Biogas to Liquid Fuels and Chemicals
Using a Methanotrophic Microorganism

WBS 2.3.2.102

2015 DOE BioEnergy Technologies Office (BETO)
Project Peer Review
March 24, 2015

Technology Area: Biochemical Conversion
Principal Investigator: Michael T. Guarnieri
Organization: National Renewable Energy Laboratory
Goal Statement

Background

- Methane-rich biogas offers a renewable alternative to natural gas as a feedstock and intermediate in bioprocesses.
- Relevant to EERE’s MYPP for developing cost-effective, integrated waste-to-energy processes for the production of bioproducts and advanced biofuels.

Goals

1. Demonstrate proof of concept for a biogas-to-liquid fuels and chemicals process.
2. Enhance carbon conversion efficiency from methane to biomass and products.

Outcome: Alternative, renewable path to hydrocarbon fuels and chemicals.

- Enables the sustainable, nationwide production of biofuels that are compatible with transportation infrastructure.
- Displaces a share of petroleum-derived fuels to reduce U.S. dependence on oil.
- Encourages the creation of a new domestic bioenergy industry.
Quad Chart Overview

Timeline

• Project start date: March, 2014
• Project end date: September, 2015
• Percent complete: 66%

Barriers

• Bt-J: Catalyst Development
• Bt-K: Biochemical Conversion
• Process Integration
• Bt-L: Biochemical/ Thermochemical Interface

Partners & Synergistic Activity

• University of Washington: subcontract to aid in metabolic flux balance analyses.
• ARPA-E: NREL, Lanzatech, Johnson Matthey, UW
• Waste-to-Energy TEA: NREL, ANL
• BCU FOA: NCSU, UW, Farmatic, Inc., Metabolon, Inc.

Budget

<table>
<thead>
<tr>
<th></th>
<th>Total Costs FY 10–FY 12</th>
<th>FY 13 Costs</th>
<th>FY 14 Costs</th>
<th>Total Planned Funding (FY 15-Project End Date)</th>
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</thead>
<tbody>
<tr>
<td>DOE Funded</td>
<td>-</td>
<td>-</td>
<td>$137,586</td>
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Project Overview

• **History:** This seed project represents the first biogas upgrading project at NREL. A related ARPA-E project exploring biological upgrading of natural gas was initiated in FY13.

• **Context:** “There is a significant near-term market entry opportunity to deploy waste-to-energy technologies in the U.S.” *
  • MSW, Landfill gas, Agriculture and WWTP waste streams represent poorly valorized domestic feedstocks
  • Biogas expands the suite of products from biorefineries, municipalities, and agricultural operations: potential to increase revenue & reduce GHG emissions.
  • Enables biological gas-to-liquids conversion and upgrading for integration with existing transportation infrastructure
  • Enables a national network of distributed power and biofuel production sites

• **Objective:** Develop a viable path for biocatalysis of biogas-derived methane to fuels and chemicals.

**Technical Approach**

**Development of Genetic Tools & Metabolic Engineering Strategy**

- CH₄ → Acetate
- CH₂O → F6P → Pyruvate
- FDH → Formate
- FPK/ACK → Acetate
- PDH/APT/ACK → Pyruvate
- ACCase → Lipids
- LDH → Lactate
- Fuels
- Chemicals

**Gas Fermentation Optimization**

**Techno-economic Analysis**

- CCE, g Acid/g CH₄
- CCE, g Biomass/g CH₄
- O₂, $/ton
- Biogas, $/ton
- N Source, $/ton
- Acid Productivity, g/L/h
- Acid Recovery Efficiency, %
- Biomass Productivity, g/L/h
- Yield from Biogas, m³/kg

**Approach:** Employ pathway analyses, fermentation engineering, and techno-economic analyses to inform hypothesis-driven strain-engineering strategies.

**Major challenges and success factors:** (i) development of methanotroph genetic tools, (ii) gas mass transfer (fermentation optimization), (iii) carbon conversion efficiency enhancements, and (iv) productivity enhancements (biomass and products)
Management Approach

- Staffing includes molecular and microbiologists, fermentation and chemical engineers, and analytical chemists
- Research guided by TEA, with related quarterly, metrified milestones
- Monthly group meetings, regular interaction with technical staff
- Synergistic interaction between Biogas seed project, ARPA-E natural gas upgrading project, and Univ. of Washington (subcontract: flux balance analysis)
Results: Development of Genetic Tools

- Unexplored genus at NREL, requiring genetic toolbox development
- Developed both replicative and integrative plasmids for constitutive and inducible gene expression and knockout in a methanotroph.
- Constitutive, strong promoters have been isolated and successfully employed for overexpression of target genes.
- Inducible operators have been identified for temporal regulation of gene expression.
Results: Development of Genetic Engineering Strategy

- *In-silico* analyses and literature review defined initial targets for strain-engineering, as reported in FY14, Q1 Milestone report.
Results: Fermentation Optimization and Lipid Accumulation

- Genetically engineered a methanotrophic bacterium to produce lactic acid and lipids from C1 substrates (methane and methanol).
  - Proof-of-concept for a methane-to-lactate and lipids bioprocess

\[
\begin{align*}
\text{CH}_4 & \rightarrow \text{CH}_2\text{O} \\
& \downarrow \text{FDH} \\
& \downarrow \text{FDH} \\
\text{Formate} & \rightarrow \text{F6P} \rightarrow \text{Pyruvate} \\
& \downarrow \text{PDH/APT/ACK} \\
& \downarrow \text{LDH} \\
\text{Acetate} & \rightarrow \text{Lactate} \\
& \downarrow \text{ACSSase} \\
\text{Lipids} & \rightarrow \text{Fuels} \rightarrow \text{Chemicals}
\end{align*}
\]

\textit{Lipid extraction and upgrading successfully developed via ARPA-E}

<table>
<thead>
<tr>
<th>Sample</th>
<th>Biomass, g/L</th>
<th>C14:0</th>
<th>C15:0</th>
<th>C16:0</th>
<th>C16:1n9</th>
<th>C16:1n7</th>
<th>C16:1n6</th>
<th>C16:1n5</th>
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<tr>
<td>@96h</td>
<td>12.4</td>
<td>6.98</td>
<td>1.12</td>
<td>12.46</td>
<td>26.22</td>
<td>24.26</td>
<td>23.43</td>
<td>4.93</td>
<td>10.7%</td>
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</table>

Fatty Acid Speciation
Results: Metabolic Engineering for Fuel & Chemical Biosynthesis

- Genetically engineered a methanotrophic bacterium to produce lipids and lactate from C1 substrates (methane and methanol).
  - Proof-of-concept for a methane-to-fuels and chemical bioprocess

(A) Pathway targets for strain-engineering (B) Colorimetric detection of lactate in culture broth of wild-type (left) and lactate dehydrogenase-overexpressing strain (right). (C) HPLC quantitation of inducible lactate biosynthesis in wild-type (left) and lactate dehydrogenase-overexpressing methanotroph (right).

- Lipid content unaltered by lactic acid production
Results: *In vitro* and *in vivo* LDH Analyses

- An array of LDH and promoter-LDH combinations were examined to define optimal pairings for methanotrophic lactate production.

- Enhanced LA production nearly two orders of magnitude over wild-type background
  - Exceeded Y1 Go/No-Go Milestone

- Subject of ROI # 15-21: Methane biocatalysis for production of lactic acid.
Results: Techno-economic Analysis

- TEA for an integrated bioprocess identified a viable biogas-to-fuels and chemicals production route.
- Capital cost (CAPEX) and operation (OPEX) cost examined using key parameters and process assumptions, including:
  - plant capacity
  - yields of core technologies
  - raw material and chemical costs
- Preliminary analyses indicate carbon conversion efficiency and gas feed costs will be primary cost drivers in the development of viable biogas-to-fuels and chemicals.

**TEA for ARPA-E encouraging for natural gas upgrading process exclusively targeting fuels:** use of biogas and coproduct credit will contribute greatly to favorable TEA and commercial success.
2014 Biochemical Upgrading FOA

- Proof-of-concept data from seed project contributed to successful application selected under the BETO Biochemical Upgrading FOA (DE-FOA-0001085), targeting biocatalysis of methane to high-value co-products and novel bioreactor design.
Relevance

- Relevant to EERE’s MYPP for developing **cost-effective, integrated waste-to-energy processes** for the production of bioproducts and advanced biofuels.
- **Specifically targets identified MYPP barriers**, including: Catalyst Development, Biochemical Conversion Process Integration, and Biochemical/ Thermochemical Interface.
- Genetic tools, biocatalysts, and TEA directly advance emerging biogas and bioenergy industry.
- Represents proof-of-concept for an array of methane biocatalysis strategies, opening the door for feedstock expansion in an emerging bioeconomy and creation of a new domestic bioenergy industry.
- **Tech transfer/marketability**: A number of commercial entities (including partners Lanzatech and Farmatic) are currently exploring biogas generation and methane upgrading technologies.
Future Work

- **Enhance flux from methane towards biomass and bioproducts**
  - Identification of flux bottlenecks via metabolomic and transcriptomic analyses
  - Strain-engineering enhancements targeting overexpression of methane oxidation genes
  - Strain-engineering enhancements targeting knockout of competitive fermentative pathways

- **FY15 Key Milestone:** >10% CCE Enhancement
Summary

• An array of biogas feedstocks with high-volume methane potential offer a versatile, renewable alternative to natural gas.
• Methanotrophic biocatalysis offers a promising path towards valorization of biogas derived from anaerobic digestion of waste streams.
• Metabolic routes and strain-engineering strategies targeting enhanced biosynthesis of fuels and high-value chemicals have been established in a methanotrophic bacterium.
  • Represents first demonstration of biological CH₄-to-fuels and chemicals
• Preliminary TEA has identified a viable commercial path
  • Sensitivity analyses indicate carbon conversion efficiency and gas feed costs will be primary cost drivers in the development of viable biogas-to-fuels and chemicals processes.
• Enhanced genetic tool development and fermentation optimization will be pursued in FY15, targeting improvements in carbon conversion efficiency from methane to biomass and products.
Acknowledgements

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Additional Slides
Presented work at 2014 SIMB Annual Meeting: “Biogas to Liquid Fuels and Chemicals Using a Methanotrophic Microorganism.”

Manuscripts in Preparation related to biogas biocatalysis to fuels and chemicals and TEA related to the conceptual integrated bioprocess.

ROI: “Methane biocatalysis to lactic acid.”