Applications of Molecular Biotechnology
Ethanol Production from Cellulosic Biomass

David R. Shonnard
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Environmental Issues in Ethanol Production and Use


1. Corn → Starch → Glucose → Ethanol
2. Cellulosic Biomass → hemicellulose+cellulose+lignin → Glucose+5-C sugars → Ethanol
Land Use: Ethanol as a Transportation Fuel

- Light Duty Fleet - 130x10^9 gallons gasoline/yr
- Equivalent Ethanol - 150x10^9 gallons ethanol/yr
- Acreage requirement - 300 to 500 x10^6 acres, about 1/4 of US total of 1800x10^6 acres.

Process to Convert Cellulosic Biomass to Ethanol

Saccharification is enzymatic hydrolysis of pretreated cellulose yielding Glucose using cellulase from *Trichoderma reesei*

History of Costs for Ethanol Production

Sequential enzymatic hydrolysis then fermentation

Improved fungal strain for cellulase production

Improved cellulase (150L) produced by Genencore

Simultaneous Saccharification-Fermentation process

More efficient cellulase

Fermentation of 6C and 5C sugars using a single microorganism


Figure 2  Progress in reducing the cost of producing ethanol from biomass based on enzymatic cellulose hydrolysis technology, as shown in 1990 dollars.
Simultaneous Saccharification + Fermentation (SSF)

The cellulase responsible for enzymatic hydrolysis of pretreated cellulosic biomass is strongly inhibited by hydrolysis products: glucose and short cellulose chains. One way to overcome cellulase inhibition is to ferment the glucose to ethanol as soon as it appears in solution. SSF combines enzymatic hydrolysis with ethanol fermentation to keep the concentration of glucose low. The accumulation of ethanol in the fermenter does not inhibit cellulase as much as high concentrations of glucose, so SSF is a good strategy for increasing the overall rate of cellulose to ethanol conversion. It is important to keep the rate limiting step in mind. In SSF the ethanol production rate is controlled by the cellulase hydrolysis rate not the glucose fermentation, so steps to increase the rate of hydrolysis will lower the cost of ethanol production via SSF. The US Department of Energy, National Renewable Energy Laboratory (NREL) is funding Genercor International, Inc. to develop low cost cellulases that will reduce the cost of cellulose breakdown by a factor of 10.
Composition of Dry Cellulosic Biomass

Dry Cellulosic Biomass

- Cellulose (35-50%)
  - Glucose
  - 6-C sugars
  - hydrolysis

- Hemicellulose (20-35%)
  - Xylose
  - Arabanose
  - Mannose
  - Galactose
  - 5-C sugars
  - hydrolysis

- Lignin (12-20%)
  - no hydrolysis
The Challenge of Fermenting all Sugars in Biomass

**Saccharomyces cervisiae**
- Ferment glucose to ethanol
- Utilize 6C sugars only
- Tolerant to ethanol
- Can these microorganisms be genetically engineered to utilize 5C sugars?

**Zymomonas mobilis**

**Escherichia coli**
- Can not ferment glucose to ethanol
- Can utilize 6C and 5C sugars
- Is it easier to genetically engineer *E. coli* to ferment ethanol?
Glycolysis
Embden-Meyerhof-Parnas (EMP) Pathway

This pathway is representative of a human muscle cell or E. coli

"Principles of Biochemistry"; Lehninger, Worth

The end product is not ethanol
Is it Easier to Genetically Engineer This Pathway into \textit{E. coli}, or

Two genes are needed. One for pyruvate decarboxylase and another for alcohol dehydrogenase. These enzymes working together in the cell will divert Pyruvate away from other fermentation products to ethanol. This would convert \textit{E. coli} into an ethanol-producing microorganism, where before it was not!

"Principles of Biochemistry", Lehninger, Worth
Is it Easier to Genetically Engineer This Pathway into *S. cerviciae* or *Z. mobilis*

This is the Pentose Phosphate pathway in *E. coli*. This pathway is obviously more complicated, containing many more enzyme-catalyzed reactions than the two-step pathway on the previous slide. The pathway for other 5C sugars (arabanose, mannose, galactose) would be similar.

"Bioprocess Engineering: Basic Concepts Shuler and Kargi, Prentice Hall, 2002"
Genetic Engineering of Ethanol Production in *E. coli*

A plasmid for Pyruvate decarboxylase (pdc)

A plasmid for Alcohol dehydrogenase (adh)

Ethanol Production in Sealed Cultures of *E. coli* TC4

High Performance Liquid Chromatography Profiles

- **G** = glucose
- **S** = succinate
- **L** = lactic acid
- **A** = acetic acid
- **U** = unknown
- **E** = ethanol