Producing Transportation Fuels via Photosynthetically-derived Ethylene

- Date: May 22, 2013
- Technology Area Review: Biochemical Conversion
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- Organization: National Renewable Energy Laboratory

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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%

**Barriers**
- Barriers addressed
  - Ft-D. Sustainable Harvesting

**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.

**Partners**
- None

![Synechocystis 6803](image)
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.
Photosynthetic Ethylene Production
The Ethylene Advantage

- Ethylene is the most produced organic compound worldwide. **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

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

### 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**
Peak ethylene production rate in lab 350 fold improvement in 2.5 years…

Will it work in real world?
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.

Photosynthesis on steroids...How does it happen?
Long term ethylene production demonstrated

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

Low productivity at night time... Can they use biomass sugars?
The wild-type strain is capable of growing on glucose.

Introduction of xylAB genes enabled growth on xylose.

Can the sugars enhance ethylene production?
A single copy *efe* gene was introduced into the *xylAB* strain…Can it use biomass-derived xylose stream?
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 °C 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](image)
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


• 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.