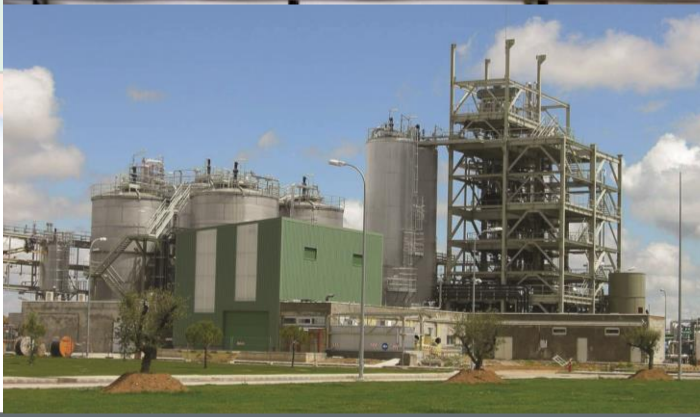
U.S. DEPARTMENT OF ENERGY | Energy Efficiency & Renewable Energy



Excellence in Bioenergy Innovation—A Presentation of 2015 R&D 100 Award Winning Projects

January 21, 2016

Bioenergy Technologies Office (BETO)

Agenda

- Introduction and BETO Overview
  - Erica Qiao, BCS, Incorporated
- Excellence in Bioenergy Innovation—A Presentation of 2015 R&D 100 Award Winning Projects
  - Dr. Jianping Yu, National Renewable Energy Laboratory
  - Douglas Elliott, Pacific Northwest National Laboratory

Questions and Comments

Please record any questions and comment you may have during the webinar and send them to eere_bioenergy@ee.doe.gov

As a follow-up to the webinar, the presenter(s) will provide responses to selected questions.

Slides from this presentation will be posted online: http://www.energy.gov/eere/bioenergy/webinars

For general questions regarding the Bioenergy Technologies Office, please email eere_bioenergy@ee.doe.gov

Bioenergy Technologies Office Webinar Series

Started in May 2010 to highlight "hot topics" in biomass and bioenergy industry.

Find past webinars and today's slides on the Office's website: http://www.energy.gov/eere/bioenergy/webinars

The Challenge and the Opportunity

THE CHALLENGE

- **More than $1 Billion** is spent every three days on U.S. crude oil imports
- Transportation accounts for **2/3rds** of petroleum consumption and **26%** of GHG emissions in the U.S.

THE OPPORTUNITY

- More than **1 Billion tons** of biomass could be sustainably produced in the U.S.
- Biomass could displace 30% of U.S. petroleum use by 2030 and reduce annual CO_2e by 550 million tons, or 10% of U.S. energy emissions

America's biomass resources can help mitigate petroleum dependence

Bioenergy Technologies Office

Mission

Accelerate commercialization of advanced bioenergy through RD&D supported by public-private partnerships

Strategy

Promote sustainable, nationwide production of infrastructure-compatible biofuels

Performance Goal

Validate at least one pathway for $3/GGE* hydrocarbon biofuel with ≥50% reduction in GHG emissions by 2017

*Mature modeled price at pilot scale.

BETO reduces risks and costs to commercialization through RD&D

BETO's Core Focus Areas

Program Portfolio Management

• Planning • Systems-Level Analysis • Performance Validation and Assessment
• MYPP • Peer Review • Merit Review • Quarterly Portfolio Review
• Competitive • Non-competitive • Lab Capabilities Matrix

Research, Development, Demonstration, & Market Transformation

Feedstock Supply & Logistics R&D

- Terrestrial
- Algae
- Product Logistics Preprocessing

Conversion R&D

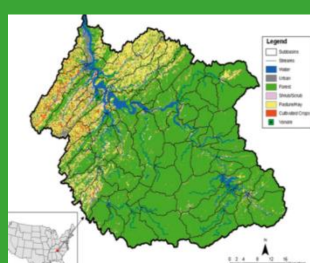
- Biochemical
- Thermochemical
- Deconstruction
- Biointermediate
- Upgrading

Demonstration & Market Transformation

- Integrated Biorefineries
- Biofuels Distribution Infrastructure

Cross Cutting

Sustainability



- Sustainability Analysis
- Sustainable System Design

Strategic Analysis

- Technology and Resource Assessment
- Market and Impact Analysis
- Model Development & Data compilation

Strategic Communications

- New Communications Vehicles & Outlets
- Awareness and Support of Office
- Benefits of Bioenergy/Bioproducts

BIO ENERGY 2015

NREL
NATIONAL RENEWABLE ENERGY LABORATORY

Cyanobacterial Bio-ethylene

Jianping Yu

January 20, 2016

BETO Webinar

•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.

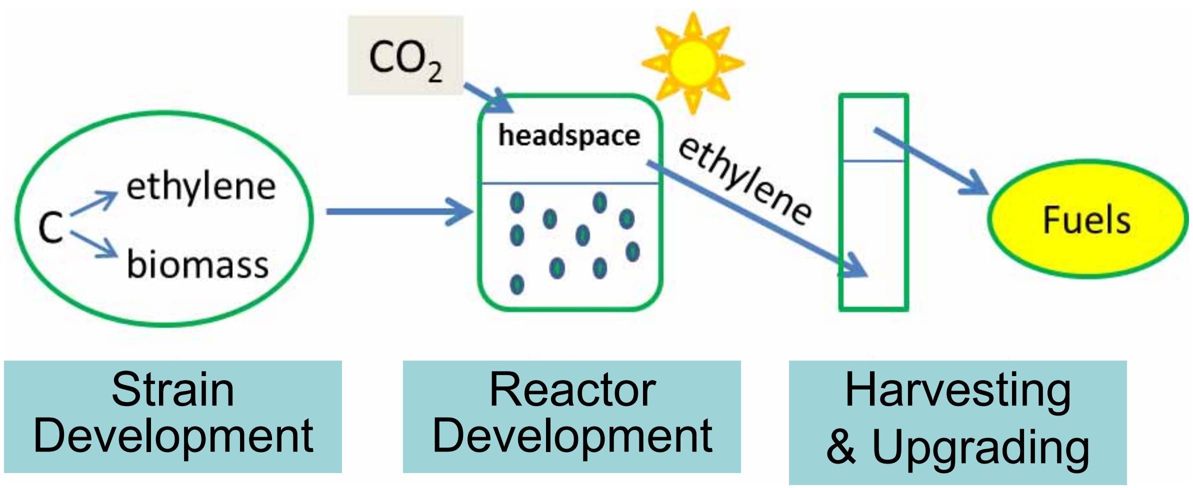
Goal Statement

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

(1) as a sustainable alternative to fossil-based feedstock;

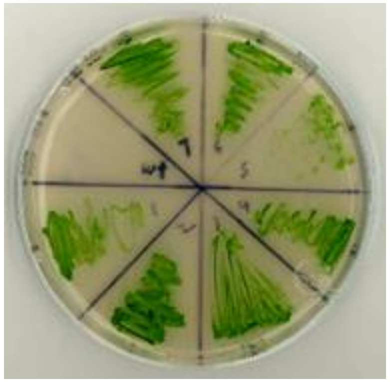
(2) with low water, CO_2 and nutrients input;

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



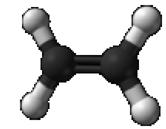
Cyanobacteria

- Cyanobacteria, aka blue-green algae, are bacteria that perform oxygenic photosynthesis, converting CO_2 to organic compounds.
- Cyanobacteria are primary solar energy converters in diverse ecosystems.



- Genetic engineering tools are well developed in some cyanobacteria, which enables introduction of new pathways into metabolism for the production of target molecules such as ethylene.

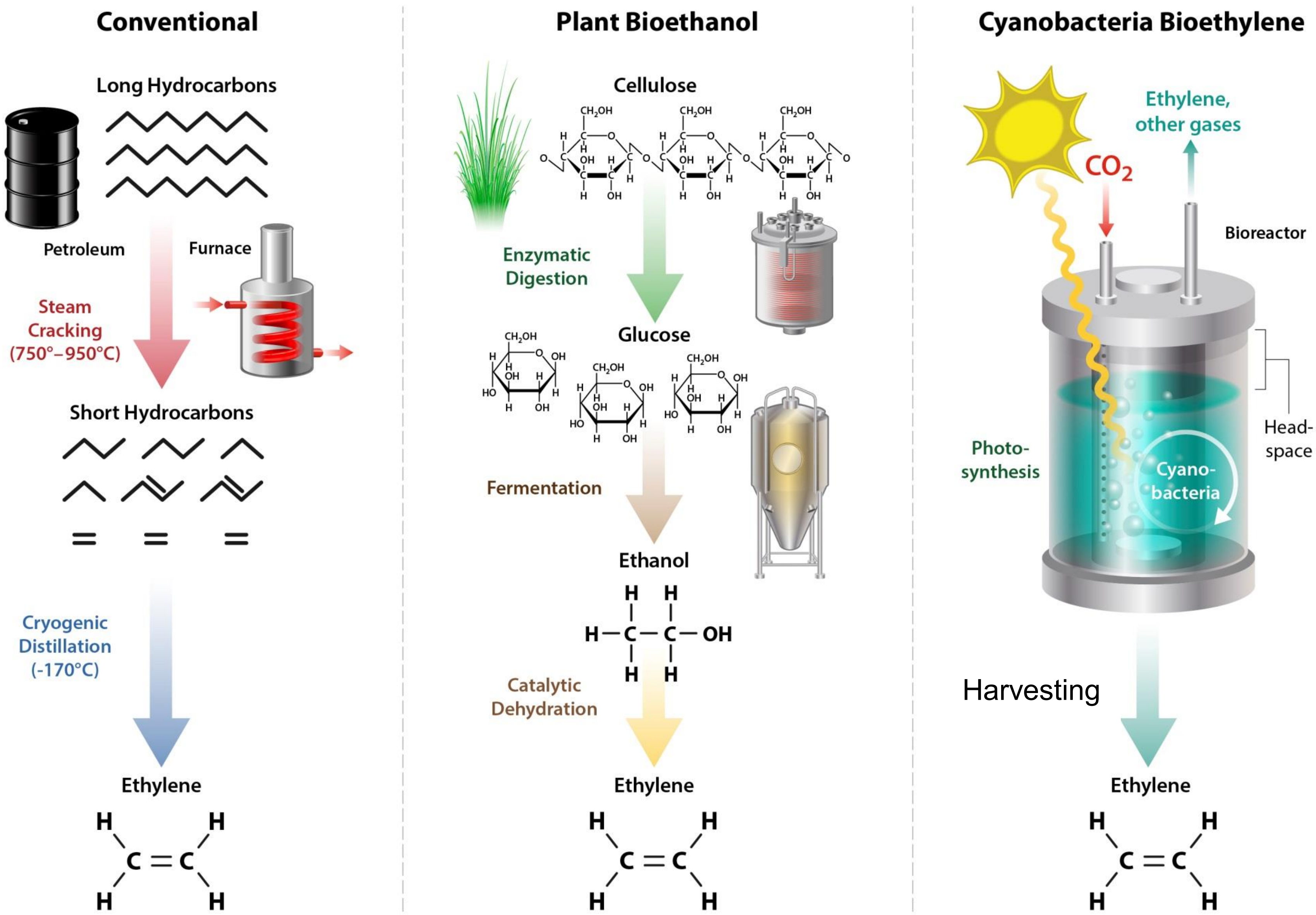
Ethylene



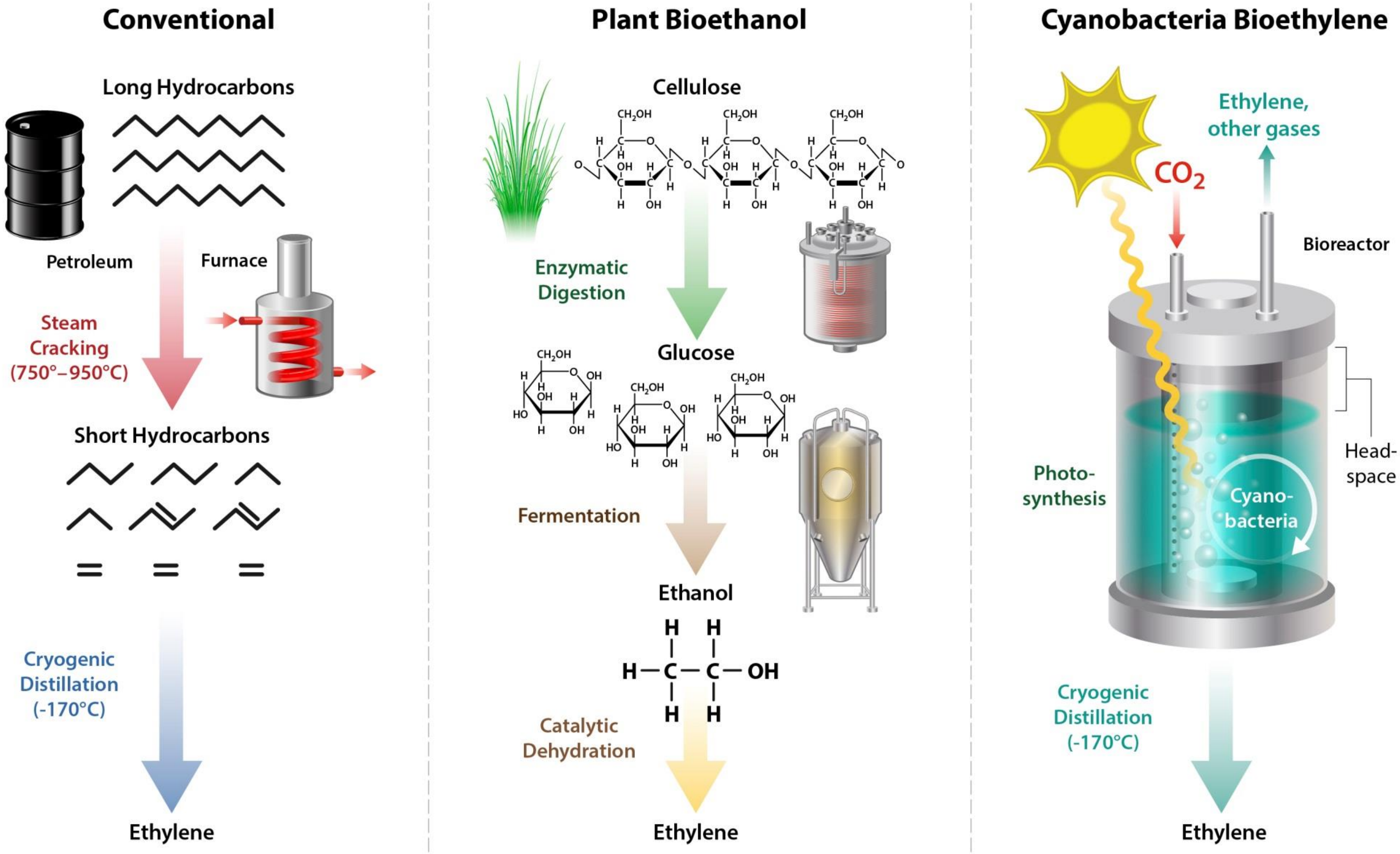
C_2H_4

- Ethylene is one of the most produced organic compounds worldwide.
- Feedstock for a wide range of materials and chemicals such as plastics.
- Can be polymerized to liquid transportation fuels.
- Currently produced from fossil resources – organic compounds derived from photosynthesis that occurred millions of years ago.
- More renewable sources of ethylene are desirable.

Ethylene Production Scenarios

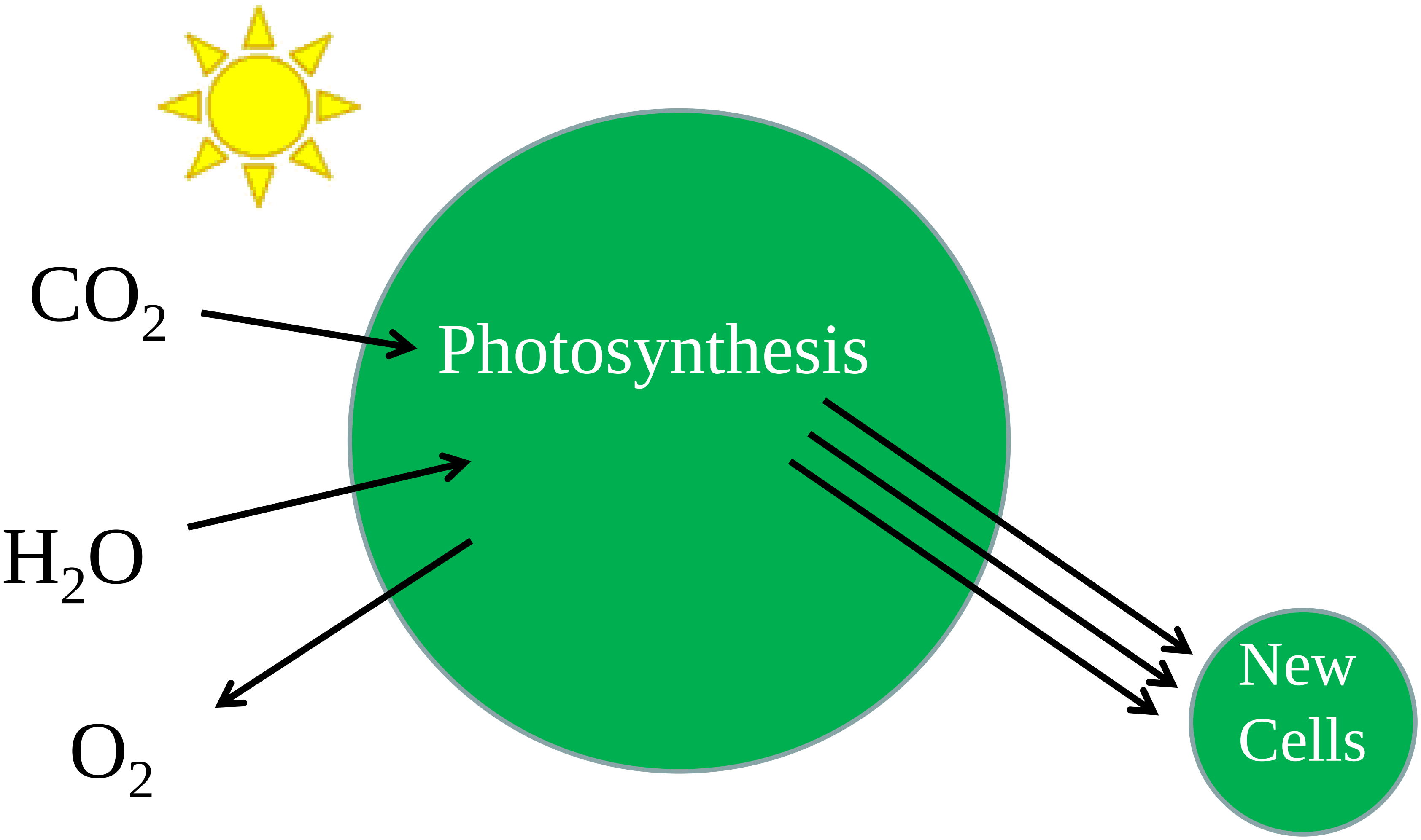


Carbon in ethylene comes from photosynthesis

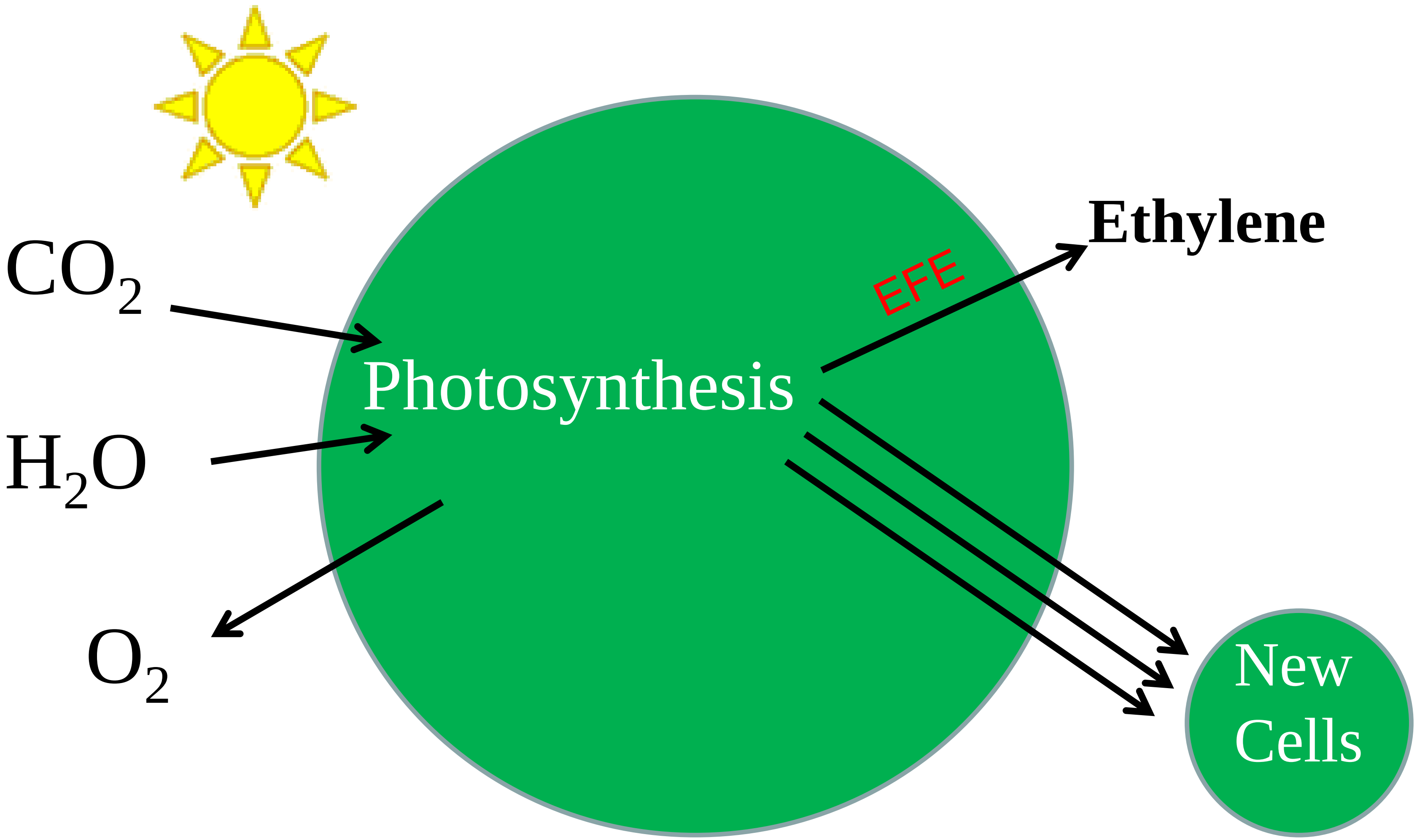


Millions of years | **Months** | **Real time**

The Cyanobacterium *Synechocystis* 6803 Wild Type



Genetically engineered ethylene producing strains



EFE: ethylene-forming enzyme. Where does it come from?

Biological Ethylene Production and EFE

- Ethylene is a plant hormone that regulates many processes in plant growth (A).
- Plant pathogens or symbiotic microbes use ethylene to weaken plant defense (B); some have EFE.
- EFE is sourced from *Pseudomonas syringae* (C).
- EFE is not well understood, a challenge for its biotech application. A reaction formula has been proposed by Fukuda *et al* 1992 (D).

D

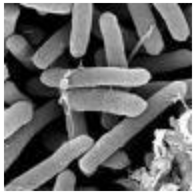
Alpha-ketoglutarate (AKG) + O_2 + L-arginine → ethylene + succinate + CO_2 + guanidine + L-delta-pyrroline-5-carboxylate (P5C)

Eckert *et al* 2014 Biotechnology for Biofuels

A

B

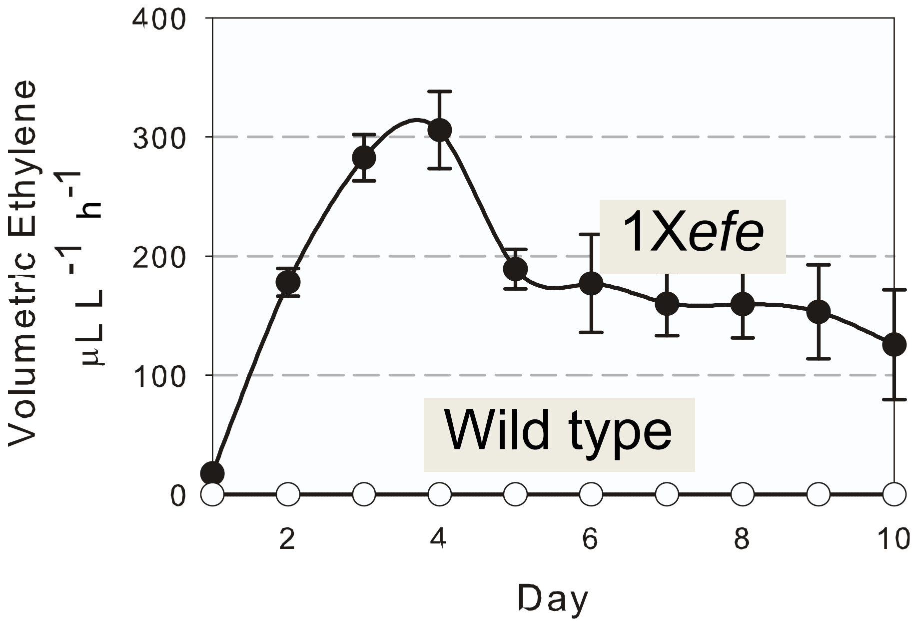
C



Ethylene is not toxic to *Synechocystis* 6803

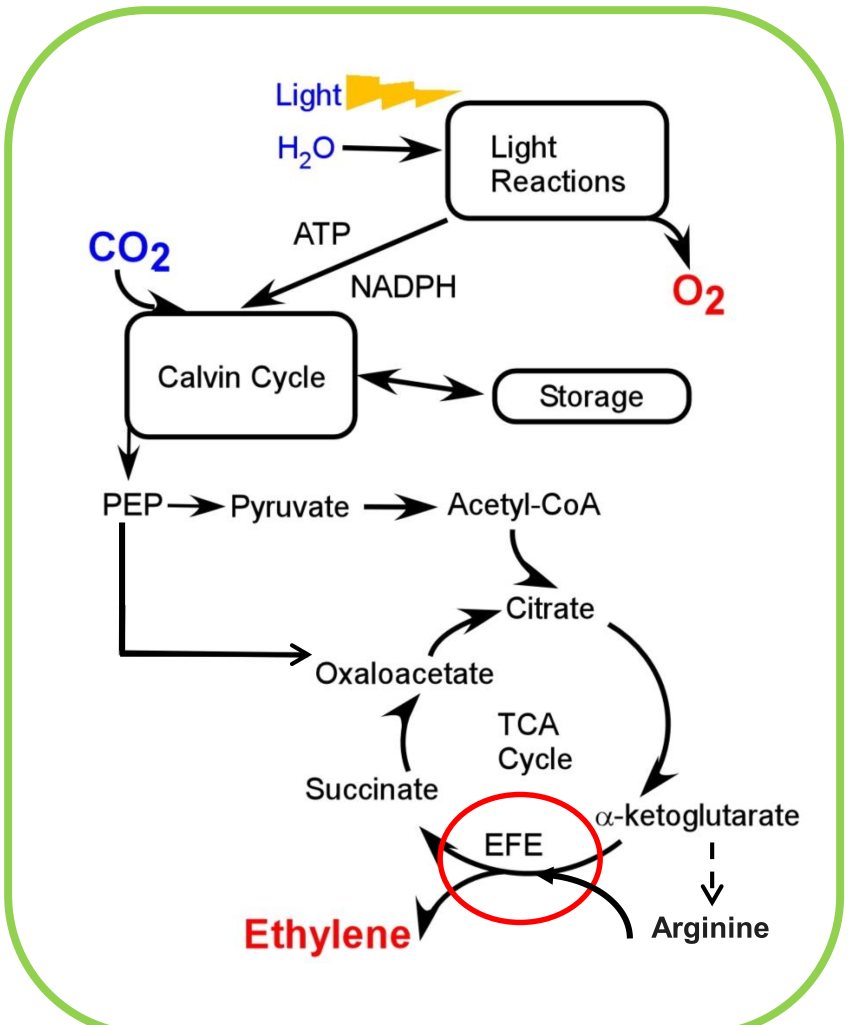
- Added ethylene has no effect on culture growth
- Ethylene-producing cultures grow as fast as WT
- Now how can we make more ethylene?
- What is the limiting factor?

Strain	Doubling time (h)
wild type	10.7 +/- 0.41
1X*efe*	11.0 +/- 0.58
2X*efe*	10.9 +/-0.63



Ungerer *et al. Energy and Environmental Science* 2012

Increasing EFE levels…



Re-design *efe* gene

↓ gene stability

Stronger promoter

↓ transcription

Multiple *efe* copy

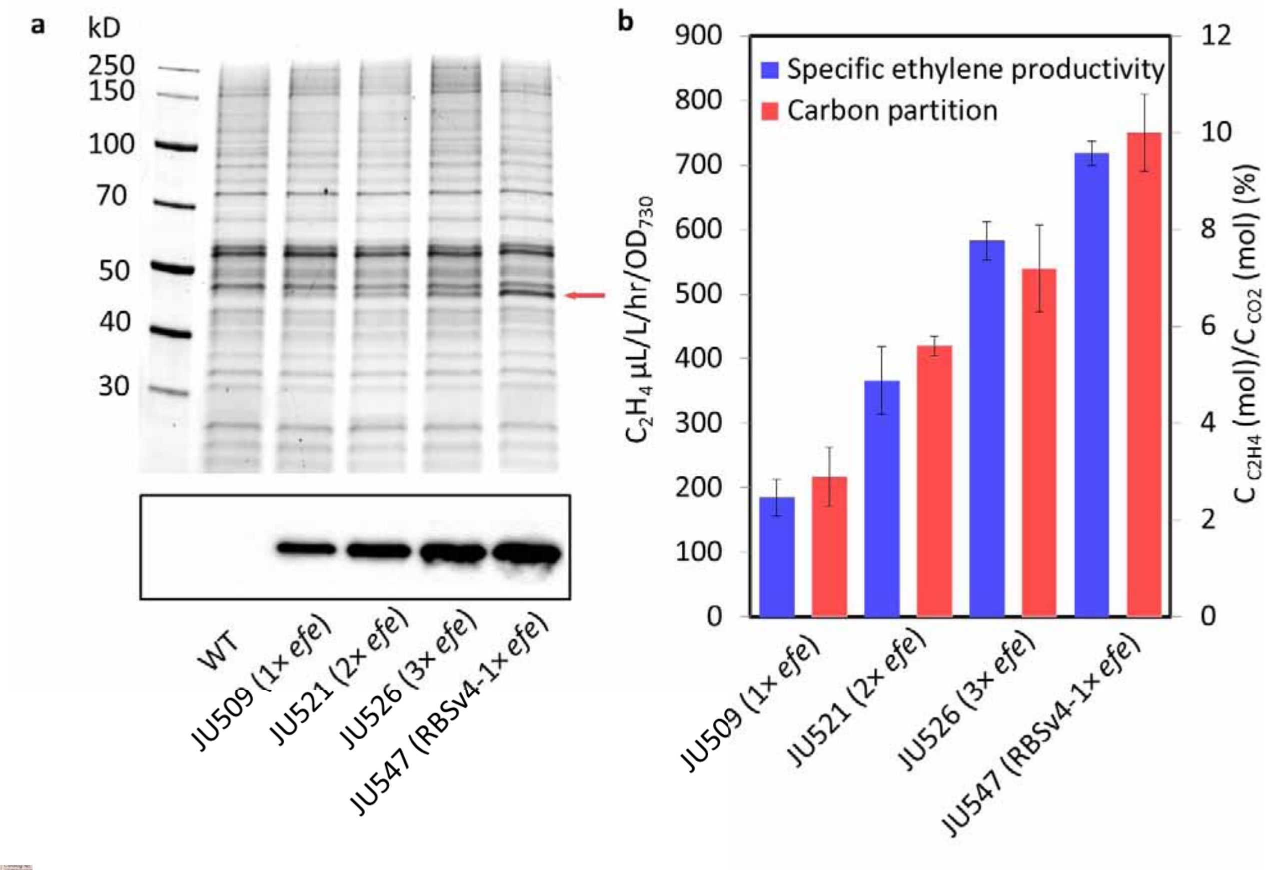
↓ gene dosage

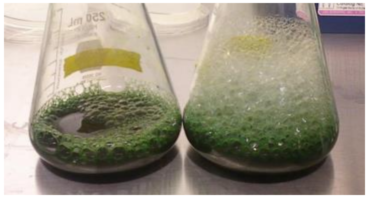
Stronger ribosome binding site (RBS)

↓ translation

Enhanced EFE synthesis

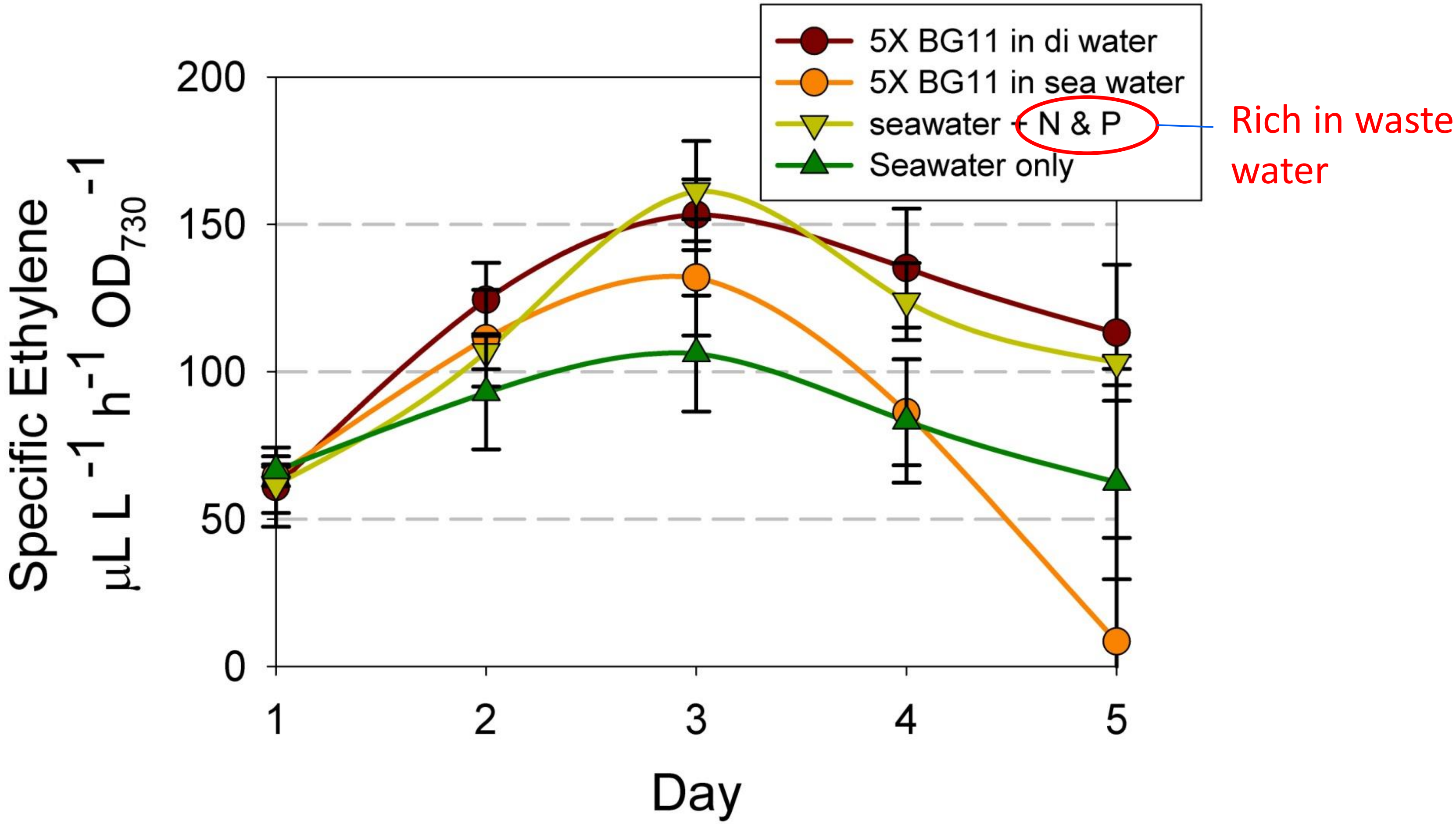
Higher ethylene productivity





- Transfer to a sealed tube and incubate
- Headspace ethylene and CO_2 are measured by GC

Use seawater for ethylene production



"Food vs fuel"

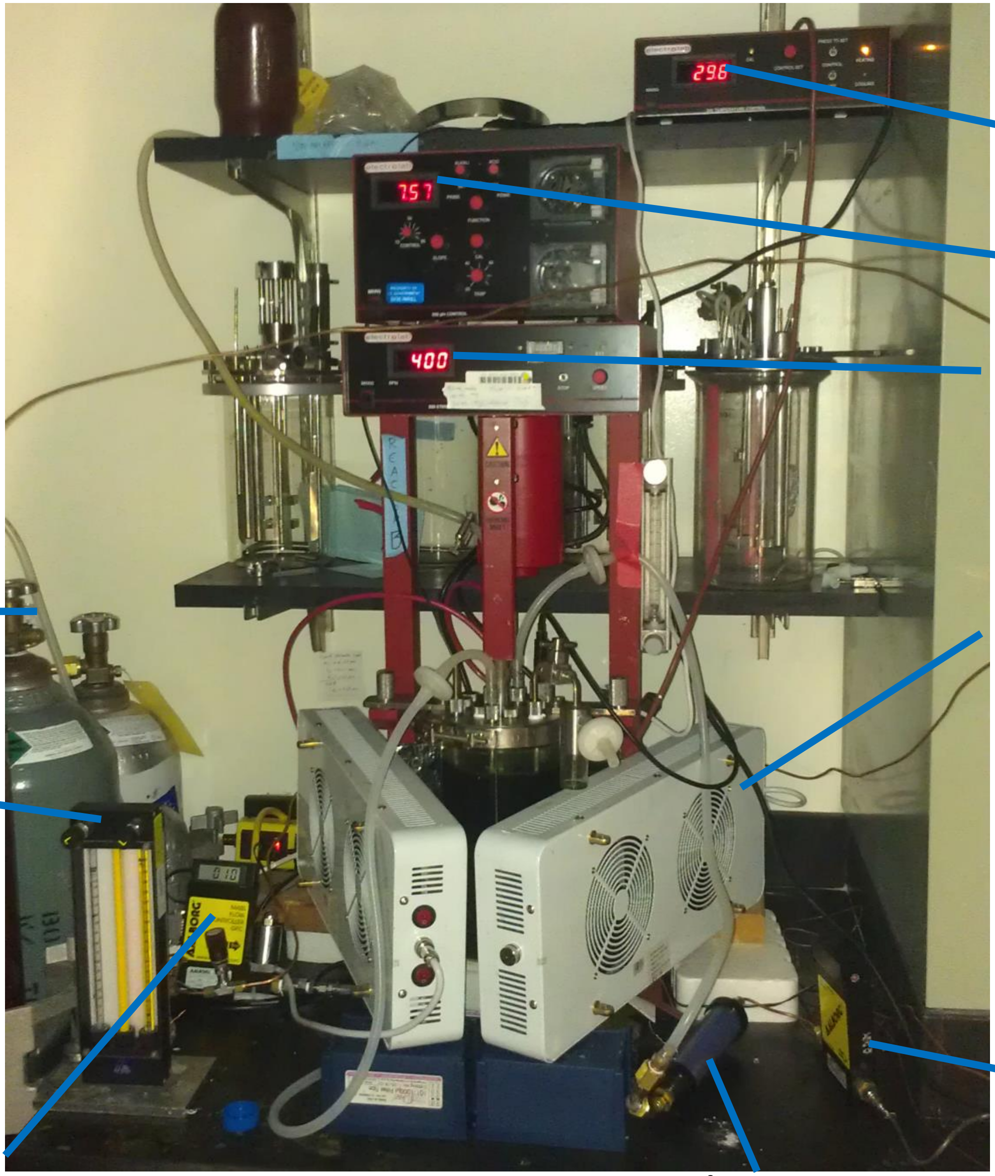
Continuous Ethylene Production

2L PBR

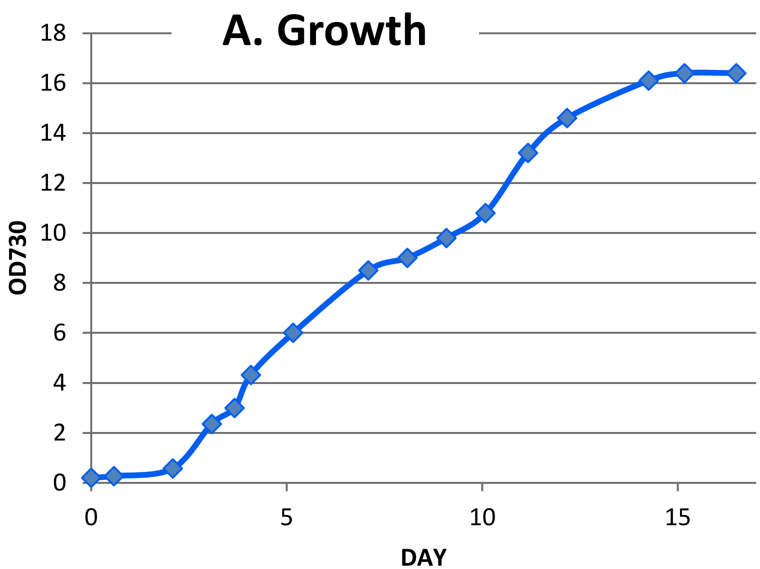
- Continuous data collection from both in stream and out stream via GC
    - CO_2
    - O_2
    - Ethylene
- Sterile sampling of culture

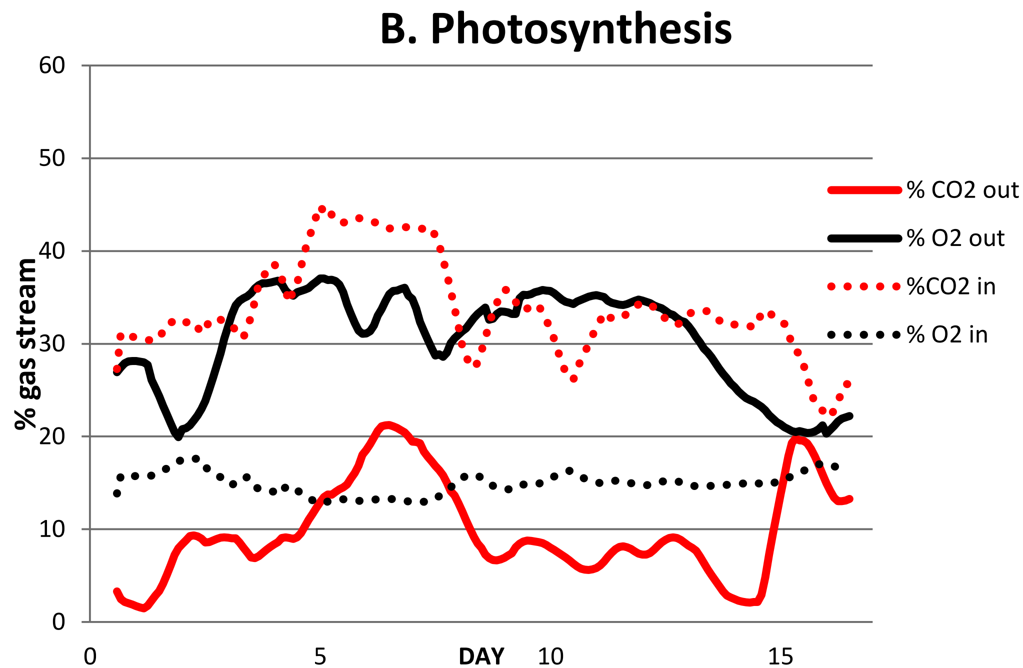
Adjustable (future optimization)

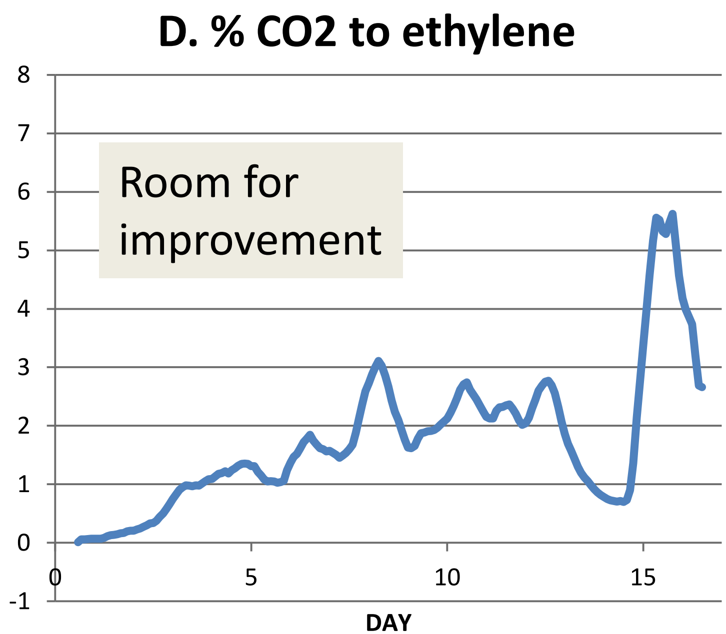
- CO_2 concentration
- light intensity
- gas flow rate
- Temperature
- stirring rate

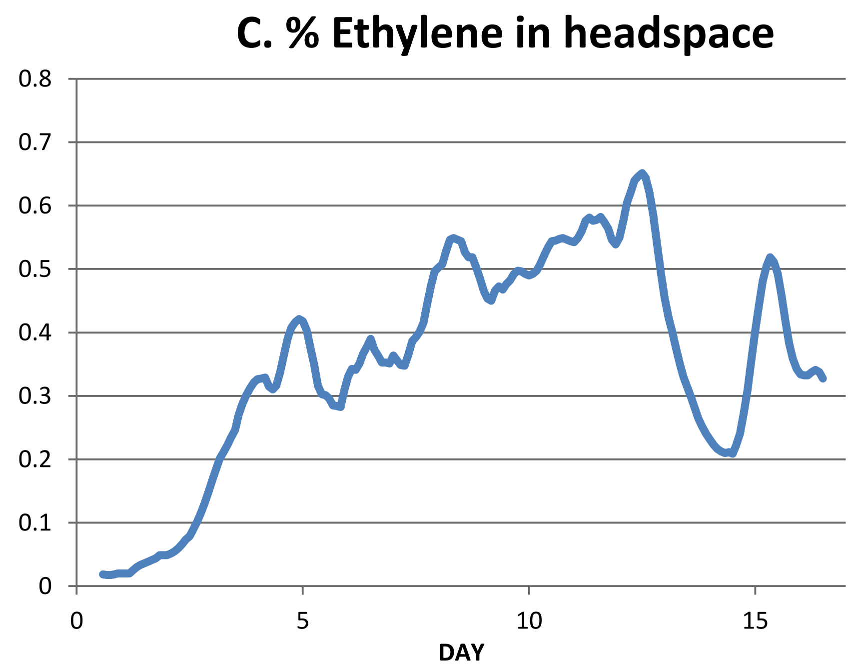


Initial Data



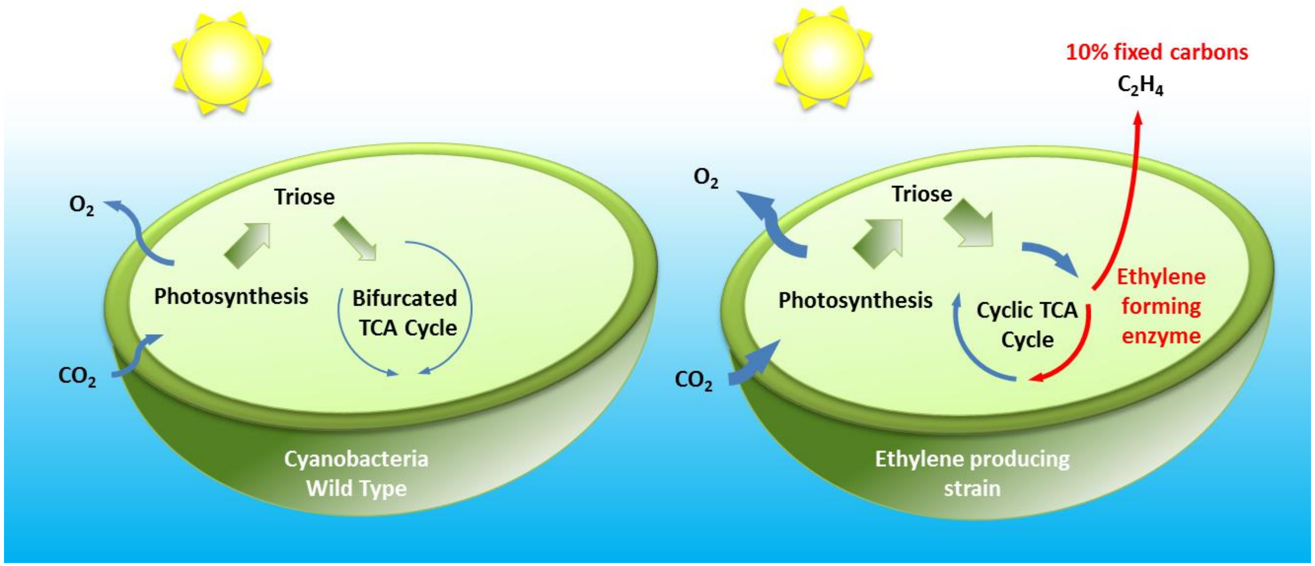






A deeper look into metabolism yields surprises

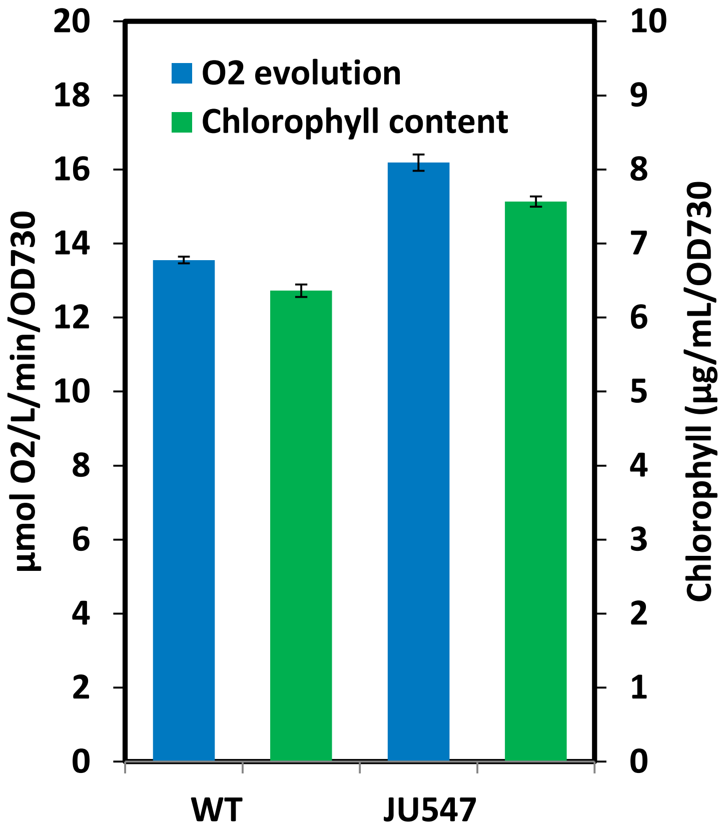
- TCA cycle operates as a cycle.
- Carbon flux to TCA cycle increased by 3X.
- Growth rate is maintained despite loss of a lot of carbon!
- Photosynthesis is stimulated to compensate for carbon loss.



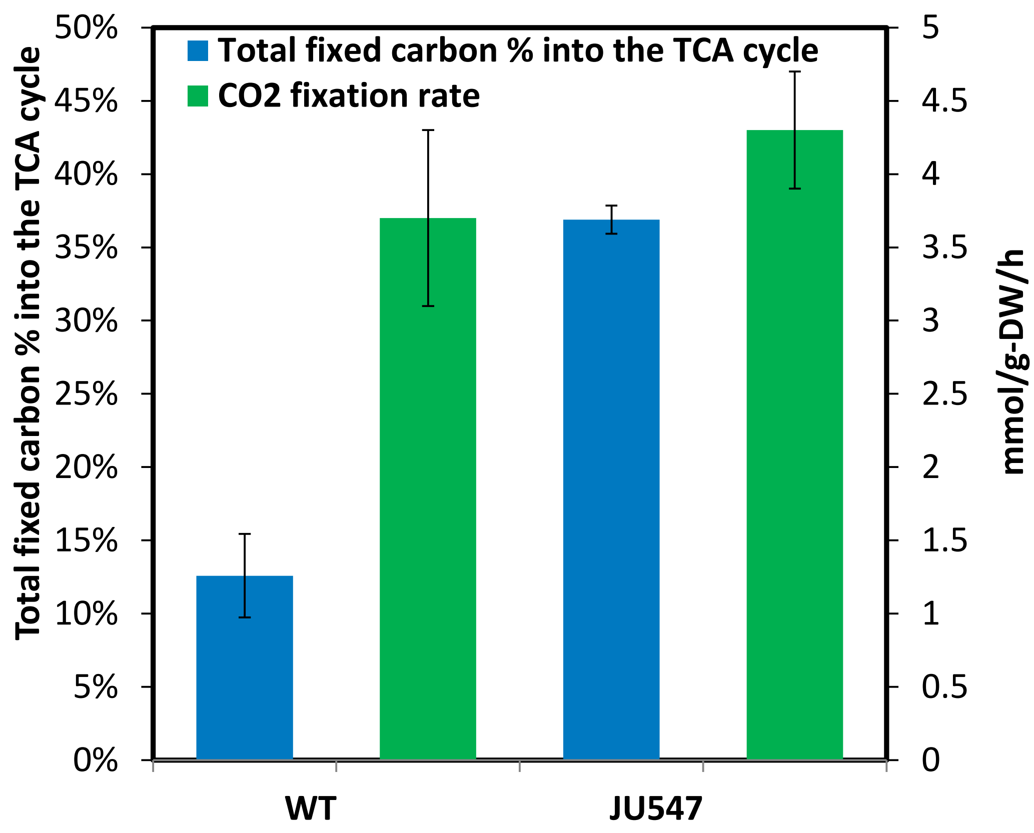
Xiong *et al.*, Nature Plants 2015a

Ethylene production stimulates photosynthesis

Light Reaction

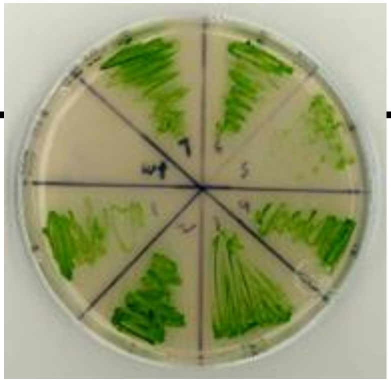


Carbon Metabolism



Rubisco activity also increased

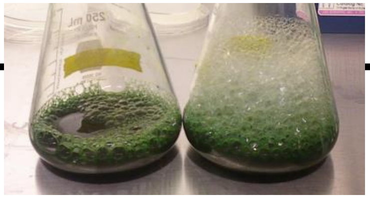
Conclusions



- A cyanobacterium has been genetically engineered to convert CO_2 to ethylene.
- Up to 10% of fixed carbons are directed to ethylene.
- Current limiting factor is EFE substrate supply*.
- The organism responds to ethylene production by rewiring metabolic network and stimulating photosynthesis.

*Overcoming EFE substrate supply limitation in *E. coli*: Lynch *et al* 2016 Biotechnology for Biofuels.

Future Directions



Strain development

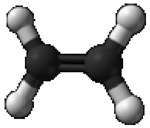
- Increase carbon conversion efficiency from up to 10% towards 90% using synthetic biology approaches
- Rate limiting factor has shifted from EFE level to substrate supply
- Increase carbon flux to EFE substrates

Reactor development and systems integration

- Develop cultivation/production/harvesting system tailored for ethylene
- Technoeconomic analysis; life cycle analysis

Lynch *et al* 2016 Biotechnology for Biofuels.

Acknowledgements



Justin Ungerer
Wei Xiong
Bo Wang

Pin-Ching Maness
Mark Davis
Phil Pienkos
Maria Ghirardi
Ling Tao
Jennifer Markham
John Fei

NREL Photobiology Group

Funding Support

DOE BETO

DOE FCTO

NREL LDRD

jianping.yu@nrel.gov

Pacific Northwest NATIONAL LABORATORY

Proudly Operated by Battelle Since 1965

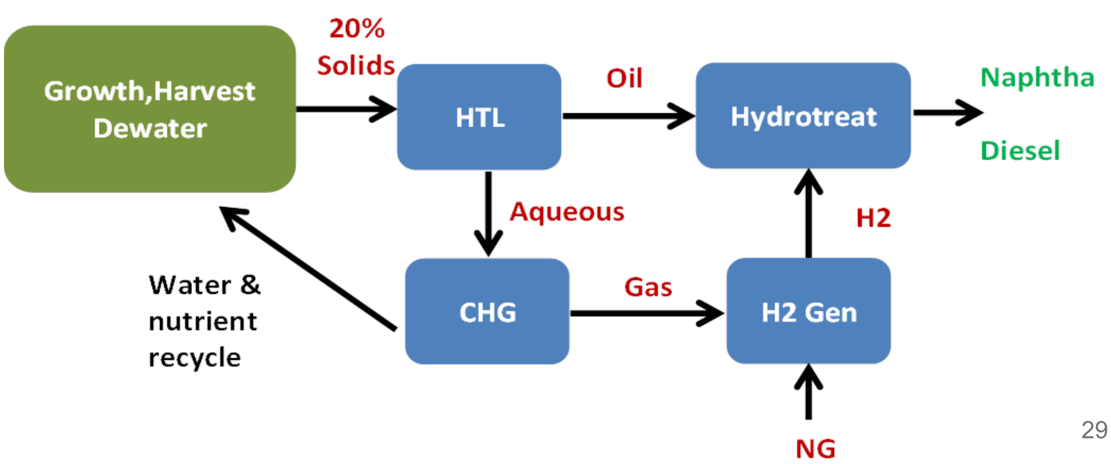
Hydrothermal process to convert wet biomass into biofuels

DOUG ELLIOTT

Excellence in Bioenergy Innovation Webinar—A Presentation of 2015 R&D 100 Award Winning Projects

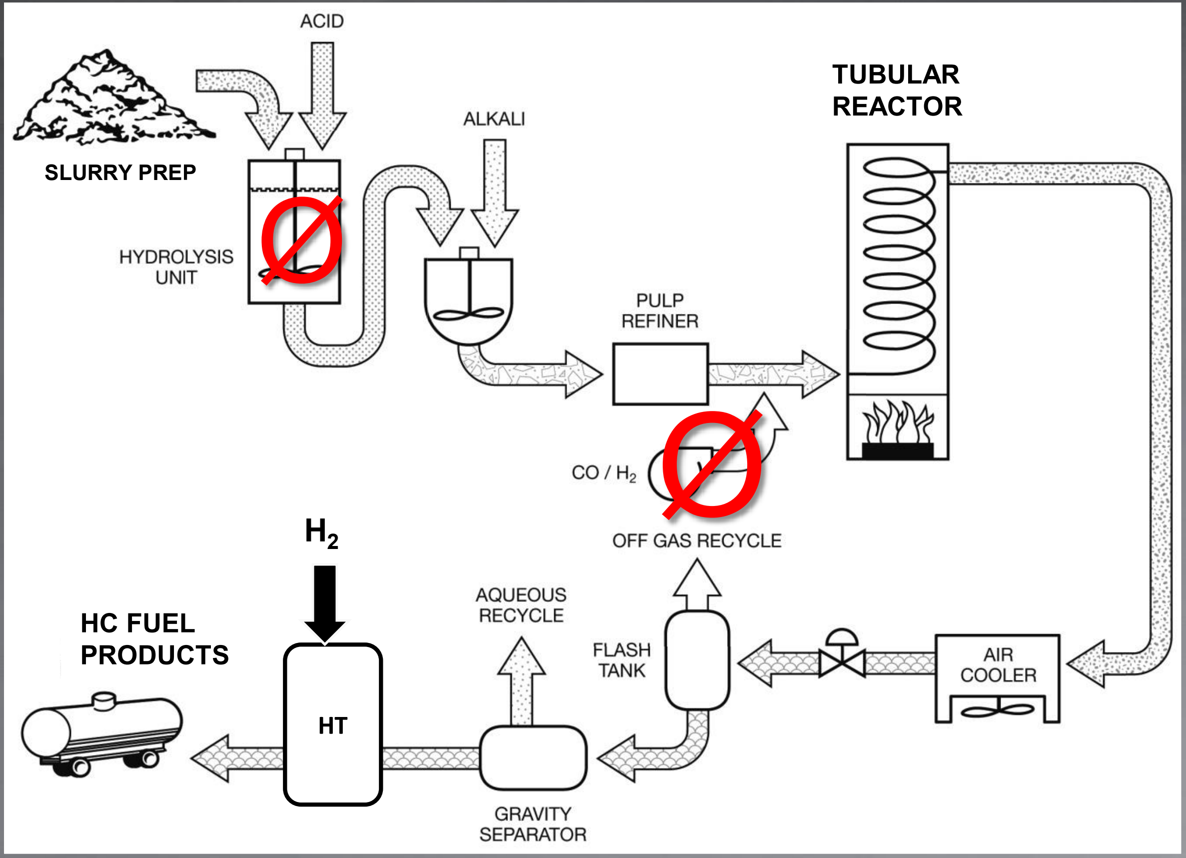
Outline

- Process overview
- HTL at PNNL
  - Continuous process
  - Range of feedstocks
  - Gravity-separable biocrude
- Upgrading of HTL biocrude
- CHG of aqueous phase
- Commercialization and future work



Hydrothermal Liquefaction and Hydrotreating

Pacific Northwest
NATIONAL LABORATORY
Proudly Operated by Battelle Since 1965



Albany, Oregon, USA
1977-1982

1 t/d Douglas fir wood

Slow pyrolysis in pH-moderated, pressurized water

HTL Overview

Pacific Northwest
NATIONAL LABORATORY
Proudly Operated by **Battelle** *Since 1965*

Hydrothermal Liquefaction (HTL)

Conversion of a biomass slurry (e.g., wood, algae, other) to biocrude and aqueous product

- 300–350°C
- 2800–3000 psig

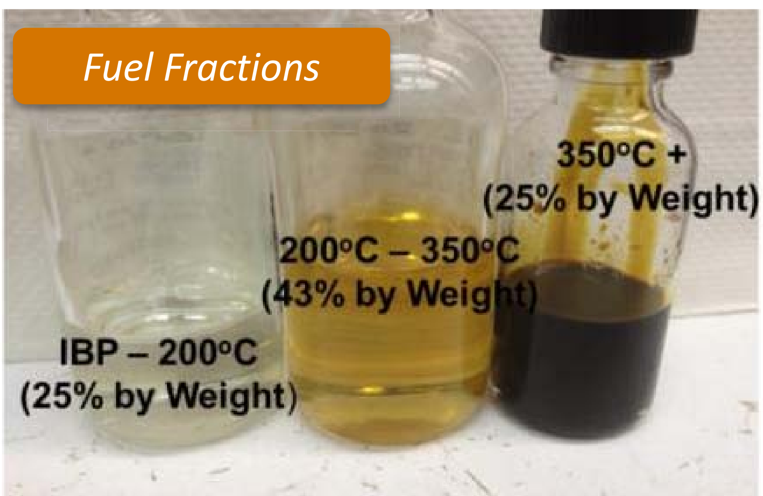

Slurry Feedstock

HTL

Biocrude Product

+

Aqueous Product (contains organics)



Bio oil product is refined via **Catalytic Hydrotreatment** and fractionated by **Distillation** to gasoline, diesel, jet fuel, and bottoms

Catalytic Hydrotreatment

Distillation

Hydrotreated Bio oil

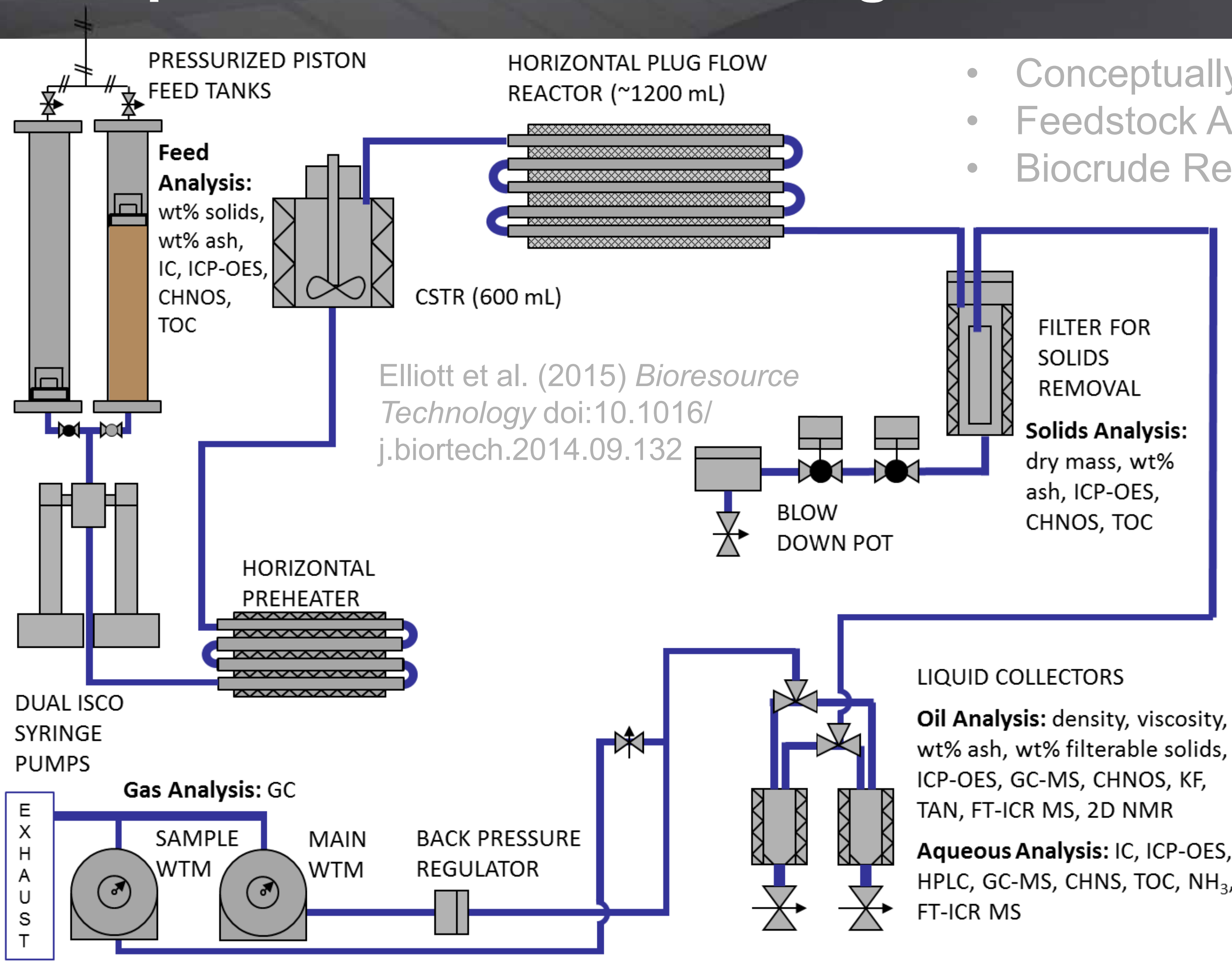
Product Description

- Oil product
  - water insoluble
  - viscosity -- fluid to cold flow
  - 25-50% mass yield, 50-70% carbon basis
  - some dissolved water
- Gas product
  - primarily carbon dioxide
  - 5-15% yield carbon basis
- Aqueous phase
  - acid components (salts)
  - some soluble organics
- Solid product
  - precipitated minerals
  - some carbon ~5%

Simplified Process Flow Diagram

Pacific Northwest NATIONAL LABORATORY
Proudly Operated by Battelle Since 1965

- Conceptually Simple
- Feedstock Agnostic
- Biocrude Readily Upgraded



Elliott et al. (2015) *Bioresource Technology* doi:10.1016/j.biortech.2014.09.132

HTL Lignocellulosic Feedstocks

Feed	Description	Solids Conc. [wt%]	Density [g/mL]
Cellulose	ARBOCEL® B800 water-insoluble cellulose (J Rettenmaier USA)	13.8	1.06
Wheat Straw	Wheat straw, 3/16 inch grind (Idaho National Laboratory)	12.7	1.03
Switchgrass	Alamo switchgrass, < #70 sieve (Oklahoma State University)	15.0	1.06
Wood	Catch Light Pine Forest Residue, <400 µm (Iowa State University)	15.2	1.04

Cellulose **Wheat Straw** **Switchgrass** **Wood**

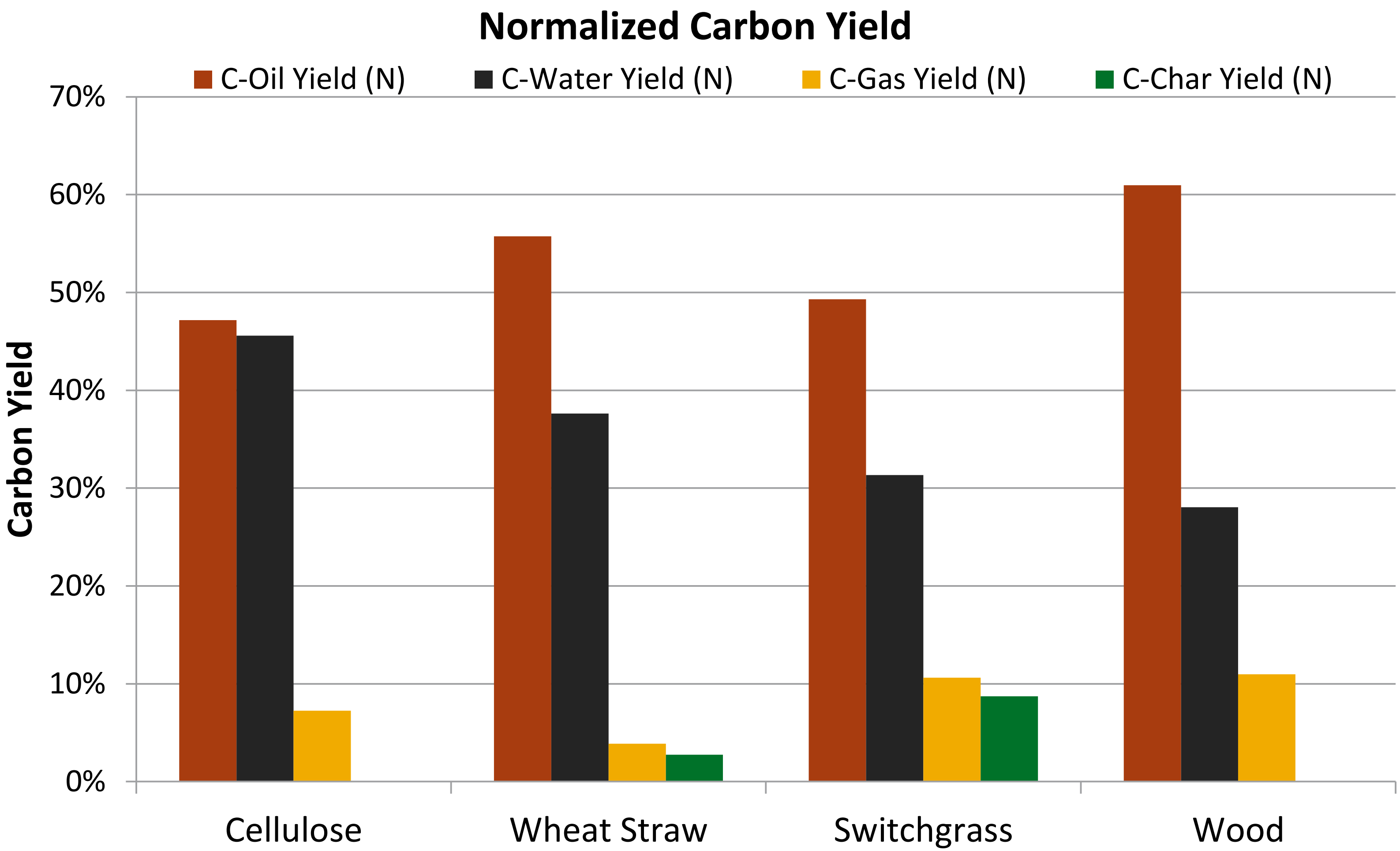
Experimental Conditions and Yields

- Steady state window duration: 2-3 hours
- Average temperature: 345-350°C
- Average pressure: 2900-2950 psig
- Slurry feed rate: 2.0 L/h (1.5 L/h wood)

	Cellulose	Wheat Straw	Switchgrass	Wood
Overall Mass Balance	99%	100%	98%	100%
Normalized Yields				
Oil Yield (dry basis) [g/g_{fd}]	22%	28%	31%	31%
Solid Yield [g/g_{fd}]	0%	1%	5%	0%
Gas Yield [g/g_{fd}]	10%	6%	18%	16%
Aqueous Yield [g/g_{fd}]*	69%	65%	46%	53%

*Aqueous yield calculated by difference

Carbon Balance and Yield



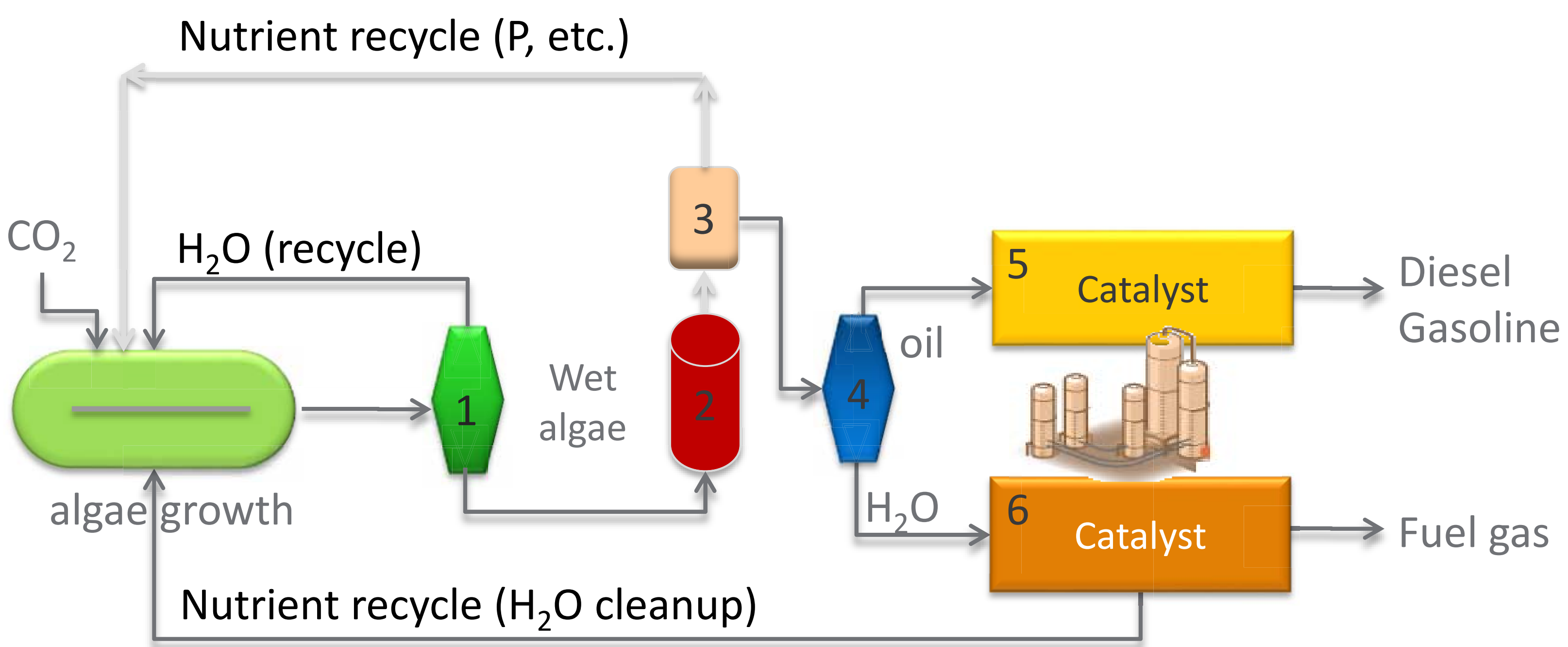
	Cellulose	Wheat Straw	Switchgrass	Wood
C-Balance	79%	85%	100%	76%
H-Balance	104%	106%	96%	105%
O-Balance	99%	100%	98%	101%

Biocrude Composition (Dry Basis)

Pacific Northwest NATIONAL LABORATORY
Proudly Operated by Battelle Since 1965

	Cellulose	Wheat Straw	Switchgrass	Wood
Carbon [wt%]	*88%*	83%	77%	78%
Hydrogen [wt%]	*7.9%*	8.5%	7.4%	7.7%
H:C [mol ratio]	*1.08*	1.21	1.14	1.18
HHV [MJ/kg, calc]	*39.5*	38.1	34.1	35.0
Oxygen [wt%]	*4.4%*	7.6%	14.4%	13.7%
Nitrogen [wt%]	0.0%	0.8%	0.9%	0.2%
Sulfur [wt%]	0.0%	0.1%	0.1%	0.0%
TAN [mg_{KOH}/g_{oil}]	33	33	43	50
Density [g/cm^3, 40°C]	1.09	1.09	1.10	1.13
Viscosity [cSt, 40°C]	1121	2443	5197	9370
Moisture [wt%, KF]	**17.0%**	8.9%	11.7%	7.9%
Ash [wt%]	0.09%	0.10%	0.23%	0.59%
Filterable Solids [wt%]	0.08%	0.10%	1.23%	0.04%

Hydrothermal Processing of Algae



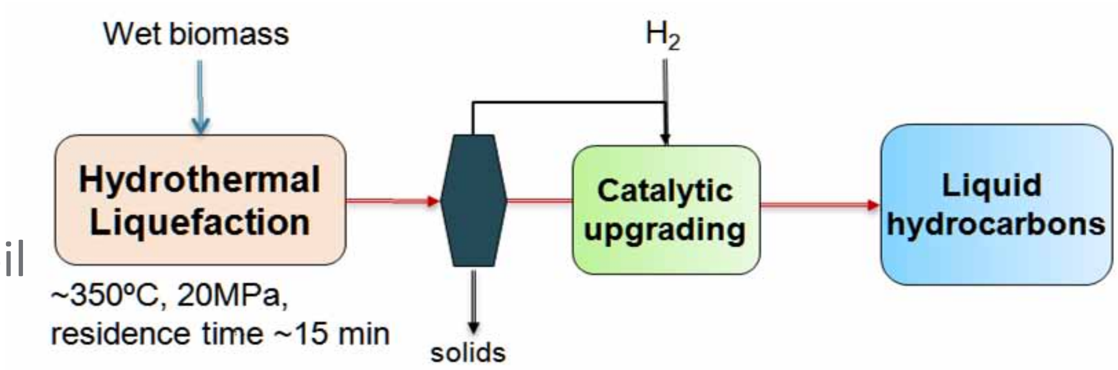
1. algae de-watered from 0.6 g/l to 100 g/L
2. hydrothermal liquefaction
3. solid precipitate separation for clean bio-oil production and phosphate capture
4. oil/water phase separate
5. oil hydrotreater to produce hydrocarbons—diesel/gasoline)
6. aqueous phase carbon is catalytically converted to fuel gas and nutrients recycled (N, K, some CO_2, etc)

Hydrothermal Liquefaction LEA or Whole Algae Biomass

Pacific Northwest NATIONAL LABORATORY
Proudly Operated by **Battelle** *Since 1965*

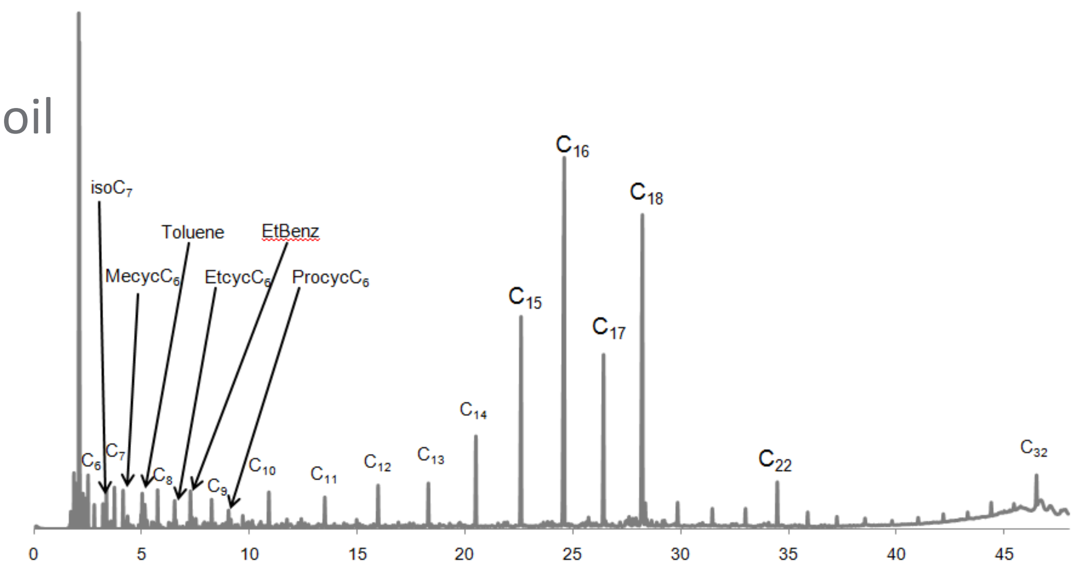
Liquefaction

- Converts wet algae slurry
- Condensed water phase
- Gravity separable bio-oil
- Low oxygen content of bio-oil (5 to 10 wt%)



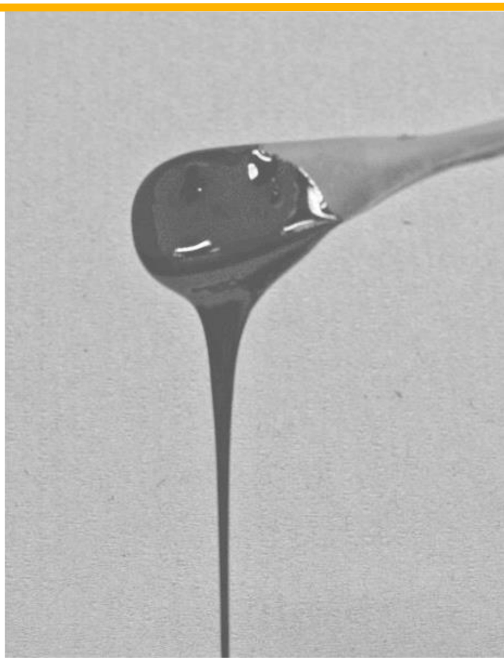
Upgrading via hydrotreatment

- Easy to hydrotreat, less H_2 required vs Fast Pyrolysis bio-oil
- Conventional hydrotreating catalyst
- Hydrotreated product = 90% volume of bio-oil
- Long chain hydrocarbons and small cyclics



HTL of Cellana Algae Summary
Nanno. Salina – low and high lipid versions

Parameter	Low lipid	High lipid
Space Velocity, L/L/h	2.2	2.2
Temperature, °C	350	348
Mass Balance	102%	97%
Total Carbon Balance	91%	96%
Oil Yield, Mass Basis (BD)	65%	64%
Oil Yield, Carbon Basis	81%	82%
Bio-Oil Composition, Dry Weight Basis		
Carbon, Wt%	77.0%	77.6%
Hydrogen, Wt%	10.4%	10.6%
Oxygen, Wt%	8.0%	7.2%
Nitrogen, Wt%	4.2%	4.0%
Sulfur, Wt%	0.3%	0.3%

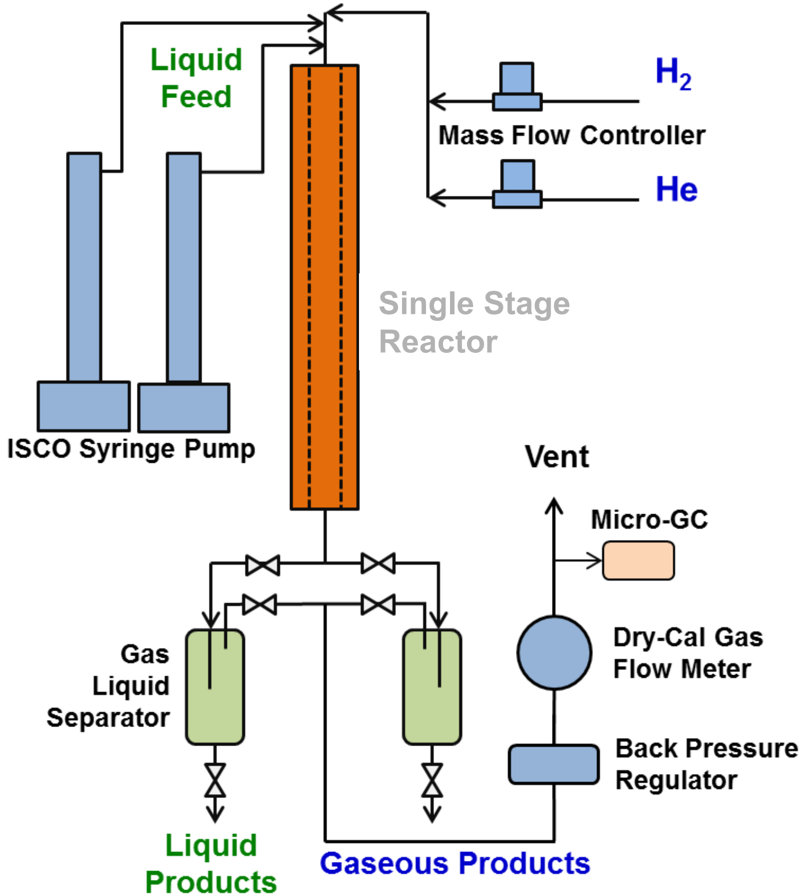


Density = 0.95 g/ml

Hydrotreating HTL Biocrude

HTL Biocrude

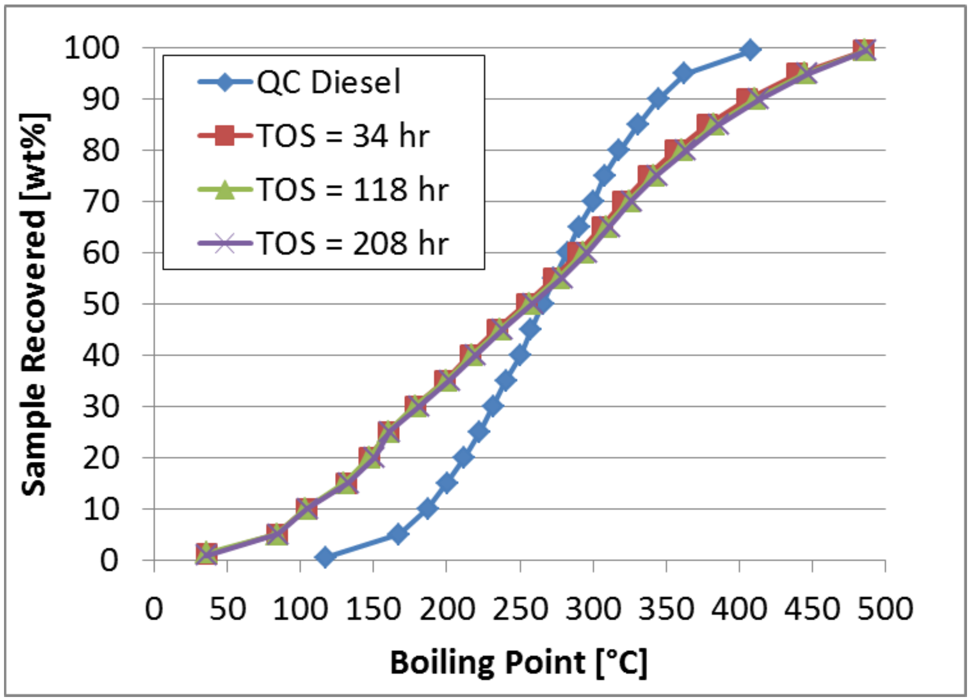
Hydrotreated Product



- Conversion and upgrading of HTL biocrudes
- Hydrotreating for O, S and N removal
- Hydrocracking/isomerization and distillation to finished fuel
- Reactor: 1.3 cm ID, 63.5 cm
- Typical operating conditions:
  - T=400°C, P=1500 psig
  - LHSV=0.15-0.25 h^{-1}
  - H_2 consumption=0.03-0.04 g $H_2/g_{dry\ feed}$
  - Commercial HT catalyst (sulfided CoMo)

Hydrotreated Biocrude

SimDis (ASTM D2887) Results from Hydrotreated HTL Biocrude from Pine Feedstock (>200 hours on stream)



~30% gasoline and ~50% diesel range

	Wood HTL Biocrude	Upgraded HT Product
LHSV (WHSV)		0.21 (0.32)
C [wt%]	78.6	88.80
H [wt%]	7.7	11.75
N [wt%]	0.16	<0.05
O [wt%]	13.5	0.86
S [wt%]†	<0.02	7 ppm
Density* [g/cm^3]	1.102	0.897
Viscosity* [cSt]	2109	4.34
Water [wt%, KF]	6.4	<0.9
TAN [mg KOH/g]	42.1	<0.01
H/C Atomic Ratio	1.2	1.58

†ASTM D4239 Biocrude, ASTM D5453 HT Product
*Biocrude at 40 C and HT Product at 20 C

Characterization of Biocrude and Product

Pacific Northwest NATIONAL LABORATORY
Proudly Operated by Battelle Since 1965

	Algae HTL Biocrude Feed	61573-62-2 TOS = 22.6 hr	61573-62-5 TOS = 58.6 hr	61573-62-8 TOS = 95.4 hr
LHSV (WHSV)			0.25 (0.34)	
Density (40°C), g/cm_3	0.987	0.777	0.781	0.784
Viscosity (40°C), cSt	243.8	1.3	1.4	1.4
C, wt% (Dry Basis)	80.4	86.5	86.6	87.0
H, wt% (Dry Basis)	9.4	15.1	15.0	14.9
N, wt% (Dry Basis)	4.9	<0.05	<0.05	<0.05
O, wt% (Dry Basis)	2.9	0.7	1.0	0.9
S, ppm (ASTM D5453)		34	24	15
H/C Atomic Ratio (Dry Basis)	1.4	2.1	2.1	2.1

Fractional Distillation Results and Yields

Pacific Northwest NATIONAL LABORATORY
Proudly Operated by Battelle Since 1965

All TOS samples from algae HTL hydrotreating test were composited for lab scale fractional distillation.

Batch Distillation Temperature Range, °C	Yield, wt%	Fraction	Density*, g/cc	Viscosity*, cSt
20-150	23.98	naptha/gasoline	0.7083	0.3524
150-265	29.77	jet	0.8046	1.6013
150-350**	68.22	jet+diesel	0.8215	3.3727
265-350	38.45	diesel	0.8337	6.5113
>350	7.78	bottoms/wax	0.8874	28.978

*** fuel cut data at 20°C, bottoms/wax data at 40°C **jet and diesel fractions recombined after distillation**

**The jet and diesel fractions were combined to produce a conventional mid-distillate for evaluation as a drop-in commercial diesel product, the jet+diesel sample with boiling point range of 150-350 C.

Analytical Data for Distillate Fractions

Pacific Northwest NATIONAL LABORATORY
Proudly Operated by Battelle Since 1965

Hydrotreated Algae HTL Biocrude Off-site Analytical	C	H	N	O	TAN	KF
61573-62-D1 Naphtha	83.23	13.75	<0.05	0.87	<0.01	<0.5
Duplicate	83.59	14.54	<0.05	0.73	<0.01	<0.5
61573-62-D2 Jet	86.05	14.14	0.1	0.55	<0.01	<0.5
Duplicate	86.33	13.2	0.12	0.64	<0.01	<0.5
61573-62-D3 Diesel	85.58	13.77	0.14	0.96	<0.01	<0.5
Duplicate	85.21	13.89	0.15	0.72	<0.01	<0.5
61573-62-D4 Bottoms	87.44	12.78	0.3	0.84	<0.01	<0.5
Duplicate	87.5	12.82	0.31	1.01	<0.01	<0.5

Fuel Property Characterization Data

Hydrotreated Algae HTL Biocrude Sample ID#	Fraction	Sulfur ASTM D5453 (ppm)	Flash Point (micro-cup) °C	Cloud Pt ASTM D5773 (°C)	Pour Pt ASTM D5949 (°C)	Freezing Pt ASTM D5972 (°C)	Cetane
61573-62-D1	naphtha/gasoline	18.1					
61573-62-D2	jet	12.6	49.5	-41.6	-48	-36.9	
61573-62-D3	diesel	9.4		3.2	3	4.2	58.7
61573-62-D2+D3	jet + diesel	7.8	TBD	-8	-9	-7.5	50.8
61573-62-D4	bottoms/wax*	NA	NA	NA	NA	NA	NA

*The bottoms/wax are semi-solid at room temperature.

Catalytic Hydrothermal Gasification

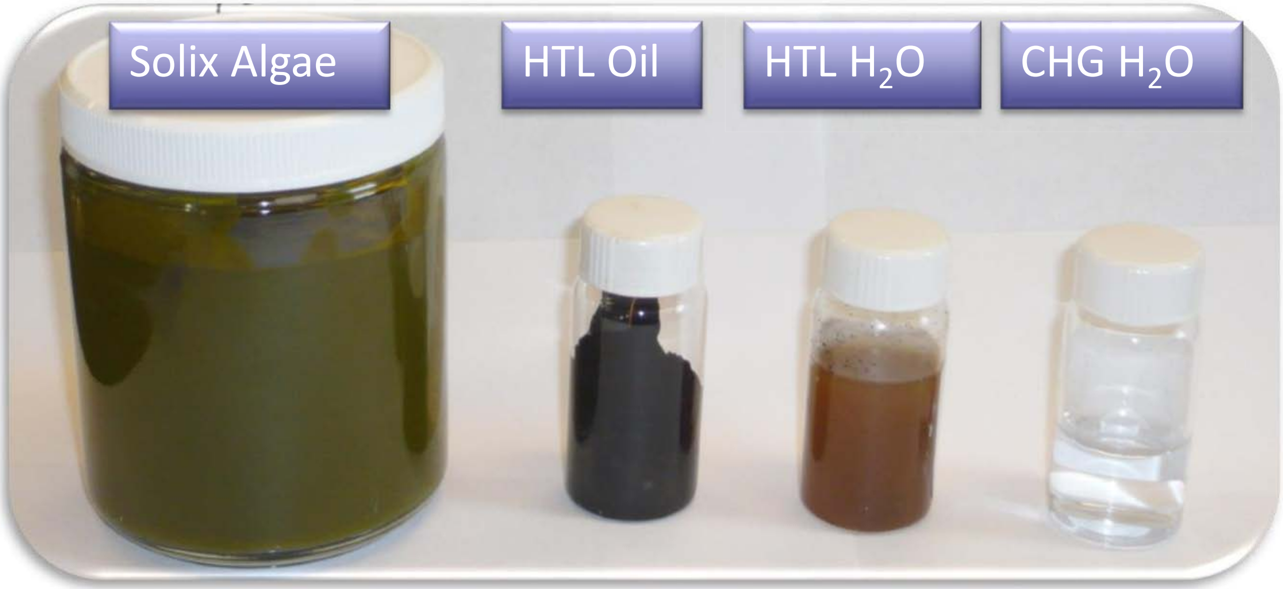
Pacific Northwest NATIONAL LABORATORY
Proudly Operated by Battelle Since 1965

Description of CHG

- "Sister technology" to Hydrothermal Liquefaction (HTL)
- Can be used on any organic rich aqueous stream
- Produces methane gas rather than oil (catalytic action)
- Compact means to do "digestion" providing a fuel gas (CH_4/CO_2) without residual sludge
- Provides potential to recycle nutrients in biomass

Samples of Materials

NAABB National Alliance For Advanced Biofuels and Bio-products



Partner

Genifuel

Scale-Up and Technology Transfer

- Updated comparison between HTL and pyrolysis (feedstock → upgraded fuel) shows comparable economics
- FLC Award for Excellence in Technology Transfer (2015)
- Engineering challenges include slurry pumping, efficient separations, and heat integration
- Third party assessment by the Harris Group
  http://www.nrel.gov/docs/fy14osti/60462.pdf

Jim Oyler with the 1000 L/day (20 wt% BDAF) continuous HTL/CHG system for algal feedstock; NAABB-Reliance-PNNL-Genifuel Hydrothermal System 2014. Genifuel is a PNNL licensee.

Scaled-up Catalytic Hydrotreater

- 9-zone fixed-bed catalytic hydrotreater (19 L)
- Atmospheric distilling column for fuel fraction collection

Current and Future Work

- Algal biomass: all types
- Wet waste: grape pomace, beet tailings, waste-water treatment sludge
- Design and build 12 L/h engineering scale reactor system skid fabrication underway
  - Delivery expected May 2016
  - Operational testing expected August 2016
- Enhanced recovery of organics from aqueous phase – TEA indicates that process economics are most sensitive to this variable
- Longer-term demonstrations of HT catalyst activity and stability (>200 hr)
- Optimize fuel finishing to meet refinery insertion points

Conclusions

- PNNL has demonstrated a **continuous** HTL process that converts a biomass slurry into a **gravity-separable** biocrude that can be upgraded by **single-step hydrotreatment** to liquid fuel-range hydrocarbons

- Biocrude can be produced from **many different feeds** including lignocellulosic, micro- and macro-algal biomass, wet food wastes, and wastewater treatment sludges

- This process has commercialization potential

Acknowledgements

Pacific Northwest NATIONAL LABORATORY
Proudly Operated by **Battelle** *Since 1965*

Much of the work summarized here was supported by U.S. Department of Energy's Bioenergy Technologies Office (BETO).

PNNL Team members are many, including

Andy Schmidt, Rich Hallen, Dan Anderson, Justin Billing, Karl Albrecht, and John Holladay.

The dedicated support from Todd Hart and Gary Maupin on feed preparation, HTL system design, and operations, including CHG tests, has been invaluable.

Hydrotreating operations include Huamin Wang, Daniel Santosa, Gary Neuenschwander, LJ Rotness, and Mariefel Olarte.

Teresa Lemmon, Marie Swita, and Beth Hofstad have provided outstanding sample analysis.

Technoeconomic assessments and life-cycle analysis is lead by Sue Jones with support from Yunhua Zhu, Lesley Snowden-Swan and Rick Skaggs' team.

Many thanks to our commercial partners and collaborators!

Genifuel

WERF
Water Environment Research Foundation
Collaboration. Innovation. Results.

metrovancouver

leidos

Summary

The creation of a robust, next -generation domestic bioenergy industry is one of the important pathways for providing Americans with sustainable, renewable energy alternatives. Through research, development, and commercialization to produce renewable fuels and products sustainably and affordably, we can provide home-grown alternatives for the transportation, energy, and bioproducts sectors.

U.S. DEPARTMENT OF ENERGY | Energy Efficiency & Renewable Energy

Questions?

Email eere_bioenergy@ee.doe.gov

Please include “R&D 100 Webinar” in the Subject Line

Thank you!