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

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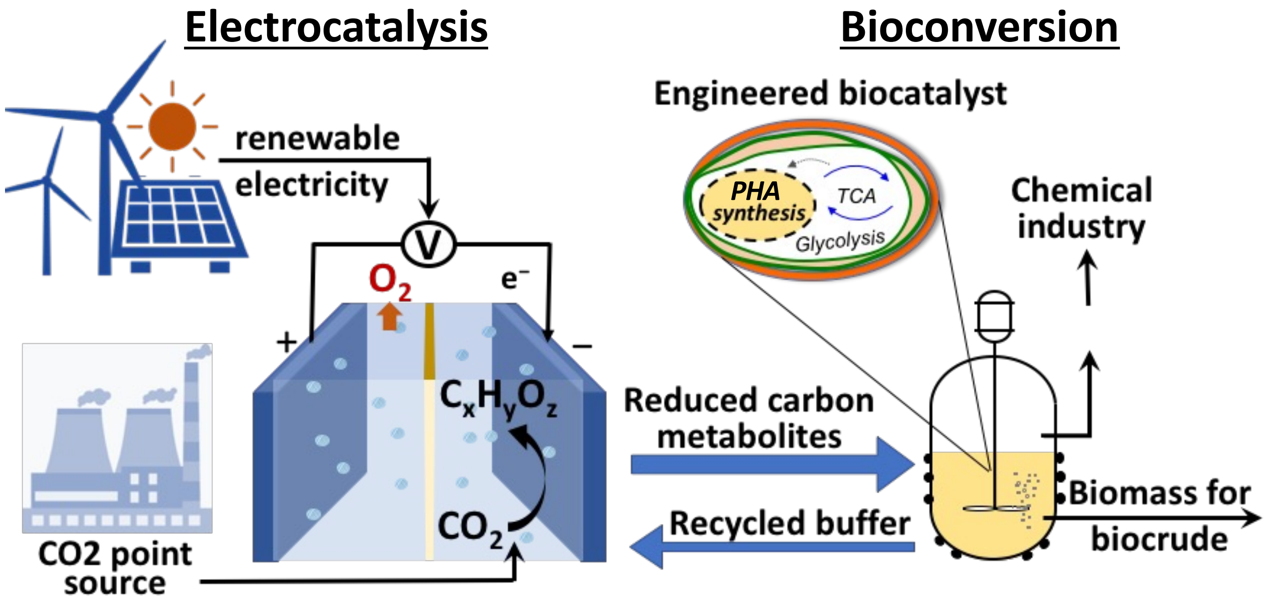
DOE Bioenergy Technologies Office (BETO)
2023 Project Peer Review
Technology Area Session: Carbon Dioxide Utilization

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 → methanol → bioproducts**



Abbreviations: polyhydroxyalkanoate (PHA), tricarboxylic acid cycle (TCA)

Quad Chart Overview

Timeline

- Start: *01 October 2018*
- End: *30 November 2023*

	FY22 Costed	Total Award
DOE Funding	$299,530	$1,419,429
Project Cost Share	$117,400	$531,910

TRL at Project Start: TRL-2
TRL at Project End: TRL-3

Project Goal

Develop a two-stage process integrating electrocatalysis and bioconversion to upcycle CO_2 into chemical intermediates and then to polyhydroxyalkanoates and biomass, which will inform both techno-economic and life cycle assessments of the complete system.

End of Project Milestone

Achieve a 37% process carbon conversion efficiency from an input CO_2 stream to polyhydroxyalkanoates 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

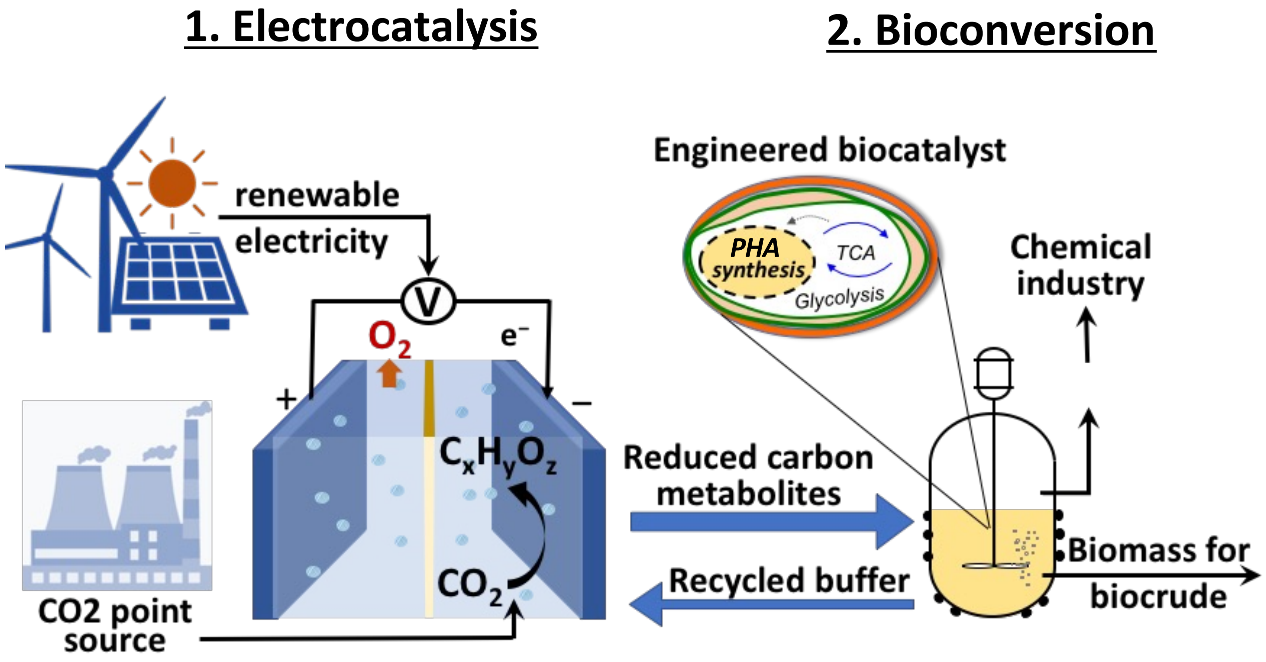
DE-FOA-0001916: Bioenergy Engineering for Products Synthesis (BEEPS) [2018]

Topic area 5: Rewiring carbon utilization

Project Partners

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

Approach
Technical Advancement and Challenges



Abbreviations: polyhydroxyalkanoate (PHA), tricarboxylic acid (TCA)

Technical Advancement

- 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 → PHA, biomass

Challenges

- Electrocatalysis: efficient conversion of CO_2 into methanol
- Bioconversion: efficient growth and chanelling of non-ideal substrates into desired products
- Integration of disparate chemical and biological conversion processes

Approach
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, product, or biomass	Demonstrate **CCE≥80%** and **FE≥70%** toward formate platform products	*Electrocatalysis*: **CCE≥50%, FE≥50%** toward methanol platform products *Bioconversion*: **CCE≥37%, yield≥0.3** on methanol
Critical because: the initial verification allowed the team to **move into BP2 to begin process development**	Critical because: it will **produce enough substrate** for integrated process	Critical because: it will enable **efficient biomass and PHA production** from CO_2 and intermediates

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Abbreviations: Faradaic efficiency (FE), carbon conversion efficiency (CCE), polyhydroxyalkanoate (PHA), budget period 2 (BP2)

Approach
Management

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 PHA

Dr. Pavlo Bohutskyi (PNNL)

Optimization

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

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

Project includes multiple women PIs plus female graduate students

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

Approach
Risk Identification and Mitigation

Dr. Chao Wang (JHU)

Risk: limited CCE toward methanol

Mitigation: recycle unused substrates for highly efficient conversion of multiple products

Dr. Marina Kalyuzhnaya (SDSU)

Risk: limited conversion of substrates to product

Mitigation: engineer metabolic pathways to improve production from alternative substrates

Dr. Pavlo Bohutskyi (PNNL)

Risk: growth varies with operating conditions

Mitigation: study range of conditions to find optimal cell performance conditions

Dr. Michael Betenbaugh (JHU)

Risk: potential toxic compounds in electrochemical effluent

Mitigation: adapt cell line and media conditions for integrated production capabilities

Dr. Sarah Jordaan (JHU)

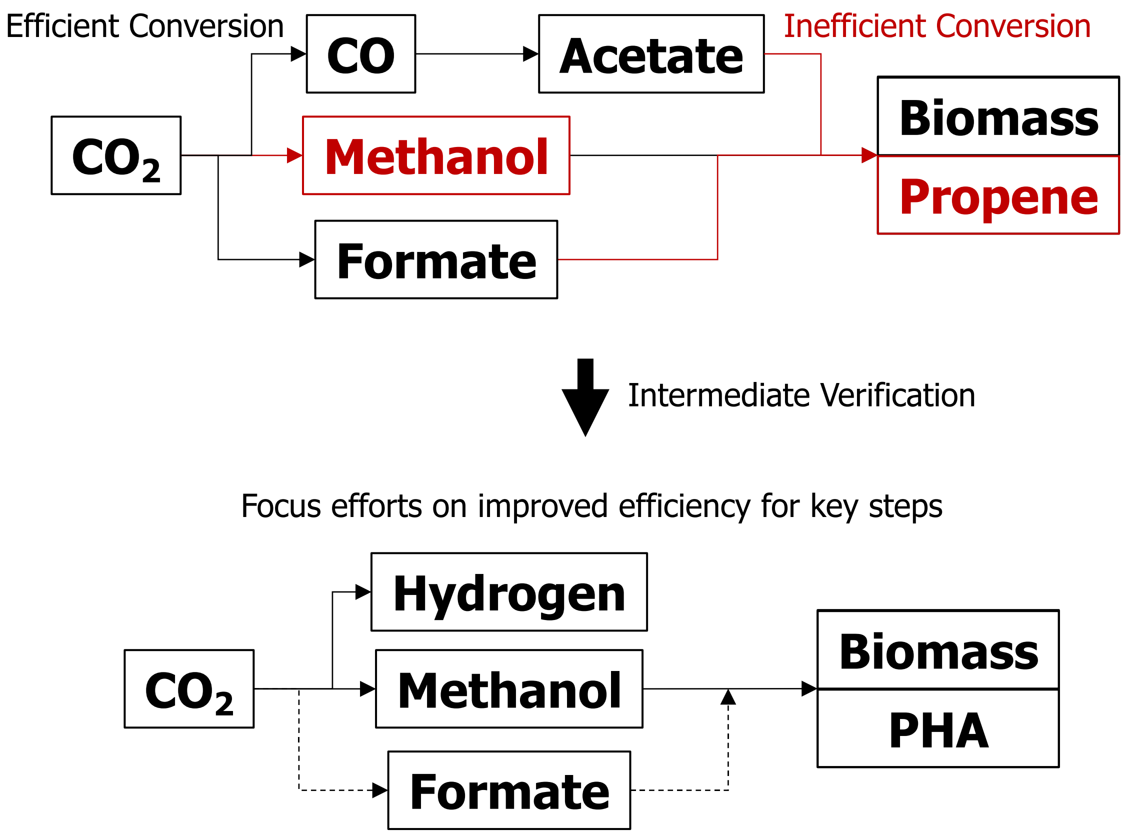
Risk: economic viability is tied to commodity markets

Mitigation: apply TEA/LCA to identify best opportunities for process adjustments to improve overall feasibility

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

Progress and Outcomes
Intermediate Verification

- Pre-Verification
  - Complex, multi-step process
  - Multiple intermediate products
  - Inefficient conversion steps
  - Propene as product of interest
- Post-Verification
  - Streamlined process
  - Primary intermediate product
  - Highly efficient conversion steps
  - PHA as product of interest



Abbreviations: polyhydroxyalkanoate (PHA)

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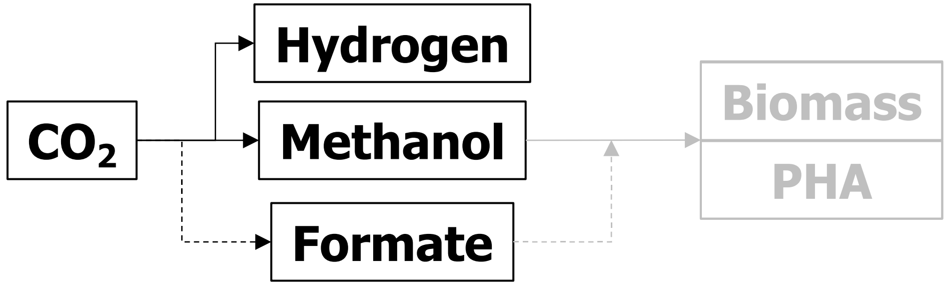
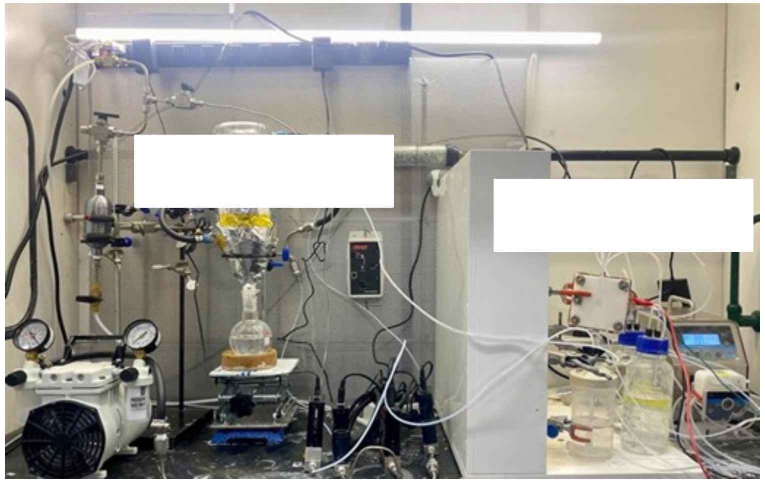
Progress and Outcomes

Electrocatalysis

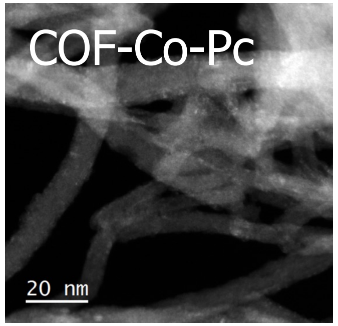


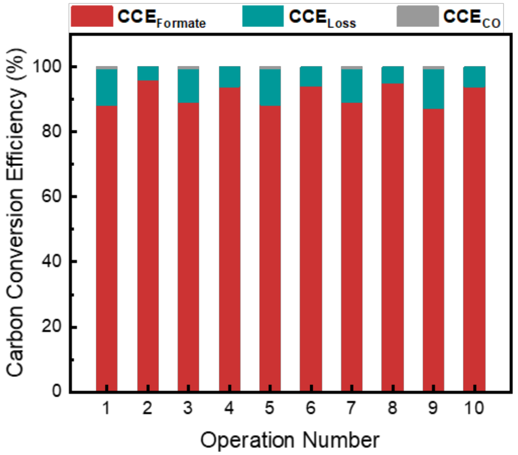
- Gas- and Liquid- recirculation system for high CCE
- Advanced stable electrocatalysts for continuous operation
- CO2–to-formate and CO2-to-methanol conversion at over 90% CCE

	CCE (formate)	CCE (methanol)	Concentration (formate and methanol)
Current Status	≥90%	≥90%	≥100 mM
Milestone Goal	≥80%	≥80%	≥100 mM

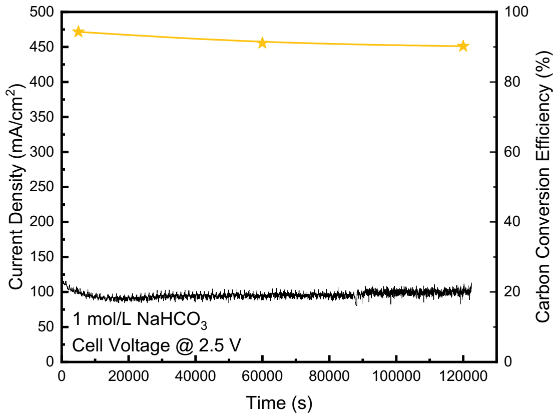


Gas Pump Flow Controls Liquid Pump





CO_2-to-Formate



CO_2-to-Methanol

Abbreviations: polyhydroxyalkanoate (PHA), carbon conversion efficiency (CCE), Faradaic efficiency (FE), coordinated organic framework (COF) coated with cobalt-phthalocyanine (Co-Pc) catalyst

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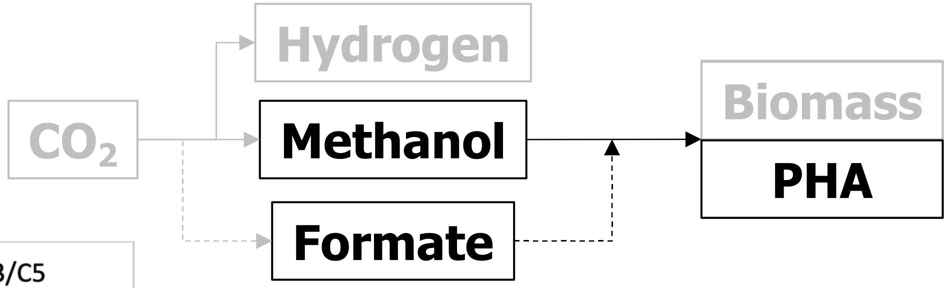
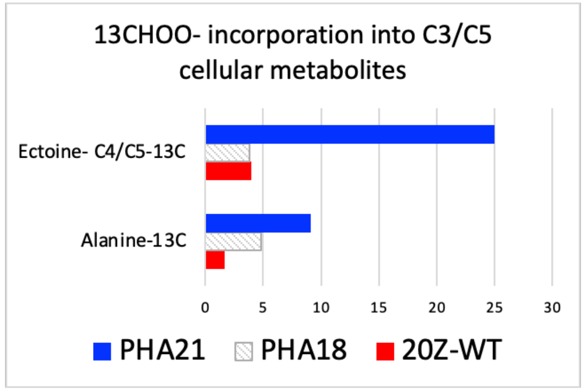
Progress and Outcomes

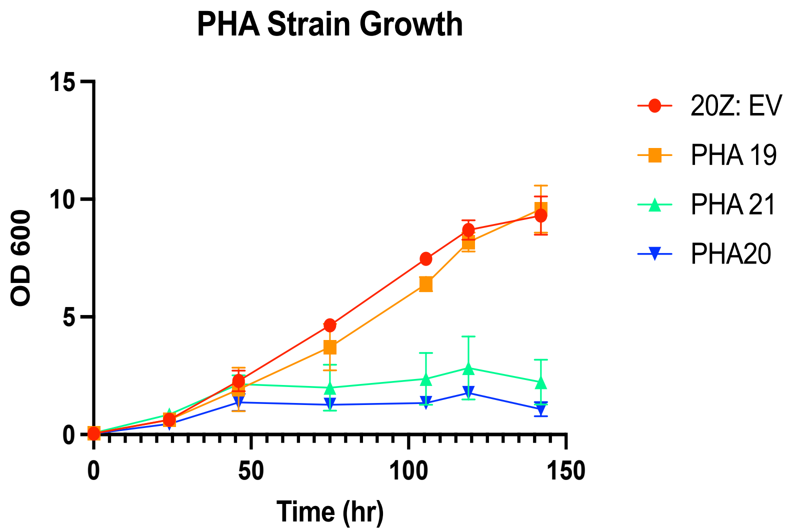
Biosynthesis



- 36 PHA-producing strains constructed and tested for desirable characteristics
- Production up to 13% PHA per gCDW
- PHA product is 114±4 kDa
- Formate utilization improved threefold in PHA producing strains

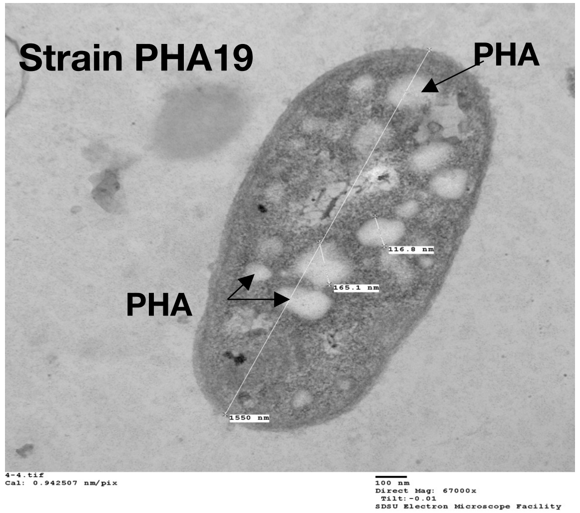
	Metabolic Engineering	Compare Strain Growth Profiles
Current Status	PHA production verified in cell line	Developed strain with high growth rate and PHA yield
Milestone Goal		

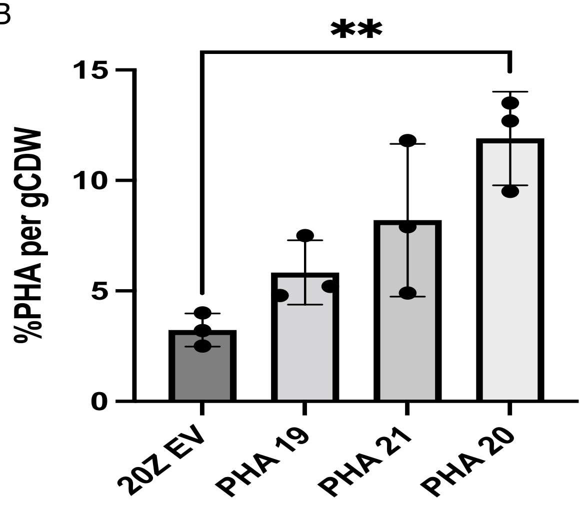




Strains

20Z EV- 20Z: Empty Vector
PHA18 - 20ZΔglg1Δglg2Δsps
PHA 19- 20Z: PmxaF PhaAC2J3B
PHA 20- 20Z Lipase Δglg1Δglg2Δsps: PmxaFPhaAC2J3B
PHA 21- 20ZΔglg1Δglg2Δsps::PmxaFPhaAC2J3B

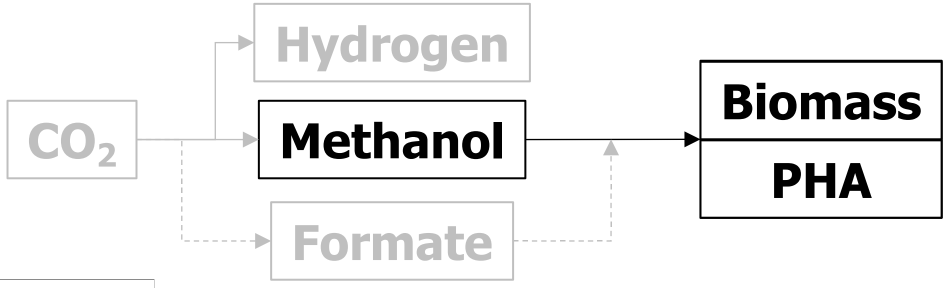




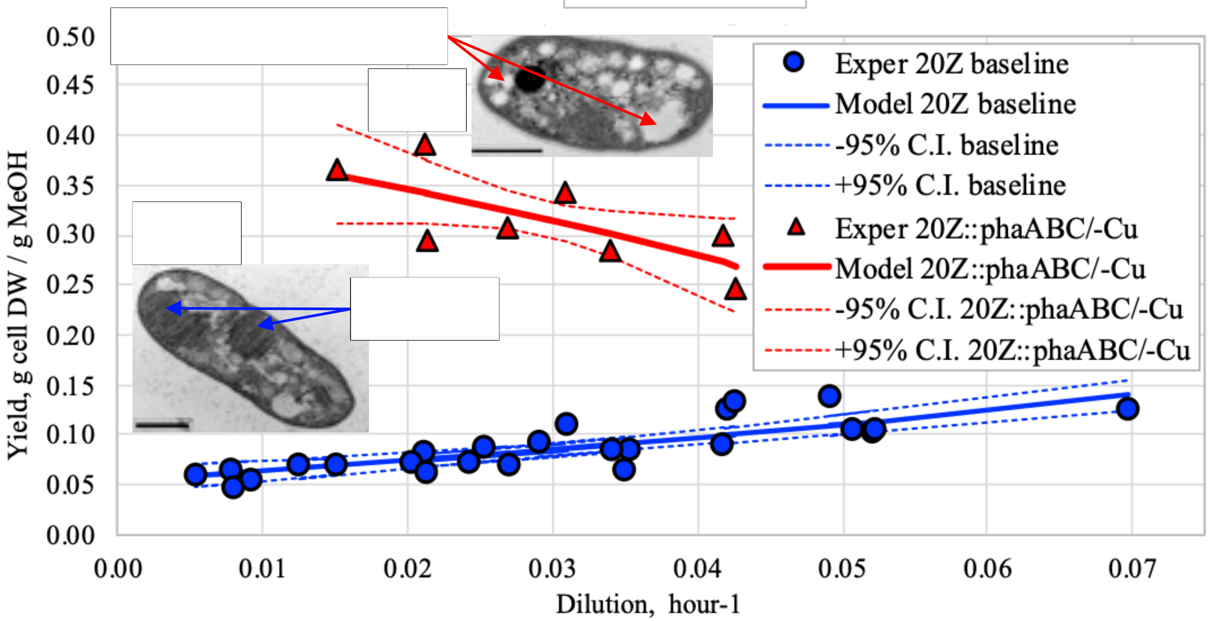
Abbreviations: polyhydroxyalkanoate (PHA), gram cell dry weight (gCDW), empty vector (EV)

Progress and Outcomes

Optimization

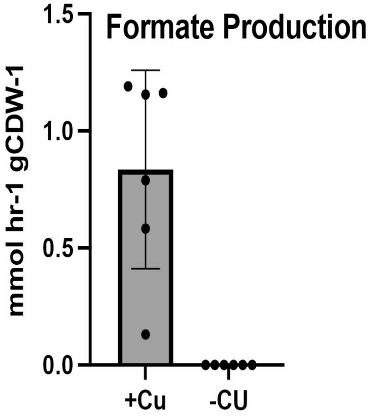


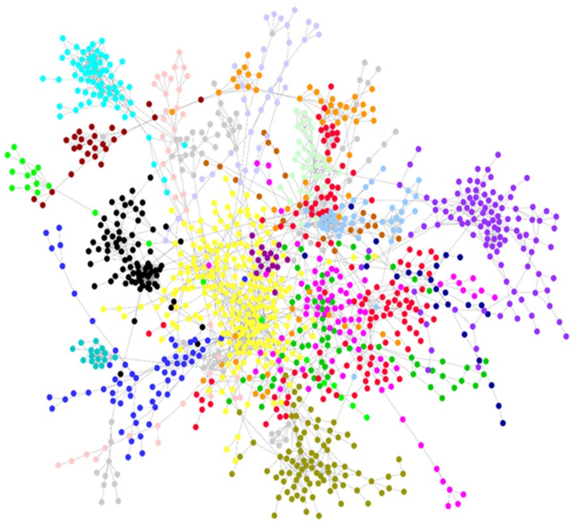
- Growth with Cu forms intracytoplasmic membranes (ICMs), reducing CCE
- Growth with Cu results in off-target formate byproducts, also reducing CCE
- Bioinformatic techniques combined with transcriptomic data identifies key regulators modulating gene expression and CCE



	Transcriptomics	Biomass +PHA CCE	Yield, g per g methanol
Current Status	Performed	≥50%	0.35
Milestone Goal	Analysis of DEG	≥53%	≥0.18

Abbreviations: carbon conversion efficiency (CCE), differentially expressed genes (DEG), intracytoplasmic membranes (ICMs)





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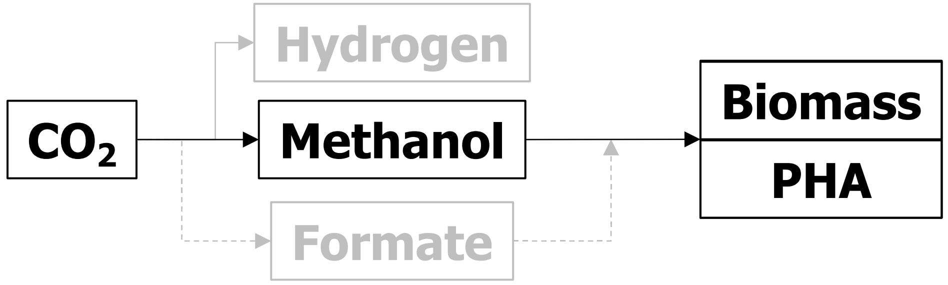
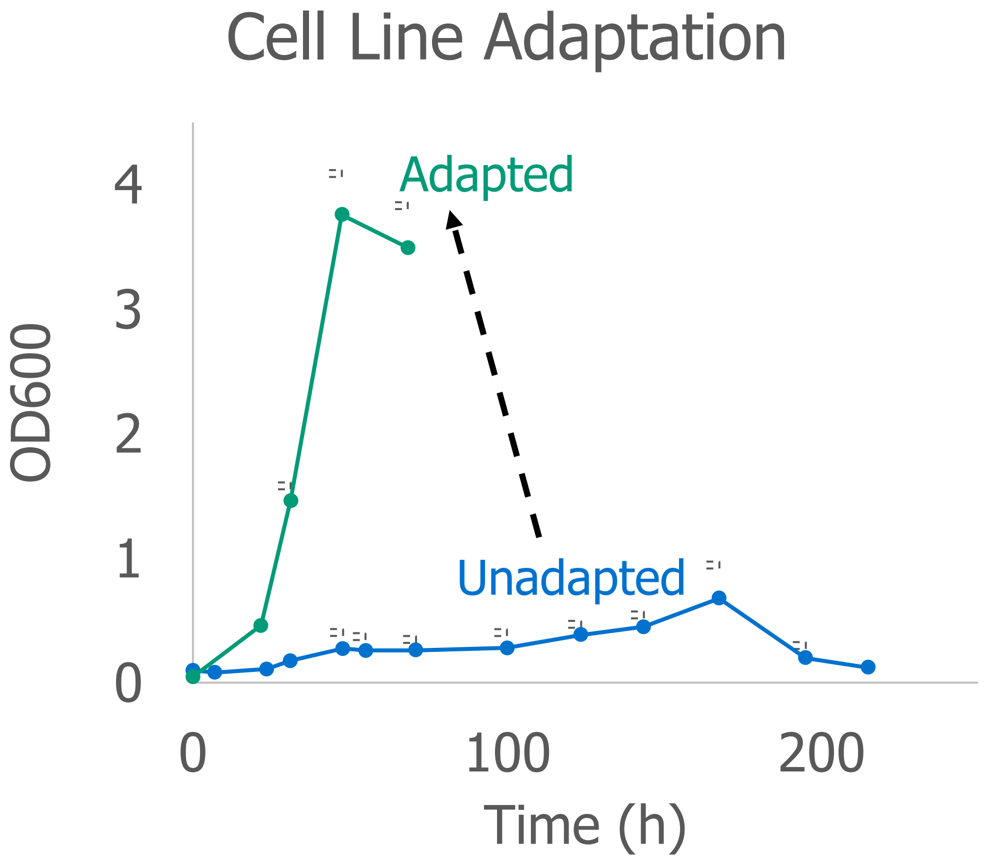
Progress and Outcomes
Integration



- High cost of methanol purification (i.e., distillation) for integrated process
- Adapt cell line to tolerate high salt concentrations optimal for upstream operations

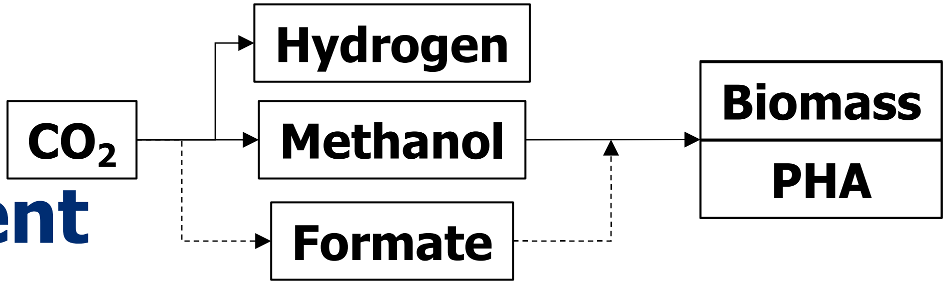


	Scale	Process CCE	Process Yield	Media
Current Status	50 mL flask	≥34%	≥0.21	Mock electrocatalysis product
Milestone Goal	1 L flask	≥37%	≥0.15	Real electrocatalysis product

Abbreviations: carbon conversion efficiency (CCE), polyhydroxyalkanoate (PHA)

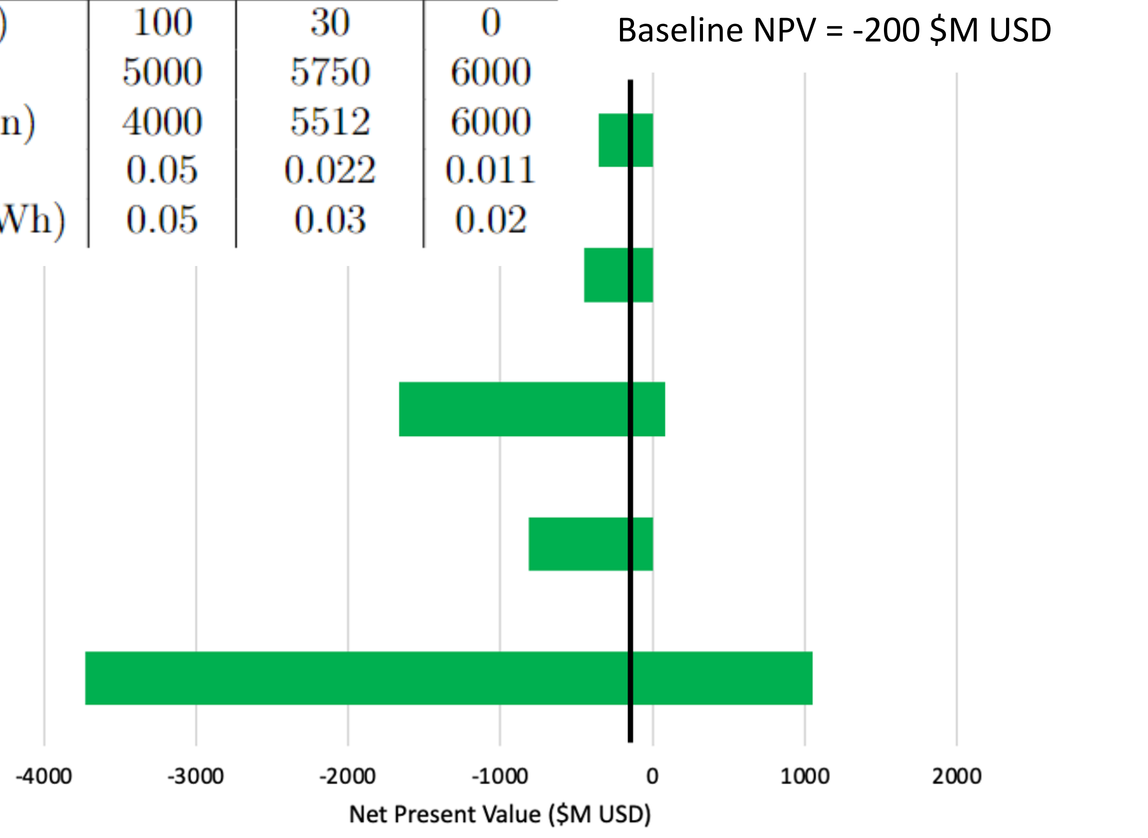
Progress and Outcomes

Techno-Economic/Life Cycle Assessment



- Process changes integrated into Aspen Plus model
- TEA completed for two technological scenarios using updated mass and energy balances
- LCA completed using US-EEIO model

Sensitivity Parameter	Worst	Baseline	Best
CO_2 Purchase ($/metric ton)	100	30	0
H_2 Sale Price ($/metric ton)	5000	5750	6000
PHA Sale Price ($/metric ton)	4000	5512	6000
Steam Utility Cost ($/kg)	0.05	0.022	0.011
Electricity Utility Cost ($/kWh)	0.05	0.03	0.02



	TEA	LCA
Current Status	TEA completed for two scenarios	LCA completed
Milestone Goal		

Abbreviations: techno-economic analysis (TEA), life cycle assessment (LCA), carbon conversion efficiency (CCE), polyhydroxyalkanoate (PHA), United States Environmentally-Extended Input-Output (US-EEIO), net present value (NPV)

Impact

4+ Publications

- Nature Catalysis
- Energy & Environmental Science
- Methods in Enzymology
- Frontiers in Microbiology

6+ Presentations

- Applied Energy Symposium
- 71st SIMB Annual Meeting
- GRC Molecular Basis of Microbial One-Carbon Metabolism

1+ Patents

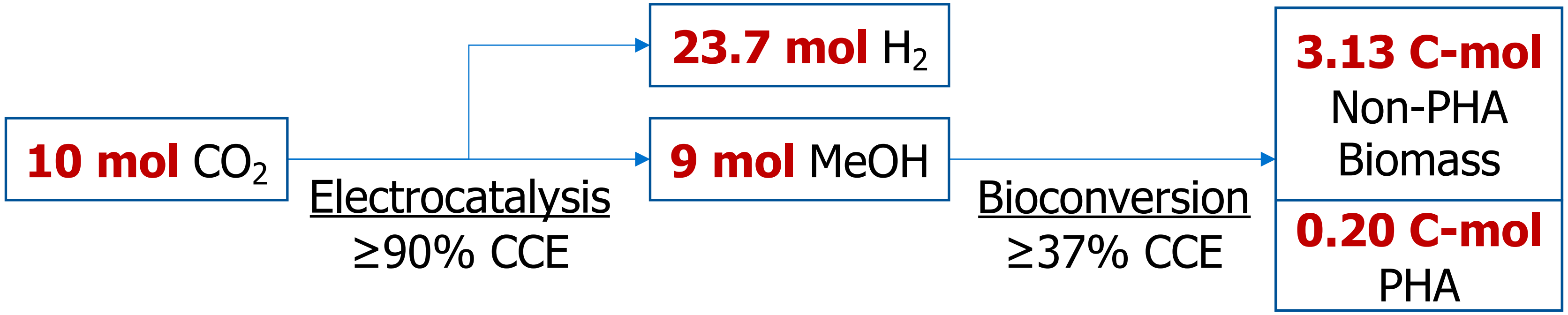
- Invention report filed with Johns Hopkins Technology Ventures Office
- Licensing in progress

nature catalysis
Energy & Environmental Science
frontiers in Microbiology
SIMB Society for Industrial Microbiology and Biotechnology
Gordon Research Conferences frontiers of science
Applied Energy Symposium
JOHNS HOPKINS TECHNOLOGY VENTURES

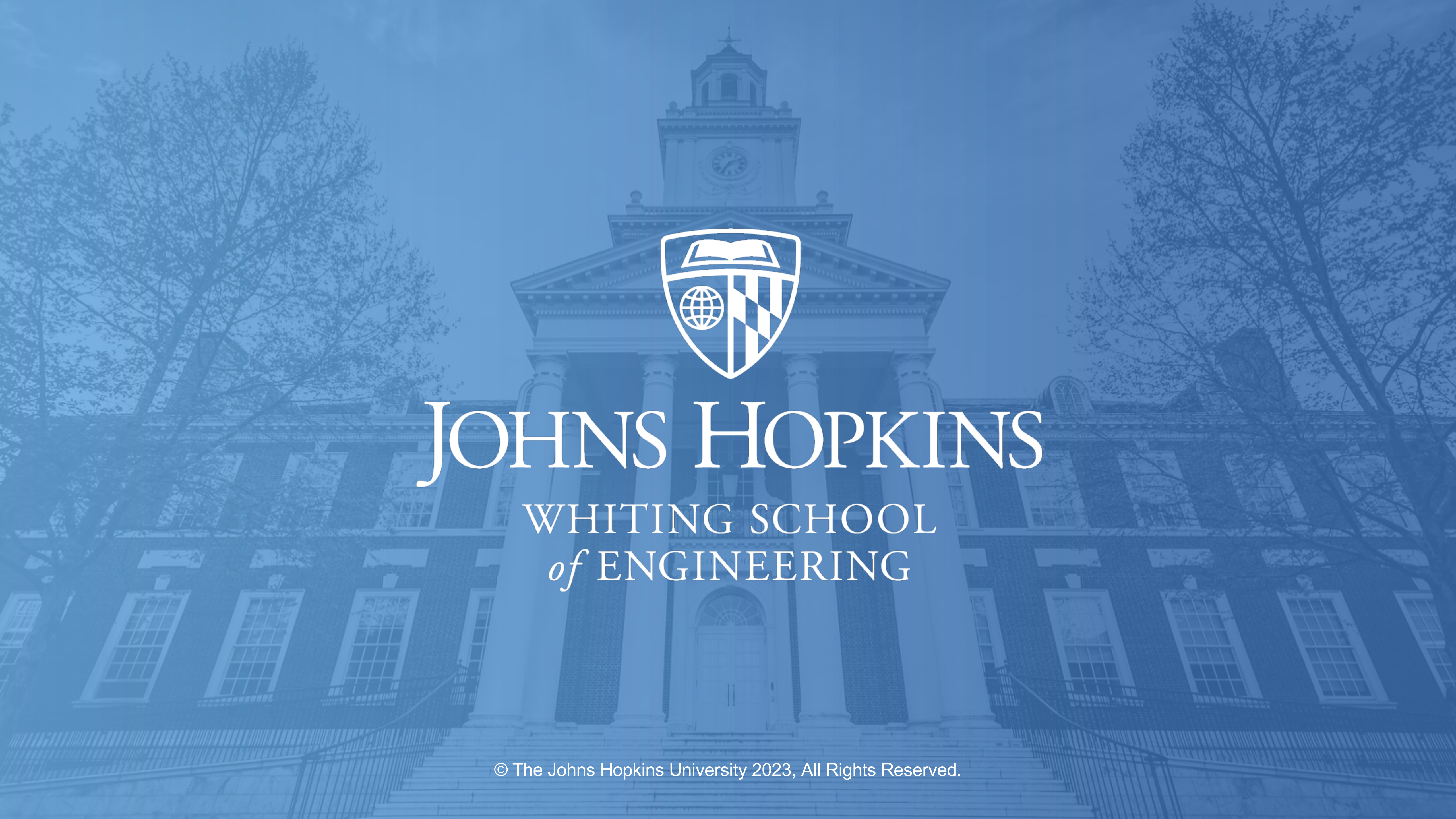
Summary

- *Approach:* Electroreduction of CO_2 to methanol and engineered microbial growth on non-ideal substrates are both the project's biggest challenges and innovations
- *Progress & Outcomes:* Each team has made significant progress toward their milestones and are on schedule to meet project goals
- *Impact:* Project success will have substantial impact as the world continues to adopt CO_2 upgrading technologies and has been demonstrated through publications, presentations, and patents

Complete Process: ≥34% CCE



Abbreviations: carbon conversion efficiency (CCE), polyhydroxyalkanoate (PHA)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Additional Slides
Responses to Previous Reviewers’ Comments

- BP2 to BP3 Go/No-Go Review:
  - Because of the high efficiency of the acetate/formate electrocatalytic processes and the biocatalyst’s strong preference for methanol, project reviewers asked us to focus primarily on methanol as an electrocatalytic product with formate as a secondary option.
  - Engineering polyhydroxybutyrate (PHB) production into the cell resulted in low yields of PHB, but high yields of a more valuable, higher-weight polyhydroxyalkanoate (PHA). After consulting with DOE mentors, the PHA being produced was deemed a suitable alternative.
- 2021 Peer Review Comments
  - The impact of the project could be strengthened by discussion of how this process will compete with existing technology for [PHA] production.
    - Further techno-economic analysis (TEA) work has helped identify whether and how this process can compete with existing polyhydroxyalkanoate (PHA) production methods. The TEA work presented as a part of this project has helped guide efforts to make the process more commercializable by eliminating the need for methanol purification and therefore making the process less energy-intensive. The TEA will also identify areas where future investments and advancements should occur to facilitate scale-up and commercialization.
  - The selection of the specific microbe and metabolic target needs more justification—for example, what are the specific advantages of this strain?
    - *Methylotuvimicrobium alcaliphilium* 20ZR is one of few cultures capable of growth in high-molarity sodium bicarbonate buffers as used in the upstream electrocatalysis portion of the process. Additionally, the strain shows robust growth on methanol, with carbon conversion efficiencies potentially attaining upwards of 60%. Lastly, a significant body of supporting research also exists for this organism, including metabolomic, transcriptomic, and proteomic data that were used to generate a computational metabolic model that can be leveraged to inform metabolic engineering efforts.

Additional Slides

Publications, Patents, Presentations, Awards, & Commercialization

- Publications
  - Jordaan, S.M. and Wang, C., 2021. Electrocatalytic conversion of carbon dioxide for the Paris goals. Nature Catalysis, 4(11), pp.915-920.
  - Ruttinger, A.W., Tavakkoli, S., Shen, H., Wang, C. and Jordaan, S.M., 2022. Designing an innovation system to support profitable electro-and bio-catalytic carbon upgrade. Energy & Environmental Science, 15(3), pp.1222-1233. https://doi.org/10.1039/D1EE03753F
  - Zachary J. Johnson, Dennis D. Krutkin, Pavlo Bohutskyi, Marina G. Kalyuzhnaya. 2021. 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. https://doi.org/10.1016/bs.mie.2021.01.046
  - Xiong W., Kalyuzhnaya M.G., Henard C.A. 2021. Editorial: Microbial C1 Metabolism and Biotechnology. Front. Microbiol., https://doi.org/10.3389/fmicb.2021.744030

- Presentations
  - Chen, I. Y., Ruttinger, A. W., Jordaan, S. M., Clancy, P. Techno-Economic and Life Cycle Assessment of an Electro- and Bio-Catalytic Carbon Upgrade Process. American Institute of Chemical Engineers (AIChE) Annual Meeting, 17 Nov 2022. Oral presentation.
  - 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.
  - Dimitri Krutkin. Utilizing Molecular Biology, Multi-Omics, and Metabolic Modeling to Bioengineer Methylomicrobium alcaliphilum 20ZR (a Methanotrophic Bacteria) for Production of Polyhydroxybutyrate. 14th Student Research Simposium, SDSU, 2021.
  - Kalyuzhnaya M.G. CO2 to Plastic: Integrating Chemical Catalysis and Biological Conversion of Carbon Intermediates into Polyhydroxyalkanoates. 71st SIMB Annual Meeting. Austin TX. (invited speaker)
  - Kalyuzhnaya M.G. Methanotroph: to Be, or Not to Be, that Is the Question. GRC Molecular Basis of Microbial One-Carbon Metabolism. Southbridge, MA, United States. (invited speaker)
  - Richard Hamilton and Marina Kalyuzhnaya. Integrating Chemical Catalysis and Biological Conversion of Carbon Intermediates into Polyhydroxyalkanoates. Bioinformatics of genome regulation and structure/Systems Biology (BGRS/SB) 2022; Novosibirsk, Russia.
  - Richard Hamilton and Marina Kalyuzhnaya. Biological Conversion of C1 Substrates to Plastics. Polymer Science and Engineering; Los Angeles, California.