Maize Silage as a Feedstock for Cellulosic Ethanol Production:

Impact of Brown Midrib Lignin Variants On Bioprocessing Performance

Darin W. Lickfeldt[1], Young-mi Kim[2], Nathan Mosier[2], Katherine Armstrong[1], Peter Friedemann[1] and Michael Ladisch[2]
[(1)] Dow AgroSciences LLC, Indianapolis, IN, [(2)] LORRE, Purdue University, West Lafayette, IN

Abstract

Effective storage of cellulosic feedstocks that preserves quality is critical for supplying biorefineries for the production of biofuels. Ensiling is a well developed technology for preserving herbaceous feedstocks for animal feed. We present the effect of *brown midrib* lignin mutations on rates and yields of cellulosic ethanol production from maize silage and dry stover. Both stover and silage from commercial sources were pretreated using liquid hot water (160-180°C) and assessed by enzymatic hydrolysis and fermentation using the glucose/xylose fermenting Purdue recombinant *S. cerevisiae* 424A (LNH-ST). At 20% solids concentration (200 g/L), silage achieved higher yields of fermentable sugars than stover. Lignin altered *bmr* stover and silage achieved higher yields of sugars than the non-*bmr* stover or silage pretreated under the same conditions. At the optimal pretreatment conditions, *bmr* silage achieved 62% of theoretical yield of glucose within 24 hours of enzymatic hydrolysis (15 FPU cellulase per gram glucan) compared to 50% yield from non-*bmr* silage. Sugars from both silage varieties fermented to ethanol at high yields using the Purdue recombinant yeast strain, indicating bmr silage may be an ideal feedstock for cellulosic ethanol production.

Materials and Methods

Silage

Whole maize plants from field plots were harvested, chopped, and ensiled in commercial silage bunkers. After the material was ensiled, 2 kg samples were taken and stored in sealed plastic bags at 4 C until processed

Pretreatment (Liquid Hot Water)

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 with KOH
Spezyme CP and Novozyme 188
(15 FPU/g glucan and 40 CBU/g beta-glucosidase)

Fermentation

Inoculated with *S. cerevisiae* (non-recombinant), ATCC 2124 (polyploid, distillery yeast)
Microaerobic conditions, orbital shaker 200 rpm, 28 C
Fermented for 24 hrs, samples analyzed by HPLC

Results

Table 1. Composition of Silage (% dry matter)

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

Figure 2. Enzymatic Yields of Glucose from Silage as a Function of Varying Conditions for Liquid Hot Water Pretreatment

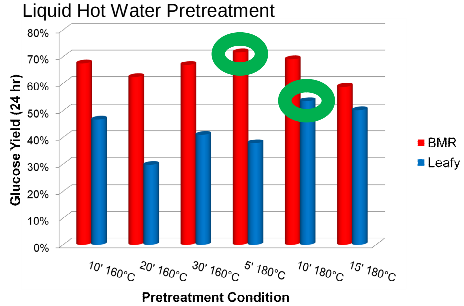


Figure 2. Deleterious Mutations to Lignin Biosynthesis Associated with *Brown Midrib* Maize

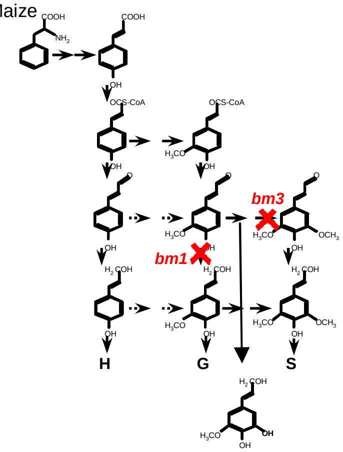


Table 2. Summary of Results

	Glucose Yield	Ethanol Titer (g/L)
BMR 5' 180C	72%	27.4
Leafy 10' 180C	54%	21.0

Figure 3. Enzymatic Yield of Glucose Over Time for Optimally Pretreated Silage

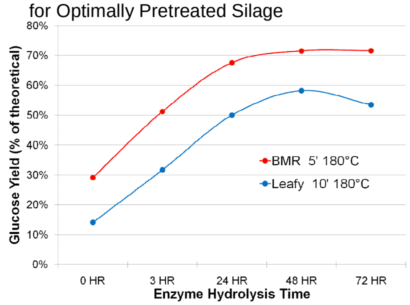
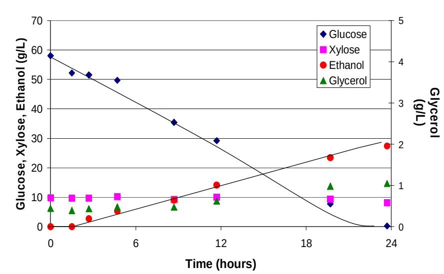


Figure 4. Fermentation Profile for Pretreated and Enzyme Hydrolyzed BMR Silage



Conclusions

- Ensiling celluosic 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*

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