DOE Bioenergy Technologies Office (BETO) 2019 Project Peer Review

2.3.2.201 BCU FOA: Biogas Valorization

Waste-to-Energy

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BCU FOA Goals

- 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 **biogas**es.”

Goal Statement

Goals: Establish a process-intensified biogas-to-liquid bioconversion platform for secretion of platform chemicals.

Outcome:

- Development, improvement and demonstration of **integrated biological upgrading technology** for the production of substitutes for petroleum-based feedstocks, products, and fuels
- Diversification of BETO portfolio via **production of chemicals from biogas**.
- Muconic acid (MA) production from methane at rates >0.5g/L/hr

Quad Chart Overview

Timeline

- **Project start date:** October, 2016
- **Merit Review Cycle:** BP1 complete
- **Percent complete:** 100% (Project Terminated)

Budget

FY16-FY18 Cost (BP1)	Total Planned Funding (FY 19->End Date)
$2.37M	$0
San Diego State University – 10% North Carolina St University – 20% Farmatic, Inc. 25% Metabolon, Inc – 10%	20% Minimal Cost-Share Requirement

Barriers

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

Objective

- Development, improvement and demonstration of integrated biological or chemical upgrading technology for the production of substitutes for petroleum-based feedstocks, products, and fuels.
- Demonstrate process-intensified bioconversion using an immobilized gas-to-liquid bioreactor configuration.

End of Project Goal

- Production of organic acid from biogas at 0.5g/L/hr productivity.
- Established robust TEA models for standalone and bolt-on technology deployment.

Project Overview

Context

- BioGTL: a scalable, modular, selective approach to biogas conversion using methanotrophic bacteria.
- Poor gas mass transfer (k_La) limits productivity.
- Biocatalyst responsiveness to biogas unknown.
- Process intensification via low-power, low-liquid volume, and high-cell density reactor operation offers a means to enhance productivity, increase product titers, and reduce up/downstream processing requirements

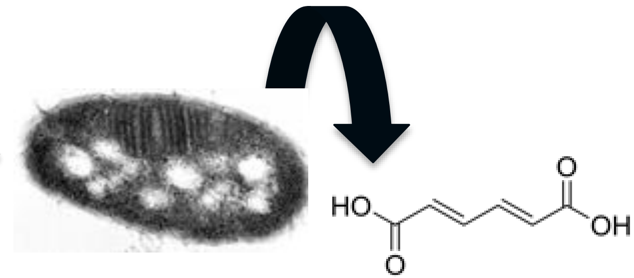
Specific Technical Goals

- Characterize biogas derived from domestic substrates and mitigate biogas toxicity.
- Generate novel MA-producing methanotrophic biocatalysts.
- Develop genome-scale metabolic models for methanotrophic biocatalysts.
- Design and implement a high-efficiency, low-power falling film reactor.
- Generate comprehensive technoeconomic models for an array of methane feedstock inputs and organic acid outputs.
- Demonstrate an integrated bioprocess for conversion of AD-biogas to MA

Approach - Management

Biogas Generation and Characterization

Falling Film Reactor Design



Strain Engineering, Metabolomics, and FBA

- Monthly group meetings, quarterly reporting, and regular interaction with BETO and technical staff.
- Synergistic interaction
  - NREL Strategic Analysis/TEA
  - Academic, government, and industrial partners conducting i) anaerobic digestion, ii) novel gas fermentation reactor design, iii) systems biology and functional genomics analyses, and iv) metabolic engineering.

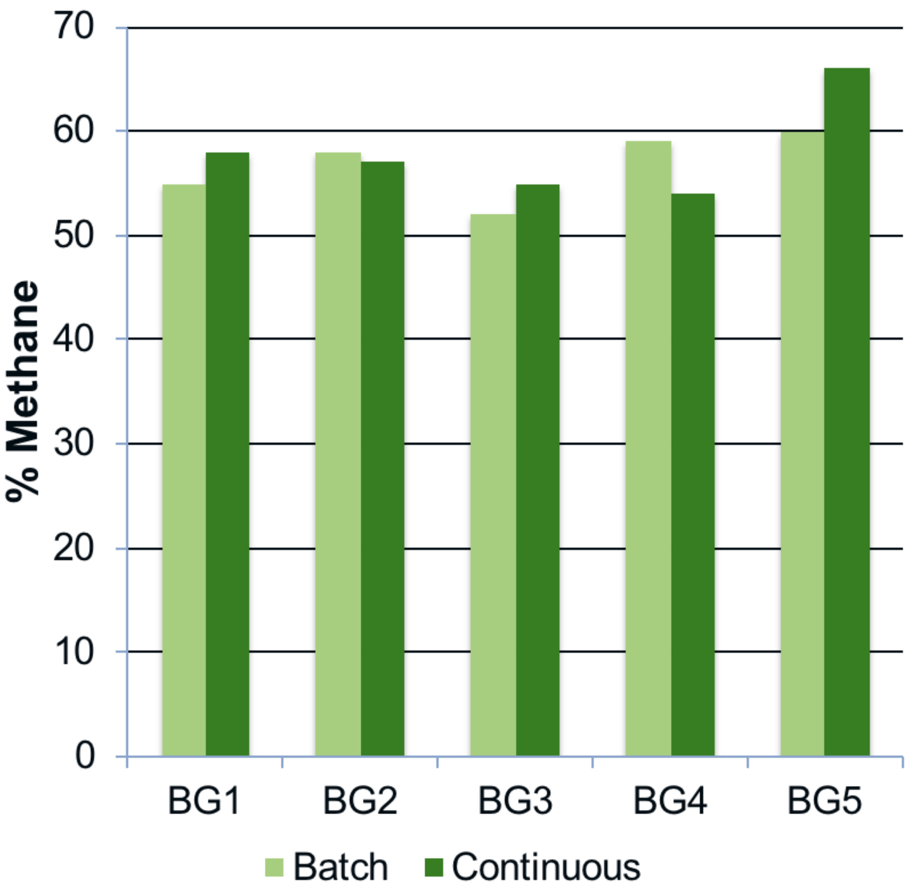
Approach - Technical

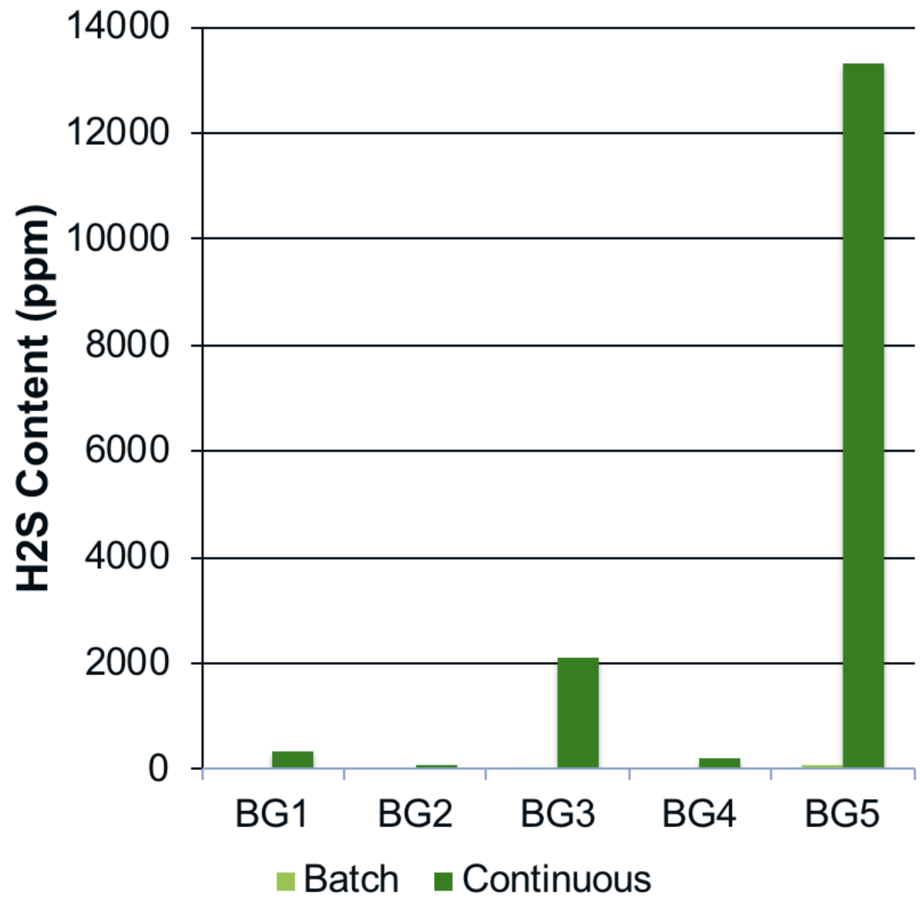
- **Approach:** Integrate AD, *in silico* modeling, metabolomics, strain engineering, and bioreactor design. Conduct iterative TEA to inform process targets and enhancements.
- **Core Tasks:**
  1. **Low-power reactor design**—cell adhesion and viability, extended performance;
  2. **Strain development**—high-productivity (titers, rates, yields (TRY) as dictated by TEA), biogas tolerance;
  3. **Process Integration**—optimization of biogas delivery, mass transfer, methane activation, and biosynthesis of MA.
- **Major challenges**
  - (i) poor gas mass transfer K_La, (ii) unknown biogas utilization capacity/toxicity, (iii) low productivity biocatalysts
- **Critical success factors:**
  - Generate methanotrophic biocatalysts with novel production capacity.
  - Sustain high productivity in immobilized methanotrophic catalysts.
  - Achieve economically-viable product titers, rates, and yields from raw biogas.

Progress-to-Date (BP1)

2016-2018

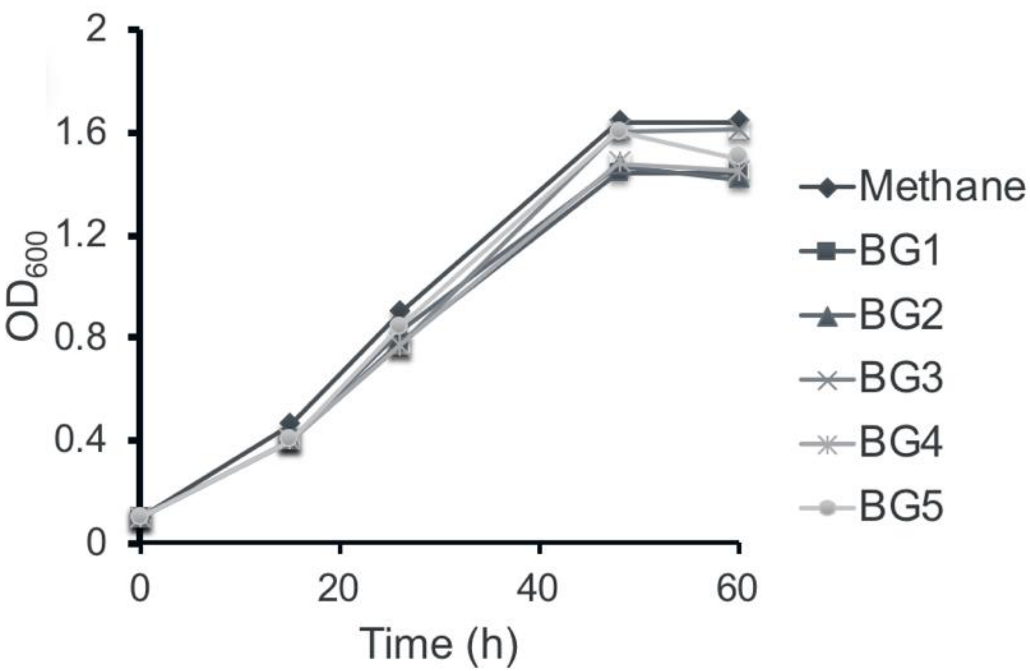
Biogas Characterization: CH_4 and H_2S Evaluation





- 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.

Biogas/H_2S Does Not Impact Growth



Carbon Source	*M. alcaliphilum* 20Z[R]	
	Y_B	T_d
CH_4	1.03	5.84
BG1	0.95	7.38
BG2	0.93	7.53
BG3	0.96	6.03
BG4	0.95	7.49
BG5	0.96	6.46

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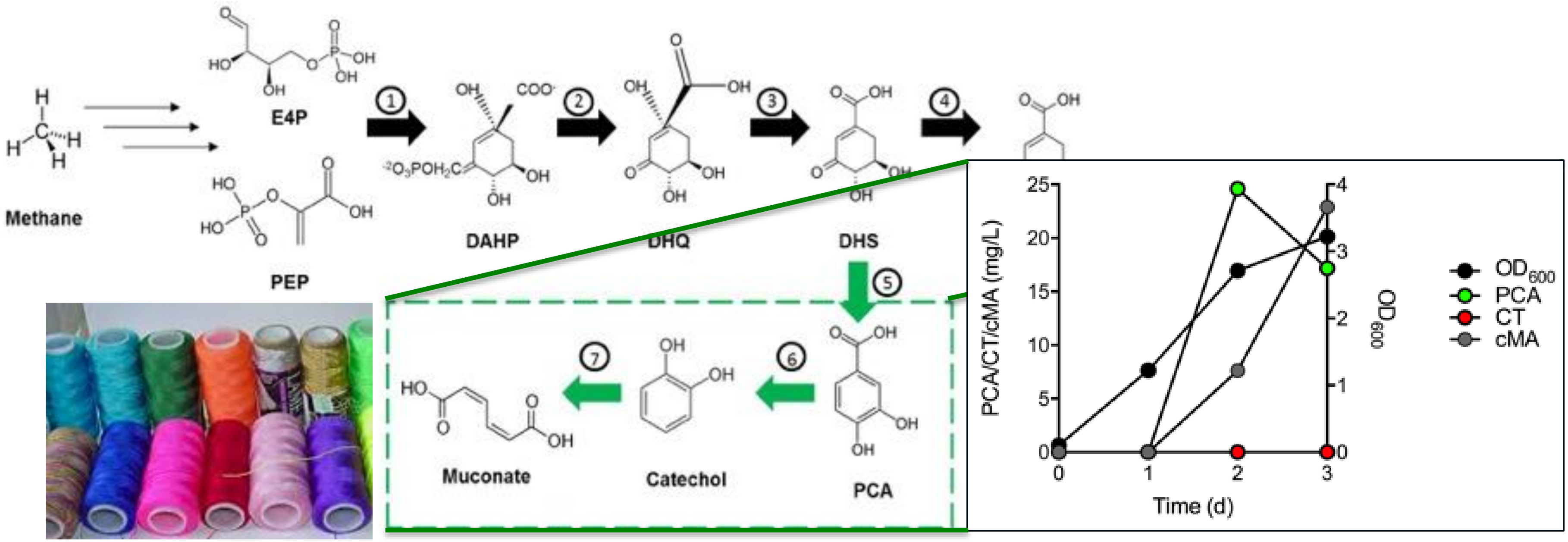
Strain Engineering: Muconate Biosynthesis

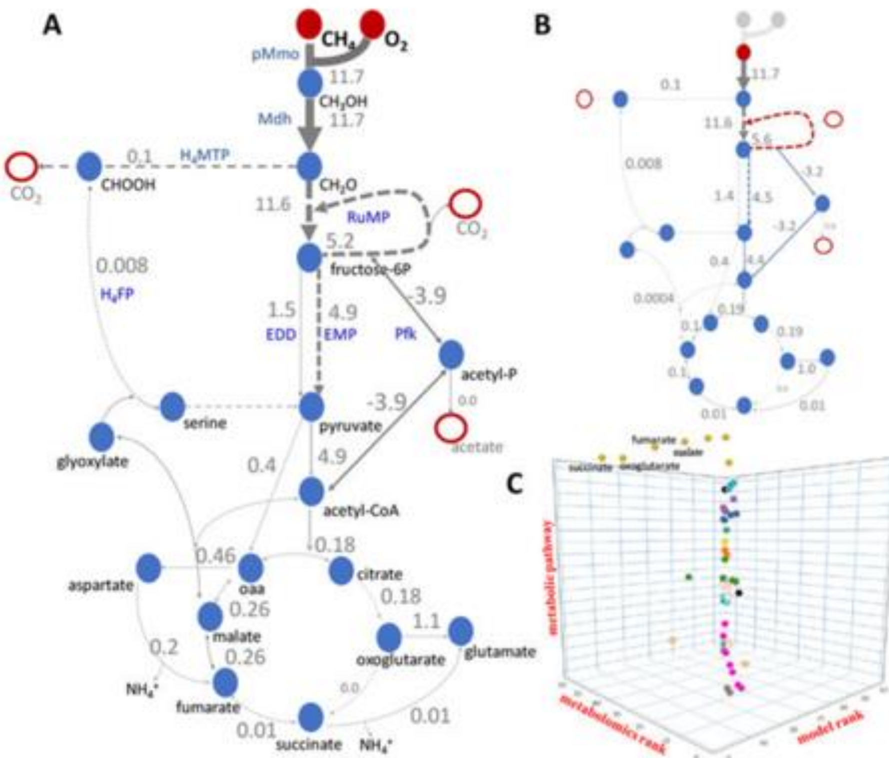


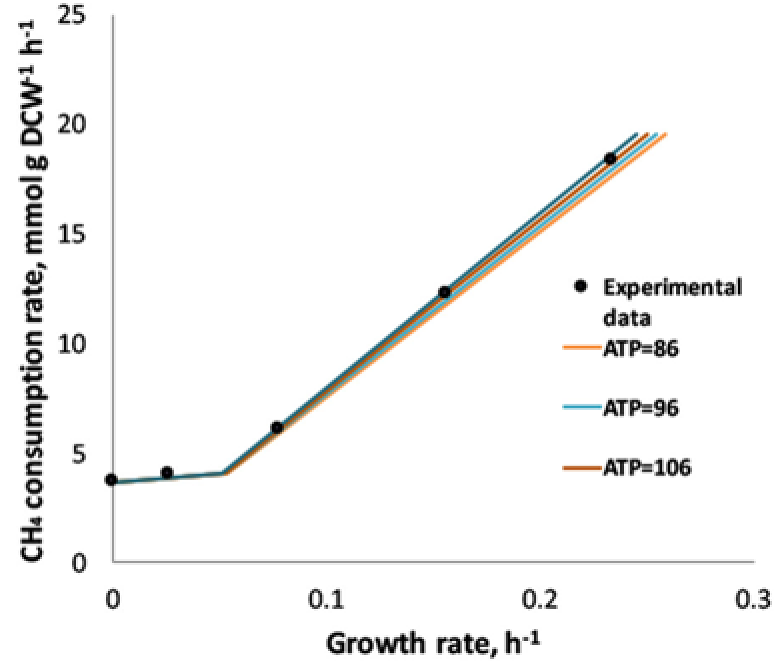
Schematic overview of synthetic muconic acid pathway.

US Patent App. 15/252,648

- MA: dicarboxylic acid that can be upgraded to produce high-value commodity chemicals, including terepthalic acid, PET.
    - Readily converted to adipic acid, a "top 50" bulk chemical; nylon-6,6 accounts for >85% of global adipic acid utilization
- Successfully achieved production of MA from CH_4 (Y1annual milestone).
- First multi-gene pathway engineered in methanotrophic bacteria.
    - US Patent App encompasses multiple organic acid production pathways.
- ***>25 cassette variants constructed and evaluated***

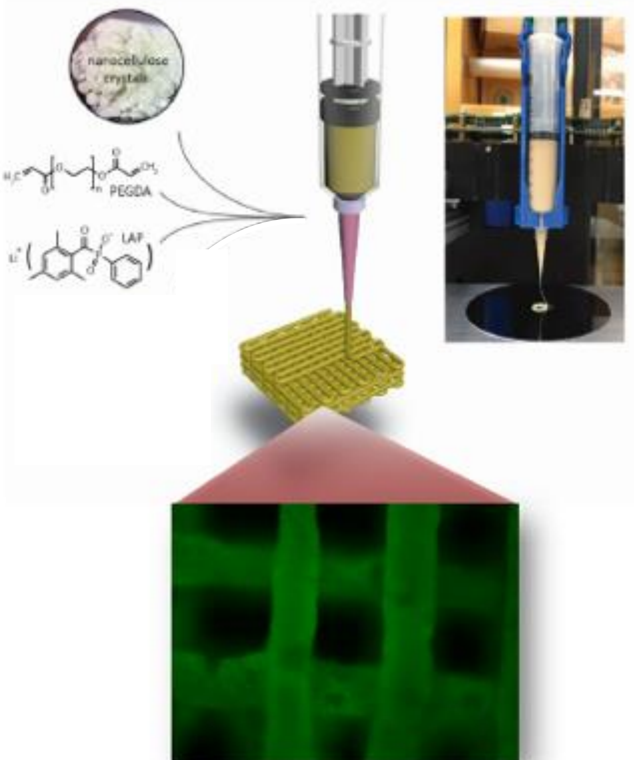
FBA and Genome Scale Modeling





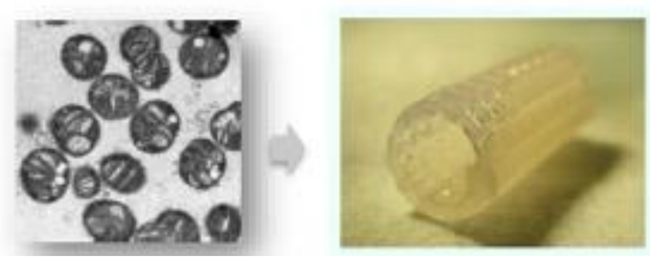
- FBA and GSMM construction enables prediction of growth rates for different CH_4 consumption rates at different level of ATP maintenance.
- Identified additional strain engineering targets for enhanced flux to shikimate pathway.
  - SDH and SDH/PDH Mutants generated
  - SDH mutant essential for high MA flux in other microbes
    - **Redundant gene(s) are present, preventing KO.**

Process Intensification via Novel Reactor Design

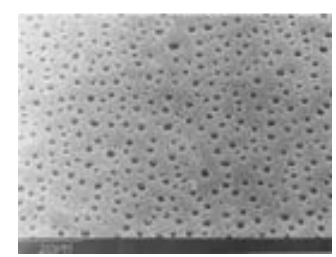


Green stain: live printed microbes

- Three immobilized reactor configurations evaluated:
  - Falling Film Reactor: cellulose immobilization
  - 3D printed scaffolding
  - Silicone embedded
- 10X $k_L a$ enhancement in FFR, but insufficient cellular immobilization.
- 3D scaffolds were ultimately selected due to production throughput, stability, lifetime, ease of handling, and cell viability.



Printed Scaffolds

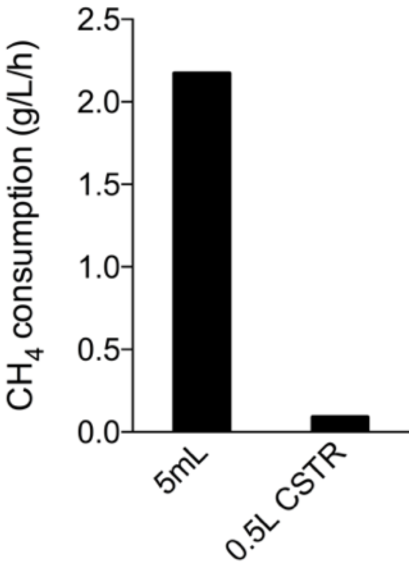


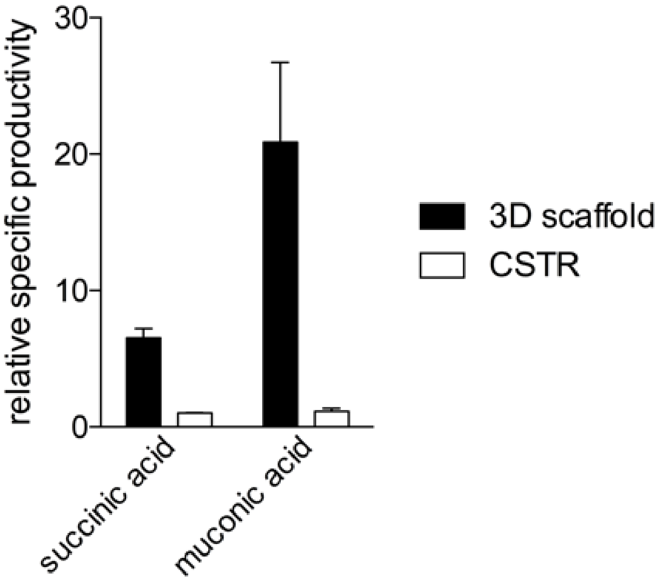
Silicone Embedded

Lawrence Livermore National Laboratory

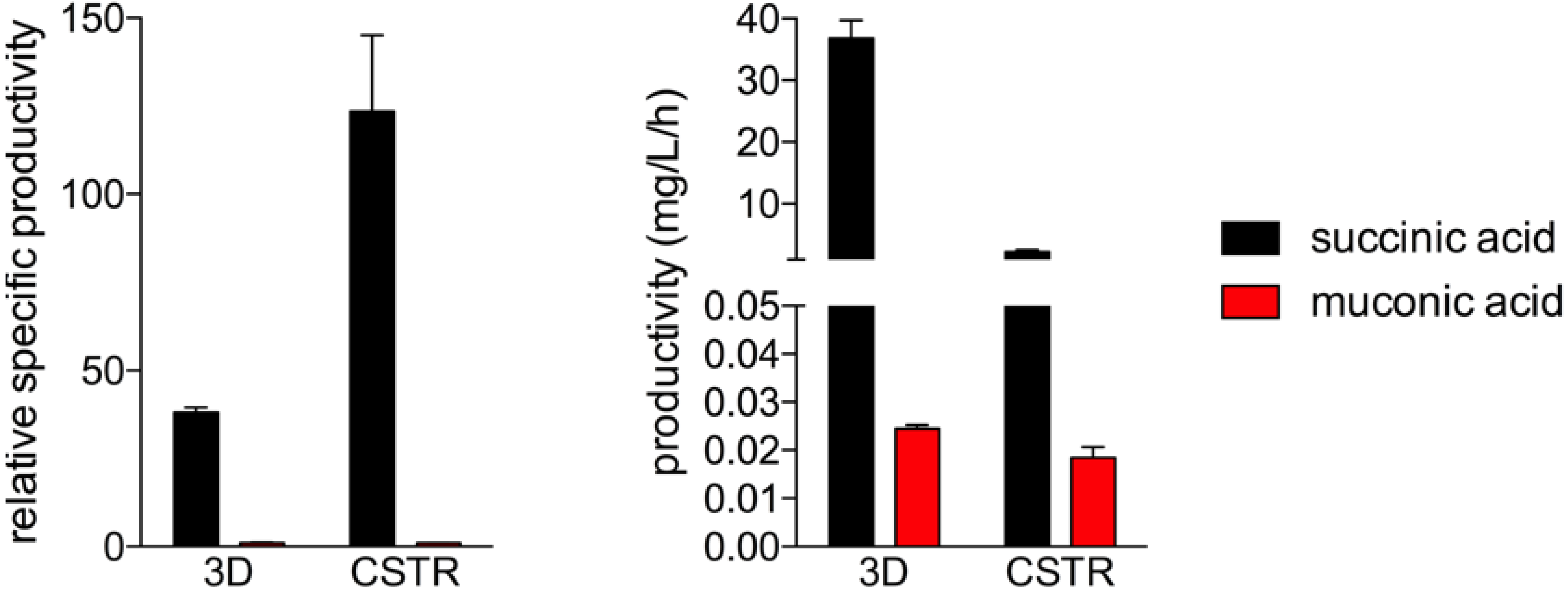
Process Intensification via Novel Reactor Design

- High density (>OD120)
- *Productivity scales linearly as a function of cell density and volume!*
- >20X Process Intensification relative to CSTR.
- >20g/L SA titer achieved at 5mL scale
- >20X specific productivity enhancement relative to CSTR!





Process Intensification via Novel Reactor Design



End-of-project productivities achieved:

- Succinic acid: 0.5g/L/hr
- Muconic acid: 0.01g/L/hr

Verification Summary

- Performance test was observed by a BETO-led verification team, including government, industry, and academic subject matter experts
- High density, scalable methane conversion achieved.
  - >20X CH_4 uptake rate relative to CSTR
  - >20X specific productivity relative to CSTR
- Titers and Rates enhanced
  - 0.01g/L/hr MA achieved at 5mL scale.
    - >0.5g/L/hr MA productivity projected to require 2-to-3 order of magnitude scale-up (5-50L).
  - >0.5g/L/hr SA productivity achieved at 10mL scale
- PI does not enhance yield to MA (substantially).
- Flux TCA > Flux PPP
  - SA flux is nearly 50X higher than MA flux
- Insufficient MA productivity led to project termination in September, 2018 following project verification.

Summary: Key Accomplishments

- **Characterization of a series of domestic biogas streams.**
  - Successful methanotrophic cultivation thereon.
- **Successful metabolic engineering for production of muconic and succinic acids from methane.**
  - Low MA flux, high TCA flux
- **Development of robust TEA models for standalone and bolt-on applications.**
- **Generated a series of scalable, process-intensified reactor designs**
  - Development of non-growth media, with sustained CH_4 uptake
  - >10x abiotic k_La enhancement.
  - Halophilic methanotrophs are poorly suited for immobilization in FFR
- **Metabolomic and *in silico* FBA and GSM integrated.**
  - Identified a series of strain engineering targets and flux bottlenecks.
  - Predictive modeling capacity for broad product suites.
- **Design principles generated here provide a path forward for an array of products from gaseous substrates.**

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Response to Reviewers' Comments 2017

- *This project has a good development pathway and has a lot of tools to achieve the targets. This is a very relevant work with large implications to industry and the Multi-Year Program Plan targets. Use of muconic acid as a target molecule gives it a general applicability to a large market…*
- *The team appears to be well-managed and highly capable on the genetic engineering side, with achievements of significant results. The project incorporates good use and interaction with TEA models. Future integration of the reactor design and biocatalyst in real-time AD will be significant if successful…*
- *The scalability of the reactor design is also something that will be good to understand in more detail…*
- *Besides some of the basic considerations regarding the actual relevance of biogas versus just regular natural gas for this type of project, my key comment is that the TEA is still quite wanting. A better analysis could help make better sense of the opportunity afforded by an otherwise highly innovative project.*

We thank the reviewers for their complimentary and constructive feedback. As noted by the Review Panel, we are excited about the broad potential impact this work will have on BETO's nascent WTE and gaseous waste carbon Technology Area, as well as the larger biogas industry and the bioeconomy as a whole. This project offers a novel route to biogas conversion via an process-intensified approach, encompassing biogas generation, biocatalyst and bioreactor engineering, in silico analyses, and techno-economic sensitivity analyses. Though the project did not achieve original MA productivity targets, we successfully produced an alternative platform chemical intermediate, succinic acid, at productivities exceeding 0.5g/L/hr, indicating high-potential for process-intensified conversion of methane to high-value chemicals. Follow-on work, if funded, would include intensified focus on TEA parameters including reactor cost, lifetime, and scalability.

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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