BETO 2021 Peer Review
2.3.2.106
Waste Carbon Gas Upgrading via Acetogens

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Conversion
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Market Trends

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

Value Proposition

- Waste carbon gas and renewable energy are substrates for microbial upgrading
- Currently microbes mainly make lower value ethanol/acetate
- What products can we make?

Differentiator

- Gaseous substrates represent a promising avenue for CO₂ conversion
- Engineering for alternative products and evaluation of engineered microbes
Quad Chart Overview

Timeline
• 10/01/2020 through 9/30/2023

<table>
<thead>
<tr>
<th>DOE Funding</th>
<th>FY20</th>
<th>Active Project</th>
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<td>(10/01/2020 – 9/30/2023)</td>
<td>$325,000 FY21</td>
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Project Goal
Evaluate Waste Carbon gas sources as a feedstock for genetically engineered microbial upgrading to novel products via non-photosynthetic mechanism.

End of Project Milestone
Production of high value product from CO and CO2 containing waste gas, with a titer of 5 g/L in a ≥5L fermentation system.

Barriers addressed

Ct-H – C1 Fermentation Development
• Unique challenges that must be overcome for gaseous feedstock such as continuous mode of operation and bioreactor configurations.

Ct-L – Advanced Bioprocess Development
• Develop robust organism via metabolic engineering to increase rate, titer, yield.

Funding Mechanism
Funded through BETO Conversion 2020 Lab call renewal
Project Overview

• Valorize waste CO₂ to C-C bonds for seasonable or long-term storage
• Leverage low-cost renewable electrons from wind and/or solar PV
• Engineer *Clostridium ljungdahlii* to produce 3-hydroxybutyrate (3HB), a building block in the carboxylate platform chemicals and bioplastic monomer.
• Synergistic with the BETO Waste-to-Energy (WTE) platform for CO₂ upgrade
• Waste carbon from gas represents a diverse and plentiful set of feedstocks
• This waste gas can be converted to syngas, a mixture of CO/H₂/CO₂
• Syngas can be microbially upgraded using anaerobic microbes called acetogens
• Acetogens use the Wood-Ljungdahl Pathway (WLP) to fix CO₂ to acetyl-CoA
• Acetyl-CoA can be biologically converted to 3-hydroxybutyrate, a C4 fuel & plastic precursor
Process idea

What feedstocks?

What organisms?

What products?

Adapted from: Gas fermentation for commodity chemicals and fuels
doi: 10.1111/1751-7915.12763
Management

Process idea

**Gas Feedstocks**
How are they generated?
What is their composition? ($\text{H}_2/\text{CO}/\text{CO}_2$)
At what prices?
How does that affect carbon efficiency?

**Microbial Syngas Conversion**
Does syngas composition effect growth?
Products?
How much is known about them?
Can they be genetically engineered towards our products of interest?

**Scaling Gas Fermentations**
Strain stability?
Performance at larger scale?
Mass transfer of syngas?
Separations?
Carbon and electron efficiency?
Yield/rate/titer?

Adapted from: Gas fermentation for commodity chemicals and fuels
doi: 10.1111/1751-7915.12763
2.5.3.701 Production of High-value Chemicals from Renewable Feedstock

2.5.4.30X Advanced Catalyst Synthesis and Characterization

2.3.2.106 Waste Carbon Gas Upgrading via Acetogens

2.1.0.111 Analysis in Support of Biofuels and Bioproducts from Organic Wet Waste Feedstocks

• 2.1.0.100 Biochemical Platform Analysis
• 2.4.1.100 Bench Scale R&D

• 2.1.0.304 Feasibility Study of Utilizing Electricity to Produce Intermediates from CO2 and Biomass
• 5.1.3.101 Integration of CO2 Electrolysis with Microbial Syngas; Upgrading to Rewire the Carbon Economy
• 2.3.2.112 Enhancing Acetogen Formate Utilization to Value-Added Products
• Royal Dutch Shell/Princeton CO2 Conversion ACT Project
Approach – Feedstocks and products

• Direct CO$_2$ conversion to C1 chemicals has a high technology readiness level, but ethanol/acetate have a low market price and limited market size.

• C1 sources have a diversity of potential feedstocks with different characteristics
  - CO$_2$ from ethanol plants (50 million tons) Colocalized to Wind Energy
  - Methane from livestock (4 millions tons) Methane $\rightarrow$ syngas
  - Iron and Steel (75 million tons) Potential CO/H2 in stream

• Developing CO$_2$ microbial conversion to valuable products
  - 3 Hydroxybutyrate as a biodegradable plastic
  - Carboxylates/alcohols as transportation fuel replacements
Approach

• What is the source of gas? Cost and yield?
  TEA/LCA analysis
  Feedstock size

• How well can microbes be engineered for conversion?
  Growth Characterization
  Metabolic Engineering

• How can gas fermentations be scaled?
  Bioreactor studies
  Improve syngas fermentation

Gas Feedstocks
How are they generated?
What is their composition? (H₂/CO/CO₂)
At what prices?
How does that affect carbon efficiency?

Microbial Syngas Conversion
Does syngas composition effect growth?
Products?
How much is known about them?
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Scaling Gas Fermentations
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Separations?
Carbon and electron efficiency?
Yield/rate/titer?
Approach - Milestones

- Fermentation performance of WT under three different syngas concentrations. (Q1)
- Compare fermentation metrics with engineered strains and understand bottlenecks (Q2). Work with industrial partners and NREL SEAC Teams to determine near term available feedstock streams.
- Omic analysis of the WT and engineered strains to determine gene candidates for strain improvements (Q3)

End of project goal/milestones (9/2023)
- Production of high value product from CO and CO2 containing waste gas, with a titer of 5 g/L in a ≥5L fermentation system.

Go/No-Go (3/2022)
- Obtain a productivity of at least 0.1 g/L/hr from H2/CO/CO2 with a genetically engineered acetogen expressing butanol/3-Hydroxybutyrate pathway
Approach: Acetogens have highest biological efficiency for CO$_2$ fixation
Acetogens non-photosynthetically, anaerobically fix CO$_2$

- Use Wood Ljungdahl Pathway (WLP), most efficient for CO$_2$ fixation
- Investigated for syngas conversion, but can use liquid C1 formate and methanol
- Can simultaneously use gases, liquids, and biomass related sugars
- Produce interesting products at high carbon and electron efficiency
- Focus on C4 product 3-hydroxybutyrate due to potential as bioplastic PHB and fuel precursor
**Progress and Outcomes**

Organism development

**C. ljungdahlii metabolism and CRISPR/Cas9 engineering**
Progress and Outcomes
Organism development

Adam Guss, ORNL

- Characterizing promoters to drive 3HB expression
- Testing different metabolic pathways to make 3HB
- Combining 3HB pathways with C. ljungdahlii deletion variants yields more 3HB
Progress and Outcomes
TechnoEconomic Analysis

2 Cases modeled:
CO₂ with electrolyzed H₂ to 3-HB
Electrochemical CO₂ reduction to CO to 3-HB

• Costs are driven by 2 factors:
  – Electrochemistry: electricity price
  – Gas fermentation: productivity and setup
• Improving productivity is paramount to reducing costs
  – CO fermentations are much faster

~$2.50/kg market 3HB price

Cost Distributions of CO₂-to-3HB (left) and CO-to-3HB (right).

Figure 1. Minimum 0.1 g/L/hr is needed for both CO₂-to-3HB and CO-to-3HB cases.

NREL - Ling Tao
Figure 3. Preliminary MFSP distribution of processing areas including hydrogen, biological conversion, 3HB purification, upgrading and outside battery limit facilities.

- CAPEX is driven by gas fermentation investment
- OPEX is driven by electrochemistry (electricity price)
- Upgrading 3-HB to 3 carbon HC fuel drops efficiency, loses CO₂, making less viable fuel
- 3-HB is a monomer for biodegradable plastic, ~$2.50/kg market
Progress and Outcomes
Process development

- Bioreactor gas scale up
  - Dynamic H₂/CO₂/CO control
  - Can run gas fermentations at ≤2L
  - Different gas diffusion strategies
  - Numerous safety controls
  - Improving yield/rate/titer
  - Capturing metrics for further TEA/LCA analysis
Progress and Outcomes
Organism scaling

Pathway insertion into genome

100% CO, 3HB (mg/L)

<table>
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<tr>
<th>Volume</th>
<th>1 mL</th>
<th>10 mL</th>
<th>100mL</th>
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<tr>
<td>Titer</td>
<td>220</td>
<td>52</td>
<td>18</td>
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Plasmid loss due to metabolic burden
Reduces 3HB titer as volume increases

Two strategies:
Phage attB integrase multiple sites
Homologous recombination
Impact – Data and Dissemination

- Project with Royal Dutch Shell/Princeton for C1 CO₂ acetogenic conversion
- Publications around microbial C1 conversion
  - Genetic engineering, metabolism, and novel strains
    - Describing new techniques and new tools
    - Insights into engineering/metabolism
    - Distributed new strains to interested parties
  - Metrics regarding C1 fermentation
    - Yield/rate/titer, scaling gas fermentation, gas mixes
  - TechnoEconomic and Life Cycle Analysis

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The Metabolism of Clostridium ljungdahliii in Phosphotransacetylase Negative Strains and Development of an Ethanologenic Strain

Jonathan Lo*, Jonathan R. Humphreys*, Joshua Jack†, Chris Urban†, Lauren Magnusson†, Wei Xiong†, Yang Gu†, Zhiyong Jason Ren§ and Pin-Ching Maness§

1 National Renewable Energy Laboratory, Golden, CO, United States; 2 Andropogon Center for Energy and Environment, Princeton University, Princeton, NJ, United States; 3 Key Laboratory of Synthetic Biology, CAS Center for Excellence in Molecular Plant Sciences, Shanghai Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, Shanghai, China.

High rate CO₂ valorization to organics via CO mediated silica nanoparticle enhanced fermentation

Joshua Jade*, Jonathan Lo†, Bryon Donohue†, Pin-Ching Maness§, Zhiyong Jason Ren‡

Directing Clostridium ljungdahliii fermentation products via hydrogen to carbon monoxide ratio in syngas

Joshua Jack†, Jonathan Lo†, Pin-Ching Maness§, Zhiyong Jason Ren‡
• Proposals to further develop process, explore variations, outside partner collaboration

• Acetogenic metabolism engineering and fundamental understanding

• Increasing knowledge of scaling gas fermentation
  – Strain stability
  – Improving mass transfer
  – Studying gas mixes

• Other implementations of acetogenic microorganisms for CO₂ efficiency
  – C1 liquids and gases, co-utilization
  – Mixotrophy (sugar + C1 gases)
  – Co-cultures
  – Expanded products and increased carbon efficiency
Summary

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

Value Proposition

- Waste Carbon Gas is a diverse and plentiful feedstock for microbial upgrading
- Syngas microbial upgrading is still a new process with limited product diversity and value

Key Accomplishments

- Microbial understanding and tool development
- Engineered production of 3HB from CO
- TEA analysis for understanding process
- Filling in knowledge gaps and disseminating knowledge among academic and industry institutions
Thank You

www.nrel.gov

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Program 2.3.2.106
Responses to Previous Reviewers’ Comments

TEA should be very valuable to direct the performers to the market size of 3HB, and what the necessary titer/productivities should be in order to commercialize.

**Response:** The preliminary TEA has revealed a 3HB minimum selling price of $1.90/kg, provided a titer of 10 g/L and a productivity of 0.2 g/L/h can be achieved. This can set research targets. A minimum selling price for coproducts like acetate and 2,3- butanediol are $1.80 and $2.20/kg, respectively. The major cost drivers are H2 and CO2 feedstock cost and capital expenditure of biological conversion. The former can be addressed through improving the production yield to near theoretical maximums and the latter through increasing productivity and product titers. These findings guide R&D efforts carried out by the project team.
