Metabolic Engineering

Re-Engineering Bacteria for Fuels and Chemicals

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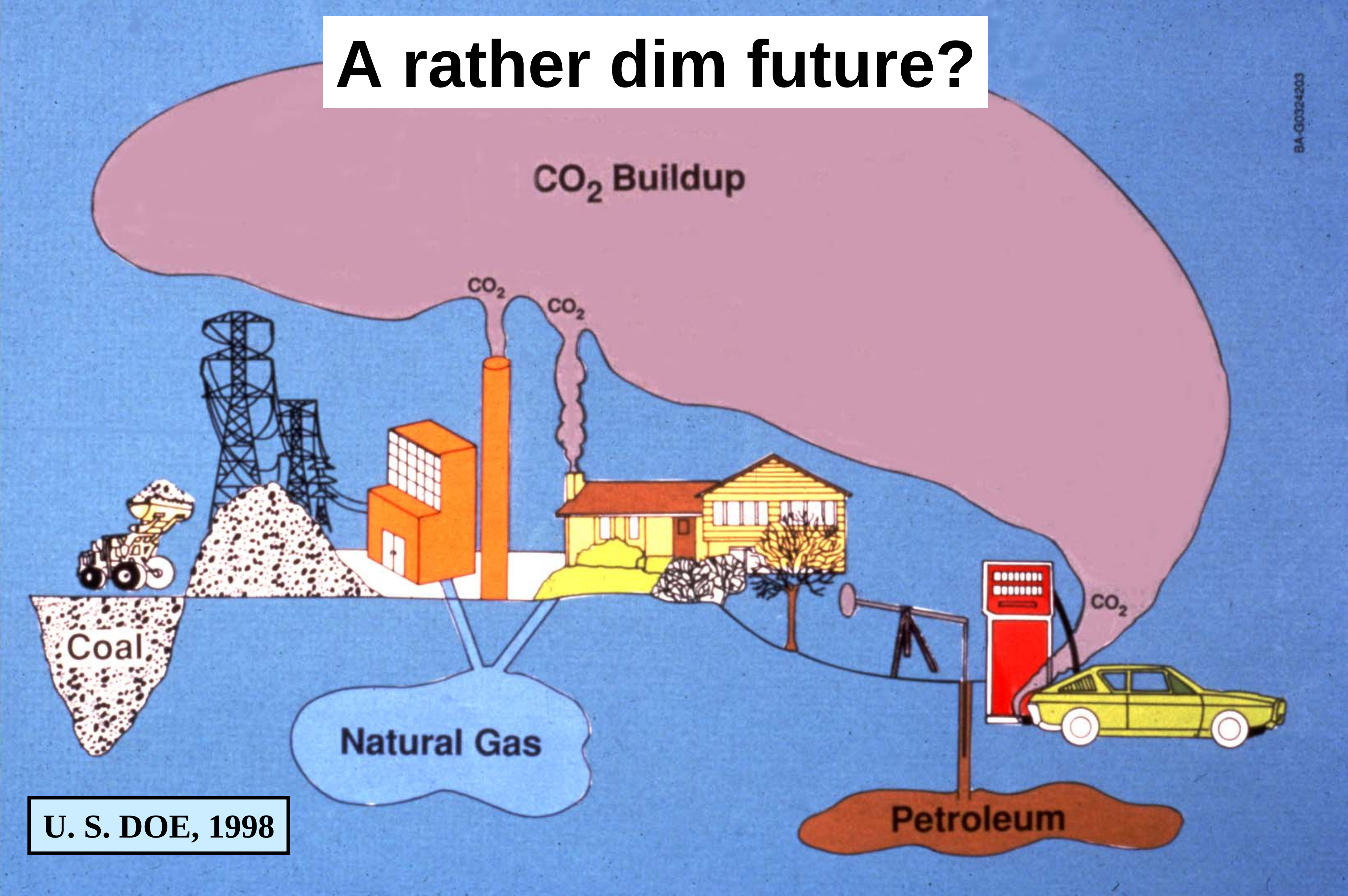
Florida Center for Renewable Chemicals

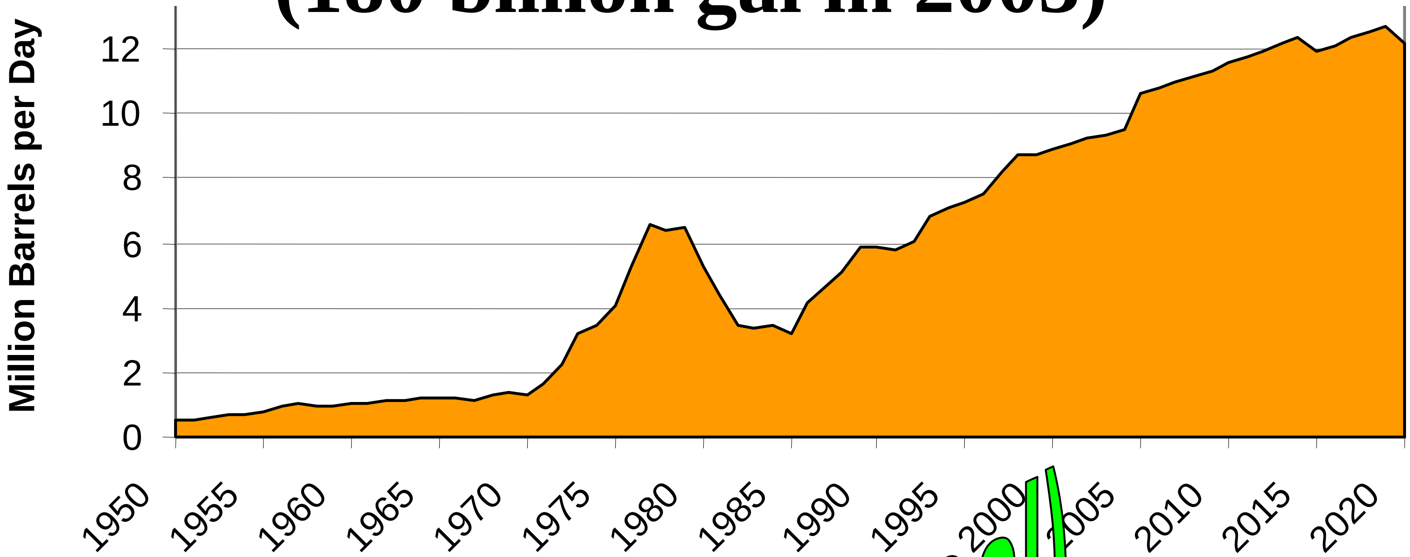
The University of Florida

A rather dim future?



U. S. DOE, 1998

U.S. Crude Oil Imports ~ Automotive Fuel
(180 billion gal in 2003)



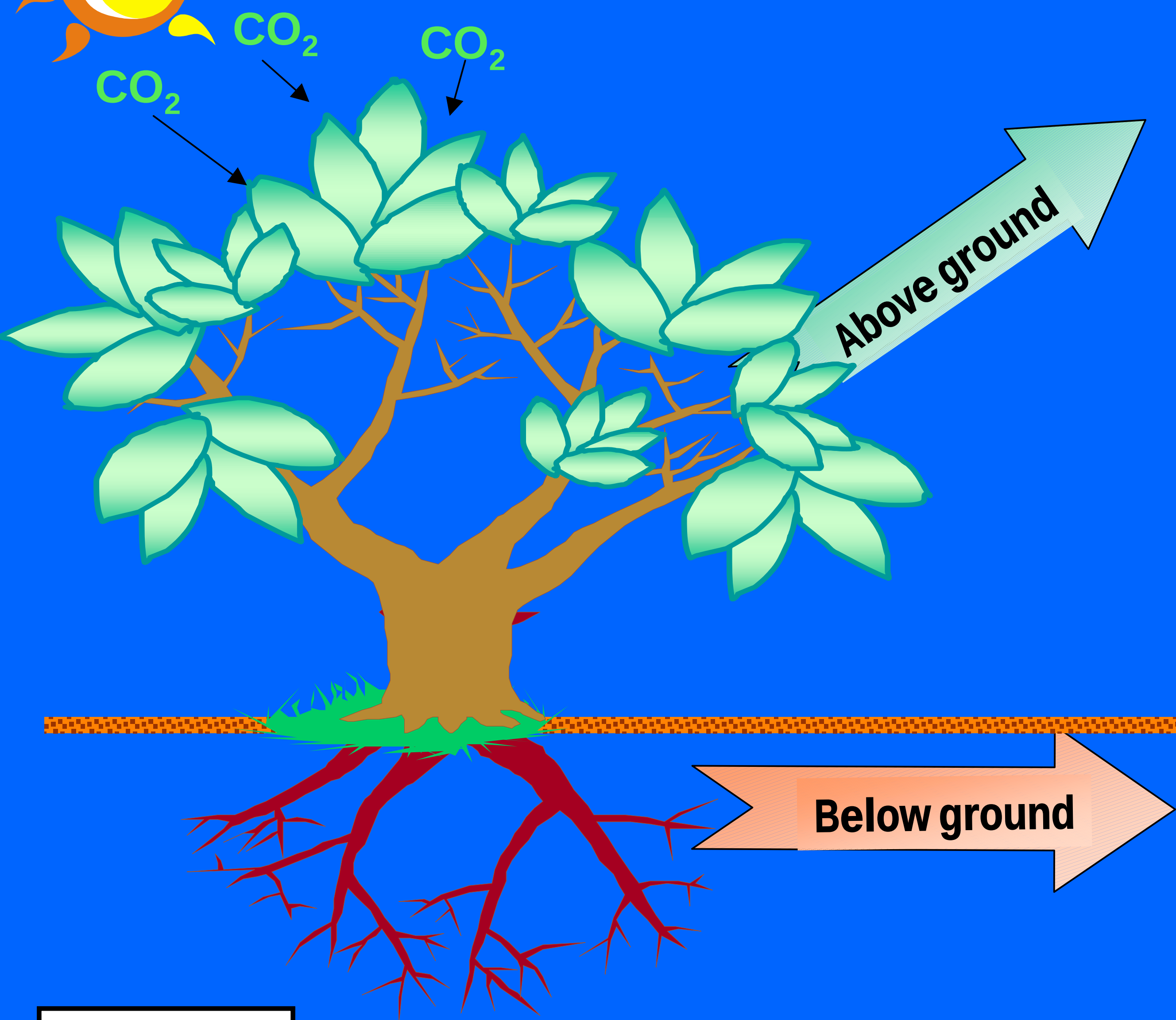
Ancient Biomass? (Oil, gas, coal)

or

Renewable Energy!

Source: U.S. Department of Energy, Energy Information Administration

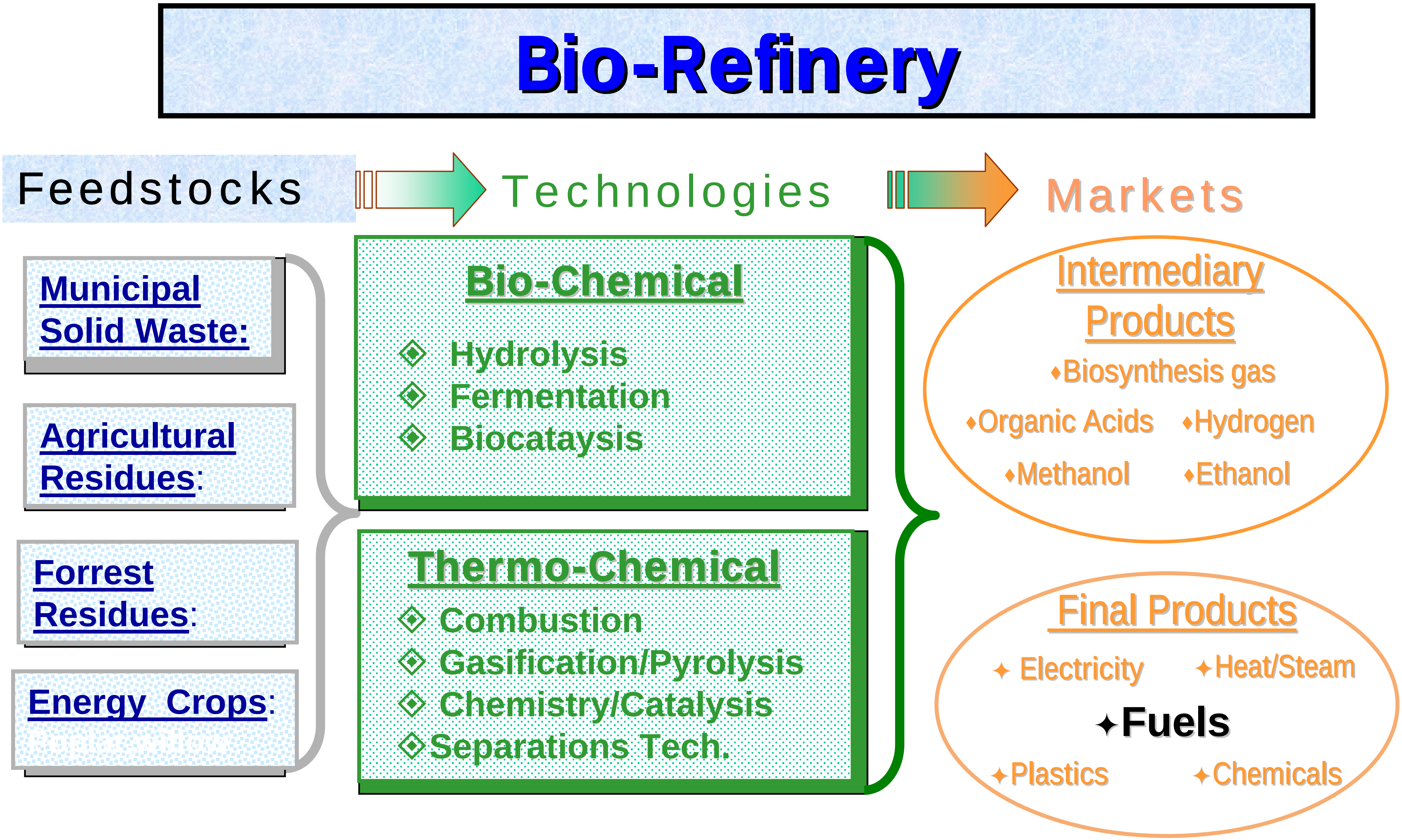
Renewable Fuels and Chemicals:



- **Recycling waste**
- **Displacement of oil**
  - Commodity chemicals
    - polylactic acid
    - 3-HP, 1-3 PD
    - Solvents, acids
  - Fuels
    - ethanol
    - biodiesel
    - power
  - Rural Employment

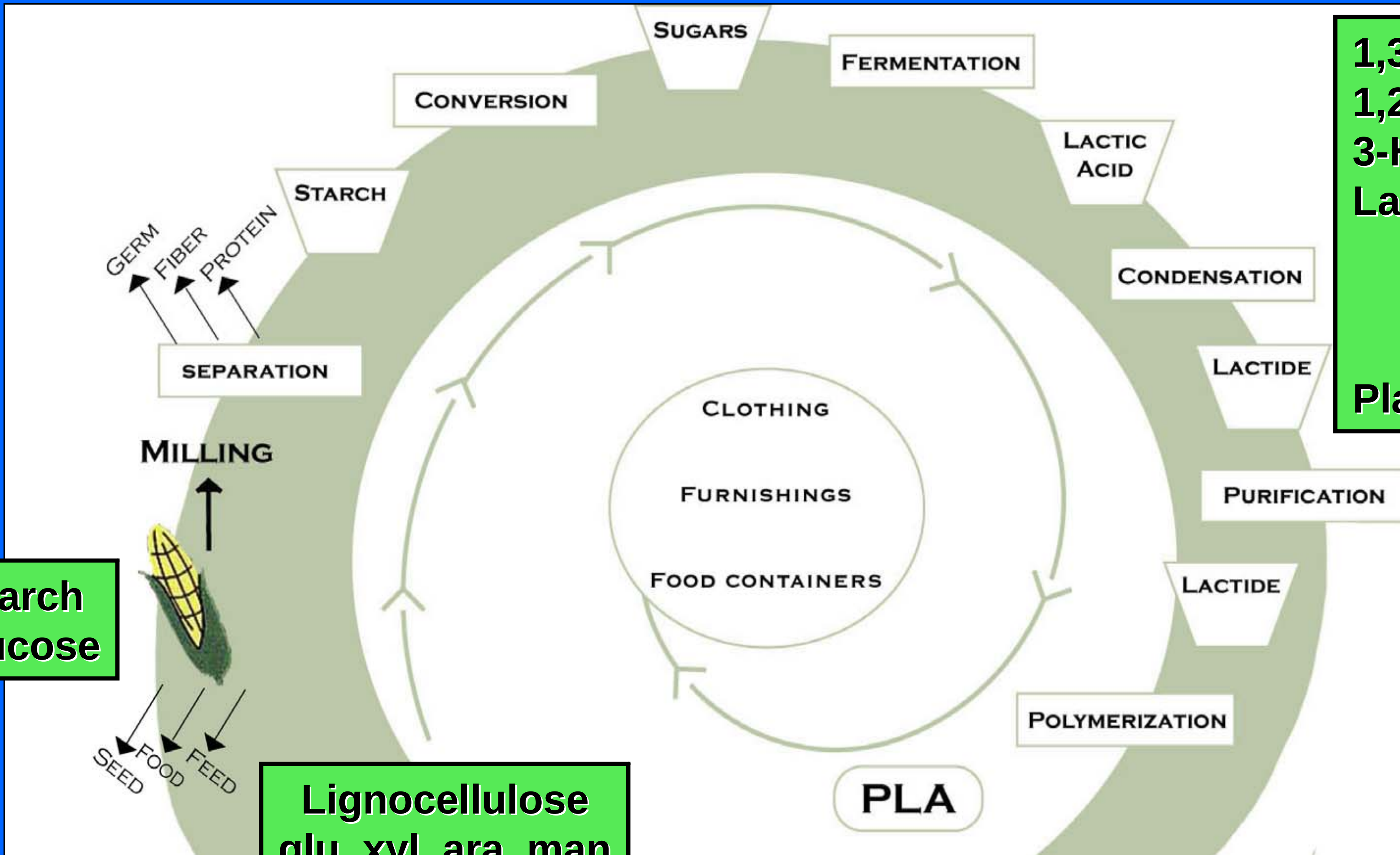
- **Carbon sequestration (short term)**

DOE, 2002


Bio-Refinery
Feedstocks
Technologies
Markets
Municipal Solid Waste:
Agricultural Residues:
Forrest Residues:
Energy Crops:
Bio-Chemical
Hydrolysis
Fermentation
Biocataysis
Thermo-Chemical
Combustion
Gasification/Pyrolysis
Chemistry/Catalysis
Separations Tech.
Intermediary Products
Biosynthesis gas
Organic Acids
Hydrogen
Methanol
Ethanol
Final Products
Electricity
Heat/Steam
Fuels
Plastics
Chemicals

(DOE, 2002)

PLA Cargill-Dow (1,3PDO Dupont/3HP Cargill)



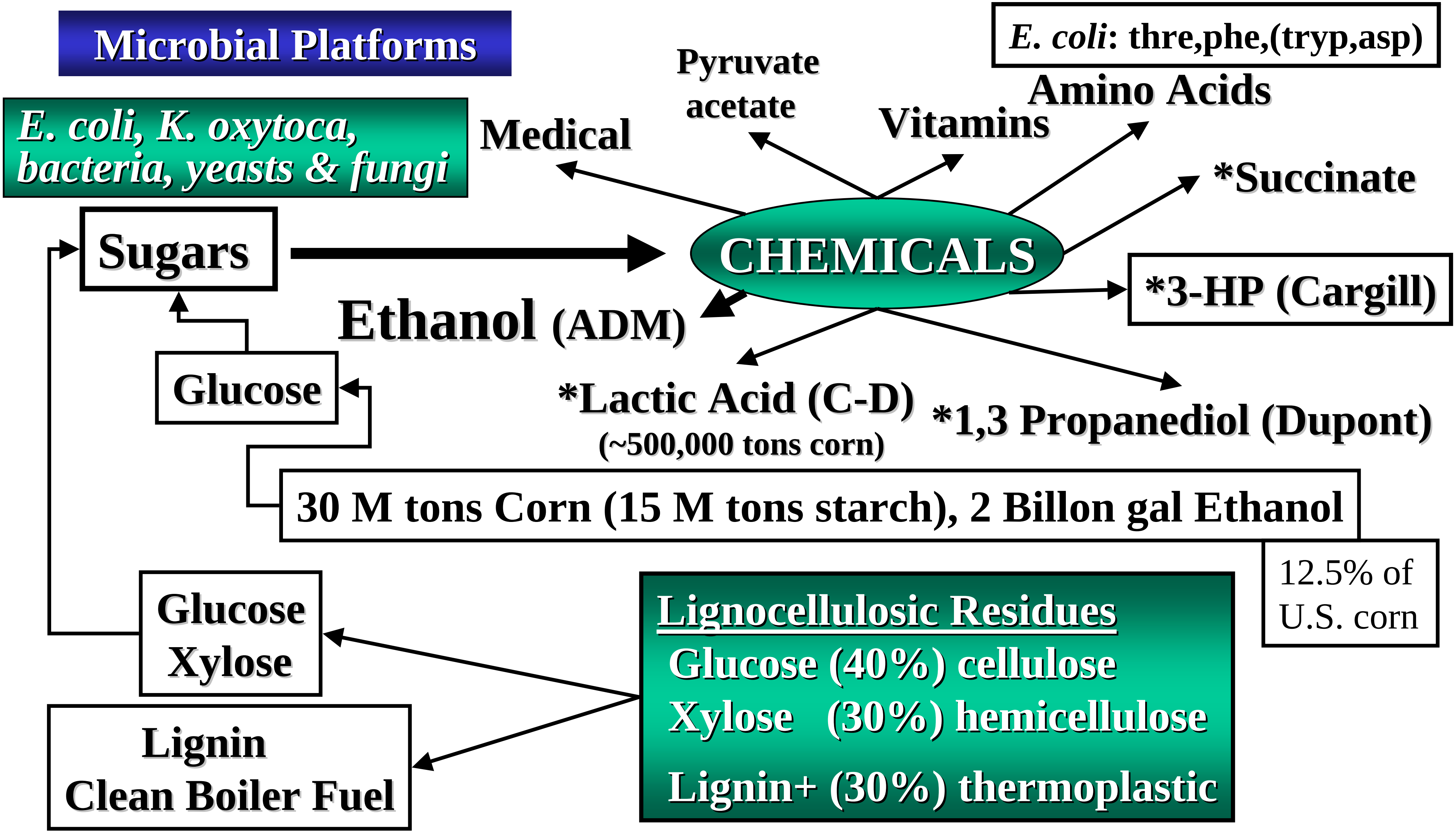
1,3 PDO
1,2 PDO
3-HP
Lactic
↓
Plastics

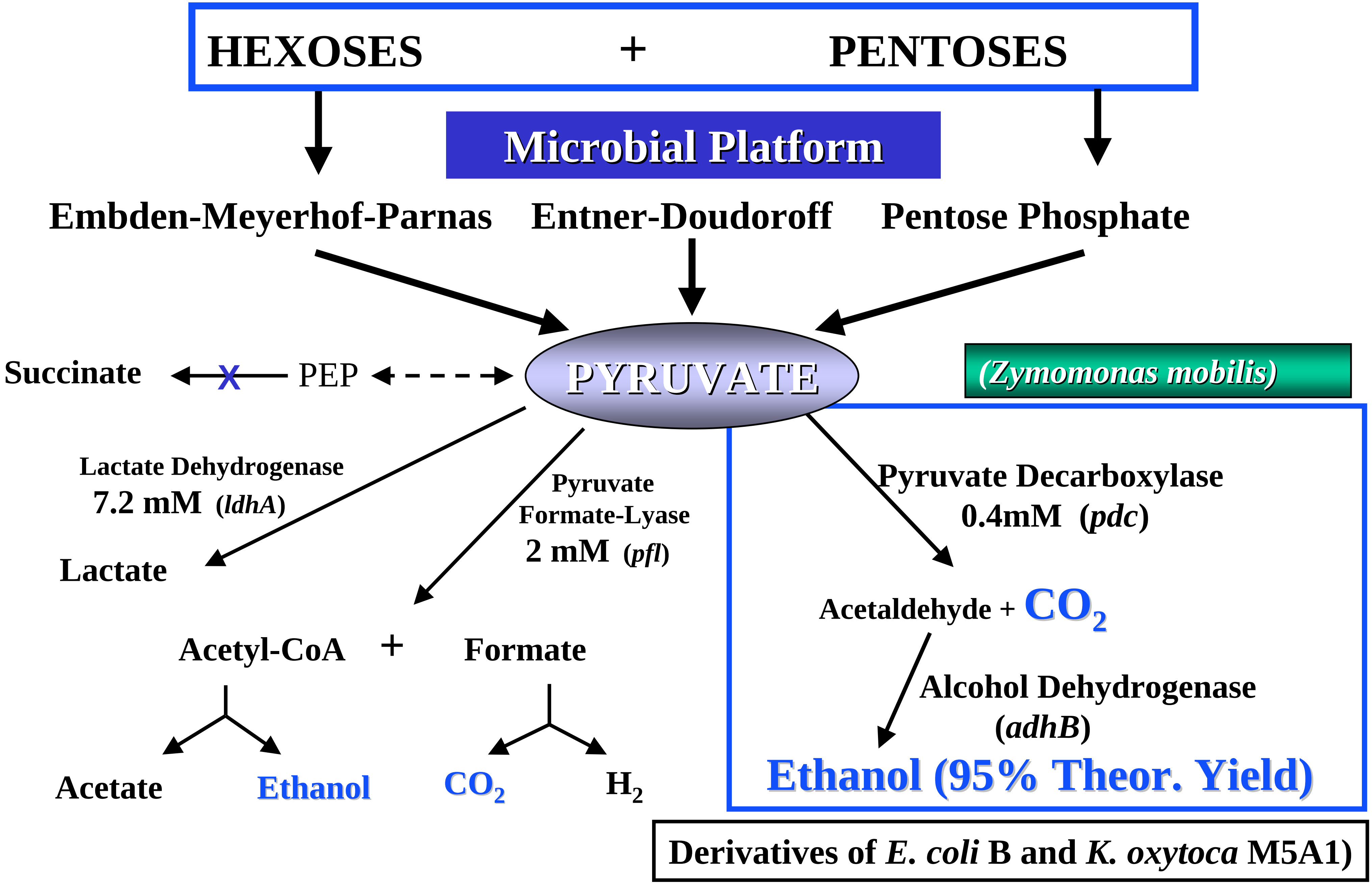
Starch glucose

Lignocellulose glu, xyl, ara, man

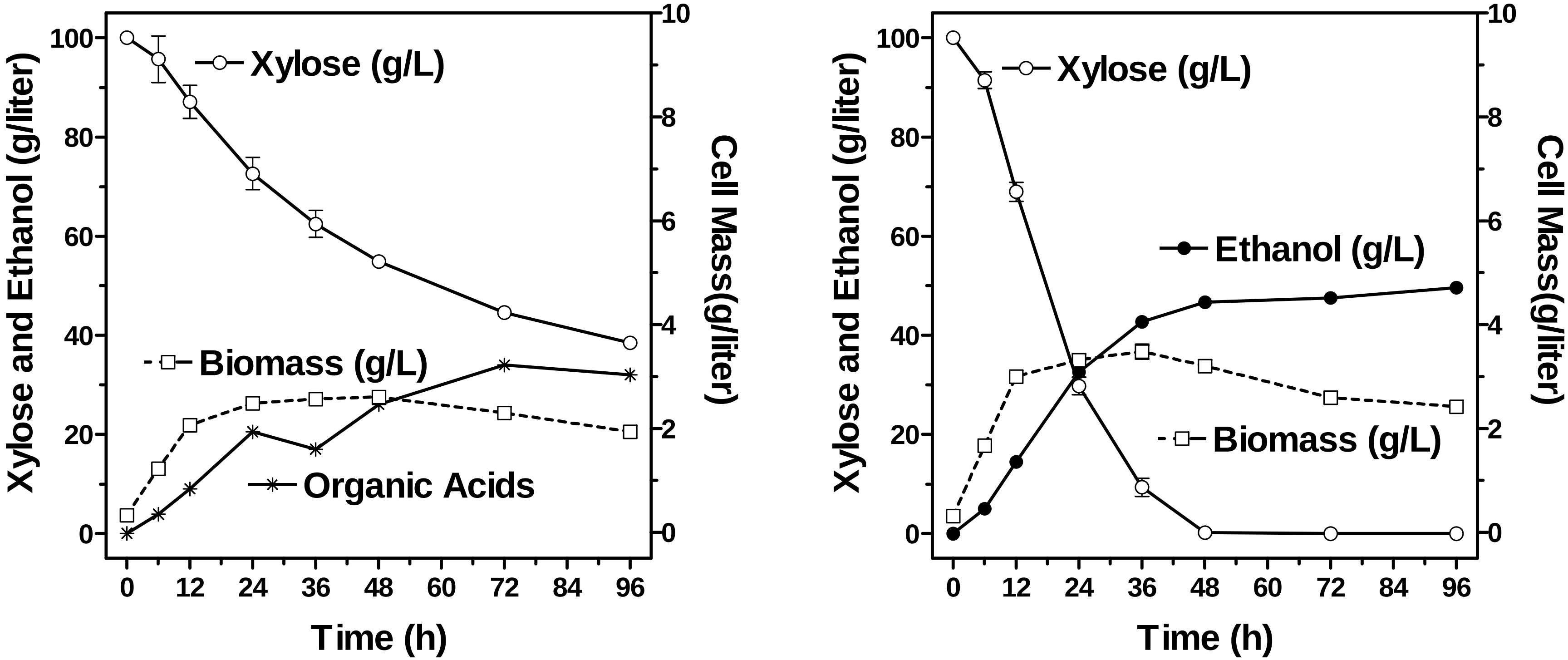
DOE, 2002

Renewable Feedstocks > Renewable Chemicals



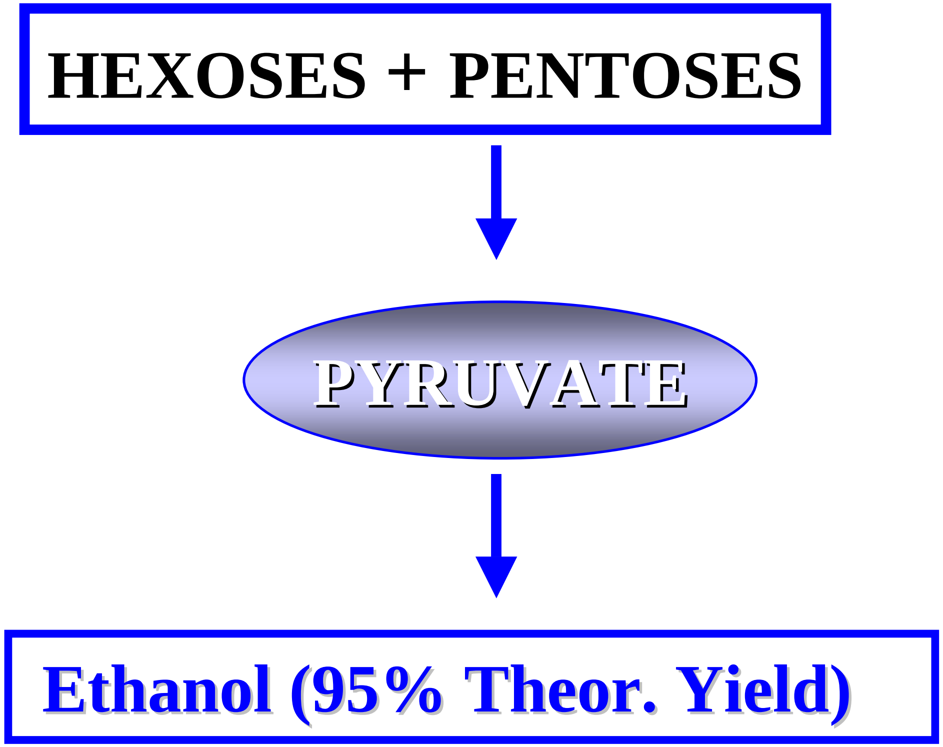

HEXOSES + PENTOSES
Microbial Platform
Embden-Meyerhof-Parnas
Entner-Doudoroff
Pentose Phosphate
PYRUVATE
Succinate
PEP
(Zymomonas mobilis)
Lactate Dehydrogenase
7.2 mM (ldhA)
Lactate
Pyruvate
Formate-Lyase
2 mM (pfl)
Acetyl-CoA + Formate
Acetate
Ethanol
CO_2
H_2
Pyruvate Decarboxylase
0.4mM (pdc)
Acetaldehyde + CO_2
Alcohol Dehydrogenase
(adhB)
Ethanol (95% Theor. Yield)
Derivatives of E. coli B and K. oxytoca M5A1)

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



Yield – 0.50 g ethanol and 0.49 g CO_2 per g xylose

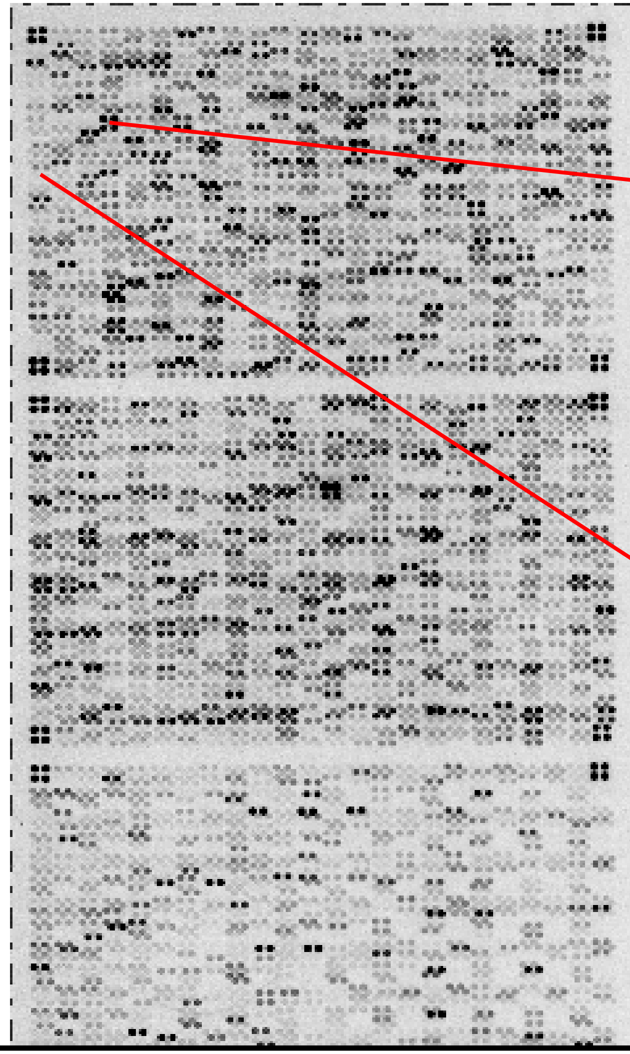
(10% Xylose, pH 6.5, 35C)



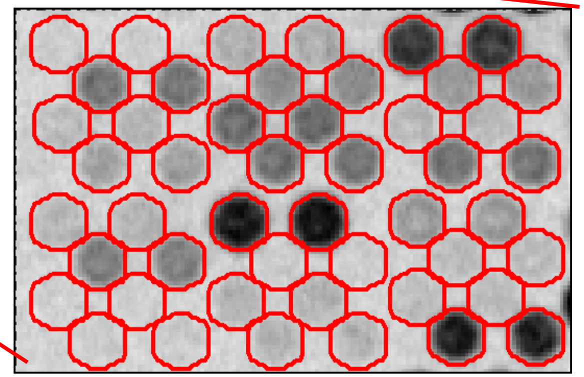
1. <u>Biocatalyst</u>
   - Feedstock/nutrients
   - Substrate range, enzymes
   - Yield
   - Rate
   - Final titer, tolerance
2. <u>Engineering</u>
   - Recovery/Purification
   - Equipment Costs

Transcriptome Analysis: cDNA Microarrays

Cells
↓
Hot Phenol
↓
DNAse I
↓
Qiagen Column
↓
Total RNA
↓
SuperScript II (Primers to 3' end, 33-P-dCTP-label)
↓
cDNA



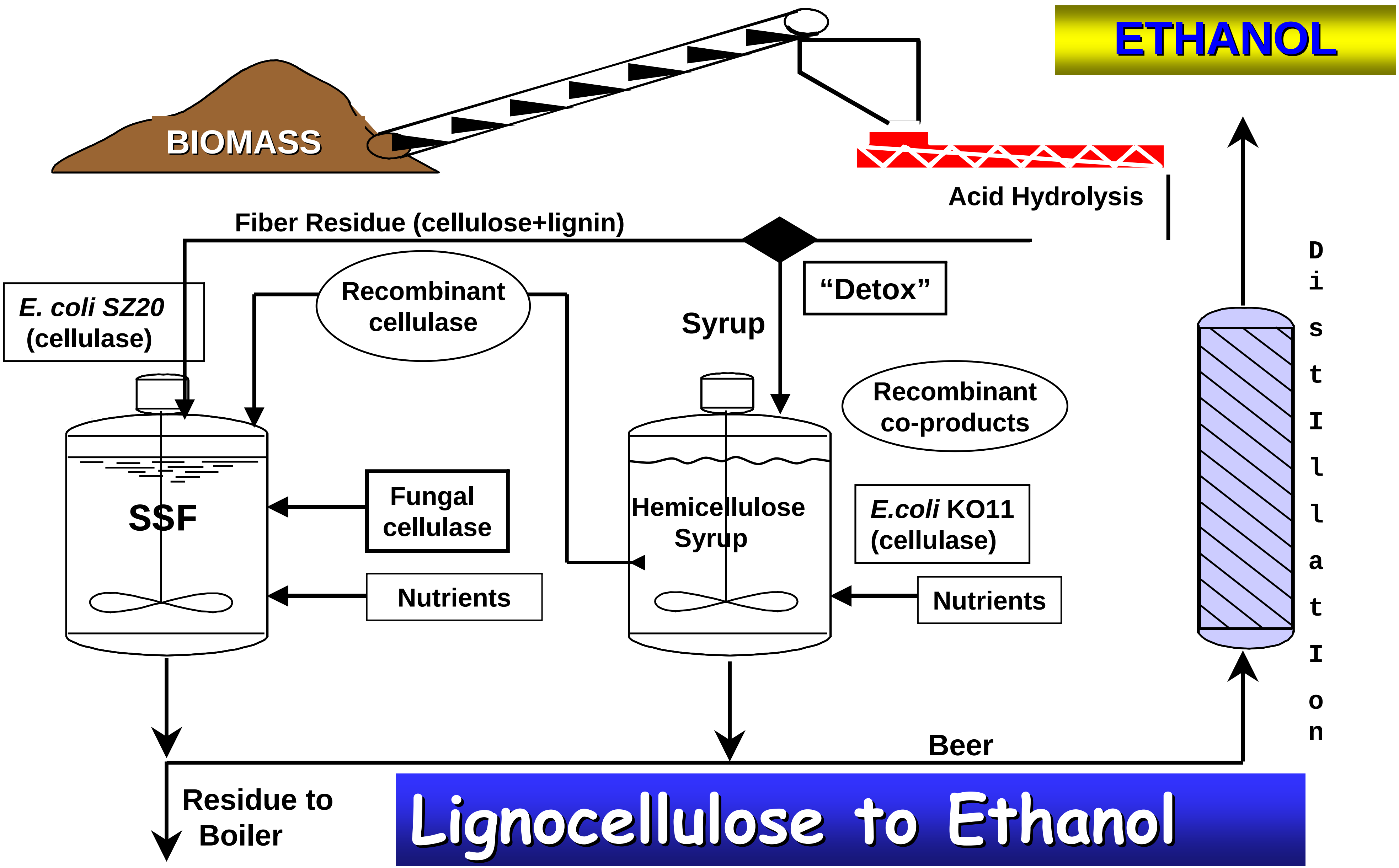
Hybridization
16 h at 65 °C



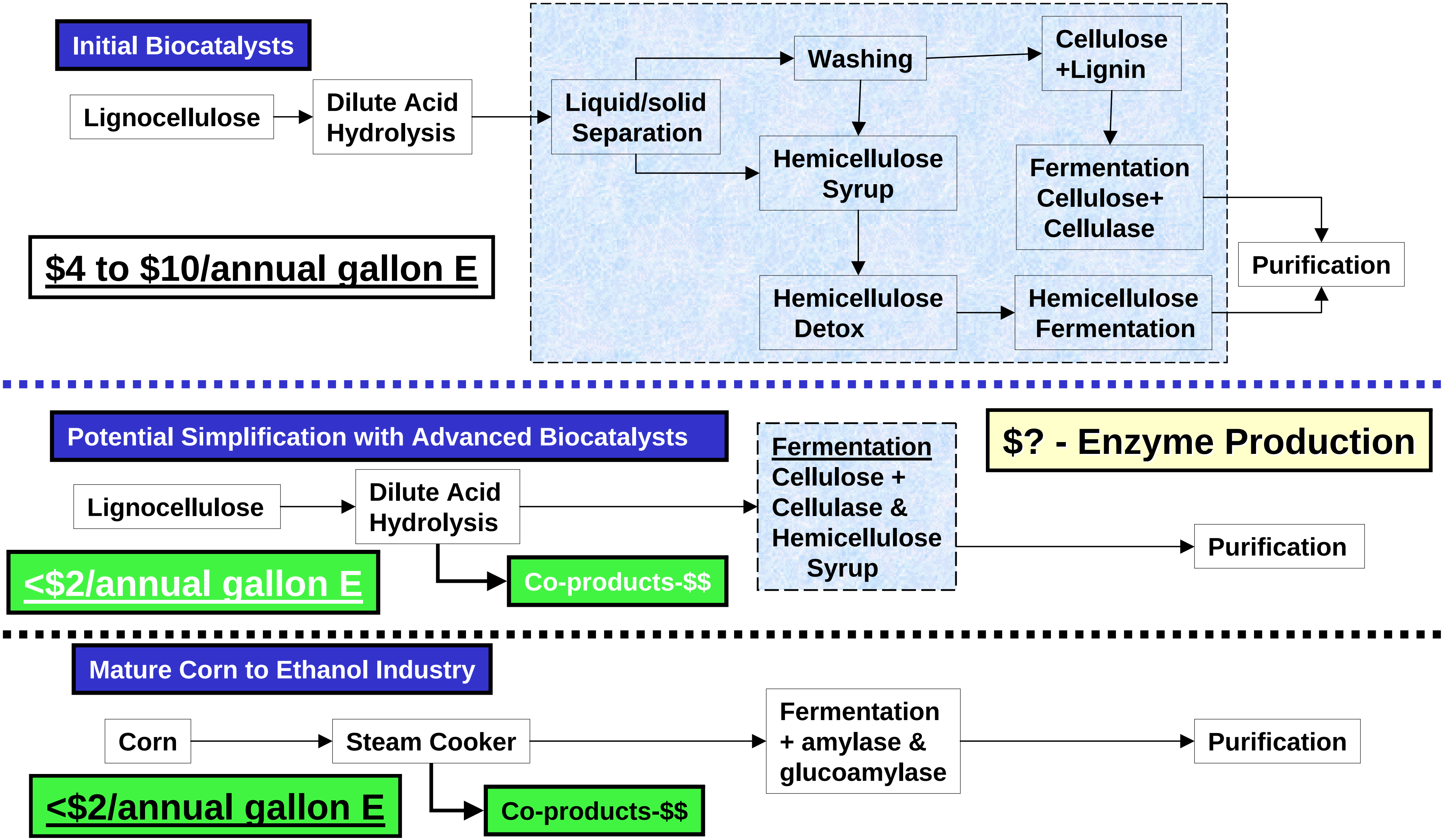
Spot Volume
Area • Intensity
% of the total

4,290 ORFs

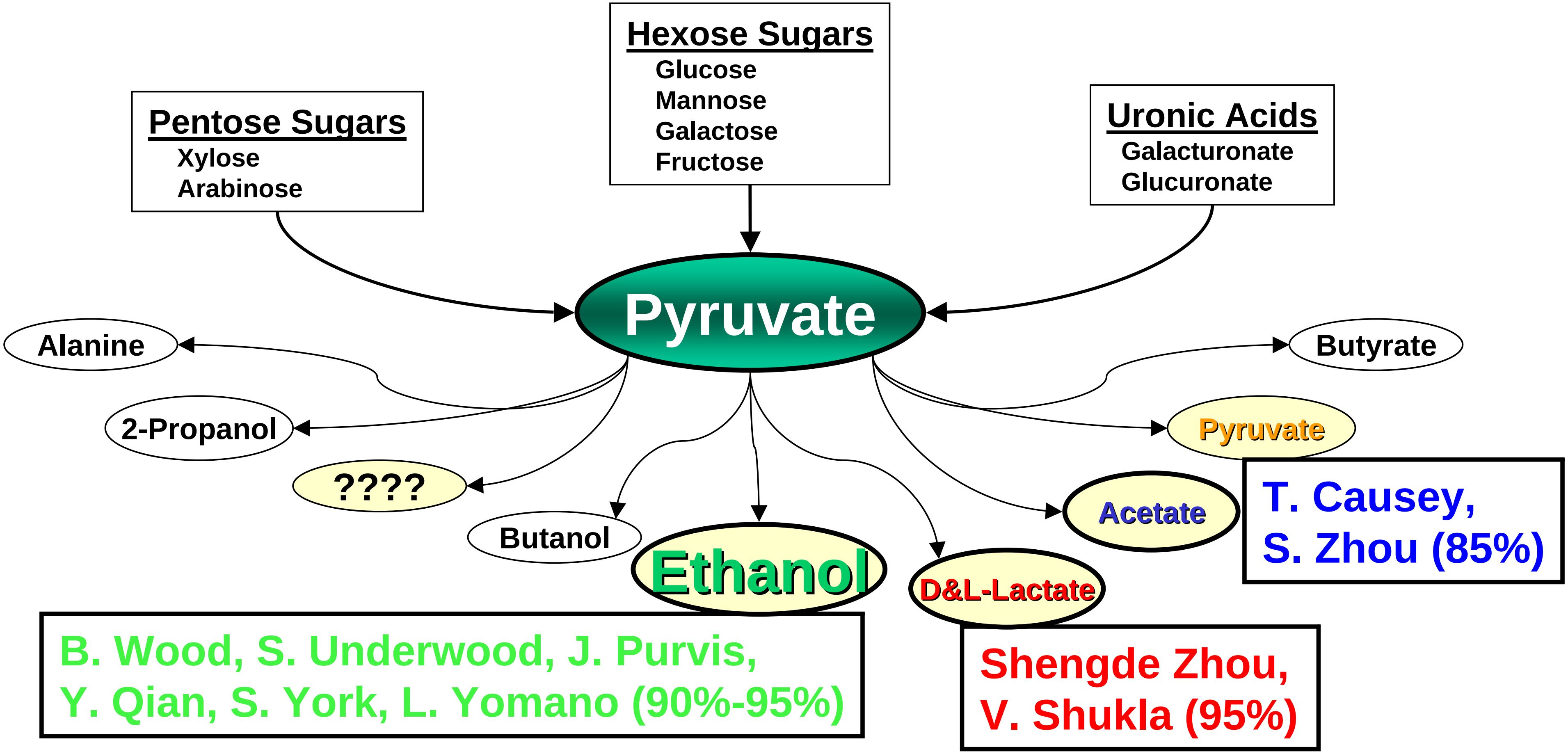
Han Tao & Ramon Gonzalez, KT Shanmugam and LOI


ETHANOL
BIOMASS
Acid Hydrolysis
Fiber Residue (cellulose+lignin)
E. coli SZ20
(cellulase)
Recombinant
cellulase
"Detox"
Syrup
Recombinant
co-products
SSF
Fungal
cellulase
Nutrients
Hemicellulose
Syrup
E.coli KO11
(cellulase)
Nutrients
Distillation
Beer
Residue to
Boiler
Lignocellulose to Ethanol

Conversion of Biomass to Fuel Ethanol & Chemicals



Higher Value Co-products



Standard Fermentation Conditions:

Mineral salts

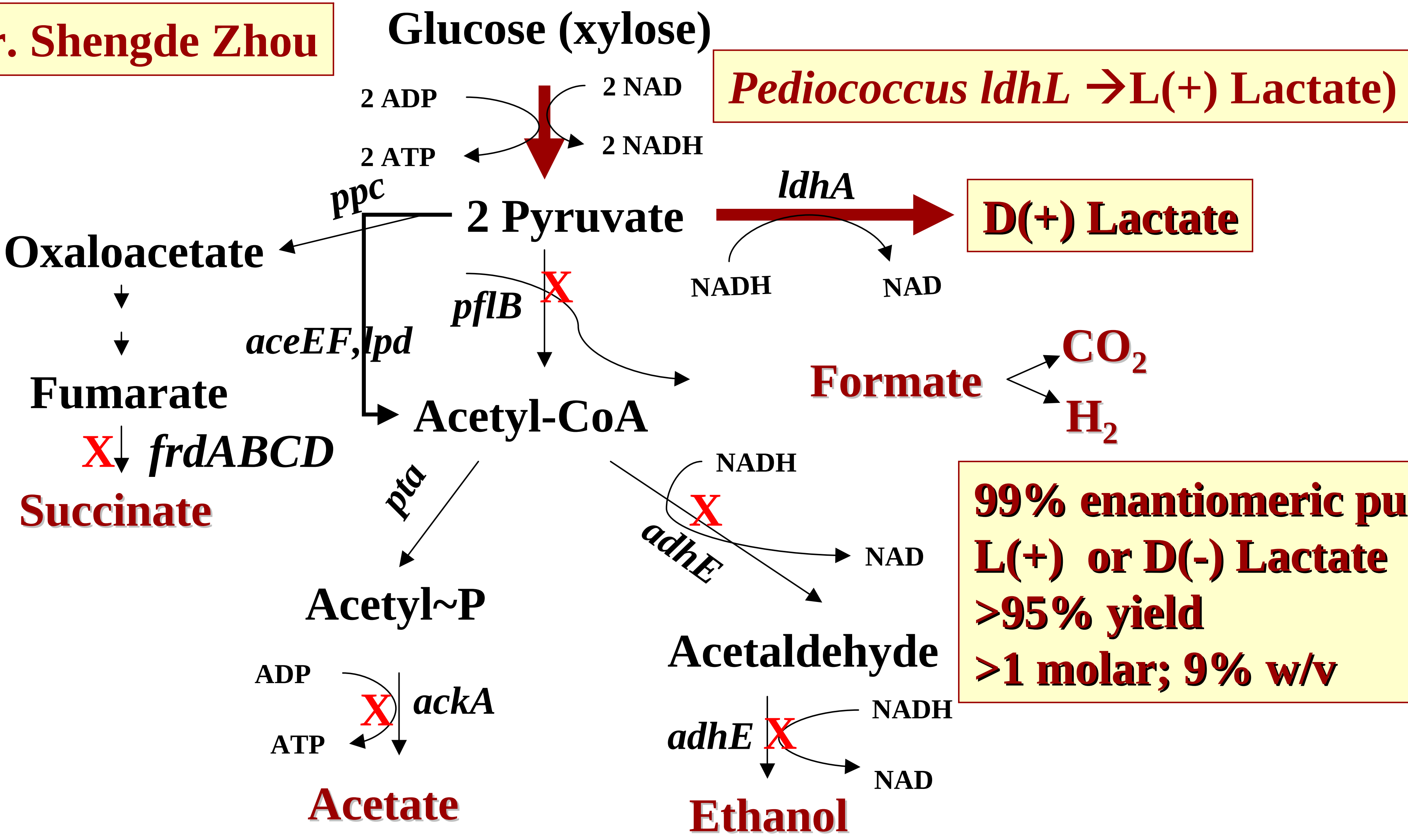
- 3 % glucose + glucose
- Starting OD_{550}=0.05
- 1% inoculum
- 5 L or 10 L volume
- Agitation set @ 350 rpm , 37^0C

- pH controlled @ 7.00 with 45% w/w KOH (11.4 M)
- +/-Gas flow 1 L • min^{-1} (0.1-0.2 vvm)

(DO controlled above 5% of air saturation by adjusting the ratio of air & O_2)

E. coli : Mixed Acid Fermentation →Lactate

Dr. Shengde Zhou



99% enantiomeric purity
L(+) or D(-) Lactate
>95% yield
>1 molar; 9% w/v

Overview of Metabolism in *E. coli*

Anaerobic, - oxygen	Aerobic, + oxygen
Glucose, $C_6H_{12}O_6$	Glucose, $C_6H_{12}O_6$

The Problem:

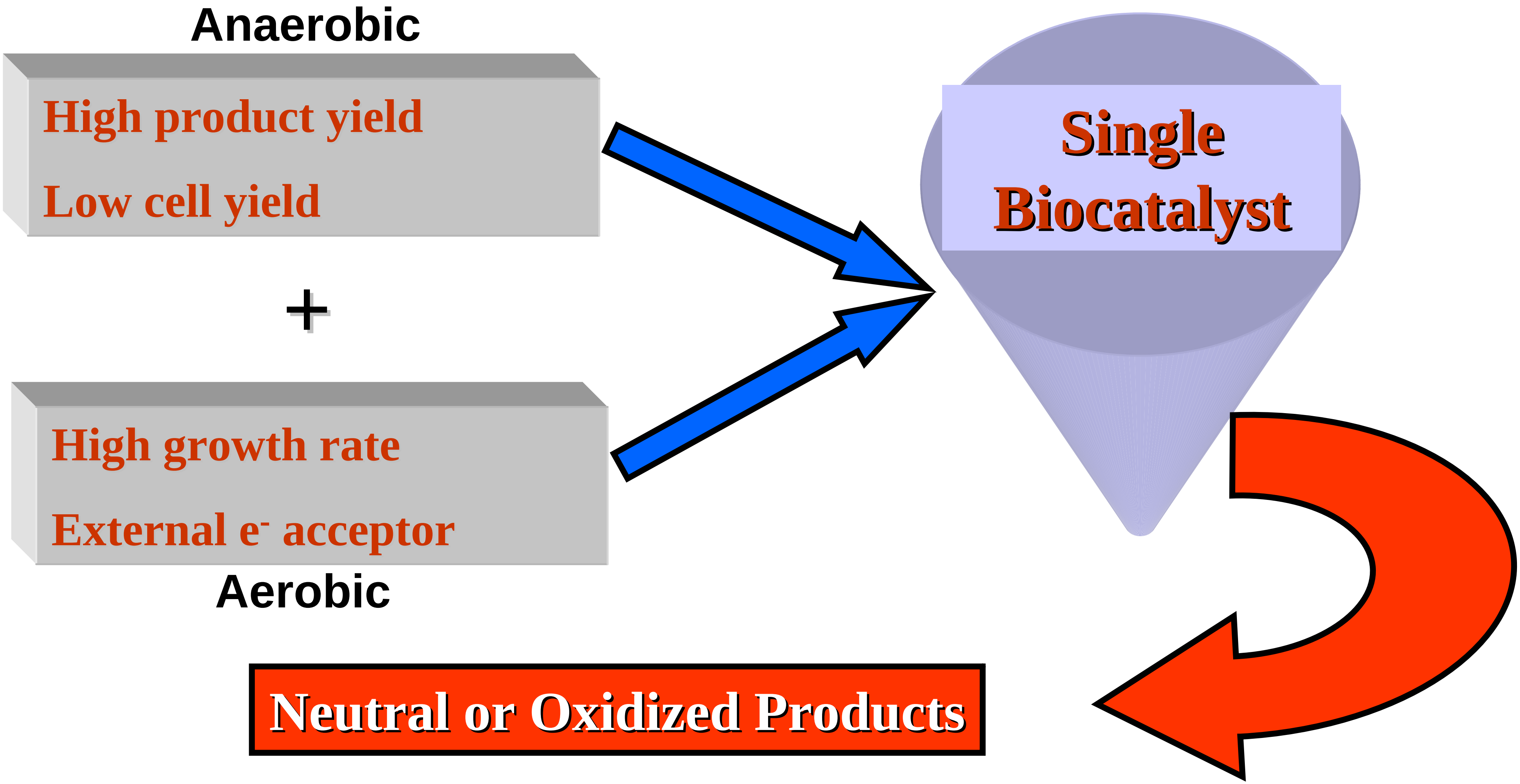
~32 ATP (aerobic) versus 2 ATP (anaerobic)

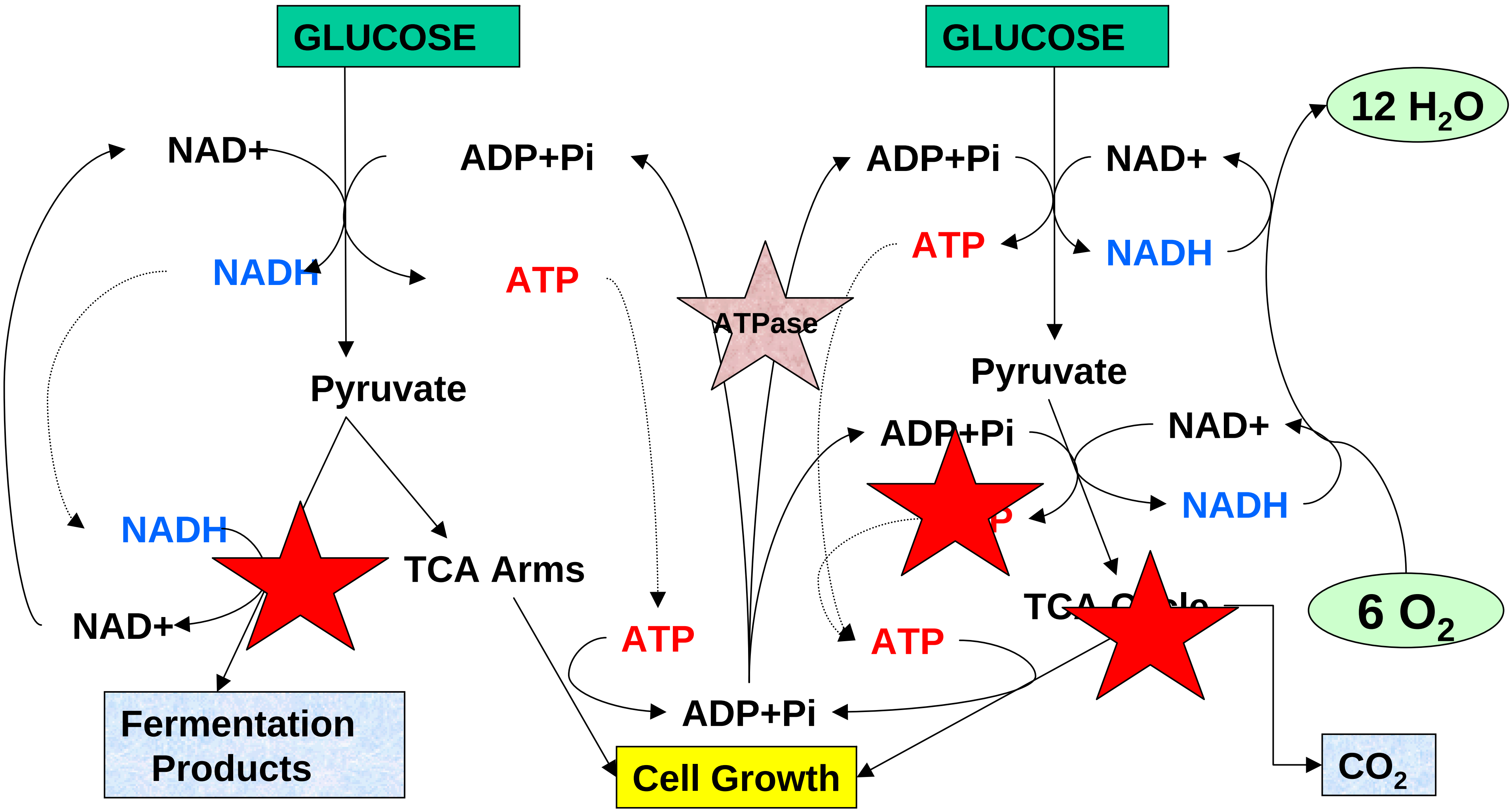
ATP promotes growth!

- External electron acceptor

- Internal electron acceptor

Goal: Combine the Attributes of Aerobic & Anaerobic Metabolism to engineer Co-Products

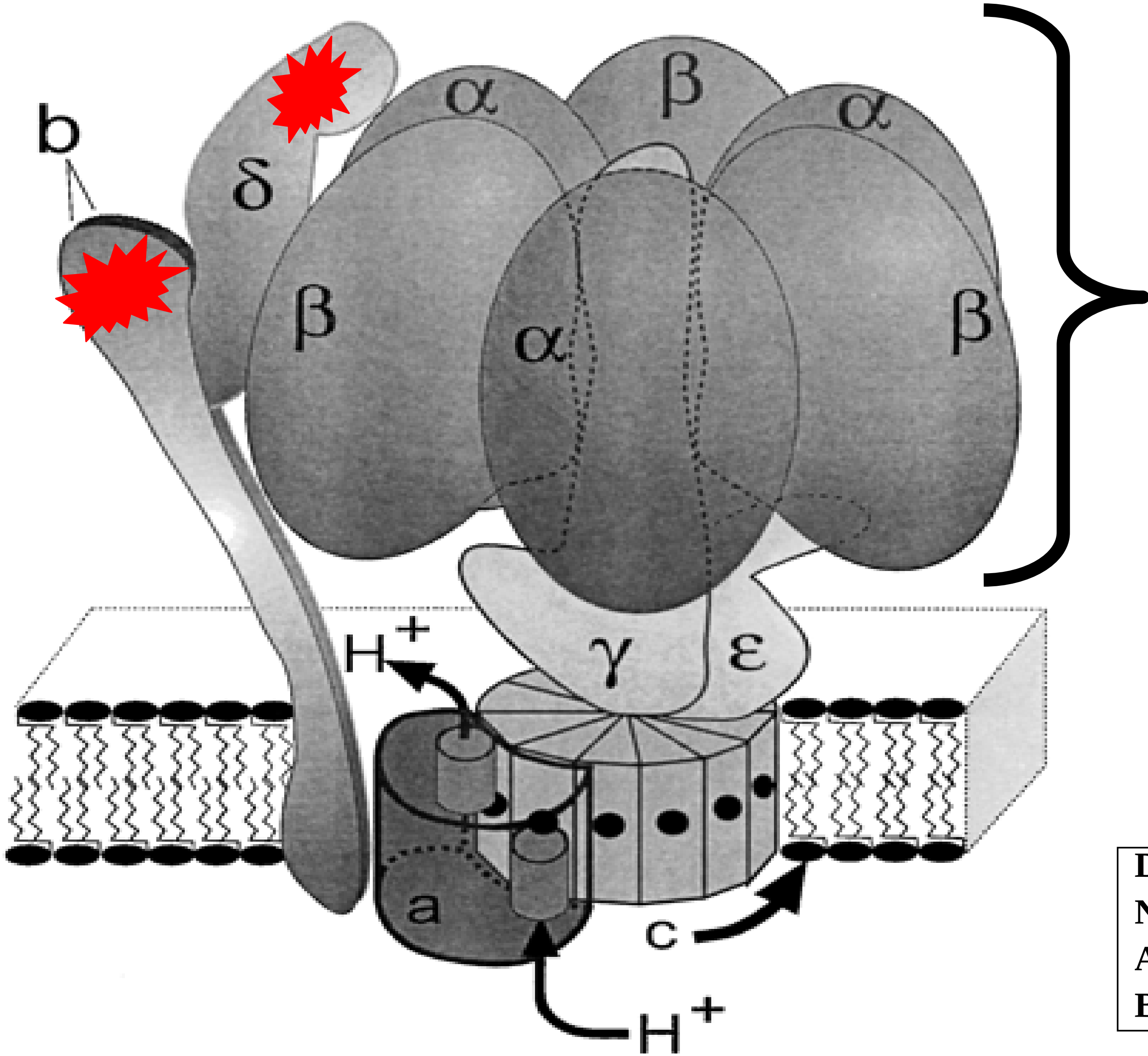




95% Carbon <5% Carbon
~2 ATP/glu

50% Carbon 50% Carbon
~33 ATP/glu

F1F0 ATPase Mutation



Active Cytoplasmic ATPase

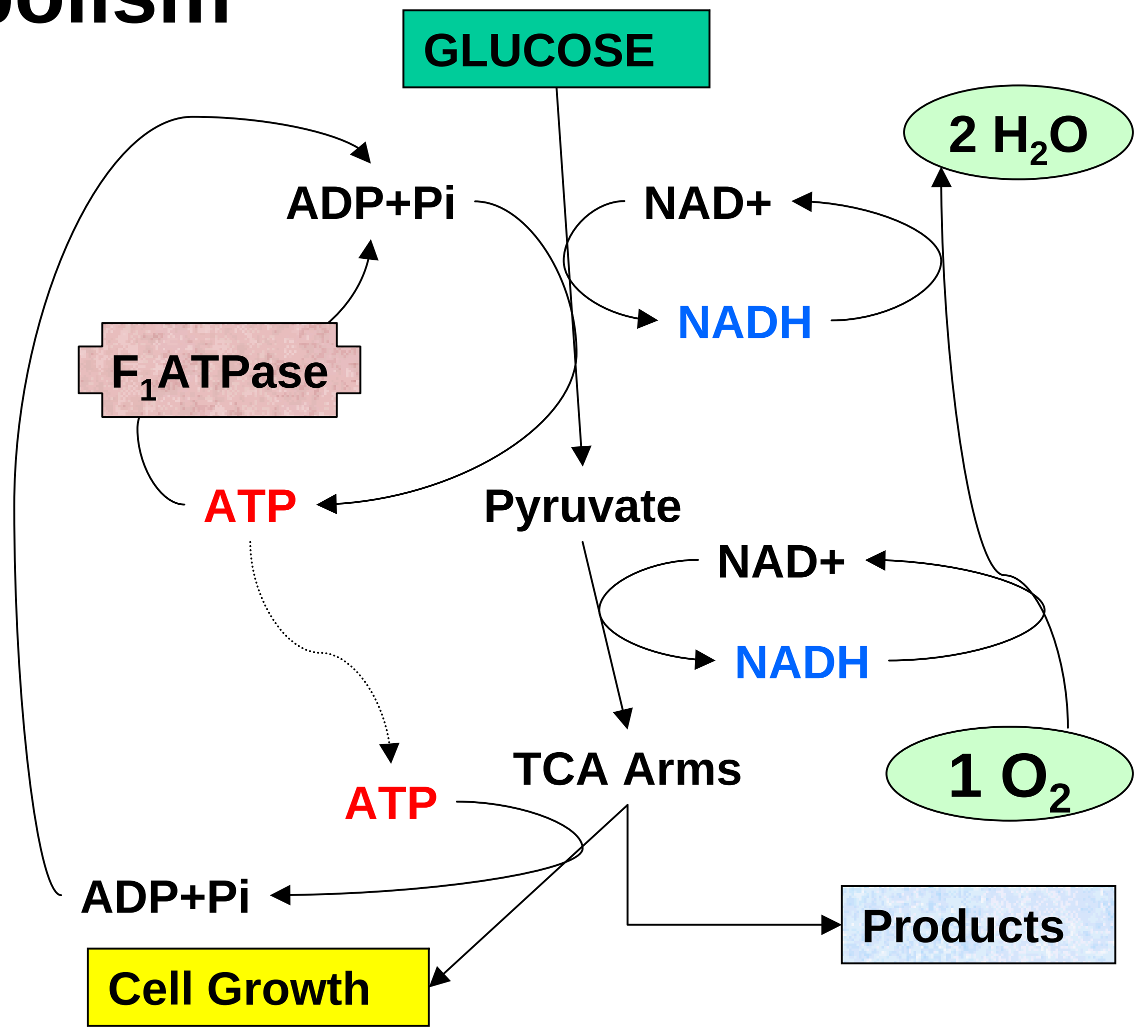
Gratuitous Hydrolysis Of ATP!

Drawing from
Nakamoto et al., 1999
Ann. Rev Biophys.
Biomol. Struct. 28:205-234.

Re-Engineered Metabolism

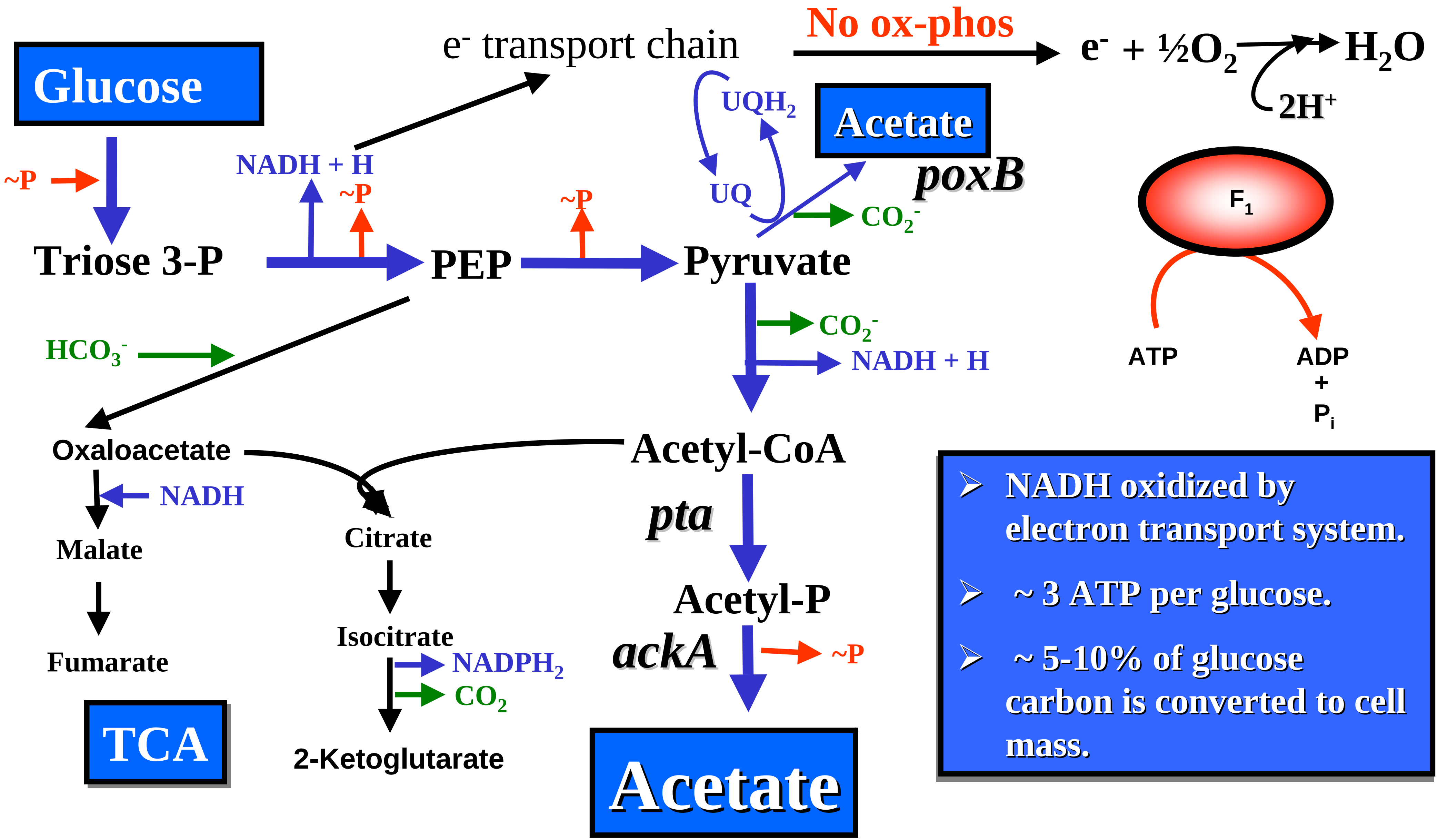
External Electron Acceptor
- **Low cell mass**
- **Low ATP yield**
- **High Product Yield**

Oxidized or Reduced Products



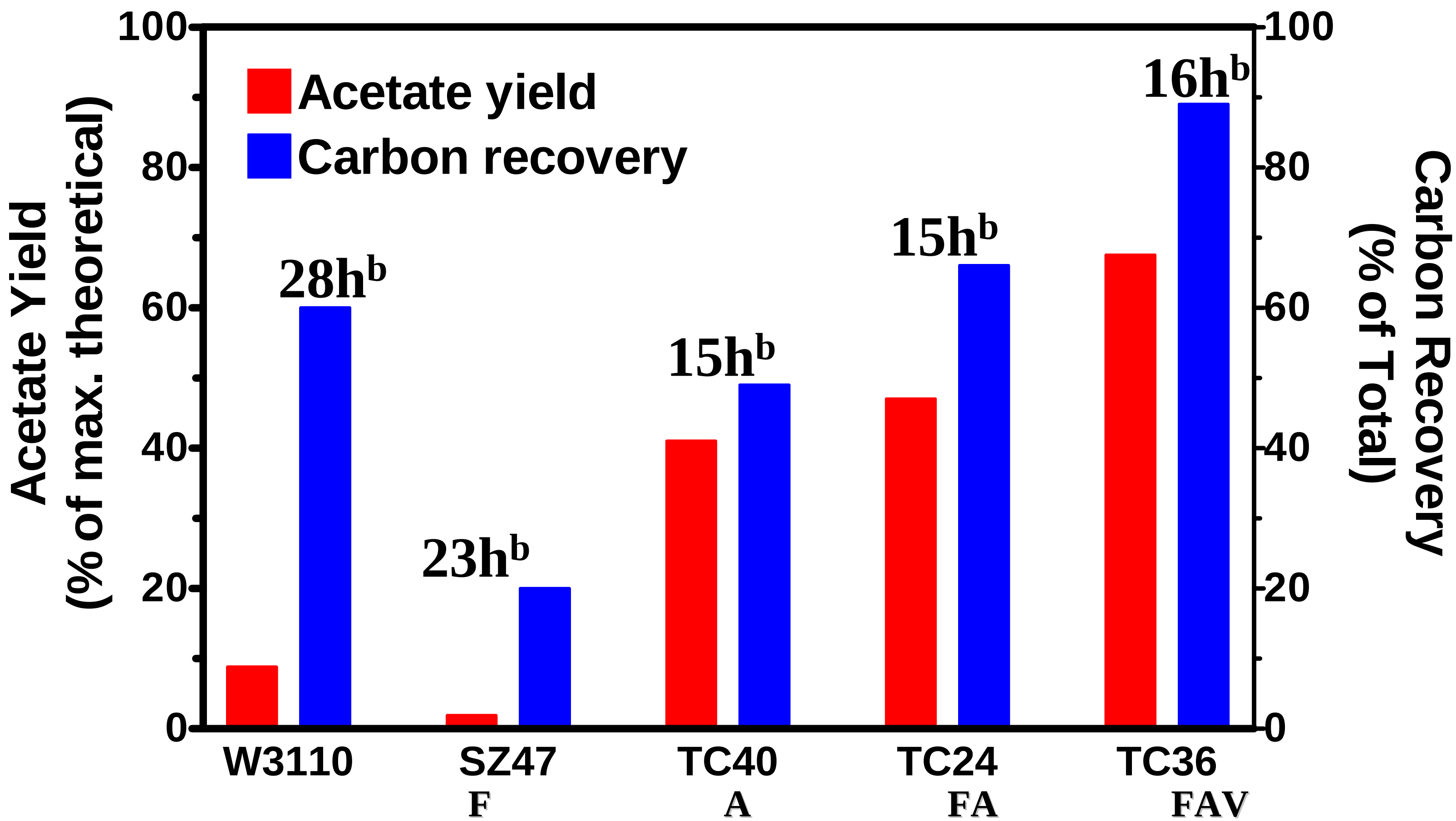
10% Carbon	90% Carbon
~2 ATP/glu	

E. coli TC36 Engineered for Acetate Production



- NADH oxidized by electron transport system.
- ~ 3 ATP per glucose.
- ~ 5-10% of glucose carbon is converted to cell mass.

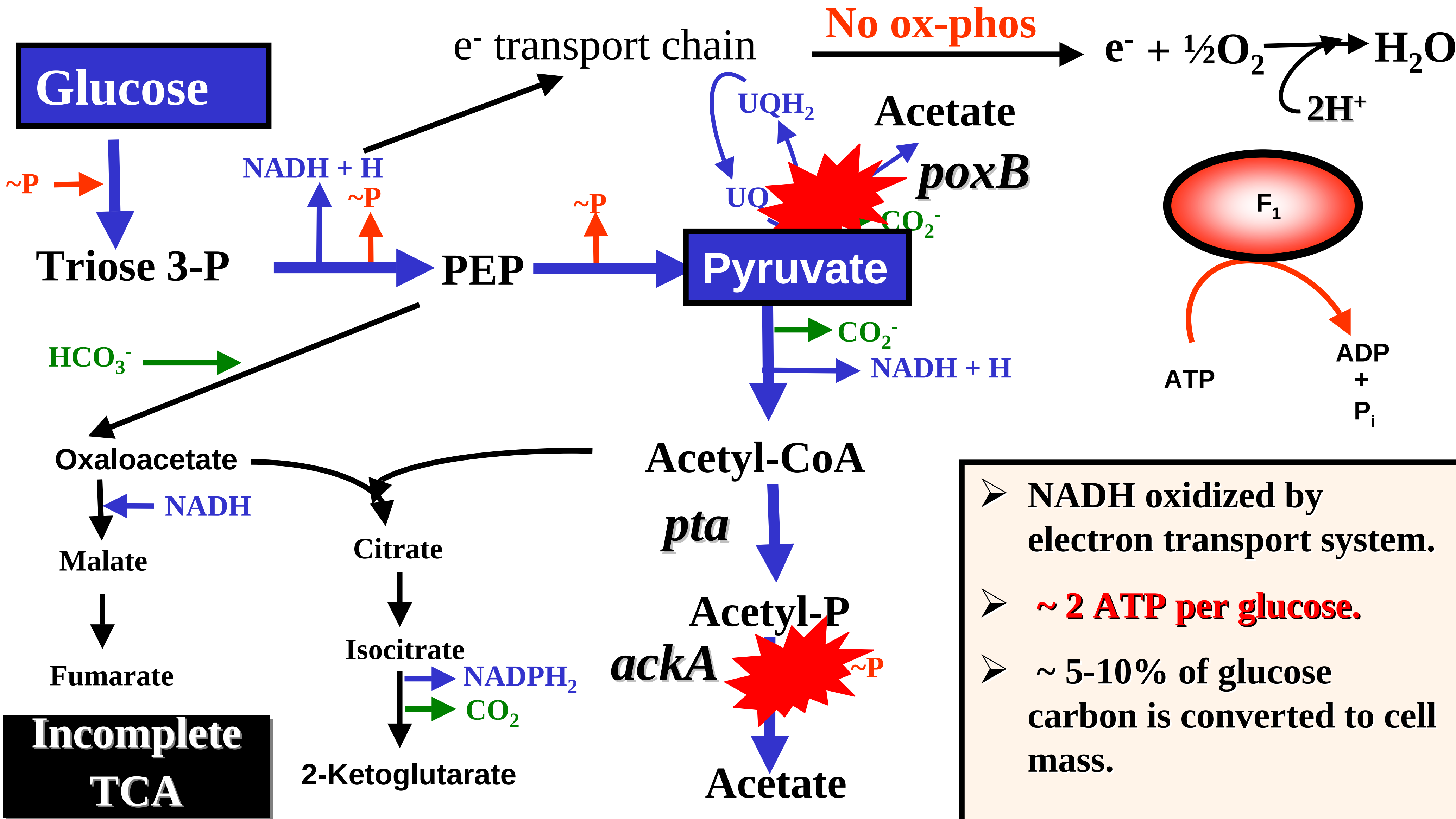
Effects of Mutations on Acetate Production and Carbon Recovery



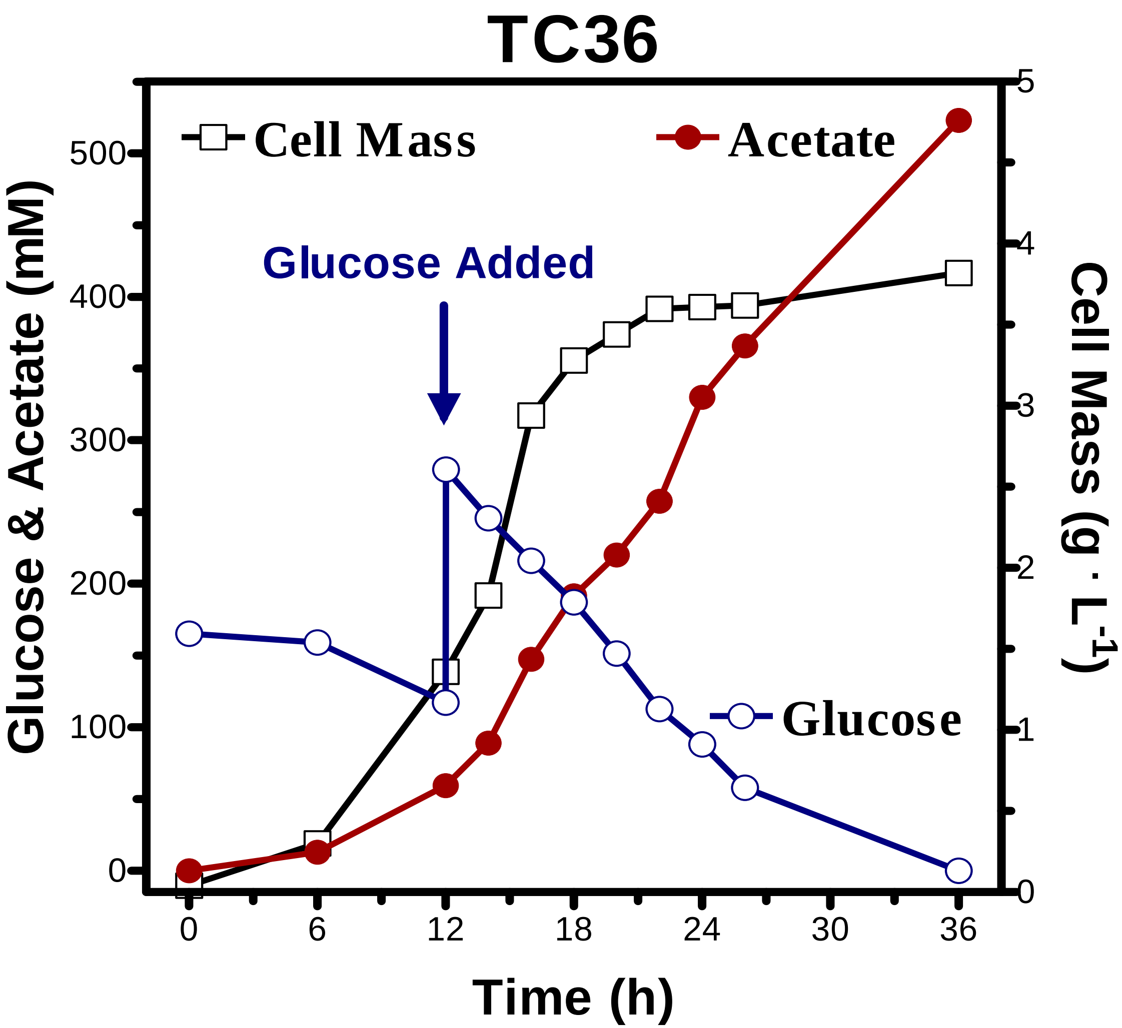
[a] % of maximum theoretical acetate yield (0.67 g g^{-1}) at glucose depletion.

[b] Time of complete glucose consumption.

E. coli TC44 Engineered for Pyruvate Production



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

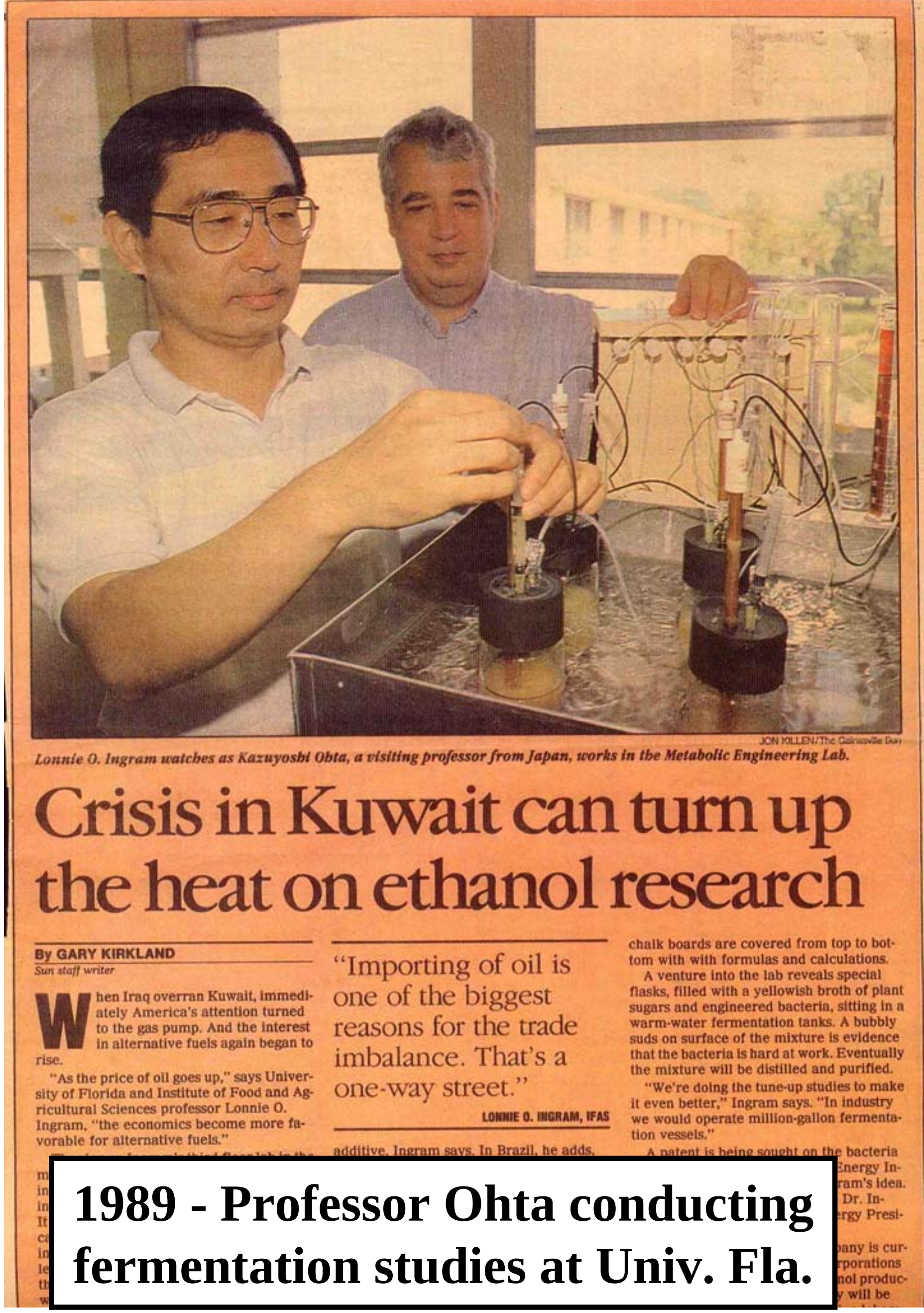


NEW RESEARCH AREAS

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

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.


JON KILLEN/The Gainesville Sun

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."

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

additive, Ingram says. In Brazil, he adds,

chalk boards are covered from top to bottom with 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

1989 - Professor Ohta conducting fermentation studies at Univ. Fla.