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
• 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

<table>
<thead>
<tr>
<th>Partners</th>
<th>FY 15 Costs</th>
<th>FY 16 Costs</th>
<th>Total Planned Funding (FY 17-&gt;End Date)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOE Funded</td>
<td>$273K</td>
<td>$927K</td>
<td>$1.3M</td>
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<tr>
<td>Farmatic, Inc</td>
<td>11% total cost share</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metabolon, Inc</td>
<td>6% total cost share</td>
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<td></td>
</tr>
<tr>
<td>NCSU</td>
<td>3% total cost share</td>
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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
• **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

- 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\textsubscript{4} and H\textsubscript{2}S Evaluation

- We evaluated a series of substrates with wide-range H\textsubscript{2}S generation potential.
- Continuous AD configuration leads to substantial H\textsubscript{2}S accumulation.
  - Potential for methanotrophic toxicity.
H$_2$S Does Not Impact Growth…but Alters Metabolism

- Minimal growth defect under high-H$_2$S 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

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

**Schematic overview of synthetic muconic acid pathway.**
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.

![MFSP and Production vs. Yield](chart.png)
Process Intensification Enhances kLa and Productivity

- > 10x organic acid titer enhancement in high-density biocomposite
- > 10x kLa enhancement in falling film reactor
- Biocomposites sustain methane uptake for >100 hours
- 100x less NO₃ than base media (0.01g/L)
- Increased CH₄ uptake rate with increased cell density
Process Intensification Enhances $k_La$ and Productivity

- > 10x organic acid titer enhancement in high-density biocomposite
- > 10x $k_La$ enhancement in falling film reactor
- Biocomposites sustain methane uptake for >100 hours
- 100x less NO$_3$ than base media (0.01g/L)
- Increased CH$_4$ uptake rate with increased cell density
Relevance

- H$_2$S-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.
• Successful production of muconic acid from methane.
  • H₂S-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.
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Danny Alexander (Metabolon, Inc.)
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CJ Duran (NCSU)
Process Intensification: Microbial Biocomposites

- 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

<table>
<thead>
<tr>
<th>Microbe</th>
<th>Gas Consumed</th>
<th>Gas or Product Evolved</th>
<th>Enzyme System</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gluconobacter</strong></td>
<td>O₂</td>
<td>L-sorbose (D → L sugar oxidation)</td>
<td>LSDH (membrane bound)</td>
</tr>
<tr>
<td><strong>Clostridia</strong></td>
<td>CO, H₂</td>
<td>EtOH, acetate</td>
<td>CODH</td>
</tr>
<tr>
<td><strong>Rhospseudomonas</strong></td>
<td>N₂</td>
<td>fixed nitrogen</td>
<td>nitrogenase</td>
</tr>
<tr>
<td></td>
<td>Ar (inert)</td>
<td>H₂</td>
<td>N₂ – limited nitrogenase</td>
</tr>
<tr>
<td><strong>Synechococcus, Synechocystis, Anabaena</strong></td>
<td>CO₂</td>
<td>O₂, carbohydrates, lipids</td>
<td>hydrogenase</td>
</tr>
<tr>
<td><strong>Chlamydomonas</strong></td>
<td>CO₂</td>
<td>H₂</td>
<td>sulfur-limited hydrogenase</td>
</tr>
</tbody>
</table>

Courtesy of Flickinger, et al, 2015
Response to Reviewers’ Comment

• This project was not subjected to prior review.
Publications, Patents, Presentations, Awards, and Commercialization

• Publications:

• Book Chapters:

• Patents:
  – Organic Acid Synthesis from C1 Substrates

• Presentations:
  – SIMB 2014
  – SIMB 2015
  – Gordon Research Conference 2016
  – ASM 2016