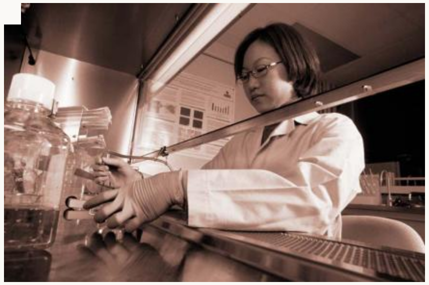
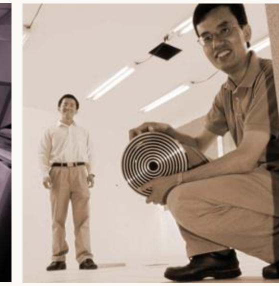
PURDUE UNIVERSITY

Liquid Hot Water Pretreatment of Corn Stover: Impact of BMR

Nathan S. Mosier
and
Wilfred Vermerris

AGRICULTURAL & BIOLOGICAL ENGINEERING

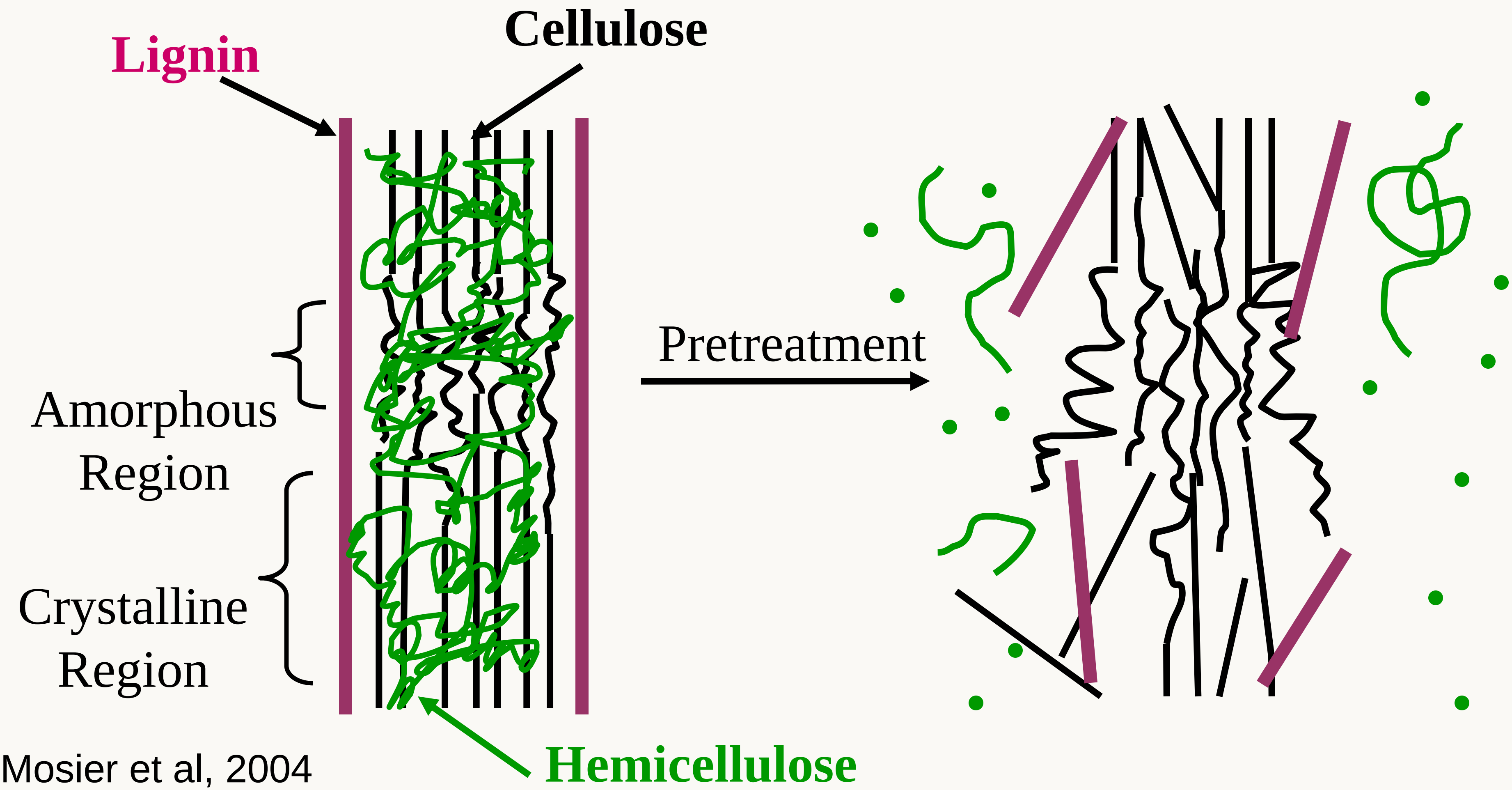
LORRE

Acknowledgements

- Research, Inc. (CPBR), U.S. Department of Energy (DOE) Prime Agreement no. DEFG36-02GO12026.
- Dow AgroSciences for funding and providing stover and silage material
- Purdue Agriculture Research Programs
- Michael Ladisch
- Yulin Lu, Arun Athmanathan, Youngmi Kim, Harini Kadambi, Lauren Quig, Erin Rosswurm, Scott Stella, Linda Liu

Effect of Pretreatment of Corn Stover

Representation of Physical Changes



Mosier et al, 2004

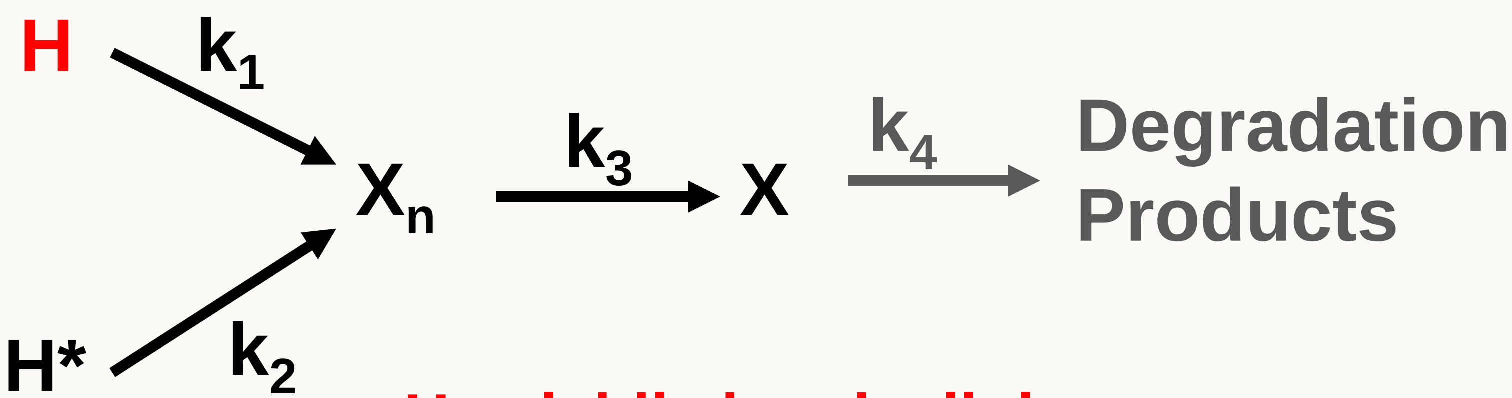
Goals:
Liquid Water Pretreatment

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



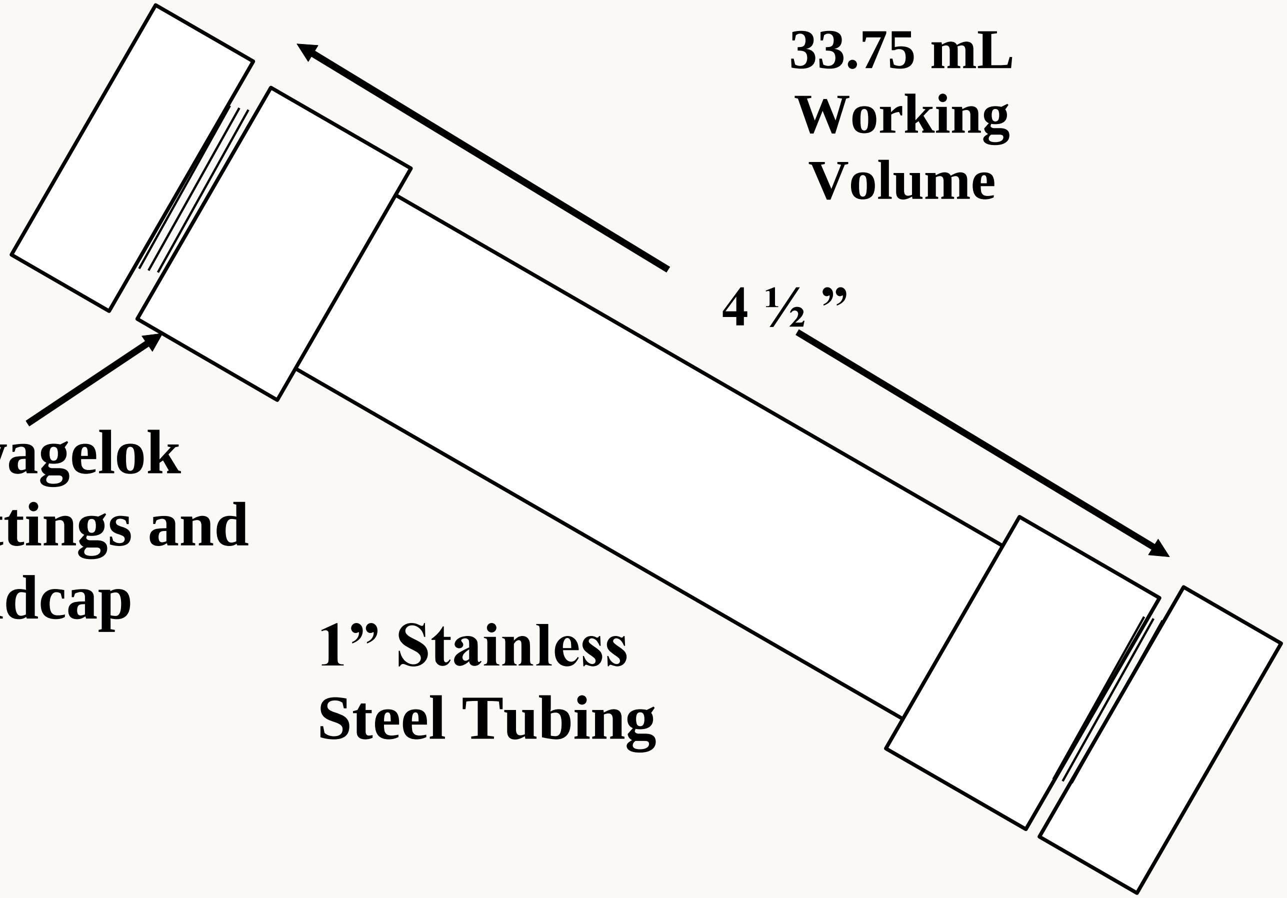
H = labile hemicellulose

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

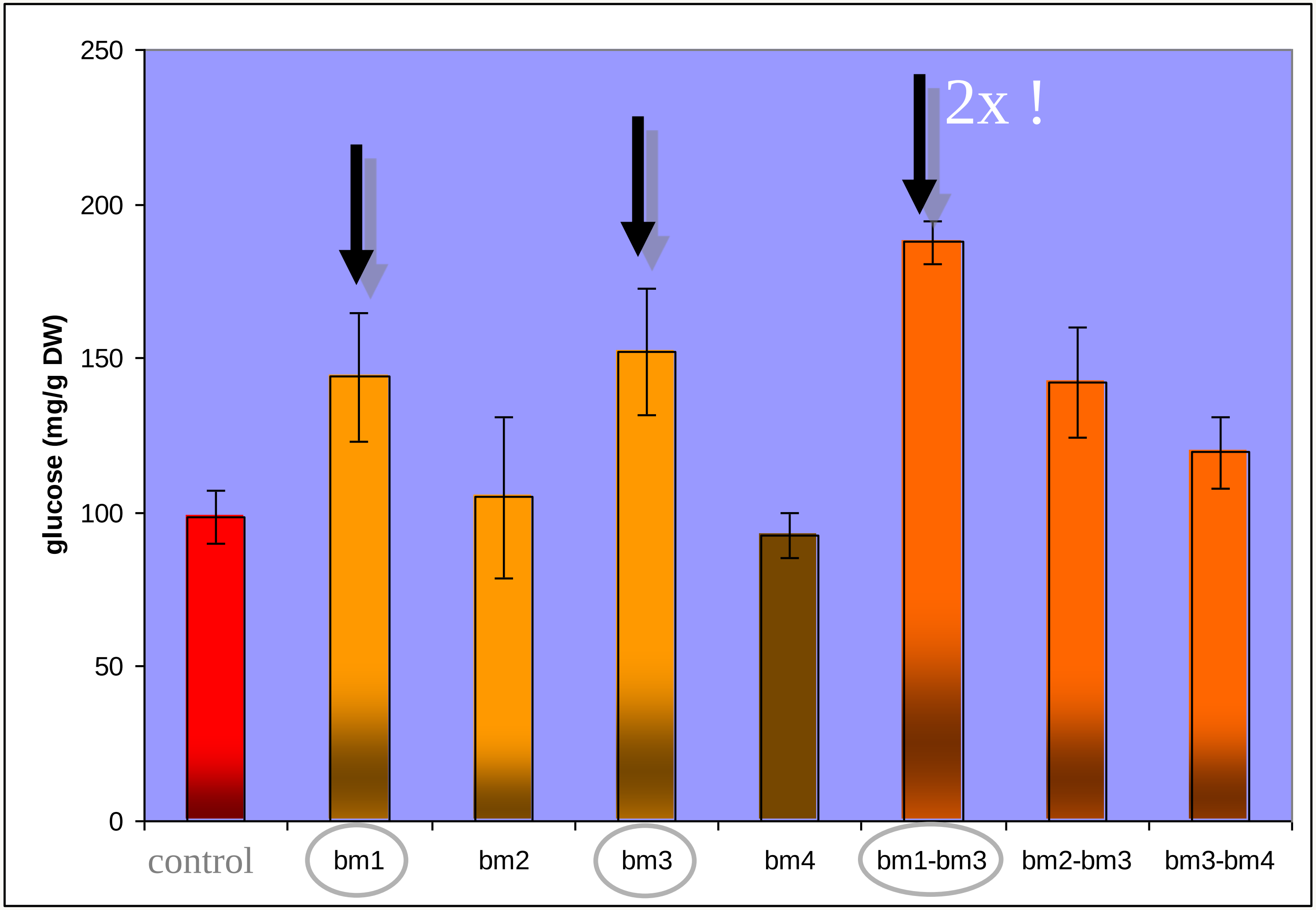
- Known to have higher digestibility in ruminants – grown commercially for silage to feed dairy cows
- Hypothesis:
  - Higher digestibility in ruminants may translate into higher enzymatic digestibility for biofuel production

AGRICULTURAL & BIOLOGICAL ENGINEERING

PURDUE UNIVERSITY

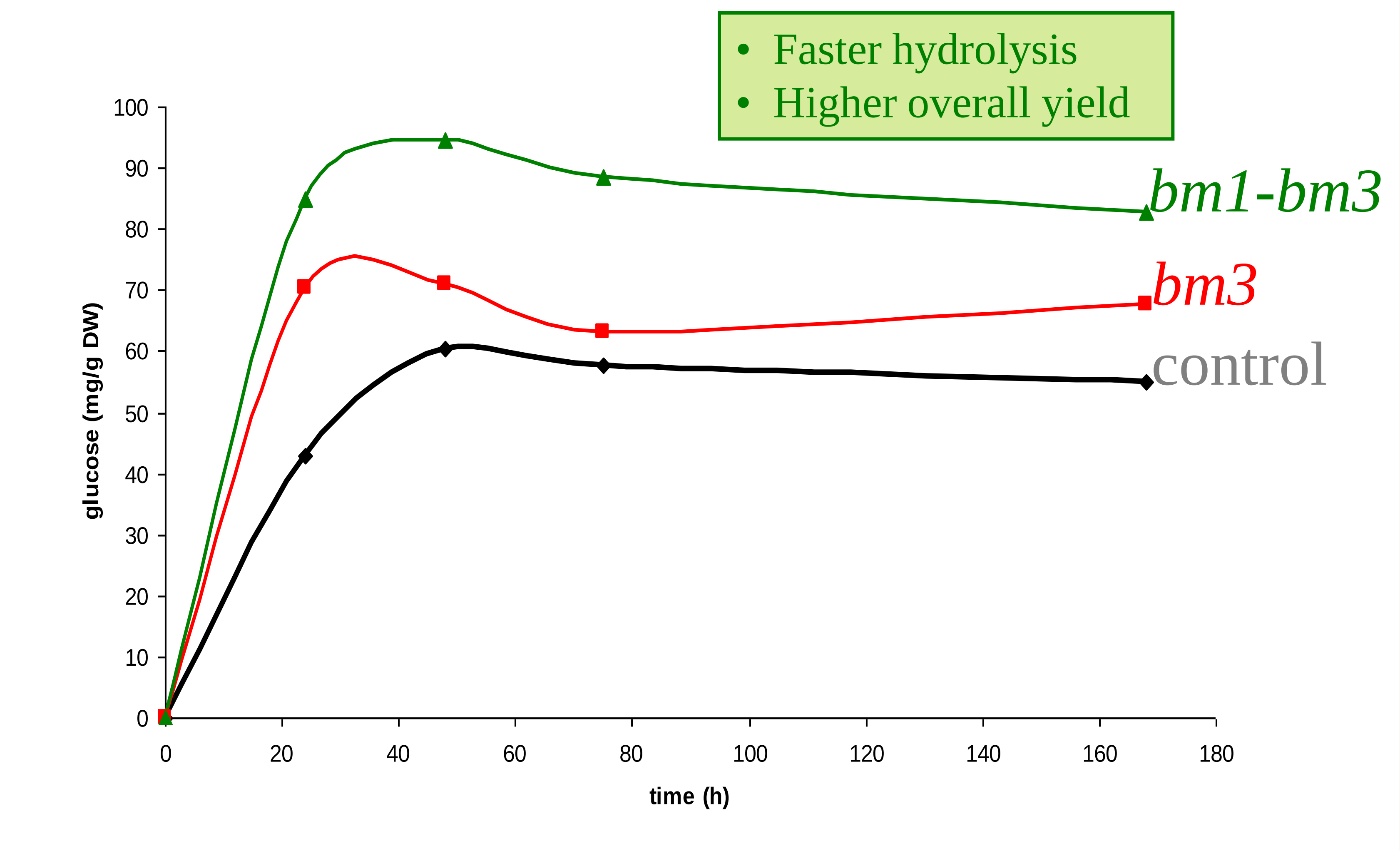
LORRE

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

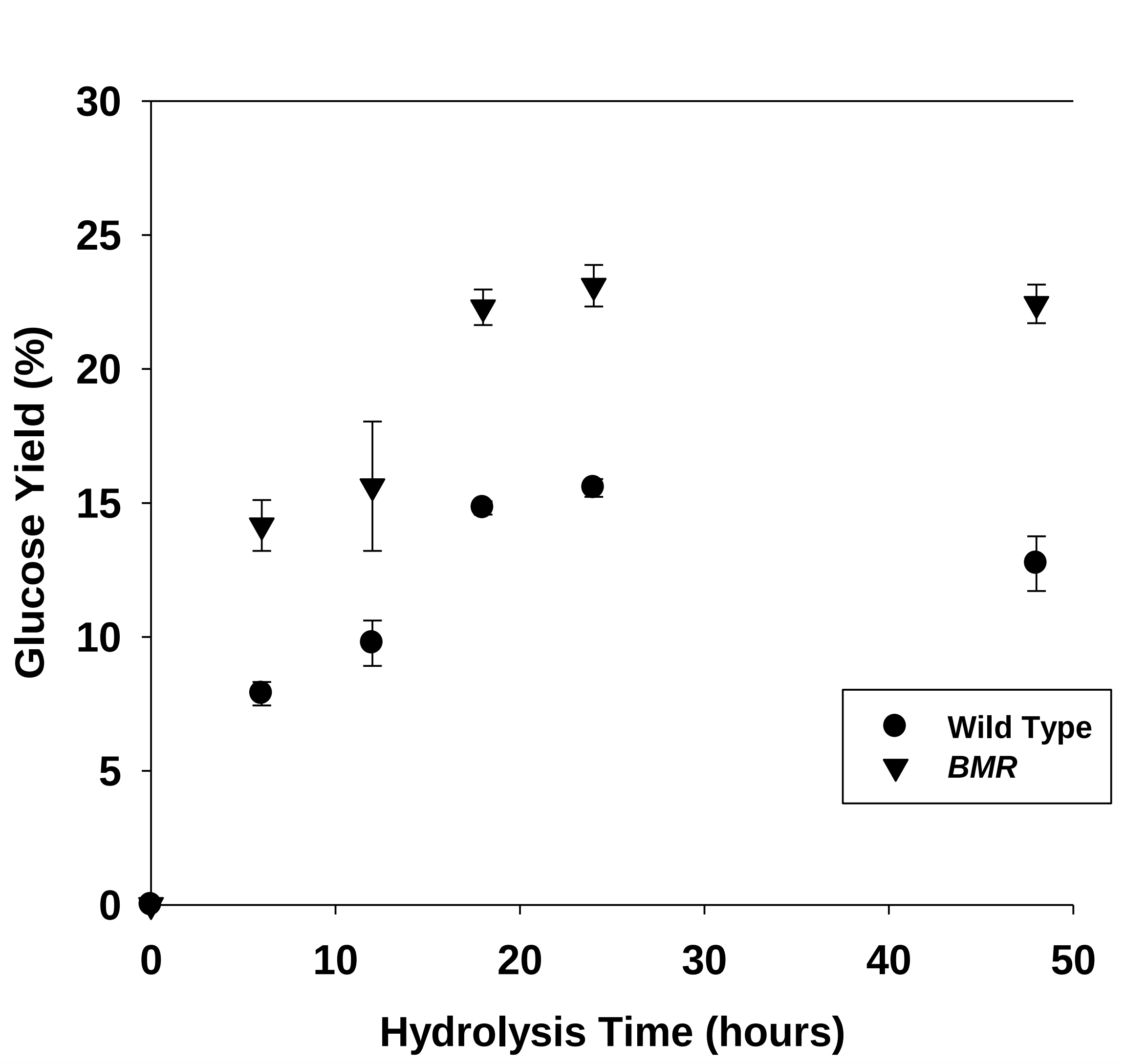


Dry matter composition of wild type and BMR maize stover: Commercial Hybrids

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.

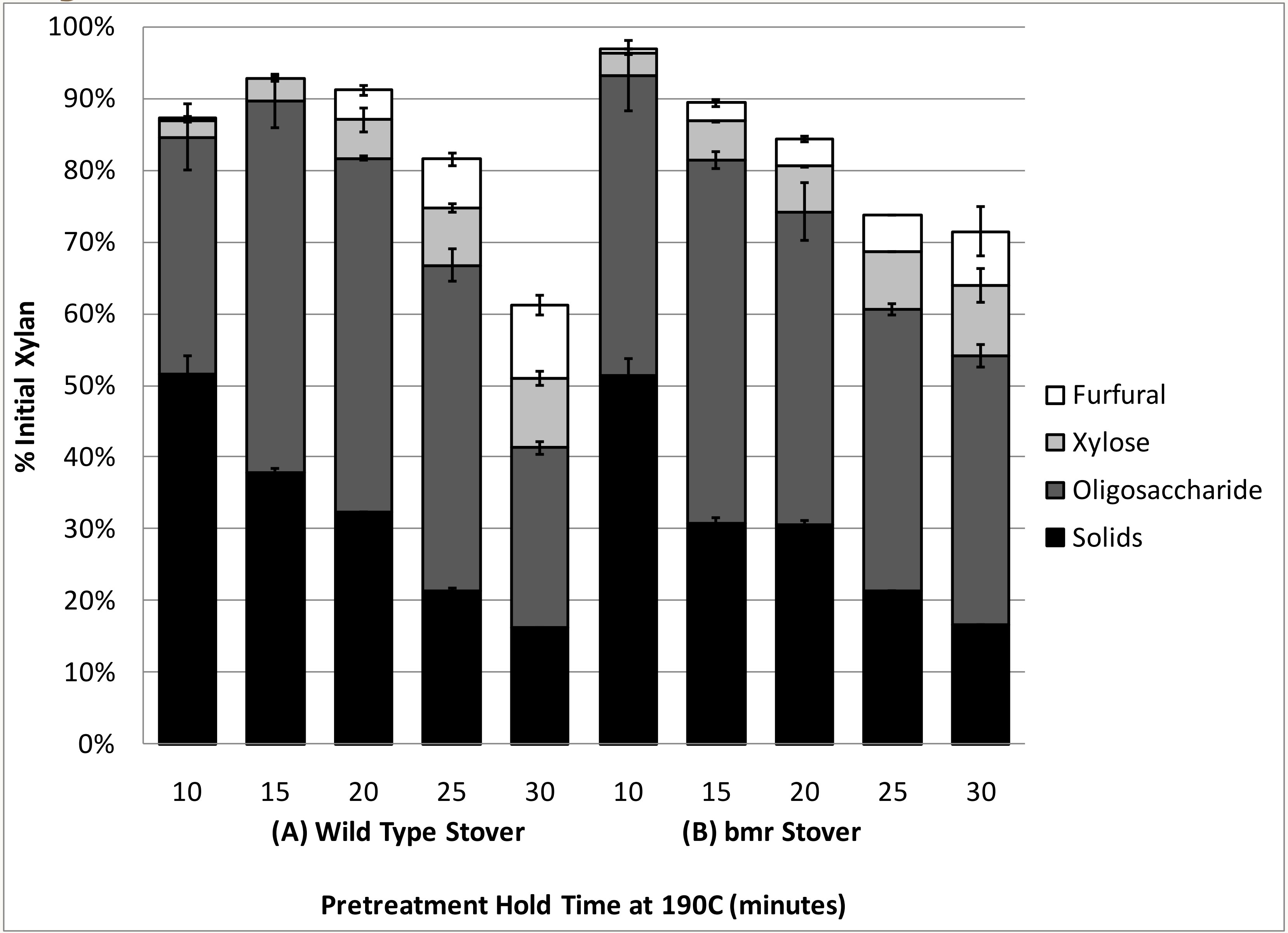
Enzymatic hydrolysis of maize stover (no pretreatment) with 15 FPU /g glucan cellulase supplemented with 40 IU β-g



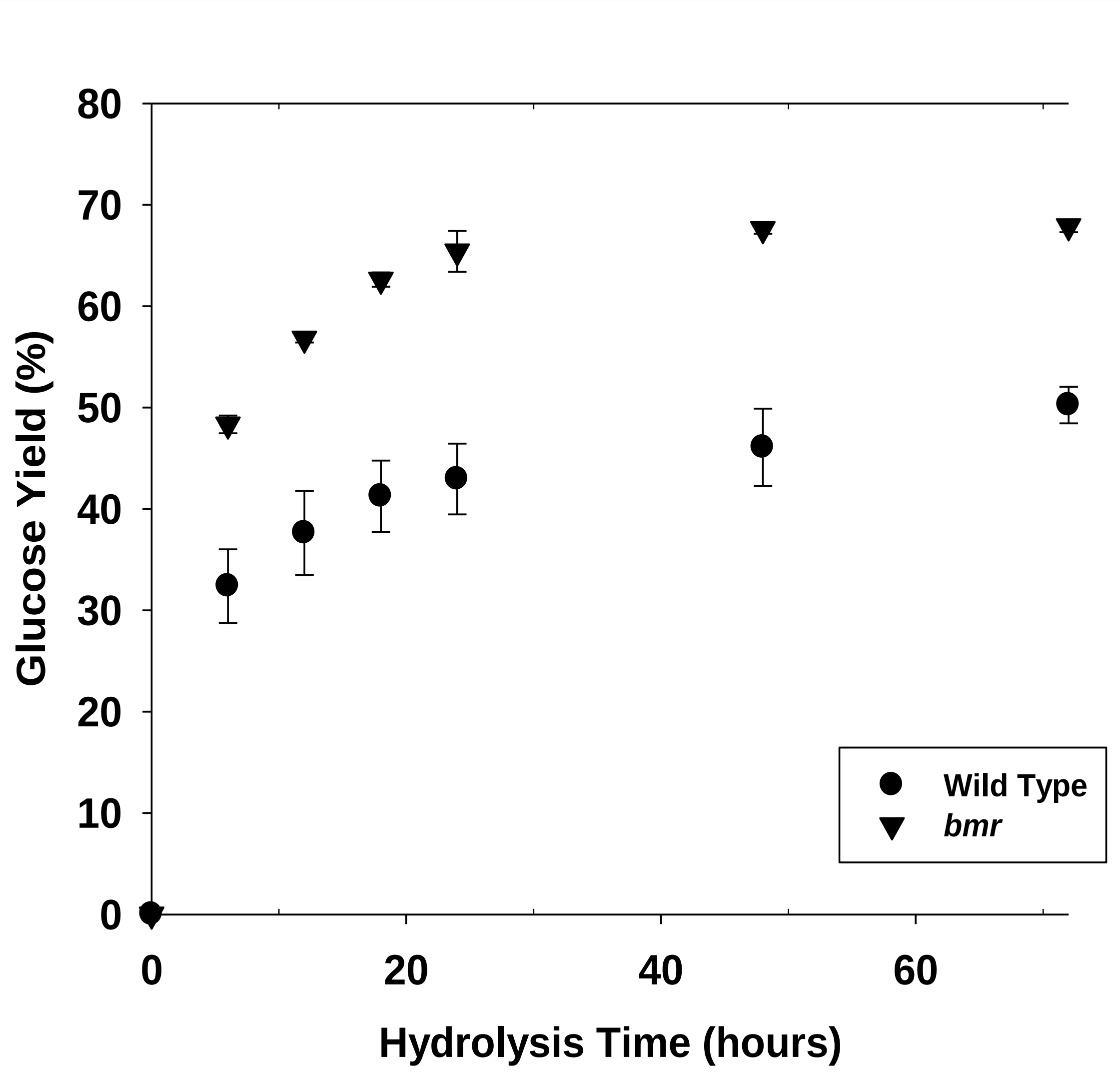
Pretreatment Method

- 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

PURDUE UNIVERSITY

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

PURDUE UNIVERSITY

LORRE

Composition of Silage

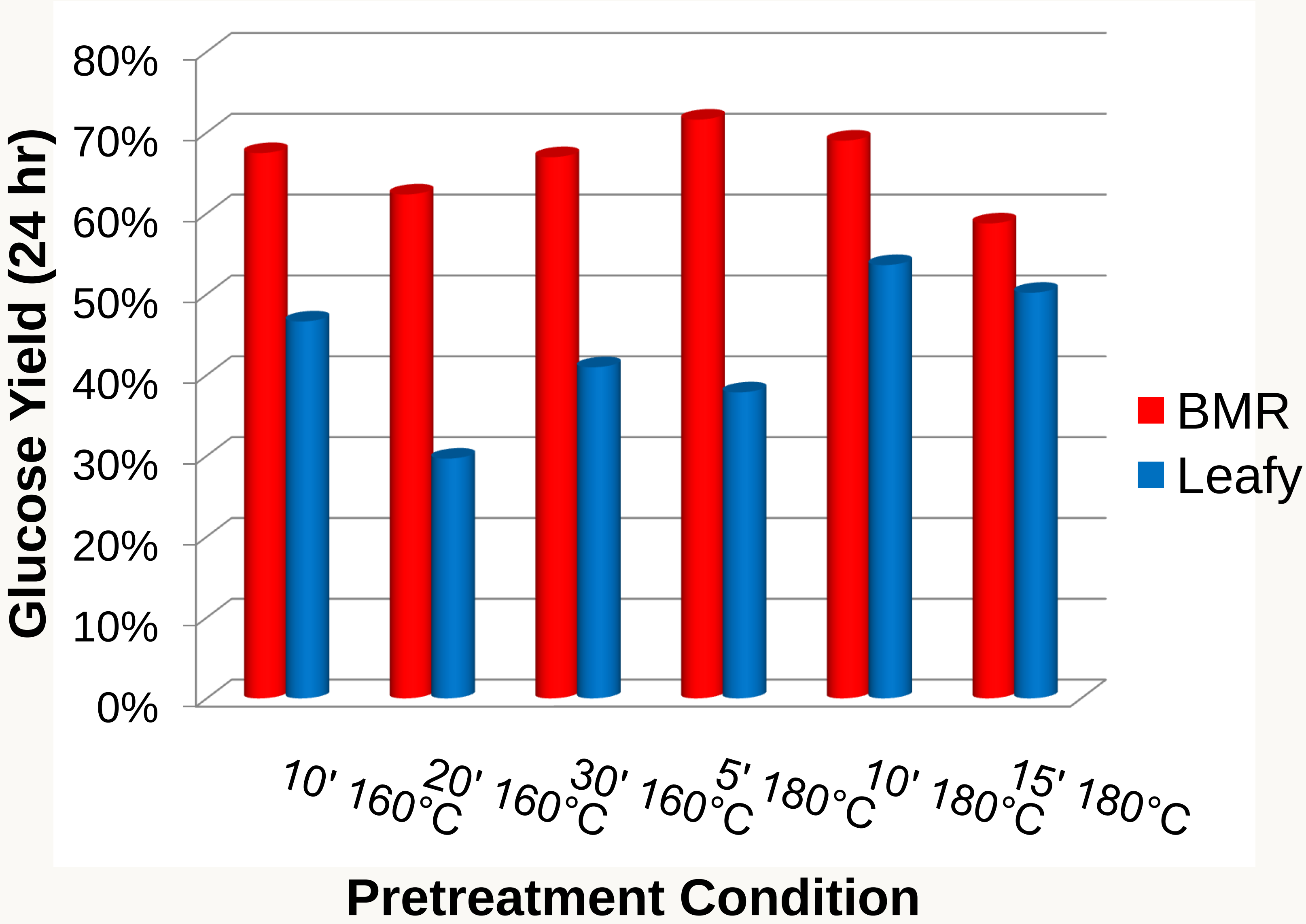
	Glucan	Xylan	Arabinan	Lignin
BMR	48.7%	27.6%	2.9%	10.4%
Leafy	59.9%	21.0%	4.0%	11.0%

PURDUE UNIVERSITY

Methods

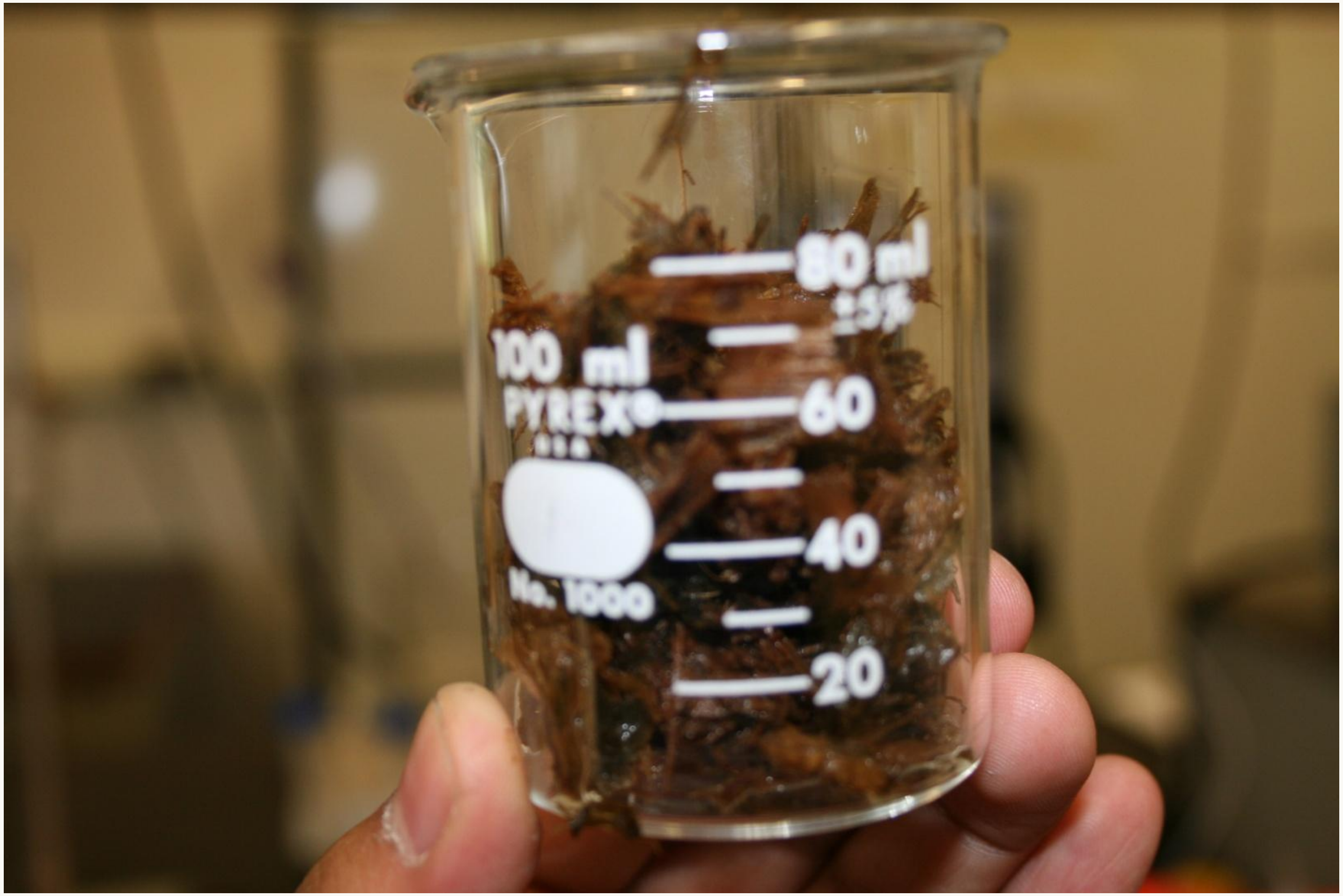
- Stainless Steel Reactors (35ml volume)
- Loading 20 w/w % (200 g/L)
- Sandbath heat up and temperature control
- Enzymatic Digestion
  - Whole slurry (undiluted)
  - pH adjustment to ~5
  - Spezyme CP and Novozyme 188 (15 FPU/g glucan and 40 CBU/g beta-glucosidase)

Optimization of Silage Pretreatment



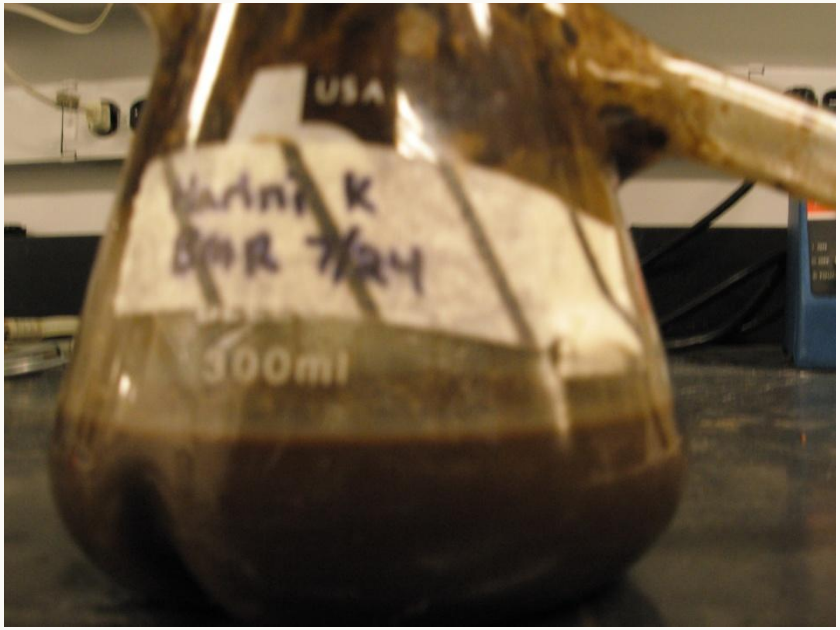
Pretreated BMR Silage

Pretreated Leafy Silage

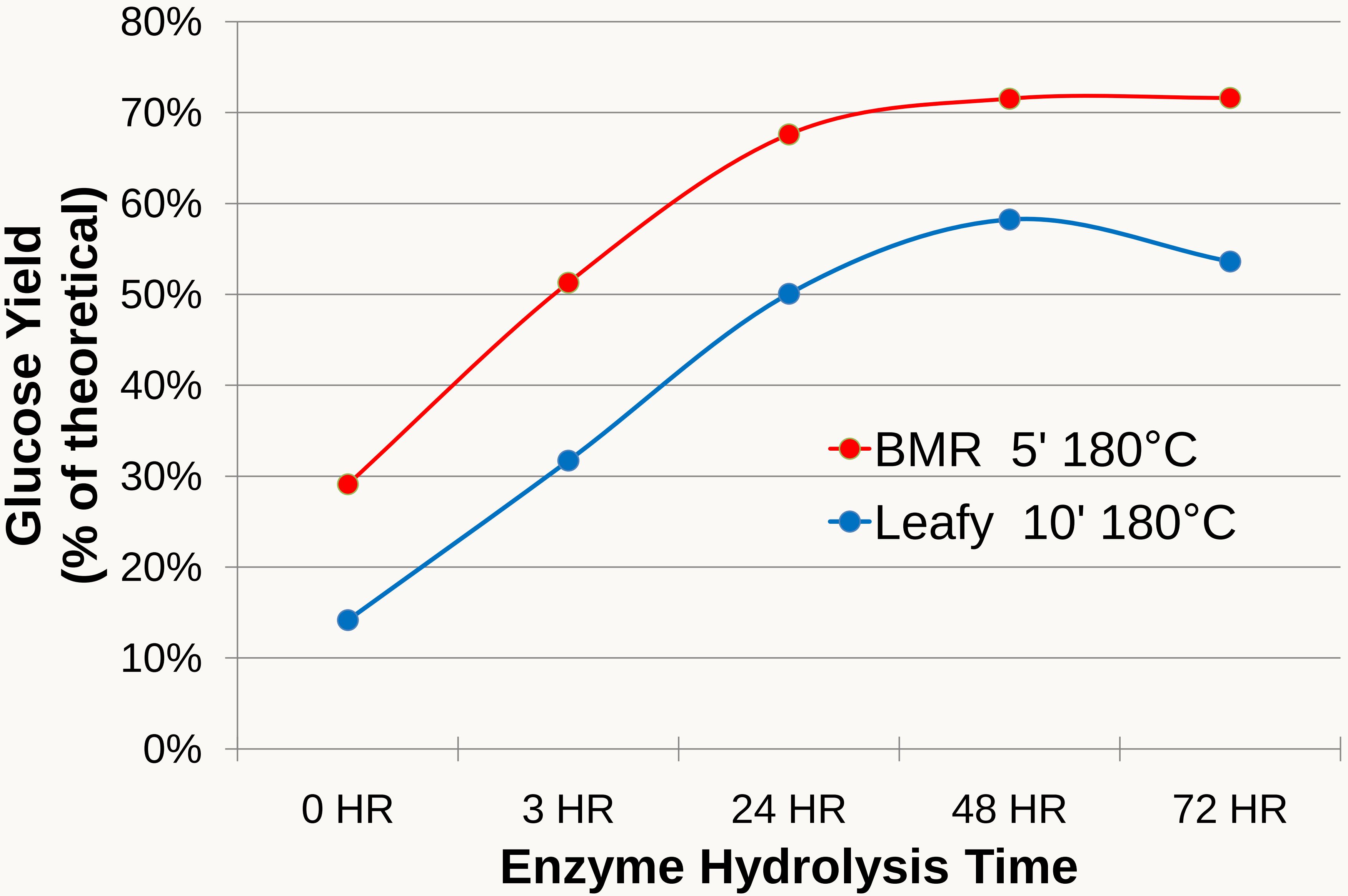


Before hydrolysis

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

PURDUE UNIVERSITY

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*

PURDUE UNIVERSITY

LORRE

Thank you!