Fermentation of Pretreated Corn Stover Hydrolysate

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Outline

› Overview – Glucose/Xylose Cofermenting *S. cerevisiae*
› Corn Stover Hydrolysates
› Conditioning of Hydrolysates
› Fermentation of Hydrolysates
› Fermentation Inhibitors
Glucose/Xylose Cofermenting Yeast

- Developed by Dr. Nancy Ho
- *Sacharomyces cerevisiae* 424A(LNH-ST)
- Parent strain has high ethanol productivity and yield
- Three enzymes that feed xylose into pentose phosphate pathway stably integrated into yeast chromosome
Corn Stover Hydrolysates

› CAFI Common Batch of Corn Stover (Kramer)

› Dilute Acid Pretreatment
  – Pretreatment liquor (liquid from pretreatment) provided by NREL

› SO2 Catalyzed Steam Explosion
  – Pretreated and enzymatically hydrolyzed by UBC
## Composition of Hydrolysates

<table>
<thead>
<tr>
<th></th>
<th>Dilute Acid</th>
<th>SO2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>24.10</td>
<td>26.9</td>
</tr>
<tr>
<td>Xylose</td>
<td>74.62</td>
<td>36.9</td>
</tr>
<tr>
<td>Furfural</td>
<td>2.09</td>
<td>0.22</td>
</tr>
<tr>
<td>HMF</td>
<td>2.73</td>
<td>0.34</td>
</tr>
<tr>
<td>Acetic Acid</td>
<td>13.31</td>
<td>4.60</td>
</tr>
</tbody>
</table>
Fermentation

› Hydrolysate adjusted to pH 6.0 with calcium hydroxide

› Seed culture was grown overnight in 100 mL of YEPD (YEP + 2% glucose)

› Inoculum harvested after 12 hours by centrifugation (3000x g, 5 min)

› Early stationary phase cells transferred to 300 mL baffled Erlenmeyer flask
  – 100 mL of hydrolysate
  – 10 mL of 10% yeast extract
  – Cell density 8.5 – 9 g/L (increases to ~9.5-10.0 g/L)

› Fermentation at 30 C, 200 rpm orbital agitation

› Samples (1 mL) were taken and analyzed by HPLC (BioRad HPX-87H column)
Fermentation of Dilute Acid Liquor

Fermentation Time (hr)

Concentration (g/L)

- ethanol
- xylose
- glucose
Control Fermentation – Pure Sugars

- Glucose
- Xylose
- Ethanol

48 hours
Fermentation of SO2 Hydrolysate

- Glucose
- Xylose
- Ethanol

Concentration (g/L) vs. Time (hours)
Fermentation Inhibitors

› Organic Acids
  - Lower pH, slow fermentation
  - Relatively high concentrations before effect seen

› Hydrophobic Compounds (Aldehydes, Phenolics, etc.)
  - Inhibitory at much lower concentrations (Furfural toxicity seen at 1mg/mL)
  - Hydrophobicity correlates to toxicity
  - Sugar degradation
  - Lignin depolymerization
Hydrolysate Conditioning

› Overliming
  - pH 9.0 – 10.0
  - Inhibitors precipitate from solution

› Hydrophobic adsorbants
  - Widely uses in chromatographic and adsorption applications
  - Packed beds with regeneration makes processing of liquid streams possible
# Summary of Conditioning – Dilute Acid

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Overliming</th>
<th>XAD4</th>
<th>Overliming &amp; XAD4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>24.10</td>
<td>24.64</td>
<td>24.10</td>
<td>22.44</td>
</tr>
<tr>
<td>Xylose</td>
<td>74.62</td>
<td>72.73</td>
<td>76.17</td>
<td>73.22</td>
</tr>
<tr>
<td>Furfural</td>
<td>2.09</td>
<td>0.74</td>
<td>0.67</td>
<td>0.0</td>
</tr>
<tr>
<td>HMF</td>
<td>2.73</td>
<td>1.01</td>
<td>2.14</td>
<td>0.97</td>
</tr>
<tr>
<td>Acetic Acid</td>
<td>13.31</td>
<td>13.44</td>
<td>12.95</td>
<td>13.30</td>
</tr>
</tbody>
</table>
# Summary of Dilute Acid Fermentation

<table>
<thead>
<tr>
<th>After 48 hrs</th>
<th>Control</th>
<th>Untreated</th>
<th>Over limed</th>
<th>XAD4</th>
<th>XAD Cascade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xylose Consumption (%)</td>
<td>98.7%</td>
<td>54.1%</td>
<td>42.4%</td>
<td>44.5%</td>
<td>41.3%</td>
</tr>
<tr>
<td>Ethanol Yield (% of theoretical for sugars consumed)</td>
<td>92.8%</td>
<td>76.8%</td>
<td>63.4%</td>
<td>79.0%</td>
<td>72.0%</td>
</tr>
</tbody>
</table>
## Summary of SO2 Fermentation

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Untreated</th>
<th>Over limed</th>
<th>XAD4</th>
<th>XAD Cascade</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>After 48 hrs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xylose Consumption (%)</td>
<td>98.7%</td>
<td>73.5%</td>
<td>59.4%</td>
<td>71.1%</td>
<td>86.6%</td>
</tr>
<tr>
<td>Ethanol Yield</td>
<td>92.8%</td>
<td>82.4%</td>
<td>53.5%</td>
<td>87.4%</td>
<td>83.3%</td>
</tr>
</tbody>
</table>
Glucose Utilization – Effect of Furfural

Fermentation Time (hrs)

Concentration (g/L)

40 g/L furfural

20 g/L furfural
Xylose Utilization – Effect of Furfural

Fermentation Time (hrs) vs. Concentration (g/L) graph showing the utilization of xylose over time in the presence of different concentrations of furfural. The graph illustrates the impact of furfural concentration on the fermentation process, with 7.5 g/L and 20 g/L furfural concentrations shown.
Glucose Utilization – Effect of HMF

30 g/L furfural

20 g/L furfural
Xylose Utilization – Effect of HMF

30 g/L furfural

15 g/L furfural
Media Detoxification

› Yeast detoxify media
  - Concentration of aldehydes drop quickly (few hours)
  - Alcohol hydrogenation detoxification products

› Ability to detoxify media limited
  - High concentrations will cause fermentation to stall
  - Residual aldehydes left
Fermentation Inhibition - Conclusions

› Xylose more sensitive than glucose
› Furfural stronger inhibitor than HMF
› Inhibition slows fermentation rate
  - Redox balance disruption
› Inhibition **stops** fermentation early
  - Cells may become depleted in key nutrient (?)
Yeast Metabolism

Glucose → Glucose-6-P → Fructose-6-P → 3-Phosphoglycerate → Phosphoenolpyruvate → Pyruvate → Acetaldehyde → Ethanol

Xylose → Xylitol → Xylulose → Xylulose-5-P → PPP

Furfural → NADH → NAD+ → Furfuryl Alcohol

HMF → NADPH → NADP+ → HMF Alcohol

TCA Cycle

NAD(P)H → NAD(P)+ → NADH → NAD+
Ongoing Work

› Pretreated Poplar Hydrolysate – more severe pretreatment, more sugar degradation

› Identification of other fermentation inhibitors (lignin derivatives)

› Synergistic effect of furfural + HMF

› Fermentation modeling – effect of inhibitors
Questions