USDA - ARS - National Center for Agricultural Utilization Research

Developing Herbaceous Energy Crops

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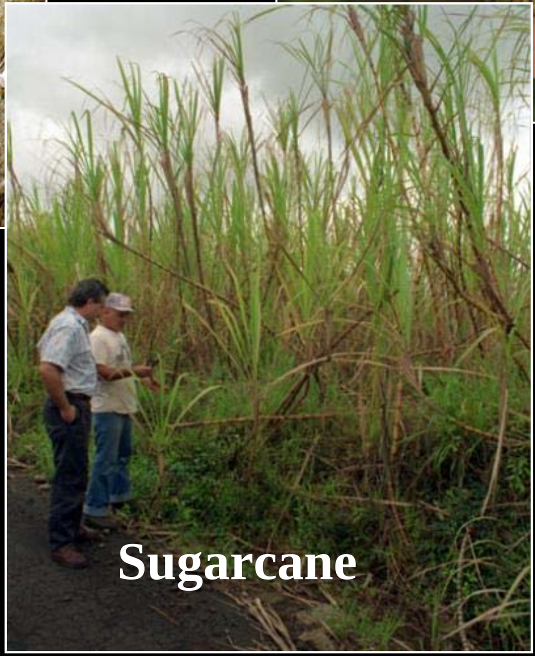
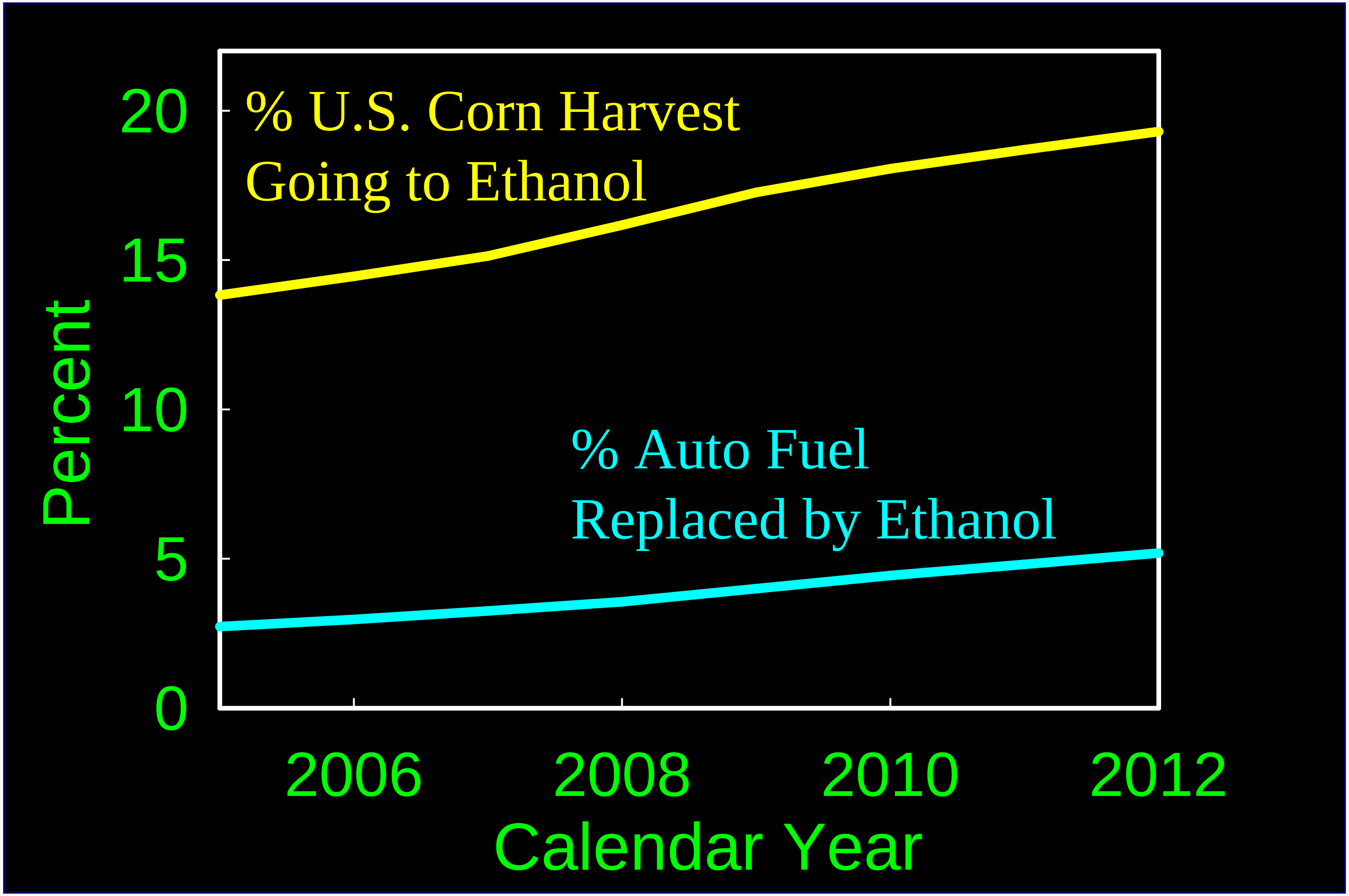
Fermentation Biotechnology Research

Current Paradigm



Potential of corn to replace oil for U.S. market



(RFA & NCGA, 2006)

Sources of Biomass

Long-term Goals of ARS Energy Crops Working Group

Develop new cultivars of herbaceous perennials that give superior ethanol yields through breeding.

Develop a better understanding of the interactions among species, maturity, and cell wall structure and the response to pretreatment and ethanol fermentation.

What can cellulosics do for us?

Feedstocks	**Million dry ton per yr**	**Billion gal.s of ethanol per yr**
Agricultural Land (selected)		
Corn Stover	**75**	**4.50**
Wheat Straw	**11**	**0.66**
CRP Biomass	**18**	**1.08**
Perennial Crops	**156**	**9.36**
Forestlands (selected)		
Logging & Processing residues	**134**	**8.04**
Total:	**4,894**	**23.6**

This is 17% of our total oil needs.

Notes: (1) 60 gal/ton ethanol yield; (2) source: http://feedstockreview.ornl.gov/pdf/billion_ton_vision.pdf

Hypothesis:

species (and plant type) and intra-species cell wall differences can influence enzymatic digestibility.

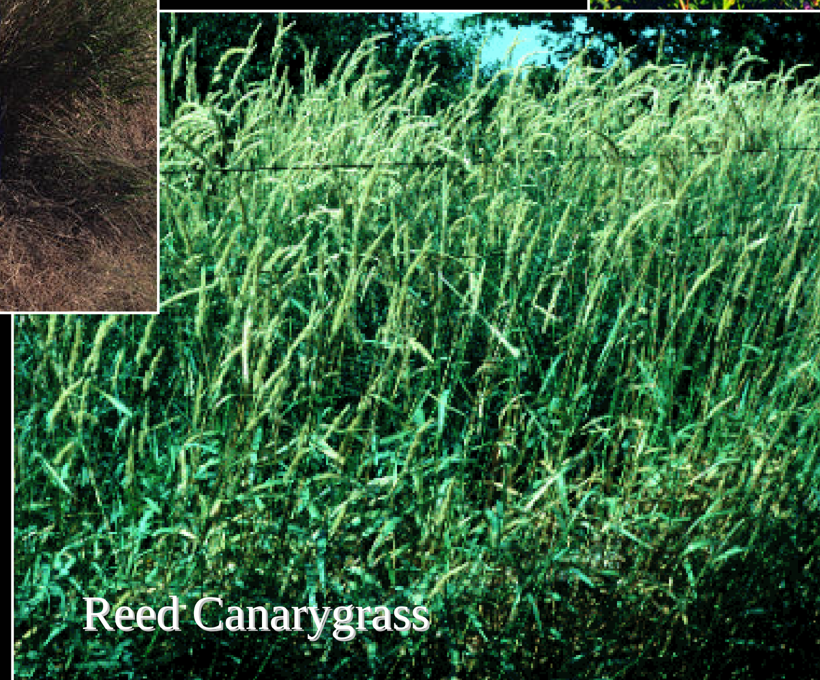
Approach:

- Vary species (plant type): **alfalfa** (legume, dicot), **reed canarygrass** (C3 cool season grass), and **switchgrass** (C4 warm season grass)

- Vary cell wall structure: by evaluating different maturities for each species


Switchgrass


Alfalfa


Reed Canarygrass

Sample Set

Species Maturity	Sample Description
Alfalfa (*Medicago sativa* L.)	
Bud (A1)	Stems, flower buds present, no open flowers
Full Flower (A2)	Stems, open flowers on all stem shoots
Reed canarygrass (*Phalaris arundinacea* L.)	
Vegetative (CG1)	Leaf blades and sheaths, no stem elongation
Ripe Seed (CG2)	Whole herbage, ripe seed
Switchgrass (*Panicum virgatum* L.)	
Pre-boot (SG1)	Leaf blades and sheaths, elongated stems
Anthesis (SG2)	Whole herbage, flower panicle on stems open
Post-Frost (SG3)	Whole herbage, ripe seed, senescent, post-frost

Samples were analyzed as follows

- Complete chemical composition using the Uppsala fiber analysis system (and detergent fiber system)
- Fiber digestibility by treating w/ dilute acid pretreatment and cellulase enzymes (using modified methods developed by NREL, DOE)
- Ethanol yields with *S. cerevisiae*, currently only for switchgrass

Overall composition of biomass (g/kg,DM)

Species/ Maturity	Ether Extracts	Crude Protein	Total Carbo	Klason Lignin	Ash	Total
Alfalfa						
Bud	9	127	563	158	81	970
Full Flower	7	88	598	175	58	950
Reed Canarygrass						
Vegetative	22	88	518	109	128	889
Ripe Seed	13	45	597	148	95	908
Switchgrass						
Pre-Boot	10	65	569	133	89	875
Anthesis	10	32	655	154	57	917
Post-Frost	16	30	650	173	57	915

Break-down of carbohydrates

Species/ Stage	Soluble	Storage	Cellulose	Xylan	Total
	g/kg, dm				
Alfalfa Stems					
Bud	55	3	275	148	481
Full Flower	49	2	306	165	522
Reed Canarygrass					
Vegetative	81	35	209	171	496
Ripe Seed	45	54	265	212	576
Switchgrass					
Pre-Boot	40	5	273	231	549
Anthesis	76	39	283	238	636
Post-Frost	27	7	322	273	629

Theoretical ethanol yields broken down by carbohydrates

Species/ Stage	Soluble	Storage	Cellulose	Xylan	Total
		gallons per dry ton			
Alfalfa Stems					
Bud	10	1	48	26	84
Full Flower	8	0	53	29	91
Reed Canarygrass					
Vegetative	14	6	36	30	86
Ripe Seed	8	9	46	38	100
Switchgrass					
Pre-Boot	7	1	47	41	96
Anthesis	13	7	49	42	111
Post-Frost	5	1	56	48	110

Corn stover = 113 gal/ton

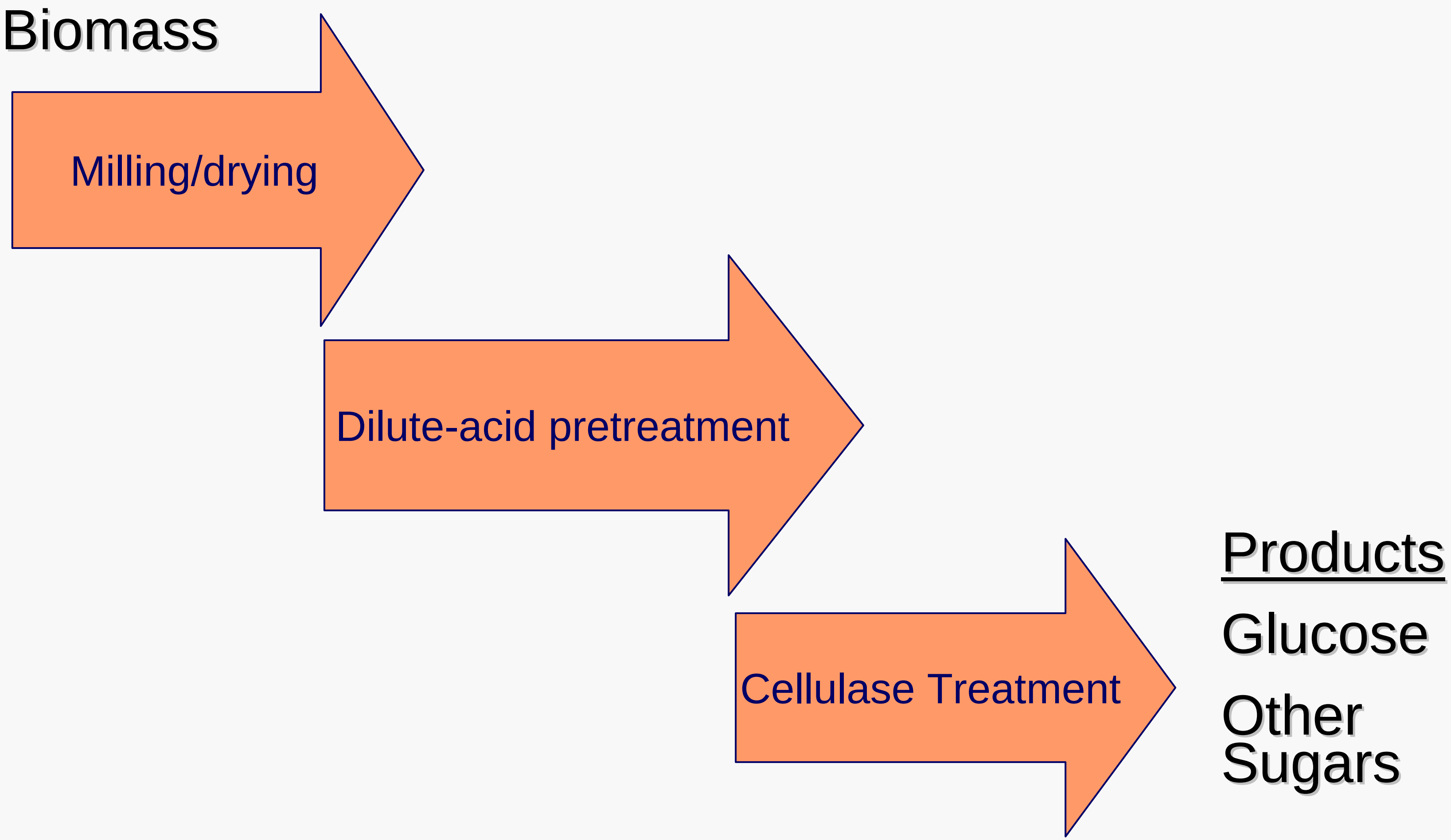
Comparison of Upsalla & Detergent Fiber systems

(selected data)

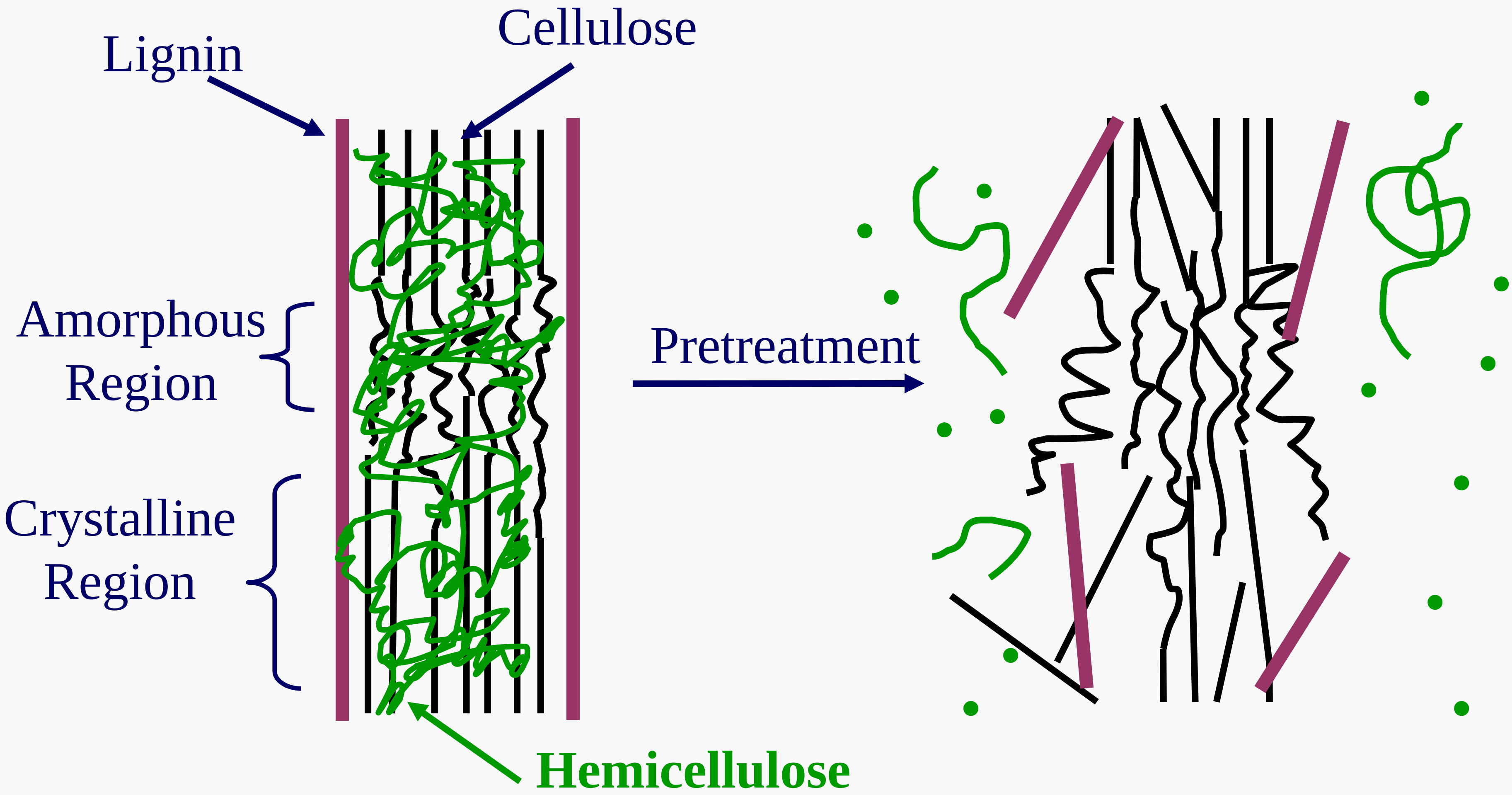
Species	Cellulose		Hemicellulose		Lignin	
Stage	Glucose	ADF-ADL	Sugars	NDF-ADF	KL	ADL
Alfalfa						
Flower	306	444	122	144	175	71
Reed Canarygrass						
Ripe Seed	265	356	218	305	148	20
Switchgrass						
Anthesis	283	340	245	301	154	23

DFS overestimates cellulose, hemicellulose and underestimates lignin

■ Measuring recoverable sugar yields



Effect of Pretreatment



Severity of Dilute Acid Pretreatment of Cellulose for Enzymatic Digestion

Combined Severity Factor (CSF):

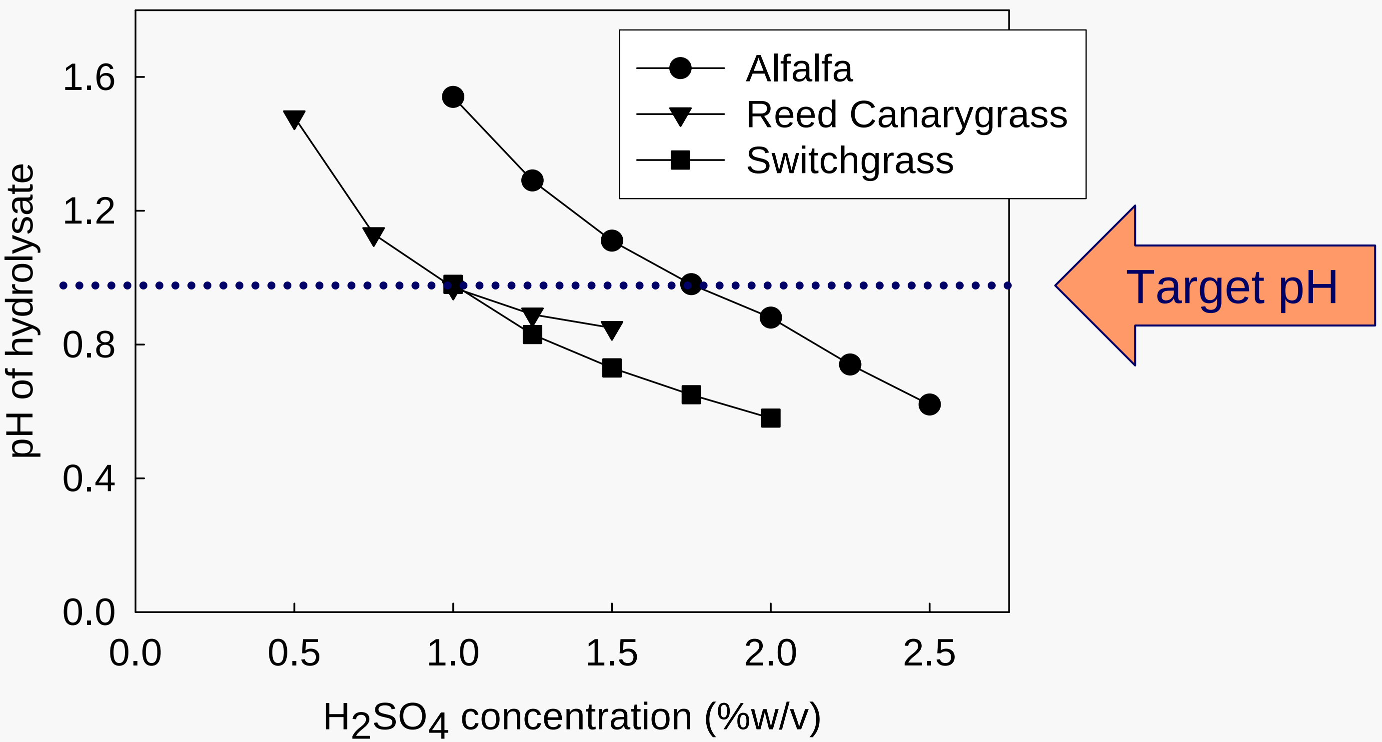
$$CSF = Log\left[t \times \exp\left(\frac{T - 100}{14.75}\right)\right] - pH$$

<u>Factors for pretreatment:</u>

- Time (at temperature)
- pH (or Acidity)
- Temperature (exponential effect!)

The lower the severity the lower the cost of pretreatment, the higher the recovery of xylan associated sugars, and more fermentable the product.

Amount of acid that needs to be added to reach similar pH's



Detailed Protocol for Measuring Sugar Yields

Milled Biomass

↓

Dilute sulfuric acid treatment at pH 1, 150°C for 20 min

↓

Cellulase treatment with 50 FPU/g cellulose at pH 4.5, 50°C for 72 hr
(note: Celluclast + Novo188 (Novozymes, Inc.)

↓

Measured released glucose and non-glucose sugars by HPLC

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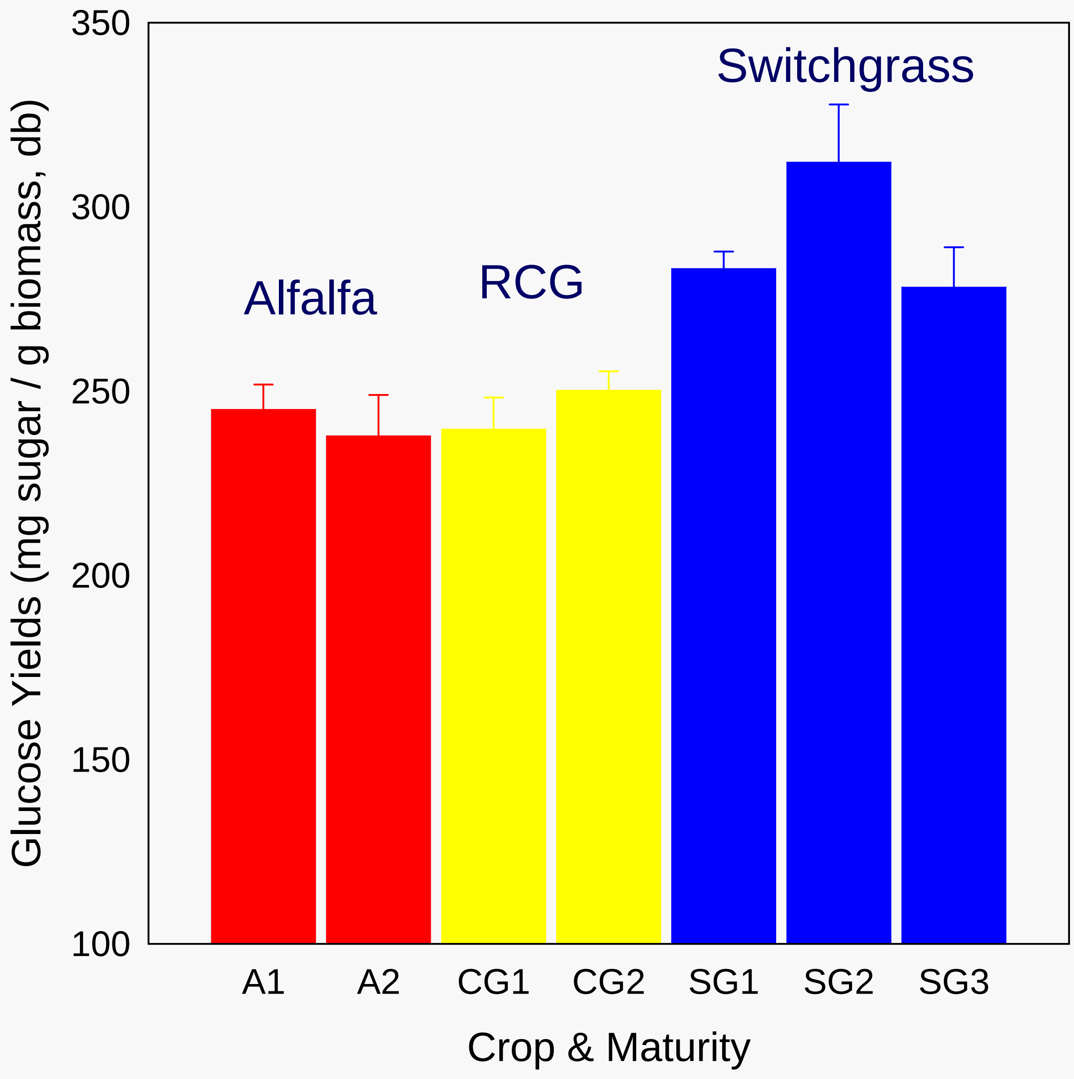
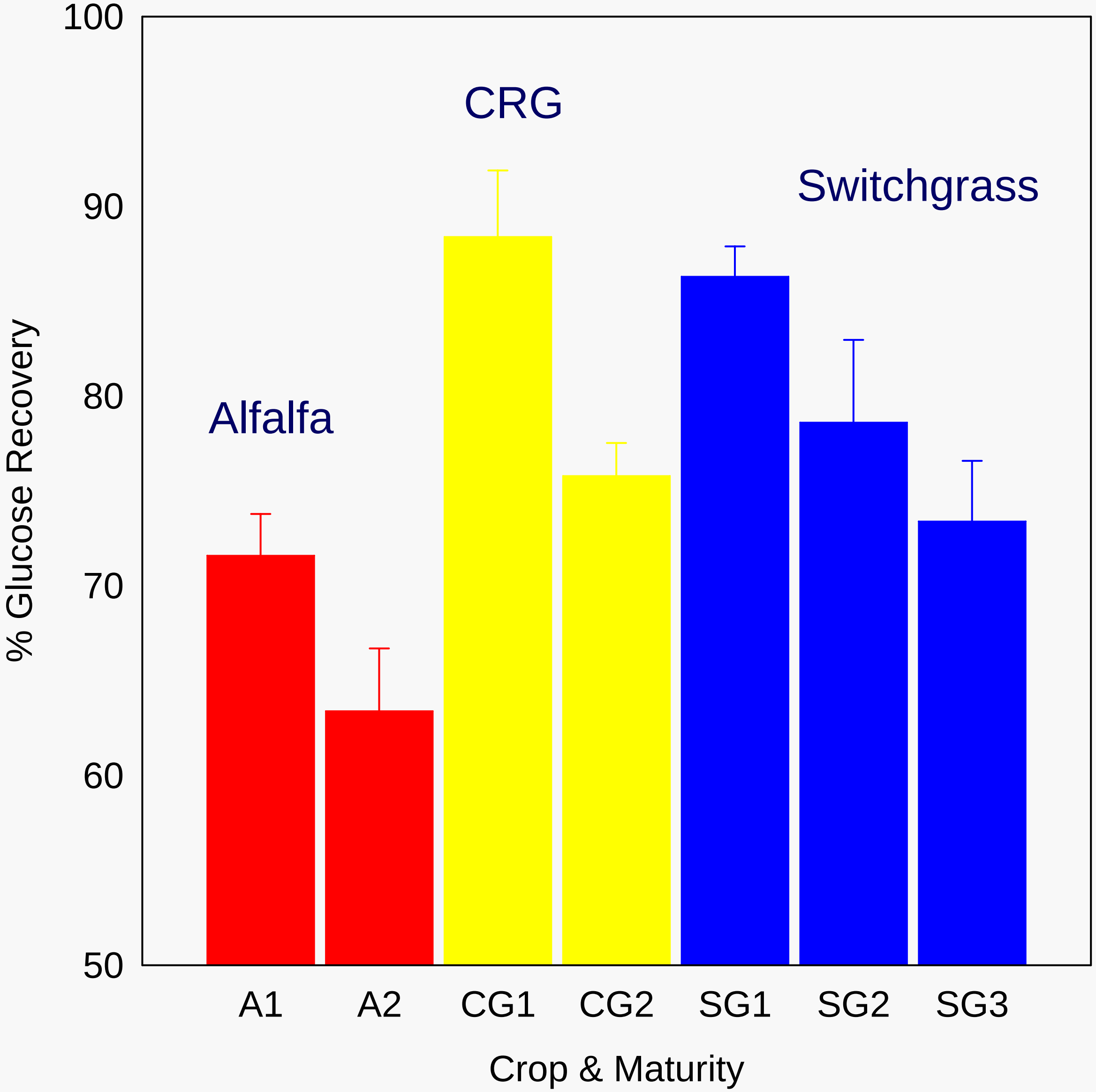
Calculate sugar yields (g/g dry biomass) & % recovery as free sugars

Changes in glucose yields with maturity and species

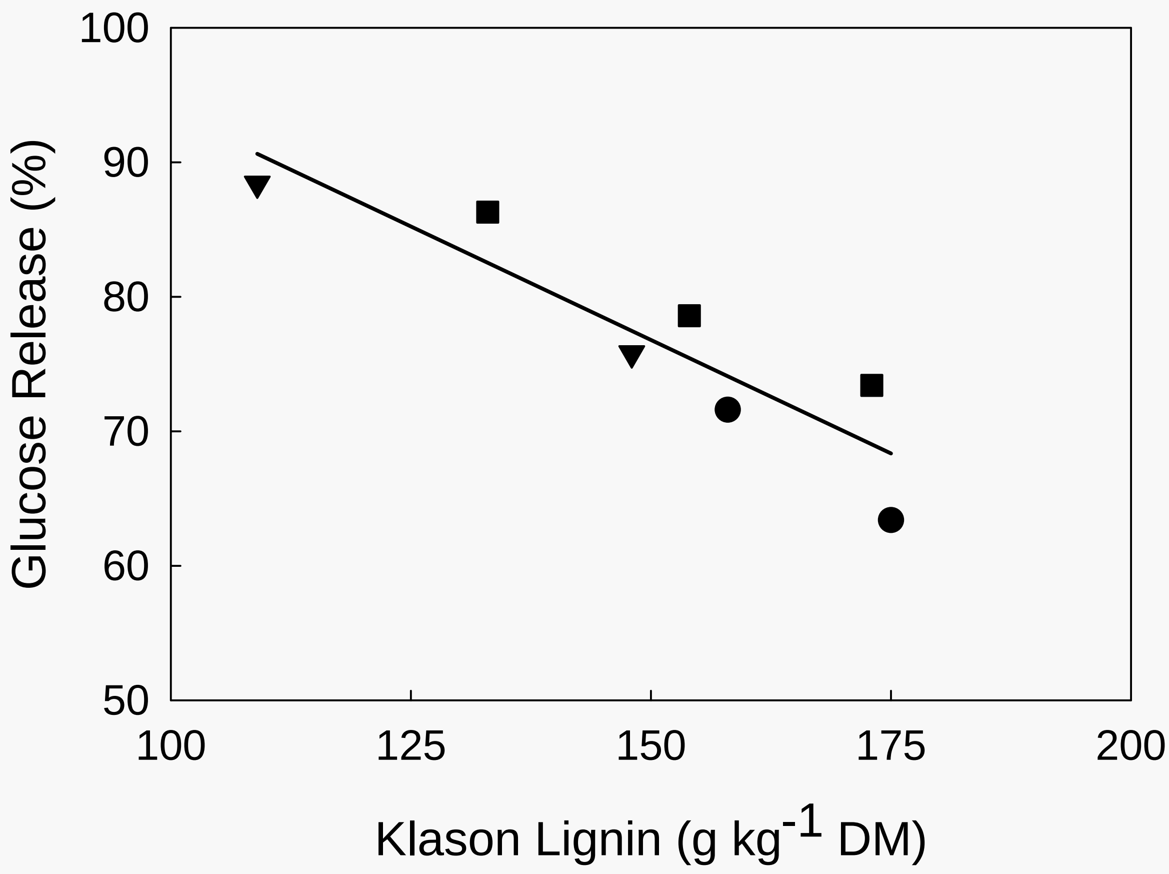


Changes in Cellulose Digestion with Species and Maturity

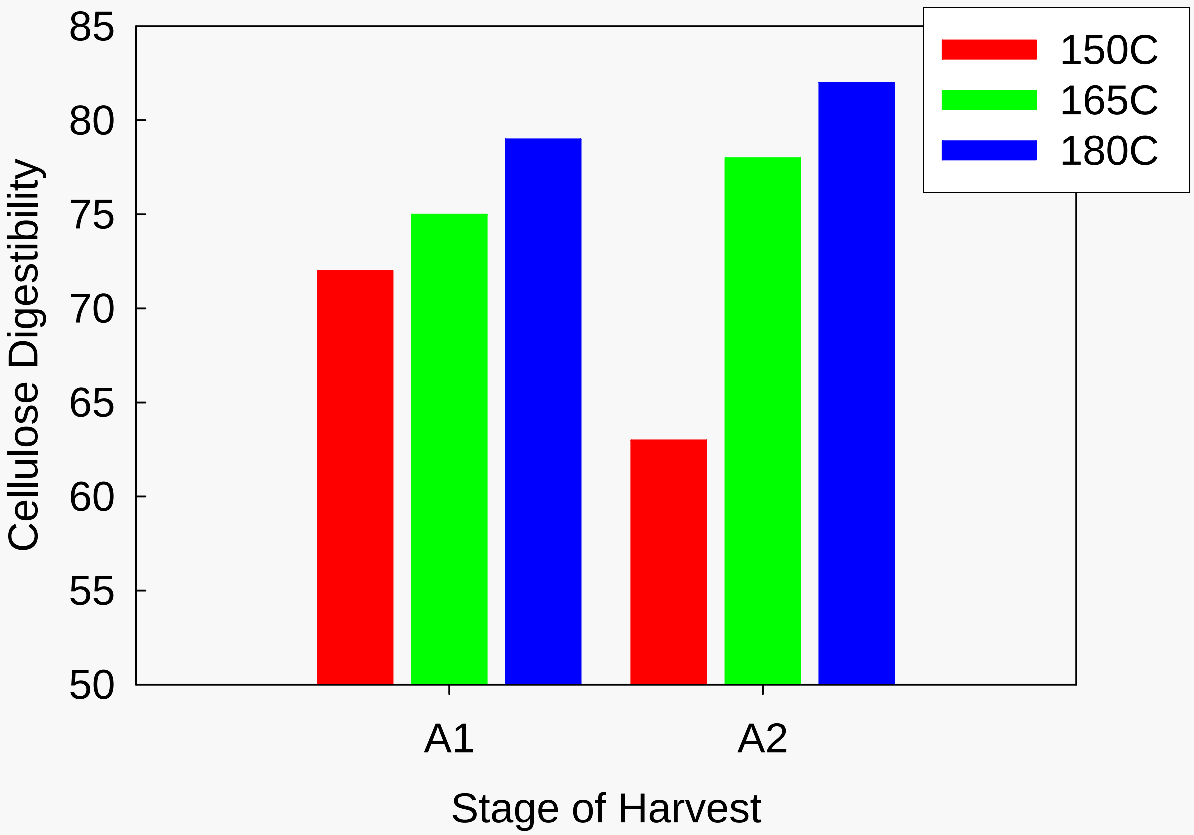


Error bars = 95% CI

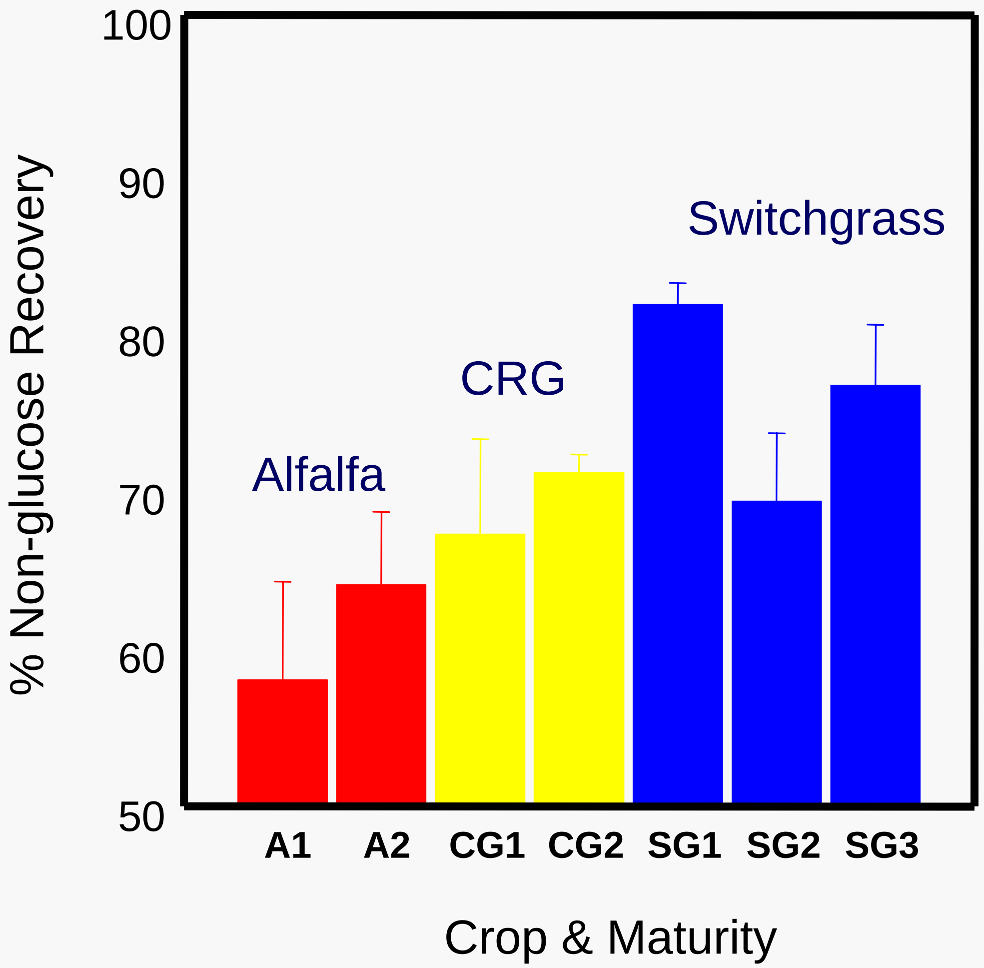
Lignin vs. glucose conversion efficiencies



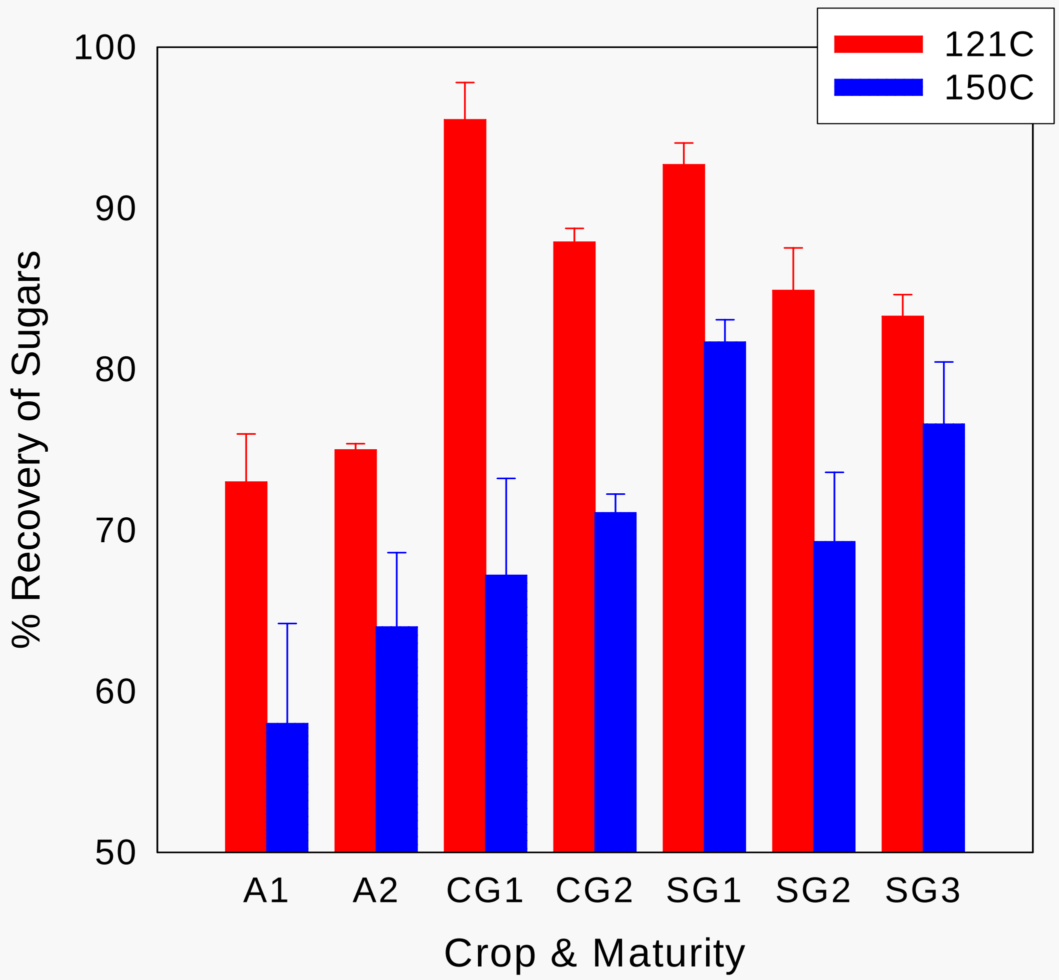
Increasing severity for alfalfa stems



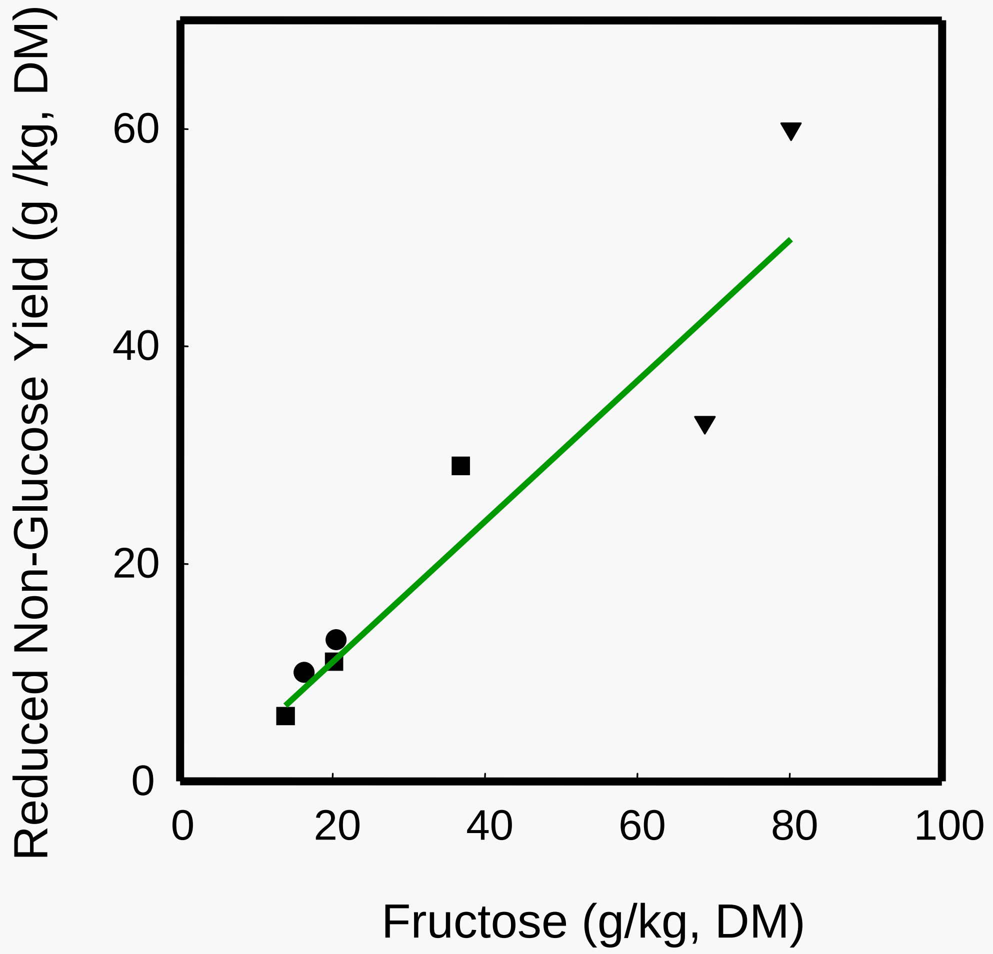
Recovery of other sugars



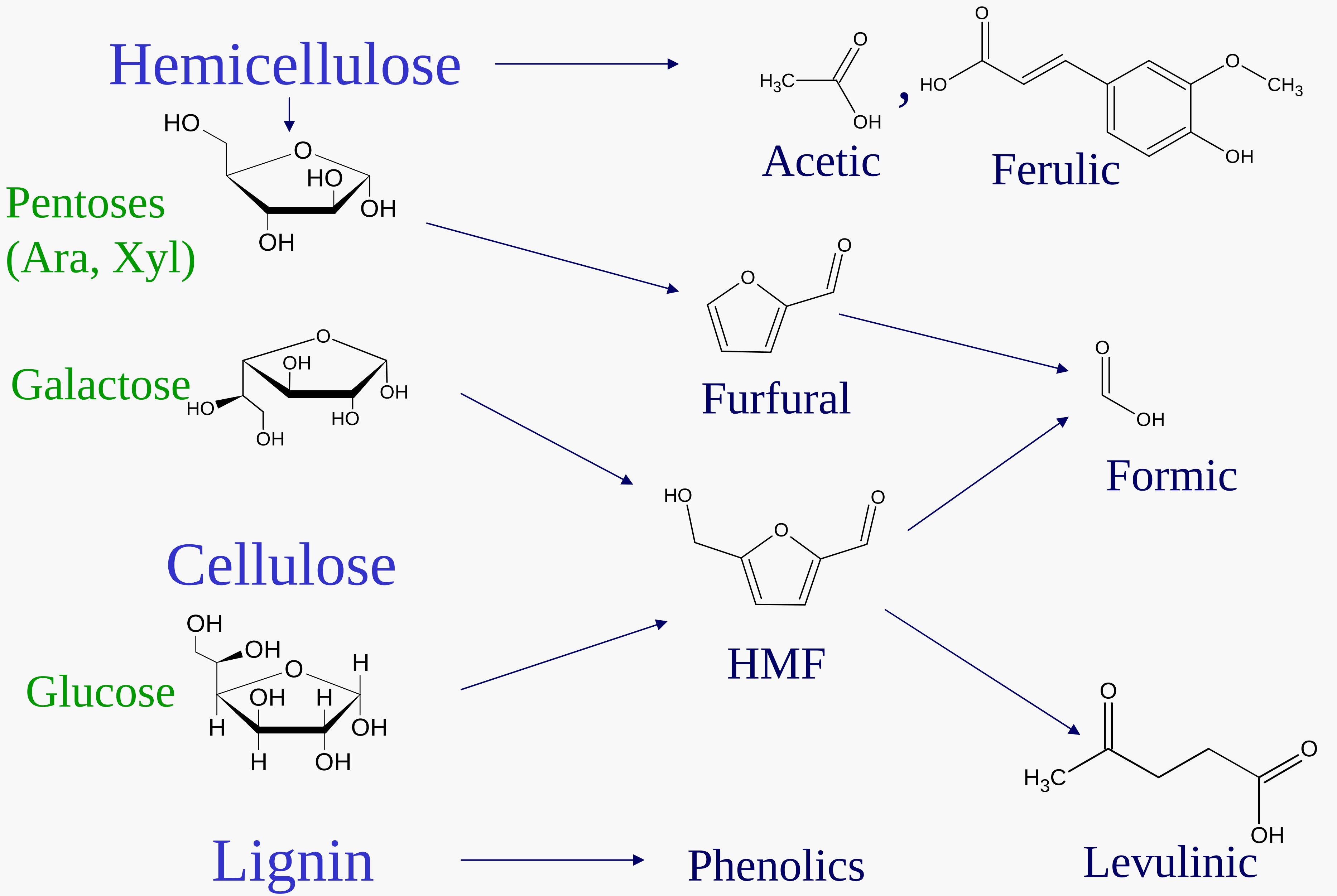
Pretreating Biomass at 121°C vs. 150°C



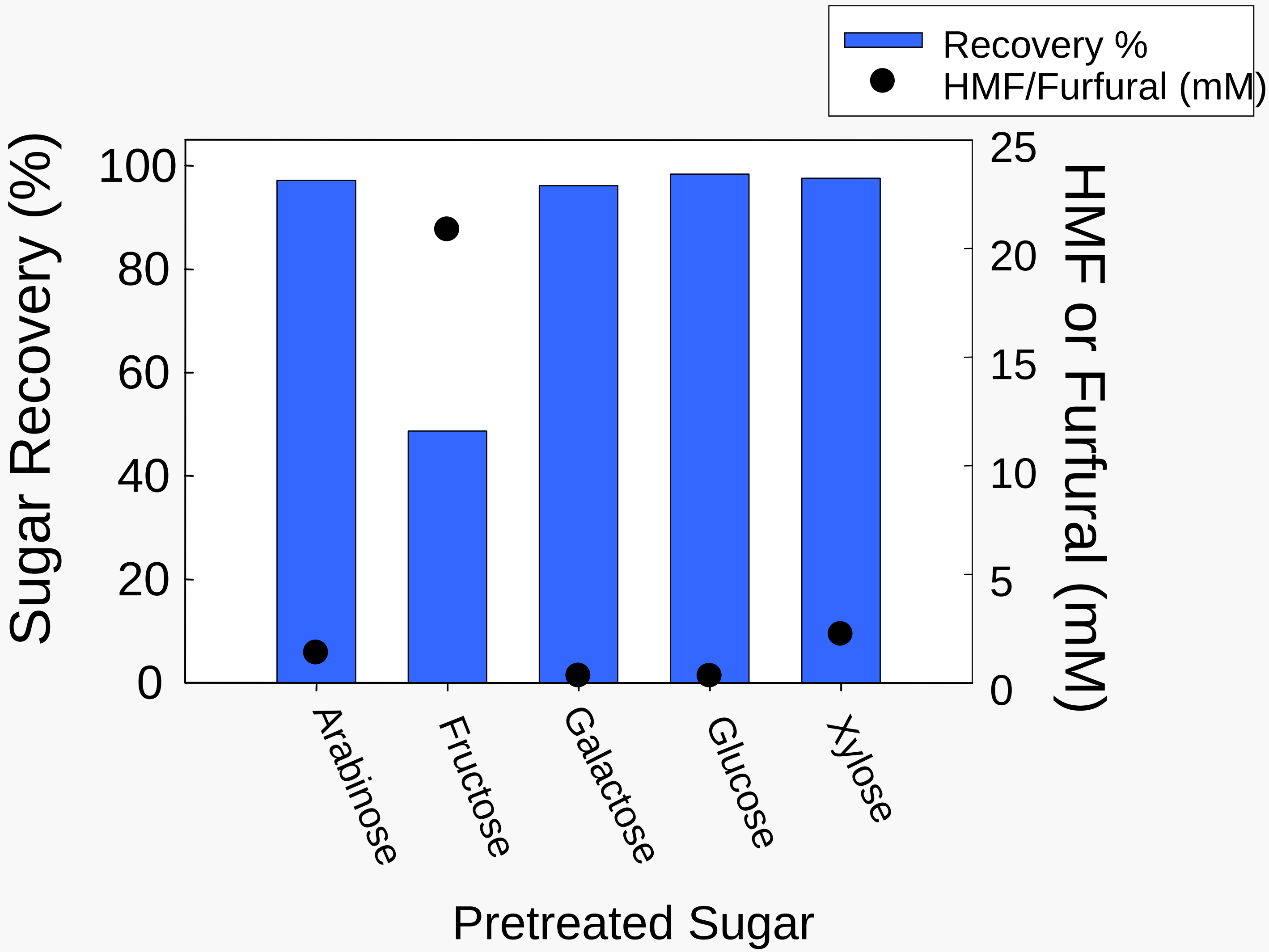
Role of fructose in reducing yields



Inhibitors formed during hydrolysis



Treatment of sugars at pH 1, 121°C for 1 hr



Future work

- Developing a screening method capable of evaluating hundreds of cultivars for *relative* cellulose fermentation efficiencies that will allow us to select the best for further development (discussed today)

Selecting other pretreatment methods for treating forage type material (not discussed today)

Proposed SSF screening method

Milled Biomass

↓

Dilute sulfuric acid treatment at 121°C for 1hr
& Neutralize to pH 4.8 with $Ca(OH)_2$

↓

Ferment w/ *S. cerevisiae* in presence of cellulase (5 FPU/g, DM)
at 35°C for 72 hr

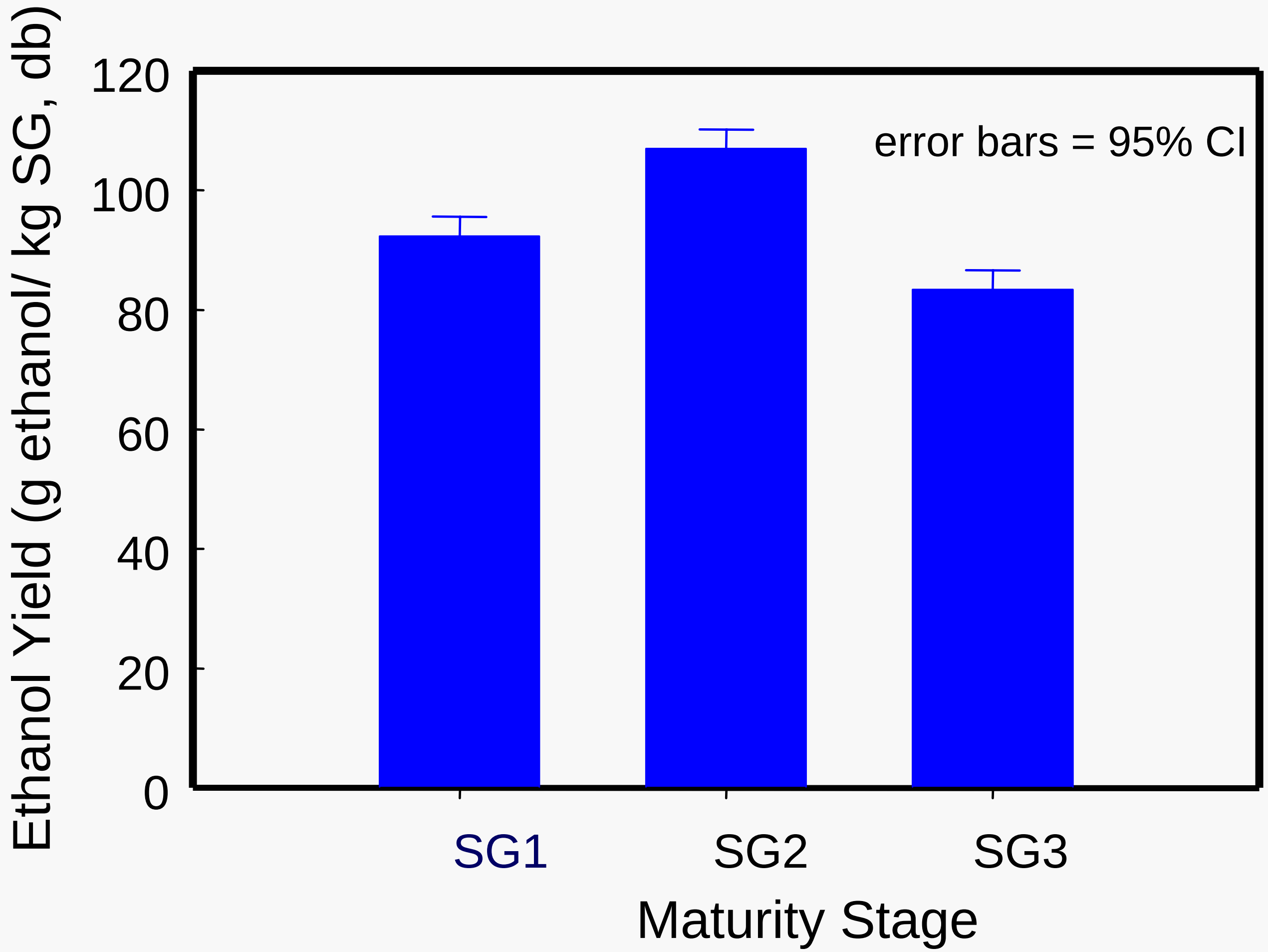
↓

Measure ethanol and non-glucose sugars by HPLC

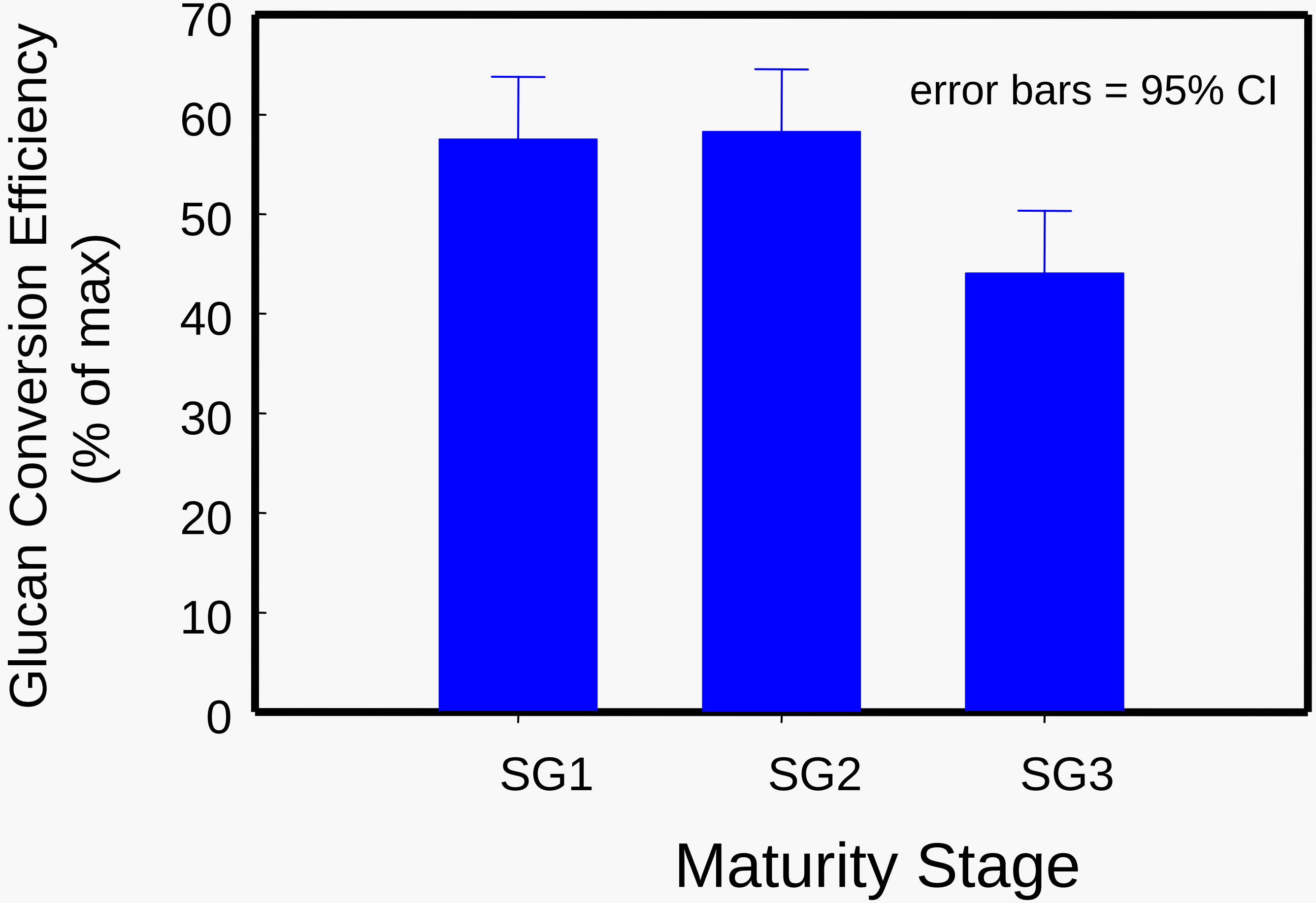
↓

Calculate Ethanol yields (g/g dry biomass) & % of theo. yield

Effect of Maturity of Switchgrass on Ethanol Yield



Effect of Maturity of Switchgrass on Glucan Conversion Efficiency to Ethanol



Conclusions

- Recoverable sugars (& ethanol yields) varies with species and maturity w/ in species
- Available glucose varied inversely with maturity and lignin content. However, total glucose yield increased with maturity due to higher cellulose contents.
- Cell wall polysaccharides, as determined by the widely applied detergent fiber system are inaccurate. Overestimates cellulose and hemicellulose and underestimates lignin.
- Soluble sugar content can be significant and may be problematic for dilute acid pretreatment, especially fructose

What’s next?

- Expand scope of samples to include additional cultivars.
- Conduct actual fermentations using conventional yeast as well as recombinant yeasts and bacteria capable of fermenting pentoses as well as hexoses.
- Develop screening tools to handle greater throughput evaluations

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