Effect of Pretreatment on Biomass Structure and Cellulose Properties

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Outline

• Why study the effect of pretreatment on biomass structure?
• What changes occur in biomass during dilute acid pretreatment?
• How do these changes affect the digestibility of cellulose?
• Summary
Cell wall is a tightly intermeshed composite

Lignin ~20%
Crosslinked phenolic polymer

Hemicellulose ~25%
Polymer of mostly xylose
Branched w/ acids and phenolics

Cellulose ~45%
Polymer of glucose
Crystalline fiber structure

## Technical Targets for Biochemical Conversion

<table>
<thead>
<tr>
<th>Barriers</th>
<th>2005 SOT</th>
<th>2012 Goals</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pretreatment</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yield (gallon/dry ton)</td>
<td>65</td>
<td>90</td>
</tr>
<tr>
<td>Solids Loading</td>
<td>30%</td>
<td>30%</td>
</tr>
<tr>
<td>Xylan to Xylose</td>
<td>63%</td>
<td>90%</td>
</tr>
<tr>
<td>Xylan to Degradation Products</td>
<td>13%</td>
<td>5%</td>
</tr>
<tr>
<td><strong>Conditioning</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xylose Sugar Loss</td>
<td>13%</td>
<td>0%</td>
</tr>
<tr>
<td>Glucose Sugar Loss</td>
<td>12%</td>
<td>0%</td>
</tr>
<tr>
<td>Enzyme Contribution* ($/gal EtOH)</td>
<td>$0.32</td>
<td>$0.10</td>
</tr>
<tr>
<td><strong>Saccharification</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saccharification &amp; Fermentation Time</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td><strong>Fermentation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall Cellulose to Ethanol</td>
<td>86%</td>
<td>86%</td>
</tr>
<tr>
<td>Xylose to Ethanol</td>
<td>76%</td>
<td>85%</td>
</tr>
<tr>
<td>Minor Sugars to Ethanol</td>
<td>0%</td>
<td>85%</td>
</tr>
</tbody>
</table>
Technical Barrier Areas for Biochemical EtOH

- Pretreatment
  - Feedstock Variation
  - Feedstock Quality
  - Feedstock Cost
  - Xylose Yield
  - Xylose Degradation
  - Solids Loading
  - Reactor Costs

- Conditioning
  - Sugar Losses

- Enzyme Production
  - Enzyme Cost

- Enzymatic Hydrolysis
  - Glucose Yield
  - Solids Loading (titer)

- Co-fermentation of C5 & C6 Sugars
  - Ethanol Yields
  - Ethanol Concentration
  - Rate
  - Hydrolyzate Toxicity

- Product Recovery

- Ethanol

- Residue Processing
  - By-products

*Hybrid Saccharification & Fermentation - HSF
What Governs Saccharification of Cellulose in Pretreated Biomass?

What makes the cellulose in substrates easier or more difficult to digest?

How can we produce much more digestible substrates?

- Targets are 85% overall yield of cellulose to EtOH in 3 days SSF at 20% solids loading

- Data from corn stover (Pioneer 34M95) pretreated with dilute acid in Sunds Reactor

- Data and samples provided by NREL Process Integration Group (Dan Schell).
What Governs Saccharification of Cellulose?

How does pretreatment affect major plant cell wall features and what is their affect on cellulose enzyme hydrolysis?

Cellulose morphology


Enzyme adsorption


Xylan/Lignin content and distribution

Enzyme Binding to Dilute Acid Pretreated Corn Stover

Dependence of SSF yields from dil. acid pretreated corn stover on enzyme accessible pore volume

Biomass porosity

Biomass particle size

mean particle diameter (µm)

Cellulose Conversion (% 120h Cel 7A)

SUNDs Reactor

Trickle Bed reactor
Xylan Hydrolysis and Redistribution

- Confocal microscopy (CFM) with a fluorescent tagged xylan anti-body probe
- CFM shows a change in xylan distribution in the cell wall during dilute acid pretreatment
- Residual xylan remains at edges of cell walls in lumen and middle lamella
- These areas have the highest concentration of lignin droplets

CS Rind Pretreated at 160 °C 1.2% H₂SO₄

Unpretreated 30s 59% xylan 60s 39% xylan 2 min 21% xylan

Lignin droplet formation

- SEM and TEM images showing lignin droplets on pretreated corn stover rind
- Formation of lignin droplets increases as temperature goes above lignin Tg,
- Formation of droplets much higher at 160 C compared to 140 C
- How does droplet formation effect digestibility of cellulose?

TEM of lignin redistribution and cell wall delamination (Stained with 1% KMnO₄)
Effect of Lignin Droplets on Saccharification of Cellulose

- Lignin droplets extracted from pretreated corn stover with aqueous dioxane
- Extraction removed droplets but did not change lignin distribution within cell wall
- Droplet removal increased digestibility ~10-15%
- Do droplets block access or adsorb enzymes

Digestibility (after 162h) of steam gun pretreated CS relative to the Xylan/Glucan (X/G) ratio in solids
Effect of Increasing Surface Area on Enzyme Hydrolysis

Grethlein compared 4 very different pretreatments, alkaline peroxide, ethylene diamine, autohydrolysis and acid prehydrolysis (3 severity levels).

Dilute acid pretreatment in NREL continuous pilot scale reactor. Weak correlation of ethanol yield with increased accessible pore volume.

**Linear dependence of initial enzymatic hydrolysis yields from pretreated hardwood on specific surface area**


**Dependence of SSF yields from dil. acid pretreated corn stover on enzyme accessible pore volume**

Effect of particle size on digestibility

Particle size decreases with increasing severity

Digestibility increases with decreasing particle size

How much of pretreatment severity decreases xylan content and how much is particle size reduction?

Is the difference between the samples due to severity, temperature, or the reactor?

Decouple affect of xylan removal and particle size reduction from digestibility

Use wet sieving to separate PCS into size fractions

Compare digestibility of PCS with different particle sizes but same xylan content

63 – 150 µm

150 - 710µm

710µm - 1.18 µm

>1180 µm
Effect of particle size on digestibility

Wet sieving used to fractionate dilute acid pretreated corn stover into 5 particle size ranges

Much larger difference in Steam Gun particle size fractions compared to Zipperclave

Pretreatment severity had larger effect on division of mass between particle size ranges in Steam Gun

Large differences in particle size distribution depending on reactor and pretreatment conditions

- Digestibility increased with decreasing particle size but xylan content also decreased
- Need to deconvolute influence of the various parameters
Effect of particle size on digestibility

Digestibility increased with decreasing particle size and xylan content
Particle size was about the same significance as xylan content
Reactors (steam gun, Sunds) incorporating size reduction could be more effective
Are higher severities more effective because of xylan removal or particle size reduction?
Could lower severity acid treatment with mechanical size reduction get high digestibility and high xylose yield?

Results of Multiple Linear Regression of Z-Scored Data

<table>
<thead>
<tr>
<th>Variable Name</th>
<th>Coefficients</th>
<th>Standard Error</th>
<th>t Stat</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>0.042</td>
<td>0.066</td>
<td>0.633</td>
<td>0.534</td>
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<tr>
<td>Mean Particle Size</td>
<td>-0.588</td>
<td>0.100</td>
<td>-5.902</td>
<td>7.391E-06</td>
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<tr>
<td>Xylan content</td>
<td>-0.570</td>
<td>0.108</td>
<td>-5.284</td>
<td>3.072E-05</td>
</tr>
<tr>
<td>Lignin Content</td>
<td>0.483</td>
<td>0.093</td>
<td>5.168</td>
<td>4.036E-05</td>
</tr>
<tr>
<td>Group</td>
<td>0.410</td>
<td>0.102</td>
<td>4.011</td>
<td>6.323E-04</td>
</tr>
<tr>
<td>Dummy variable</td>
<td>0.027</td>
<td>0.070</td>
<td>0.381</td>
<td>0.707</td>
</tr>
<tr>
<td>Reactor</td>
<td>0.009</td>
<td>0.083</td>
<td>0.112</td>
<td>0.912</td>
</tr>
</tbody>
</table>

$R^2 = 0.912$
Effect of Pretreatment on Cellulose Dp

Cellulose Carbanilation Procedure

- Carbanilation solubilizes individual celloextrin chains
- Minimal interference from xylan and lignin
- Gel Permeation Chromatography of cellulose carbanilates in THF
- 5 Columns used with polystyrene calibration from $10^2$ - $10^7$
Effect of Pretreatment on Cellulose Dp

Organosolv Pretreated Corn Stover
Trickle-bed reactor

Dilute Acid Pretreated Corn Stover
SUNDS reactor

<table>
<thead>
<tr>
<th>Log Ro</th>
<th>T °C</th>
<th>t min</th>
<th>Acid %</th>
<th>Solvent</th>
<th>DPw</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.88</td>
<td>130</td>
<td>56</td>
<td>0.5</td>
<td>OS</td>
<td>4800</td>
</tr>
<tr>
<td>1.13</td>
<td>140</td>
<td>56</td>
<td>0.5</td>
<td>OS</td>
<td>4900</td>
</tr>
<tr>
<td>1.13</td>
<td>140</td>
<td>56</td>
<td>0.5</td>
<td>H2O</td>
<td>4670</td>
</tr>
<tr>
<td>1.24</td>
<td>140</td>
<td>28</td>
<td>1.0</td>
<td>OS</td>
<td>4800</td>
</tr>
<tr>
<td>1.73</td>
<td>160</td>
<td>56</td>
<td>0.5</td>
<td>OS</td>
<td>2570</td>
</tr>
</tbody>
</table>

OS = Organosolv (MiBK:EtOH:H2O 1:2:3)

Log Ro = Severity Factor incl. pH
Effect of acid treatment on α-cellulose Dp

Very mild acid treatment decreases Dp_w of α-cellulose until level-off Dp reached
10X reduction Dp_w in appears to have little effect on digestibility
Mean particle size diameter decreases as severity of acid treatment increases
Cellulose crystallinity increases as severity increases

Can we tell which property of cellulose has the greatest affect on cellulose digestibility?
It is not clear that any of these parameters control digestibility
Higher crystallinity would be expected to decrease digestibility while lower Dp and smaller particle size should increase digestibility.
Possibly all parameters have an affect
Does isolated cellulose behave the same as the cellulose in biomass?
Effect of acid treatment on Dp of different celluloses

Regardless of starting cellulose Dp very mild acid treatment decreased Dp of the celluloses to the level-off Dp

Acid treatment caused a smaller change in mean particle size diameter

<table>
<thead>
<tr>
<th></th>
<th>Solka Floc</th>
<th>α-cellulose</th>
<th>Cotton Linters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>1110</td>
<td>1850</td>
<td>6000</td>
</tr>
<tr>
<td>Level-off DP&lt;sub&gt;w&lt;/sub&gt;</td>
<td>140-180</td>
<td>160-210</td>
<td>250-270</td>
</tr>
</tbody>
</table>

![Graph showing effect of acid treatment on cellulose DP](image1)

![Graph showing effect of acid treatment on mean particle diameter (MPD)](image2)
Digestibility of acid treated $\alpha$-cellulose

- Despite large differences in the properties of acid treated cellulose there was little difference in cellulose conversion.
- The biggest change in properties occurred at the start of the digestion, especially in mean particle diameter, crystallinity index.
- Changes during the digestion were relatively small.
Effect of Cellulose Morphology on Digestibility

- Does cellulose morphology affect its digestibility?
- Treatment of cellulose with liquid NH$_3$ converts it to cellulose III, but final T determines crystallinity.
- Treatment of cellulose with NaOH (16.5%) converts it to cellulose II, but with low CI.

<table>
<thead>
<tr>
<th>Cellulose samples</th>
<th>DP$_n$</th>
<th>DP$_w$</th>
<th>C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avicel PH 101</td>
<td>90</td>
<td>450</td>
<td>74</td>
</tr>
<tr>
<td>α-cellulose</td>
<td>190</td>
<td>1850</td>
<td>57</td>
</tr>
<tr>
<td>Cotton Linters (IFC)</td>
<td>1040</td>
<td>6000</td>
<td>73</td>
</tr>
<tr>
<td>Corn stover</td>
<td>170</td>
<td>3100</td>
<td>50</td>
</tr>
</tbody>
</table>
Effect of Cellulose Morphology on Digestibility

- Cellulose conversion at 16, 24, 48 & 72 h most closely correlated with amorphous content
- Slight correlation with initial cellulose
- Morphology almost orthogonal to digestibility
Summary

- Dilute acid pretreatments must have a high level of xylan hydrolysis to produce substrates with good enzymatic digestibility.
- Several other changes occur in biomass during dilute acid pretreatment that could be expected to affect digestibility.
- Lignin phase separates from carbohydrates:
  - droplets may block or adsorb enzymes
  - but, cell wall disruption may increase accessibility of enzymes
- Particle size decreases with increasing severity and corn stover does not appear strong enough to produce a highly porous substrate.
- Substrates high in amorphous cellulose are more digestible.
- Cellulose Dp is reduced under pretreatment conditions, but it is not clear that it affects the digestibility of the cellulose.
- It is still very difficult to deconvolute these multiple effects to determine which are the most important.
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Questions?