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cyanobacteria as biocatalysts for solar-driven biofuel production

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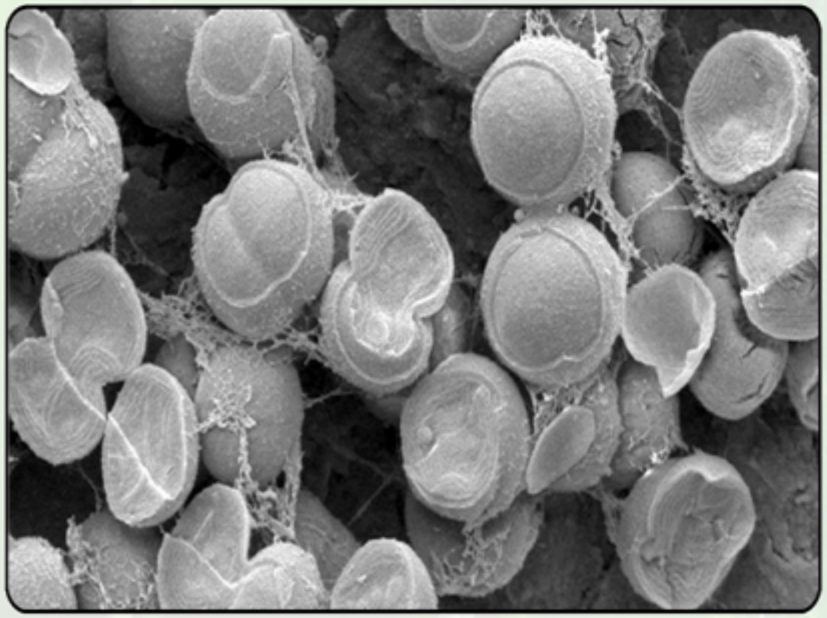
Summary and outlook

Sunlight and CO_2 to Fuels via Photosynthesis

- Keep cyanobacteria in stationary phase: “no” biomass generated, no net mineral use, maximum biofuel generation
- Secrete the product, and separate it from the culture
- Other obvious products: alcohols, hydrocarbons, etc.
- Minimize biochemical steps in solar-to-fuel bioconversion
- CO_2 can come from the air

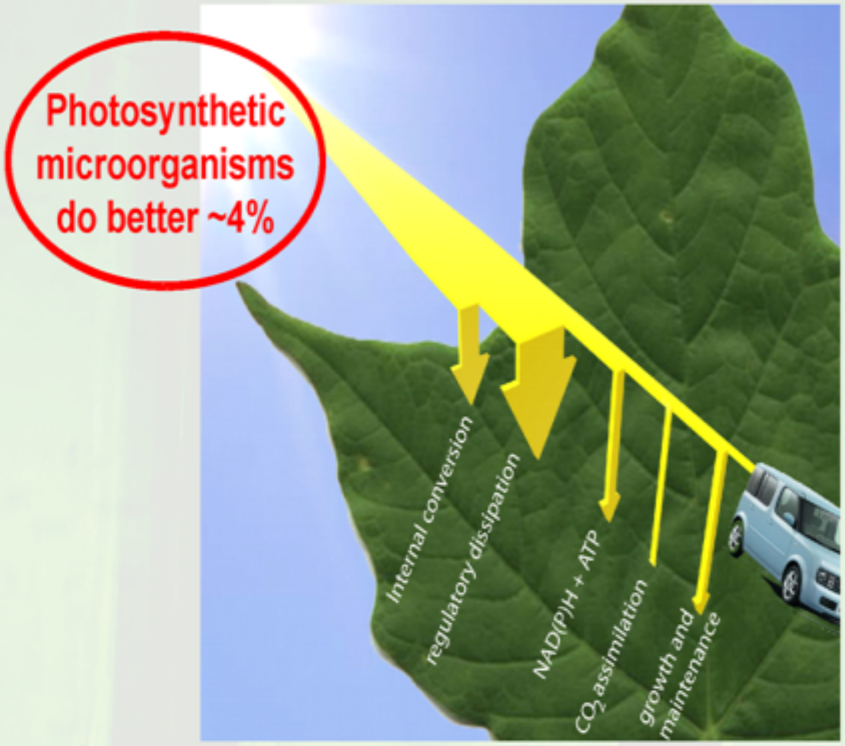
Important factors:

- Biocompatibility of product
- Availability of enzymes / genes
- Stoichiometry of NADPH and ATP use
- Scalability
- Ability to secrete but no way to utilize the product



Cyanobacterial platform for biofuel production

Plant photosynthesis is (much) less than 1% efficient

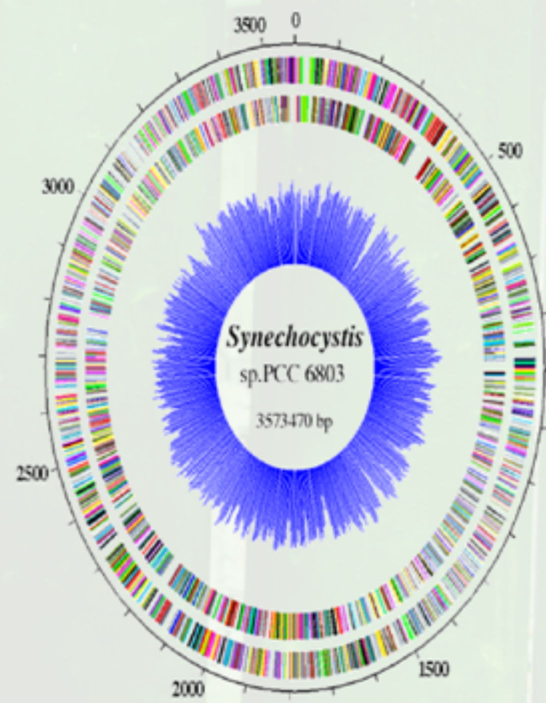


Main reasons:

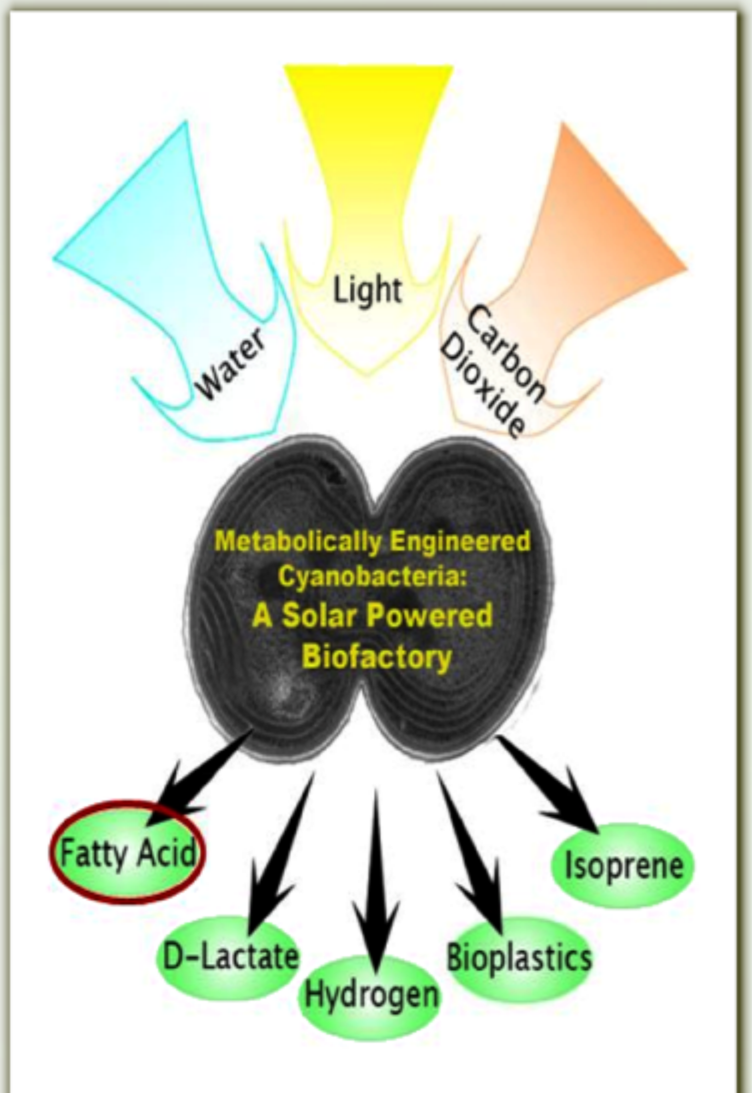
- CO_2 is soluble in water; converts to HCO_3^- at high pH that cyanobacteria prefer
- Essentially free-living chloroplasts; no leaves or stems

Synechocystis sp. PCC 6803

- Naturally transformable
- Known genome
- Salt tolerant (0-1.2 M NaCl)
- Temperature tolerant (up to 45ºC)
- Efficient light harvesting

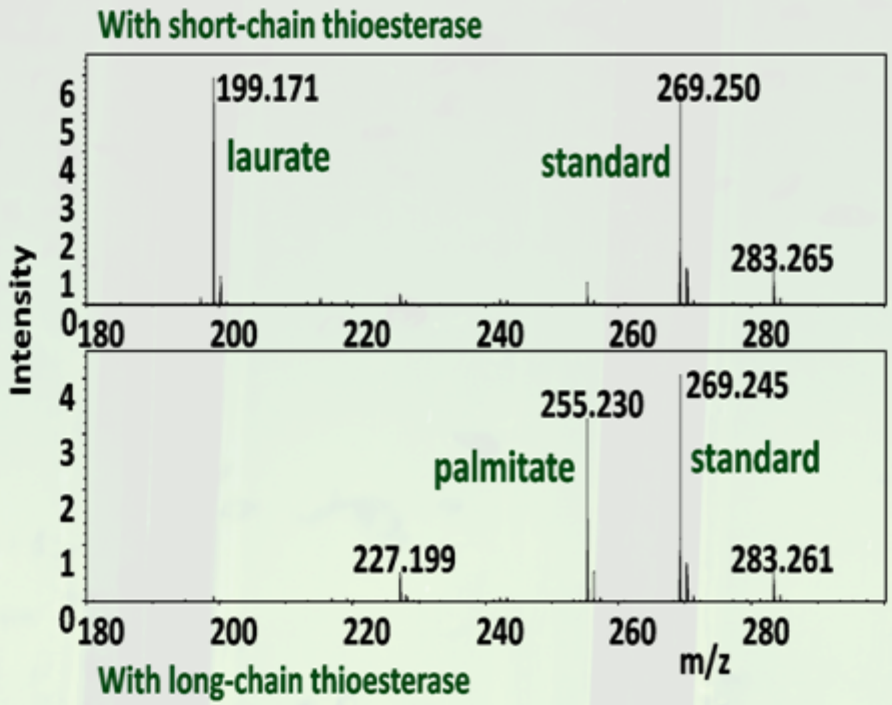


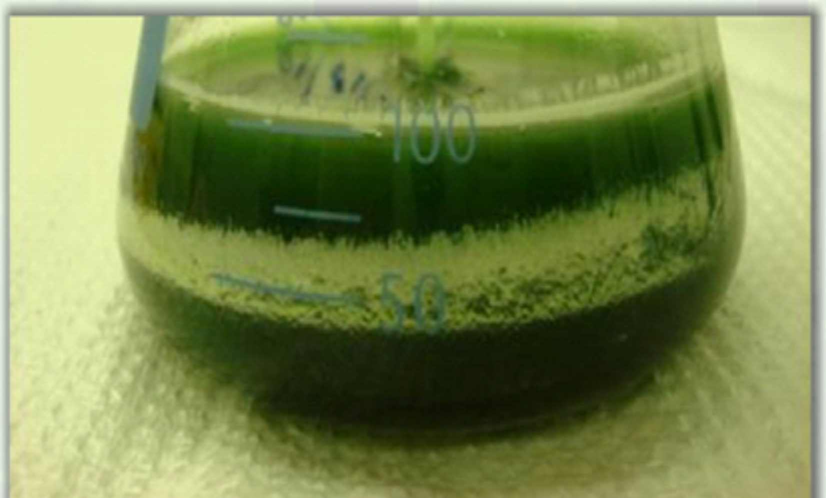
Synechocystis as a biocatalyst

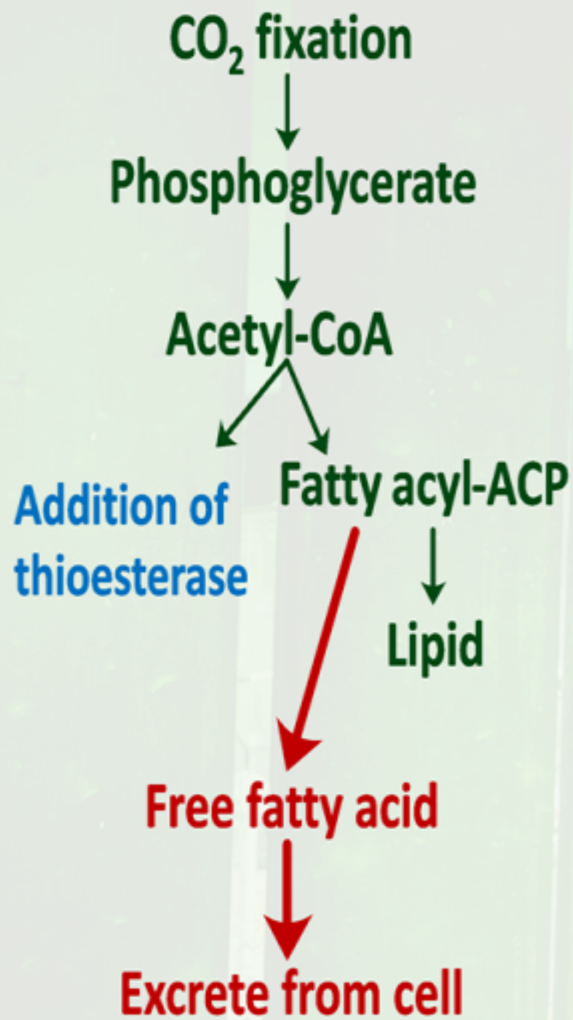


- The cyanobacterium can serve as a solar-powered, CO_2-converting biocatalyst to make and secrete:
  - Fatty acids
  - Isoprenoids
  - Alkanes
  - Bioplastics
  - Alcohols, etc.
- Focus in this project is on solar-powered fatty acid production and secretion, followed by chemical decarboxylation and isomerization to make fuel-compatible alkanes
- Biomass generation is minimized

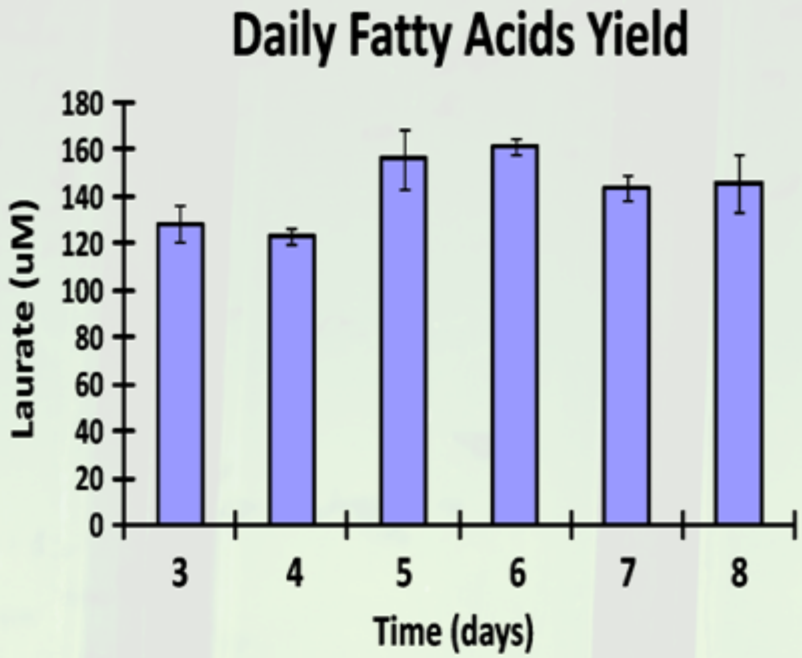
Producing secreted fatty acids







Efficient light energy conversion



Energy Conversion Efficiency:

- About 3% energy conversion efficiency
- longer-term target: 10%

Carbon fixation:

Net: 12 CO_2 + 47 ATP + 36 NADPH -> C12 fatty acid + 47 ADP + 47 P_i + 36 NADP

Light reactions (linear electron flow):

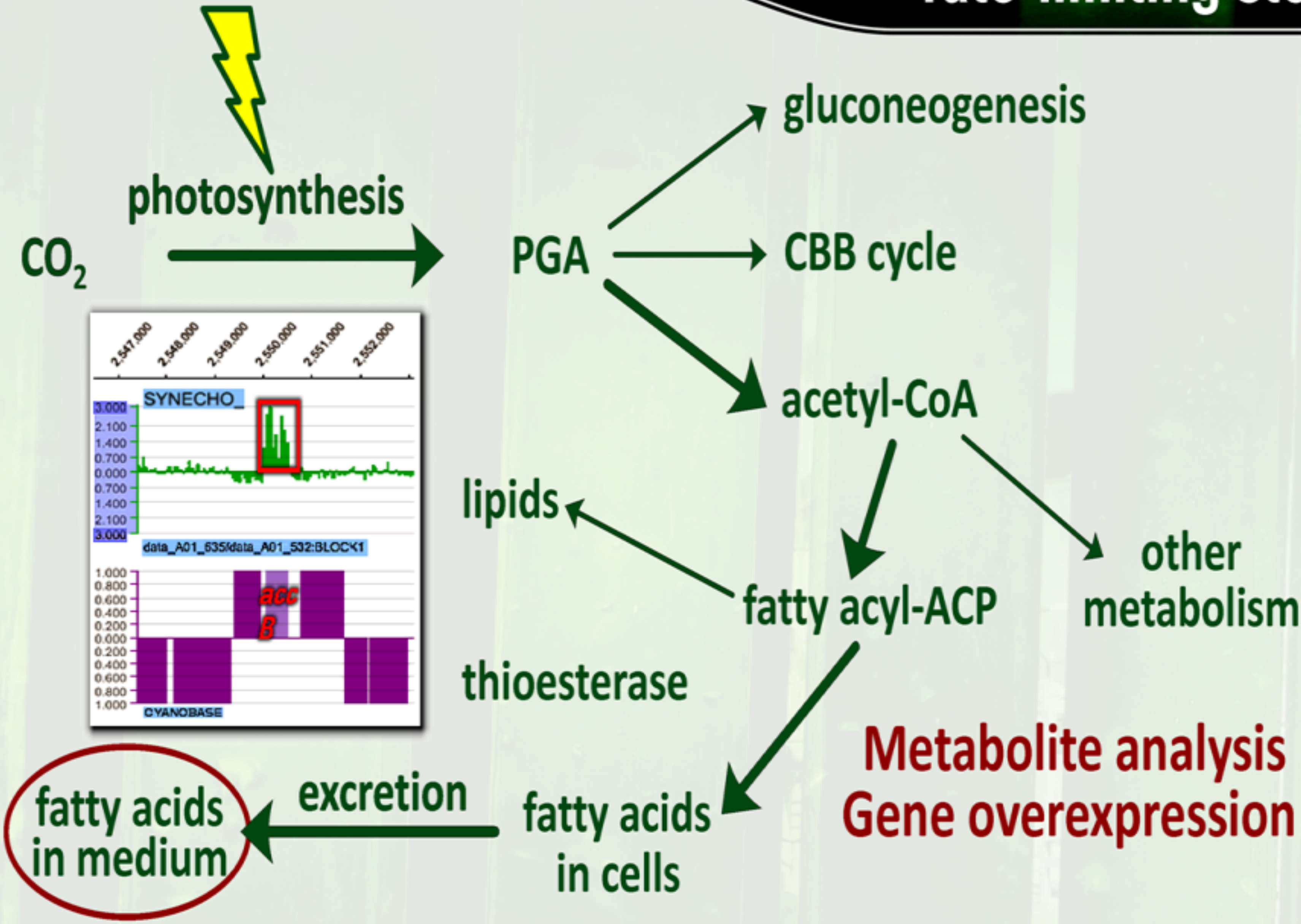
36 H_2O + 36 NADP + 144 hn + 48 ADP/P_i -> 36 NADPH + 18 O_2 + 48 ATP

A mole of quanta of 680 nm light: 177 kJ

A mole of C_{12} fatty acid: 7300 kJ

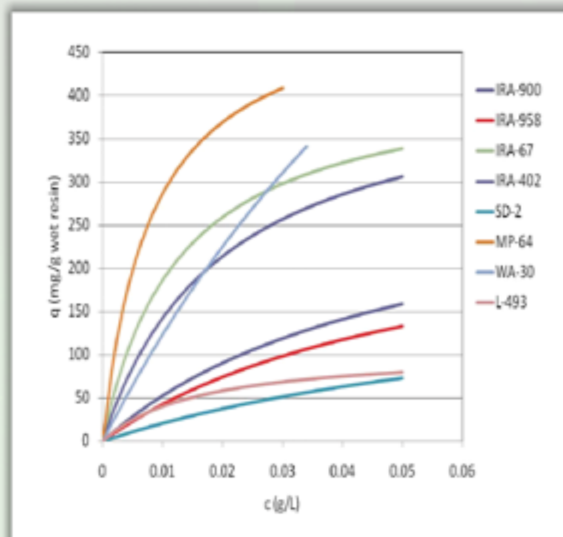
Maximum energy efficiency (680nm light): 7300x100%/(177x144) = 28%

Eliminate rate-limiting steps



Metabolite analysis
Gene overexpression

Fatty acid harvesting

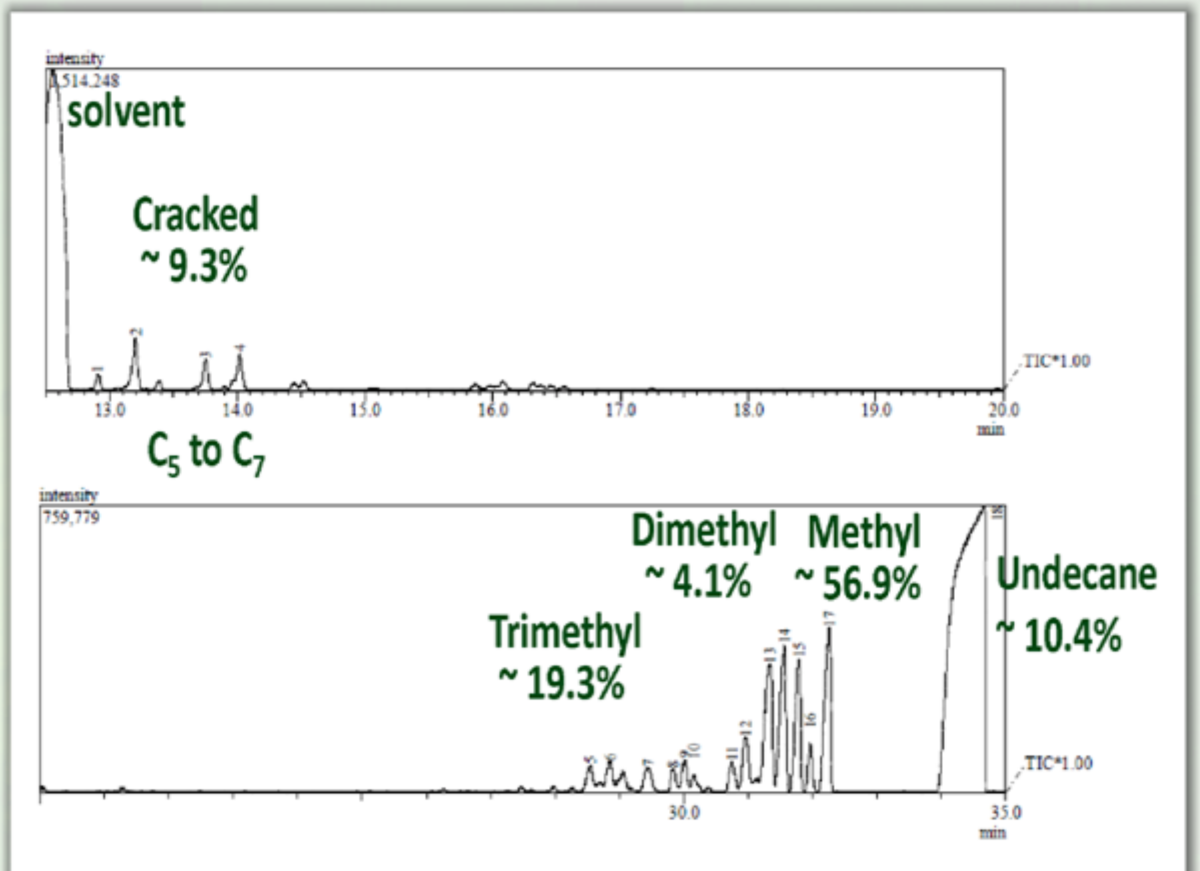


Langmuir constants determined for screened resins.

Resin	q_{max} (mg laurate/ g wet resin)	K_d (mg laurate/ mL)
IRA-900	319	0.050
IRA-958	280	0.055
IRA-67	426	0.013
IRA-402	430	0.020
MP64	519	0.008
WA30	1273	0.093
SD-2	194	0.083
L-493	105	0.016

- Resin in expanded-bed column adsorbs fatty acid
- Excellent fatty acid recovery
- Resin is reusable

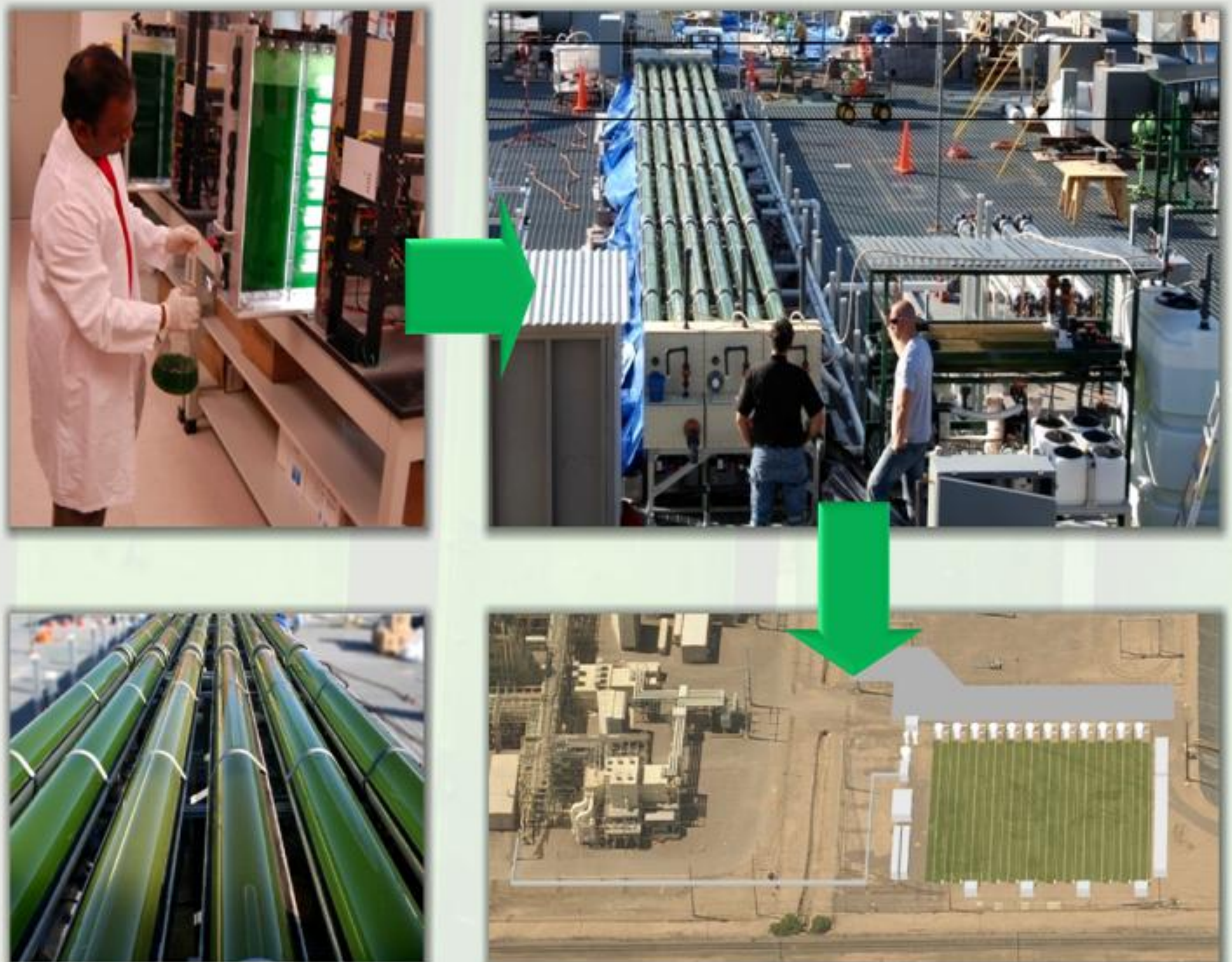
Decarboxylation and isomerization



Properties	
Freeze Point*	-35°C
Flash Point	37°C
Mass Conversion	83%

* As low as we can test

Scale-up scenario



Comprehensive cost analysis