BETO 2021 Peer Review

Synthetic C1 Condensation Cycle for Formate-Mediated ElectroSynthesis

March 11, 2021

Technology Area Session: **CO_2 Utilization**

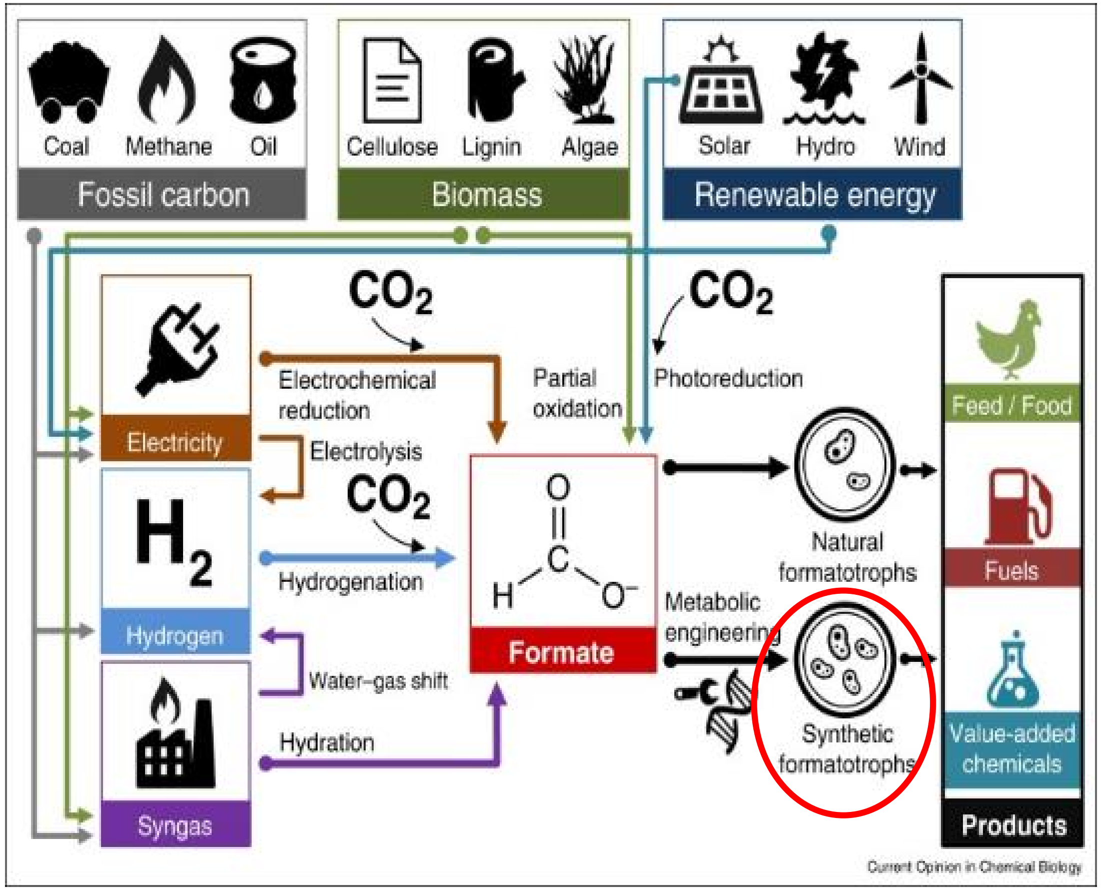
Principal Investigator: **Wei Xiong**

Organization: **National Renewable Energy Laboratory**

Project Overview

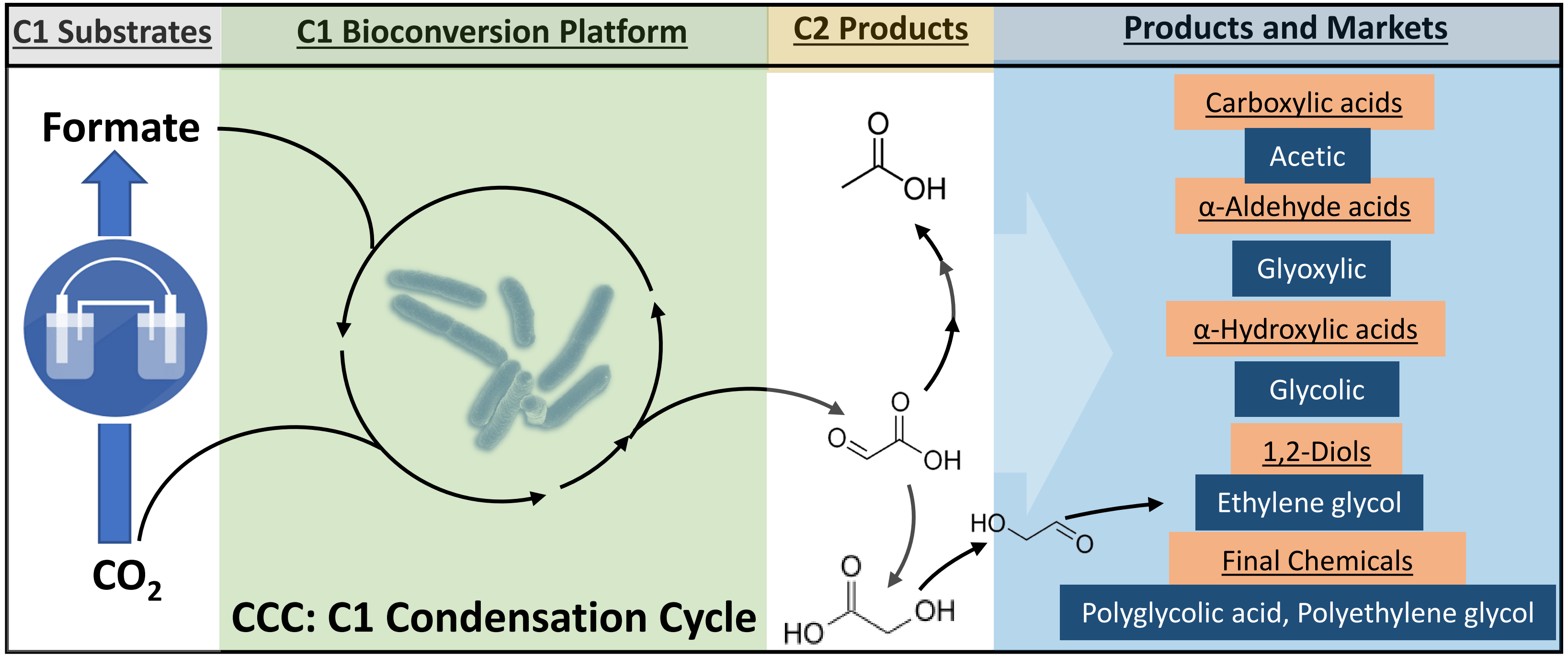
CO_2 utilization via formate

- **The goal of this project** is *to develop a practical microbial technology for upgrading of* ***formate*** *to bio-commodities;*
- *to construct efficient C1 utilization as a high carbon conversion efficiency (CCE) and cost-effective process.*



Yishai, O. et al., *Curr Opin Chem Biol*. 2016, 35, 1-9.

Project Overview



Why *E. coli* ?

- A model industrial microorganism;
- Metabolic adaptability;
- Successful synthetic C1 pathways by using *E. coli.*

Why Glycolate?

- A model and multifunctional C2 compound;
- Applicable in energy, textile, food and pharmaceutical industry;
- Growing market size; (*The glycolate market was USD **93.3 million** in 2011 and USD **203 million** in 2018).

Market Trends

Product

- Anticipated decrease in gasoline/ethanol demand; diesel demand steady
- Increasing demand for aviation and marine fuel
- Demand for higher-performance products
- Increasing demand for renewable/recyclable materials

Feedstock

- Sustained low oil prices
- Decreasing cost of renewable electricity
- Sustainable waste management
- Expanding availability of green H_2
- Closing the carbon cycle

Capital

- Risk of greenfield investments
- Challenges and costs of biorefinery start-up
- Availability of depreciated and underutilized capital equipment

Social Responsibility

- Carbon intensity reduction
- Access to clean air and water
- Environmental equity

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Value Proposition

- CO_2 utilization via soluble C1 intermediate (the overarching goal for Formate Lab Call)
- Carbon neutral technology to reduce GHG emissions
- Improve Carbon Conversion Efficiency (CCE)

Key Differentiators

- **Innovativeness**

  Novel pathway design for formate upgrading

  Utilization of state-of-the-art synthetic biology

- **Key Success Factors**

  Recent progress in construction synthetic formatotroph

  BETO SynBio Facility Agile BioFoundry

  Systems Biology Guidance (computational pathway analysis and ^{13}C-flux)

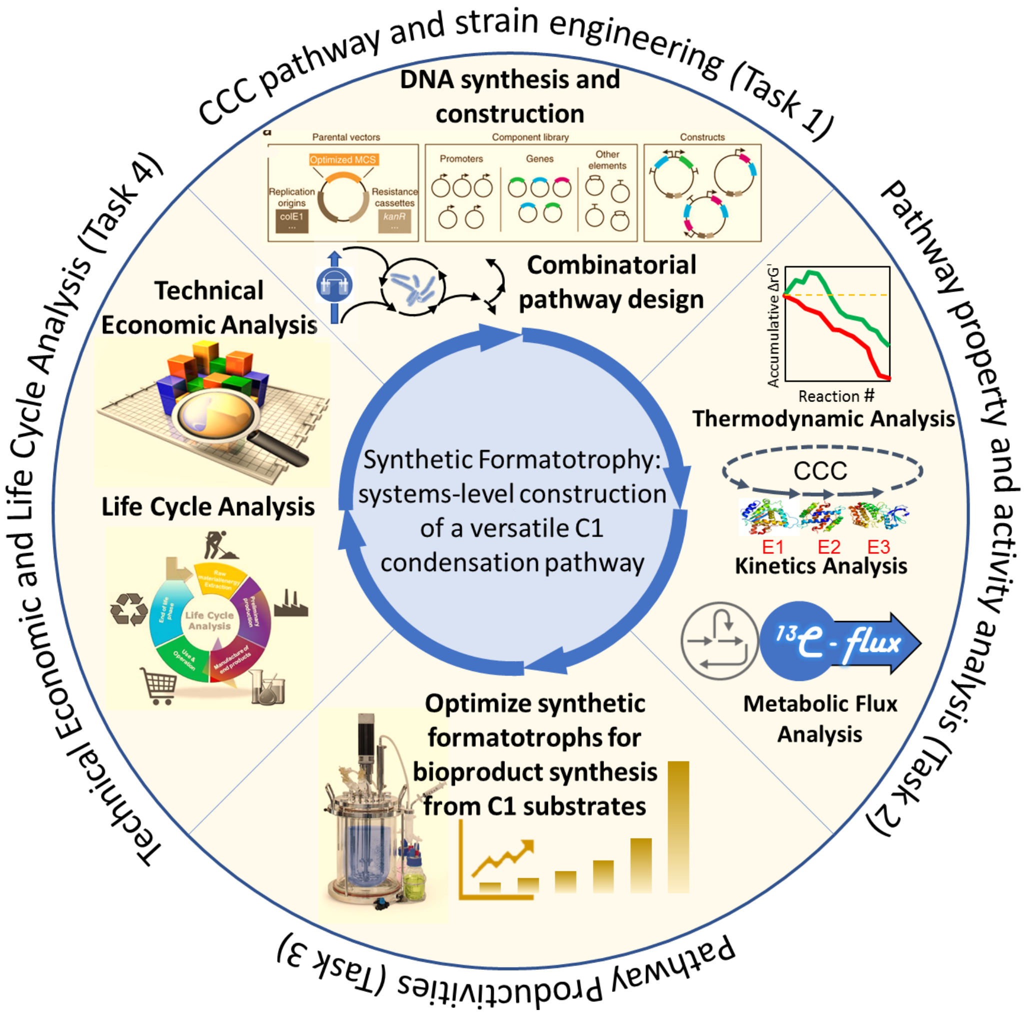
  Commercial feasibility ensured by Technical Economic Analysis

Management

Potential risks

- Technical challenges in microbial engineering for efficient formate utilization
- Strain/pathway instability
- Possibly lower glycolate productivity than industrial requirement
- Other key cost drivers or technology bottlenecks potentially reducing the industrial feasibility

De-risk Plan



Strain Engineering and Analysis

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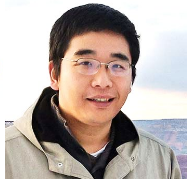
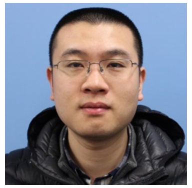


Wei Xiong, PI

Xiang Gao,
Strain engineering



Chao Wu,
Thermodynamics and ^{13}C flux analysis

Chris Urban,
Lab evolution

Automatic DNA Synthesis

Automated and high-throughput gene synthesis

Nathan Hillson

BERKELEY LAB
Lawrence Berkeley National Laboratory

TEA and LCA

TEA and LCA

Ling Tao

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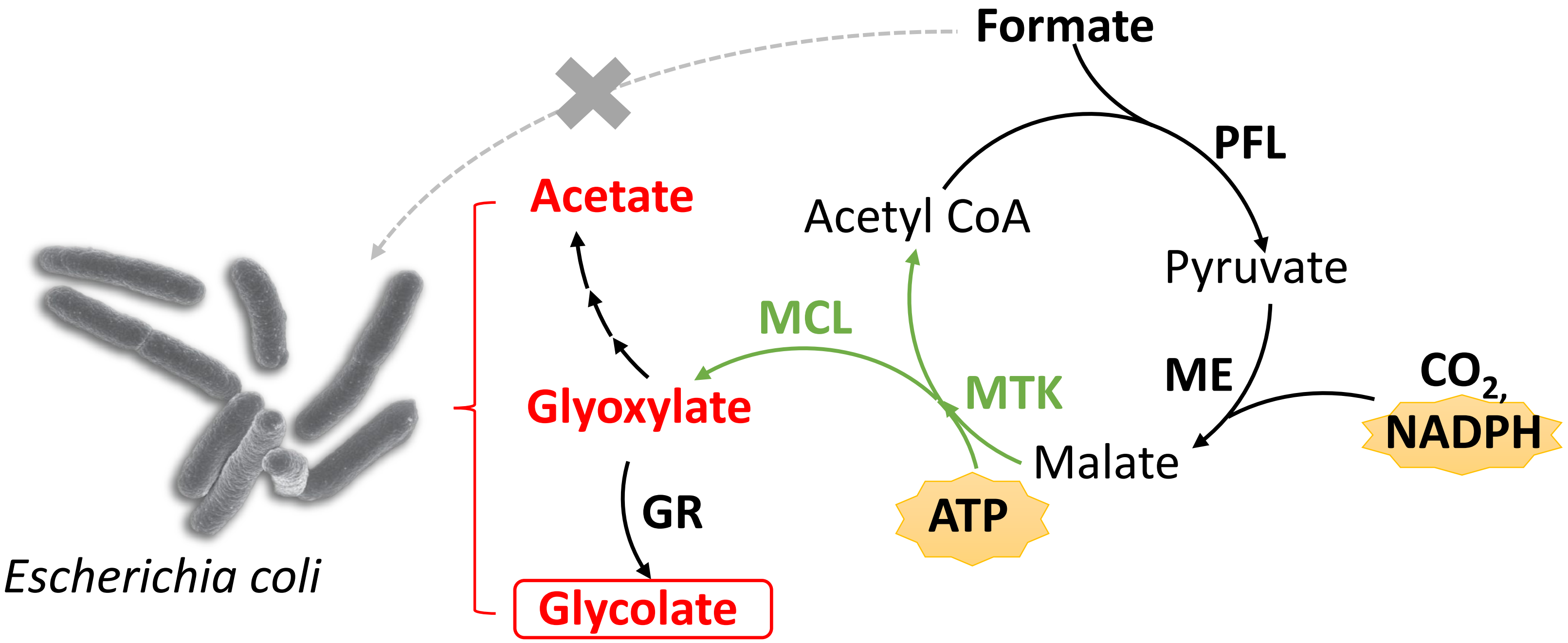
Approaches



CCC: C1 Condensation Cycle

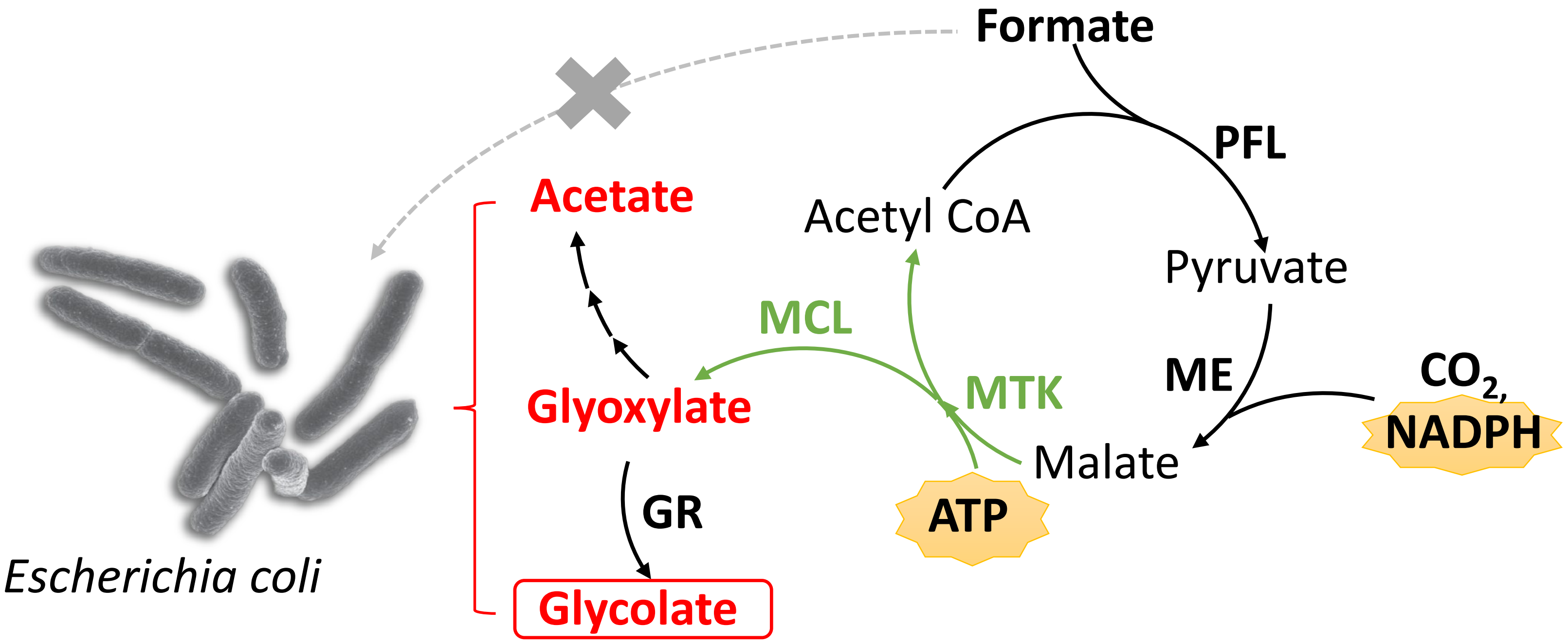
C1 Condensation Cycle

- Pathway novelty and feasibility;
- Bioenergetics feasibility;
- Pathway simplicity;
- Intermediates of industrial interest.

Technical Metrics

- Construct a “formatotrophic microbe” capable of utilizing formate/CO_2 as the major carbon source.
- Build up a novel biological pathway upgrading formate to glycolate at **1 g/L/d production rate**.

Impact



CCC: C1 Condensation Cycle

Impact and Relevance

- Success could reduce green house gas (GHG) (e.g., CO_2) emissions, specifically in current fermentation industry.
- Increase the carbon conversion efficiency (CCE) in final products, thus reducing production cost.
- Provide a new technology to couple electrochemical reduction of CO_2 to formate.
- Scalable strategy compatible to existing industry.

Progress and Outcomes 1: Flux Balance and Thermodynamics Analysis

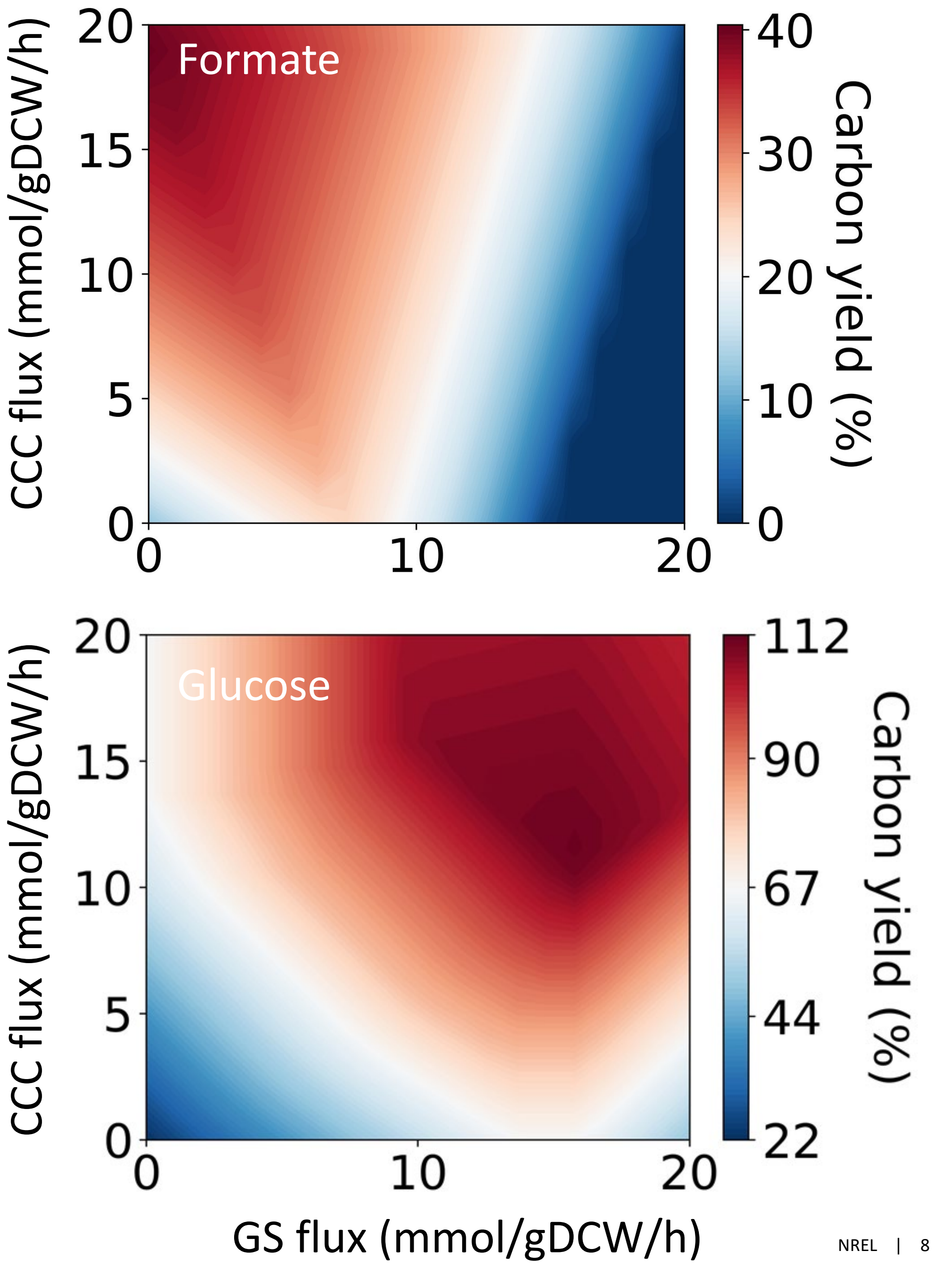
Computational Validation:

- Genome-scale flux balance analysis demonstrates that the CCC pathway we designed can realize efficient bioproduction of glycolate using formate as the feedstock; In addition, the CCC can achieve >100% carbon yield for glycolate production when using glucose as the carbon source.

  Genome-scale model iAF1260 of E. coli with 2077 reactions is used for this analysis (Feist et al. *Molecular Systems Biology*. 2007)

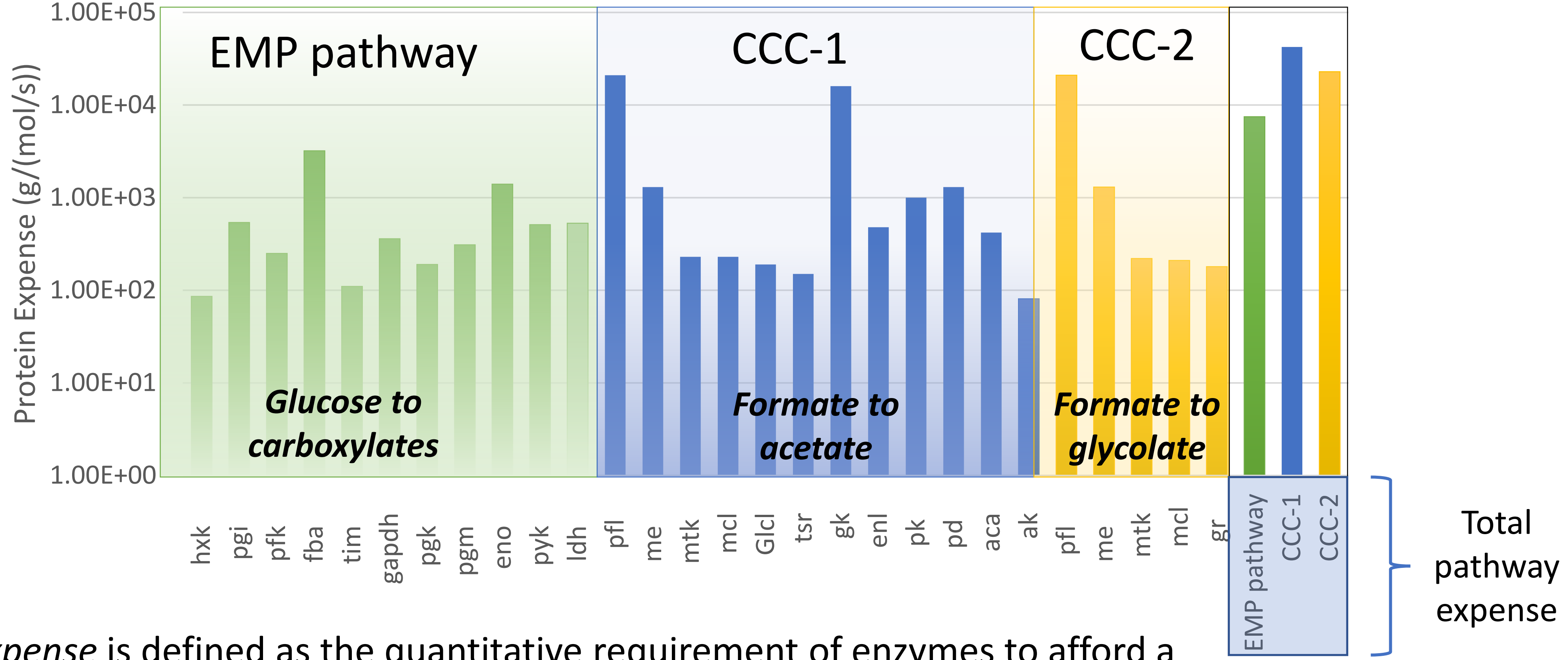
- Thermodynamic optimization shows that physiological concentration ranges of metabolic intermediates allow negative Gibbs energy (ΔG' <0) for all CCC reactions, indicating the pathway feasibility.

  Our algorithm and method were published in Wu et al. Metabolic Engineering. 2020.



CCC: C1 Condensation Cycle (Synthetic); GS: Glyoxylate Shunt (Native)

Progress and Outcomes 2: Analyzed Catalytic Kinetics and Enzyme Expense for Formate-to-Glycolate Conversion

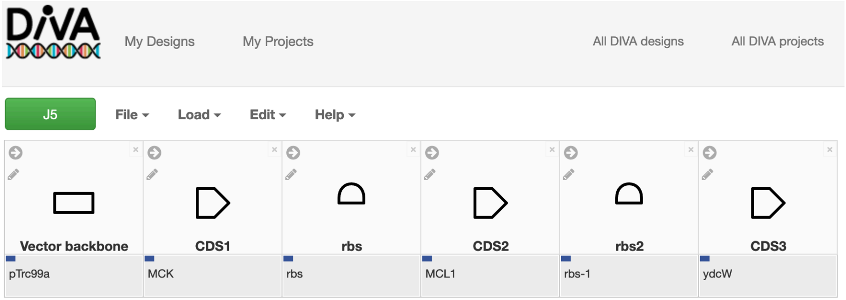


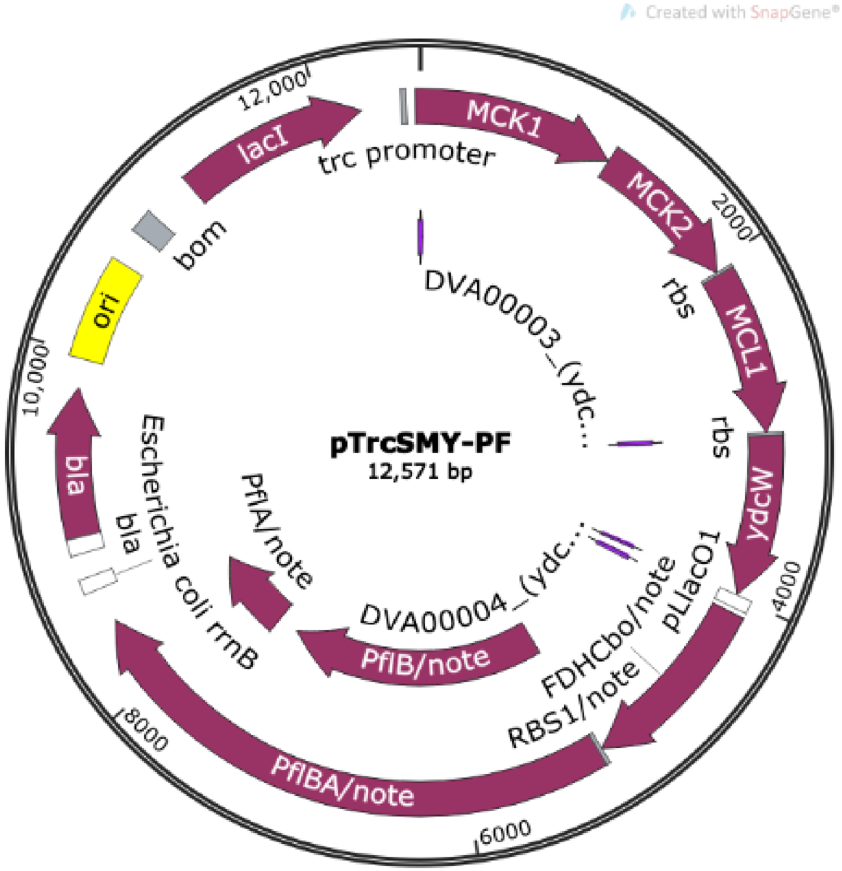
- *Enzyme expense* is defined as the quantitative requirement of enzymes to afford a pathway in certain flux rate (*e.g.,* for glycolate production).
- It is analyzed by enzymatic kinetics and thermodynamics modeling.
- Our results show that the formate upgrading may expends 3-6 fold more enzymes than canonical sugar-derived glycolysis pathway (e.g. EMP), indicating a theoretical ceiling of this approach as well as the goal of metabolic engineering.

Progress and Outcomes 3: Construction of the Pathway in *E. coli* Chassis

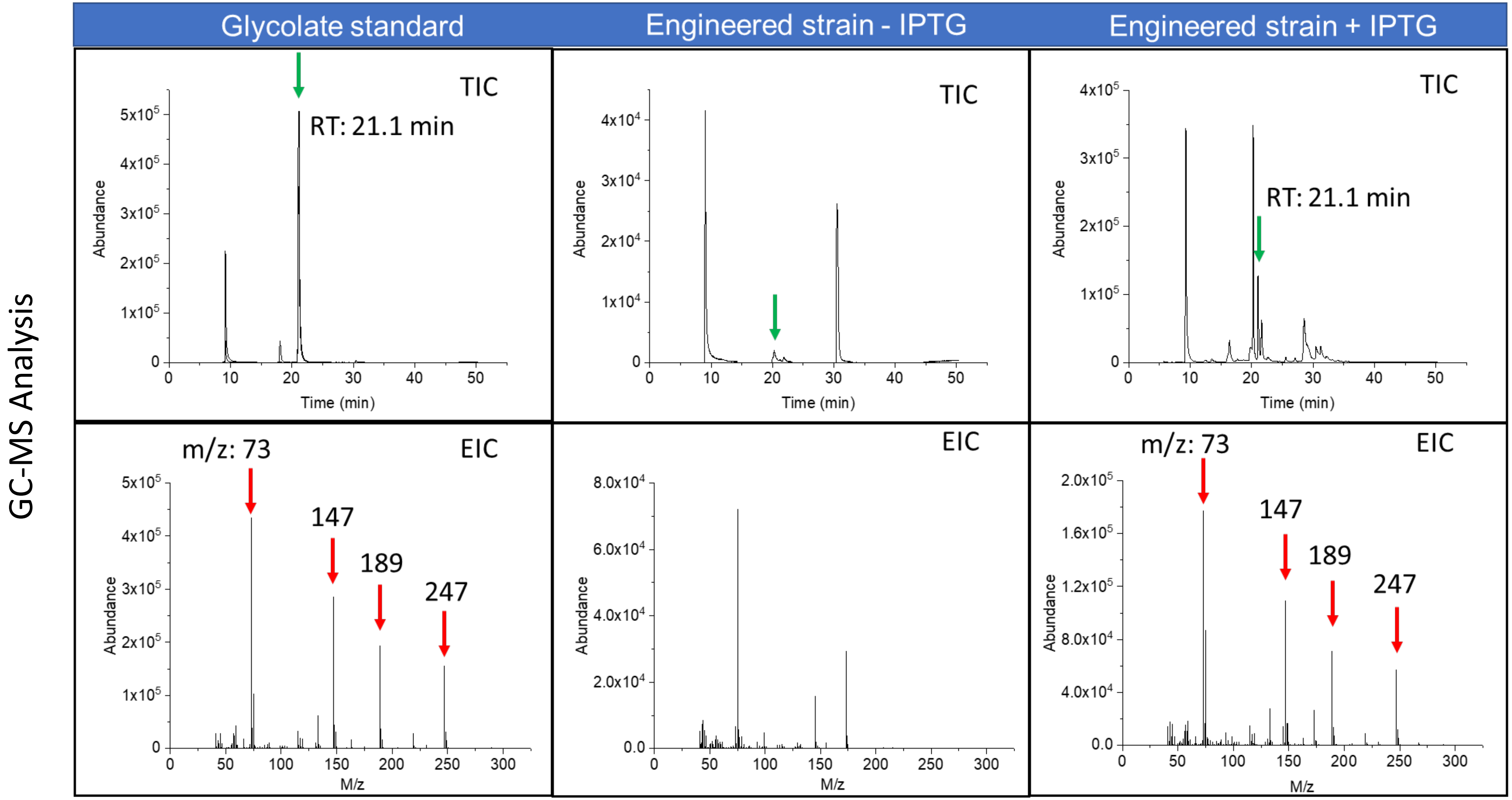
- Leveraging ABF infrastructure (DIVA) in LBNL for plasmid analysis/construction
- Knocking out native genes that compete the C1 Condensation Cycle

Plasmids	Genotype	Purpose
pTrcSMY	pTrc99A origin, pTrc MTK MCL ycdW	Introduce synthetic C1 pathway
p184AKY	pACYC184 origin, pTrc aceAK ycdW	Overexpression glycolate pathway
pTrcSMY-PF	pTrc99A origin, pTrc MTK MCL ycdW, PLacO1 PFL FDH	Introduce synthetic C1 pathway with PFL and FDH
Strains		
XG300	E. coli BW25113, ΔaceB ΔglcB ΔglcD	Delete the competitive pathway
XG400	E. coli BW25113, ΔaceB ΔglcB ΔglcD ΔlipA	Delete the competitive pathway
XG301	XG300/ pTrcSMY	Introduce synthetic C1 pathway
XG303	XG300/ p184AKY	Introduce synthetic C1 pathway
XG308	XG300/ pTrcSMY-PF	Introduce synthetic C1 pathway
XG401	XG400/TrcSMY	Introduce synthetic C1 pathway
XG403	XG400/p184AKY	Introduce synthetic C1 pathway
XG408	XG400/ pTrcSMY-PF	Introduce synthetic C1 pathway





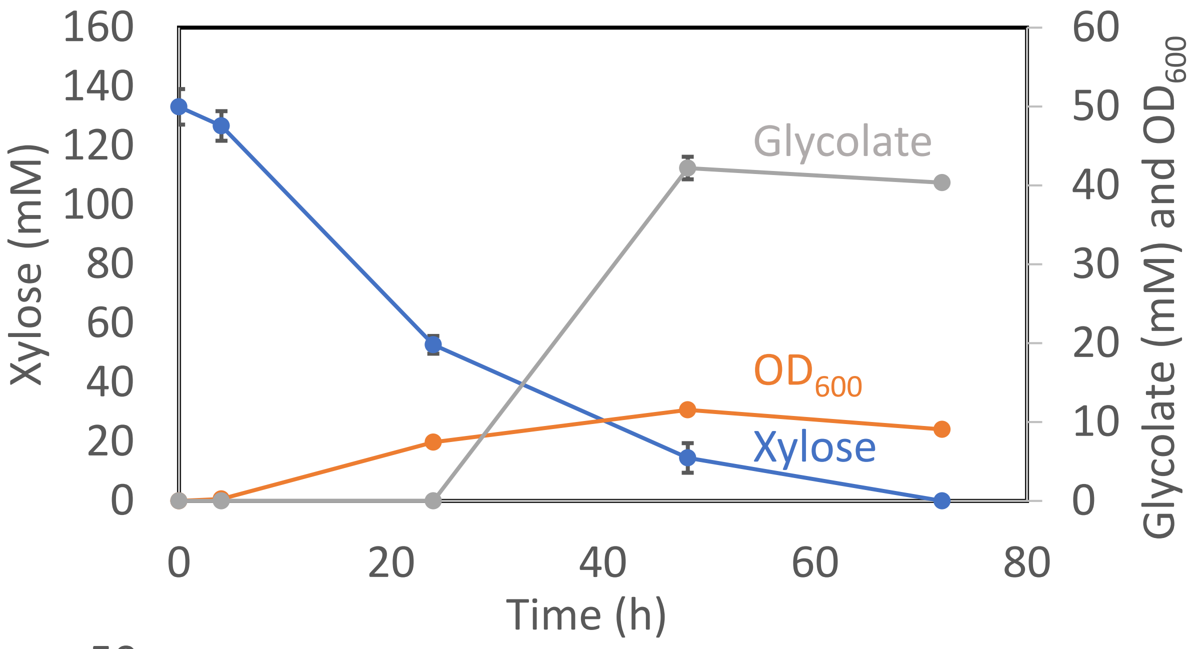
Progress and Outcomes 4: Detected Glycolate Production from the Intermediate in Engineered Pathway

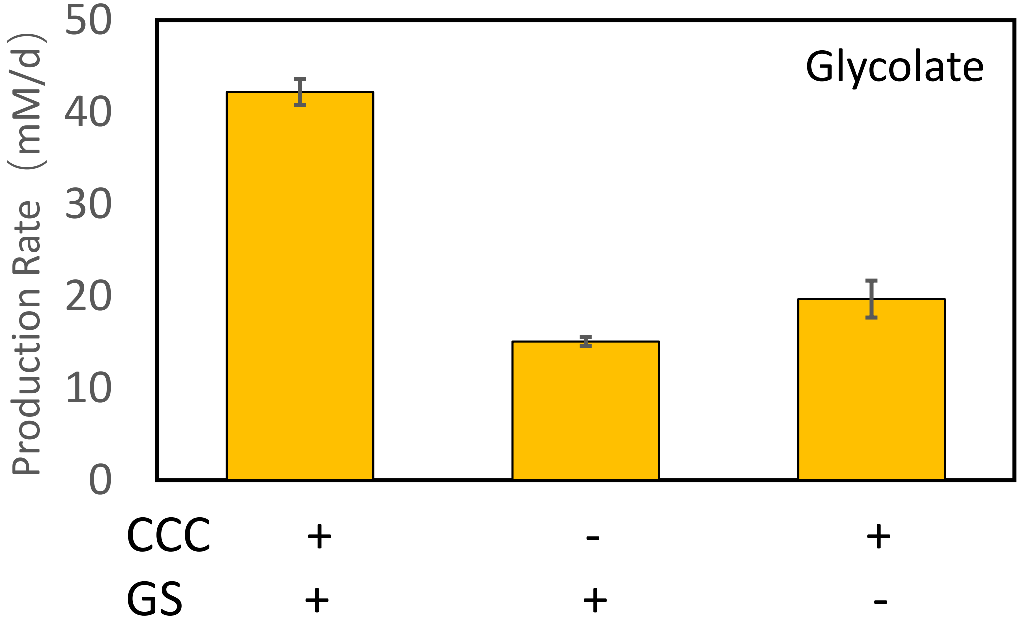


Engineered strain: pTrc99A-SMR in *aceB*::kan/ BW25113, **Intermediate**: Malate

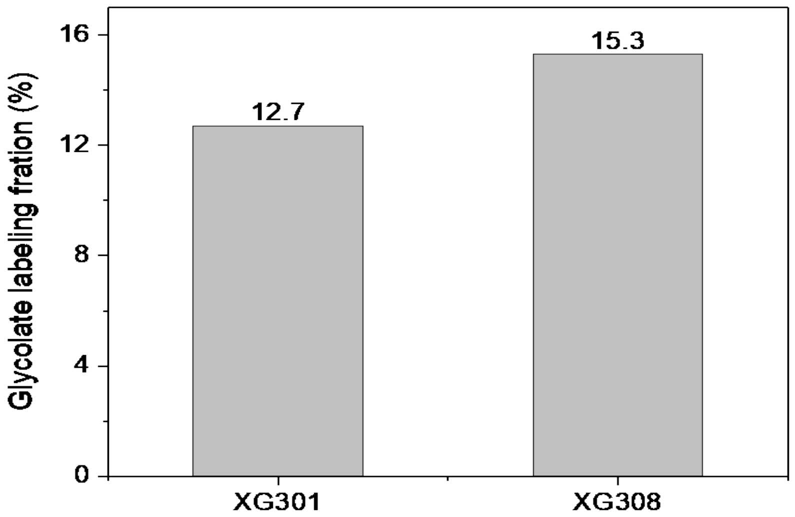
Progress and Outcomes 5: High Glycolate Yield is Attainable

- Glycolate production was associated with xylose and C1 utilization in shaking flasks.
- A titer of ~4.1 g/L glycolate is achievable at flask level.
- The production rate of glycolate depends on the pathway availability.
- The designed CCC pathway and the native glyoxylate shunt (GS) are in synergy to achieve higher glycolate production rate than single pathway standalone.
- The yield, titer and productivity of glycolate can be further improved in a better controlled reacting environment and the bioreactor experiment is ongoing.

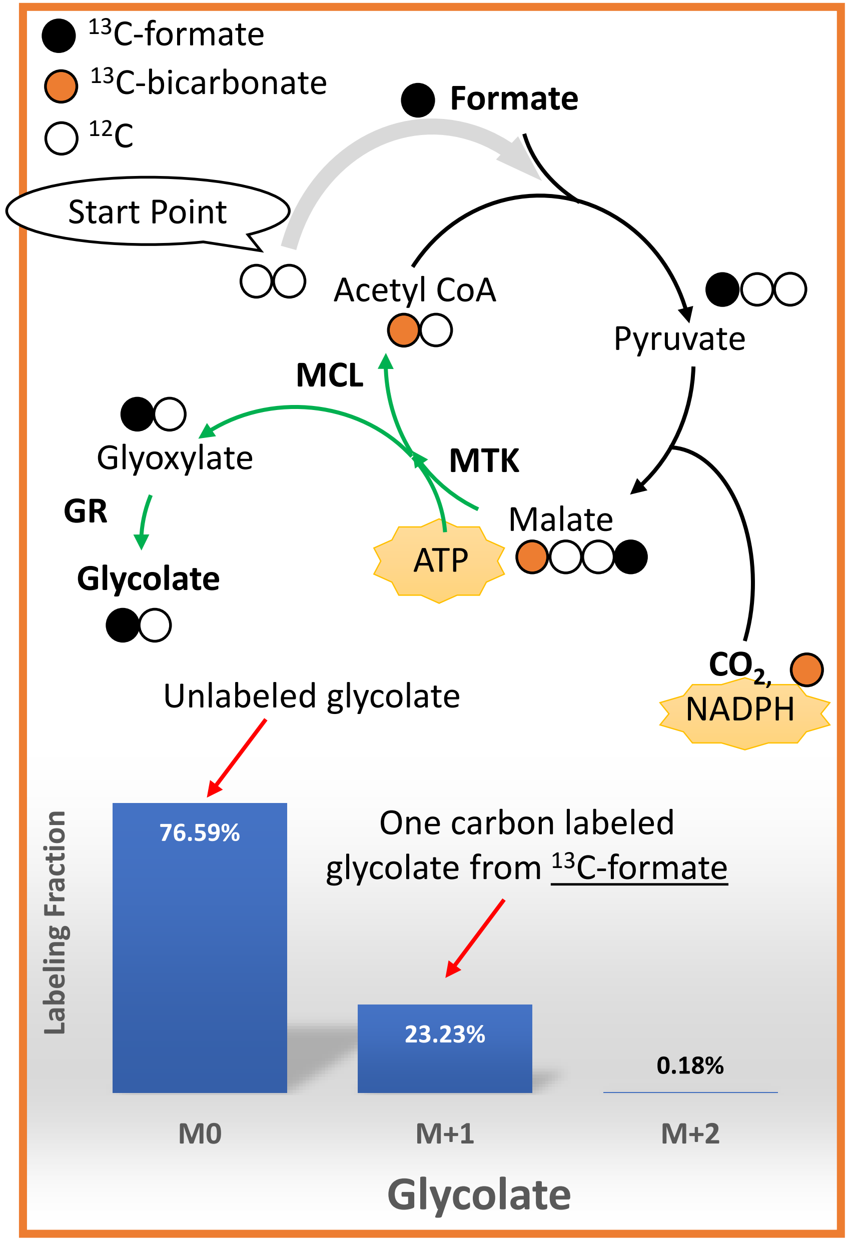




Progress and Outcomes 6: Detected Glycolate Production from Formate

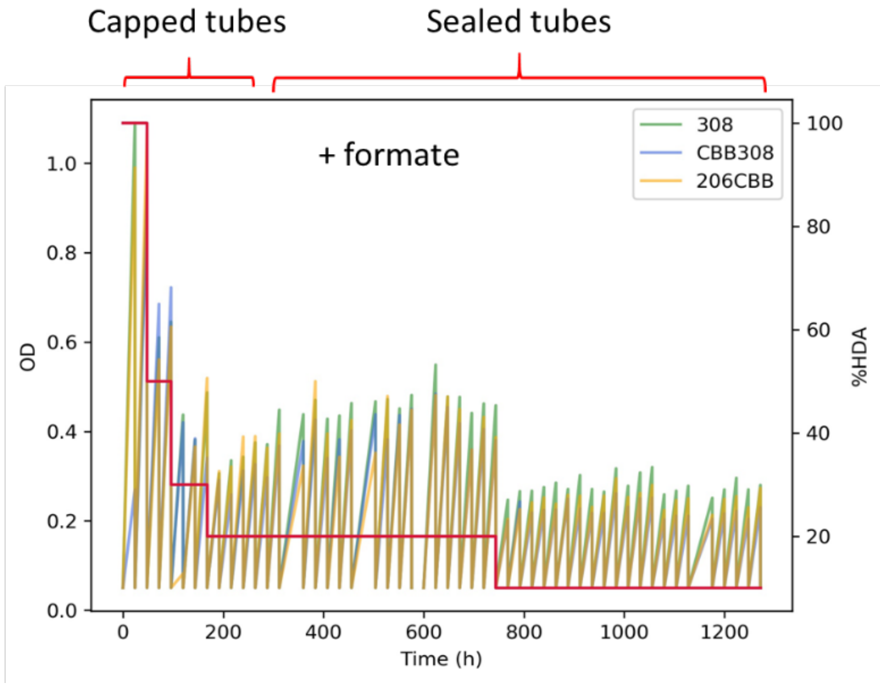


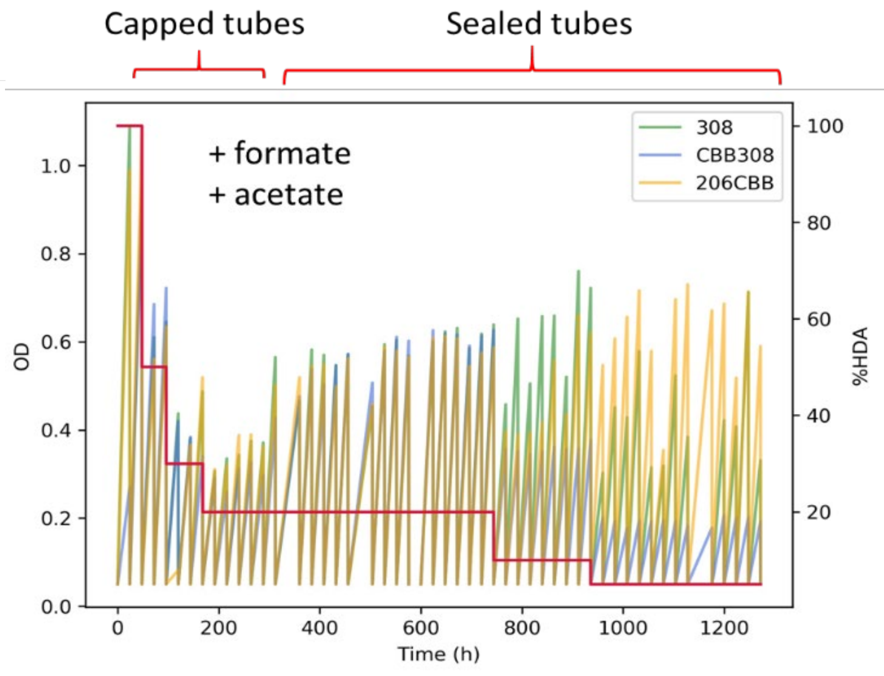
- Leveraging ^{13}C-tracing technique, we detected labeled glycolate from ^{13}C-substrate (^{13}C-formate and bicarbonate).
- Partially labeled glycolate was detected, indicating at the highest so far, ~15% glycolate can be produced from formate and rest glycolate was produced from glucose or other feedstocks. (Please see the figure above for ^{13}C-labeling fraction by strain XG308)



Progress and Outcomes 7: Laboratory Evolution toward Formatotroph

- Laboratory evolution is performed for adaptive growth of engineered E.coli strains upon formate.
- We chose three engineered strains grown on formate as well as a complex nutrient solution, the concentration of which is gradually reduced during evolution.
- Now the nutrient solution is reduced to 2.5-5% of its original level, and cells can still grow (ΔOD > 0.2).

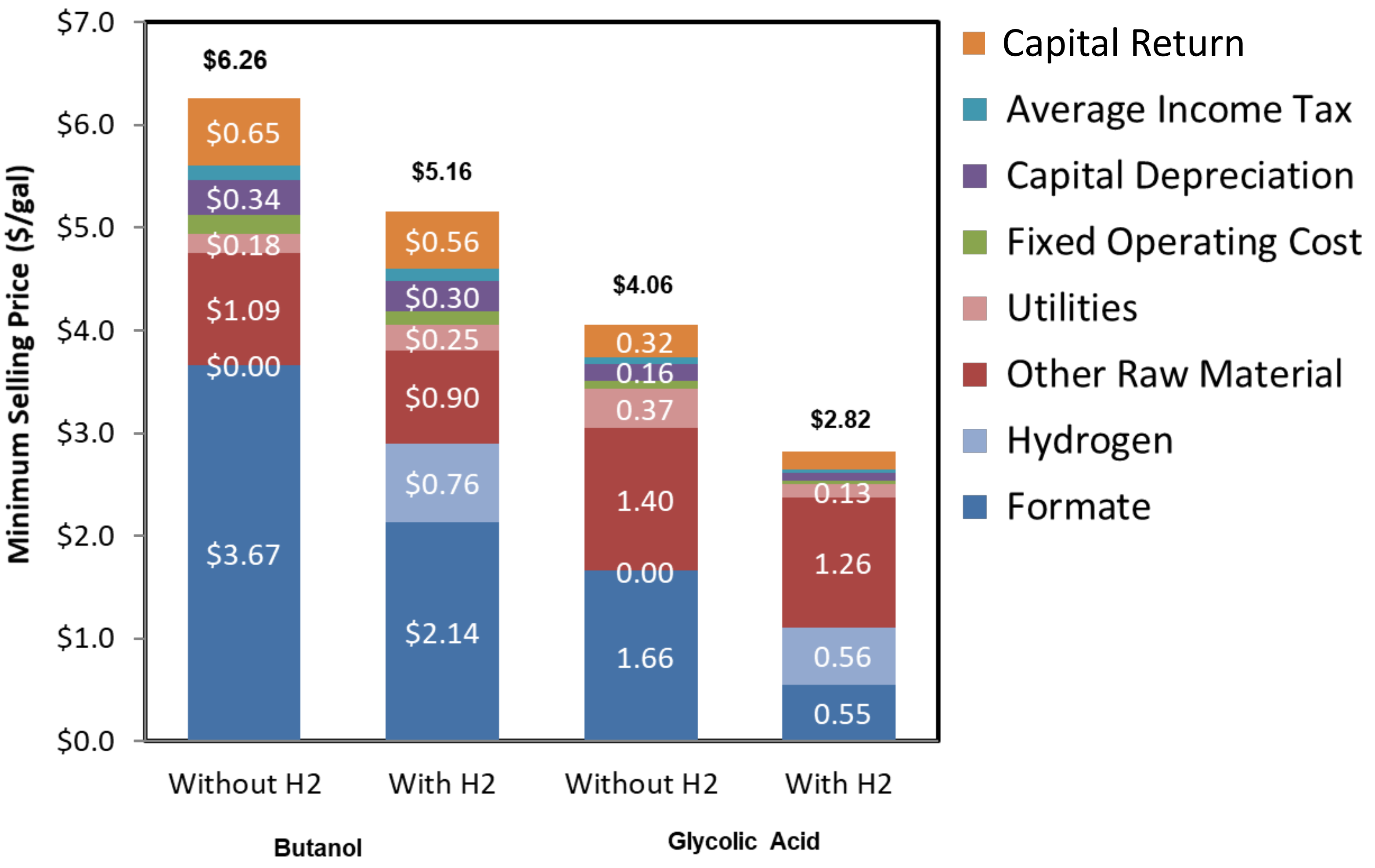




	Genetic background	Plasmid 1 (expressing C1 condensation cycle)	Plasmid 2 (expressing CBB cycle)
308 CBB	ΔaceB-glcB-glcD	99A-SMG-PFL-FDH	pTwist-CBB
308	ΔaceB-glcB-glcD	99A-SMG-PFL-FDH	
206 CBB	wild type	99A-SMG-PFL-FDH	pTwist-CBB

Progress and Outcomes 8: Elementary Technical Economic Analysis

- Elementary TEA suggests commercial feasibility of this technology for producing glycolate.
- Comparative TEA analysis shows lower glycolic acid price ($/gal) than the reference product (butanol).
- The major cost contributors are the cost for the substrate (formate) and other raw material, indicating the importance of the feedstock's economy.
- Additional electron supply (e.g., H_2) to formate can further improve the production yield and thus reduce the cost.



*Detailed TEA report please see our Quarterly report.

Summary

Product		Anticipated decrease in gasoline/ethanol demand; diesel demand steady
		Increasing demand for aviation and marine fuel
		Demand for higher-performance products
		Increasing demand for renewable/recyclable materials
Feedstock		Sustained low oil prices
		Decreasing cost of renewable electricity
		Sustainable waste management
		Expanding availability of green H_2
		Closing the carbon cycle
Capital		Risk of greenfield investments
		Challenges and costs of biorefinery start-up
		Availability of depreciated and underutilized capital equipment
Social Responsibility		Carbon intensity reduction
		Access to clean air and water
		Environmental equity

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Key Accomplishments

- Designed and constructed a novel synthetic pathway (CCC) in E. coli for formate utilization.
- The CCC pathway can convert formate and other feedstocks (e.g., glucose and xylose) to glycolate.
- In flask level, ~4 g/L glycolate titer can be achieved within 24 hours when xylose and C1 substrates are both provided as the carbon source.
- 15% of glycolate production can be derived from C1 substrates (e.g., CO_2 and formate).
- Absolute formatotrophic growth of engineered E. coli has not achieved yet, while we are approaching this target by laboratory evolution.
- TEA analysis supports the industrial feasibility and indicates key cost drivers of this new technology.

Summary

Product	Anticipated decrease in gasoline/ethanol demand; diesel demand steady
Product	Increasing demand for aviation and marine fuel
Product	Demand for higher-performance products
Product	Increasing demand for renewable/recyclable materials
Feedstock	Sustained low oil prices
Feedstock	Decreasing cost of renewable electricity
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Feedstock	Expanding availability of green H_2
Feedstock	Closing the carbon cycle
Capital	Risk of greenfield investments
Capital	Challenges and costs of biorefinery start-up
Capital	Availability of depreciated and underutilized capital equipment
Social Responsibility	Carbon intensity reduction
Social Responsibility	Access to clean air and water
Social Responsibility	Environmental equity

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

Publication Highlights

- Chao Wu, Huaiguang Jiang, Isha Kalra, Xin Wang, Melissa Cano, PinChing Maness, Jianping Yu, Wei Xiong*. A generalized computational framework to streamline thermodynamics and kinetics analysis of metabolic pathways. ***Metabolic Engineering***. 2020,57,140-150 (*This work developed a new thermodynamics and kinetics approach to analyze synthetic formate pathway*.)
- Chao Wu, Chia-hsin Chen, Jonathan Lo, Williem Michener, Pinching Maness, Wei Xiong*. EMUlator: an elementary metabolite unit (EMU) based isotope simulator enabled by adjacency matrix. ***Frontiers in Microbiology***. 2019,10: 3389. (*This work developed a new technique for this project: modeling metabolic flux from experimental ^{13}C-labeling data*.)
- Xiang Gao, Chao Wu, Matt Wecker, Bin Yang, Nathan Hillson, Wei Xiong*. A synthetic carbon-fixing pathway improves carbon conservation in microbial glycolate production. (In preparation) (*This article will report our latest progress of how the C1 Condensation Cycle can improve carbon yield in glycolate production*.)

Q&A and Thank you!

Ian Rowe

www.nrel.gov

Xiang Gao

Chris Urban

Matt Wecker

Chao Wu

Ling Tao

Nathan J. Hillson

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Quad Chart Overview

Timeline

- Project start date: **Oct 1, 2018**
- Project end date: **Sep 30, 2021**

	FY20	Active Project
DOE Funding	(10/01/2019 – 9/30/2020) $ 269,100	$ 740,000

Project Partners*

- Lawrence Berkeley National Laboratory

Barriers addressed

Ct-H. Efficient Catalytic Upgrading of Sugars/Aromatics, Gaseous and Bio-Oil Intermediates to Fuels and Chemicals

Project Goal

This project aims to design and engineer one-carbon substrate utilization in E. coli. A synthetic and orthogonal C1 Condensation Cycle (CCC) will be constructed for the conversion of formate to C2 carboxylates. Leveraging this innovative C1 bioconversion platform, we propose high yield production of value-added glycolate.

End of Project Milestone

- Achieve 1 g/L glycolate production from formate in 5-liter benchtop bioreactor within 24 hours.
- The Technical Economic Analysis for this technology.

Funding Mechanism

BETO Formate Lab Call, 2018

**Only fill out if applicable.*