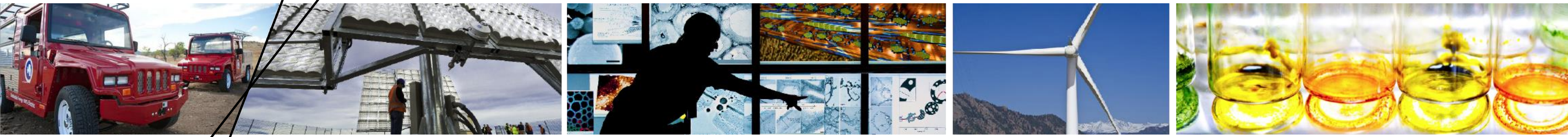
2013 DOE Bioenergy Technologies Office (BETO) Project Peer Review

Producing Transportation Fuels via Photosynthetically-derived Ethylene



- Date: May 22, 2013
- Technology Area Review: Biochemical Conversion

- Principal Investigator: Jianping Yu
- Organization: National Renewable Energy Laboratory

This presentation does not contain any proprietary, confidential, or otherwise restricted information

•NREL is a national laboratory of the U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy, operated by the Alliance for Sustainable Energy, LLC.

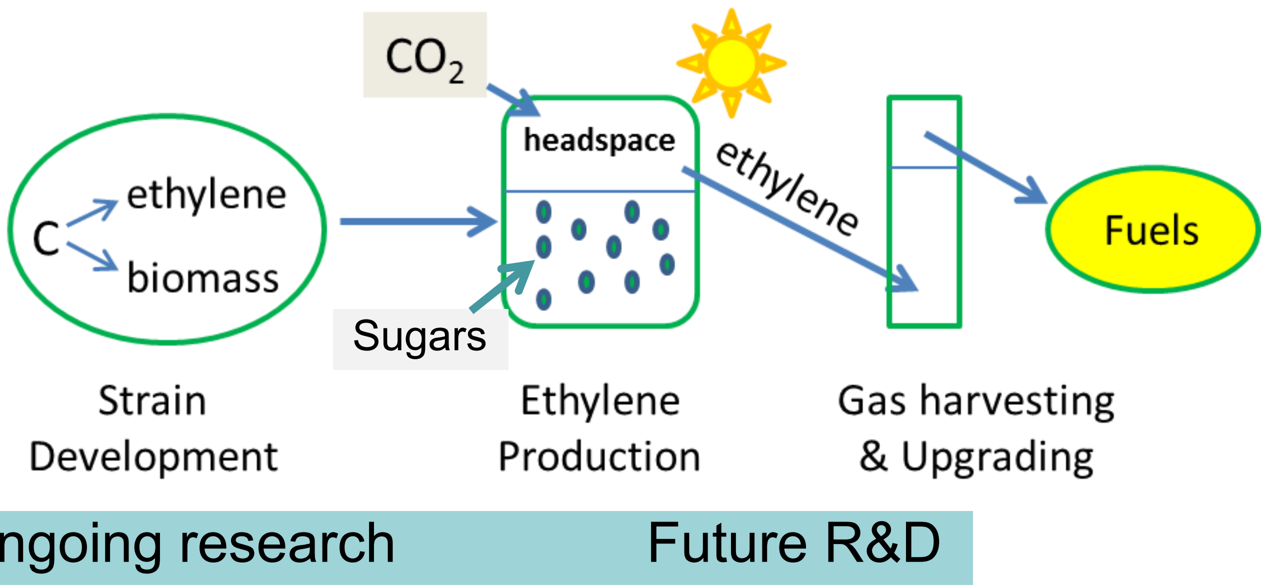
Goal Statement

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

(1) at higher areal productivity than terrestrial plants;

(2) not competing with agriculture for arable land and fresh water;

(3) as a component of an integrated biomass conversion system to use the CO_2 from fermentation, as well as biomass sugars.



Quad Chart Overview

Timeline

- Project start: October 2010
- Project end: September 2018
- Percent complete: 40%

Budget

- Funding for FY11(DOE seed project $150K)
- Funding for FY12(DOE seed project $150K)
- Funding for FY13 (DOE $180K)
- The project has been funded for 3 years / average annual funding $160K.

Barriers

- Barriers addressed
  - Ft-D. Sustainable Harvesting

Partners

- None

Synechocystis 6803

Project Overview

- Early-stage new technology development as DOE shifts focus from ethanol to hydrocarbon.
- Ethylene can be converted to liquid fuels.
- Ethylene can be synthesized by ethylene-forming enzyme (EFE, encoded by *efe* gene).
- Previous efforts in expressing *efe* in a transgenic cyanobacterium *Synechococcus* 7942 encountered genetic instability; production did not last.
- We started to develop the excellent genetic model *Synechocystis* 6803 into an ethylene producer as a seed project in FY11.

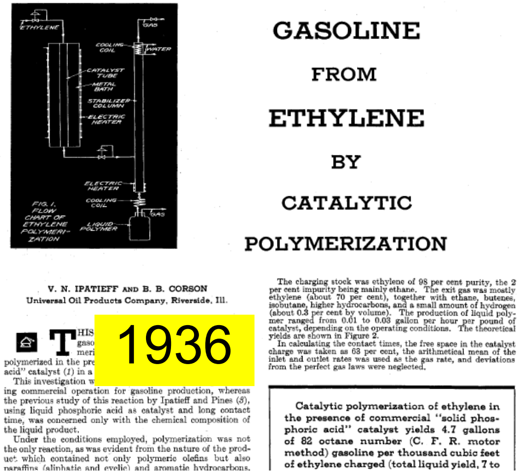

GASOLINE FROM ETHYLENE BY CATALYTIC POLYMERIZATION

FIG. 1. FLOW CHART OF ETHYLENE POLYMERIZATION

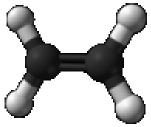
V. N. IPATIEFF AND B. B. CORSON
Universal Oil Products Company, Riverside, Ill.

1936

THIS gaso... meri... polymerized in the pre... acid" catalyst (1) in a ...
This investigation w... ing commercial operation for gasoline production, whereas the previous study of this reaction by Ipatieff and Pines (5), using liquid phosphoric acid as catalyst and long contact time, was concerned only with the chemical composition of the liquid product.
Under the conditions employed, polymerization was not the only reaction, as was evident from the nature of the product which contained not only polymeric olefins but also paraffins (aliphatic and cyclic) and aromatic hydrocarbons.

The charging stock was ethylene of 98 per cent purity, the 2 per cent impurity being mainly ethane. The exit gas was mostly ethylene (about 70 per cent), together with ethane, butenes, isobutane, higher hydrocarbons, and a small amount of hydrogen (about 0.3 per cent by volume). The production of liquid polymer ranged from 0.01 to 0.03 gallon per hour per pound of catalyst, depending on the operating conditions. The theoretical yields are shown in Figure 2.
In calculating the contact times, the free space in the catalyst charge was taken as 63 per cent, the arithmetical mean of the inlet and outlet rates was used as the gas rate, and deviations from the perfect gas laws were neglected.

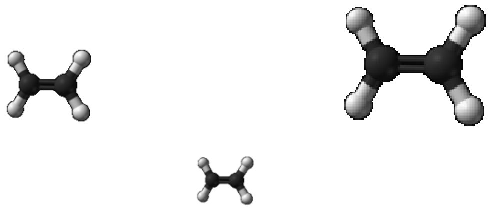
Catalytic polymerization of ethylene in the presence of commercial "solid phosphoric acid" catalyst yields 4.7 gallons of 82 octane number (C. F. R. motor method) gasoline per thousand cubic feet of ethylene charged (total liquid yield, 7 to

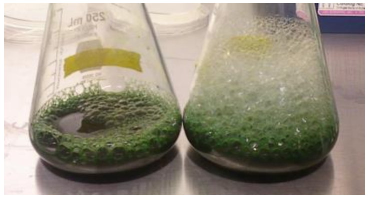


C_2H_4

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 compared to algal lipids production.
- Direct, aerobic, continuous CO_2 /sugars to ethylene conversion.
- Not a food source for common microbes; reduces feeding and contamination problems.
- No toxicity to the microbe thus affording higher productivity.





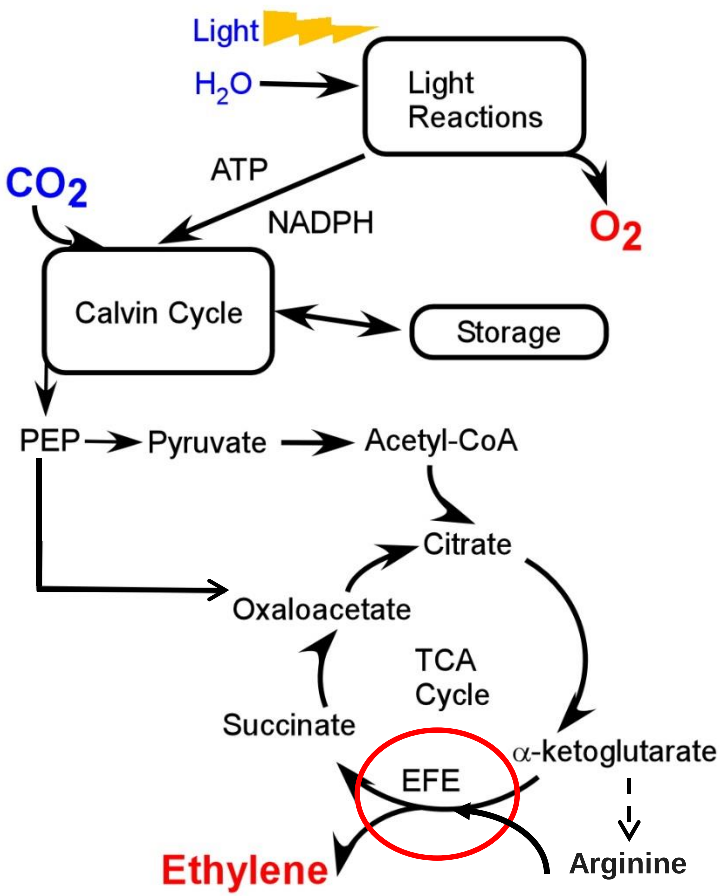
1 - Approach

Milestones	Prior Literature	FY11 (100% completed)	FY12 (100%)	FY13 (50%)	FY14 (0%)
Peak rate (mg/L/Hr)	unstable	Stable	**10**	**15**	**20**
Approach		Redesign *efe* gene	Increase copy #	Redesign RBS	Metabolic flux analysis

Also studied:

- Ethylene toxicity in *Synechocystis* 6803. *Result: not toxic*
- Sea water as growth medium and nutrient requirements. *Result: sea water with added N and P supports ethylene production.*
- Carbon partition into ethylene synthesis versus biomass growth.
- Photosynthesis in ethylene-producing strains.
- Long-term ethylene productivity in day/night cycles.
- Biomass sugars to ethylene conversion.

Strategies to enhance EFE synthesis and photosynthetic ethylene production



Re-design *efe* gene

↓ gene stability

Stronger promoter

↓ transcription

Multiple *efe* copy

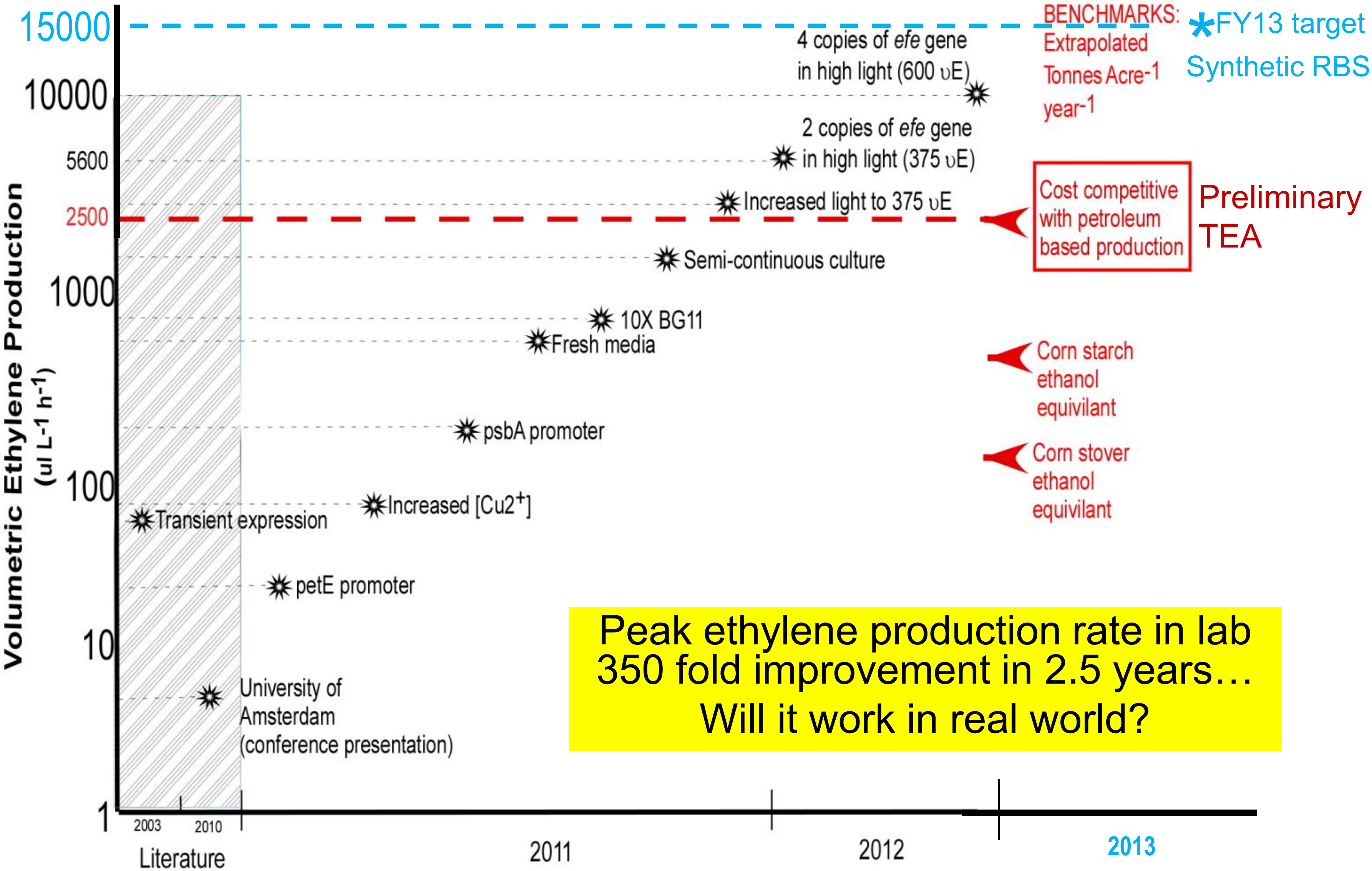
↓ gene dosage

Stronger ribosome binding site (RBS)

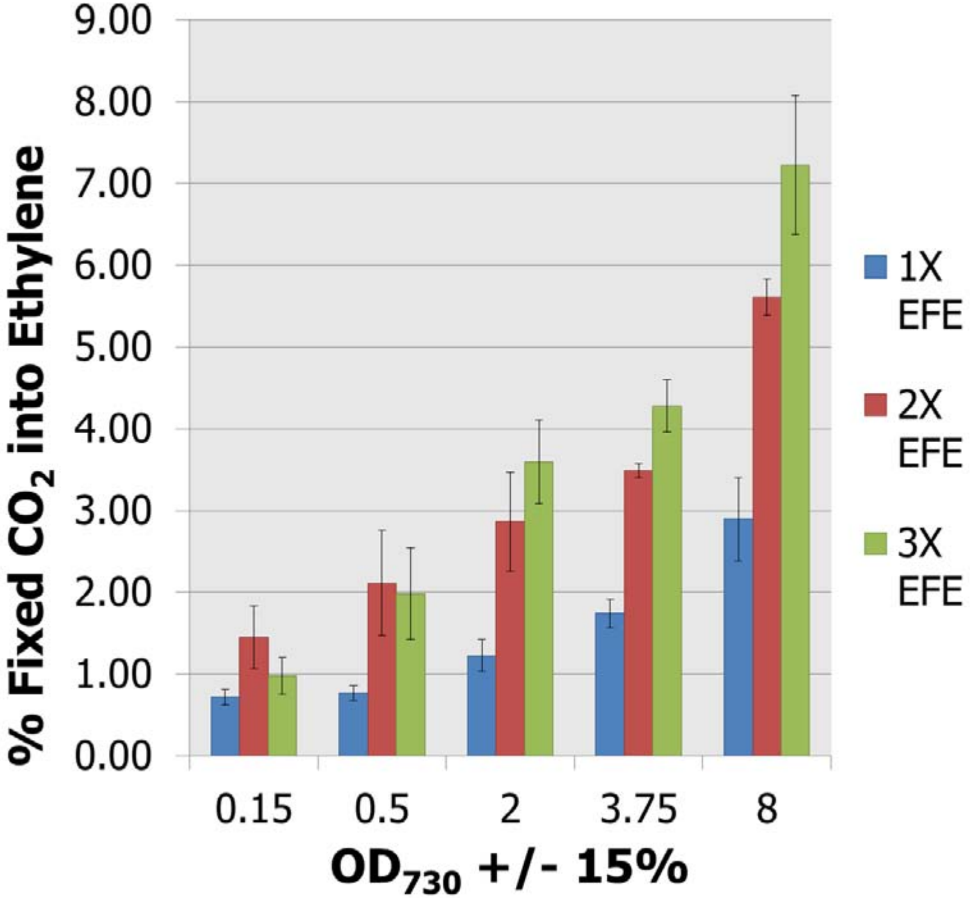
↓ translation

Enhanced EFE synthesis

2 - Technical Accomplishments/ Progress/Results



Carbon partition into ethylene increases with increasing culture density

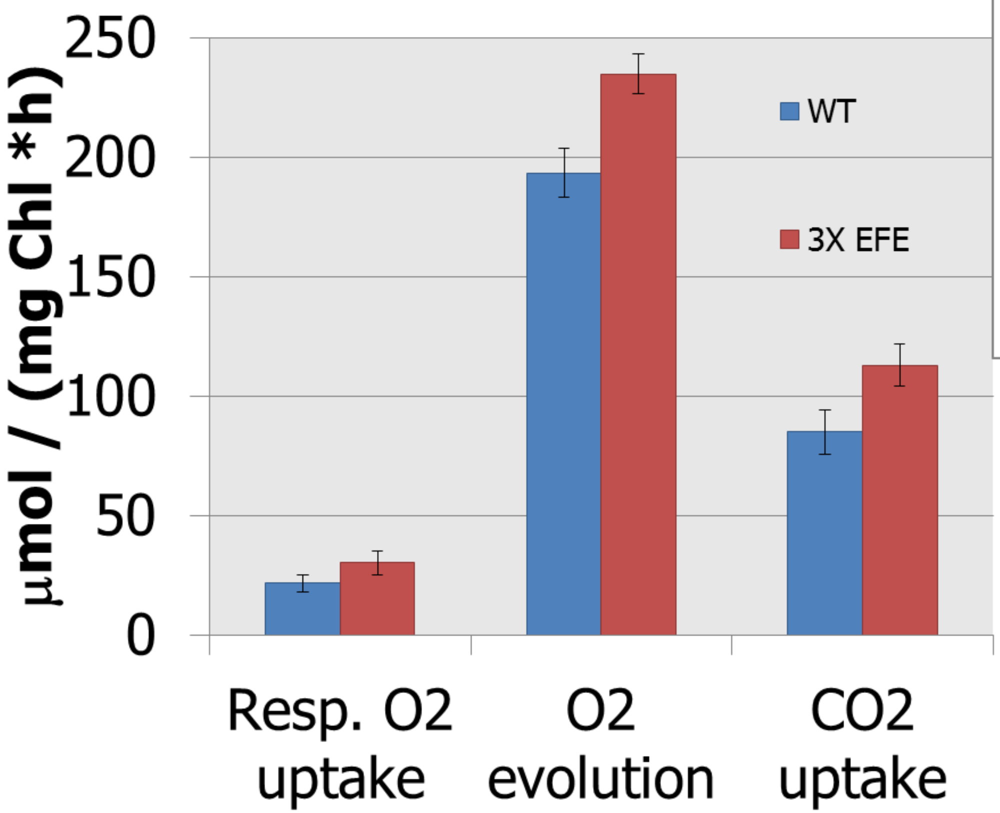


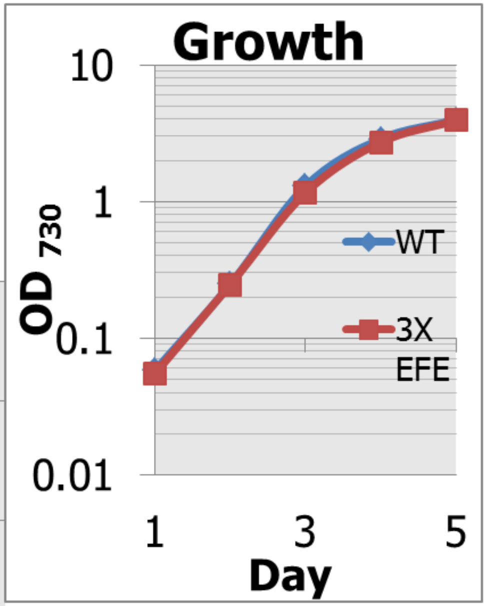
Strains with increasing copy numbers of *efe* gene

Large room for improvement…using immobilized culture?

Ethylene production enhances photosynthesis

Growth rates are the same between WT and ethylene producing strains. Increased photosynthesis allows high level ethylene production without sacrificing biomass production.





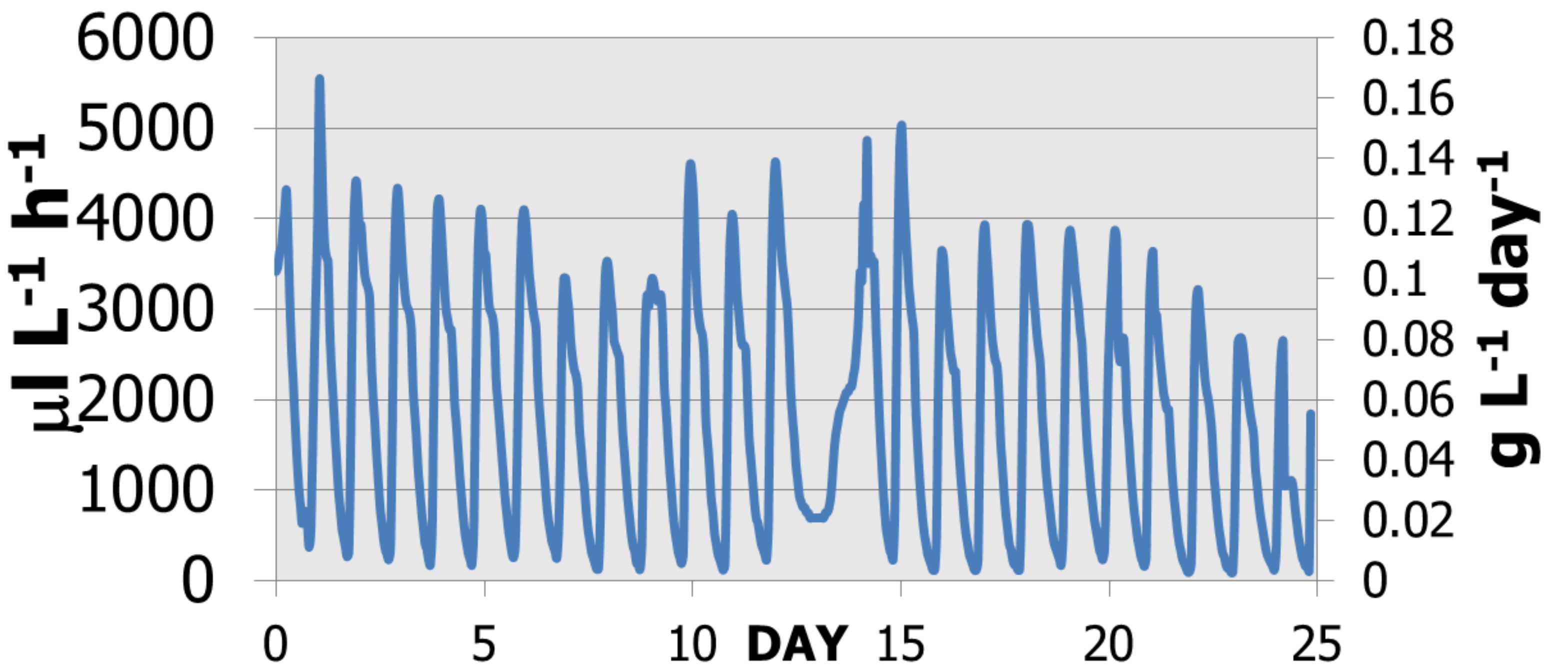
Rubisco activity

WT:
0.78 +/- 0.01 U/mg total soluble protein

3X EFE:
0.87 +/- 0.06 U/mg total soluble protein

Photosynthesis on steroids…How does it happen?

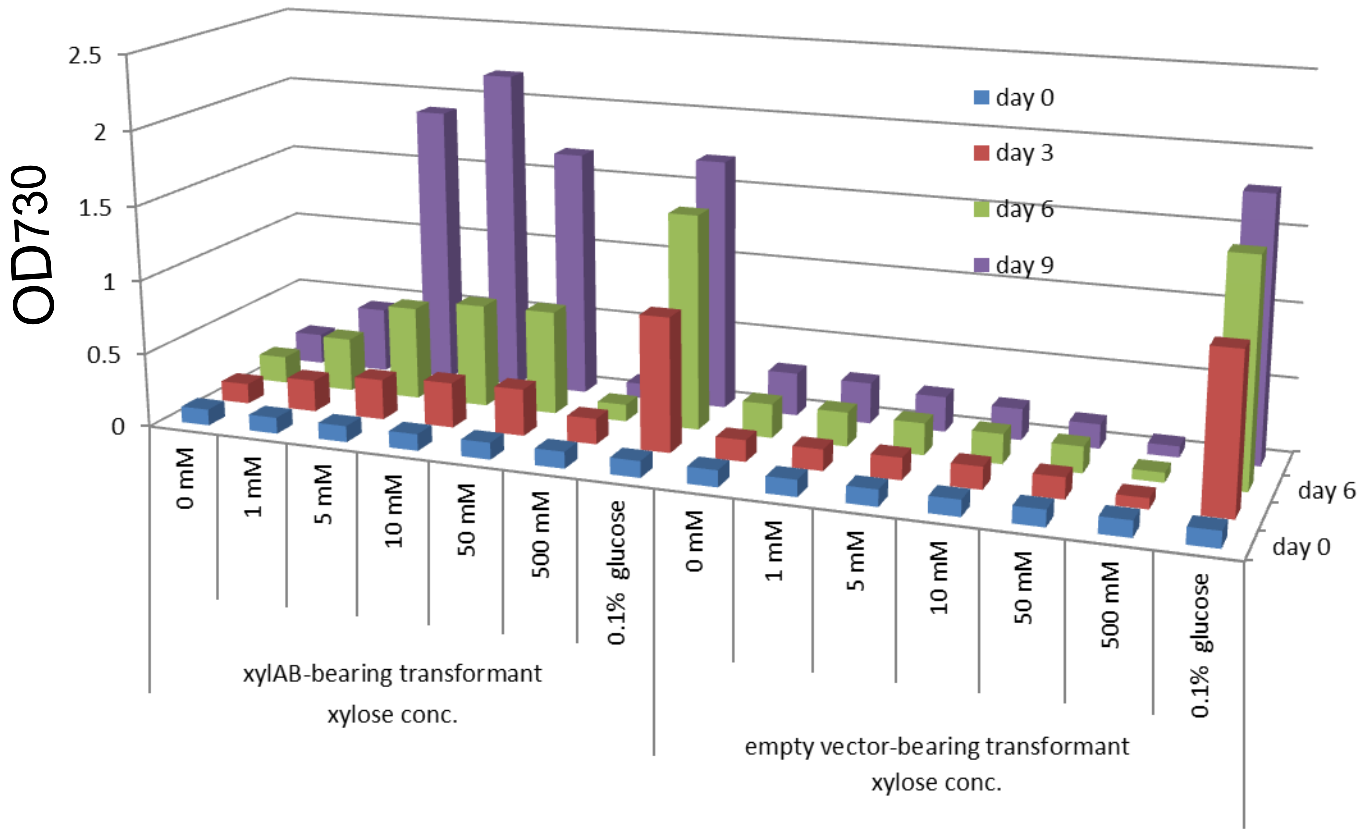
Long term ethylene production demonstrated



- 2L photobioreactor in12/12h diurnal cycles
- Online GC measurement

Low productivity at night time…Can they use biomass sugars?

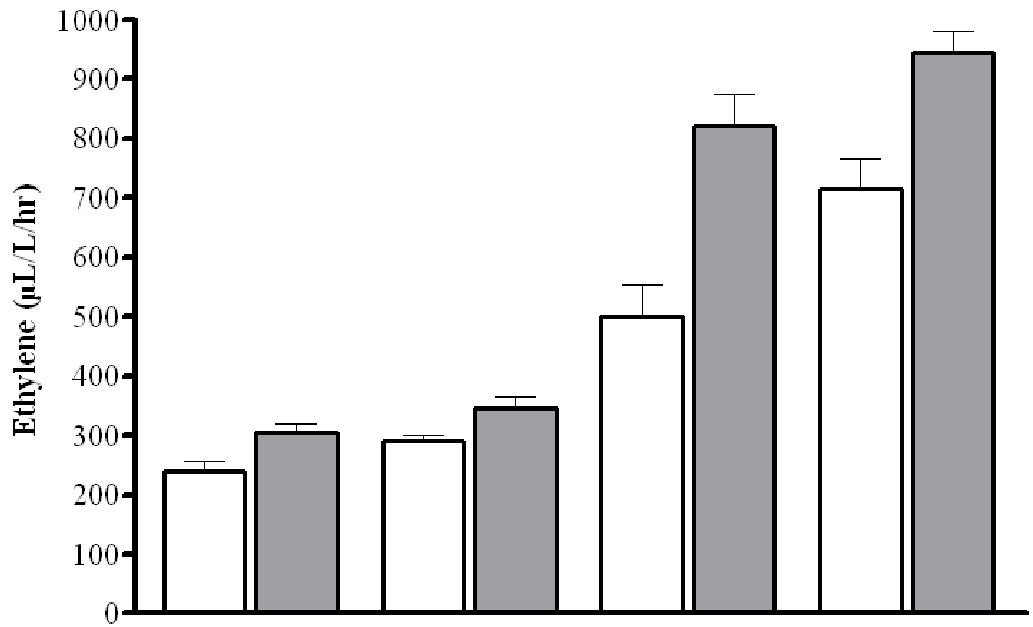
Generation of *Synechocystis* 6803 strain capable of utilizing xylose and glucose



- The wild-type strain is capable of growing on glucose
- Introduction of xylAB genes enabled growth on xylose
- Can the sugars enhance ethylene production?

Xylose utilization enhances ethylene production

A single copy *efe* gene was introduced into the *xylAB* strain…Can it use biomass-derived xylose stream?

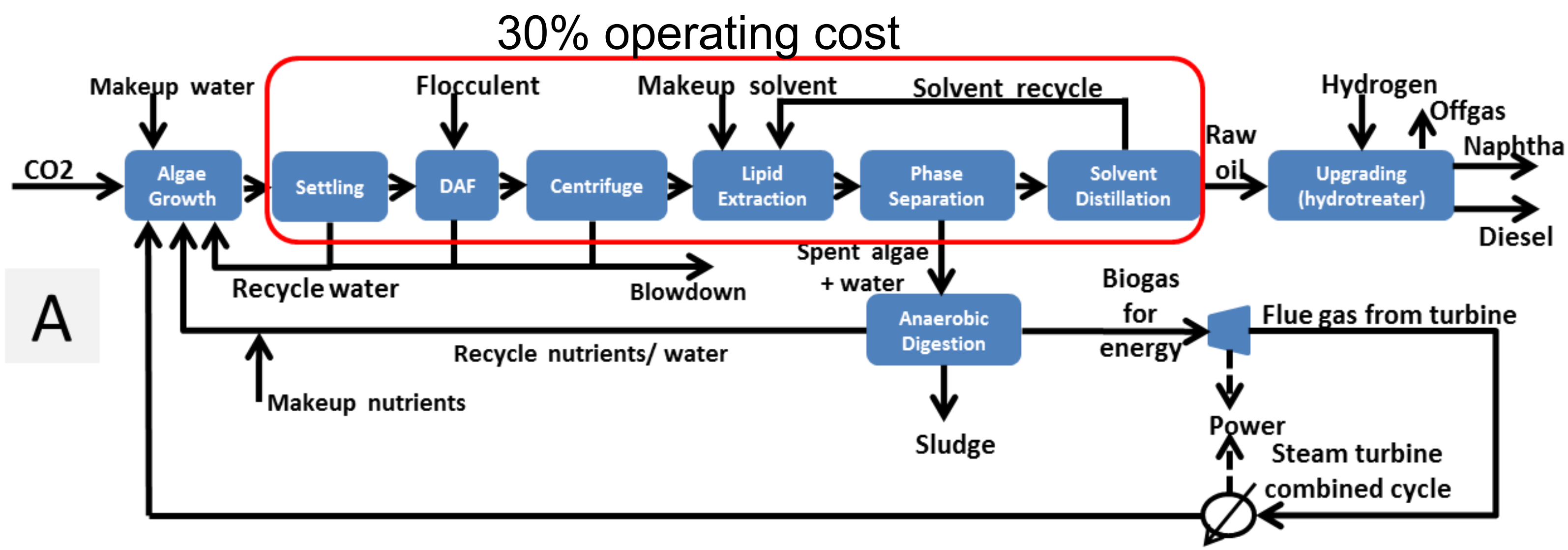


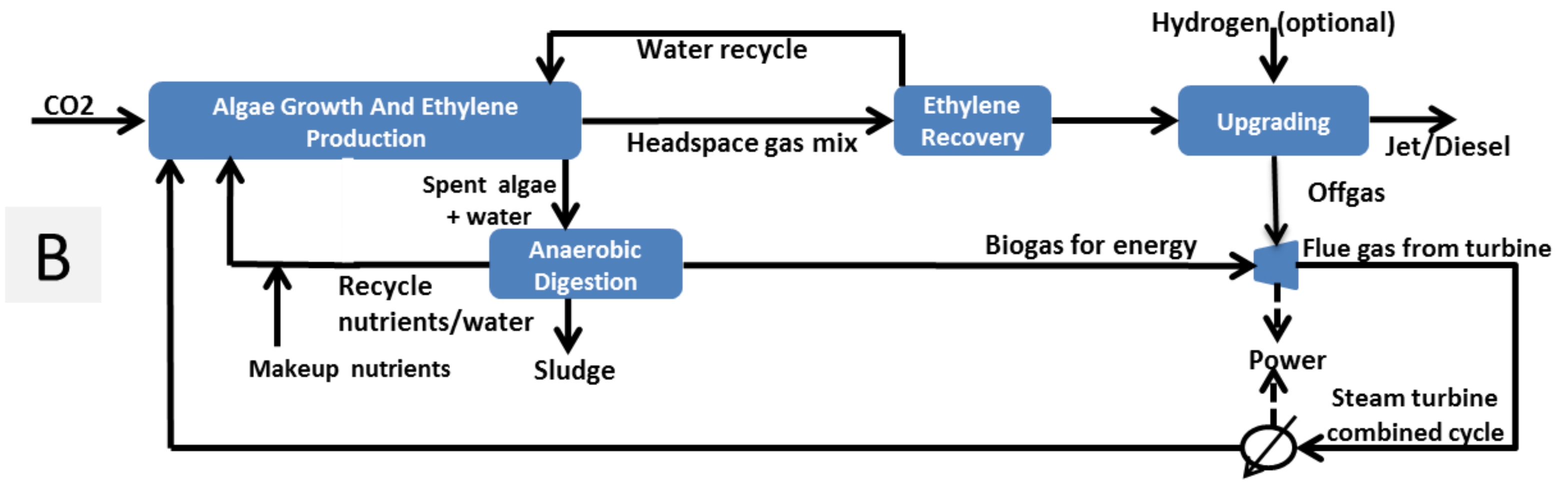
D-xylose (50 mM):	-	+	-	+	-	+	-	+
Light intensity ($\mu Em^{-2}s^{-1}$):	0	0	5	5	40	40	110	110
Starting OD_{730}:	5.34	5.34	5.34	5.34	5.34	5.34	5.34	5.34
Final OD_{730}:	4.64±0.18	6.48±0.30	4.77±0.12	6.44±0.22	5.45±0.22	7.09±0.49	5.96±0.20	8.61±0.18
Total ethylene in 12 hrs (μL/L):	2894±178	3676±164	3474±139	4155±215	6003±626	9856±627	8580±593	11338±433
Total ethylene (μL/L/hr):	241±15	306±14	290±12	346±18	500±52	821±52	715±52	945±36
Ethylene from D-xylose:	65		57		321		230	
Fold increase of ethylene:	1.27		1.19		1.64		1.32	

3 - Relevance

- Direct photosynthetic conversion of CO_2 and biomass sugars to ethylene provides an alternative design for the production of fuels and chemicals, that has potential to be highly productive, cost competitive and sustainable.
- Can be a component of an integrated biomass conversion system, using both CO_2 from fermentation as well as biomass sugars.
- Infrastructure for ethylene conversion is already in place.
- Ethylene collected from headspace is essentially free of contaminants (such as metal, sulfur), will produce cleaning-burning fuels and high-purity chemicals.
- Use sea water for fuels and chemicals production.
- Ethylene is a versatile feedstock –replacing the whole barrel.

Photosynthetic lipid (A) versus ethylene (B) production processes



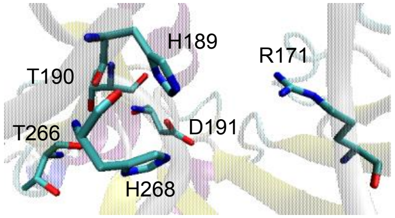


4 - Critical Success Factors

- Real-world productivity of the strains should be tested in various closed photobioreactors, including as immobilized culture, in order to identify environmental factors limiting productivity and to reduce cost of production.

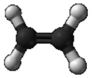
- Carbon partition (currently up to 10%) into ethylene versus biomass can be much improved by optimization of *efe* expression, identification of future bottlenecks using metabolic flux analysis, and deletion of competing metabolic sinks.

- EFE enzyme shows peak activity at 25 ºC and loses activity quickly in higher temperatures. Enzyme engineering could improve EFE performance in higher temperatures, to increase productivity and reduce operating cost.



Putative EFE Fe(II) binding site

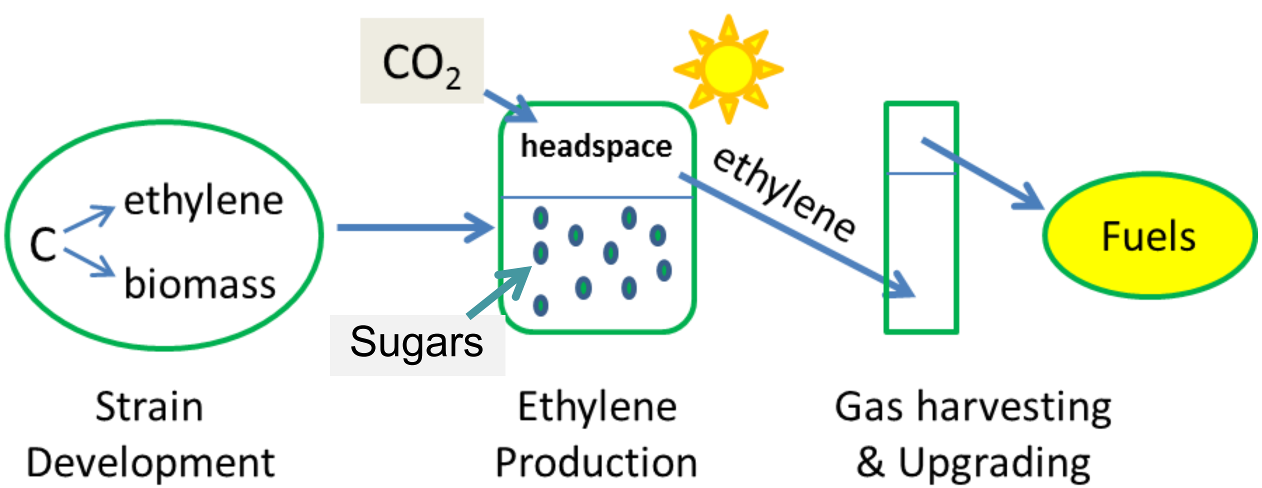
5. Future Work



- Optimize *efe* gene expression levels using synthetic RBS, to demonstrate peak ethylene production rate of 15 mg/L/Hr as FY13 milestone).
- Identify additional bottlenecks using metabolic flux analysis and sink mutants, in order to achieve peak production rate of 20 mg/L/Hr as FY14 milestone.
- Understand how ethylene production enhances photosynthesis.
- Test ethylene production with biomass-derived xylose stream.
- Engineer EFE enzyme to improve thermal stability.
- Look for partners to test ethylene production in various closed photobioreactors, including from immobilized culture.
- Contribute to ethylene techno-economic analysis (TEA) at NREL.

Summary

- Started in FY11 as a seed project, we have demonstrated sustained photosynthetic CO_2 to ethylene conversion.
- Current ethylene productivity of 10-15 mg/L/Hr is among the highest algal biofuels productivities reported.
- Enhancing *efe* expression is key to increasing rate of ethylene production.
- Biomass sugars including xylose can supplement photosynthesis for ethylene production.
- Ethylene can be readily converted to a wide variety of clean-burning fuels and high-purity chemicals by commercial processes.



Additional Slides

Publications, Presentations, and Commercialization

- Published a paper: Ungerer *et al*. (2012) Sustained photosynthetic conversion of CO_2 to ethylene in recombinant cyanobacterium *Synechocystis* 6803. *Energy & Environmental Science* 5: 8998-9006.

- Filed a utility patent application.

- EERE Accelerating Innovation webinar presentation in August 2012; http://techportal.eere.energy.gov/about/webinar_series.

- Oral presentation at Algal Biomass Biofuels Bioproducts June 2012.

- Several poster presentations at international meetings 2011-2013.