Fuel Choices Summit 2016

Habima Theater

Tel Aviv, Israel

3 November, 2016

Engineering microbes for production of biofuels and chemicals

Gregory Stephanopoulos

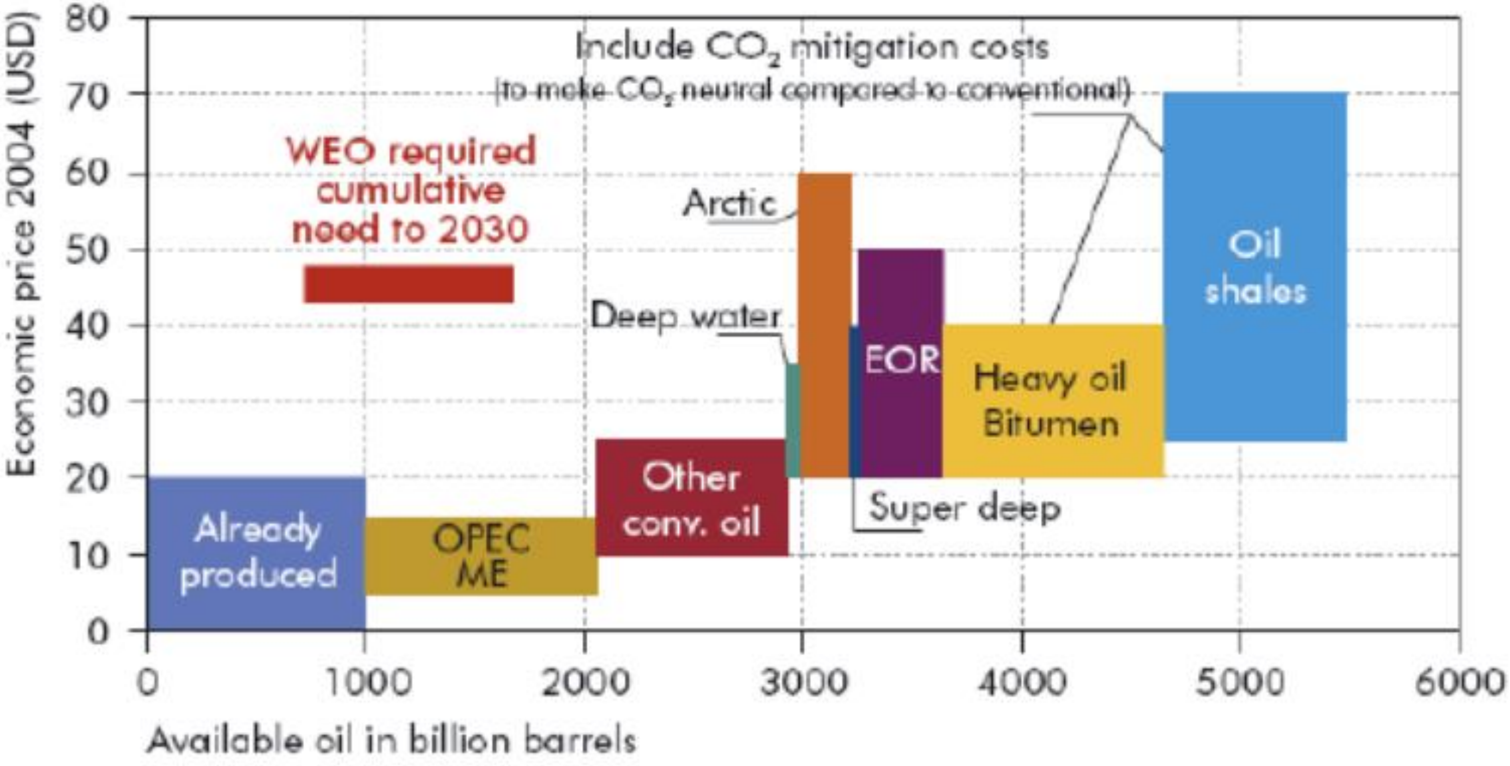
MIT

Forces of change

What has changed drastically during the past 25-30 years?

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

Oil supply and cost curve



Source: IEA (2005)

U.S. DEPARTMENT OF ENERGY

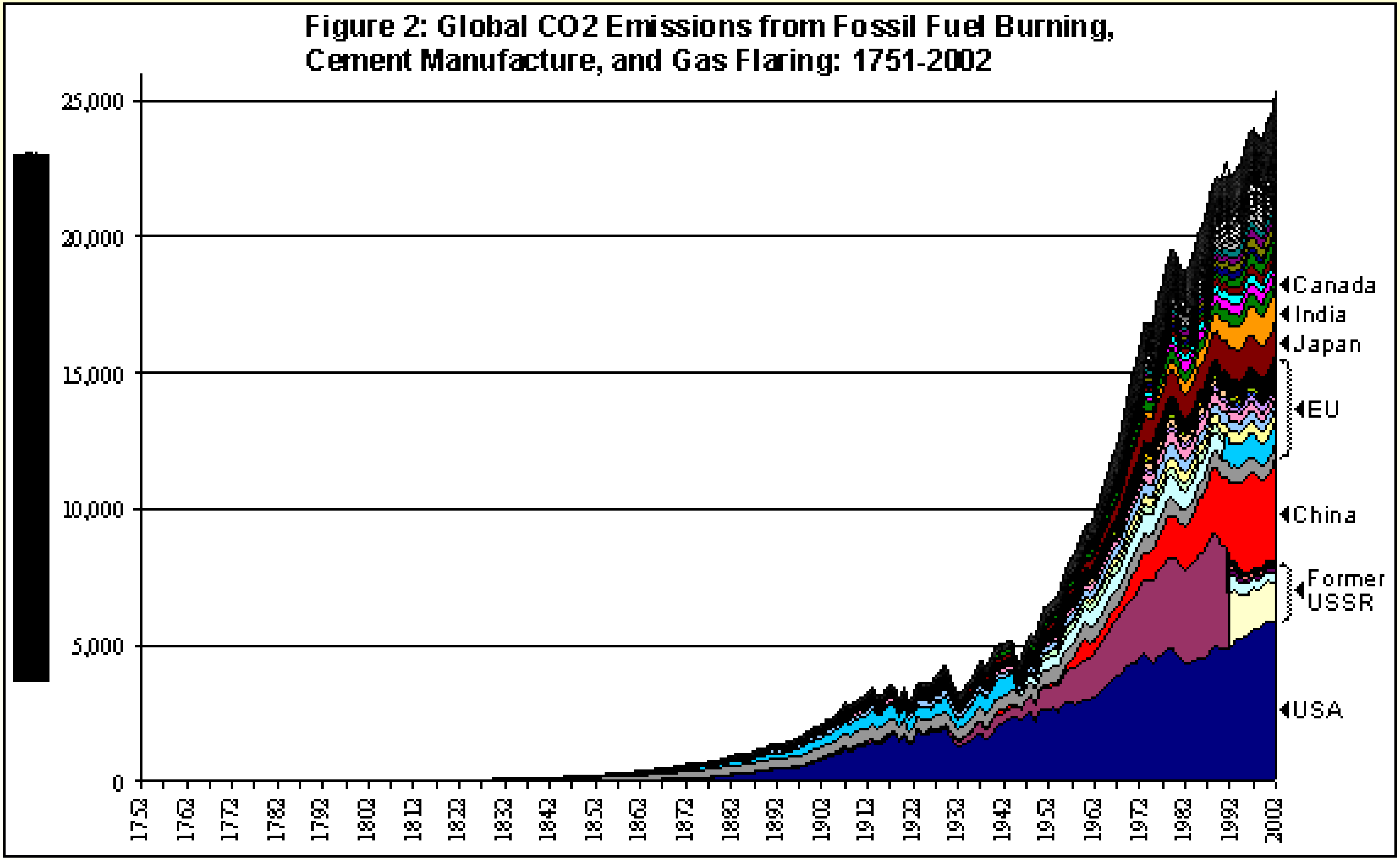
What has changed drastically during the past 25-30 years?

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

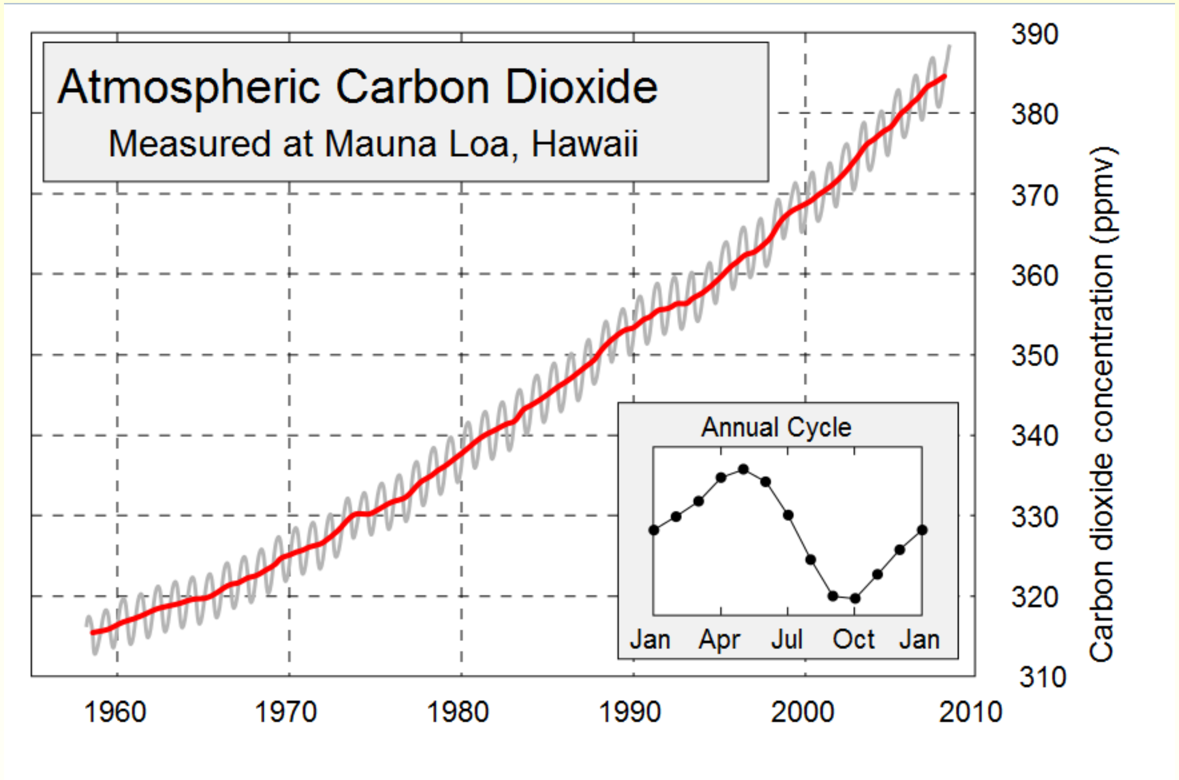
- **Strategic challenges in securing the required amounts of fuels and raw materials**

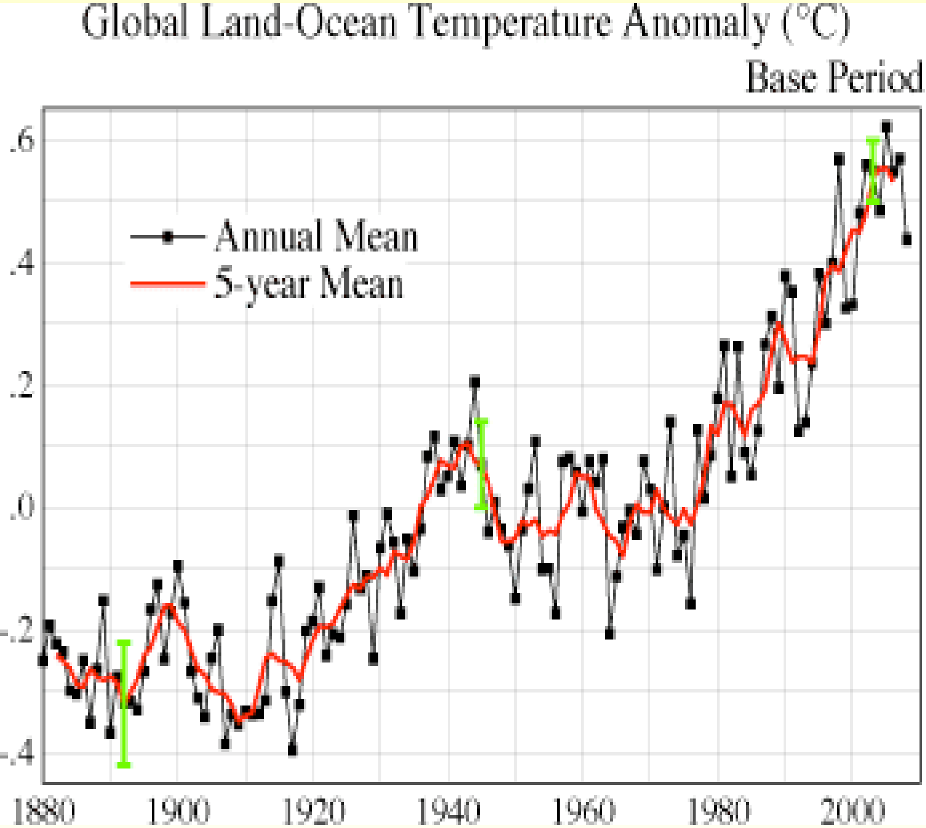
- **Grave consequences for climate change**

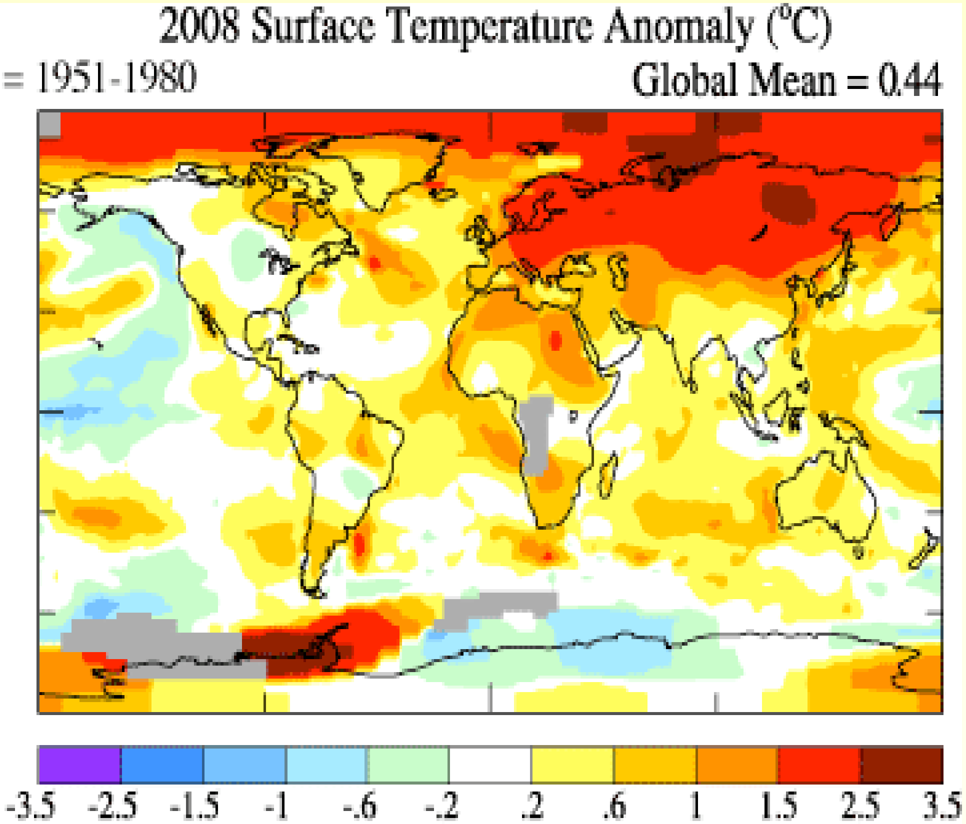
Global CO2 emissions



Atmospheric Carbon Dioxide




Global Land-Ocean Temperature Anomaly (°C)
Annual Mean
5-year Mean
.6
.4
.2
.0
-.2
-.4
1880
1900
1920
1940
1960
1980
2000


2008 Surface Temperature Anomaly (°C)
Base Period = 1951-1980
Global Mean = 0.44
-3.5
-2.5
-1.5
-1
-.6
-.2
.2
.6
1
1.5
2.5
3.5

What has changed drastically during the past 25-30 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**

Technology advances:

Engineering the metabolism of microbes to convert them to ***chemical factories*** for the production of *biofuels and* chemicals

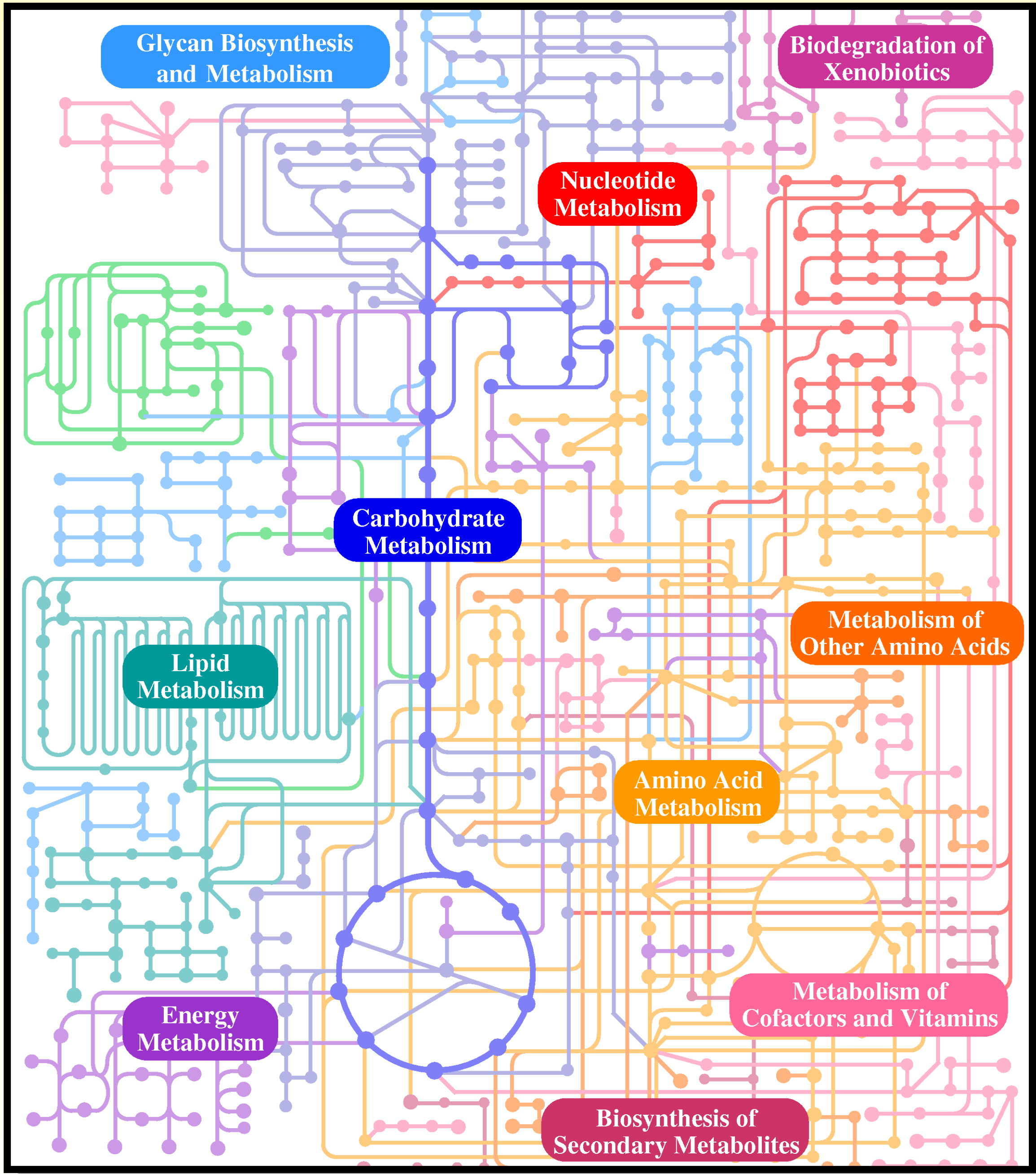
Biotechnology beyond medicine

Cells:

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

Main substrate: Sugars

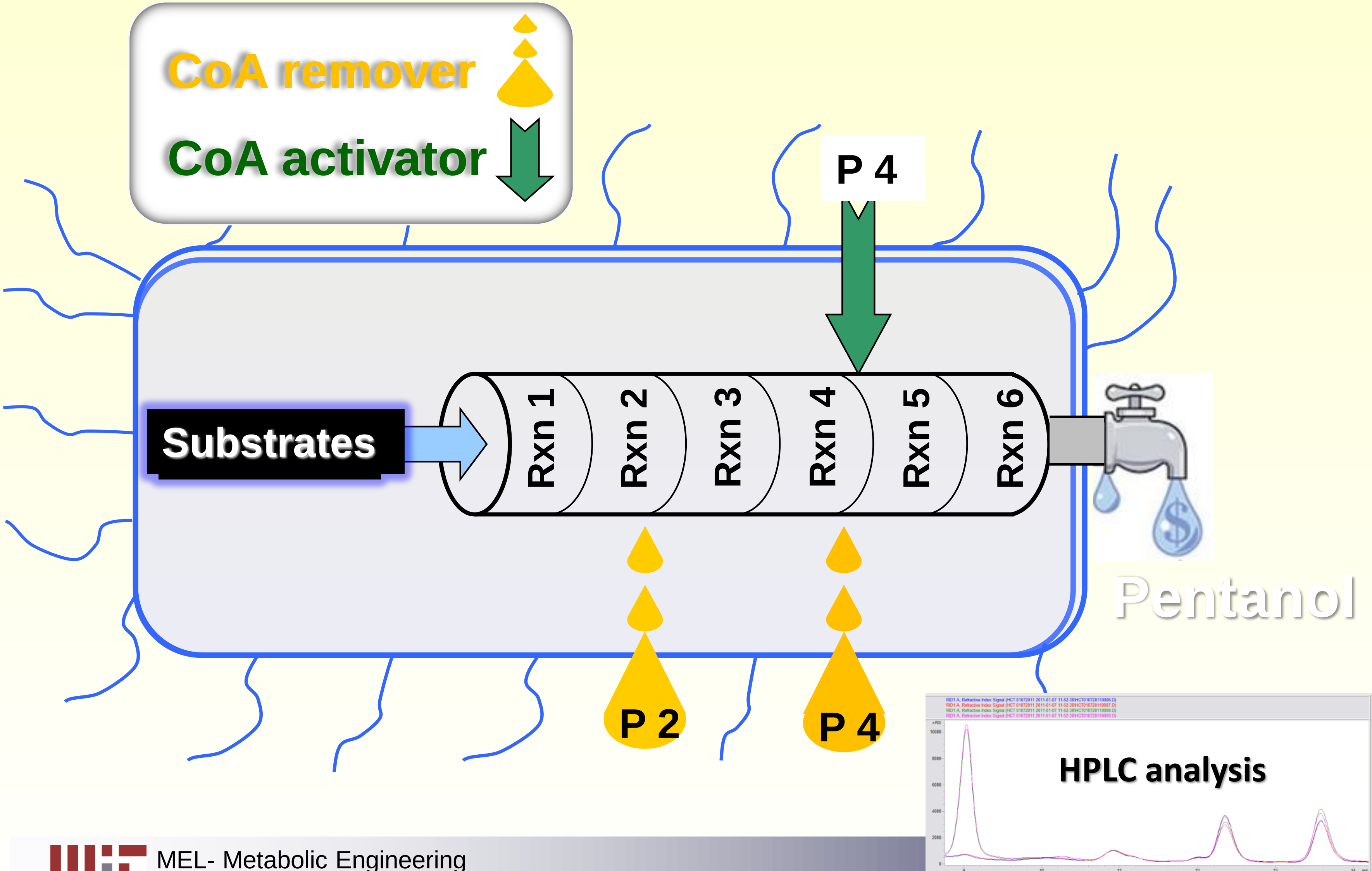
Products: Virtually infinite



Microorganisms

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

Engineering microbes to produce any product



Types of biofuels and biofuel feedstocks

- Ethanol from corn
- Biodiesel from plant seeds and vegetable oils

- Ethanol from sugarcane

- Other feedstocks (not competing with food): cellulosics, algae
- Other biofuels than ethanol (butanol, lipids, hydrocarbons)

Contributions from my lab

1. Improving ethanol tolerance of yeast

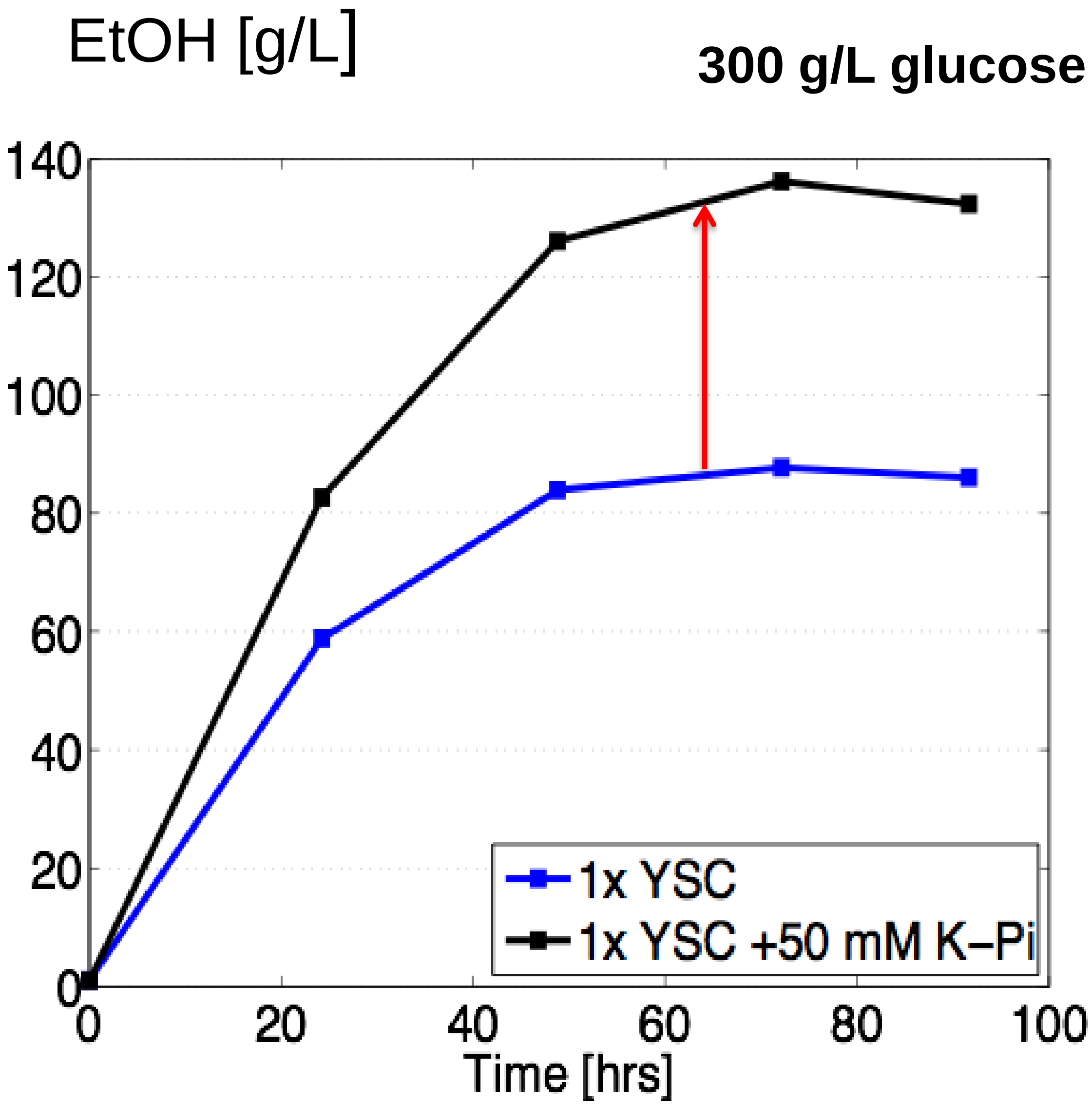
Extensions:
Improving microbial tolerance to toxicity

1. F.H. Lam, A. Ghaderi, G.R. Fink and G. Stephanopoulos, "Engineering alcohol tolerance in yeast," *Science,* 346: 71-75 (2014)
2. H. Alper, J. Moxley, E. Nevoigt, G.R. Fink and G. Stephanopoulos, "Engineering yeast transcription machinery for improved bioethanol tolerance and production," *Science,* 314: 1565-1568 (2006)

Product **toxicity** is a major problem in engineering microbes for production of biofuels and biochemical products

It is important that studies aiming at improving tolerance are conducted under **bioprocess-relevant** conditions

EtOH increased upon K-Pi supplementation

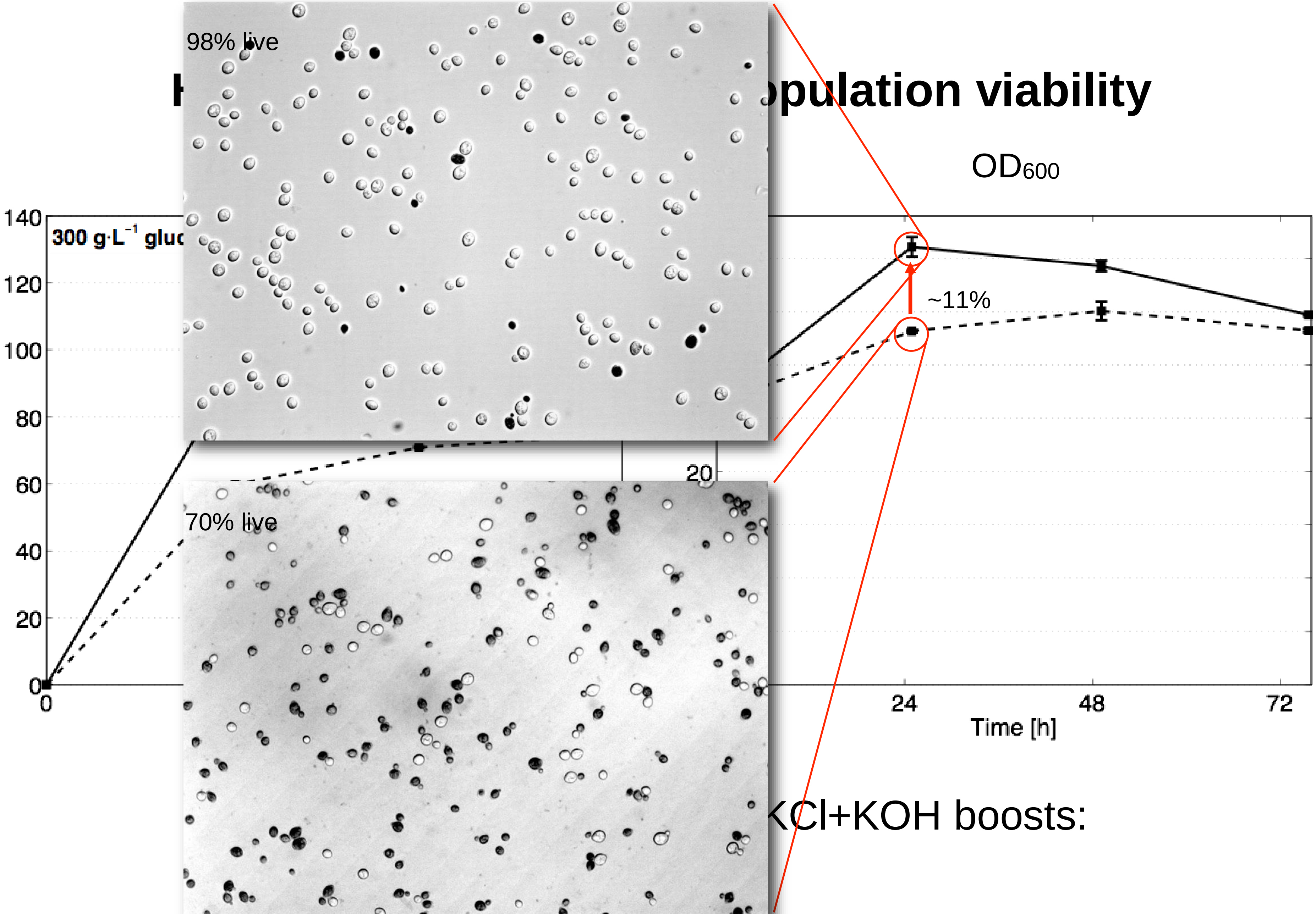


K-P_i supplementation and pump engineering enhances:

I. Growth despite accumulating EtOH

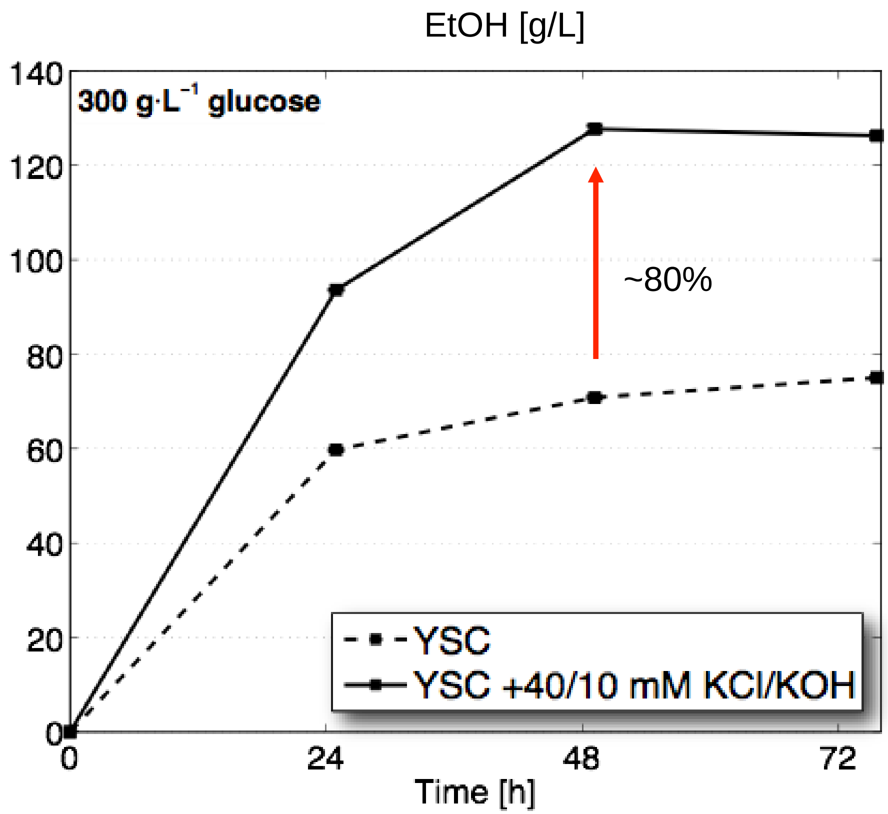
II. Tolerance despite accumulating EtOH

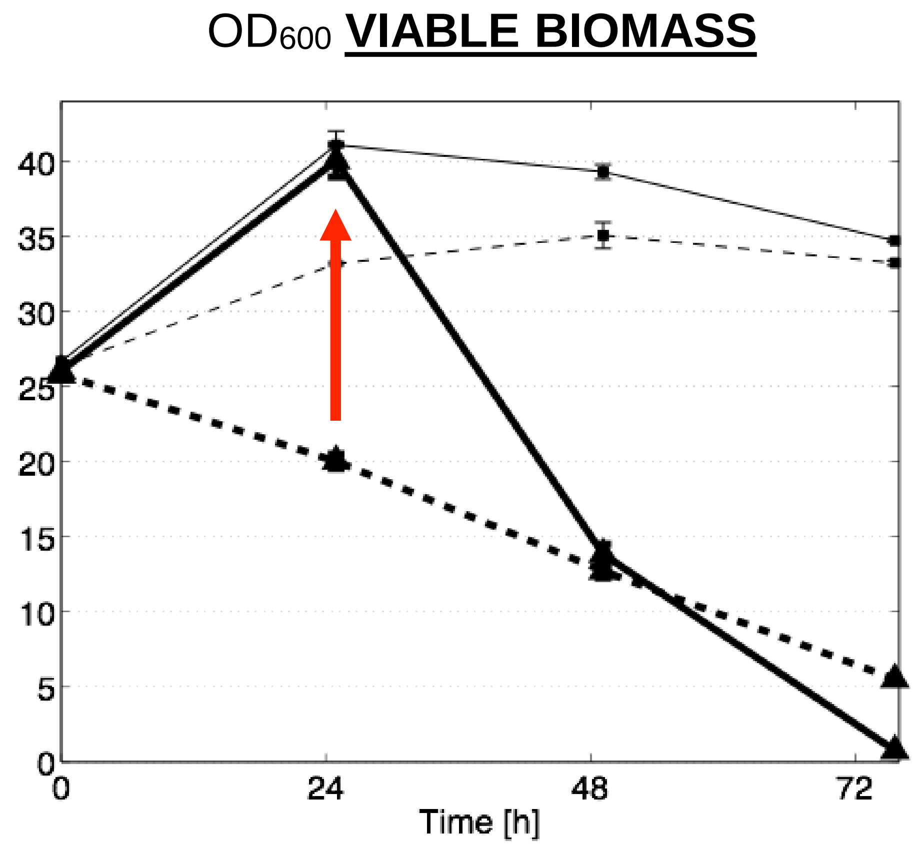
H... opulation viability



KCl+KOH boosts:

High KCl+KOH enhance population viability



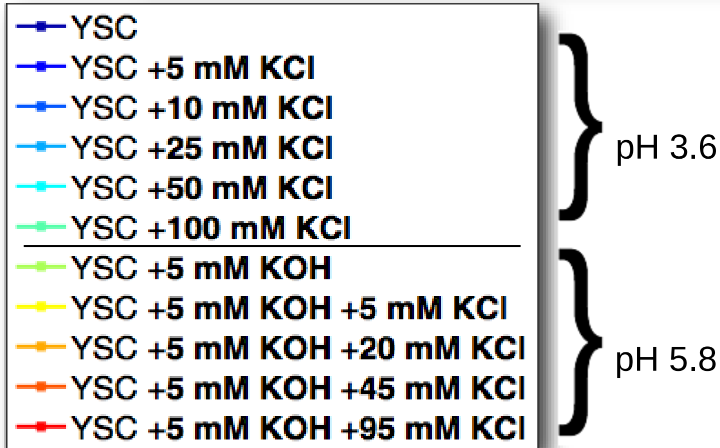


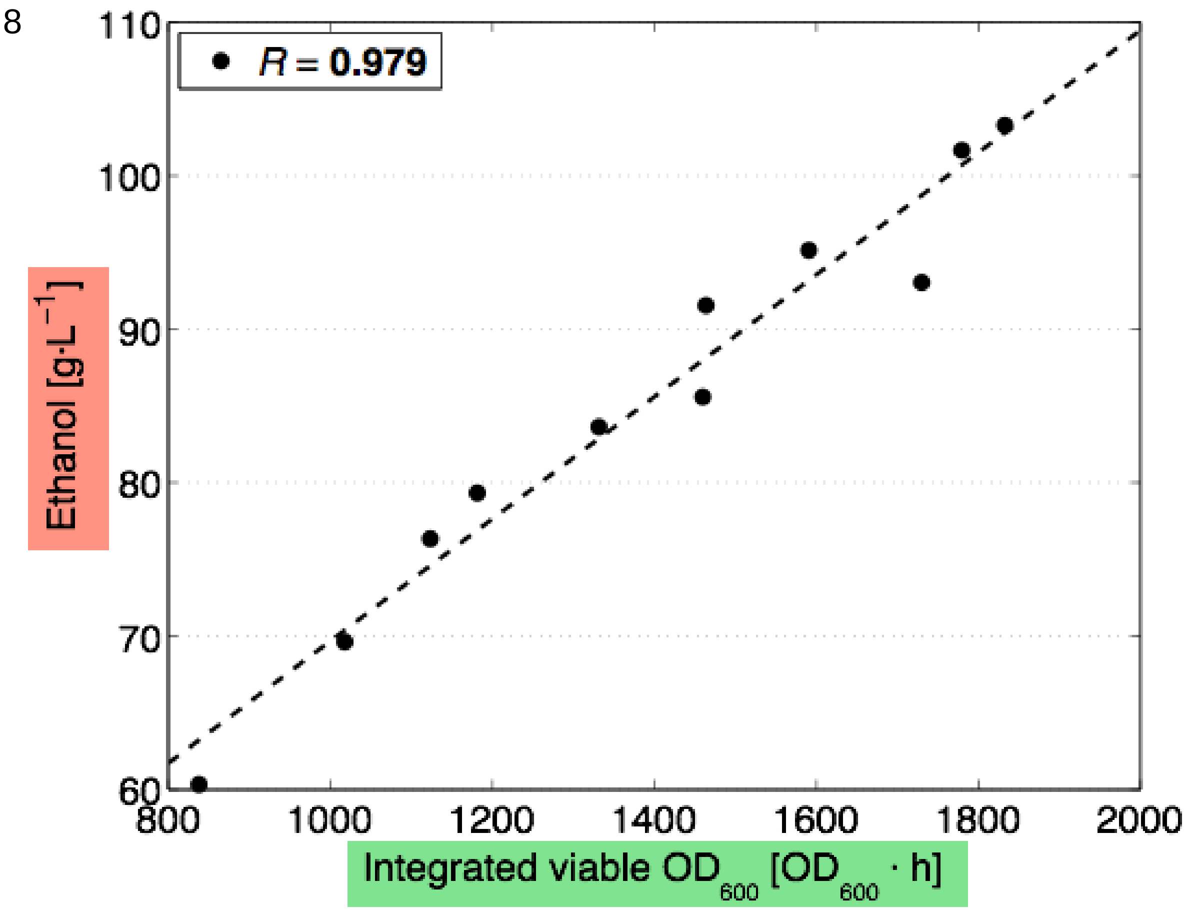
Despite accumulating EtOH, KCl+KOH boosts:

I. Cell growth

II. Tolerance

Tolerance and titer highly correlated





F.H. Lam, A. Ghaderi, G.R. Fink and G. Stephanopoulos, "Engineering alcohol tolerance in yeast," *Science*, 346: 71-75 (2014)

Product **toxicity** is a major problem in engineering microbes for production of biofuels and biochemical products

It is important that studies aiming at improving tolerance are conducted under **bioprocess-relevant** conditions

Contributions from my lab

2. Engineering xenobiotic pathways to prevent contamination

Extensions: Eliminating the need for the use of antibiotics

A.J. Shaw, F.H. Lam, M. Hamilton, A. Consiglio, K. KacEwen, E. E. Brevnova, E. Greenhagen, W.G LaTouf, C. R. South, H. van Dijken, V. Rajgarhia and G. Stephanopoulos, "Engineering contamination resistance in industrial biosystems," *Science*, **353:** 583-586 (2016)

Contributions from my lab

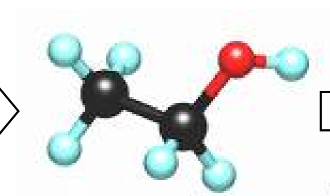
3. Engineering yeast to metabolize *all* sugars from biomass hydrolysis

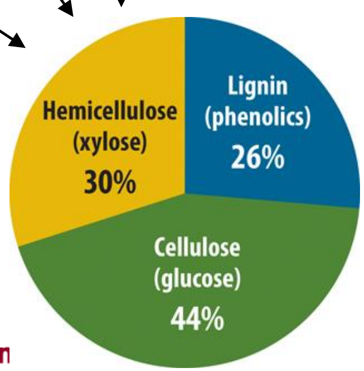
Extensions:
Use of vast amounts of lingo-cellulosics for biofuels

Hang Zhou, J.-S. Cheng, B. Wang, G. R. Fink and G. Stephanopoulos, "Xylose isomerase overexpression along with engineering of the pentose phosphate pathway and evolutionary engineering enable rapid xylose utilization and ethanol production by *Saccharomyces cerevisiae,*" *Metabolic Engineering,* **14:** 611-622 (2012)

Biofuel (ethanol) from renewables

- **Fuel ethanol from corn starch or sugar**
  - Used as such or blended with gasoline





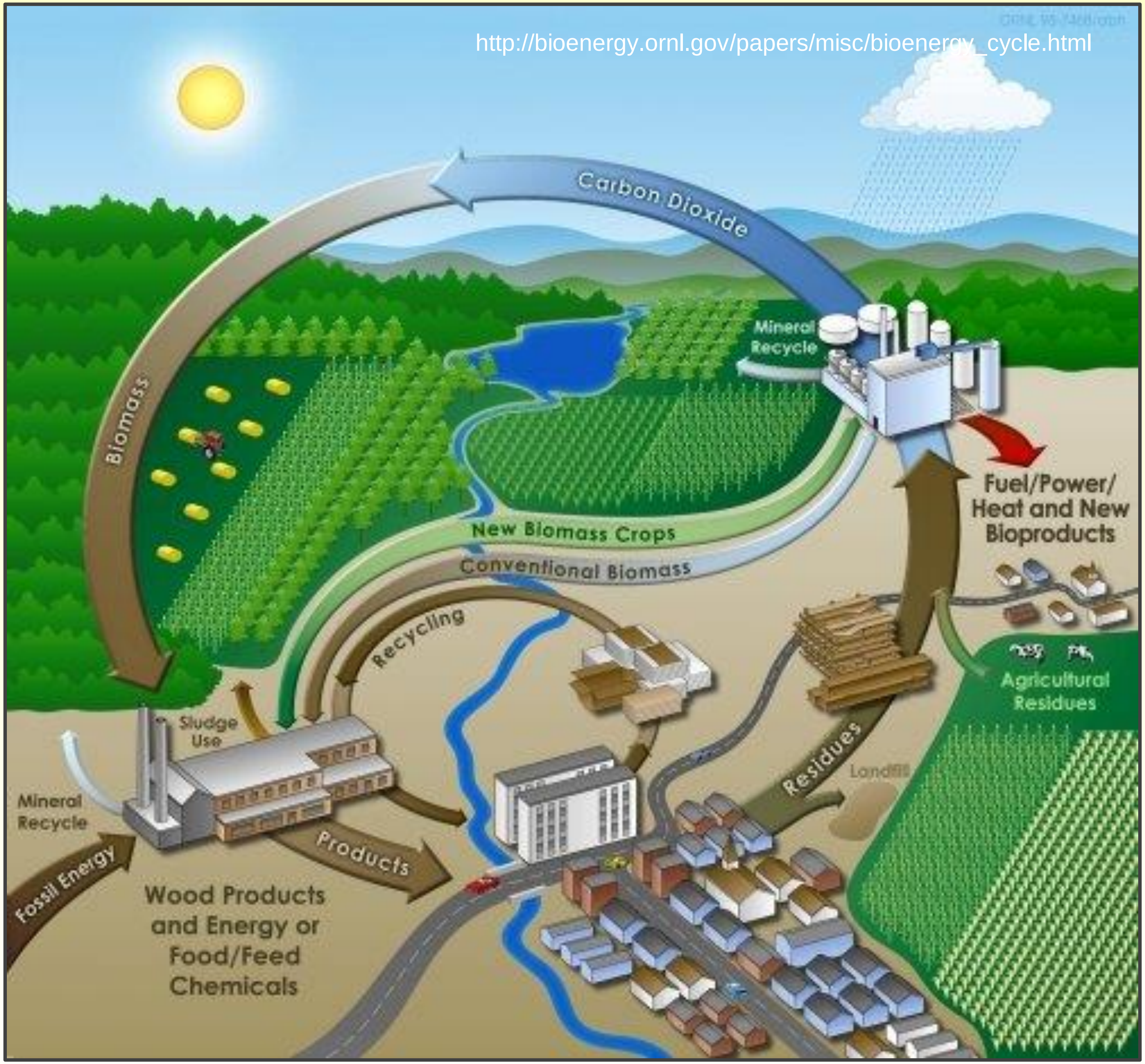
Biofuels

- **Ethanol from plant biomass**
  - Readily fermentable starch and sucrose
    - Resistant lignocellulosic fractions

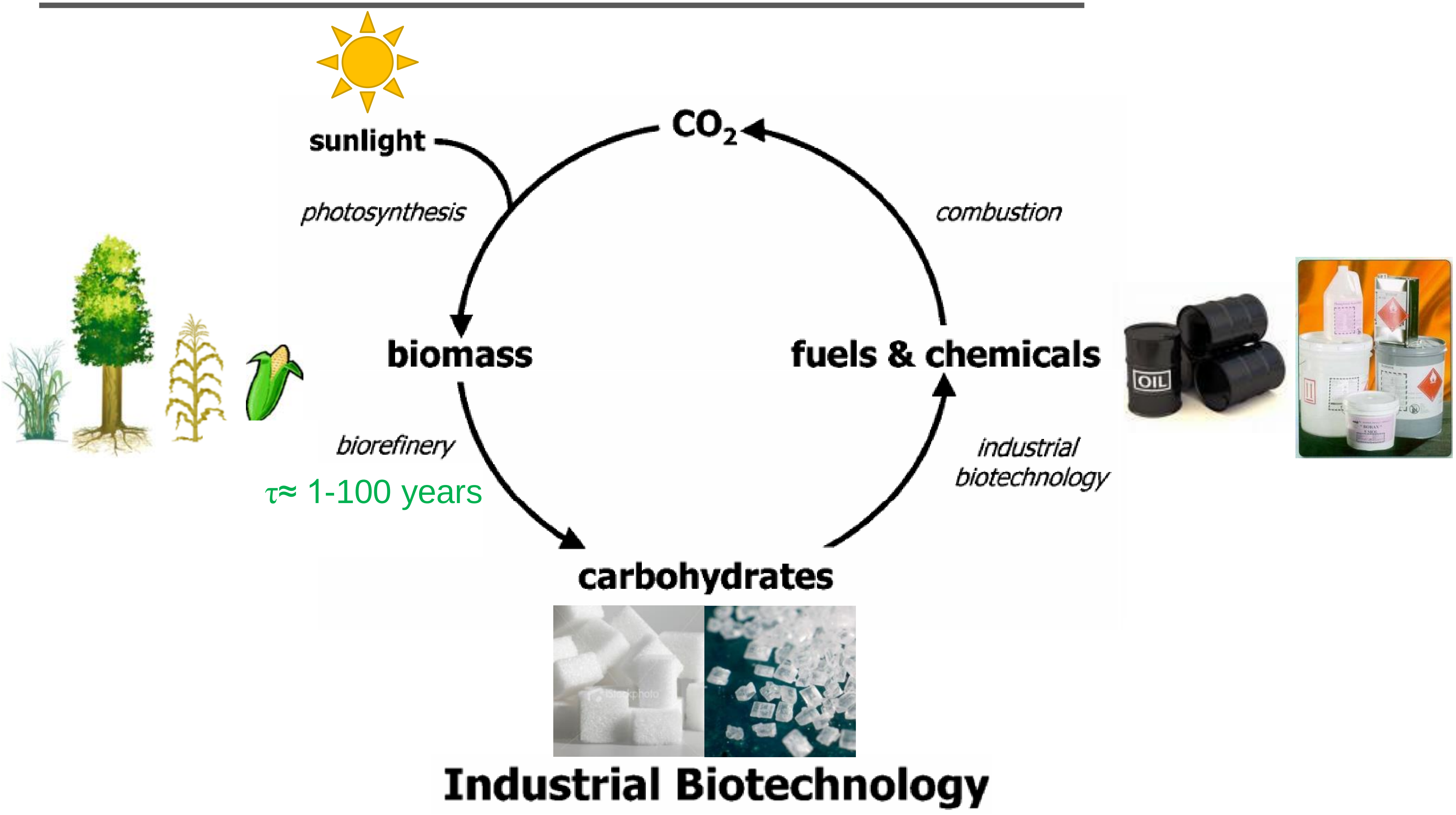
Performance of the *S. cerevisiae* strains

Strain	Description	Conditions	Yields g/g		Ethanol production $g \cdot g^{-1} h^{-1}$	Xylose consumption $g \cdot g^{-1} h^{-1}$	μ_{max} h^{-1}
			Ethanol	Xylitol			
H131-A31	*XylA, PsXyl3, PsTal1, TKL1, RPE1, RKI1*	Aerobic batch, SDX	N/A	N/A	N/A	N/A	0.031±0.022
H131E1-A31	Selection of H131-A31, aerobic sequential batch	Aerobic batch, SDX	0.200	<0.01	0.034	0.169	0.197±0.006
H131E3-A31	Selection of H131E1-A31, micro-aerobic sequential batch	Anaerobic batch, 2×YNB, 4% xylose	0.440	<0.01	0.120	0.273	0.061±0.002
H131E5-A31	Selection of H131E3-A31, anaerobic sequential batch	Anaerobic batch, 2×YNB, 4% xylose	0.410	<0.01	0.233	0.568	0.073±0.002
H131E8-A31	Selection of H131E5-A31, anaerobic chemostat	Anaerobic batch, 2×YNB, 4% xylose	0.440	<0.01	0.383	0.870	0.120±0.004
H131E8-A31	Anaerobic chemostat of H131E5-A31	Anaerobic chemostat, YNBX	0.438	<0.01	0.641	1.464	0.148
RWB 217	*XylA, XKS1, TAL1, TKL1, RPE1, RKI1, gre3Δ*	Anaerobic batch, synthetic medium	0.43	0.003	0.46	1.06	0.09
RWB 218	Selection of RWB 217	Anaerobic batch, synthetic medium	0.41	0.001	0.49	1.2	0.12

Towards *integrated and complete* processes for biofuel and chemical production from renewable feedstocks


http://bioenergy.ornl.gov/papers/misc/bioenergy_cycle.html
Carbon Dioxide
Biomass
Mineral Recycle
New Biomass Crops
Conventional Biomass
Fuel/Power/ Heat and New Bioproducts
Recycling
Sludge Use
Agricultural Residues
Mineral Recycle
Residues
Landfill
Products
Fossil Energy
Wood Products and Energy or Food/Feed Chemicals

Carbon cycle



First and foremost, biofuels are a feedstock story

Feedstocks must be cheap and aggregated

Examples:

1. Lignocellulosic biomass
2. Waste solids
3. Waste gases
4. Algae (cheap?)

Potential of biofuels (USA)

- 70-100 gallons ethanol/dry ton of biomass
- 42-60 B gallons Ethanol/year or
  28-40 B gallons of gasoline equivalent
  20-30% of gasoline used

(1 ton of ethanol = 333 gallons, or
1 Gallon = 3 kgs, or 1 B Gallons = 3 M tons)

NEW Contributions from my lab

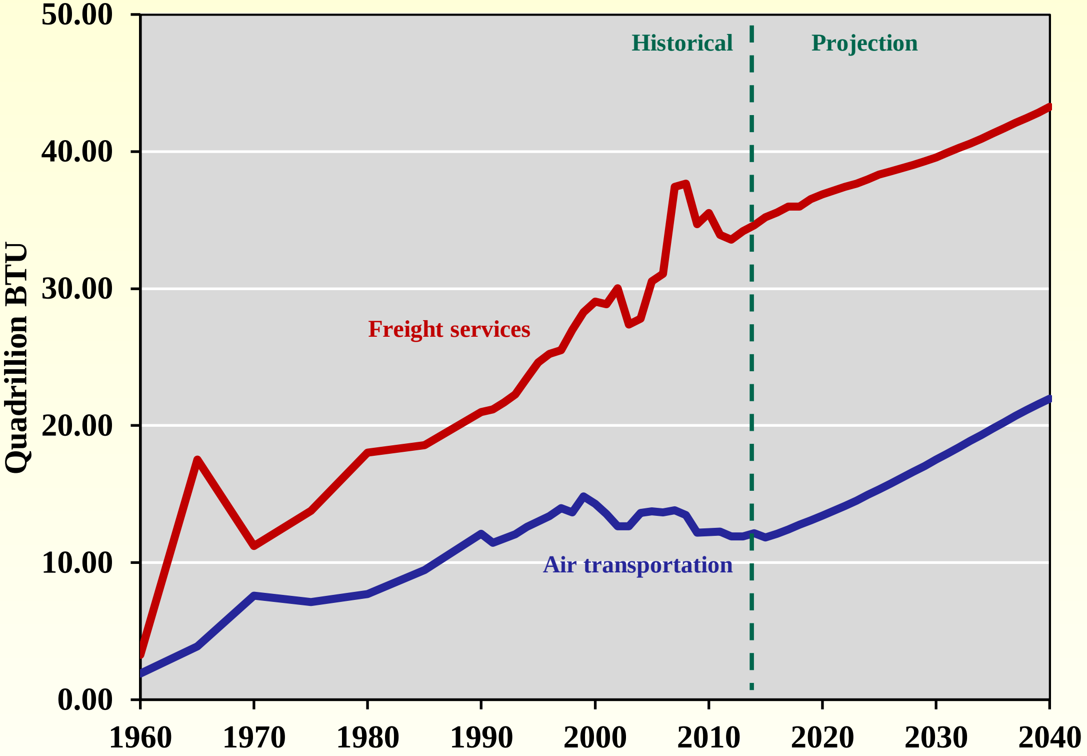
4. Engineering oleaginous yeast for overproduction of lipids

Extensions:
Open up the potential for Green Diesel or Renewable Diesel from biomass or waste

K.J. Qiao, T.M. Wasylenko, K. Zhou, P. Hu and G. Stephanopoulos, "Rewiring metabolism to maximize lipid production in *Yarrowia lipolytica," Nature Biotechnology* (in press) (2016)

Peng Hu*, S.* Chakraborty*,* A. Kumar, B. Woolston, H. Liu, D. Emerson, and G. Stephanopoulos, **"**Integrated system for biological conversion of gaseous substrates to lipids," *Proceedings of the National Academy of Sciences, PNAS,* doi/10.1073/pnas.1516867113 (2016)

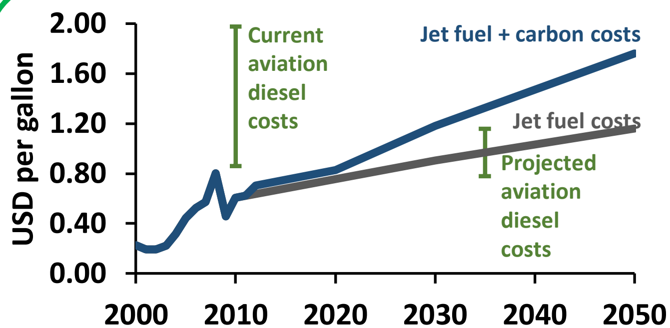
Rising global diesel demand



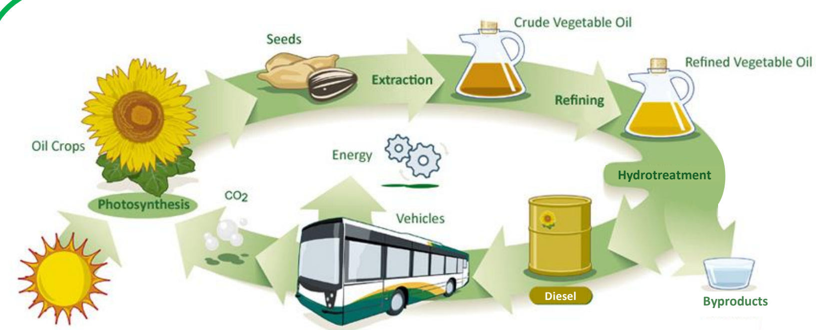
- High demands in diesel consumption in both industries
- Diesel consumption in the U.S. projected to grow at 400-500 million gallons per year

US department of transportation, 2016.
US energy information administration, 2016.

Benefits of green diesel

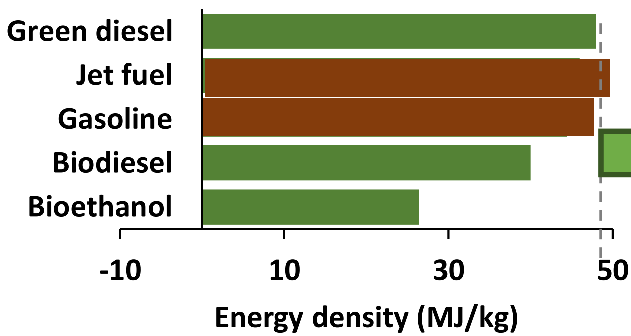


Economical

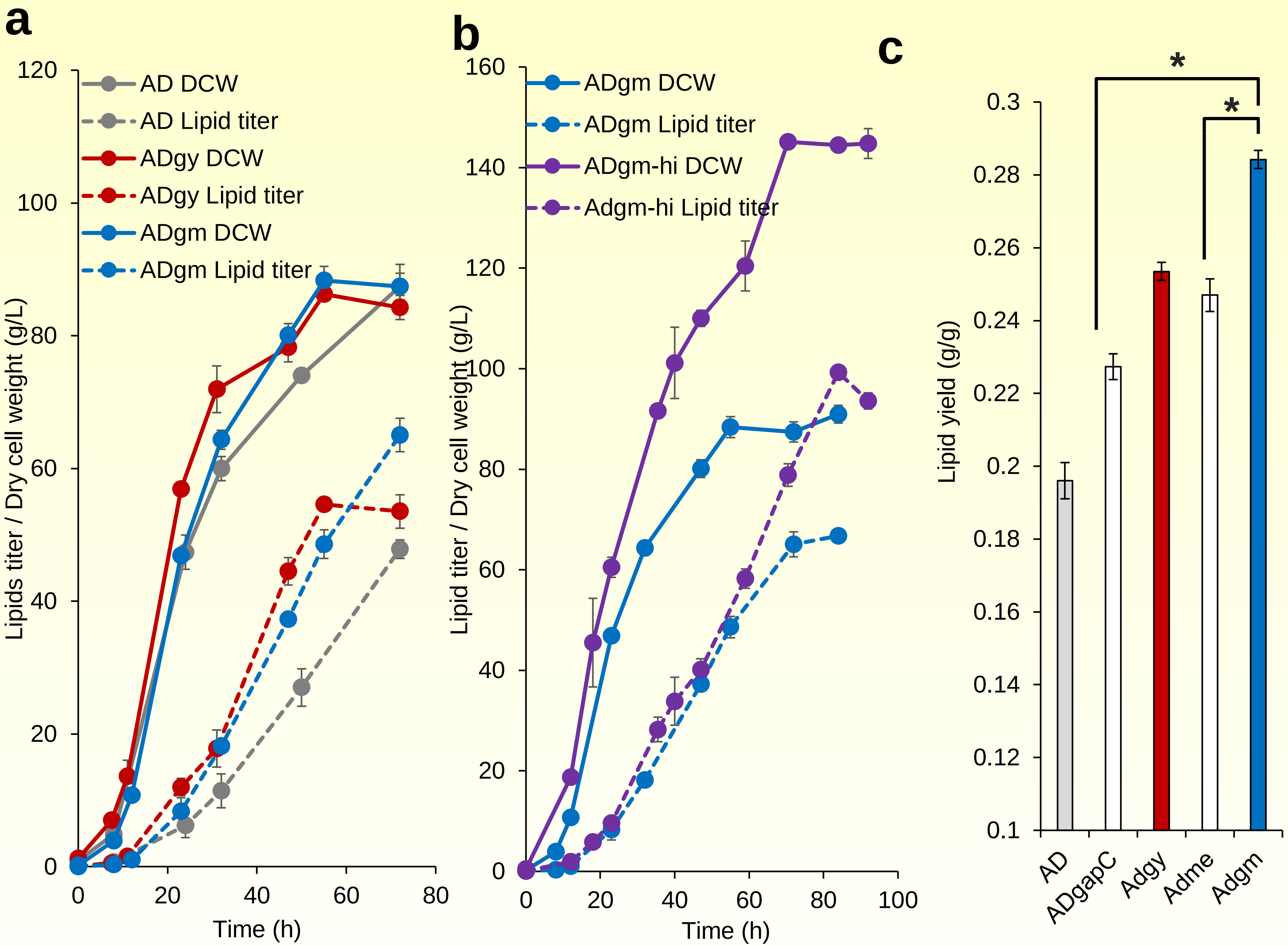


Environmental

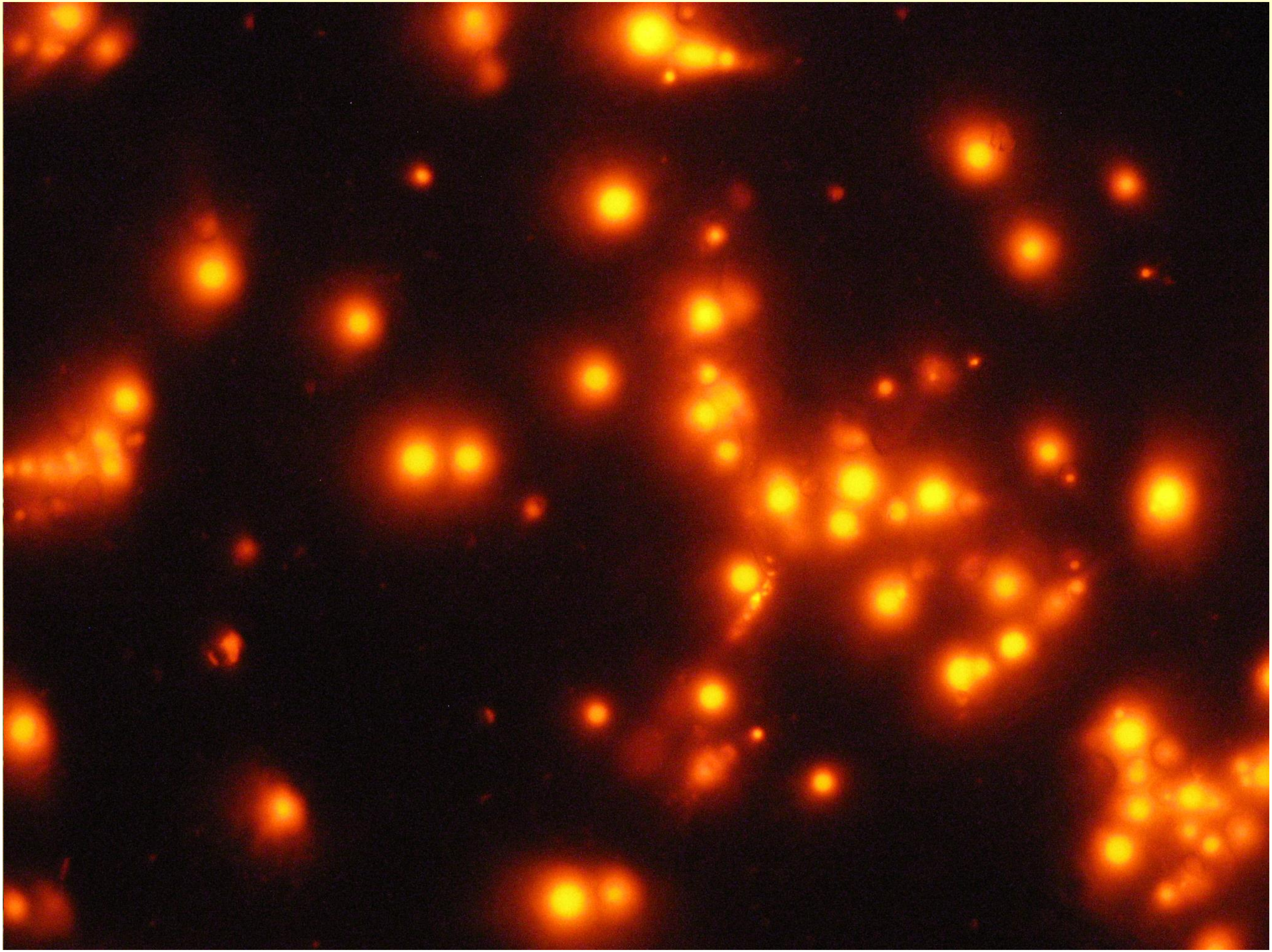
Ideal physical and chemical properties



Air transport action group, 2011
US energy information administration, 2016
Alternative energy news, 2016



a
AD DCW
AD Lipid titer
ADgy DCW
ADgy Lipid titer
ADgm DCW
ADgm Lipid titer
Lipids titer / Dry cell weight (g/L)
Time (h)
b
ADgm DCW
ADgm Lipid titer
ADgm-hi DCW
Adgm-hi Lipid titer
Lipid titer / Dry cell weight (g/L)
Time (h)
c
Lipid yield (g/g)
AD
ADgapC
Adgy
Adme
Adgm
*
*



Importance in advancing Renewable Diesel

Base case: Sugars at $200/ton (~9c/lb)

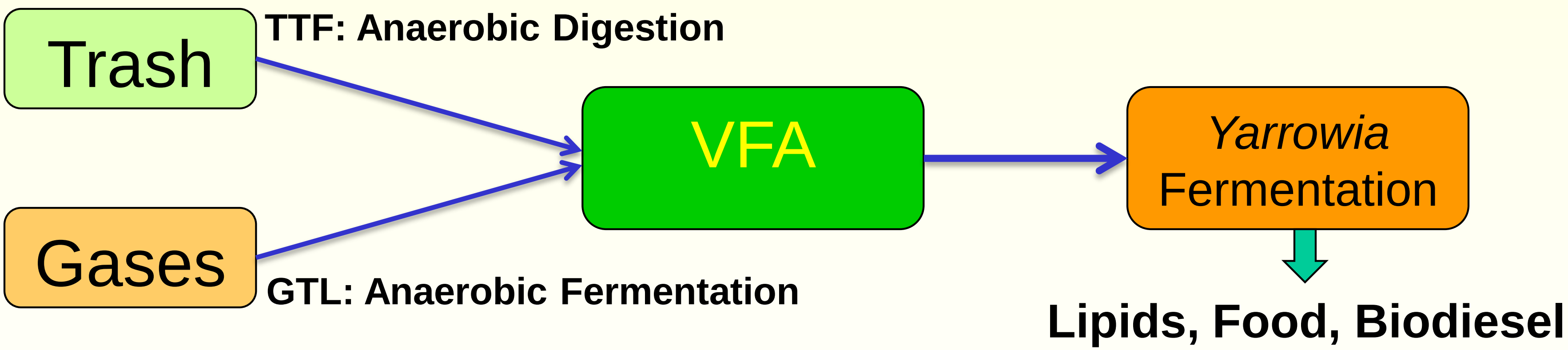
Feedstock cost of lipids produced from sugars from renewable biomass:

1. At a yield of 0.18g/g (state of the art):$1,100/ton
2. At a yield of 0.30g/g (our work): $660/ton

3. Oil selling price range: $700-1,100

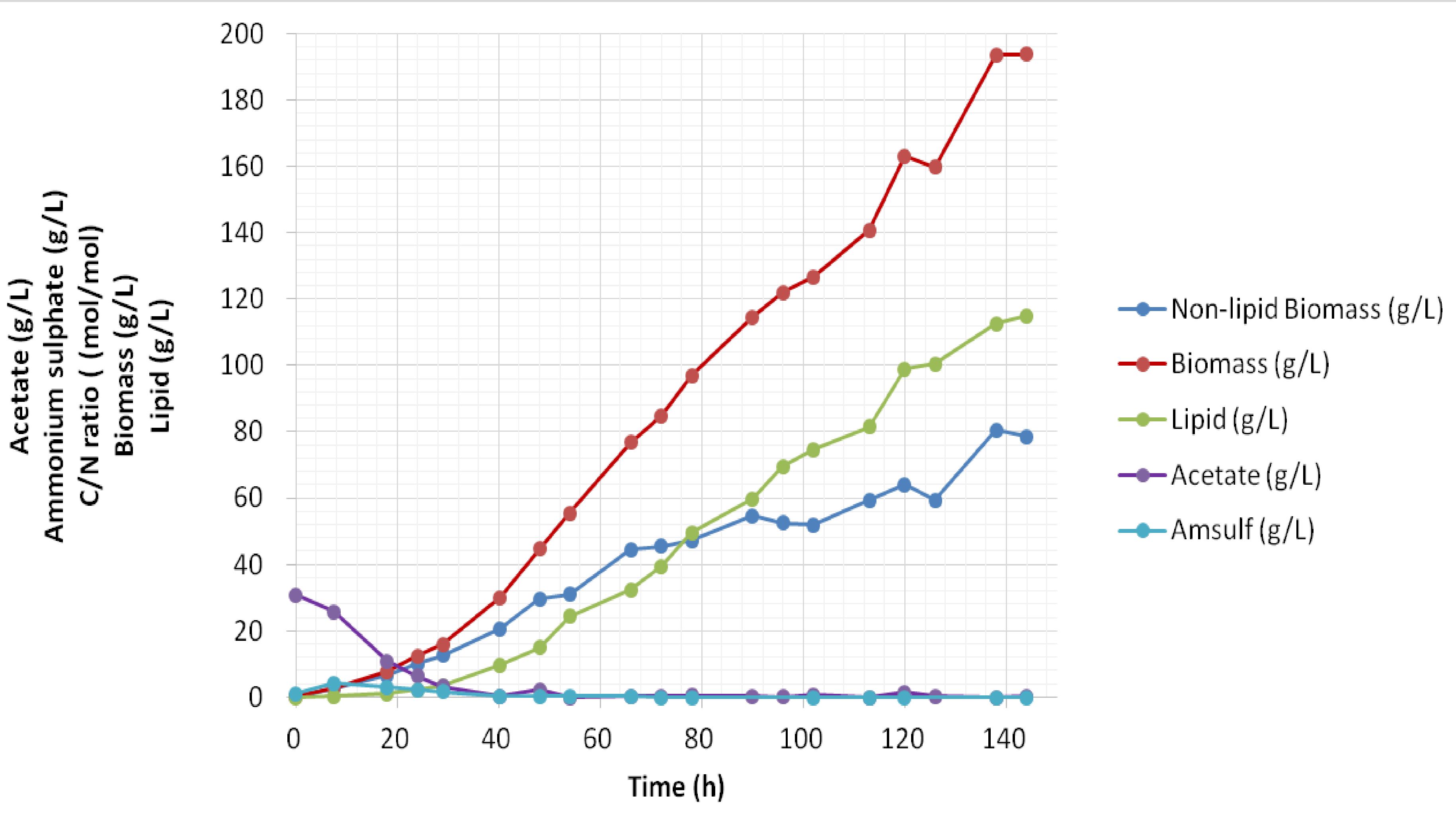
Alternative feed stocks

- Glucose is expensive
- Acetic acid is interesting alternative. Can be supplied at large volumes from
  - Anaerobic digestion
  - Fixation of CO2 with CO or Hydrogen using anaerobic acetogenic bacteria



4. Optimization of Nitrogen feed based on RQ/CTR feedback control

- Working volume 1.5 L
- Maintain carbon at zero



Importance of waste utilization

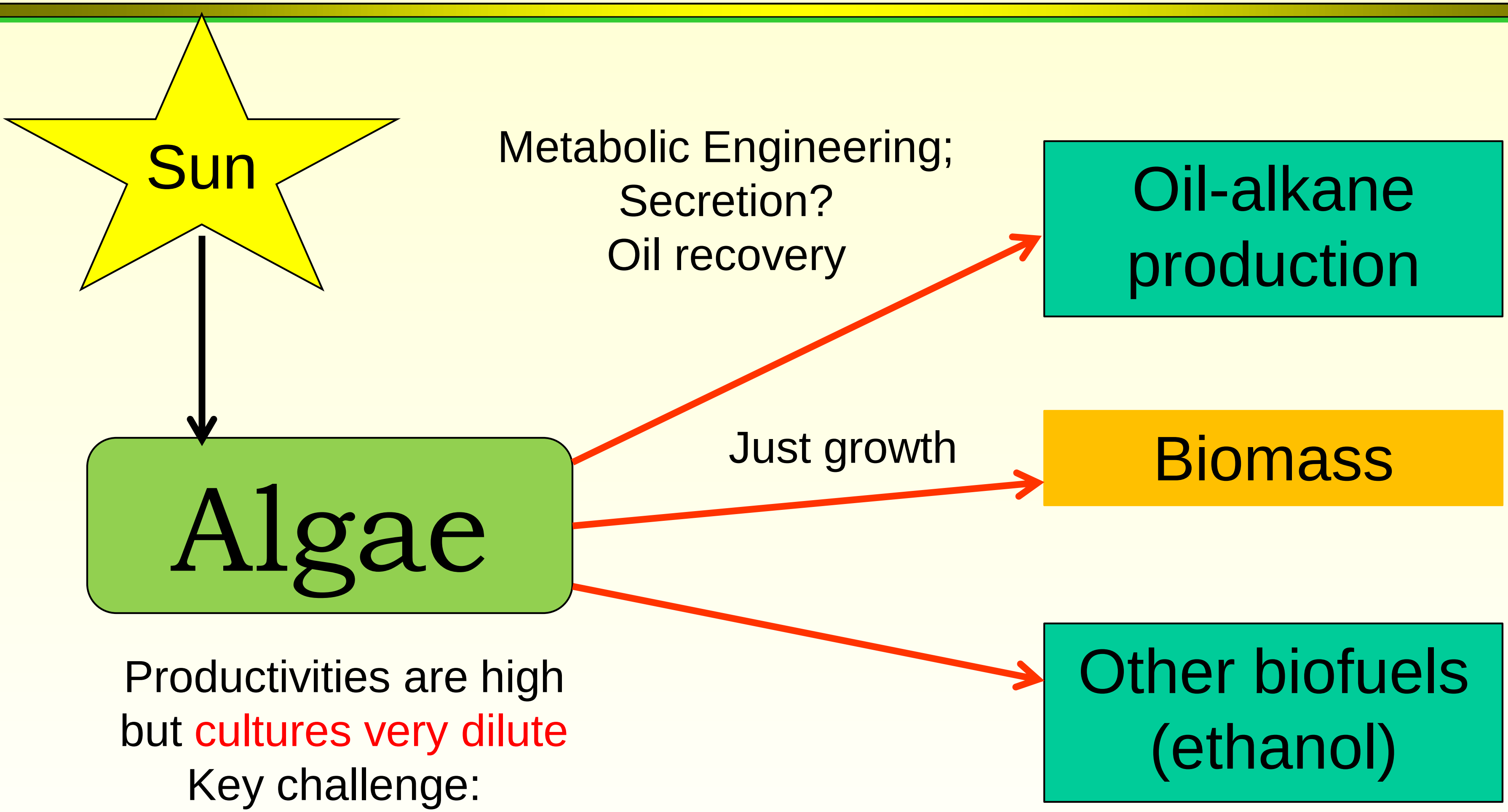
1. Waste generation: 1 ton/person (US, 2011)
2. Fermentable fraction: 25% (US)-50% (China) (use 35%)
3. Potential for 3-5B gallons diesel/year (USA)
4. Cost of waste: can be *negative* at $100/ton

5. Potential depends on capacity to *aggregate* waste economically

Algae

	Gallons GE/acre/year
Soybeans	48
Sesame	74
Jatropha	202
Cellulosic (for ethanol production)	533
Sugarcane (for ethanol)	566
Algae	~6,000

Biofuel production by direct photosynthesis



Productivities are high
but cultures very dilute
Key challenge:
Cost-effective dewatering

A final word about chemicals

It is now possible to produce commodity chemicals (as well as, of course, specialty chemicals):

1. With cost-effective processes
2. Using renewable feedstocks
3. Small, efficient specialized units

4. Examples: Ethylene glycol, glycolic acid, biopolymers, organic acids (diacids), others

B. Pereira, Zheng-Jun Li, M. De Mey, C.G. Lim, H. Zhang, C. Hoeltgen and Gregory Stephanopoulos, "Efficient utilization of pentoses for bioproduction of the renewable two-carbon compounds ethylene glycol and glycolate," *Metabolic Engineering,* **34:** 80-87, (2016); dx.doi.org/10.1016/j.ymben.2015.12.004 (2015)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