

NIST



Metabolic Engineering for Fuels and Chemicals

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**Metabolic
Engineering**

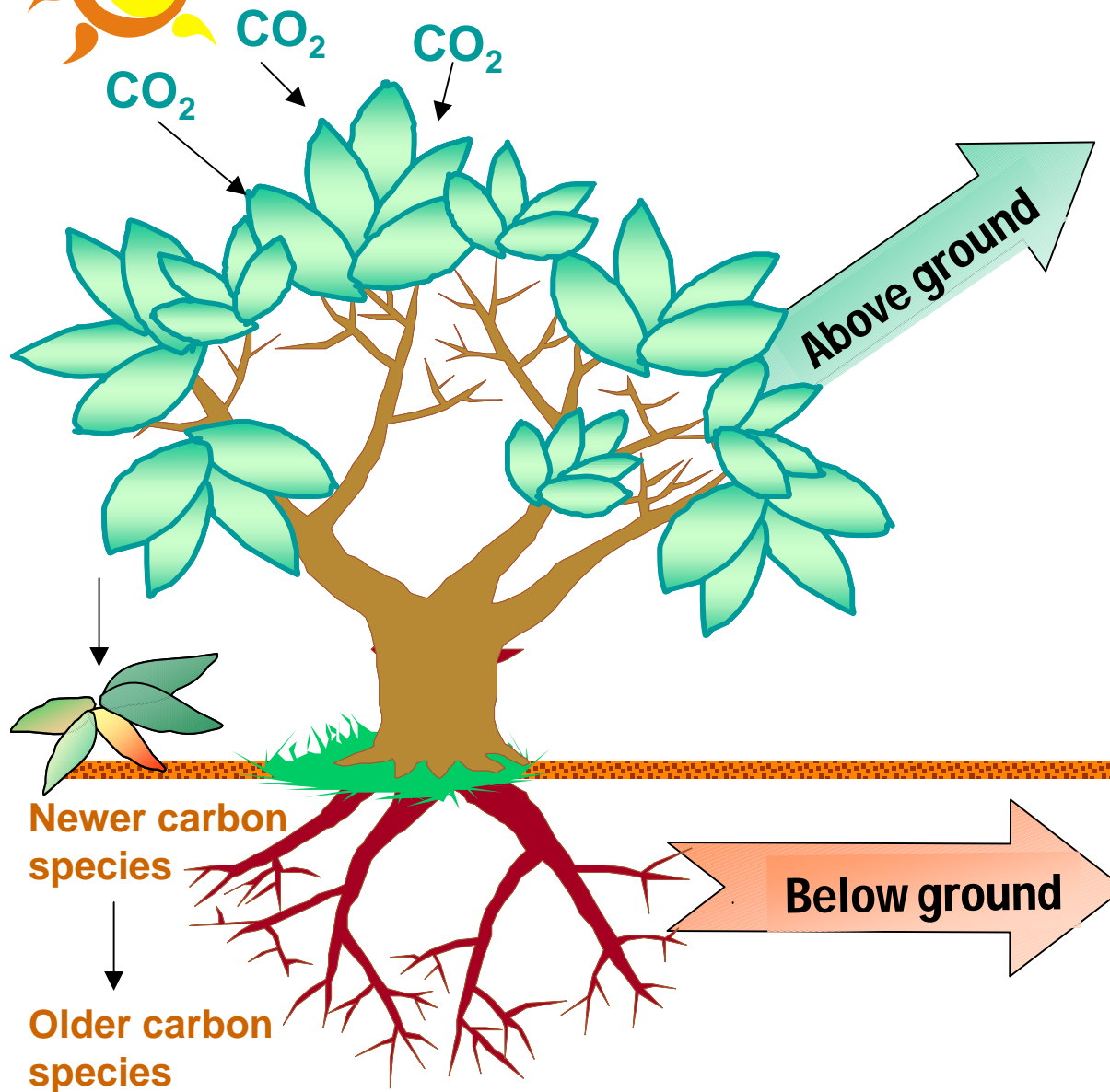


**Renewable
Biomass
to
Chemicals
&
Fuels**

Dr. Lonnie O'Neal Ingram, Director



RENEWABLE FUELS AND CHEMICALS



❖ Displacement of oil

- **Commodity chemicals**
 - polylactic acid
 - solvents
 - acids
- **Fuels**
 - ethanol
 - biodiesel
 - power
- **Rural Employment**

**Carbon
Sequestration
in soil**

PROPOSED BIOMASS-DERIVED COMPOUNDS

- ❖ **Ethanol**
- ❖ **Lactic acid**
- ❖ **Succinic acid**
- ❖ **1,2-Propandiol**
- ❖ **1,3-Propandiol**
- ❖ **Polyhydroxybutyrate**

**Reduced compounds produced
under anaerobic conditions**

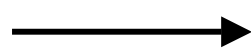
CONVERSION OF LIGNOCELLULOSICS TO ETHANOL

FEEDSTOCK → **PROCESS** → **ETHANOL (CHEMICALS)**

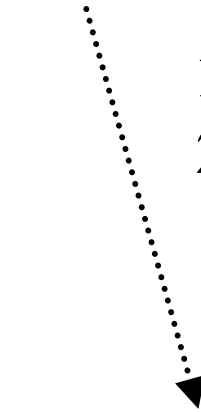
1. **Choice**
2. **Availability**
3. **Cost**
4. **Quality**



Depolymerization



1. **H⁺**
2. **Cellulases**
3. **Hemicellulases**
4. **Inhibitors**



Biocatalyst

1. **Recovery**
2. **Waste Disposal**
Solid
Liquid

Depolymerization

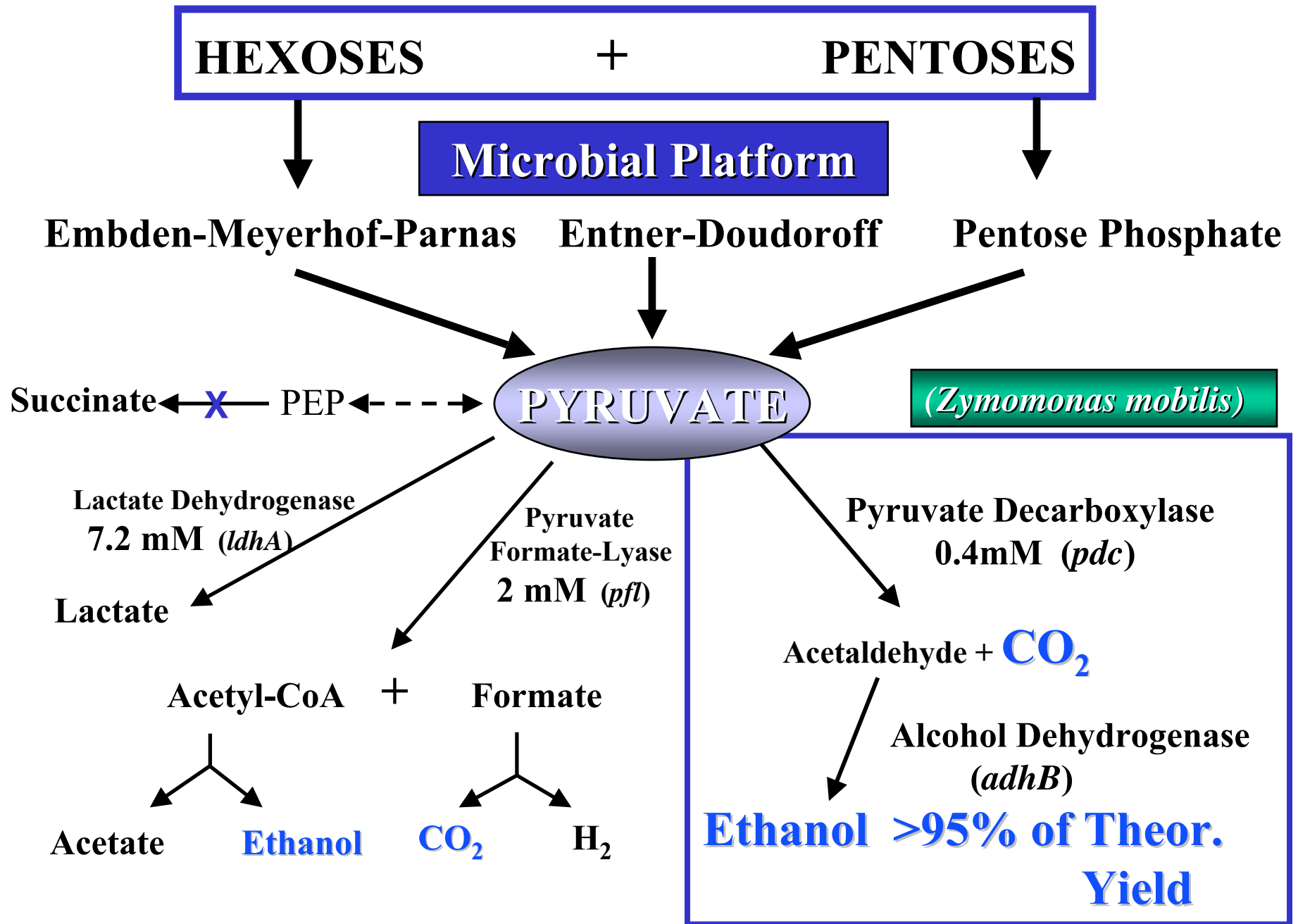
- 1. Cellulose**
Cellulases
Optimize with the Biocatalyst
- 2. Xylose**
Xylanases, Xylosidases
- 3. Glucuronoxylan**
 α -Glucuronidase; Xylosidase
- 4. Acid Hydrolysis**

BIOCATALYST

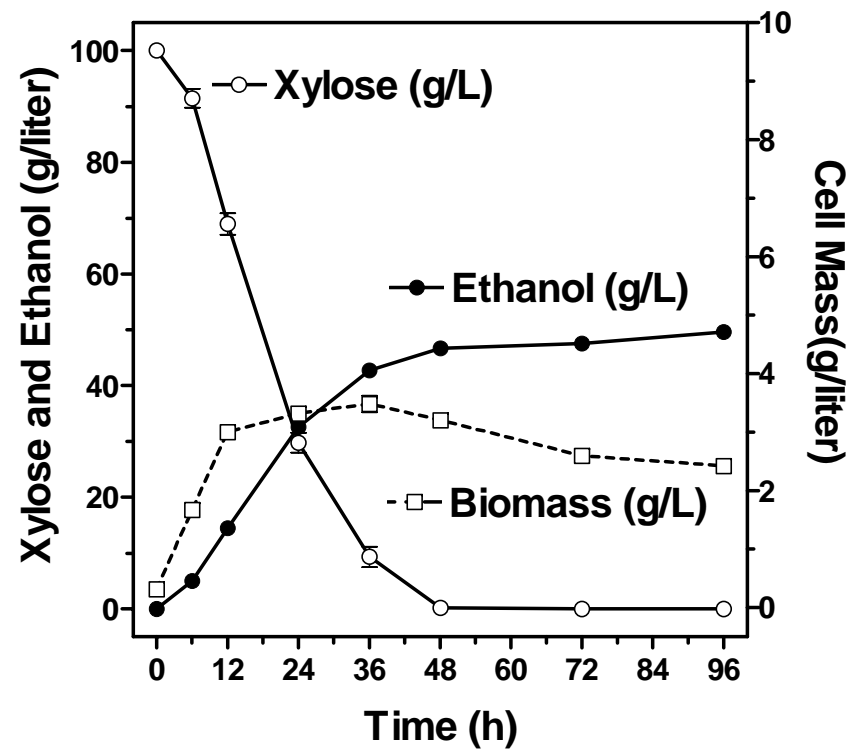
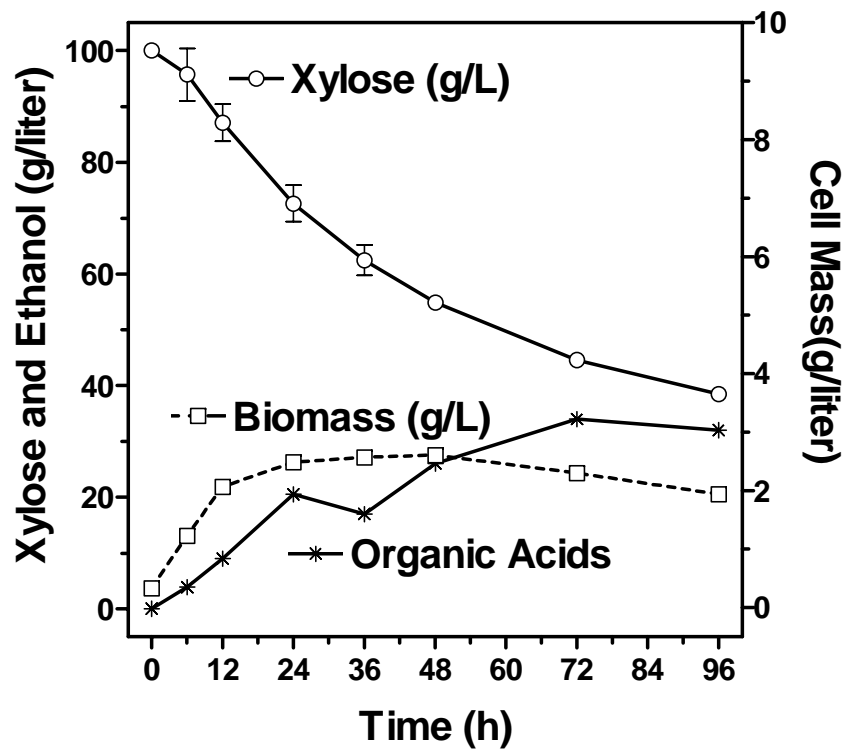
- 1. High Growth Rate**
- 2. High Cell Yield**
- 3. High Product Yield**
 - Volumetric Productivity**
 - Specific Productivity**
- 4. Purity of the Product**
 - Optical**
 - Chemical**
- 5. Minimal Growth Requirements**
- 6. Metabolic Versatility**
- 7. Co-utilization of Various Sugars**
- 8. Tolerate High Sugar Concentration**
- 9. Resistance to Inhibitors**
- 10. Insensitive to Product Inhibition**
- 11. High-value Co-products**
- 12. Amenable to Genetic Engineering**
- 13. Robust**
- 14. Cellulases**
- 15. Xylan degradation**

***E. coli*: Potential Industrial Platform for Renewable Fuels and Chemicals**

- 1. Safety, reliability, and industrial experience.**
- 2. Uses broad range of sugars derived from biomass (hexose, pentose, sugar alcohol and sugar acid; expanded to – cellobiose and xylobiose).**
- 3. Simple nutrient requirements.**
- 4. Well understood physiology and established tools for genetic manipulation.**



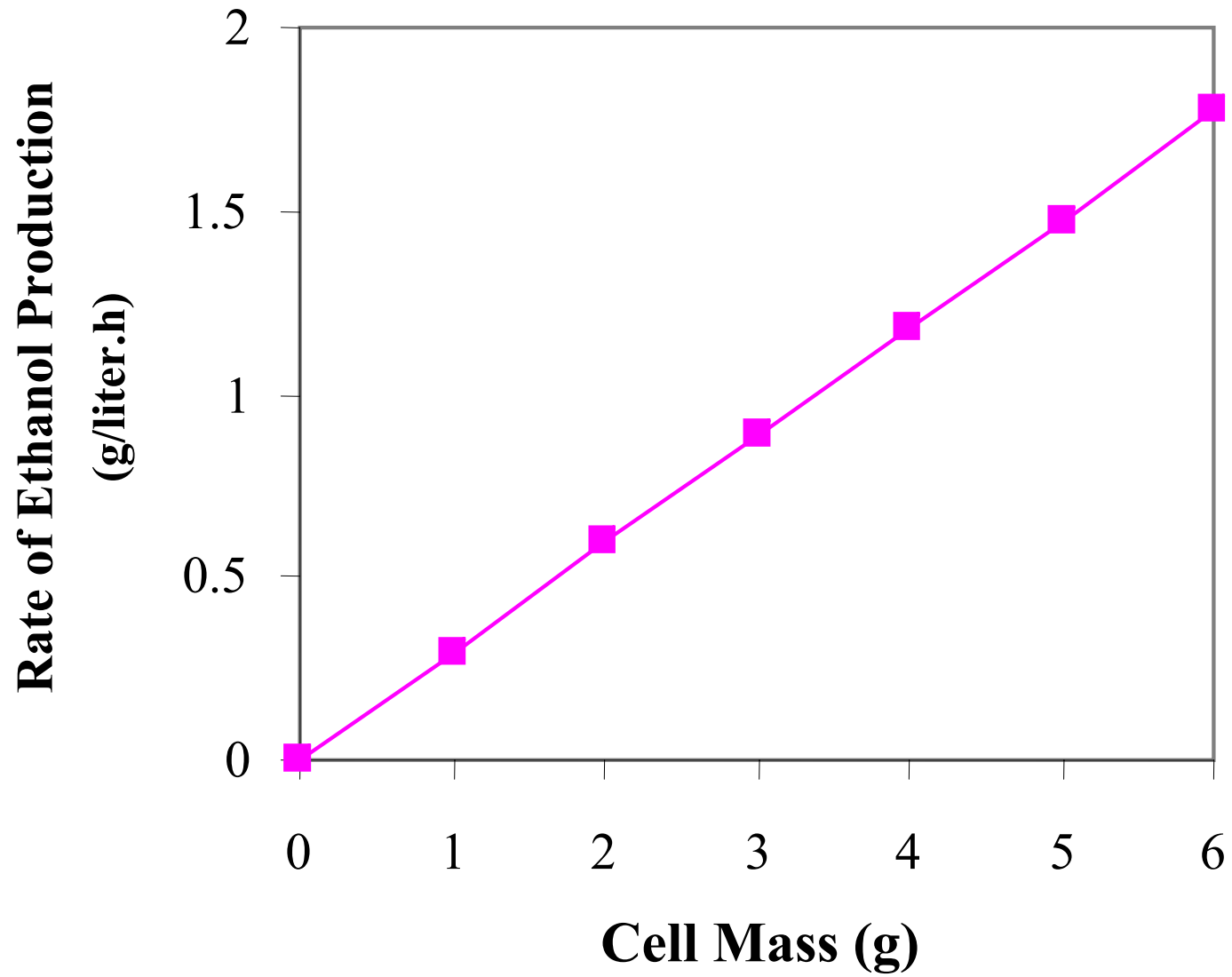
***E. coli* B (organic acids) and KO11 (ethanol)**



Yield – 0.50 g ethanol and 0.49 g CO₂ per g xylose

(10% Xylose, pH 6.5, 35C)

PRODUCTIVITY IS RELATED TO CELL MASS



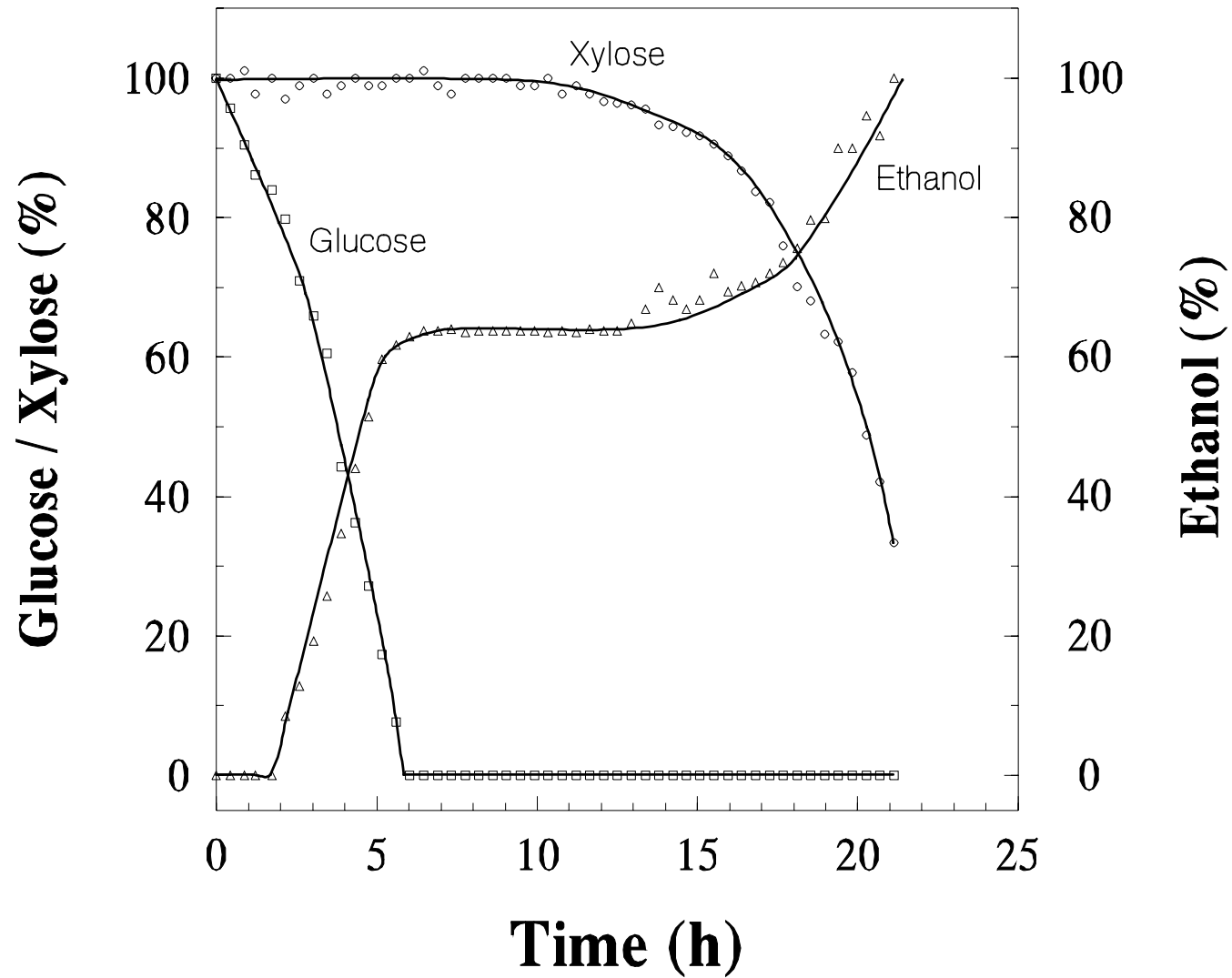
SUGAR UTILIZATION and SSCF

CELLULOSE: GLUCOSE

HEMICELLULOSE: XYLOSE

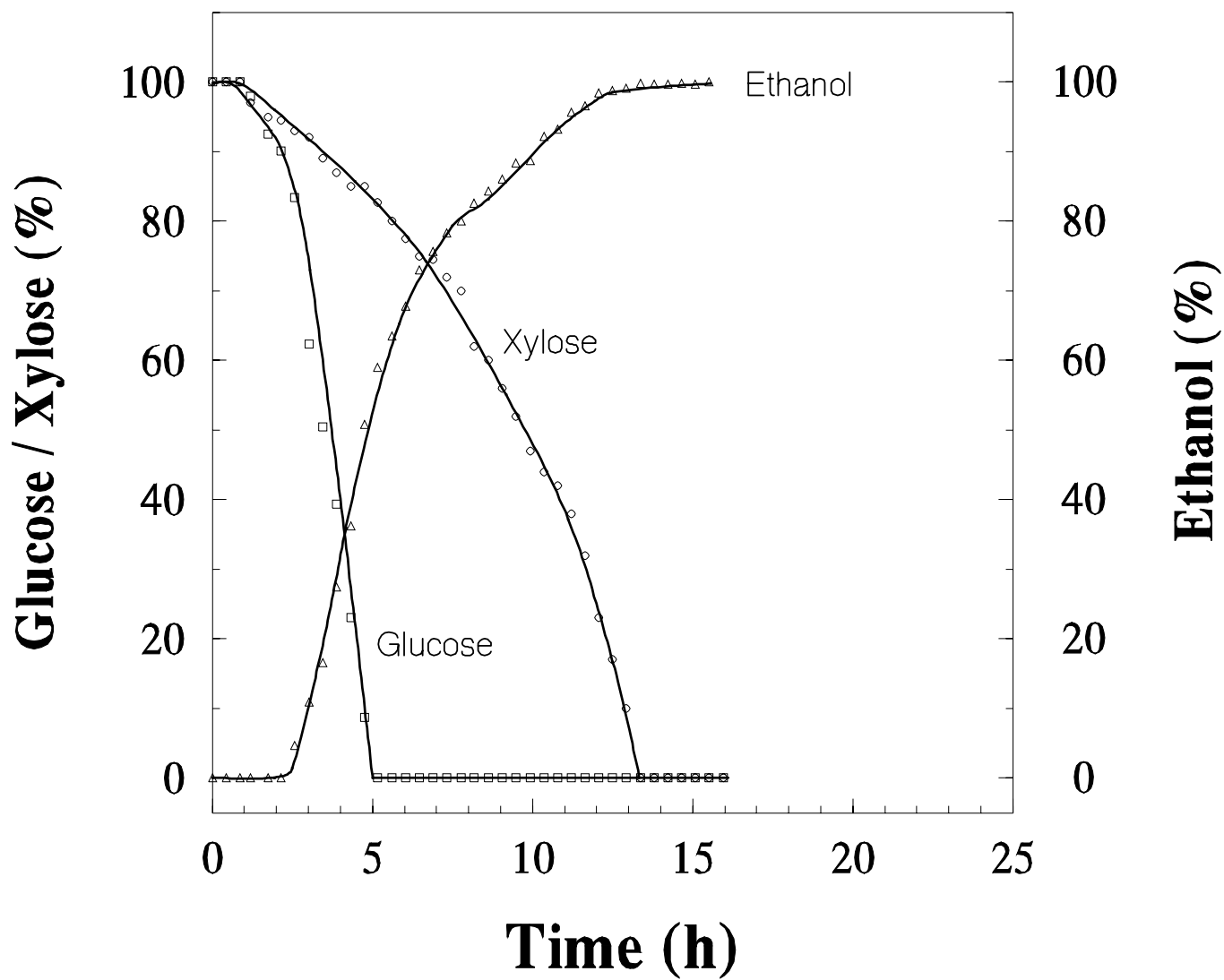
SEQUENTIAL – Catabolite Repression
SIMULTANEOUS

E. coli B (pLOI555)



Culture was grown with $^{13}\text{C}_1$ - glucose and $^{13}\text{C}_1$ - xylose at 37C in the NMR without pH control.

E. coli KO11

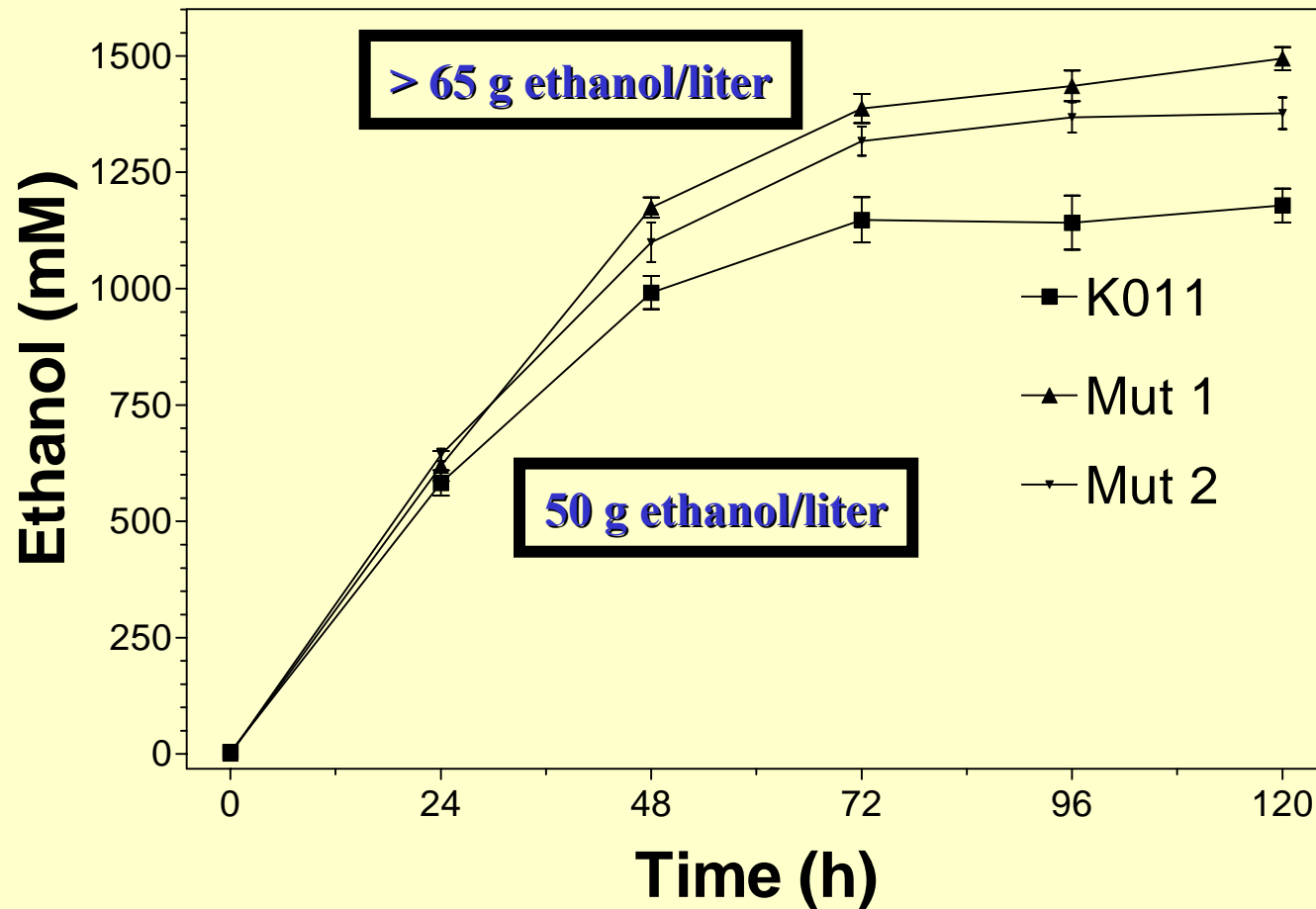


TOLERANCE TO HIGHER LEVEL OF ETHANOL

Higher Product Yield

Lower Product Cost

Ethanol Tolerance: Mutants reach over 6.5% w/v ethanol (14% xylose, 35C, pH 6.5, 100 rpm, Luria Broth)



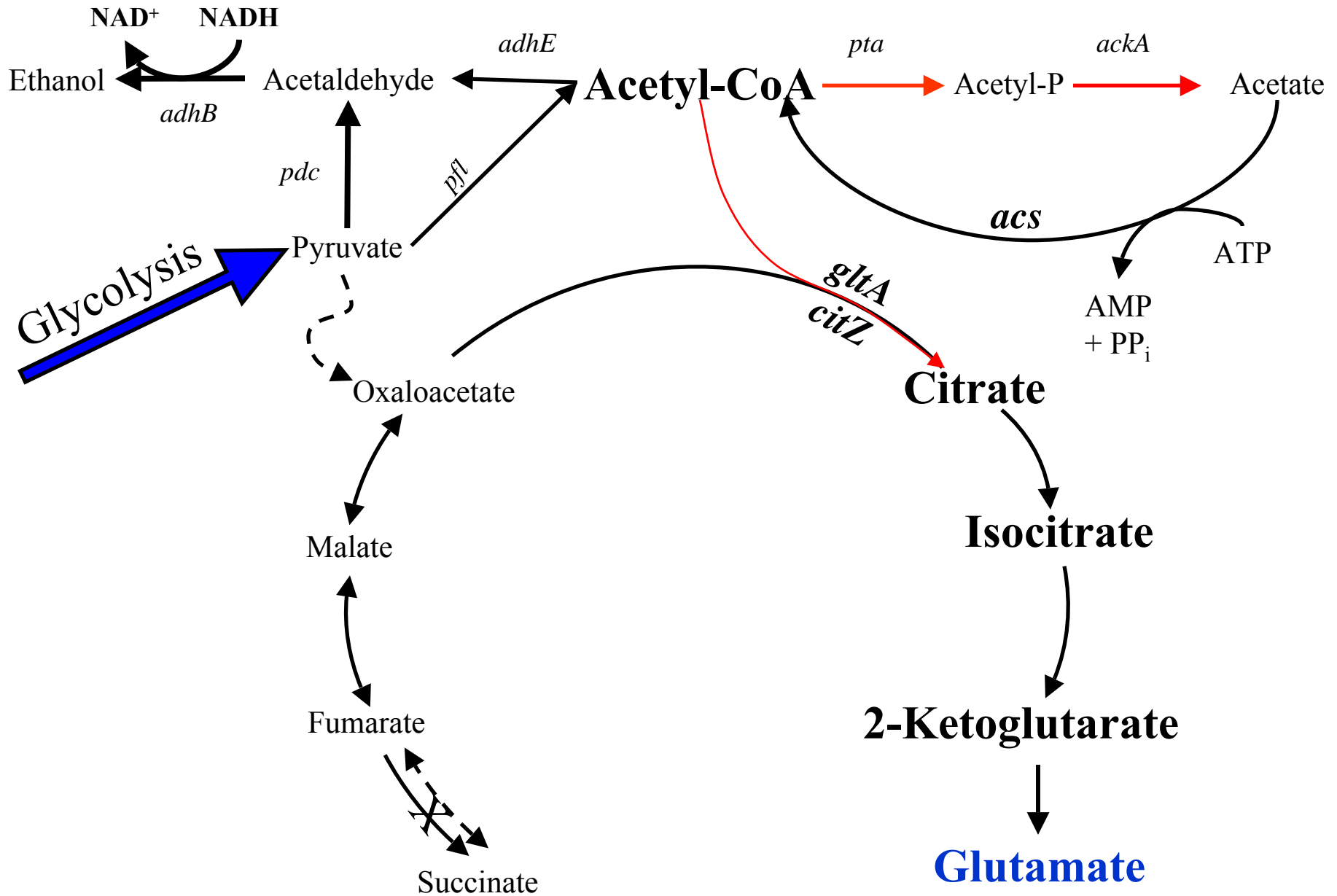
FERMENTATIONS AT HIGH SUGAR CONCENTRATIONS

Expect: Higher Product Yield

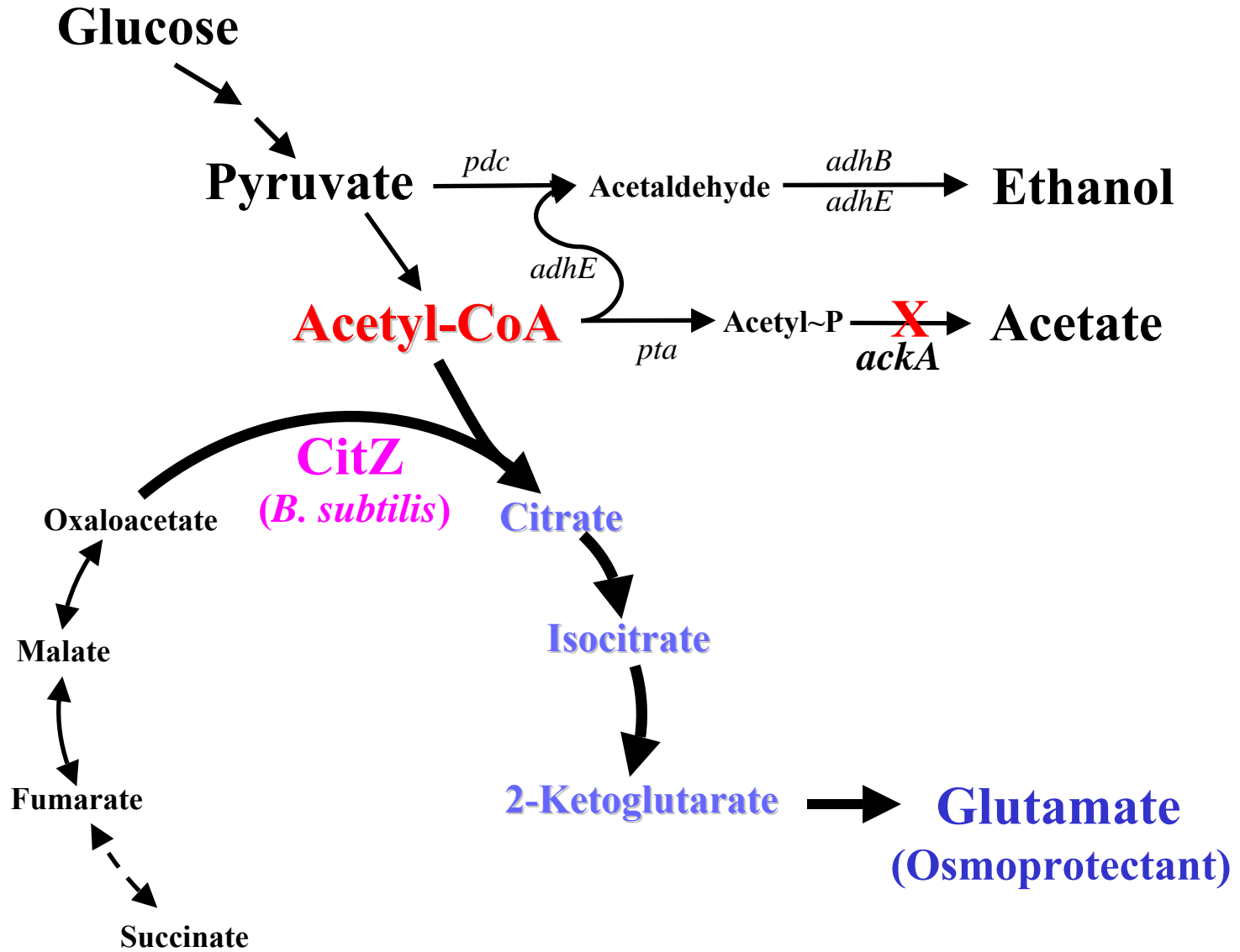
Observed: Lower Growth Rate and Cell Yield of KO11

Cause: Osmotic Effect

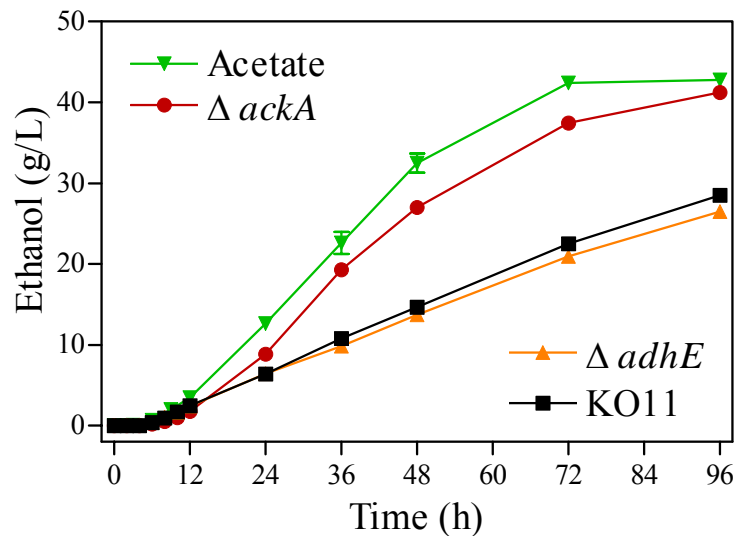
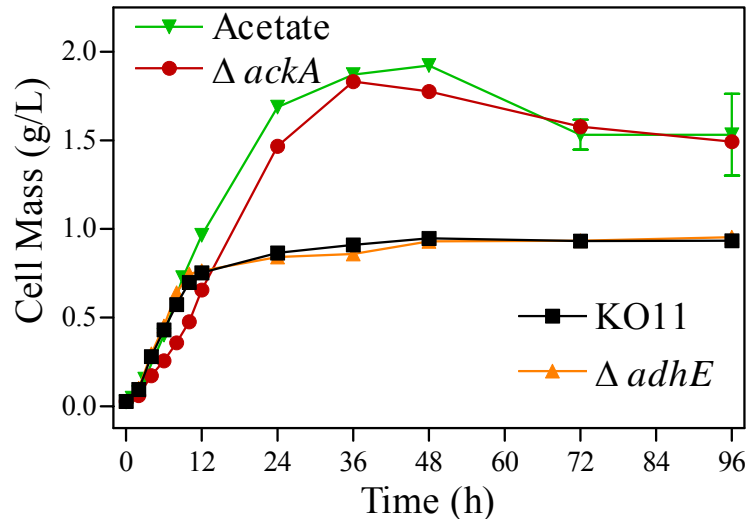
Limiting Acetyl-CoA Pool



Genetic solution



Fermentations with $\Delta ackA$ and $\Delta adhE$



- Deletion of *ackA* eliminates conversion of acetyl-CoA to acetate.
- This resulted in a stimulation of growth and ethanol production similar to acetate supplementation.
- Ethanol yield by $\Delta ackA$, 0.47 g/g total xylose (92%).
- Average volumetric productivity for $\Delta ackA$ increased (0.57 g/L/h), compared to KO11 (0.33 g/L/h).
- Average specific productivity for $\Delta ackA$ (0.38 g/g/h), similar to KO11 (0.36 g/g/h).
- The combination ($\Delta ackA \Delta adhE$) was no better than the $\Delta ackA$.

***E. coli* Citrate Synthase**

Inhibited by NADH & 2-ketoglutarate

70% inhibition at 50 μ M NADH and 0.16mM Acetyl-CoA

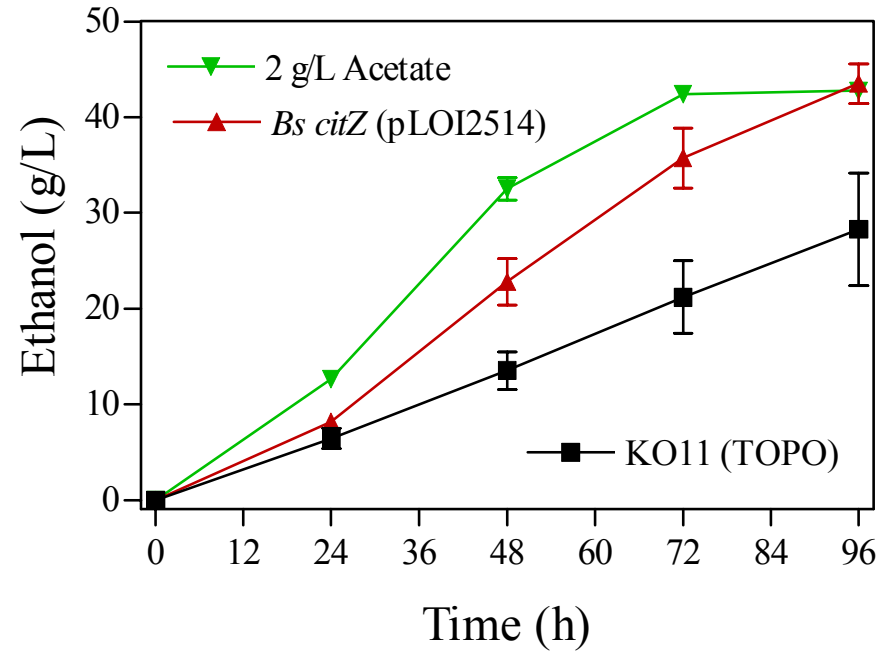
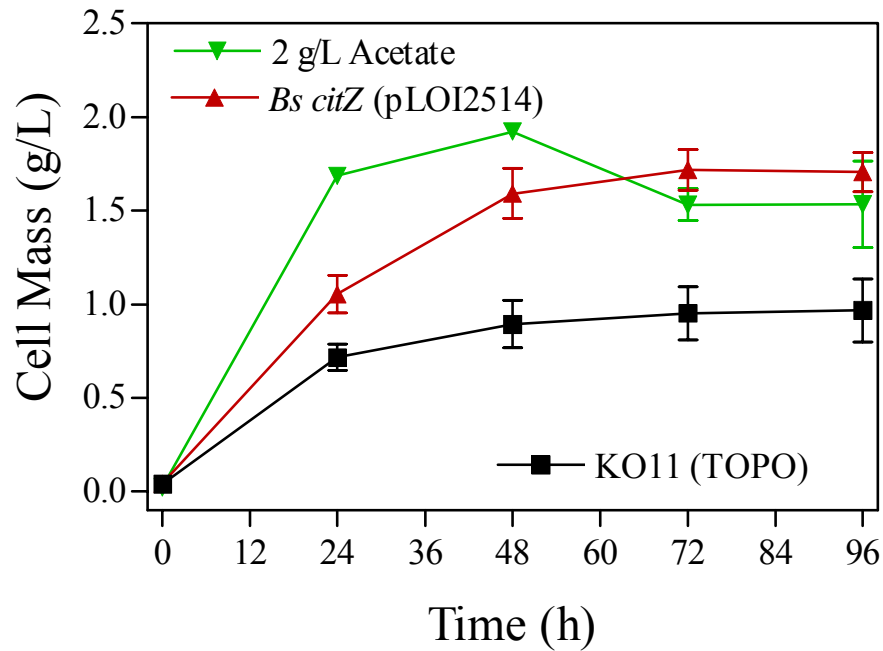
(Weitzman, PDJ. 1966. Biochim. Biophys. Acta 128:213-215)

***B. subtilis* Citrate Synthase**

Inhibited by ATP

2 mM NADH – No effect

Expression of *B. subtilis* *citZ* in KO11



Sugars, Oligosaccharides

**Microbial Zoo
(*E. coli*)**

**Ethanol
& other
products**



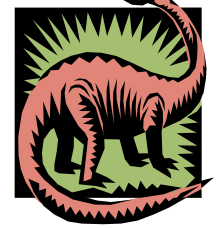
Erwinia
~33kb secretion genes
2 cellulases
pectate lyase



Klebsiella
2 PTS cellobiose genes
2 xylobiose genes



Bacillus
citrate synthase

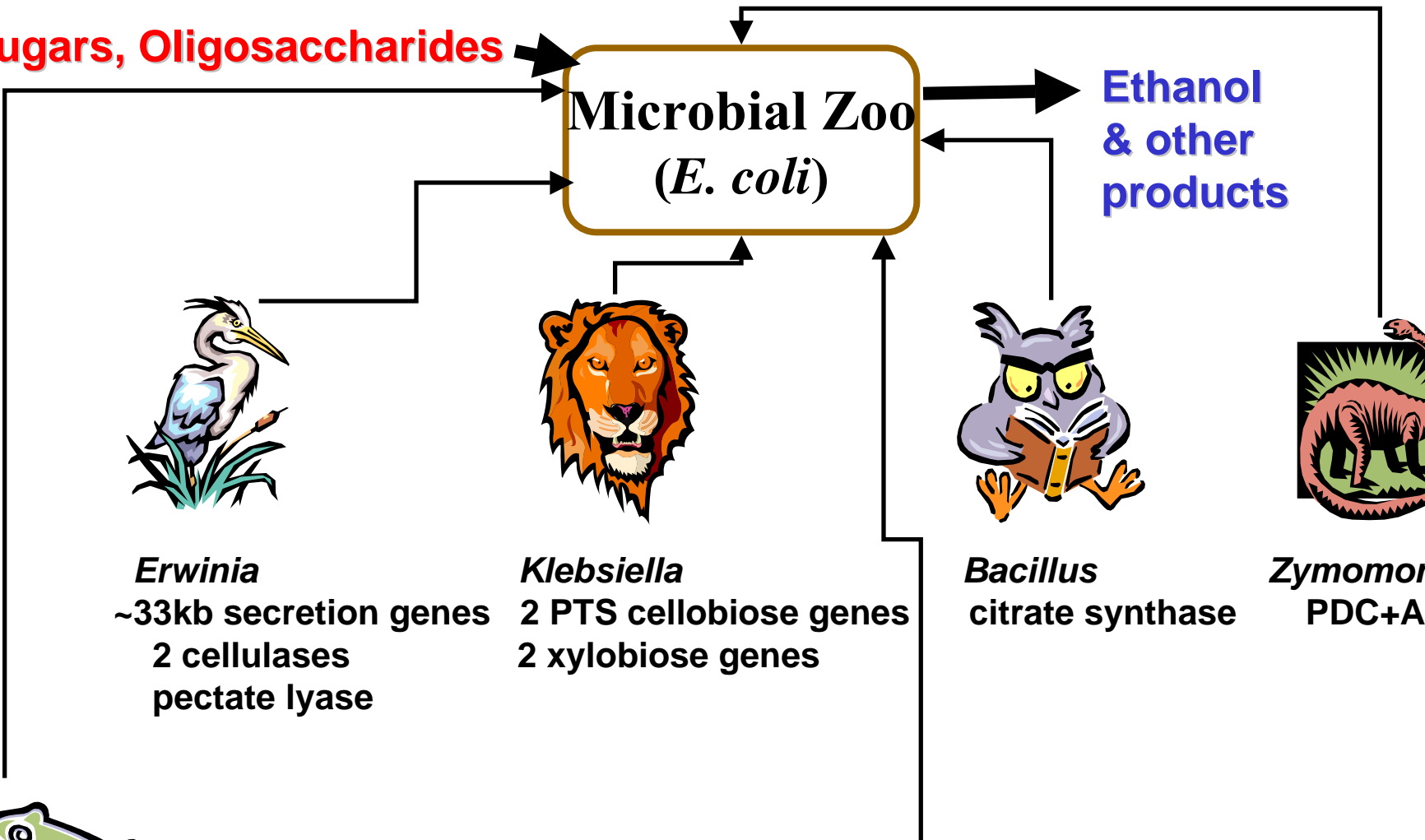


Zymomonas
PDC+ADH



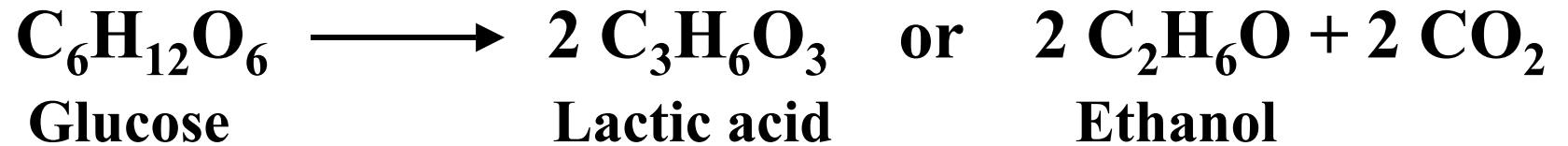
Pseudomonas
esterase for
ethyl acetate

**Who knows what
the future will bring?**



PRODUCTION OF OXIDIZED COMPOUNDS

Anaerobic: Redox Neutral or Reduced Compounds



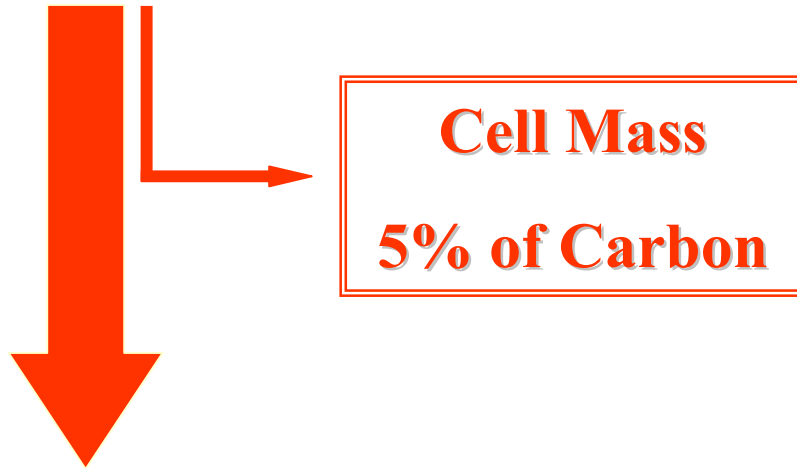
Aerobic: Oxidized Compounds



Overview of Metabolism in *E. coli*

Anaerobic

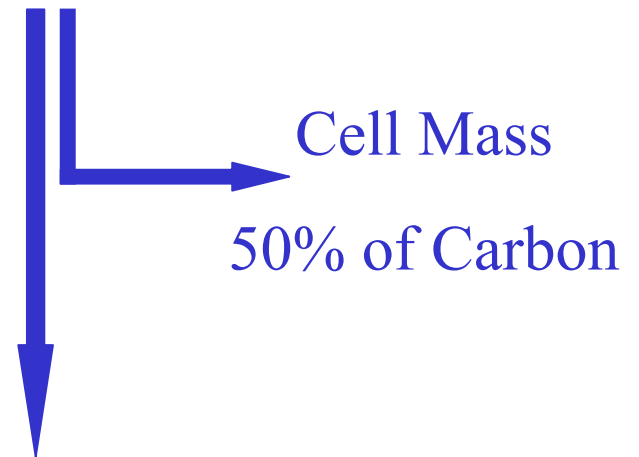
Glucose, $C_6H_{12}O_6$



- Up to 95 % of carbon converted to products (low CO₂ production)
- 2.5 ATP produced
- Low growth rate
- Internal electron acceptor

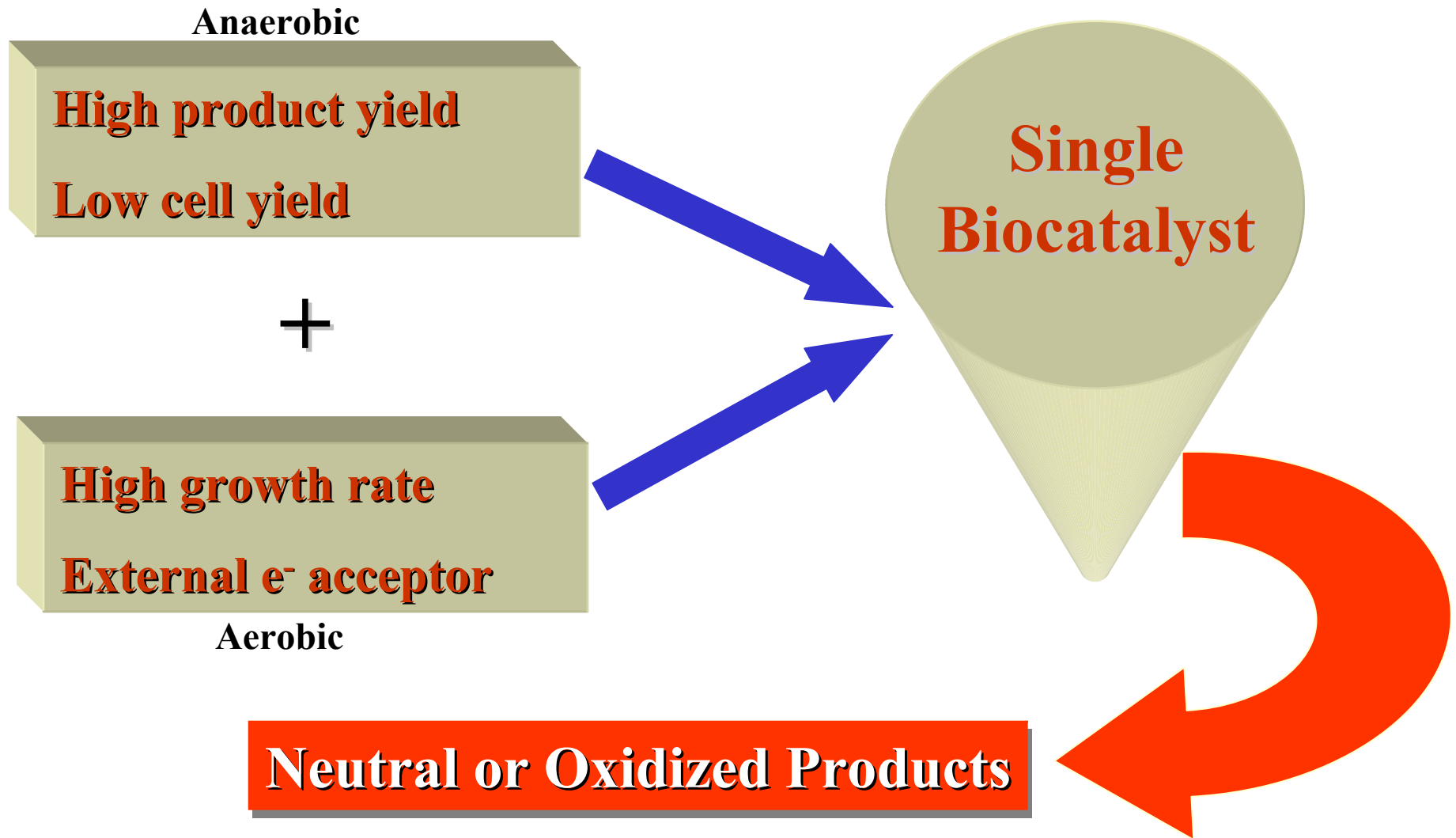
Aerobic

Glucose, $C_6H_{12}O_6$

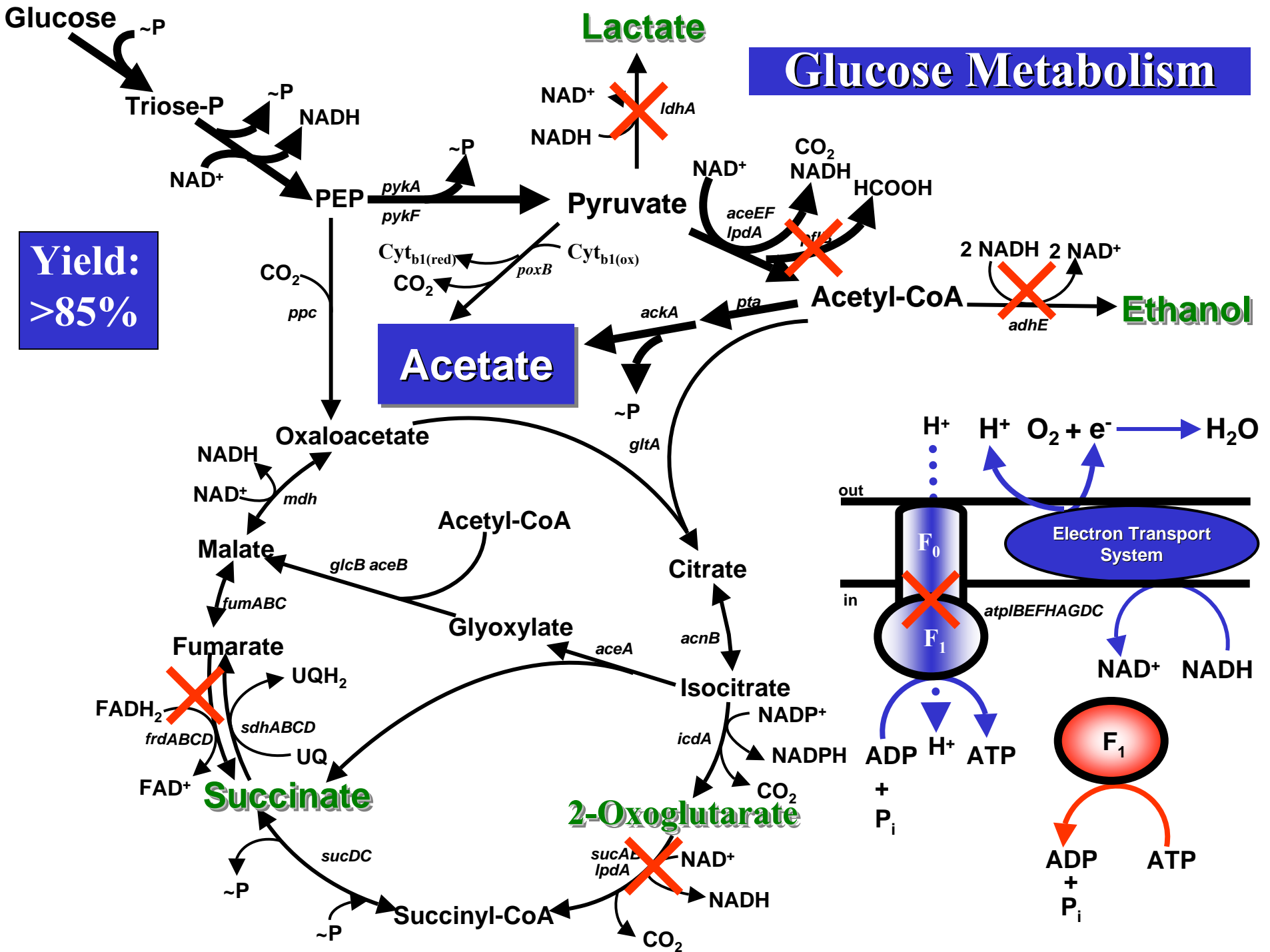


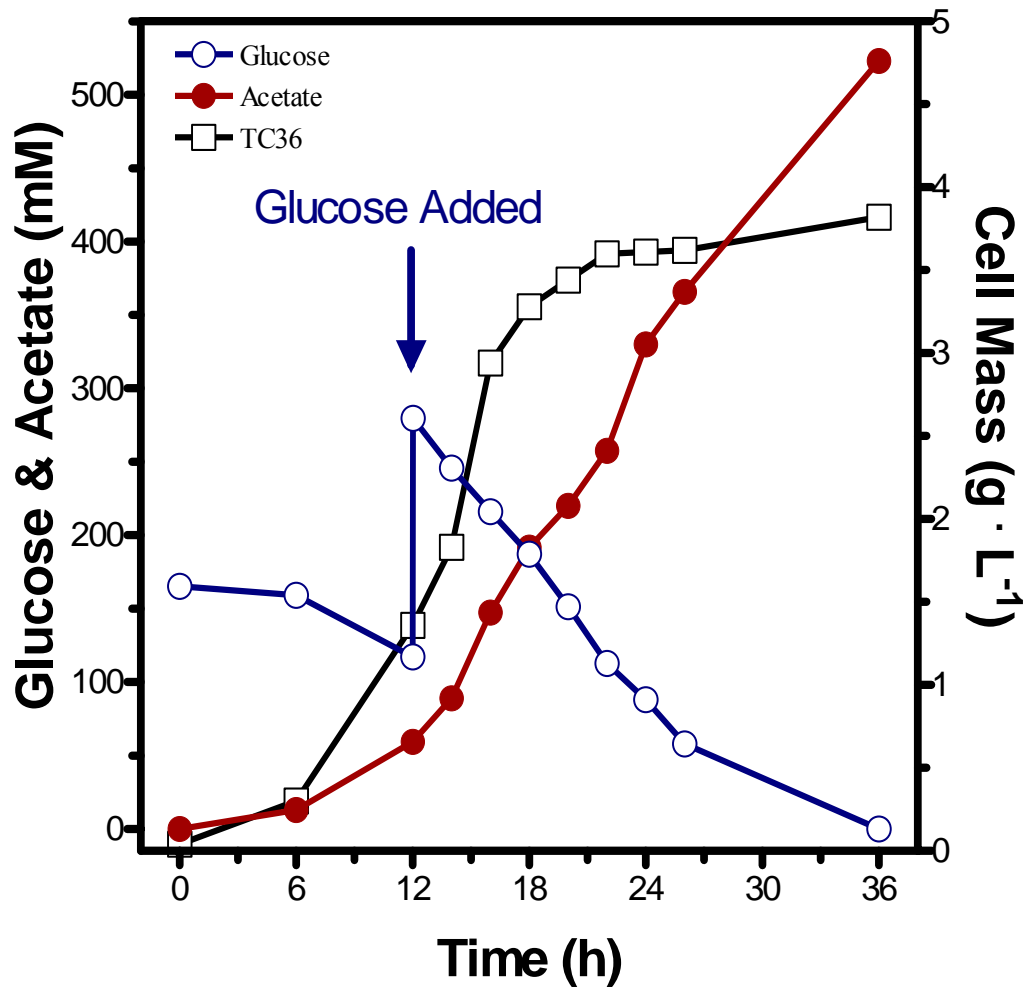
- 50% of carbon converted to CO₂
- 33 ATP (calc.) produced
- High growth rate
- External electron acceptor

Goal: Combine the Attributes of Aerobic & Anaerobic Metabolism



Glucose Metabolism





NEW RESEARCH AREAS

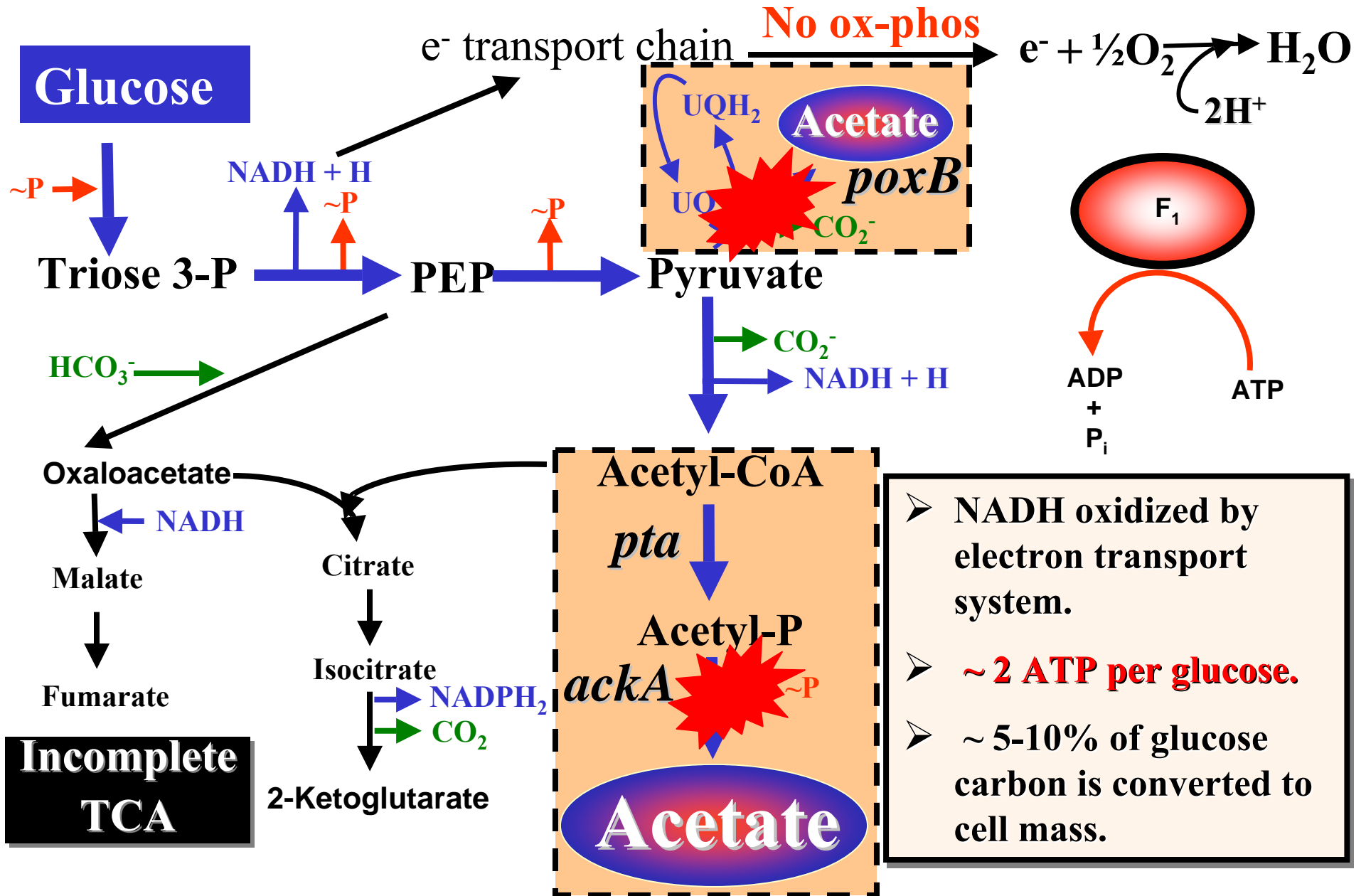
- Limits for Glycolytic Flux?
- Control of Carbon Partitioning?
- Limits for Growth Rate?
- Maximum Cell Density?

Isogenic Strains:

(Mixed acid, ethanol, lactate, acetate, pyruvate, glutamate, succinate, alanine, citrate)

- ATP/ADP?
- NADH/NAD?
- Metabolomics
- Proteomics
- Transcriptome Analysis

Engineered *E. coli* TC44 Metabolism



- **NADH oxidized by electron transport system.**
- **~ 2 ATP per glucose.**
- **$\sim 5-10\%$ of glucose carbon is converted to cell mass.**

BIOCATALYST

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Future Studies



BioRefinery

Gene Array Investigations:

Global regulators for carbon metabolism
(mutations in *mlc*, *crp*, *csrA*)

Global regulators for redox control
(mutations in *fnr*, *arcA*)

Prolonging the growth phase and metabolism
(comparing ethanol/lactic acid)

Improvements for Ethanol and Other Chemicals:

Ethanol tolerance, Process simplification

Carbon partitioning/production costs

Rates and yields

Cellulases, cellobiose/triose; Xylanases, xylobiose/triose

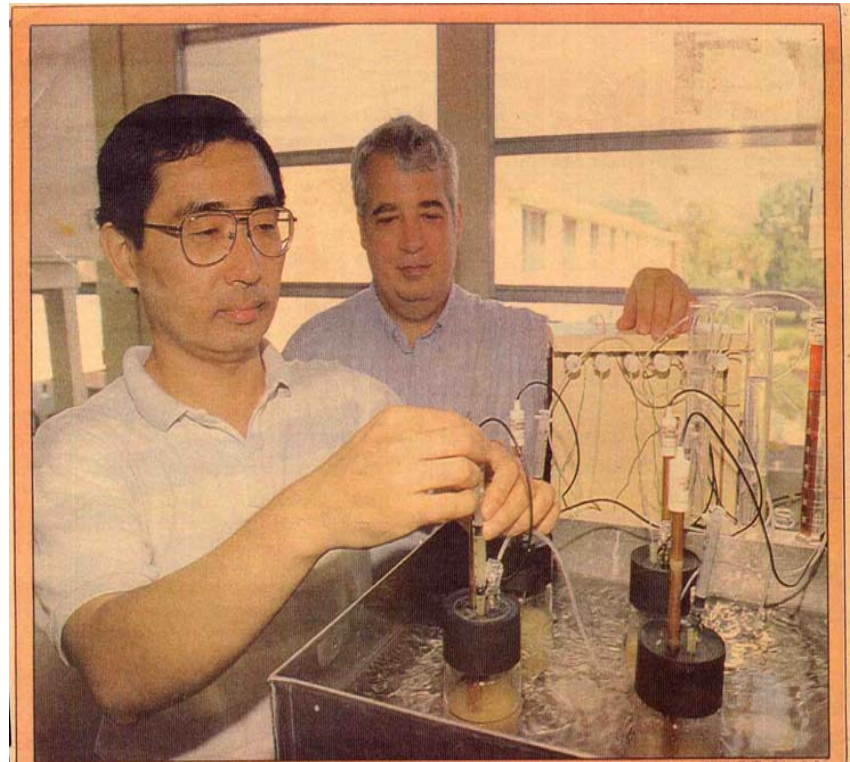
Metabolic Engineering for Higher Value Products:

L(+)-lactic acid and D(-)-lactic acid

Acetic acid, pyruvic acid, succinate, glutamate, citrate

Dependence on petroleum remains as the single most important factor affecting the world distribution of wealth, global conflict, human health, and environmental quality.

Reversing this dependence would increase employment, preserve our environment, and facilitate investments that improve the health and living conditions for all.



Lonnie O. Ingram watches as Kazuyoshi Ohta, a visiting professor from Japan, works in the Metabolic Engineering Lab.

Crisis in Kuwait can turn up the heat on ethanol research

By GARY KIRKLAND
Sun staff writer

When Iraq overran Kuwait, immediately America's attention turned to the gas pump. And the interest in alternative fuels again began to rise.

"As the price of oil goes up," says University of Florida and Institute of Food and Agricultural Sciences professor Lonnie O. Ingram, "the economics become more favorable for alternative fuels."

The micro-organisms in the fermentation process are being used to produce ethanol. It was called ethanol because it leaves the fat and waste...

"Importing of oil is one of the biggest reasons for the trade imbalance. That's a one-way street."

Lonnie O. Ingram, UFAS

additive, Ingram says. In Brazil, he adds,

chalk boards are covered from top to bottom with formulas and calculations. A venture into the lab reveals special flasks, filled with a yellowish broth of plant sugars and engineered bacteria, sitting in a warm-water fermentation tanks. A bubbly suds on surface of the mixture is evidence that the bacteria is hard at work. Eventually the mixture will be distilled and purified. "We're doing the tune-up studies to make it even better," Ingram says. "In industry we would operate million-gallon fermentation vessels."

A patent is being sought on the bacteria. InEnergy Ingram's idea. Dr. InEnergy Presi- company is cur- corporations ethanol produc- ergy will be

Professor Ohta conducting fermentation studies at the University of Florida