NATIONAL RENEWABLE ENERGY LABORATORY

Biological and Chemical Upgrading for Advanced Biofuels and Products – FOA 0001085

Biogas Valorization: Development of a Biogas-to-Muconic Acid Bioprocess
WBS 2.3.2.201

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

FOA Summary

- Targeted “development, improvement and demonstration of integrated biological or chemical upgrading technology for the production of substitutes for petroleum-based feedstocks, products, and fuels.”

- Diversification of BETO portfolio via… “production of chemicals from biologically or chemically derived intermediate feed streams, including biogases.”

Goal Statement

- **Project Goal:**
  - Establish a novel gas fermentation bioprocess for secretion of an array of fuel and chemical intermediates.
    - Develop a novel methanotrophic biocatalyst and fermentation configuration for the production of muconic acid from renewable biogas.
- **Outcome:**
  - Demonstration of an integrated, AD-biogas biological conversion process for the production of platform chemicals.
    - Achieve industrially-relevant production (>0.5g/L/hr) of muconic acid from biogas.
- **Relevance to Bioenergy Industry:**
  - Biological methane conversion offers a scalable, modular, and selective approach to biogas upgrading.
    - Deployment advantages over physical and chemical conversion strategies.
  - Development of robust biocatalysts and a high-efficiency, low-power reactor will enable facile integration with AD infrastructure and offers substantial biogas valorization potential.
  - Offers an alternative biochemical route to target enhanced yield via development of a novel reactor with an immobilized biocatalyst.
    - Non-growth state, enhanced mass transfer, low-power
    - Applicable to an array of gaseous substrates, including syngas, natural gas, CO2, etc.

Quad Chart Overview

Timeline

- Project start date: July, 2015
- Project end date: June, 2018
- Percent complete: 50%

Barriers

- Bt-J: Catalyst Development
  - *Novel methanotrophic biocatalyst generation*
- Bt-K: Biochemical Conversion Process Integration
  - *Process-intensified configuration with immobilized biocatalyst FFR*

Budget

	FY 15 Costs	FY 16 Costs	Total Planned Funding (FY 17->End Date)
DOE Funded	$273K	$927K	$1.3M
Farmatic	11 % total cost share		
Metabolon	6% total cost share		
NCSU	3% total cost share		

Partners

- **NREL (30%):** Strain development, TEA
- **Farmatic, Inc (33%):** AD-biogas provision and analysis.
- **NC State University (20%):** falling film reactor design
- **Metabolon, Inc (10%):** metabolomics and pathways mapping
- **San Diego State University (7%)**: metabolic flux balance analyses.
- **All:** Process Integration

Project Overview

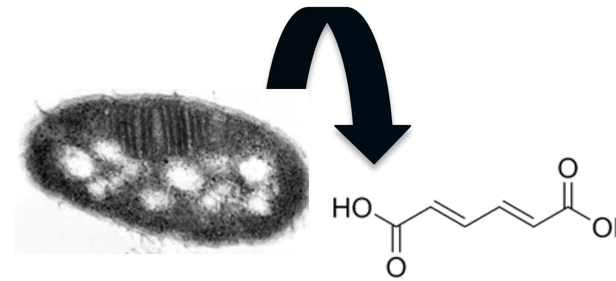
- **Context:**
  - Process intensification offers an alternative means to target CCE.
  - Bioconversion offers advantages related to scalability, modularity, and selectivity.
  - Targeting production of muconic acid, which can be readily upgraded to adipic acid, a nylon precursor and critical GHG contributor.
  - This project leverages prior work conducted under ARPA-E and synergizes with other NREL BC project targeting production of organic acids.

- **Specific Project Goals:**
  - Characterization of biogas derived from domestic substrates and mitigation of biogas toxicity.
  - Generation of novel MA-producing methanotrophic biocatalysts
  - Development of genome-scale metabolic models for methanotrophic biocatalysts
  - Design and implementation of a high-efficiency, low power falling film reactor.
  - Generation of comprehensive techno-economic models for an array of methane feedstock inputs and organic acid outputs.
  - Demonstration of an integrated bioprocess for conversion of AD-biogas to MA

Management Approach

Biogas Generation & Characterization

Falling Film Reactor Design



Strain Engineering, Metabolomics, & FBA

- Research guided by TEA, with related quarterly milestone metrics.
- Monthly team/quarterly WTE meetings, regular interaction with BETO and tech. staff.
- BETO Validation: initial (FY15), intermediate (FY17), and final (FY18).
- Synergistic interaction between BCU FOA, Biogas AOP, Strategic Analysis/WTE Program, carboxylate and lignin platforms, and related external activities (industry and interlab interaction).

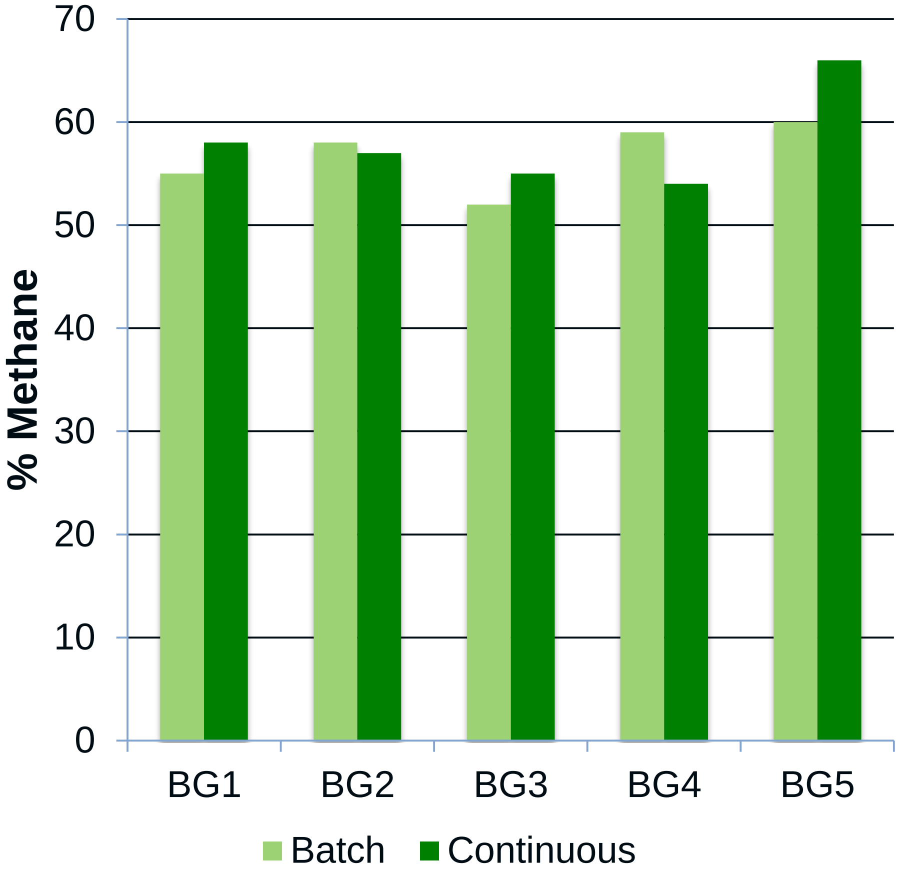
Technical Approach

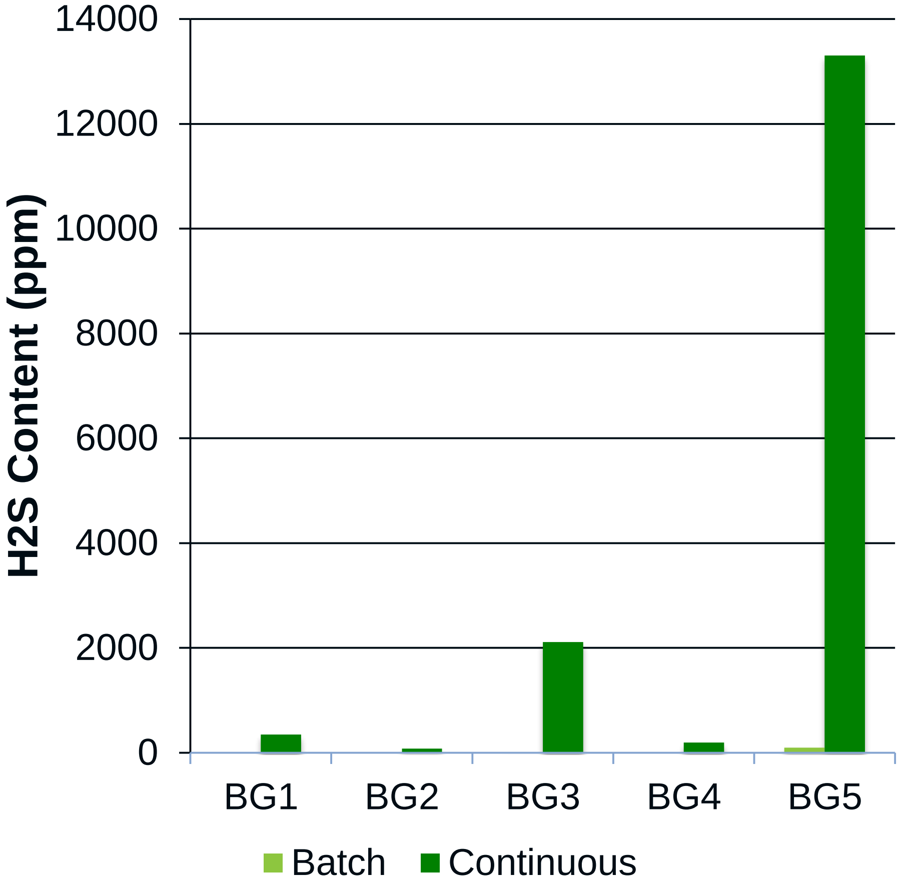
- **Approach:** Integrate AD, *in silico* modeling, metabolomics, strain engineering, and bioreactor design. Conduct iterative TEA to inform process targets and enhancements.

- **Major challenges:**
  - Low power biocomposite reactor design: cell adhesion and viability, extended performance.
  - Strain development: high-productivity (T, R, Y, as dictated by TEA), biogas tolerance.
  - Process Integration: optimization of biogas delivery, mass transfer, methane activation, and biosynthesis of MA.

- **Critical Success Factors:**
  - Develop a methanotrophic biocatalyst with muconic acid biosynthetic capacity.
  - Achieve enhanced mass transfer and process intensification via novel reactor deployment.
  - Demonstrate a bioconversion process integrated with real-time AD biogas production.

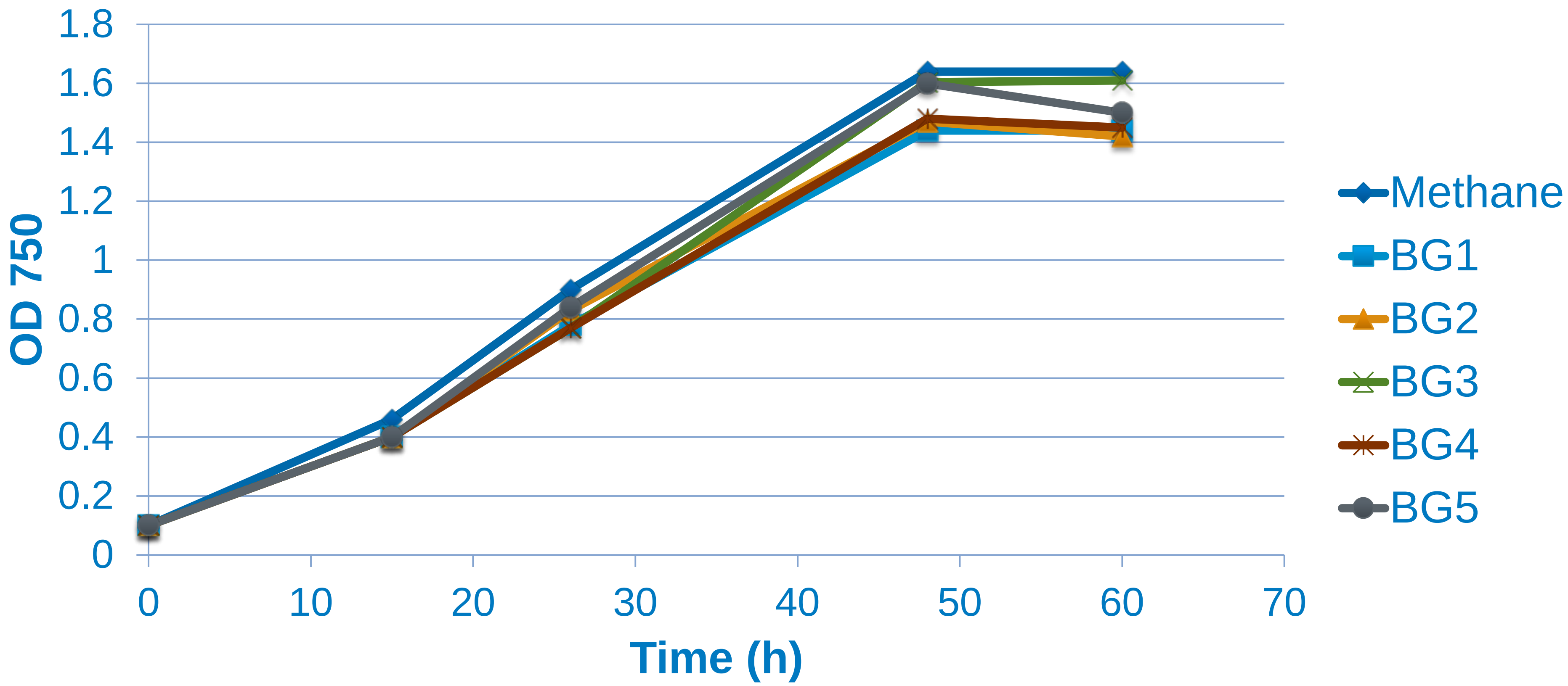
Biogas Characterization: CH_4 and H_2S Evaluation





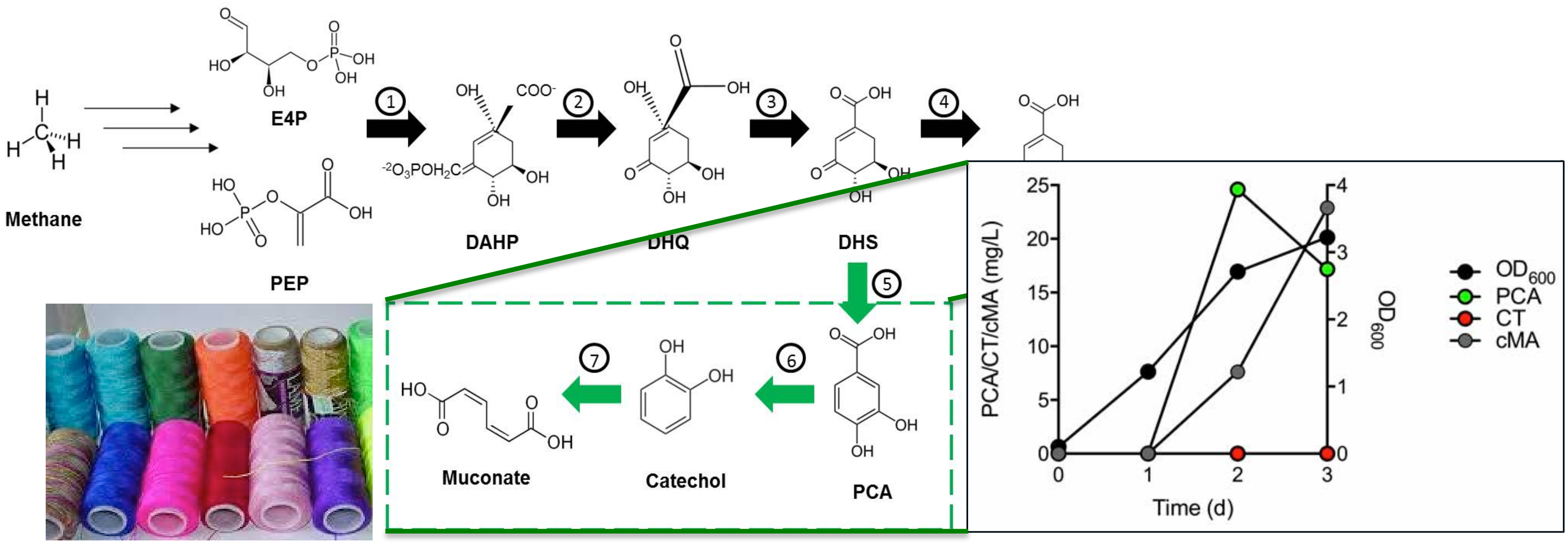
- We evaluated a series of substrates with wide-range H_2S generation potential.
- Continuous AD configuration leads to substantial H_2S accumulation.
  - Potential for methanotrophic toxicity.

H_2S Does Not Impact Growth…but Alters Metabolism



- Minimal growth defect under high-H_2S cultivation conditions.
- Comparative metabolomics indicated dramatic metabolic rearrangement.
  - Strain adaptation and engineering underway to mitigate potential exacerbation and flux alterations at scale.

Proof-of-Concept Muconic Acid Biosynthesis

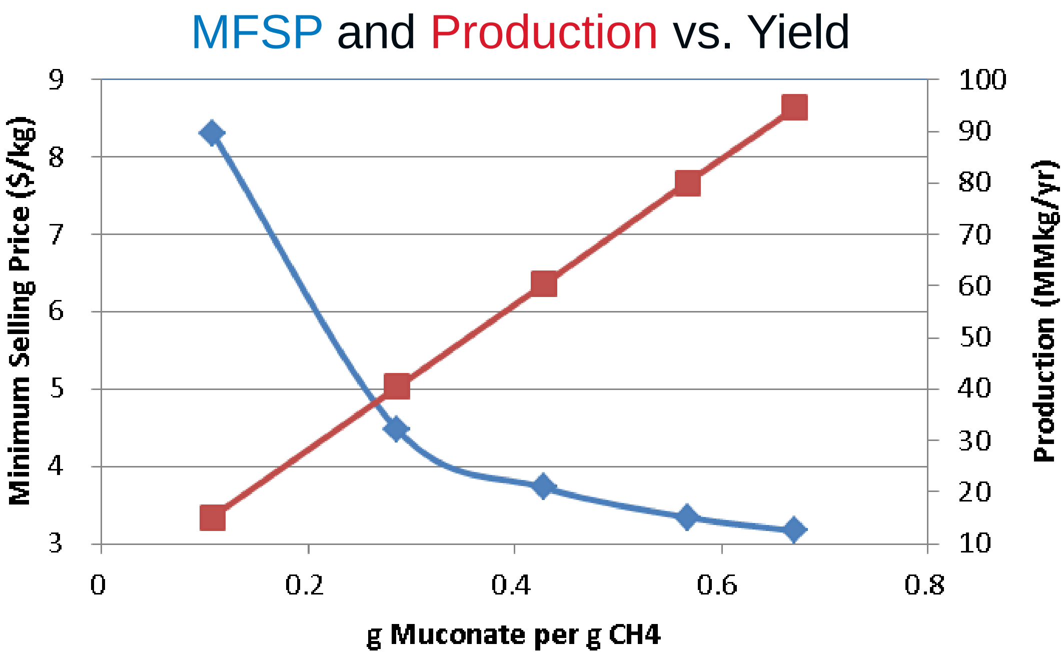


Schematic overview of synthetic muconic acid pathway.

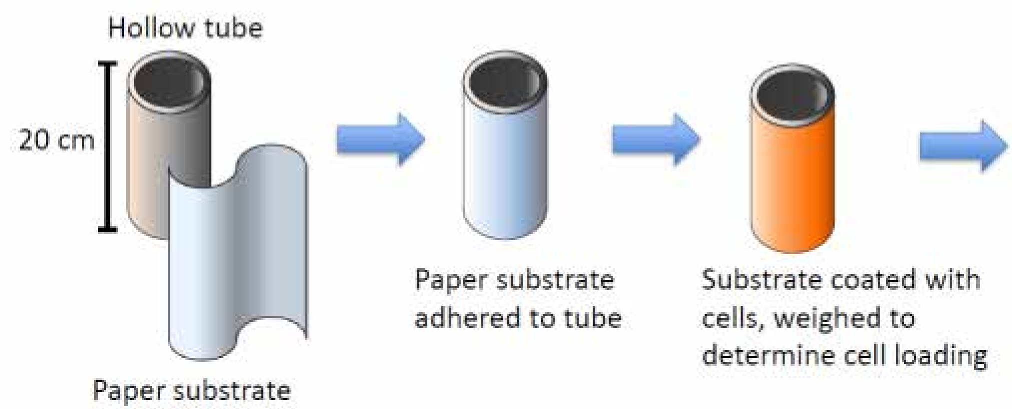
- Successfully achieved production of MA from methane (annual milestone).
- First multi-gene pathway engineered in methanotrophic bacteria.
- Pathway optimization and fermentation scale-up underway.

Techno-economic Analysis

- Preliminary analyses indicate **yield** remains a primary cost driver in the development of a viable biogas-to-fuels and chemicals processes.
  - Process intensification enhancements will specifically target CCE.
- kLa and volumetric productivity are interrelated and must be balanced to avoid CAPEX expenditures related to gas recycle and compression.



Process Intensification Enhances kLa and Productivity



- **> 10x organic acid titer enhancement in high-density biocomposite**
- **> 10x $k_L a$ enhancement in falling film reactor**
- Biocomposites sustain methane uptake for >100hours
- 100x less NO_3 than base media (0.01g/L)
- Increased CH_4 uptake rate with increased cell density

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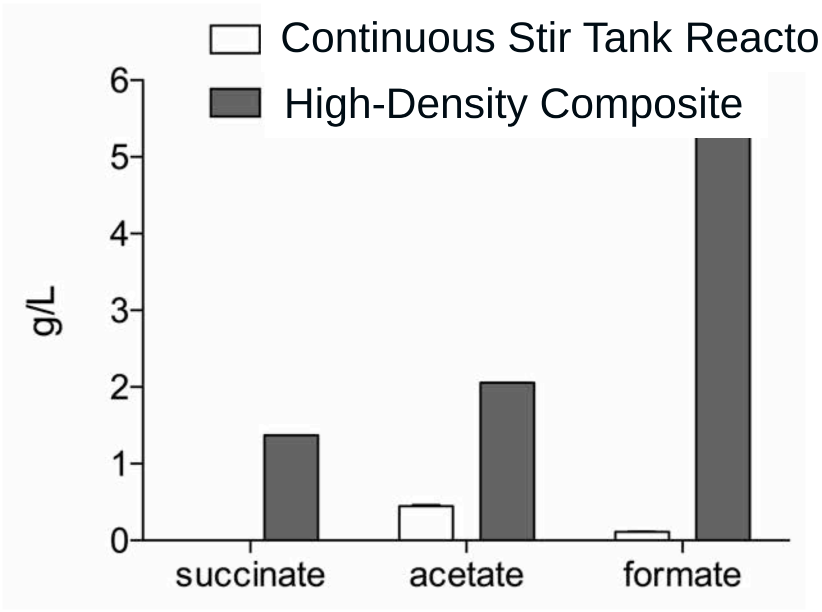

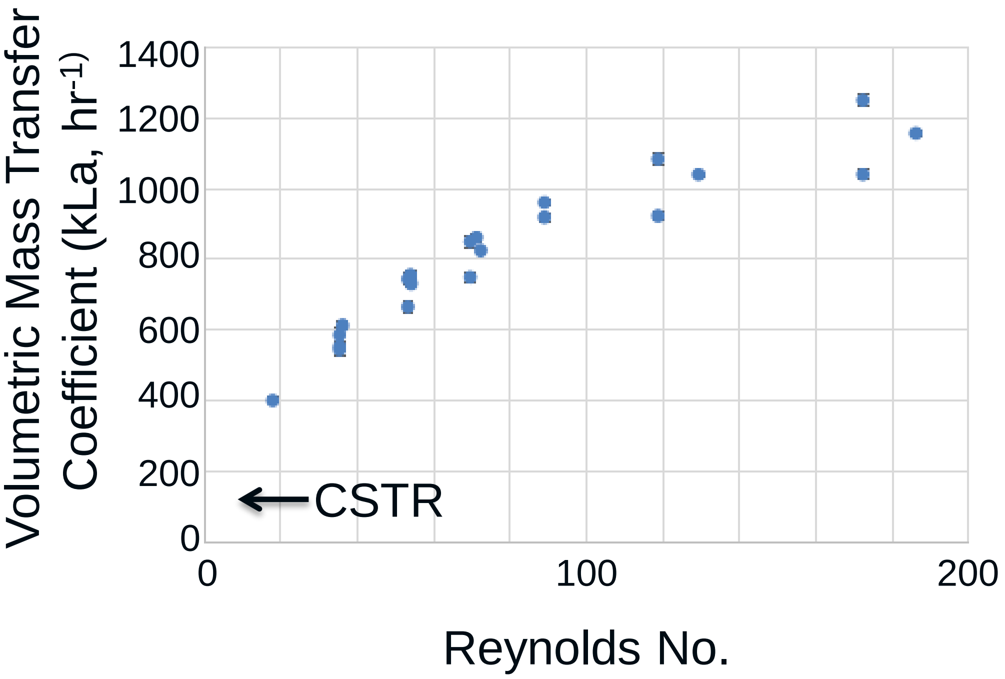

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Relevance

- H_2S-tolerant methanotroph opens the door for “feedstock agnostic” biogas valorization
- Novel reactor design enables process intensification for secreted products, with low water and power inputs, enhanced mass transfer and CCE.
- Tech transfer/marketability: represents proof-of-concept for an array of methane biocatalysis strategies.
- Applicable to an array of gaseous substrates.
- Relevant to EERE’s MYPP for developing cost-effective, integrated waste-to-energy processes for the production of bioproducts.

Future Work

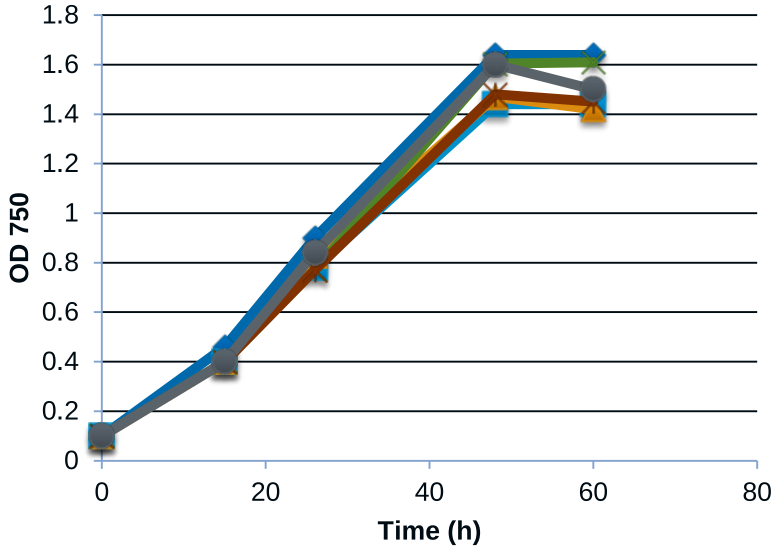
- **Future work will primarily target strain and reactor improvements for enhanced productivity and bioprocess integration.**
  - Incorporation of strain modifications informed by metabolomics and FBA.
    - MA pathway optimization via fine-tuned overexpression.
  - Covalent cell immobilization on biocomposites and initiation of trials with top MA production strain.
  - Updated TEA models with Y2 productivity metrics.

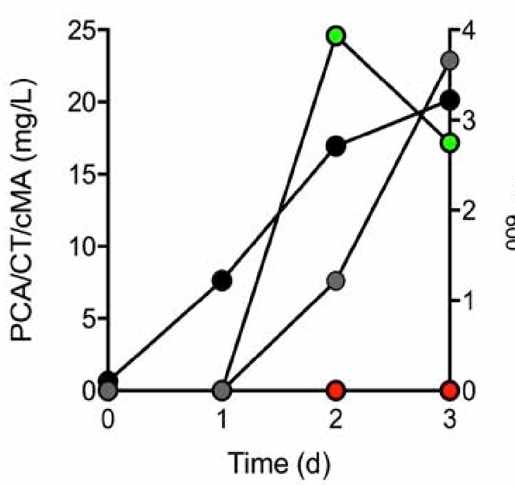
- **FY17 Go/No-Go:** Demonstrate 0.1g/L/hr productivity and reactor viability >96 hrs.

- **FY18 Targets & Beyond:** Integrate reactor and MA biocatalyst with real-time AD production.
  - Identify new opportunities for this platform and integrate with AOP activities.

Summary

- **Successful production of muconic acid from methane.**
  - H_2S-tolerant strain capable of cultivation on an array of biogas streams.
- We have developed an integrated approach and bioprocess for production of fuel and chemical intermediates from biogas.
  - Novel reactor design substantially enhances process efficiency.
- Significant potential to impact rapidly emerging methane conversion industry.
- Widespread applicability to an array of gas fermentation technologies.





Acknowledgements

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Process Intensification: Microbial Biocomposites

Cyanobacterial (top) and *Rps. palustris* (bottom)

Microbe	Gas Consumed	Gas or Product Evolved	Enzyme System
Gluconobacter	O_2	L-sorbose (D → L sugar oxidation)	LSDH (membrane bound)
Clostridia	CO, H_2	EtOH, acetate	CODH
Rhopseudomonas	N_2	fixed nitrogen	nitrogenase
	Ar (inert)	H_2	N_2 – limited nitrogenase
Synechococcus, Synechocystis, Anabaena	CO_2	O_2 , carbohydrates, lipids	hydrogenase
Chlamydomonas	CO_2	H_2	sulfur-limited hydrogenase

Courtesy of Flickinger, et al, 2015

- Dramatic productivity enhancements
  - Enhanced mass transfer; >10x improvements in gas uptake rate
  - Reactive for >6 months
  - Minimal media requirements; non-growth media
  - Applicable to an array of microbes, fuels, and co-products

Response to Reviewers' Comment

- This project was not subjected to prior review.

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