Enzymatic liquefaction of pericarp and corn stover

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Outline

Cellulose Ethanol in a Corn to Ethanol Plant

Corn Fractionation

Enzyme Liquefaction of Pericarp and Cornstover

Achieving high solids

Conclusions
Dry Grind Process with Cellulose Conversion

Corn → Milling → Water → Enzymes → Cooking → Enzymes → Hydrolyze → Pretreat → Backset → DG → DDGS → Ethanol

Distillation / Dehydration

Kim et al, Bioresource Technology, 99(12) 2008
Dry Grind Process with Kernel Fractionation

Corn → Enzymes → Cooking → Enzymes → Ferment → CO₂ → Ethanol

Enzymes

Water

Pericarp

Hydrolyze → Change a core assumption

Backset

Distillation / Dehydration

DG

DDGS
### Corn Kernel

<table>
<thead>
<tr>
<th>Component</th>
<th>% of total by weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tip cap</td>
<td>0.9</td>
</tr>
<tr>
<td>Pericarp (Cellulose &amp; Hemicellulose)</td>
<td>5.3</td>
</tr>
<tr>
<td>Endosperm (Starch)</td>
<td>81.9</td>
</tr>
<tr>
<td>Germ (Oil)</td>
<td>11.9</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

Gulati et al., 1996
Fractionation of Corn Kernel (Gives Identifiable Starch, Oil, Pericarp Fractions)

Added Enzyme enables 85% starch release from kernel in 12 hrs
And separation of oil (germ) and pericarp components of the kernel

Separated by density & size

![Graph showing starch release over time](image)

- **Oil**
- **Sugars**
- **Protein**
- **Starch**
- **Pericarp**

**Starch, g/L**

**Time (h)**

0 4 8 12

- **Corn + Enzyme**
- **Control**
### Pericarp Composition

<table>
<thead>
<tr>
<th>Dry weight basis composition (%)</th>
<th>Corn pericarp</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>As is</td>
<td>After Extraction</td>
</tr>
<tr>
<td>Cellulose</td>
<td>25.7</td>
<td>42.6</td>
</tr>
<tr>
<td>Xylan</td>
<td>15.5</td>
<td>25.8</td>
</tr>
<tr>
<td>Arabinan</td>
<td>8.2</td>
<td>13.7</td>
</tr>
<tr>
<td>Acetate</td>
<td>1.8</td>
<td>3.0</td>
</tr>
<tr>
<td>Lignin</td>
<td>4.5</td>
<td>7.4</td>
</tr>
<tr>
<td>Ash</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Extractives</td>
<td>39.7</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>95.5</td>
<td>92.6</td>
</tr>
</tbody>
</table>
# Enzymes for Pericarp Liquefaction

Multifect (Pectinase) and Spezyme CP (Cellulase)

<table>
<thead>
<tr>
<th>Enzyme component</th>
<th>Pectinase</th>
<th>Cellulase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endo-glucanase</td>
<td>577</td>
<td>1142</td>
</tr>
<tr>
<td>Xylanase</td>
<td>947</td>
<td>943</td>
</tr>
<tr>
<td>β-glucosidase</td>
<td>176</td>
<td>3</td>
</tr>
<tr>
<td>β-xylosidase</td>
<td>35</td>
<td>10</td>
</tr>
</tbody>
</table>

Activity = 1 µmol reducing sugar or p-nitrophenol / min at 50 °C, pH 4.8, (Dien et al., 2008)
Long cellulose chains in pericarp contribute to high viscosity slurries.

Viscosity may be reduced by through the use of a cellulolytic enzyme. The action of endoglucanase catalyzes the hydrolysis of cellulose specifically at the internal locations of the chain.

Kumar, Wyman et al., 2004, 2008
Enzyme Inhibitors

Lignin derived inhibitors could interfere with Liquefaction

Ximenes et al., 2011. Kim et al, Ko et al, Mhlongo et al., 2015
Tannic acid is a major inhibitor

Inhibition occurs at the mg level

- Filter Paper hydrolysis (FPase activity)
- Carboxymethyl Cellulose hydrolysis (CMCase activity)

Endoglucanases are more resistant to inhibitors

![Graph showing the remaining cellulase activities in Spezyme CP as a function of the ratio of phenolic compounds to cellulase protein.](image)

Inhibitors from hardwood, Kim et al, 2011, 2015
Enzyme Spezyme CP

- FPAase, CMCase: 48 h
- CBH: 48 h
- Beta G: 6 h
- Beta G: 24 h
- Beta G: 48 h
High solids needed (Goal is 300 g/L)

- Ethanol g / L
- Fiber Loading g / L
- Time, hrs

Min. concentration for efficient distillation

Wet Pericarp + Germ

Fed batch addition to enzyme solution, pH 4.8
Laboratory Experiment

Inputs

<table>
<thead>
<tr>
<th>t=0 (h)</th>
<th>Enzyme Pericarp</th>
</tr>
</thead>
<tbody>
<tr>
<td>t=0</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td></td>
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</table>

12 to 72 hrs

Buffer pH 4.8

- 2 g
- 2 g
- 2 g
- 2 g
- 2 g
- 2 g
- 3 g

Liquified pericarp

- 50 °C
- 290 rpm
- 12 hrs

- 50 °C
- 290 rpm
- 60 hrs
Mass Balance on Pericarp

Maximum of 28.4 lbs of ethanol from 100 lbs of pericarp, as is. If extracted pericarp is used, yield per unit weight is 1.7x higher. Assume both hexoses and pentoses ferment to ethanol.

Stoichiometric: Glucan to Glucose = 1.111; Xylan to Xylose = 1.136; Sugar to ethanol = 0.511
Mass Balance (Maximum Yields Possible)

1 Ton Corn (Dry basis)

1648 lb Starch
106 lb Pericarp
840 lb EtOH
30 lb EtOH
870 lb EtOH
130 gal

1 Bu Corn
Theoretical Maximum
3.1 gal EtOH

With other sources of fiber in the kernel, increase in ethanol yields of 6%
Sugars from pericarp are readily fermented

- 20 g/L of glucoses, minimal inhibitors
- 1 g/L of inoculation (*S. cerevisiae*), 28°C, 200 rpm, for 48h, 50 mM sodium citrate buffer pH 4.8
Corn Stover

Corn kernels

Corn stover: lignocellulose

Leaves, stalks, cobs (without kernel)
<table>
<thead>
<tr>
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<th><strong>Corn stover</strong></th>
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<td>Extractive free</td>
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Opportunity for Biology + Engineering

**Upstream Engineering and Process Biology**
- Bioreactor scale-up
- Engineering robust operations based on living systems
- Microbial and biochemical catalyst production
- Understanding (re-discovering) how to manage low carbon footprint, biomass feedstocks

Pictures of corn stover at 22% weight of solids / volume of water
Shear stress vs shear rate data for corn stover at different solid concentrations results in data for apparent viscosity ($\mu_a$) of a non-newtonian fluid.

$$Re_i = \frac{\rho N D_i^2}{\mu_a}$$

The impeller speed ($N$) is determined for the $\mu_a$ found to keep a turbulent regime ($Re > 10^4$) that maximize enzyme-solid contact.

$$M = \frac{cD_i^3}{2\pi} \mu_a N$$

Having $N$ and $\mu_a$ needed to reach a turbulent regime is possible to calculate the torque ($M$) required.

M and N is used to calculate the power required by the impeller to maintain a turbulent regime.

Slurries have shear thinning properties

\[
\log \eta = c + A \log \gamma.
\]

\[A_{24h} = -0.660,\]
\[A_{48h} = -0.692\]

Sugar cane bagasse

Cunha et al., 2014
Conclusions

Cellulose ethanol production in corn to ethanol plants is possible and beginning to occur.

Enzyme-assisted processing may be used to achieve mixable / pumpable slurries
  Mixtures of cellulase / hemicellulase used
  Effect of phenol inhibition must be considered

High solids loadings needed but materials handling (viscosities) must be addressed
  Shear thinning fluids
  Power requirements for mixing