Butanol: A Second Generation Biofuel

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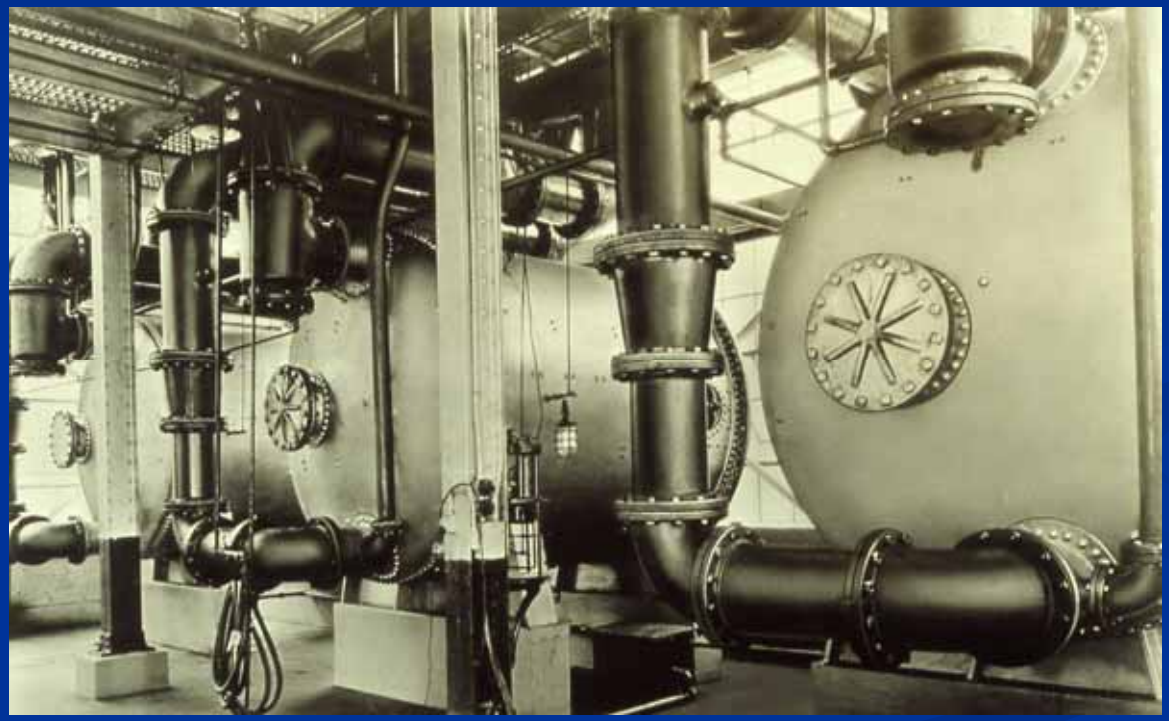
Outline

- 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
- Foodgrade 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

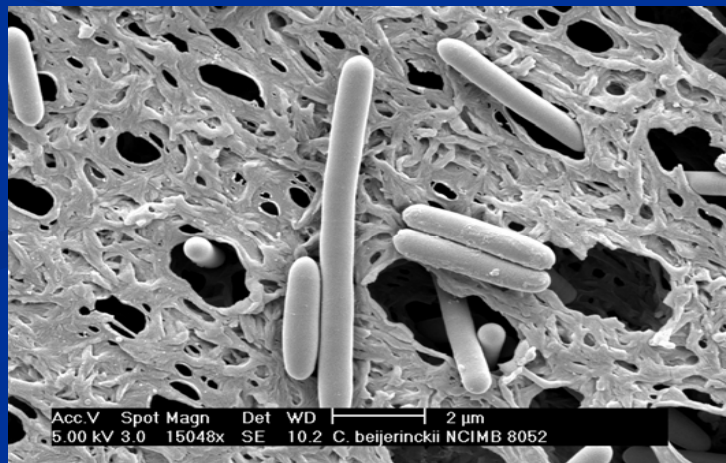
Characteristic	Gasoline	Diesel	Methanol	Ethanol	Butanol
Formula	C4-C12 hydrocarbons	C14-C20 hydrocarbons	CH_3OH	CH_3CH_2OH	$CH_3(CH_2)_3OH$
Boiling Point					
^oC	32-210	204-343	65	78	**118**
^oF	90-410	400-650	149	173	**244**
Lower heating value*					
MJ/kg	44.5	43.0	19.6	26.9	**33.1**
Btu/gal	114,800	140,000	55,610	76,100	**96,100**

C. beijerinckii BA101: Characteristics

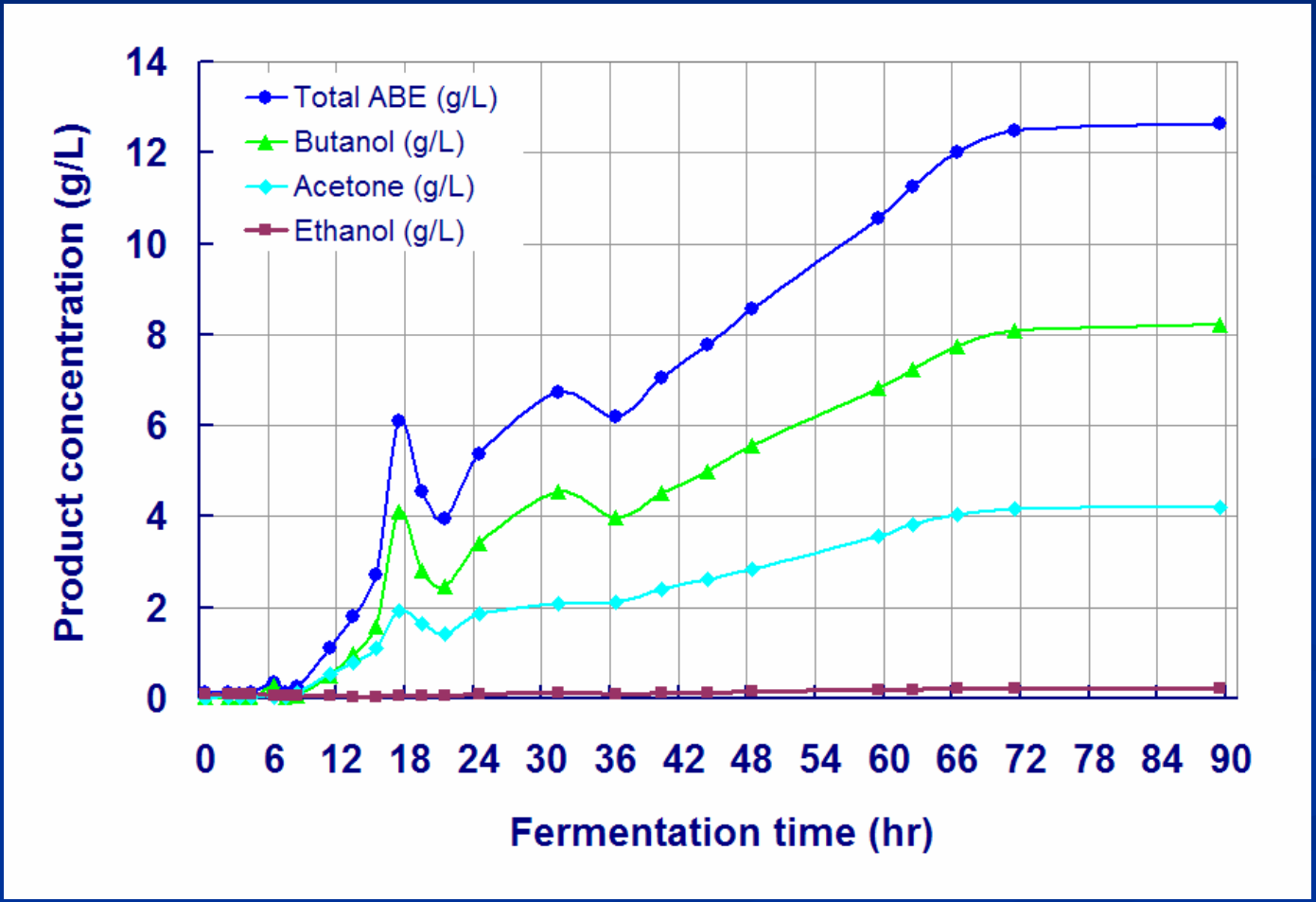
C. beijerinckii BA101* is able to produce up to 20g butanol/l and 33 g/L total solvents

- Hyper amylolytic
- Hyper butanol producing
- Low acid producing
- Stable

*U.S. Patent 6,358,717

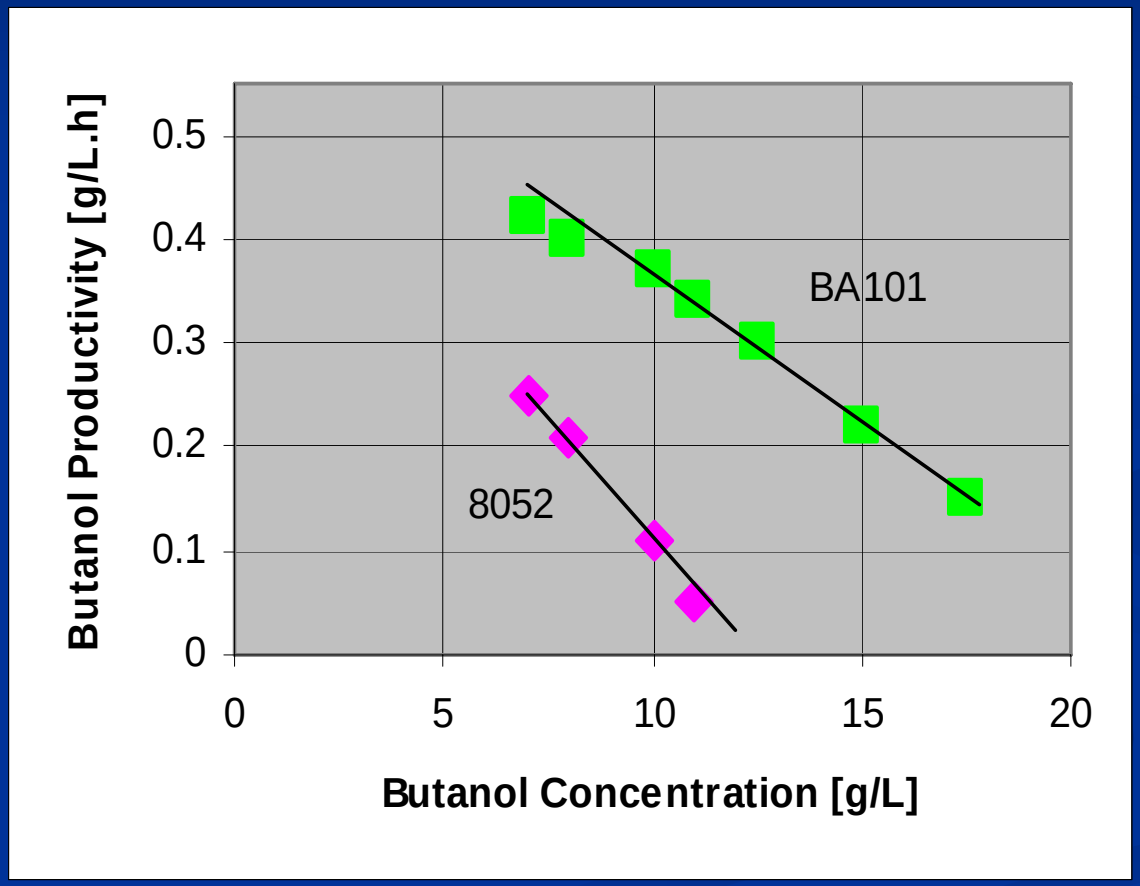


Solvent production using solventogenic *Clostridia*

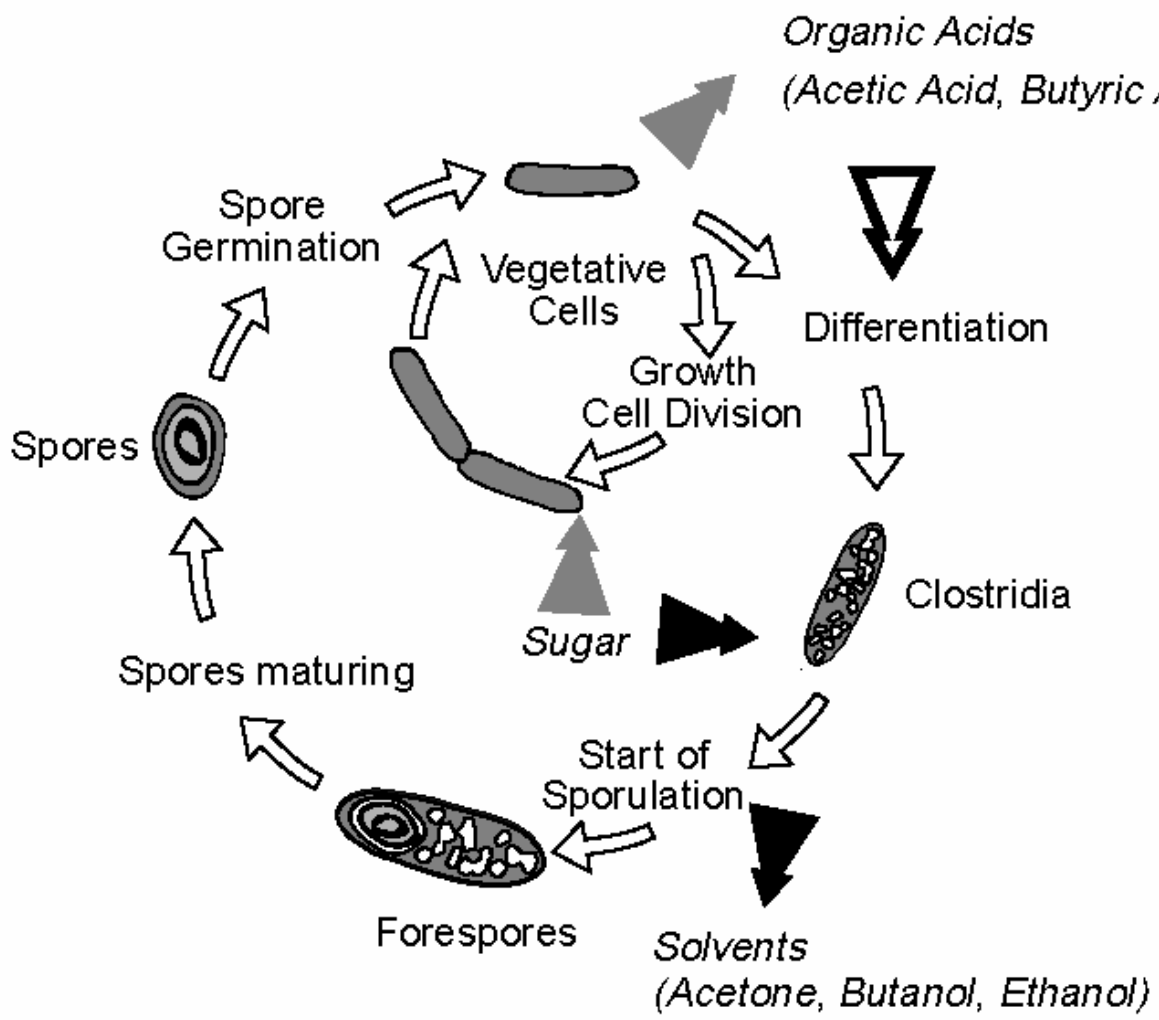


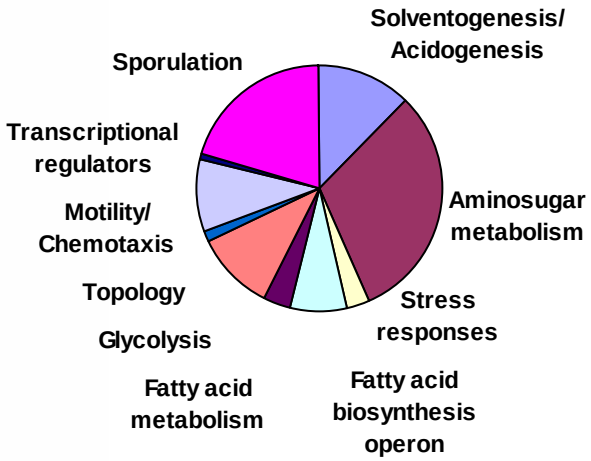
Fermentation by *C. beijerinckii* NCIMB 8052 in laboratory batch culture typically yields butanol, acetone and ethanol in a 6:3:1 ratio.

Bio-Butanol: Fermentation strain comparison



Clostridial life cycle





Genes profiled in *C. beijerinckii* microarray

Genomic analysis of *C. beijerinckii* NCIMB 8052

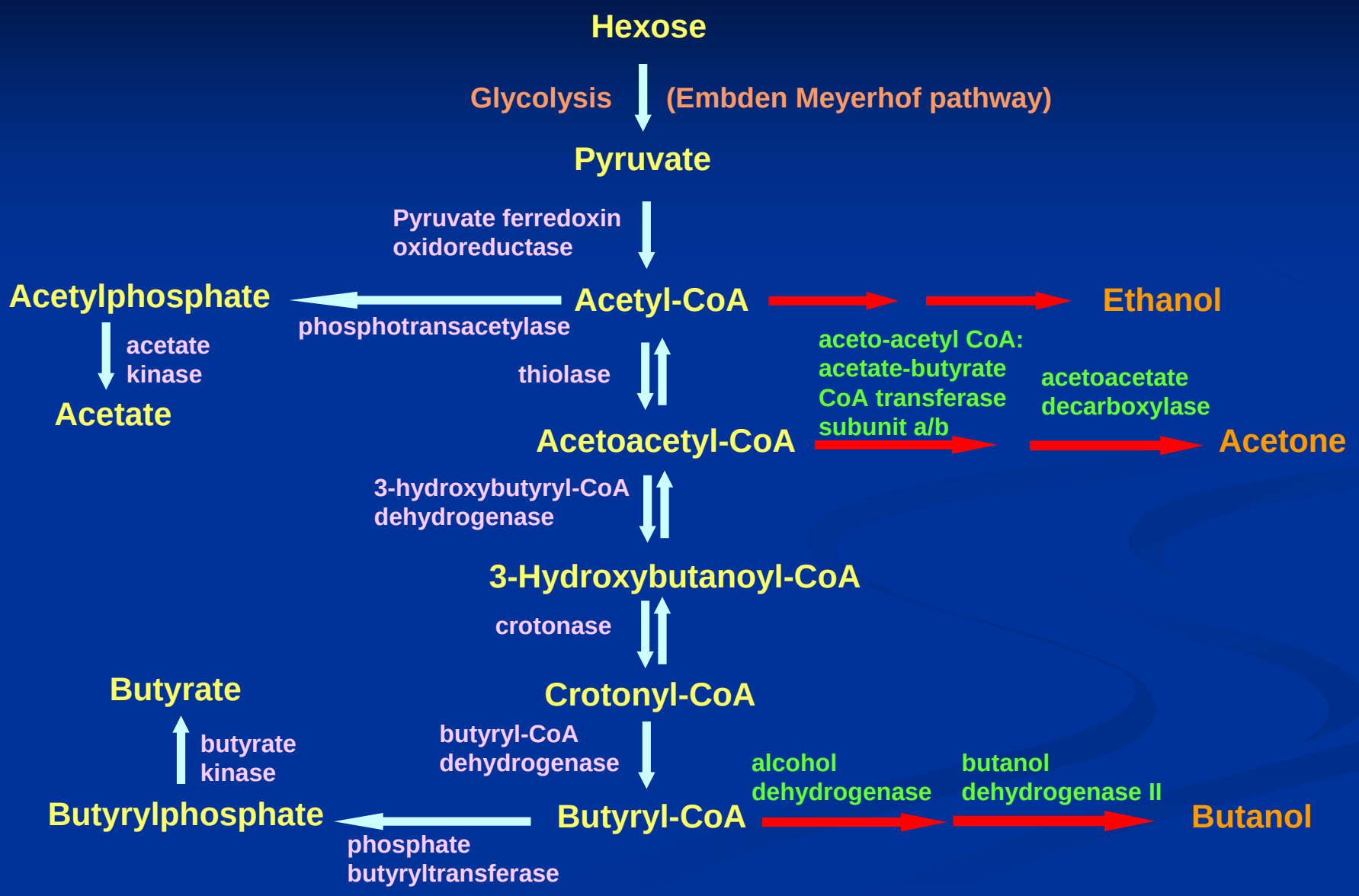
- JGI-DOE sequenced the *C. beijerinckii* NCIMB 8052 genome
- Determination of Gene Functions

www.jgi.doe.gov/JGI_microbial/html/

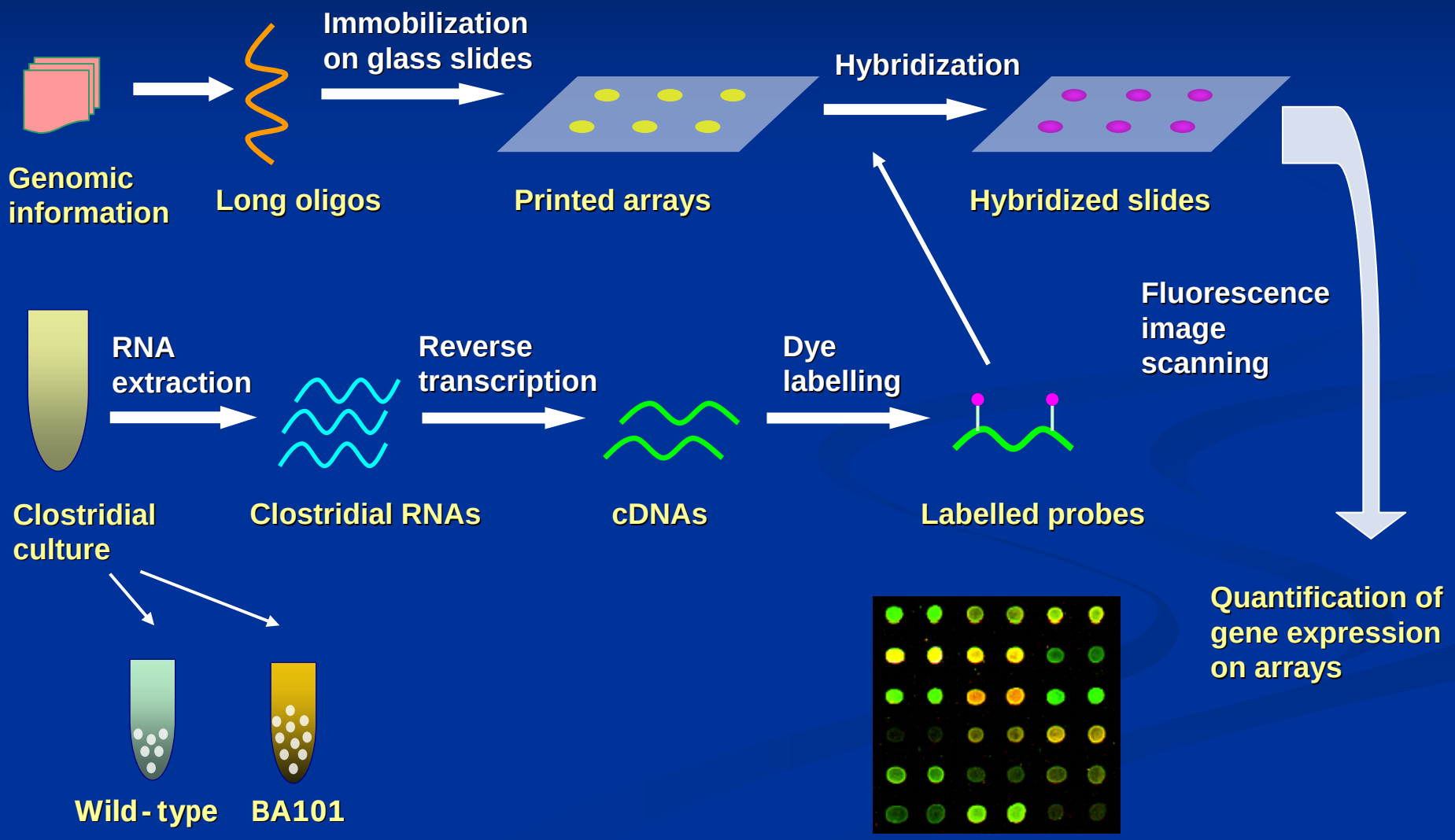
Post-Genomic Analysis

- 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

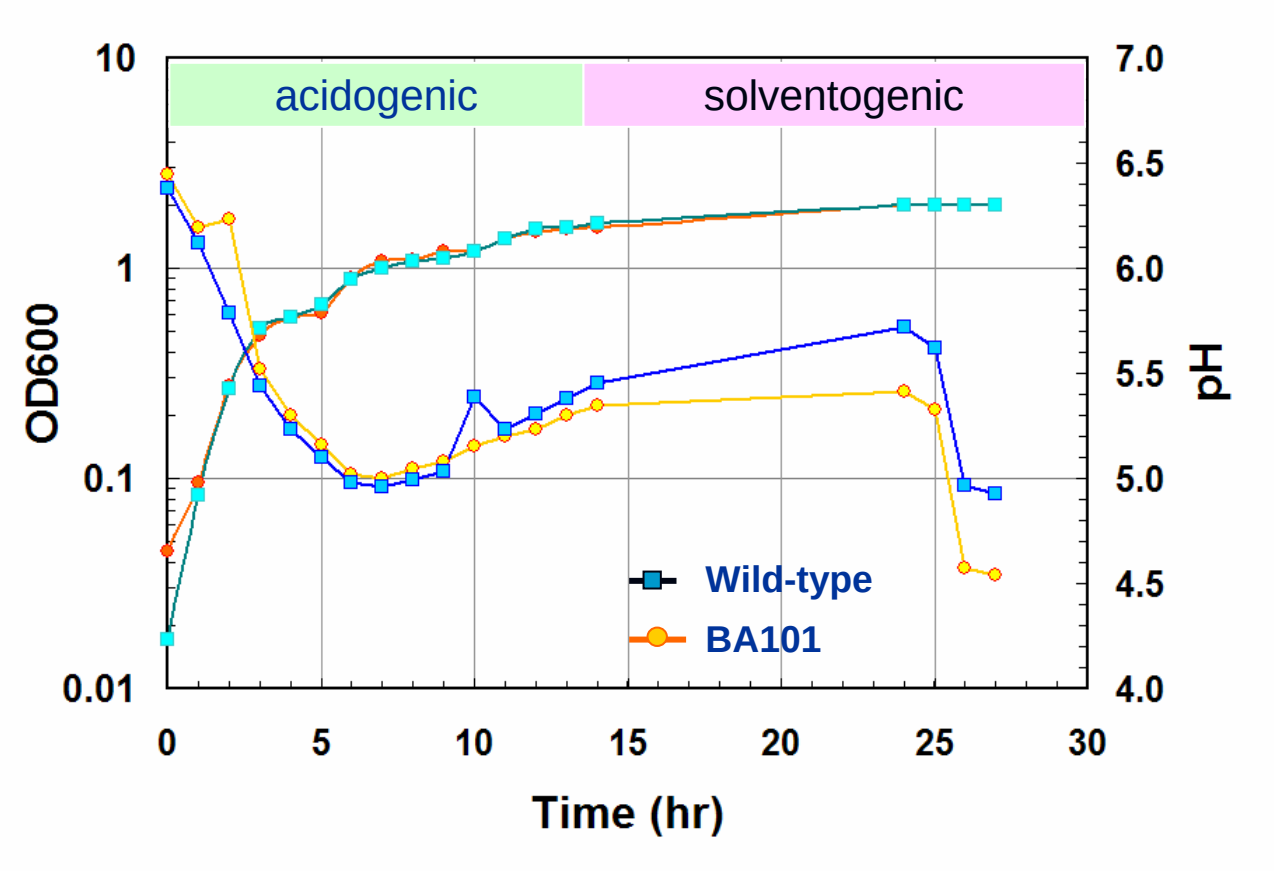
Clostridial pathway reactions for solvent production



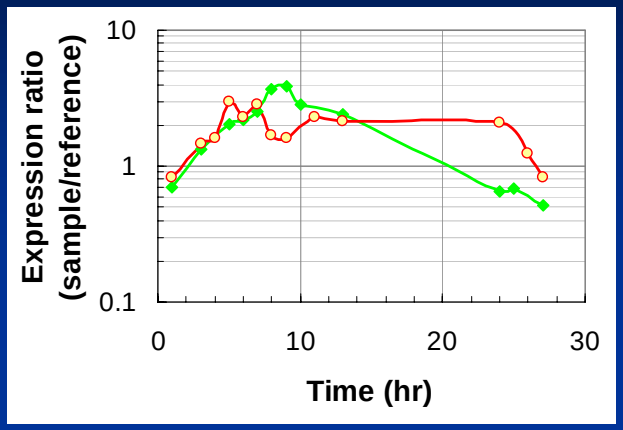
Transcriptional analysis of *C. beijerinckii*



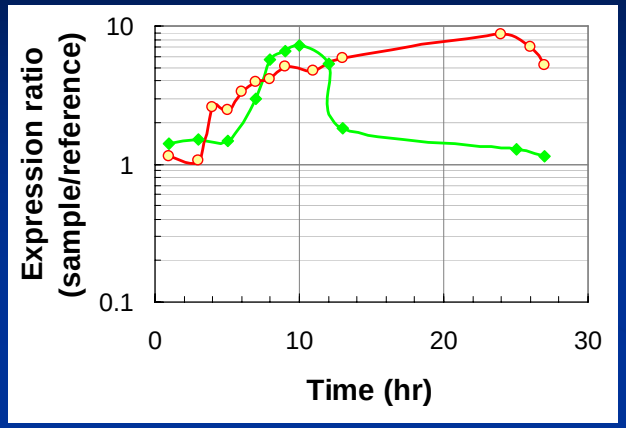
Growth Response of *C. beijerinckii* 8052 and hyper-butanol producing BA101 mutant



Solventogenic/acidogenic genes – butanol production



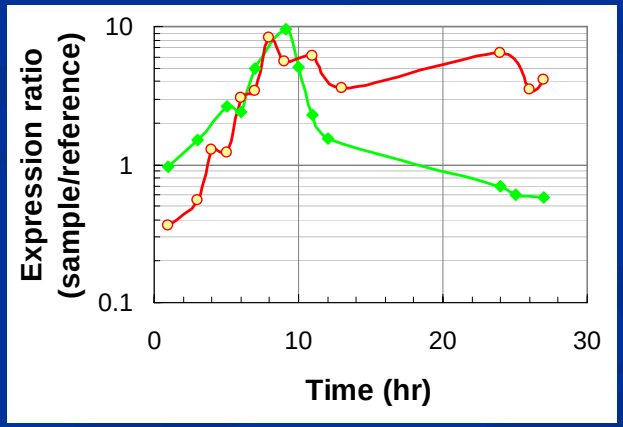
NADH-dependent butanol dehydrogenase II (BDHII)



Alcohol dehydrogenase

Expression of alcohol dehydrogenases:

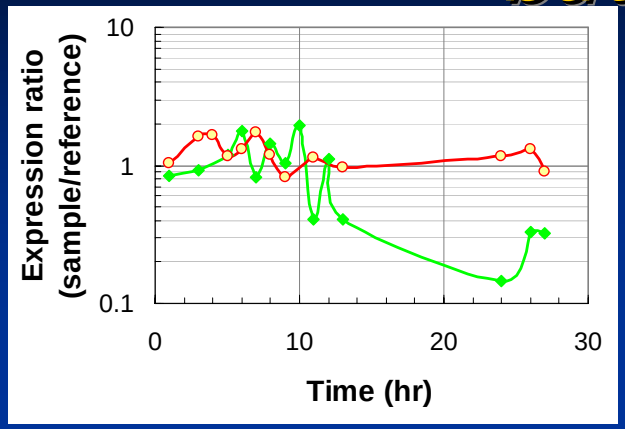
- Increase by ~10 fold to a maximal level during acidogenic phase in wild-type and BA101.
- Expression is at maximal level in BA101 during solventogenic phase, but down-regulated to basal level in wild-type.



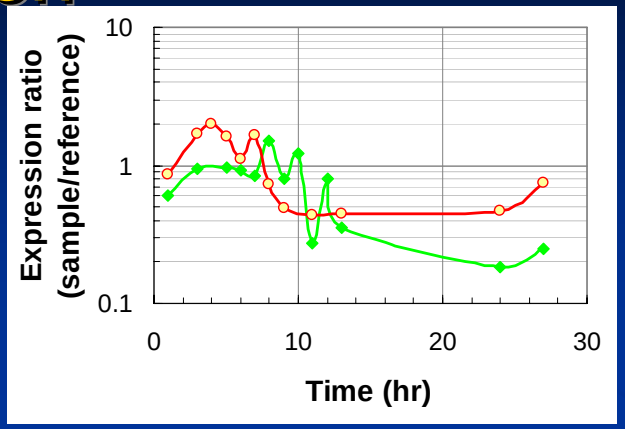
Alcohol dehydrogenase

Wild-type BA101

Solventogenic/acidogenic genes – *ptb-butK* operon

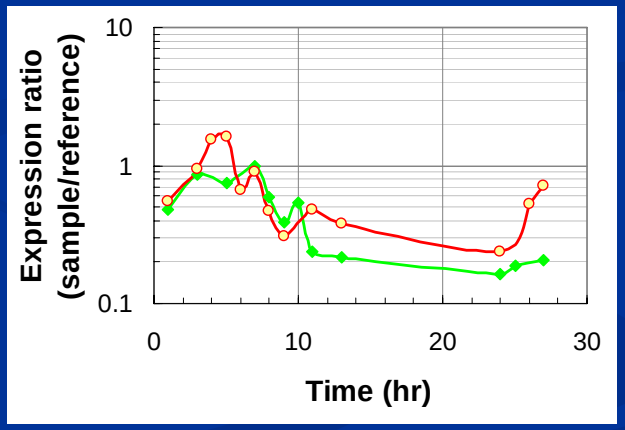


Butyrate kinase (bk)



Acetate kinase (ak)

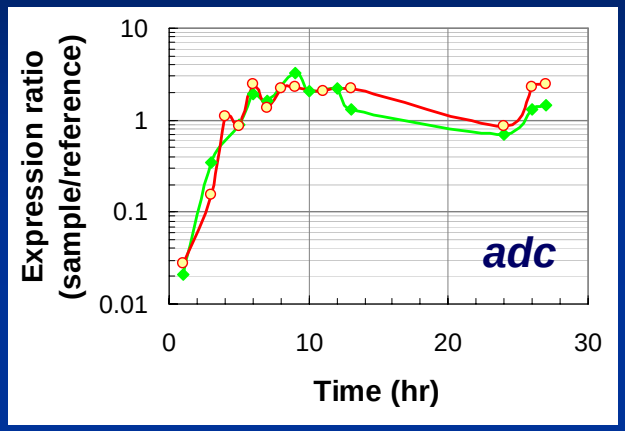
- Expression of bk, ak and PTA is similar during acidogenic phase for both wild-type and BA101, while expression level is higher for BA101 by up to 10 fold during solventogenic phase vs. wild-type.
- Expression remains constant in BA101, but decreases by ~10 fold in solventogenic phase for wild-type.
- Ak and PTA expression is lower during solventogenesis, although less pronounced in BA101 than in wild-type.

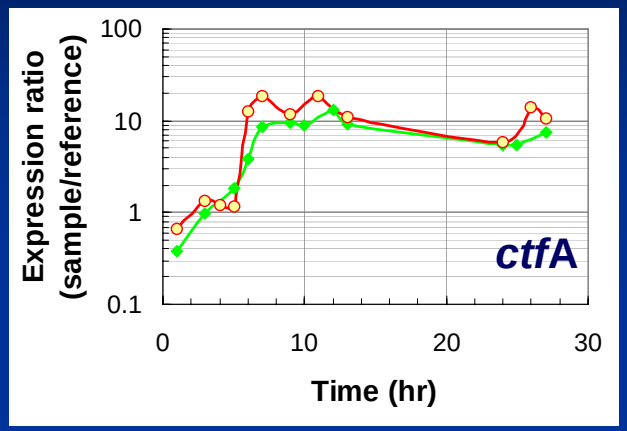


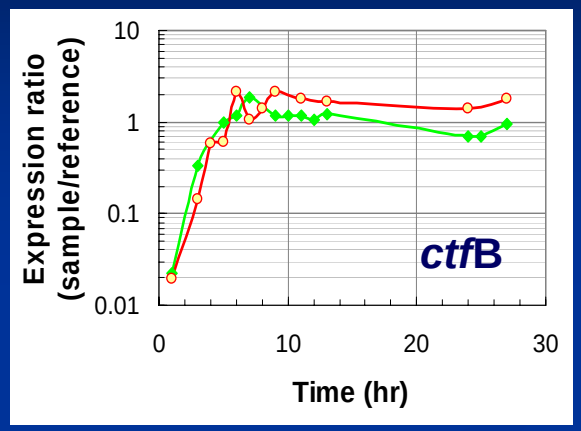
Phosphotransacetylase (PTA)

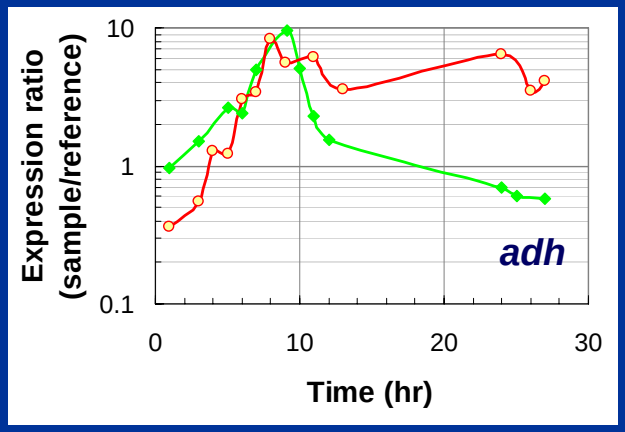
Wild-type BA101

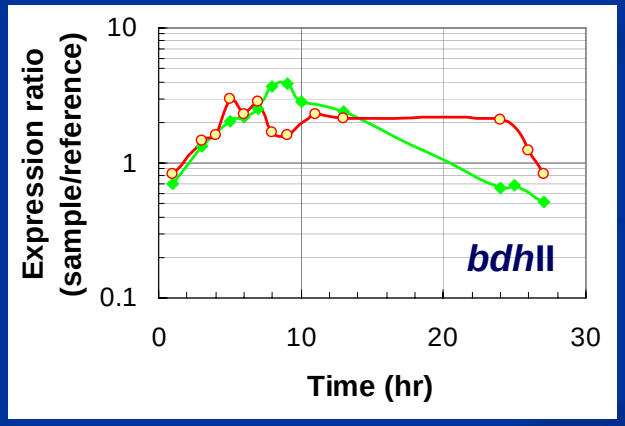
Gene expression for terminal enzymes in solvent formation pathway







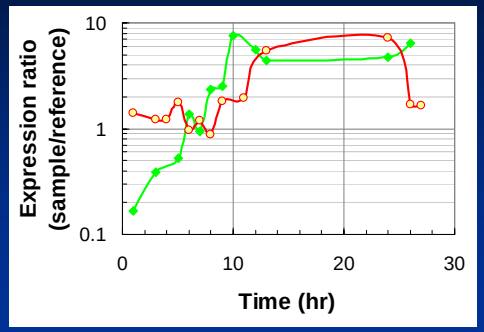




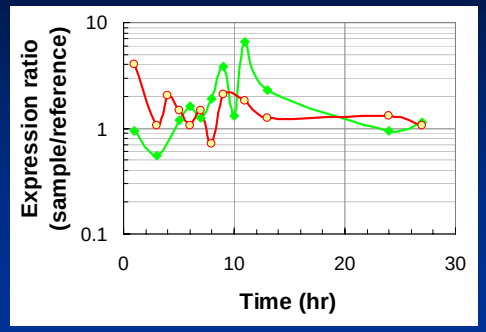
Wild-type

Mutant

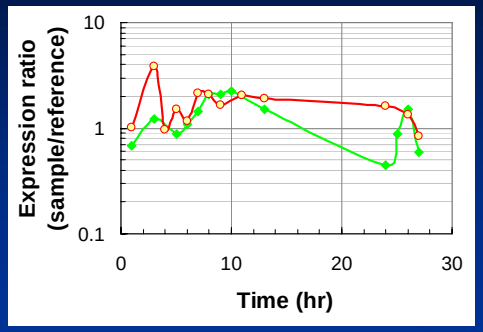
Gene expression for pyruvate conversion to solvents



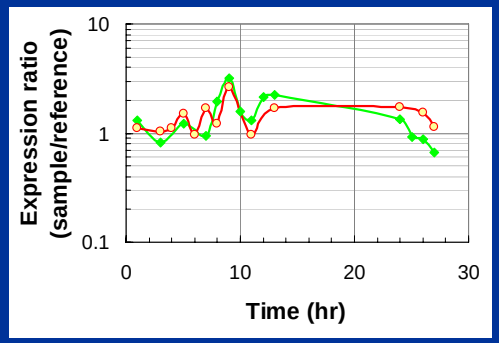
pyruvate ferredoxin oxidoreductase



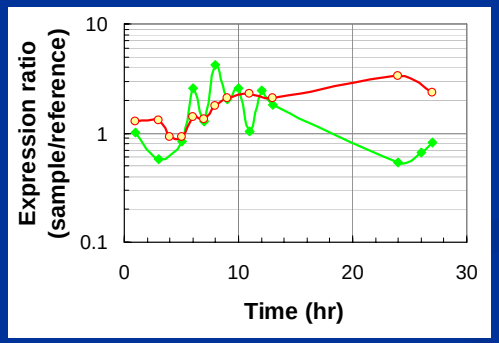
thiolase



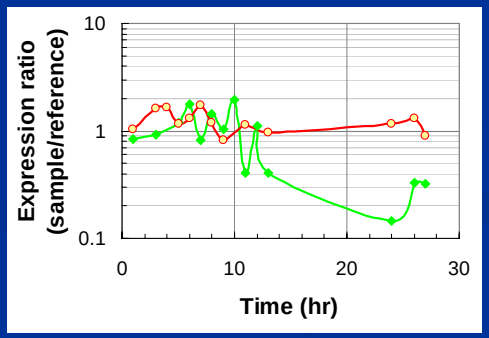
3-hydroxybutyryl-CoA dehydrogenase



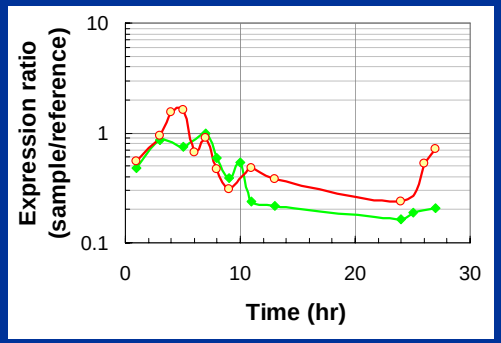
crotonase



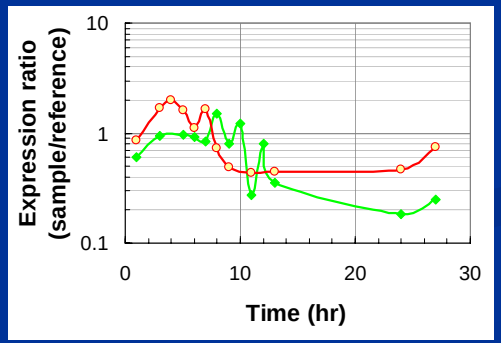
butyryl-CoA dehydrogenase



butyrate kinase



Phosphotransacetylase

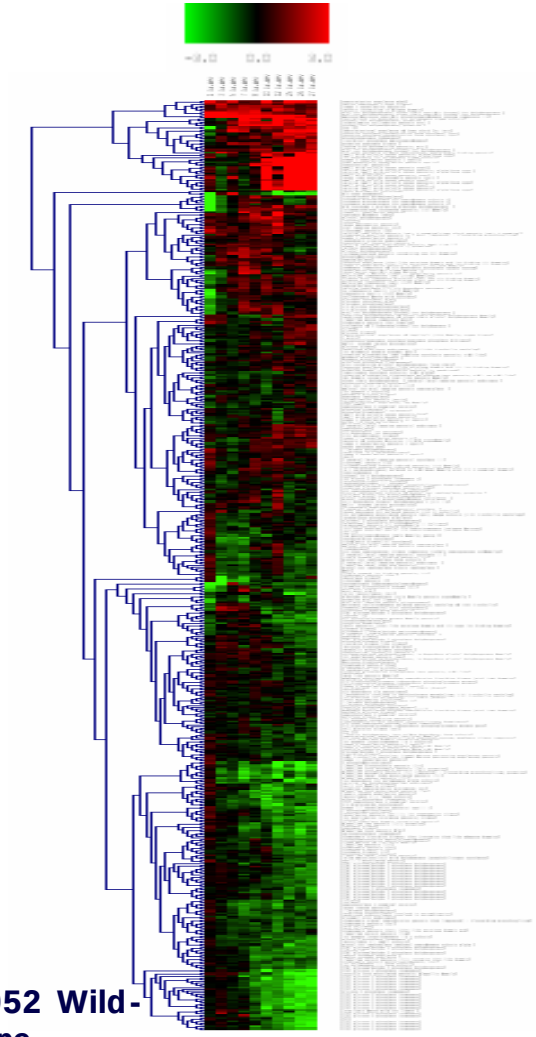


Acetate kinase

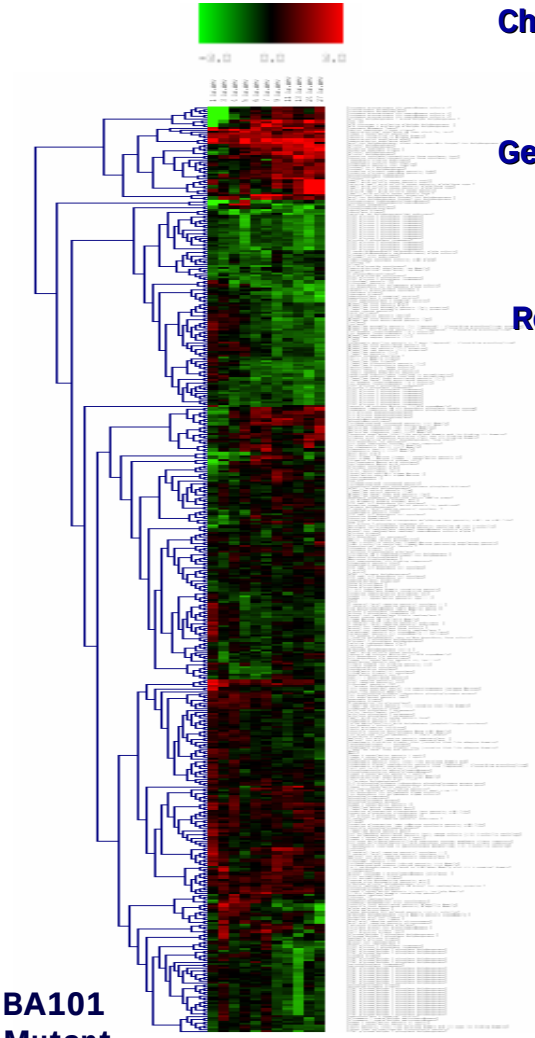
Wild-type

Mutant

Temporal profiling of gene expression during the ABE fermentation by *C. beijerinckii*



8052 Wild-type

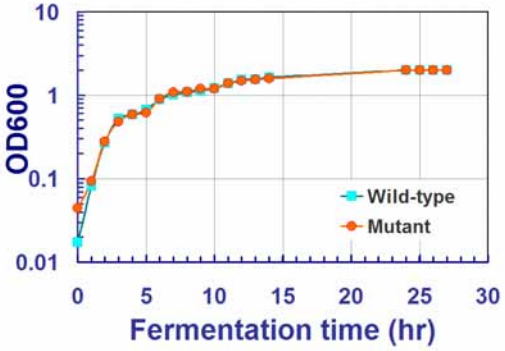


BA101 Mutant

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.



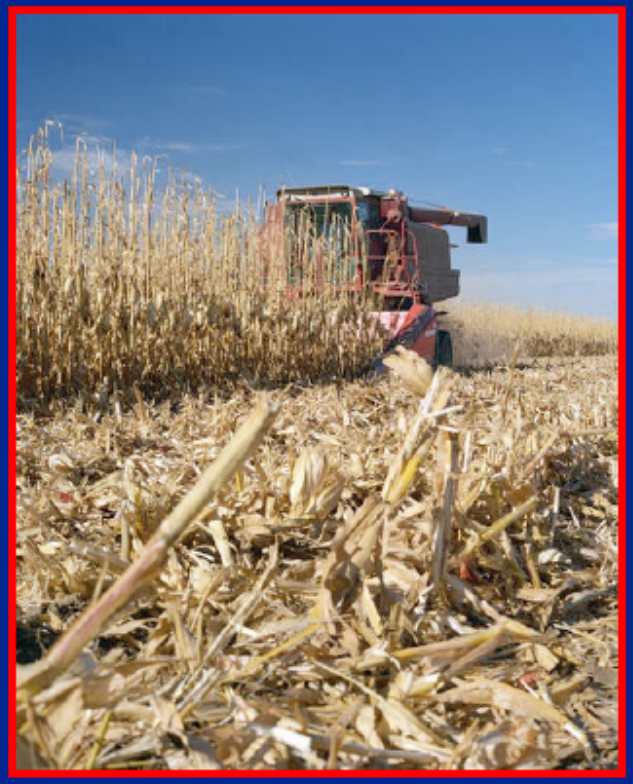
Genomic organization of terminal enzymes in the solventogenic pathway

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

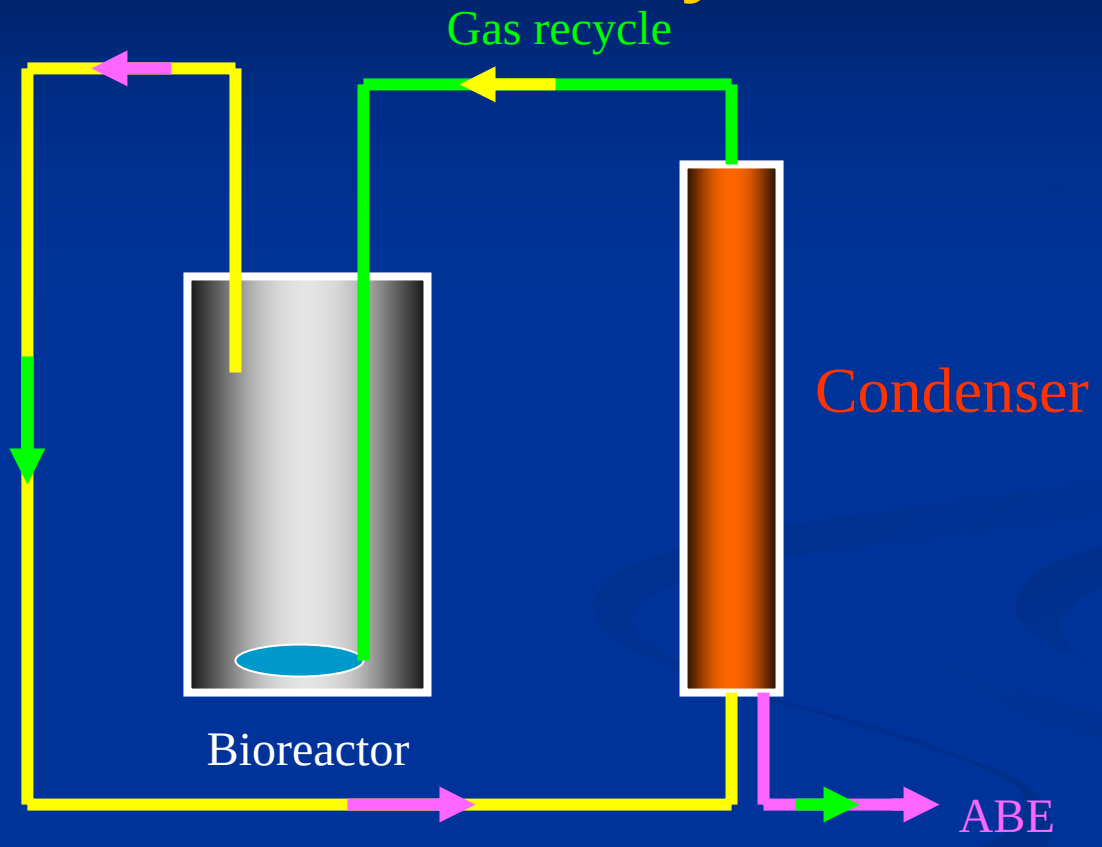
Substrates/Co-Products

- 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

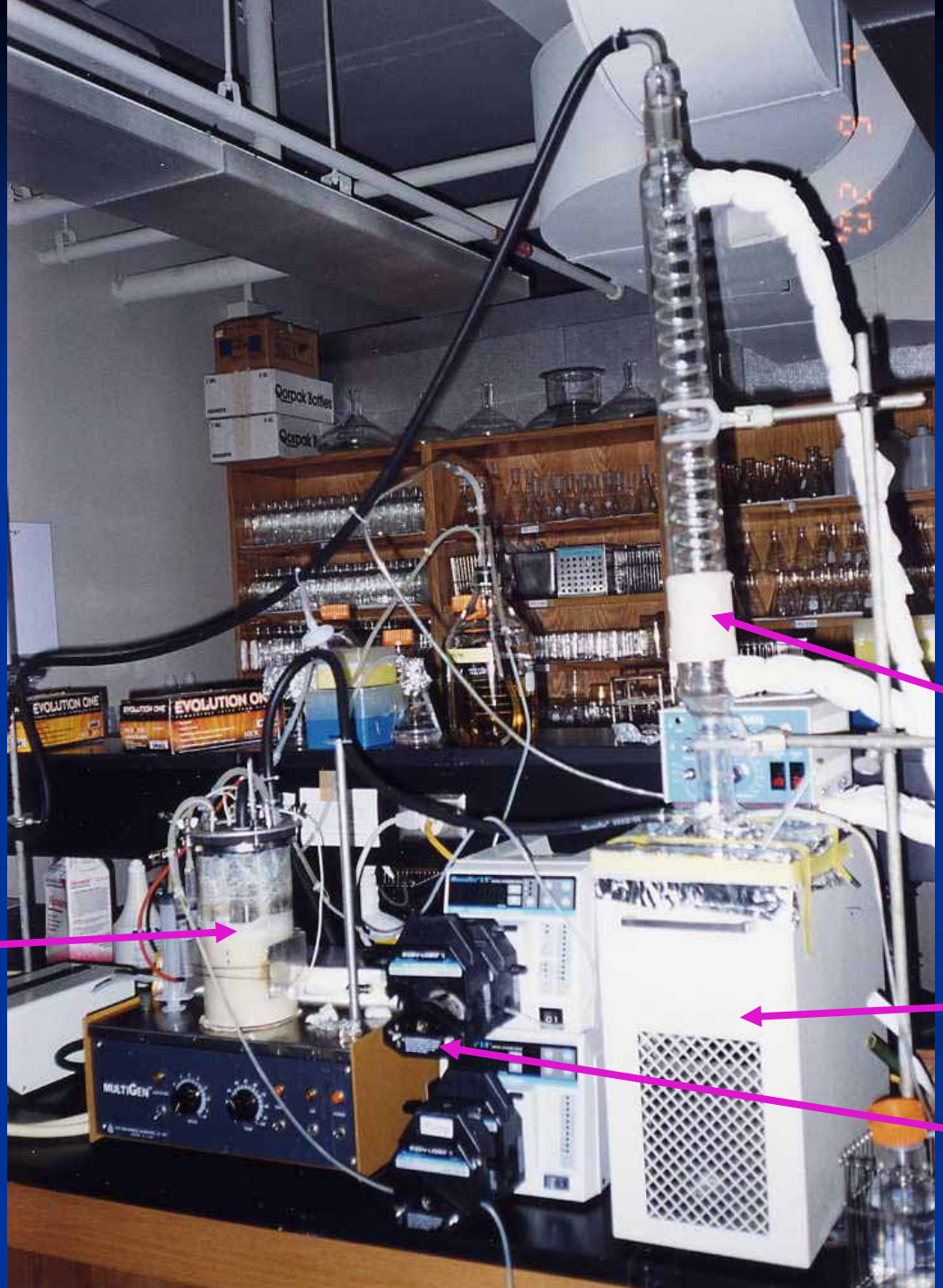
- Eliminate product inhibition (butanol toxicity)
- Achieve high productivity
- Produce concentrated products (acetone-butanol)
- Utilize concentrated substrates - to allow for a reduction in process volume
- Better economics

Gas stripping: Integrated process for production and recovery



Gas is bubbled through the fermentation broth and then circulated though 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



condenser

vessel

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.

  - N. Qureshi and H.P. Blaschek. 2001. ABE production from corn: A recent economic evaluation. J. Industrial Microbiol. & Biotechnol. 27:292-297.

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.