Effect of Liquid Hot Water Pretreatment on Switchgrass Hydrolysis

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Presented at 2008 AIChE meeting
Philadelphia, PA Nov 20, 2008
Acknowledgements

Material in this work supported by US Department of Energy Office of the Biomass Program, Contract DE-FG36-04GO14017

For gifts of enzymes
   Genencor, a Danisco Division

Switchgrass
   Ceres

CAFI Team Collaborators

LORRE Staff
Linda Liu, Rick Hendrickson
Switchgrass

- A plentiful, warm-season perennial grass
- Low fertility requirement
- Tolerant of poor soils
- High yield (6-8 ton/acre)

Photos courtesy of Department of Agronomy, Purdue University
## Switchgrass Composition

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Shawnee (% dry wt)</th>
<th>Alamo (% dry wt)</th>
<th>Dacotah (% dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucan</td>
<td>30.8</td>
<td>33.2</td>
<td>35</td>
</tr>
<tr>
<td>Xylan</td>
<td>21</td>
<td>21</td>
<td>21.8</td>
</tr>
<tr>
<td>Arabinan</td>
<td>2.2</td>
<td>3.2</td>
<td>3.5</td>
</tr>
<tr>
<td>Sucrose</td>
<td>2.9</td>
<td>4</td>
<td>1.5</td>
</tr>
<tr>
<td>Acetyl</td>
<td>2.5</td>
<td>2.5</td>
<td>2.8</td>
</tr>
<tr>
<td>Lignin</td>
<td>17.6</td>
<td>17.9</td>
<td>21.4</td>
</tr>
<tr>
<td>Protein</td>
<td>4.5</td>
<td>5.7</td>
<td>1.4</td>
</tr>
<tr>
<td>Extractives</td>
<td>11.6</td>
<td>10.2</td>
<td>8.1</td>
</tr>
<tr>
<td>Ash</td>
<td>4.4</td>
<td>3.7</td>
<td>3.3</td>
</tr>
<tr>
<td>Total</td>
<td>97.5</td>
<td>101.4</td>
<td>98.80</td>
</tr>
</tbody>
</table>

*(Compositions provided from Ceres)*
Controlled pH, Liquid Hot Water (LHW) Pretreatment

- pH is controlled to 4-7 to minimize formation of monomeric sugars and sugar decomposition products during the pretreatment
- LHW pretreatment enhances enzymatic digestibility of biomass by
  a) dissolving hemicellulose fraction of biomass as soluble oligomers
  b) minimizing formation of monomeric sugars and sugar degradation products
Liquid Hot Water Pretreatment Optimization

- Substrate: Shawnee switchgrass
- % solids loading: 15% dry solids (150 g/L)
- Temperature: 180, 190, 200ºC
- Pretreatment time: 5, 10, 15, 20 min
- No external pH control

Hydrolysis of whole pretreatment slurry:
- Diluted to ~4.5% dry solids (~1% glucan)
- 15 FPU Cellulase and 40 IU β-glucosidase per g cellulose
- 50 ºC, 48 hrs, pH 4.8
pH of LHW Pretreated Poplar

- 180 C
- 190 C
- 200 C

pH of Pretreated Switchgrass

Pretreatment Time (min)

Unpretreated Switchgrass
Solid Dissolution

Solubilization by Pretreatment and Enzymatic Hydrolysis (%)

- 180 C
- 190 C
- 200 C

Unpretreated Switchgrass

Pretreatment Time (min)
Glucose Yields

Enzymatic Hydrolysis of Pretreated Switchgrass, Glucose Yield (%)

Pretreatment Time (min)

- 180 C
- 190 C
- 200 C

Unpretreated Switchgrass

0 5 10 15 20 25

0 10 20 30 40 50 60 70 80 90 100
Enzymatic Hydrolysis of Pretreated Switchgrass, Xylose/Galactose Yield (%)

Pretreatment Time (min)

Xylose Yields

Unpretreated Switchgrass

- 180 C
- 190 C
- 200 C
Total Sugar Yields

Pretreatment Time (min)

Unpretreated Switchgrass

200°C, 5min  200°C, 10min  190°C, 15min  190°C, 20min

Total Sugar Yield (Glucose+Xylose/Galactose) (%)

- 180°C
- 190°C
- 200°C
Sugar Degradation Products (HMF+Furfural) (in pretreatment media, not including polymerized furans)

[Graph showing concentration of sugar degradation products (HMF+Furfural) g/L vs. pretreatment time (min) for different temperatures: 180°C, 190°C, 200°C.]

- 180°C
- 190°C
- 200°C

Key points:
- 200°C, 10min
- 200°C, 5min
- 190°C, 15min
- 190°C, 20min
Optimal LHW Pretreatment Conditions for Switchgrass

- Total sugar yields were similar at the following conditions:
  - 200°C, 5 min or 10 min, 190°C, 15 min or 20 min
- Sugar decomposition concentration (measured HMF+furfural) was 1-3 g/L (equivalent to <1% glucose degradation, 5-12% xylose degradation)
- Sugar loss to degradation might be reduced further by adjusting pH 4-7 during the LHW pretreatment with addition of a base.
- 200°C, 5 min and 200°C, 10 min pretreatment were chosen for further study.
Distribution of Glucan and Xylan in the LHW Pretreated Switchgrass Slurry

<table>
<thead>
<tr>
<th></th>
<th>Glucan</th>
<th>Xylan</th>
<th>Glucan</th>
<th>Xylan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soluble oligomers</td>
<td>6.4%</td>
<td>0.7%</td>
<td>0.6%</td>
<td>0.7%</td>
</tr>
<tr>
<td>Monomeric sugar</td>
<td>12.6%</td>
<td>12.1%</td>
<td>20.1%</td>
<td></td>
</tr>
<tr>
<td>Sugar loss to degradation</td>
<td>91.5%</td>
<td>69.8%</td>
<td>84.3%</td>
<td>66.2%</td>
</tr>
<tr>
<td>Remaining in solids</td>
<td>15.1%</td>
<td>66.2%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

200°C, 5 min

200°C, 10 min
Cellulase Dose vs Sugar Yields

Substrate: Dacotah Switchgrass (35% Glucan, 22% Xylan) milled to 40 mesh (moisture=5.5%)

Pretreatment: 200 C, 10 min or 200C, 5 min
150 g dry solids/L

Hydrolysis of whole pretreatment slurry:
Pretreated slurry was diluted to 30 g dry solids/L (=10 g glucan/L).
Hydrolyzed at pH 4.8, 50 C, 800 rpm using microplate mixer/incubator. 72 hr hydrolysis.

Enzyme: Spezyme CP (0.5-150 mg protein/g glucan)
(0.5, 1, 1.5, 3, 6, 15, 30, 60, 100 FPU/g glucan)
Novozym188 (fixed ratio of 2:1 CBU:FPU,
0.2-40 mg protein/g glucan)
Glucose Yields vs Cellulase+β-G Loadings

15 FPU Cellulase+30 CBU β-G/g glucan

- 200 °C, 10 min
- 77.0%
- 200 °C, 5 min
- 61.2%
Xylose Yields vs Cellulase Loadings

Cellulase Loading (mg total protein/g glucan)

Xylose Yield (%)

15 FPU Cellulase per g glucan

68.7%

70.0%

200°C, 5 min

200°C, 10 min
Cellulase Dose vs Sugar Yields of Pretreated and Hot-Washed Switchgrass Substrate: Dacotah Switchgrass (35% Glucan, 22% Xylan) milled to 40 mesh (moisture=5.5%)

Pretreatment: 200 C, 10 min
150 g dry solids/L

Hydrolysis of pretreated and hot-water washed solids:
Pretreated/hot washed solids was diluted to 30 g dry solids/L (=10 g glucan/L).
Hydrolyzed at pH 4.8, 50 C, 800 rpm using microplate mixer/incubator. 72 hr hydrolysis.

Enzyme: Spezyme CP (0.5-150 mg protein/g glucan of pretreated/hot washed solids) (0.5, 1, 1.5, 3, 6, 15, 30, 60, 100 FPU/g glucan)
Effect of Hot-Washing on Glucose Yields at Different Cellulase Loadings
Glucose Yields at Different Cellulase Loadings
(Accellerase 1000 vs Spezyme CP)
Pretreated and Un-Washed Switchgrass
Glucose Yields at Different Cellulase Loadings (Accelerase 1000 vs Spezyme CP)
Pretreated and Hot-Washed Switchgrass
High Solids Hydrolysis
(Alamo switchgrass, Pretreated at 200C, 5 min, 48 hr hydrolysis of whole pretreated slurry, 15 FPU Cellulase +40 CBU β-G/g glucan)
Summary and Conclusions

- LWH pretreatment improves enzymatic digestibility of switchgrass
- Above ~40 mg protein/g glucan (30 FPU/g), there is no significant yield improvement
- Presence of pretreatment liquid (inhibitors) has negative impact on sugar yield
- Optimal LHW pretreatment conditions need to be determined by considering energy requirement and enzyme dose
- High solids hydrolysis of switchgrass requires improvement
Thank You.