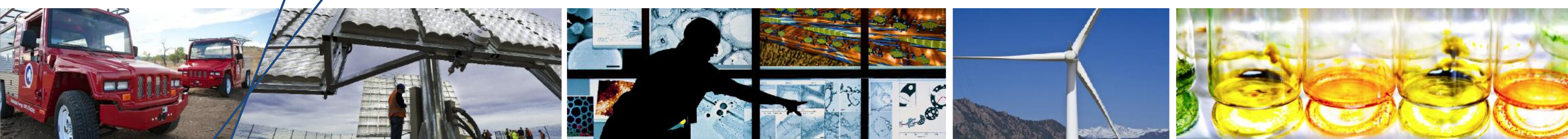
NATIONAL RENEWABLE ENERGY LABORATORY

Photosynthetic Conversion of CO_2 to Fuels and Chemicals using Cyanobacteria



Accelerating Innovation Webinar

August 8, 2012

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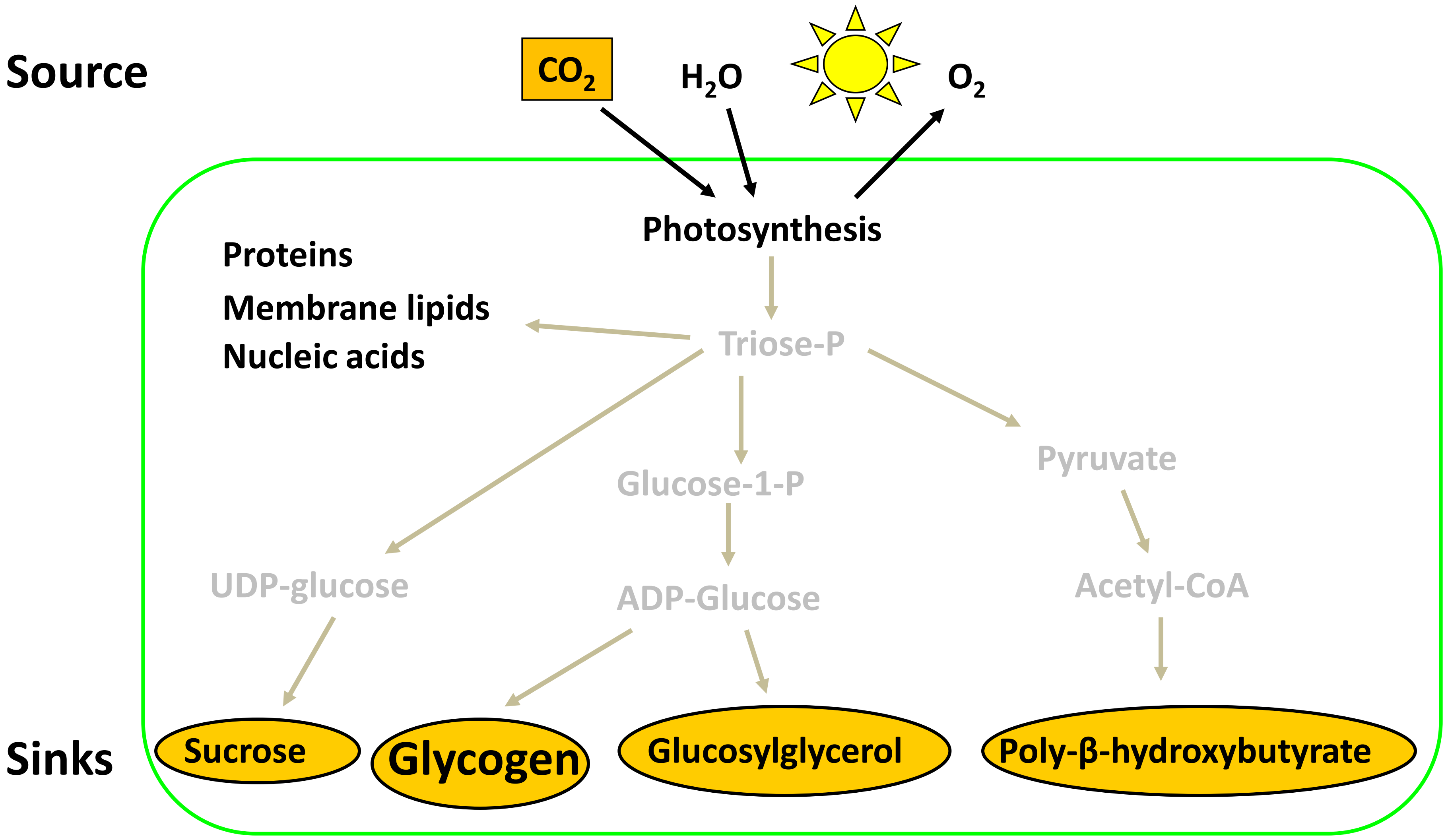
Algae Biofuels Opportunities and Challenges

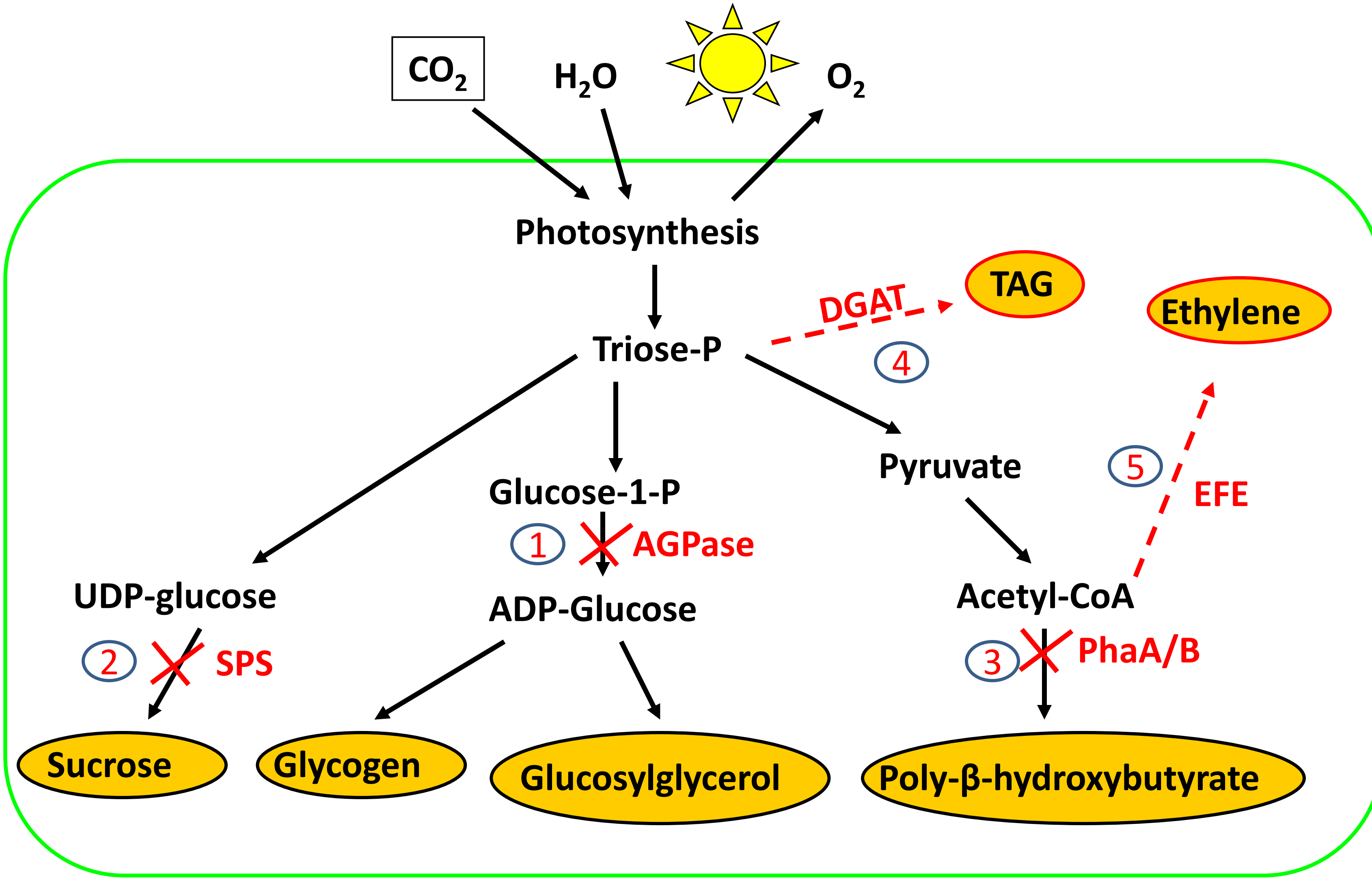
Synechocystis 6803

- **Many eukaryotic algae accumulate triacylglycerols (TAG), a biodiesel precursor.**
- **Genetic system in eukaryotic algae lags behind; while cyanobacteria offer good genetic tools that facilitate fundamental understanding and they ability to manipulate pathways.**
- **Harvesting TAG accumulated inside algae cells requires large energy input; products that separate naturally from the cells and the culture medium could lower operating cost.**
- **Many algae require fresh water for growth; using sea water and waste water may bypass this resource limitation.**
- **Contamination by other microbes is a major challenge; products that are not food sources will reduce the risk.**

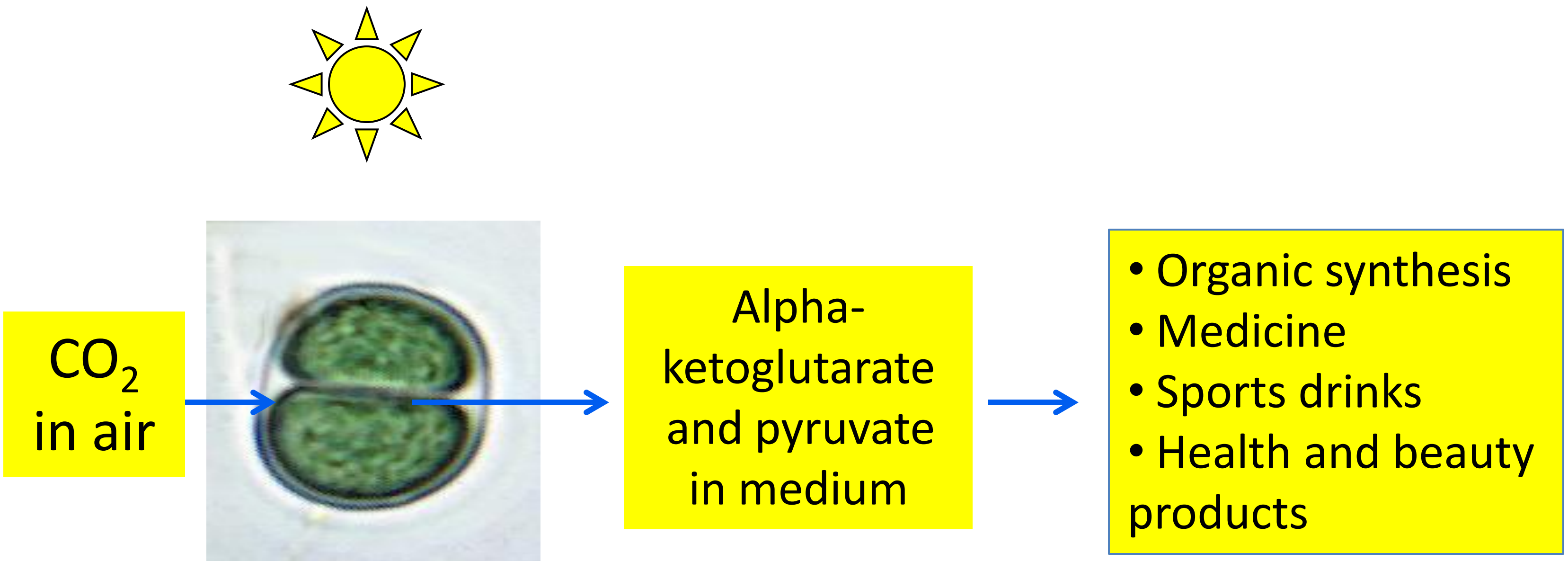
Synechocystis 6803 Carbon Sinks



Removing native carbon sinks and creating new sinks to produce biofuels and platform chemicals



I. Solar Bio-catalyst Producing Platform Chemicals

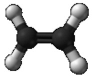
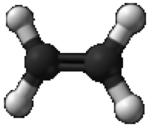
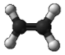


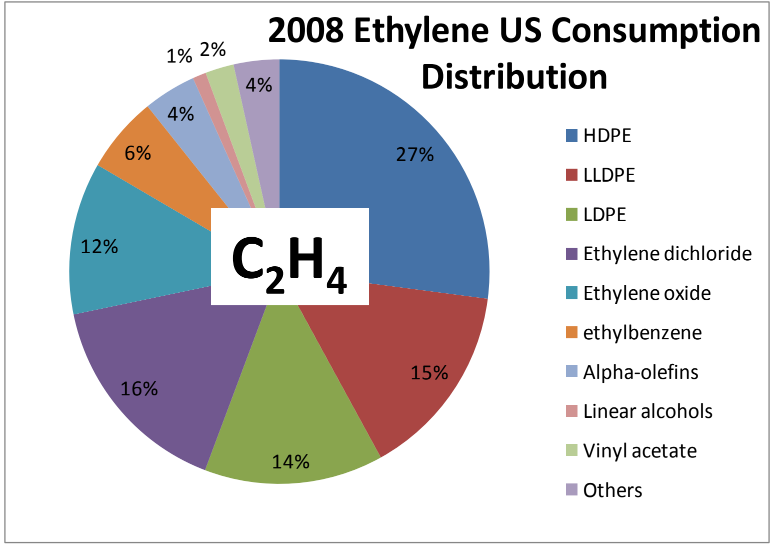
- A glycogen synthesis mutant strain is capable of halting growth yet continues photosynthesis when a nutrient is limiting.
- CO_2 is converted into the organic acids that naturally leave the cells and accumulate in the medium.
- More products than cell biomass in days.
- Possible continuous "milking" system.

II. Photosynthetic Ethylene Production

The Ethylene Advantage

- **Ethylene is the most produced organic compound world wide. Infrastructure for ethylene utilization is already in place.**
- **Versatile feedstock for fuels, plastics, and chemicals.**
- **Ethylene is a gas, can be harvested directly from the headspace of photobioreactor, saving cost and energy in harvesting and extraction.**
- **Direct, aerobic, continuous CO_2 to ethylene conversion.**
- **Not a food source for microbes; reducing feeding and contamination problems.**
- **No toxicity to the microbe thus affording higher productivity.**



EFE (ethylene forming enzyme) Expression in *Synechococcus* 7942 Could not Sustain (previous work)

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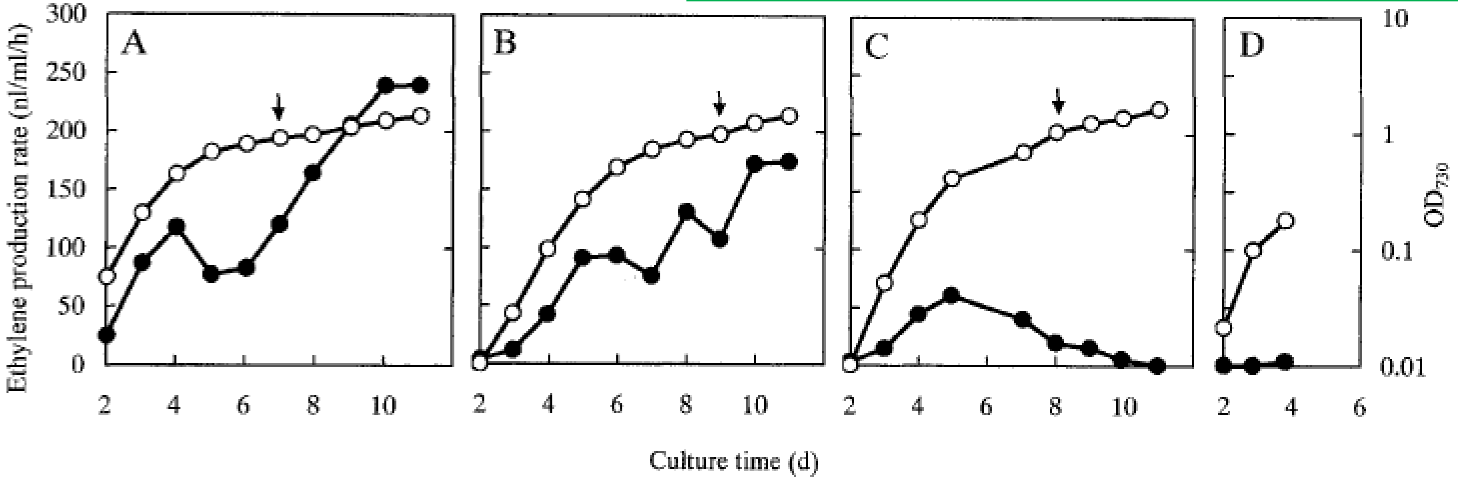
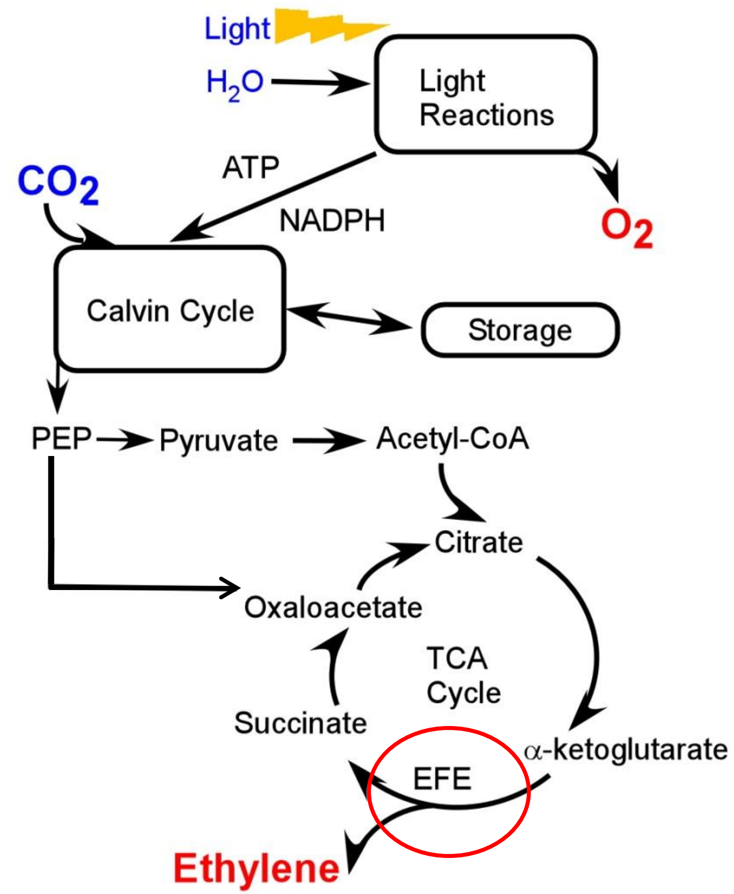


- *efe* gene from the plant pathogenic bacterium *Pseudomonas syringae*.
- Inactivation of *efe* lead to loss of ethylene production.
- Small, yellow-green colonies suggests metabolic stress or toxicity.

Our Approach

- **Tested ethylene toxicity in *Synechocystis* 6803**
- **Designed a new version (*Sy-efe*) of the gene to improve stability**
- **Introduced into *Synechocystis* 6803**
- **Studied ethylene production stability**
- **Studied limiting factors**



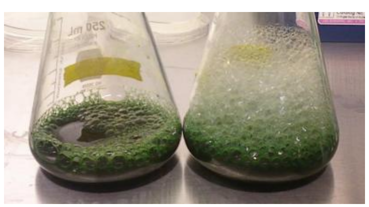
Photosynthetic ethylene production

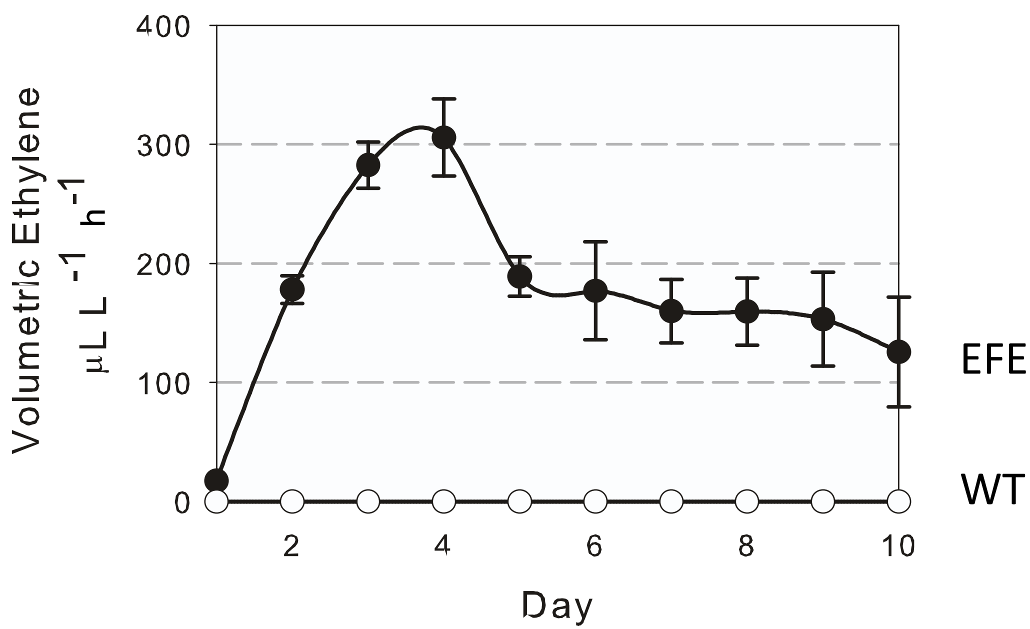
Energy Environ. Sci., DOI:10.1039/C2EE22555G. Accepted.

Ethylene Is Not Toxic to *Synechocystis*

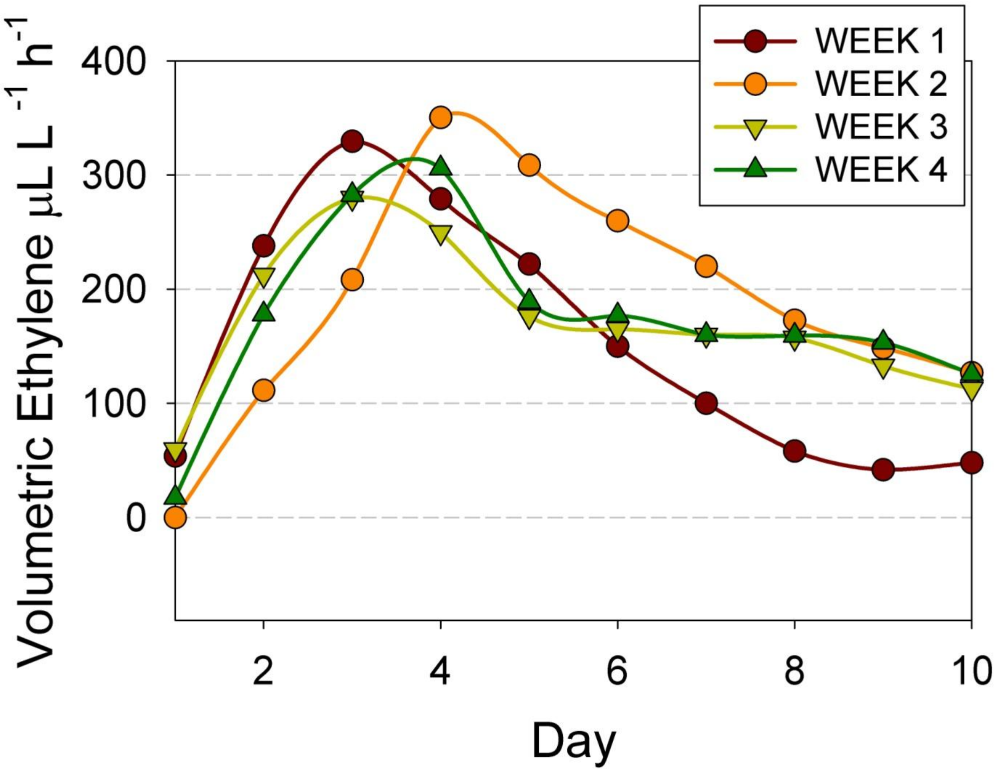
- **Added ethylene (95%) has no effect on WT culture growth**
- **Ethylene-producing cultures grow as fast as WT; look the same**

Strain	Generation time (h)
wild type	10.7 +/- 0.41
psbA:*Sy-efe*	11.0 +/- 0.58
2X psbA:*Sy-efe*	10.9 +/-0.63



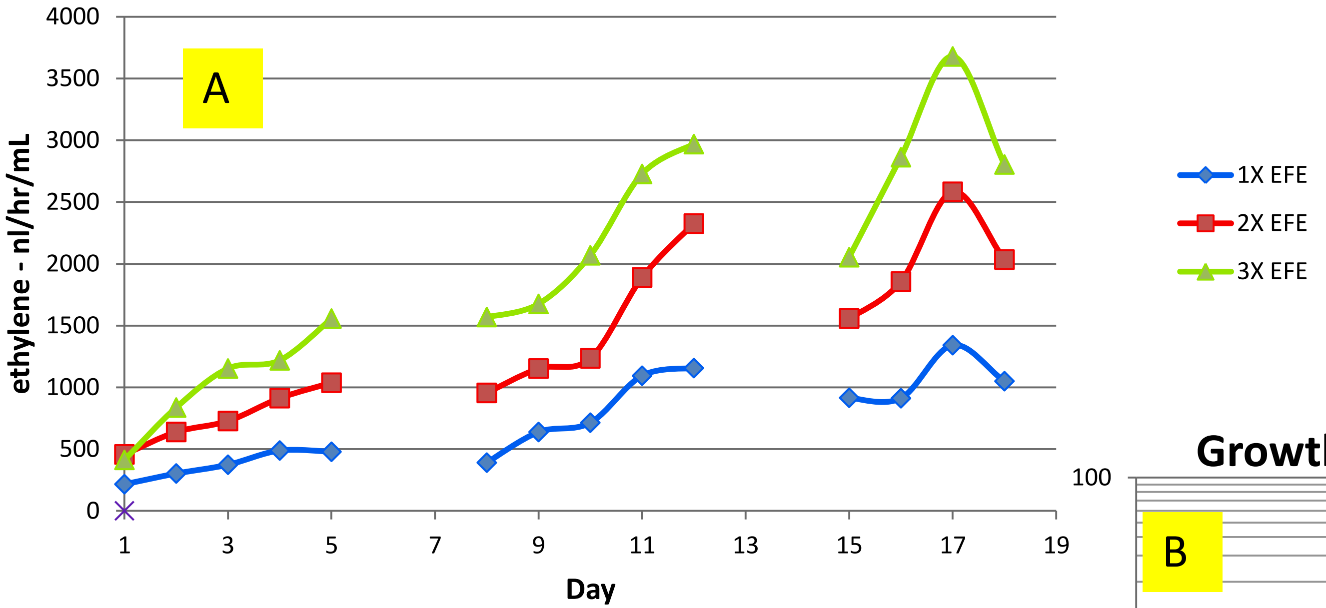


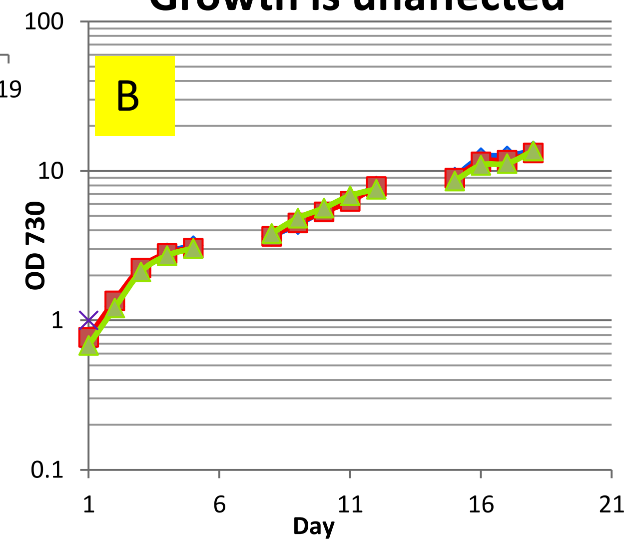
Sy-efe Is Stable in *Synechocystis*



- Ethylene production is linked to culture growth.
- Nutrient depletion slows down growth and ethylene production.
- Fresh medium restores both growth and ethylene production rates.

EFE Expression Limits Ethylene Production

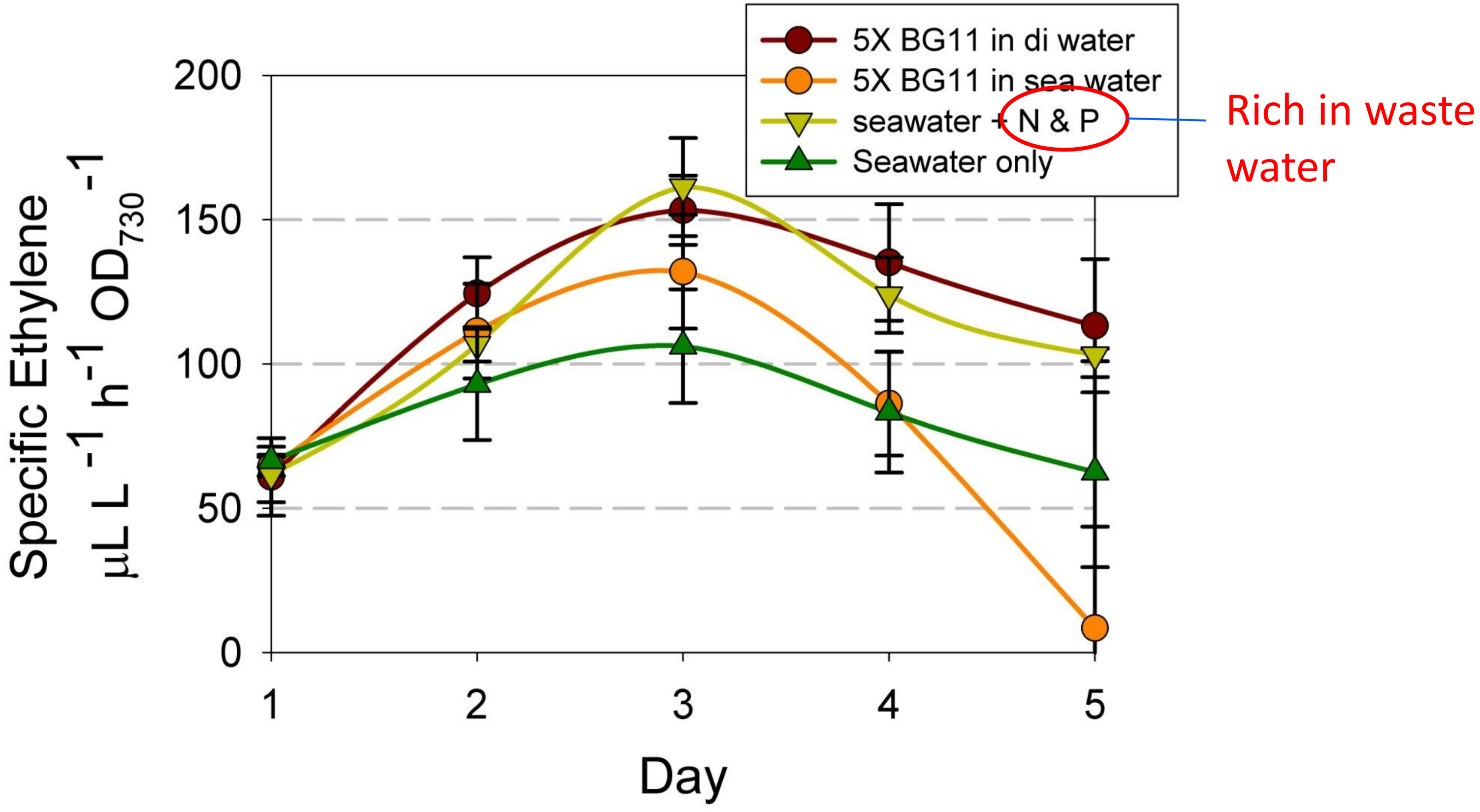




- Peak ethylene production rate is 10 ml/L/Hr.
- Preliminary TEA shows photosynthetic ethylene production has potential to be cost competitive with petroleum based production, while reducing CO_2 emission.

Seawater Supports Ethylene Production

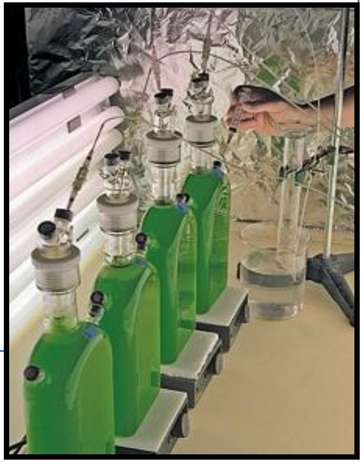
Overcoming fresh water resource limitation



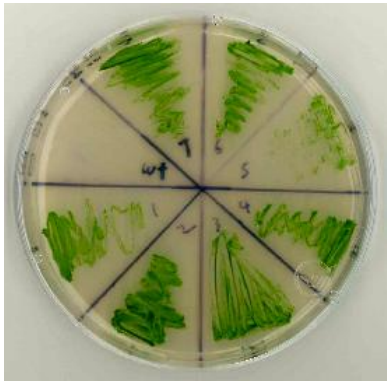
Future Directions

- **Biology**
- Goal: redirect carbon to ethylene. Currently less than 10% of carbon is converted to ethylene.
- Enhance EFE expression.
- Understand EFE structure and reaction mechanism.
- Understand how ethylene production impacts photosynthesis.
- Delete/limit competing pathways guided by metabolic flux analysis.

- **Engineering**
- Photobioreactor design and operation.
- Ethylene adsorption and/or conversion.



Acknowledgements



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Funding from

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NREL LDRD

DOE Office of Science BES

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Thank you for your attention