Butanol: A Second Generation Biofuel

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Outline

- Introduction
  - History
  - Rationale
- Microbe Development and Characterization
  - Genetic and Post-genomic Characterization
  - Substrate (Co-Product) Utilization
- Downstream Processing and Recovery
  - Integrated process
- Scale-up and Commercialization of ABE
Production of Butanol from Corn

- Commercial Plants were operational in the USA to produce butanol from corn
Commercial Solvents Corp.
Terra Haute, Indiana
Rationale

- Butanol is an important industrial chemical and feedstock
- Superior fuel extender/replacement
- Food grade extractant
BioButanol: A Second Generation Liquid Fuel

- Higher energy content than ethanol
- Can be stored under humid conditions unlike ethanol
  - Lack of solubility with water (higher flash point and lower vapor pressure)
- Can be used in internal combustion and diesel engines; less corrosive
- Can be shipped through existing pipelines
- Replacement for gasoline or as a solvent
# Liquid Fuel Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Gasoline</th>
<th>Diesel</th>
<th>Methanol</th>
<th>Ethanol</th>
<th>Butanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formula</td>
<td>C4-C12 hydrocarbons</td>
<td>C14-C20 hydrocarbons</td>
<td>CH₃OH</td>
<td>CH₃CH₂OH</td>
<td>CH₃(CH₂)₃OH</td>
</tr>
<tr>
<td>Boiling Point</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>°C</td>
<td>32-210</td>
<td>204-343</td>
<td>65</td>
<td>78</td>
<td>118</td>
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<tr>
<td>°F</td>
<td>90-410</td>
<td>400-650</td>
<td>149</td>
<td>173</td>
<td>244</td>
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<tr>
<td>Lower heating value*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MJ/kg</td>
<td>44.5</td>
<td>43.0</td>
<td>19.6</td>
<td>26.9</td>
<td>33.1</td>
</tr>
<tr>
<td>Btu/gal</td>
<td>114,800</td>
<td>140,000</td>
<td>55,610</td>
<td>76,100</td>
<td>96,100</td>
</tr>
</tbody>
</table>
**C. beijerinckii BA101: Characteristics**

*C. beijerinckii* BA101* is able to produce up to 20g butanol/l and 33 g/L total solvents
- Hyper amylolytic
- Hyper butanol producing
- Low acid producing
- Stable

*U.S. Patent 6,358,717*
Solvent production using solventogenic *Clostridia*

Fermentation by *C. beijerinckii* NCIMB 8052 in laboratory batch culture typically yields butanol, acetone and ethanol in a 6:3:1 ratio.
Bio-Butanol: Fermentation strain comparison

![Graph showing Butanol Productivity vs Butanol Concentration for strains BA101 and 8052. The graph indicates a decrease in Butanol Productivity as the Butanol Concentration increases.]
Clostridial life cycle

Clostridial metabolism involves the following processes:

- **Sporulation Genes**
- **Aminosugar metabolism**
- **Sporulation**
- **Solenogenesis/ Acidogenesis**
- **Stress responses**
- **Transcriptional regulators**
- **Motility/ Chemotaxis**
- **Topology**
- **Glycolysis**
- **Fatty acid metabolism**
- **Fatty acid biosynthesis operon**

**Genes profiled in C. beijerinckii microarray**

- **Organic Acids** (Acetic Acid, Butyric Acid)
- **Solvents** (Acetone, Butanol, Ethanol)
Genomic analysis of *C. beijerinckii* NCIMB 8052

- JGI-DOE sequenced the *C. beijerinckii* NCIMB 8052 genome
- Determination of Gene Functions

www.jgi.doe.gov/JGI_microbial/html/
Post-Genomic Analysis

- Post transcriptional analysis of global shift in gene expression during transition from acidogenesis to solventogenesis
- Examination of differences in gene expression between hyper-butanol producing *C. beijerinckii* BA101 and wild type 8052 strain
- Roadmap for development of next generation fermentation microorganisms
Clostridial pathway reactions for solvent production

Hexose

Glycolysis (Embden Meyerhof pathway)

Pyruvate

Pyruvate ferredoxin oxidoreductase

Acetyl-CoA

thiolase

3-hydroxybutyryl-CoA dehydrogenase

Acetoacetate decarboxylase

Butyrylphosphate

Acetate kinase

Acetylphosphate

Acetyl-CoA

phosphotransacetylase

Acetoacetyl-CoA

crotonase

3-hydroxybutanoyl-CoA

crotonyl-CoA

3-hydroxybutyryl-CoA dehydrogenase

Butyryl-CoA

butyryl-CoA dehydrogenase

Butyrate

butyrate kinase

Butyryl-CoA

phosphate butyryltransferase

Butyrylphosphate

Acetone

Ethanol

Hexose

Glycolysis (Embden Meyerhof pathway)
Transcriptional analysis of *C. beijerinckii*

1. Genomic information
2. Immobilization on glass slides
3. Long oligos
4. Printed arrays
5. Hybridization
6. Hybridized slides
7. Reverse transcription
8. Dye labelling
9. Labelled probes
10. RNA extraction
11. Clostridial culture
12. Clostridial RNAs
13. cDNAs
14. Wild-type
15. BA101
16. Fluorescence image scanning
17. Quantification of gene expression on arrays
Growth Response of *C. beijerinckii* 8052 and hyper-butanol producing BA101 mutant
Solventogenic/acidogenic genes - butanol production

Expression of alcohol dehydrogenases:

- Increase by ~10 fold to a maximal level during acidogenic phase in wild-type and BA101.
- Expression is at maximal level in BA101 during solventogenic phase, but down-regulated to basal level in wild-type.
Solventogenic/acidogenic genes - \textit{ptb-butK} operon

- Expression of \(\text{bk}, \text{ak}\), and PTA is similar during acidogenic phase for both wild-type and BA101, while expression level is higher for BA101 by up to 10 fold during solventogenic phase vs. wild-type.
- Expression remains constant in BA101, but decreases by \(~10\) fold in solventogenic phase for wild-type.
- \(\text{ak}\) and PTA expression is lower during solventogenesis, although less pronounced in BA101 than in wild-type.
Gene expression for terminal enzymes in solvent formation pathway

- **adc**
- **ctfA**
- **ctfB**
- **adh**
- **bdhII**

**Wild-type**

**Mutant**
Gene expression for pyruvate conversion to solvents

- pyruvate ferredoxin oxidoreductase
- thiolase
- 3-hydroxybutyryl-CoA dehydrogenase
- crotonase
- butyryl-CoA dehydrogenase
- butyrate kinase

Phosphotransacetylase

Acetate kinase

Wild-type
Mutant
Temporal profiling of gene expression during the ABE fermentation by *C. beijerinckii*

Changes in global gene expression appear more robust in 8052 wild-type cells than in the BA101 mutant.

Gene activation, mostly in sporulation during the solventogenic phase is prominent in the wild-type, but less distinct in mutant.

Repression of genes, many in glycolysis, during solventogenic phase is pronounced in wild-type, less so in mutant.
Acetone production enzymes ctfA/ctfB and adc are co-located in a single operon in the *C. beijerinckii* genome, consistent with their clustering in expression analysis.
Biological Conversion

- Fermentation - utilization of 5 & 6 carbon sugars
- Examples:
  - *Zymomonas mobilis* engineered to ferment xylose and arabinose,
  - *S. cerevisiae* strains engineered to ferment arabinose,
  - Solventogenic clostridia with ability to simultaneously saccharify and ferment.
Turning Corn Stover into Value-Added Products

Could be collected as used as a substrate

Current Treatment Technologies:
Pretreatment with steam and acid followed by enzymatic hydrolysis to produce 90 M tons of fermentable sugars available for conversion to liquid fuels & chemicals
Substrates/Co-Products

- DDGS and Corn Fiber (DOE/Midwest Consortium)
- Starch-based Packaging Peanuts (Ezeji et al., 2003)
- Agricultural Waste Streams (Jesse et al., 2002)
- De-germed Corn (Campos et al., 2002)
- Soy Molasses (Qureshi et al., 2001)
Integrated Process

- Eliminate product inhibition (butanol toxicity)
- Achieve high productivity
- Produce concentrated products (acetone-butanol)
- Utilize concentrated substrates - to allow for a reduction in process volume
- Better economics
Gas is bubbled through the fermentation broth and then circulated through the condenser. ABE captured in reactor is condensed in condenser and gas is recycled back to the reactor. ABE is recovered as liquid.
2 Liters

vessel

condenser

cooler

Circulating pump
Modeling Studies

- Economic modeling studies indicated that the use of *C. beijerinckii* BA101 hyper-butanol producing strain when used in combination with improved recovery technologies can be competitive to the petrochemical route for producing butanol.

Summary

- Genes encoding terminal solventogenic enzymes exhibit rapid and large fold increases in message levels (up to 100-fold) during early exponential phase growth and the high expression level is sustained throughout solventogenic phase.

- In the *C. beijerinckii* BA101 hyper-butanol producing mutant, expression of a large number of genes including alcohol dehydrogenases is maintained throughout the solventogenic stage, while these genes are repressed during the solventogenic phase in the 8052 wild-type culture.

- Repression of various sporulation genes is stronger in the BA101 mutant than in wild-type strain.

- Potential exists for utilization of 5 and 6 carbon sugars obtained from lignocellulosic-based hydrolysates.

- Integrated approach for bio-butanol production and simultaneous product removal is promising and is currently being scaled up.