Optimization of Controlled pH Liquid Hot Water Pretreatment of Corn Fiber and Stover

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Biomass Refining CAFI
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USDA IFAFS Project Overview

Multi-institutional effort funded by USDA Initiative for Future Agriculture and Food Systems (IFAFS) Program to develop comparative information on cellulosic biomass pretreatment by leading options with common source of cellulosic biomass

– Aqueous ammonia recycle pretreatment - YY Lee, Auburn University
– Water only and dilute acid hydrolysis by co-current and flowthrough systems - Charles Wyman, Dartmouth
– Ammonia fiber explosion - Bruce Dale, Michigan State
– Controlled pH pretreatment - Michael Ladisch, Purdue
– Lime pretreatment - Mark Holtzapple, Texas A&M

Logistical support and economic analysis - Rick Elander/Tim Eggeman, NREL

Biomass Refining CAFI
What is corn stover?

- NREL supplied corn stover to CAFI (source: BioMass AgriProducts, Harlan IA)
- Stover washed and dried in small commercial operation, knife milled to pass ¼ inch round screen
Corn Stover
Where does corn fiber come from?

A KERNEL OF CORN

ENDOSPERM
(81.9%)

STARCH

STARCH AND GLUTEN

TIP CAP
(0.8%)

GERM
(11.9%)

HULL AND FIBER
(5.3%)

(Rutenberg, 1989)
Corn Fiber
## Compositions

<table>
<thead>
<tr>
<th></th>
<th>Corn Stover</th>
<th>Corn Fiber</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucan (cellulose)</td>
<td>36.2%</td>
<td>14.3%</td>
</tr>
<tr>
<td>Glucan (starch)</td>
<td>0.0</td>
<td>23.7</td>
</tr>
<tr>
<td>Xylan</td>
<td>21.4</td>
<td>16.8</td>
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<tr>
<td>Arabinan</td>
<td>3.5</td>
<td>10.8</td>
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<tr>
<td>Mannan</td>
<td>1.8</td>
<td>NA</td>
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<tr>
<td>Galactan</td>
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<td>NA</td>
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<tr>
<td>Lignin</td>
<td>17.2</td>
<td>8.4</td>
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<tr>
<td>Protein</td>
<td>4.0</td>
<td>11.8</td>
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<tr>
<td>Acetyl</td>
<td>3.2</td>
<td>NA</td>
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<tr>
<td>Ash</td>
<td>7.1</td>
<td>0.4</td>
</tr>
<tr>
<td>Uronic Acid</td>
<td>3.6</td>
<td>NA</td>
</tr>
<tr>
<td>Non structural sugars</td>
<td>1.2</td>
<td>0.0</td>
</tr>
</tbody>
</table>
Goals:
Water Pretreatment at Controlled pH

Determine conditions that:
1. during pretreatment, minimize hydrolysis.
2. after pretreatment, maximize hydrolysis.
3. develop mechanistic explanations and optimize pretreatment conditions.
Controlled pH Liquid Hot Water Pretreatment

- pH control through buffer capacity of liquid
- No fermentation inhibitors, no wash stream
- Minimize hydrolysis to monosaccharides thereby minimizing degradation
Pretreatment Conditions for this Work

Fiber or Corn Stover : Water Ratio  
   dry basis = 0.15 : 1 to 0.20:1

Temperature and Hold Time  
   160 to 200 °C hold for 10 to 30 min

Saccharify liquid and solid  
   Cellulase enzyme supplemented with cellobiase

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Effect of Pretreatment

Lignin

Amorphous Region

Crystalline Region

Cellulose

Pretreatment

Hemicellulose
Chromatogram of Oligosaccharides
Formed upon Pretreatment of Fiber

Comparison of Pretreatment Liquid and Stillage

- Pretreatment
  - 10x Dilute

- Stillage
  - 10x Dilute

Oligosaccharides
- Glucose
- Glycerol
Comparison of Pretreatment Liquid and Stillage

Bio-Rad HPX-87H 300mm x 7.8mm HPLC Column
5mM H₂SO₄ Buffer
60°C, 0.6 mL/min

Pretreatment
10x Dilute

Stillage
10x Dilute

Minimal degradation products
Example of Effect of Pretreatment on Enzyme Hydrolysis

Solids Reduction (%) vs. Hydrolysis Time (hours)

- Pretreated
  - Pretreated (a)
  - Pretreated (b)
- Not pretreated
  - Fiber (a)
  - Fiber (b)

10 FPU/g Celluclast 1.5L + Novozyme 188
During Pretreatment
Water acts as Acid

Liquid water dissociation constants

\[ k_w = 0.01 \times 10^{-12} \text{ (at 20 °C)} \]
\[ \text{to } 6.0 \times 10^{-12} \text{ (at 230 °C)} \]
Autohydrolysis during Pretreatment of Cellulose at 190 C

\[ C \xrightarrow{k_1} C^* \xleftarrow{k_2} K \]

\[ G_n \xrightarrow{k_3} G \xrightarrow{k_4} \text{Degradation Products} \]

C = native cellulose
C* = hydrated cellulose
G_n = glucans (oligosaccharides)
G = glucose (monomer)
Autohydrolysis during Pretreatment
(follows path of least resistance)

\[ C \xrightarrow{k_1} C^* \xrightarrow{k_2} G_n \xrightarrow{k_3} G \xrightarrow{k_4} \text{Degradation Products} \]

\[ \text{C} = \text{native cellulose} \]
\[ \text{C}^* = \text{hydrated cellulose} \]
\[ G_n = \text{glucans (oligosaccharides)} \]
\[ G = \text{glucose (monomer)} \]
Autohydrolysis and Sugar Degradation during Pretreatment

\[ C \xrightarrow{K} G \xrightarrow{k_3} G \xrightarrow{k_4} \text{Degradation Products} \]

\[ k_2, k_3, >> k_1 \]

at high temperatures \( k_4 = k_3 \)

Degradation products:
- **organic acids** that catalyze further hydrolysis and degradation
- **aldehydes** that inhibit both bacterial and yeast fermentations

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Pretreatment Model:
Goal for this work

*separate*
pretreatment *(a physical change)*
from hydrolysis *(a chemical change)*

*Rationale*
Avoid hydrolysis, degradation products
1. Pretreatment
(carry out at high temperature)

Minimize hydrolysis

$K \xrightarrow{k_1} C \xleftarrow{k_2} C^*$

$C = \text{native cellulose}$

$C^* = \text{hydrated cellulose}$

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2. Hydrolysis
(at low temperature, using enzymes)

Maximize hydrolysis

\[ C \xrightarrow{k_1} G_n \xrightarrow{k_3} G \xrightarrow{k_4} \text{Degradation Products} \]

- \( C = \text{native cellulose} \)
- \( C^* = \text{hydrated cellulose} \)
- \( G_n = \text{glucans (oligosaccharides)} \)
- \( G = \text{glucose (monomer)} \)
Pretreatment Tube
Heat in Sandbath

33.75 mL Working Volume

4 ½ ”

1” Stainless Steel Tubing

Swagelok Fittings and Endcap

Corn Stover (¼ “ Mesh, 10-13% MC) loaded into tube at 12% (dry basis) in water

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Hemicellulose Solubilization from Pretreated Corn Stover – pH of 4

(after enzyme hydrolysis followed by NREL LAP 14 to determine Xylan oligomers in solution)
SEM of Corn Stover before Pretreatment
SEM of Corn Stover after Pretreatment
SEM
Pretreated Corn Stover
¼” Mesh
8000x

1 μm

~ 4.5 μm
Fermentability
Hydrolyzed Pretreatment Liquid

Data from Nacy Ho and Miroslav Sedlak, LORRE, Purdue University
Fermentability confirmed by Bruce Dien, USDA-NCAUR
Stepwise Process Yields & Mass Balance for 7.5 FPU Spezyme

Water
620 lb
Stover
100 lb (dry basis)
37.5 lb glucan
22.4 lb xylan

Cellulase Enzyme
1105 FPU per lb stover (30 FPU/ml)

Controlled pH
Liquid Hot Water

Treated
62.8 lb undissolved solids
Stover
37.2 lb dissolved solids
Slurry
620 lb water

Hydrolysis

Hydrolyzate
Liquid
30.01 lb glucose
18.67 lb xylose

Residual
28.6 lb
Solids

Fermentation

Ethanol
21.8 lb

72.0% total glucan conversion (raw stover basis)
73.4% total xylan conversion (raw stover basis)
88% of theoretical ethanol yield from glucose + xylose
Stepwise Process Yields & Mass Balance for 15 FPU Spezyme

Water
- 620 lb

Stover
- 100 lb (dry basis)
  - 36.1 lb glucan
  - 21.4 lb xylan

Cellulase Enzyme
- 2209 FPU per lb stover (30 FPU/ml)

Hydrolysis

Residual Solids
- 22.0 lb

Ethanol
- 25.2 lb

Fermentation

Hydrolyzate
- Liquid
  - 36.32 lb glucose
  - 19.89 lb xylose

Controlled pH

Liquid Hot Water

Treated
- 62.8 lb undissolved solids
- 37.2 lb dissolved solids

Slurry
- 620 lb water

90.54% total glucan conversion (raw stover basis)
81.80% total xylan conversion (raw stover basis)
88% of theoretical ethanol yield from glucose + xylose
Stepwise Process Yields & Mass Balance for 60 FPU Spezyme

Water
620 lb
Stover
100 lb (dry basis)
36.1 lb glucan
21.4 lb xylan

Cellulase Enzyme
8836 FPU per lb stover (30 FPU/ml)

Hydrolysis

Controlled pH
Liquid Hot Water

Treated Stover
62.8 lb undissolved solids
37.2 lb dissolved solids
620 lb water

Residual Solids
20.5 lb

Cellobiase Enzyme
5891 IU per lb stover (309 IU/ml)

Fermentation

Hydrolyzate

Liquid
37.47 lb glucose
19.78 lb xylose

Ethanol
25.7 lb

93.42% total glucan conversion (raw stover basis)
81.33% total xylan conversion (raw stover basis)
88% of theoretical ethanol yield from glucose + xylose
Conclusions

Water is effective in pretreating corn stover and corn fiber when pH is maintained at 4. Minimizing hydrolysis during pretreatment minimizes degradation inhibitors. 90% yields of fermentable sugars from cornstover are possible. Sugars yields translate to 78 gal ethanol/ton.
### Pretreatment at 190 C, 15 min

<table>
<thead>
<tr>
<th>Compound</th>
<th>NREL %</th>
<th>Wt. In 13.8306 g dry stover initial</th>
<th>Pretreatment Liquid (g)</th>
<th>4 Day Enzyme Liquid (g)</th>
<th>4 Day Enzyme Solids (g)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucan</td>
<td>37.5</td>
<td>5.1864</td>
<td>0.2809</td>
<td>2.9617</td>
<td>1.9025</td>
<td>99.20</td>
</tr>
<tr>
<td>Xylan/Galn</td>
<td>22.4</td>
<td>3.0981</td>
<td>1.8529</td>
<td>0.6701</td>
<td>0.5586</td>
<td>99.47</td>
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<tr>
<td>Arabinan</td>
<td>2.7</td>
<td>0.3734</td>
<td>0.1878</td>
<td>0.1402</td>
<td>0.0691</td>
<td>106.35</td>
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<tr>
<td>Lignin</td>
<td>17.6</td>
<td>2.4342</td>
<td>NA</td>
<td>NA</td>
<td>2.7623</td>
<td>113.48</td>
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<tr>
<td>Protein</td>
<td>2.9</td>
<td>0.4011</td>
<td>0.1654</td>
<td>NA</td>
<td>0.4133</td>
<td>144.28</td>
</tr>
<tr>
<td>Acetyl</td>
<td>2.2</td>
<td>0.3043</td>
<td>0.2307</td>
<td>0.0332</td>
<td>0.035</td>
<td>98.23</td>
</tr>
<tr>
<td>Ash</td>
<td>6.7</td>
<td>0.9267</td>
<td>0.3358</td>
<td>0.053</td>
<td>0.5324</td>
<td>99.41</td>
</tr>
</tbody>
</table>

This experiment was run in duplicate with similar results. There was no ethanol extraction step so the fats and waxes would show up as lignin. We also found that some of the protein added as enzyme bound to the solids and could not be washed out resulting in much higher measured recovery.