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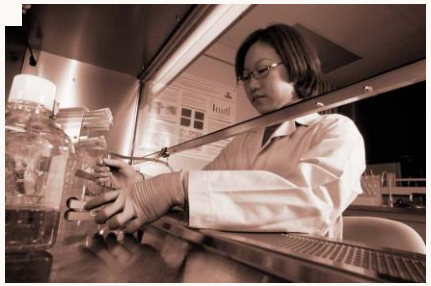


Liquid Hot Water Pretreatment of Corn Stover: Impact of BMR

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AGRICULTURAL & BIOLOGICAL ENGINEERING

LORRE

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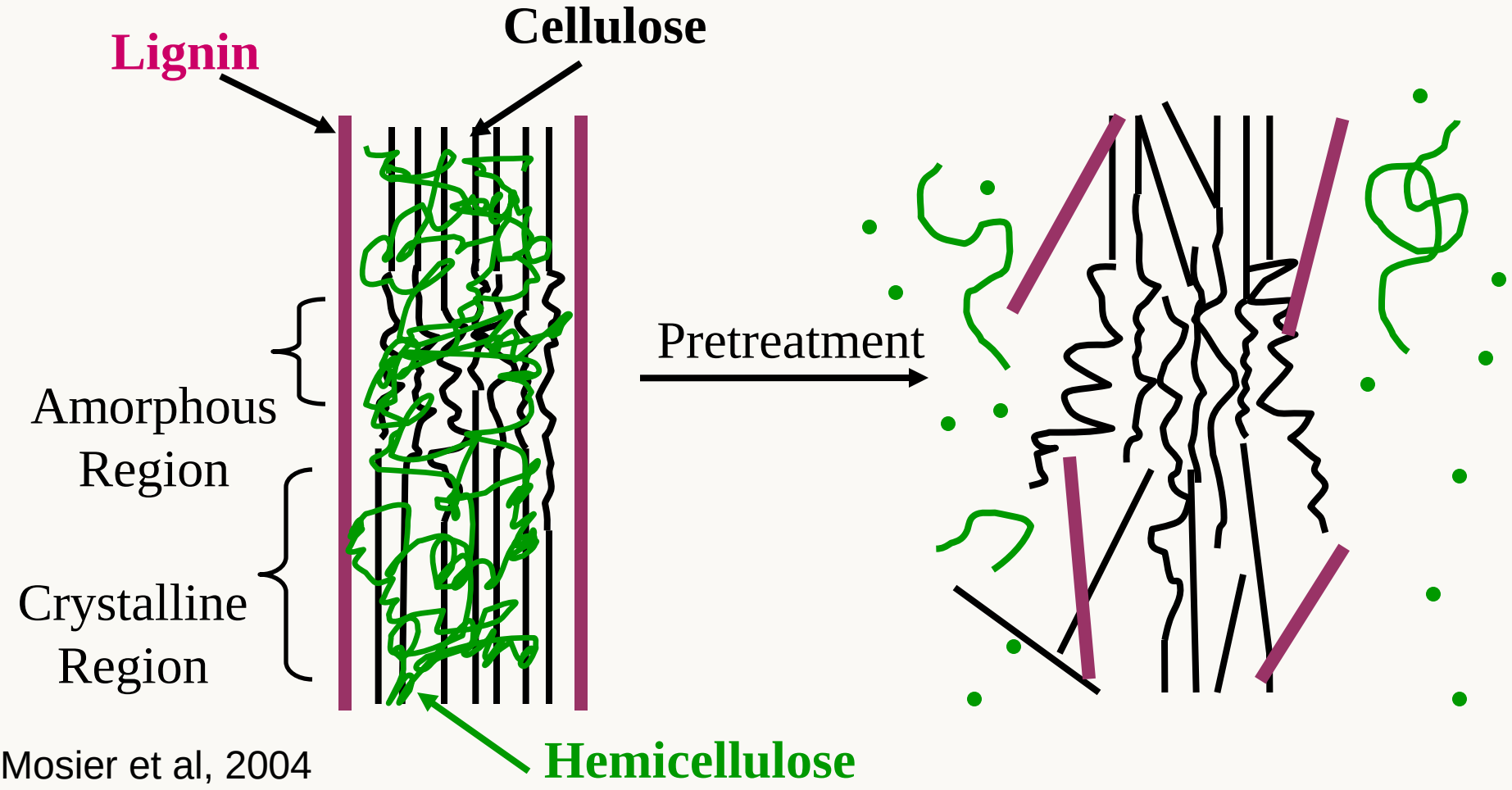
Effect of Pretreatment of Corn Stover
Representation of Physical Changes



Mosier et al, 2004

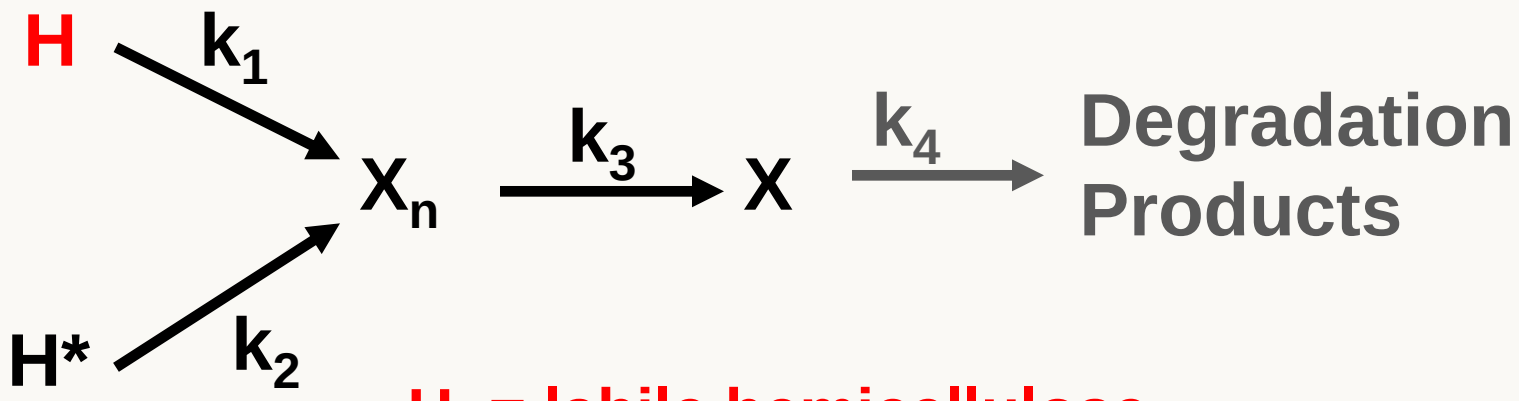
Goals:
Liquid Water Pretreatment

Determine conditions that:

1. **During** pretreatment
    1. Minimize hydrolysis to simple sugars
    2. Maximize disruption of lignin

2. **After** pretreatment
    1. Maximize hydrolysis to simple sugars
    2. Maximize fermentation of sugars to ethanol

Autohydrolysis during Pretreatment of Lignocellulose at 190 C



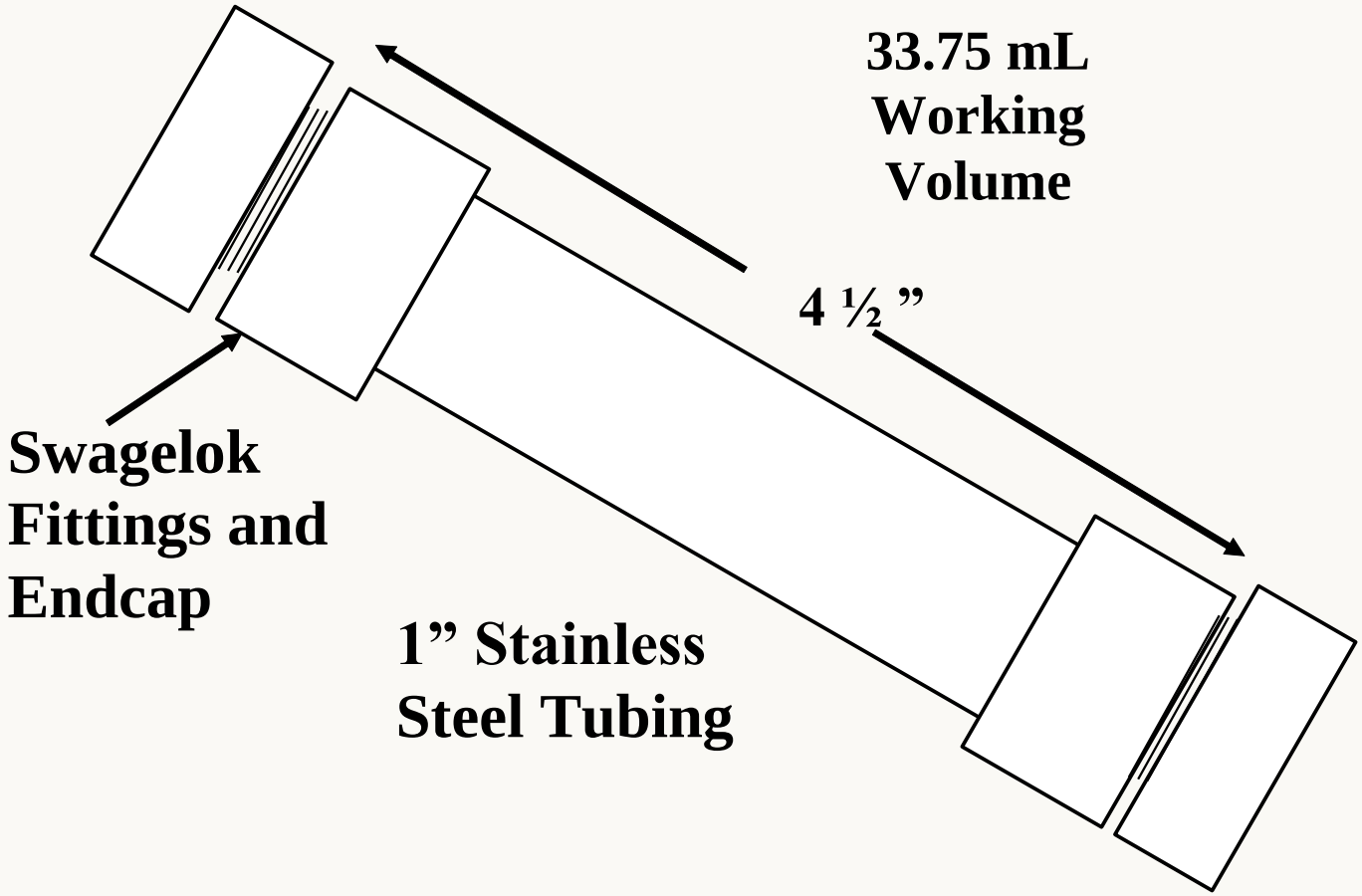
H = labile hemicellulose

H* = recalcitrant hemicellulose

X_n = soluble xylans (oligosaccharides)

X = xylose (monomer**)**

Liquid Hot Water Pretreatment at Lab Scale



Maize *brown midrib* mutants: naturally occurring lignin mutants

- At least four different genetic loci:

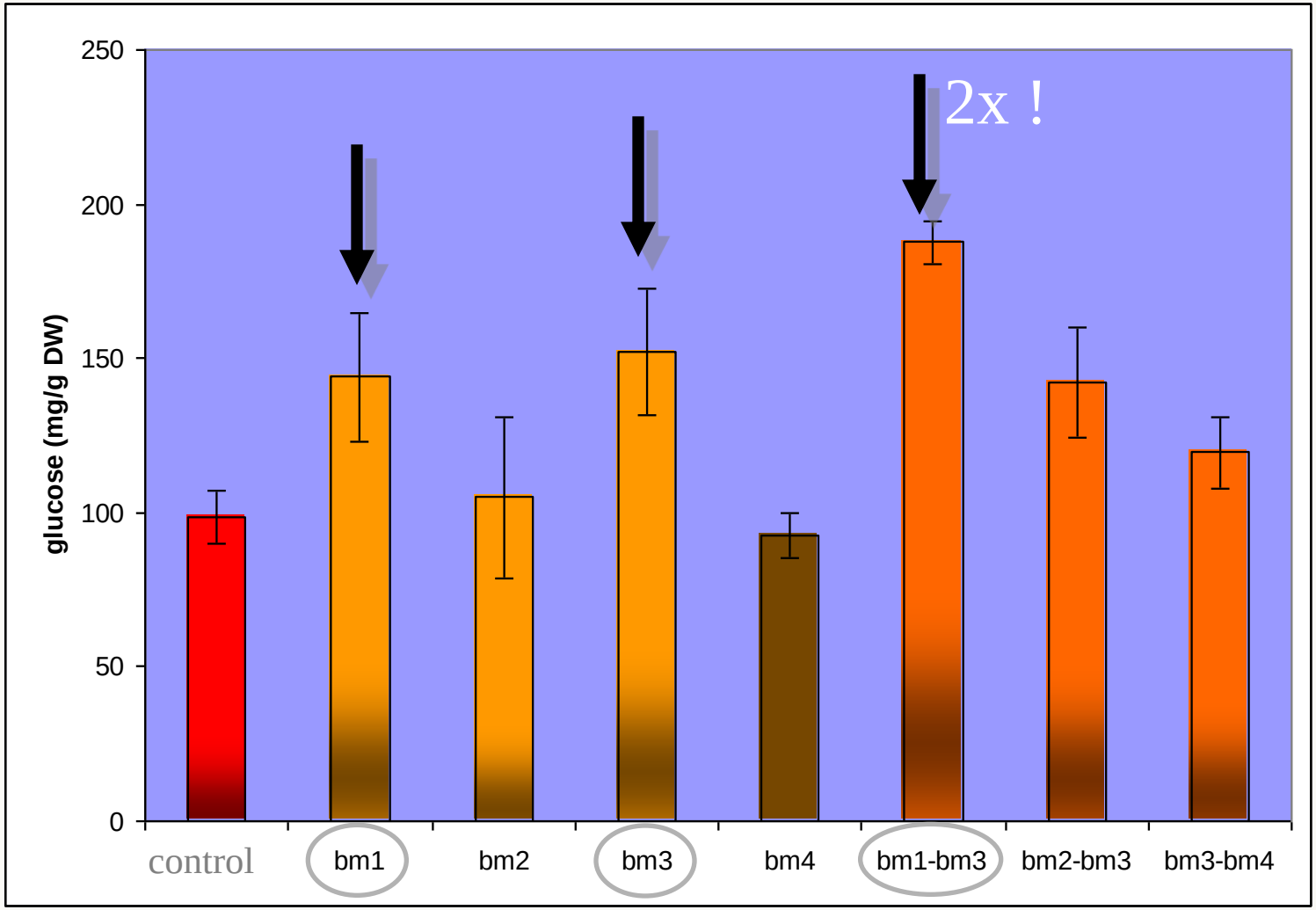
  Bm1, Bm2, Bm3, Bm4

- Maize and Sorghum BMR known
- BMR maize commercially available
- Mutants have
  - defective copies of the genes in biosynthesis of lignin monomers
  - brown vascular tissue
  - alterations in lignin chemical composition
- Maize BMR varieties were discovered between 1924 and 1947

Brown Midrib Maize

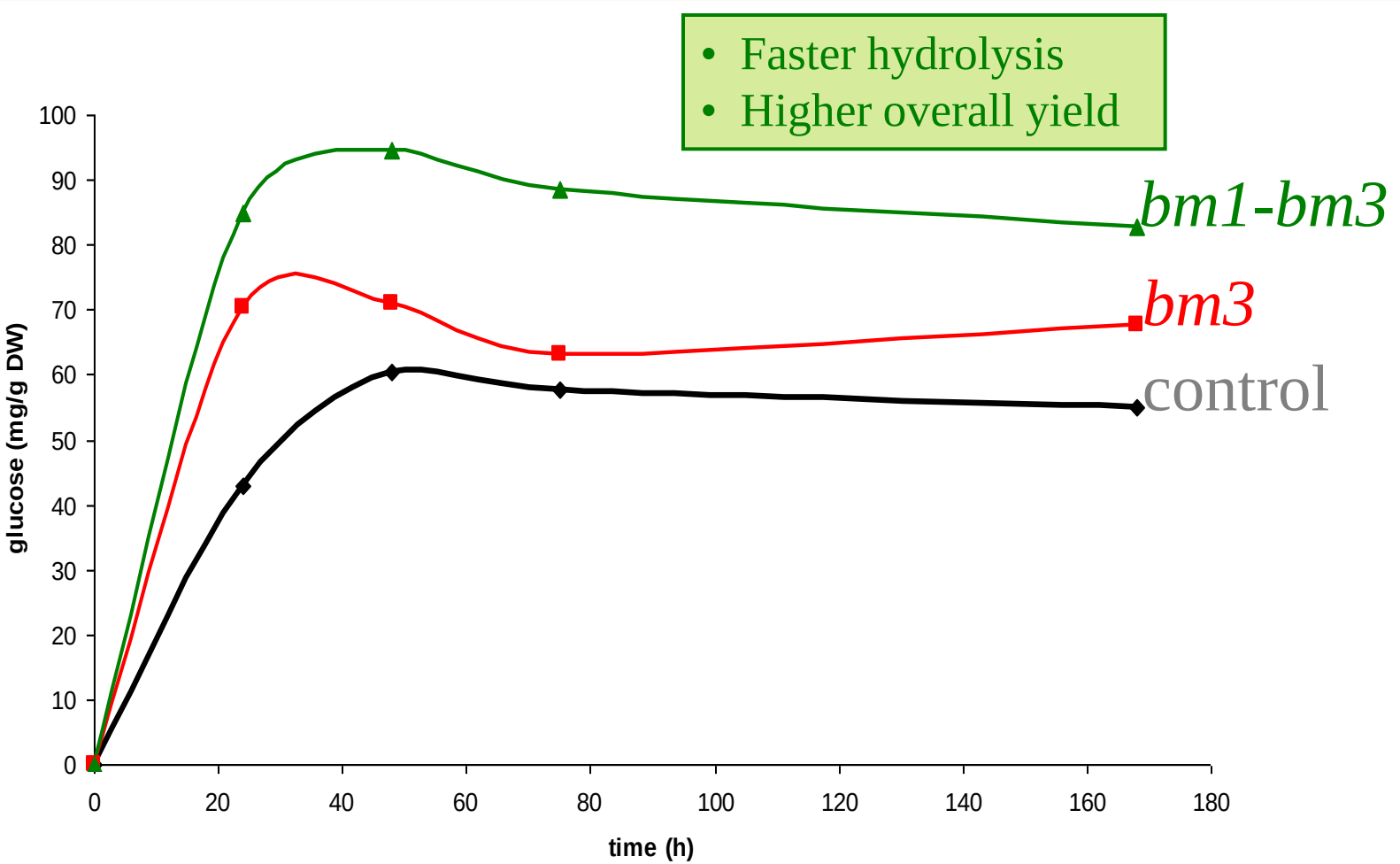
- Known to have higher digestibility in ruminants – grown commercially for silage to feed dairy cows
- Hypothesis:
  - Higher digestibility in ruminants may translate into higher enzymatic digestibility for biofuel production

Improved yield of fermentable sugars from *brown midrib* corn stover (no pretreatment)



All mutants in the same genetic background (A619)

Enzymatic Hydrolysis of Corn Stover

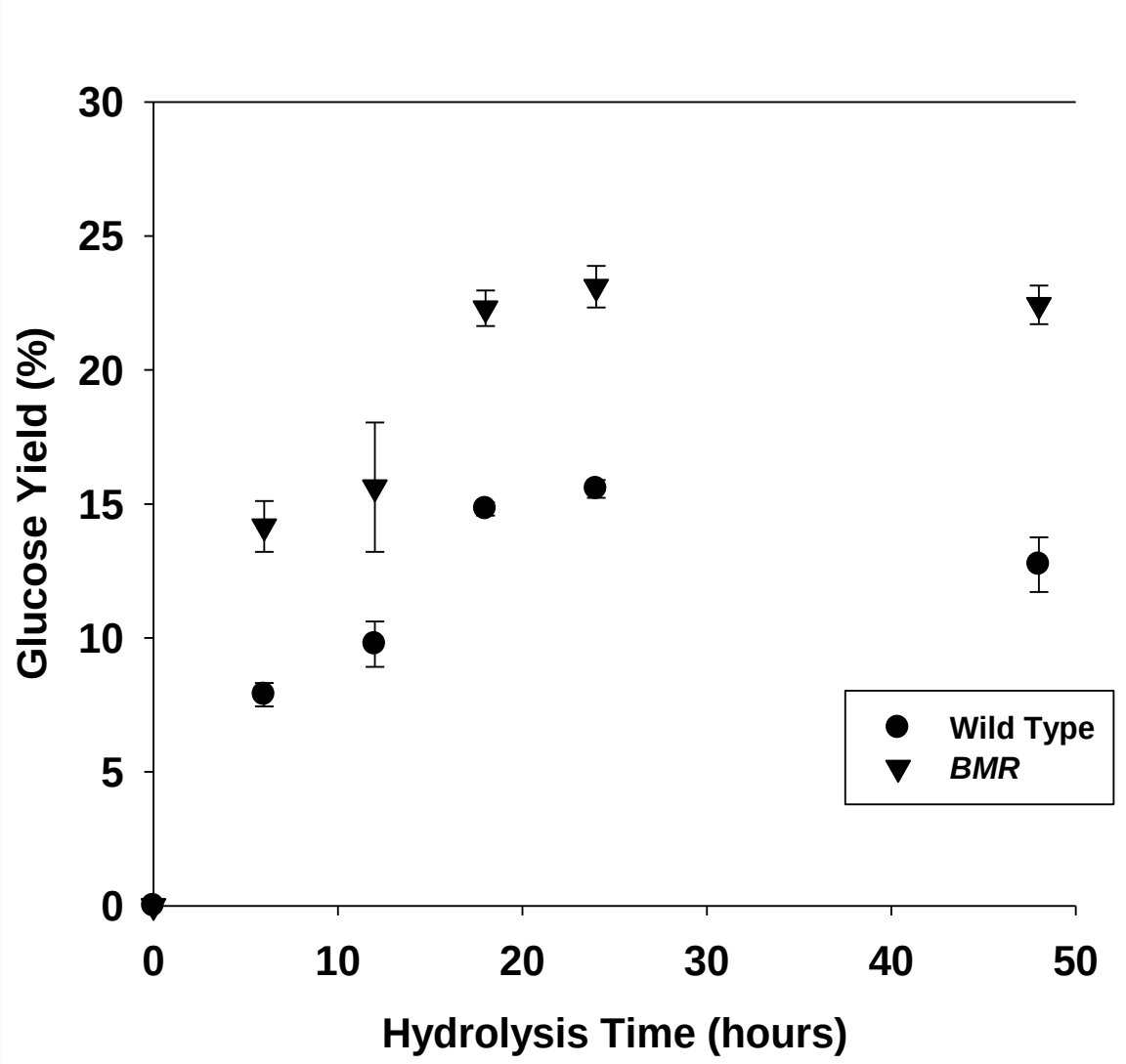


Dry matter composition of wild type and BMR maize stover: Commercial Hybrids

Composition (% dry mass)	Wild Type	BMR	CAFI Maize Stover*
Glucan	39%	41%	34%
Xylan + Galactan	25%	27%	24%
Arabinan	2.8%	3.1%	4.2%
Acetyl	3.9%	3.5%	5.6%
Lignin	22%	20%	17%
Ash	4.1%	4.9%	6.1%
Total	96.8%	98.5%	90.9%

* Mosier et al., 2005a.

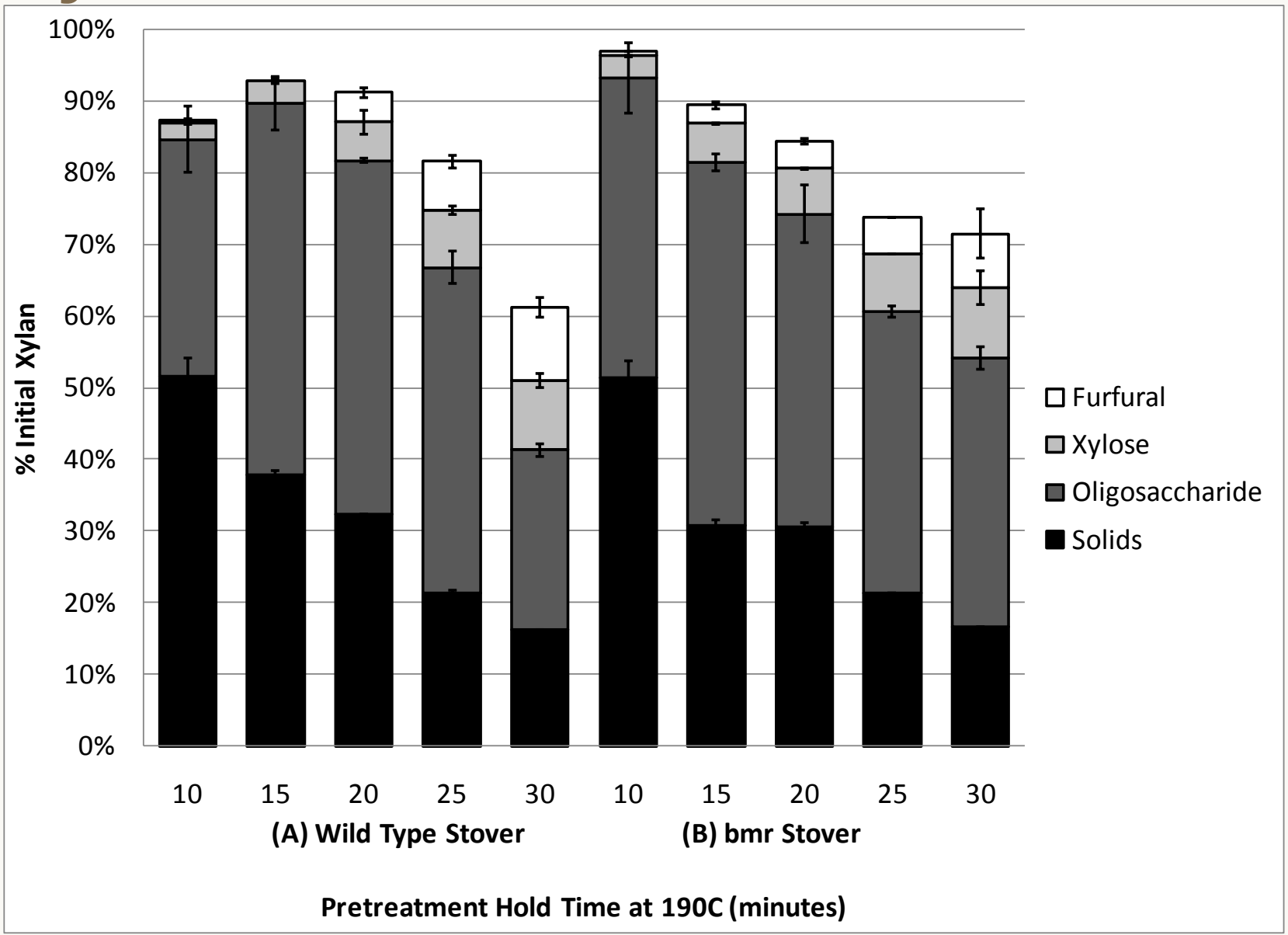
Enzymatic hydrolysis of maize stover (no pretreatment) with 15 FPU /g glucan cellulase supplemented with 40 IU β-g



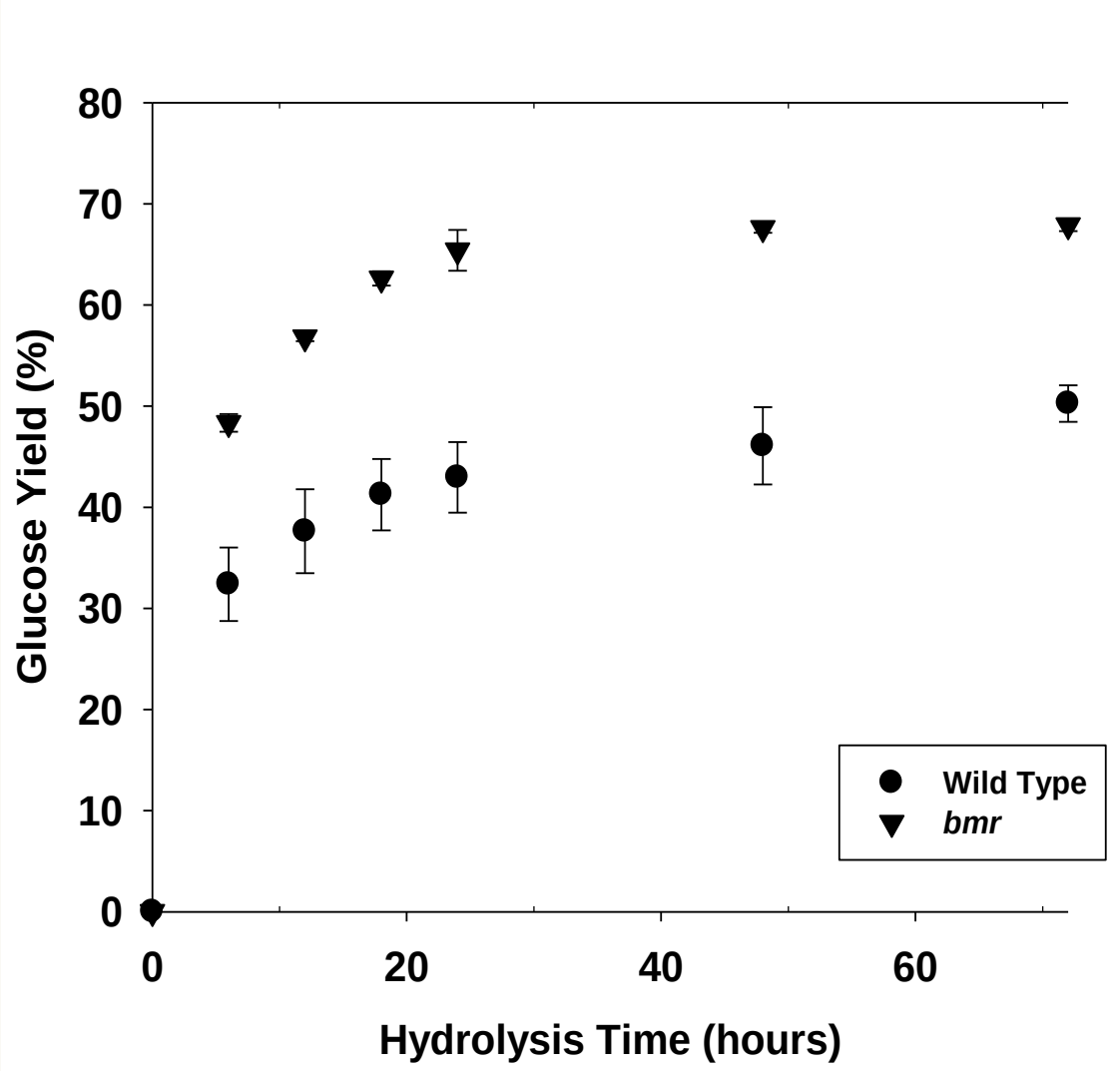
Pretreatment Method

- Liquid Hot Water Pretreatment
  - 15% Solid Loading (150 g/L)
  - 190 ° C, varying times
  - 1 inch stainless steel tube

Mass Balance Around Pretreatment: Xylan



Enzymatic Hydrolysis of Unwashed, Pretreated Maize Stover Slurry 15 FPU/g glucan



Summary of BMR Stover

- Optimum pretreatment conditions same for both types
- Lignin structure, but not amount, accounts for difference in enzymatic hydrolysis of untreated stover
- Differences observed after pretreatment
  - BMR stover digestibility 70%
  - Wild type digestibility 50%
  - BMR results in 40% improvement in sugar yield

Materials and Methods

- Silage
  - Whole maize plants from field plots were harvested, chopped, and ensiled in commercial silage bunkers.
  - Ensiling is a fermentative preservation method: microbial activity produces organic acids that lower the pH and generate anaerobic conditions to preserve biomass for long term storage.
  - Samples were taken after 6 months and stored at 4 C until processed

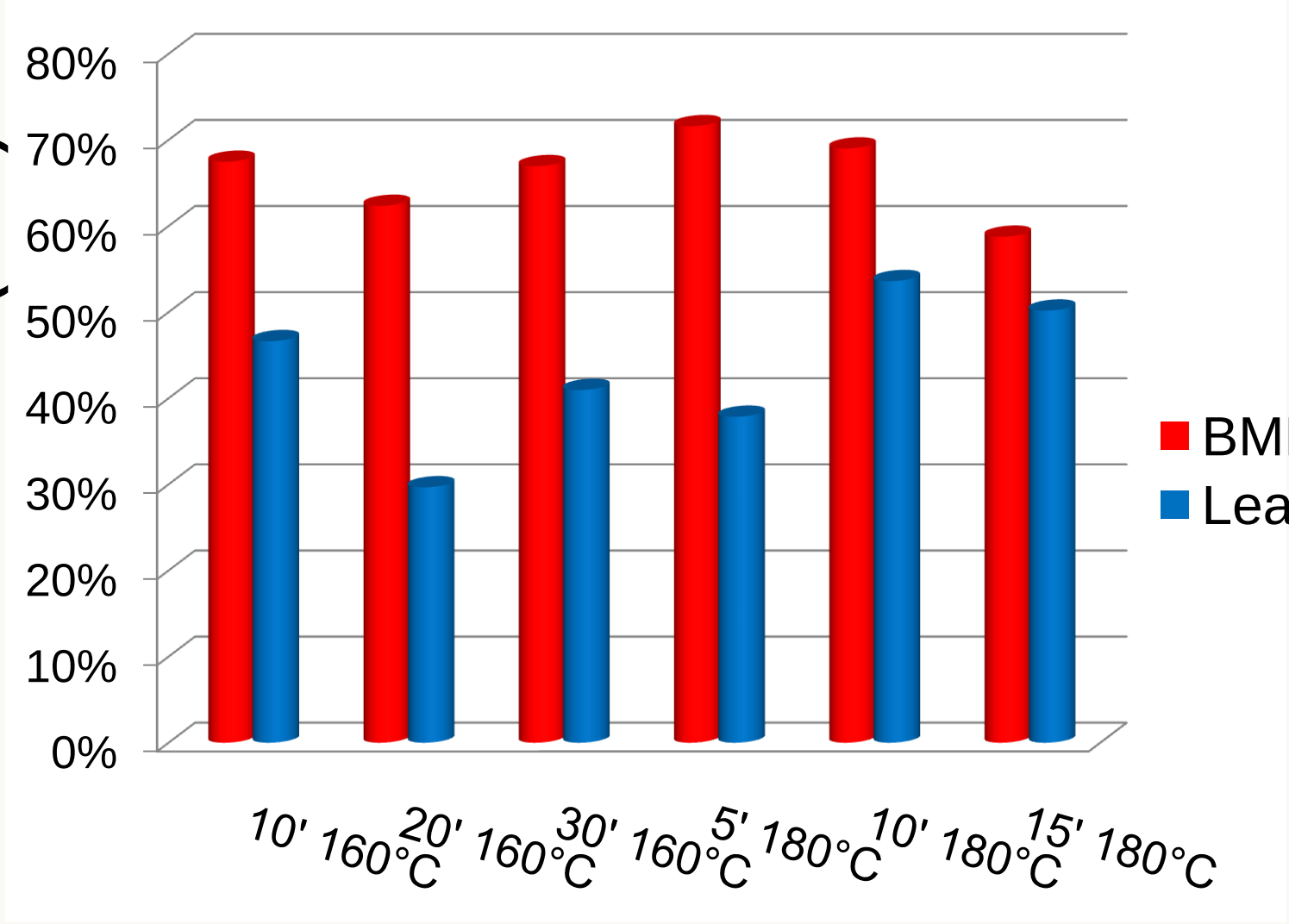
Composition of Silage

	Glucan	Xylan	Arabinan	Lignin
BMR	48.7%	27.6%	2.9%	10.4%
Leafy	59.9%	21.0%	4.0%	11.0%

Methods

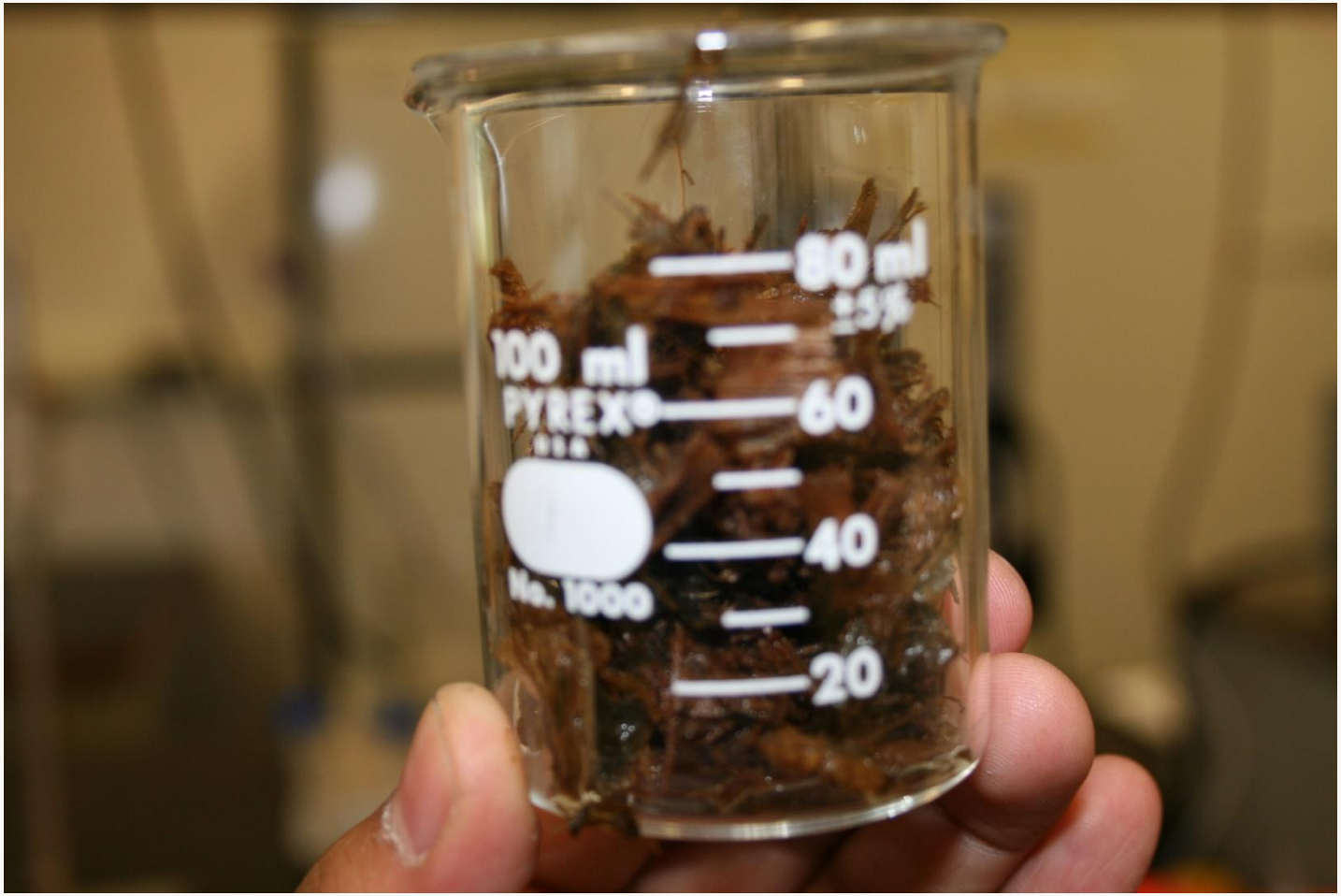
- Stainless Steel Reactors (35ml volume)
- Loading 20 w/w % (200 g/L)
- Sandbath heat up and temperature control
- Enzymatic Digestion
  - Whole slurry (undiluted)
  - pH adjustment to ~5
  - Spezyme CP and Novozyme 188 (15 FPU/g glucan and 40 CBU/g beta-glucosidase)

Optimization of Silage Pretreatment



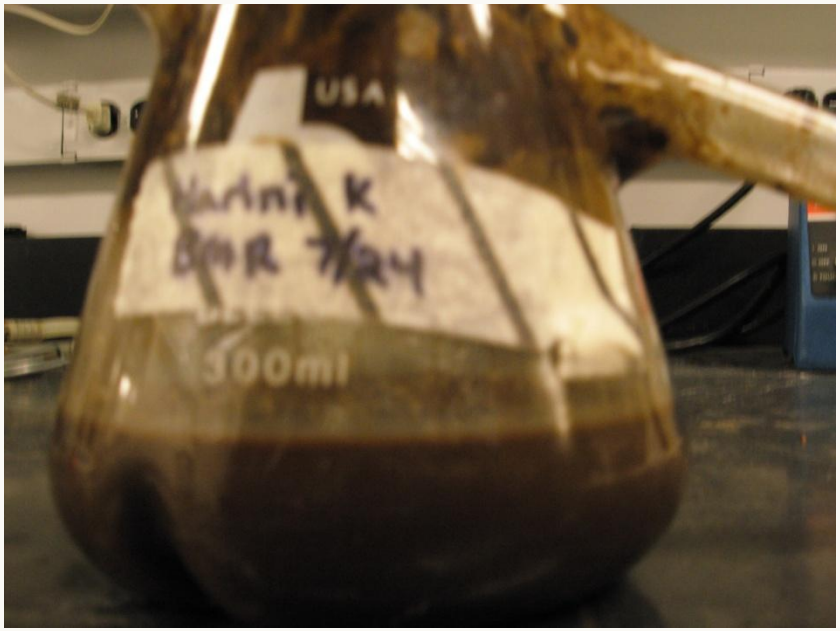
Pretreated BMR Silage

Pretreated Leafy Silage

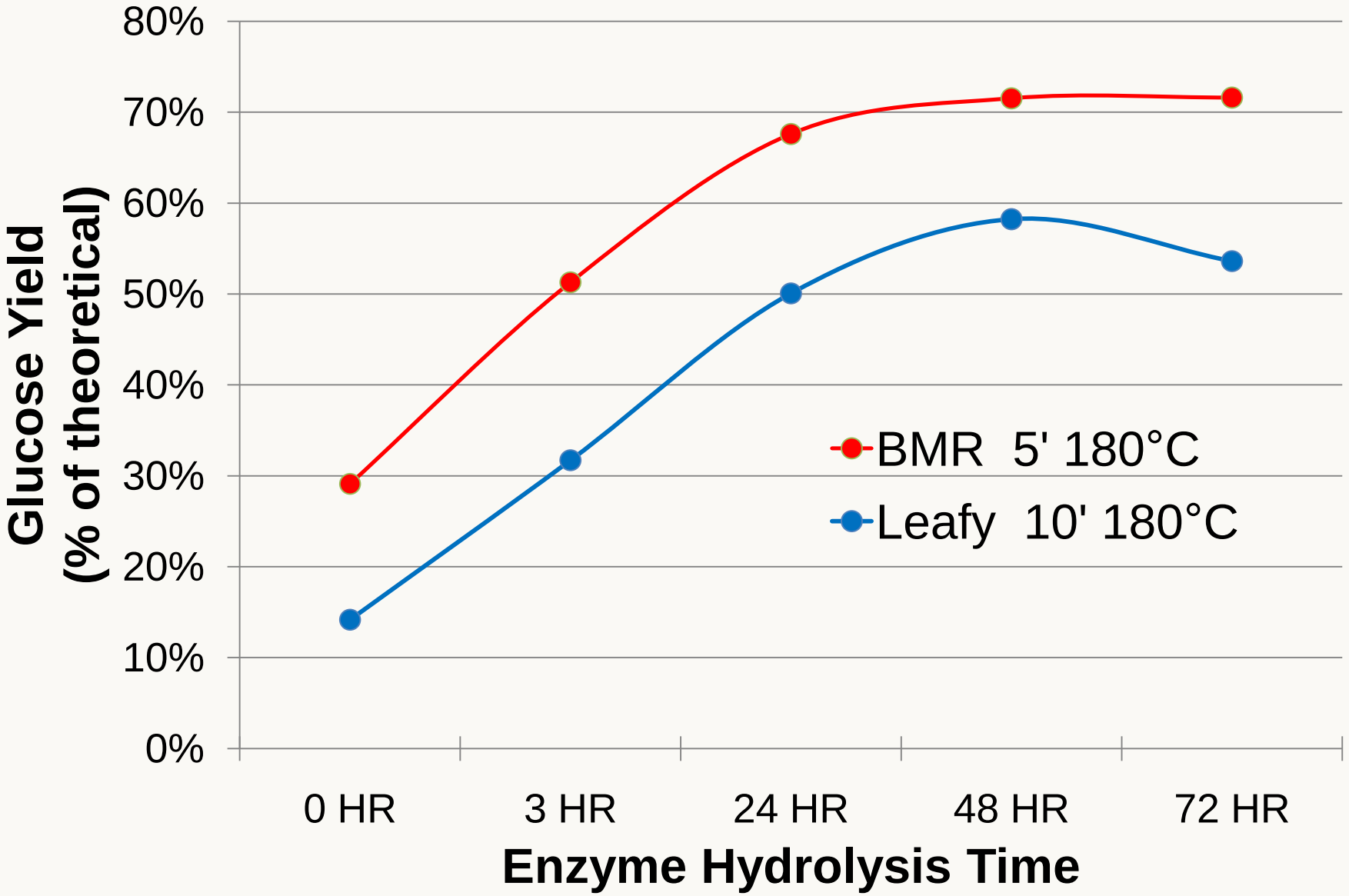


Before hydrolysis

After hydrolysis



Saccharification of Pretreated Silage 15 FPU/g glucan Spezyme CP



Fermentation Results

	Ethanol Titer (g/L)
BMR 5’ 180C	27.4
Leafy 10’ 180C	21.0

24 hrs of hydrolysis at 50 C
+
24 hrs of fermentation at 30 C

Summary

- Ensiling cellulosic biomass has potential as a way to preserve feedstock quality for biofuels production between harvest seasons.
- Liquid hot water pretreatment of maize silage requires less severity than dry maize stover (180C rather than 190C)
- *Brown Midrib* variety of maize silage result in higher yields of glucose than “leafy” variety of silage after pretreatment and enzyme hydrolysis
- Glucose released by enzymatic hydrolysis of pretreated silage is readily fermented to ethanol by *S. cerevisiae*

Thank you!