Biogas to Liquid Fuels and Chemicals Using a Methanotrophic Microorganism

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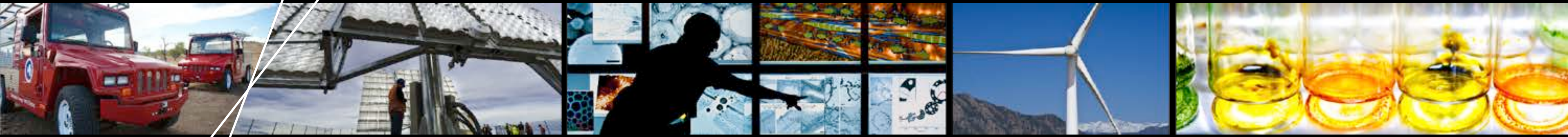


2015 DOE BioEnergy Technologies Office (BETO)
Project Peer Review
March 24, 2015

Technology Area: Biochemical Conversion
Principal Investigator: Michael T. Guarnieri
Organization: National Renewable Energy Laboratory

Goal Statement

Background

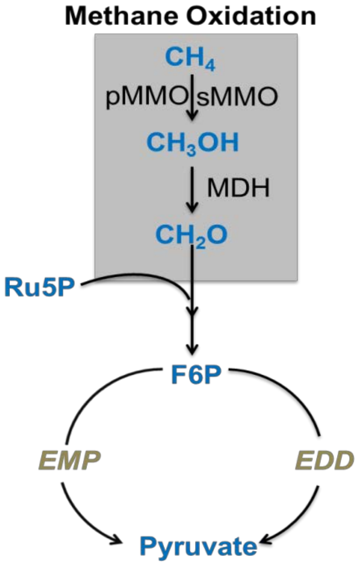
- Methane-rich biogas offers a renewable alternative to natural gas as a feedstock and intermediate in bioprocesses
- Relevant to EERE's MYPP for developing cost-effective, integrated waste-to-energy processes for the production of bioproducts and advanced biofuels.

Goals

1. Demonstrate proof of concept for a biogas-to-liquid fuels and chemicals process.
2. Enhance carbon conversion efficiency from methane to biomass and products.

Outcome: Alternative, renewable path to hydrocarbon fuels and chemicals.

- Enables the sustainable, nationwide production of biofuels that are compatible with transportation infrastructure.
- Displaces a share of petroleum-derived fuels to reduce U.S. dependence on oil.
- Encourages the creation of a new domestic bioenergy industry.



Quad Chart Overview

Timeline

- **Project start date:** March, 2014
- **Project end date:** September, 2015
- **Percent complete:** 66%

Barriers

- Bt-J: Catalyst Development
- Bt-K: Biochemical Conversion Process Integration
- Bt-L: Biochemical/ Thermochemical Interface

Budget

	Total Costs FY 10 –FY 12	FY 13 Costs	FY 14 Costs	Total Planned Funding (FY 15-Project End Date
DOE Funded	-	-	$137, 586	$262,414

Partners & Synergistic Activity

- **University of Washington**: subcontract to aid in metabolic flux balance analyses.
- **ARPA-E:** NREL, Lanzatech, Johnson Matthey, UW
- **Waste-to-Energy TEA:** NREL, ANL
- **BCU FOA:** NCSU, UW, Farmatic, Inc., Metabolon, Inc.

Project Overview

- **History:** This seed project represents the first biogas upgrading project at NREL. A related ARPA-E project exploring biological upgrading of natural gas was initiated in FY13.

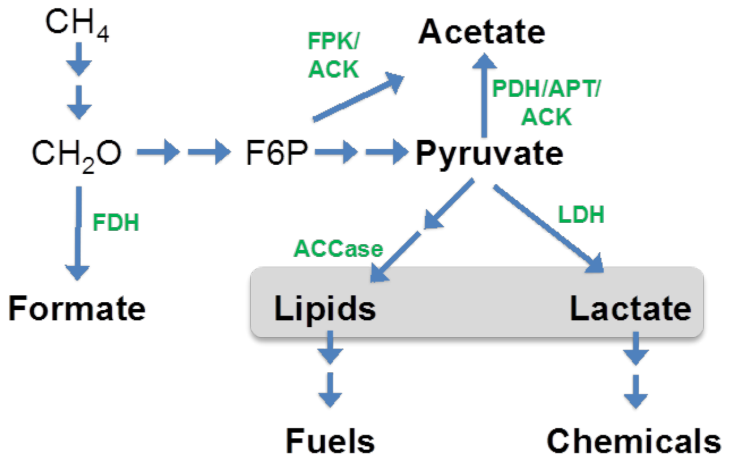
- **Context:** "*There is a significant near-term market entry opportunity to deploy waste-to-energy technologies in the U.S.*" *
  - MSW, Landfill gas, Agriculture and WWTP waste streams represent poorly valorized domestic feedstocks
  - Biogas expands the suite of products from biorefineries, municipalities, and agricultural operations: potential to increase revenue & reduce GHG emissions.
  - Enables biological gas-to-liquids conversion and upgrading for integration with existing transportation infrastructure
  - Enables a national network of distributed power and biofuel production sites

- **Objective:** Develop a viable path for biocatalysis of biogas-derived methane to fuels and chemicals.

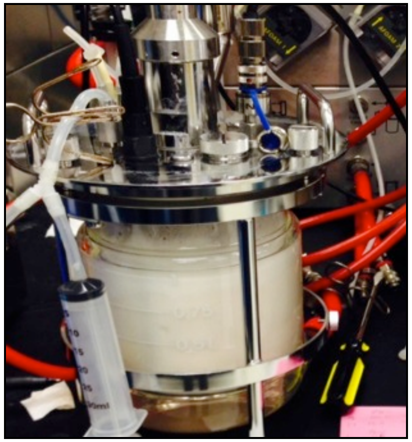
*Male, J. *Waste to Energy (*2014) http://energy.gov/sites/prod/files/2014/11/f19/male_waste_to_energy_2014.pdf

Technical Approach

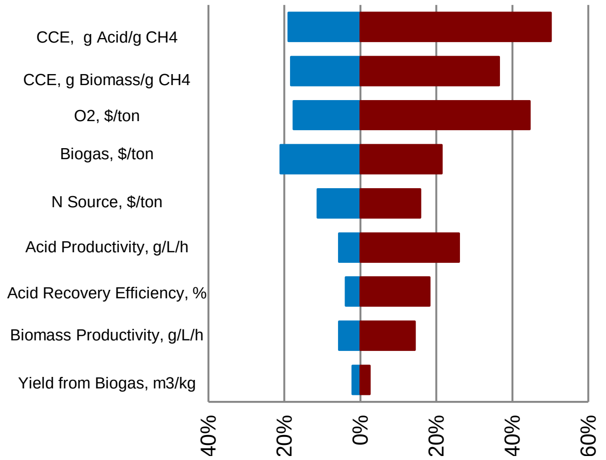
Development of Genetic Tools & Metabolic Engineering Strategy



Gas Fermentation Optimization



Techno-economic Analysis

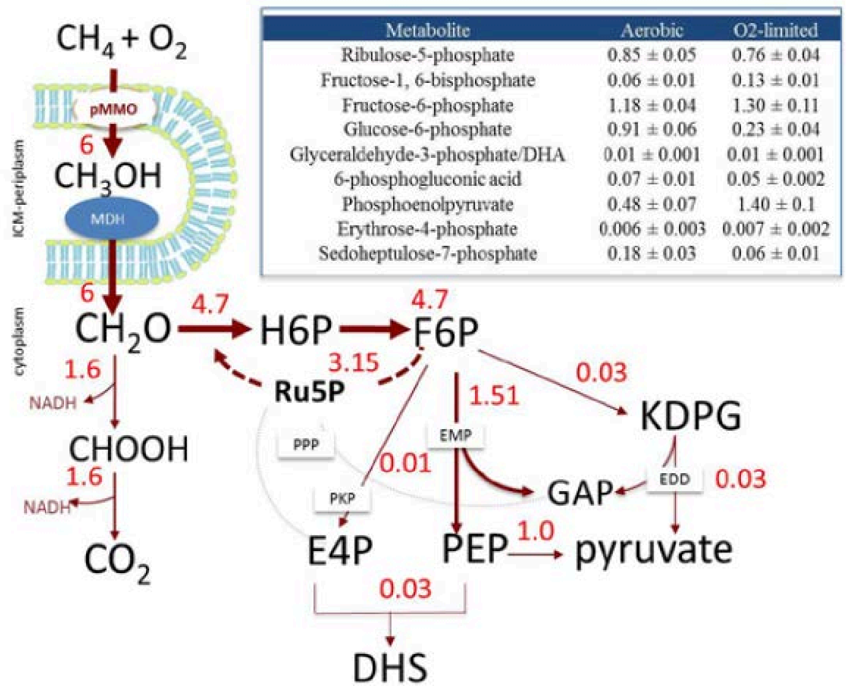


- **Approach:** Employ pathway analyses, fermentation engineering, and techno-economic analyses to inform hypothesis-driven strain-engineering strategies.

- **Major challenges and success factors:** (**i**) development of methanotroph genetic tools, (**ii**) gas mass transfer (fermentation optimization), (**iii**) carbon conversion efficiency enhancements, and (**iv**) productivity enhancements (biomass and products)

Management Approach

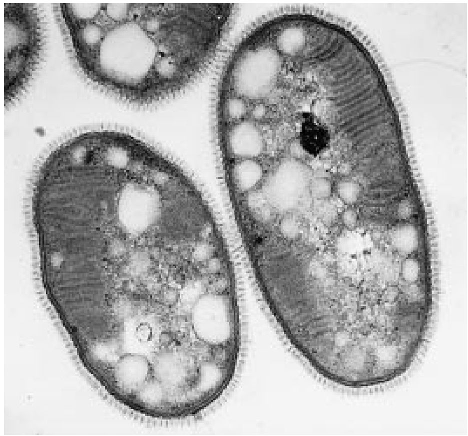
- Staffing includes molecular and microbiologists, fermentation and chemical engineers, and analytical chemists
- Research guided by TEA, with related quarterly, metrified milestones
- Monthly group meetings, regular interaction with technical staff
- Synergistic interaction between Biogas seed project, ARPA-E natural gas upgrading project, and Univ. of Washington (subcontract: flux balance analysis)

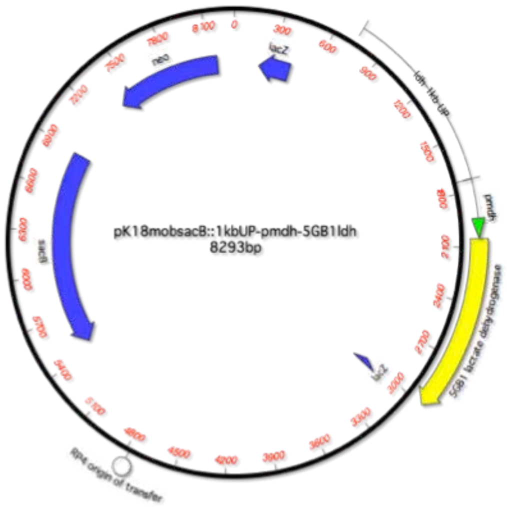


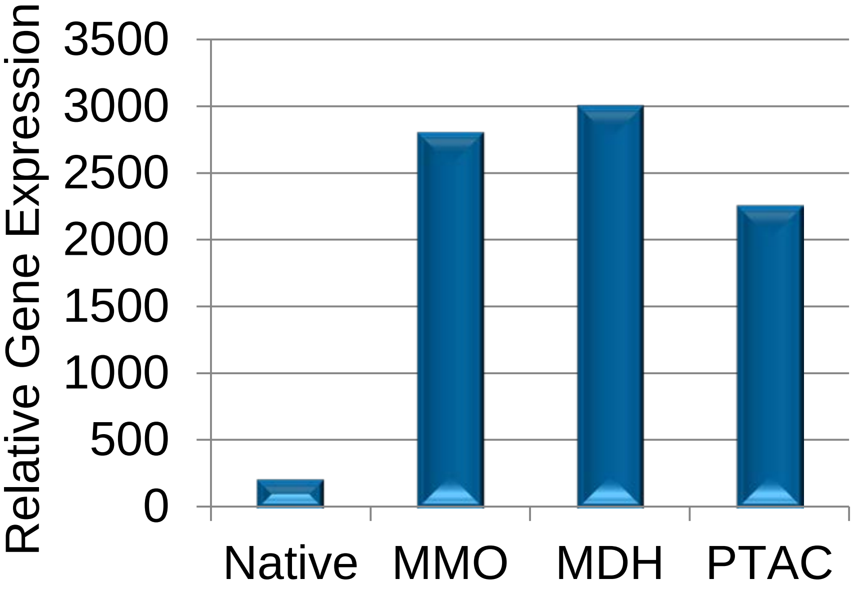
Metabolite	Aerobic	O2-limited
Ribulose-5-phosphate	0.85 ± 0.05	0.76 ± 0.04
Fructose-1, 6-bisphosphate	0.06 ± 0.01	0.13 ± 0.01
Fructose-6-phosphate	1.18 ± 0.04	1.30 ± 0.11
Glucose-6-phosphate	0.91 ± 0.06	0.23 ± 0.04
Glyceraldehyde-3-phosphate/DHA	0.01 ± 0.001	0.01 ± 0.001
6-phosphogluconic acid	0.07 ± 0.01	0.05 ± 0.002
Phosphoenolpyruvate	0.48 ± 0.07	1.40 ± 0.1
Erythrose-4-phosphate	0.006 ± 0.003	0.007 ± 0.002
Sedoheptulose-7-phosphate	0.18 ± 0.03	0.06 ± 0.01

Results: Development of Genetic Tools

- Unexplored genus at NREL, requiring genetic toolbox development
- Developed both replicative and integrative plasmids for constitutive and inducible gene expression and knockout in a methanotroph.
- Constitutive, strong promoters have been isolated and successfully employed for overexpression of target genes.
- Inducible operators have been identified for temporal regulation of gene expression.

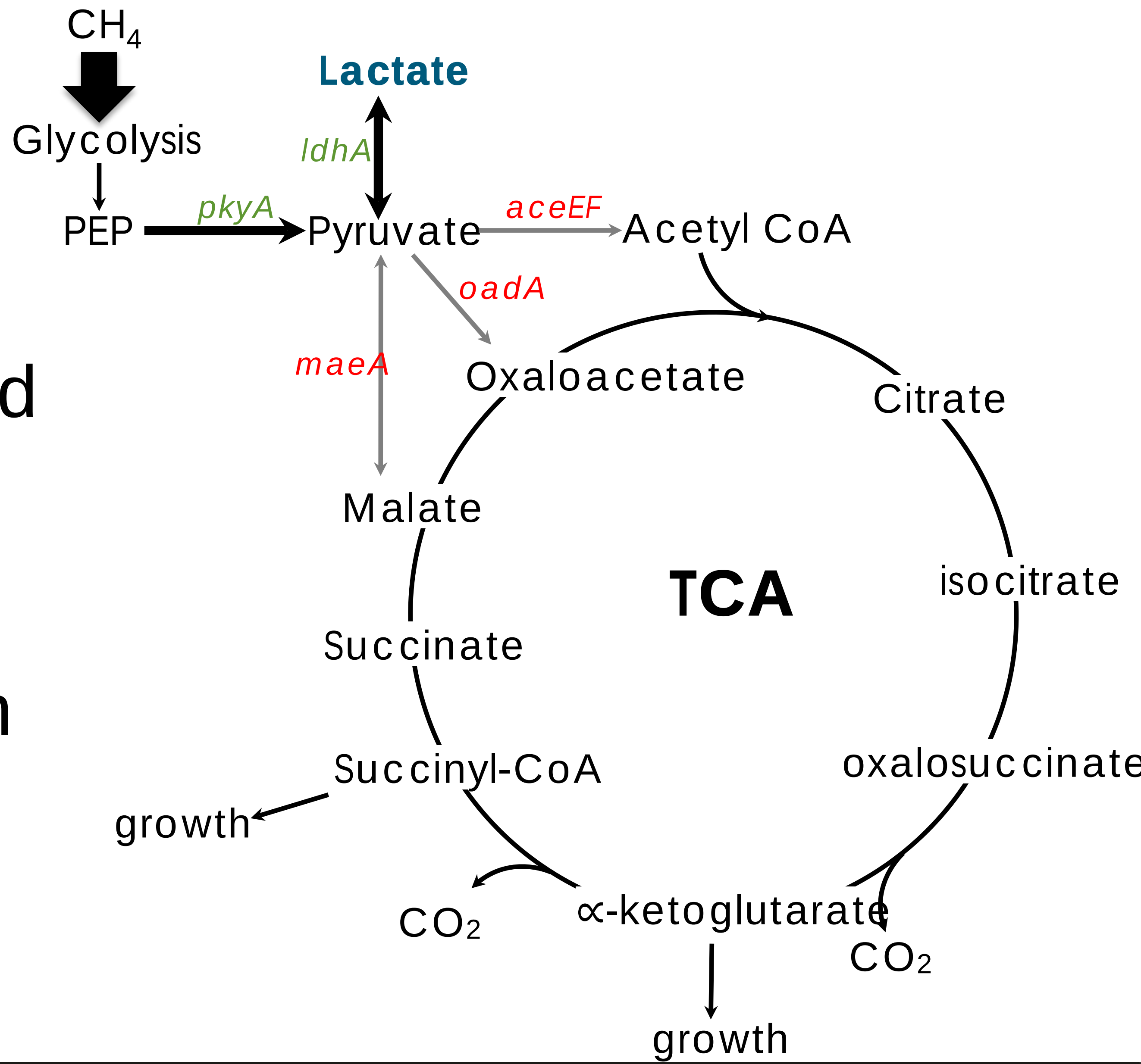






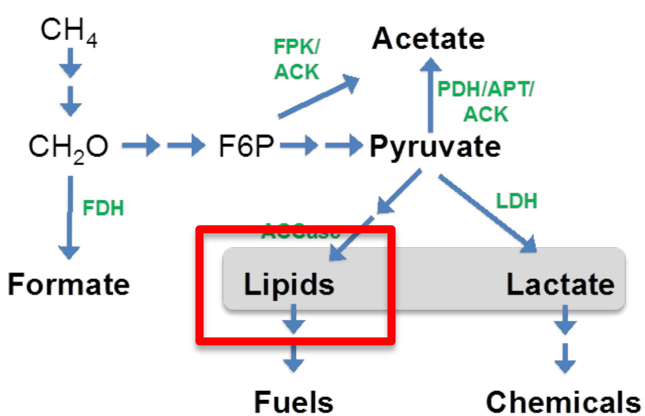
Results: Development of Genetic Engineering Strategy

- *In-silico* analyses and literature review defined initial targets for strain-engineering, as reported in FY14, Q1 Milestone report.

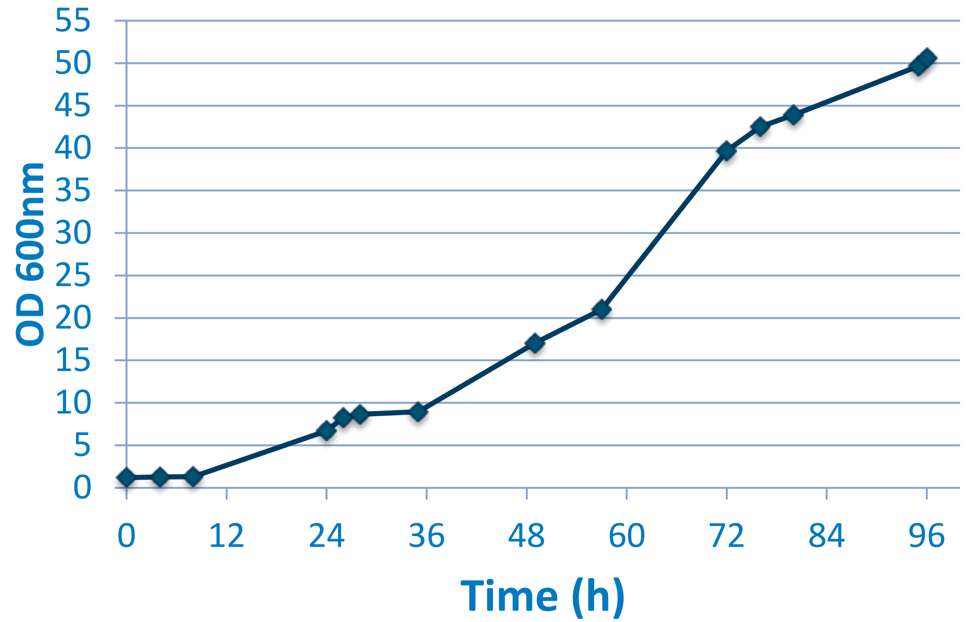


Results: Fermentation Optimization and Lipid Accumulation

- Genetically engineered a methanotrophic bacterium to produce <u>lactic acid and lipids from C1 substrates</u> (methane and methanol).
  - Proof-of-concept for a methane-to-lactate and lipids bioprocess



Lipid extraction and upgrading successfully developed via ARPA-E

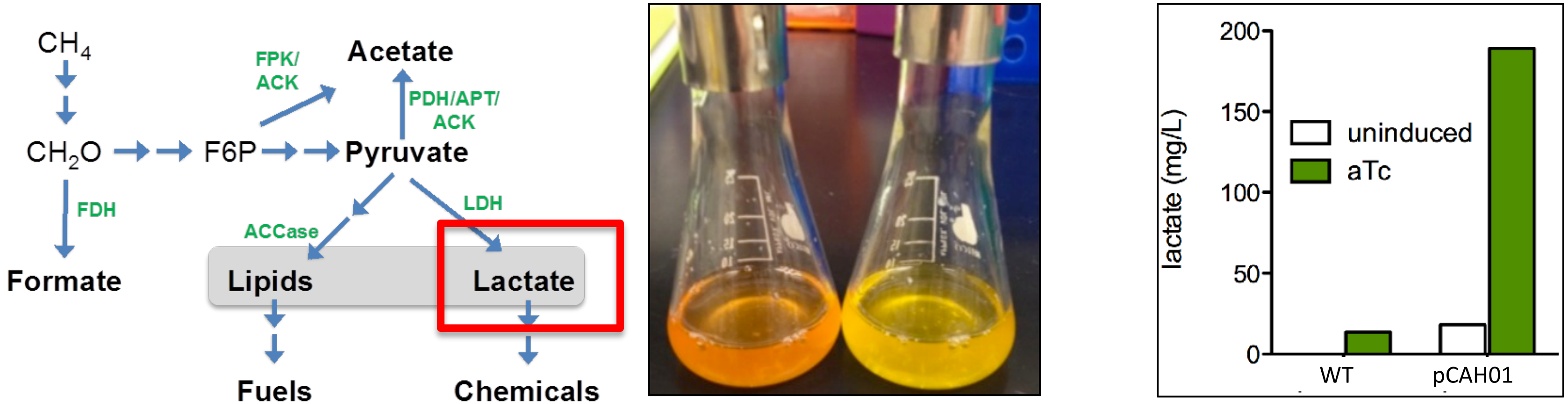


Fatty Acid Speciation

Sample	Biomass, g/L	C14:0	C15:0	C16:0	C16:1n9	C16:1n7	C16:1n6	C16:1n5	Total FAME
@96h	12.4	6.98	1.12	12.46	26.22	24.26	23.43	4.93	**10.7%**

Results: Metabolic Engineering for Fuel & Chemical Biosynthesis

- Genetically engineered a methanotrophic bacterium to produce lipids and lactate from C1 substrates (methane and methanol).
    - Proof-of-concept for a methane-to-fuels and chemical bioprocess

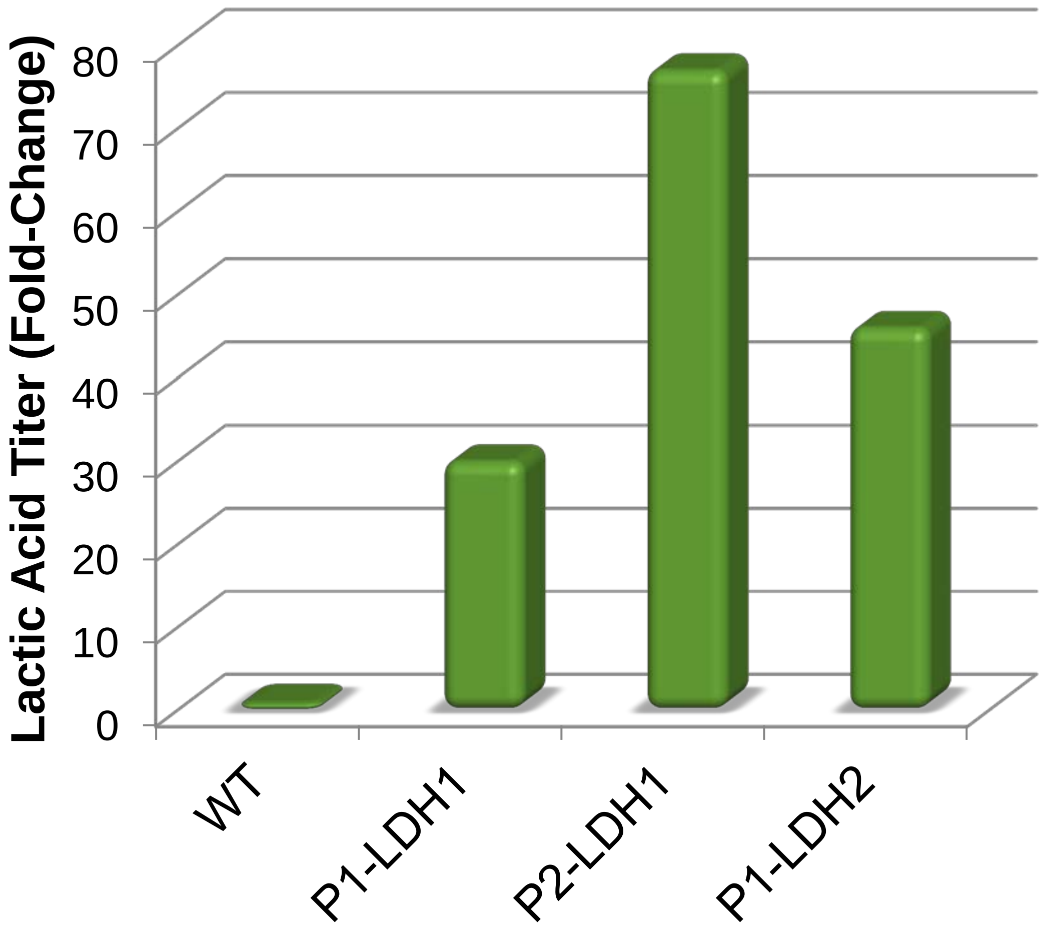


(A) Pathway targets for strain-engineering **(B)** Colorimetric detection of lactate in culture broth of wild-type (left) and lactate dehydrogenase-overexpressing strain (right). **(C)** HPLC quantitation of inducible lactate biosynthesis in wild-type (left) and lactate dehydrogenase-overexpressing methanotroph (right).

- **Lipid content unaltered by lactic acid production**

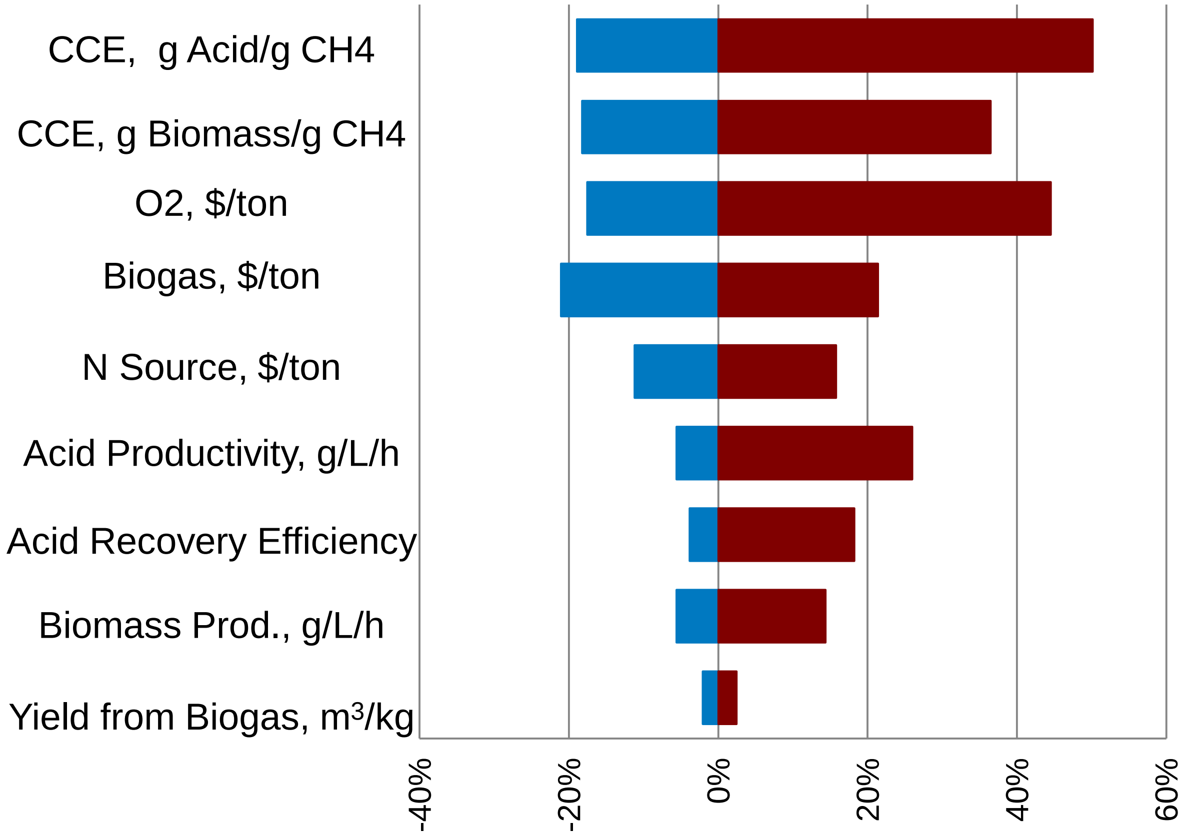
Results: *In vitro* and *in vivo* LDH Analyses

- An array of LDH and promoter-LDH combinations were examined to define optimal pairings for methanotrophic lactate production.
- **Enhanced LA production nearly two orders of magnitude over wild-type background**
  - Exceeded Y1 Go/No-Go Milestone
- Subject of ROI # 15-21: Methane biocatalysis for production of lactic acid.



Results: Techno-economic Analysis

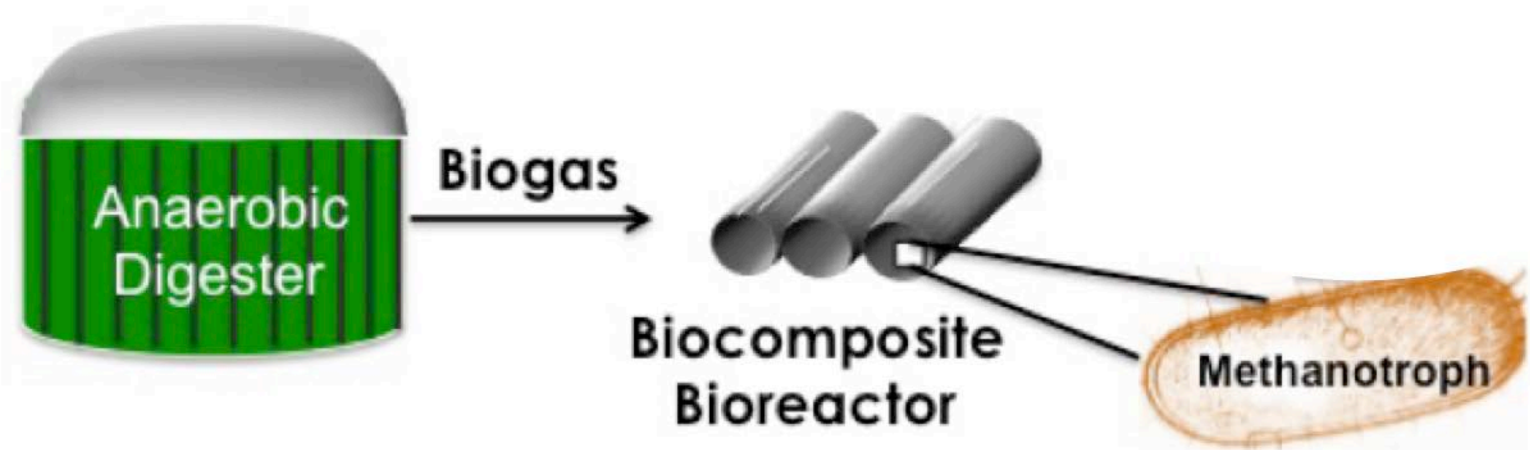
- TEA for an integrated bioprocess identified a viable biogas-to-fuels and chemicals production route
- Capital cost (CAPEX) and operation (OPEX) cost examined using key parameters and process assumptions, including
  - plant capacity
  - yields of core technologies
  - raw material and chemical costs
- Preliminary analyses indicate **carbon conversion efficiency** and **gas feed costs** will be primary cost drivers in the development of viable biogas-to-fuels and chemicals processes



TEA for ARPA-E encouraging for natural gas upgrading process exclusively targeting fuels: use of biogas and coproduct credit will contribute greatly to favorable TEA and commercial success.

2014 Biochemical Upgrading FOA

- Proof-of-concept data from seed project contributed to successful application selected under the BETO Biochemical Upgrading FOA (DE-FOA-0001085), targeting biocatalysis of methane to high-value co-products and novel bioreactor design.



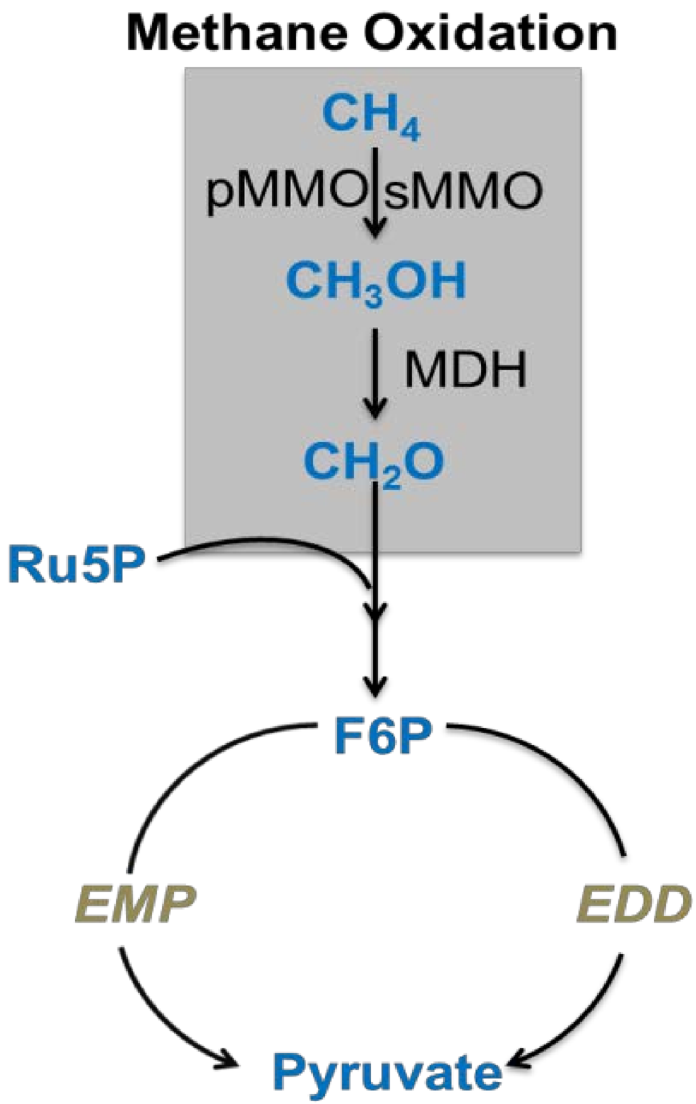
Relevance

- Relevant to EERE's MYPP for developing **cost-effective, integrated waste-to-energy processes** for the production of bioproducts and advanced biofuels.
- **Specifically targets identified MYPP barriers,** including*:* Catalyst Development, Biochemical Conversion Process Integration, and Biochemical/ Thermochemical Interface.
- Genetic tools, biocatalysts, and TEA directly advance emerging biogas and bioenergy industry.
- Represents proof-of-concept for an array of methane biocatalysis strategies, opening the door for feedstock expansion in an emerging bioeconomy and creation of a new domestic bioenergy industry.
- **Tech transfer/marketability:** A number of commercial entities (including partners Lanzatech and Farmatic) are currently exploring biogas generation and methane upgrading technologies.

Future Work

- **Enhance flux from methane towards biomass and bioproducts**
  - Identification of flux bottlenecks via metabolomic and transcriptomic analyses
  - Strain-engineering enhancements targeting overexpression of methane oxidation genes
  - Strain-engineering enhancements targeting knockout of competitive fermentative pathways

- **FY15 Key Milestone:** >10% CCE Enhancement



Summary

- An array of biogas feedstocks with high-volume methane potential offer a versatile, renewable alternative to natural gas.
- Methanotrophic biocatalysis offers a promising path towards valorization of biogas derived from anaerobic digestion of waste streams.
- Metabolic routes and strain-engineering strategies targeting enhanced biosynthesis of fuels and high-value chemicals have been established in a methanotrophic bacterium.
  - Represents first demonstration of biological CH_4-to-fuels and chemicals
- Preliminary TEA has identified a viable commercial path
  - Sensitivity analyses indicate carbon conversion efficiency and gas feed costs will be primary cost drivers in the development of viable biogas-to-fuels and chemicals processes.
- Enhanced genetic tool development and fermentation optimization will be pursued in FY15, targeting improvements in carbon conversion efficiency from methane to biomass and products.

Acknowledgements

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Additional Slides

Publications, Patents, Presentations, Awards, and Commercialization

- **Presented work at 2014 SIMB Annual Meeting: "**Biogas to Liquid Fuels and Chemicals Using a Methanotrophic Microorganism."
- **Manuscripts in Preparation** related to biogas biocatalysis to fuels and chemicals and TEA related to the conceptual integrated bioprocess.
- **ROI:** "Methane biocatalysis to lactic acid."