Fuel Ethanol Dates Back to First Cars
Some Milestones in Ethanol Production Research at NCAUR

• Patented process for corn cob conversion to ethanol and furfural
• Alkali Peroxide pretreatment agricultural biomass
• Pentose fermenting yeast for ethanol
• Characterization of enzymes for biomass hydrolysis
• Recombinant ethanol producing bacteria
Benefits of Fuel Ethanol

- Lowers dependence on imported oil (170 million barrels in 2005).
- Lowers pollutants and greenhouse gas emissions, including CO, CO2, and VOC.
- Is an environmentally friendly replacement for oxygenate MTBE.
- Creates a market for corn (13% of U.S. corn harvest in 2005).
- Increased farm income by $4.5 billion and led to creation of 200,000 jobs (1994).

Annual Production: 3.9 B gal (2005)
Renewable Fuel Standard: 7.5 B gal (2012)
Potential of corn to replace oil for U.S. market

- % U.S. Corn Harvest Going to Ethanol
- % Auto Fuel Replaced by Ethanol

(RFA & NCGA, 2006)
## Potential of lignocellulosic biomass to replace oil for U.S. market

<table>
<thead>
<tr>
<th>Feedstocks</th>
<th>Million dry ton per yr</th>
<th>Billion gal of ethanol per yr</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Agricultural Land (selected)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn Stover &amp; Wheat Straw</td>
<td>75</td>
<td>5.22</td>
</tr>
<tr>
<td>Corn Fiber &amp; DDGS</td>
<td>13</td>
<td>0.83</td>
</tr>
<tr>
<td>CRP Biomass</td>
<td>18</td>
<td>1.08</td>
</tr>
<tr>
<td>Perennial Crops</td>
<td>156</td>
<td>9.36</td>
</tr>
<tr>
<td><strong>Forestlands (selected)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Logging &amp; Processing Residues</td>
<td>134</td>
<td>8.04</td>
</tr>
<tr>
<td>Total:</td>
<td>4,907</td>
<td>24.4</td>
</tr>
</tbody>
</table>

*This is 17% of our total oil needs.*

Notes: (1) 60 gal/ton ethanol yield; (2) source: [http://feedstockreview.ornl.gov/pdf/billion_ton_vision.pdf](http://feedstockreview.ornl.gov/pdf/billion_ton_vision.pdf)
Corn Fiber: lignocellulosic biomass conversion to ethanol.

- Dilute-acid pretreated corn fiber
- Buffered hot-water pretreated corn fiber
Structure of corn kernel

A KERNEL OF CORN

ENDOSPERM (81.9%)

STARCH

STARCH AND GLUTEN

TIP CAP (0.8%)

GERM (11.9%)

HULL AND FIBER (5.3%)
Why Corn fiber?

• Could be a first step leading to other sources of lignocellulose
• Easy to digest and ferment because it contains no lignin
• Centrally located, so no collection fees
• Lower capital & labor costs, usually located at pre-existing ethanol fermentation facility
### Ethanol Yield from a Bushel of Corn

<table>
<thead>
<tr>
<th>Product</th>
<th>Ethanol Yield (gallons)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch (32 lbs)</td>
<td>2.5 - 2.7</td>
</tr>
<tr>
<td>Fiber* (4.5 lbs)</td>
<td>0.30</td>
</tr>
</tbody>
</table>

One bushel of corn weighs 56 lbs
One gallon of ethanol = 3.785 L = 6.58 lbs
*contained in DDGS; ref. Gulati et al., 1996
## Fibrous Biomass vs. Corn Compositions

<table>
<thead>
<tr>
<th>From:</th>
<th>Starch (%w/w)</th>
<th>Cellulose (%w/w)</th>
<th>Hemicellulose (%w/w)</th>
<th>Total (%w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar</td>
<td>Glucose</td>
<td>Glucose</td>
<td>Arabinose</td>
<td>Xylose</td>
</tr>
<tr>
<td>Corn Fiber</td>
<td>20</td>
<td>14</td>
<td>11</td>
<td>18</td>
</tr>
<tr>
<td>DDG</td>
<td>3</td>
<td>27</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>Corn Stover</td>
<td>none</td>
<td>38</td>
<td>3</td>
<td>18</td>
</tr>
<tr>
<td>Corn</td>
<td>78</td>
<td>na</td>
<td>na</td>
<td>na</td>
</tr>
</tbody>
</table>

Data: corn fiber Grohmann and Bothast, 1997; corn stover Wiselogel et al., 1996)
Challenges to processing fibrous biomass compared to grains

- High bulk mat’l (wood less so)
- 2-phase reactions (β-glucan insoluble for > 10 d.p.)
- Complex cell wall structure & lignin (e.g. storage vs. structural CHO’s)
- Xylan related sugars not fermented by *Saccharomyces*

<table>
<thead>
<tr>
<th>Moisture</th>
<th>Corn</th>
<th>Fiber</th>
<th>DWG</th>
<th>Stover</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15%</td>
<td>46%</td>
<td>64%</td>
<td>5%</td>
</tr>
</tbody>
</table>
Designing Process

• Select pretreatment
  – Hydrolyze hemicellulose
  – Prepare cellulose for enzymatic digestion

• Select Hydrolytic enzymes
  – Hemicellulases
  – Cellulases

• Select ethanol producing biocatalyst
  – *Saccharomyces* does not ferment xylose!
## Selected Pretreatment Strategies

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Pentoses</th>
<th>Inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strong Acid</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Dilute Acid</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Hot Water</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>AFEX</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alkaline Peroxide</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Corn Fiber to Ethanol Process

Corn Fiber

Dilute Acid Pretreatment

Centrifugation

Syrup (glucose and pentoses)
Fermentation
Ethanol Recovery

Solids (cellulose)
Liq. Residues
Dry
Animal Feed
Corn Fiber Reactor

Corn Fiber Slurry Enters Reactor

Steam

To jacket
Rate of Corn Fiber Hydrolysis

Sugar Concentration (%w/v)

Time (min)

Xyl

Glu

Ara
Ethanol Producing Strains Capable of Fermenting Pentoses

**Engineered to use pentoses**
- Zymomonas mobilis
- Saccharomyces

**Engineered to make ethanol**
- Escherichia coli
- Klebsiella oxytoca

**Natural microorganisms**
- Pentose fermenting yeast
- Thermophiles
- Saccharomyces + xylose isomerase
Metabolic Engineering an ethanologenic bacterium

I. *K12* converts glucose to mixed acids

\[
\text{Glucose} \rightarrow \text{wild-type K12} \rightarrow \text{Lactic Acid, Ethanol, Acetic Acid, Formic}
\]

II. Mutant does not ferment glucose (*Dr. Clark, SIU*)

\[
\text{Glucose} \rightarrow \text{pfl-, ldh-} \rightarrow \text{No Growth}
\]

III. *FBR5* ferments glucose selectively to ethanol (*Dr. Ingram, U.Fl.*)

\[
\text{Glucose} \rightarrow \text{PET Operon} \rightarrow \text{Ethanol}
\]
Ethanol Production Restores Anaerobic Growth

$pfl-, ldh- + pet$ genes
Ethanol Fermentation Of Corn Fiber Hydrolysate by *E. coli* FBR5

![Graph showing concentrations of various sugars and ethanol over time.](image-url)
## Fermenting Fibrous Components produced by Corn Milling

<table>
<thead>
<tr>
<th>Feed stock</th>
<th>Sugars %w/v</th>
<th>Max. Ethanol %w/v</th>
<th>Ethanol Yield g/g</th>
<th>Ethanol Prod. g/l/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>DWG</td>
<td>3.98</td>
<td>2.12</td>
<td>0.49</td>
<td>0.71</td>
</tr>
<tr>
<td>Germ</td>
<td>4.15</td>
<td>2.19</td>
<td>0.50</td>
<td>0.56</td>
</tr>
<tr>
<td>Fiber</td>
<td>8.50</td>
<td>3.74</td>
<td>0.46</td>
<td>0.77</td>
</tr>
</tbody>
</table>
Comparison of Laboratory Microorganisms for Fermenting Biomass Hydrolysates

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Strain</th>
<th>Max ethanol (g/l)</th>
<th>Yield¹ (g/g)</th>
<th>Max. productivity (g/l/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilute acid</td>
<td><em>E. coli</em> K011</td>
<td>34.7</td>
<td>0.41</td>
<td>1.16</td>
</tr>
<tr>
<td>Dilute acid</td>
<td><em>E. coli</em> SL40</td>
<td>31.7</td>
<td>0.42</td>
<td>1.12</td>
</tr>
<tr>
<td>Dilute acid</td>
<td><em>E. coli</em> FBR3</td>
<td>28.0</td>
<td>0.46</td>
<td>0.97</td>
</tr>
<tr>
<td>Dilute acid</td>
<td><em>Zymomonas</em> CP4 (pZB5)</td>
<td>22.6</td>
<td>0.47</td>
<td>1.05</td>
</tr>
<tr>
<td>AFEX</td>
<td><em>Saccharomyces</em> 1400 (pLNH32)</td>
<td>21.0</td>
<td>0.50</td>
<td>1.60</td>
</tr>
</tbody>
</table>

¹grams ethanol per grams sugar consumed; maximum possible is 0.51 g/g.
A review of more current work: Buffered hot-water pretreatment of corn fiber demonstration at Aventine Bioenergy

Collaborators:
Mike Ladisch (PI), N. Mosier, Purdue U.
G. Welch, Aventine
B. Dien, NCAUR
A. Arden, DOE
Purdue’s Buffered hot-water pretreatment

Advantages over dilute-acid:
- Lower capital costs
- Easy to integrate into process
- Maintain water-balance
- Does not generate gympsum
- Does not change color of corn gluten feed

Disadvantages compared to dilute acid:
- Does not completely hydrolyze hemicellulose

Conclusion: existing plant concerns trumped pretreatment concerns.
Principles of Liquid Water Pretreatment

a. Control (maintain) pH to prevent complete hydrolysis of the hemicellulose sugars – reduces formation of inhibitors

b. Use high temperature (160 – 180°C) to ensure disruption of cell wall and swelling of cellulose fibers

(Ladisch, et al.)
Pretreatment Flow Diagram

1. Fiber from Vetter press
2. Stillage
3. Steam
4. Cake
5. Liquid 50 lbm/min

Pump
Hold Tank
Recycle
43 gpm
41.5 gpm w/dissolved solids
Heat Exchanger
Back-pressure Regulator
Centrifuge

(Ladisch, et al.)
“Snake-coil” Plug Flow Pretreatment Coil

(Ladisch, et al.)
Release of sugars from corn fiber when treated with hot-water

- Glucose
- Xylose
- Arabinose

Yields (% of maximum)

Pretreatment Time (min)

Soluble CHO

Monosaccharides
Complex Mixture of Enzymes Needed to Degrade Arabinoxylan

Selinger et al., 1996
Digesting hot-water treated corn fiber with commercial enzyme

![Graph showing the effect of enzyme loading on monosaccharide yield. The x-axis represents enzyme loading (%v/v) and the y-axis represents monosaccharide yield (% of max). The graph shows two curves: one for glucose and another for Ara & Xyl. The glucose curve starts at 0 at 0.0% enzyme loading and reaches 100% at around 2.5% enzyme loading. The Ara & Xyl curve also starts at 0 but reaches around 60% at 2.5% enzyme loading.]
## Preparing custom enzyme preparations by culturing fungi on corn fiber

<table>
<thead>
<tr>
<th>Enzyme Preparation</th>
<th>Corn Fiber Pretreat.</th>
<th>Protein (mg/ml)</th>
<th>Xylanase (U/ml)</th>
<th>Cellulase (U/ml)</th>
<th>FE Activity (uM/m/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. niger 2001</em></td>
<td>HW</td>
<td>1.24</td>
<td>113.5</td>
<td>5.0</td>
<td>2.25</td>
</tr>
<tr>
<td><em>A. niger 2001</em></td>
<td>Untreated</td>
<td>0.49</td>
<td>72.2</td>
<td>4.8</td>
<td>1.15</td>
</tr>
<tr>
<td><em>T. reesei RUT C30</em></td>
<td>HW</td>
<td>1.35</td>
<td>64.4</td>
<td>7.0</td>
<td>nd</td>
</tr>
<tr>
<td><em>T. reesei RUT C30</em></td>
<td>Untreated</td>
<td>1.42</td>
<td>46.3</td>
<td>8.3</td>
<td>nd</td>
</tr>
</tbody>
</table>
Enzymatic treatment of hot-water treated corn fiber

% Xylan released as Ara & Xyl

Protein Loading (mg protein per g DSCF)

- T. reesei only
- A. niger only
- Both (same loading)
Factorial Design to Optimize Sugar Yields

Improved Saccharification of DSCF

A = increase from 20’ to 30’ pretreatment; B = glucohydrolyases; C = feruolyl esterase
Fermentation of enzyme released sugars to ethanol

![Graph showing the fermentation of enzymes released sugars to ethanol. The graph plots concentration (g/l) of Glucose, Xylose, Arabinose, and Ethanol against time (hr). The x-axis represents time from 0 to 48 hours, and the y-axis represents concentration from 0 to 14 g/l. The graphs show the decrease in Glucose and Xylose concentrations over time, while Arabinose concentration increases. Ethanol concentration starts at 0 and increases significantly from 24 hours onwards.](image_url)
Summary

- Ethanol yield from corn can be increased 10% by converting fibers from the germ and pericarp into ethanol.

- Corn fiber can be converted to ethanol by treating with dilute sulfuric acid and fermenting with ethanologenic *E. coli*.

- Corn fiber can also be converted by pretreating with hot-water, but further work is needed to develop more efficient hemicellulases.
Some Future Trends

- High-solids pretreatment of biomass
  - Lower chemical usage (e.g. acid or alkali)
  - Less energy required for heating
- High-solids saccharification or fed-batch SSF
  - More concentrated ethanol
  - Smaller unit operations
  - Possibility for using high-temperature enzymes
Acknowledgments

From NCAUR:
Nancy Nichols
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Patricia O’Bryan
Loren Iten
Xin Li

Aventine/Purdue/NCAUR project:
Gary Welch, Nathan Mosier, Rick Hendrickson, Rich Dreschel, Michael Ladisch, and Andy Aden
Enzymatic treatment of HW-CF w/ mixture

Sugar Yields (% of maximum)

- Glucose
- Xylose
- Arabinose

A. niger & T. reesei Protein Loadings (mg/g DSCF of each)
**Process Description**
Pretreat Fiber and Liquid/Solid Separation

- Fiber → Pretreat
- Pretreat → Centrifuge
- Centrifuge → Stillage (Water)
- Stillage (Water) → To drier or hydrolysis
- Centrifuge → Solids
- Solids → To fermentation
- Liquid → To drier or hydrolysis

*(Ladisch, et al.)*
What is expected of a pretreatment?

Allow cellulase access to cellulose polymers by disrupting cell wall structure

- Dissolve Hemicellulose
- Displace Lignin
- Swell Cellulose Bundles
Chemical Mechanisms

Hemicellulose
Acid hydrolyzes, alkali dissolves, hot-water acts as week acid

Lignin
Molecular oxygen, ozone, peroxide break lignin ether bonds, alkali sponifies ferulic/arabinose ester bonds

Cellulose
Ammonia disrupts H bonds, solvents & conc. acid dissolves cellulose polymer