Ethanol production

MCB 113

Ethanol production

- Originated 5000 years ago with the manufacture of alcoholic beverages
- Wine was often more pure than water
- Produced by fermentation of sugars
- In addition to its use in beverages, ethanol is used for a fuel and for industrial purposes (solvent, additive, chemical feedstock)

Ethanol as a fuel

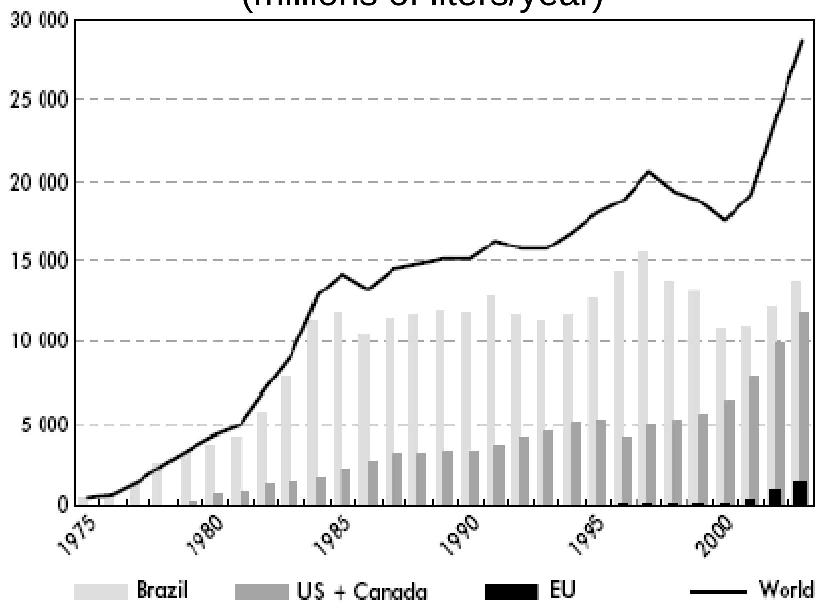
- Lead-free fuel
- Its combustion produces lower amount of NOx and CO than regular gasoline.
- No added CO_2
- Ethanol was used to fuel cars in the 1920’s and 1930’s
- After WWII, petroleum fuels became available in large quantities
- In the late 1970’s, interest in alcohol as a fuel renewed due to the energy crisis.

Global ethanol production

- World ethanol production (all categories, fuel, industrial and beverage) was estimated to be 33.3 billion liters in 1998, up from 28.7 billion liters in 1993.
- The ethanol demand: fuel, 68%; industrial use, 21%; and beverages, 11% .
- Production: 9% is produced synthetically, whereas fermentation is responsible for the remaining 91%.
- The production by continents were (in billion liters) America: 22.3, Asia: 5.7, Europe: 4.6, Africa: 0.5, Oceania: 0.2 in 1998.

World Ethanol Production

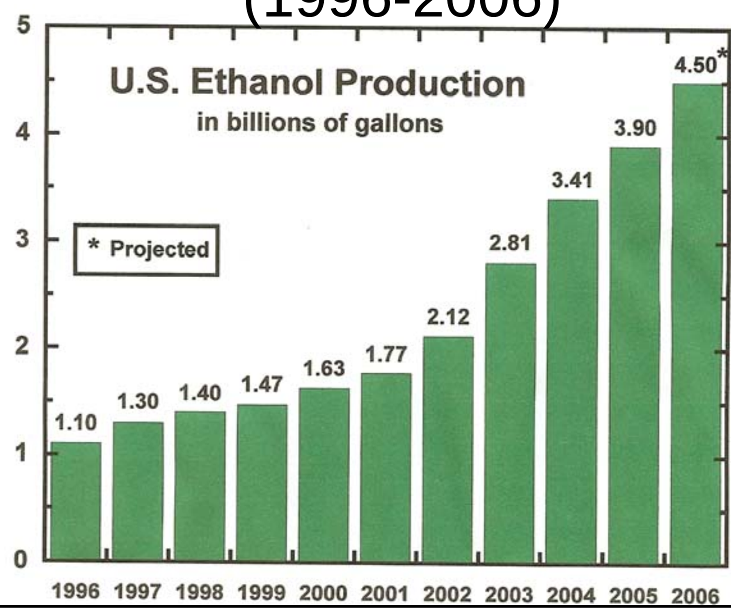
(millions of liters/year)



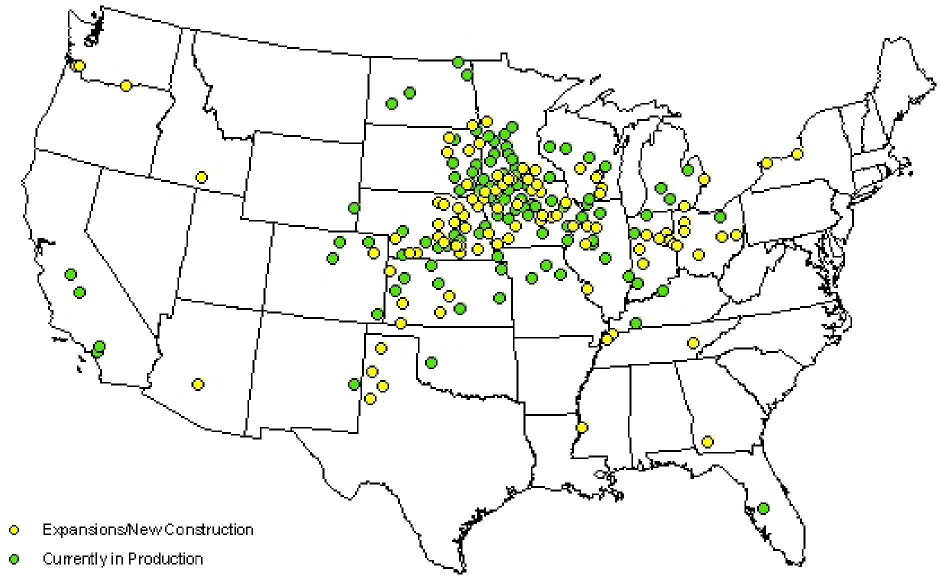
Ethanol in the US

- In the US, the total capacity of ethanol production was estimated to be 6.5 billion liters in 1994, 88% of which was produced by fermentation.
- 17% of the demand was for industrial applications, 5% was in the beverage market, and 78% of the ethanol demand in the US was for fuel purposes.
- The market for fuel-grade ethanol has grown substantially in recent years because
  - US dependence on foreign oil supplies
  - a desire to increase the octane of unleaded gasoline
  - surplus production of corn
  - concerns over air pollution.

US fuel ethanol production (1996-2006)

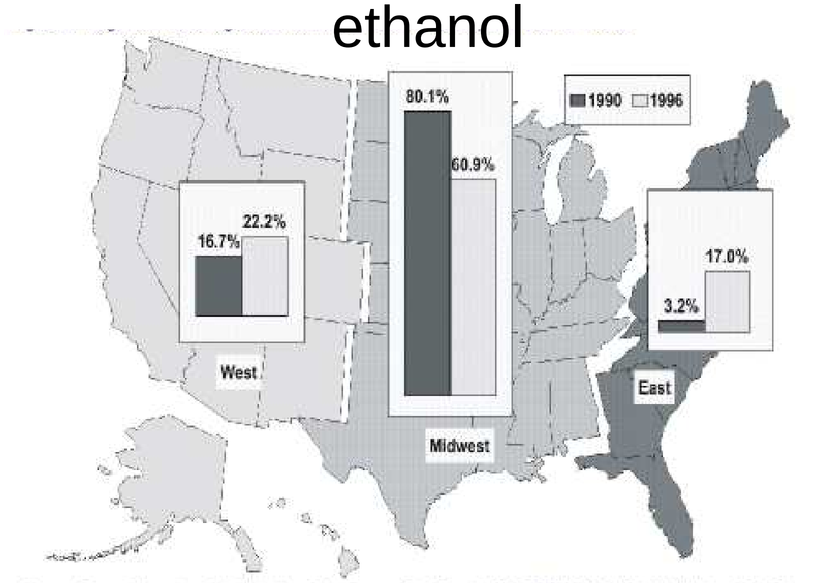


Ethanol Plants in US



- The government has set a tax exemption of 5.4 cents per gallon for a minimum of 10% ethanol blend.
- Corn is the major feedstock in the US for ethanol production.
- The U.S. demand for ethanol could increase even more dramatically as a result of proposed government regulation on use of renewable resources in production of oxygenates.

Regional consumption of ethanol



Source: Energy Information Administration, *State Energy Data Report 1996*, DOE/EIA-0214(96) (Washington, DC, February 1999).

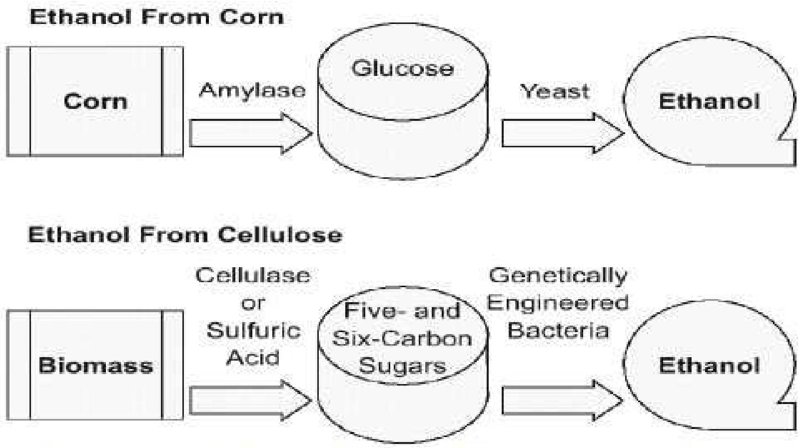
Fermentative ethanol production

- Agricultural raw materials for ethanol production can be classified into three categories:
  - sugars
    - Sugars (from sugar cane, sugar beet, molasses or fruit), may be converted directly to ethanol.
    - Sucrose, found in sugar beets and cane, can be converted into glucose and fructose by yeast invertase.
  - starch
    - Starches (from grains, potatoes or root crops) need first to be hydrolyzed to sugars before conversion to ethanol.
  - cellulose.
    - Cellulose and hemicellulose (from wood, agricultural residues or any other lignocellulose resource) need to be converted to sugar before fermentation, but more severe treatment is normally needed to achieve the hydrolysis.

Fermentation (continued)

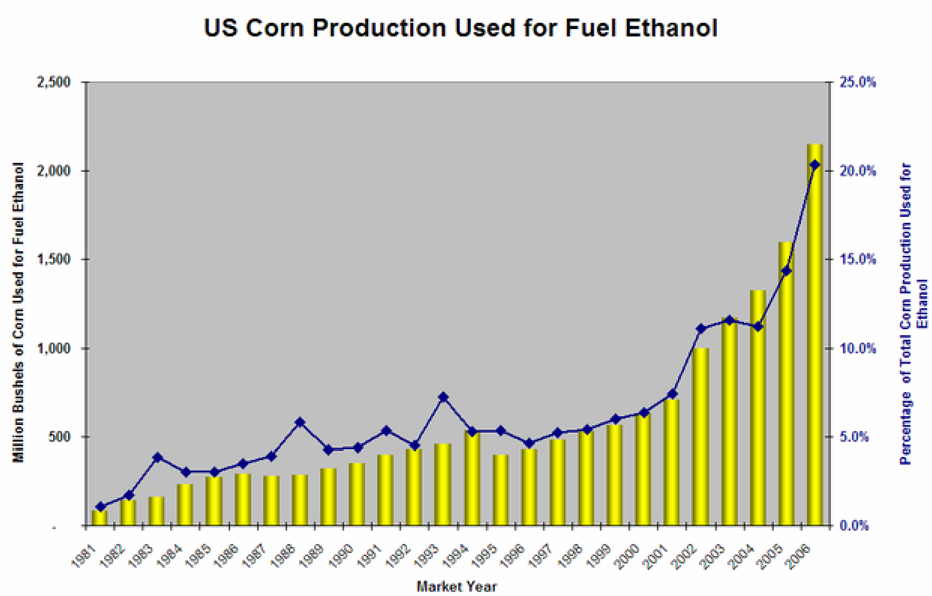
- Although lignocellulosic materials are available at prices lower than refined sugars, transportation costs limit their usage.
- Consequently, ethanol production must be based on available raw material relatively close to the site of production.

Ethanol production from corn and cellulose

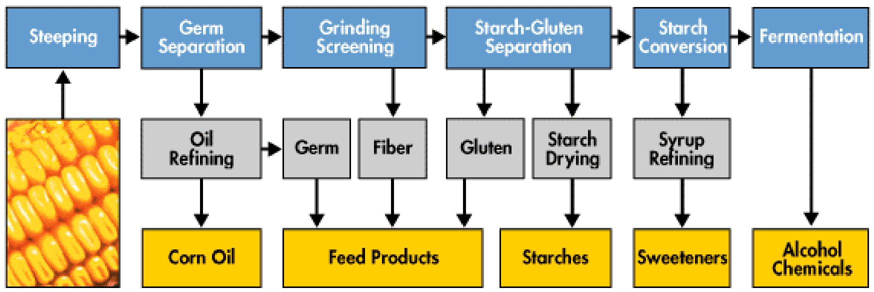


Source: M. McCoy, "Biomass Ethanol Inches Forward," *Chemical And Engineering News* (December 7, 1998), p. 29.

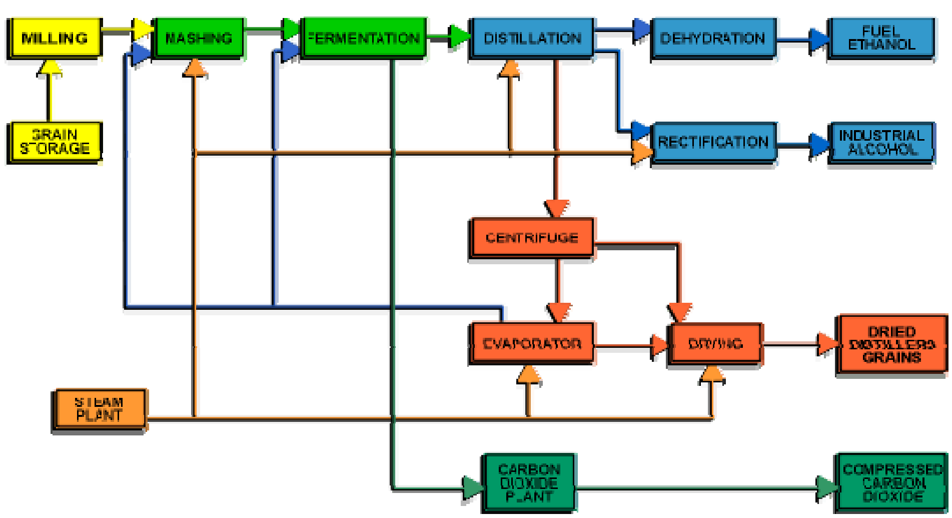
Corn and ethanol production



Ethanol production process



Fermentation process



Fermentation process (continued)

- **Milling:**
  - The corn first passes through hammer mills, which grind it into a fine powder called meal.
  - The meal is then fed to the mashing system
- **Mashing:**
  - The meal is mixed with water and alpha-amylase, and passes through cookers.
  - The action of heat liquifies the starch, and enzymes begin the process of breaking down the starch to sugars.
  - The mash from the cookers is then cooled and pumped to a fermenter.

Fermentation process (continued)

- **Fermentation:**
  - Yeast is added to the mash to convert the sugars to ethanol and carbon dioxide.
  - Using a continuous process, the fermenting mash is allowed to flow, or cascade, through several fermenters, until the mash leaving the final tank is fully fermented.
- **Distillation:**
  - The fermented mash, now called "beer", contains about 10% alcohol, as well as all the non-fermentable solids from the corn and the yeast cells.
  - The mash is then pumped to the continuous flow, multi-column distillation system, where the alcohol is removed from the solids and water.

Fermentation process (continued)

- **Distillation (continued):**
  - The alcohol leaves the top of the final column at about 96% strength, and the residue mash, called stillage, is transferred from the base of the column to the co-product processing area.
- **Dehydration:**
  - The alcohol from the top of the column passes through a dehydration system (a molecular sieve dehydrator), where the remaining water is removed.
  - The alcohol product at this stage is called anhydrous (pure) alcohol or ethanol.
- **Rectification:**
  - The ethanol can be further purified (or 'rectified') to produce industrial and beverage grade alcohol.

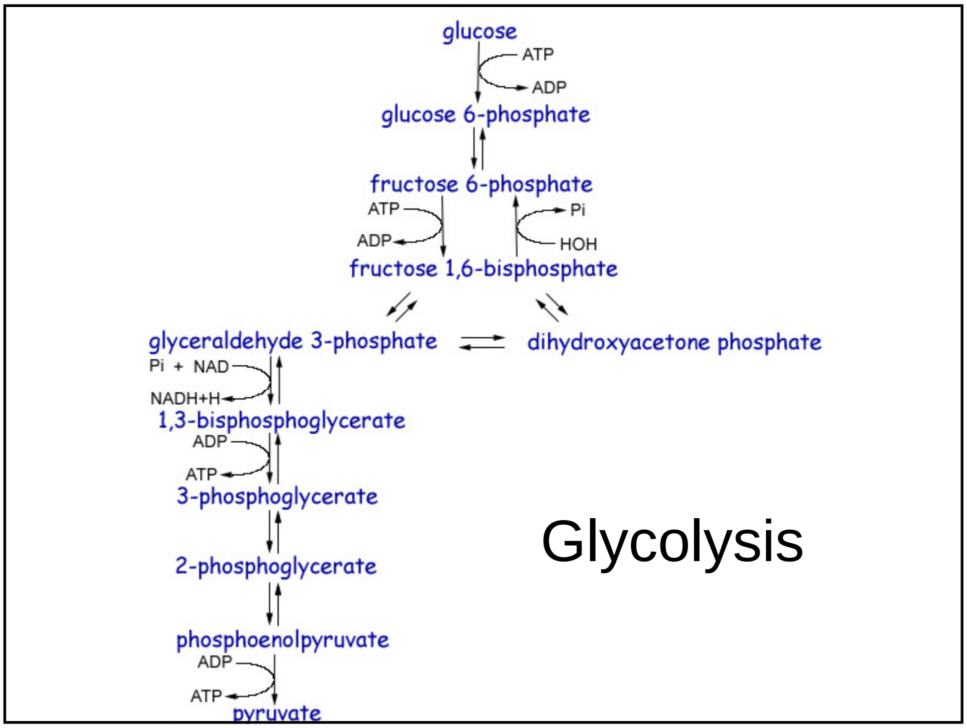
Some ethanol production plants

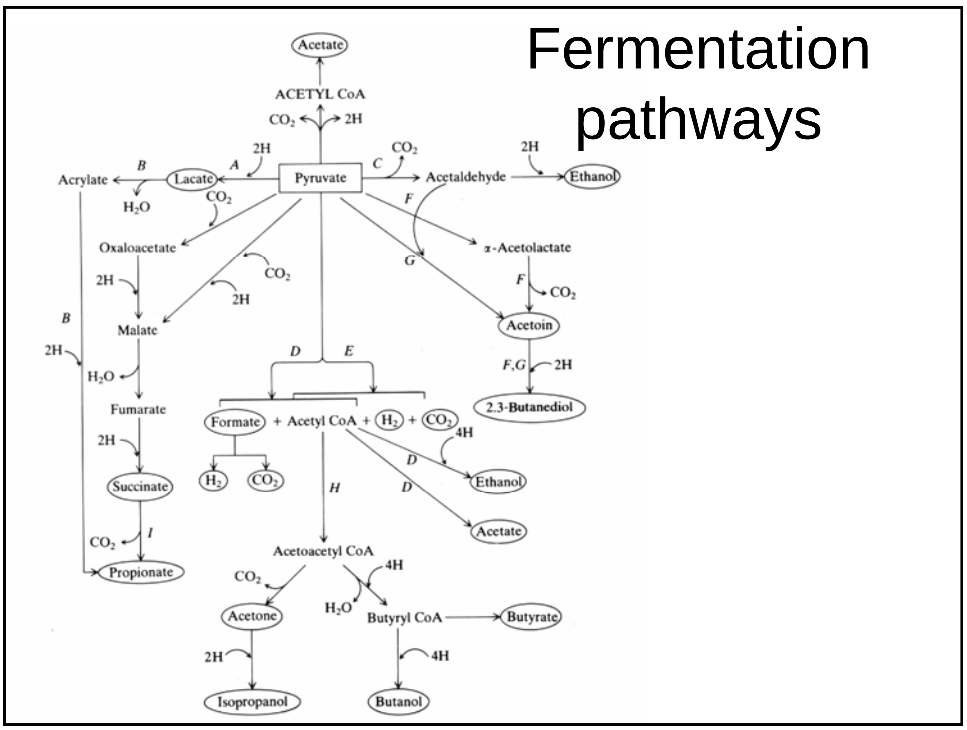
Fermentations

- Occur when no electron acceptor is present
- Fermentation products differ from organism to organism
- Fermentation products can also differ depending on the growth condition

Fermentation of sugars to alcohol

- Yeasts
  - Industrial processes use *Saccharomyces cerevisiae*
  - Substrates include glucose, galactose, lactose, maltose, sucrose, xylose, and xylulose
- Bacteria
  - *Zymomonas mobilis, Clostridium thermocellum, Thermoanaerobium brockii*
  - Substrates include glucose, fructose, sucrose, cellobiose, starch


Glycolysis
glucose
ATP
ADP
glucose 6-phosphate
fructose 6-phosphate
ATP
ADP
Pi
HOH
fructose 1,6-bisphosphate
glyceraldehyde 3-phosphate
dihydroxyacetone phosphate
Pi + NAD
NADH+H
1,3-bisphosphoglycerate
ADP
ATP
3-phosphoglycerate
2-phosphoglycerate
phosphoenolpyruvate
ADP
ATP
pyruvate

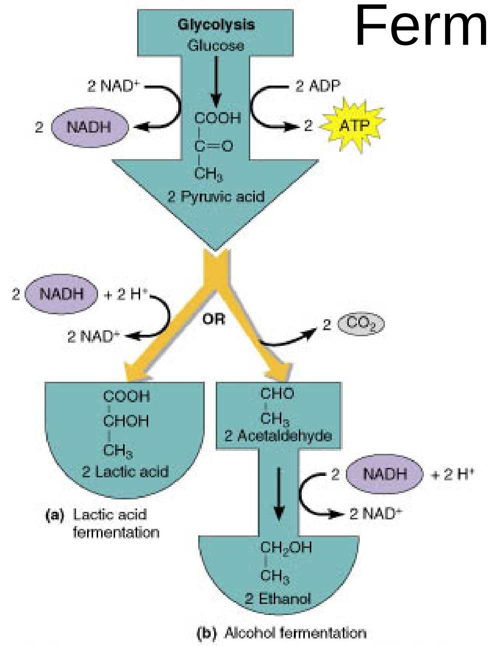

Fermentation pathways
Acetate
ACETYL CoA
CO2
2H
Pyruvate
Lacate
Acrylate
H2O
Acetaldehyde
Ethanol
α-Acetolactate
Oxaloacetate
Malate
Fumarate
Succinate
Propionate
Acetoin
2,3-Butanediol
Formate + Acetyl CoA + H2 + CO2
H2
CO2
Ethanol
Acetate
4H
Acetoacetyl CoA
Acetone
Isopropanol
Butyryl CoA
Butyrate
Butanol

Bacterial sugar fermentations

Fermentation	Principal products	Bacteria
Homolactic	Lactic acid	*Lactobacillus*
Mixed acid	Lactic acid, acetic acid, succinic acid, formic acid, ethanol	Enteric bacteria (*E. coli*)
Butyric acid	Butyric acid, acetic acid	*Clostridium*
Propionic acid	Propionic acid, acetic acid, succinic acid	*Propionibacterium*

Some yeasts and bacteria that produce significant quantities of EtOH

Organism	Substrate
Saccharomyces cerevisiae	Glucose, fructose, galactose, maltose, xylulose
S. carlsbergensis	Glucose, fructose, galactose, maltose, xylulose
Kluyveromyces lactis	Glucose, galactose, lactose
Zymomonas mobilis	Glucose, fructose, sucrose
Clostridium thermocellum	Glucose, cellobiose, cellulose



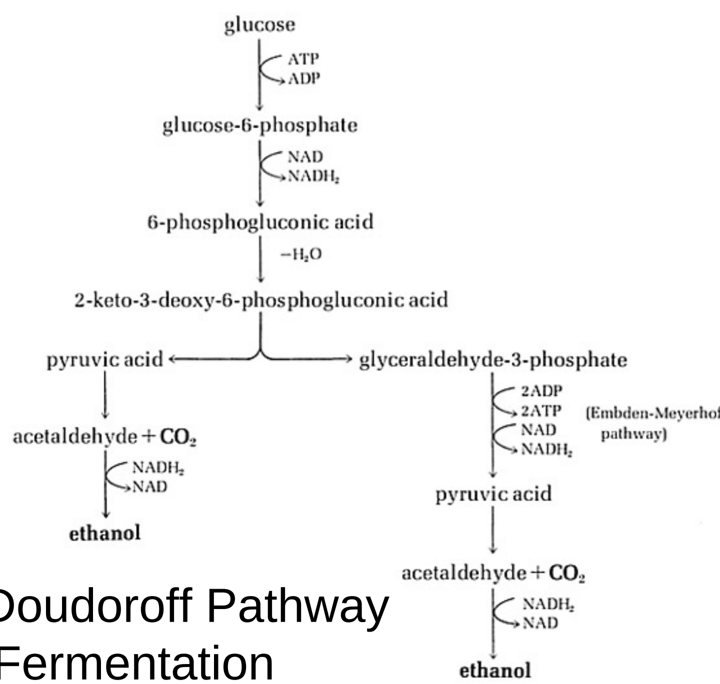
Fermentation

Zymomonas mobilis

- Anaerobic, Gram-negative rods
- Can ferment a broad range of carbohydrate substrates
- Can tolerate and produce high concentrations of ethanol (up to 13%)
- Few by-products produced
- Osmotolerant (can tolerate up to 40% glucose)
- High cell viability for recycling
- Can flocculate and sediment easily

Z. mobilis uses the ED pathway

- *Z. mobilis* uses the Entner-Doudoroff pathway, rather than the glycolytic pathway, for metabolism of glucose.
- Glucose and fructose are taken up by facilitated diffusion.
- The ED pathway produces 1 mol ATP per mol glucose, whereas the glycolytic pathway produces 2 mol ATP per mol glucose.
- Approximately half the cytoplasmic mass of *Z. mobilis* is enzymes involved in the ED pathway and ethanologenic enzymes.
- The yield of ethanol from glucose is 95% of theoretical and from fructose is 90% of theoretical.
  - Due to side reactions that occur during growth on fructose



Entner-Doudoroff Pathway of Fermentation

End product yields in microbial fermentations

Pathway	Key enzyme	Ethanol	Lactic Acid	CO_2	ATP
Embden-Meyerhof *Saccharomyces*	fructose 1,6-diP aldolase	2	0	2	2
Embden-Meyerhof *Lactobacillus*	fructose 1,6-diP aldolase	0	2	0	2
Heterolactic *Streptococcus*	phosphoketolase	1	1	1	1
Entner-Doudoroff *Zymomonas*	KDPG aldolase	2	0	2	1

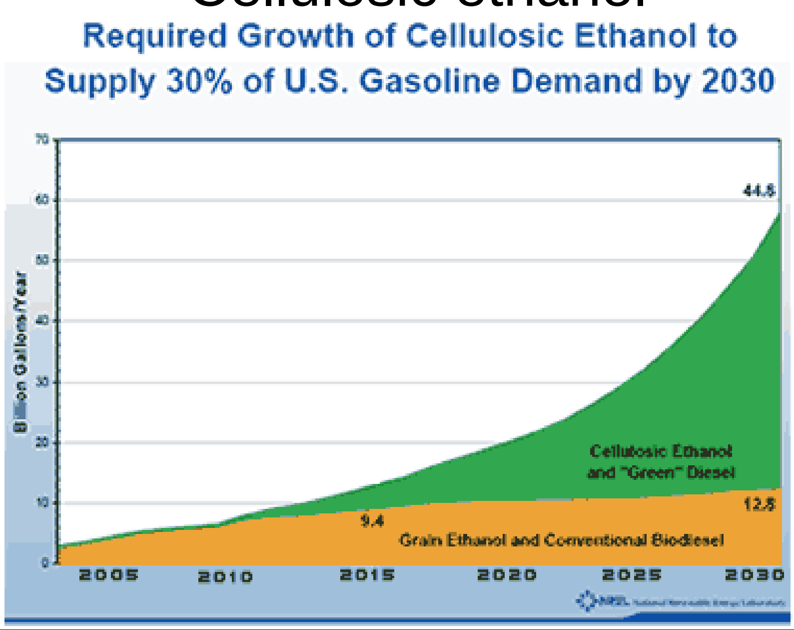
Zymomonas ethanol tolerance

- Cell membrane is rich in mono-unsaturated fatty acids.
- Fatty acid chains are longer by 1 carbon than in other Gram-negative bacteria.
- Contains hopanoids (functional analogs of sterols) in the membrane.

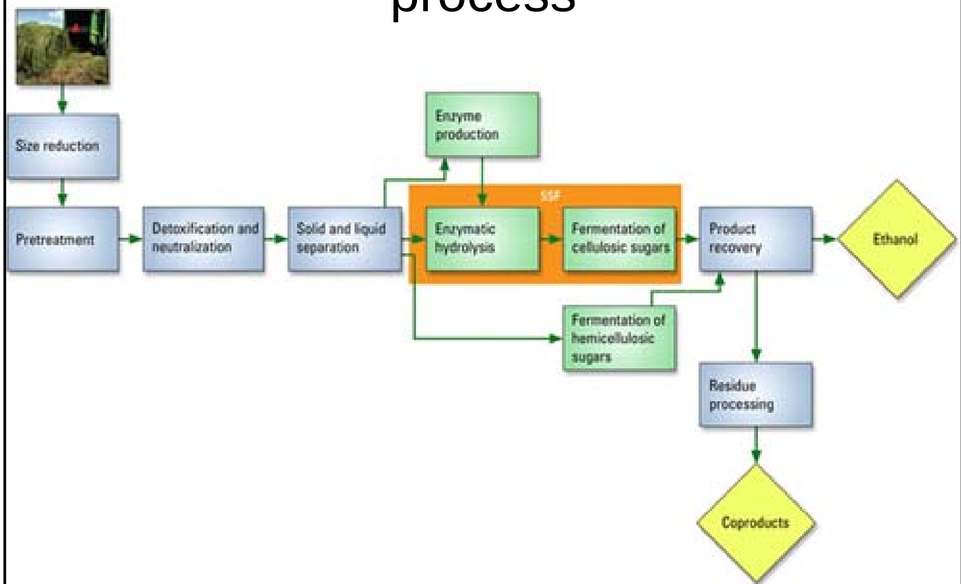
Simultaneous saccharification and fermentation

- Hydrolysis of the polysaccharides and fermentation of monosaccharides are carried out in one vessel.
  - Glucoamylase is added when corn syrup is the substrate
  - *T. reesei* cellulases are added when cellulose is the substrate

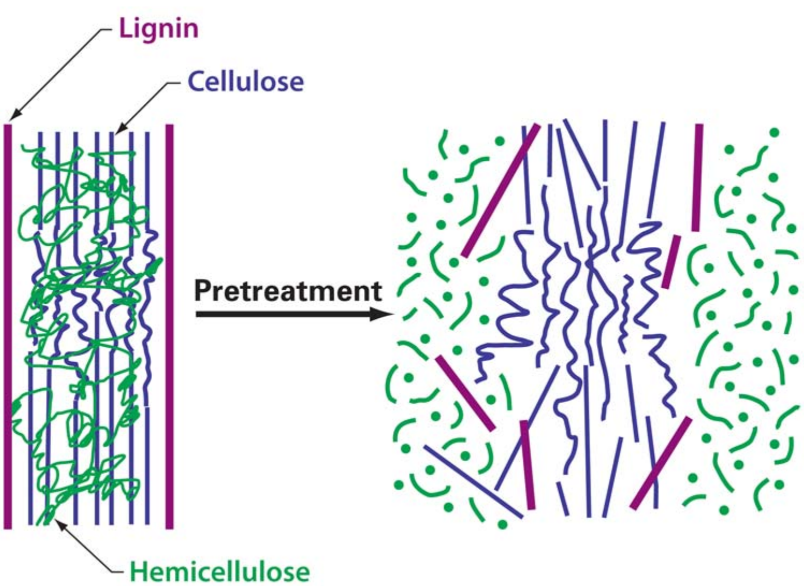
Cellulosic ethanol



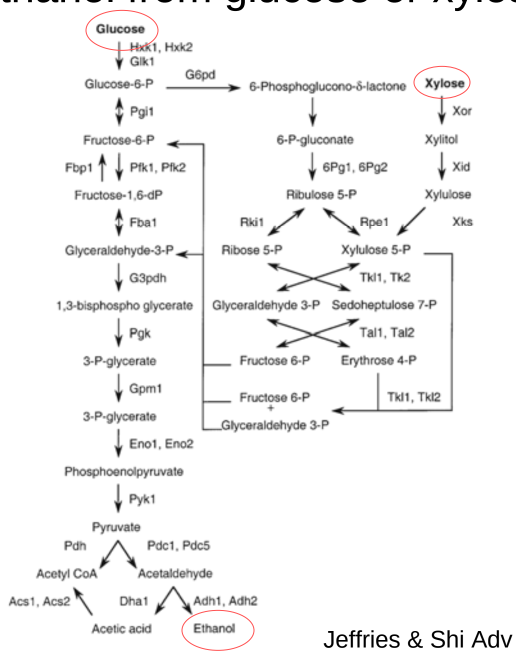
Cellulosic ethanol production process



Cellulosic ethanol



Ethanol from glucose or xylose



Jeffries & Shi Adv Bioch Eng 65,118

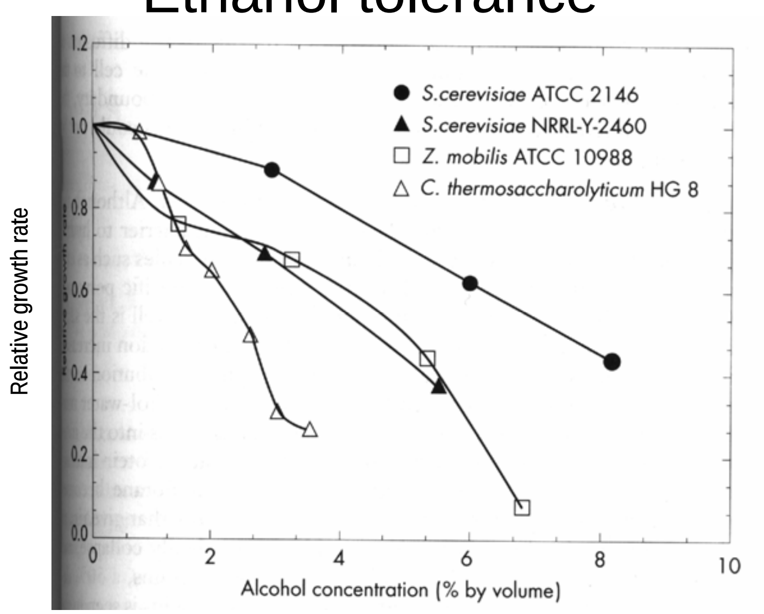
Clostridial fermentations

- Direct conversion of cellulosic biomass to ethanol by anaerobic bacteria
  - Potentially cheaper than using enzymes with yeasts
- Takes advantage of the cellulosomes produced by various Clostridial strains
  - *Clostridium thermocellum*
  - *Clostridium thermosaccharolyticum*
  - *Clostridium thermohydrosulfuricum*
- Grow optimally at high temperatures (55 - 75°C)
- By-products include large amounts of organic acids
  - Highest molar ratio of ethanol to organic acids is about 2.3

Ethanol tolerance

- Ethanol is toxic to yeast at concentrations ranging from 8 – 18%, depending on the strain.
- Ethanol can pass freely across the membrane
- Ethanol disrupts the structure of water, so the entropic contribution to the stabilization of lipid bilayer membranes is lower in alcohol-water mixtures than in water alone.
- It also disturbs lipid-lipid and lipid-protein interactions in the membrane
- As ethanol increases, membranes become progressively more leaky.
  - Ion gradients across membranes are depleted and proton gradient-dependent transport ceases

Ethanol tolerance



Membrane composition and ethanol tolerance

- Membranes of cells grown in the presence of high concentrations of ethanol contain higher concentrations of unsaturated, long-chain fatty acids
  – Small amounts of oxygen (microaerophilic growth) are necessary for production of unsaturated fatty acids
- The membranes also contain higher concentrations of ergosterol

Flocculation

- Cells tend to flocculate during fermentation
  – Cells stick together because mannans on the cell walls bind calcium
- Flocculation aids in cell settling
- Cells can be recycled, increasing the yield of ethanol
- Not often used because of the high cost of separating cells from the broth

The future of ethanol

- Can ethanol replace fossil fuels?
- Corn productivity (in heat of combustion produced per unit time divided by the land area) is 0.6 W/m^2 (or 0.34 W/m^2 for the grain).
- About 30% of the output energy must be used to produce it -> net 0.24 W/m^2
- If the corncobs and stocks are collected for input energy, the output/input ratio is 1.3.
- For sugar cane, the output/input ratio is 2.

Energy inputs per hectare for corn and sugar cane

Input	**Corn (10^3 kcal)**	**Sugar Cane (10^3 kcal)**
Labor	7	21
Machinery	1485	1944
Fuel	1255	3788
N	3192	3318
P	473	611
KCl	240	373
Limestone	134	353
Seed	520	802
Insecticides	150	250
Herbicides	200	620
Electricity	100	--
Transport	89	146
Total	7845	12226
Output	26000	24618
Output/Input	3.31	2.01

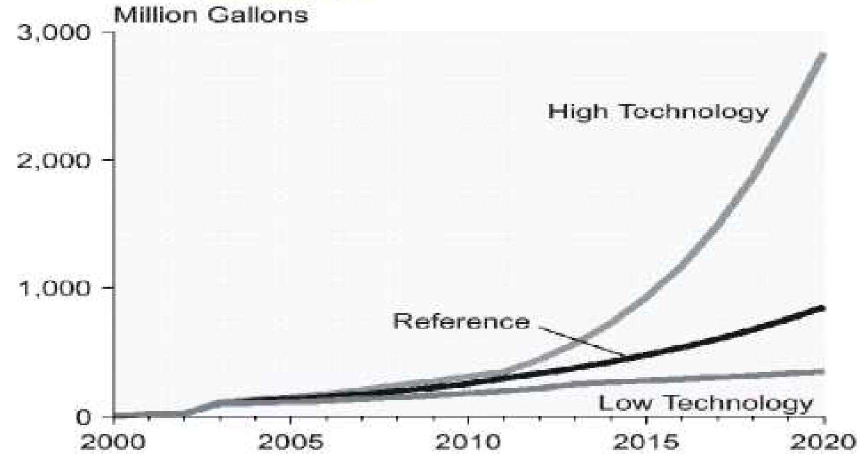
Energy inputs per 1000L EtOH from corn and sugar cane

Input	Corn (10^3 kcal)	Sugar Cane (10^3 kcal)
Corn (2700)	3259	
Sugar cane (14000 kg)		1945
Transport	325	400
Water	90	70
Stainless steel	89	45
Steel	139	46
Cement	60	15
Coal	4617	
Bagasse		7600
Total input	8579 (3,962)	10121 (2,521)
Output	5130	5130
Output/input	0.6 (1.29)	0.51 (2.03)

Can ethanol replace fossil fuels?

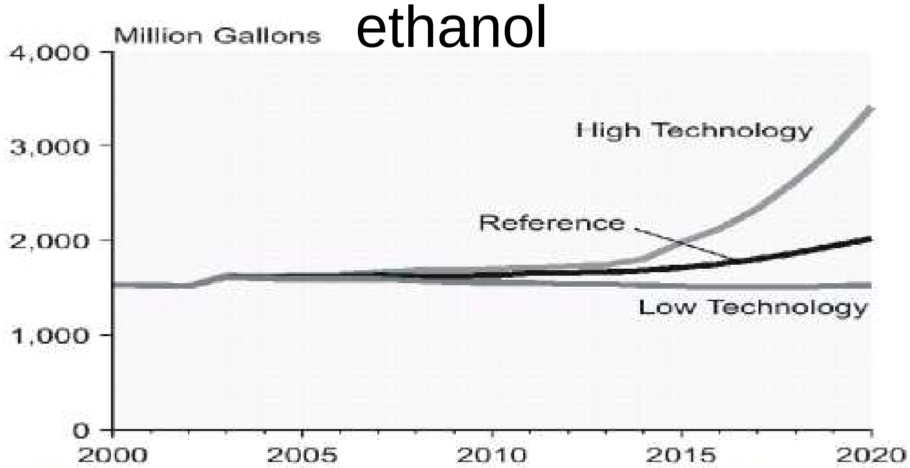
- Energy consumption
  - 2.03 W/m^2 in Japan
  - 0.97 W/m^2 in Northern Europe
  - 0.32 W/m^2 in the US
- The rate of energy consumption exceeds the average production of energy in the biosphere and is greater than the rate of energy generation in food crops produced by modern agriculture
- To replace the current world oil consumption with ethanol from corn, an area approximately 4.5 times the total land area of the US would be needed.
  - About 2 X the total arable land used for food crop production worldwide

Projected ethanol production



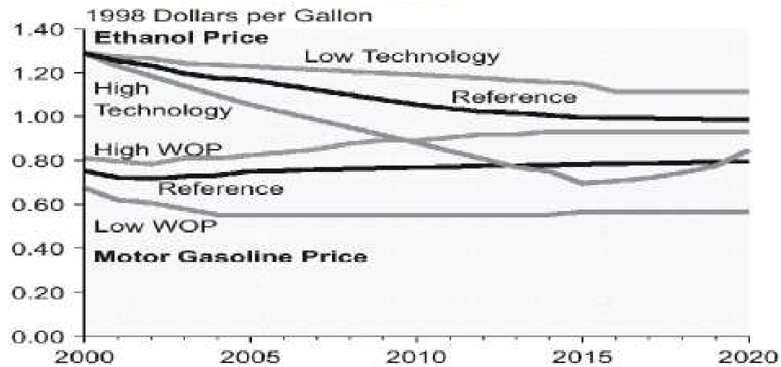
Note: Includes ethanol subsidies.
Sources: **Reference Case:** AEO2000 National Energy Modeling System, run REFER.D122199A. **High Technology Case:** Run HITECH.D122999A. **Low Technology Case: Run FRZTECH.D122199F.**

Gasoline blending with ethanol



Notes: Includes conventional, oxygenated, and reformulated gasolines. Includes ethanol subsidies.
Sources: **Reference Case:** AEO2000 National Energy Modeling System, run REFER.D122199A. **High Technology Case:** Run HITECH.D122999A. **Low Technology Case: Run FRZTECH.D122199F.**

Ethanol and gasoline prices (2000 – 2020)



Notes: WOP = world oil price. Terminal prices exclude taxes, subsidies, distribution costs, and retail markups.

Sources: **Ethanol Price:** Reference Case—AEO2000 National Energy Modeling System, run REFER.D122199A; High Technology Case—run HITECH.D122999A; Low Technology Case—run FRZTECH.D122199F. **Motor Gasoline Price:** AEO2000 National Energy Modeling System, run REFER.D122199A, HWOP2K.D100199A, and LWOP2K.D100199A.