Bio-ethanol Production from Corn Stover using Low Moisture Anhydrous Ammonia (LMAA) Pretreatment and SSCF

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Website: http://www.ILoveDrKim.com/
Schematic of a biochemical cellulosic ethanol production process

(Source: NREL web site, accessed in March 2011)
Pretreatment?
Pretreatment

Purpose:

- It is required for efficient enzymatic hydrolysis of biomass because of the physical and chemical barriers that inhibit the accessibility of enzyme to the cellulose substrate.
- Open up the rigid structure.
- Pretreatment is one of the key elements in the bioconversion of lignocellulosic materials.

Common Effects:

- Decrease of lignin, hemicellulose, and extraneous components.
- Increase of surface area, porosity, and pore size.
- Reduce the crystallinity of cellulose
- Enhance the accessibility of enzyme to the cellulosic substrate

(Source: Kim, T.H. Book Chapter: Pretreatment of lignocellulosic biomass, In-press, Book title- Bioprocessing Technology, John Wiley&Sons)
Ideal biomass pretreatment

- **High** enzymatic hydrolysis rate and high yields of ethanol/butanol (products).
- **Minimal** degradation of the carbohydrate fractions.
- **No** production of compounds that are inhibitory to microorganisms used in the fermentation step.
- **Inexpensive** materials of construction.
- **Mild** process conditions to reduce the capital costs.
- **Recycle** of chemicals to reduce the operating costs.
- **Minimal** wastes.

Problems of conventional pretreatment methods

• High liquid input: high energy cost
  ❖ High chemical loading
  ❖ Washing step is required

• Can “low-liquid pretreatment” be possible?

• Pretreatment which uses no-additional water and low

• Low moisture anhydrous ammonia (LMAA) pretreatment
## Chemical and water inputs in various pretreatment methods and their ethanol yields

**Water input does not include washing water**

<table>
<thead>
<tr>
<th>Methods</th>
<th>Reaction conditions</th>
<th>Chemical input</th>
<th>Water input</th>
<th>Max. theoretical EtOH yield &amp; conc.</th>
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<td>190 °C, 2.0 min, 2.6 g-liquid/g-biomass, 1.9 wt.% H₂SO₄</td>
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<td>2.5</td>
<td>?</td>
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<td>ARP</td>
<td>170 °C, 10 min, 3.3 g-liquid/g-solid, 15 wt.% NH₃</td>
<td>0.5</td>
<td>2.8</td>
<td>?</td>
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<td>SAA</td>
<td>60 °C, 12 h, 6.0 g-liquid/g-solid, 15 wt.% NH₃</td>
<td>0.9</td>
<td>5.1</td>
<td>?</td>
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<tr>
<td>LMAA</td>
<td>80 °C, 84-96 h, 1.1 g-liquid/g-biomass, 50-70% MC</td>
<td>0.1</td>
<td>1.0~2.3</td>
<td>?</td>
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</tbody>
</table>

**Note:** 1. Water input does not include washing water; 2. Maximum ethanol yield and concentration are based on the optimal reaction conditions; ethanol yields are calculated based on total glucan and xylan in untreated corn stover; 3. Dilute acid (Kazi et al., 2010, NREL Report); 4. ARP: Ammonia recycle percolation (Kim et al., 2006); 5. SAA: Soaking in aqueous ammonia (Kim and Lee, 2005; Kim and Lee, 2007); 6. LMAA: Low moisture anhydrous ammonia (Yoo et al., 2011)
Schematic of corn stover-to-ethanol conversion using LMAA and SSCF
# LMAA process conditions

## Substrate
Corn stover (central Iowa, 2009)

## Ammoniation
30 – 70 % MC, 10 min, ~10 psi
Bench-scale reactor (2.85 in. ID×6.5 in. L, 690 ml of internal volume)

## Pretreatment
20-140 °C, 24-144 h
Batch reactor (0.93 in. ID×6 in. L, 67 ml of internal volume)

## Evaporation
Ambient condition, 12 h
Ammonia as a pretreatment reagent

- Highly selective and effective in **delignifying** the biomass.
- Minimal interaction with cellulose and hemicellulose
- Prevention of fungal growth
- Easy to recover and reuse because of **high volatility**.
- One of the most widely used commodity chemicals (one-fourth the cost of sulfuric acid).
- **Non-polluting** and **non-corrosive** chemical.
Lab-scale LMAA experiments setup

Ammoniation

- Purge
- PG: Press. Gauge
- TG: Temp. Gauge
- N₂ gas

Pretreatment

- TG
- Pretreatment setup

Press. Gauge
Temp. Gauge
1. Effects of LMAA Pretreatment on Ethanol Fermentation
Effects of LMAA pretreatment temperature on corn stover composition (96 hour pretreated corn stover)

Note. Conditions: Anhydrous Ammonia treatment; reaction conditions: 70% MC, 40 – 140 °C, 96 hours; All numbers are based on dried untreated corn stover

ASL: Acid soluble lignin / AIL: Acid insoluble lignin
### Microorganism
Recombinant *Escherichia coli KO11 ATCC® 55124*

### Medium
LB medium (0.5% of Yeast extract, 1% of Tryptone)

### Substrate
LMAA pretreated corn stover (50-70 % MC, 40-120 °C, 24-144 h)
3% w/v glucan loading

### Enzyme loading
15 FPU of GC220 + 30 CBU of Novozyme188 + 1,000 GXU of Multifect xylanase (5 mg protein) per g-glucan
Effects of xylanase on SSCF of LMAA-treated solid

Note. Conditions: (1) Anhydrous Ammonia treatment; reaction conditions: 50% MC, 80°C, 84 hours; (2) Anhydrous Ammonia treatment; reaction conditions: 70% MC, 80°C, 84 hours; SSF/SSCF condition: 3% glucan loading; 15 FPU GC220, 30 CBU Novo188; E.Coli KO11, 37°C, 150 rpm, pH 7.0 (1) with Multifect xylanase 5mg protein/g-glucan; (2) without xylanase
Effects of pretreatment conditions on ethanol fermentation

Ethanol conc.  =  -6.92969 + 0.24235 * Treatment time + 0.38824 * Treatment temp. - 6.38021E-004 * Treatment time * Treatment temp. - 9.41277E-004 * Treatment time^2 - 1.49433E-003 * Treatment temp.^2

The optimum reaction conditions:
- Reaction time = 92.3 h
- Reaction temp. = 109.8 °C.
- Ethanol production = 25.5 g/l.
Images of biomass structure

Untreated Corn Stover (20X objectives with dark-field)

LMAA treated Corn Stover (20X objectives with dark-field)

Note. Conditions: Anhydrous Ammonia treatment; reaction conditions: 70% MC, 110°C, 92 hours. Images are taken by DXR Raman Microscope (Thermo scientific, IA, USA)
2. Effect of Residual Ammonia Content on Ethanol Fermentation
Moisture contents vs. Residual ammonia in the Ammoniated Corn Stover

Moisture contents of untreated corn stover [%]

Residual ammonia contents in ammoniated corn stover [%]
Effect of moisture content in untreated C.S. on 120-h fermentation and residual ammonia content of LMAA-treated biomass

Note: Pretreatment condition: 30 - 70 % of MC, 110 °C, 92 h, SSCF condition: 3 % w/v glucan loading/50 ml working volume; recombinant *Escherichia. coli* KO11 (ATCC® 55124); 15 FPU of GC 220/g-glucan, 30 CBU of Novozyme 188/g-glucan, 1,000 GXU of Multifect xylanase /g-glucan; LB medium; anaerobic condition; 37°C, 150 rpm. Ammonia content is based on dry biomass weight.
Effects of residual ammonia on ethanol fermentation

\[ y = 17.194x + 2.8142 \]
\[ R^2 = 0.8109 \]
Raman spectroscopic image of the untreated, LMAA, and SAA pretreated corn stover

Note. Pretreatment Conditions: (1) Anhydrous Ammonia treatment; reaction conditions: 70% MC, 110°C, 92 hours; (2) Soaking in Aqueous Ammonia; reaction conditions: 15 wt.% aqueous ammonia, 60°C, 24 hours, solid : liquid=1:8
DXR Raman microscope (Thermo Scientific, Barrington, IL)
Overall mass balance of LMAA process
(50% moisture content case)

Corn Stover

Water 1,000 kg
Anhydrous NH\textsubscript{3} (gas) ~117 kg

Glucan: 387 kg
Xylan: 233 kg
Lignin: 171 kg

Ammoniation
10 psi, 10 min

Glucan: 387 kg
Xylan: 233 kg
Lignin: 171 kg

Pretreatment
80 °C, 84 h, 50% M.C.

Glucan: 387 kg
Xylan: 233 kg
Lignin: 171 kg

Evaporation
Ambient temp., 12 h

Glucan: 387 kg
Xylan: 233 kg
Lignin: 171 kg

Enzymes KO11 \textit{E. coli}

SSCF
37 °C, pH=7.0, anaerobic

Glucan: 39 kg
Xylan: 23 kg
Lignin: 171 kg

Ethanol 320 kg
CO\textsubscript{2} 306 kg

Solid residues

NH\textsubscript{3}: 101 kg
Water: 513 kg

Glucan: 387 kg
Xylan: 233 kg
Lignin: 171 kg

Glucan: 387 kg
Xylan: 233 kg
Lignin: 171 kg

Glucan: 39 kg
Xylan: 23 kg
Lignin: 171 kg
Chemical and water inputs in various pretreatment methods and their ethanol yields

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<td>0.05</td>
<td>2.5</td>
<td><strong>67 (18.5)</strong></td>
</tr>
<tr>
<td>ARP</td>
<td>170 °C, 10 min, 3.3 g-liquid/g-solid, 15 wt.% NH₃</td>
<td>0.5</td>
<td>2.8</td>
<td>71 (19.4)</td>
</tr>
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<td>60 °C, 12 h, <strong>6.0 g-liquid/g-solid</strong>, 15 wt.% NH₃</td>
<td>0.9</td>
<td>5.1</td>
<td><strong>70 (19.2)</strong></td>
</tr>
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<td>80 °C, 84-96 h, <strong>1.1 g-liquid/g biomass</strong>, 50-70% MC</td>
<td>0.1</td>
<td>1.0</td>
<td><strong>89 (24.9)</strong></td>
</tr>
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Note: 1. Water input does not include washing water; 2. Maximum ethanol yield and concentration are based on the optimal reaction conditions; ethanol yields are calculated based on total glucan and xylan in untreated corn stover; 3. Dilute acid (Kazi et al., 2010, NREL Report); 4. ARP: Ammonia recycle percolation (Kim et al., 2006); 5. SAA: Soaking in aqueous ammonia (Kim and Lee, 2005; Kim and Lee, 2007); 6. LMAA: Low moisture anhydrous ammonia (Yoo et al., 2011)
“Very rough” Estimated ethanol production cost (2,000 ton corn stover/day basis)

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<th>Dilute Acid</th>
<th>SAA</th>
<th>LMAA</th>
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<tbody>
<tr>
<td>Estimated EtOH Cost</td>
<td>[$/gal EtOH]</td>
<td>3.39</td>
<td>3.31</td>
<td>2.54</td>
</tr>
<tr>
<td>Corn stover Input</td>
<td>[ton/day]</td>
<td>2,000</td>
<td>2,000</td>
<td>2,000</td>
</tr>
<tr>
<td>EtOH production</td>
<td>[MMGal/year]</td>
<td>73</td>
<td>66</td>
<td>81</td>
</tr>
<tr>
<td>Operating cost</td>
<td>[$/gal EtOH]</td>
<td>2.78</td>
<td>2.68</td>
<td>2.07</td>
</tr>
<tr>
<td>- Pretreatment</td>
<td>[$/year]</td>
<td>34,870,659</td>
<td>15,641,239</td>
<td>7,566,725</td>
</tr>
<tr>
<td>- Chemical &amp; Water recovery + WWT</td>
<td>[$/year]</td>
<td>13,347,286</td>
<td>14,252,993</td>
<td>2,592,294</td>
</tr>
<tr>
<td>Capital cost</td>
<td>[$/gal EtOH]</td>
<td>0.61</td>
<td>0.63</td>
<td>0.47</td>
</tr>
<tr>
<td>- Pretreatment</td>
<td>[$/year]</td>
<td>36,200,000</td>
<td>17,244,807</td>
<td>3,975,780</td>
</tr>
<tr>
<td>- Chemical &amp; Water recovery + WWT</td>
<td>[$/year]</td>
<td>3,500,000</td>
<td>10,137,892*</td>
<td>10,137,892*</td>
</tr>
</tbody>
</table>

* same numbers are applied.
(by C.G. Yoo and T.H. Kim, 2011)
Conclusions

- **Ammonia and water inputs** were significantly reduced using LMAA pretreatment (~ 1:1 of biomass : liquid).
- LMAA pretreatment process resulted in high fermentation yield (**89-91%** theoretical maximum ethanol yield based on glucan + xylan in corn stover) with enzymes (15 FPU + 30 CBU + 1,000 GXU/g-glucan) and *E. coli* KO11 strain.
- There is a strong relationship between residual ammonia contents and ethanol fermentation yield - Ammoniation can supply assimilable nitrogen for microbial growth in the fermentor.
- **Moisture content, residual ammonia content, pretreatment temperature, and pretreatment time** are important factors affecting ethanol fermentation.
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Pretreatment of corn stover using low-moisture anhydrous ammonia (LMAA) process

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Thank you!