Biogas to Liquid Fuels and Chemicals Using a Methanotrophic Microorganism
WBS 2.3.2.102

2017 DOE BioEnergy Technologies Office
Project Peer Review
March 7, 2017

Technology Area: Waste-to-Energy
Principal Investigator: Mike Guarnieri
Organization: National Renewable Energy Laboratory

Goal Statement

- **Project Goals:**
  - Develop an economically viable biological approach to convert biogas-derived methane to hydrocarbon fuels and platform chemicals.
  - Enhance biological carbon conversion efficiency via TEA-informed metabolic and fermentation engineering strategies.

- **Outcome:** Generation of carbon-efficient methanotrophic biocatalysts, with enhanced methane conversion and yield to products.

- **Relevance to Bioenergy Industry:**
  - CH_4 is the 1^o component of natural gas and AD-derived biogas
  - > 4Quad btu can be generated from biogas domestically
  - Gaseous state of CH_4 prevents facile integration with transportation and industrial infrastructure.
  - Biological GTL offers a scalable, modular, and selective approach to CH_4 conversion.
    - Superior economies of scale vs. conventional conversion routes.

Quad Chart Overview

Timeline

- Project start date: October, 2015
- Project end date: September, 2018
- Percent complete: 50%

Barriers

- Bt-J: Catalyst Development
  - *Development of carbon-efficient methanotrophic biocatalysts*
- BETO WTE Assessment
  - *Enhanced CH4 activation; complete biogas utilization.*

Budget

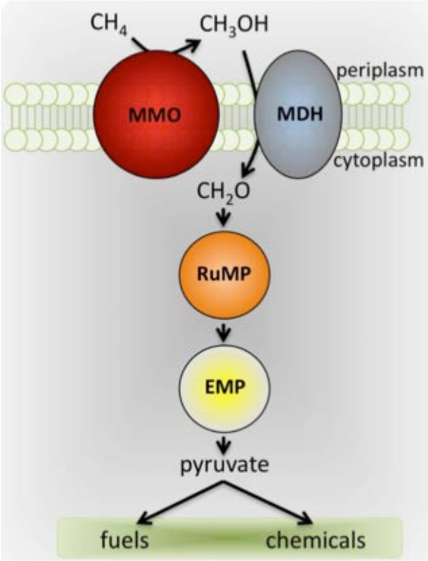
Total Costs FY12 –FY 14	FY 15 Costs	FY 16 Costs	Total Planned Funding (FY 17->End Date)
$150,000	$250,000	$250,000	$900,000

Partners & Synergistic Activity

- **Strategic Analysis Support/Strategic TEA:** NREL
- **BCU FOA:** NCSU, SDSU, Farmatic, Inc., Metabolon, Inc.
- **Alberta Innovates:** Univ. Alberta, Mango Materials
- **San Diego State University**: subcontract to aid in metabolic flux balance analyses.

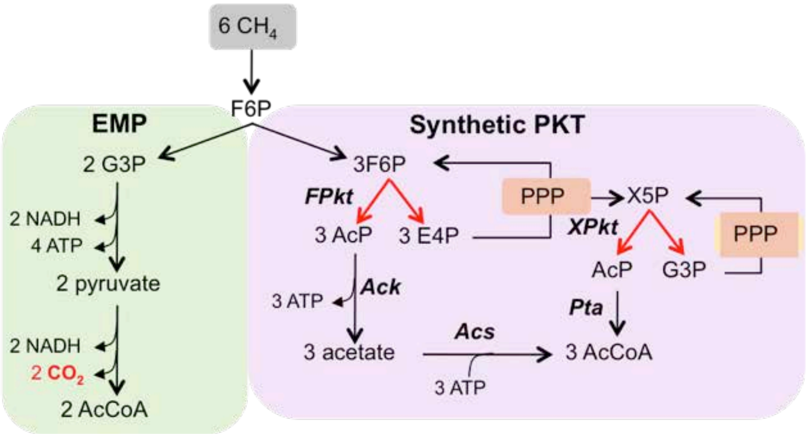
Project Overview

- **Context:**
  - This project represents the first biogas conversion project at NREL. A related ARPA-E project exploring biological upgrading of natural gas was initiated in FY13 and a BETO-funded BCU FOA was awarded in FY15.
    - ARPA-E laid the foundation for conversion of methanotrophic lipids to hydrocarbon fuels.
  - Following conversion from seed AOP to full AOP, this project underwent a TEA-driven re-scope to focus on process barriers, including enhanced CCE.
- **Objective:** Develop an economically-viable platform for biological methane conversion to fuel and chemical intermediates.
  - Develop a carbon-efficient methanotrophic biocatalyst.
- **Specific Project Goals:**
  - o Enhance yield from CH_4 to fuel intermediates to >1g/g.
  - o Improve CH_4 uptake rate >20% via ferm and strain engineering.
  - o Develop an industrially-relevant methanotroph culture collection.
  - o Evaluate limitations in mass transfer vs. methane oxidation.
  - o Target complete biogas utilization.
  - o Establish viable high-value co-product targets.

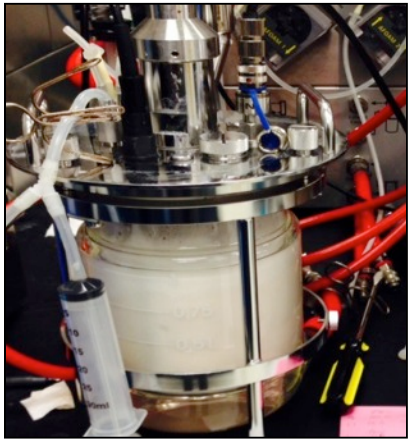


Management Approach

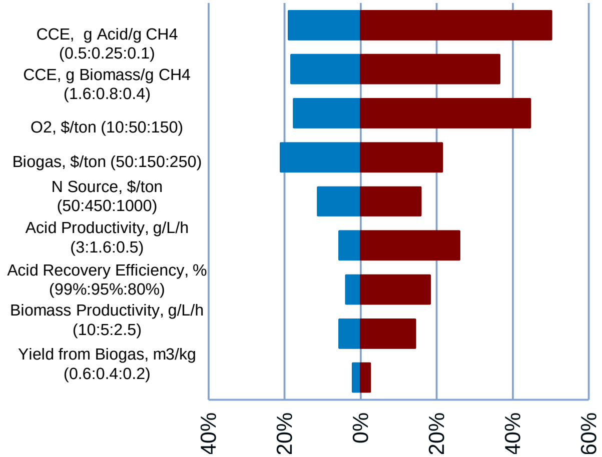
Genetic Tool Development, Metabolic & Protein Engineering



Gas Fermentation Optimization



Techno-economic Analysis



- Staffing includes:
  - **Molecular & microbiologists:** conduct pathway analyses, strain/protein engineering
  - **Fermentation engineers:** gas fermentation optimization
  - **Chem. & Process Engineers:** techno-economic analysis
- Research guided by TEA, with related quarterly milestone metrics.
- Monthly group meetings, quarterly WTE meetings, regular interaction with BETO and technical staff.
- Synergistic interaction between Biogas AOP, BCU FOA, NREL Strategic Analysis/WTE program, and related external activities.

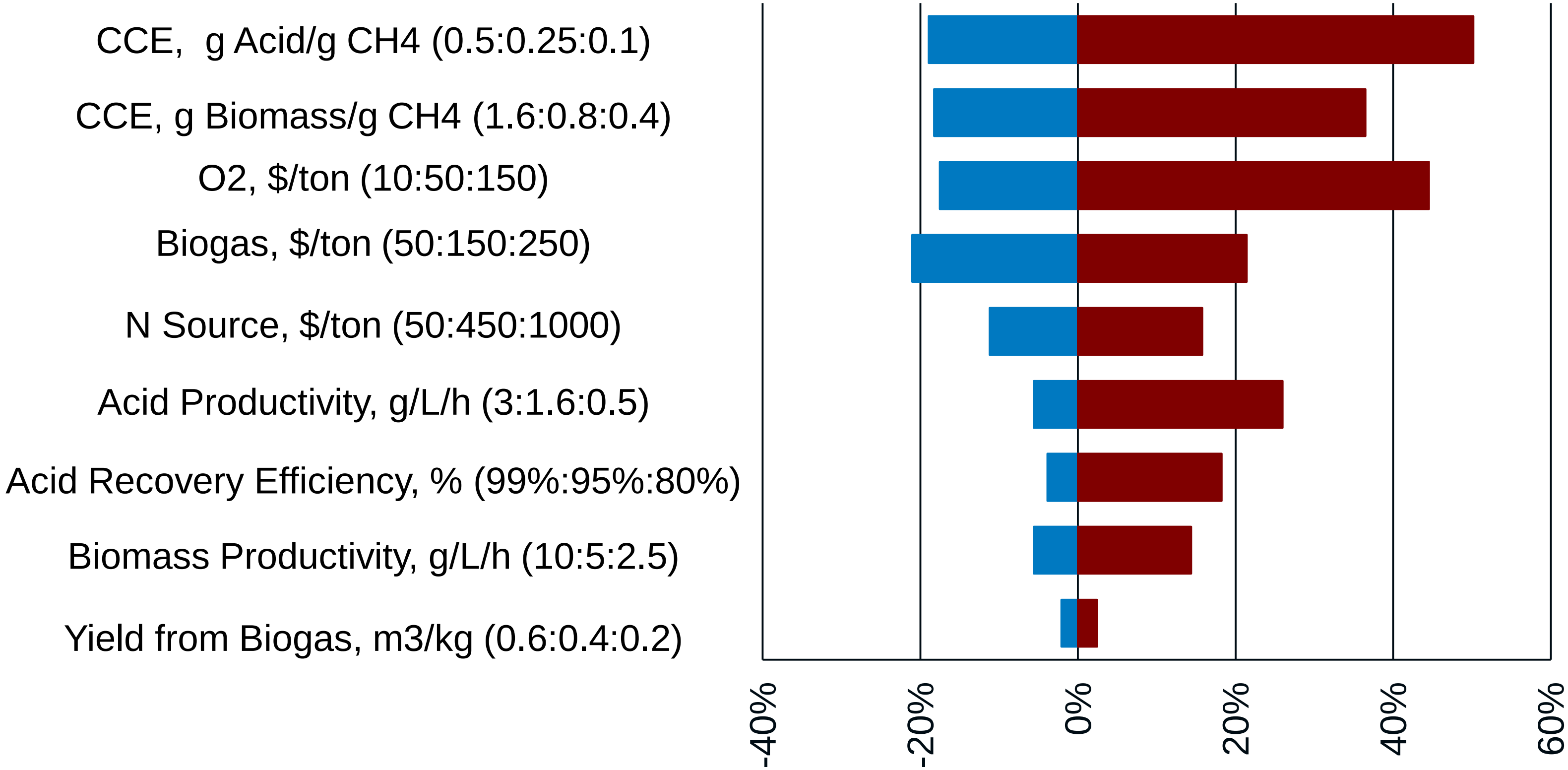
Technical Approach

- **Approach:** Integrate metabolic and fermentation engineering with TEA to inform hypothesis-driven strain-development strategies.

- **Major challenges**
  - **Technical:** (i) limited methanotroph genetic tools, (ii) gas mass transfer limitations (iii) low carbon conversion efficiency
  - **Market:** Conduct laboratory testing and field demonstrations to reduce risk to early adopters

- **Critical success factors:**
  - Demonstrate facile metabolic engineering capacity for targeted strain improvement.
  - Generate methanotrophic biocatalysts and fermentation engineering strategies enabling economically-viable CCE and methane conversion.
  - Develop and demonstrate a viable path to commercialization via integration with commercial anaerobic digestion facilities.

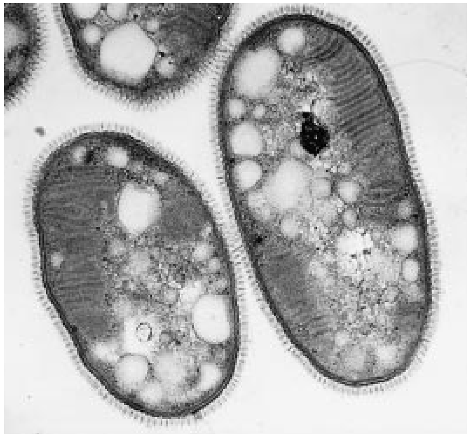
Techno-economic Analysis Highlights the Impact of Yield



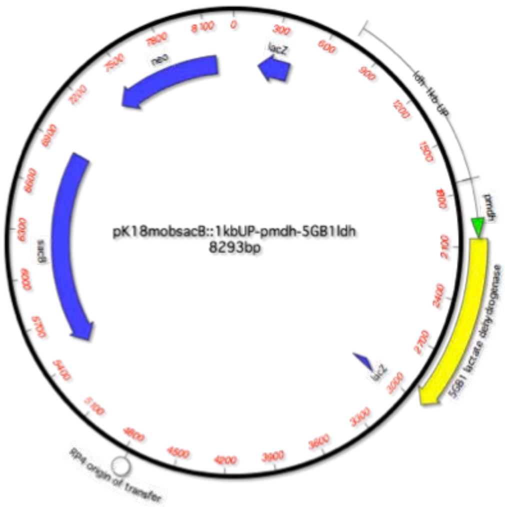
- Preliminary analyses indicate **carbon conversion efficiency** is the primary cost driver in the development of a viable biogas-to-fuels and chemicals processes.
  - Impacts reactor volume and quantity, gas recycle and compression, gas sourcing, etc.
  - Fundamental to all methane bioconversion processes.
- TEA for an integrated bioprocess identified a viable biogas-to-fuels and chemicals production route.

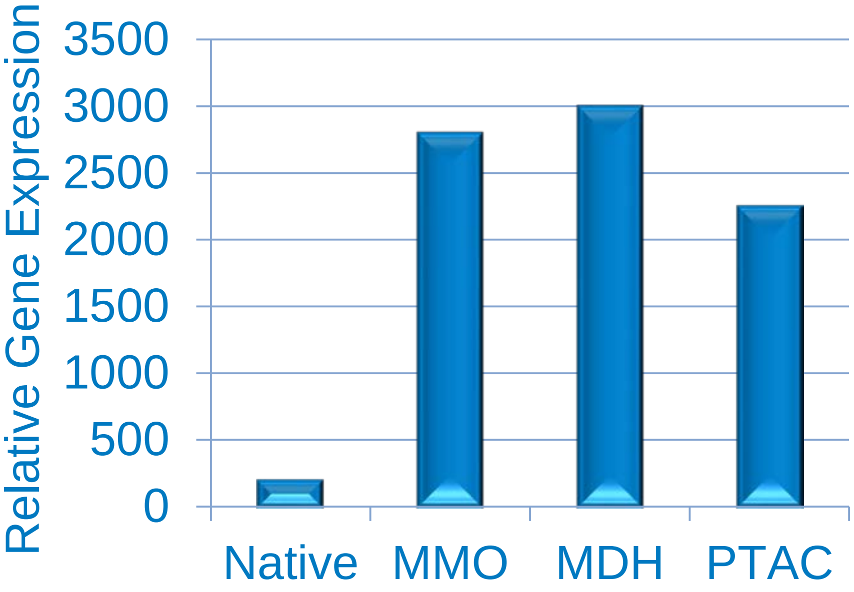
Successful Genetic Tool Development

- Unexplored genus at NREL, requiring genetic toolbox development
  - Limited tractability, with few reports of successful metabolic engineering
- Developed both replicative and integrative plasmids for gene expression and knockout in a methanotroph.
  - Inducible operators have been identified for temporal regulation of gene expression.
- Genetic tools will enable metabolic engineering strategies for efficient methane utilization and conversion in platform production strains.



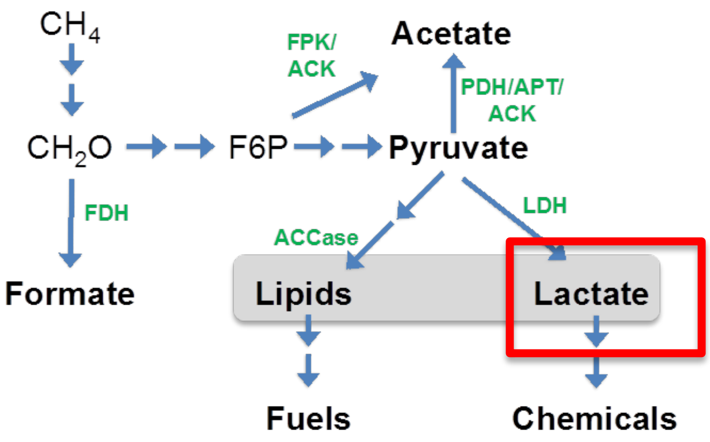
Methylomicrobium spp.

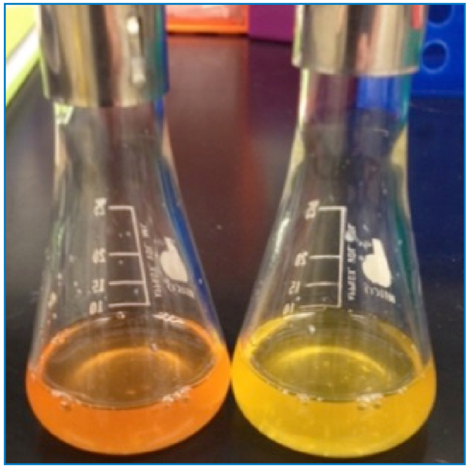




Metabolic Engineering for Fuel & Chemical Biosynthesis

- **FY15 Target:** Demonstrate proof-of-concept co-production of fuel and chemical intermediates from methane using an engineered methanotrophic biocatalyst.
- **Approach:** Employ genetic tools for inducible expression of a heterologous lactate dehydrogenase.





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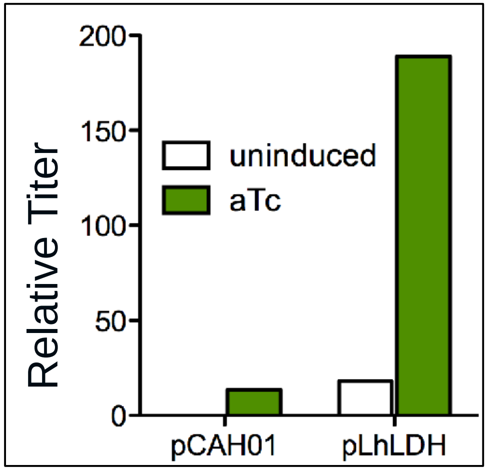
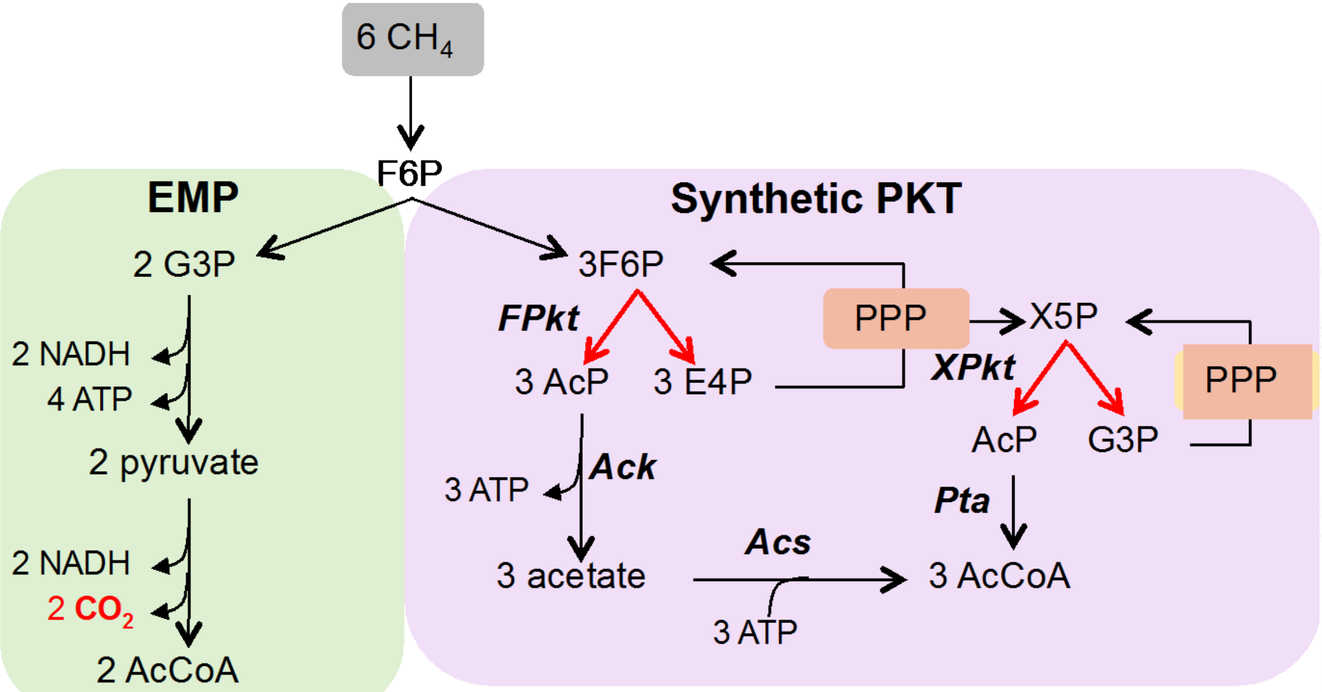
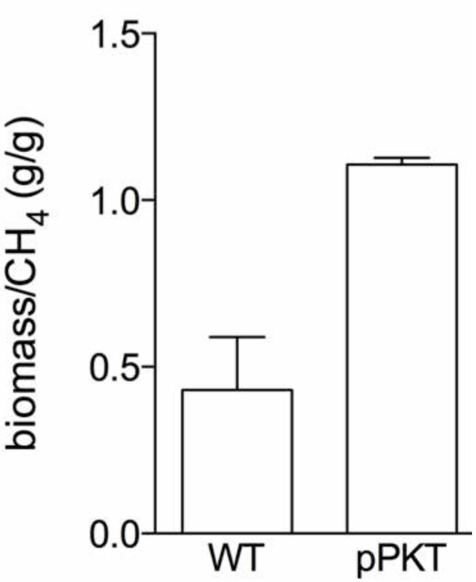


- **Result:** Genetically engineered a methanotrophic bacterium to produce <u>lipids and lactate from methane</u>.
  - >1g/L titers achieved in Y1; no alteration to lipid productivity.
  - Proof-of-concept for a methane-to-fuels and chemical bioprocess.

CCE Enhancement via Metabolic Engineering

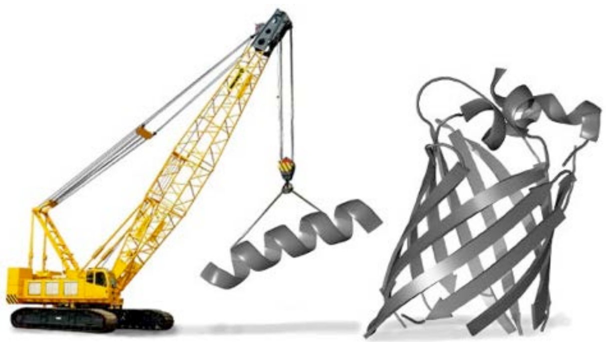
- **TEA:** yield from methane to biomass and products represents key cost driver in a biogas-to-fuels and chemicals process.
- **FY16 Target:** Achieve 20% yield enhancement from CH4 biomass & lipids.
- **Approach:** PKT pathway engineering
- **Result:** >2-fold yield enhancement from C1 substrates to biomass and lipids
  - *Most carbon-efficient methanotroph reported to date; applicable an array of AcCoA-derived products.*
  - ***Effective reduction of >$1/GGE***

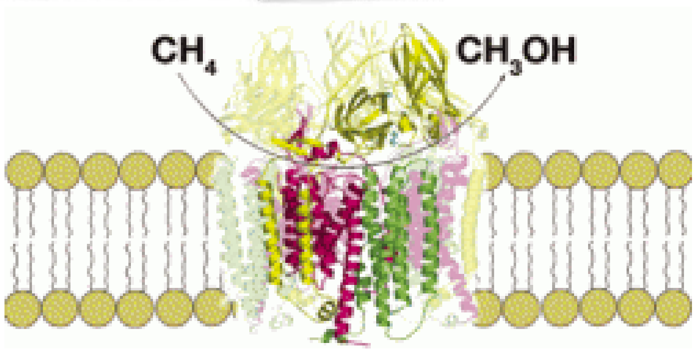


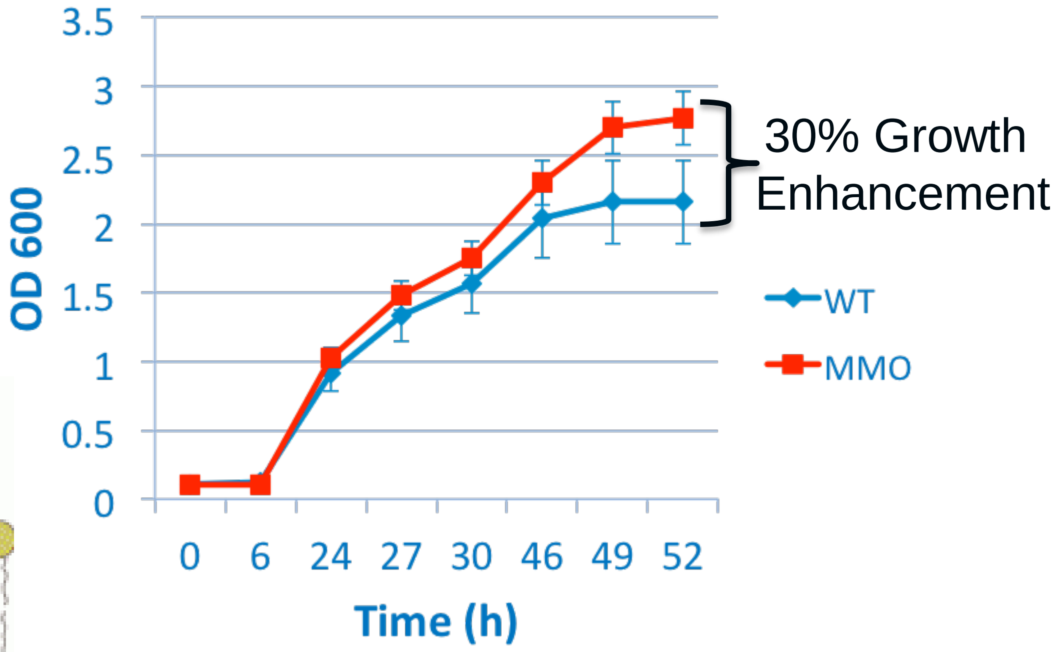


Enhanced Methane Activation via Protein Engineering

- **FY17 Target:** 20% enhancement in methane oxidation.
- Methane monooxygenase catalyzes oxidation of methane to methanol.
  - Unknown mechanism; low activity represents a potential bottleneck.
- **Approach:** Generation of MMO mutant libraries (>2,000 variants).
- **Result:** 30% growth enhancement with no alteration to composition.
  - *Represents highest growth/oxidation enhancement reported to date for methanotrophic bacteria.*
  - Combinatorial strain engineering approaches underway.



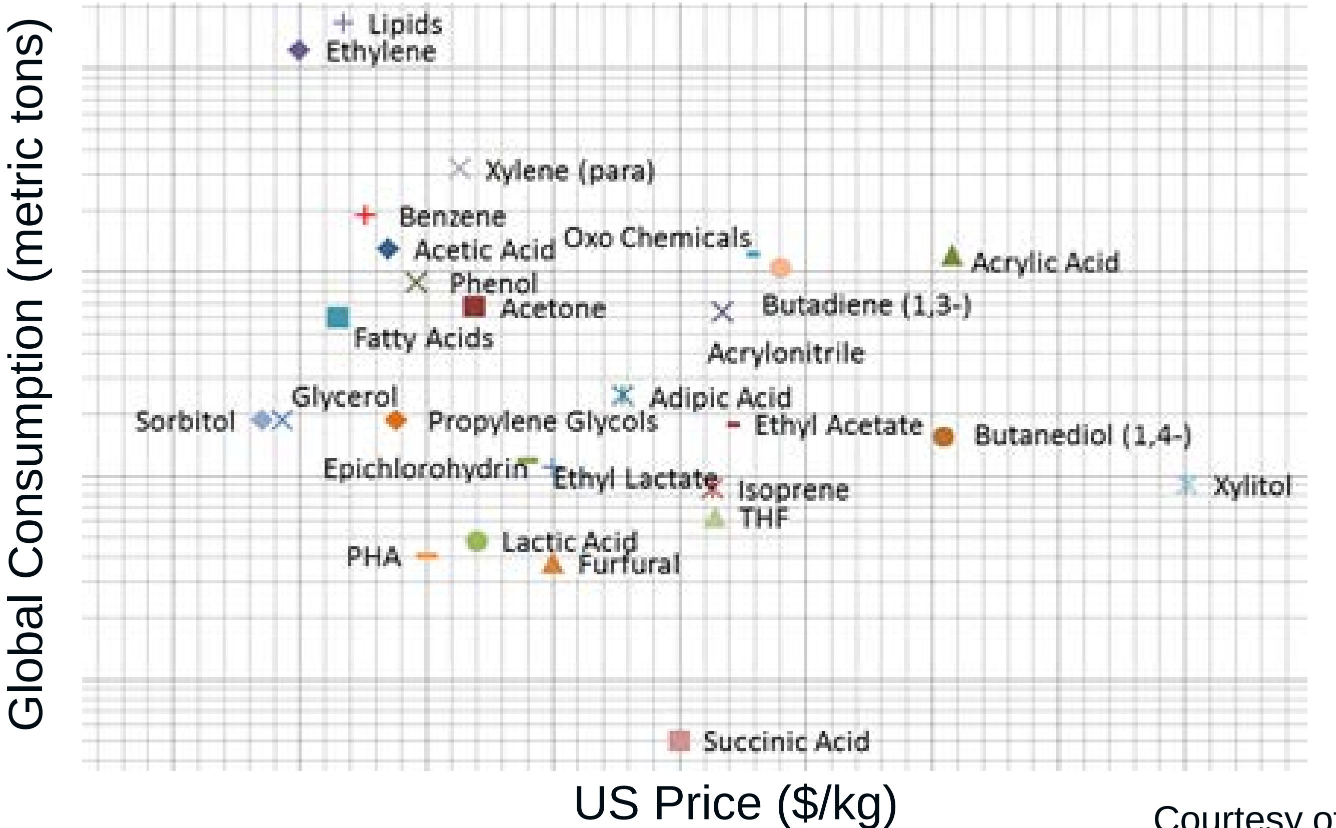




Verification at 1L scale underway

Strategic TEA for Novel Co-product Identification

- Down-selection of alternative fuel and chemical product suites
  - In coordination with the strategic analysis team, TEA and metabolic evaluation was conducted to identify top-candidate fuel and chemical intermediates.
  - FY18 efforts will target methanotrophic strain engineering for biocatalysis of top-candidate molecules.



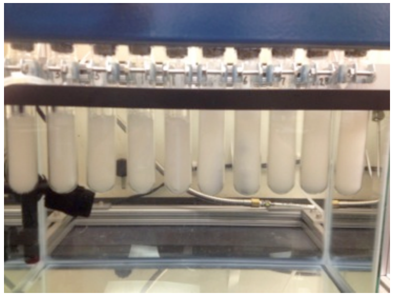
Courtesy of Biddy, et al., 2015

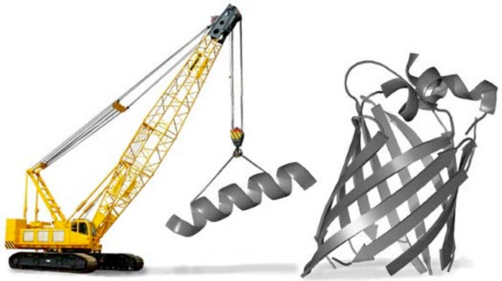
Relevance

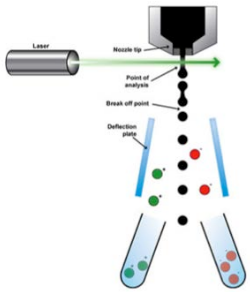
- MSW, landfill gas, agriculture and WWTP waste streams represent poorly valorized domestic feedstocks.
  - >4 Quad BTU energy potential for biogas-derived methane, with large-scale GHG reduction potential
  - Bioconversion offers a down-scalable, modular, and selective option
- Tech transfer/marketability: this work represents proof-of-concept for an array of methane biocatalysis strategies and opens door for feedstock expansion.
  - A number of commercial entities are currently targeting **biogas generation** and **methane upgrading** technologies.
  - This work represents and early commercialization scenario.
- Encourages the creation of a new domestic bioenergy industry.
- Relevant to EERE’s MYPP for developing cost-effective, integrated waste-to-energy processes for the production of bioproducts and advanced biofuels.

Future Work

- **FY17 Target:** Enhance methane uptake rate 20% via integrated strain and fermentation engineering strategies.
  - Initiate efforts for complete biogas utilization.
  - Develop industrially-relevant methanotrophic culture collection.
  - Evaluation of alternative fermentation configurations and optimization thereof.
- **FY18 Targets & Beyond:** Development of a suite of TEA-informed hydrocarbon and chemical co-production biocatalysts; integration of methanotrophic biocatalyst with real-time generation of biogas at an industrial scale AD unit.







Successful implementation will generate the most robust methanotrophic biocatalyst reported to date.

We are actively engaging with industry to facilitate technology transfer.

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.
- Achieved proof-of-concept for a fuel and chemical co-production process.
- TEA indicates CCE will be the primary cost driver in the development of a viable biogas-to-fuels and chemicals process
- TEA-informed metabolic engineering has generated the most carbon-efficient methanotrophic biocatalyst reported to date.
  - > 2-fold enhancement in yield.
  - > $1 GGE reduction in TEA models
  - >30% enhancement in cell density
- Future work will target integrated strain- and fermentation-engineering approaches to enable development of carbon- and energy-efficient methanotrophic biocatalysts for production of TEA-informed fuel and chemical intermediates.

Acknowledgements

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Response to Reviewers' Comment

- We thank the Reviewers for their positive and encouraging assessment.
- Following Reviewer guidance, we have continued to pursue the path laid out in FY15, targeting the development of robust, efficient methanotrophic biocatalysts via TEA-informed strain-engineering strategies.

Publications, Patents, Presentations, Awards, and Commercialization

- Publications:
  - Henard, et al. 2017, Phosphoketolase overexpression increases the efficiency of methane utilization by an obligate methanotrophic biocatalyst. *Met. Eng. Manuscript in Revision.*
  - Henard, et al. 2016, Bioconversion of methane to lactate by an obligate methanotrophic bacterium. *Sci. Rep 6:21585*
  - Henard, et al. 2015, Phosphoketolase pathway engineering for carbon-efficient biocatalysis. *Curr Opin Biotechnol.* 36:183-8.
  - Fei, et al. 2014, Bioconversion of natural gas to liquid fuel: opportunities and challenges. *Biotechnol Adv.* 32(3):596-614.
- Book Chapters:
  - 'Metabolic Engineering of Methanotrophic Bacteria for the Production of Fuels and High-Value Chemicals.' in *Methane Biocatalysis: Paving the Way to Sustainability*, Editor: Kalyuzhnaya, Springer Publishing.
- Patents:
  - Organic Acid Synthesis from C1 Substrates
- Presentations:
  - SIMB 2014
  - SIMB 2015
  - Gordon Research Conference 2016
  - ASM 2016