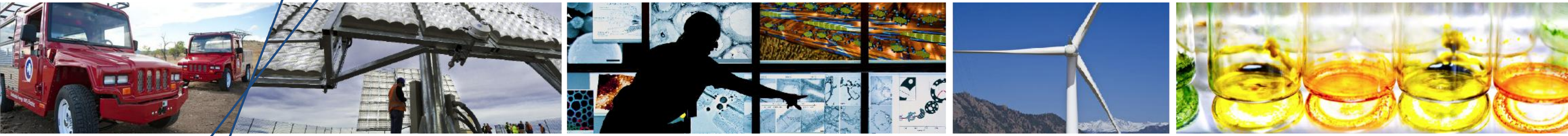
DOE Bioenergy Technologies Office (BETO) 2015 Project Peer Review

Producing Transportation Fuels via Photosynthetically-derived Ethylene



March 23, 2015

Technology Area Review: Algae

Principal Investigator: Jianping Yu

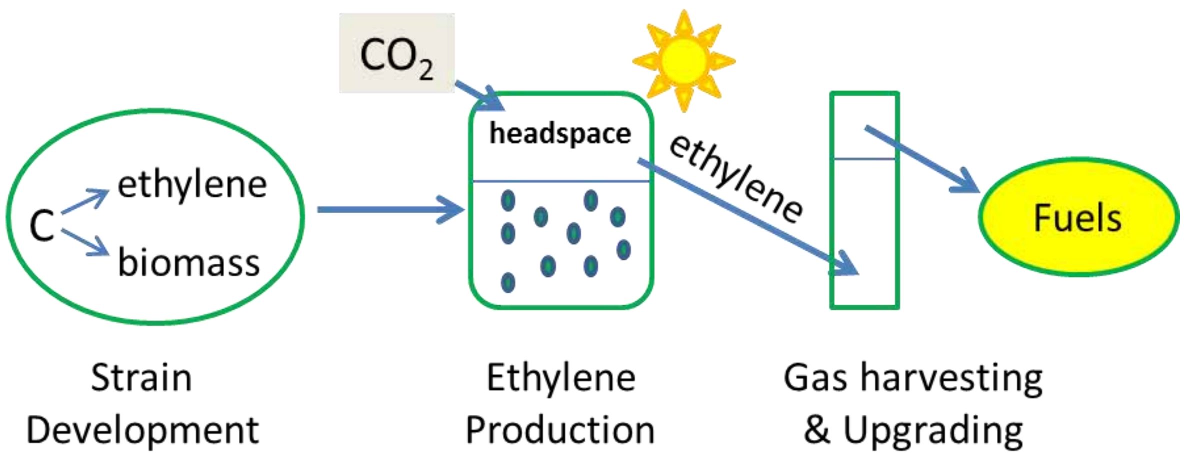
Goal Statement

To develop a novel photosynthetic ethylene production technology using cyanobacteria. This technology has potential to produce biofuels and green chemicals

(1) at cost that is competitive with conventional ethylene and derivatives production using fossil-based feedstock;

(2) reducing water and nutrients input, and CO_2 emission;

(3) not competing with agriculture for arable land and fresh water.



Ongoing research   Future R&D

Quad Chart Overview

Timeline

- 10/1/2013
- 9/30/2015
- 60% completion
- Sun-setting?

Budget

	Total Costs FY 10 –FY 12	FY 13 Costs	FY 14 Costs	Total Planned Funding (FY 15-Project End Date
DOE Funded	$345K	$155K	$250K	$400K

Barriers

- Barriers addressed
- Aft-D. Sustainable Harvesting
- Aft-E. Algal Biomass Characterization, Quality, and Monitoring
- Aft-G. Algal feedstock material properties

Partners

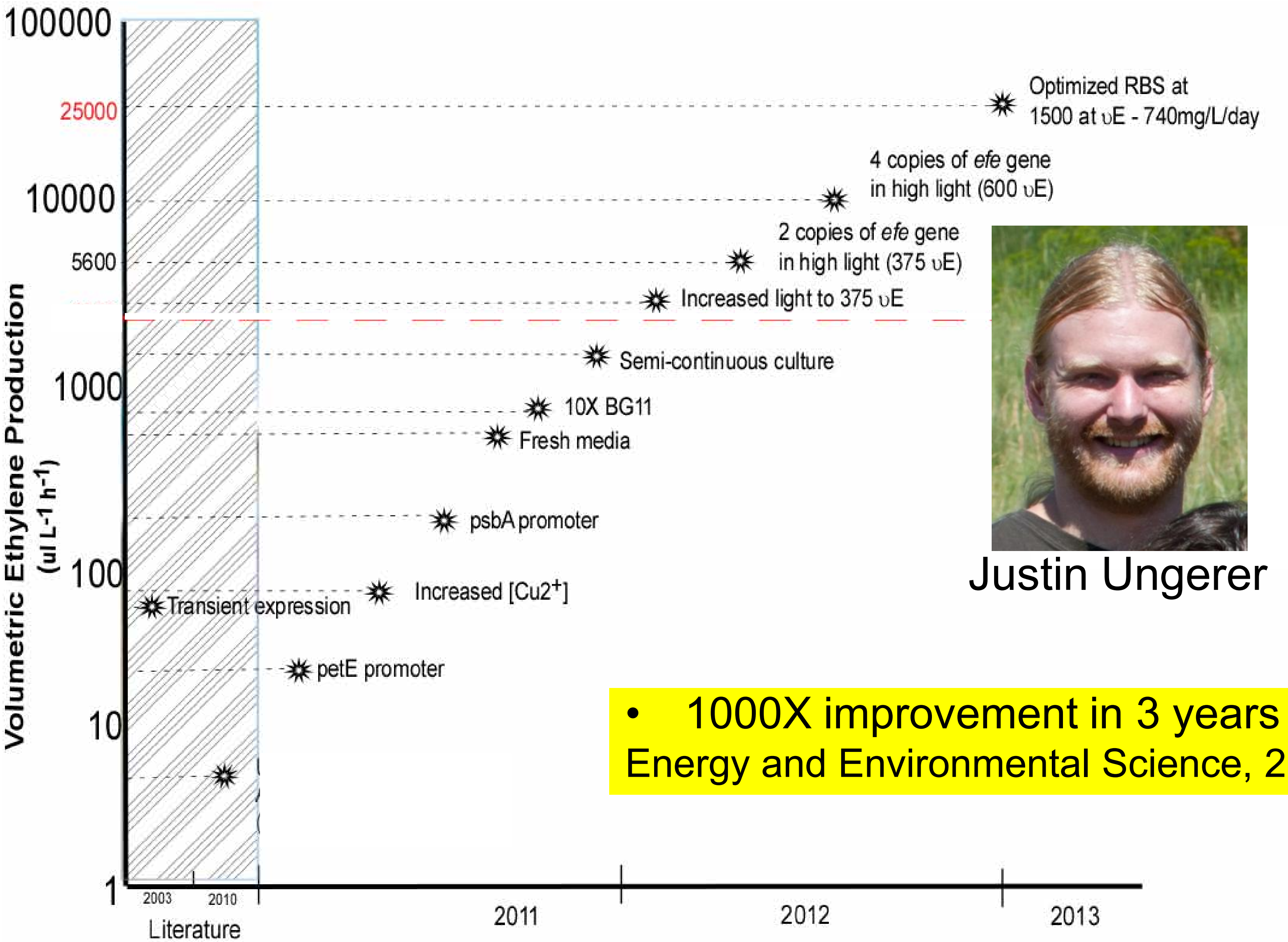
- Partners NA
- Other interactions/collaborations
  - NREL: TEA; algal biomass conversion; carbon regulation; metabolic flux analysis; EFE structure
  - A major chemical company
  - An algal cultivation company
  - Cornell University (ARPA-E)
  - Purdue University (BER)
  - Oklahoma State University (BES)
  - Michigan State University (NIH)
  - University of Colorado (BER)
  - University of Louisiana (NSF)

1. Project Overview

Synechocystis 6803

- Ethylene can be converted to liquid fuels
- Ethylene can separate itself from cells as gas
- Ethylene can be synthesized by ethylene-forming enzyme (EFE, encoded by *efe* gene)
- We are developing the model cyanobacterium *Synechocystis* 6803 into an ethylene production strain.
- We showed that photosynthetic ethylene production can be supported by sea water with the addition of N and P nutrients.
- We showed that ethylene productivity can be sustained for at least 1 month.

Progress of Photobiological-ethylene Research

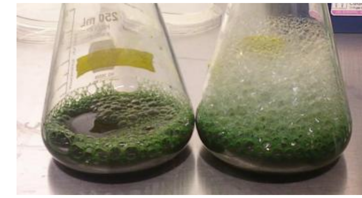


Justin Ungerer

- 1000X improvement in 3 years

Energy and Environmental Science, 2012

2 – Approach (Technical)



- Increase productivity by identifying and overcoming limiting factors in ethylene production – EFE levels
- Increase EFE levels by optimizing gene copy, promoter, and ribosome binding site
- Identify changes in central metabolic network using isotope labeling
- Understand relationship between photosynthesis and biofuel production by analyzing photosynthesis in ethylene producing strains
- Establish conceptual ethylene production process and economics, including algal biomass conversion .

2 – Approach (Management)

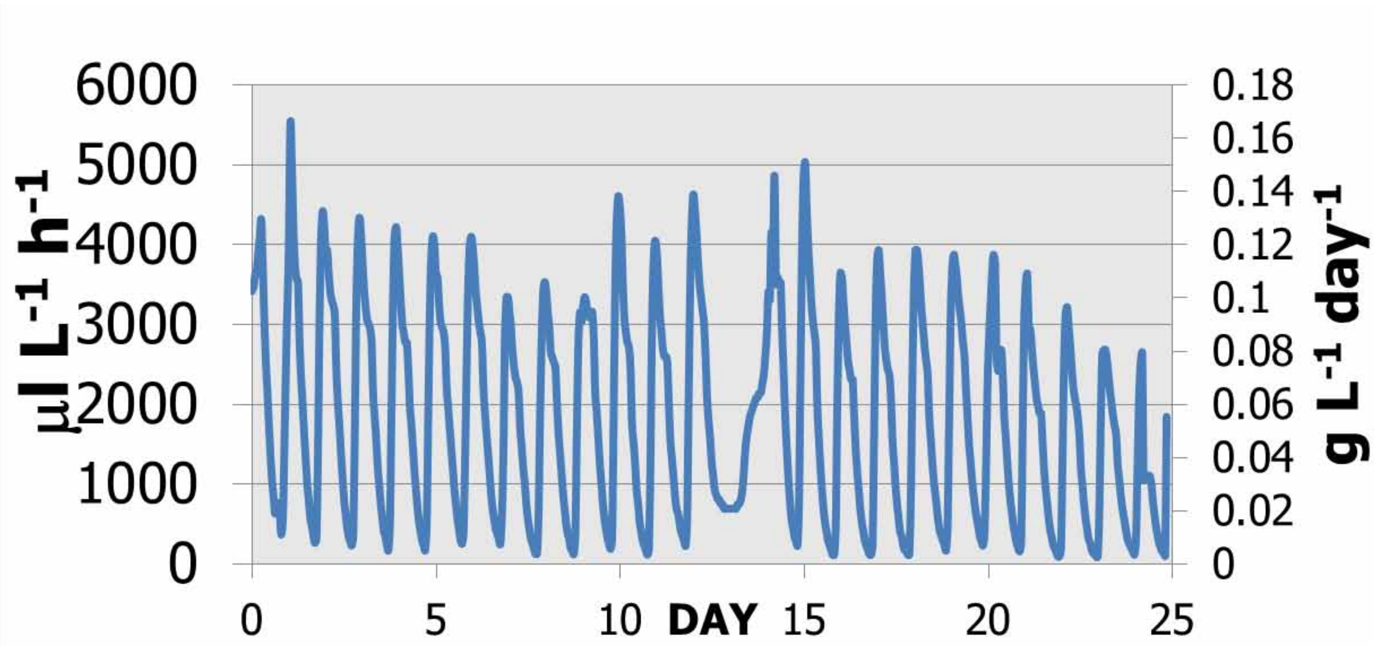
- Use milestones to track progress
- Use TEA to set research priorities
- Integrate with algal biomass conversion project
- Seek input from industry
- *E. coli* work guides future strain improvement

Critical success factors

- Industry showed strong interest in bioethylene; not ready to "pick it up" yet.
- Photobioreactor development is needed; biofilm reactor may have advantages. –future work?
- Carbon partition (currently up to 10%) into ethylene needs to be much improved (to ultimately 90% or more)

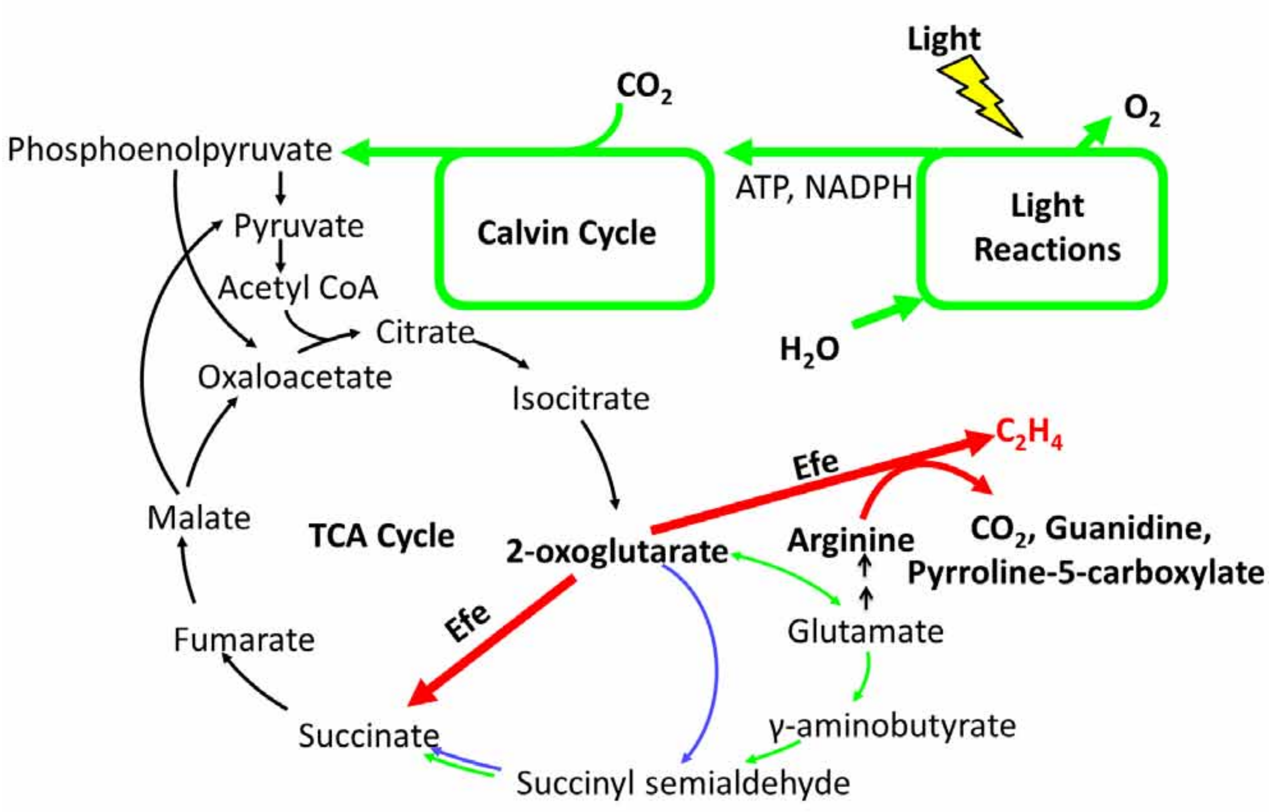
Milestones	FY13	FY14	FY15
Peak rate (mg/L/Hr)	**15**	**40**	**50**
Approach	Redesign RBS	Efe gene copies	Efe gene copies

3 – Technical Accomplishments/ Progress/Results



Longevity of light-dependent ethylene production in a 2L photobioreactor operated under 12h/12h diurnal cycles. Longest test was 72 days under continuous light where productivity was maintained.

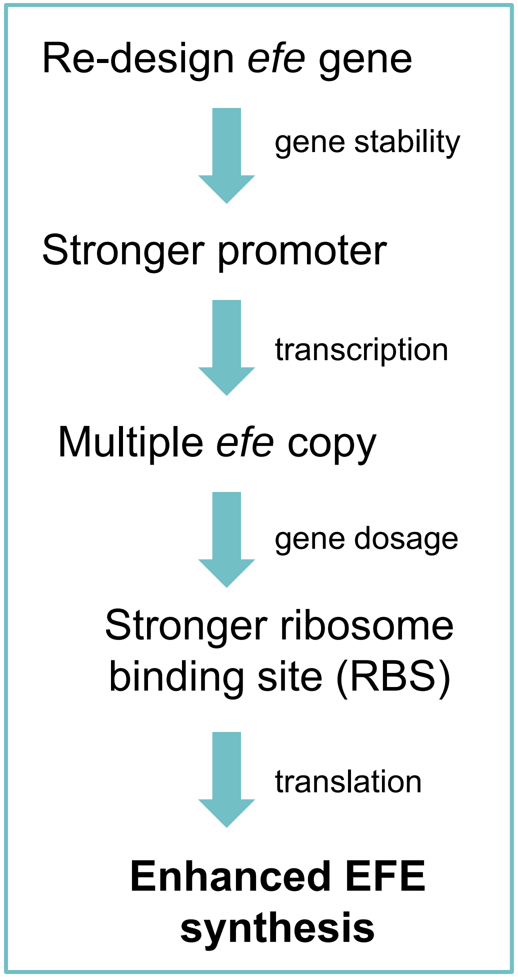
Photosynthetic Ethylene Production Metabolic Pathway

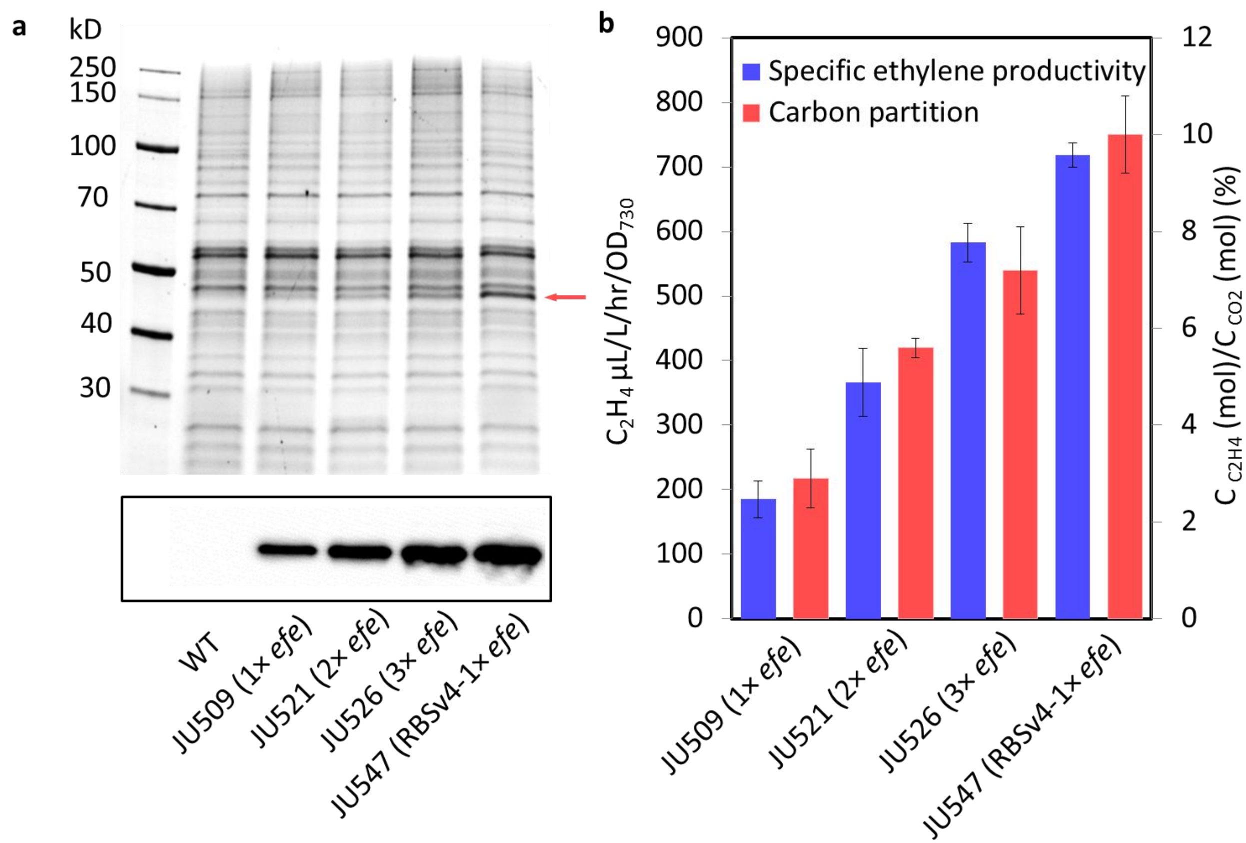


Wei Xiong
Director's PD fellowship

How does EFE impact overall metabolism?

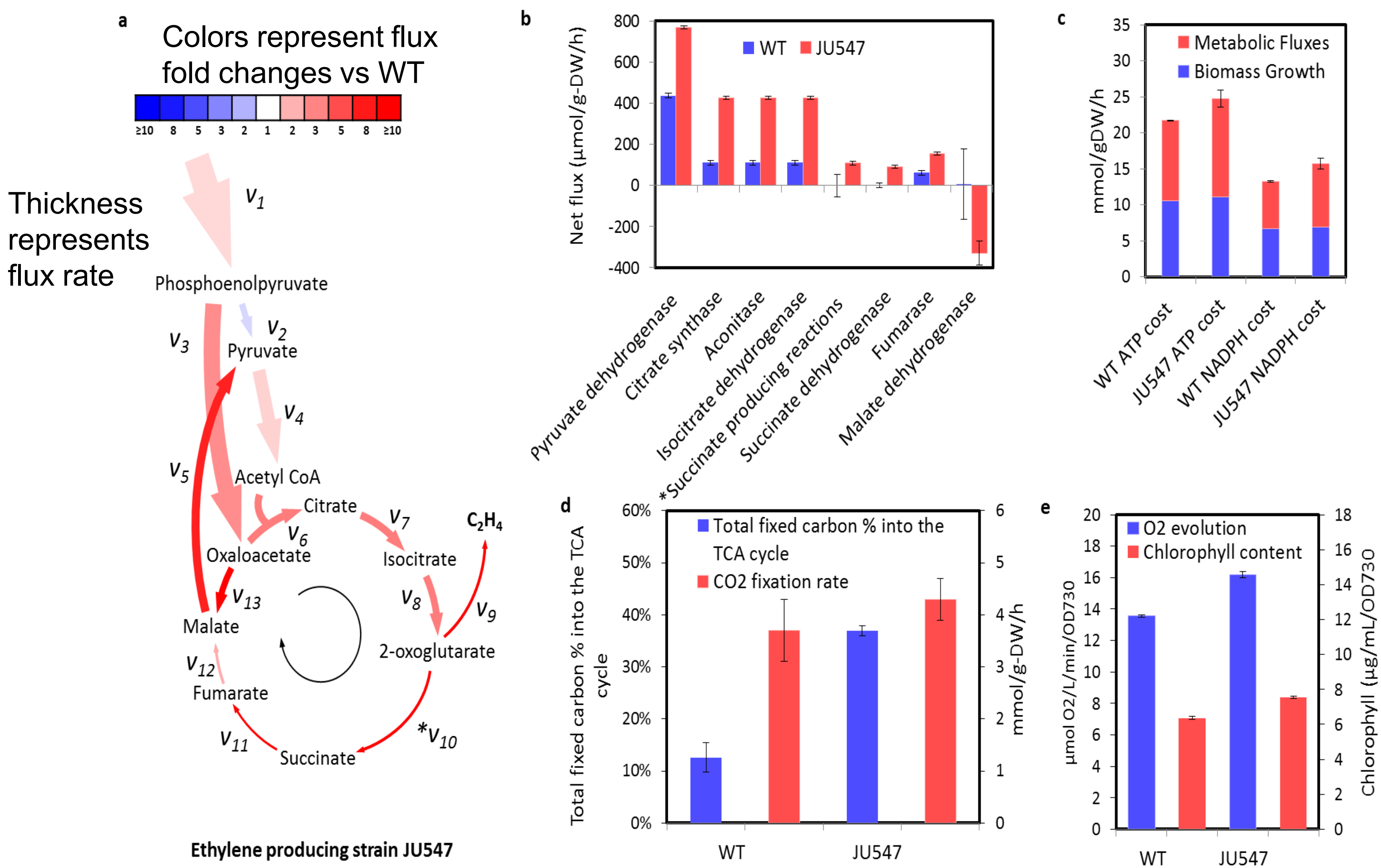
Improving EFE Expression Led to Higher Ethylene Productivity





The ethylene producing strains grow as fast as WT, despite of losing up to 10% carbons as ethylene. What's going on with carbon metabolism and photosynthesis?

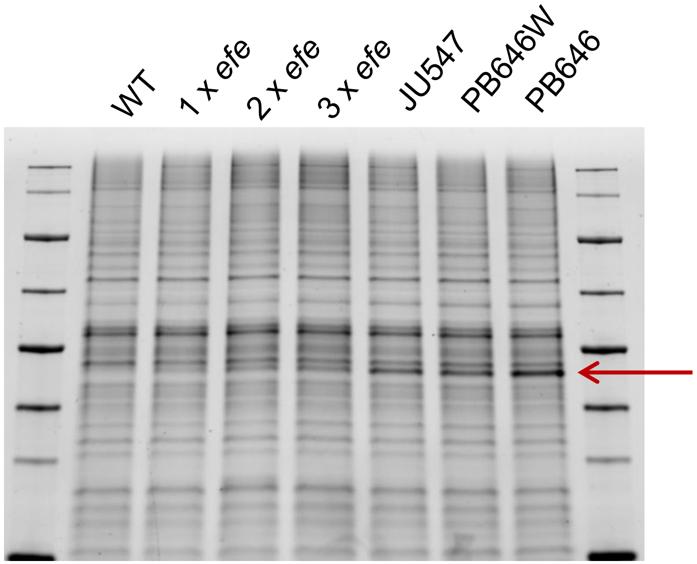
The organism is cooperating: Ethylene production is supported by metabolic network rewiring and stimulation of photosynthesis



Xiong *et al.*, Nature Plants, accepted

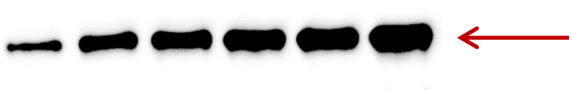
Current Best Strain PB646 Has Two Copies of efe

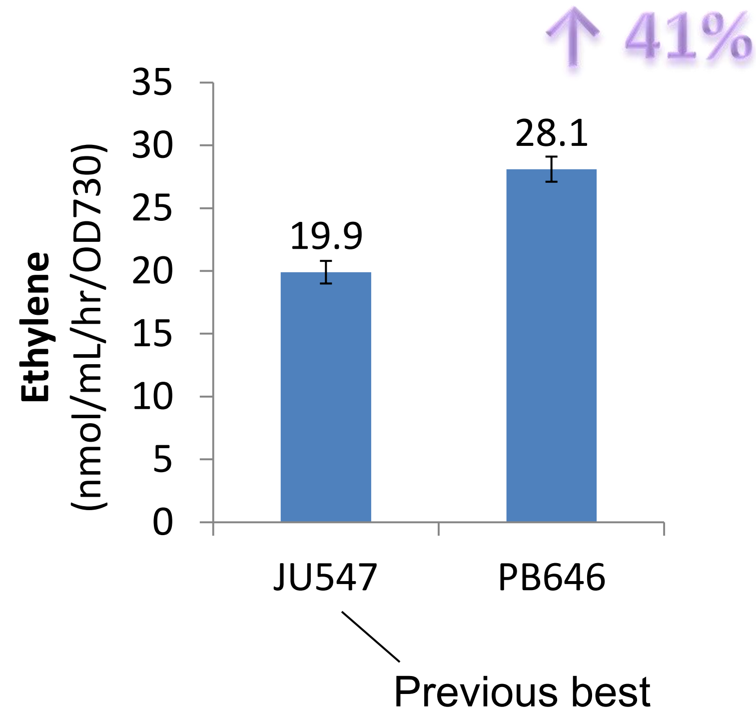
Bo Wang



Band intensity 1 : 3 : 5 : 9 : 10 : 16

EFE level increase: 70%

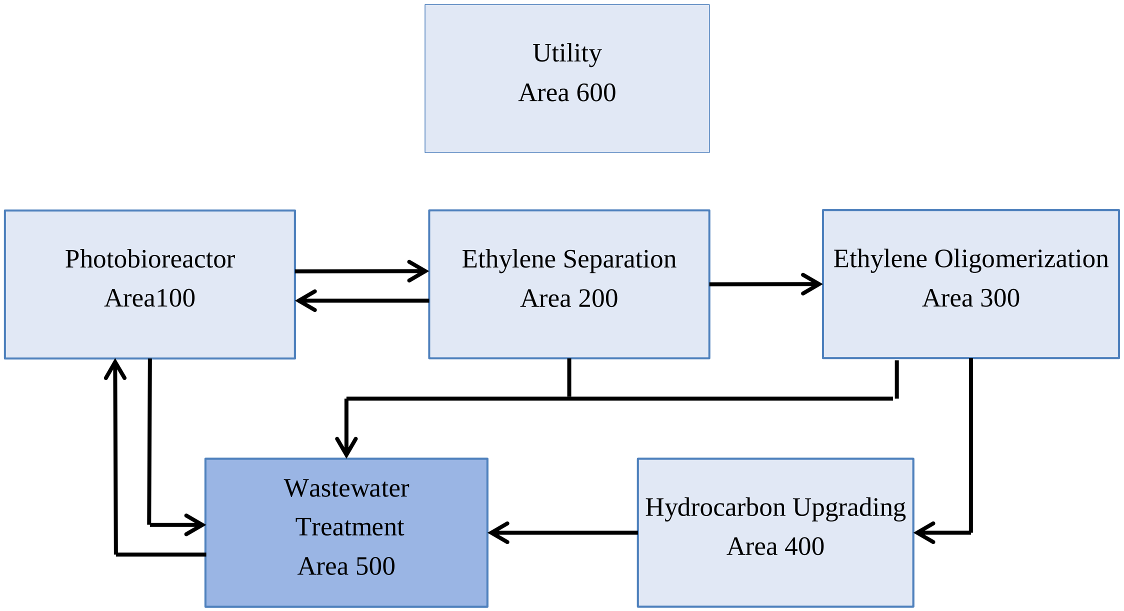




- The 70% increase in EFE and 41% increase in ethylene productivity suggest that EFE is no longer the only limiting factor
- Peak productivity has reached 35 mg/L/Hr.

Bioethylene to Fuels Process (conceptual)

Ling Tao



Initial cost analysis

- The major cost contributor is photobioreactor (industry comment on plastic cost)
- The most important variable is photosynthetic productivity
- The model is being reviewed by several industrial partners
- Long term (9-10 years) fuel cost target is $4-5/gge, dependent on R&D funding.

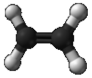
4 – Relevance

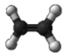
- Using advanced biology to increase photosynthetic productivity and carbon utilization.
- Let sunlight do most of the work in the organism, including separation, and minimize the use of resources such as water and nutrients.
- Ethylene production stimulates photosynthesis, generates fundamental knowledge on regulation of photosynthesis and algal metabolism. Strong synergy with HFCT, BES, BER projects.
- Strain requests (we shared published strains) indicate that bio-ethylene research is expanding in Europe (Denmark, Finland, Czech) and in China.

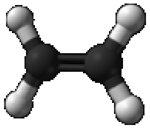
Interest from Industry

- Many on-site visits from chemical companies and cultivation companies.
- Numerous conference calls and webinars.
- Small funds for cost analysis and for licensing evaluation.
- Joint grant applications with very favorable reviews.
- Request for published strains.
- Request for preliminary TEA model; comment on cost of PBRs and on harvesting technologies.
- Letter of support for R&D100 Award.

- This novel technology is at early stage, needs further development before industry will “pick it up”.







Summary

- We have demonstrated sustained photosynthetic CO_2 to ethylene conversion for at least one month.
- Current peak productivity is 35 mg/L/Hr. FY13 goal was 15 mg/L/Hr.
- Enhanced EFE production to the point that something else in metabolism is becoming the rate limiting factor. BER work in *E. coli* provided strategies to overcome substrate limitation.
- The organism cooperates with ethylene production by re-wiring central metabolism and stimulating photosynthesis. Great news for biofuels production.
- Ethylene is the only organic compound in head space.
- Built an initial TEA model; received industry inputs.

Additional Slides

Responses to Previous Reviewers' Comments

- Need to develop TEA model- We have developed an initial model and will continue to improve it based on inputs from industry
- Do not work more on biomass sugars as feedstock- We are publishing the initial study and have not done more on that topic.
- Think about ethylene harvesting- We have evaluated several harvesting technologies, and have received inputs from industry.
- How EFE impact overall metabolism- We performed a detailed study and were thrilled to find that overall metabolism is stimulated to support ethylene production.

Publications, Patents, Presentations, Awards, and Commercialization

- Ungerer, J. *et al.* Sustained photosynthetic conversion of CO_2 to ethylene in recombinant cyanobacterium *Synechocystis* 6803. (2012) *Energ Environ Sci* **5**, 8998-9006.
- Eckert, C *et al.* Ethylene-forming enzyme and bioethylene production. (2014) *Biotechnology for Biofuels* **7**:33.
- Xiong, W *et al*. The plasticity of cyanobacterial metabolism supports direct CO_2 to ethylene conversion. In revision for *Nature Plants*.
- Lee, T *et al*. Xylose utilization enhances bio-products productivity in the cyanobacterium *Synechocystis* 6803. In revision for *Metabolic Engineering*.
- US patent application document. Biological Production of Organic Compounds. 2013-0203136.
- Finalist, Katerva Awards 2013.
- Young Investigator Award, Justin Ungerer, ABS 2012.
- Presentations at ABS, ABBB, ACS, SIMB, Gordon Conference, Michigan State University, Oklahoma State University, major chemical companies.
- Major chemical companies reviewed NREL TEA draft and provided inputs on ethylene harvesting technology.