ARPA-E Workshop

Bio-technologies for Methane to Liquids conversion: *Bio-GTL*

Washington, DC, December 5, 2012

Microbial biofuel technologies

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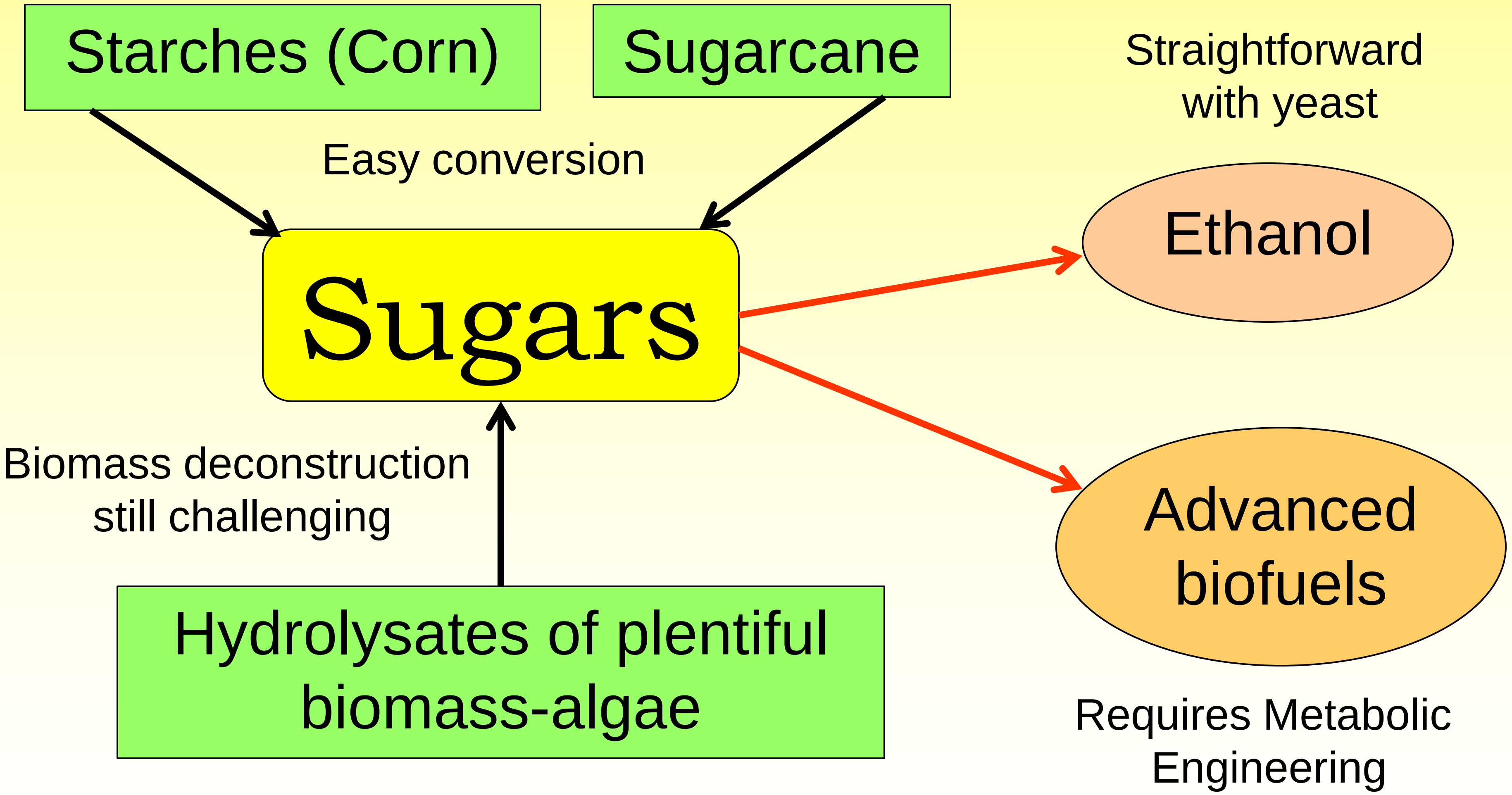
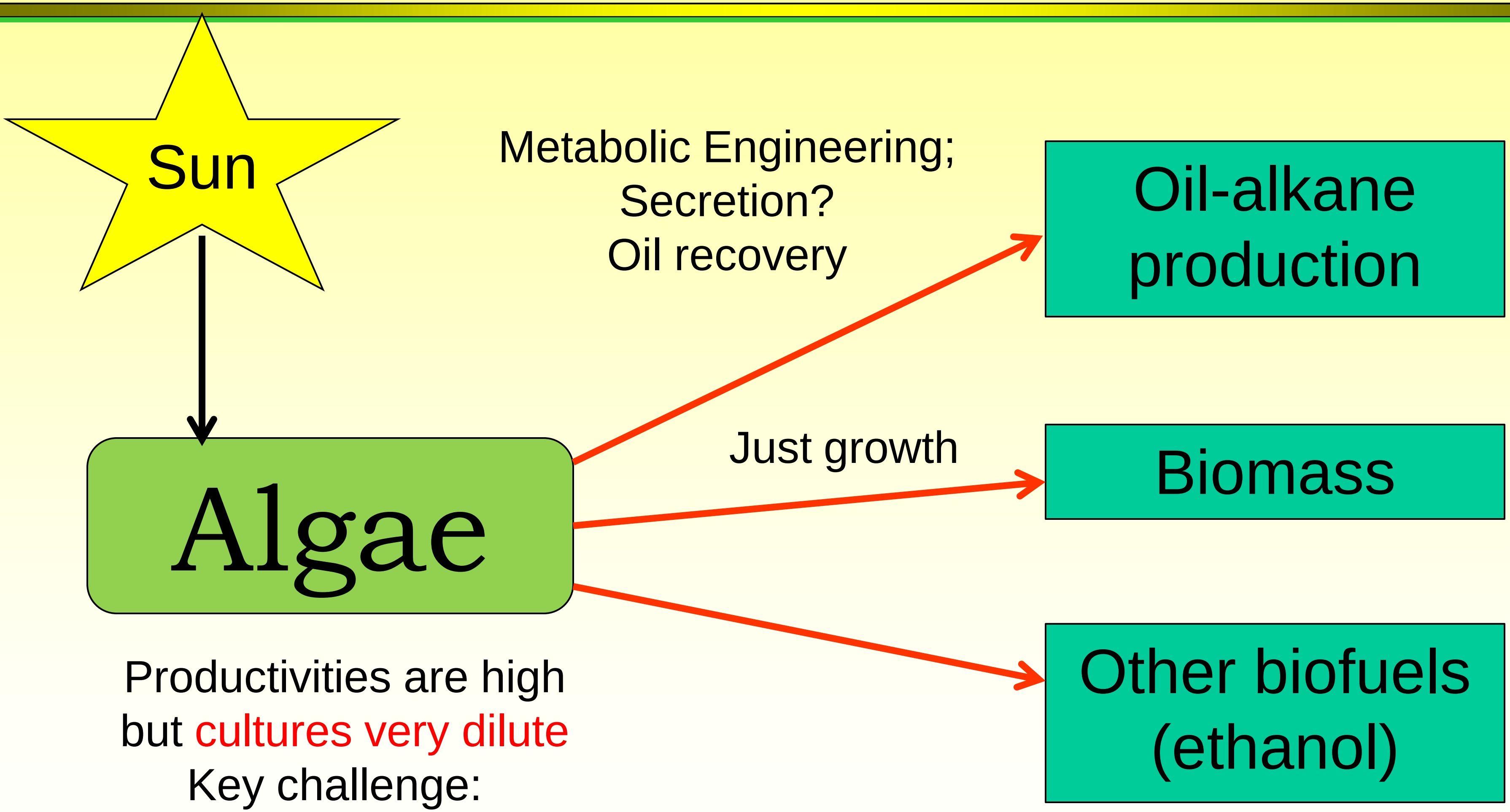
1. Sugar platform

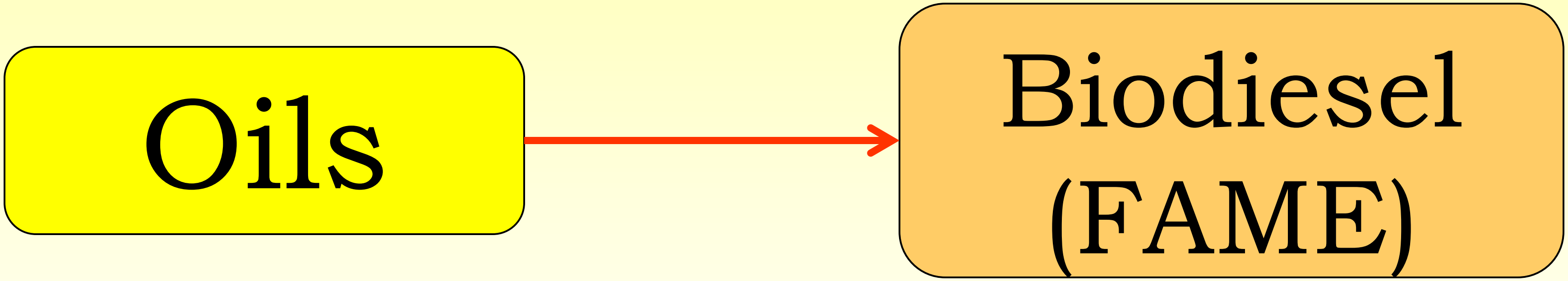


2. Biofuel production by direct photosynthesis



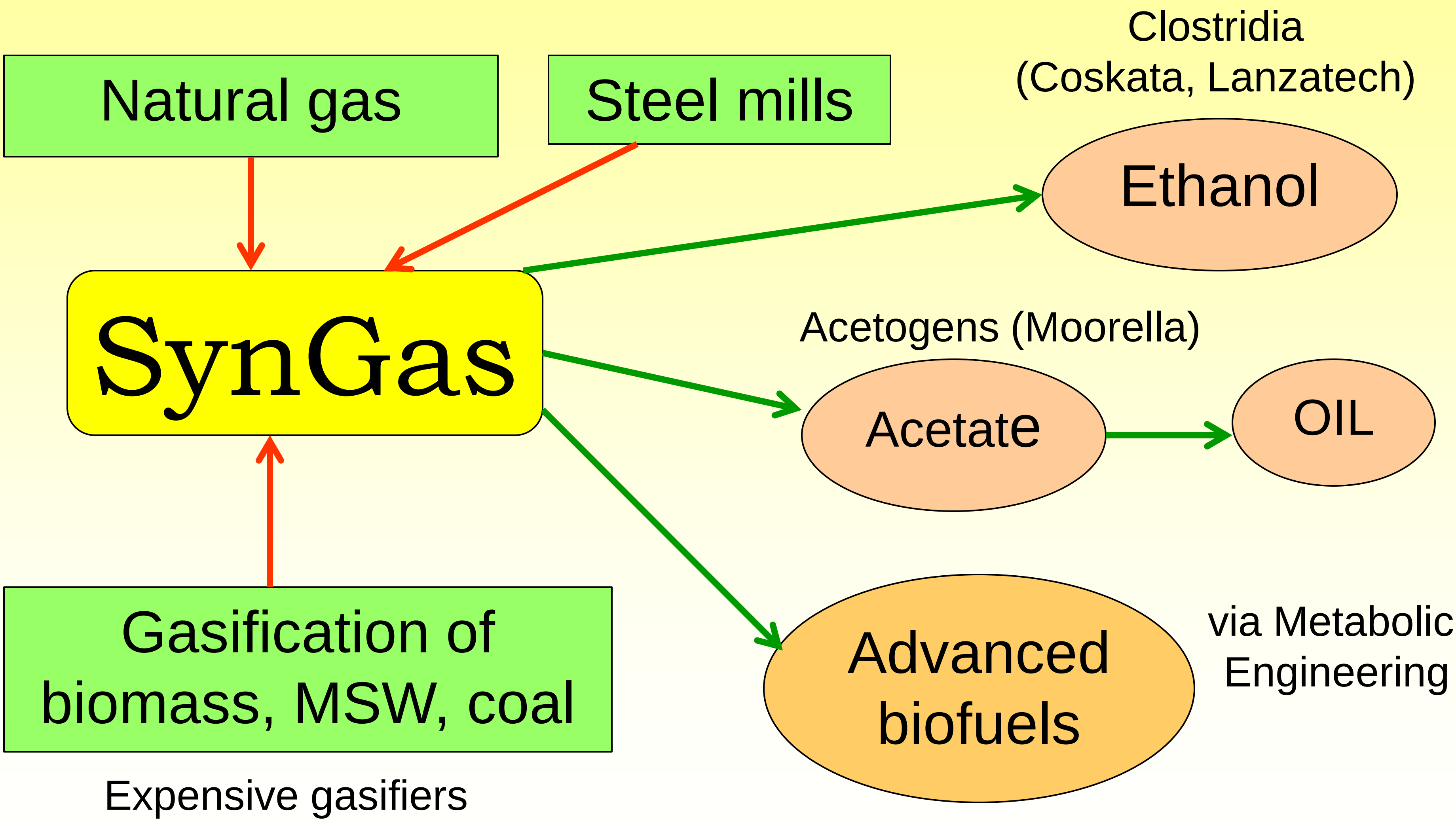
Productivities are high
but cultures very dilute
Key challenge:
Cost-effective dewatering

3. Biodiesel



Simple trans-esterification reaction
Key issues: Feedstock cost and availability

4. Bio-GTL


Natural gas
Steel mills
Clostridia
(Coskata, Lanzatech)
Ethanol
SynGas
Acetogens (Moorella)
Acetate
OIL
Gasification of
biomass, MSW, coal
Advanced
biofuels
via Metabolic
Engineering
Expensive gasifiers

Drivers for explosive growth of biotech in the 21st century:

- Push for Process Sustainability
- Technology advances
  - Metabolic Engineering
  - Engineering microbes for *any* conversion at very high *selectivity*
- Opportunity for resource utilization and rural development

Key points

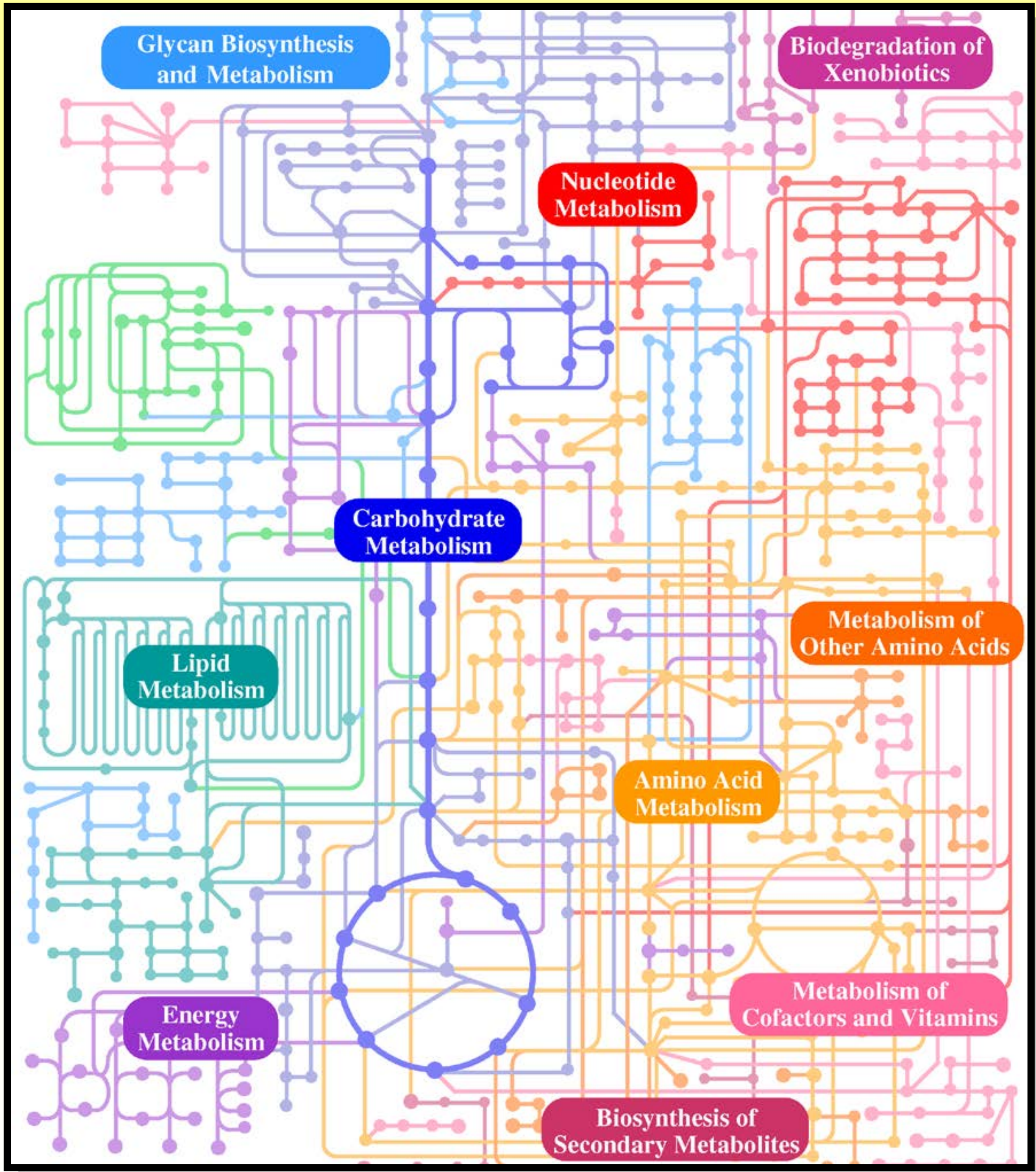
- Modern era of Metabolic Engineering
- ME of the Future
- Biological vs. Thermochemical processes
- Accelerating pathway engineering
- Special issues with gas substrates: Mass transfer but also product stripping

Cells:

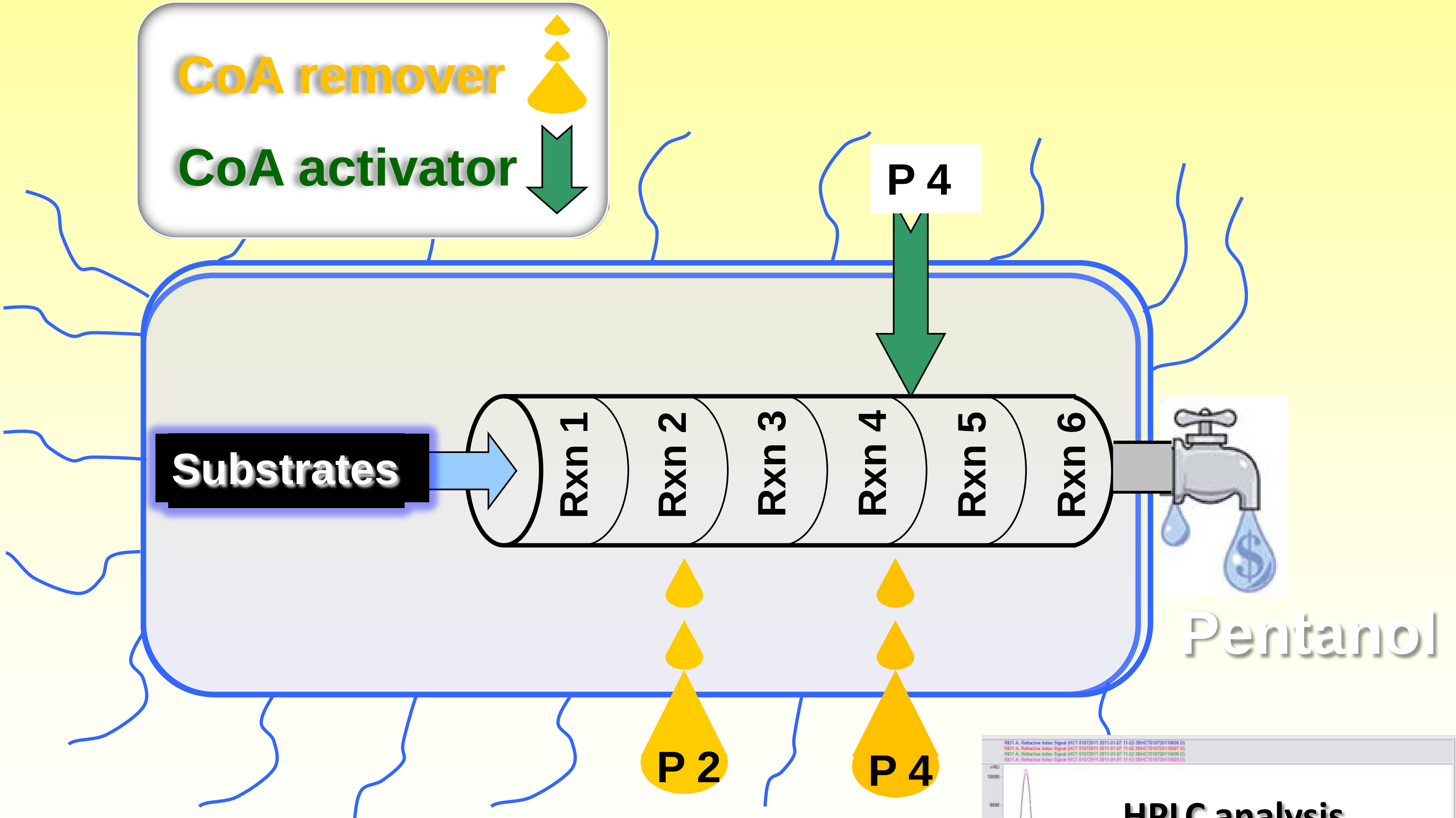
Little chemical factories with thousands of chemical compounds interconverted through thousands of chemical reactions

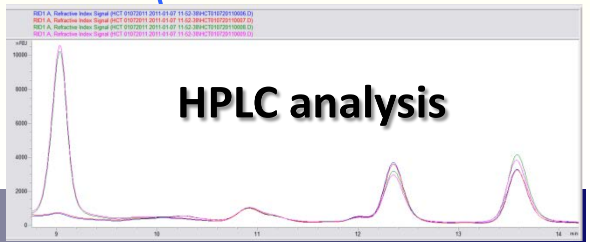
Main substrate: Sugars

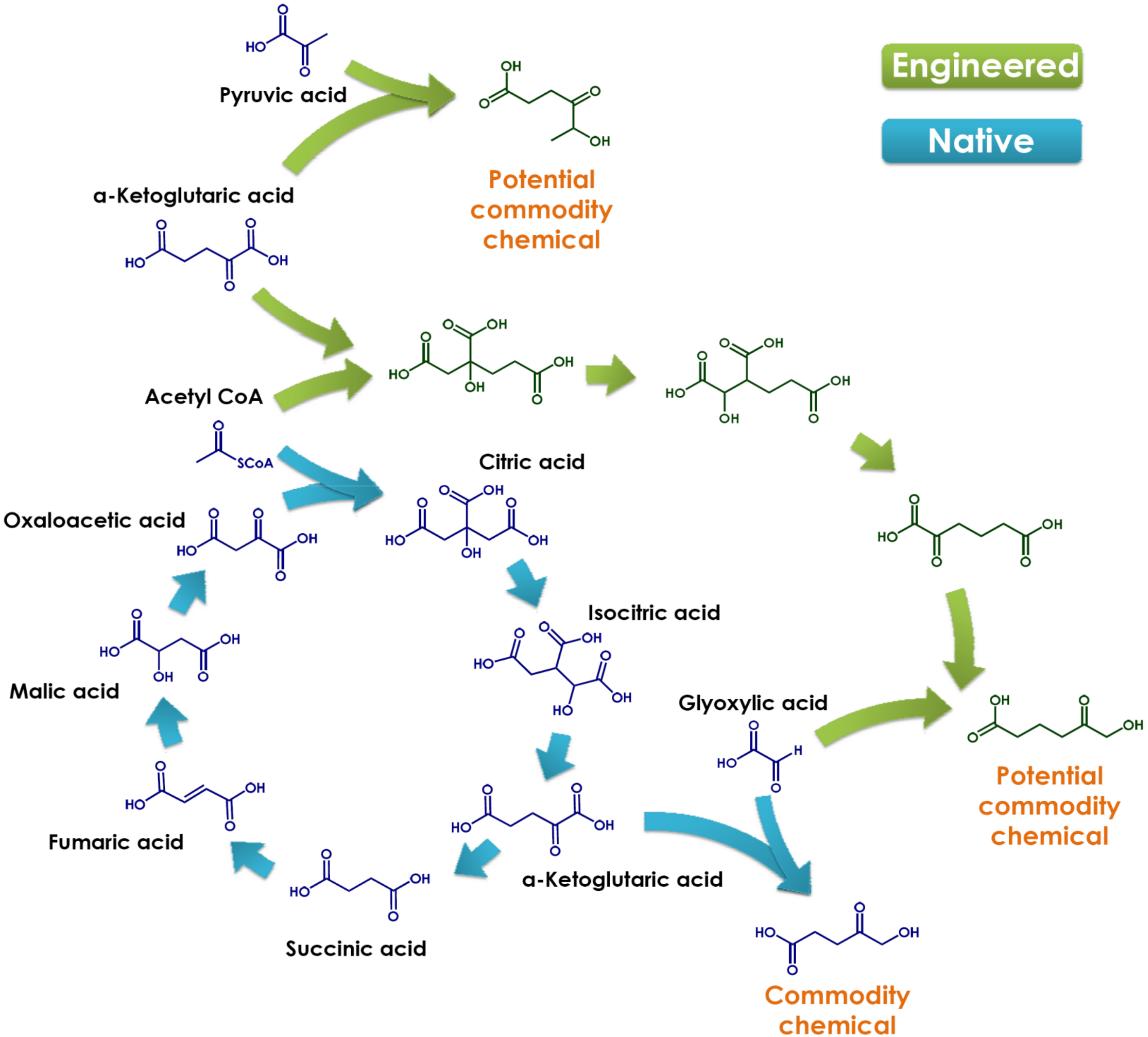
Products: Virtually infinite



Engineering microbes to produce any product






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

Microorganisms

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

Metabolic Engineering, the biotech revolution, and the chemical-fuels industry (White Biotech)

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

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Future Metabolic Engineering

- Unlimited synthetic pathways and non-natural products
- Introduction of global metabolic controls to self regulate (toxic) product accumulation (courtecy of Synthetic Biology)
- Engineering or synthesizing *de novo* special cellular compartments
- Use of scaffolds for enhancing local metabolite concentrations-channeling
- Synthesis of novel enzymes and new chemistry

Sophisticated pathway and microbe engineering is required to create biocatalysts for converting sugars to advanced biofuels

Coupled with

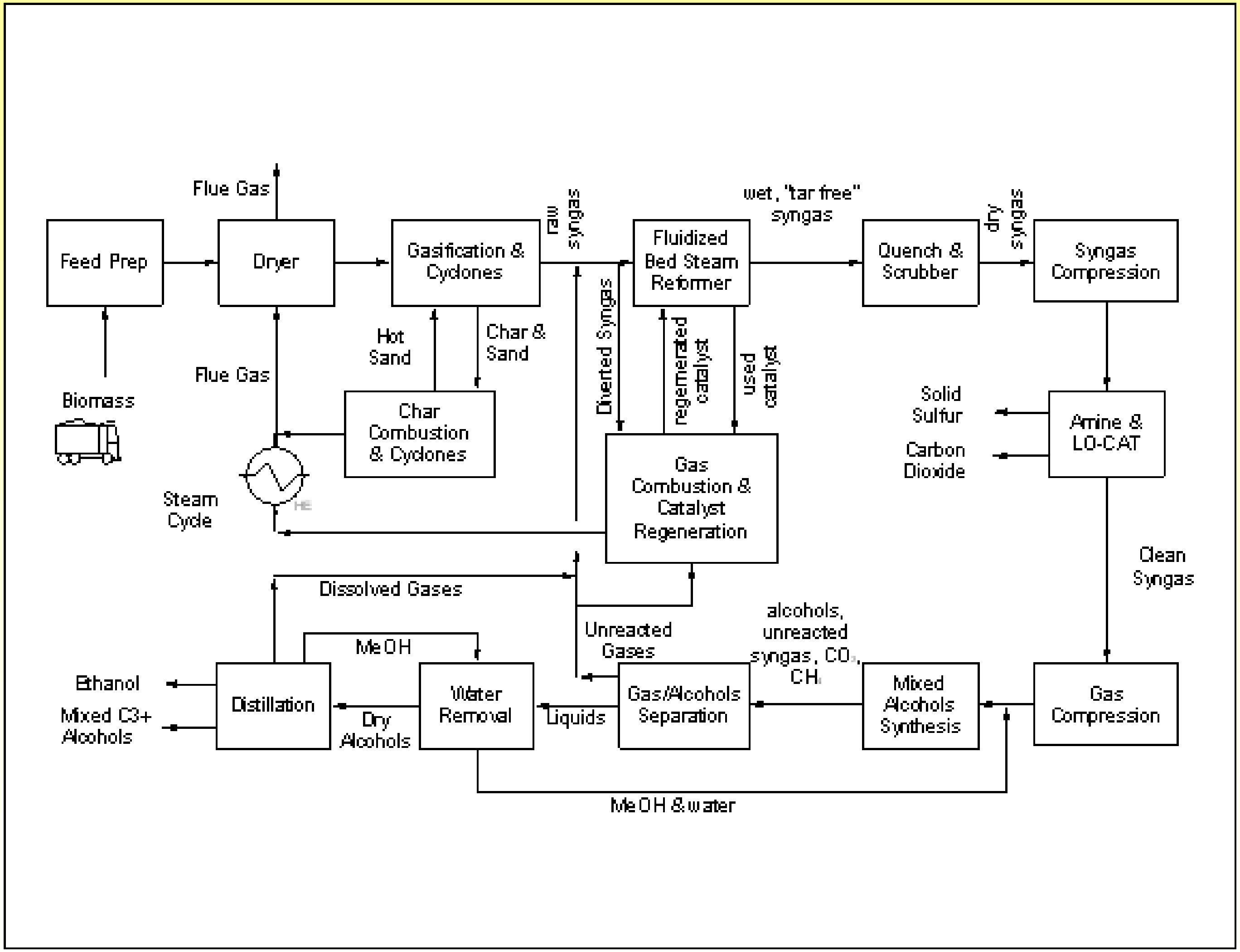
Advanced bioprocessing

Key points

- ❑ Modern era of Metabolic Engineering
- ❑ ME of the Future
- ❑ **Biological vs. Thermochemical processes**
- ❑ **Accelerating pathway engineering**
- ❑ **Special issues with gas substrates: Mass transfer but also product stripping**

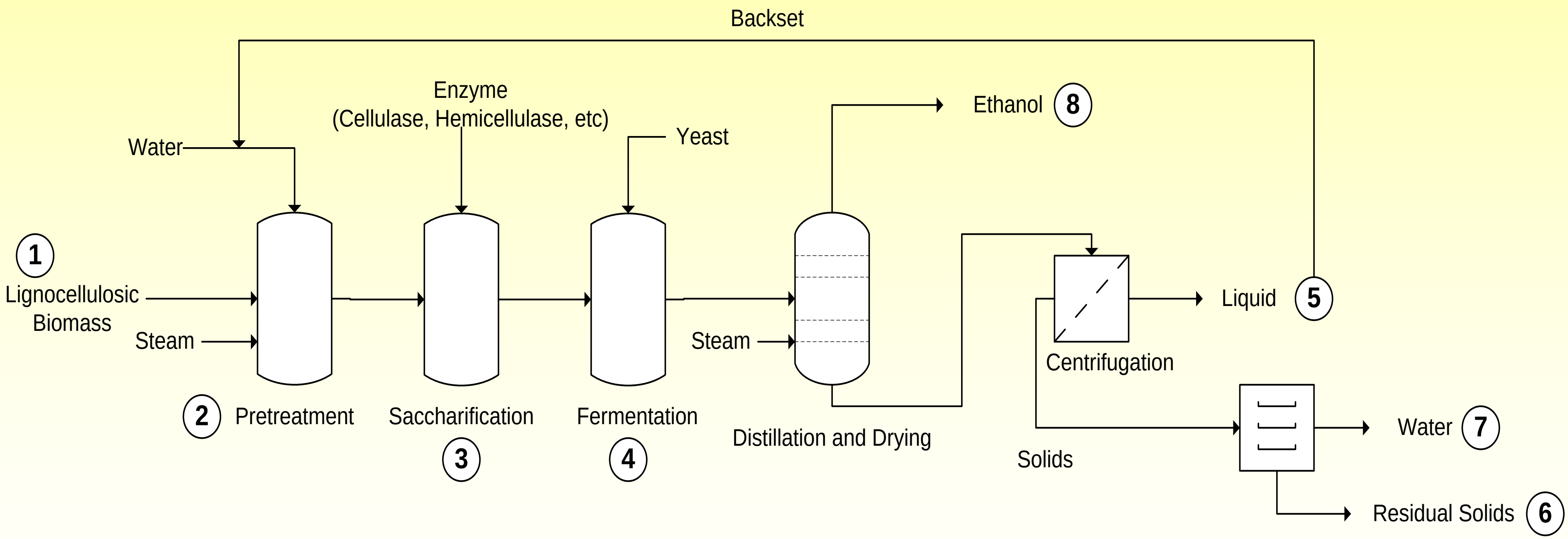
Biochemical
vs.
Thermochemical

Process Block Flow Diagram for FT ethanol



Poplar -> Ethanol

Cellulosic Ethanol Process Flow Diagram



Biochemical vs. thermochemical

- Thermochemical (via syn gas)
  - Advantage: Feedstock agnostic
  - Disadvantage: Requires large, integrated plant to offset large capital costs
- Biochemical (mainly sugar platform)
  - Advantage: Simple, linear process, high selectivity, smaller plants
  - Disadvantage: Depends on availability of cheap sugars

Key points

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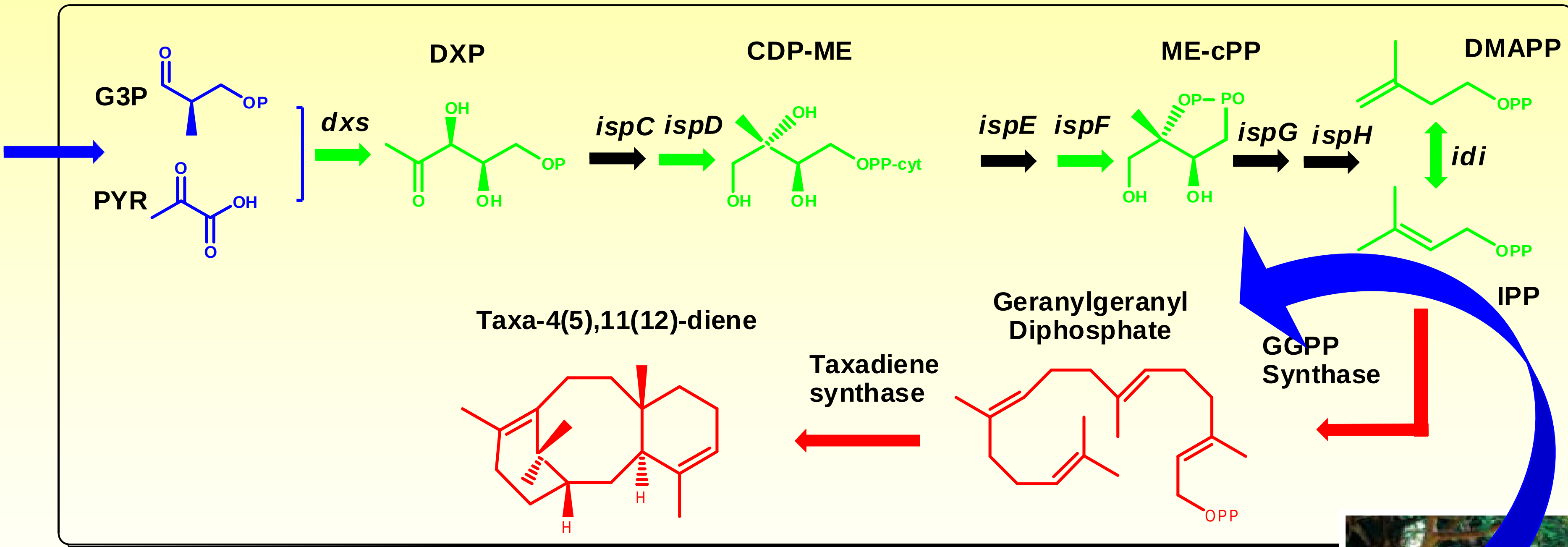
Engineering Taxol biosynthetic pathway in *E. coli*

– most challenging and complex chemistry in natural products

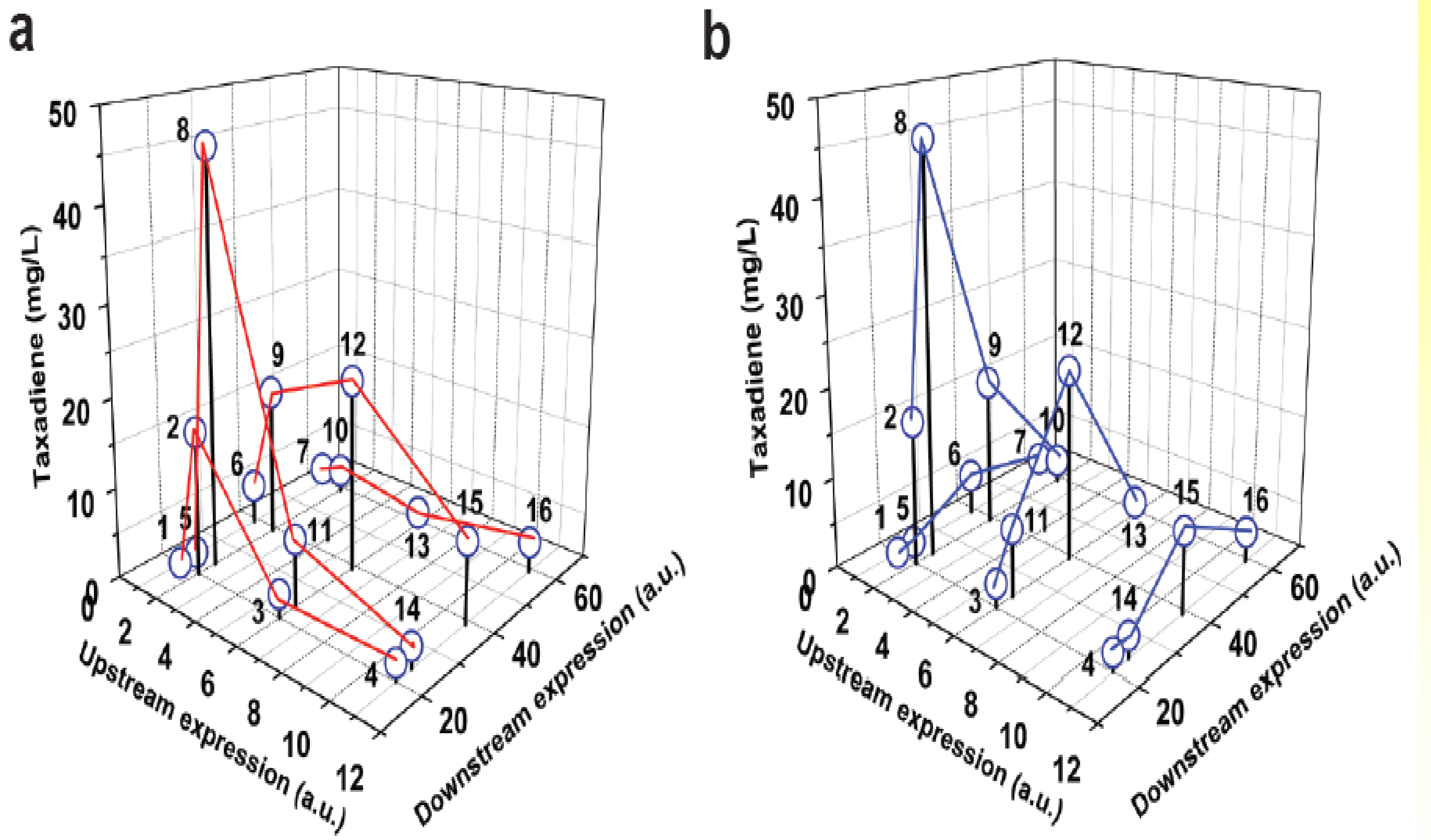
Upstream pathway



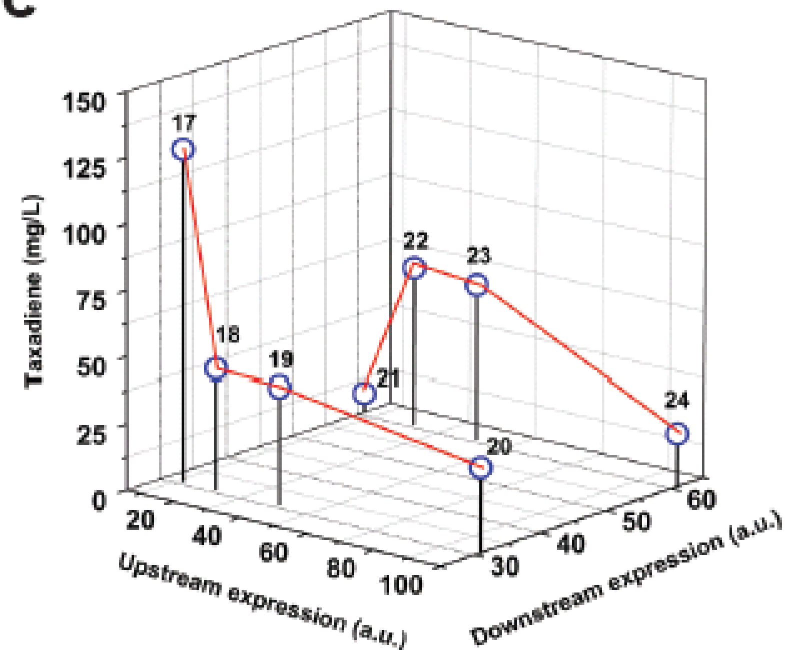
19- enzymes from IPP and DMAPP

Downstream pathway

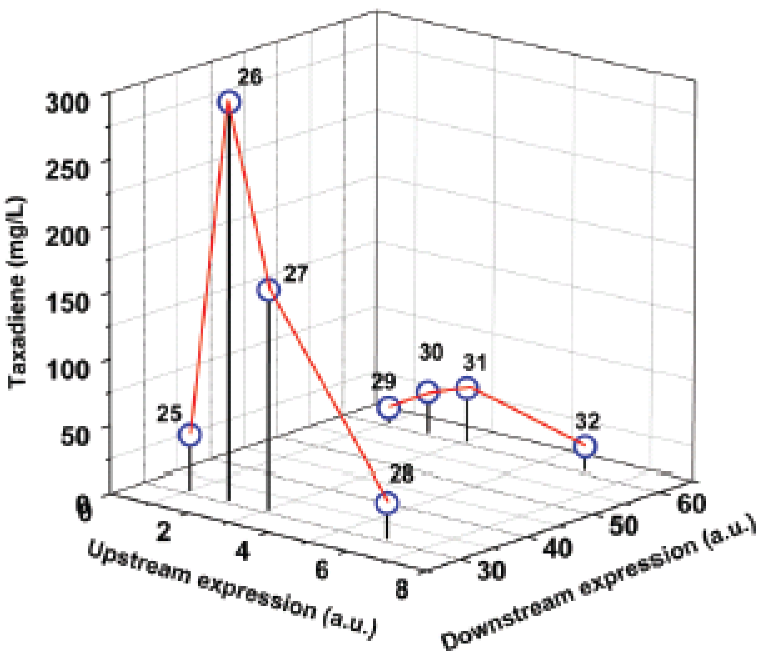
Two genes, GGPP synthase and Taxadiene synthase from taxus Pacific yew are grafted into *E. coli* isoprenoid pathway


a
b
Taxadiene (mg/L)
Upstream expression (a.u.)
Downstream expression (a.u.)

c



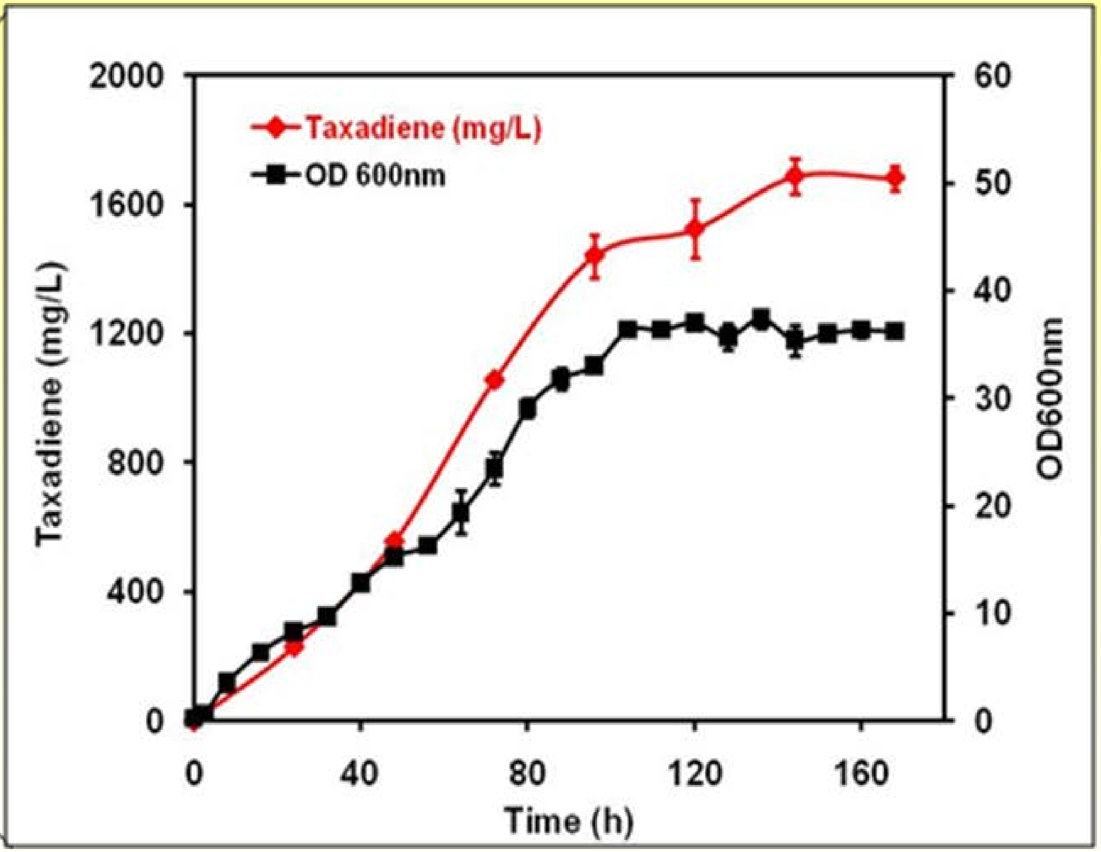
d



e

1. Ep20TrcGT
2. ECh1TrcMEPp20GT
3. Ep5TrcMEPp20TrcGT
4. Ep10TrcMEPp20TrcGT
5. Ep20TrcTG
6. Ep20T5GT
7. Ep20T5GTTrcT
8. ECh1TrcMEPp20TrcTG
9. ECh1TrcMEPp20T5GT
10. ECh1TrcMEPp20T5GTTrcT
11. Ep5TrcMEPp20TrcTG
12. Ep5TrcMEPp20T5GT
13. Ep5TrcMEPp20T5GTTrcT
14. Ep10TrcMEPp20TrcTG
15. Ep10TrcMEPp20T5GT
16. Ep10TrcMEPp20T5GTTrcT
17. EDE3p10TrcMEPp5T7TG
18. EDE3p20TrcMEPp5T7TG
19. EDE3p20T5MEPp5T7TG
20. EDE3p20T7MEPp5T7TG
21. EDE3p5TrcMEPp10T7TG
22. EDE3p20TrcMEPp10T7TG
23. EDE3p20T5MEPp10T7TG
24. EDE3p20T7MEPp10T7TG
25. EDE3p5T7TG
26. EDE3Ch1TrcMEPp5T7TG
27. EDE3Ch1T5MEPp5T7TG
28. EDE3Ch1T7MEPp5T7TG
29. EDE3p10T7TG
30. EDE3Ch1TrcMEPp10T7TG
31. EDE3Ch1T5MEPp10T7TG
32. EDE3Ch1T7MEPp10T7TG

Fermentation of taxadiene producing strain AP2T7TG



Science, 330: 70-74 (2010)

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

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Carbon dioxide fixation with CO/Hydrogen

Electron production

Acetyl-CoA pathway

$$\mathbf{2\,CO_2 + 8\,e^- \rightarrow C_2H_4O_2}$$

$$H_2 \xrightarrow{\textit{hydrogenase}} 2\,H^+ + 2\,e^{-1}$$

4 moles needed

$$CO + H_2O \xrightarrow{\textit{CODH}} CO_2 + 2\,H^+ + 2\,e^{-1}$$

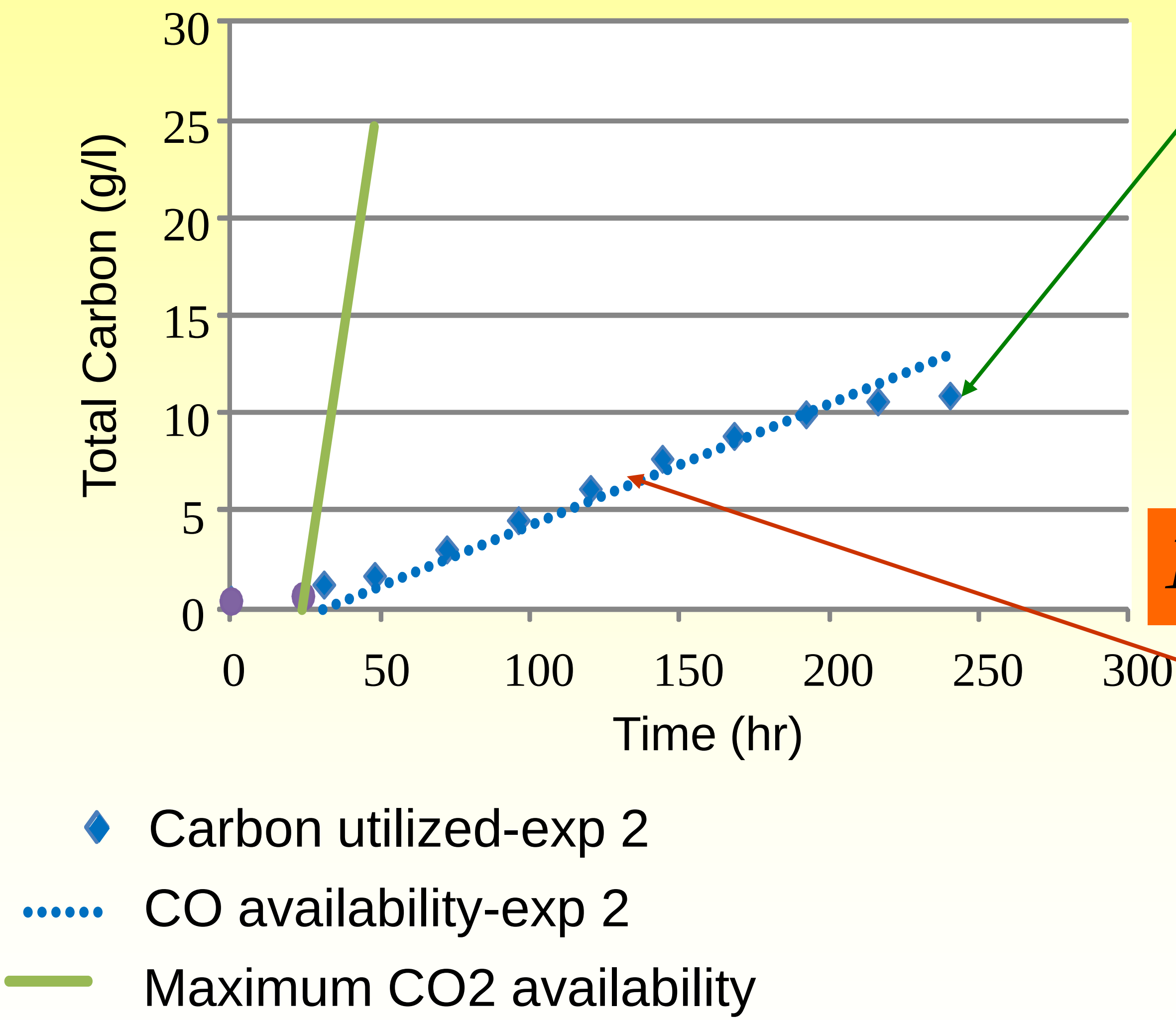
If electrons from H_2

$$2\,CO_2 + 4\,H_2 \rightarrow C_2H_4O_2 + 2\,H_2O$$

If electrons from CO

$$4\,CO + 2\,H_2O \rightarrow C_2H_4O_2 + 2\,CO_2$$

Carbon utilization: Experiment 1

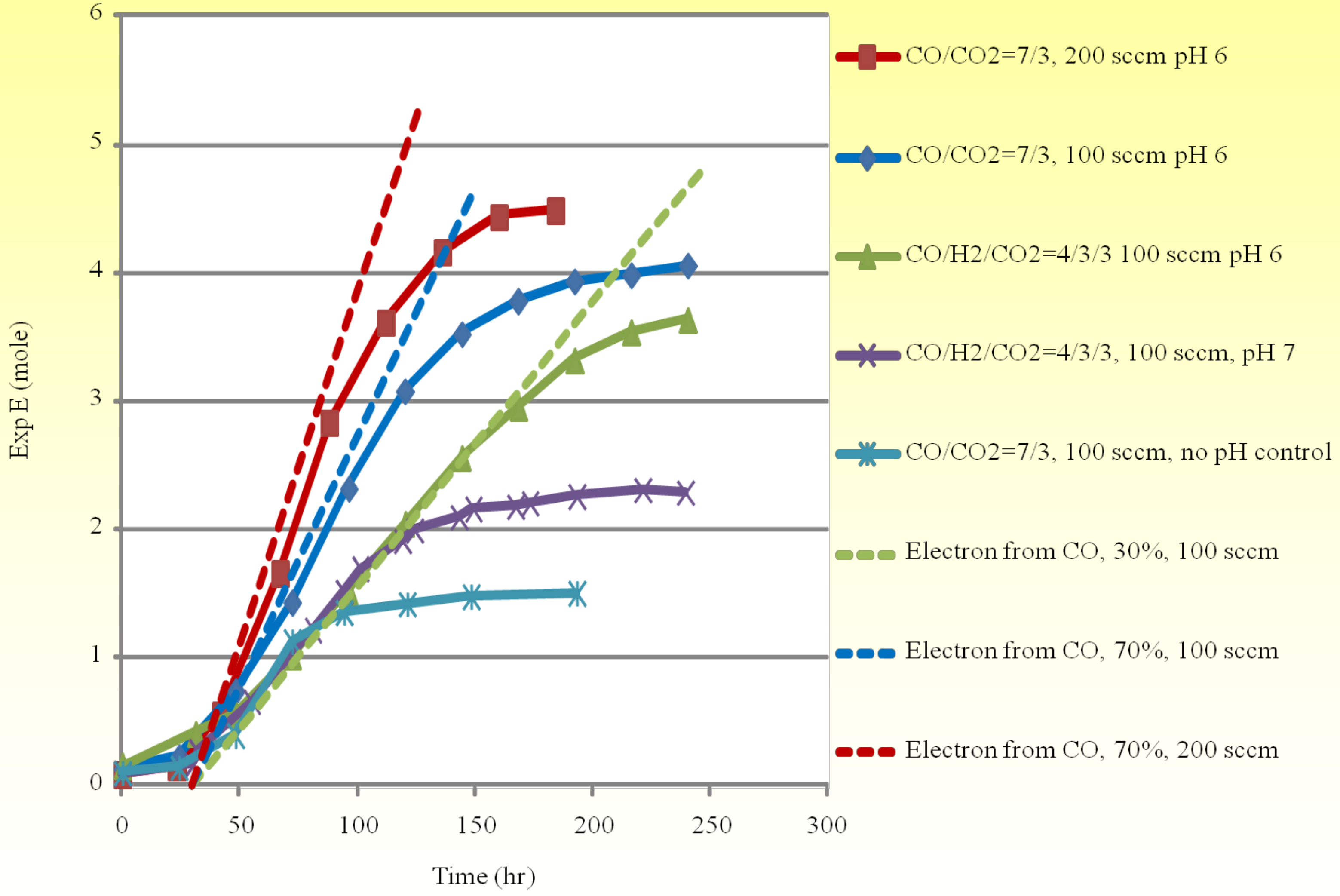


Utilization = 0.5 cell mass + 0.4 $C_{acetate}$ (g/l)

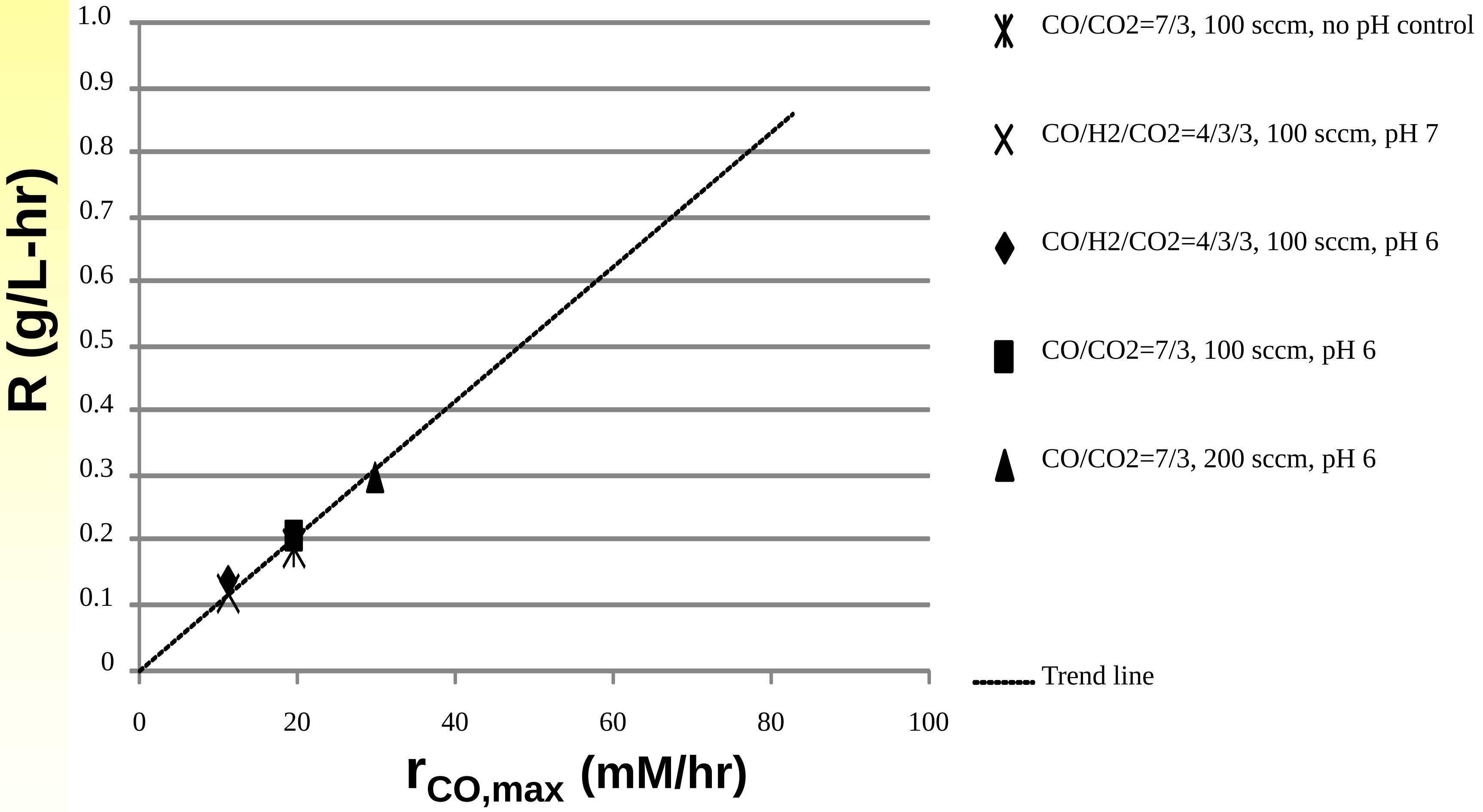
$$N_A = k_L a(C^* - C_t)$$

Availability of Gas = 12 $k_L a$ C^* (g/l/hr)

Electrons utilized are calculated from experimental data and the maximum available electrons from carbon monoxide transferred as determined from mass transfer modeling



Correlation between acetic acid productivity and maximum mass transfer rate of carbon monoxide



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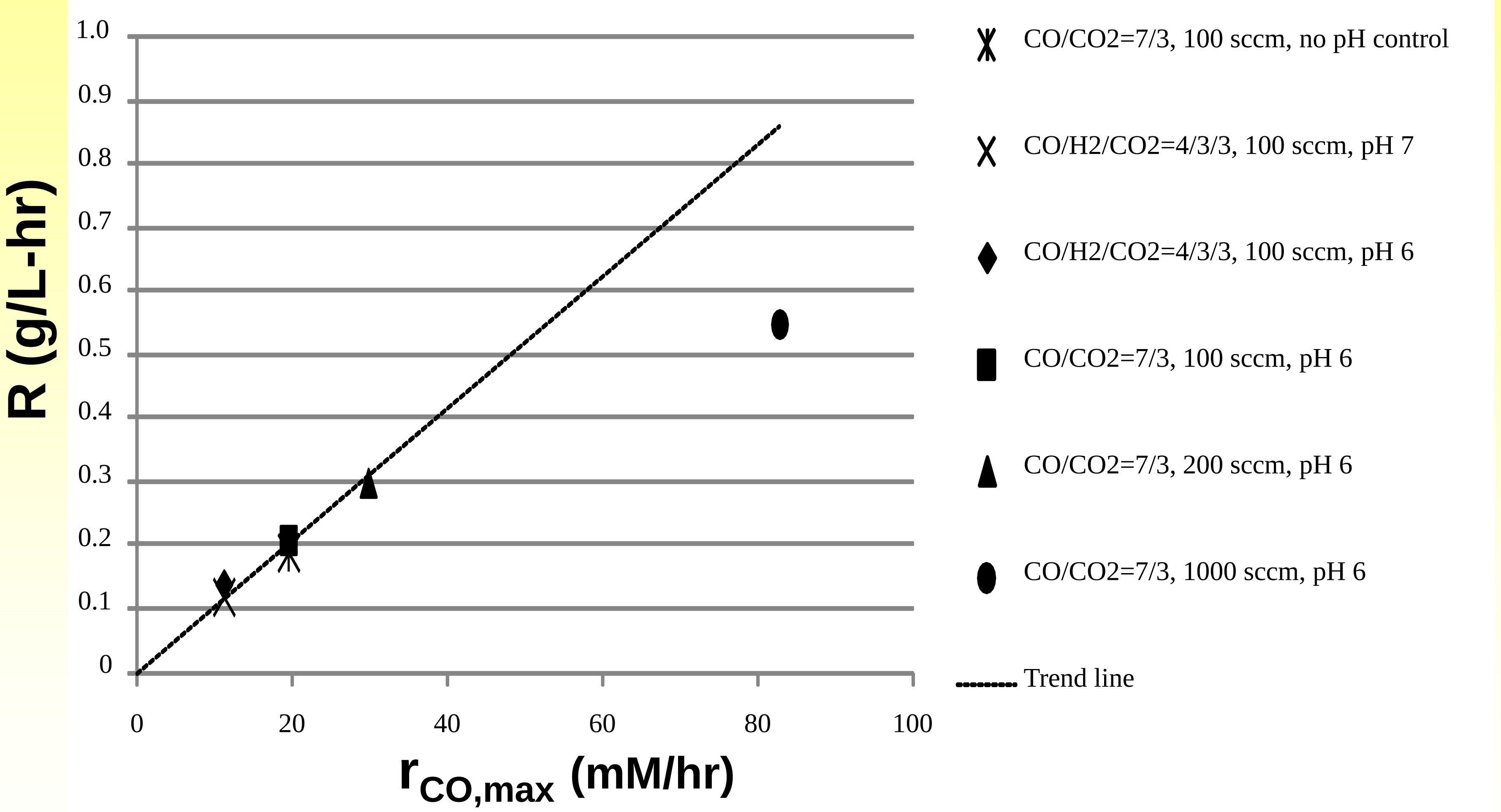

IV. What is in the future?

Future applications drivers

- **New technology push:**
  - **Chemical synthesis of heterologous genes**
  - **Increased appreciation of systemic approaches to pathway engineering (mind-frame of *Systems Biology*)**
  - **Increased experimentation with pathway construction harboring random DNA combinations (Synthetic DNA)**
  - **Inverse Metabolic Engineering**
  - **Development of High-Throughput screens for chemicals production**

Future directions of ME-1

- **Expand portfolio with numerous new applications:**
  - Sustained interest in renewable resource utilization
  - Expansion to the core of the chemical industry at oil prices greater than $100/bbl (xylenes, terpenes, isoprene, butadiene,…)
  - Best technology for specialty chemicals (specific oxidations, acylations, amidations, stereo-specific compounds, API's, …)
  - Tremendous *diversity* of new products (isoprenoid pathway, glycosylated compounds)

The end