DOE FOA 0001916 BioEnergy Engineering for Products Synthesis (BEEPS)

Integrating Chemical Catalysis and Biological Conversion of Carbon Intermediates for Deriving Value Added Products from Carbon Dioxide

Dr. Michael Betenbaugh, Johns Hopkins University

Dr. Chao Wang, Johns Hopkins University

Dr. Sarah Jordaan, Johns Hopkins University

Dr. Marina Kalyuzhnaya, San Diego State University

Dr. Pavlo Bohutskyi, Pacific Northwest National Laboratory

Dr. Alexander Beliaev, Pacific Northwest National Laboratory

March 11th, 2021

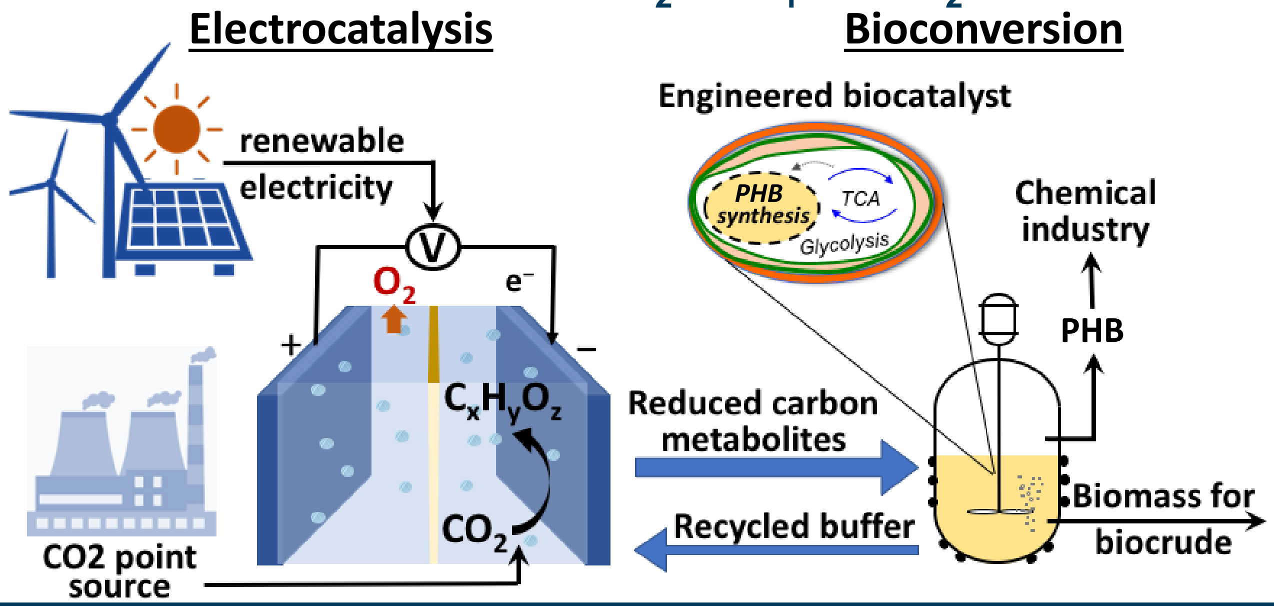
This presentation does not contain any proprietary, confidential, or otherwise restricted information

Johns Hopkins University

Whiting School of Engineering
Chemical and Biomolecular Engineering

Project Overview

- CO_2 capture is increasingly viewed as an important technology towards meeting climate goals
- Electrocatalytic systems excel at converting CO_2 into C_1 and C_2 compounds, but are challenged in forming additional carbon bonds
- Biological systems can build complex carbon compounds from simple carbon compounds, but are less efficient at upcycling CO_2 due to gas diffusion limitations and limited conversion to long carbon chain biomolecules
- **Project goal: Develop an integrated platform that takes advantage of the strengths of electrocatalysis and bioconversion to convert CO_2 → C_1 and C_2 intermediates → bioproducts**



Abbreviations: polyhydroxybutryate (PHB), tricarboxylic acid cycle (TCA)

Quad Chart Overview

Timeline
- Start: 01 October 2018
- End: 01 September 2022

	FY20 Costed	Total Award
DOE Funding	$472,106	$1,419,429
Project Cost Share	$66,420	$531,910

Project Partners
- Johns Hopkins University
- Pacific Northwest National Laboratory
- San Diego State University

Project Goal
Develop a two-stage process integrating electrocatalysis and bioconversion to upcycle CO_2 to intermediates to polyhydroxybutyrate and biomass, which will inform both techno-economic and life cycle analyses of the complete system.

End of Project Milestone
Achieve a 37% process carbon conversion efficiency from an input CO_2 stream to polyhydroxybutyrate and biomass for biocrude using microbial bioconversion; develop an accompanying techno-economic and life-cycle analysis to assess barriers to cost-competitive product generation and sensitivity of the system to market dynamics.

Funding Mechanism
DE-FOA-0001916: Bioenergy Engineering for Products Synthesis (BEEPS)
Topic area 5: Rewiring carbon utilization
Year: 2018

Structure and Team

Dr. Chao Wang (JHU)

Electrocatalysis

Optimization of electrocatalytic conversion of CO_2 to reduced carbon compounds

Dr. Marina Kalyuzhnaya (SDSU)

Biosynthesis

Engineering of *M. alcaliphilum* 20Z^R to convert reduced carbon intermediates to PHB

Dr. Alex Beliaev Dr. Pavlo Bohutskyi (PNNL)

Optimization

Characterization of engineered *M. alcaliphilum* 20Z^R and optimization of the biological production of PHB

Dr. Michael Betenbaugh (JHU)

Integration

Integration of electrocatalytic reduction and bioconversion processes

Dr. Sarah Jordaan (JHU)

TEA/LCA

Techno-economic and life cycle analysis of complete process to assess commercial viability

Abbreviations: Johns Hopkins University (JHU), San Diego State University (SDSU), Pacific Northwest National Laboratory (PNNL), polyhydroxybutryate (PHB), techno-economic and life cycle analysis (TEA/LCA)

Structure and Team

Dr. Chao Wang (JHU)

Risk: low CCE toward methanol

Mitigation: co-product production (e.g., formate, acetate)

Dr. Marina Kalyuzhnaya (SDSU)

Risk: low conversion of substrates to product

Mitigation: consider variable inputs and engineer metabolic pathways

Dr. Alex Beliaev
Dr. Pavlo Bohutskyi (PNNL)

Risk: growth varies with operating conditions

Mitigation: study range of conditions to find optimum

Dr. Michael Betenbaugh (JHU)

Risk: potential toxic compounds in electrochemical effluent

Mitigation: adjust chemical feedstocks for biological use

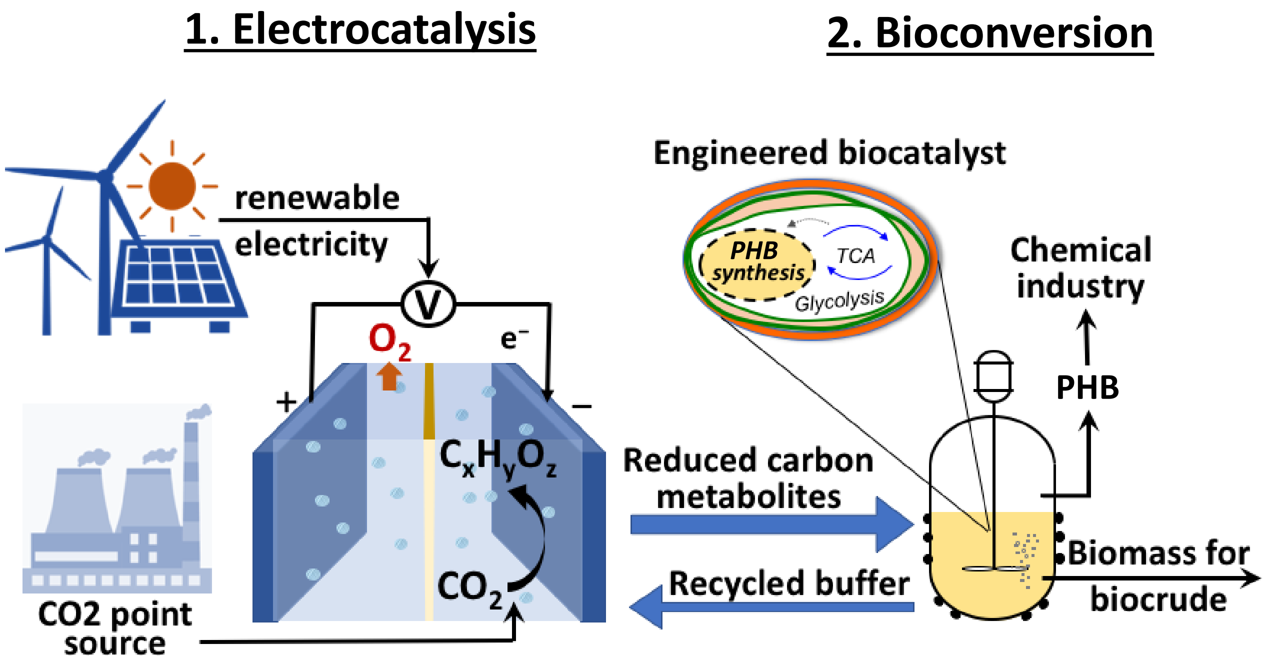
Dr. Sarah Jordaan (JHU)

Risk: economic viability is tied to commodity markets

Mitigation: apply TEA/LCA to identify best targets and opportunities

Abbreviations: Johns Hopkins University (JHU), San Diego State University (SDSU), Pacific Northwest National Laboratory (PNNL), polyhydroxybutryate (PHB), techno-economic and life cycle analysis (TEA/LCA)

Background, Approach, and Challenges



Abbreviations: polyhydroxybutyrate (PHB)

Background

- Few studies on integrated electrocatalytic-biocatalytic systems
- Limited work on selective electroreduction of CO_2 to methanol
- Anaerobes can capture industrial off-gases and upcycle them to short carbon chain chemicals/fuels

Technical Approach

- Two-step process
  1. Electrocatalysis: use of electricity and inorganic catalysts to convert chemicals
  2. Bioconversion: use of bacteria to convert chemicals
- CO_2 → methanol, acetate, formate → PHB, biomass

Challenges

- Electrocatalysis: efficient conversion of CO_2 into methanol
- Bioconversion: efficient growth and chanelling of non-ideal substrates into valuable products

Johns Hopkins University
Whiting School of Engineering
Chemical and Biomolecular Engineering

Go/No-Go Decision Points

Budget Period 1	Budget Period 2	Budget Period 3
Demonstrate potential to achieve **CCE>37%** from CO_2 to fuel or product	Demonstrate **FE≥70%** and **CCE≥80%** toward formate platform products	*Electrocatalysis*: **FE≥50%, CCE≥50%** toward methanol platform products *Bioconversion*: **CCE≥40%, yield≥0.3** on methanol
Critical because: it will enable **PHB and biomass production at proof-of-concept CCE**	Critical because: it will **produce enough substrate** for integrated process	Critical because: it will enable **efficient biomass and PHB production**

Abbreviations: Faradaic efficiency (FE), carbon conversion efficiency (CCE), polyhydroxybutyrate (PHB)

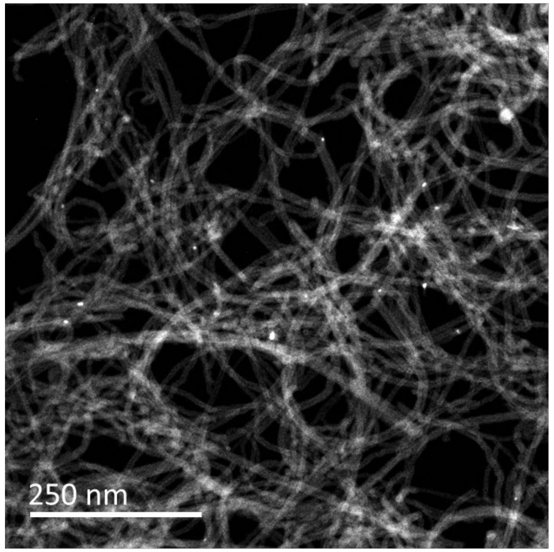
Key Metrics

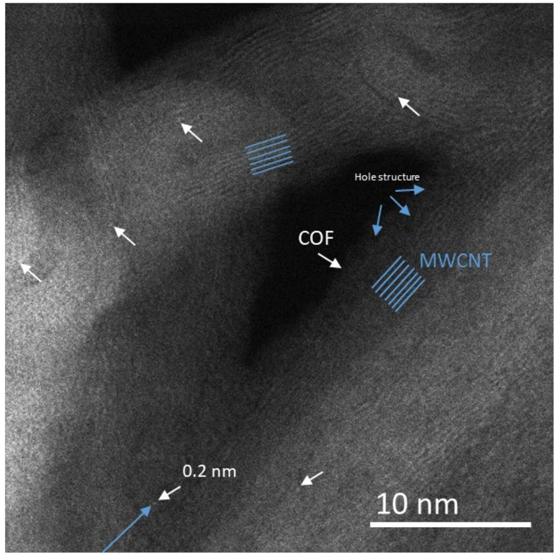
Team	Metric	Assumptions	Analyses
Electrocatalysis	Faradaic efficiency	Electrocatalyst design can improve FE	GC-MS, NMR
	Carbon conversion efficiency	Co-products will improve CCE	GC-MS, NMR, flow control
	Product concentration and rate	Recirculation can improve concentration	NMR, flow control (gas, liquid)
Biosynthesis	Recombinant protein expression	Proteins are expressed & active	SDS-PAGE, enzyme activities
	Yield (g PHB/g cell dry weight)	Engineering can improve yield	Extraction; cell dry weight
	Carbon distribution	Metabolic tuning can improve	Metabolomics, NMR, GC-MS
Optimization	Yield (g biomass/g substrate)	Dependent on dilution rate	Extraction; cell dry weight
	Carbon conversion efficiency	Dependent on media composition	NMR, HPLC, GC
Integration	Operating mode (batch, semibatch)	Culture behavior is consistent	Reactor design
	Yield (g biomass+PHB/g substrate)	Dependent on methanol	Extraction; cell dry weight
	Carbon conversion efficiency	Substrate utilization pathways	NMR, HPLC, GC
TEA/LCA	Net present value	Material costs, selling prices	Aspen process simulation
	CO_2 emissions	Process parameters	Emissions modeling

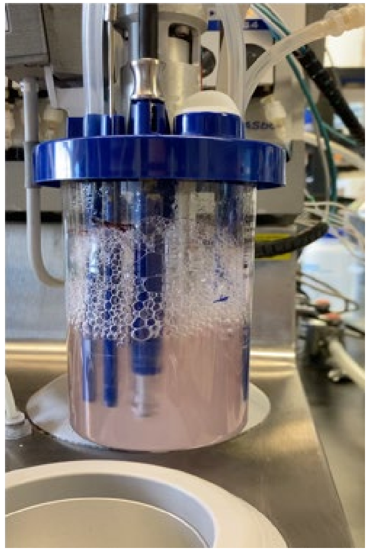
Abbreviations: techno-economic and life cycle analysis (TEA/LCA), sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), nuclear magnetic resonance (NMR), high performance liquid chromatography (HPLC), gas chromatography (GC), gas chromatography-mass spectrometry (GC-MS), Faradaic efficiency (FE)

Technological Impact

- New electrochemical reduction process and catalyst for high efficiency energy conversion and chemical transformation
- Efficient microbial catalyst for upcycling carbon intermediates
- Integrated platform for CO_2 electroreduction and bioconversion



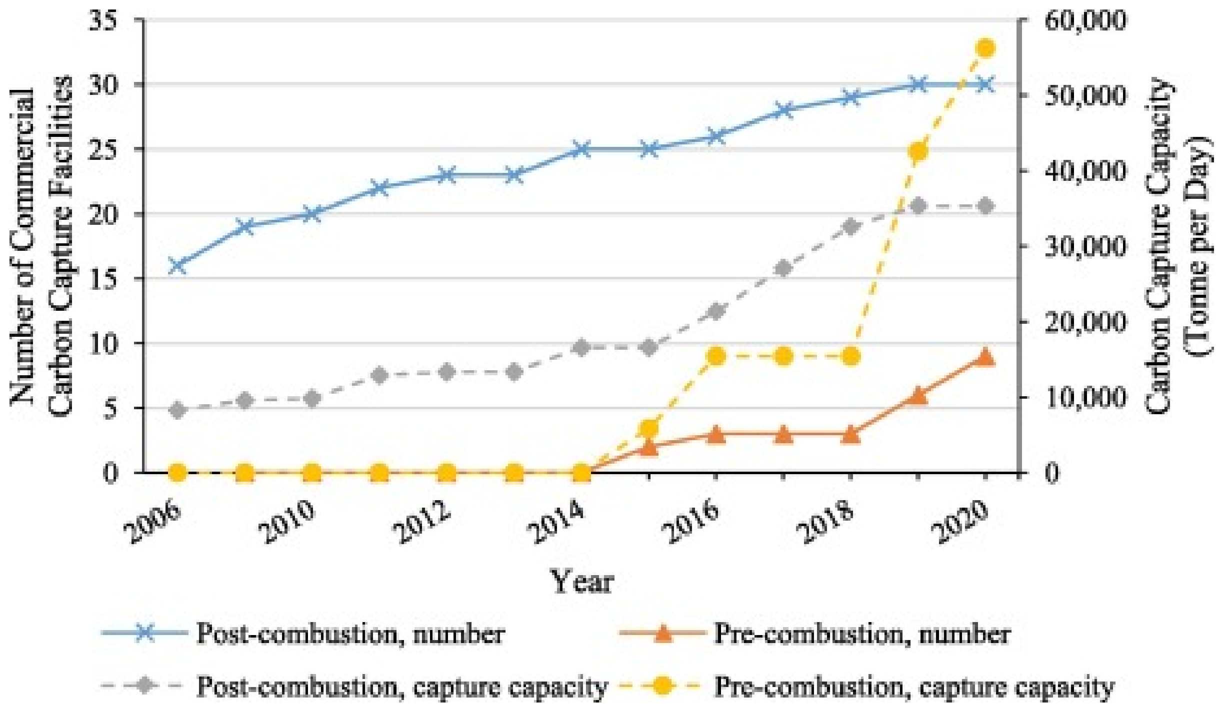




Abbreviations: coordinated organic framework (COF), multi-wall carbon nanotubes (MWCNT)

Potential Industrial Impact

- Companies working to improve CO_2 capture, electroreduction
- Project will demonstrate potential to electroreduce CO_2 into valuable products
- TEA will identify key areas for growth to ensure competitiveness
- Project will demonstrate feasibility of linking electrochemical and biological processes to generate useful products



Abbreviations: techno-economic analysis (TEA)

Graph: W. L. Theo, J. S. Lim, H. Hashim, A. A. Mustaffa, W. S. Ho, Appl. Energy. 183, 1633–1663 (2016).

Academic Impact

- Conferences
  - Ruttinger, A. W., Tavakkoli, S., Jordaan, S. M. Evaluating Technology and Market Scenarios for the Deployment of a Profitable Carbon Capture, Utilization, and Storage Process. Applied Energy Symposium, MIT A+B, Virtual. 13-14 Aug 2020. Oral presentation.
  - Betenbaugh, M., et al. Integrating Chemical Catalysis and Biological Conversion of Carbon Intermediates for Deriving Value Added Products from Carbon Dioxide. U.S. DOE BETO Project Peer Review 2021, Virtual. 8-26 Mar 2021. Oral presentation.
- Publications
  - Ruttinger, A. W., Tavakkoli, S., and Jordaan, S. M. Evaluating Technology and Market Scenarios for the Deployment of a Profitable Carbon Capture and Utilization Process. [In Submission]
  - Zachary J. Johnson, Dennis D. Krutkin, Pavlo Bohutskyi, Marina G. Kalyuzhnaya. Metals and Methylotrophy: via Global Gene Expression Studies. In Rare-earth element biochemistry, biology, and bio-applications (Ed. J.ECotruvo). Methods in Enzymology. Volume 650. Chapter 22 (in press).

Applied Energy Symposium

2021 PROJECT PEER REVIEW

U.S. DEPARTMENT OF ENERGY
BIOENERGY TECHNOLOGIES OFFICE

Key Milestones (Budget Period 2)

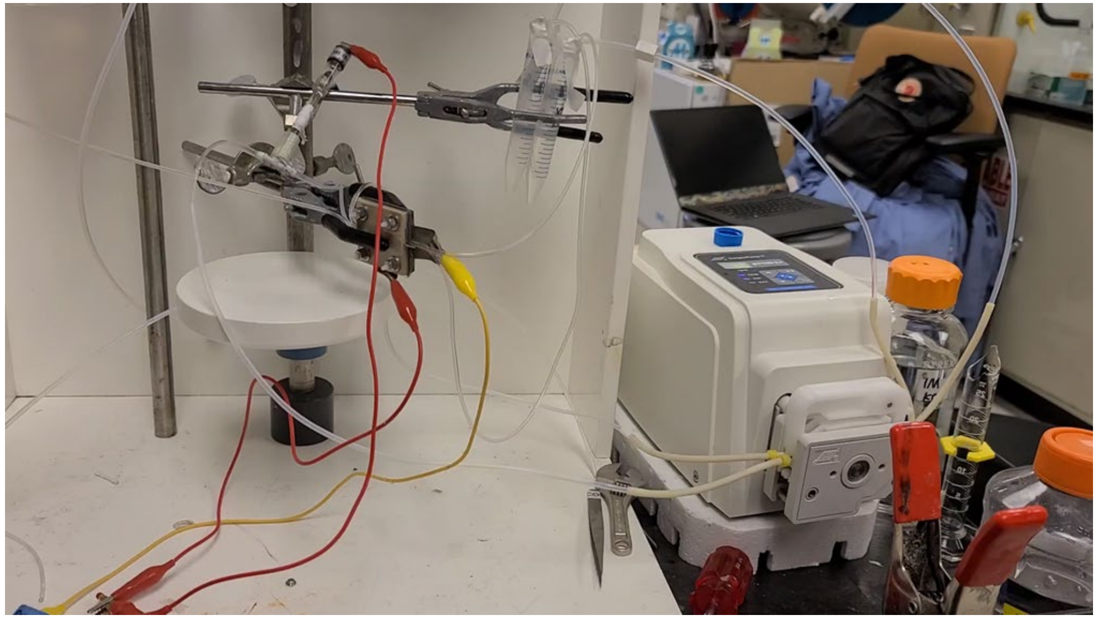
Team	Key Milestone	Current State	Status
Electrocatalysis	**1 mM formate** at 2 ml/min, **≥60% FE**, **≥70% CCE**	**21 mM, >90% FE** at 100 mA/cm^2, **>90% CCE**	>100%
	1 mM methanol and other C_1 and C_{2+} metabolites at 2 ml/min, **≥40% FE**, **≥40% CCE**	**C1 Pathway (Methanol)** **55 mM, 20% FE at 100 mA/cm^2, 20% CCE**	90%
		C1/C2 Pathway (Methanol+Acetate) **55 mM methanol, 19 mM acetate, 70% FE, >50% CCE**	
Bioconversion	*M. alcaliphilum* 20Z expressing **PHB biosynthesis** pathway	Individual **PHB pathway** genes into biocatalyst; enzyme activities verified; integration of **pathway under assessment**	75%
Optimization	Experimental biocatalyst **CCE (%)** and **yield (g biomass per g substrate)**	Highest biomass yield: **0.11-0.13 g DW per g MeOH** Highest CCE to biomass: **>20%** (with MeOH as a substrate)	100%
Integration	**Batch-to-batch** operating mode, total **CCE≥25%** (toward biomass), yield **≥0.20 g of biomass per g CO_2**	**Integrated batch-to-batch** growth characterization Total CCE: **~12%** Total yield: **~0.07 g biomass/g CO_2**	50%
TEA/LCA	Complete **Aspen model**, according to integrated technology for all cases.	**Aspen process model integrated** for all cases proposed **TEA completed** for all cases proposed **Acetate scenarios** and emissions analysis pending	80%

Abbreviations: techno-economic and life cycle analysis (TEA/LCA), Faradaic efficiency (FE), carbon conversion efficiency (CCE), polyhydroxybutyrate (PHB), dry weight (DW)

Technical Achievements: Electrocatalysis

CO_2 → Methanol, Acetate

- Methanol (CH_3OH)
  - 55 mM methanol
  - 20% FE toward methanol
  - 20% CCE toward methanol
- Methanol + Acetate ($C_2H_3O^-$)
  - 55 mM methanol
  - 19 mM acetate
  - 70% FE toward methanol/acetate
  - >50% CCE toward methanol/acetate

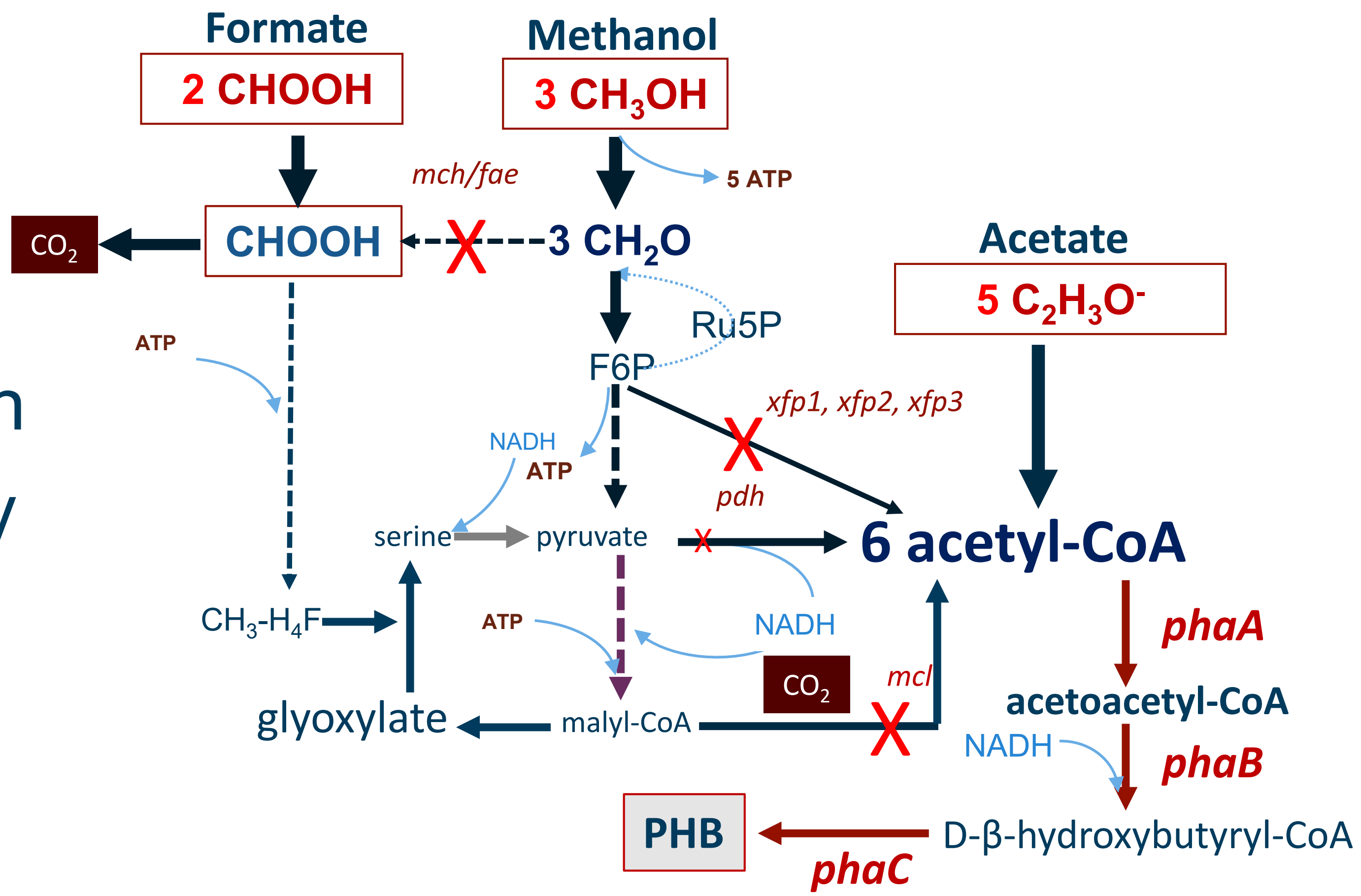


Abbreviations: Faradaic efficiency (FE), carbon conversion efficiency (CCE)

Technical Achievements: Biosynthesis

Methanol, Acetate → PHB

- Completed construction of glyoxylate shunt
- Improving formate utilization
- Expression of PHB pathway with active enzymes

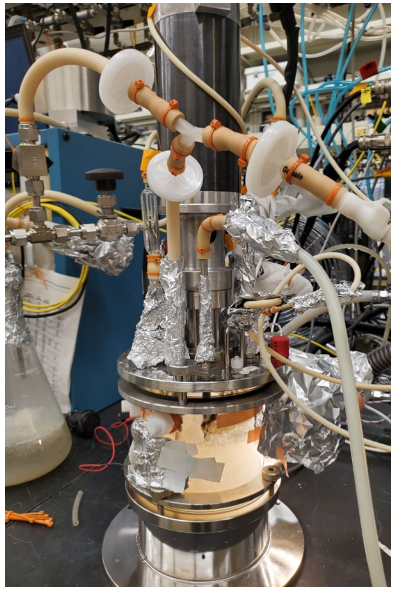


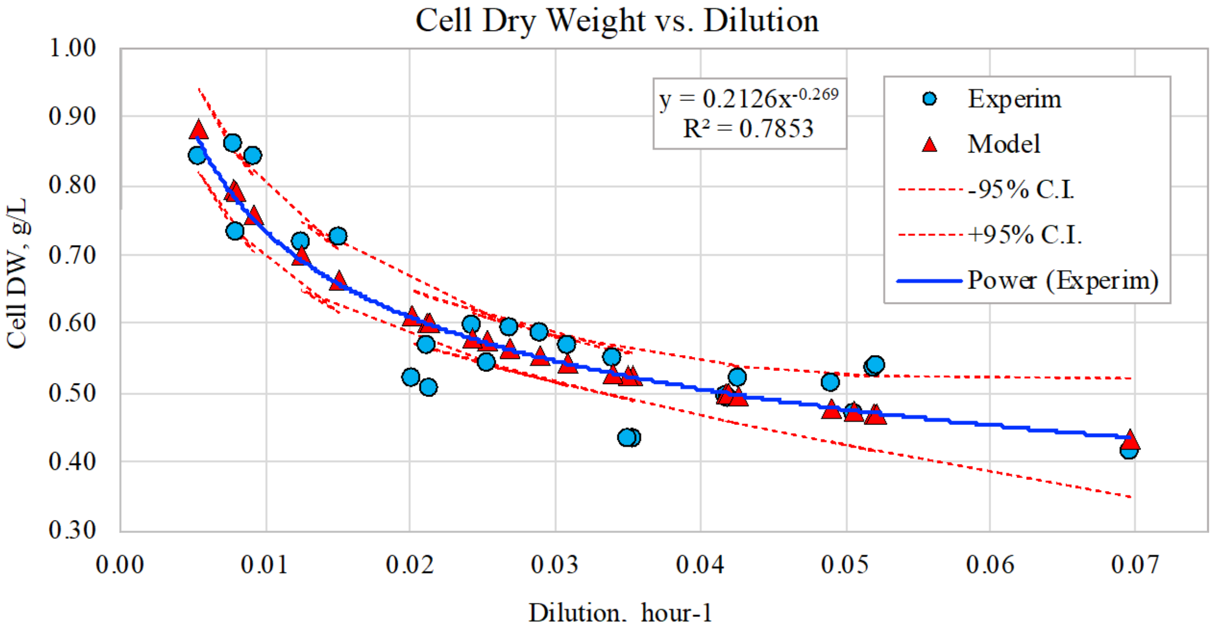
Abbreviations: polyhydroxybutyrate (PHB), methenyl-cyclohydrolase and formaldehyde-activating enzyme (mch/fae), malyl-CoA lyase (mcl), pyruvate dehydrogenase (pdh), phosphoketolase 1, 2, and 3 (xfp1, xfp2, xfp3), 3-ketothiolase (phaA), acetoacetyl coenzyme A reductase (phaB), polyhydroxyalkanoate synthase (phaC), adenosine triphosphate (ATP), nicotinamide adenine dinucleotide (NAD), fructose 6-phosphate (F6P), ribulose 5-phosphate (Ru5P), coenzyme A (CoA),

Technical Achievements: Optimization

Methanol, Acetate → Biomass, PHB

- >20% baseline CCE to biomass on methanol
- 0.13 baseline yield (g dry weight/g methanol)
- Effect of dilution rate on growth characteristics in continuous culture





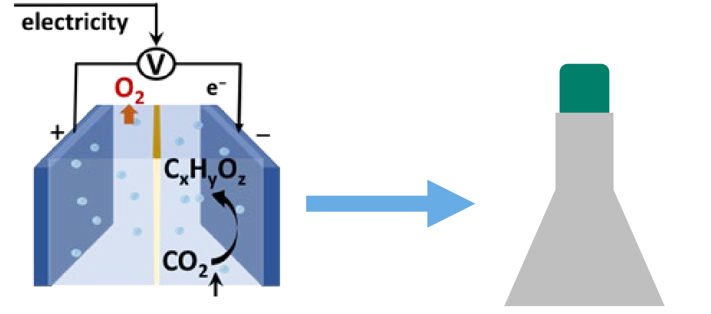
Abbreviations: polyhydroxybutyrate (PHB), carbon conversion efficiency (CCE)

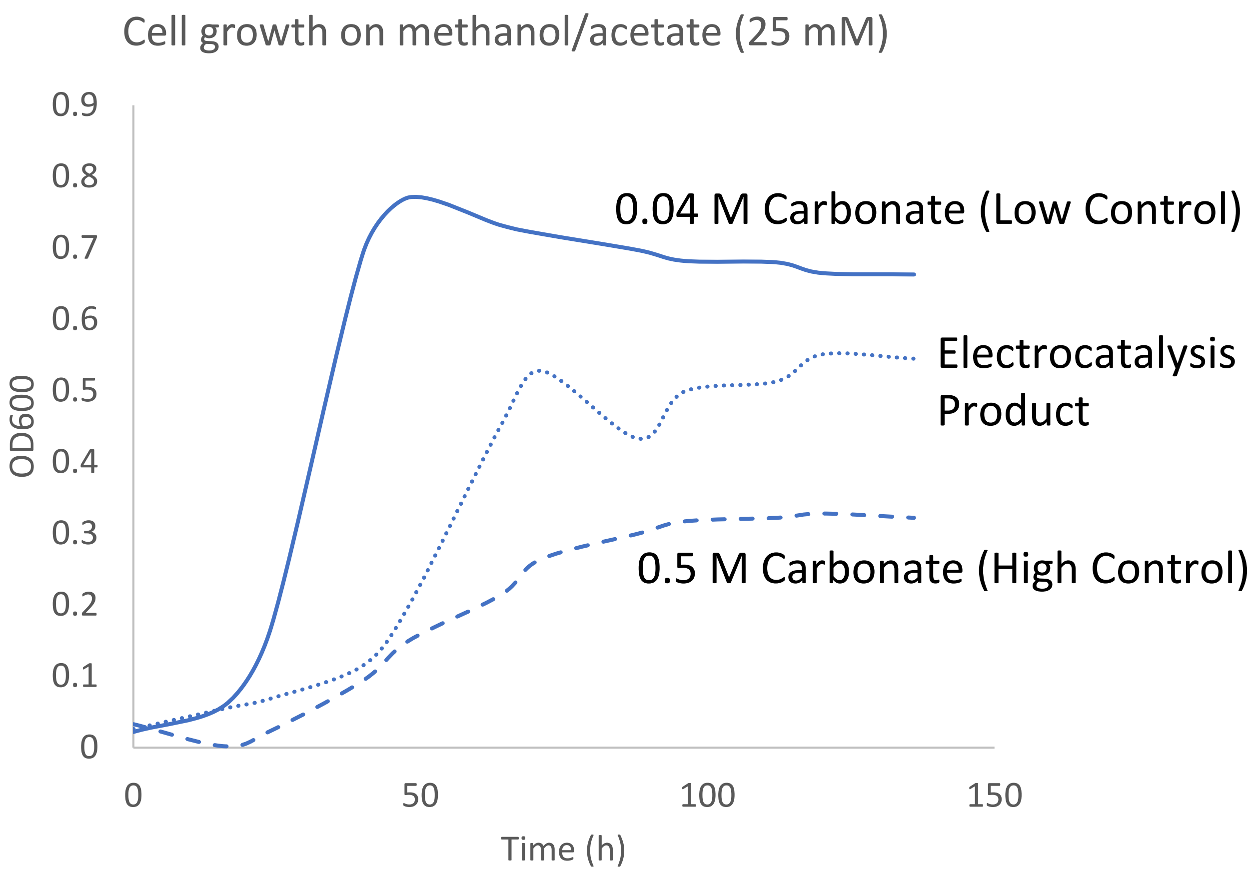
JOHNS HOPKINS UNIVERSITY
Whiting School of Engineering
Chemical and Biomolecular Engineering

Technical Achievements: Integration

CO_2 → Methanol, Acetate → Biomass, PHB

- CCE: ~12%
- Yield: ~0.07 g biomass/g CO_2
- Demonstration of biomass production on electrocatalysis product



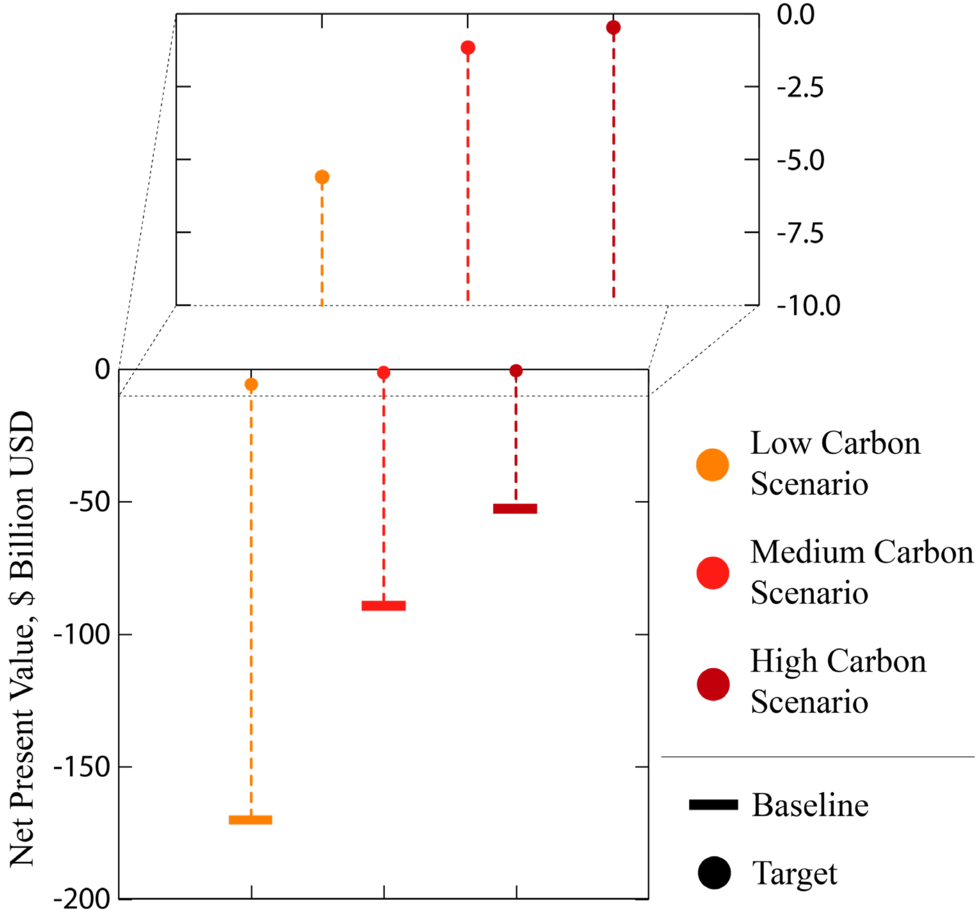


Abbreviations: polyhydroxybutyrate (PHB), carbon conversion efficiency (CCE)

Johns Hopkins University
Whiting School of Engineering
Chemical and Biomolecular Engineering

Technical Achievements: TEA/LCA

- ASPEN model developed
- TEA completed for proposed scenarios
- Currently analyzing impact of acetate production
- Evaluating emissions implications



Abbreviations: polyhydroxybutyrate (PHB), techno-economic analysis and life cycle analysis (TEA/LCA)

Johns Hopkins University
Whiting School of Engineering
Chemical and Biomolecular Engineering

Summary

- *Overview:* Project seeks to develop an efficient and integrated electrocatalytic-bioconversion process to upgrade CO_2
- *Management:* Process development and risk mitigation are split by topic expertise
- *Approach:* Electroreduction of CO_2 to methanol and engineered microbial growth on non-ideal substrates are both the project's biggest challenges and innovations
- *Impact:* Project success will have substantial impact as the world continues to adopt CO_2 upgrading technologies
- *Progress & Outcomes:* Each team has made significant progress toward their milestones goals and are addressing risks appropriately

Additional Slides

For DOE evaluation

Responses to Previous Reviewers' Comments

Not applicable; project has not been previously peer-reviewed.

Publications, Patents, Presentations, Awards, and Commercialization

- Conferences
  - Ruttinger, A. W., Tavakkoli, S., Jordaan, S. M. Evaluating Technology and Market Scenarios for the Deployment of a Profitable Carbon Capture, Utilization, and Storage Process. Applied Energy Symposium, MIT A+B, Virtual. 13-14 Aug 2020. Oral presentation. Video recording URL: https://www.bilibili.com/video/BV1CK4y1Y7vN
  - Betenbaugh, M., et al. Integrating Chemical Catalysis and Biological Conversion of Carbon Intermediates for Deriving Value Added Products from Carbon Dioxide. U.S. DOE BETO Project Peer Review 2021, Virtual. 8-26 Mar 2021. Oral presentation.
- Publications
  - Ruttinger, A. W., Tavakkoli, S., and Jordaan, S. M. Evaluating Technology and Market Scenarios for the Deployment of a Profitable Carbon Capture and Utilization Process. [In Submission]
  - Zachary J. Johnson, Dennis D. Krutkin, Pavlo Bohutskyi, Marina G. Kalyuzhnaya. Metals and Methylotrophy: via Global Gene Expression Studies. In Rare-earth element biochemistry, biology, and bio-applications (Ed. J.ECotruvo). Methods in Enzymology. Volume 650. Chapter 22 (in press).
- Current technology transfer or commercialization efforts awaiting proof-of-concept