Liquid Hot Water Pretreatment of Corn Stover: Impact of BMR

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and
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Effect of Pretreatment of Corn Stover Representation of Physical Changes

Lignin

Cellulose

Amorphous Region

Crystalline Region

Hemicellulose

Mosier et al, 2004

Pretreatment
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

\[ \begin{align*} 
H & \xrightarrow{k_1} X_n \\
H^* & \xrightarrow{k_2} X_n \\
X_n & \xrightarrow{k_3} X \\
X & \xrightarrow{k_4} \text{Degradation Products} 
\end{align*} \]

\[ \begin{align*} 
H & = \text{labile hemicellulose} \\
H^* & = \text{recalcitrant hemicellulose} \\
X_n & = \text{soluble xylans (oligosaccharides)} \\
X & = \text{xylose (monomer)} 
\end{align*} \]
Liquid Hot Water Pretreatment at Lab Scale

Swagelok Fittings and Endcap

1" Stainless Steel Tubing

33.75 mL Working Volume

4 ½”
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)

![Graph showing glucose levels for different mutants in the same genetic background (A619).]

All mutants in the same genetic background (A619)
Enzymatic Hydrolysis of Corn Stover

- Faster hydrolysis
- Higher overall yield

\[ \text{bm1-bm3} \]
\[ \text{bm3} \]
\[ \text{control} \]
Dry matter composition of wild type and BMR maize stover: Commercial Hybrids

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

* 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

[Graph showing the mass balance of Xylan in Wild Type Stover and bmr Stover with varying pretreatment hold times at 190°C.]
Enzymatic Hydrolysis of Unwashed, Pretreated Maize Stover Slurry
15 FPU/g glucan

![Graph showing glucose yield vs. hydrolysis time for 'Wild Type' and 'bmr']
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

<table>
<thead>
<tr>
<th></th>
<th>Glucan</th>
<th>Xylan</th>
<th>Arabinan</th>
<th>Lignin</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMR</td>
<td>48.7%</td>
<td>27.6%</td>
<td>2.9%</td>
<td>10.4%</td>
</tr>
<tr>
<td>Leafy</td>
<td>59.9%</td>
<td>21.0%</td>
<td>4.0%</td>
<td>11.0%</td>
</tr>
</tbody>
</table>
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

Glucose Yield (24 hr)

- BMR
- Leafy

Pretreatment Condition:
- 10°C
- 160°C for 10', 20', 30', 5', 10', 15', 180°C
Pretreated BMR Silage
Pretreated Leafy Silage
Before hydrolysis

After hydrolysis
Saccharification of Pretreated Silage
15 FPU/g glucan Spezyme CP

Glucose Yield (% of theoretical)

Enzyme Hydrolysis Time
0 HR 3 HR 24 HR 48 HR 72 HR

BMR 5' 180°C
Leafy 10' 180°C
Fermentation Results

<table>
<thead>
<tr>
<th></th>
<th>Ethanol Titer (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMR 5’ 180C</td>
<td>27.4</td>
</tr>
<tr>
<td>Leafy 10’ 180C</td>
<td>21.0</td>
</tr>
</tbody>
</table>

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!