Enhancing Acetogen Formate Utilization to Value-Added Products

March 11, 2021
Conversion
Jonathan Lo
National Renewable Energy Laboratory
### Market Trends

#### Product
- Anticipated decrease in gasoline/ethanol demand; diesel demand steady
- Increasing demand for aviation and marine fuel

#### Feedstock
- Decreasing cost of renewable electricity
- Sustainable waste management
- Expanding availability of green H₂

#### Capital
- Closing the carbon cycle
- Challenges and costs of biorefinery start-up

#### Social Responsibility
- Carbon intensity reduction

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

### Value Proposition
- How do we utilize CO₂ with cheaper renewable energy?
- What products can we make?

### Differentiator
- **Liquid C1 compounds as medium for microbial upgrading**
- Diversity of potential inputs
- Long term temporal storage, easy transport
Quad Chart Overview

**Timeline**
- 10/01/2018 through 9/30/2021

<table>
<thead>
<tr>
<th>DOE Funding</th>
<th>FY20</th>
<th>Active Project</th>
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</thead>
<tbody>
<tr>
<td>(10/01/2018 – 9/30/2021)</td>
<td>$850,000 for 3 years</td>
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**Barriers addressed**

**Ct-H – C1 Fermentation Development**
Liquid C1s are a novel and promising avenue for microbial conversion to bioproducts, but little is known about C1 liquid fermentation processes

**Ct-D – Advanced Bioprocess Development**
Liquid C1s can be derived from a variety of renewable feedstocks and utilized in a variety of ways, but no industrial process exists

**Project Goal**
- Develop acetogens as a platform for renewable liquid C1 conversion to value-added products
- Perform TEA/LCA analysis to understand CO₂ and economic considerations

**End of Project Milestone**
- Demonstrate production of 2 g/L of C4 compound C1 feedstocks
- TEA/LCA analysis of potential process with generated fermentation metrics

**Funding Mechanism**
Funded through BETO Conversion 2018 Lab call
**Project Overview**

- **Liquid C1 compounds** represent an understudied avenue for **renewable energy capture and CO₂ bioconversion**
  - Electro/thermochemical approaches have focused on syngas (H₂, CO)
  - CO₂ capture to liquid feedstock (methanol/formate)
  - **High energy density** – Formate = 53 g/L H₂, methanol = 100g/L H₂
  - **Easily stored/transported**
  - **Miscible** - Avoiding mass transfer limits

- **Goal**: Develop a biological approach to convert liquid C1 into products

- Combine renewable **chemical CO₂ reduction** with **biological upgrading**

Renewable energy conversion of CO₂ combined with biological upgrading
**Project Overview**

- **Relevance:** Chemicals with CO$_2$ and low-cost energy as feedstocks
  - Low-cost electricity to chemically reduce CO$_2$ to formate/methanol
  - Scalable strategy as a stand-alone process or value add to existing industry

- **Outcomes:**
  - **Proof of concept:**
    - General process outline
    - Feedstocks and Organism Identification/Characterization
    - Soluble C1 Conversion to C4 compounds at 2 g/L titer
  - **Life cycle (LCA) and technoeconomic analysis (TEA):**
    - Identify cost drivers and synergies with existing technologies

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**Species Table**

<table>
<thead>
<tr>
<th>Species</th>
<th>Rate of Formation$^a$</th>
<th>Selectivity$^a$</th>
<th>Energy Efficiency$^a$</th>
<th>Current TRL$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon Monoxide</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Ethylene</td>
<td>High</td>
<td>Medium</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>Formate</td>
<td>Medium</td>
<td>High</td>
<td>Medium</td>
<td>Low</td>
</tr>
<tr>
<td>Methane</td>
<td>High</td>
<td>High</td>
<td>Medium</td>
<td>High</td>
</tr>
<tr>
<td>Acetate</td>
<td>Low</td>
<td>High</td>
<td>Medium</td>
<td>Low</td>
</tr>
<tr>
<td>Methanol</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
</tr>
</tbody>
</table>

$^a$ High: >200 mAh/cm$^2$ (or commensurate TCE), Medium: 20-200 mAh/cm$^2$, Low: <100 mAh/cm$^2$

Qualitative evaluation of product ease of formation. From "Transforming the carbon economy: challenges and opportunities in the convergence of low-cost electricity and reductive CO$_2$ utilization"
An industrial perspective on catalysts for low-temperature CO₂ electrolysis

Richard I. Masel, Zengcai Liu, Hongzhou Yang, Jerry J. Kaczur, Daniel Carrillo, Shaoxuan Ren, Danielle Salvatore & Curtis P. Berlinguette

*Nature Nanotechnology* (2021) | Cite this article

“Units to convert CO₂ to formic acid are projected to reach pilot scale in the next year.”
**Management**

**Feedstocks**
NREL- C1 Electrochemist
Kenneth Neyerlin
Carbon Recycling International - MeOH producer

**Products**
NREL- TEA/LCA analysis Ling Tao

**Related Industry Contacts**
Liquid C1 Conversion Startups
Royal Dutch Shell C1 conversion

**C1 Organisms**
NREL- Microbiologist Jonathan Lo
**Methanol and Formate Feedstocks**

How are they generated?
H\(_2\)/CO\(_2\) synergy?
At what prices?

**C1 Microbes**

Who can use them? How well?
What do they make?
Can they be genetically engineered?

**Products**

What products can be made?
Cost? Market price?
Carbon and electron efficiency?
Upgrading paths and market size?
2.1.0.111 Analysis in Support of Biofuels and Bioproducts from Organic Wet Waste Feedstocks

2.3.2.112 Enhancing Acetogen Formate Utilization to Value-Added Products

2.5.3.701 Production of High-value Chemicals from Renewable Feedstock

2.5.4.30X Advanced Catalyst Synthesis and Characterization

• 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
• Royal Dutch Shell/Princeton CO2 Conversion ACT Project
Approach: Highest biological efficiency for CO₂ fixation

Renewable methanol and formate as microbial feedstocks
Charles AR Cotton¹, Nico J Claassens¹, Sara Benito-Vaquerizo² and Arren Bar-Even

Current Opinion in Biotechnology 2020, 62:168–180

“Bioproduction with acetogens is thoroughly researched and commercially exploited using gaseous C₁ feedstocks...only a small number of acetogens have been tested for growth on methanol and formate...miscible carbon sources support higher energetic efficiencies of bioproduction”
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
- Avoids gas mass transfer issue, easier to store and transport
- Can simultaneously use gases, liquids, and biomass related sugars
- Produce interesting products at high carbon and electron efficiency
- Focus on C4 products (butanol/butyrate) due to their ease of upgrading to fuels
Approach - Milestones

• Transform C4 overexpression pathway into acetogen to boost yield of C4 products (Q1)

• Growth ≥1L reactor for TEA/LCA metrics analysis (Q2)

• TEA/LCA analysis of butanol/butyrate production with different feedstock mixes to determine cost drivers, carbon efficiencies, product separation, purification, and upgrading (Q3)

End goal

• Proof of concept: Demonstrate production of 2 g/L of a C4 compound in an engineered acetogen using 1-carbon feedstocks (Q4)
Progress and Outcomes
TechnoEconomic Analysis

Assumptions for CO₂ Reduction to Formate

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell Voltage (V)</td>
<td>2.08</td>
</tr>
<tr>
<td>Current Density (mA/cm²)</td>
<td>300</td>
</tr>
<tr>
<td>Faradaic Efficiency (%)</td>
<td>95</td>
</tr>
<tr>
<td>CO₂ Single pass Conversion (%)</td>
<td>90</td>
</tr>
<tr>
<td>CO Faradaic Efficiency (%)</td>
<td>0</td>
</tr>
<tr>
<td>H₂ Faradaic Efficiency (%)</td>
<td>5</td>
</tr>
<tr>
<td>Electrolyzer Capital Cost ($/m²)</td>
<td>10,000</td>
</tr>
<tr>
<td>Electricity Price ($/kWh)</td>
<td>0.03</td>
</tr>
<tr>
<td>CO Market Price ($/kg)</td>
<td>0.23</td>
</tr>
<tr>
<td>H₂ Price ($/kg)</td>
<td>1.57</td>
</tr>
<tr>
<td>Water Price ($/kg)</td>
<td>0.00022</td>
</tr>
<tr>
<td>CO₂ Capture Price ($/ton)</td>
<td>20</td>
</tr>
</tbody>
</table>

- Formate is a poor electron source
- H₂ improves Carbon yield
- Butyrate versus butanol?

- Methanol is cheap, electron rich, soluble
- From methane or electrochemically
- Potential cosubstrate
- C₁ miscible

Equation

\[
\text{C}_6\text{H}_{12}\text{O}_6 \text{ (sugar)} \rightarrow \text{C}_4\text{H}_{10}\text{O}_2 \text{ (butanol)} + 2\text{CO}_2 + \text{H}_2
\]
\[\text{MW} \quad \text{Mass yield} \quad \text{Input cost $ per kg} \quad \% \text{ Cost reduction}\]

<table>
<thead>
<tr>
<th></th>
<th>USD/kg $</th>
<th>MW</th>
<th>Mass yield g/g</th>
<th>Input cost $ per kg</th>
<th>% Cost reduction</th>
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</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>$0.29</td>
<td>32.04</td>
<td>0.58</td>
<td>$0.49</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>$0.29</td>
<td>180.16</td>
<td>0.41</td>
<td>$0.70</td>
<td>30%</td>
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<tr>
<td>Butanol</td>
<td>$1.20</td>
<td>74.12</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Preliminary Minimum Butanol Selling Price ($/gal)

- Capitall Return
- Average Income Tax
- Capital Depreciation
- Fixed Operating Cost
- Utilities
- Other Raw Material
- Hydrogen
- Formate

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Progress and Outcomes
C1 conversion

- **Butyribacterium methylotrophicum (Bm)**
  - Can use formate/methanol alone
  - No genetic tools
  - No metabolic models
  - Acetate/ethanol/butyrate/butanol

Growth and production on formate/methanol
Progress and Outcomes
C1 conversion specifics

- **Bm** can naturally make C4 (butyrate/butanol)
  - 100 mM formate 200 mM MeOH
  - 41 mM (3.6 g/L) butyrate

- Methanol seems to drive formation for butyrate, but does not make butanol

- Different C1 mixtures and conditions for different product formation

- Single substrate C1 fermentation is slow

- C1 mix is synergistic
  - Tolerance (~500mM formate, >1M methanol)
  - Consumption kinetics (yields, rates, titers)
Progress and Outcomes
Organism development

• Genetics tools development
  – Protocol established
  – Building CRISPR/Cas9 plasmids
  – Butanol production strategies
  – Promoter testing

• Metabolic analysis
  – RNAseq analysis
  – Substrate specificity
  – Product profile
  – Linking genes to products

Plasmids expressing different alcohol dehydrogenases (AdhE). AdhE is known to convert aldehydes into alcohols and is needed to create ethanol and butanol.

Growth of *B. methyltrophicum* engineered strains on 90 mM formate and 100 mM methanol.
Progress and Outcomes
Organism development

• Test tube scale
  – 3.6 g/L butyrate
  – 50% Carbon efficiency

• Bioreactor scale up
  – Fermentation control
  – Experimenting with conditions
  – Feeding strategies
  – Improving yield/rate/titer
  – Capturing metrics for second TEA/LCA analysis
Direct CO\textsubscript{2} conversion to C1 chemicals has a high technology readiness level, but C1 chemicals have a low market price.

Larger compounds C2-C4 have a higher value.

Direct contact with Royal Dutch Shell, and renewable Methanol Carbon Recycling International (CRI)

Several forthcoming publications around microbial C1 conversion.

- Genetic engineering and describing metabolism
  - Describing techniques, new tools, analysis
- Metrics regarding C1 fermentation
  - Yield/rate/titer, fermentation strategies to change products
- Fermentation process using real liquid C1s from CO\textsubscript{2}

<table>
<thead>
<tr>
<th>Feedstocks</th>
<th>USD/kg</th>
<th>$/mole</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1 Methanol</td>
<td>$0.29</td>
<td>$0.009</td>
</tr>
<tr>
<td>C1 CO\textsubscript{2}</td>
<td>$0.00</td>
<td>$0.000</td>
</tr>
<tr>
<td>C1 Formate</td>
<td>$1.00</td>
<td>$0.046</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Products</th>
<th>USD/kg</th>
<th>$/mole</th>
</tr>
</thead>
<tbody>
<tr>
<td>C2 Acetate</td>
<td>$0.80</td>
<td>$0.048</td>
</tr>
<tr>
<td>C4 Butyrate</td>
<td>$1.50</td>
<td>$0.132</td>
</tr>
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</table>

Feedstock and product market costs.
Impact – Future Work

• Liquid C1 fermentation strategies
  – Implementing real CO$_2$ reduction streams
  – Fed batch, continuous, in situ extraction
  – Supplement CO/H$_2$ for better growth and carbon efficiency?

• Direct production of methanol conversion to C4?
  – Methanol is cheap ($276/MT), readily available, derived from methane, or electrochemically renewable from CO2 (Vulcanol).

• Engineering *B. methyltrophicum* to make C4 (butyrate /butanol) at higher yield
  – Target native pathways, adding C4 pathways, planning RNAseq experiment
  – CRISPR/Cas9 gene deletion, genomic integration

• Proposals to further develop process, explore variations, outside partner collaboration
Summary

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

Value Proposition
- CO$_2$ conversion to liquid C1 for microbial upgrading represents an interesting proposition for renewable energy integration with CO$_2$ as a feedstock

Key Accomplishments
- Identified and developed microbial C1 conversion to C2 and C4 products
- Developed a TEA/LCA analysis for understanding the process
- Filling in knowledge gaps and disseminating knowledge among academic and industry institutions
Thank You

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Jonathan.Lo@nrel.gov

Program 2.3.2.112

This work was authored by the National Renewable Energy Laboratory, operated by Alliance for Sustainable Energy, LLC, for the U.S. Department of Energy (DOE) under Contract No. DE-AC36-08GO28308. Funding provided by DOE Bioenergy Technologies Office. The views expressed in the article do not necessarily represent the views of the DOE or the U.S. Government. The U.S. Government retains and the publisher, by accepting the article for publication, acknowledges that the U.S. Government retains a nonexclusive, paid-up, irrevocable, worldwide license to publish or reproduce the published form of this work, or allow others to do so, for U.S. Government purposes.
This project aims to convert formate to butanol using Clostridia ljungdahlii. Formate is one of the target intermediates that can be produced via chemical synthesis. It is not clear whether butanol is the best target product considering their value and subsequent separation issues etc. Thus, a better system engineering should be incorporated to fully evaluate the proposed technology.

**Response:** The TEA analysis is utilized to evaluate which products could best be made from formate. In our system, formate is first converted into acetyl-CoA, which is a precursor to many other products that could be made instead of formate, including ethanol, butyrate, and mevalonate, which could be made instead of butanol.

As a benchmark, assuming 100% formate conversion and 3 V for the electrochemical cell producing formate, the electricity demand will be ~26 kWh per kg butanol. The energy demand for state-of-the-art formate producing cells is substantially more than the thermodynamic minimum. Even if electricity is cheap, the extra cost of an inefficient energy conversion may make the process uncompetitive.

**Response:** Having high conversion to product is an important consideration for reaching economically feasible. It may be that we need higher efficiency of formate conversion to products, and that may be through co feeding other substrates like H2/CO to better efficiencies so that the formate carbon and electrons are better matched towards products.
We anticipate at least 2 publications from this project in progress:

*Butyribacterium methylotrophicum* C1 liquid conversion characterization via RNAseq analysis and bioreactor data

Development and Genetic Engineering of *Butyribacterium methylotrophicum* as a chassis organism for conversion of C1 compounds to Value Added products