NREL
Transforming ENERGY

Biogas Biocatalysis

WBS 2.3.2.102

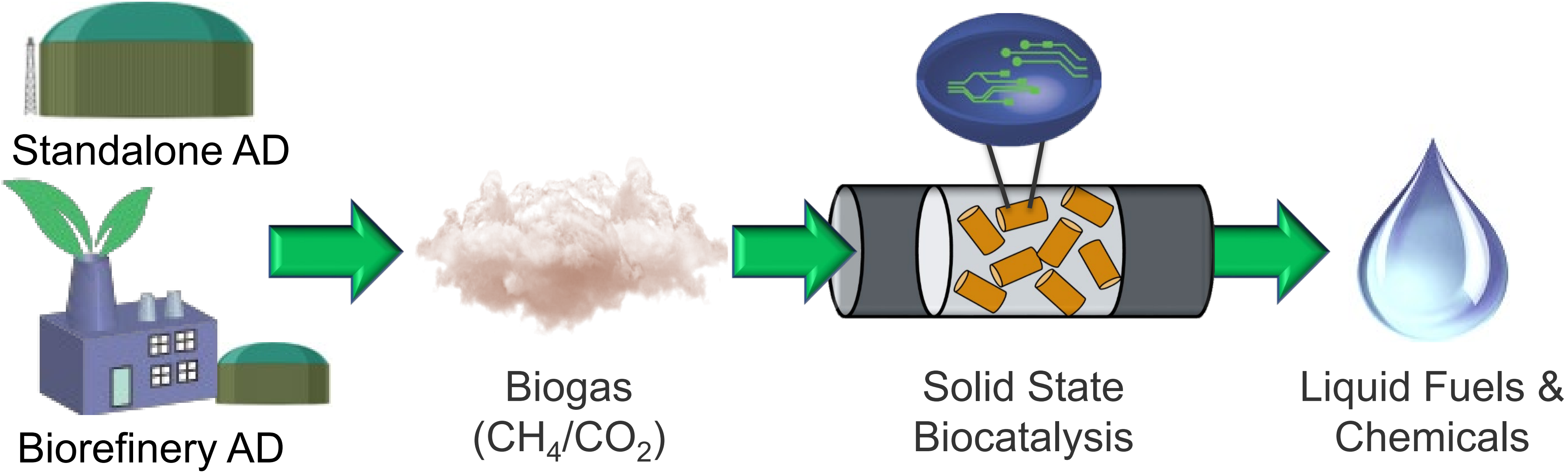
March 10, 2021

Technology Area Session: Organic Waste

Principal Investigator: Mike Guarnieri
Organization: NREL

Project Overview Snapshot

Big Picture: Biological gas-to-liquid conversion offers a means to valorize biogas, improve bioprocess sustainability, and reduce risk of waste and biomass processing.



Value: Biogas presents large market and energy value: > 35B GGE (> 4 Quad btu)
SOT: Biogas is primarily flared or used to produce combined heat and power (CHP)
Goal: Develop biocatalysts and gas fermentation tech to enable gas-to-liquid conversion achieving biogas valorization and improved process economics and sustainability.
Risk(s): Poor mass transfer and gas conversion metrics = unviable space-time yields

Market Trends

Product	Anticipated decrease in gasoline/ethanol demand; diesel demand steady
Product	Increasing demand for aviation and marine fuel
Product	Demand for higher-performance products
Product	Increasing demand for renewable/recyclable materials
Feedstock	Sustained low oil prices
Feedstock	Decreasing cost of renewable electricity
Feedstock	Sustainable waste management
Feedstock	Expanding availability of green H_2
Feedstock	Closing the carbon cycle
Capital	Risk of greenfield investments
Capital	Challenges and costs of biorefinery start-up
Capital	Availability of depreciated and underutilized capital equipment
Social Responsibility	Carbon intensity reduction
Social Responsibility	Access to clean air and water
Social Responsibility	Environmental equity

NREL's Bioenergy Program Is Enabling a Sustainable Energy Future by Responding to Key Market Needs

Value Proposition

- **Expand BETO's feedstock portfolio**: reducing exposure to commodity risk
- **Improve economics and C-intensity** of waste conversion and biorefining
- Enable first-in-class **bioprocess intensification for** an array of **gas conversion processes**

Key Differentiators

- **Biological gas-to-liquid** conversion: a scalable, modular, selective approach to biogas conversion using methanotrophic bacteria.
- Leverages **unique National lab capabilities** in methanotrophic metabolic engineering and gas fermentation

1. Management

U.S. DEPARTMENT OF ENERGY | Energy Efficiency & Renewable Energy

BIOENERGY TECHNOLOGIES OFFICE

PM, TM: Beau Hoffman, Mark Philbrick

NREL Transforming ENERGY

PI: Mike Guarnieri

Platform Lead: Rick Elander

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Techno-Economic Analysis

Leads: Ling Tao, Ryan Davis

Scientific Collaborators and Advisors

San Diego State University 1897

UNT

iMiCROBES

tradewater

MANGO MATERIALS

ZYMOCHEM

NREL Transforming ENERGY

Task 1: Metabolic Engineering

Lead: Mike Guarnieri

Lawrence Livermore National Laboratory

Task 2: Reactor Development

Lead: Sarah Baker

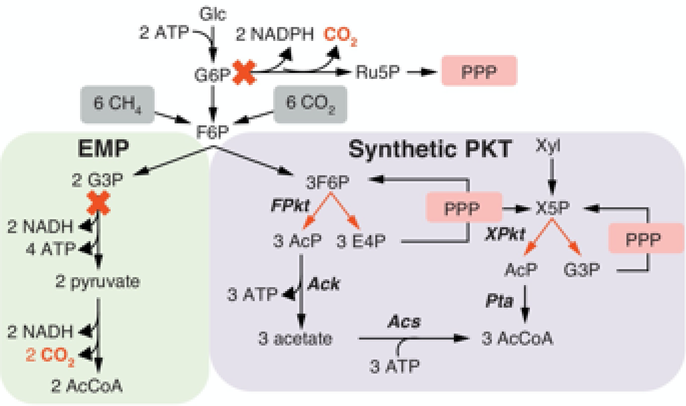
1. Management

- **Diverse Staffing Plan Enables Multi-disciplinary Approach**
  - *Strain Engineering*: molecular microbiologists conduct systems biology and strain engineering
  - *Reactor engineering*: fermentation engineers and materials scientists lead gas ferm optimization
  - *Chem/Process Engineers* conduct chemical catalysis and TEA/LCA
- **Research guided by TEA/LCA**, with related quarterly milestone metrics & reporting.
- **Team and Industry Engagement:** group (weekly), SAC and Platform (bi-monthly)
  - *Constant communication/collaboration with related projects and scientific advisors*
- **Risk I.D. and Mitigation**
  - TEA-informed Annual SMART and Go/No-Go decision points target key cost drivers
  - Leads are empowered to make minor changes to the research plan (no milestone impact)
  - Decisions resulting in a major shift require approval of the PI and Platform Lead
  - Team review is deployed for risk assessment, mitigation, and evaluation of the affect the change will have on the **S**chedule, **D**eliverables, and **B**udget.
  - DOE engagement to refine/approve proposed major changes and execute Change Control.

2. Approach

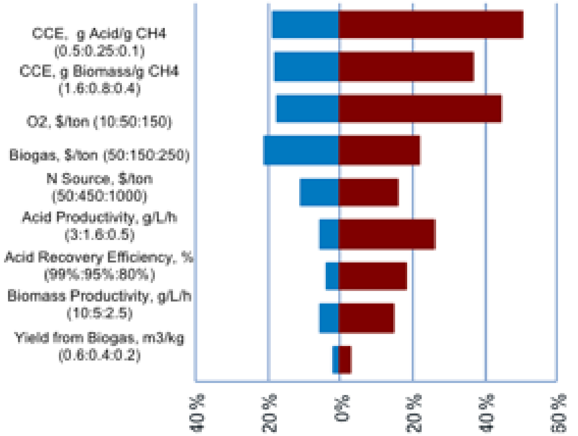
Approach: Integrate metabolic engineering, novel reactor design, and TEA to inform hypothesis-driven strain- and fermentation-development strategies.

- **Task 1**: Develop biocatalysts with high-yield CH_4/CO_2 conversion to broad product suites.
- **Task 2:** Design and fabricate a first-in-class solid state gas fermentation reactor.



Strain Engineering

Fermentation Engineering



TEA/LCA

2. Approach

Major challenges

- (i) CCE (FY17-19), (ii) end-product tolerance, (iii) CO_2 utilization, (iv) gas-liquid mass transfer

Critical success factors:

- Achieve economically-viable product titers, rates, and yields via CH_4/CO_2 co-utilization
- Achieve >10X process intensification via solid state gas fermentation relative to SOT

FY19-FY21 Overarching Project Goals:

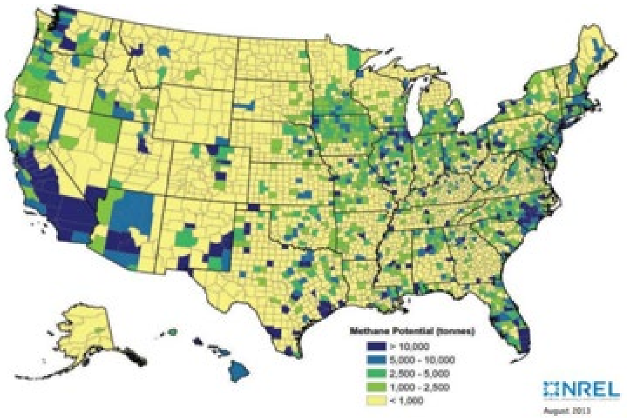
1. Achieve C- and energy-efficient biogas bioconversion
2. Generate biocatalysts with CH_4/CO_2 co-utilization capacity
3. Develop first-in-class solid state gas bioreactor tech (integration with FY20 Seed)
4. Establish a TEA baseline for biogas biocatalysis

Go/No-Go (FY20): Complete TEA to i.d. performance "TRY" metrics required to incur a net TEA benefit of >$0.25/GGE reduction and >5% carbon yield enhancement relative to a biorefinery baseline that diverts biogas to CHP. Establish biocatalyst baseline and down-select to 2 bioproducts for further development and reactor integration.

End Project Target: Achieve >$0.25/GGE reduction and >5% carbon yield-increase relative to baseline for BC Platform (biogas→power).

3. Impact

- **<u>CH_4 is the primary component of anaerobic digestion biogas</u>**, landfill gas, and natural gas (NG), and second most abundant GHG.
  - > 2,000 AD units in the U.S.



- Flared, stranded, and remote gas presents **<u>large market and energy value</u>**
  - > 35 Billion Gallons of Gasoline Equivalent (> 5 Quad btu)
  - Sufficient to displace 46% of current NG consumption in the electric power sector and the entirety of NG consumption in the transportation sector

- **<u>20% input biomass C in biorefinery →Wastewater A.D</u>**.

- *Despite market and energy potential, biogas is generally incompatible with transportation and manufacturing infrastructure!*

3. Impact

Successful implementation of the Biogas Biocatalysis project plan will:

- Expand BETO's feedstock portfolio: reducing exposure to commodity risk
- Directly targets BETO MYPP Barriers (please refer to Quad Chart)
- Improve economics and C-intensity of waste conversion and biorefining
- Establish first-in-class bioprocess intensification for myriad gas conversion processes

Strain, Tool, and Data Dissemination

- Publications, patents, presentations (please refer to Additional Slides)
- > 10 Material Transfer Agreements executed for strains and tools

Commercialization Potential

- Industry targeting **biogas generation** and **C1 gas upgrading** technologies.
- Biocatalysts and reactors developed here = "game-changer" process improvements
- Partnership has been initiated with biogas providers and gas upgrading industry with frequent engagement to assess collaborative and market entry opportunities/barriers.
  - ***FY17-20 established robust cultivation capacity on raw biogas***
  - Commercial technology piloting opportunities have been established

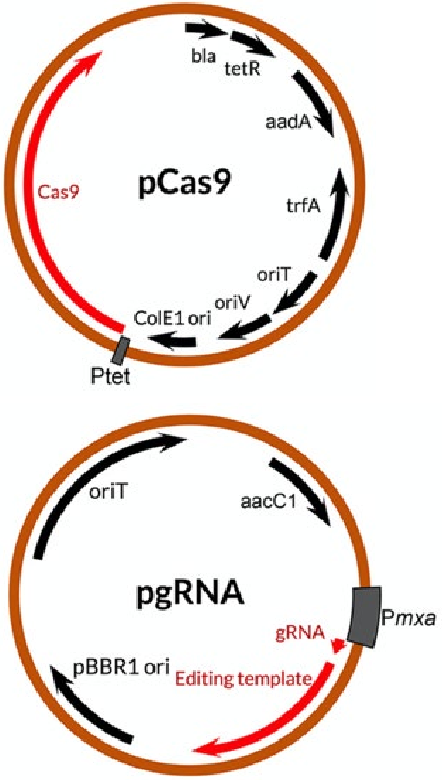
4. Progress and Outcomes: Snapshot

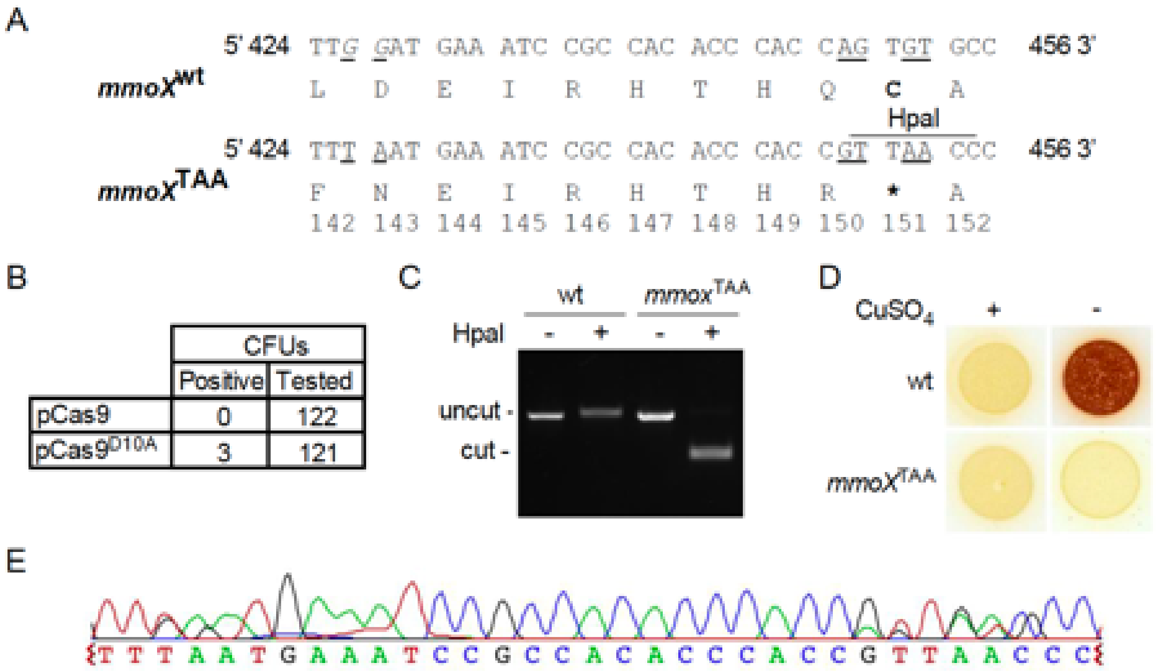
First-in-class technical advances

- Achieved the **highest carbon conversion efficiency** reported to date
  - > 90% theoretical yield, > 1g biomass/g CH_4
- Developed first methanotrophic **CRISPR genome editing** system
  - Enables rapid, multi-target metabolic engineering
- Achieved **CH_4/CO_2 co-utilization** via targeted strain engineering
  - > 30% of biomass derived from CO_2
- Conducted Adaptive Laboratory Evolution to increase acidotolerance
  - **Highest reported methanotroph acid tolerance** to date: > 20g/L
- Metabolic engineering for production of > 10 fuel- and polymer intermediates
- Design and fabrication of a solid-state gas fermentation vessel
  - Achieved **highest reported methane conversion rate** to date: > 5g/L/hr
  - **>10X Process intensification** relative to current SOT (>10X K_La increase)

4. Developed First Methanotrophic CRISPR Toolbox

This advance enables multi-target *in vivo* genome editing

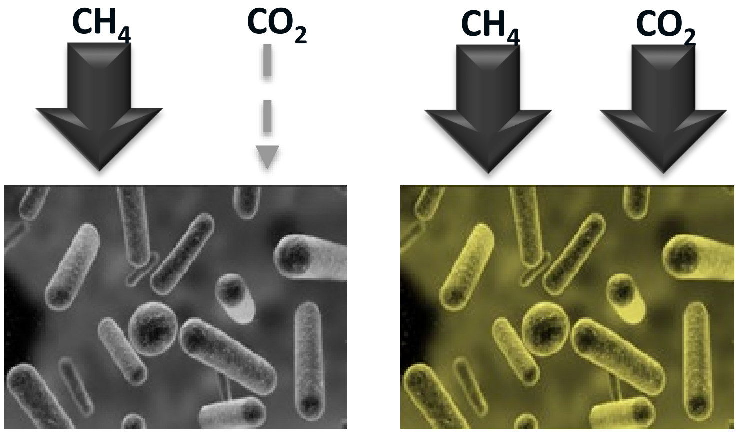




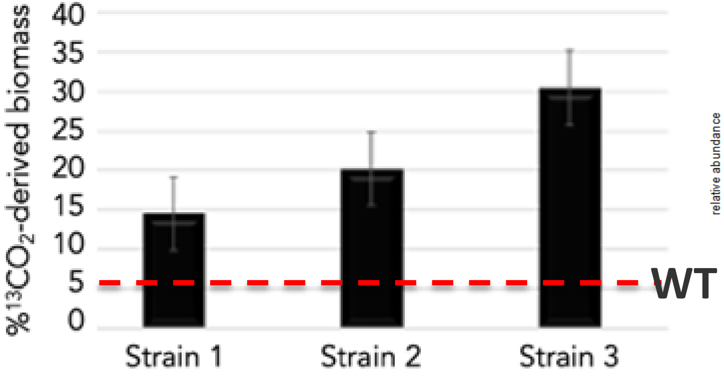
4. Achieved CH_4/CO_2 Co-Utilization

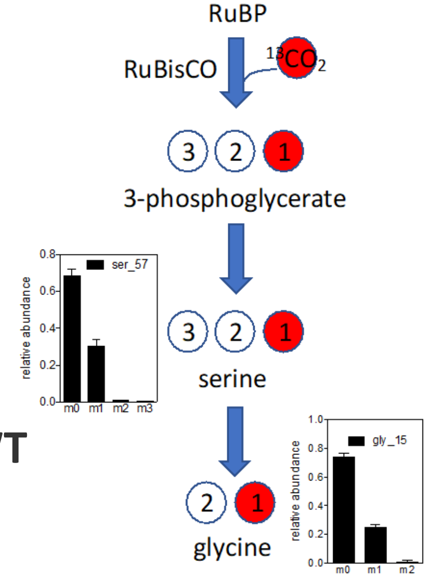
This advance enables complete biogas utilization

- Biogas is comprised of 25-50% CO_2
- Strain engineering achieved **> 30% biomass carbon derived from CO_2**
- Conducted metabolic flux analysis (MFA) to determine CO_2 flux node(s)



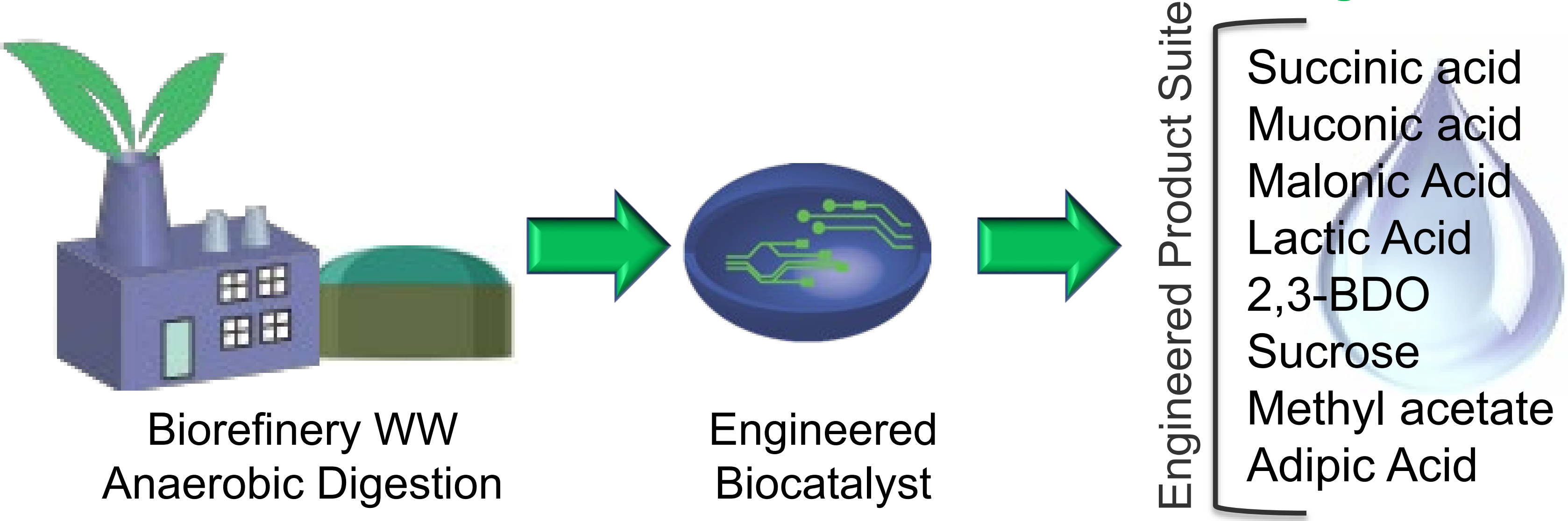
Wild-Type Methanotroph Engineered Methanotroph





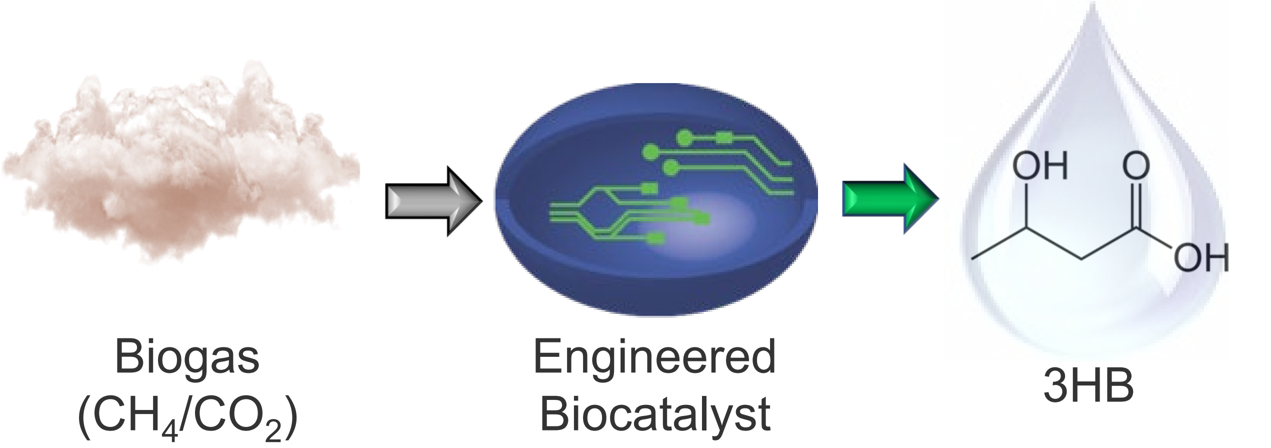
4. Generation of High-Value Co-Product Suites

- 10-20% total biomass carbon ends up in WWT.
- TEA and metabolic evaluation was conducted to identify top-candidate fuel and chemical intermediates
  - **>$1/GGE cost-reduction potential** for cellulosic fuel processes.
- Successful production and baselining of 10 candidate target liquid products
- **FY19-20 Go/No-Go led to TEA-informed down-selection to target molecule(s).**



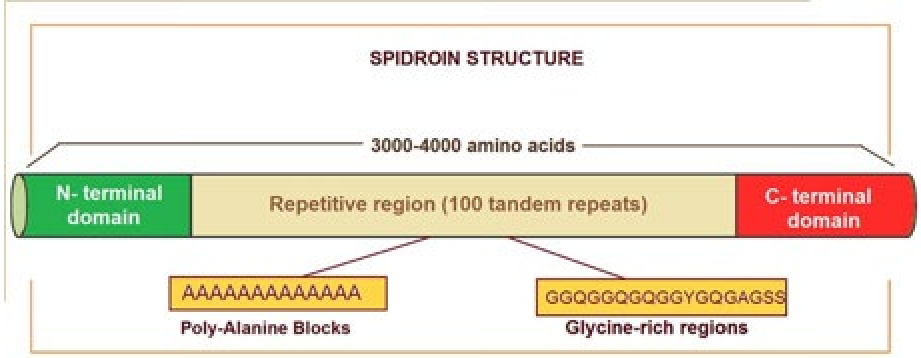
4. Established a High-Yield Biogas-to-Chemical Platform

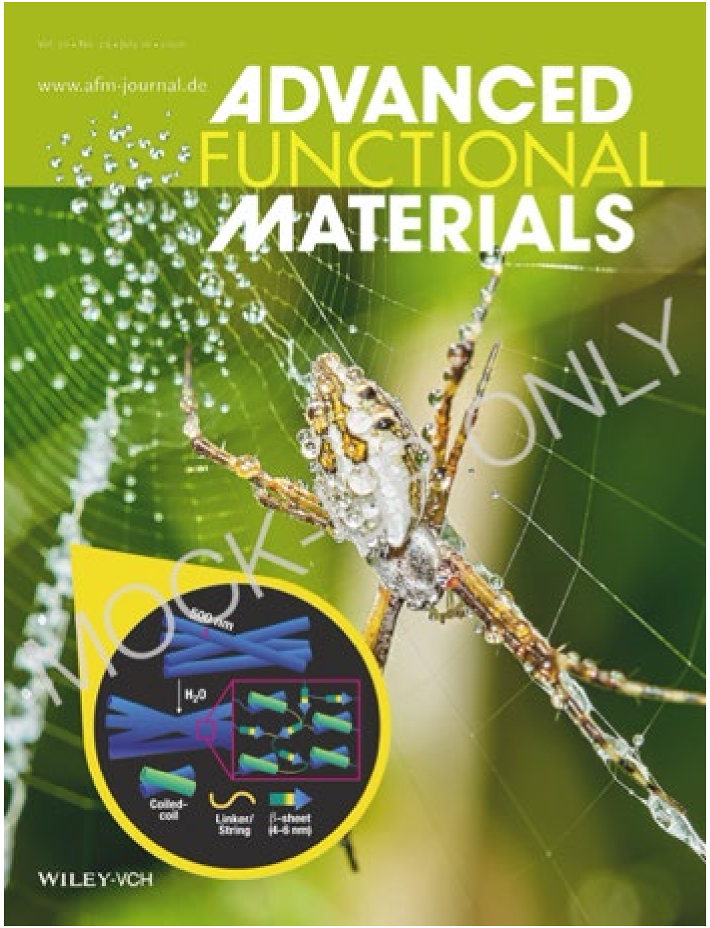
- >50% dry cell weight is intracellular polyhydroxybutyrate (PHB)
- Metabolic rewiring achieved >50% yield (g/g) to excreted 3-hydroxybutyrate (3HB)
  - 3HB is a precursor for an array of polymers, commodity, and fine chemicals
- **Q1 Milestone:** Achieve >2-fold productivity enhancement to 3HB via genetic knockout of 3HB dehydrogenase
  - ***Achieved highest reported organic acid titer to date in methanotrophic biocatalysts***
- Current titer (> 5g/L) is suitable for direct catalytic upgrading to propene.



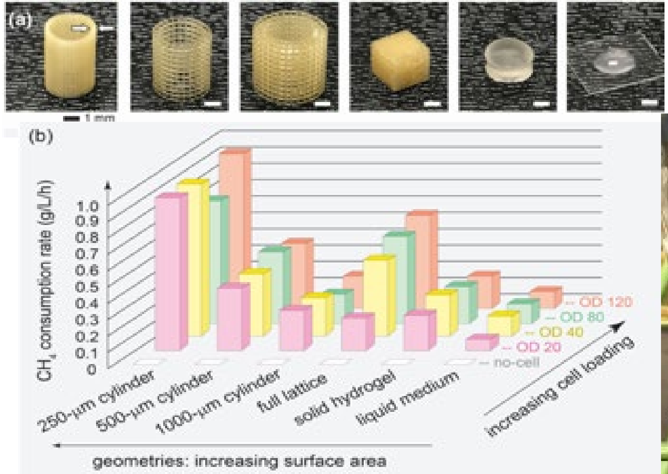
4. Established a Biogas-to-Proteopolymer Platform

- 30-50% methanotrophic biomass is protein
- **Target**: divert >5% protein flux to **<u>spidroin</u>**
  - Suitable for production of diverse functional materials (e.g. renewable Kevlar)
  - High-value, sustainable bioproduct
- *Presents a route to complete biomass valorization*

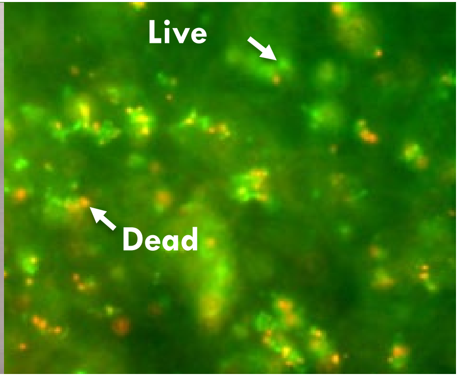




4. Designed and Fabricated Solid State Gas Fermentation



This advance enables >10X Process Intensification

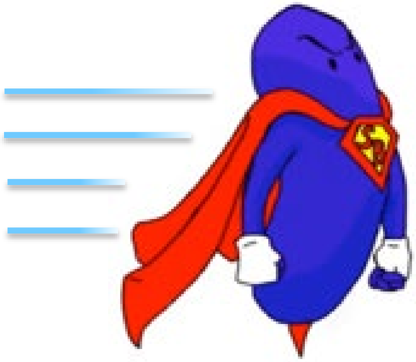


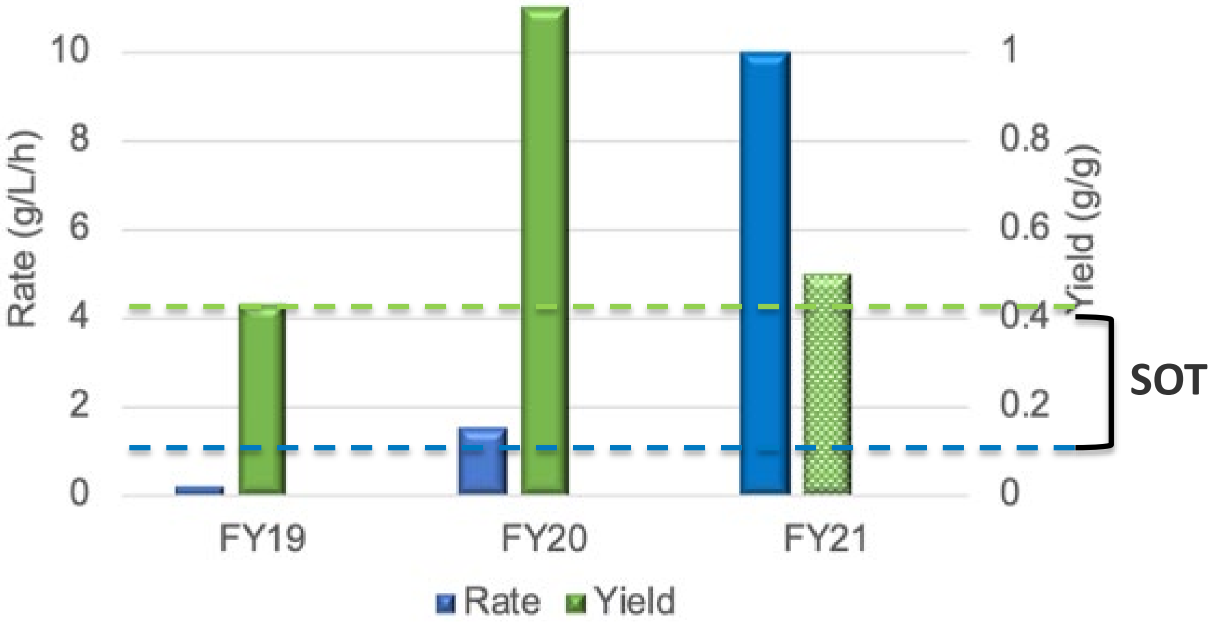
- > 10X conversion rate and organic acid titer enhancement relative to SOT
  - **<u>Highest reported methane conversion rate reported to date</u>** (> 5g/L/hr)
- No liquid inputs, low-to-no power inputs
- Non-growth conversion = no nutrient input = maximal flux to product
- Linear scalability – suitable for small- and large-scale digester integration
- > 3-month biocatalyst reactor lifetime
- *In situ* product separations and recovery

FY19-21 State of Technology Progress

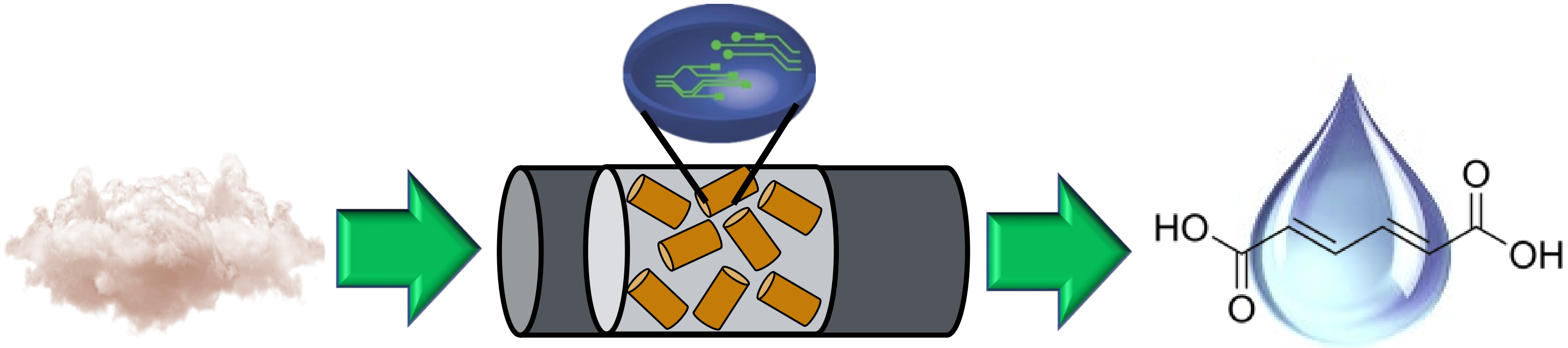
We have successfully targeted Critical Success Factors in order to achieve:

- > 50X enhancement to C1 conversion rate.
- > 90% theoretical yield from C1 to biomass.
- > 50% yield from C1 to 3HB.





Future Directions: Process Integration



- **Q2 Milestone:** Integrate top candidate biocatalyst(s) into solid state reactor and demonstrate continuous biogas uptake for >96hours, achieving >50% biological yield from biogas to 3HB.
- **End Period of Performance Goal:** Achieve >$0.25/GGE reduction and >5% carbon yield-increase relative to biorefinery baseline (biogas→power).
- **Next Period of Performance:**
  - Systems and synthetic biology to maximize flux to target product(s)
  - Reactor optimization and prototyping
  - *Pilot-scale deployment in partnership with commercial A.D. operators and industrial biogas upgrading partners.*

Summary

- **Management**
  - Multi-disciplinary staffing plan
  - Frequent and Iterative Team and Industry Engagement
  - Comprehensive risk management plans ensures agile execution
- **Approach**
  - TEA-informed strain and fermentation engineering
  - Dual pronged task structure targets strain and reactor enhancements
- **Impact**
  - Development of potential “game changer” technology to enable valorization of high-volume, high-energy gaseous waste
  - Frequent industry engagement and data/strain/tool transfer to facilitate commercialization
- **Progress**
  - Highest reported carbon conversion efficiency to date
  - CH_4/CO_2 co-utilization
  - Biocatalysts with diverse product suites
  - >10X process intensification and highest reported methane conversion rate to date

Market Trends

Product	Anticipated decrease in gasoline/ethanol demand; diesel demand steady
	Increasing demand for aviation and marine fuel
	Demand for higher-performance products
	Increasing demand for renewable/recyclable materials
Feedstock	Sustained low oil prices
	Decreasing cost of renewable electricity
	Sustainable waste management
	Expanding availability of green H_2
	Closing the carbon cycle
Capital	Risk of greenfield investments
	Challenges and costs of biorefinery start-up
	Availability of depreciated and underutilized capital equipment
Social Responsibility	Carbon intensity reduction
	Access to clean air and water
	Environmental equity

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Value Proposition

- **Expand BETO's feedstock portfolio**: reducing exposure to commodity risk
- **Improve economics and C-intensity** of waste conversion and biorefining
- Enable first-in-class **bioprocess intensification for** an array of **gas conversion processes**

Key Accomplishments

- Achieved TEA-informed biocatalyst and gas fermentation engineering enhancements enabling **>10X process intensification, CH_4/CO_2 co-utilization capacity, and first-in-class biogas-derived chemical product suites**.

Acknowledgements

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Ellsbeth Webb

Additional Slides

Quad Chart Overview

Timeline
- Project start date: 10/1/18
- Project end date: 9/30/21

	FY20	Active Project
DOE Funding	(10/01/2019 – 9/30/2020)	$400,000

Project Partners
- Lawrence Livermore National Lab (Sarah Baker)

Barriers addressed
Ct-H. Gas Fermentation Development
Ct-D. Advanced Bioprocess Development
Ct-F. Increasing the Yield from Catalytic Processes

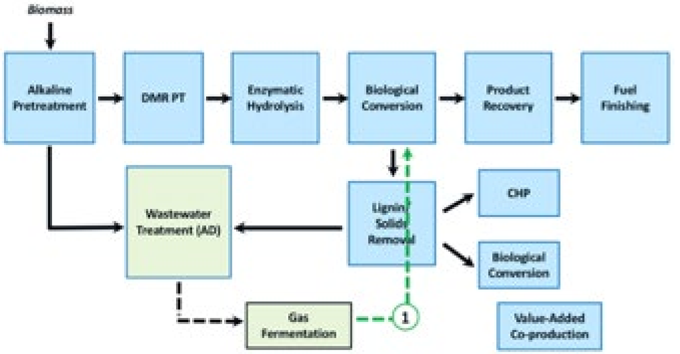
Project Goal
The Biogas Biocatalysis AOP aim to develop a carbon- and energy-efficient biogas bioconversion process via techno-economic-informed strain and fermentation engineering strategies.

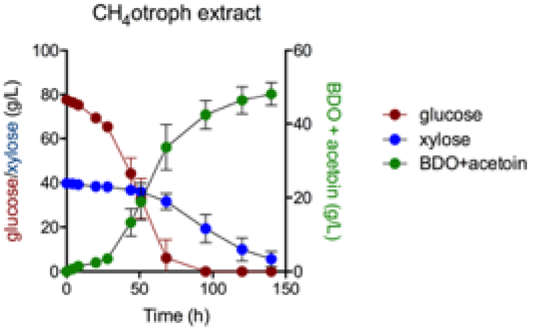
End of Project Milestone
Achieve >$0.25/GGE reduction and >5% carbon yield-increase relative to baseline for BC Platform conceptual biorefinery design (biogas→power) via conversion of biogas to value-added liquid fuels and/or chemicals.

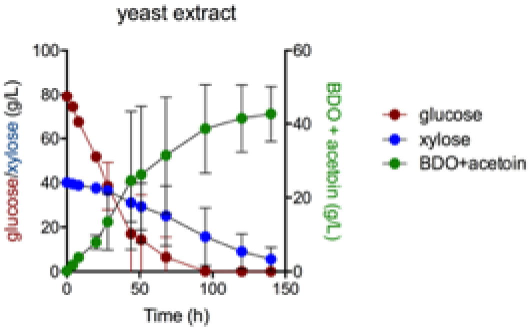
Funding Mechanism
Direct funding AOP

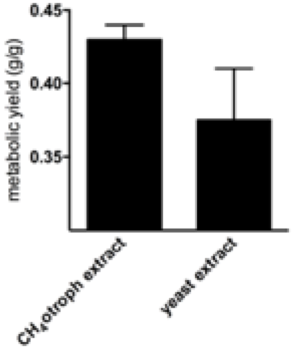
Lignocellulosic Biorefinery Integration

- Biomass recycle leads to >15% TRY enhancement
- **Estimated >$1 reduction in MFSP**









BETO Relevance

- Relevant to EERE's MYPP for developing cost-effective, integrated waste-to-energy processes for the production of bioproducts and advanced biofuels.
- Targets key MYPP Barriers:
  - Ct-H. Gas Fermentation Development
  - Ct-D. Advanced Bioprocess Development
  - Ct-F. Increasing the Yield from Catalytic Processes
- 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.
- MSW, landfill gas, agriculture and WWTP waste streams represent poorly valorized domestic feedstocks.

Response to Reviewers' Comments 2019

- We thank the Reviewers for their positive and encouraging assessment.
- Following Reviewer guidance, we have:
  - Continued to target the development of robust, carbon-efficient methanotrophic biocatalysts and gas fermentation process intensification via TEA-informed strategies.
  - Defined SMART milestone targets to explicitly metrify biorefinery economic and sustainability enhancements
  - Expanded engagement with existing stakeholders to include biogas host site owners, biogas/biomethane project developers
  - Refined TEA to more accurately model process improvements: FY20 Go/No-Go directly targeted TEA-informed down-selection.

Publications, Patents, Presentations, Recognition, and Commercialization

Publications (FY19-21 ONLY):

1. Henard, et al. *PNAS, Under Review*
2. Jesser, et al 2020. *Metabolic Engineering, Under Review*
3. Fei, et al 2020. *Biochemical Engineering Journal* 158, 107500
4. Grim, et al. 2020. *Energy & Environmental Science* 13 (2), 472-494
5. Tapscottt, et al 2019. *Applied and environmental microbiology* 85 (11)
6. Qian, F. eet al 2019. *Nano Letters* 19 (9), 5829-5835
7. Henard, et al. 2019. *Green Chemistry* 21 (24), 6731-6737
8. Guarnieri, 2018. *Systems Biology* 83, 117-132
9. Henard, et al. 2018. *Frontiers in Microbiology*, 9, 2610
10. Tays, et al. 2018. *Frontiers in Microbiology,* 9, 2239

Patents:

- US Patent 10,889,821: Organic acid synthesis from C1 substrates
- US Patent 10,435,693: Organic acid synthesis from C1 substrates

Presentations (NREL Invited Only, FY19-21)

- SIMB 2014-2020
- AIChE Annual Meeting 2019-2020
- SBFC 2018-2020
- Gordon Research Conference 2018

Press:

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

Material Transfer Agreements and Data Dissemination:

- Over a dozen MTA have been executed encompassing engineered strains and tools, with >10 active MTA.
- Tools (plasmids, primers, and associated sequence files) have been deposited at Addgene to facilitate rapid, easy access.