DOE Bioenergy Technologies Office (BETO) 2019 Project Peer Review

2.3.2.102 Biogas Biocatalysis

Waste-to-Energy

March 5, 2019

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National Renewable Energy Laboratory

Goal Statement

Goal: Develop an economically-viable biological platform to convert biogas to hydrocarbon fuel- and chemical intermediates using methanotrophic biocatalysts.

Outcome:

- Enable BETO MFSP goals of <$2.50/GGE
  - >$0.25/GGE cost reduction via biogas valorization
  - >5% refinery carbon conversion efficiency enhancement
- Achieve complete biogas conversion: CH_4 and CO_2 co-utilization
  - Novel, non-photosynthetic CO_2 conversion pathway
- Define a path to commercialization for AD biogas valorization technology.

Relevance to Bioenergy Industry:

- Carbon-efficient biocatalysts with broad product suite capacity will enable biogas valorization and enhance sustainability of industrial infrastructure.
- Improved process economics and efficiency of (i) lignocellulosic biorefineries and (ii) extant standalone anaerobic digestion infrastructure.

Quad Chart Overview

Timeline

- **Project start date:** October, 2016
- **Merit Review Cycle:** 2019-2021
- **Percent complete:** 15% current cycle

Budget

Total Costs Pre FY17	FY 17 Costs	FY 18 Costs	Total Planned Funding (FY 19->End Date)
$250,000	$300,000	$350,000	$1,200,000

Barriers

- Ct-H. Gas Fermentation Development
- Ct-J. Identification and Evaluation of Potential Co-products
- Ct-D. Advanced bioprocess development

Objective

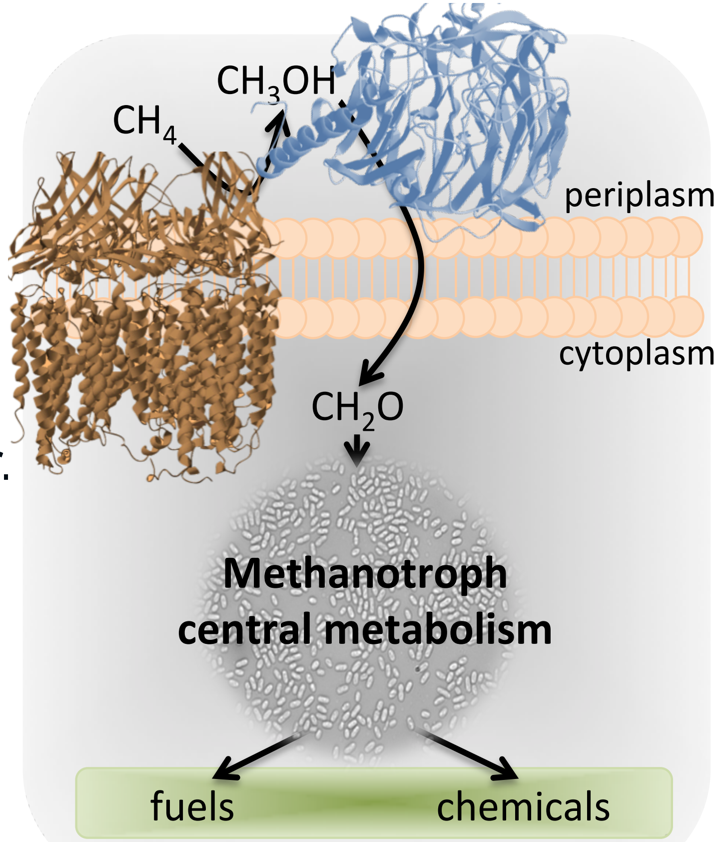
- Develop a carbon- and energy-efficient biogas bioconversion process via TEA-informed metabolic engineering strategies.

End of Project Goal

- Define a path to commercialization for AD biogas valorization technology.
- Achieve >$0.25/GGE cost reduction and >5% carbon conversion efficiency enhancement.

Project Overview

- **Context**
  - >4Quad btu can be generated from biogas domestically
  - Gaseous state of biogas prevents facile integration with transportation and industrial infrastructure.
  - Biogas currently has low market value; primarily utilized for combined heat and power.
  - BioGTL: a scalable, modular, selective approach to biogas conversion using methanotrophic bacteria.



- **Specific Project Goals**
  - Achieve complete biogas utilization.
  - Contribute to MFSP goals of <$2.50/GGE
    - Establish viable high-value co-product targets and/or carbon recycling strategies for lignocellulosic refining and standalone AD biogas.

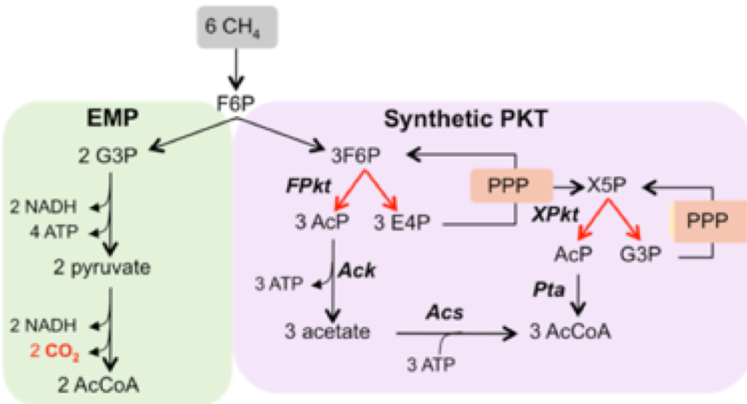
Approach - Management

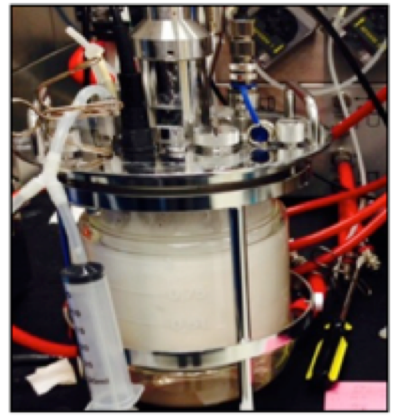
- Diverse Staffing Plan:
  - Molecular & microbiologists: conduct pathway analyses, strain/protein engineering
  - Fermentation engineers: gas fermentation optimization
  - Chem. & Process Engineers: techno-economic analysis
- **Research guided by TEA**, with related quarterly milestone metrics.
- Monthly group meetings, quarterly WTE meetings, quarterly reporting, and regular interaction with BETO and technical staff.
- Synergistic interaction
  - NREL Strategic Analysis/TEA, Biochemical and WTE Platform Project interactivity
  - Academic, government, and industrial partners synergize via contributions in systems biology, gas fermentation reactor design, and mapping a path to commercialization.

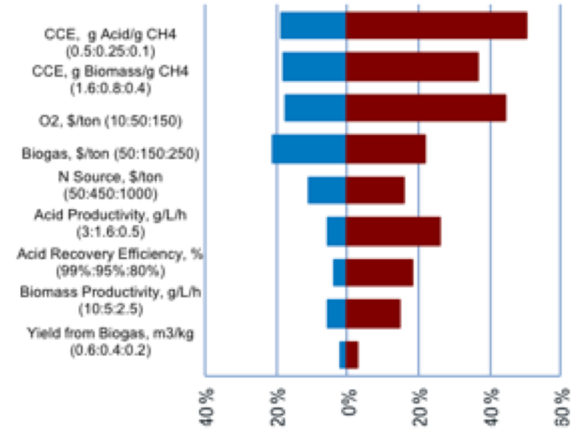
WBS 4.1.1.30 Strategic Support
WBS 2.1.0.100 BC Platform
WBS 2.3.2.201 BCU FOA
WBS 2.3.2.105 BUS
WTE: AD and Biopower

Approach - Technical

Genetic Tool Development, Metabolic & Protein Engineering	Gas Fermentation Optimization	Techno-economic Analysis





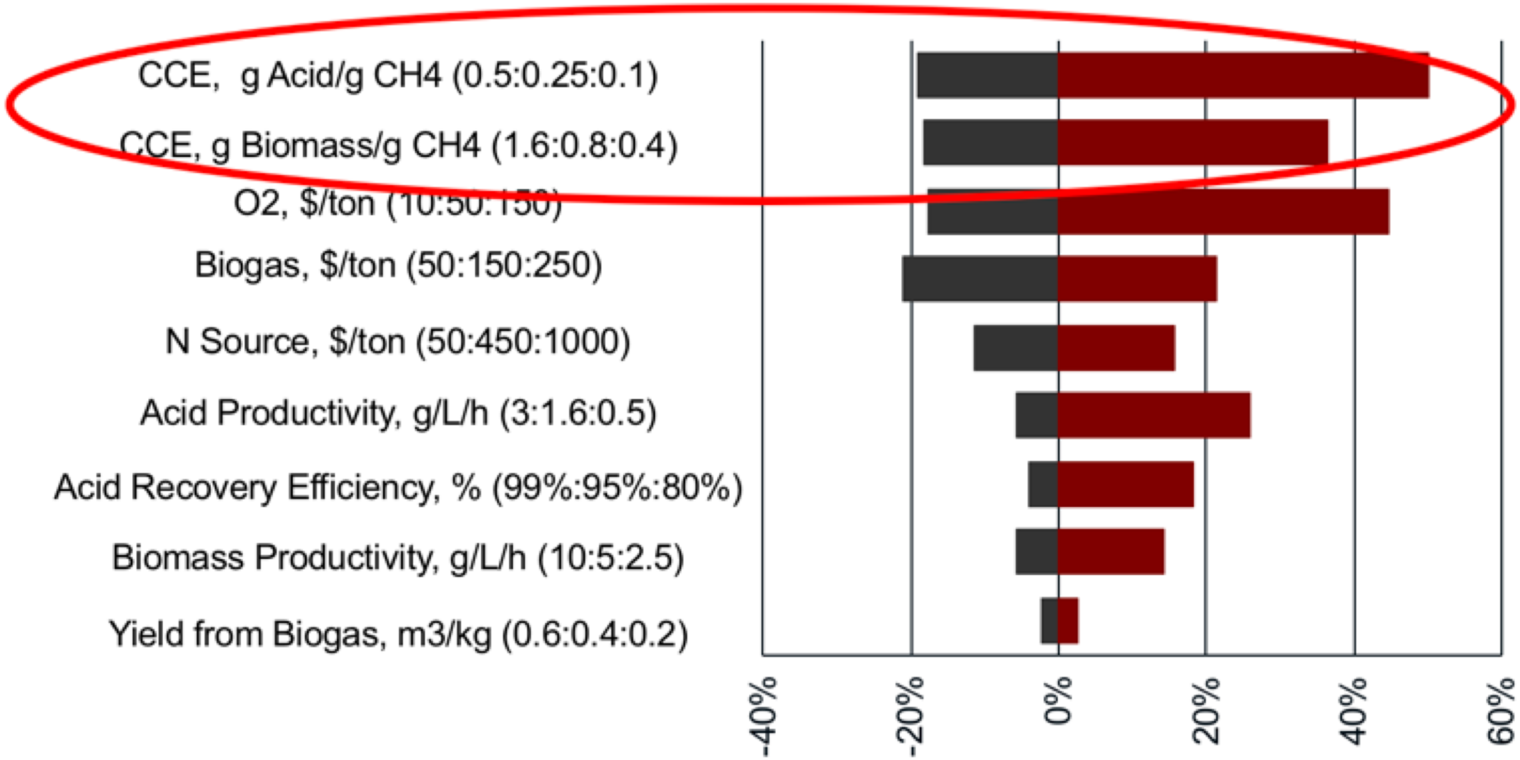


- **Approach:** Integrate metabolic engineering, synthetic biology, and functional genomics with TEA to inform hypothesis-driven strain-development strategies.
  - Evaluate mechanisms of CH_4 and CO_2 utilization in methanotrophs.
  - Develop methanotrophic biocatalysts with high-yield, broad product suite capacity.
- **Major challenges**
  - (i) limited methanotroph genetic tools, (ii) low CCE, (iii) no CO_2 utilization
- **Critical success factors:**
  - Demonstrate facile metabolic engineering capacity for targeted strain improvement.
  - Achieve economically-viable product titers, rates, and yields
  - Generate biocatalysts with CH_4/CO_2 co-utilization capacity

Progress-to-Date

Merit Review Cycle: 2016-2018

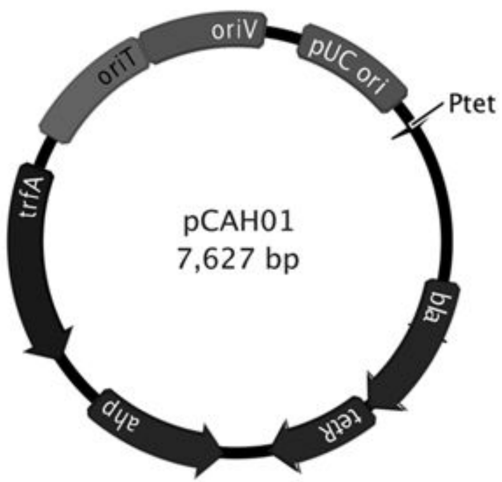
TEA Highlights the Impact of Yield

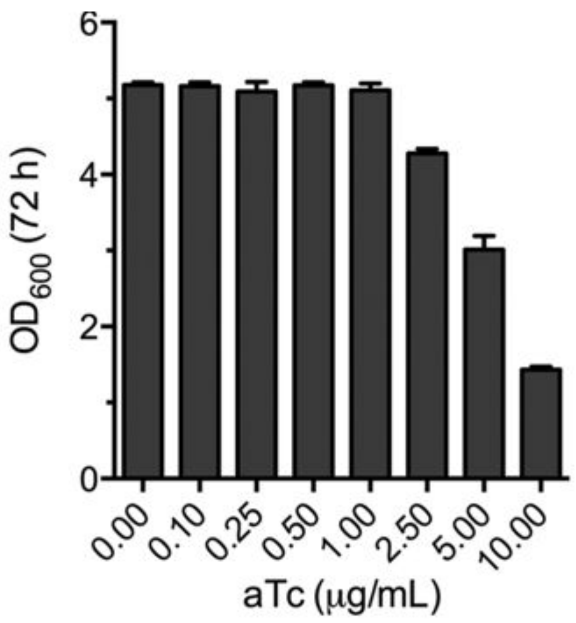


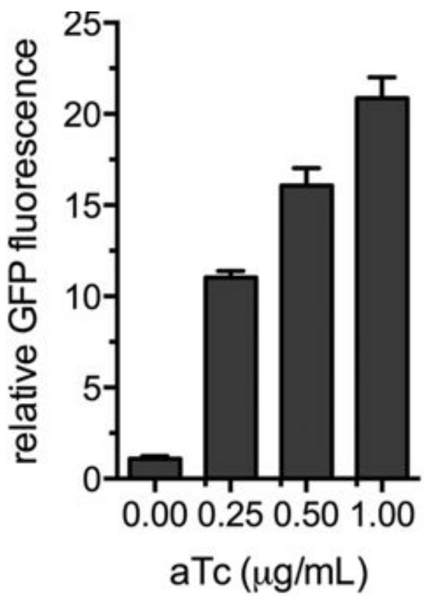
- Preliminary analyses indicate **carbon conversion efficiency** is the primary cost driver in the development of a viable biogas-to-fuels and chemicals processes.
  - Impacts reactor volume and quantity, gas recycle and compression, gas sourcing, etc.
  - Fundamental to all methane bioconversion processes.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

Successful Genetic Tool Development

- **Genetic tools will enable targeted metabolic engineering.**
- Developed both replicative and integrative plasmids for gene expression and knockout in a methanotroph.
- CRISPR-mediated genome editing has been initiated.



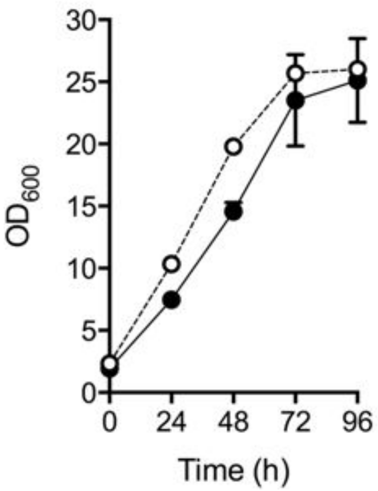


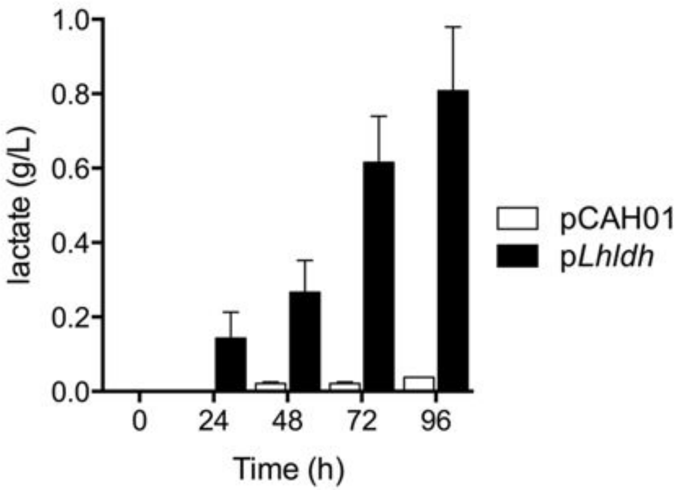


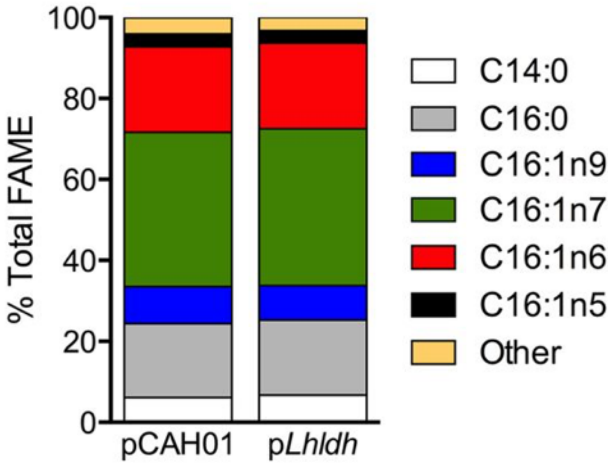
Scientific Reports **6**, 21585 (2016)

Proof-of-Concept Platform Chemical Production

- **Genetic tools will enable targeted metabolic engineering.**
- Heterologous expression of lactate dehydrogenase enabled co-production of lactic acid and lipid-fuel intermediates.



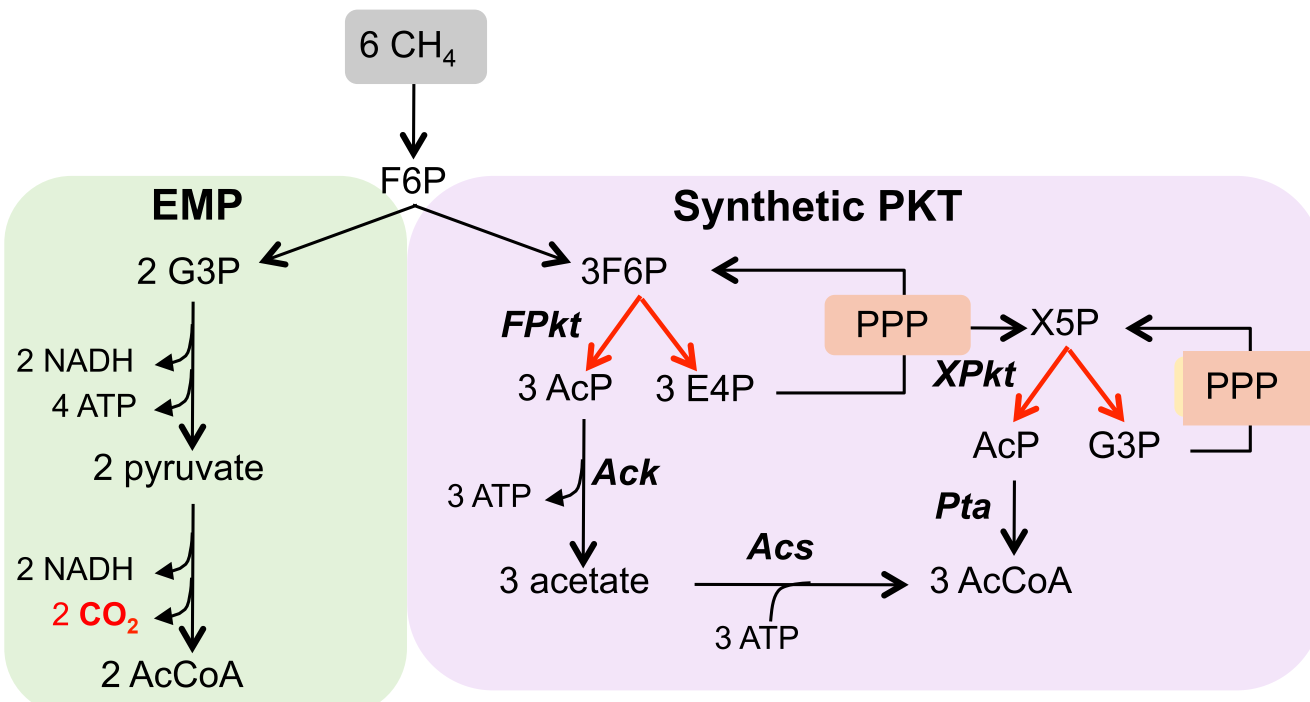


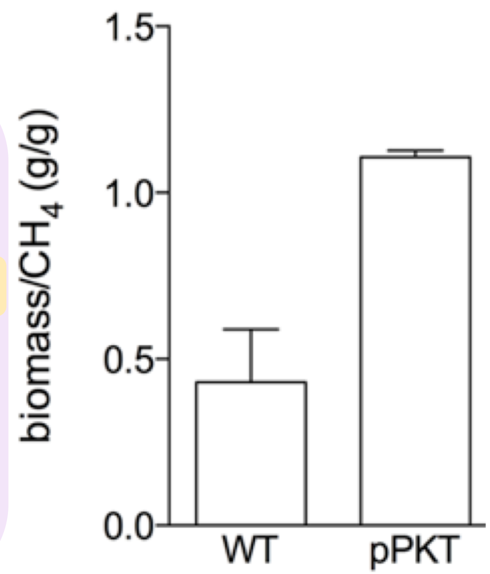


Scientific Reports **6**, 21585 (2016)

CCE Enhancement via Metabolic Engineering

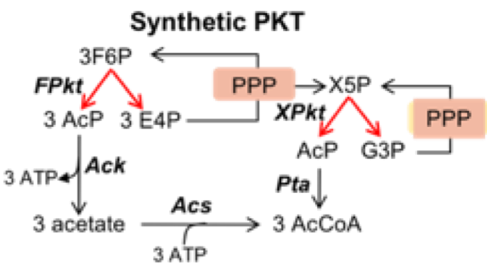
- **TEA:** yield from methane to biomass and products represents key cost driver in a biogas-to-fuels and chemicals process.
- **FY16 Target:** Achieve 20% yield enhancement from CH_4 biomass & lipids.
- **Approach:** PKT pathway engineering
- **Result:** >200% yield enhancement from C1 substrates to biomass and lipids
  - *Most carbon-efficient methanotroph reported to date; applicable an array of AcCoA-derived products.*

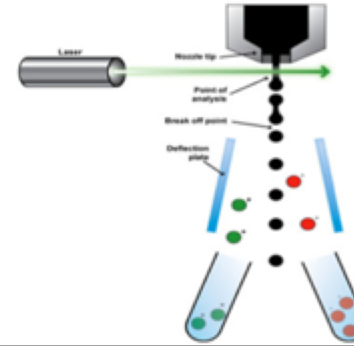




Metabolic Engineering (2017) **41**, 152-158

2016-2018: Moving the SOT Needle



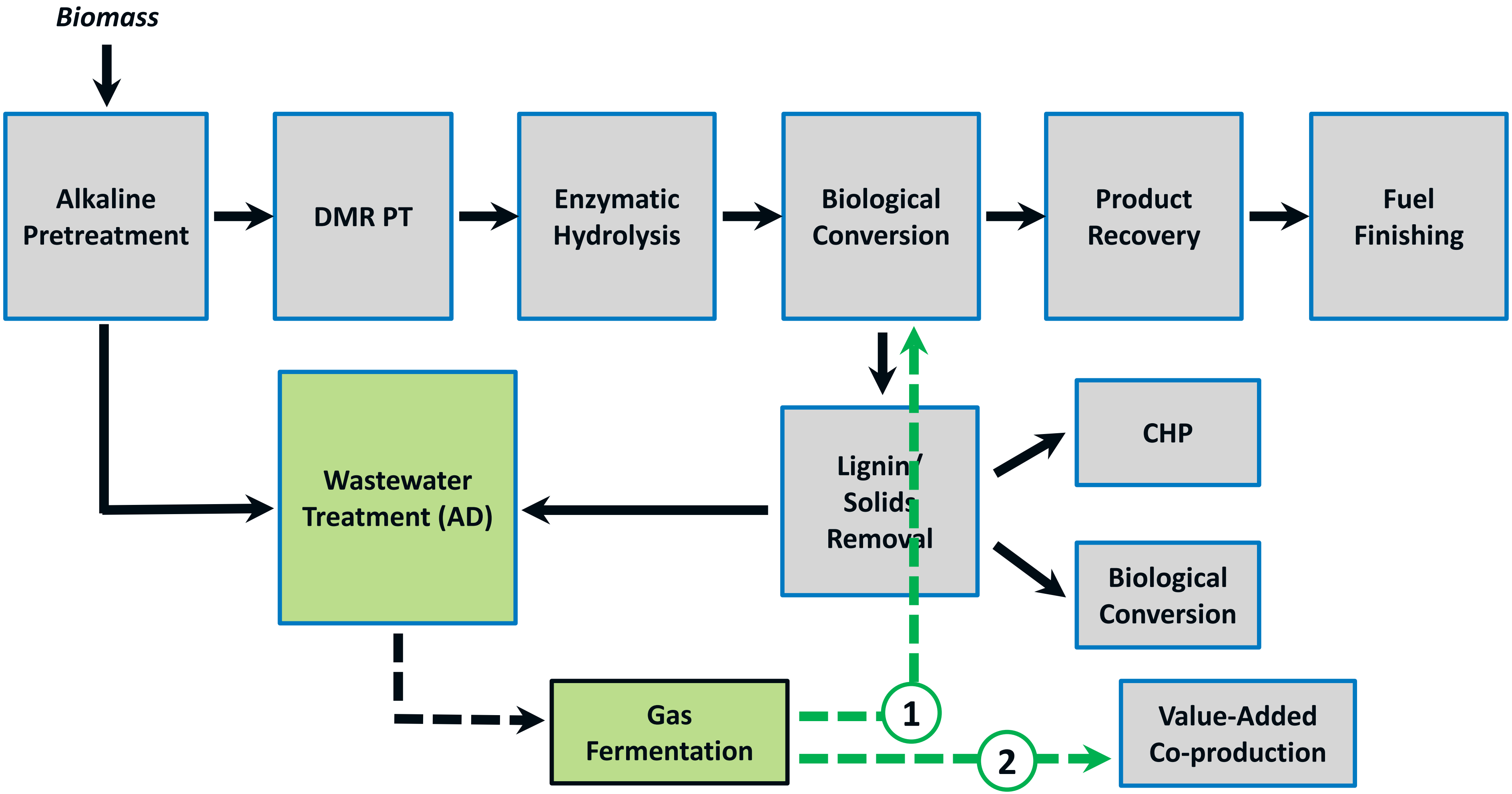


	2016	2017	2018
Organic Acid **Titer**	0.2g/L (native)	>1g/L	>10g/L
CH_4 conversion **Rate**	0.05g/L/hr	0.35g/L/hr	>0.5g/L/hr
Yield to Biomass*	0.25g/g	0.6g/g	0.6g/g

***Achieved ~90% theoretical yield.**

Merit Review Cycle 2019-2021

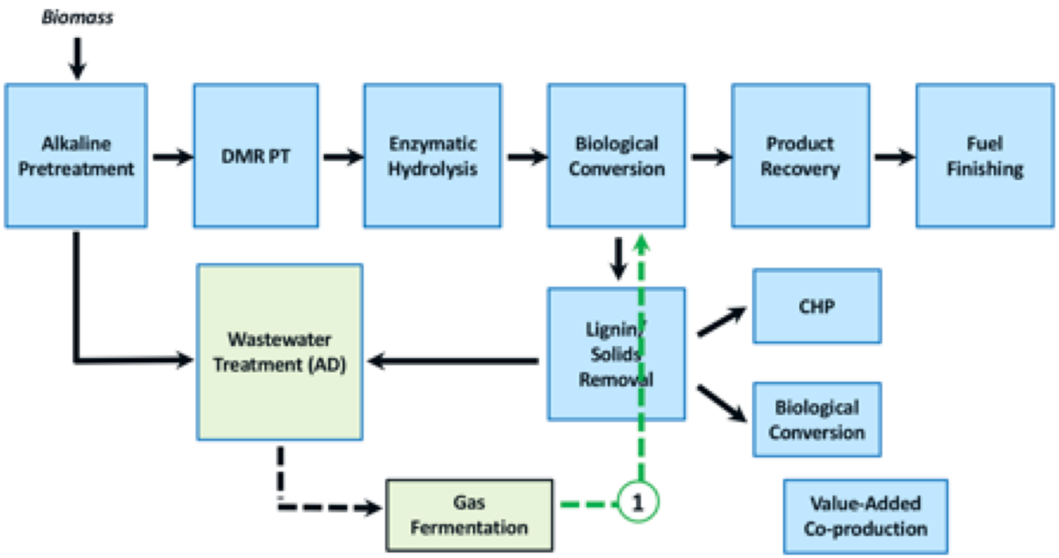
Lignocellulosic Biorefinery Integration

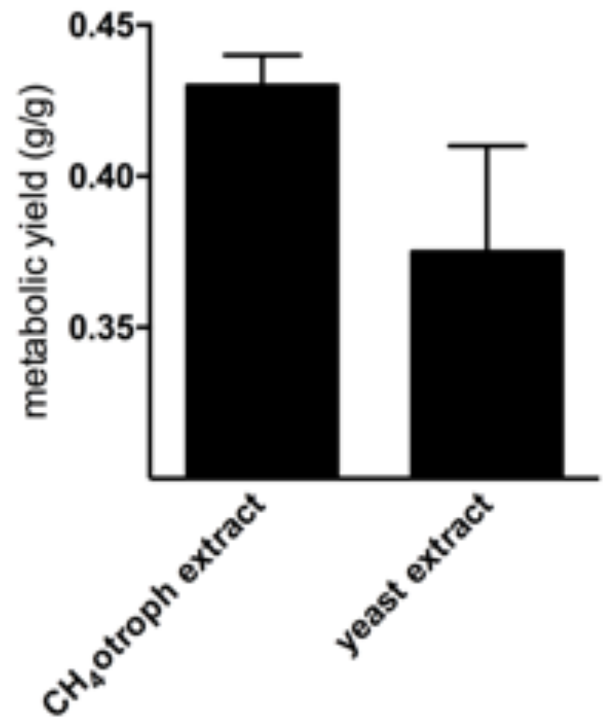


- **10-20% total biomass carbon ends up in WWT.**
- Biogas conversion offers a means to increase economics and sustainability.

Lignocellulosic Biorefinery Integration

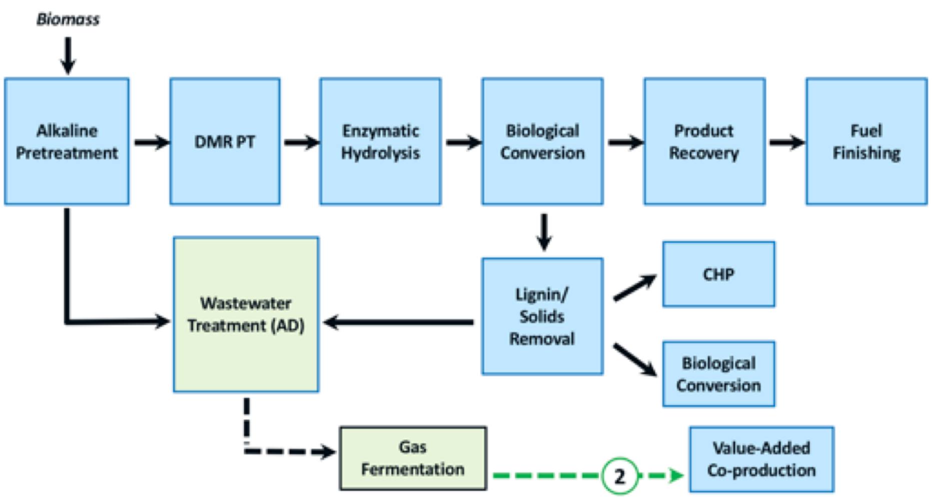
- Biogas conversion provide BC Platform with a significant economic benefit.
- Cell recycling leads to >15% TRY enhancement
- **Estimated >$1 reduction in MFSP**

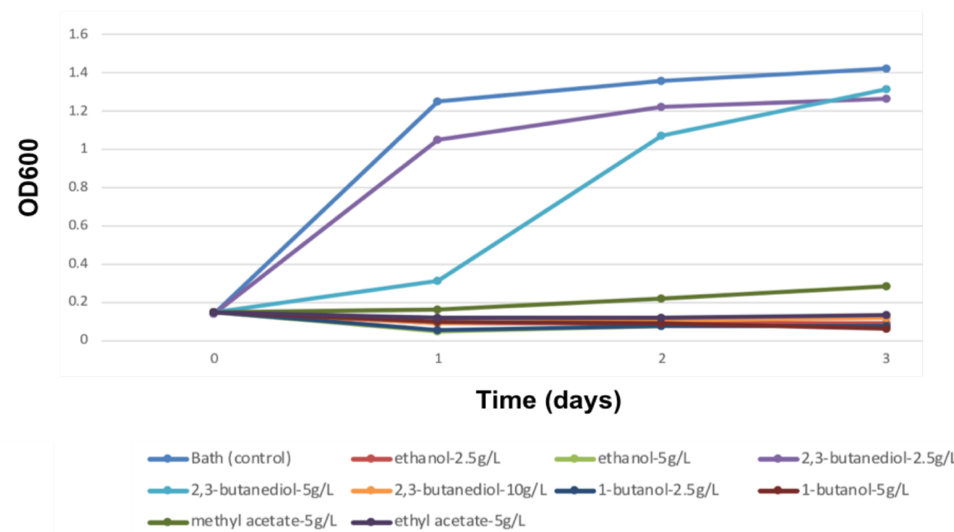




Generation of High-Value Co-Product Suites

- TEA and metabolic evaluation was conducted to identify top-candidate fuel and chemical intermediates.
- End-product toxicity assessed across diverse genera to select product-strain pairings for metabolic engineering pursuits.
- Successful metabolic engineering for production of seven candidate molecules: organic acids, higher alcohols, esters

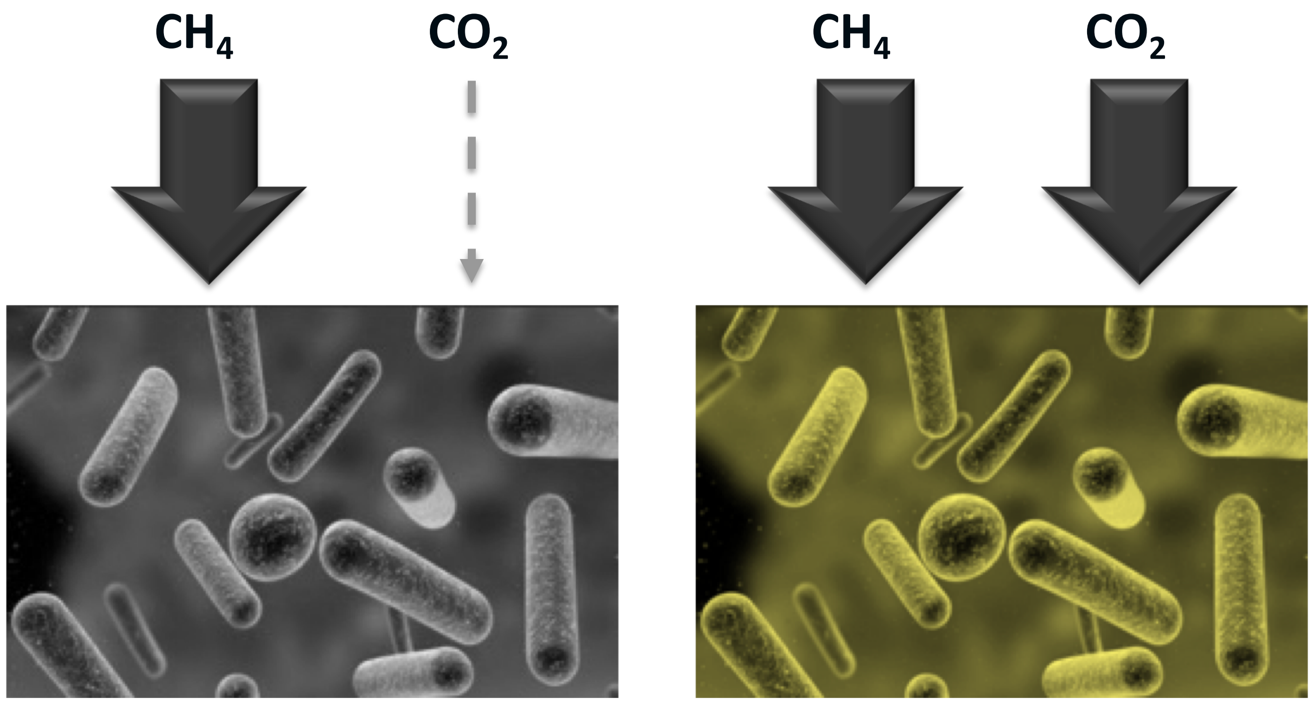




- *FY19 will lead to TEA-informed down-selection to a single target molecule.*

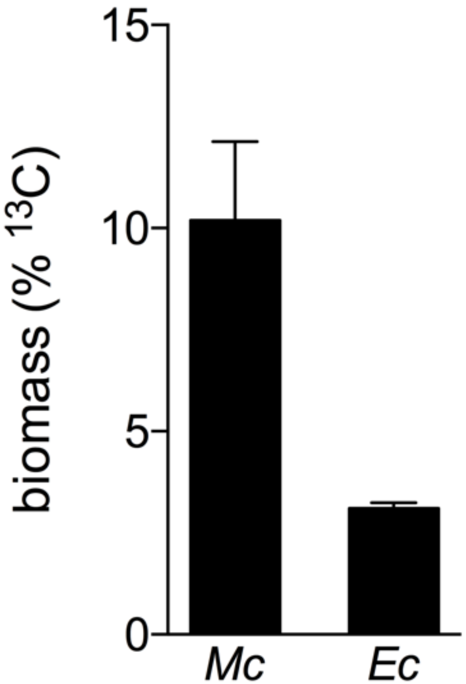
Towards Complete Biogas Utilization

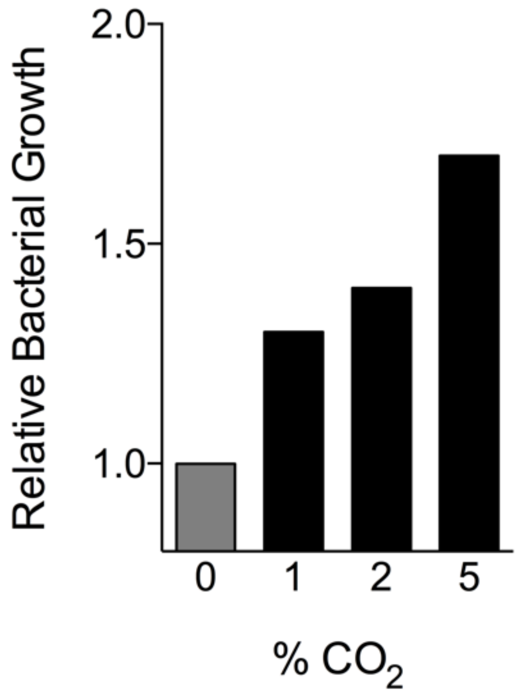
- Biogas is comprised of 25-50% CO_2
- Goal: Establish CH_4/CO_2 co-utilization.

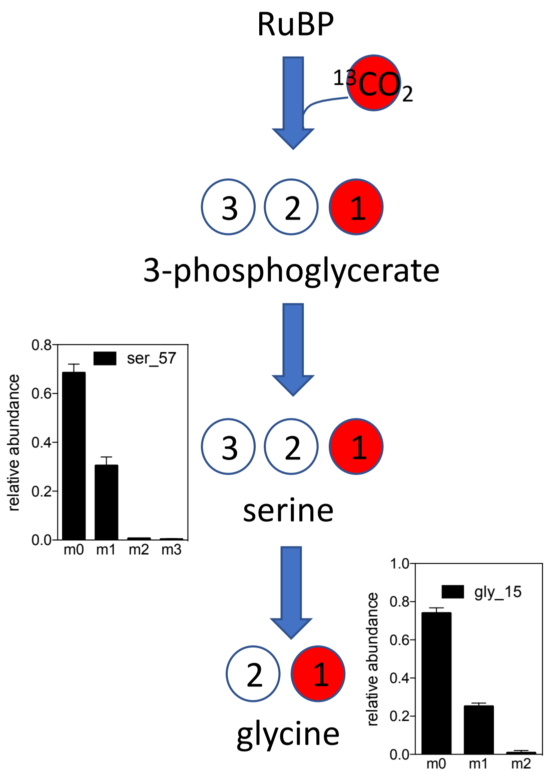


Wild-Type Methanotroph **Engineered Methanotroph**

Achieved non-photosynthetic, aerobic CO_2 assimilation







- **First-in-class CH_4/CO_2 co-utilization achieved.**
- Conditional carboxylase knockouts can be complemented with ectopic expression; highlight essentiality for bacterial growth.
- Conducted metabolic flux analysis (MFA) to determine CO_2 flux node(s)
- Strain engineering initiated to enhance CO_2 uptake and flux to target products.

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
- Goal: Develop an economically-viable biological platform to convert biogas to hydrocarbon fuel- and chemical intermediates using methanotrophic biocatalysts.
- Relevant to EERE's MYPP for developing cost-effective, integrated waste-to-energy processes for the production of bioproducts and advanced biofuels.
- Valorization of waste biogas streams will be integral to achieving BETO lignocellulosic biorefinery MFSP and efficiency goals, as well as establishing an alternative route to capture and convert standalone AD-derived biogas.

Relevance

Stakeholder Outreach and Engagement:

- 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.
  - Collaborative engagement with biogas providers and methane upgrading industry to evaluate and define a path to commercialization.

Future Work

- **FY19 Target:** Generate TRY metrics associated with baseline target products and employ data as TEA inputs.
  - Identify the performance metrics required to incur a net TEA and LCA benefit.
- **FY20 G/NG Target:** Achieve TRY production metrics at >25% targets
- **FY21 Target:** Demonstrate >$0.25/GGE reduction and >5% carbon yield enhancement relative to baselines excluding this step

- Additional efforts supporting the above milestones will encompass **i**) advanced genetic tool development for multiplex genome editing, **ii**) system biology approaches to elucidate and rewire mechanisms governing C1 utilization, and **iii**) targeted strain engineering for enhanced biocatalyst productivity.
- Successful implementation will generate the most robust methanotrophic biocatalysts reported to date.
- We will continue to actively engage with industry to refine a path to commercialization and facilitate technology transfer.

Summary

- **Overview**
  - AD-derived biogas offers high energetic and economic potential.
  - Methanotrophic biocatalysis offers a promising path towards valorization of biogas derived from anaerobic digestion of waste streams.
- **Approach:** Integrate TEA, metabolic and gas ferm engineering, and systems biology to develop carbon-efficient, methanotrophic biocatalysts with novel biosynthetic capacity.
- **Accomplishments:**
  - Successfully generated biocatalysts with CH_4/CO_2 co-utilization capacity.
  - Achieved proof-of-concept for high-value co-production of fuel- and chemical intermediates.
  - TEA-informed metabolic engineering has generated the most carbon-efficient methanotrophic biocatalysts reported to date.
    - > 2-fold enhancement in yield.
    - > $1 MFSP reduction in TEA models
- **Future work:**
  - Enable viable economics for lignocellulosic refineries and standalone AD infrastructure via targeted strain engineering to enhance methane conversion efficiency and productivity.

Acknowledgements

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Ilya Aberkin (SDSU)

Response to Reviewers' Comments 2017

- *…exciting project with great progress already. Toolbox production for methanotrophs is a very important work and very relevant for the application and biotech industry in general. The skilled and experienced project team has been very effective where others have made slow progress using relevant adjacent organism integrations.*
- *This approach to producing liquid fuels from biogas is sound and has the potential to be impactful.*
- *The project team appears to be well-managed and has produced some impressive genetic engineering results to date.*

We thank the Reviewers for their positive feedback. We have continued to pursue the path laid out in FY17, targeting the development of robust, carbon-efficient methanotrophic biocatalysts via TEA-informed strain-engineering strategies.

- *To use biogas, one needs to address the use of whole biogas (CH4 and CO2)…*

We agree with the Reviewers whole-heartedly and have thus made "complete biogas utilization" a primary project focus. We have made exciting progress on this front, demonstrating successful co-utilization of CH_4 and CO_2 in a series of methanotrophic biocatalysts,. We will continue to pursue strategies to enhance CO_2 utilization in industrially-relevant strains with novel biosynthetic capacity.

Publications, Patents, Presentations, Awards

- **Publications**

1. Tapscott, et al. 2019, *Appl. Environ. Micro, Ms under review*
2. Qian, et al. 2018. *Nano Letters, In Press*
3. Henard, et al. 2018. *Frontiers in Microbiology,* 9, 2610
4. Tays, et al. 2018. *Frontiers in Microbiology,* 9, 2239
5. Henard, et al. 2017. *Metabolic Engineering* 41, 152-158.
6. Flickinger, et a. 2017. *J. Coatings Technol Res*, 14:791-808.
7. Akberdin, et al. 2017. *Scientific Reports* 8 (1), 2512
8. Schulte, et al. 2017*. PLoS One*, 10.1371.
9. Flickinger, et al. 2017 *Integrated Continuous Biomanufacturing*, 76
10. Schulte, et al. 2016*, Biotechnology and Bioengineering*, 113, 9
11. Henard, et al. 2015. *Curr Opin Biotechnol.* 36:183-8.

- **Book Chapter:**

1. "Metabolic Engineering of Methanotrophic Bacteria for Industrial Biomanufacturing" in *Methane Biocatalysis: Paving the Way to Sustainability*, Editor: Kalyuzhnaya, Springer Publishing.

- **Patents and Records of Invention**
  – Organic Acid Synthesis from C1 Substrates, US Patent App. 15/252,648

- **Presentations (Invited)**
  – SIMB 2014-2018
  – SBFC 2018
  – WERF 2017
  – Gordon Research Conference 2016, 2018
  – ASM 2018

- **Press**

Feature article *Biofuels Digest,* August, 2017
Feature article *R&D Magazine,* February 2018

Thank You

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