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 7th 2019

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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

	Total Costs Pre FY17**	FY 17 Costs	FY 18 Costs	Total Planned Funding (FY 19-Project End Date)
DOE Funded	0	0	0	$850k

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

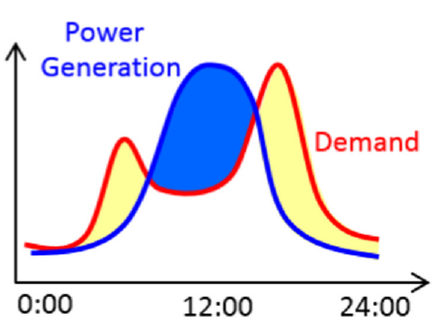
TEA/LCA for the integrated concept of formate to butanol, with biological conversion, co-utilization of electrosynthesis products, and final product separation/purification.

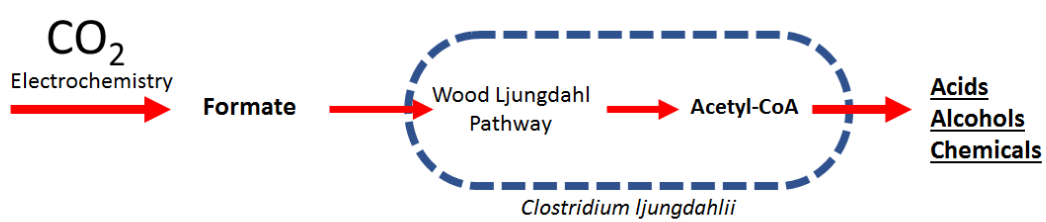
Project Overview

- **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**





Approach - Management

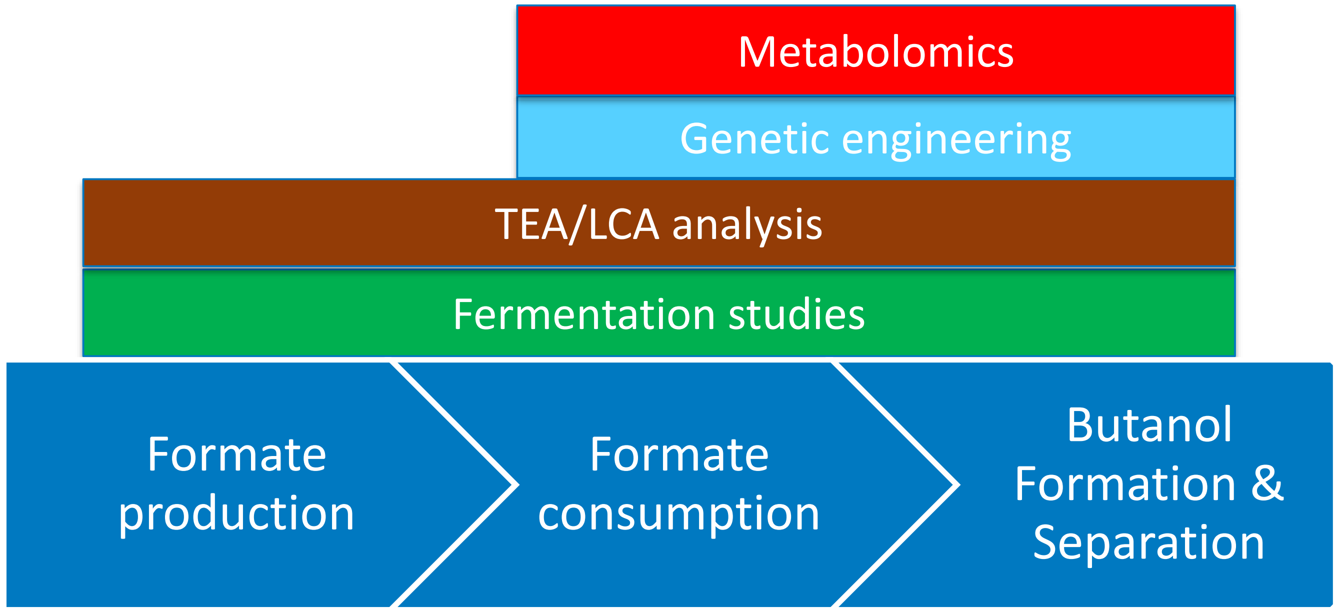
Understanding bacterial metabolism: Using metabolomics and genetic engineering to improve formate consumption and butanol formation.

- Expertise in *Clostridia* with analysis of metabolic pathways using **13C 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



Approach - Management

2.3.2.112 Enhancing Acetogen Formate Utilization to Value-Added Products

Agile BioFoundry

2.5.3.701 Production of High-value Chemicals from Renewable Feedstock

Waste-to-Energy

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

Catalysis

2.5.4.30X Advanced Catalyst Synthesis and Characterization

Biochemical Conversion

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

CO_2

- 2.1.0.304 Feasibility Study of Utilizing Electricity to Produce Intermediates from CO_2 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 CO_2 Electrolysis with Microbial Syngas; Upgrading to Rewire the Carbon Economy
- 2.3.2.106 CO2 Valorization via Rewiring Metabolic Network

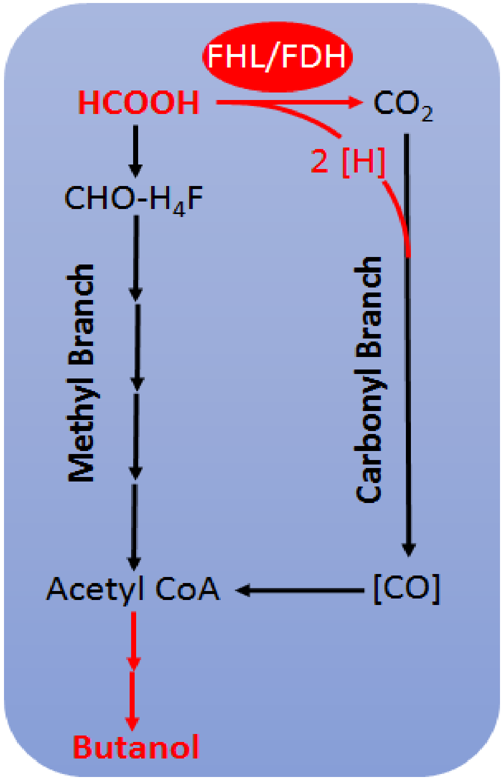
Approach - Technical

- *C. ljungdahlii*
  - **Wood Ljungdahl Pathway (WLP)** convert formate (as well as H_2+CO_2/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**



$$CO_2 + \text{reduced ferredoxin} \rightarrow CO$$

through enzymes like **Formate dehydrogenase (FDH)**

$$2\ HCOO^- + \text{oxidized ferredoxin} + NAD^+ \rightarrow 2\ CO_2 + \text{reduced ferredoxin} + NADH + H^+$$

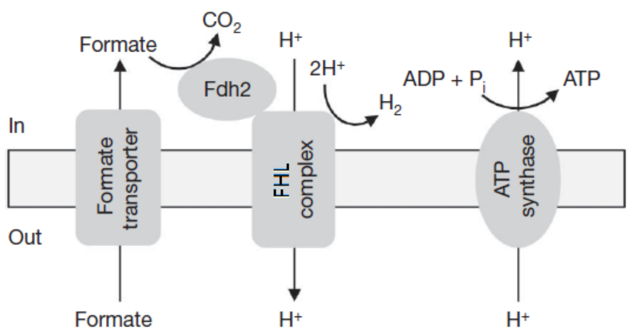
Approach - Technical

Aim 1: Robust formate utilizing strain

Approach:

Formate utilization genes

- Formate hydrogen lyase (FHL): Formate → $H_2 + CO_2$



- Electron bifurcating formate dehydrogenase (EBFDH)

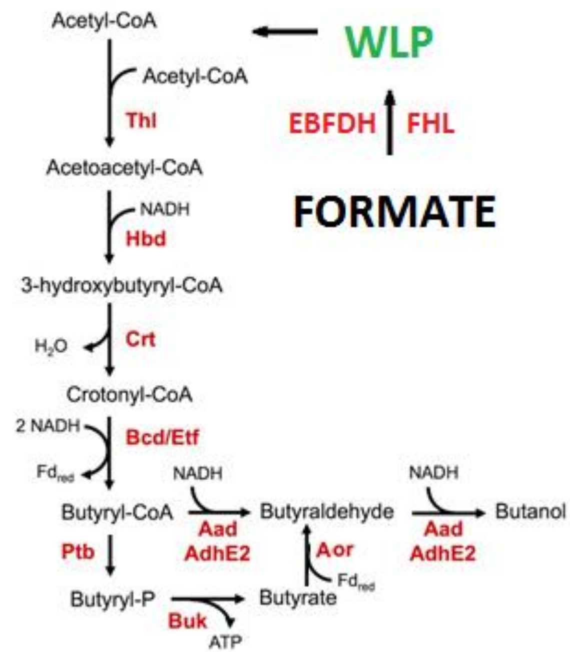
Track formate utilization

- 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

Description of Risk	Mitigation Plan
Metabolic engineering may compromise microbial strain stability as formate is both a feedstock and intermediate	^{13}C-fluxomics will guide an informed strategy to ensure metabolic balance to attain higher titers of targeted products in stable chassis hosts
Formate at high levels could be toxic to C. ljungdahlii	Formate can be fed continuously into the bioreactor. Formate utilization pathways should improve tolerance. Genetic engineering can improve tolerance.

Redirect metabolism from native products to butanol

Description of Risk	Mitigation Plan
Increasing alcohol concentration toxicity may be an issue for C. ljungdahlii	Various in situ recovery methods exist to remove alcohol as it is being formed. Alternatively, butanol tolerance can be improved by genetic engineering
Butanol may be not practical under TEA	The formate conversion platform is product agnostic; other products can be engineered into C. ljungdahlii.

Accomplishments

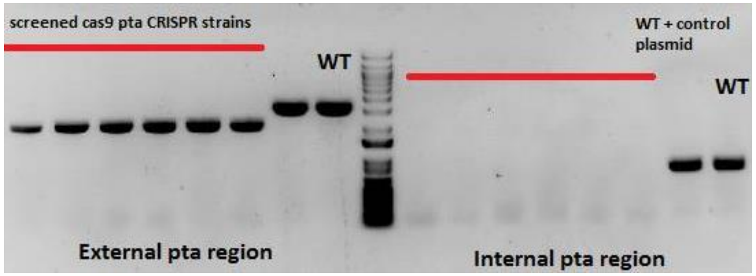
Aim 1: Develop robust formate utilizing strain

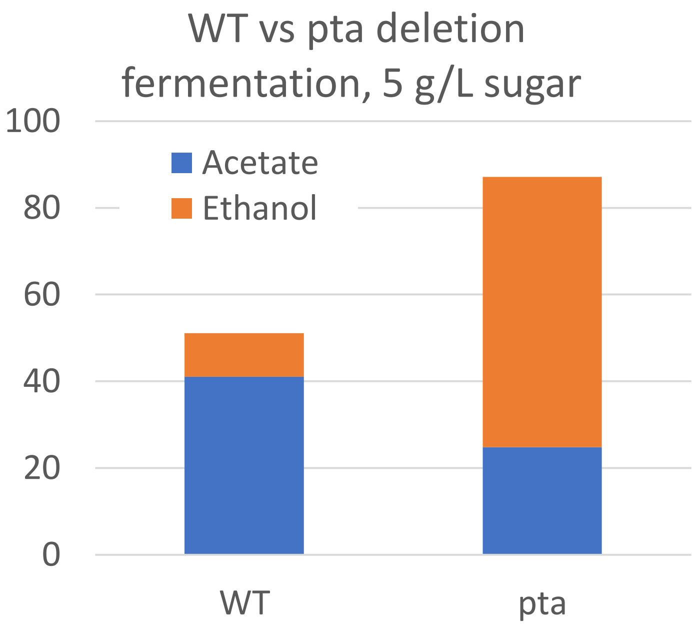
Native formate utilization

Growth condition	Formate remaining (mM)
No bacteria control	15
Formate alone	6.5
$H_2 + CO_2$	4.75
Formate + CO	0
Formate + Sugar	0

Aim 2: Produce butanol

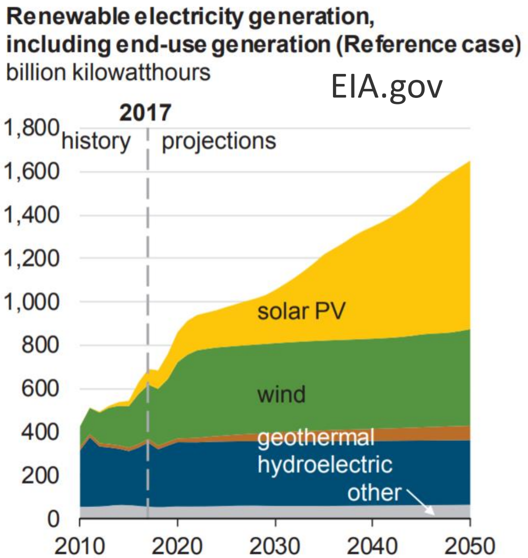
- Gene editing using Cas9, deleting *pta*.
- Less acetate more alcohol





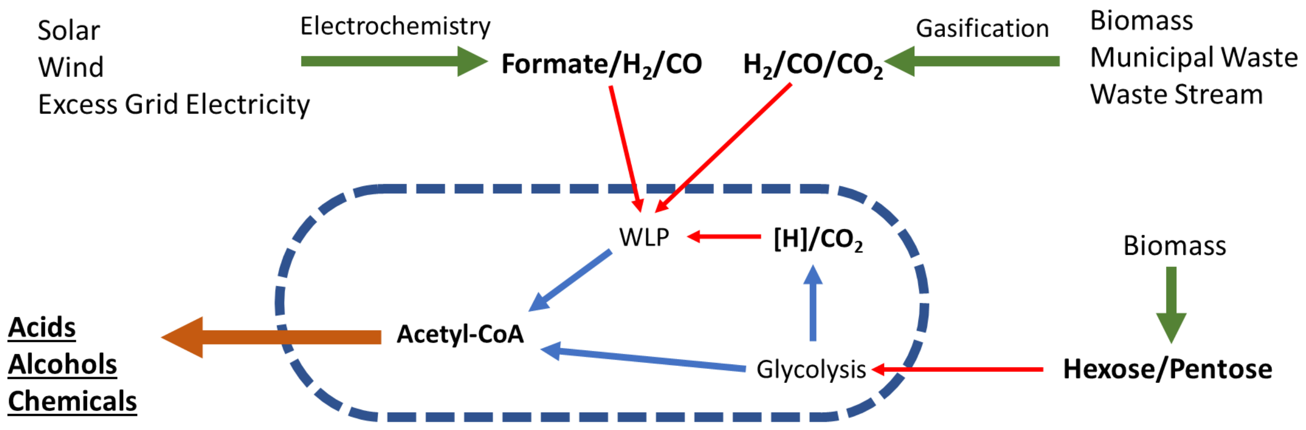
Relevance

- 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 CO_2 reduction side products H_2/CO can be used
  - A variety of ≥C2 chemicals can be accessed by genetic engineering
  - Utilizes variety of substrates like biomass sugars, H_2, CO, CO_2, 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*.

Future Work

Aim 1: Develop formate utilizing strain

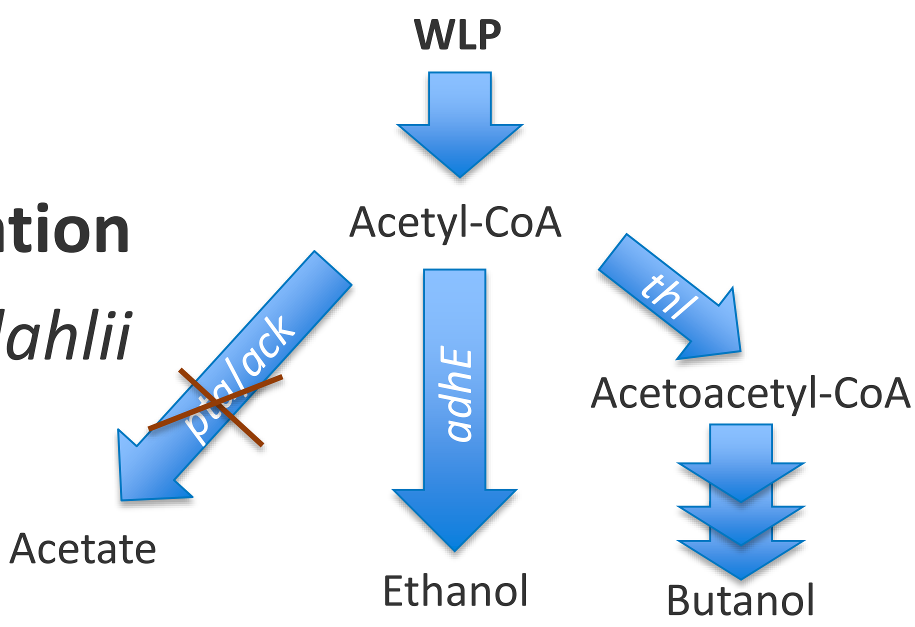
- Track formate metabolism using 13C labeling
- Genetically engineer *C. ljungdahlii* to better utilize formate
  - Introduce novel formate utilization genes

Milestone	Description	End Date	Type
Flux mapping of formate utilization	^{13}C-tracer studies (feeding ^{13}C-formate) to map carbon fluxes of formate	3/31/2019	Quarterly Progress
New formate pathways	Input formate utilization genes and obtain a titer of 1 g/L products with formate as a sole carbon source.	9/30/2019	SMART Milestone
Formate conversion to product	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	3/31/2020	Go/No Go

Future Work

Aim 2: Introduce butanol formation

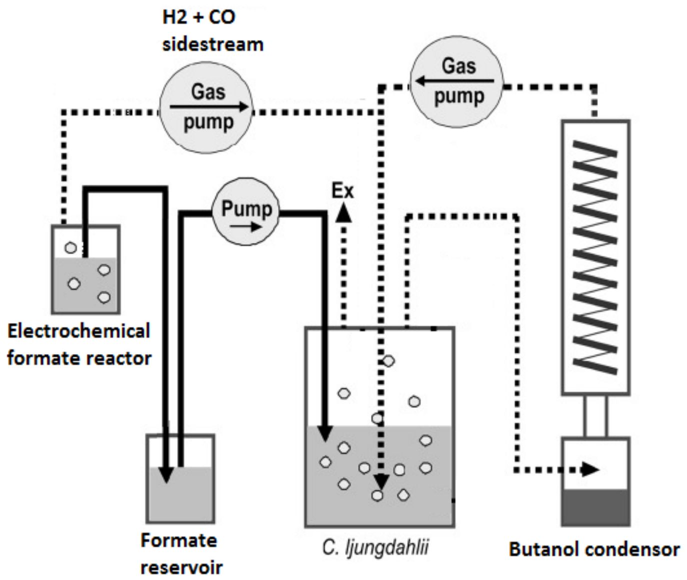
- Genetically engineer *C. ljungdahlii*
  - Introduce butanol genes
  - Curb native products



Milestone	Description	End Date	Type
Butanol production	Generate a titer of 200 mg/L of butanol from formate as a sole carbon source	9/30/2020	Annual Milestone
Produce butanol from formate	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.	9/30/2021	Project End

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.

Milestone	Description	End Date	Type
TEA of butanol production from formate	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.	3/31/2020 (mid point)	Go/No Go
TEA/LCA of overall integrated concept	Perform detailed TEA/LCA for the integrated concept from formate to butanol, biological conversion, co-utilization of electrosynthesis products, final product separation and purification.	9/30/2021	Project End

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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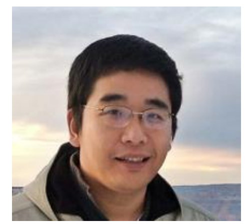

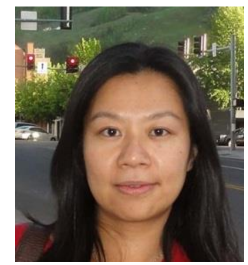

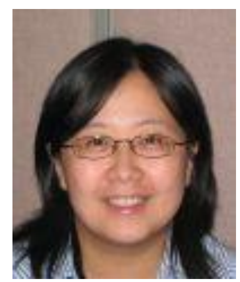

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