield (g/kg of DDGS)		
LHW Pretreated DDGS					
-	-	-	197	27	19
+	-	-	193	44	25
-	+	-	204	81	41
+	+	-	196	76	39
-	+	+	209	93	44

Note: cellulase mixture added to each reaction



Midwest Consortium for Biobased
Products & Bioenergy



Summary of AFEX Results

Multifect Xylanase	Multifect Pectinase FE	DEPOL 740	<u>Glucose</u>	<u>Xylose</u>	<u>Arabinose</u>
			Yield (g/kg of DDGS)		
AFEX Pretreated DDGS					
-	-	-	214	20	16
+	-	-	203	25	17
-	+	-	236	75	49
+	+	-	224	70	47
-	+	+	232	92	61

Note: cellulase mixture added to each reaction



Midwest Consortium for Biobased Products & Bioenergy



Higher Conc. (20% w/w) Experiments

	<u>AFEX DDGS</u>	<u>LHW DDGS</u>
Final Sugar Conc. (g/l):		
Glucose	42.2	36.2
Xylose	15.1	14.9
Arabinose	10.6	7.1
Total	68.0	58.2
Yields (g/kg DDGS, db):		
Glucose	173	160
Xylose	62	66
Arabinose	43	33
Total	278	261



Midwest Consortium for Biobased
Products & Bioenergy



Possible Reasons for Lower Yields at Higher Solids

- End product inhibition
- Mass transfer limits
- Xylan solubility/association effects

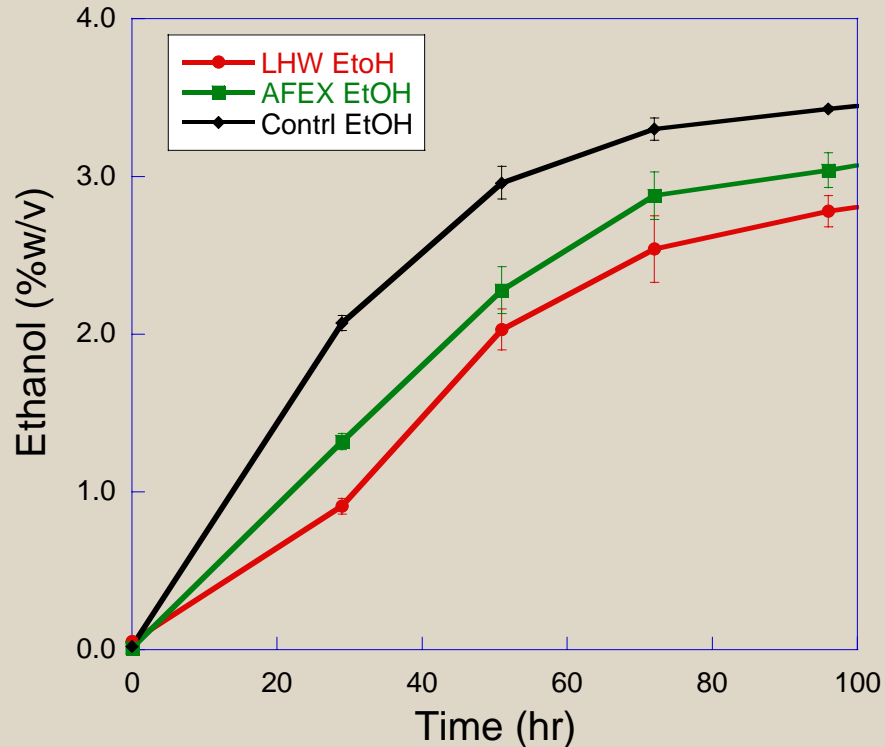


Midwest Consortium for Biobased
Products & Bioenergy

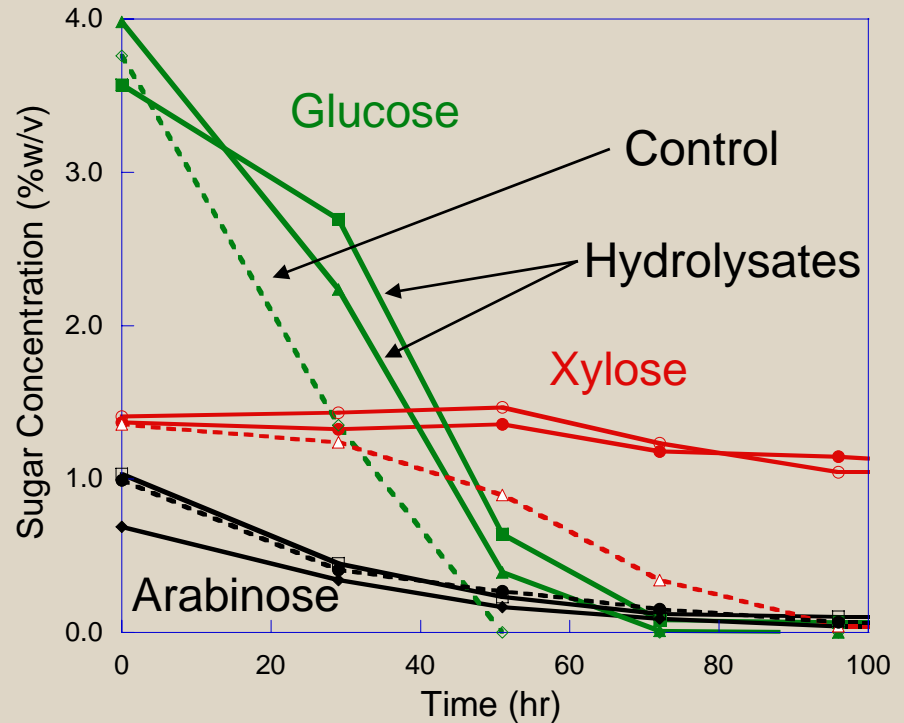


Fermentation w/ Bacterial Ethanologen

Ethanol Profile



Sugar Profiles



Midwest Consortium for Biobased
Products & Bioenergy



Poster: Producing Enzymes from DDGS

Paper submitted at Symposium for Biotechnology for Fuels and Chemicals

Enzyme Production by Industrially Relevant Fungi Cultured on Co-Product from Corn Dry Grind Ethanol Plants

By
Eduardo Ximenes, et al.

- Cultured on HW-DDGS for enzyme production: *Trichoderma reesei* Rut C-30, *Aspergillus niger* NRRL 2001, *Aurobasidium pullulans*
- Profiled spectra of enzymes produced by each fungi
- Demonstrated production in 5 L bioreactor
- Evaluated enzyme preparations for digestion of HW-DDGS



Midwest Consortium for Biobased
Products & Bioenergy



Conclusions for digestion work

- Commercial cellulases give poor yields of xylan monosaccharides
- Adding commercial xylanases did not improve these yields
- However, adding in pectinase and FAE gave high yields (346 g/kg & 385 g/kg DDGS)
- Sugars produced are fermentable, though xylose utilization remains problematic



**Midwest Consortium for Biobased
Products & Bioenergy**



Future Goals

- Lower enzyme loading by applying purified enzymes
- Increase sugar yields at higher-solids
- Increase ethanol yield by culturing in automatic pH control fermentations



Midwest Consortium for Biobased
Products & Bioenergy

