NREL
NATIONAL RENEWABLE ENERGY LABORATORY

DOE Bioenergy Technologies Office (BETO) 2019 Project Peer Review

NL0034713 (2.3.2.111) Improving formate upgrading by *Cupriavidus necator*

CO_2 Utilization Technology Session

March 7, 2019

Christopher W. Johnson

National Renewable Energy Laboratory

Goal Statement

Goals

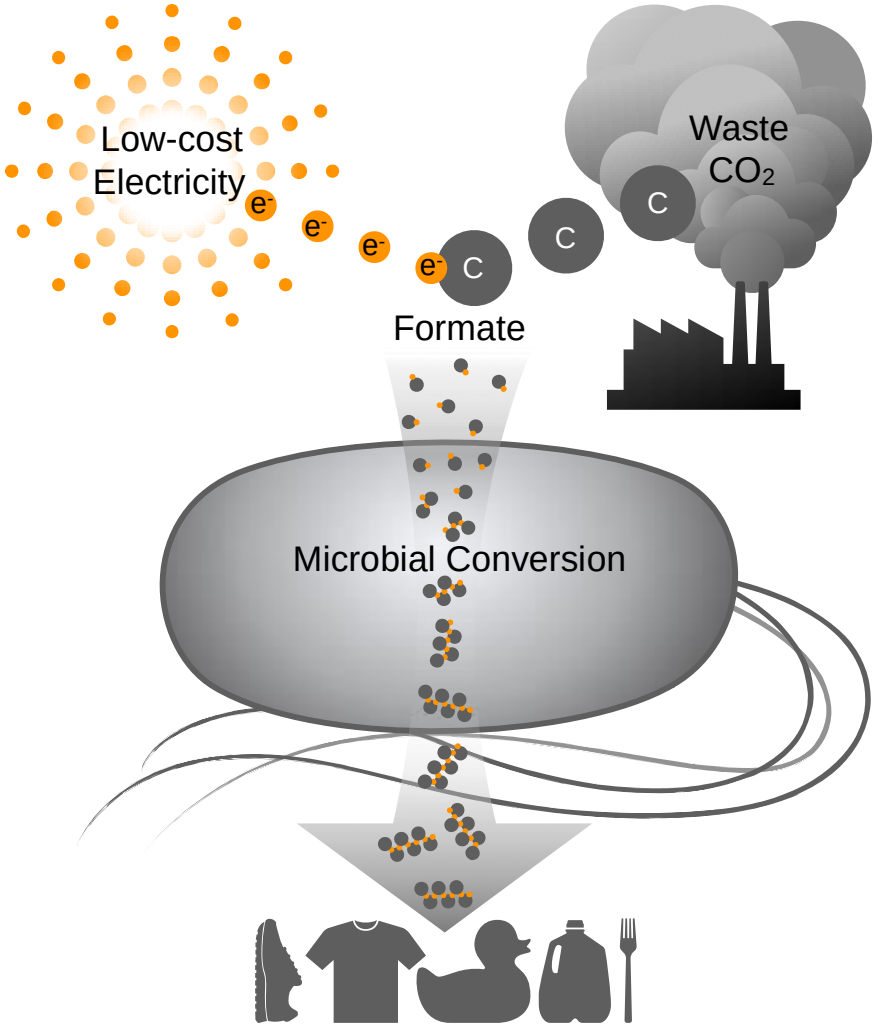
- Develop the natural formatotroph, *Cupriavidus necator*, as a robust microbial chassis for efficient conversion of formate to value-added products
- Demonstrate production of 2 g/L of the polymer precursor β-ketoadipate using formate as the sole source of carbon and energy

Outcome

- Support efforts to capture and convert CO_2 using low-cost electricity

Relevance

- Improve the economics of energy generation technologies that emit waste CO_2, including biorefining



Quad Chart Overview

Timeline

- Start: FY2018
- Merit review cycle: FY2017-2019
- 17% complete of review cycle

	Total Costs Pre FY17**	FY 17 Costs	FY 18 Costs	Total Planned Funding (FY 19-Project End Date)
DOE Funded	$0	$0	$0	$960,000

Barriers addressed

- Ct-D. Advanced Bioprocess Development
- Ct-H. Gas Fermentation Development
- Ct-J. Identification and Evaluation of Potential Bioproducts
- At-A. Analysis to Inform Strategic Direction

Objective

- Develop the natural formatotroph, *C. necator*, as a robust microbial chassis for efficient formate conversion to value-added products in support of CO_2 capture.

End of Project Goals

- Demonstrate the amenability of *C. necator* as a powerful host for the upgrading of formate to value-added products by producing at least 2 g/L of β-ketoadipate using formate as the sole source of carbon and energy.
- Generate TEA/LCA to enable the DOE and other stake holders to evaluate the economic and environmental impact of this project and similar work pursued elsewhere.

Project Overview

History

- This is a new project (10/01/18) funded by a “Rewiring the Bioeconomy: Biological Formate Upgrading Lab Call” issued by BETO in response to guidance from EERE leadership and congress.

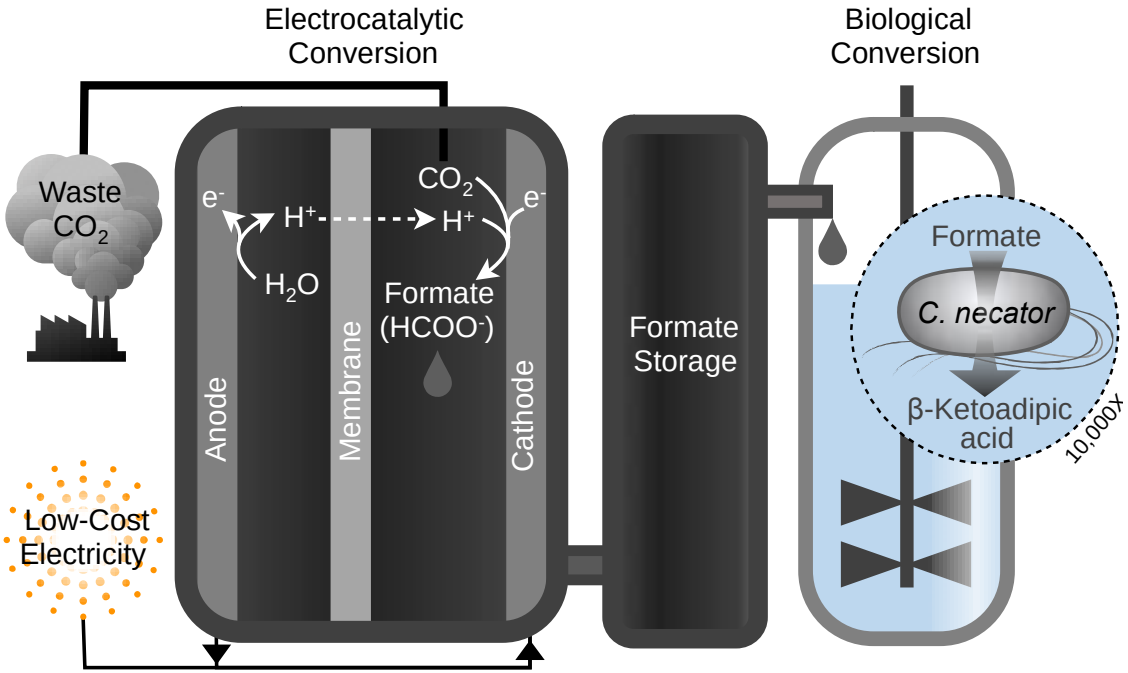
- “Rewiring” the carbon cycle refers to producing reduced forms of carbon from CO_2 using electricity rather than solar energy, bypassing photosynthesis.

- NREL (with OPX Bio and Johnson Matthey) previously received funding from ARPA-E’s Electrofuels program that aimed to engineer *C. necator* to produce free fatty acids from CO_2 and H_2.

Project Overview

Context:

- Waste CO_2 is generated by virtually all energy technologies.
- CO_2 can be electrocatalytically reduced to generate formate.
- Formate has been proposed as a soluble intermediate compound to store energy and carbon for microbial conversion to fuels and chemicals.



- *C. necator* natively assimilates formate and can be engineered to convert it value added products.

Project Overview

Project Goals

- Improve formate assimilation via the native CBB cycle (≥ 20%).
- Improve formate assimilation via a more efficient synthetic pathway (≥ 10%).
  - The above goals will demonstrate that formate assimilation can be improved upon and generate findings to advance the development of microbial conversion of formate.
- Demonstrate improved conversion of formate to the polymer precursor β-ketoadipic acid (≥ 2 g/L).
  - This will demonstrate that improvements to formate assimilation can leveraged to produce a value added product and achieve titers higher the 1.4 g/L previously achieved for production of higher alcohols using the native formate pathway (Li, et al. 2012. Science 335, 1596–1596.)
- Perform techno-economic analysis (TEA) and life-cycle assessment (LCA).
  - The findings of this analysis could be used to identify cost drivers, informing the direction and foci of this project and related formate and CO_2 conversion technologies.

Approach - Management

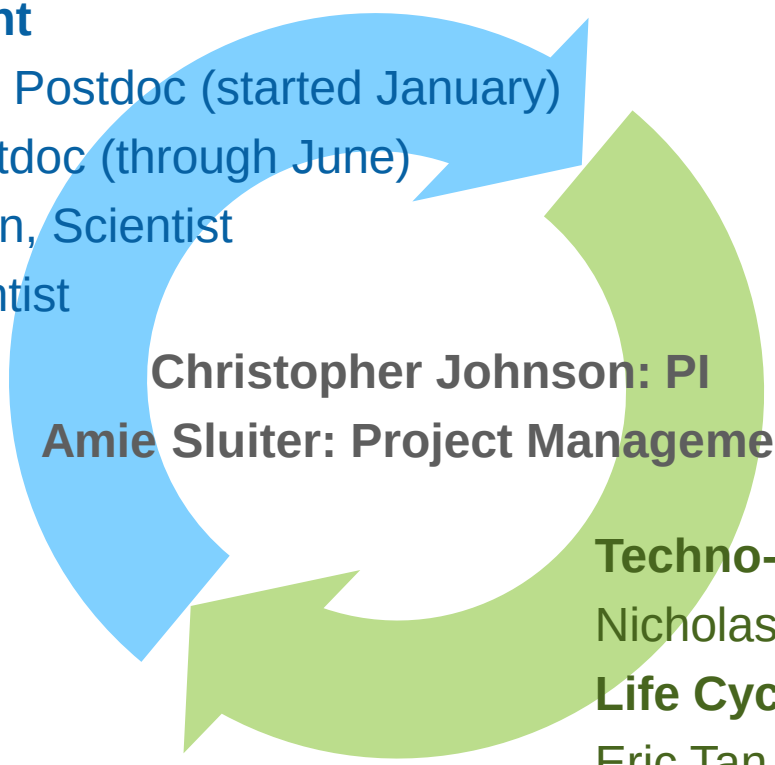
Strain Development

Christopher Calvey, Postdoc (started January)

Joan Marcano, Postdoc (through June)

Christopher Johnson, Scientist

Carrie Eckert, Scientist



Relevant experience

C. necator engineering

CBB cycle engineering

Laboratory evolution

β-ketoadipate production

Project management

Techno-Economic Analysis

Nicholas Grundl

Life Cycle Assessment

Eric Tan

Project Interfaces

Strain development meetings biweekly

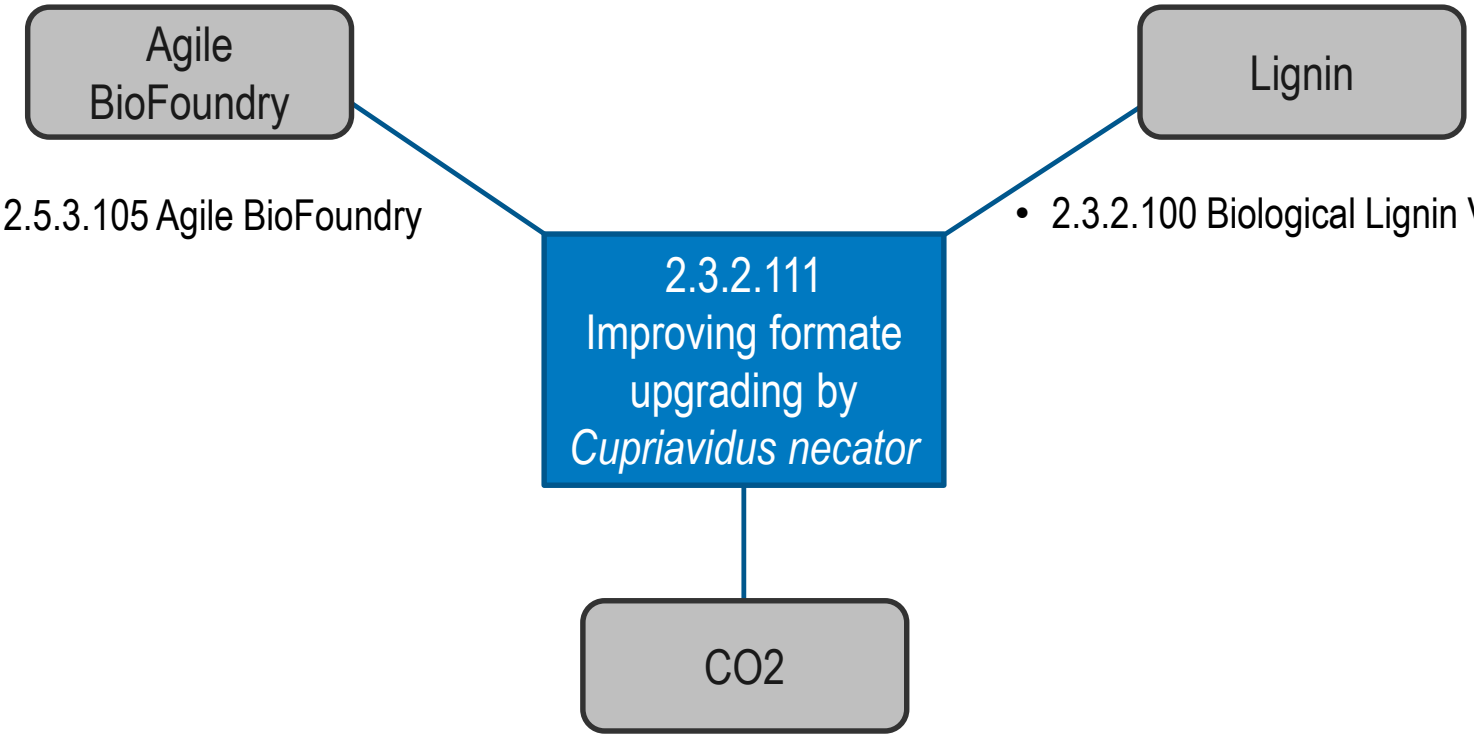
Meetings with other formate conversion projects quarterly

Meetings with TEA/LCA team quarterly (and as needed)

Milestones

Demonstrate feasibility and progress toward strain development objectives

Approach - Management



- 2.5.3.105 Agile BioFoundry
- 2.3.2.100 Biological Lignin Valorization

- 2.1.0.304 Feasibility Study of Utilizing Electricity to Produce Intermediates from CO_2 and Biomass
- 2.3.2.112 Enhancing Acetogen Formate Utilization to Value-Added Products
- 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 CO_2 Valorization via Rewiring Metabolic Network

Approach - Technical

AIM 1. Improve formate assimilation via the native CBB cycle

- Rational engineering
- Directed evolution
- Adaptive laboratory evolution

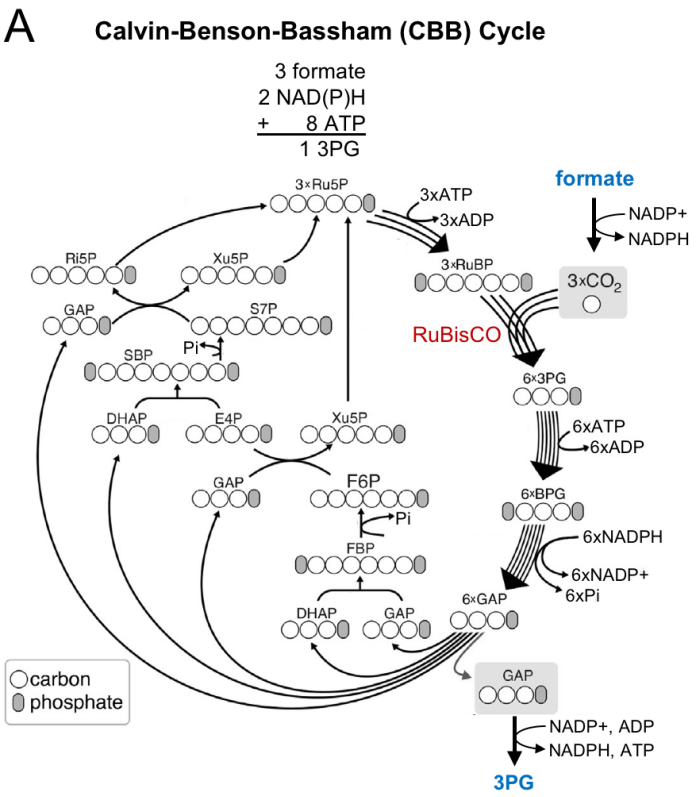


Figure adapted from Antonovsky, N., et al., 2017. Curr. Opin. Biotechnol. 47, 83–91. doi:10.1016/j.copbio.2017.06.006

AIM 2. Improve formate assimilation via a more efficient, synthetic pathway

- Introduce the formolase pathway
- Directed evolution
- Adaptive laboratory evolution

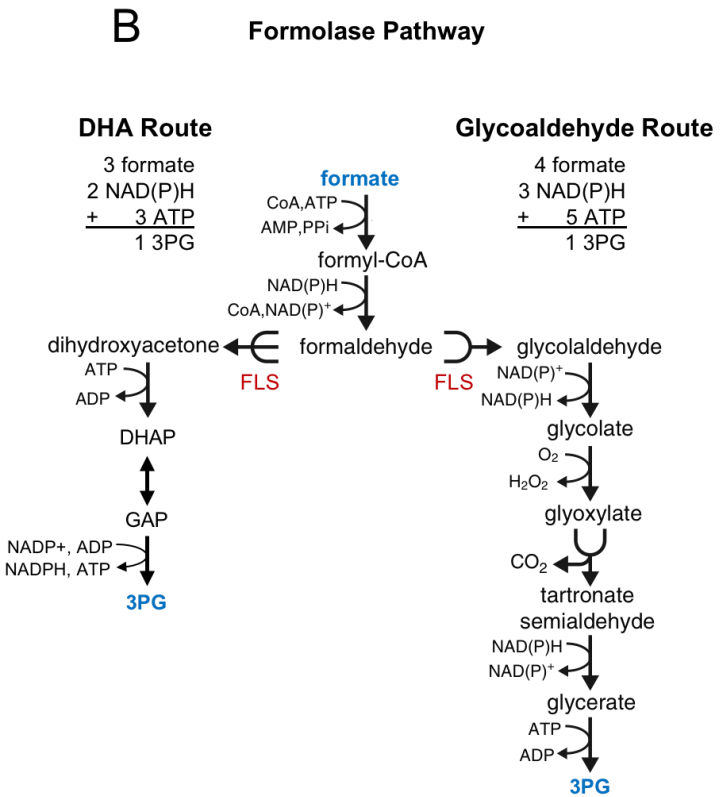
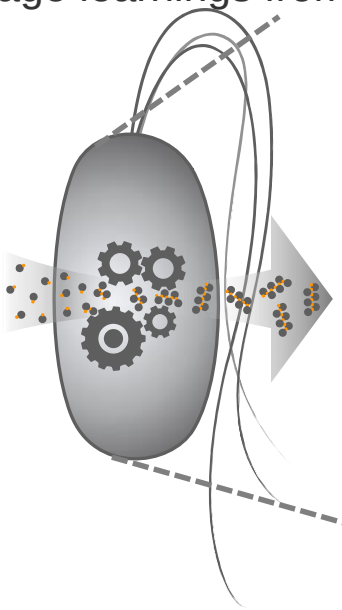


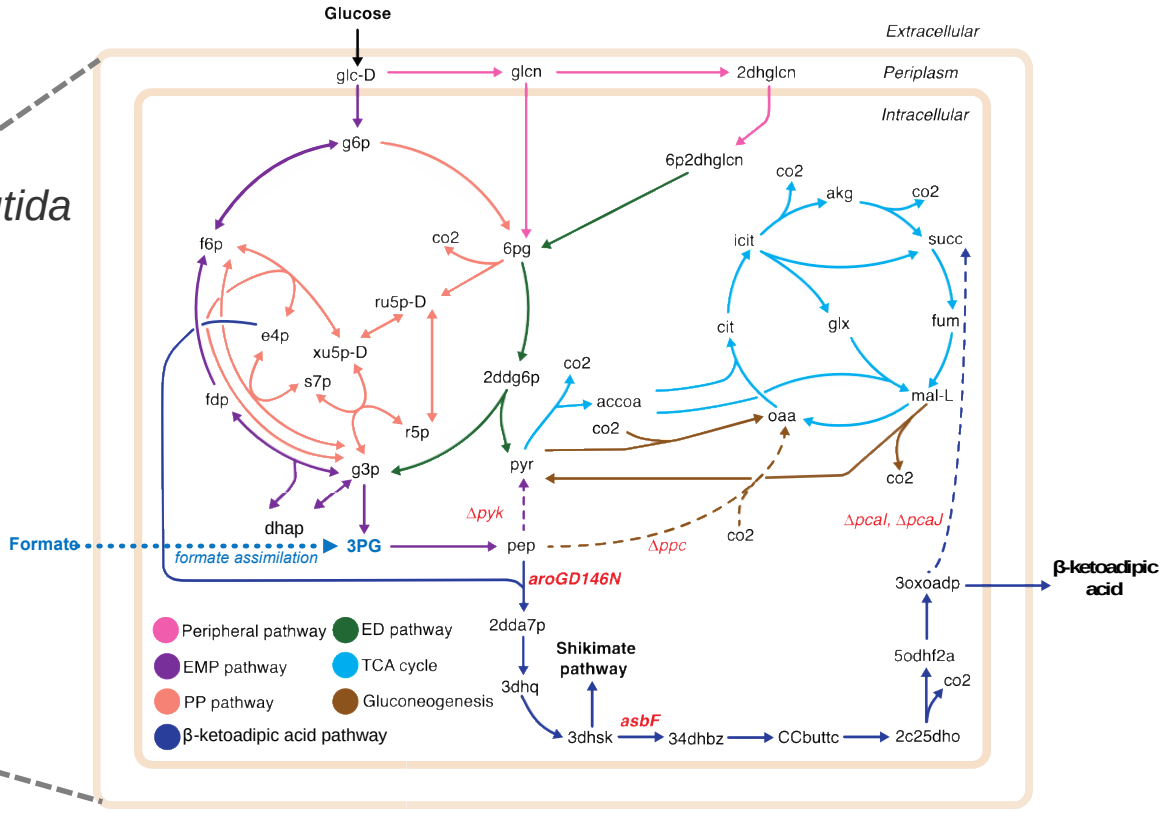
Figure adapted from Yishai, O., et al., 2016. The formate bio-economy. Curr Opin Chem Biol 35, 1–9. doi:10.1016/j.cbpa.2016.07.005

Approach - Technical

AIM 3. Demonstrate improved conversion of formate to the polymer precursor β-ketoadipic acid

- Introduce production pathway
- Delete competing pathways
- Leverage learnings from *P. putida*





Adapted from a figure by Jon Magnuson *et al.*, PNNL

AIM 4. Generate techno-economic analysis (TEA) and life-cycle assessment (LCA)

- Can inform the foci and direction of this and related technologies for biological formate conversion.

Approach - Technical

Critical success factors

- TEA to define the rates, titers, and yields that would need to be achieved for microbial conversion of formate to be commercially viable
- Industrially relevant strains capable of meeting these metrics
- Improved formate conversion beyond what is capable using native pathways

Potential challenges

- Transformation efficiency may be too low to screen large genetic libraries.

  Mitigation:

  - Work by Adam Guss at ORNL as part of an Agile BioFoundry CRADA with Kiverdi aims to improve transformation efficiency.
  - DNA can also be introduced by conjugation at higher efficiency.
- Native CBB pathway may not be able to be improved.

  Mitigation:

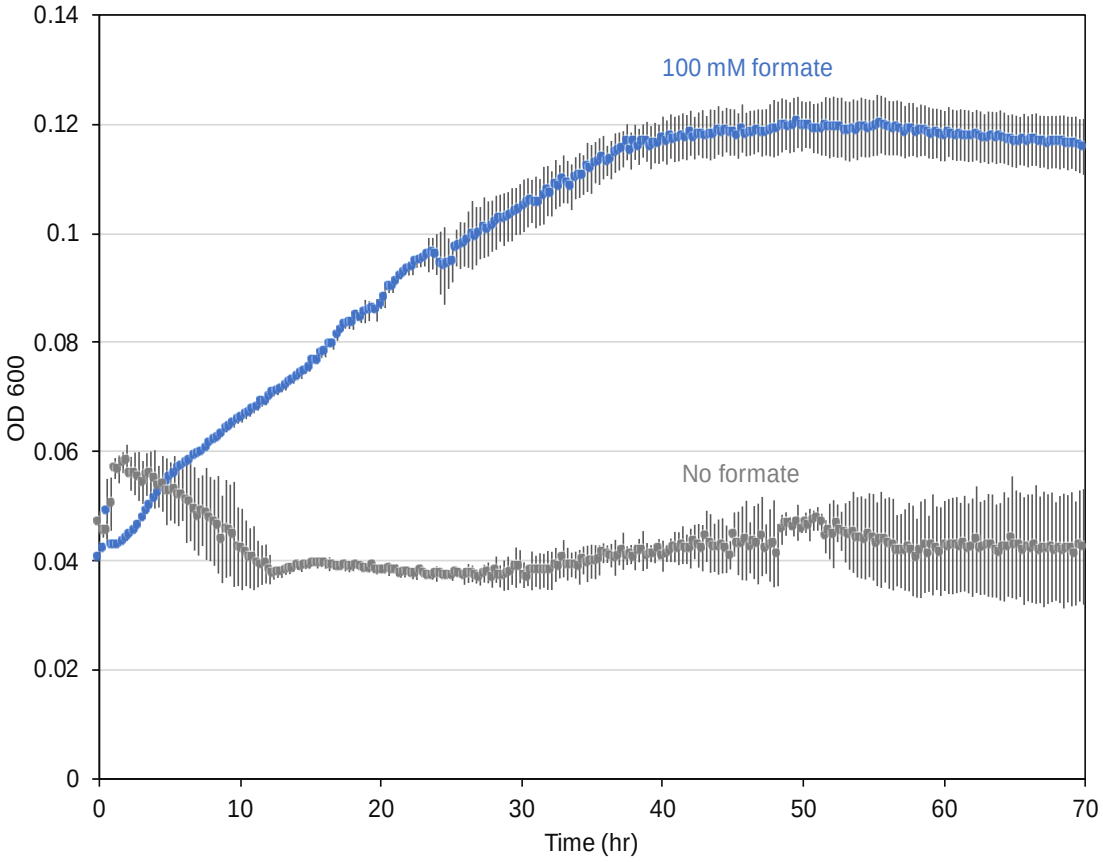
  - Concurrent introduction and evolution of the formolase pathway or another synthetic or non-native pathway could still improve formate assimilation.
- Activity of the synthetic formolase pathway may be insufficient.

  Mitigation:

  - Concurrent improvement of the native CBB cycle or another synthetic or non-native pathway could still improve formate assimilation.
  - Based on midpoint go/no-go, resources can be redirected toward further enhancing the native CBB cycle or introducing another promising, non-native pathway.

Accomplishments

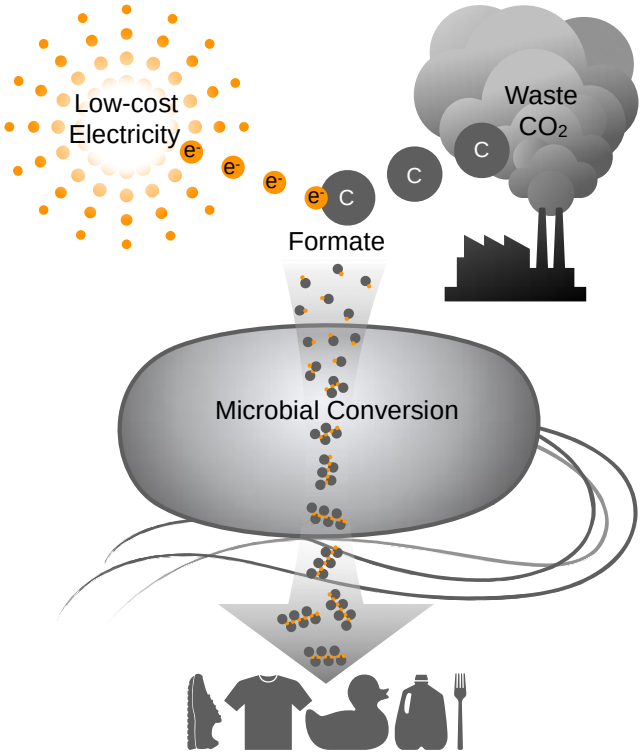
- Growth of WT *C. necator* H16 was demonstrated in a plate-reader on minimal media containing 100 mM (4.6 g/L) formate as a sole source of carbon and energy.
- *C. necator* grew from OD_{600} 0.04 to 0.12 in about 50 hours with a maximum growth rate (μ) of 0.031 h-1.



Level	Performance Measure	Date
Quarterly Progress Measure	Determine growth rate and biomass yield of wild-type *C. necator* grown on formate as the sole source of carbon and energy.	12/31/18

Relevance

GOAL: Develop the natural formatotroph, *C. necator*, as a robust microbial chassis for efficient conversion of formate to value-added products



Developing strains with improved formate conversion supports efforts to capture and convert CO_2 using low-cost electricity and increase the carbon efficiency of the economy.

Relevance

GOAL: Develop the natural formatotroph, *C. necator*, as a robust microbial chassis for efficient conversion of formate to value-added products

- Supports broad interest by DOE in CO_2 capture and conversion.

  From Senate Report 115-132, July 20, 2017:

  "The Committee encourages the Bioenergy Technologies Office to continue its collaboration with the Office of Fossil Energy on bioenergy with carbon capture sequestration [BECCS] research, as well as research to advance net carbon-negative transportation fuels."

  "The Committee encourages the Office of Fossil Energy to collaborate with the Bioenergy Technologies program within EERE to support projects that utilize carbon dioxide in the production of algae and other potentially marketable products."

- CO_2 is produced by virtually all human activity, including all forms of energy generation, so there is likely to be interest from a variety of stakeholders in creating value from this waste.

Future Work

- Rational metabolic engineering will be used to improve the native pathway for formate assimilation.

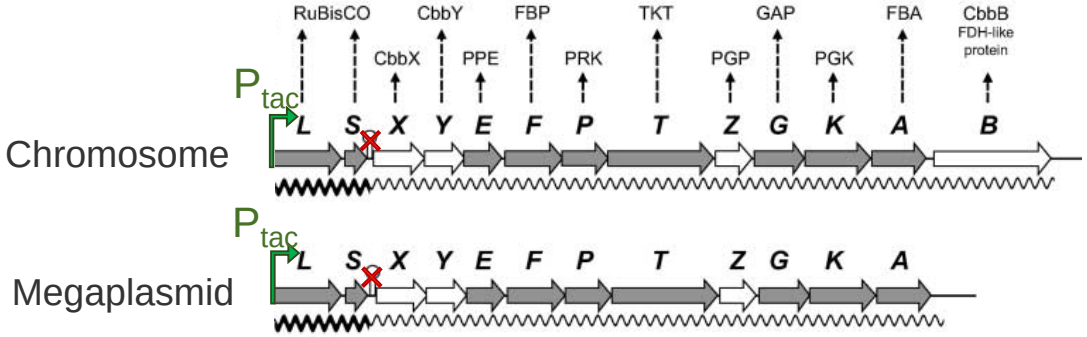


Figure adapted from Bowien, B., Kusian, B., 2002. Archives of Microbiology 178, 85–93. doi:10.1007/s00203-002-0441-3

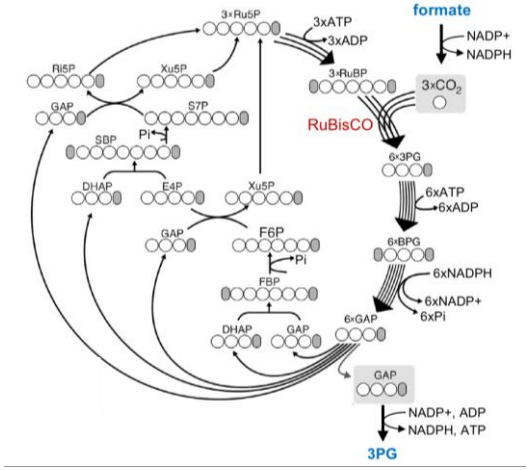


Figure adapted from Antonovsky, N., et al., 2017. Curr. Opin. Biotechnol. 47, 83–91. doi:10.1016/j.copbio.2017.06.006

- Genes encoding the formolase pathway will be introduced into *C. necator*.
  - acetyl-Co synthetase (ACS)
  - acetaldehyde dehydrogenase (ACDH)
  - formolase enzyme (FLS)
  - dihydroxyacetone kinase (DHAK)

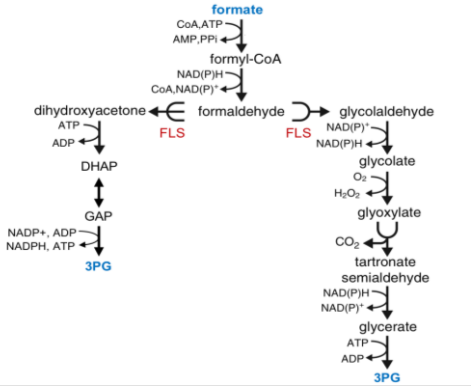
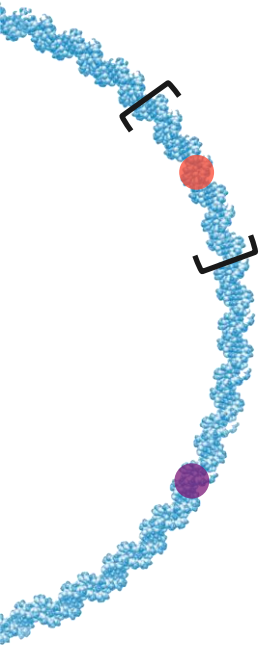


Figure adapted from Yishai, O., et al., 2016. The formate bio-economy. Curr Opin Chem Biol 35, 1–9. doi:10.1016/j.cbpa.2016.07.005

Level	Performance Measure	Date
Quarterly Progress Measure	Introduce synthetic formolase enzyme into *C. necator*, perform initial evaluation.	3/31/19

Future Work

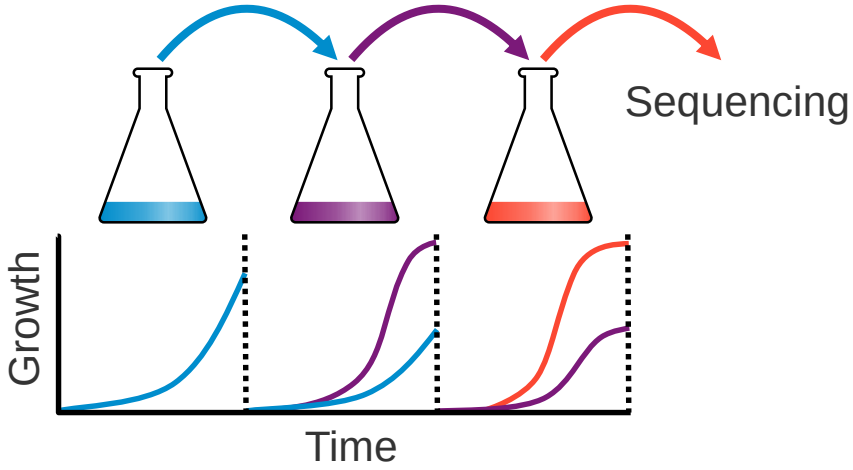
- Directed evolution and adaptive laboratory evolution will be used to improve the native and synthetic pathway for formate assimilation.



Directed evolution introduces mutations within the gene(s) of interest.

Adaptive laboratory evolution allows natural mutations to occur anywhere in the genome.

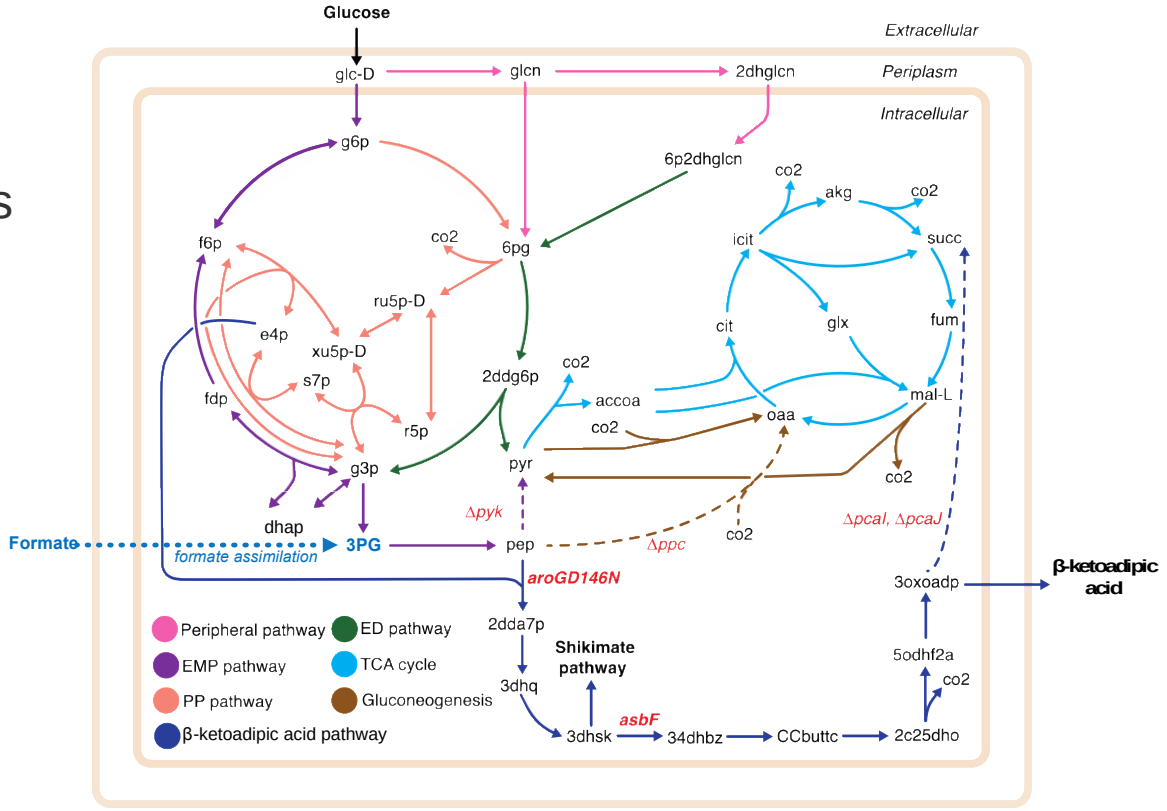
Serial transfer allows mutations that enable faster growth on formate to be identified.



Level	Performance Measure	Date
Annual SMART Milestone	Demonstrate 1.2-fold improvement of growth on formate	9/30/19
Go/No Go	Demonstrate 1.1-fold benefit from incorporation of the formolase pathway on growth rate or biomass yield during growth of *C. necator* on formate. If no benefit can be demonstrated, resources will be redirected toward further enhancing the native CBB cycle or introducing another promising, non-native pathway.	3/31/20 (midpoint)

Future Work

- The pathway for production of β-ketoadipate will be introduced into *C. necator.*
  - *aroG*^{D146N}
  - *asbF*
- Competing pathways will be deleted.
  - *pyk*
  - *ppc*
  - *pcaIJ*

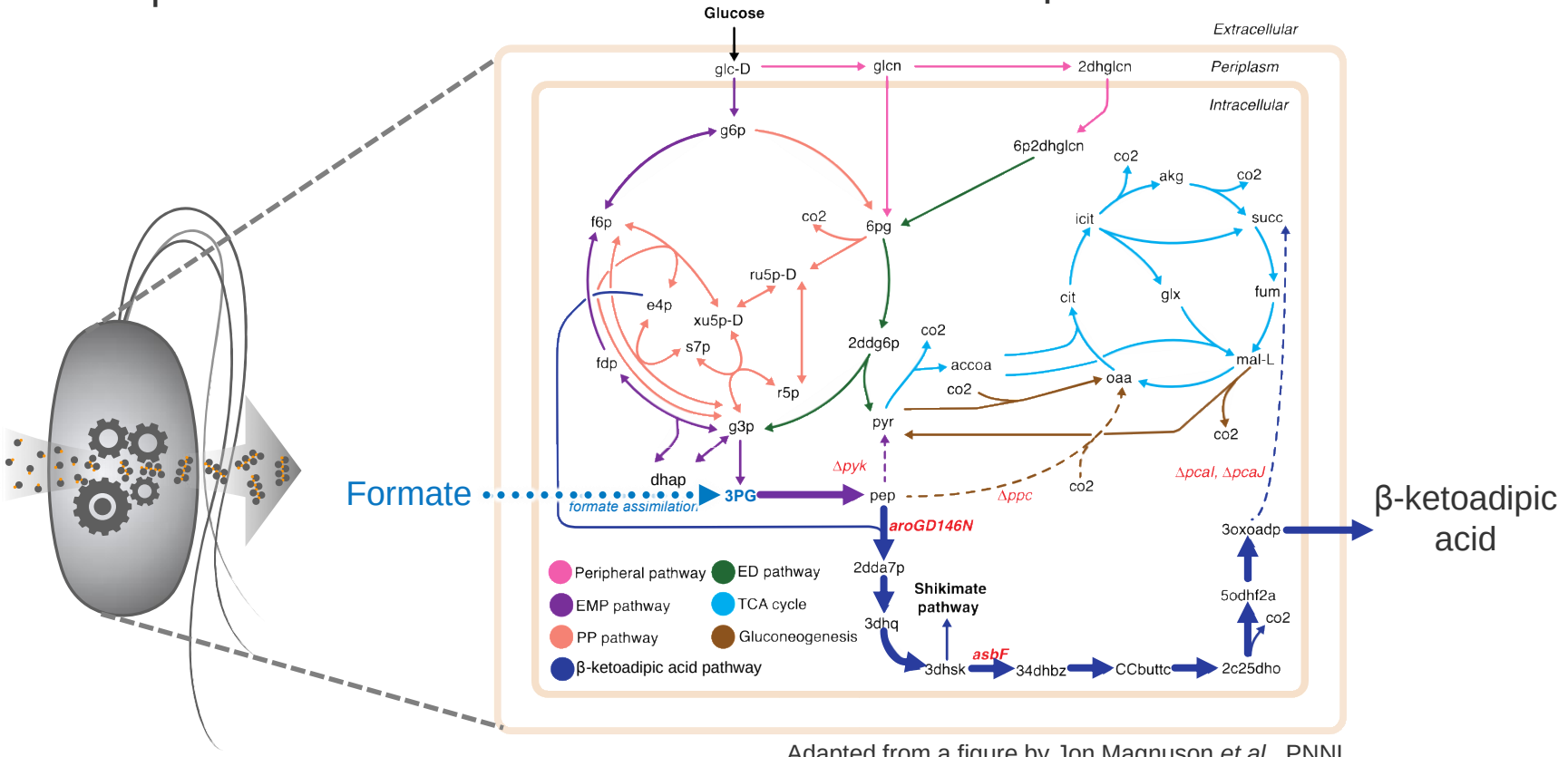


Adapted from a figure by Jon Magnuson *et al.*, PNNL

Level	Performance Measure	Date
Quarterly Progress Measure	Demonstrate production of 0.2 g/L β-ketoadipate by *C. necator* using formate as the sole source of carbon and energy	6/30/19

Future Work

- Merge efforts by deploying the β-ketoadipate production pathway in strains with improved formate assimilation to demonstrate improved conversion.



Adapted from a figure by Jon Magnuson *et al.*, PNNL

Level	Performance Measure	Date
End of Project Milestone	Demonstrate production of 2 g/L of β-ketoadipate by *C. necator* using formate as the sole source of carbon and energy. Generate final TEA/LCA on biological production of β-ketoadipate from formate.	9/30/21

Summary

- **Overview:** The natural formatotroph, *C. necator*, will be developed as a robust microbial chassis for improved production of value-added products from formate, which can be produced by electrocatalytic reduction of CO_2.
- **Approach:** Formate assimilation will be improved via the native pathway and a more efficient synthetic pathway using a combination of rational metabolic engineering and evolution and further engineering these improved strains to produce β-ketoadipate. Data generated with these strains will inform TEA and LCA.
- **Technical Accomplishments:** We have demonstrated growth of wild-type *C. necator* on 100 mM formate as the sole source of carbon and energy in conditions that are amenable to the high-throughput strain evaluation that will be required by this project.
- **Relevance:** This work supports broad interest by DOE an others in the capture and conversion of CO_2, a waste generated by virtually all human activity.
- **Future work:** In the near future, the synthetic formolase pathway will be introduced into *C. necator*, strategies to improve formate assimilation via the native CBB Cycle will be initiated, and conversion of formate to β-ketoadipate will be demonstrated.

NREL

- Carrie Eckert
- Christopher Calvey
- Joan Marcano
- Amie Sluiter
- Michelle Reed

BETO

- Ian Rowe

U.S. DEPARTMENT OF ENERGY | Energy Efficiency & Renewable Energy

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Thank You

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Christopher.Johnson@nrel.gov

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Milestones

Level	Performance Measure	Date
Quarterly Progress Measure	**Determine growth rate and biomass yield of wild-type C. necator grown on formate as the sole source of carbon and energy.** Expected to be μ_{max}: 0.18 h^{-1} and 0.10 Cmole Cmole^{-1} based on published literature. This will be used to establish baselines by which to measure improvements in formate assimilation and will inform the initial TEA.	12/31/18
Quarterly Progress Measure	**Introduce synthetic formolase enzyme into C. necator, perform initial evaluation.** This will demonstrate engineering of C. necator and enable us to determine a base-line case for formolase activity, which may initially be undetectable.	3/31/19
Quarterly Progress Measure	**Demonstrate production of 0.2 g/L β-ketoadipate in C. necator using formate as the sole source of carbon and energy.** This will serve as further evidence that C. necator can be efficiently engineered for upgrading of formate to value-added products.	6/30/19
Annual SMART Milestone	**Demonstrate 1.2-fold improvement of growth on formate** (e.g. growth rate of μ_{max}: 0.22 h^{-1} or biomass yield of 0.12 Cmole Cmole^{-1} if literature values are correct) by an engineered /evolved C. necator strain. This will serve as an important demonstration of the ability to improve formatotrophy in C. necator.	9/30/19
Go/No Go	**Demonstrate 1.1-fold benefit from incorporation of the formolase pathway on growth rate or biomass yield during growth of C. necator on formate** relative to an identical strain lacking the formolase pathway. This will serve to demonstrate the in vivo feasibility of the pathway as a means of formatotrophy. If no benefit can be demonstrated, resources will be redirected toward further enhancing the native CBB cycle or introducing another promising, non-native pathway. The strategy chosen will inform the conditions assumed in final TEA/LCA analysis.	3/31/20 (midpoint)
End of Project Milestone	**Demonstrate production of 2 g/L of β-ketoadipate in C. necator using formate as the sole source of carbon and energy.** This is substantially higher than previously reported for higher alcohols produced in C. necator from formate alone (1.4 g/L)[14] and will demonstrate the amenability of this organism as a powerful cassis for the upgrading of formate to value-added products. Generate final TEA/LCA on biological production of β-ketoadipate from formate. This analysis will enable the DOE and other stake holders to evaluate the economic and environmental impact of this project and similar work pursued elsewhere.	9/30/21