Biofuels from Cellulosic Feedstocks

BE 247C

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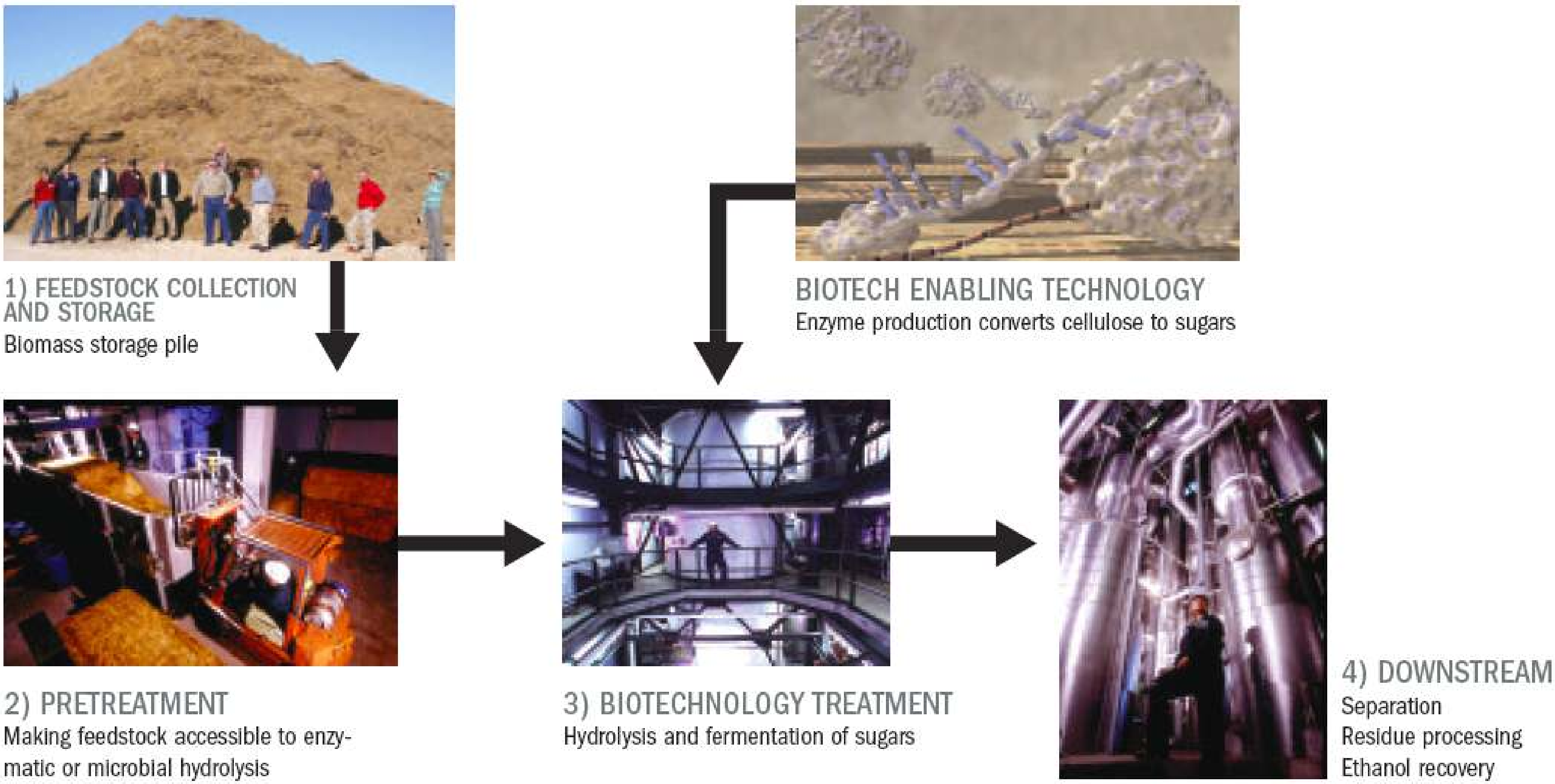
http://www.seco.cpa.state.tx.us/re_biomass-crops.htm

Why Cellulose

- Fossil fuels are nonrenewable
  - Oil=45 yrs; Gas=72 yrs; Coal=252 yrs
  - Carbon emissions
- Cellulose sources are renewable, currently underutilized
- Methods
  - Co-firing (traditional) - burn with coal
  - Gaseous, liquid, solid fuels
    - Thermochemical - heat but not burned
    - Biochemical - bacteria, yeasts, enzymes

http://en.wikipedia.org/wiki/Fossil_fuel

Lifecycle Overview

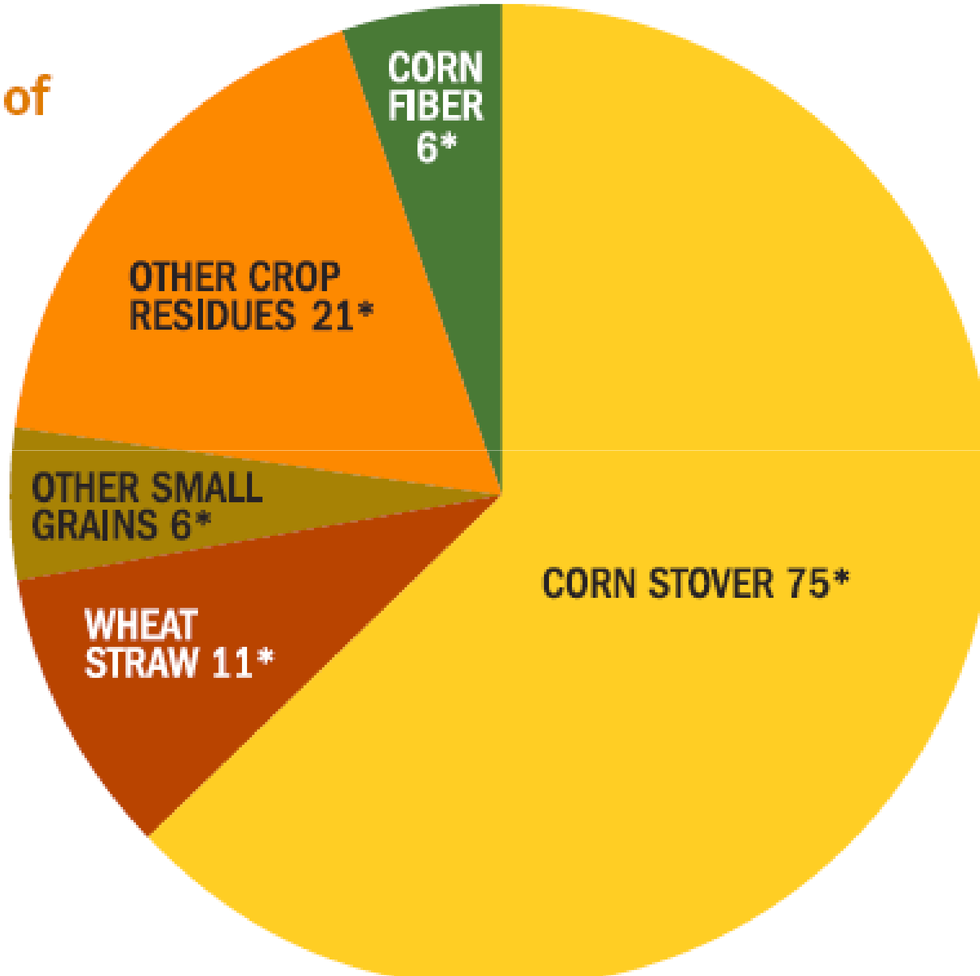


Sources: J. Hettenhaus, Iogen Corporation, National Renewable Energy Laboratory

Hettenhaus 2006

Types of Biomass

Figure 2:
Pie Chart of
Data from
Table 1

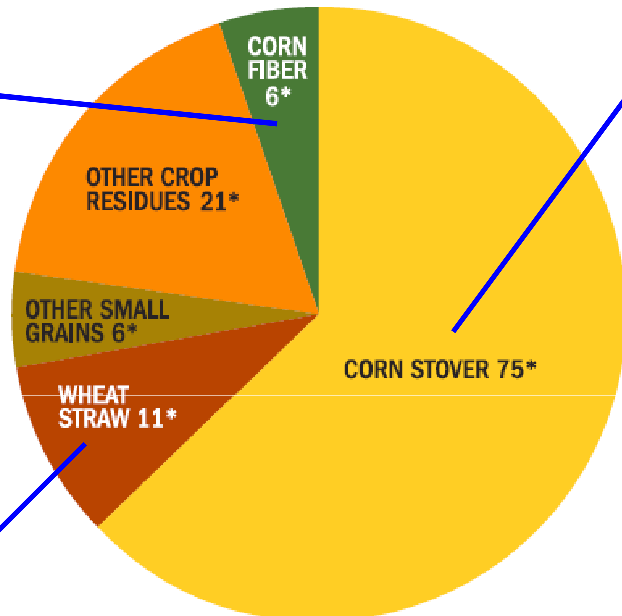


* Figures above represent millions of dry tons per year.

Hettenhaus 2006

Types of Biomass

- Corn fiber
  - Co-product of corn dry mill ethanol operations
  - Already collected
  - Much less lignin than stover



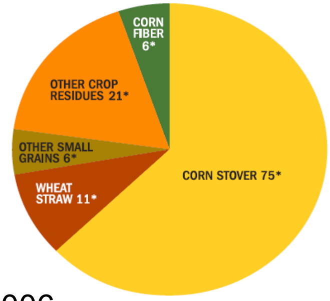
- Corn Stover
  - Stalks, cobs and leaves on ground following corn harvest
  - Collection methods need to be developed
  - 50% moisture, needs to dry before harvest

- Wheat Straw
  - Collection methods well developed

Hettenhaus 2006

Types of Biomass

- Soybean Stubble
  - Leftover from soybean harvest
- Bagasse
  - Remains of sugar cane plants after sucrose extraction
- Process Waste
  - Cotton gin trash, Paper mill sludge



Hettenhaus 2006

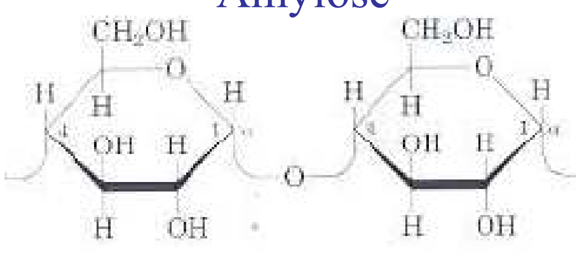
- Energy Crops
  - Grasses
    - Switchgrass, elephant grass
    - harvested for up to 10 yrs b/f replanting
  - Trees
    - Eucalyptus, Hybrid Poplar/Willows
    - Coppicing
      - Grow back after being cut off close to ground
    - Short-rotation woody crops
      - Harvested every 3-8yrs for 20-30yrs b/f replanting
      - Grow 40 feet high b/t harvests

http://www.ucsusa.org/clean_energy/renewable_energy_basics/offmen-how-biomass-energy-works.html

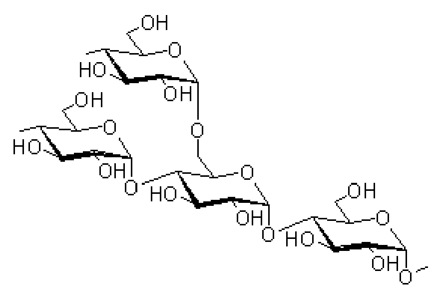
Starch vs. Cellulose

- Starch
  - Storage of fuel monosaccharides
  - 2 types D-glucose polymers
  - Amylose: unbranched chains, ($\alpha 1 \rightarrow 4$) linkage
  - Amylopectin: branch points every 24-30 residues: $\alpha 1 \rightarrow 6$
- Cellulose
  - Structural element in plant walls: fibrous, touch and water insoluble
  - Long unbranced D-glucose
  - Beta Conformation:
    - ($\beta 1 \rightarrow 4$) glycosidic bonds

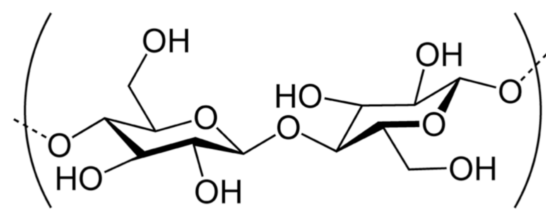
Amylose



Amylopectin



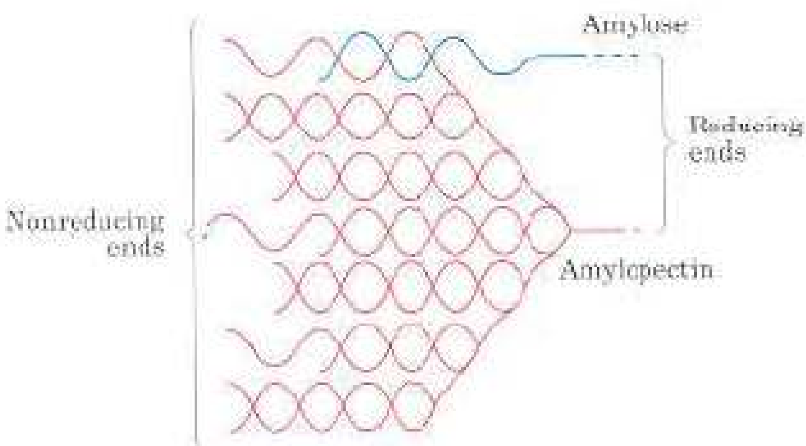
Beta Conformation:Cellulose

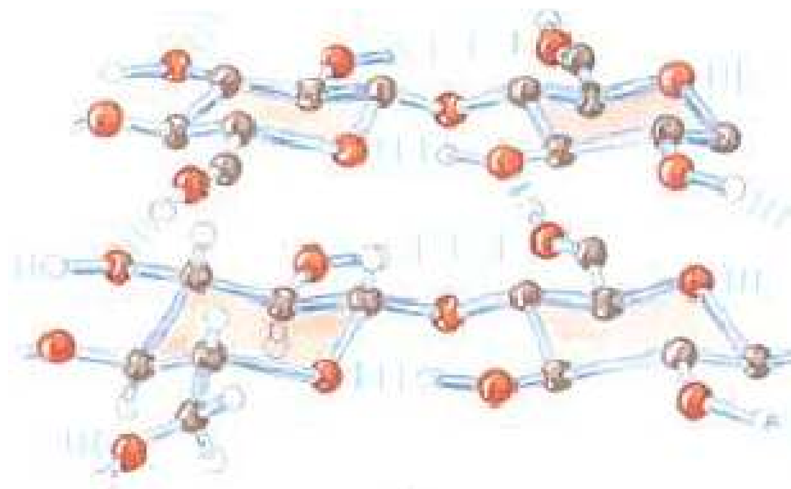


Nelson 2005

Starch vs. Cellulose

- Starch
  - 60 degree stable conformation
  - Helical formation: 6 residues/turn
  - Heavily hydrated
- Cellulose
  - 180 degree stable conformation
  - Straight extended chains
  - H Bonds between neighbors: supramolecular strength
  - Hydrolyzed by **Cellulase**





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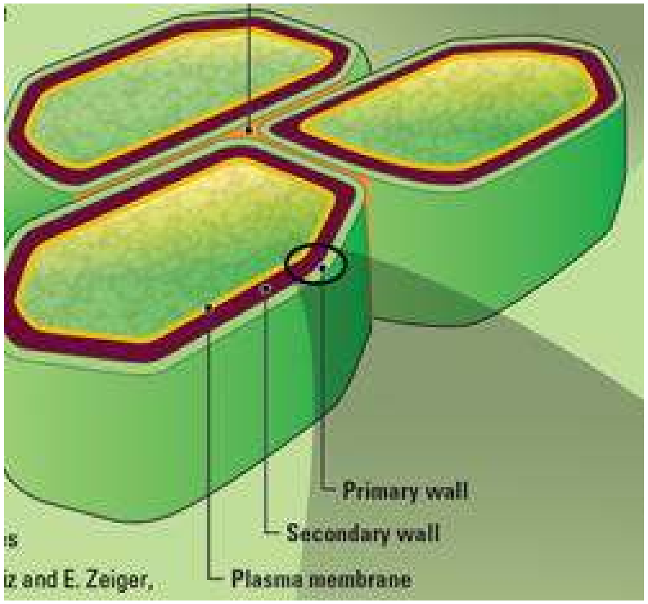
Pretreatment

- Cellulosic biomass: ~40-50% cellulose, ~25-35% hemicellulose, 15-20% lignin
- Lignin believed to be a major hindrance to enzymatic hydrolysis
- Goal: Accessibility to glycosidic bonds to cellulase enzymes $\beta 1 \rightarrow 4$
- Disrupt cell wall physical barriers and cellulose crystallinity and association with lignin



•Improves enzyme digestibility and downstream ethanol production

•Operations cost effects:

1. Biomass size reduction before pretreatment
2. Reduce use of expensive enzymes
3. Downstream costs by determining fermentation toxicity, enzymatic hydrolysis rates, enzyme loadings, mixing power, product concentrations, product purification, waste treatment demands, power generation,

Wyman 2005

Pretreatment Comparison

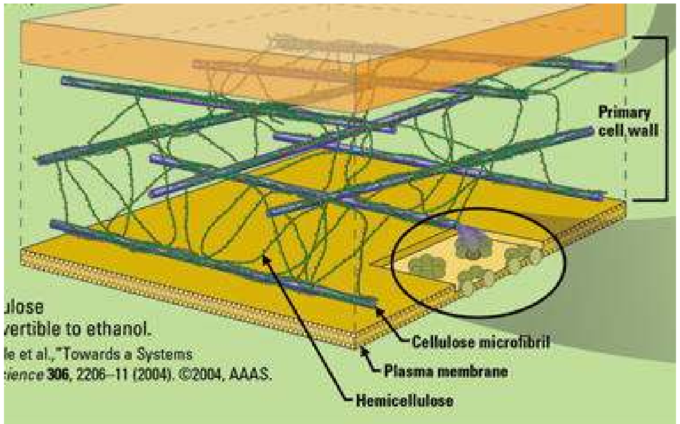
- Dilute Acid 0(~.5-3%.01 sulfuric acid), (~140-190°c)

  -90% hemicellulose -> dissolved sugars,

  -little lignin removal but disrupted

  -downside: costly construction materials,

  high pressures, binding of enzymes to lignin

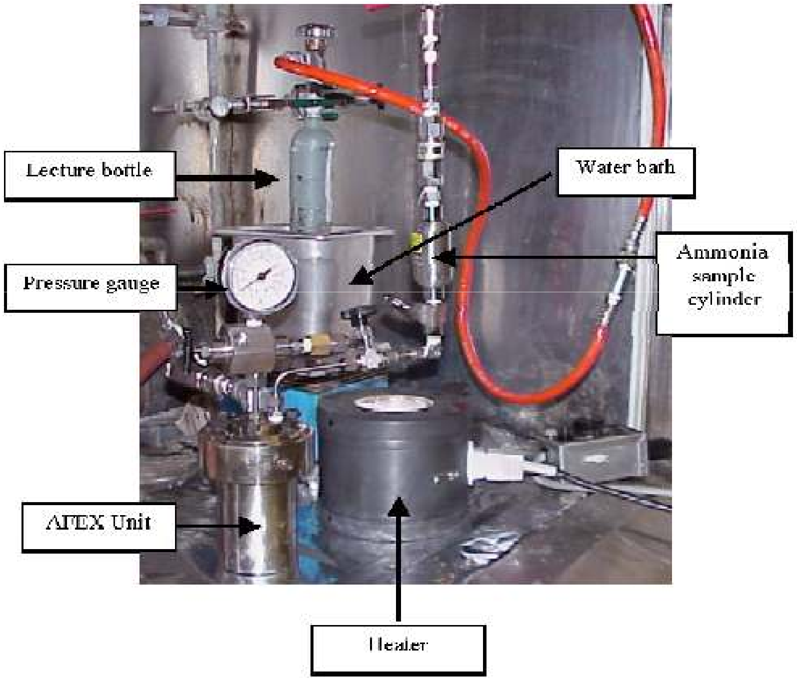


- Autohydrolysis:
  - Less hemicellulose sugar yields
- Flowthrough percolation (~190-200°c):
  - Enhance hemicellulose and lignin removal without acid
  - Difficult to implement commercially, energy expensive

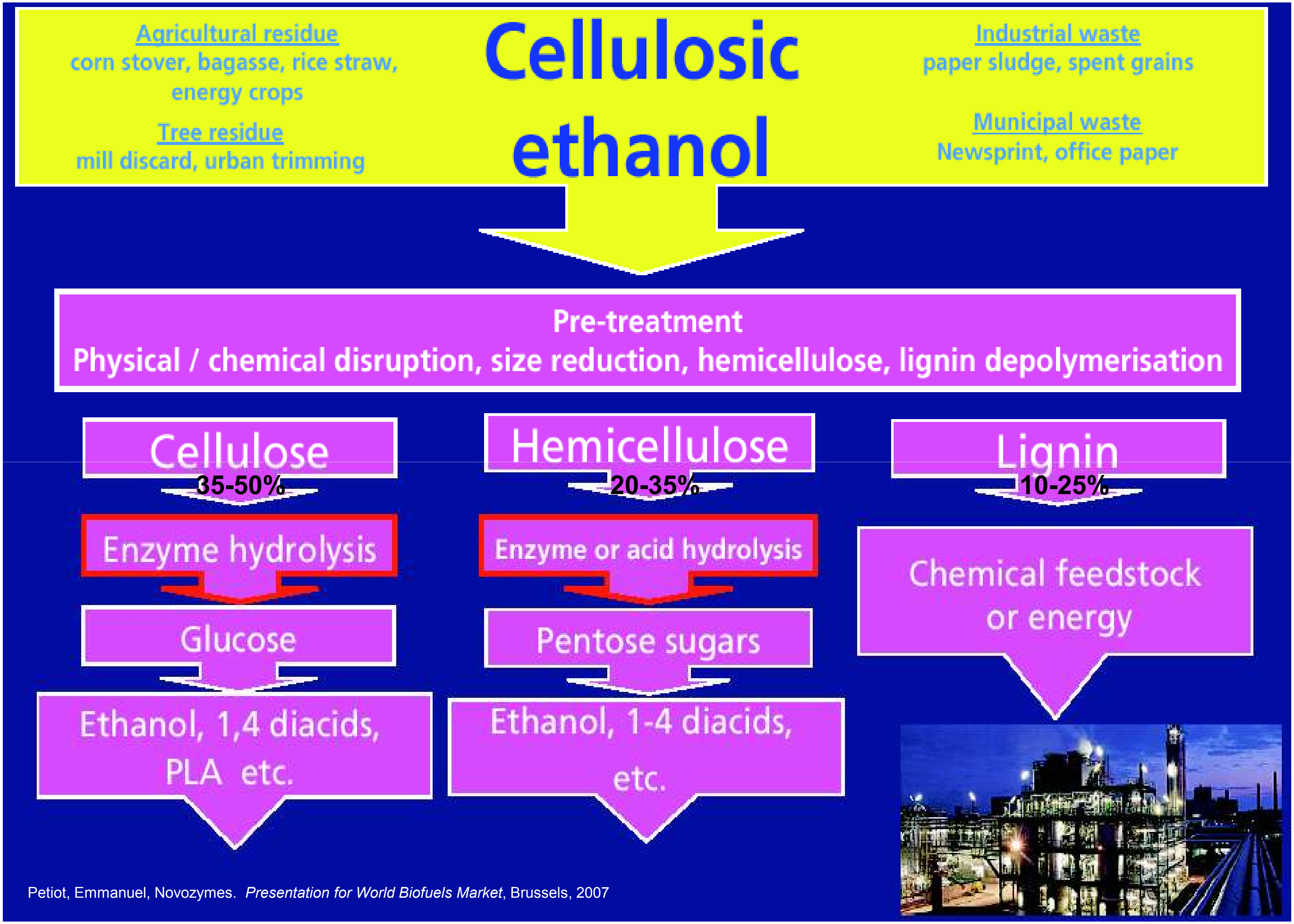
Wyman 2005

Pretreatment Comparison

- AFEX: ammonia fiber explosion (70-90 ° C)
  - Alters lignan structure and depolymerizes hemicellulose to oligomers
  - Cellulose decrystalizes
  - lower cost pressure vessels than dilute acid, high yields at low enzyme loadings
- Lime (25-130°C)
  - Low cost alternative
  - may take hours (130 °C) to weeks (25 °C)
  - removes 33% lignin ~100% acetyl groups
  - switchgrass digestable
- Lime + air
  - O_2 increases lignin removal (~80%)
  - higher "woody materials" like poplar
  - slower than ammonia but low cost and safe handling



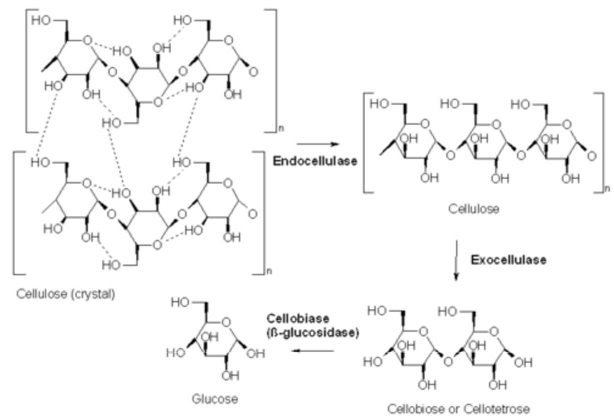
Wyman 2005

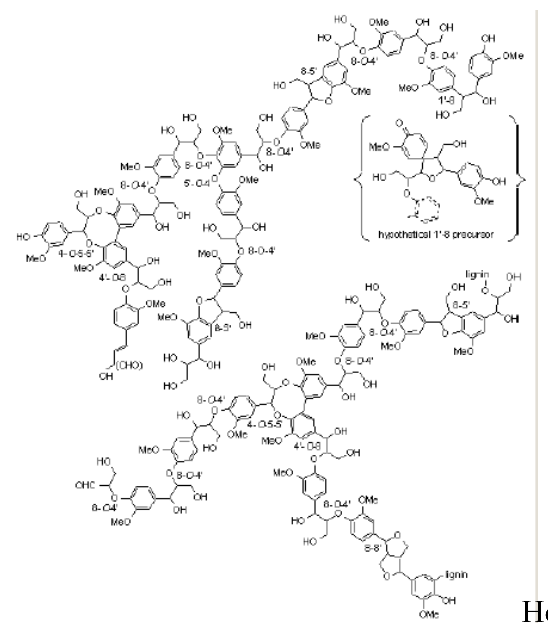


Saha, B. C. 2004. "Lignocellulose Biodegradation and Applications in Biotechnology," pp. 2–34 in *Lignocellulose Biodegradation*, American Chemical Society, Washington, D.C.

Commercial Cellulase Preparations

- Enzymes synthesized by fungi/bacteria
- Soluble enzymes vs. crystalline cellulose
  - Rate-limiting step
- Mixtures of glycosyl hydrolases
  - Endocellulase – breaks internal crystalline bonds
  - Exocellulase – cleaves 2-glucose units from smaller chains
  - Cellobiase – cleaves beta linkage in dissacharide
  - Endo/exoxylanase – hydrolyses ogliosaccharaides

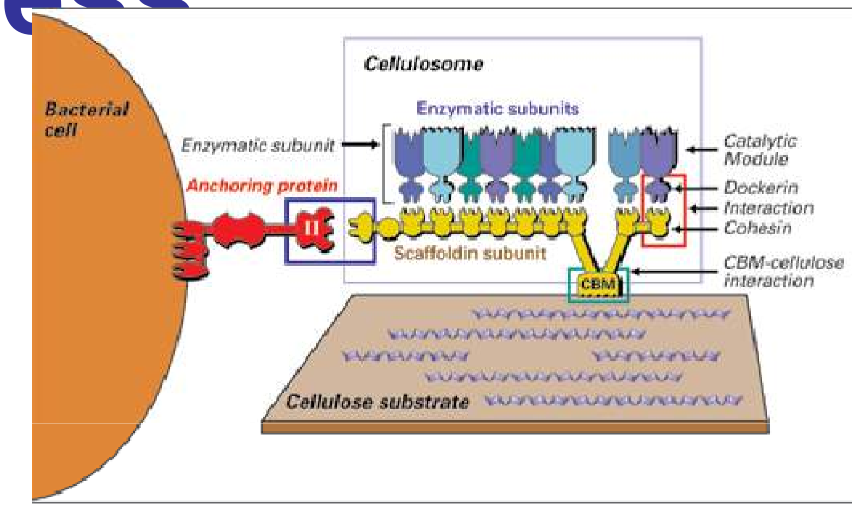


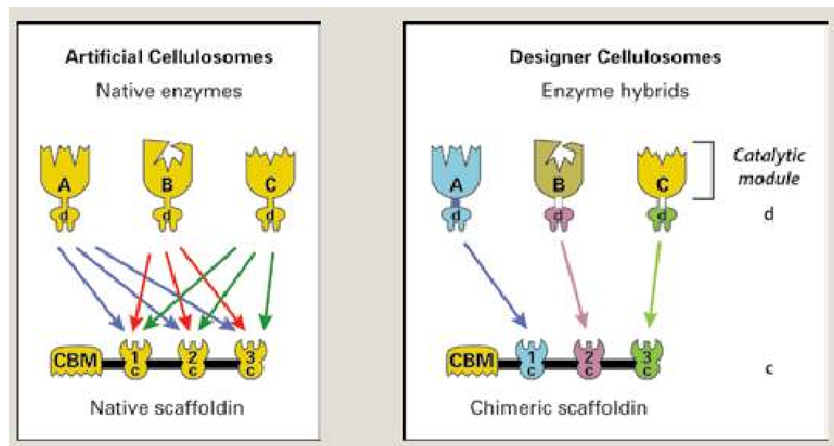


Houghton, John

Improving Cellulase Process

- Reduce cost
  - Endo/exoglucanase + β-glucosidases
  - 30-fold cost reduction (~$3.50 to $0.12/gal)
- Designer cellulosomes
  - Addition of scaffoldins, cohesions, dockerins, etc.
  - Improvement of hybrid enzymes
  - Targeted cellulosomes





DOE National Renewable Energy Laboratory, 2004.

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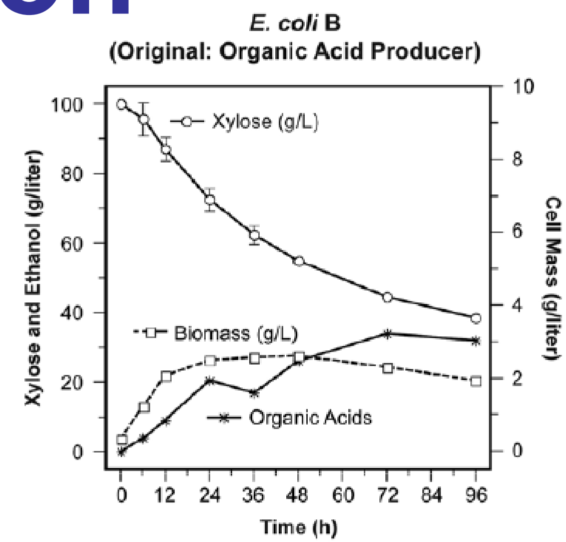
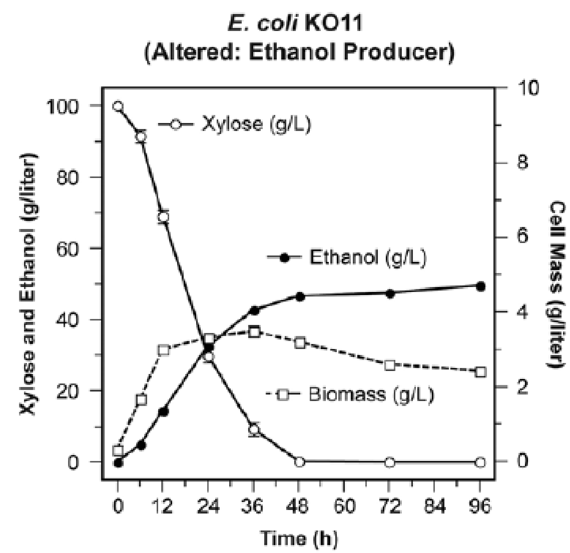
Fermentation

- Most popular sugar-ethanol process
- Yeast (glucose only)
  - High ethanol yield (90%)
  - High titers (10 to 14 wt %)
  - Reasonable rates (~2 g/L/hr)
- Recombinant (yeast, *E. Coli*, *Z. mobilis*)
  - Both glucose & xylose
  - Low natural ethanol yields (6%)
  - Modified to produce higher yields





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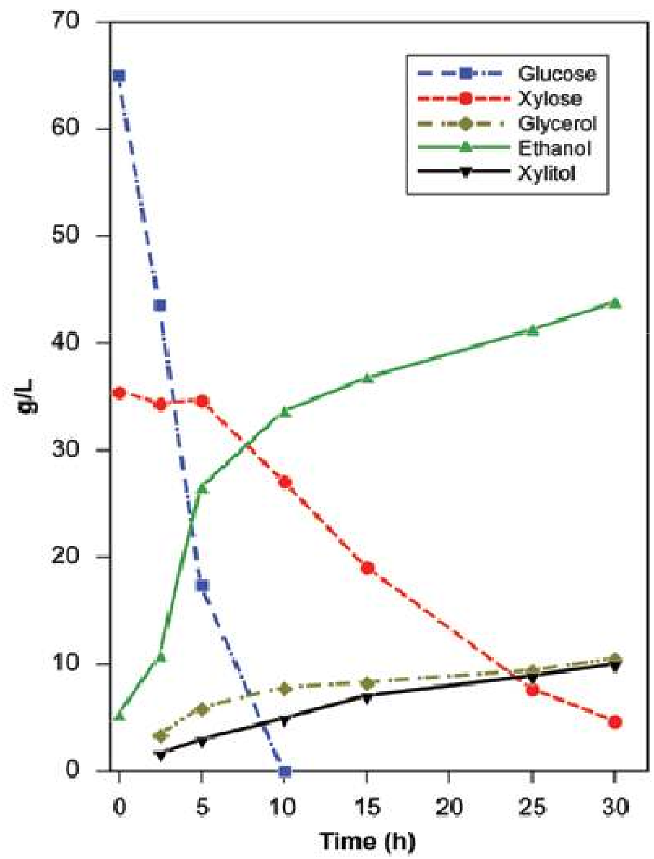
Improving Fermentation

- Criteria
  - High yield (95%)/min biproduct
  - High Ethanol titers (10-15%)
  - High Productivity (2-5 g/L/hr)
  - Minimal media or on actual hydrolysates
  - Utilize both 5 and 6-C sugars
  - Re-use of CO_2 biproduct
  - Low capital and operating cost
  - Engineering of organisms tolerant of high [Ethanol]
- Recombinant Yeast



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Integrated Bioprocessing

- Genetically modified, multifunctional organism
  – Robust host/novel genes
  – Native host (process-related properties)
  – Stable mixed culture
- *Clostridium thermocellum*
  – Anaerobic bacteria
  – Hydrolyses cellulose & ferments glucose -> ethanol
  – Low yield, slow conversion
  – Uses cellulosome

Bayer, E. A. and R. L. Lamed

Considerations

- Farming practices
- Demand vs. supply
- Greenhouse gasses
- Climate
- Transportation
- Land

Farming Practices

- Development of one-pass harvesting
- Transition from conventional → no till
- Carbon models to predetermine residue collection
- Cover crops
  - Soil quality
  - Erosion prevention
- Reduction in nitrogen fertilizer use

Mulch-Till

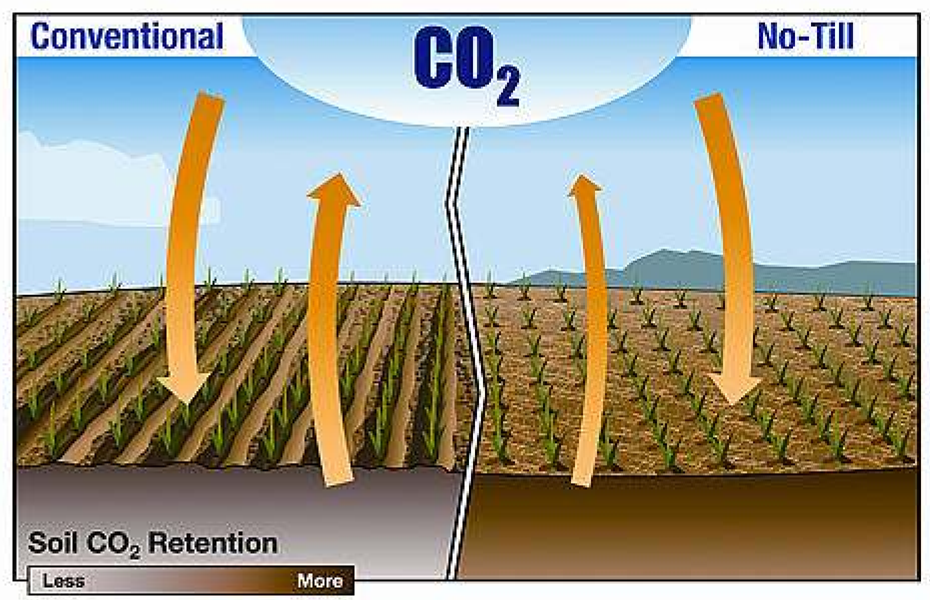
No-Till

Supply and Demand

- 7 billion gallons of ethanol in 2007
- Goal: 60 billion by 2030 (30% replacement of petroleum)
- Currently: 65 gallons ethanol per dry ton
- In order to meet goals:
  - 428 million dry tons form crop residues
  - 377 million dry tons from energy crops

Greenhouse Gases

- Net Carbon Cycle
  - No till reduces rate at which carbon is removed from the atmosphere
  - Outweighed by reduction in fossil carbon emissions gained by using biomass vs. fossil-based feedstocks
- Potential to sell carbon credits
- Combined greenhouse gas benefits would offset the growth in US emissions in 2004



Climate

- Residue collection could be limited in dry seasons
  - Limited Growth
  - Soil moisture retention
- Extremely wet seasons prohibit collection

Transportation & Land Considerations

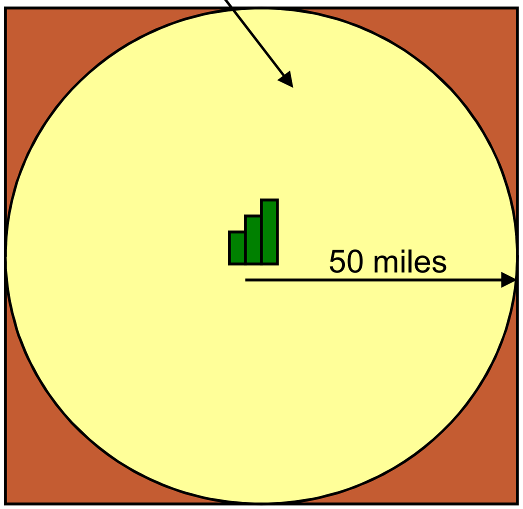
Need railroads to supply stover to within 50 mile radius of the biorefinery

100 MGPY EtOH per refinery per MM tons biomass → 8,000 square miles

Table 1: Current Sustainable Availability of Cellulosic Biomass from Agricultural Lands

Source	Currently available biomass (million dry tons per year)
Corn stover	75
Wheat straw	11
Other small grains	6
Other crop residues (oil seeds, soybeans, sugar crops, root crops)	21
Corn fiber	6

Source: Perlack, Wright, et al., 2005.



~ 600,000 sq. miles needed for the 75 refineries for 30% gasoline replacement (could be reduced 10x w/ better collection)

Equivalent land area equal to all of CO, WY, KS, NB, OK, IL, IA, AK

Energy Balances (perspective #1)

Some Basic Facts…

1. EtOH = 85,000 BTU/gallon vs. 125,000 BTU/gallon for gasoline
2. US uses 400,000,000 gallons of gasoline per day

Basis: 1000L Ethanol	Corn	Switchgrass	Wood	Soybeans	Sunflower
Energy Input (Btu)	6,597	7,455	8,061	11,878	19,599
Energy Output (Btu)	5,130	5,130	5,130	9,000	9,000
Energy Yield	-29 %	-45%	-57%	-27%	-118%

Source: D. Pimentel & Tad W. Patzek   U.C. Berkeley & Cornell

3. Distillation takes a lot of energy to go from 8% ethanol to 99.5% ethanol
4. Steam and electricity account for ~50% of that energetic separation procedure
5. Note that we haven't cited the energy yield for stover, but it would be < corn

Energy Balances (perspective #2)

1. USDA and some government labs say that ethanol energy yield is about +30%

2. Assumptions and energy requirements built into models vary greatly

Reference	Year	Feed stock	Region/ country	Energy output/input
Marland and Turhollow	1991	Corn	U.S.	1.14
				1.28
Pimentel	1991	Corn	U.S.	0.58
				0.69
Keeney and DeLuca	1992	Corn	U.S.	0.83
				0.92
Morris and Ahmed	1992	Corn	U.S.	1.01
				1.51
Lorenz and Morris	1995	Corn	U.S.	1.04
				1.33
Shapouri et al.	1995	Corn	U.S.	1.01
				1.24
Venendaal et al.	1997	Winter wheat	Germany	1.1-1.7
				4.0-5-0
	1997	Winter wheat	Belgium	1.1-5.9
	1997	Winter wheat	France	1.3
Macedo	1998	Sugarcane	Brazil	9.2
McLaughlin et al.	1998	Corn	U.S.	1.21
		Switchgrass	U.S.	4.43
Bernesson	2004	Winter wheat	Sweden	1.1-1.13
Börjesson	2004	Winter wheat	Sweden	1.31
				2.05
Punter et al.	2004	Winter wheat	U.K.	0.68-2.22
Pimentel and Patzek	2005	Switchgrass	U.S.	0.69
		Wood cellulose	U.S.	0.64
Nielsen et al.	2005	Corn	U.S.	1.9

Assumptions make a big difference!

Lots of variability

Need better ways to (1) separate EtOH from water and (2) use enzymes that can perform catalysis at ambient temperatures

Source: N. Bentsen, C. Felby, K. Ipsen Energy Balance of 2nd Generation Bioethanol Production in Denmark

Where does that leave us?

- Genetically engineer to reduce pretreatment
  - Low lignin trees/plants
    - DNA vector with anti-sense genes to limit enzymes in lignin biosynthesis pathway
    - Ethanol yield comparison:
      - Corn: 400gal EtOH/acre yr
      - Regular Hybrid Poplar: 700gal EtOH/acre yr
      - Low Lignin Poplar: 1000gal EtOH/acre yr
    - Cons: reduced defense against insects, fungi, bacteria
- Enzymes
  - Combine all the steps into one organism (or two coexisting organisms)
    - Design cellulase superstructures to match structure of specific biomass

http://www.purdue.edu/UNS/html4ever/2006/060823.Chapple.poplar.html

Conclusions

- Energy Balance can be region dependent
- Many steps would need to be improved
- Research has linearly progressed
- Data presented is for 30% replacement of petroleum vs. 100%, numbers astronomical

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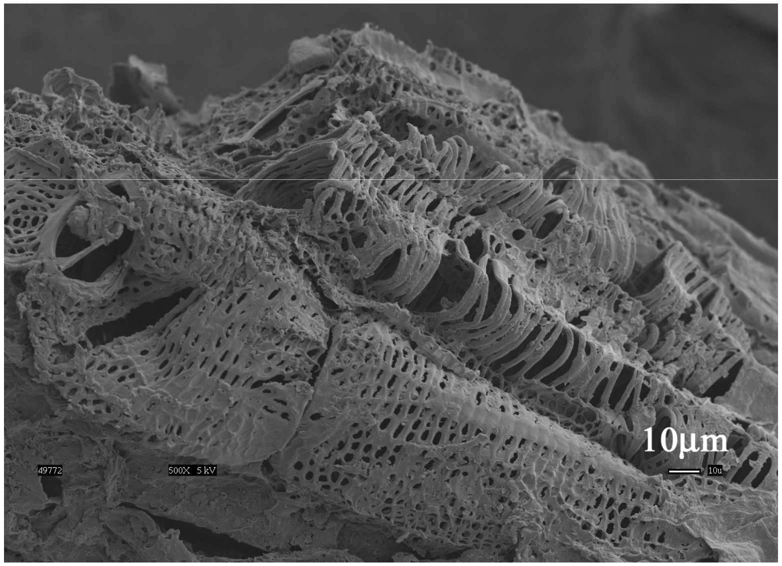
- Amylopectin. http://en.wikipedia.org/wiki/Amylopectin

Novel Solutions - pretreatment

- Pretreating corn plant tissue with hot water
  - Exposes pores in cell wall
  - Increases surface area for enzyme reactions
  - 3x to 4x ethanol yield



Magnified image of cornstalk particle

Zeng, Meijuan *et al.*

Possible Solutions

Research into ligninase enzymes. Mechanism not well understood right now, although it is known to be an oxygenase, requiring hydrogen peroxide. Ligninase from (source listed in bibliography) are shear sensitive and the enzyme actually loses activity when agitated in a tank of flask.

Look at Fuelzyme from a company called Diversa. They made a super alpha amylase by using extremophiles. Maybe they can do the same sort of thing for ligninase?

Find a way to separate ethanol from water without having to use distillation

Find a way to get the enzymes to perform catalysis at ambient temperature. The steam and electricity costs are what holds this back the most.

Energy Crops & Feedstock Options

Corn stover and cereal straw (wheat and rice)

- 80% of currently available residues
- 50% of corn biomass left in field (about 250 million tons) = 16 BGPY EtOH
- stover collection not well developed unlike wheat and rice

Soybean Stubble

- might be irrelevant if farmers adopt corn stover

Sugar Cane

- limited supply, sucrose has already been extracted

Switchgrass

- yields of 8 tons per acre been demonstrated

Sunflower & Soybeans

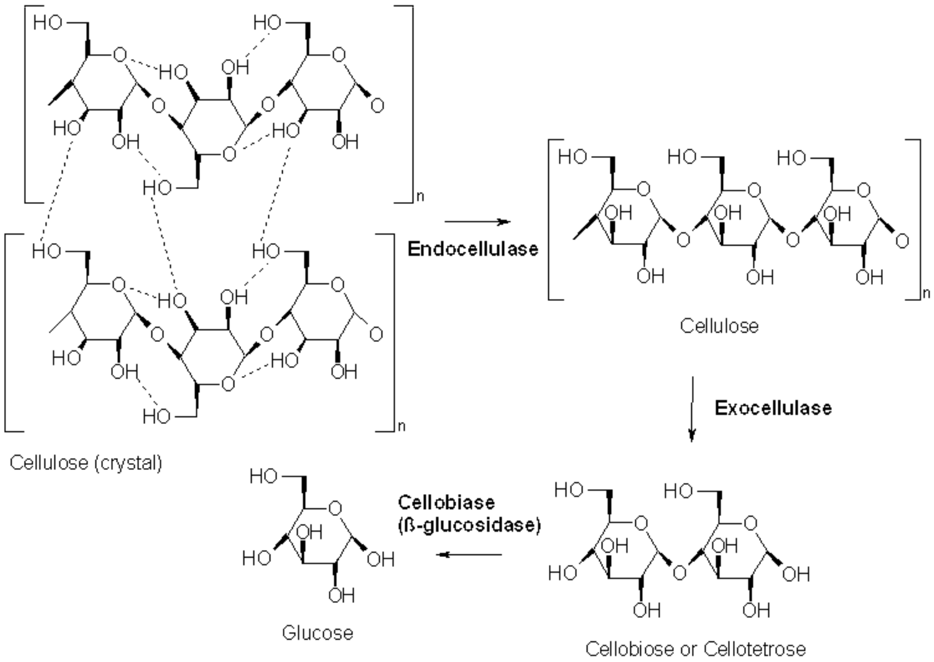
- biodiesel

Source: 2006 Biotechnology Organization

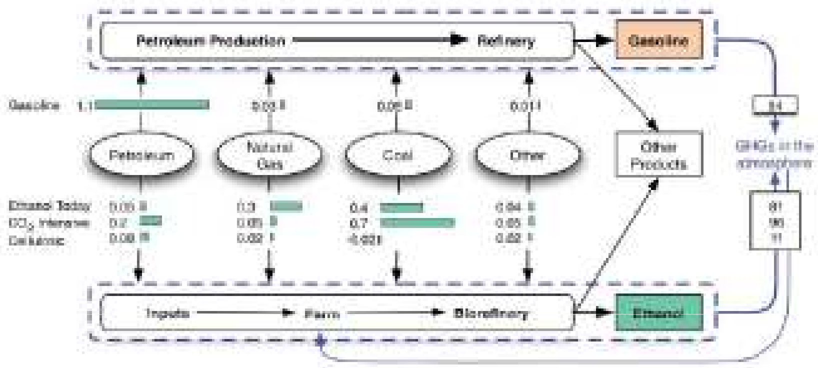
Stover consists of stalks, cobs and leaves usually left on the ground following harvest. Special equipment needed to collect this stover since it is currently not used.

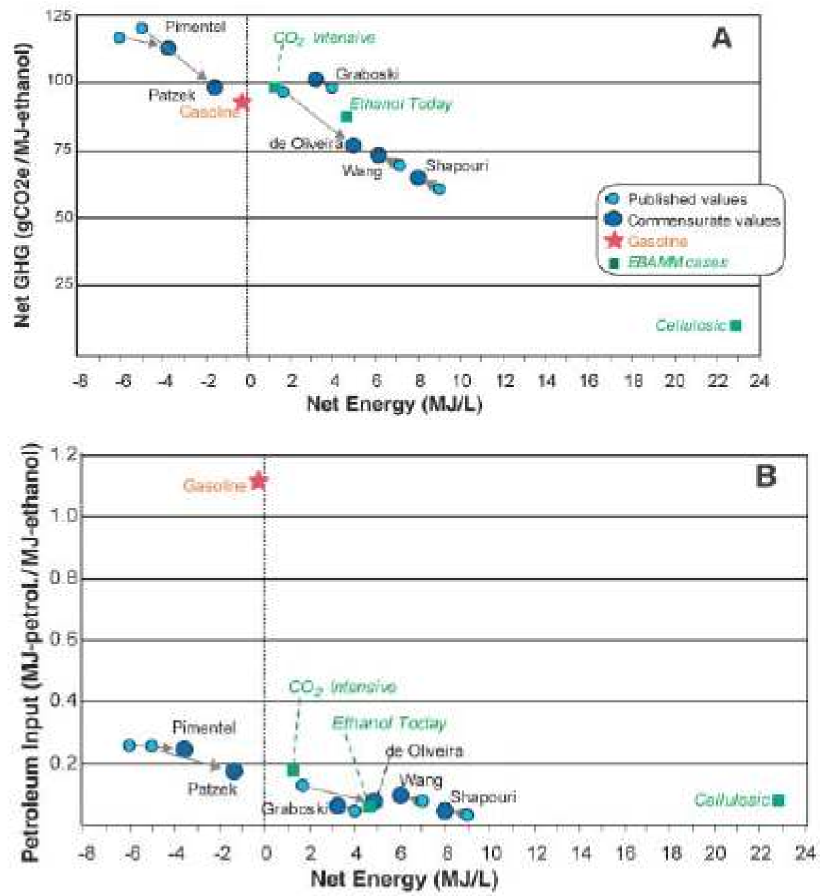
Cellulase

- Endocellulase
  - Disrupt structure
- Exocellulase
  - Cleaves 2-4 units from end
- Cellobiase or beta-glucosidase:
  - hydrolyses the endo-cellulase product
- Oxidative cellulases
  - Depolymerize by radical reactions
- Cellulose phosphorylases
  - Depolymerize using phosphates



Energy balance slide (extra)





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Science **311,** 506 (2006);