EASTERN REGIONAL RESEARCH CENTER

Bioethanol Technology and Future Opportunities

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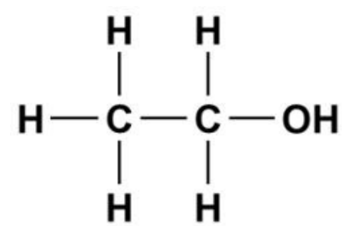
Part 1. Bioethanol from Sugar and Starch Feedstocks

1^{st} 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

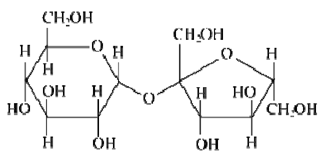


ethanol
Fuel For Clean Air

Source: F.O. Licht

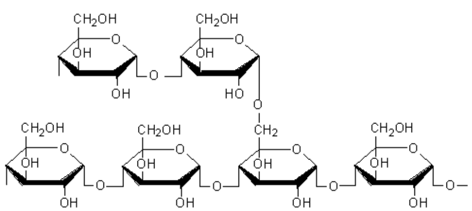
1st Generation Feedstocks for Ethanol

- Substrates for Ethanol Fermentation:
  - Sucrose (Sugar): From Cane or Beet



$$\textbf{Sucrose} \xrightarrow[\text{Invertase}]{} \textbf{Glucose + Fructose}$$

  - Starch: From Corn, Milo, Wheat, Cassava



$$\textbf{Starch} \xrightarrow[\alpha\text{-Amylase}]{} \textbf{Dextrins} \xrightarrow[\text{Glucoamylase}]{} \textbf{Glucose}$$

- Sucrose and Starch are Readily Converted to Simple Sugars that can be Fermented to Ethanol by the Yeast *Saccharomyces cerevisiae*

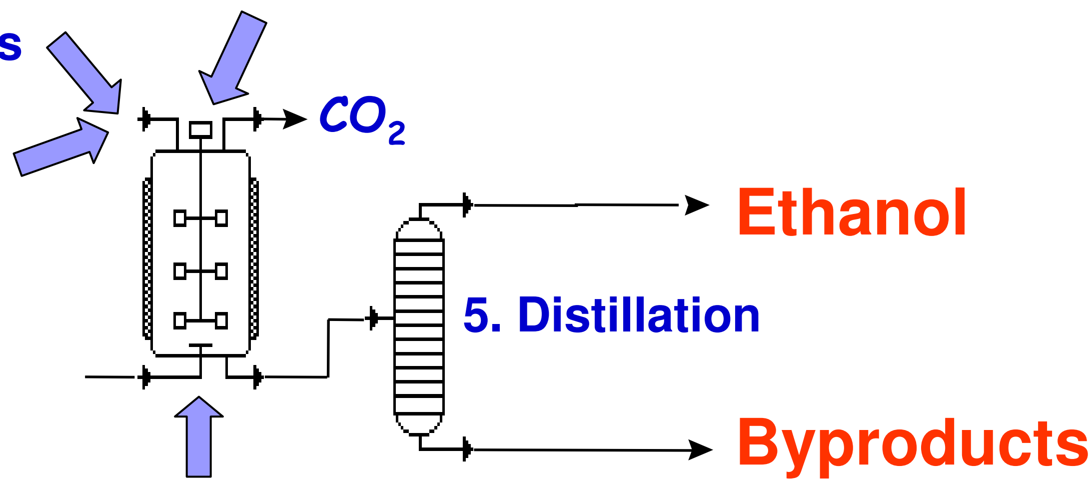
Bioethanol is Simple to Produce from Sugar Feedstocks

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

3. Add Yeast

1. Add sugar or molasses

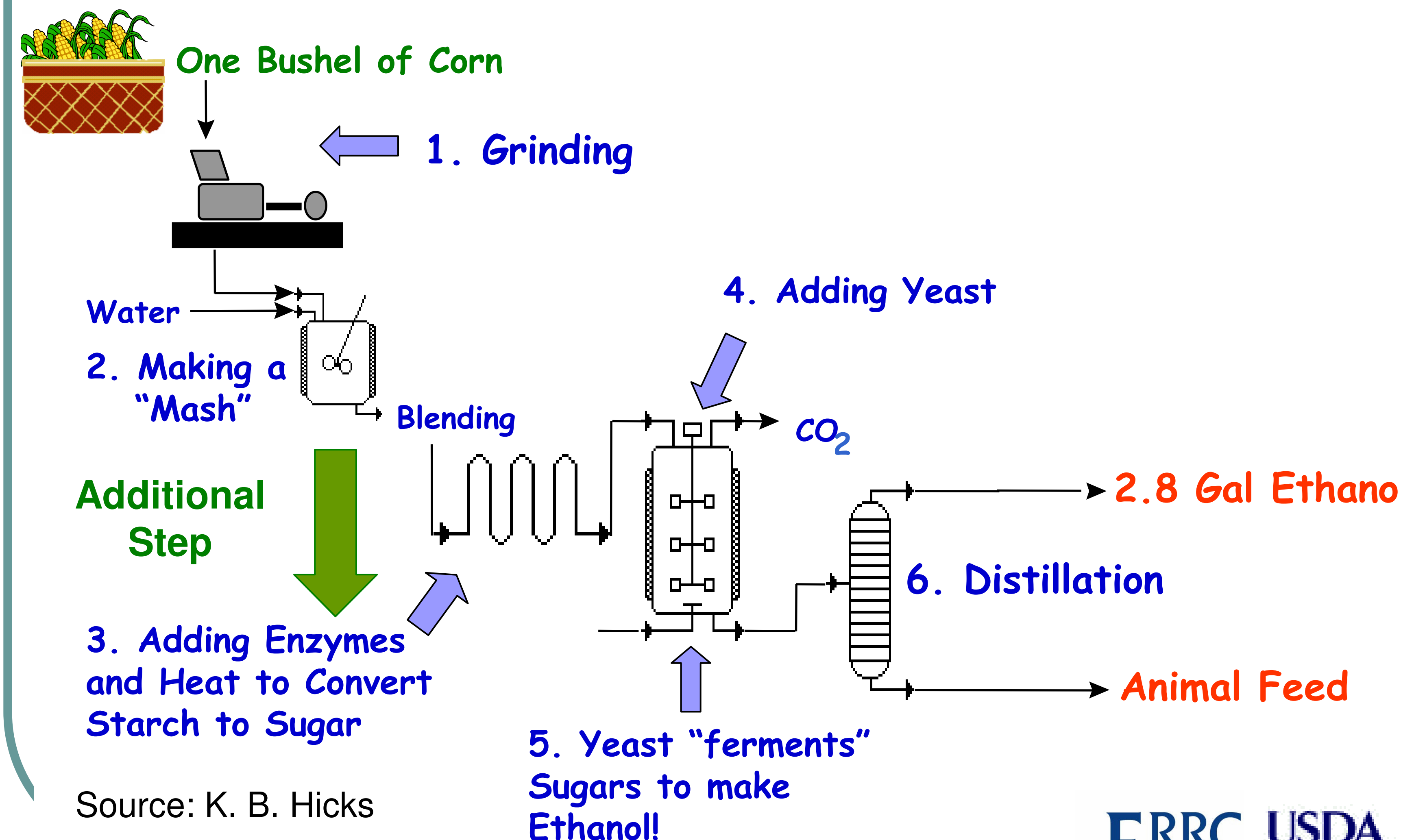
2. Add water and nutrients



4. Yeast ferments sugar to make ethanol

Source: K. B. Hicks

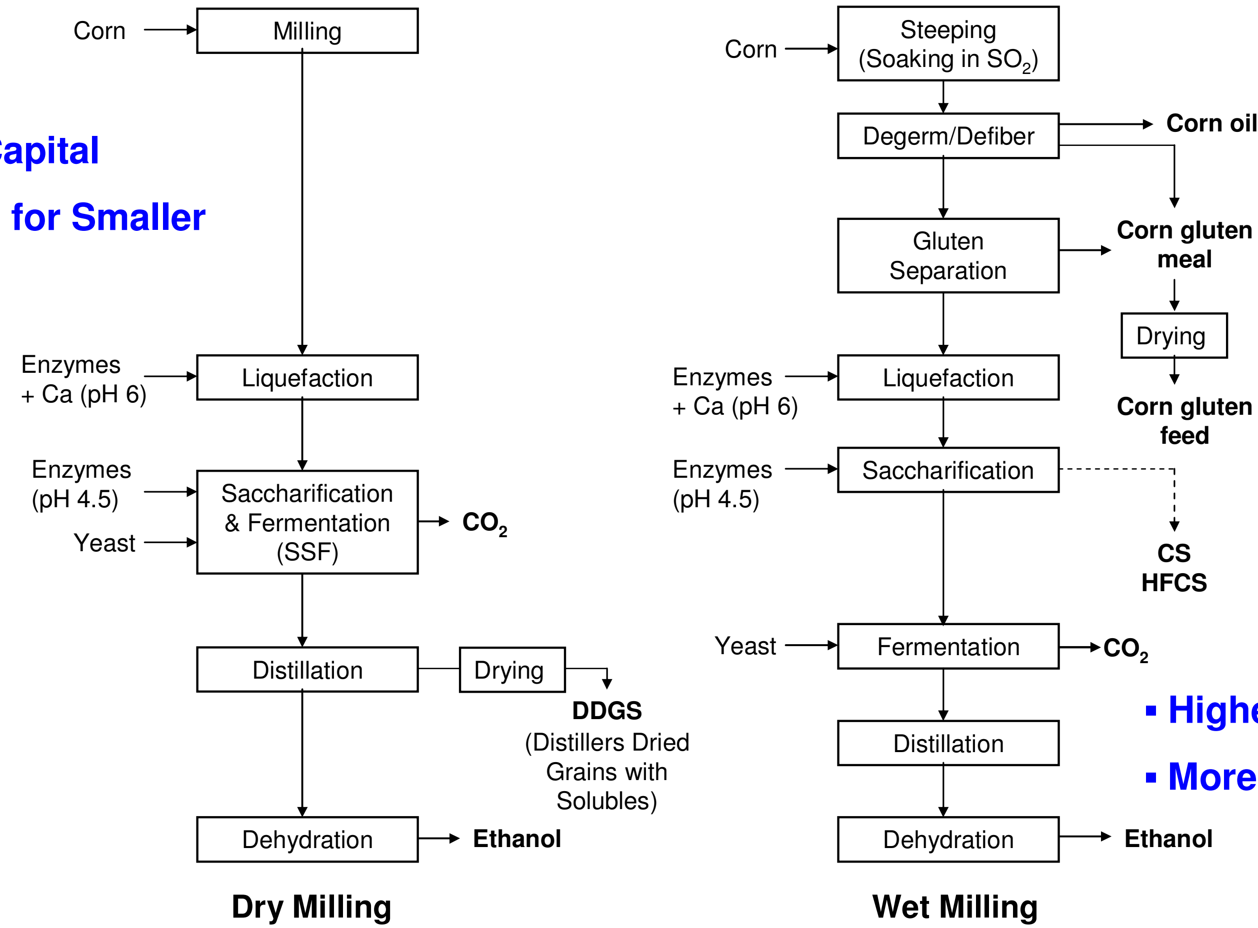
Making Ethanol from Starchy Crops is Slightly More Complex than from Sugar



Source: K. B. Hicks

Ethanol from Corn: Wet and Dry Milling

- **Lower Capital**
- **Suitable for Smaller Plants**



- **Higher Capital**
- **More Co-products**

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 α-amylase corn
    - Enzyme activated with water at 70^{o}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 α-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 α-amylase effective at pH 4.5
      - Only a single pH adjustment needed

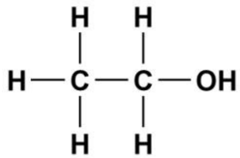
Recent Developments in Starch Ethanol Technology

- 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 SO_2 to obtain starch yield equivalent to a conventional wet-milling process
    - But low levels of SO_2 still are needed for antimicrobial control

Part 2. Bioethanol from Cellulosic Feedstocks

2^{nd} Generation Bioethanol

- "Cellulosic Ethanol"

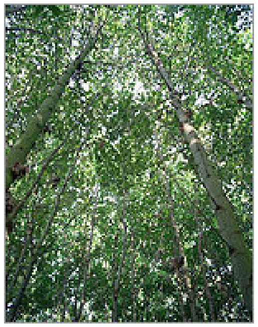


  - Ethanol Made from Lignocellulosic Biomass
    - Forest residues
    - Agricultural residues
      - Corn stover
      - Bagasse
    - Dedicated energy crops
      - Switchgrass
      - Hybrid poplar

Wood Chips

Corn Stover

Switchgrass

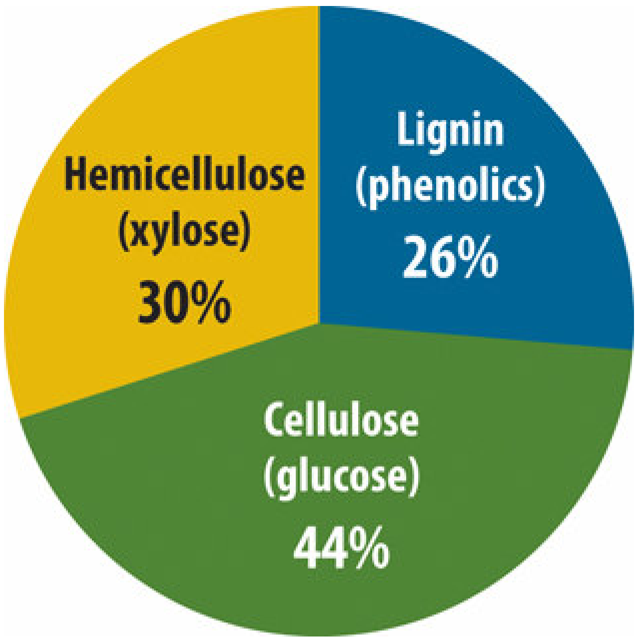


Hybrid Poplar

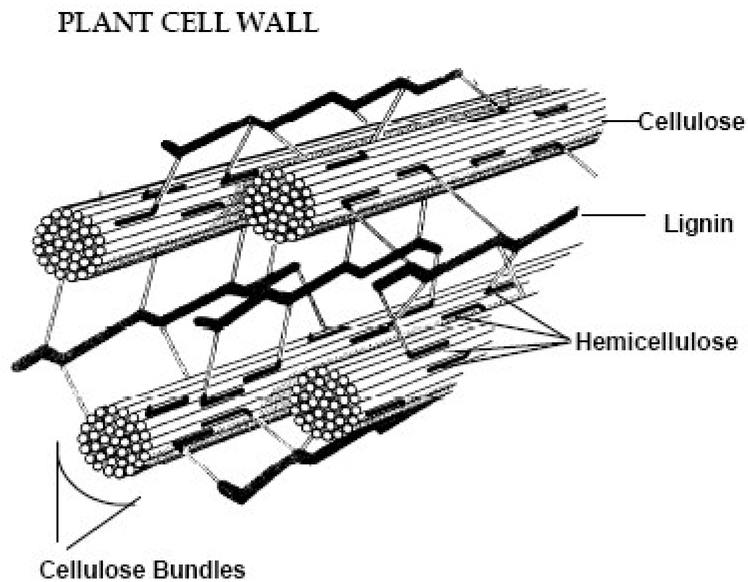
Source: NREL

Lignocellulosic Biomass

- Composition and Structure



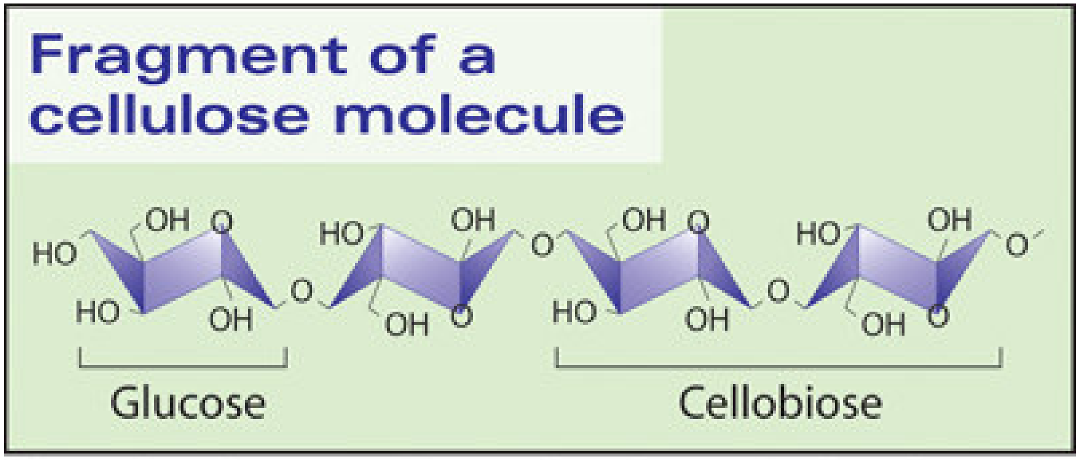
Source: US DOE



Source: C. N. Hamelinck et al., Universiteit Utrecht

Lignocellulosic Biomass

- Cellulose



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

$$\text{Cellulose} \xrightarrow[\text{Celullase}]{} \text{Cellobiose} \xrightarrow[\text{Cellobiase } (\beta\text{-Glucosidase})]{} \text{Glucose}$$

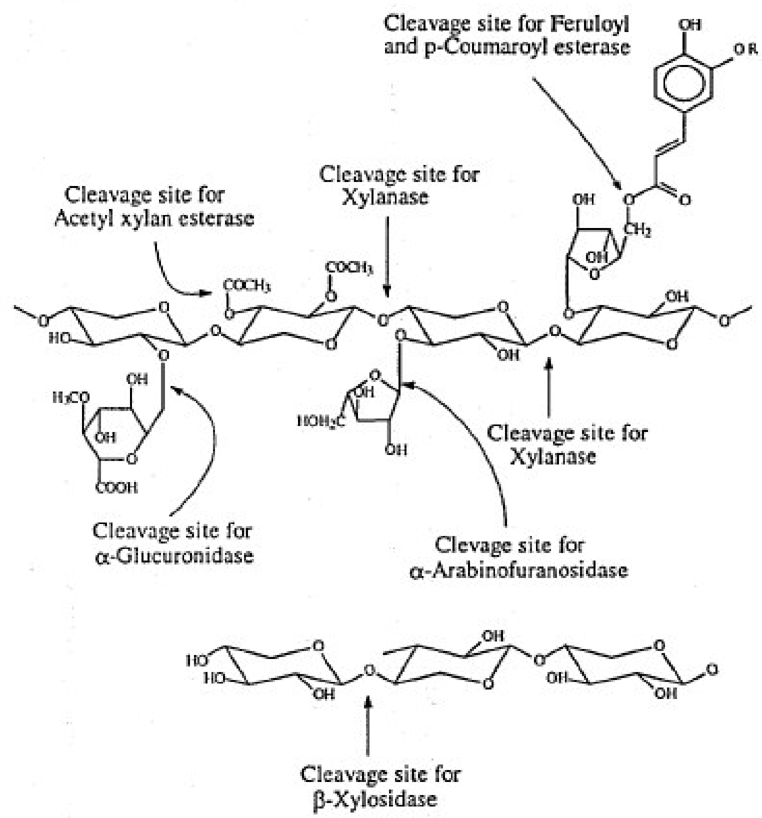
- Most commercial cellulases have some cellobiase activity but not enough
  - Cellobiase must be added

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

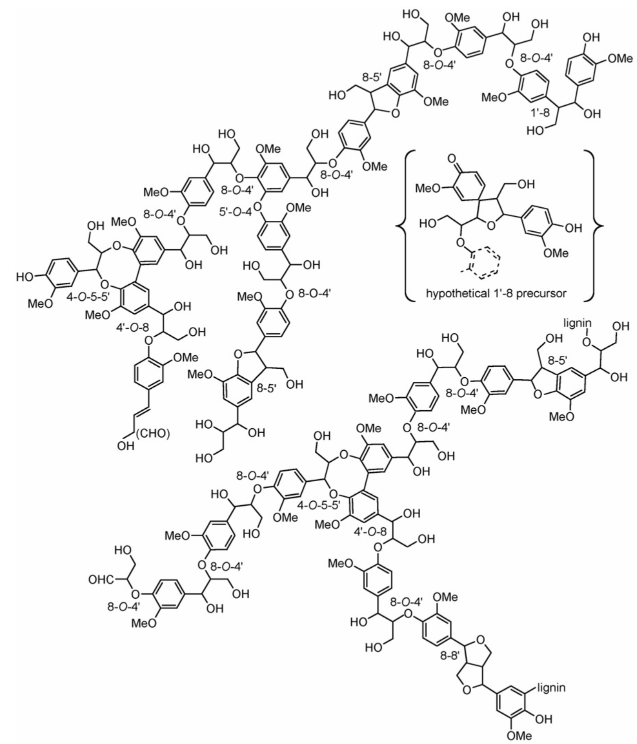


Wheat Straw Hemicellulose Hydrolysis

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

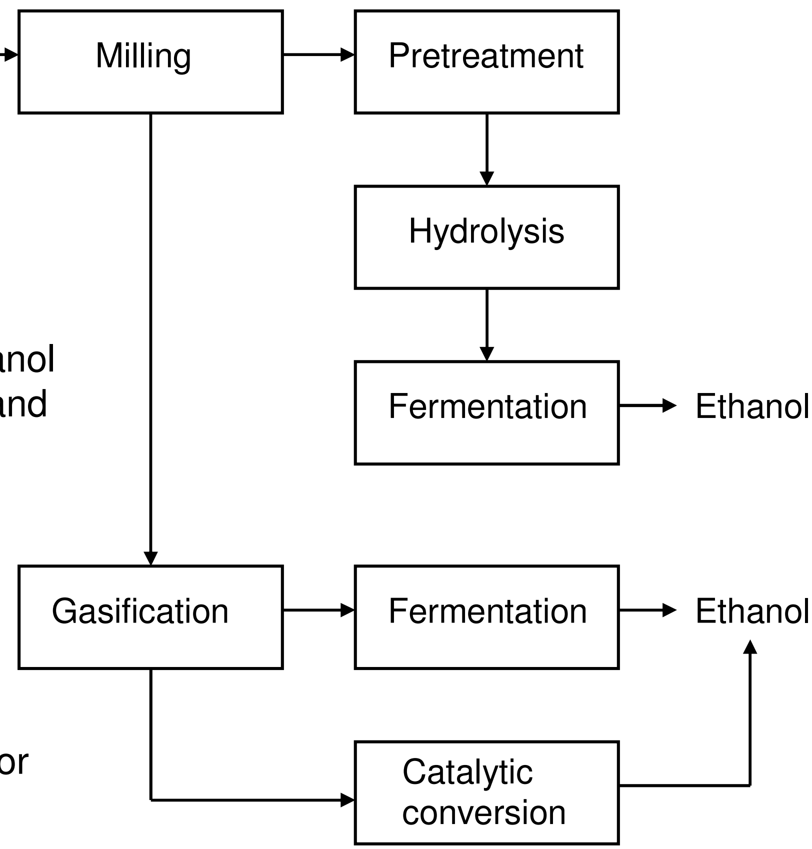


Source: US DOE

Biomass Ethanol Technology

- Basic conversion concept

Lignocellulosic biomass



Sugar Platform

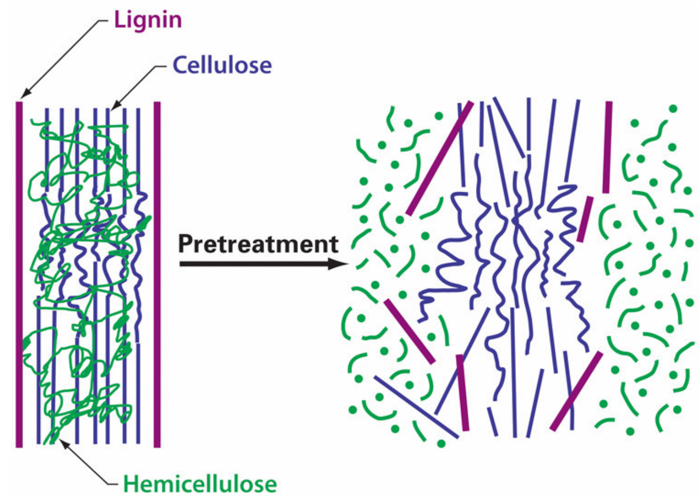
- Biochemical process
- Only carbohydrates go to ethanol
- Lignin is considered a waste and burned for energy

Syngas Platform

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

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

Pretreatment Process	Company
Concentrated Sulfuric Acid (Arkenol)	Blue Fire Ethanol
Dilute Sulfuric Acid (NREL)	
Steam Explosion	Abengoa, Iogen
Soaking in Aqueous Ammonia (Auburn U)	
Ammonia Fiber Explosion (Michigan State U)	
Lime Treatment (Texas A&M U)	
Alkaline Peroxide Treatment	
Wet Oxidation	BioGasol
Organosolv Fractionation	

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	**Company**
Zymomonas mobilis[1] (NREL)	DuPont, Poet
Saccharomyces cerevisiae[1] (Purdue U)	Iogen
Saccharomyces cerevisiae[1] (Delf U)	Nedalco, Mascoma, SunOpta
Escherichia coli[1] (U Florida)	Verenium, BioEthanol Japan
Klebsiella oxytoca[1] (U Florida)	Verenium Corp.
Bacillus stearothermophilus[2]	Colusa Biomass Energy Corp.
Thermoanaerobacter mathranii[2] (Danish Technical U)	Biogasol
Clostridium phytofermentans[3] (U Mass)	SunEthanol

(1) Mesophilic organisms

(2) Thermophilic organisms

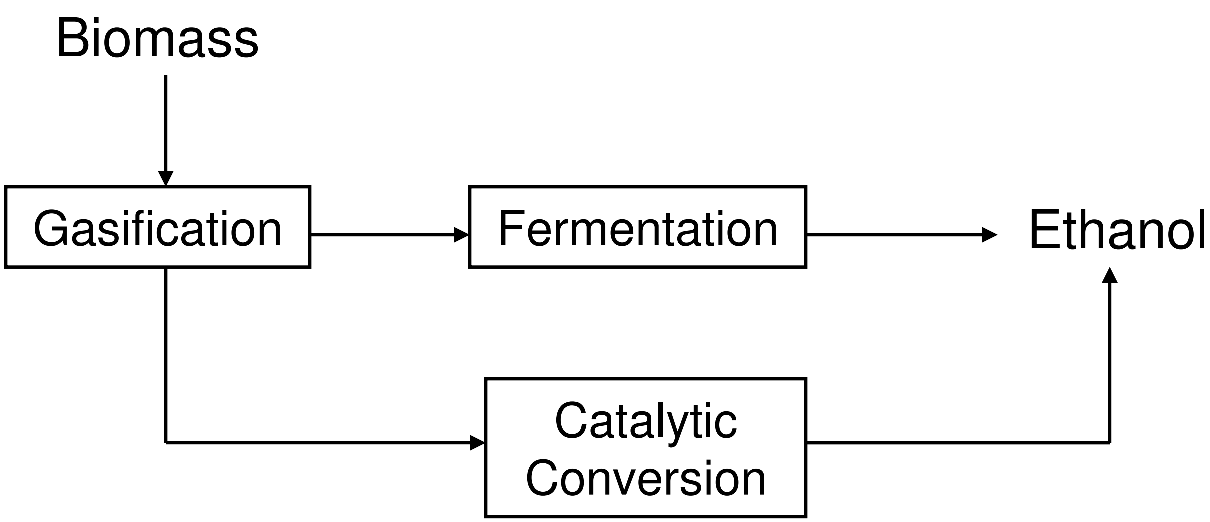
(3) Cellulolytic and xylanolytic organism

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



Major components of syngas are CO, CO_2, and H_2

The Syngas Platform: Current Status

- Several types of commercial gasifiers are available
- Strains of *Clostridium ljungdahlii* 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

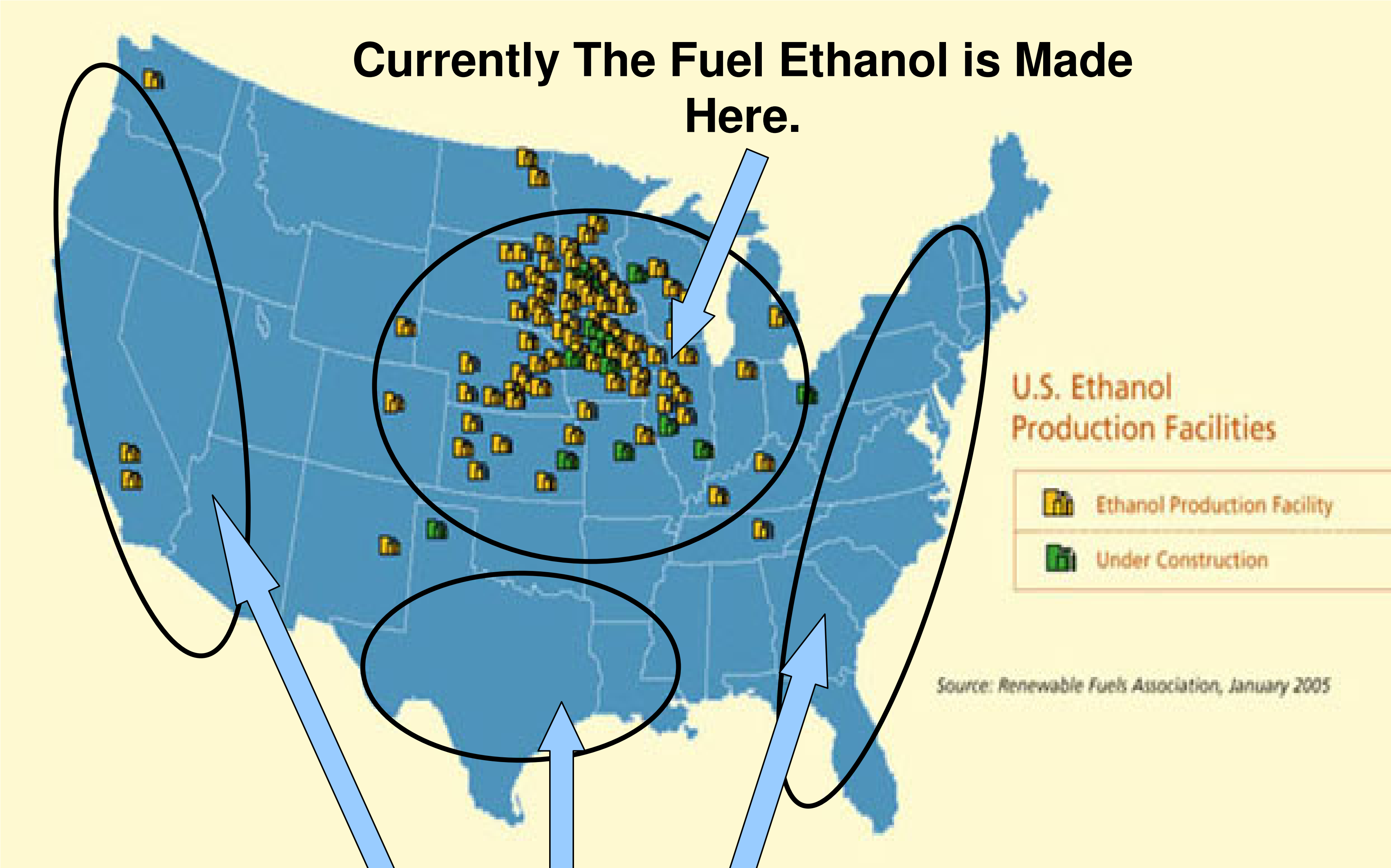
- Microorganisms
  - Robust strains
    - Capable of effective metabolism of cellobiose and high ethanol yields
      - Reduce or eliminate needs for cellobiase
    - Suitable for Consolidated Bioprocessing
      - Direct cellulose conversion
      - Reduce or eliminate needs for cellulase

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

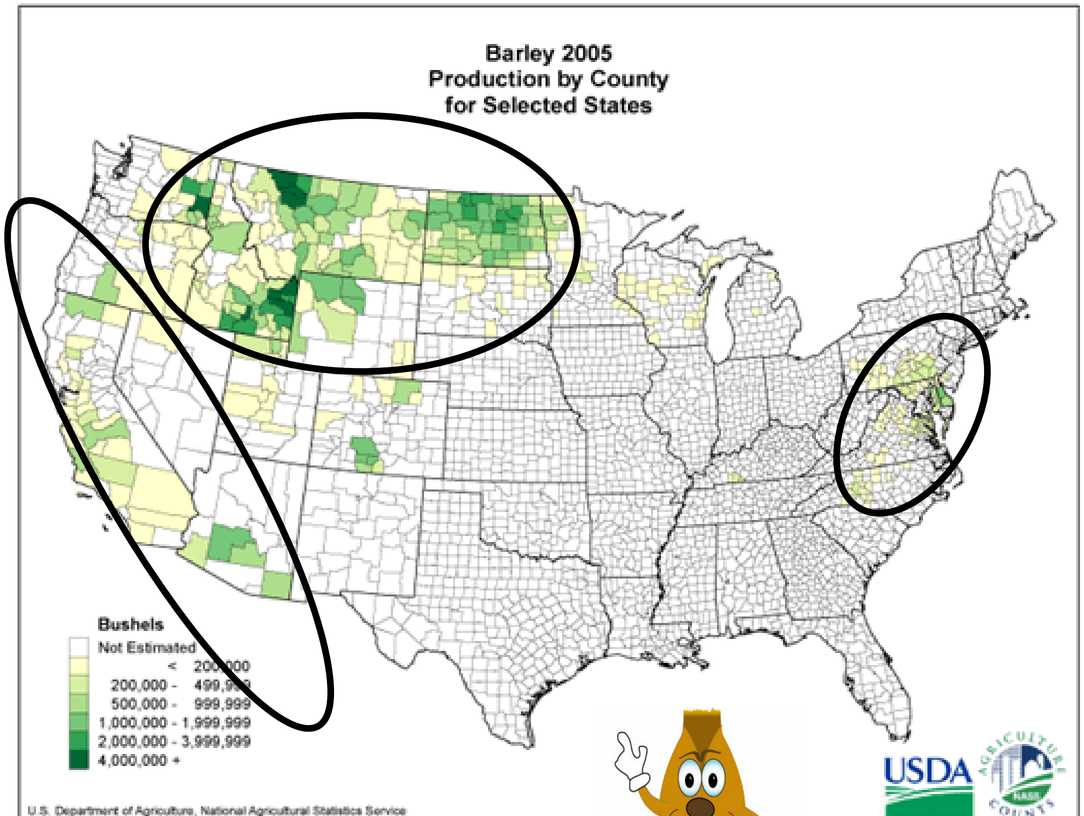
Starch Ethanol R&D Needs

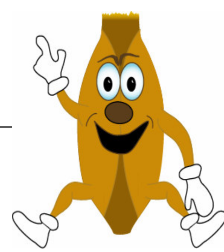
Currently The Fuel Ethanol is Made Here.



But We Have the Major Markets for Fuels Here!!

Barley is A Crop Grown Outside the Corn Belt

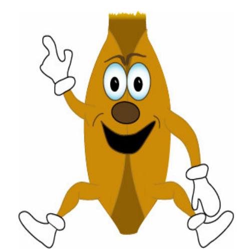




These "barley belts" can provide feedstock for ethanol plants outside the corn belt where transportation fuels are needed!

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 **<u>β-glucans</u>** – 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

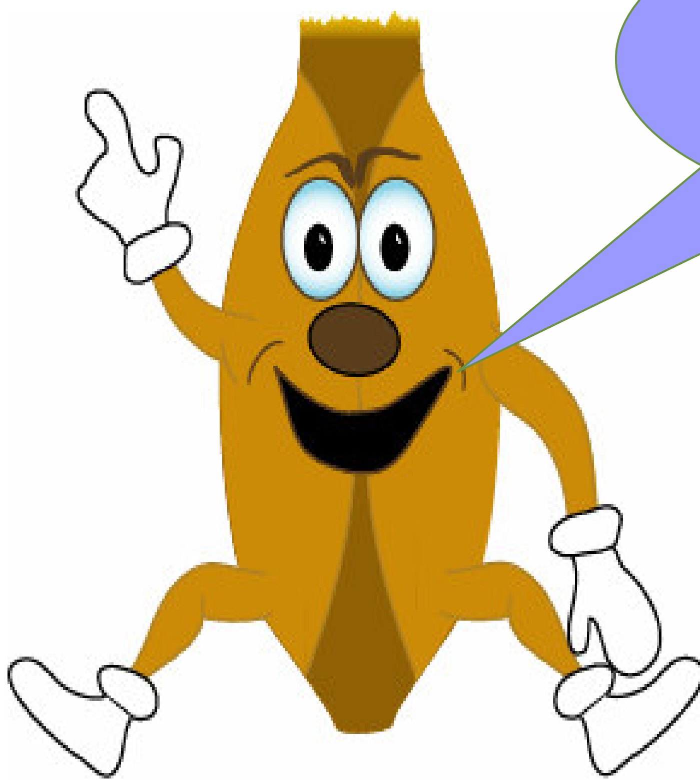
Bottom Line on Barley

- 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!