Bioethanol Technology and Future Opportunities

Nhuan P. Nghiem
Eastern Regional Research Center, ARS/USDA
600 E. Mermaid Lane
Wyndmoor, PA 19038
John.Nghiem@ars.usda.gov

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Part 1. Bioethanol from Sugar and Starch Feedstocks
1st Generation Bioethanol

Fuel Ethanol is the #1 Biofuel in the World Today
- 13.5 Billion Gallons Produced in 2006
- ~70% Produced in USA and Brazil
- Feedstocks are corn in the U.S. and sugar cane in Brazil

Source: F.O. Licht
1st Generation Feedstocks for Ethanol

- Substrates for Ethanol Fermentation:
  - Sucrose (Sugar): From Cane or Beet
    - Sucrose → Glucose + Fructose
    - Invertase
  - Starch: From Corn, Milo, Wheat, Cassava
    - Starch → Dextrins → Glucose
    - $\alpha$-Amylase
    - Glucoamylase

- Sucrose and Starch are Readily Converted to Simple Sugars that can be Fermented to Ethanol by the Yeast *Saccharomyces cerevisiae*
Bioethanol is Simple to Produce from Sugar Feedstocks

A Simple Batch or Continuous Process with Cell Recycle Can Be Used

1. Add sugar or molasses
2. Add water and nutrients
3. Add Yeast
4. Yeast ferments sugar to make ethanol
5. Distillation

Source: K. B. Hicks
Making Ethanol from Starchy Crops is Slightly More Complex than from Sugar

1. Grinding

2. Making a “Mash”

3. Adding Enzymes and Heat to Convert Starch to Sugar

4. Adding Yeast

5. Yeast “ferments” Sugars to make Ethanol!

6. Distillation

2.8 Gal Ethanol

Animal Feed

Source: K. B. Hicks
Ethanol from Corn: Wet and Dry Milling

- Lower Capital
- Suitable for Smaller Plants

Dry Milling
- Corn → Milling → Liquefaction → Saccharification & Fermentation (SSF) → Distillation → Drying → Dehydration → Ethanol

Wet Milling
- Corn → Steeping (Soaking in SO₂) → Degerm/Defiber → Gluten Separation → Drying → Corn gluten feed → Corn gluten meal → Drying → Corn oil → CS HFCS
- Enzymes + Ca (pH 6) → Liquefaction → Saccharification → Fermentation → Distillation → Dehydration → Ethanol

Source: Bothast & Schlicher
Recent Developments in Starch Ethanol Technology

- **New Corn Feedstocks**
  - Pioneer’s corn hybrids
    - “High Total Fermentables” (HTF)
    - 4% increase in ethanol yield
  - Monsanto’s “Processor Preferred High Fermentable Corn”
    - 2.7% increase in ethanol yield
  - Syngenta’s $\alpha$-amylase corn
    - Enzyme activated with water at 70°C
  - Renessen’s Mavera™
    - High oil and lysine contents
    - Highly Fermentable Fraction (HFF)
    - High Oil Fraction (HOF)
Recent Developments in Starch Ethanol Technology

- **New Enzymes for Starch Hydrolysis**
  - Genencor’s Stargen™
    - Contains $\alpha$-amylase and glucoamylase
    - Synergistic effects allow hydrolysis at fermentation temperature
      - Eliminate cooking step
    - Elimination of Ca requirement
      - Reduce scaling problems
  - Novozymes also developed enzymes for starch hydrolysis at low temperatures
  - Innovase’s Ultra-Thin
    - Hybrid $\alpha$-amylase effective at pH 4.5
      - Only a single pH adjustment needed
Recent Developments in Starch Ethanol Technology

- New/Improved Production Processes
  - Poet’s BFRAC™ and BPX processes
    - Equipment for corn fractionation developed by Satake
    - Use Novozymes enzymes
    - Has been implemented at 11 Poet plants – total capacity of 525 MM gal/yr
    - Reported average 20% increase in ethanol concentration
  - Continuous dry-grind and stripping process (ARS ERRC)
  - Enzymatic milling (E Milling) process (ARS ERRC)
    - Soak corn in water for ~ 6 hours
    - Coarse grind
    - Addition of protease to release the starch granules from the endosperm
    - Does not require SO₂ to obtain starch yield equivalent to a conventional wet-milling process
    - But low levels of SO₂ still are needed for antimicrobial control
Part 2. Bioethanol from Cellulosic Feedstocks
2nd Generation Bioethanol

“Cellulosic Ethanol”

- Ethanol Made from Lignocellulosic Biomass
  - Forest residues
  - Agricultural residues
    - Corn stover
    - Bagasse
  - Dedicated energy crops
    - Switchgrass
    - Hybrid poplar

Source: NREL
Lignocellulosic Biomass

**Composition and Structure**

- **Hemicellulose (xylose)**: 30%
- **Lignin (phenolics)**: 26%
- **Cellulose (glucose)**: 44%

*Source: US DOE*

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*Source: C. N. Hamelinck et al., Universiteit Utrecht*
Lignocellulosic Biomass

- Cellulose

Fragment of a cellulose molecule

Alternating glucose residues are in an inverted orientation so the cellobiose (a disaccharide) is the repeating structural unit.

Source: US DOE
Cellulose Hydrolysis

- Two enzymes are needed

\[
\text{Cellulose} \xrightarrow{\text{Celullase}} \text{Cellobiose} \xrightarrow{\text{Cellobiase}} \text{Glucose}
\]

- Most commercial cellulases have some cellobiase activity but not enough
  - Cellobiase must be added
Lignocellulosic Biomass

- **Hemicellulose**
  - Short and highly branched chains
    - C-5 sugars (xylose, arabinose)
    - C-6 sugars (glucose, galactose, mannose)
    - Non-sugars (acetyl groups)
Hemicellulose Hydrolysis

- Amorphous form makes hydrolysis relatively easy
- But the process is more complex because of heterogeneous structure
- Complete hydrolysis requires action of several enzymes
- Commercial products contain some but not all enzymes needed
- Chemical hydrolysis (dilute acid or hot water) is another option

Wheat Straw Hemicellulose Hydrolysis

Source: A. S. Schmidt et al.
Lignocellulosic Biomass

- Lignin
  - Complex polymer of phenyl propane and methoxy groups
  - Encrusts the cell walls and cements the cells together
  - Highly resistant to biodegradation

Source: US DOE
Biomass Ethanol Technology

**Basic conversion concept**

- **Lignocellulosic biomass**
  - Milling
  - Pretreatment

**Sugar Platform**
- Biochemical process
- Only carbohydrates go to ethanol
- Lignin is considered a waste and burned for energy

**Syngas Platform**
- Thermo/Biochemical process
- All components can be used for ethanol production

- **Gasification**
  - Hydrolysis
  - Fermentation
  - Ethanol

- **Fermentation**
  - Catalytic conversion
  - Ethanol
The Sugar Platform: Biomass Pretreatment

- To make biomass more accessible to enzyme hydrolysis
  - Partial removal of lignin
  - Decrease crystallinity
  - Increase amorphous regions
- Desired characteristics of a good pretreatment process
  - Operate at moderate T and P
  - No needs for expensive materials of construction
  - Preserve the carbohydrates
  - Allow production of fermentable sugars at high rates and yields
  - Generate no inhibitory compounds
  - Generate minimal wastes
  - Narrow range of pH adjustment
  - Allow recycle of chemicals

Source: U.S. DOE
The Sugar Platform: Biomass Pretreatment

- Several processes have been developed but no clear winner
- Leading pretreatment technologies

<table>
<thead>
<tr>
<th>Pretreatment Process</th>
<th>Company</th>
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<tbody>
<tr>
<td>Concentrated Sulfuric Acid (Arkenol)</td>
<td>Blue Fire Ethanol</td>
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<tr>
<td>Dilute Sulfuric Acid (NREL)</td>
<td>Abengoa, logen</td>
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<td>Steam Explosion</td>
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<tr>
<td>Soaking in Aqueous Ammonia (Auburn U)</td>
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<td>Ammonia Fiber Explosion (Michigan State U)</td>
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<td>Lime Treatment (Texas A&amp;M U)</td>
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<tr>
<td>Alkaline Peroxide Treatment</td>
<td>BioGasol</td>
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<tr>
<td>Wet Oxidation</td>
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<td>Organosolv Fractionation</td>
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Microorganisms for Biomass Sugars Fermentation

- Desired Characteristics
  - Capability of fermenting both C-5 and C-6 sugars to high ethanol yields
    - Genetic modifications are required to
      - Add C-5 metabolism capability (Zymomonas mobilis, Saccharomyces cerevisiae)
      - Improve ethanol yield by elimination of by-products (Escherichia coli, Klebsiella oxytoca)
  - Genetically stable
  - High ethanol tolerance
  - Low pH optimum
  - Robustness
# Microorganisms for Biomass Sugars Fermentation

## Microbial Strains

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>(Institution)</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Zymomonas mobilis</em>&lt;sup&gt;1&lt;/sup&gt;</td>
<td>(NREL)</td>
<td>DuPont, Poet</td>
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<tr>
<td><em>Saccharomyces cerevisiae</em>&lt;sup&gt;1&lt;/sup&gt;</td>
<td>(Purdue U)</td>
<td>Iogen</td>
</tr>
<tr>
<td><em>Saccharomyces cerevisiae</em>&lt;sup&gt;1&lt;/sup&gt;</td>
<td>(Delf U)</td>
<td>Nedalco, Mascom, SunOpta</td>
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<tr>
<td><em>Escherichia coli</em>&lt;sup&gt;1&lt;/sup&gt;</td>
<td>(U Florida)</td>
<td>Verenium, BioEthanol Japan</td>
</tr>
<tr>
<td><em>Klebsiella oxytoca</em>&lt;sup&gt;1&lt;/sup&gt;</td>
<td>(U Florida)</td>
<td>Verenium Corp.</td>
</tr>
<tr>
<td><em>Bacillus stearothermophilus</em>&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td>Colusa Biomass Energy Corp.</td>
</tr>
<tr>
<td><em>Thermoanaerobacter mathranii</em>&lt;sup&gt;2&lt;/sup&gt;</td>
<td>(Danish Technical U)</td>
<td>Biogasol</td>
</tr>
<tr>
<td><em>Clostridium phytofermentans</em>&lt;sup&gt;3&lt;/sup&gt;</td>
<td>(U Mass)</td>
<td>SunEthanol</td>
</tr>
</tbody>
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1. Mesophilic organisms
2. Thermophilic organisms
3. Cellulolytic and xylanolytic organism
The Sugar Platform: Process Development

- Important Factors for Process Design Considerations
  - Feedstock availability and cost, including transportation cost
  - Suitable pretreatment process for selected feedstock
  - Technical efficiency and economic feasibility of bioconversion process for the pretreated feedstock
  - Wastewater treatment and process water recycle
The Syngas Platform for Biomass Ethanol Production

- General Process Concept

Biomass

\[ \text{Gasification} \rightarrow \text{Fermentation} \rightarrow \text{Ethanol} \]

\[ \text{Catalytic Conversion} \]

Major components of syngas are CO, CO$_2$, and H$_2$
Several types of commercial gasifiers are available

Strains of *Clostridium ljungdahliii* developed for syngas fermentation (U Arkansas)
- Commercialization on going (Bioengineering Resources, Inc.)

Development of catalysts for conversion of syngas to ethanol
- Commercialization on going (Syntech Biofuel Research, Inc.)
Part 3. Opportunities for Bioethanol R&D
Biomass Ethanol Research Needs

- Feedstock Development
  - High yield per acre
  - High yield of fermentable sugars
  - Production and sustainable collection
  - Distribution and transportation

- Pretreatment
  - Improve process economics

- Cellulase enzymes
  - Lower manufacturing cost
  - Higher cellobiase activity
Biomass Ethanol Research Needs

- Microorganisms
  - Robust strains
    - Capable of effective metabolism of cellobiose and high ethanol yields
      - Reduce or eliminate needs for cellobiase
    - Suitable for Consolidated Bioprocessing
      - Direct cellulose conversion
      - Reduce or eliminate needs for cellulase
Biomass Ethanol R&D Needs

- Fermentation Process
  - Increase solid loading
    - Higher final ethanol concentrations
- Development of co-products
  - Xylose oligomers for food applications
  - High-value lignin-based products
- Process Integration
Starch Ethanol R&D Needs

Don’t forget other grains .... like me, please

Mr. Joe Barley
Currently The Fuel Ethanol is Made Here.

But We Have the Major Markets for Fuels Here!!
Barley is a crop grown outside the Corn Belt. These “barley belts” can provide feedstock for ethanol plants outside the corn belt where transportation fuels are needed!
Technical Issues with Barley as a Fuel Ethanol Feedstock

- Abrasive hull – damages milling equipment
- Low starch content (~50-55%) compared to corn’s (~70%) – results in low ethanol yields
- High viscosity of mash due to \( \beta \)-glucans – makes processing difficult and expensive and limits the feed use of the ethanol co-products, DDGS, on monogastric animals.
ERRC/ARS Has A Major Barley Research Program to Solve These Technical Issues

- Working with breeders (Virginia Tech) to develop better hull-less and hulled barley for fuel ethanol production.
- Developing dry fractionation processes to separate barley grain into fermentable and non-fermentable fractions.
- Working with Genencor International (a division of Danisco) to use new enzymes to eliminate viscosity, increase ethanol yield, and develop energy saving fuel ethanol processes.
Bottom Line on Barley

- Production of fuel ethanol from barley can lead to another 1-2 billion gallons of ethanol from the grain plus another 1-2 billion gallons from the straw when cellulosic ethanol processes are commercial.
- Farmers and rural economy outside the corn belt will benefit.
- In many areas of the U.S., winter barley can be “double cropped” with soy, providing more grain from the same land.
- Barley as a cover crop prevents erosion and loss of nitrates/phosphates into watershed and improves the environment.
Back to the Future?

- Rudolph Diesel ran his engine on peanut oil
- Henry Ford developed the Model T to run on ethanol
Let’s work together to make the renewable fuels of the future using the inspiration from the past.
Thanks For Your Attention!