Biogas Biocatalysis

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March 10, 2021
Technology Area Session: Organic Waste
Principal Investigator: Mike Guarnieri
Organization: NREL

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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

- 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
- Sustained low oil prices
- Decreasing cost of renewable electricity
- Sustainable waste management
- Expanding availability of green H₂
- Closing the carbon cycle
- Risk of greenfield investments
- Challenges and costs of biorefinery start-up
- Availability of depreciated and underutilized capital equipment
- Carbon intensity reduction
- Access to clean air and water
- 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

PM, TM: Beau Hoffman, Mark Philbrick

PI: Mike Guarnieri
Platform Lead: Rick Elander

Techno-Economic Analysis
Leads: Ling Tao, Ryan Davis

Task 1: Metabolic Engineering
Lead: Mike Guarnieri

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 **Schedule**, **Deliverables**, and **Budget**.
  - 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.
2. Approach

Major challenges
- (i) CCE (FY17-19), (ii) end-product tolerance, (iii) CO₂ utilization, (iv) gas-liquid mass transfer

Critical success factors:
- Achieve economically-viable product titers, rates, and yields via CH₄/CO₂ 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 CH₄/CO₂ 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

- **CH₄** is the primary component of anaerobic digestion biogas, 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 **large market and energy value**
  - > 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

- **20% input biomass C in biorefinery → Wastewater A.D.**

- *Despite market and energy potential, biogas is generally incompatible with transportation and manufacturing infrastructure!*
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 $K_L$ a increase)
4. Developed First Methanotrophic CRISPR Toolbox

This advance enables multi-target \textit{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).

Biorefinery WW Anaerobic Digestion → Engineered Biocatalyst → Engineered Product Suite

- Succinic acid
- Muconic acid
- Malonic Acid
- Lactic Acid
- 2,3-BDO
- Sucrose
- Methyl acetate
- Adipic Acid
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 **spidroin**
  - 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
  - **Highest reported methane conversion rate reported to date** (> 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 >96 hours, 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
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
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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.
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Additional Slides
**Timeline**
- Project start date: 10/1/18
- Project end date: 9/30/21

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<tr>
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<th>FY20</th>
<th>Active Project</th>
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<tr>
<td>DOE Funding</td>
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**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.

**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

**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.
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 (FY19-21 ONLY):

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.