3rd Bioeconomy Forum:

Policies and the Environment for Innovation and Business in Brazil

Sao Paulo, SP, Brazil

23 October, 2014

The impact of metabolic engineering and synthetic biology: paradigm shift on the 21st century business, Or,

Developing the bioprocesses of a sustainable bioeconomy using Biotechnology and Metabolic Engineering

Gregory Stephanopoulos

MIT

Main thesis

- **Global economic and population growth is creating new tensions in the supply of energy and raw materials leading to serious environmental issues**
- **Navigating through the inevitable changes is a profound challenge and responsibility of politicians, scientists and businessmen**
- ***Technology* is a powerful force, but attitude adjustments will also be necessary**
- **Bioprocesses are the key technology of a future sustainable bioeconomy**

Messages of this talk

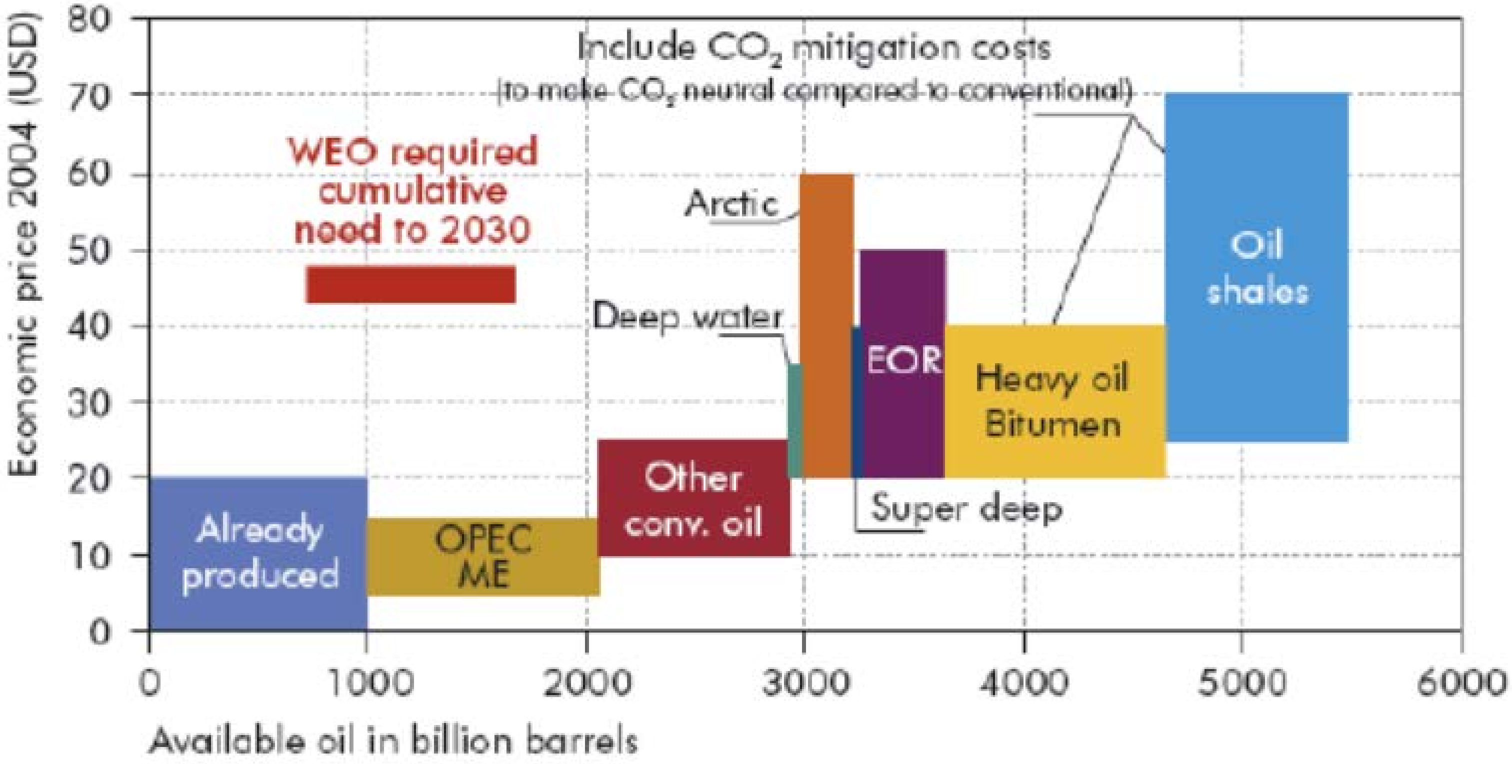
1. **Drivers of bio-economy**
2. **Technologies of change: Metabolic Engineering and Synthetic Biology**
3. **Can biotechnology compete with chemistry**
4. **Examples**
5. **Necessary (but *not* sufficient) ingredients of a successful ecosystem**
6. **Role of Brazil**

Forces of change

What has changed drastically during the past 15-20 years?

- **Continuous increase of the cost of fuels and raw materials**

Oil supply and cost curve



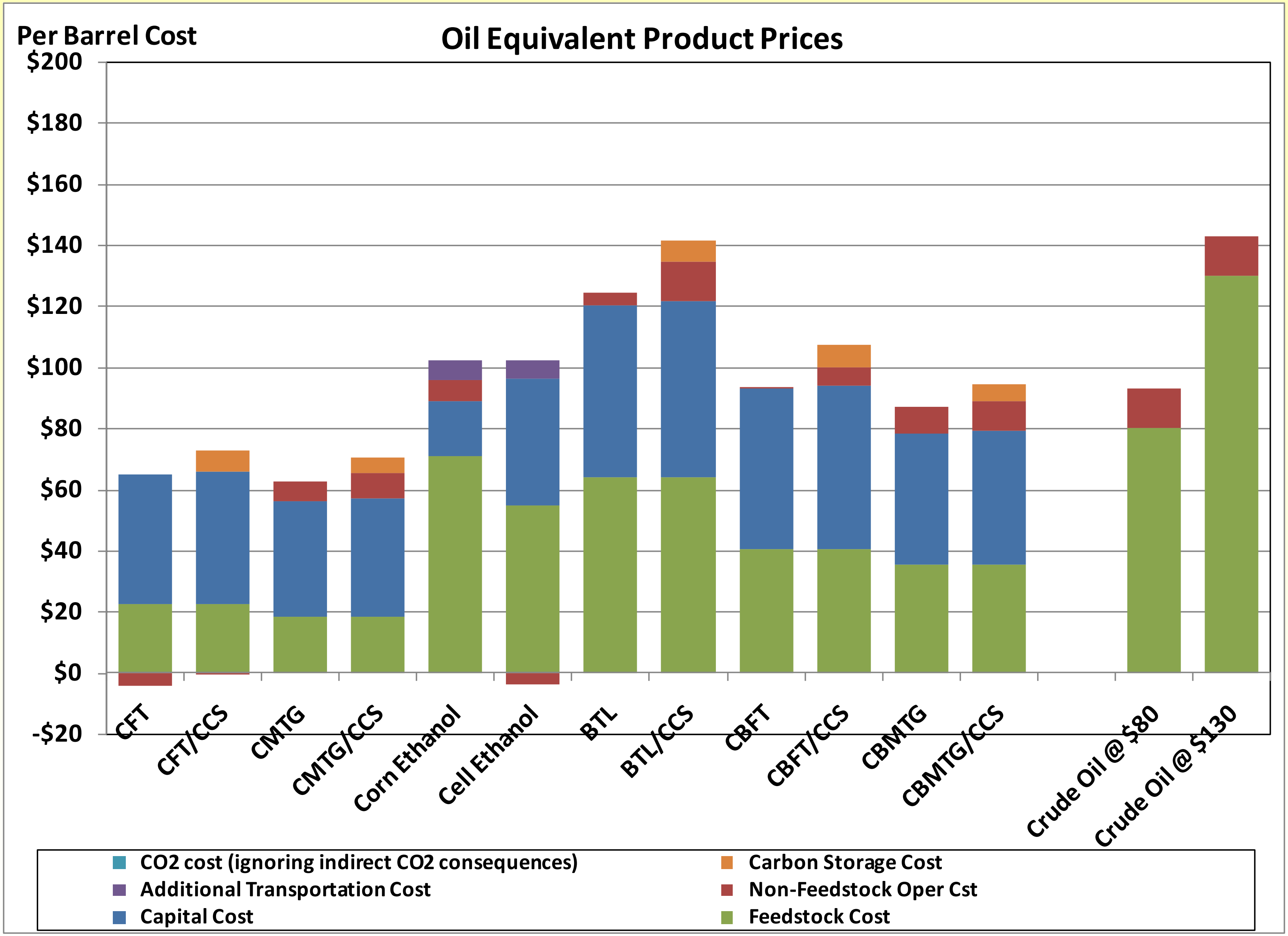
Source: IEA (2005)

U.S. DEPARTMENT OF ENERGY

What has changed drastically during the past 15-20 years?

- **Continuous increase of the cost of fuels and raw materials**

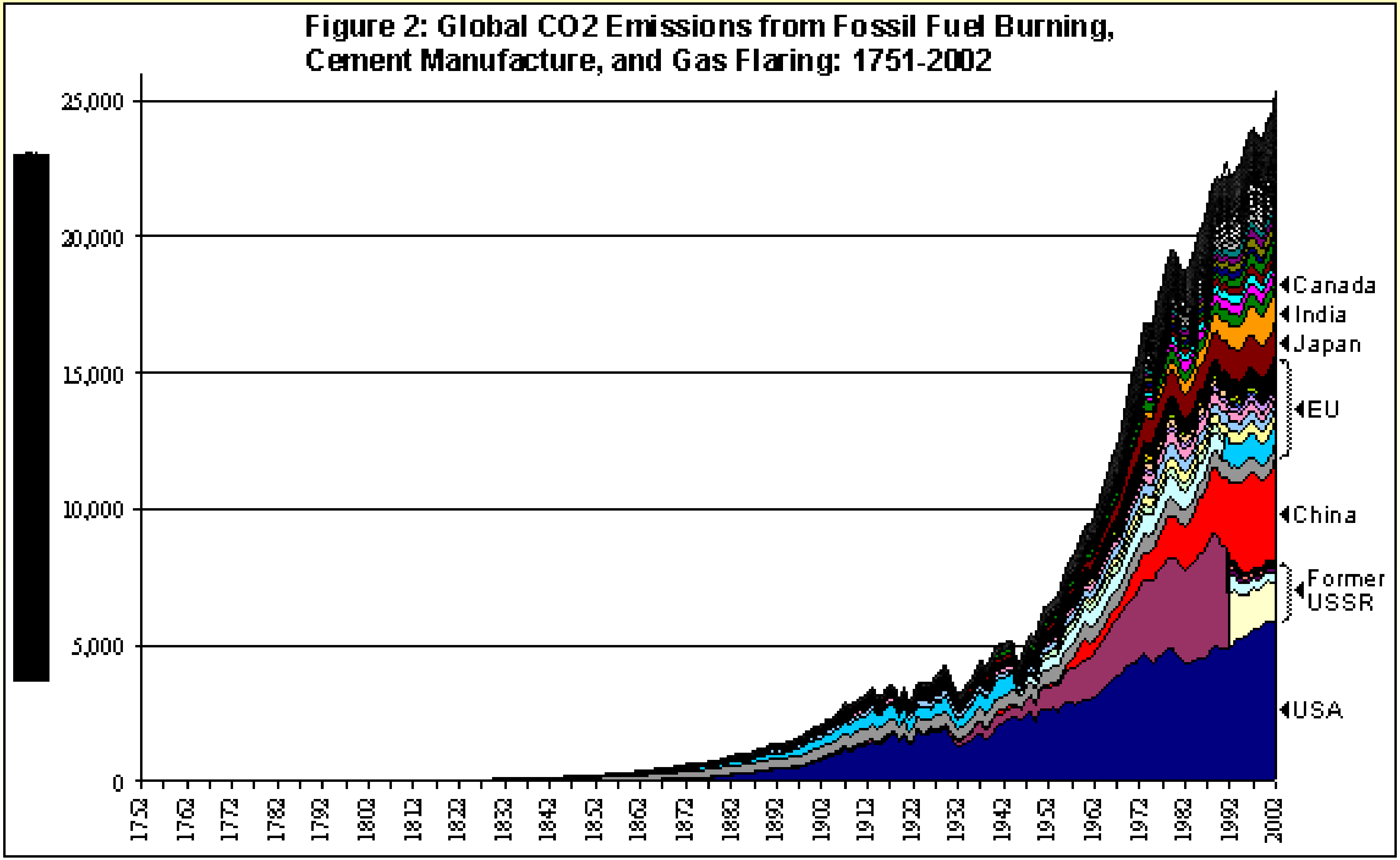
- **Strategic challenges in securing the required amounts of fuels and raw materials**


Per Barrel Cost
Oil Equivalent Product Prices
$200
$180
$160
$140
$120
$100
$80
$60
$40
$20
$0
-$20
CFT
CFT/CCS
CMTG
CMTG/CCS
Corn Ethanol
Cell Ethanol
BTL
BTL/CCS
CBFT
CBFT/CCS
CBMTG
CBMTG/CCS
Crude Oil @ $80
Crude Oil @ $130
CO2 cost (ignoring indirect CO2 consequences)
Carbon Storage Cost
Additional Transportation Cost
Non-Feedstock Oper Cst
Capital Cost
Feedstock Cost

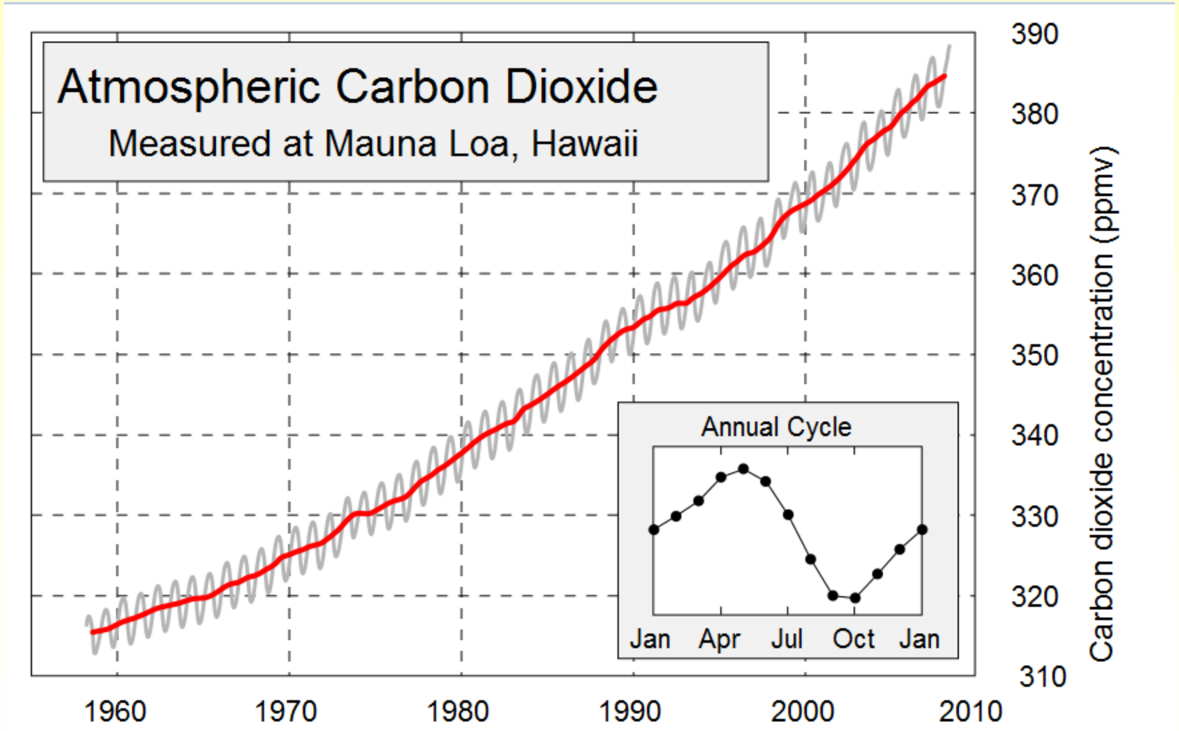
What has changed drastically during the past 15-20 years?

- **Continuous increase of the cost of fuels and raw materials**
- **Strategic challenges in securing the required amounts of fuels and raw materials**
- **Grave consequences for climate change**

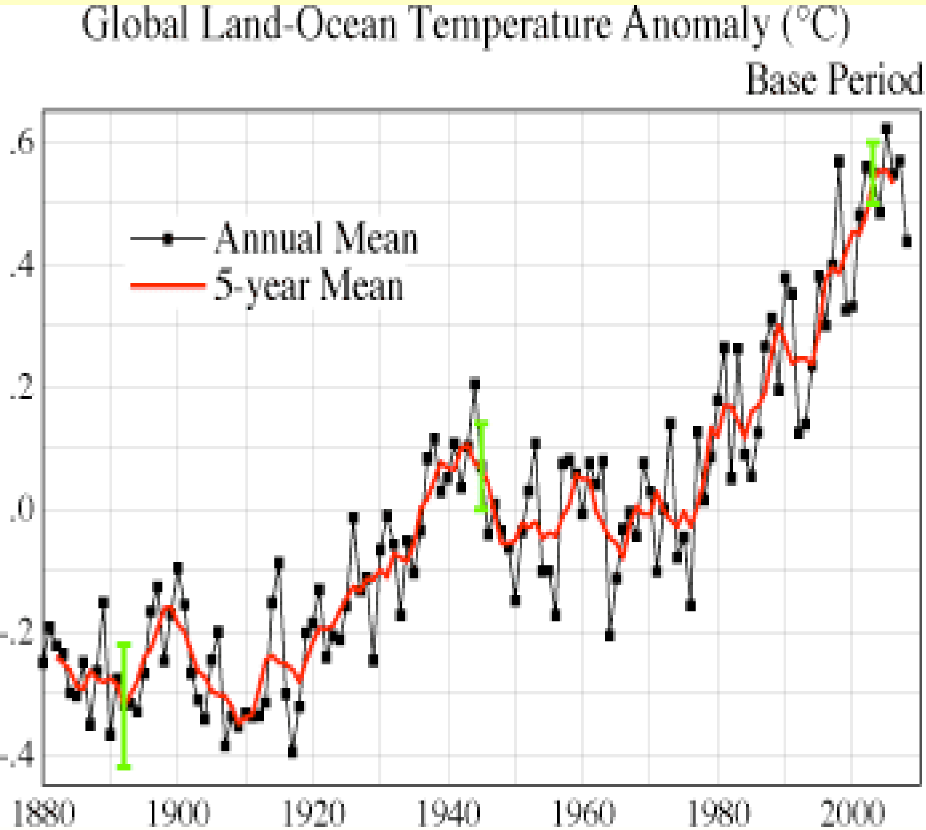
CO2 emissions

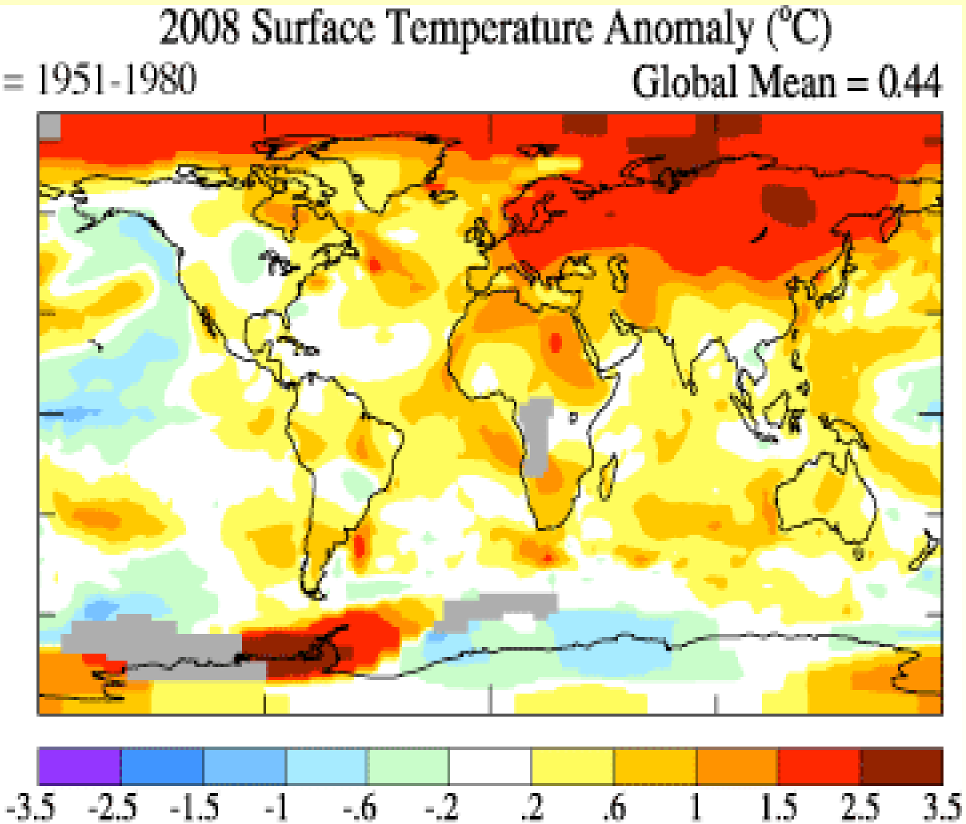


Atmospheric Carbon Dioxide

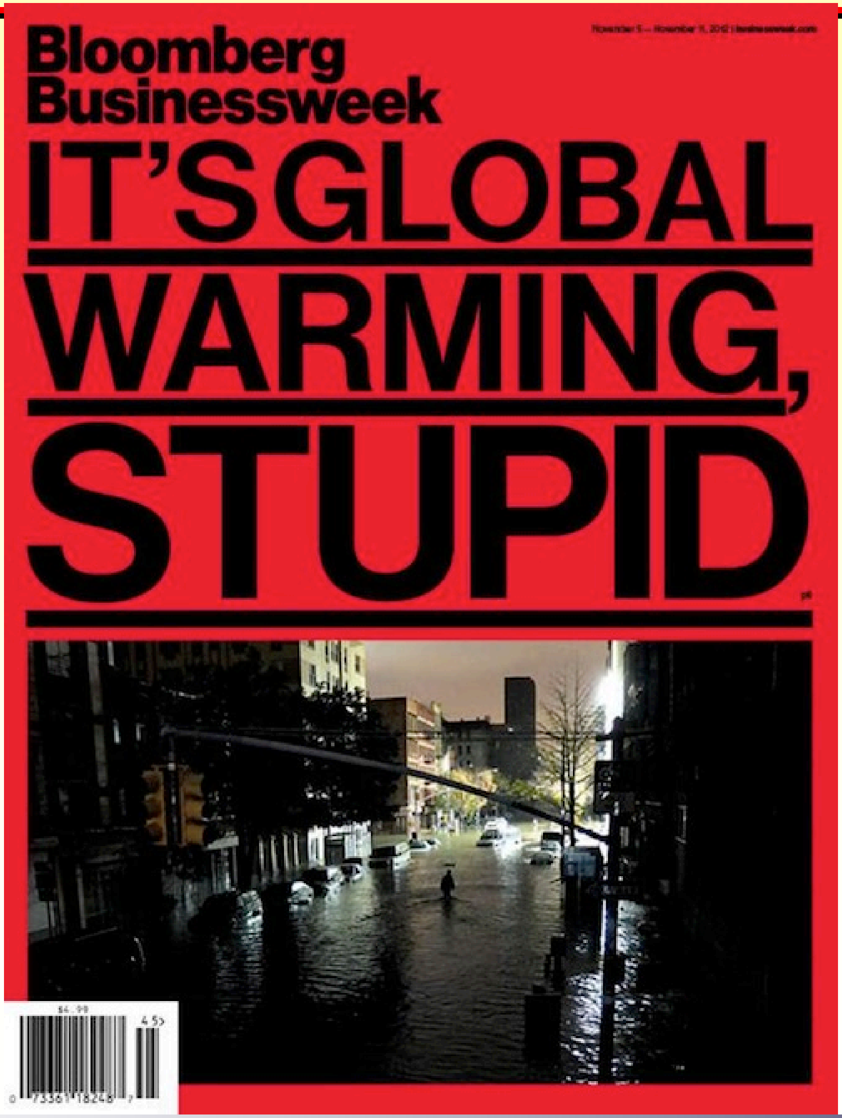


Global temperature change





After hurricane Sandy…



Sustainability: Not much of a choice…

Must begin transitioning to a sustainable bio-based economy

- **Good use of resources and energy. Energy cost must reflect *all costs* of a sustainable system**
- **Rational land use**
- **Development and acceptance of new technologies**

What has changed drastically during the past 15-20 years?

- **Continuous increase of the cost of fuels and raw materials**

- **Strategic challenges in securing the required amounts of fuels and raw materials**

- **Serious concerns about climate change**

- **Development of Biotechnology and Metabolic Engineering: Core technologies for converting renewable resources to fuels and chemicals**

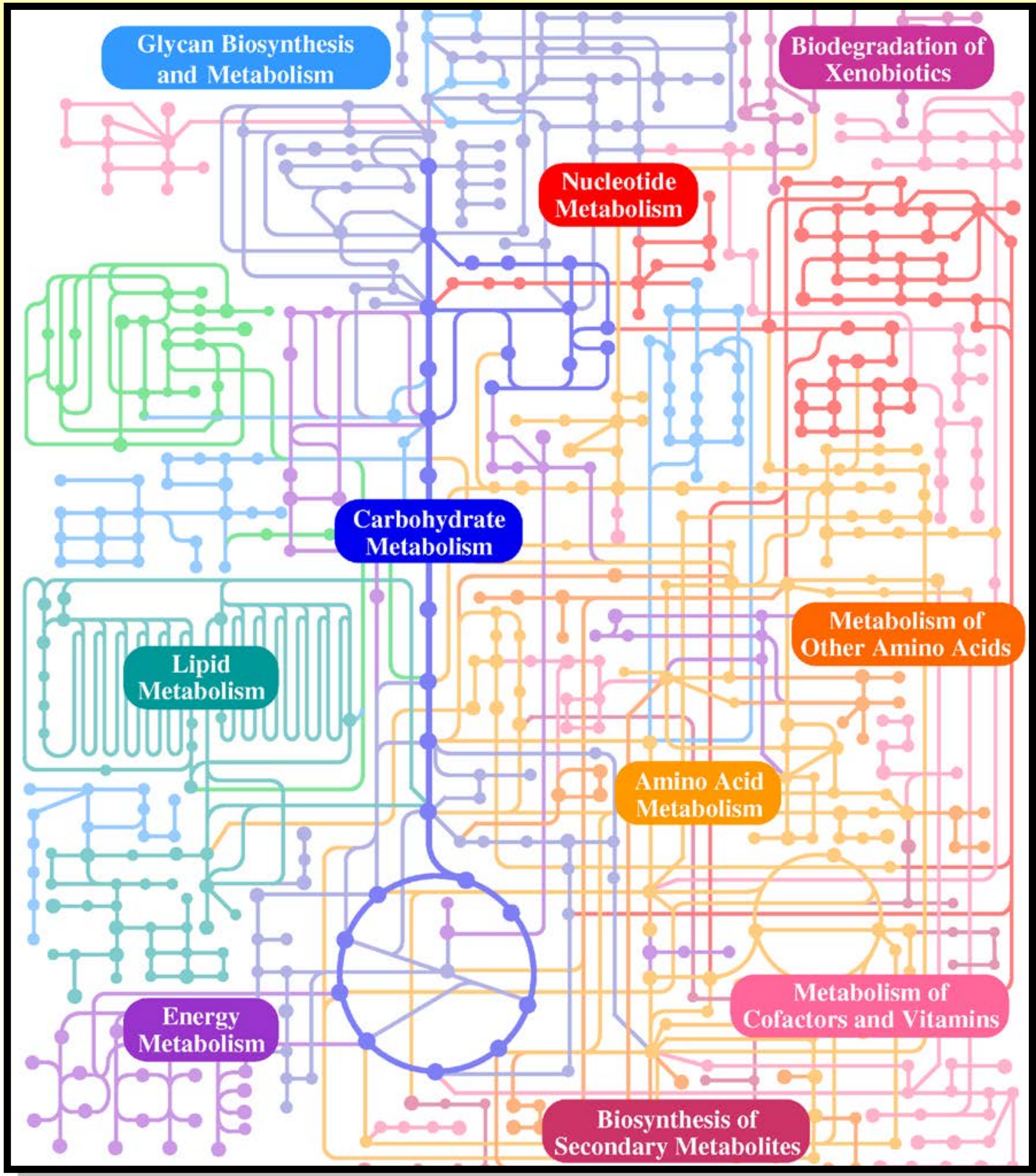
Biotechnology and Metabolic Engineering: Enabling technologies of a bio-based economy

Metabolic Engineering

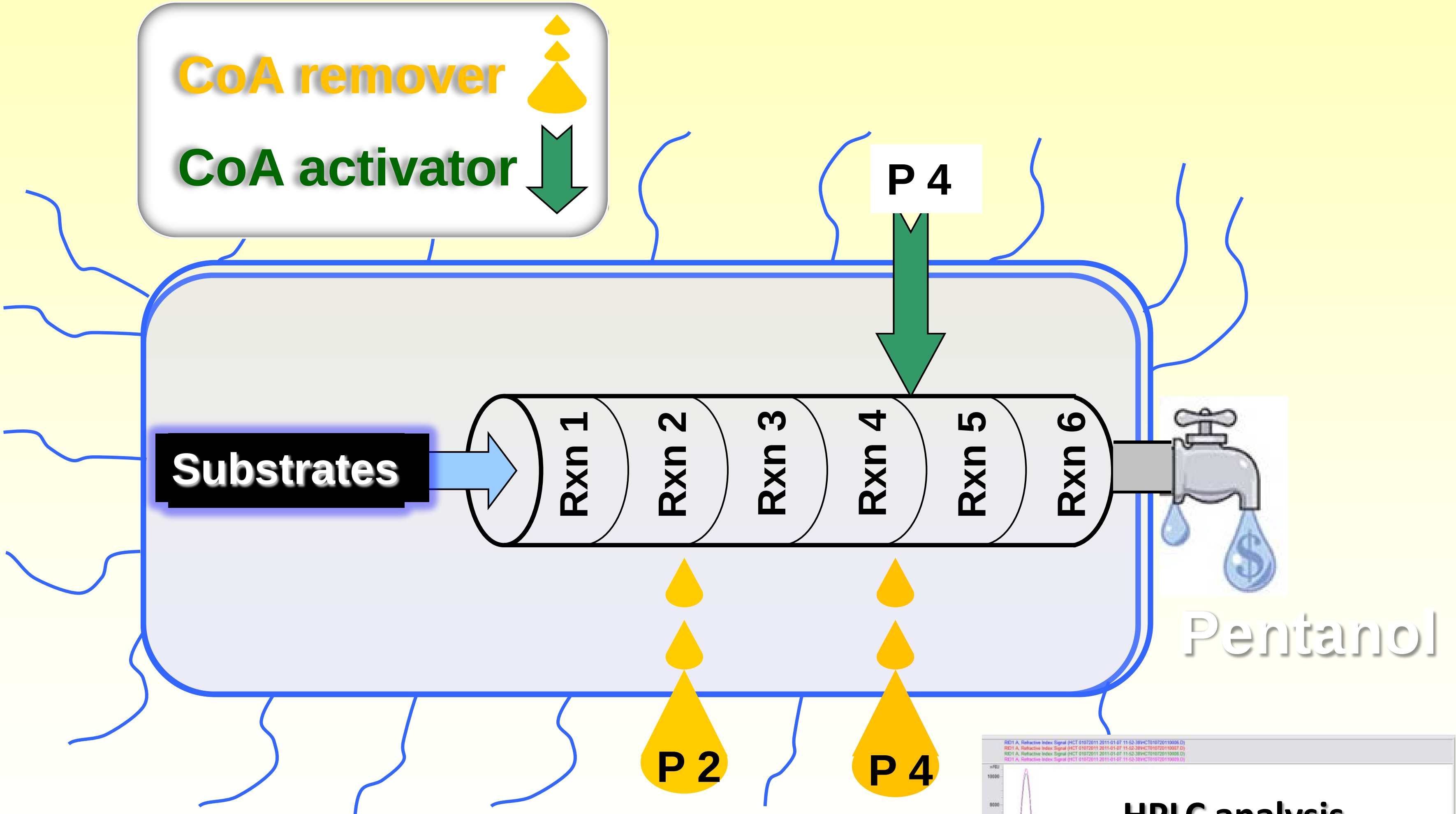
Cells:
Little chemical factories with thousands of chemical compounds interconverted through thousands of chemical reactions

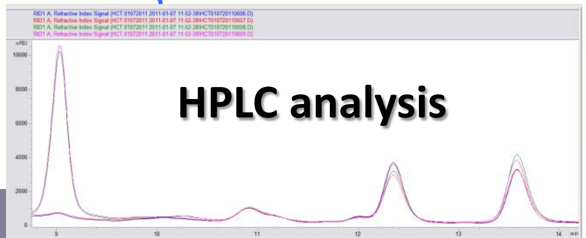
Main substrate: Sugars

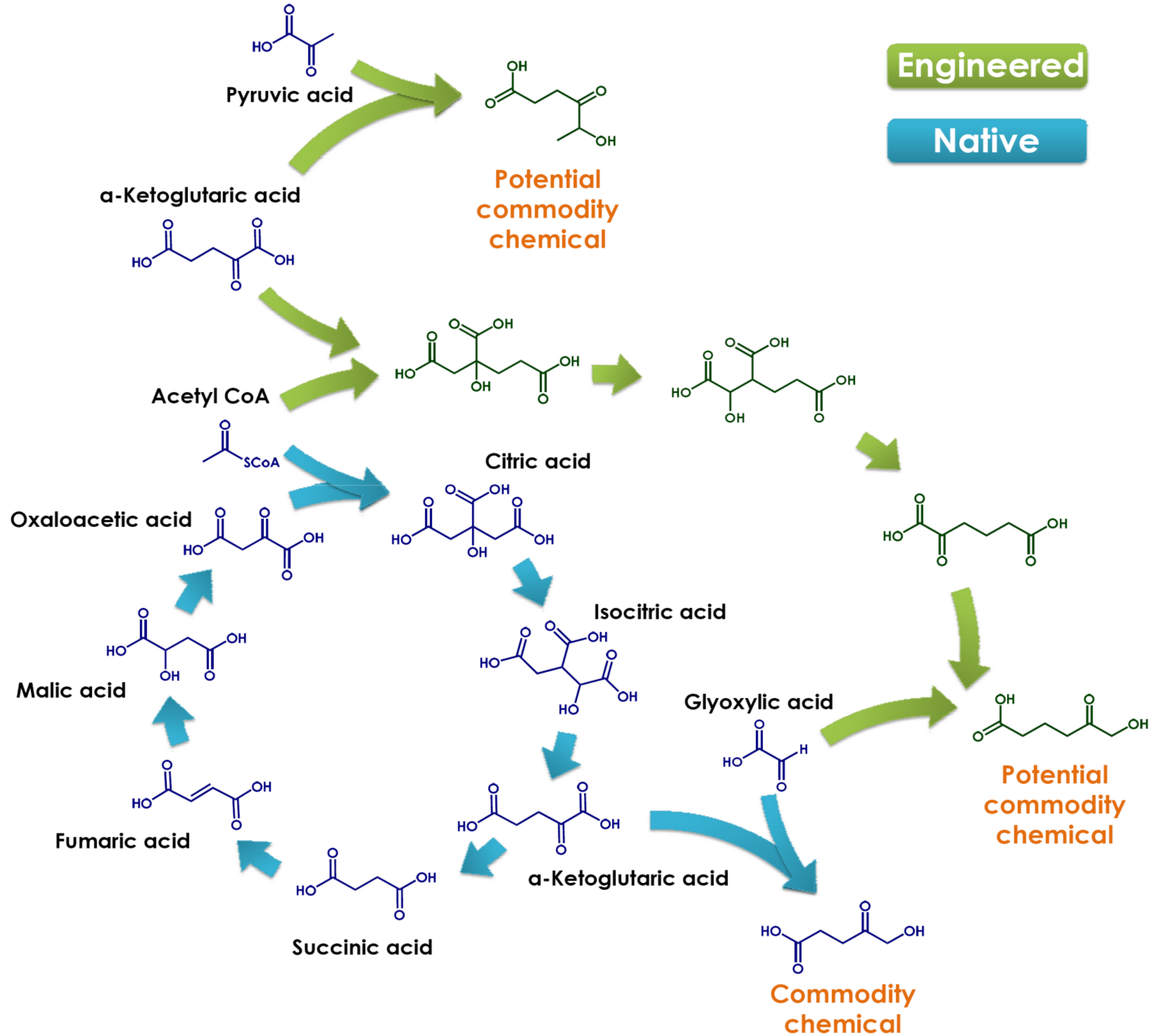
Products: Virtually infinite



Engineering microbes to produce any product






Engineered
Native
Pyruvic acid
a-Ketoglutaric acid
Potential commodity chemical
Acetyl CoA
Citric acid
Oxaloacetic acid
Isocitric acid
Malic acid
Glyoxylic acid
Fumaric acid
a-Ketoglutaric acid
Succinic acid
Potential commodity chemical
Commodity chemical
HO
OH
SCoA
H

Metabolic Engineering as a new Organic Chemistry

Metabolic Engineering: Making improved biocatalysts capable of:

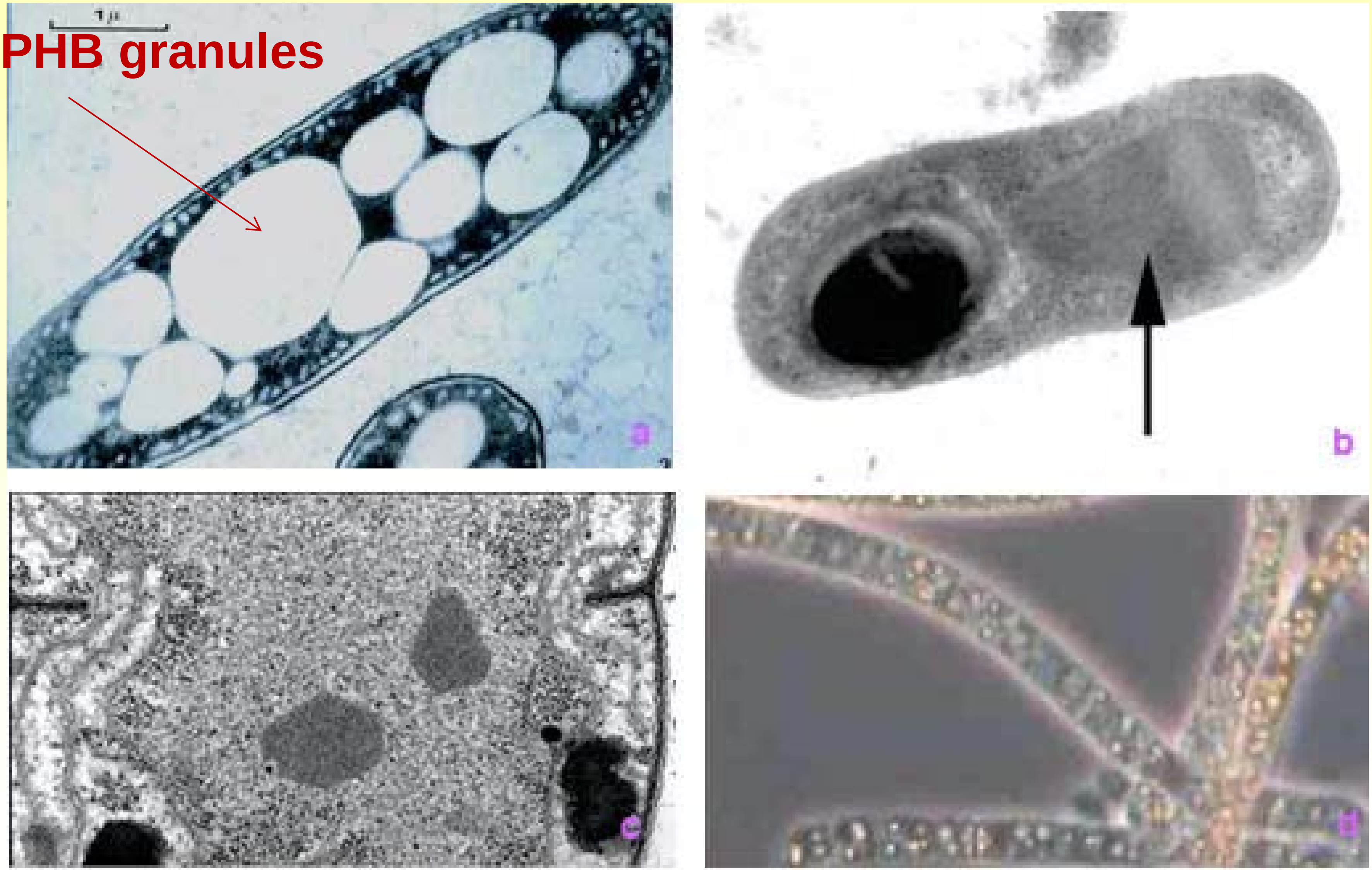
- **Enhanced production of a *native* product to a microorganism**
- **Formation of a product that is *new* to the microorganism**
- **Synthesizing *novel products***

How does Metabolic Engineering differ from Genetic Engineering?

… Metabolic engineering differs from Genetic Engineering and related molecular biological sciences in that it concerns itself with the properties of the *entire metabolic network* as opposed to individual genes and enzymes.

"Metabolic Engineering: Issues and Methodologies," *Trends in Biotechnology,* Vol. 11, pp. 392-396 (1993)

Παραγωγή βιο-αποικοδομήσιμων πολυμερών



Isoprene production for tires

Microorganisms

They are found everywhere, from the human gut to the hot springs of Yellowstone Park

Engineering microbes for *any* conversion at very high *selectivity*

Biotechnology beyond biofuels

- Propylene
- Acrylic, adipic, lactic acids
- Terephthalic acid (PET)
- Succinic acid, BDO, PDO
- Isoprene
- Biopolymers (PLA, PHB,…)
- Fats, fatty alcohols, detergents
- Polysaccharides, gums

The biotechnology revolution, and the chemical-fuels industry (White Biotech)

- Fuels and chemicals were the initial biotech target
  - Cetus (Chiron), Genex, Biogen, Genentech
- More *challenging* technical problem than insulin
  - Switch of emphasis to medical applications
- Changing boundary conditions
  - Emphasis on renewable resources
  - Robust US federal funding ⇒ Applied mol. biology
  - Genomics
  - Systems Biology: a new mindframe in biological research
  - Metabolic Engineering
- Exploit applications of biology beyond medicine

Creative destruction:

Replace depreciated low-cost chemical plants with modern high-biotech processes

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Why?

1. Selectivity of bioprocesses

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6. **Sensitivity of catalysts**
7. **High temperatures, P avoided**

Illustrative examples from MIT lab

Example 1:

Ethylene glycol

MEG from Xylose: C2 Fermentation Pathway

C5 sugar → C2 inter + C3 intermediate

C2 intermediate → MEG

C3 intermediate → MEG + CO_2

Glucose:

Six carbon sugar derived from multiple sources including corn, cane sugar, biomass

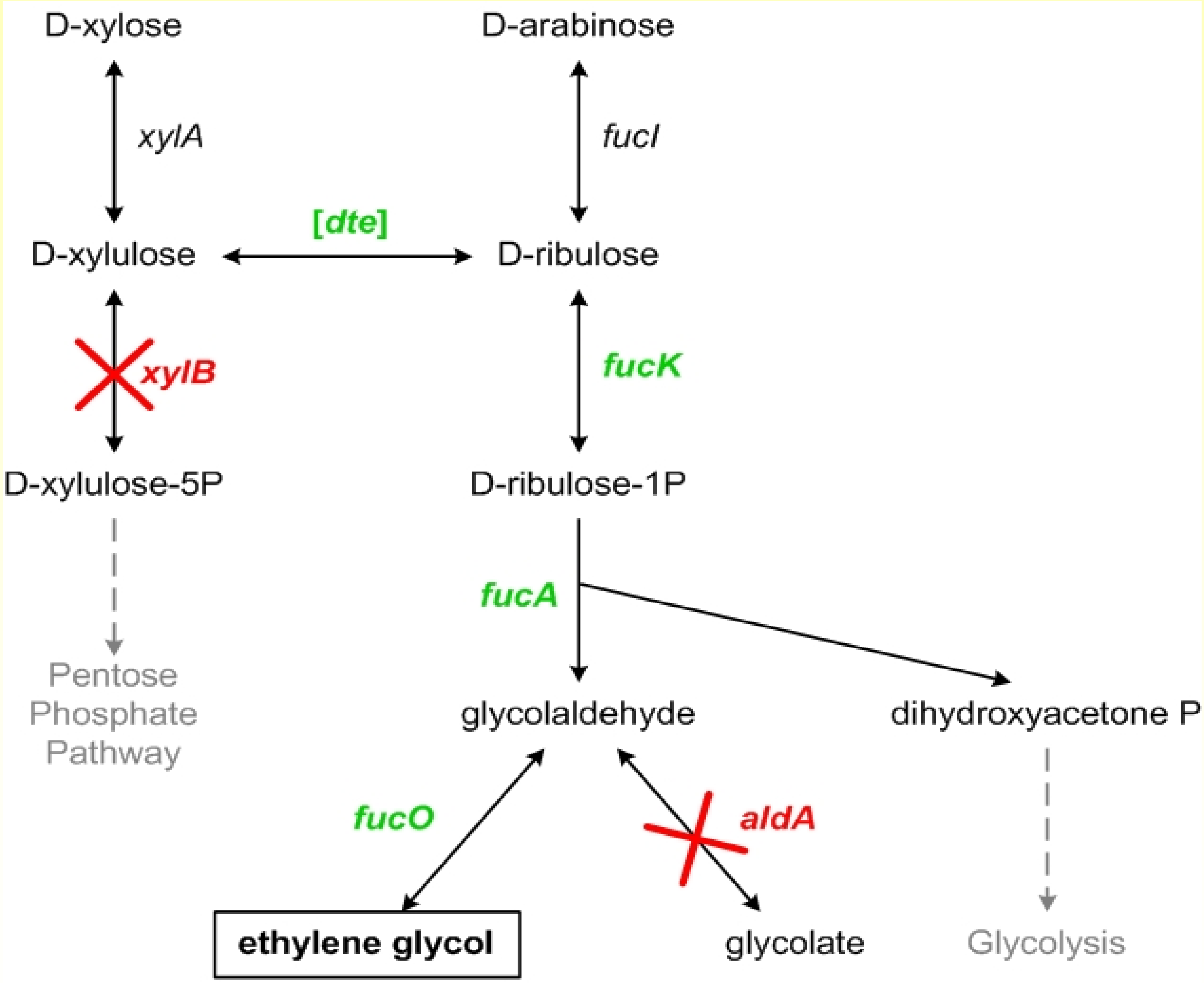
Glucose conversion to MEG

C6 sugar → 2 C3 intermediate

C3 intermediate → MEG + CO_2

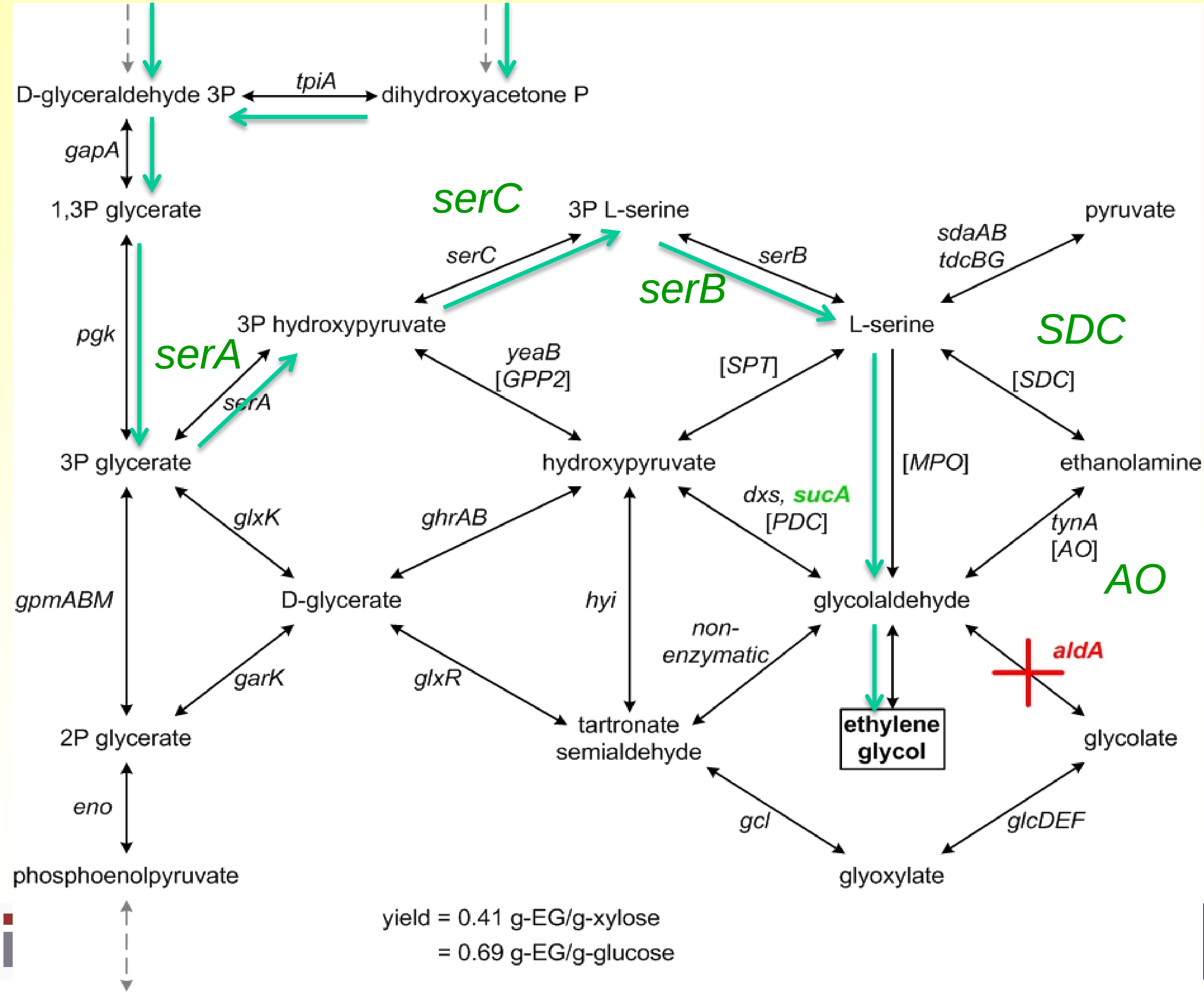
ARTEK

EG from the C2 pathway



yield = 0.41 g-EG/g-xylose

EG from the C3 pathway



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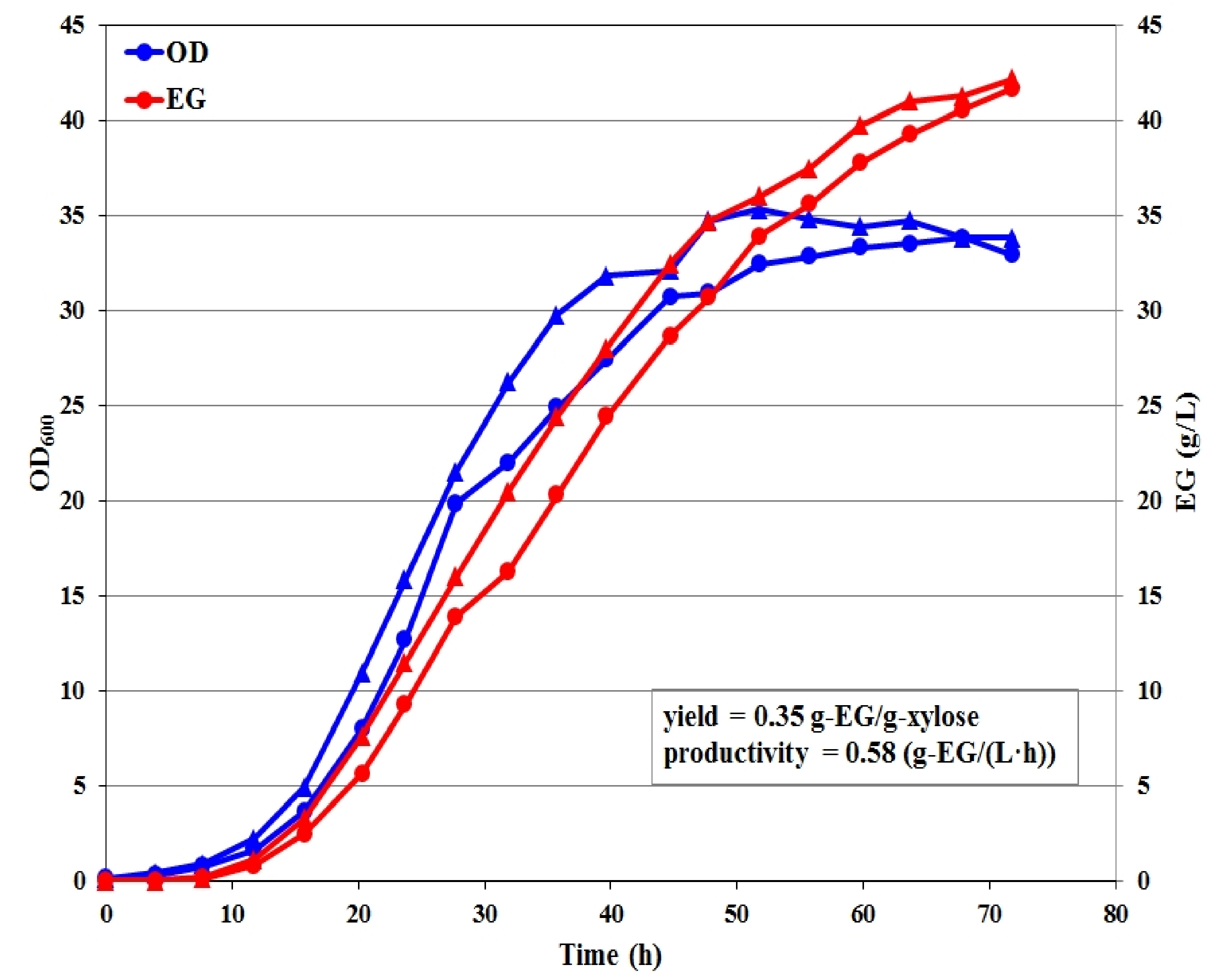
2014

Xylose Fermentation Data



C5 pathway:

- Fermentation Organism: E. Coli
- Yield: 0.40 kg MEG/kg xylose
- Titer: 40 g MEG /liter
- Carbon Source: Xylose

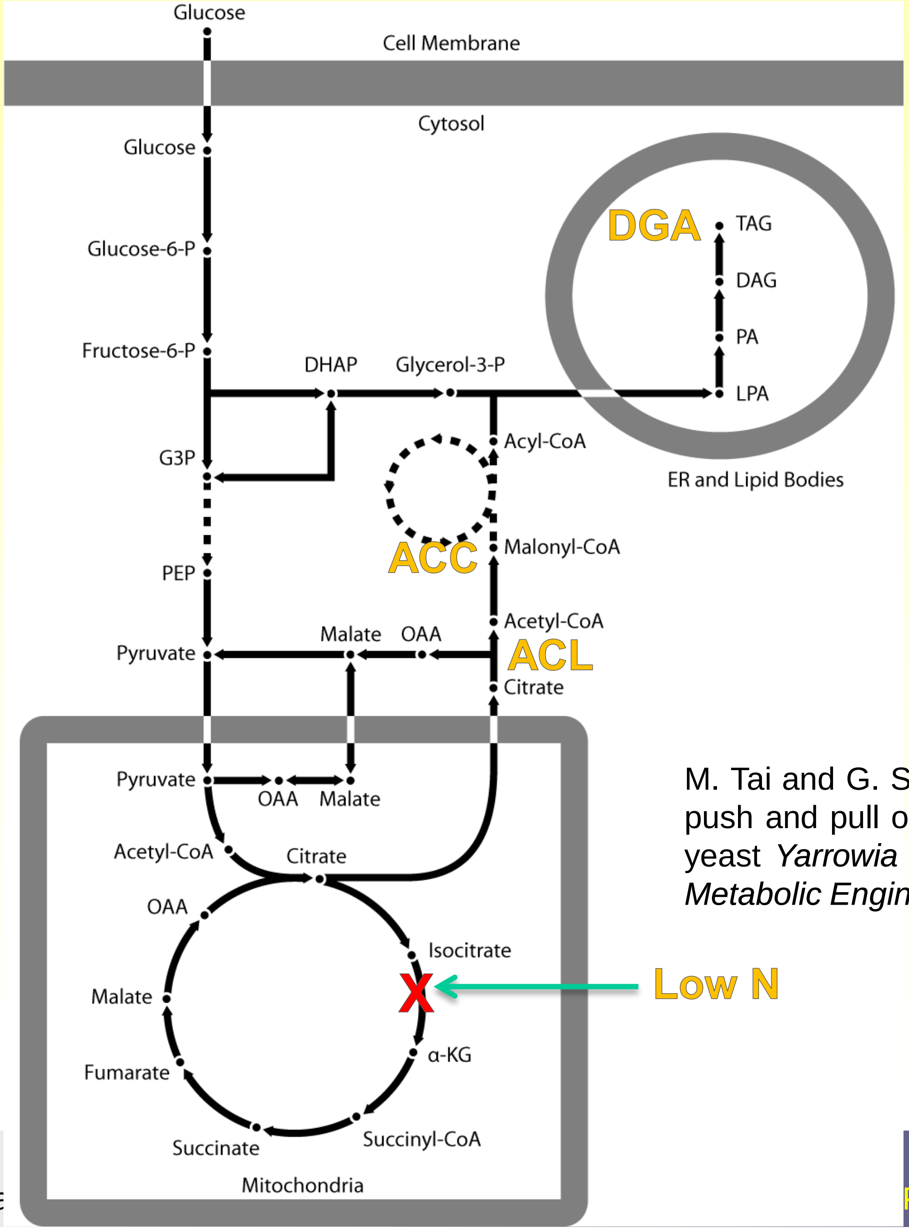
Conclusions:

- A proven pathway to MEG from sugar today
- Industrially relevant rates, yields and titers
- Technology patented

Example 2:

Carbohydrates to lipids for biodiesel production

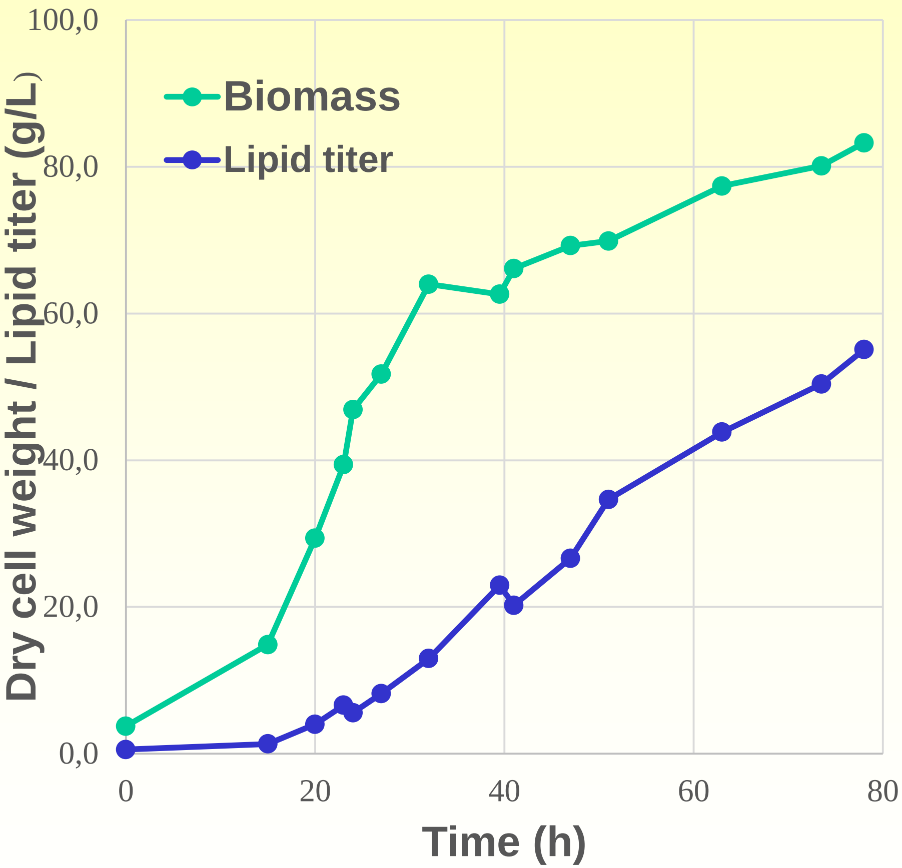
Lipid biosynthesis pathway: role of ACL



M. Tai and G. Stephanopoulos, "Engineering the push and pull of lipid biosynthesis in oleaginous yeast *Yarrowia lipolytica* for biofuel production," *Metabolic Engineering,* 15: 1-9, (2013)

Optimization of AD9 fermentation 3: Lipid production

Biomass & lipid production of AD9 in 1.6-liter bioreactor



Lipid titer (g/l)	56.1
Dry cell weight (g/l)	83.2
Lipid content	66.2%
consumed Glu (g/l)	236.2
Yield (g/g)	0.24
Productivity (g/l/h)	0.707
Time (h)	78

Maximum lipid production during lipid formation phase

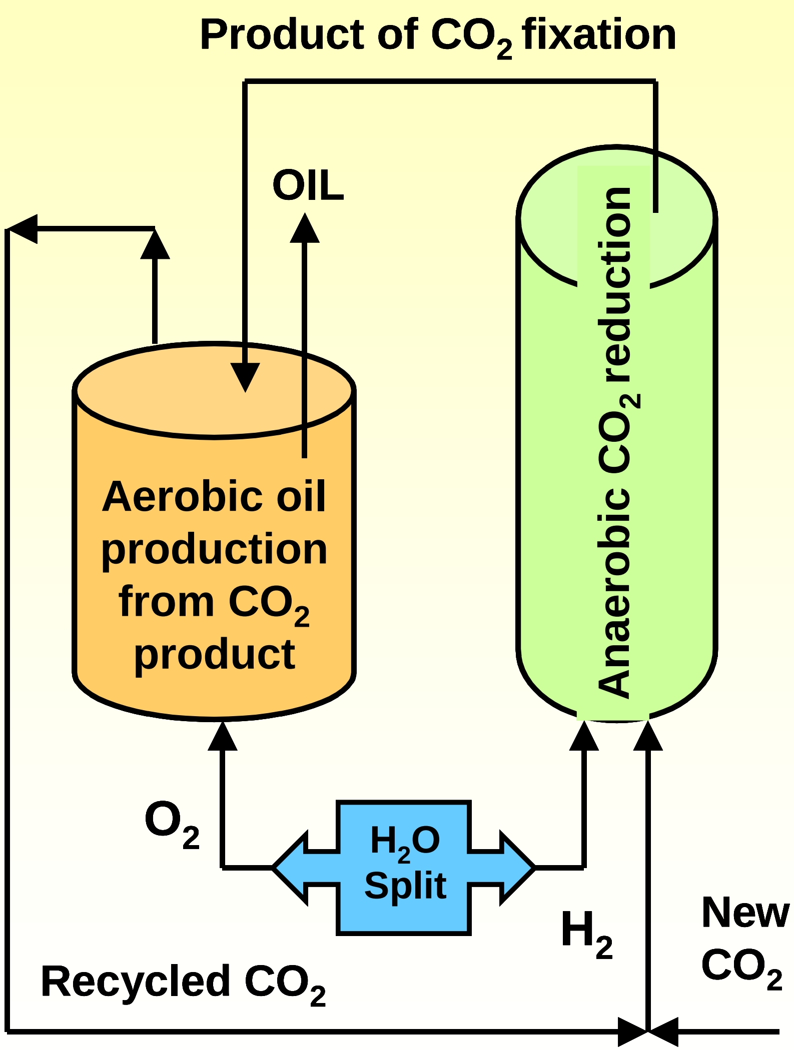
Lipid prod'n phase	41-78 h
Consumed Glu (g/l)	130.85
Yield (g/g)	0.2664
Productivity (g/l/h)	0.942

Patent pending

Example 3:

Gases to lipids for biodiesel production

A two-stage system for converting syn gas to lipids

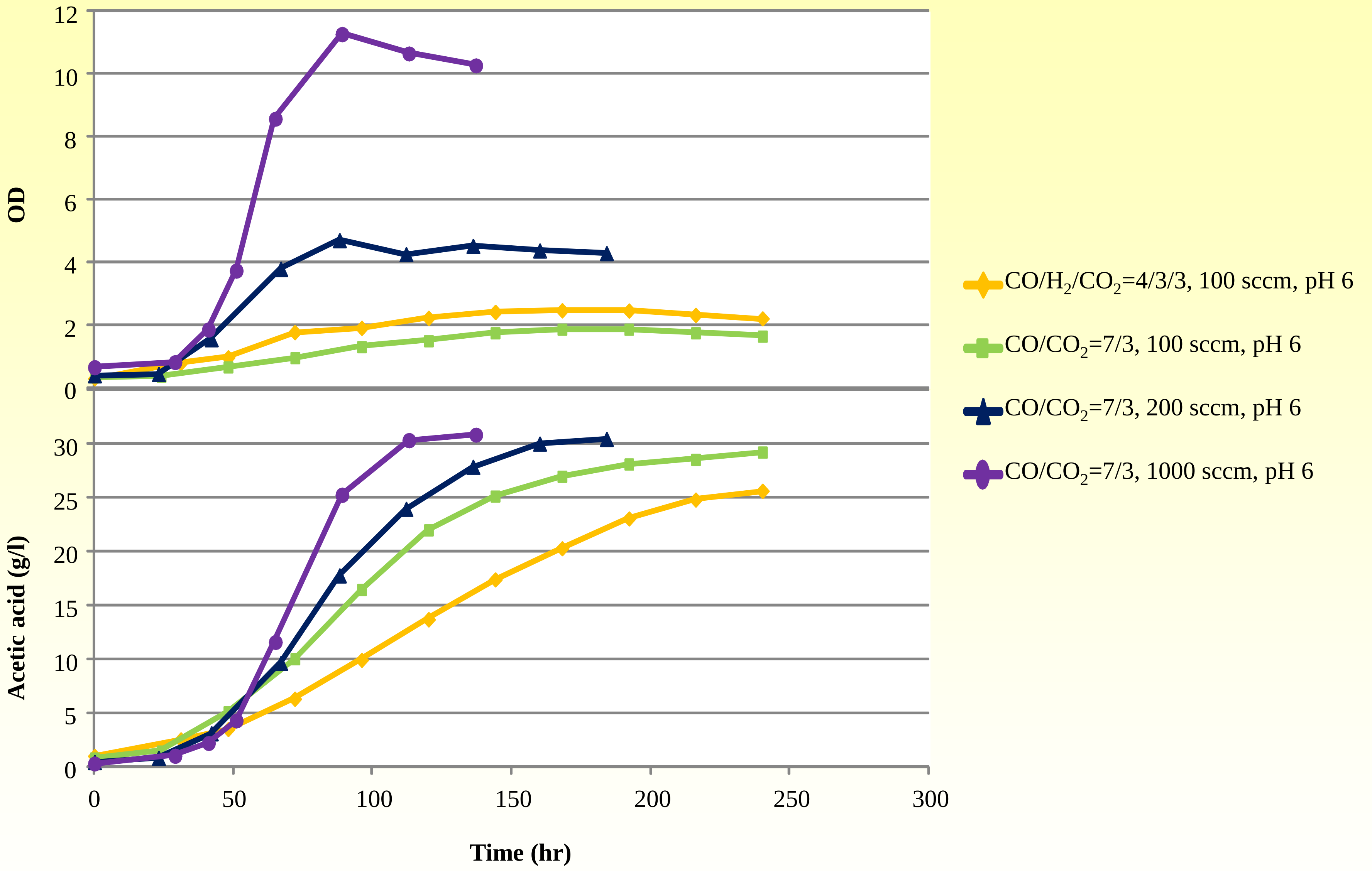


Goal: Produce an infrastructure compatible fuel (biodiesel) from CO_2 and H_2

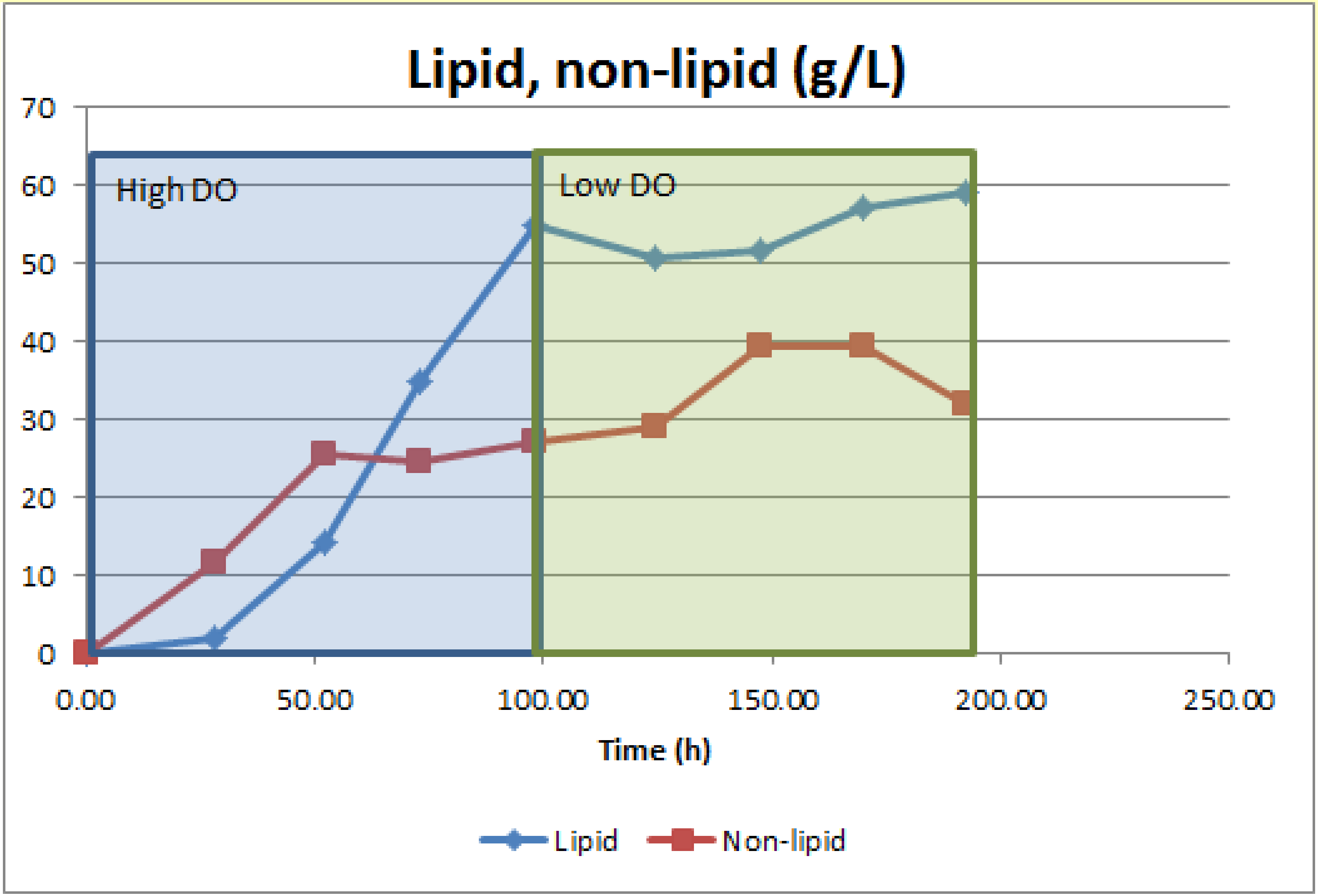
Asset: Oleaginous microbe with extremely high yields, productivities, and titers

Strategy: Fix CO_2 and H_2 by acetogenic bacteria and feed **acetate** so produced to Oleaginous microbe

Challenges: Achieve high rates of growth of acetogenic bacteria, and acetate production


OD
12
10
8
6
4
2
0
Acetic acid (g/l)
30
25
20
15
10
5
0
0
50
100
150
200
250
300
Time (hr)
CO/H2/CO2=4/3/3, 100 sccm, pH 6
CO/CO2=7/3, 100 sccm, pH 6
CO/CO2=7/3, 200 sccm, pH 6
CO/CO2=7/3, 1000 sccm, pH 6

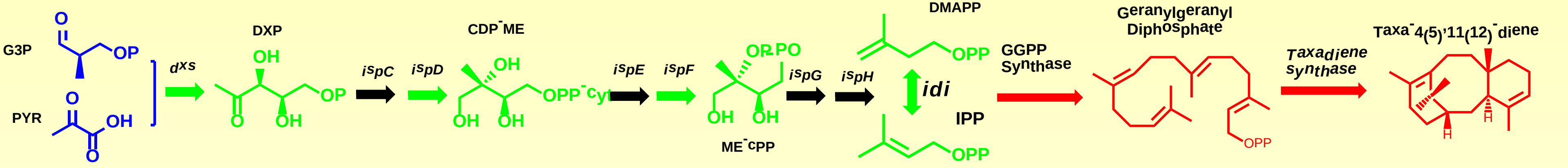
Lipid and non-lipid time courses

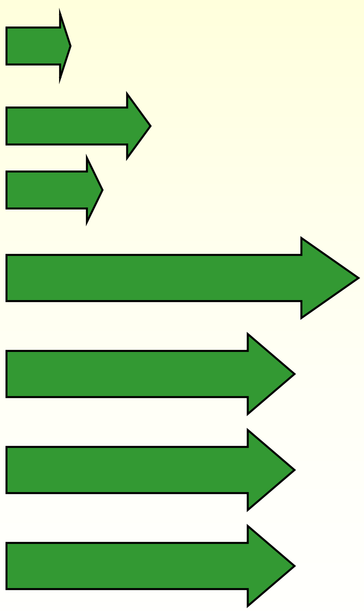


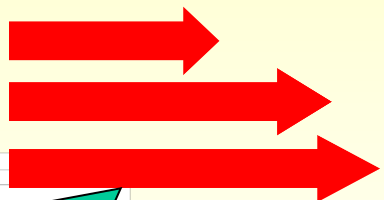
Example 4:

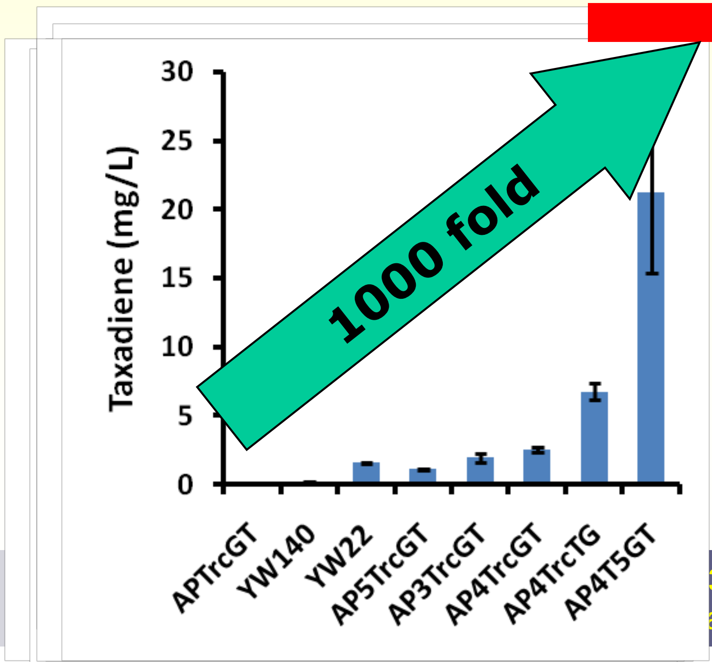
Isoprenoids

II.6 Modulating the upstream and downstream pathway for amplifying taxadiene production

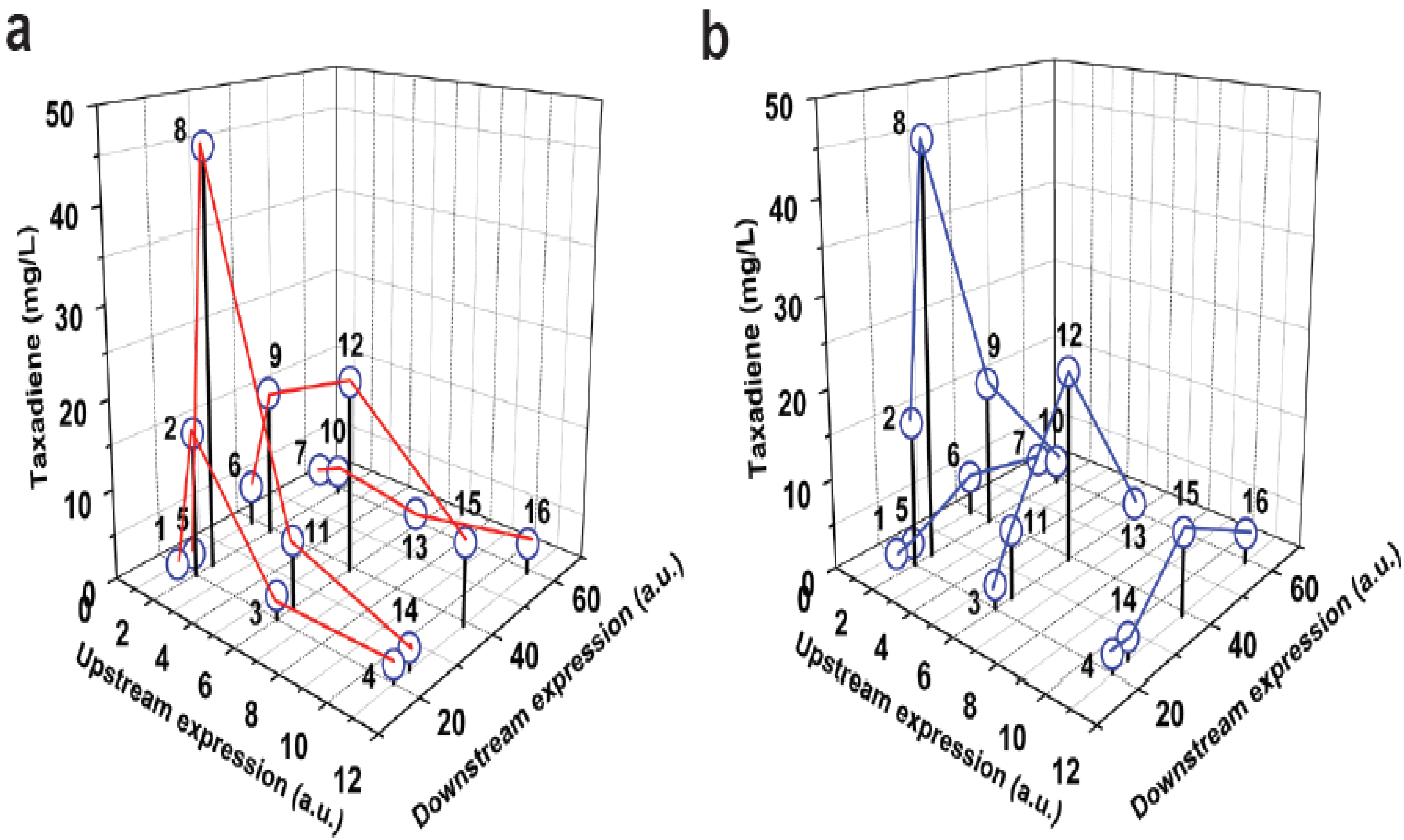




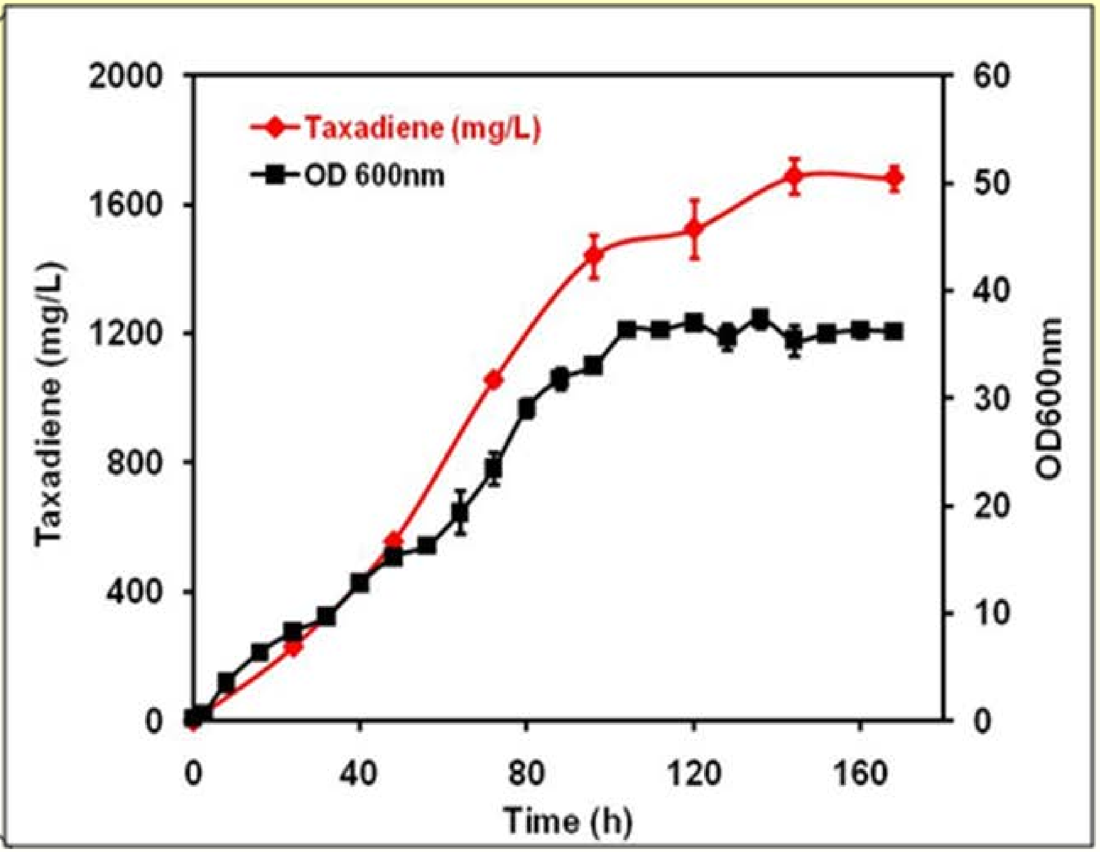




YW140=T5 single
YW22=T7, single
AP5TrcGT=single
AP3TrcGT=10 copies
AP4TrcGT=5 copies
AP4TrcTG=5 copies
AP4T5GT=5 copies


a
b
Taxadiene (mg/L)
Upstream expression (a.u.)
Downstream expression (a.u.)
0
10
20
30
40
50
2
4
6
8
10
12
20
40
60

Fermentation of taxadiene producing strain AP2T7TG



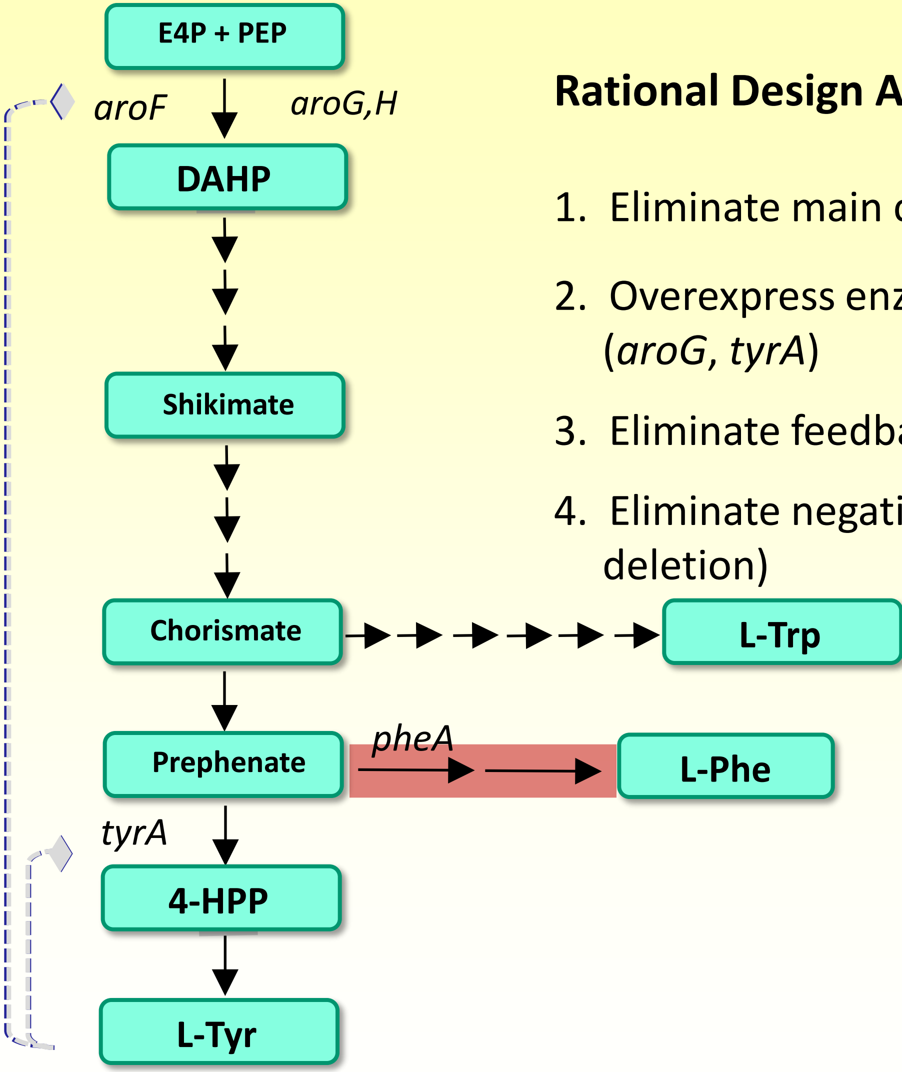
Science, 330: 70-74 (2010)

- **Taxadiene production: ~1,700 mg/L**

Example 5:

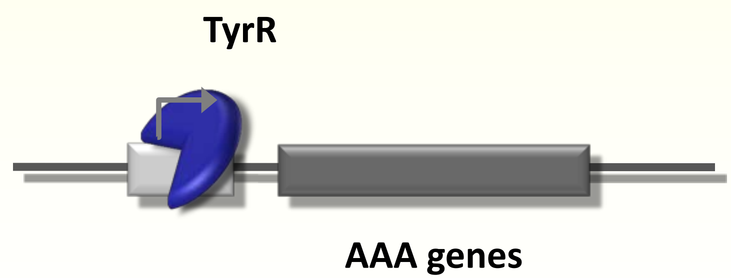
Engineering *Escherichia coli* to overproduce tyrosine directly from glucose

Aromatic amino acid biosynthetic pathway



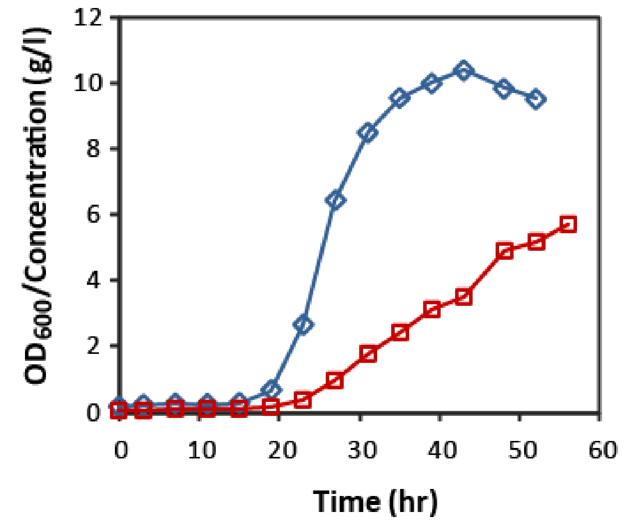
Rational Design Approaches

1. Eliminate main competing reactions (*pheA* deletion)
2. Overexpress enzymes constituting major bottlenecks (*aroG*, *tyrA*)
3. Eliminate feedback repression of enzymes (*aroG*^{fbr}, *tyrA*^{fbr})
4. Eliminate negative transcriptional regulator (TyrR deletion)

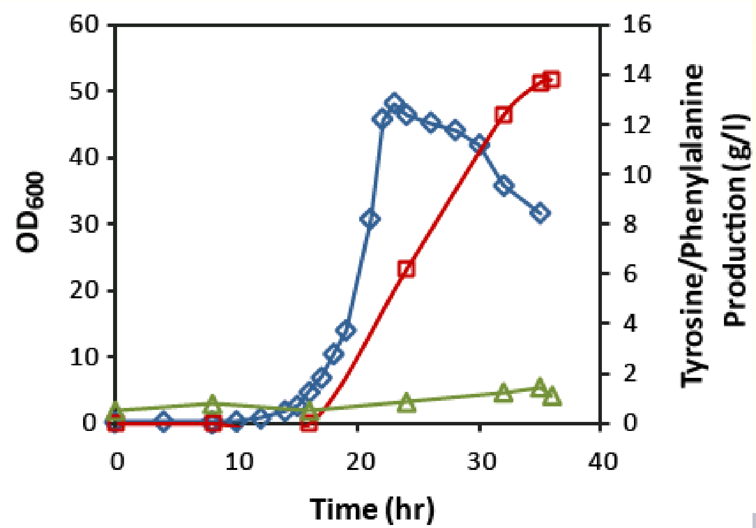


1.5-l Fermentations in MOPS and R media

MOPS minimal medium



R medium



Media Formulation	MOPS	MOPS	R
Fermentation scale	50 ml	1.5-l	1.5-l
L-tyrosine	902 mg/l	5.71 g/l	13.8 g/l
Glucose consumed (g)	5 g	28	115
Yield (g Tyr/g Glc)	0.180	0.204	0.120
Maximum Productivity (mg Tyr/g DCW/hr)	-	92.6	188
Maximum Productivity (g Tyr/L/hr)	-	0.280	2.06
Growth rate (hr^{-1})	0.296	0.275	0.405
Maximum OD_{600}	3.72	10.4	48.1

Trade-off between yields and maximum productivities/titers

◇ OD_{600} □ L-tyrosine △ L-phenylalanine

Example 6:

Muconic (and adipic) acid

Necessary (but not sufficient) ingredients of a successful biotech ecosystem – the MIT experience

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- **First-class academic institution**

MIT

- Private University, founded in 1861
- Endowment ~$10B (450M (1985))
- Tuition: $42,050 + $12,000 (room & board)
- Research budget: ~$750M/yr ('12)
- Revenue of Technology Licensing Office from Stock cash out, royalties, fees: ~$80/year (2012)
- Rate of return: 10.7%

MIT

- If you counted the number of companies founded by MIT faculty and Alumni the past 25 years you would have:
  - ~25,000 εταιρίες, employing,
  - ~1,600,000 employees, generating
  - ~$1.5T in goods and services (equivalent to a G20 country)

Necessary (but not sufficient) ingredients of a successful biotech ecosystem – the MIT experience

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- **Reward innovation**
- **High risk capital-competitive tax treatment**
- **Legal-financial infrastructure**
- **Government support (remove obstacles)**

Biofuels and chemicals are first and foremost a feedstock story

- **Key competitive advantages**
  - **Land availability**
  - **Plentiful water**
  - **Sunlight and highly productive land**

Industrial Biotech and Brazil

- **A natural**

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- **Biotech and Metabolic Engineering: technologies of the 21st century**

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- **Present negative outlook: An opportunity to move in and make up for lost ground!**

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- **Biotech and overall economy: can contribute 5-10% of GDP, lift rural development, substitute imports**

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- Biotech and Metabolic Engineering: technologies of the 21st century
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- Present negative outlook: An opportunity to move in and make up for lost ground!
- Biotech and overall economy: can contribute 5-10% of GDP, lift rural development, substitute imports
- Very promising technology: unlimited products

What is in the future?

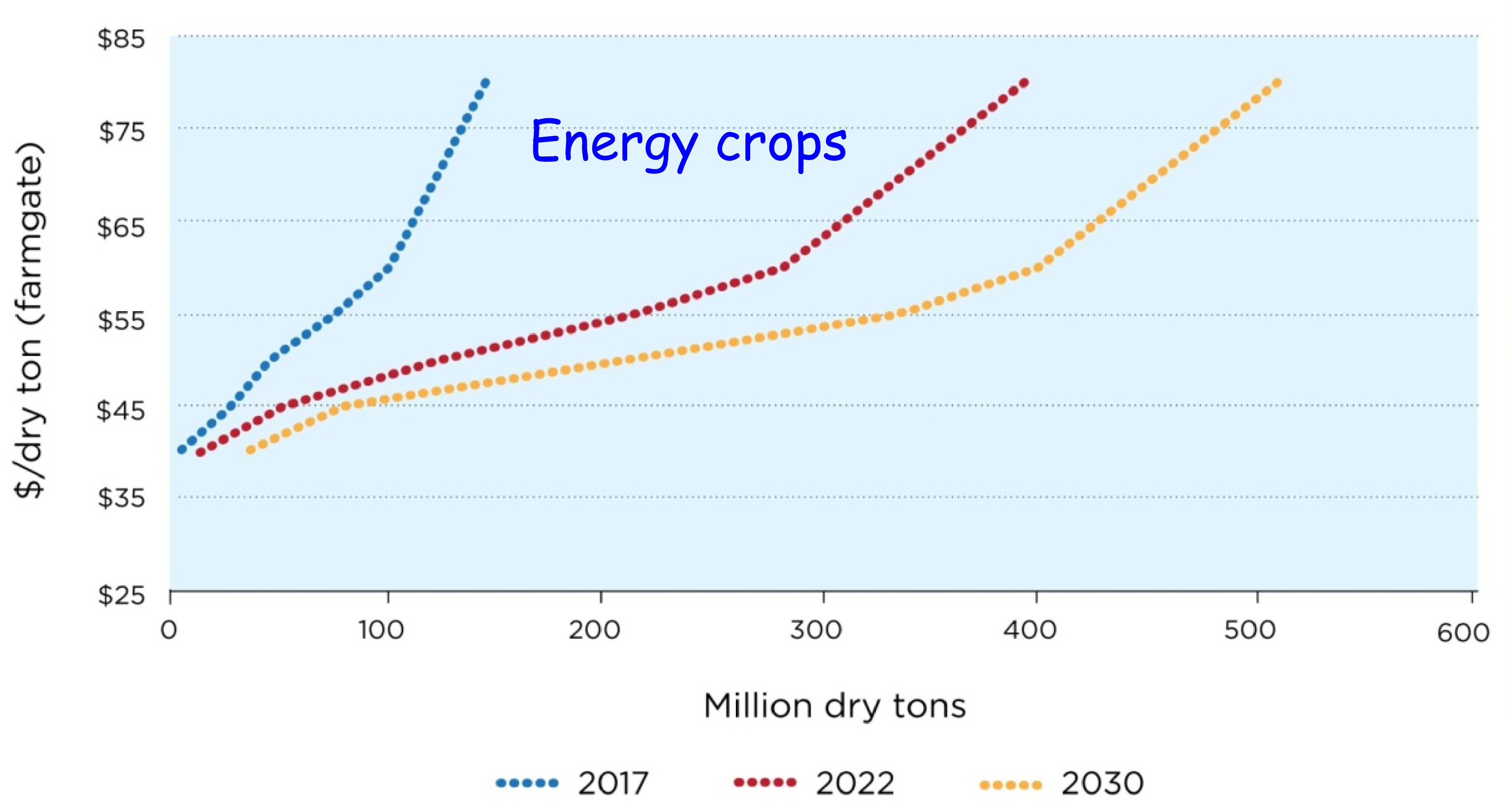
Future applications drivers

- **Sustained interest in utilization of renewable resources**
    - **Pressure on commodities will continue**
    - **Climate change concerns will persist**
    - **Biotechnology is better than chemistry in utilizing carbohydrates**

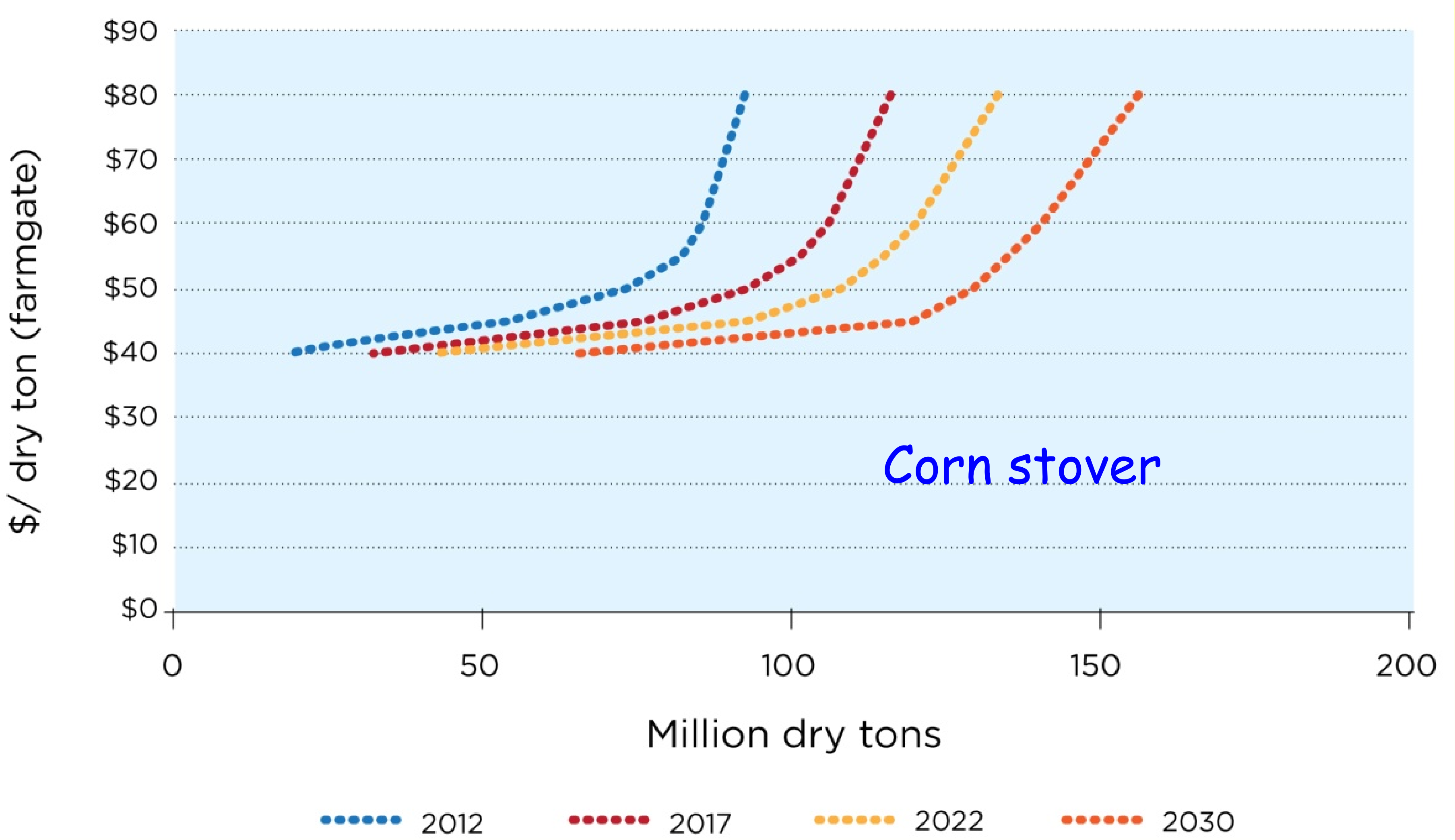

MCMXVI
MASSACHVSETTS INSTITVTE OF TECHNOLOGY

Expected supply curves for biomass

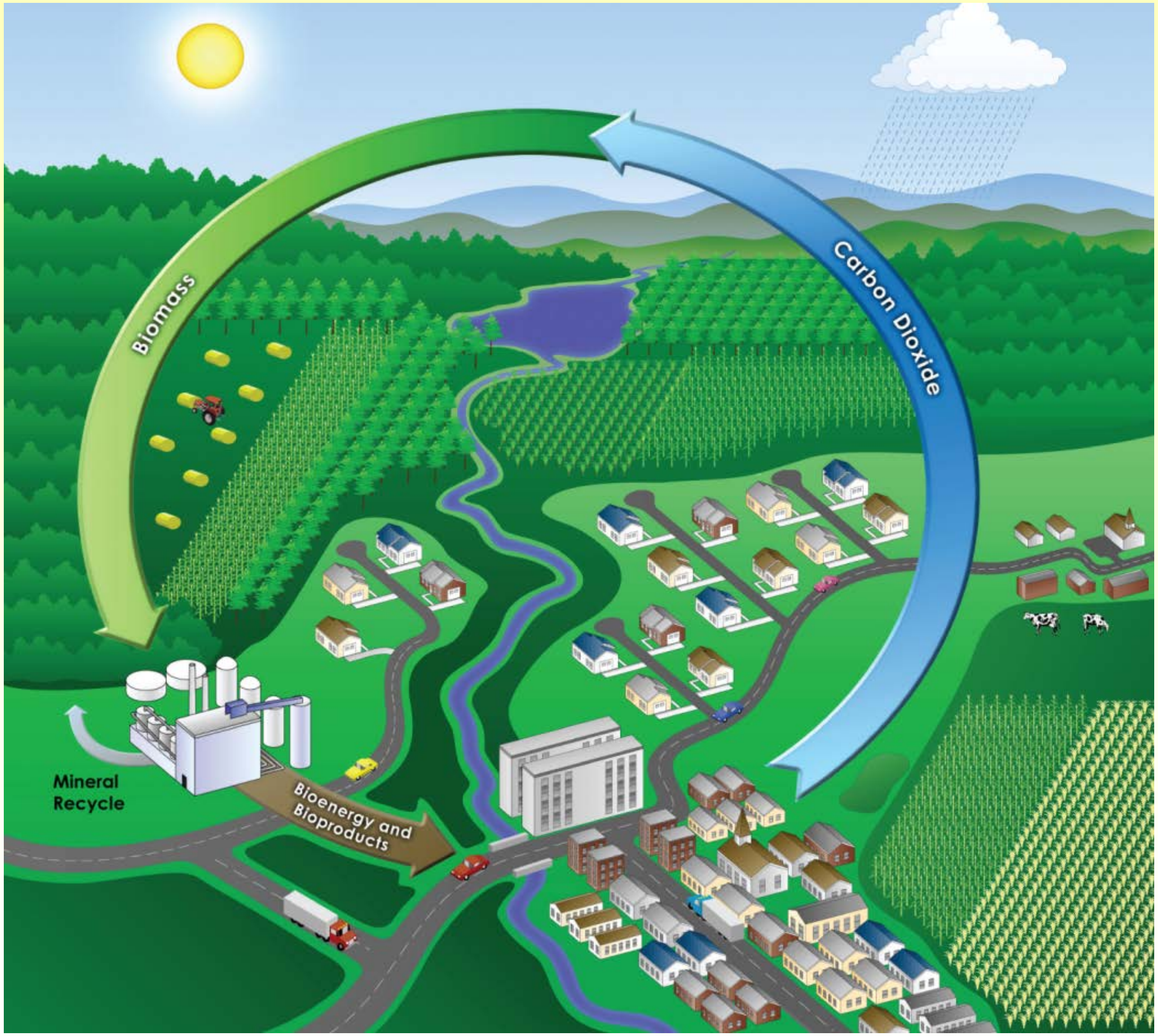
http://www1.eere.energy.gov/biomass/pdfs/billion_ton_update.pdf



Expected supply curves for biomass



Need a sustainable bio-economy



Can land-based renewables replace sustainably fossil fuels?

- Yes for the production of chemicals
- Fuel production must involve lignocellulosics, MSW or gases

Global Biotech business

	2010	2015
• Food	$80B	100
• Medical	125	225
• Agricultural	15	25
• Marine	10	20
• Industrial	80	180
• TOTAL	$310B	$550B

Sources: Frost & Sullivan, BCC Research