


Enzymatic Extraction of Sugars from AFEX and Liquid Hot-Water Pretreated Distiller's Grains and Their Conversion to Ethanol

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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

Ms. Patricia O'Bryan

Mr. Loren Iten

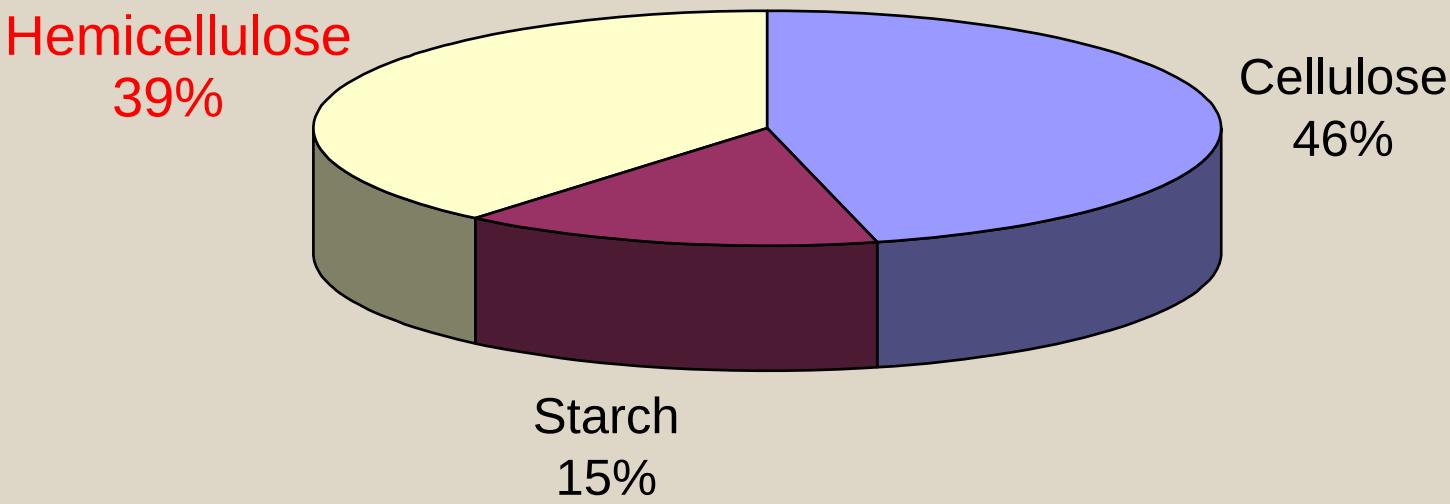
Dr. Mohammed Moniruzzaman

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

Why Hemicellulases?

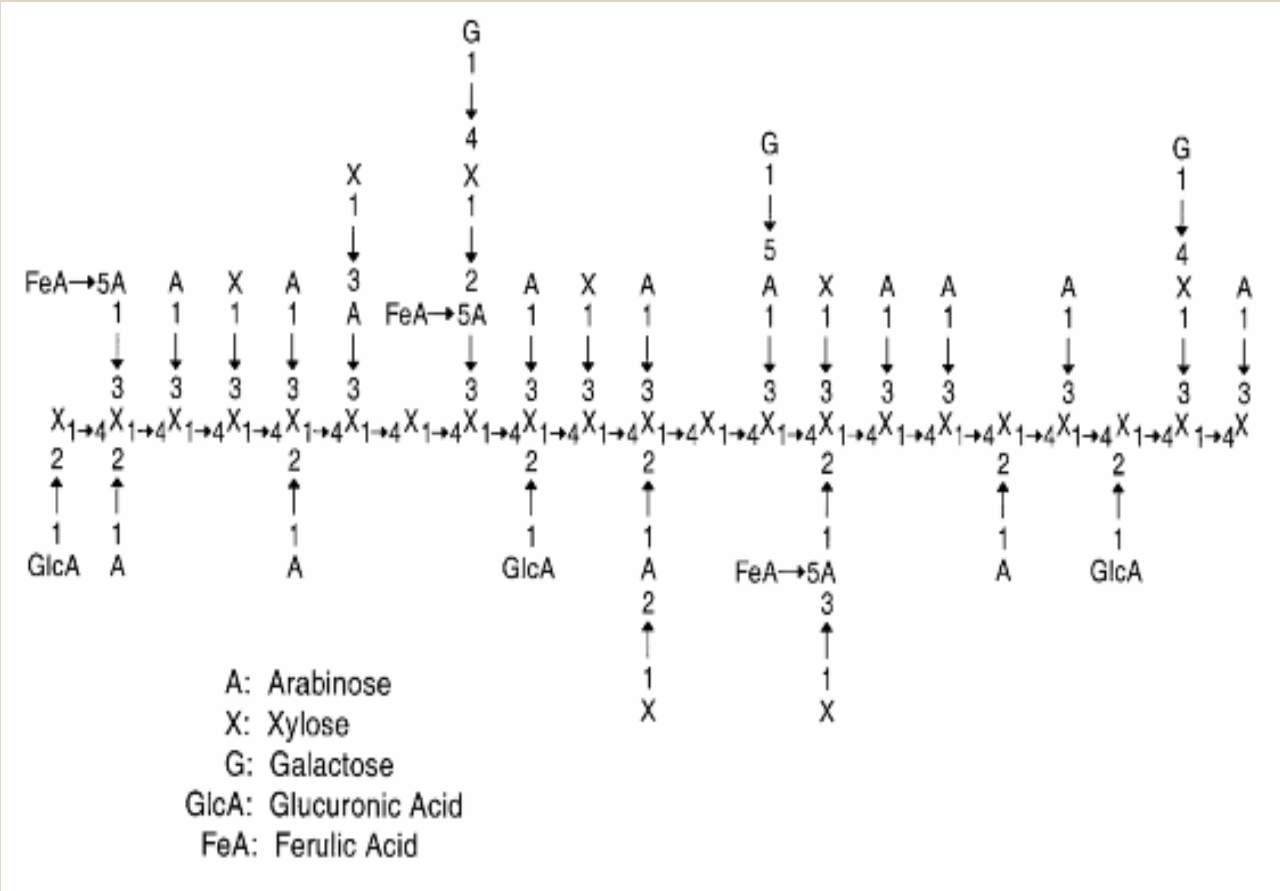
- Hemicellulases are needed to convert AFEX & LHW pretreated xylan into fermentable monosaccharides.
- Hemicellulases may be beneficial auxiliary enzymes for cellulases in some cases.

Pentose sugars are significant

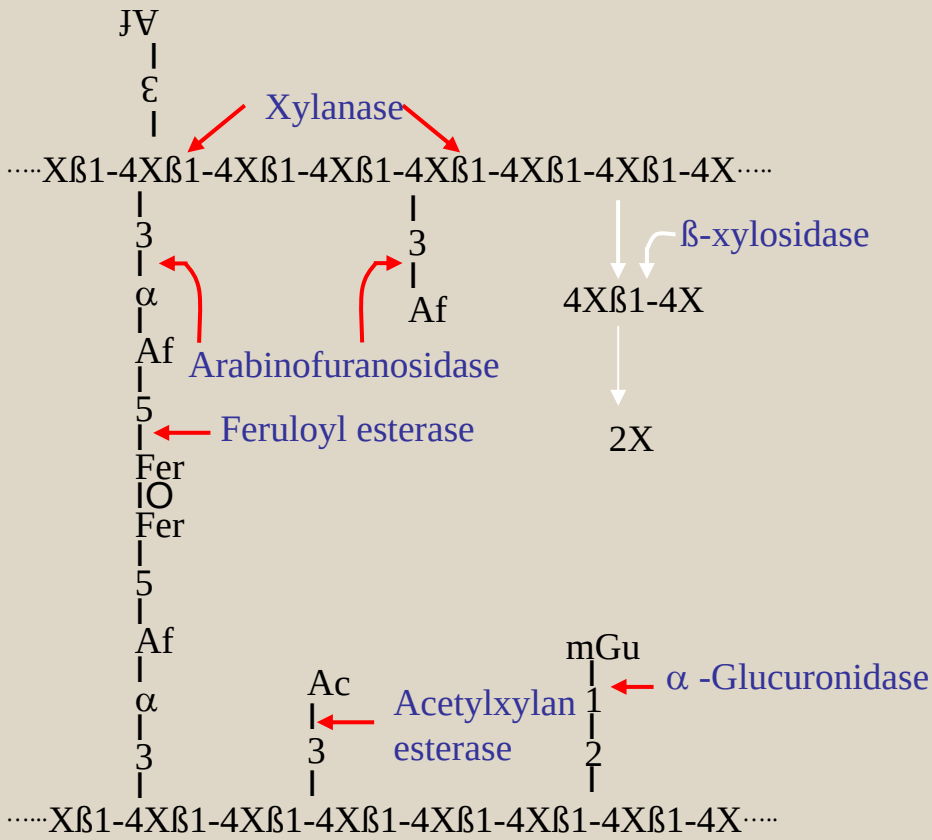


Relative Amounts of Carbohydrates

Corn Fiber Heteroxylan



Complex Mixture of Enzymes Needed to Degrade Arabinoxylan



Selinger et al., 1996

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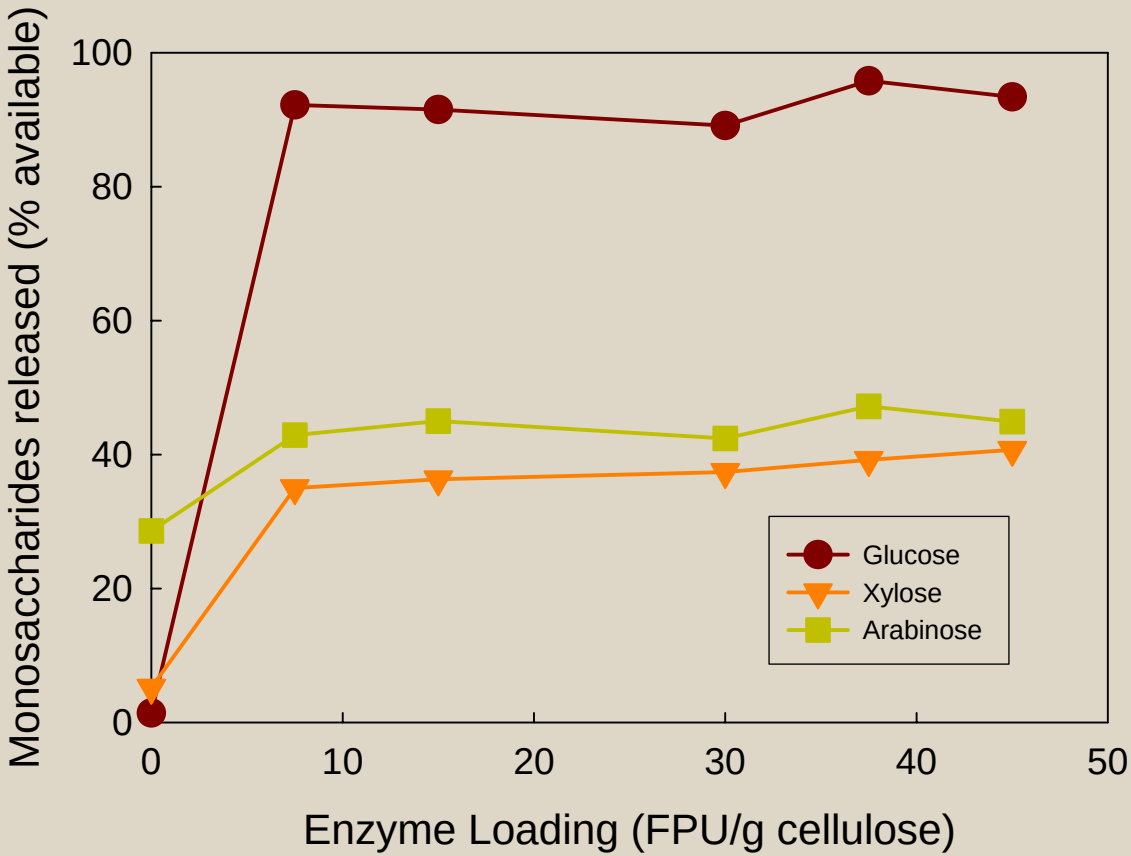
Commercial Cellulases Blends have significant xylanase activities

Activity	GC220	Spezyme CP	Novo 188
		Acitivity U/ml	
Cellulase (FPU)	92.8	58.2	8.5
β-glucosidase	99.7	128	665
Xylanase (OSX)	2782	2622	123
β-xylosidase	23.3	7.3	16.6

Xylanase activities are shaded in red. Activities were all measured at pH 4.8 and 50°C.

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



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

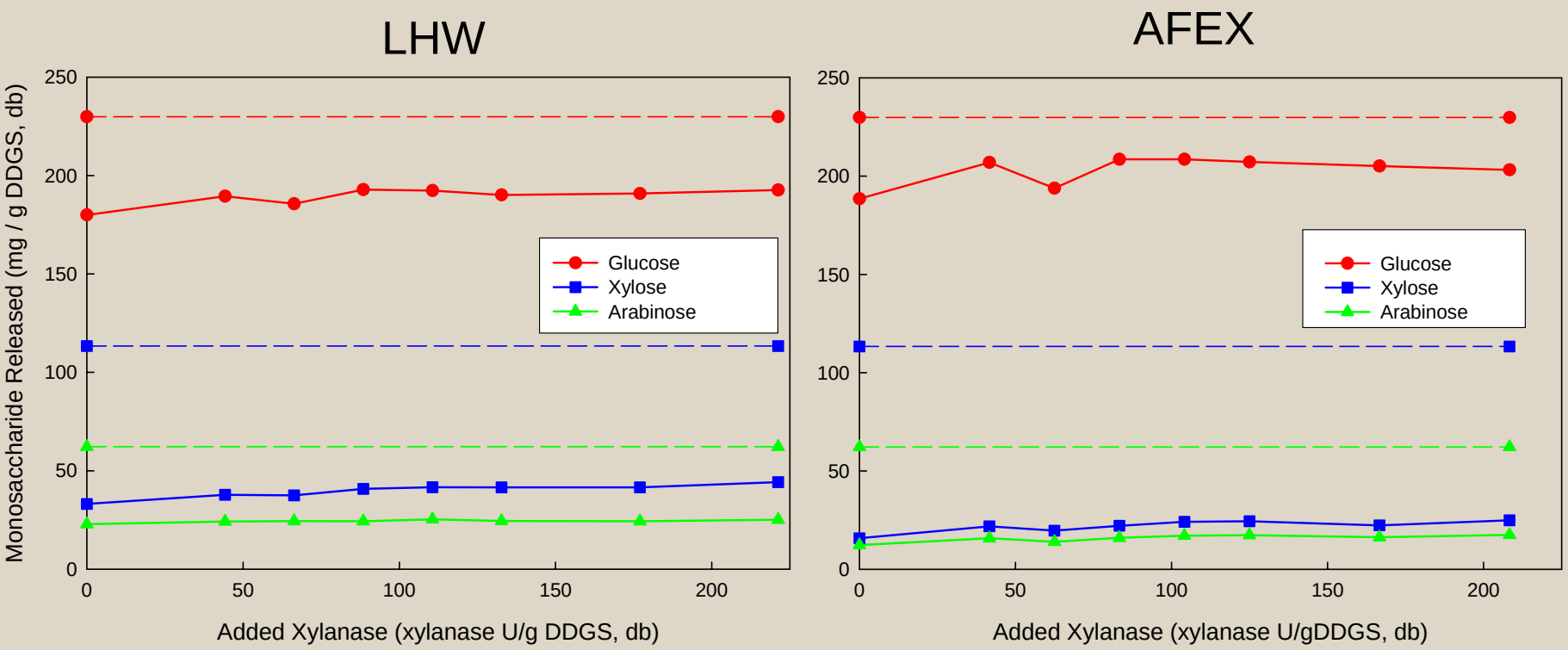
Spectra of Activities in Genencor Enzyme Prep.s (pH 4.8, 50ºC)

Activity	GC220	Spezyme CP	Novo 188	Multifect Xylanse	Multifect Pectinase
			Acitivity U/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

Digestion Assay

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

I. + *Xylanase*


LHW
AFEX
Monosaccharide Released (mg / g DDGS, db)
Glucose
Xylose
Arabinose
0
50
100
150
200
250
Added Xylanase (xylanase U/g DDGS, db)
Added Xylanase (xylanase U/gDDGS, db)

Commercial Xylanase Applied to Pretreated LHW DDGS

GC260

Multifect A40

Multifect XL

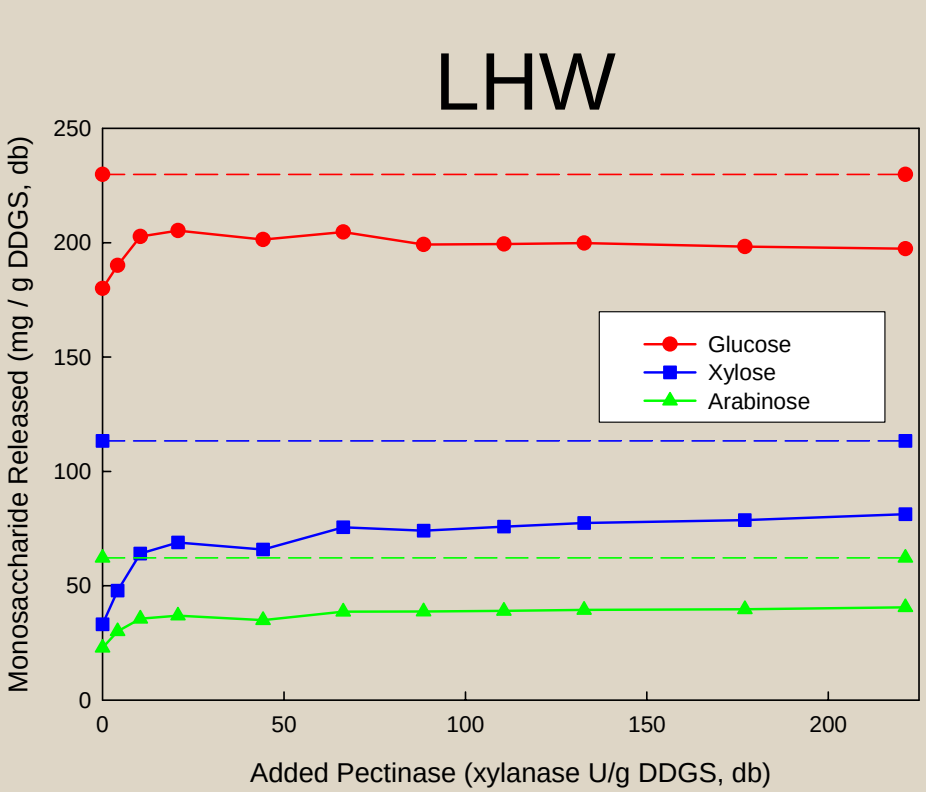
Multifect Xylanase

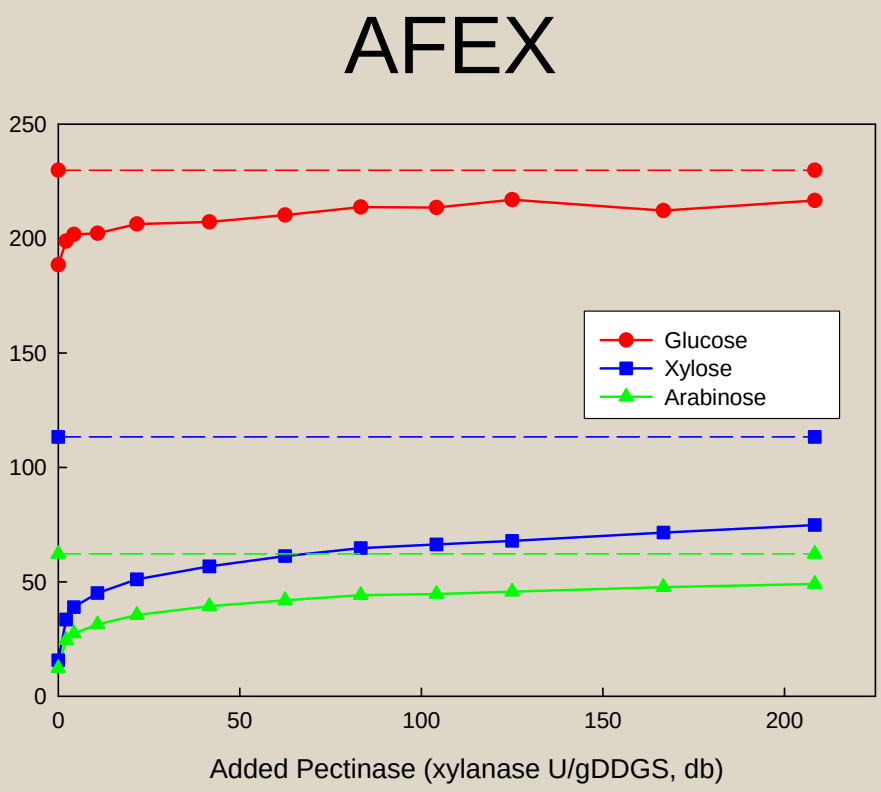
Optimash BG

Optimash XL

Result: None had any appreciable effect on monosaccharide yields

II. + *Pectinase*



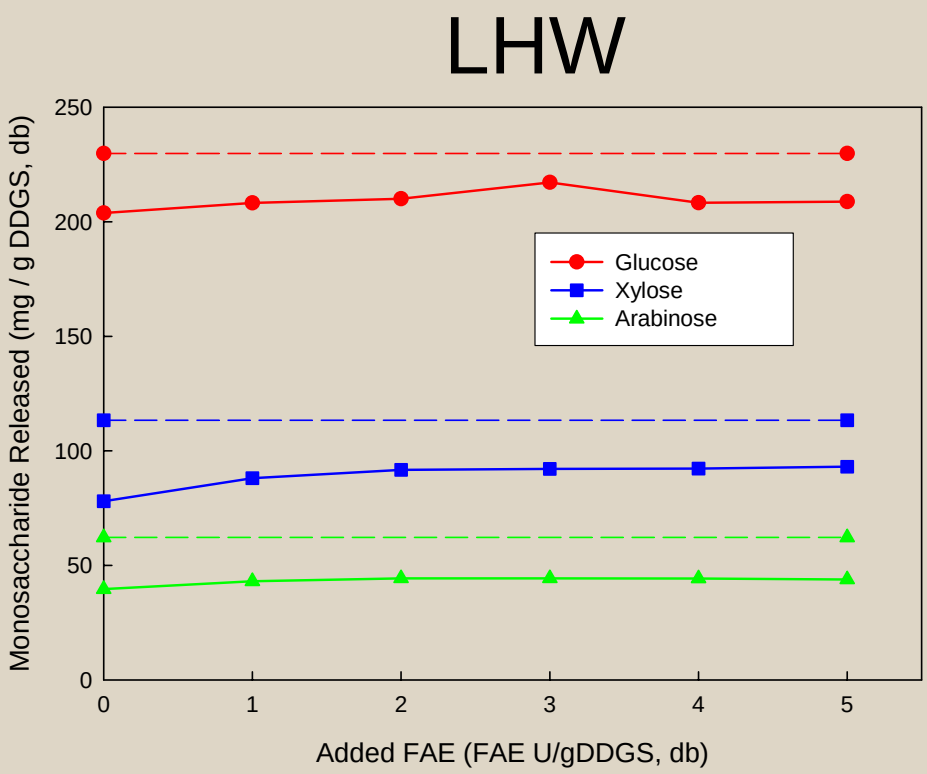


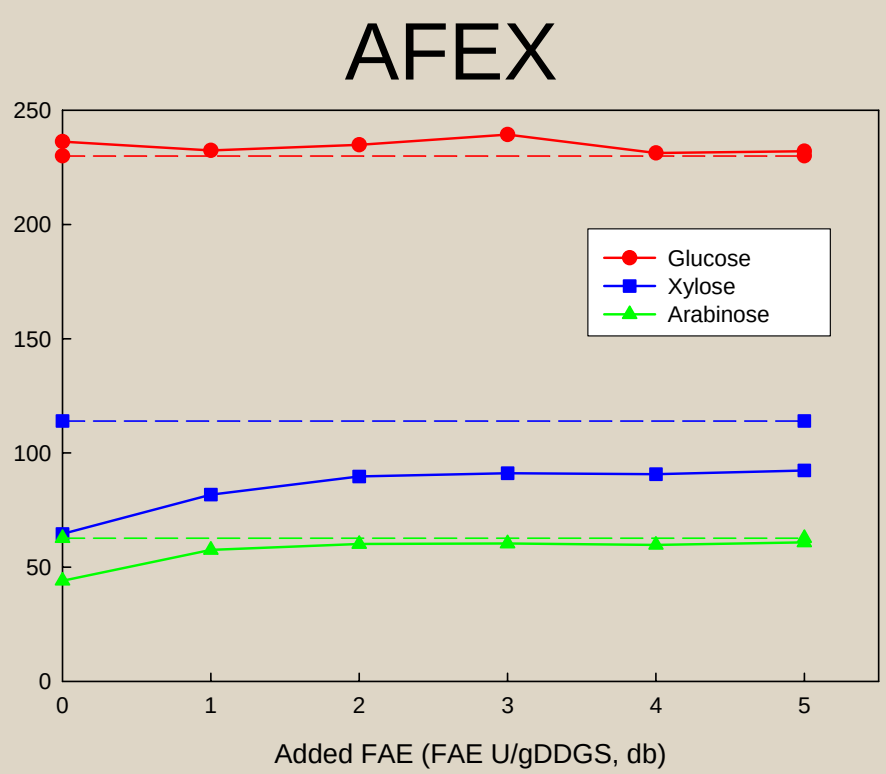
Commercial Source of FAE (Depol 740L)

Activity	U/ml
feruloyl esterase	15.6
p-coumaroyl esterase	13.8

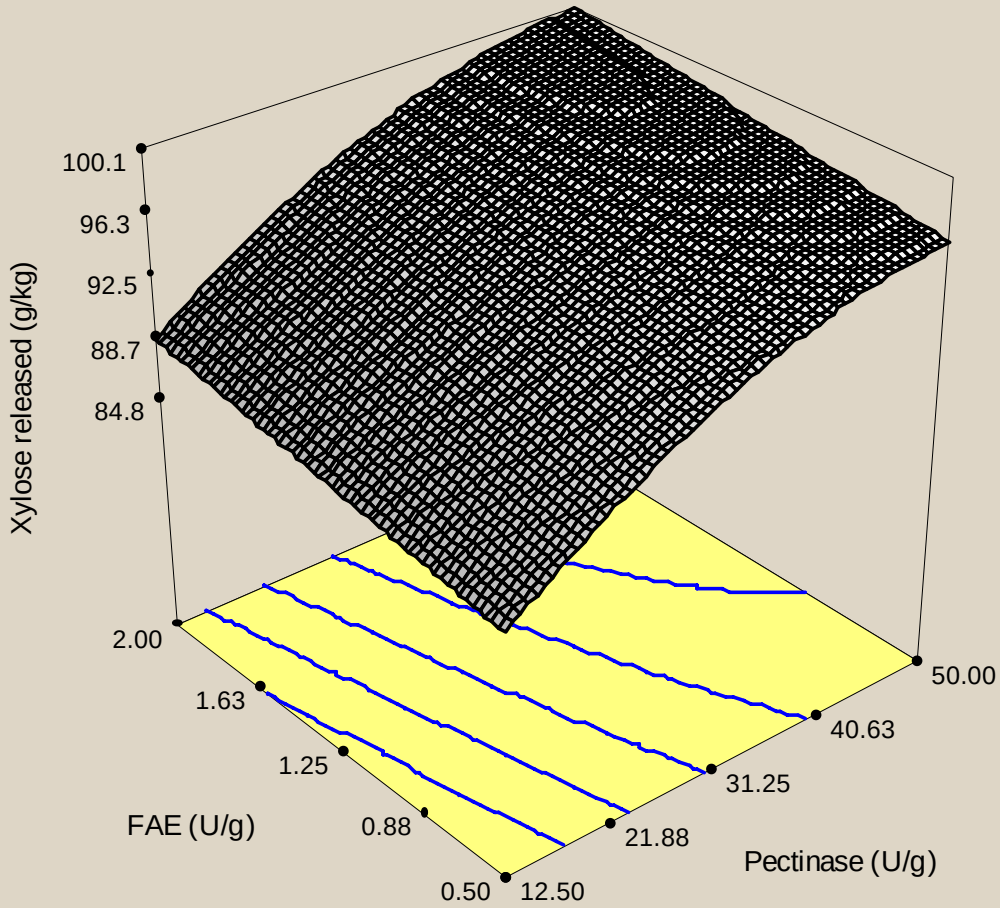
Note: Rxn conditions: pH 4.8, 50ºC

III. + *Pectinase* + *FAE*





LHW Response Surface



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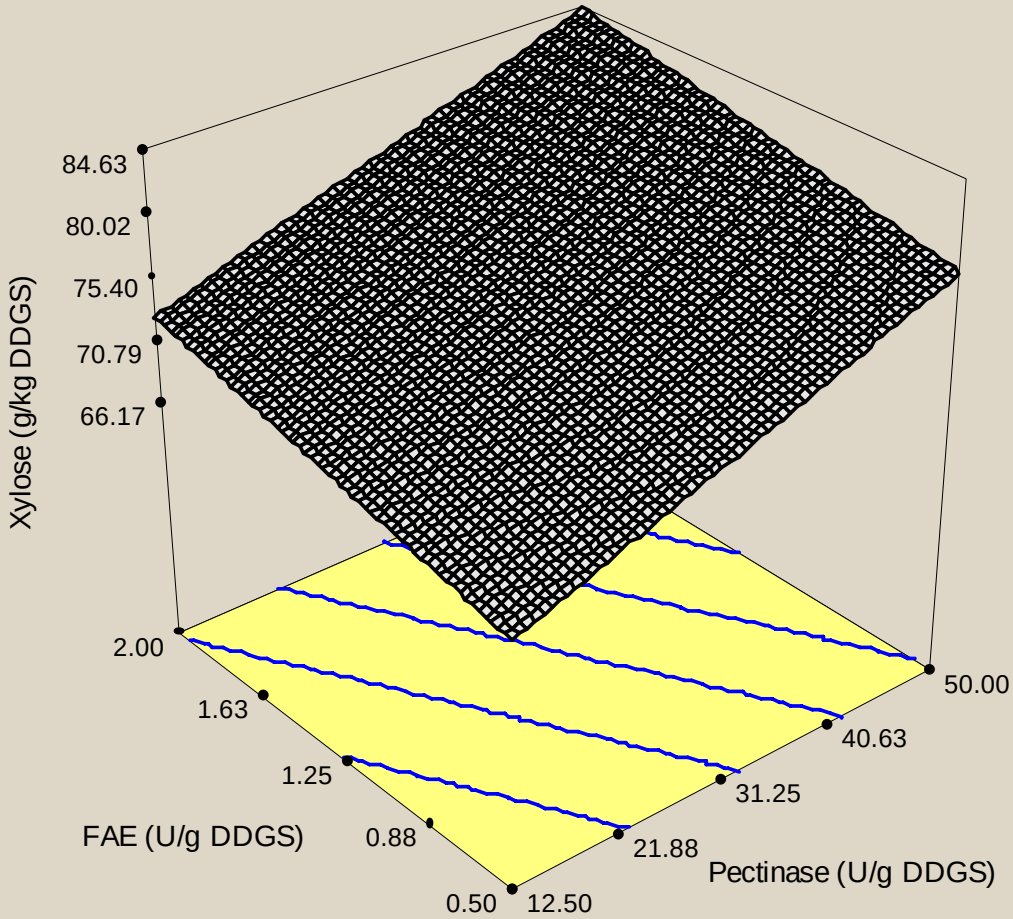
AFEX Response Surface



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Summary of LHW Results

Multifect Xylanase	Multifect Pectinase FE	DEPOL 740	Glucose	Xylose	Arabinose
			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

Summary of AFEX Results

Multifect Xylanase	Multifect Pectinase FE	DEPOL 740	Glucose	Xylose	Arabinose
			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

Higher Conc. (20% w/w) Experiments

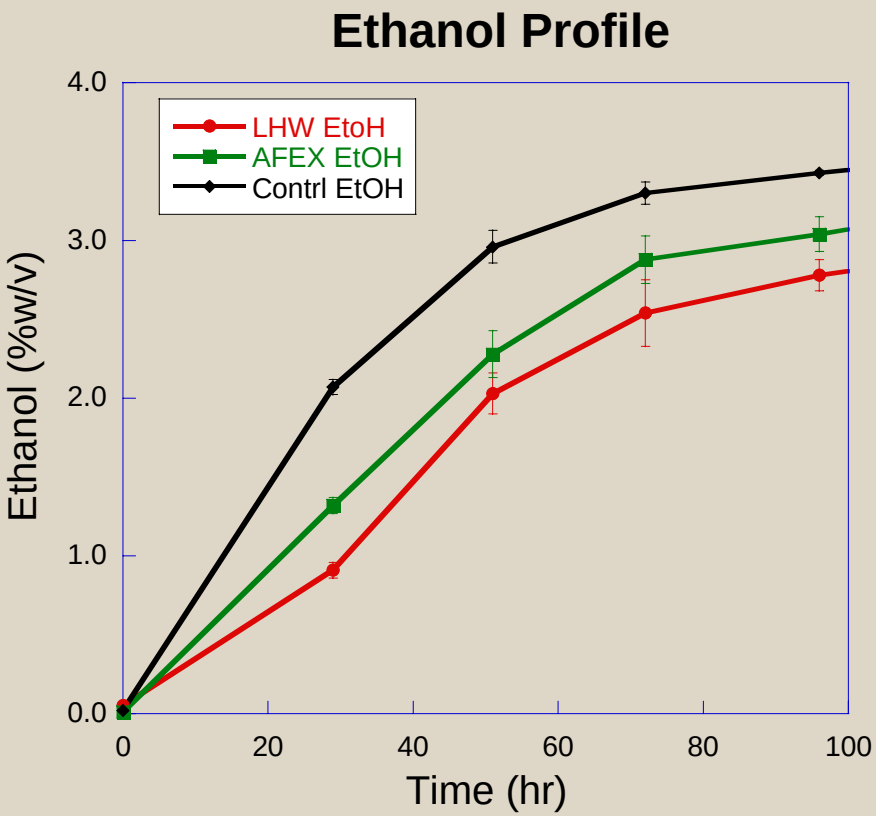
	AFEX DDGS	LHW DDGS
Final Sugar Conc. (g/l):		
Glucose	42.2	36.2
Xylose	15.1	14.9
Arabinose	10.6	7.1
Total	68.0	58.2
Yields (g/kg DDGS, db):		
Glucose	173	160
Xylose	62	66
Arabinose	43	33
Total	278	261

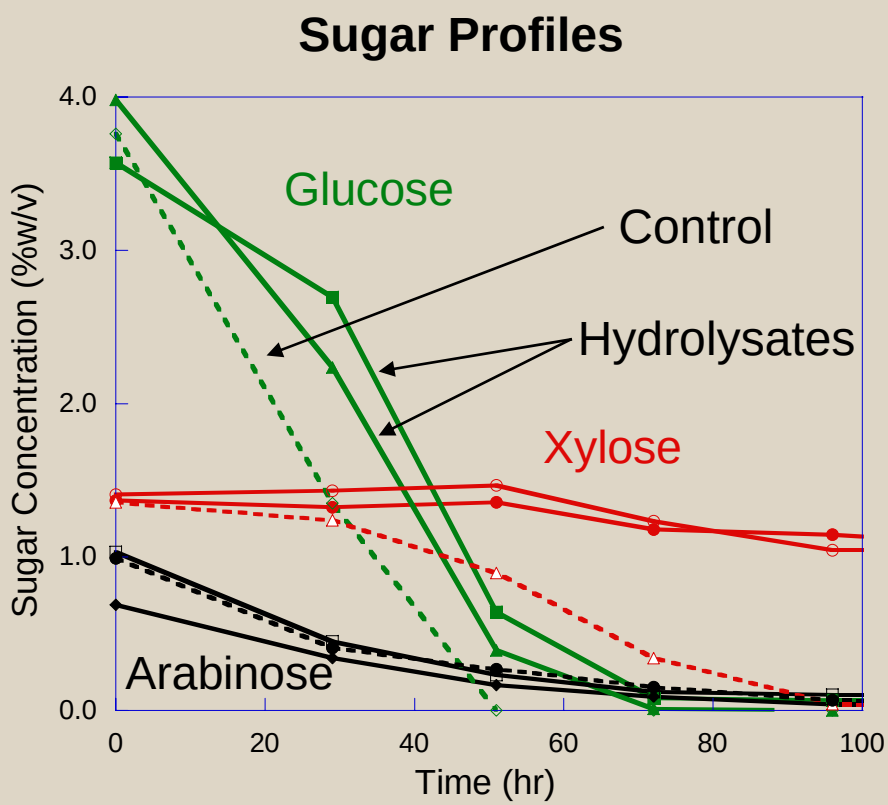
The chosen enzyme loadings were: cellulase (15 FPU/g cellulose), β-glucosidase (40 U/g cellulose), pectinase (40 U xylanase /g DDGS (db), and FAE (1.2 U/ g DDGS (db)). Rxn Time/Temp = 72 hr at 50C.

Possible Reasons for Lower Yields at Higher Solids

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

Fermentation w/ Bacterial Ethanologen





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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

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