DOE Bioenergy Technologies Office (BETO)  
2019 Project Peer Review

WBS 2.3.2.112  
Enhancing Acetogen Formate Utilization to Value-Added Products

CO$_2$ Utilization Technology Session  
March 7$^{th}$ 2019

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National Renewable Energy Laboratory
Goal Statement

- **Goals:** Develop a biological approach to convert formate into products
  - Combine efficiency of **electrochemical CO\(_2\) reduction to formate** with proficiency of **biological carbon upgrading**
- **Relevance:** Success could enable chemical production with CO\(_2\) and low cost energy as feedstocks
  - **Low cost electricity** to electrochemically reduce CO\(_2\) to formate
  - **Scalable strategy** that could be a **stand-alone process** or **value add** to existing industry
  - CO\(_2\) capture/fixation using **soluble feedstock**, avoiding mass transfer limitations
- **Outcomes:**
  - **Proof of concept:** Engineer *Clostridium ljungdahlii* conversion of formate to butanol with **titer and rate targets** (2 g/L at 0.18 g/L/h (10% of max titer/productivity in literature))
  - **Life cycle and technoeconomic analysis:** Identify **cost drivers** and **synergies** with existing technologies
Quad Chart Overview

Timeline
• Start: 10/1/2018
• End: 9/30/2021
• 10% complete

<table>
<thead>
<tr>
<th>Total Costs Pre FY17**</th>
<th>FY 17 Costs</th>
<th>FY 18 Costs</th>
<th>Total Planned Funding (FY 19-Project End Date)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOE Funded</td>
<td>0</td>
<td>0</td>
<td>$850k</td>
</tr>
</tbody>
</table>

Barriers addressed:
**Ct-D. Advanced Bioprocess Development**

**Ct-H. Gas Fermentation Development**

Objective
Develop a carbon-based domestic industry based on electrochemically produced formate from CO\(_2\).

End of Project Goal
Produce a titer of 2 g/L of butanol from formate as a sole carbon source, with productivity of 0.18 g/L/h

TEA/LCA for the integrated concept of formate to butanol, with biological conversion, co-utilization of electrosynthesis products, and final product separation/purification.
**Project Overview**

- **History:** BETO is interested in innovative approaches for using CO$_2$ in the production of biofuels and bioproducts.

  “BETO to focus FY18 efforts to develop **biological upgrading efforts on aqueous C1 intermediates** to compliment CO$_2$ catalysis efforts...The use of electrocatalytically-generated **C1 intermediates as a feedstock for biological upgrading** is an appealing route for CO$_2$ utilization because it avoids requiring biology to perform carbon fixation while still leveraging the proficiency of carbon upgrading through biological routes.”

- **Context:** Cheap electricity is low cost but intermittently generated
  - Used to electrochemically reduce CO$_2$ to soluble formate
  - Formate can be fed to bacteria to upgrade to higher value chemicals

- **Project Objectives:**
  - Develop *Clostridium ljungdahlii* for formate conversion into butanol
  - Focus efforts towards **titer and rate targets**
  - TEA/LCA to **identify cost drivers** and **synergies with existing technologies**
Understanding bacterial metabolism: Using metabolomics and genetic engineering to improve formate consumption and butanol formation.

- Expertise in Clostridia with analysis of metabolic pathways using $^{13}$C labeled carbon, and using genetic tools for making novel products

TEA/LCA Analysis: Informs potential targets for research.

- Fermentation studies provide data, leveraging in-house anaerobic fermentation experience.

Following SMART Milestones and Go/NoGo decisions
2.3.2.112 Enhancing Acetogen Formate Utilization to Value-Added Products

2.5.4.30X Advanced Catalyst Synthesis and Characterization

2.5.3.701 Production of High-value Chemicals from Renewable Feedstock

2.1.0.100 Biochemical Platform Analysis

2.4.1.100 Bench Scale R&D

Agile BioFoundry

Waste-to-Energy

Biochemical Conversion

CO₂

- 2.1.0.304 Feasibility Study of Utilizing Electricity to Produce Intermediates from CO₂ and Biomass
- 2.3.2.111 Improving formate upgrading by *Cupriavidus necator*
- 2.3.2.113 Synthetic C1 Condensation Cycle for Formate-Mediated ElectroSynthesis
- 5.1.3.101 Integration of CO₂ Electrolysis with Microbial Syngas; Upgrading to Rewire the Carbon Economy
- 2.3.2.106 CO₂ Valorization via Rewiring Metabolic Network
Approach - Technical

- *C. ljungdahlii*
  - Wood Ljungdahl Pathway (WLP) convert formate (as well as $\text{H}_2+\text{CO}_2/\text{CO}$) to acetyl-CoA
  - Acetyl-CoA can be converted to butanol

- WLP two parts:
  - methyl and carbonyl branches

- Formate is a methyl branch intermediate

- Formate can supply the carbonyl[CO] branch by generating reduced ferredoxin

\[
\text{CO}_2 + \text{reduced ferredoxin} \rightarrow \text{CO}
\]

through enzymes like *Formate dehydrogenase (FDH)*

\[
2\text{HCOO}^- + \text{oxidized ferredoxin} + \text{NAD}^+ \rightarrow 2\text{CO}_2 + \text{reduced ferredoxin} + \text{NADH} + \text{H}^+
\]
**Approach - Technical**

**Aim 1:** Robust formate utilizing strain

**Approach:**

**Formate utilization genes**
- Formate hydrogen lyase (FHL): Formate $\rightarrow$ H$_2$ + CO$_2$
  - Electron bifurcating formate dehydrogenase (EBFDH)

**Track formate utilization**
- Fermentation studies
- 13C labeled formate metabolomics

**Aim 2:** Produce butanol

**Approach:**
- Introduce butanol formation genes from *Clostridium acetobutylicum*
  - Delete native competing pathways
## Approach - Technical

### Primary challenges and success factors:

**Formate utilization and improving uptake/conversion rates**

<table>
<thead>
<tr>
<th>Description of Risk</th>
<th>Mitigation Plan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolic engineering may compromise microbial strain stability as formate is both a feedstock and intermediate</td>
<td>$^{13}$C-fluxomics will guide an informed strategy to ensure metabolic balance to attain higher titers of targeted products in stable chassis hosts</td>
</tr>
</tbody>
</table>

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<tr>
<th>Description of Risk</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Formate at high levels could be toxic to <em>C. ljungdahlii</em></td>
<td>Formate can be fed continuously into the bioreactor. Formate utilization pathways should improve tolerance. Genetic engineering can improve tolerance.</td>
</tr>
</tbody>
</table>

**Redirect metabolism from native products to butanol**

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<tbody>
<tr>
<td>Increasing alcohol concentration toxicity may be an issue for <em>C. ljungdahlii</em></td>
<td>Various in situ recovery methods exist to remove alcohol as it is being formed. Alternatively, butanol tolerance can be improved by genetic engineering</td>
</tr>
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</table>

<table>
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</tr>
</thead>
<tbody>
<tr>
<td>Butanol may be not practical under TEA</td>
<td>The formate conversion platform is product agnostic; other products can be engineered into <em>C. ljungdahlii</em>.</td>
</tr>
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</table>
Accomplishments

**Aim 1:** Develop robust formate utilizing strain

**Native formate utilization**

<table>
<thead>
<tr>
<th>Growth condition</th>
<th>Formate remaining (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No bacteria control</td>
<td>15</td>
</tr>
<tr>
<td>Formate alone</td>
<td>6.5</td>
</tr>
<tr>
<td>H2 + CO2</td>
<td>4.75</td>
</tr>
<tr>
<td>Formate + CO</td>
<td>0</td>
</tr>
<tr>
<td>Formate + Sugar</td>
<td>0</td>
</tr>
</tbody>
</table>

**Aim 2:** Produce butanol
- Gene editing using Cas9, deleting *pta*.
- Less acetate more alcohol

![WT vs pta deletion fermentation, 5 g/L sugar](chart.png)
BETO’s Conversion Program goal is to develop biological and chemical technologies to convert feedstocks into energy-dense, fungible liquid transportation fuels and biopower.

Lowering production cost through increased efficiency and yield, can be accomplished by capturing waste CO$_2$ as formate and converting it to butanol.

Butanol formation from waste CO$_2$ can reduce emissions and price, helping meet BETO goals (2022) of a 50% reduction in emissions relative to petroleum fuels at modeled fuel price of $3/gasoline gallon equivalent.

Feedstocks contribute up to 79% of butanol costs, and 33% C is lost as CO$_2$.

Electrochemical CO$_2$ reduction is driven by price of electricity & increasing energy supply will reduce prices.

Electrochemical reduction to formate is appealing:
- Scalable and storable at standard temperature/pressure
- Can take advantage of fluctuating electricity supply/price
- Avoids mass transfer problems of gaseous CO$_2$/CO
Relevance

• Formate utilization by *C. ljungdahlii* has several advantages
  – Electrochemical CO₂ reduction side products H₂/CO can be used
  – A variety of ≥C2 chemicals can be accessed by genetic engineering
  – Utilizes variety of substrates like biomass sugars, H₂, CO, CO₂, formate
  – Formate conversion to acetyl-CoA integrates with other products, acetyl-CoA is a precursor to many chemicals (carboxylic acids, alcohols, terpenes)

• Collaboration with Visolis on an Agile BioFoundry project for products from acetyl-CoA from *Clostridium ljungdahlii*. 
Aim 1: Develop formate utilizing strain

- Track formate metabolism using $^{13}$C labeling
- Genetically engineer *C. ljungdahlii* to better utilize formate
  - Introduce novel formate utilization genes

<table>
<thead>
<tr>
<th>Milestone</th>
<th>Description</th>
<th>End Date</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flux mapping of formate utilization</td>
<td>$^{13}$C-tracer studies (feeding $^{13}$C-formate) to map carbon fluxes of formate</td>
<td>3/31/2019</td>
<td>Quarterly Progress</td>
</tr>
<tr>
<td>New formate pathways</td>
<td>Input formate utilization genes and obtain a titer of 1 g/L products with formate as a sole carbon source.</td>
<td>9/30/2019</td>
<td>SMART Milestone</td>
</tr>
<tr>
<td>Formate conversion to product</td>
<td>Demonstrate consumption of at least 2 g/L formate as a sole carbon source via expression of formate utilization genes, with conversion to fermentation products at a rate of 0.18 g/L/h</td>
<td>3/31/2020</td>
<td>Go/No Go</td>
</tr>
</tbody>
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Future Work

Aim 2: Introduce butanol formation

- Genetically engineer *C. ljungdahlii*
  - Introduce butanol genes
  - Curb native products

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<th>Milestone</th>
<th>Description</th>
<th>End Date</th>
<th>Type</th>
</tr>
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<tbody>
<tr>
<td>Butanol production</td>
<td>Generate a titer of 200 mg/L of butanol from formate as a sole carbon source</td>
<td>9/30/2020</td>
<td>Annual Milestone</td>
</tr>
<tr>
<td>Produce butanol from formate</td>
<td>Produce a titer of 2 g/L of butanol from formate as a sole carbon source, with butanol productivity of 0.18 g/L/h.</td>
<td>9/30/2021</td>
<td>Project End</td>
</tr>
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Future Work

Aim 3: TechnoEconomic Analysis (TEA) and Life Cycle Analysis (LCA)

Potential integrated formate conversion combines production, fermentation, and separation. Adapted from Richter et al 2016.

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<th>End Date</th>
<th>Type</th>
</tr>
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<tr>
<td>TEA of butanol production from formate</td>
<td>TEA will determine whether formate conversion to butanol is economically feasible as a technology going forward, as well as guide of genetic engineering priorities for formate conversion to butanol.</td>
<td>3/31/2020 (mid point)</td>
<td>Go/No Go</td>
</tr>
<tr>
<td>TEA/LCA of overall integrated concept</td>
<td>Perform detailed TEA/LCA for the integrated concept from formate to butanol, biological conversion, co-utilization of electrosynthesis products, final product separation and purification.</td>
<td>9/30/2021</td>
<td>Project End</td>
</tr>
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</table>
Summary

- **Overview:** Electrochemical CO$_2$ reduction produces a soluble feedstock that captures CO$_2$ using cheap electricity
- **Approach:** Formate can be converted to butanol using genetically modified *C. ljungdahlii*
- **Results:** *C. ljungdahlii* has been genetically engineered to produce more alcohol and consumes formate under some conditions
- **Relevance:** Conversion of waste CO$_2$ to formate could fit into a variety of industrial processes as value add or stand alone
- **Future work:**
  - Understanding formate metabolism with 13C flux analysis
  - Targeting of native pathways
  - Improving formate utilization
  - Heterologous gene expression of butanol forming genes
  - Fermentation studies
  - TEA/LCA analysis of process
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www.nrel.gov

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