SAND2011-4651C

Characterization of Genetically Engineered *Synechococcus elongatus* PCC 7942 for Biofuel Production

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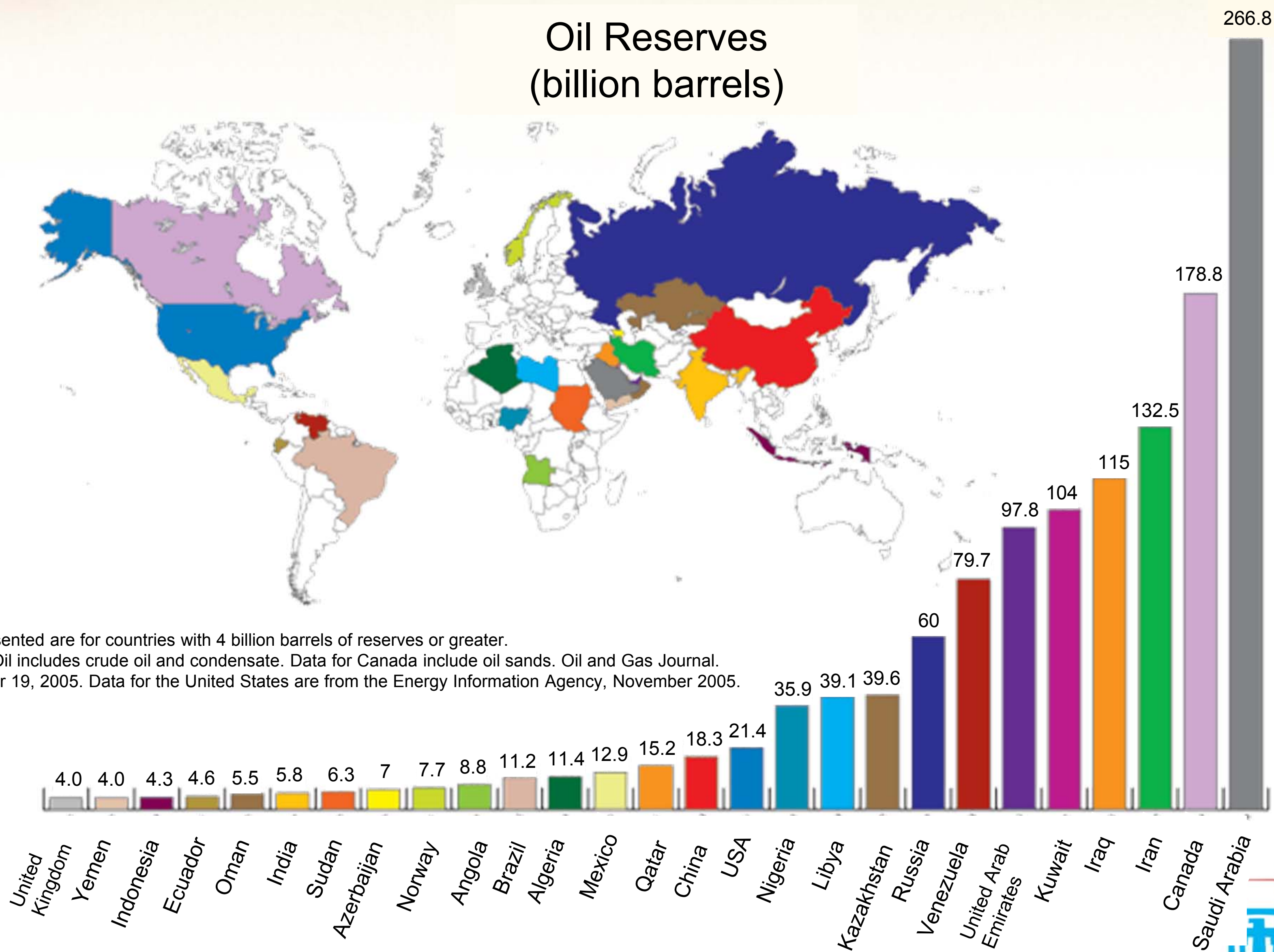
Truman Fellow

Sandia National Laboratories

Phycological Society of America Annual Meeting

July 16, 2011

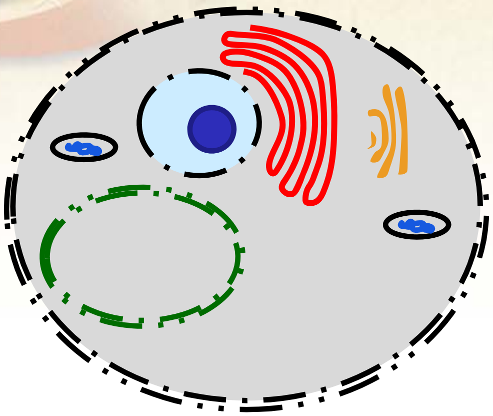
Energy: An Issue of National Security



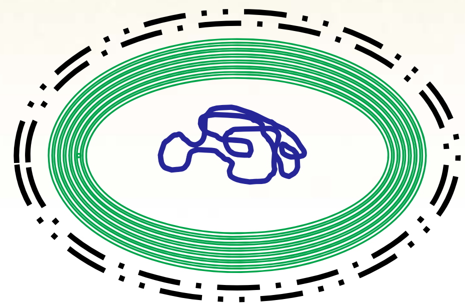
Data presented are for countries with 4 billion barrels of reserves or greater.
Source: Oil includes crude oil and condensate. Data for Canada include oil sands. Oil and Gas Journal. December 19, 2005. Data for the United States are from the Energy Information Agency, November 2005.

http://lugar.senate.gov/energy/graphs/worldoil.html

Why Cyanobacteria?



Eukaryotic Algae

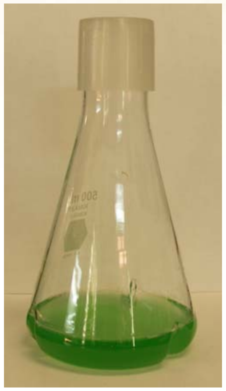


Cyanobacteria

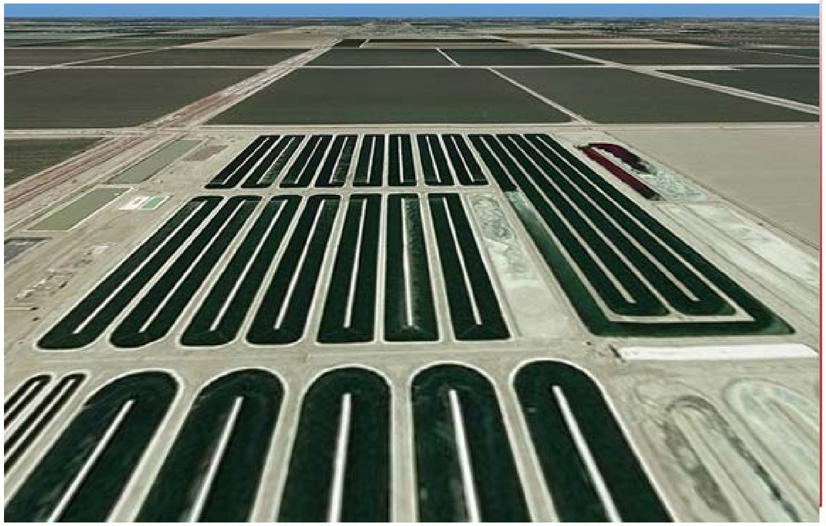
Eukaryotic Algae	Cyanobacteria
Intracellular accumulation of TAG	Natural secretion of FFA
Additional downstream processing	Simplified downstream processing
Batch operation	Continuous operation possible
Genetic manipulation is difficult	Genetic manipulation is straightforward
Multiple membrane barriers	Facile DNA uptake
Few genetic tools available	Genetic tools developed since 1980’s
Gene expression and gene knockouts have proven to be difficult	Many successful examples of genetic manipulation and multiple mutations

Why Not Cyanobacteria?

Possible Advantages of Cyanobacteria-Based Fuel



1. Fast growth rates compared to plants (similar to algae)
2. Harvest energy from sunlight (renewable)
3. Reduce greenhouse gas emissions (carbon fixation)
4. Fuel secretion - simplified downstream processing and continuous cultivation (reduced operational costs)
5. Potential for greater fuel production and other desirable traits for large scale processing (genetic engineering)
   - Prevent 'escape' of GMO's
   - Reduce contamination
   - Improve rate of photosynthesis
   - Increase light penetration
   - Enhanced CO_2 uptake
   - Temperature resistance



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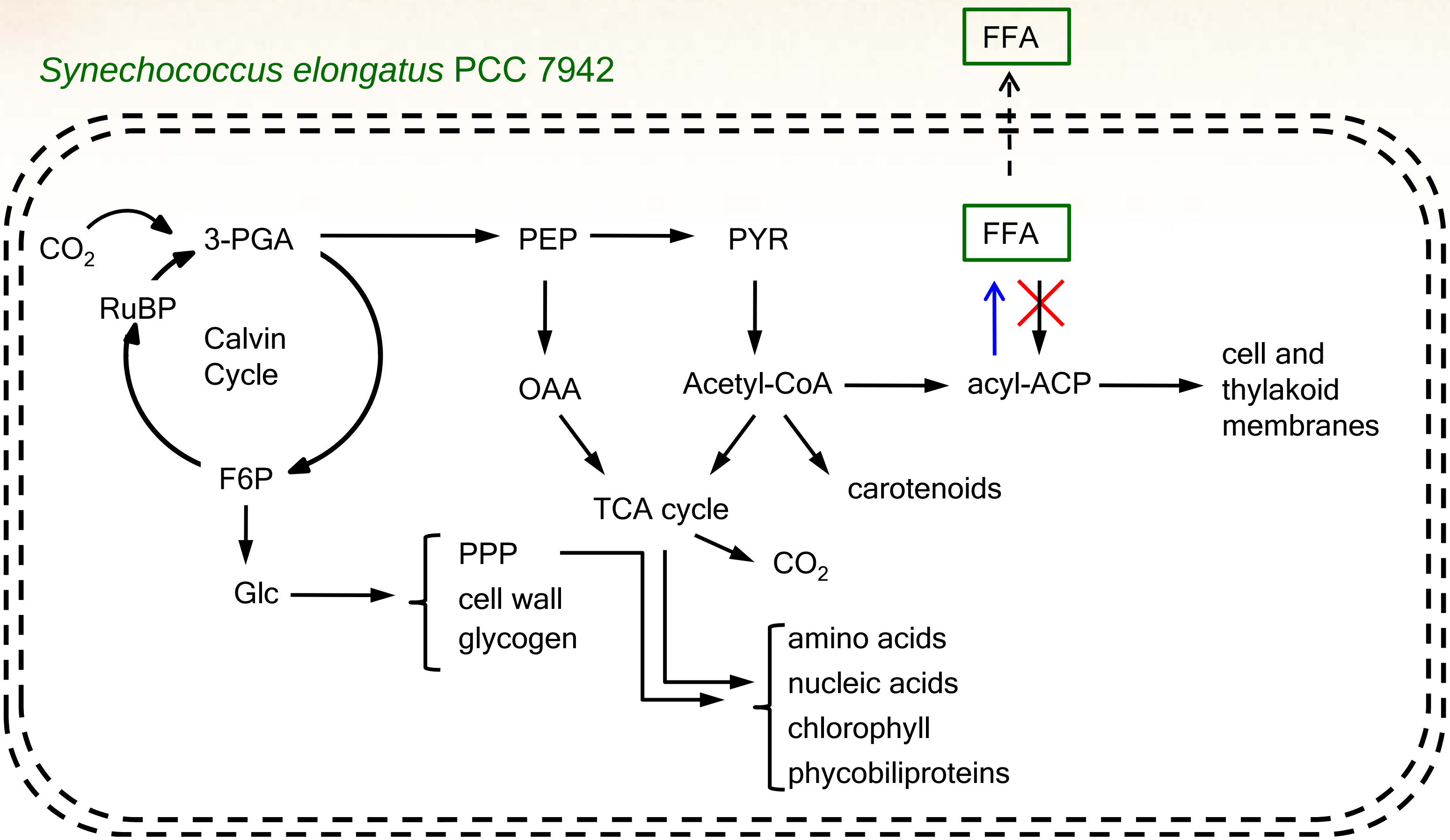
Genetic Engineering of Cyanobacteria to Produce FFA



7942: Wild type

SE01: Knockout of acyl-ACP synthetase

SE02: Knockout of acyl-ACP synthetase; expression of thioesterase (‘*tesA* from *E. coli*)

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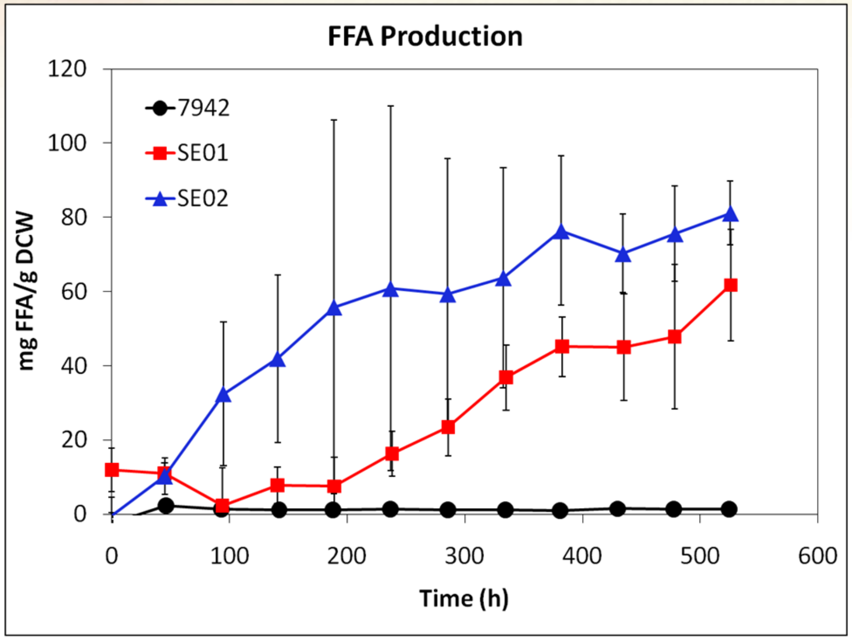
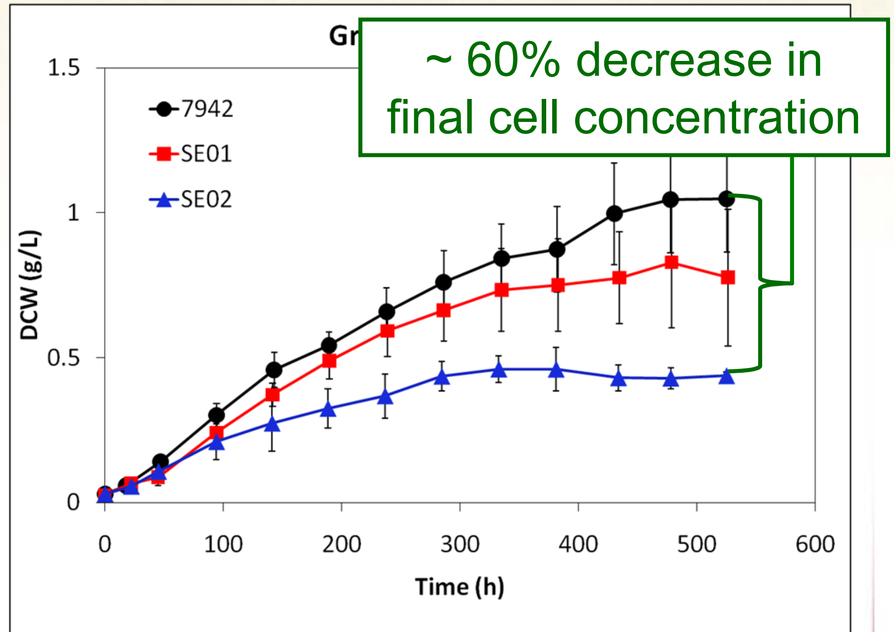
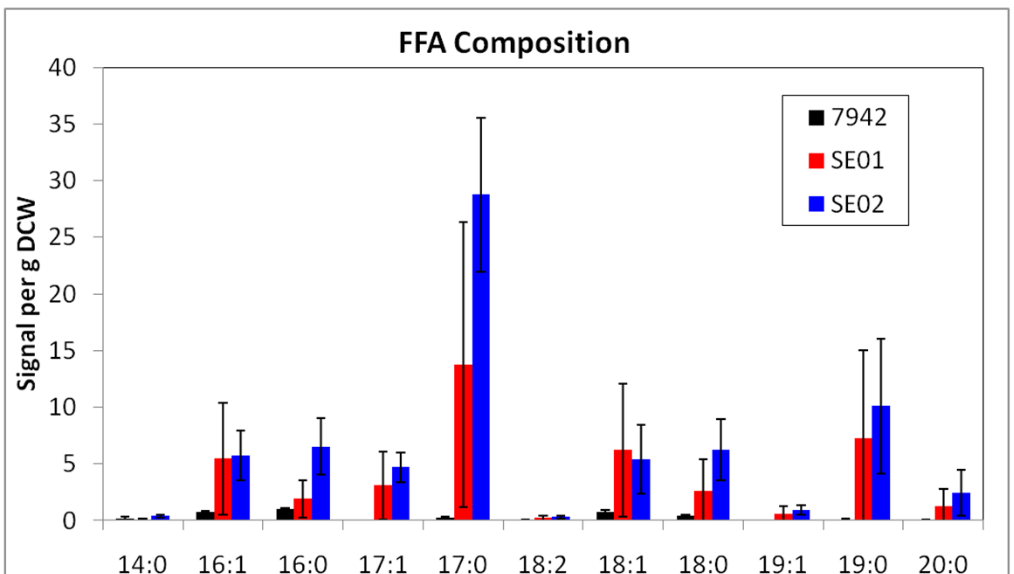
FFA Production and Growth of Engineered Strains







- Negligible FFA production in wild-type
- SE02 produces up to 80 mg FFA/g DCW
- Predominantly saturated and monounsaturated C16 – C19 FFA are produced
- FFA-producing strains have decreased growth

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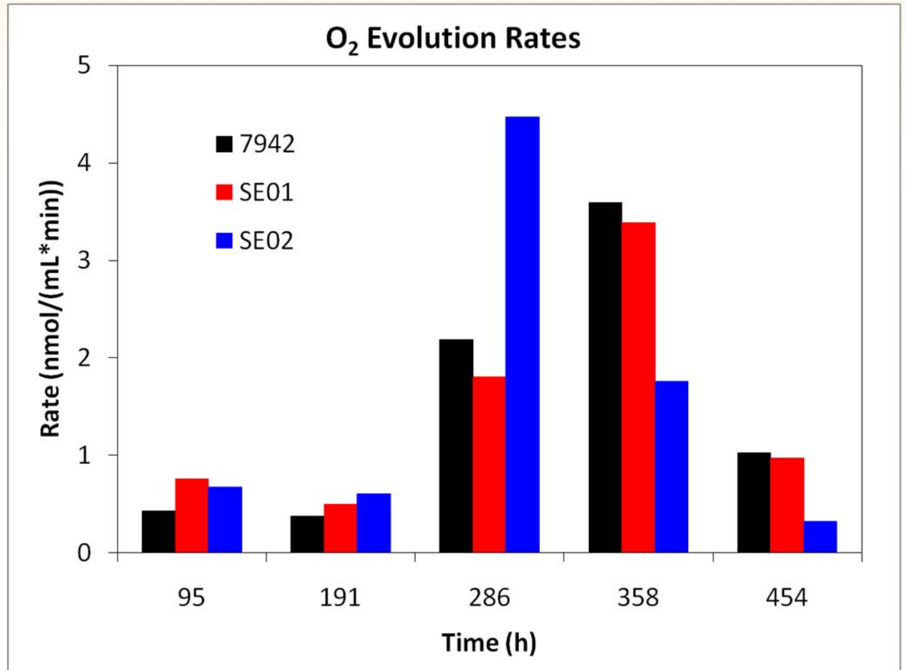
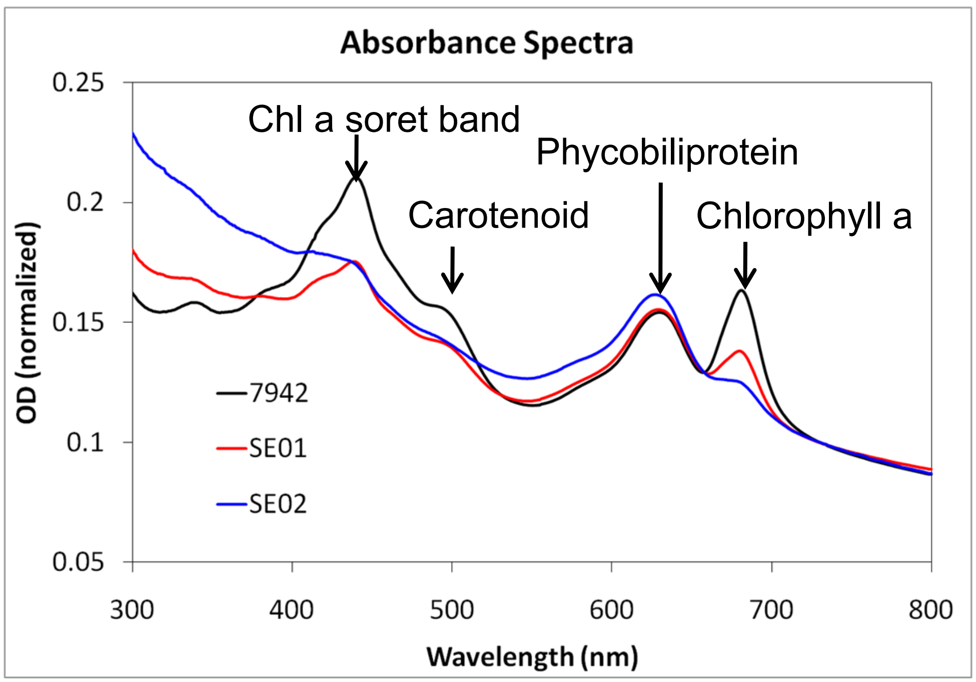
FFA Production: Effect on Photosynthesis and Photosynthetic Pigments

- Negligible change in rate of O_2 evolution for SE01 compared to wild-type
- Increase in O_2 evolution rate for SE02 at mid-point of FFA synthesis reaction
- Decreased rates of O_2 evolution for SE02 late in the experiment





- Decreased chlorophyll a in FFA-producing strains
- Slight increase in phycobiliprotein

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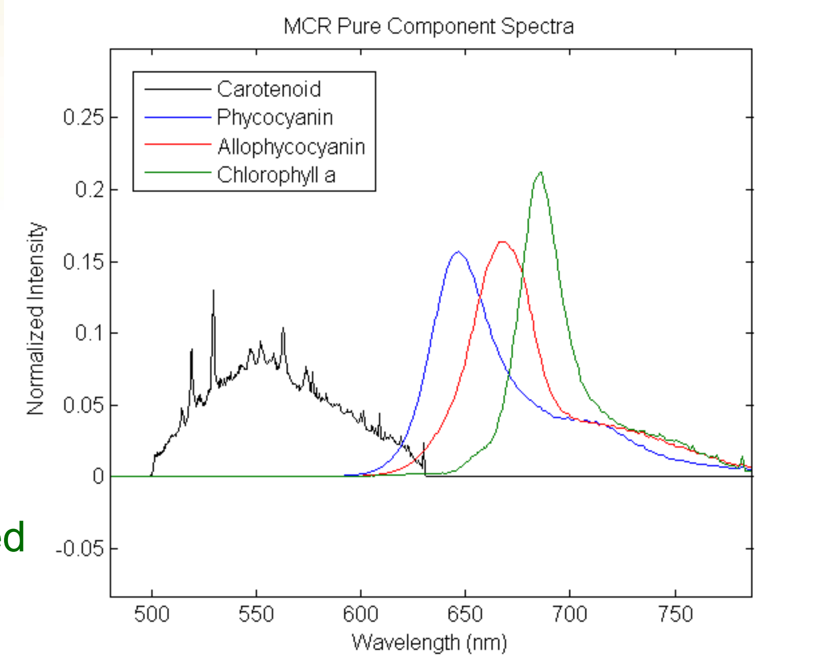
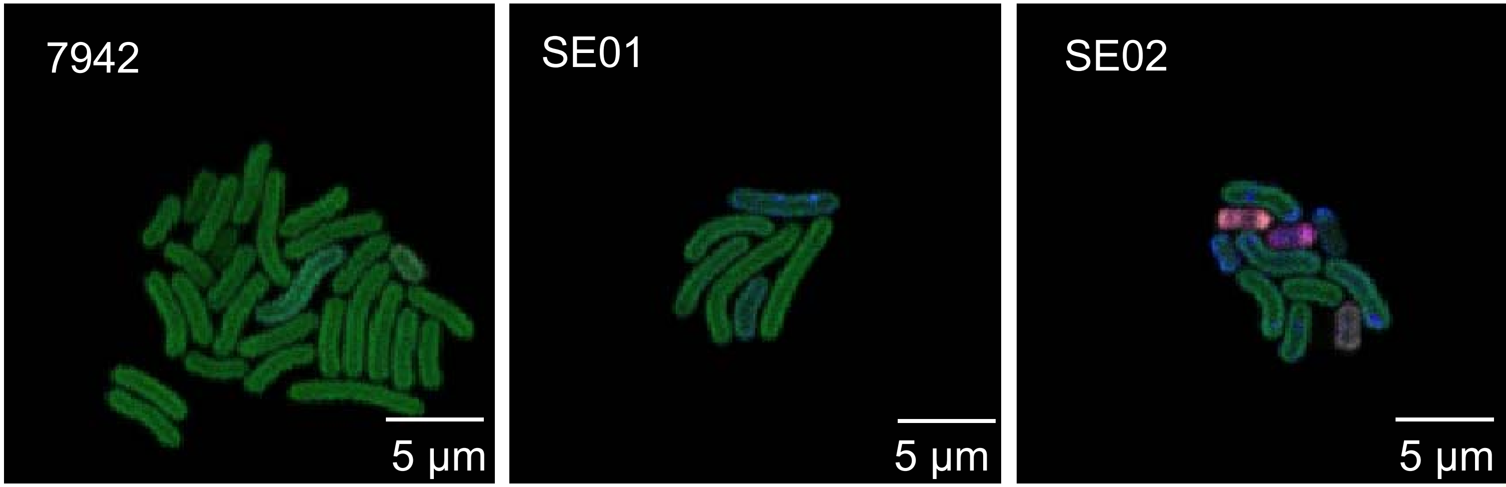
FFA Production: Effect on Subcellular Pigment Localization

Hyperspectral Confocal Fluorescence Imaging

- Fluorescence emission spectrum for each pixel collected with 488 nm excitation
- Multivariate curve resolution (MCR) algorithms pull out pure component spectra
- Wild-type (7942) has high levels of Chl a with pigments localized in thylakoid membranes
- Engineered strains show reduced Chl a, increased phycocyanin and allophycocyanin, and pigment aggregation at cell poles





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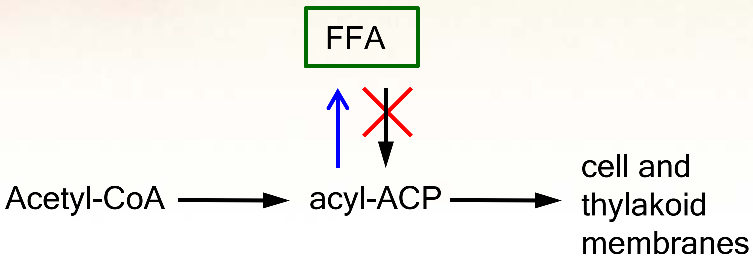
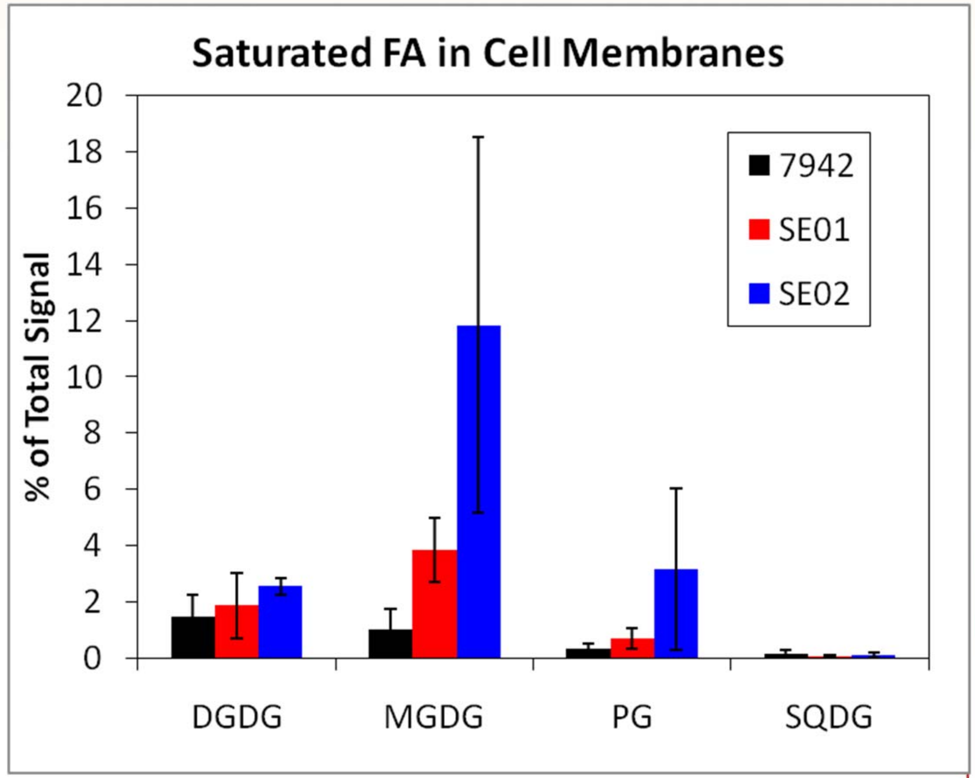
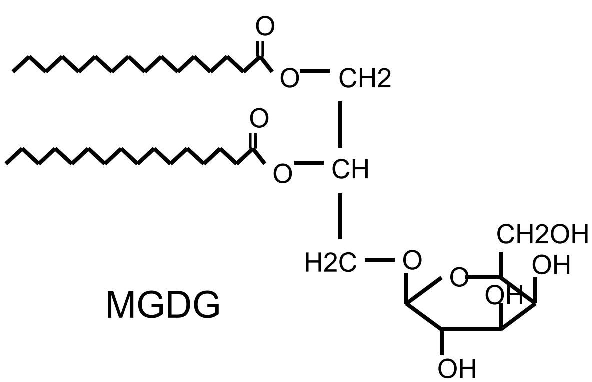
FFA Production: Effect on Thylakoid and Cell Membranes

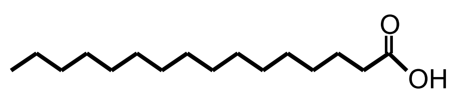


- Increase in % composition of saturated FA in thylakoid and cell membranes of engineered strains
- Increase in FA saturation → increased membrane viscosity
- Possible effect on attachment of phycobilisomes to thylakoid membrane

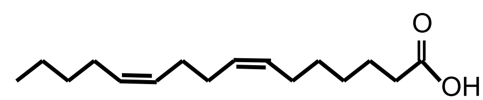




MGDG



Saturated FA



Unsaturated FA

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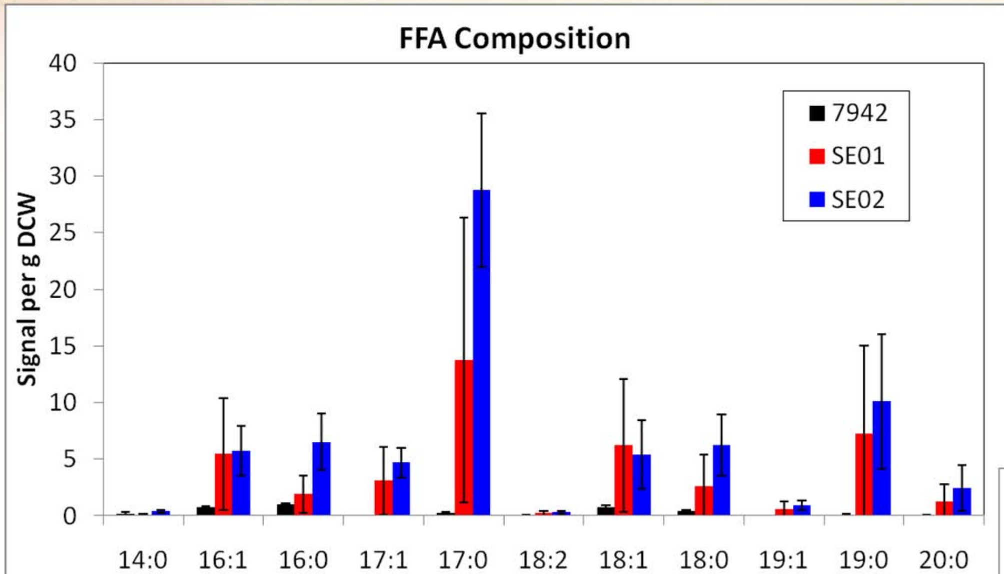
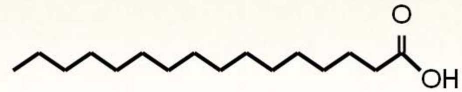
FFA Toxicity: Effect of Saturated FFA

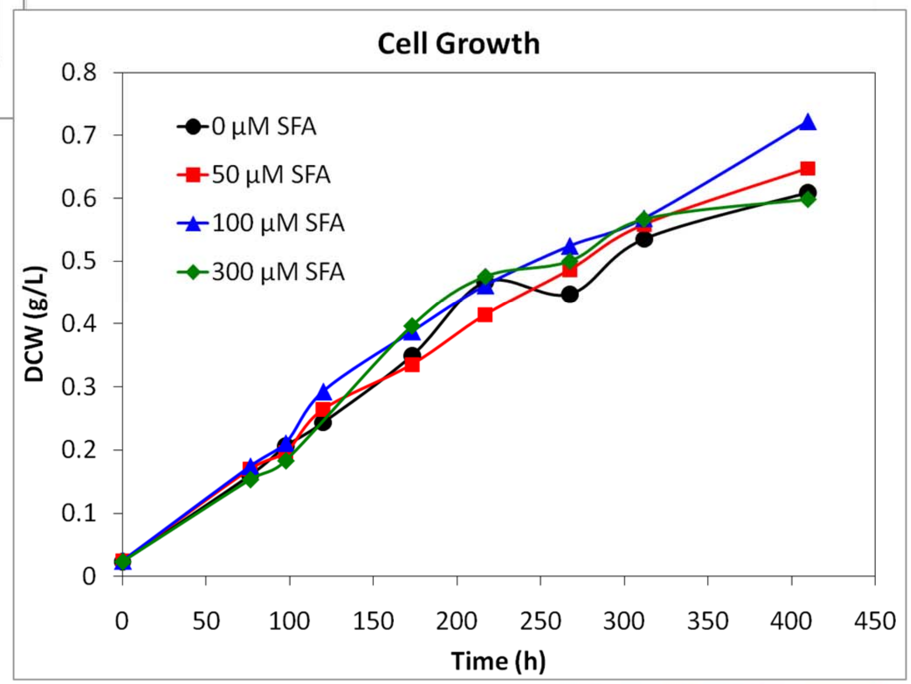




Palmitic Acid (30%)

Stearic Acid (70%)

- FFA produced by engineered strains are C16-C18 monounsaturated and saturated
- Added exogenous mixture of palmitic (C16:0) and stearic (C18:0) acids to wild-type (7942) to determine toxicity
- Saturated FFA has negligible effect on cell growth and photosynthetic pigments



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FFA Toxicity: Effect of Unsaturated FFA

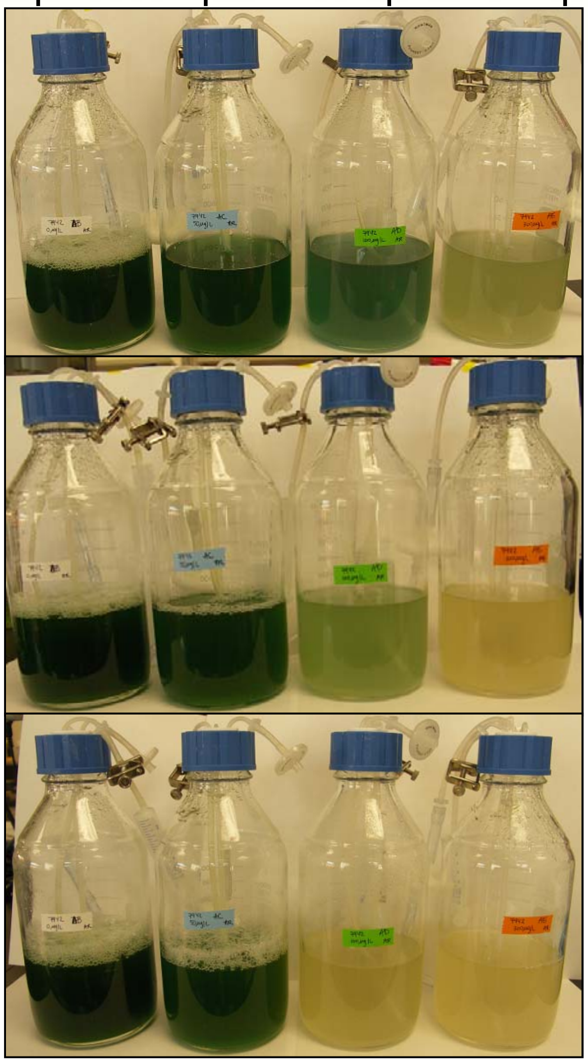
Linolenic Acid (LA)

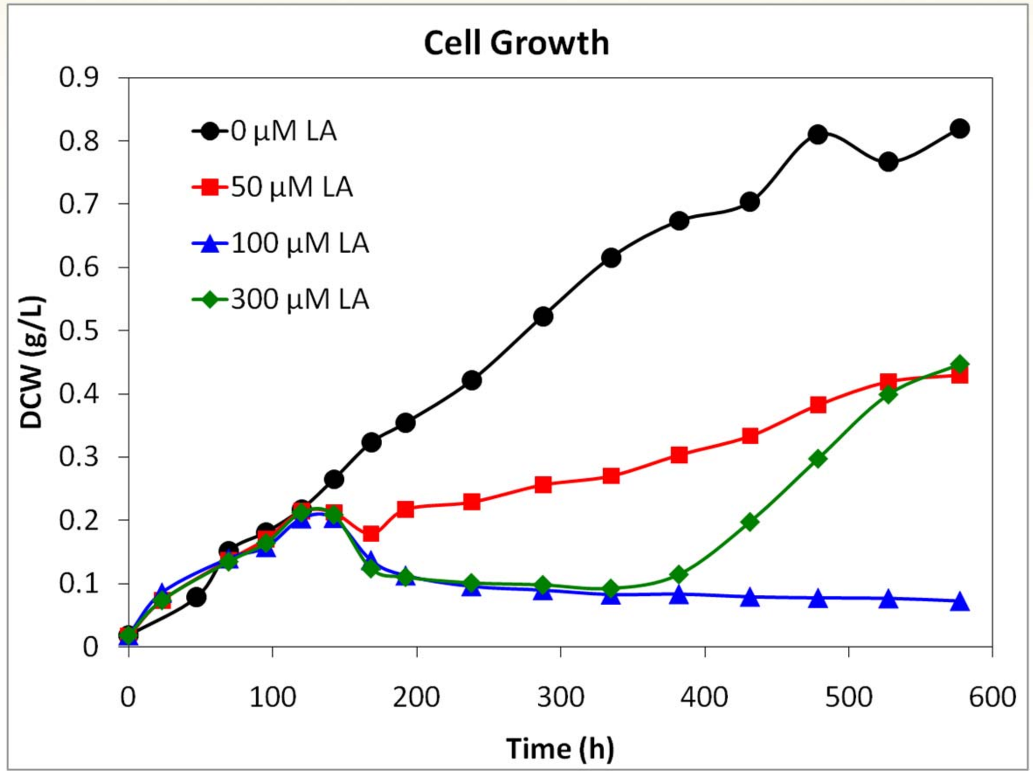
0µM 50µM 100µM 300µM

Day 2

Day 3

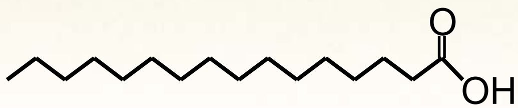
Day 4

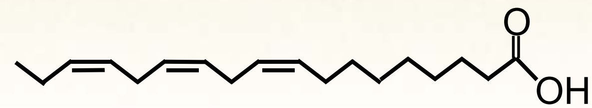




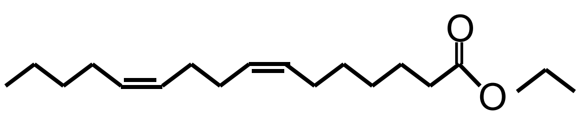
- Added exogenous mixture of linolenic (C18:3) acid to wild-type (7942) to determine toxicity
- Unsaturated FFA has toxic effect, leading to reduced cell growth and degradation of photosynthetic pigments

Saturated vs. Unsaturated FFA for Biodiesel





Saturated		Unsaturated	
Property	Pros/Cons	Property	Pros/Cons
Increased viscosity	• Cold start problems	Reduced viscosity	• Good flow properties
Oxidatively stable	• Good long-term storage	Oxidatively unstable	• Fuel degradation • Decreased fuel quality • Storage problems • Increased viscosity - clogged fuel lines and pumps



Unsaturated FAEE (Biodiesel) + O_2 → (*Heat*, *Light*, *Metals*)

Hydroperoxides
Aldehydes
Acids
High molecular weight compounds
Keto compounds
Epoxy compounds
Di- and trihydroxy compounds
Hydroperoxy epidioxides

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4. Knothe, G. (2007) Fuel Processing Tech. 88: 669-77.

Conclusions and Future Work

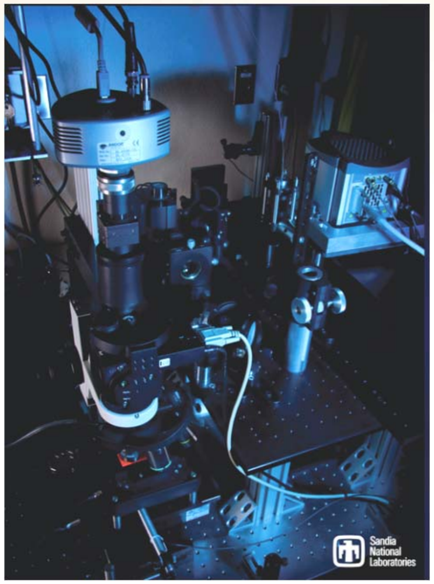
Conclusions

- Engineered cyanobacteria are promising candidates for biofuel production
- FFA production has a toxic effect on *S. elongatus* PCC 7942
  - Reduced cell growth
  - Decrease in chlorophyll a
  - Disruption of thylakoid membrane structure
- Toxic effect of FFA production is, at least in part, due to unsaturated FA
  - Unsaturated FA are oxidatively unstable
  - Exogenously added unsaturated FFA produces toxic effect (reduced cell growth and degradation of photosynthetic pigments)

Future work

- Identify an acyl-ACP thioesterase for the preferential release of SFA
- Investigate the physiological effects of other biodiesel precursors (i.e. long chain alkanes or alcohols)

Acknowledgements



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Anthony Martino

Eric Ackerman

President Harry S. Truman Fellowship in National Security Science and Engineering

Kansas Lipidomics Research Center (KLRC)

Kansas State University

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