Development of a scalable, robust electrocatalytic technology for conversion of CO_2 to formate salt via graded microstructures and development of a bioengineered C1 pathway for subsequent upconversion to ethylene glycol

DOE Bioenergy Technologies Office (BETO) 2021 Project Peer Review

April 7, 2023

Catalysis / Upconversion

BioEnergy Engineering for Products Synthesis (BEEPS)

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MONTANA STATE UNIVERSITY

USF UNIVERSITY OF SOUTH FLORIDA

Project Overview

- *Response to FOA 0001916 BioEnergy Engineering for Products Synthesis (BEEPS) Topic area 5: Rewiring Carbon Utilization*
- *Use catalytic methods to reduce CO_2 to single carbon intermediates*
- *Follow with biological upconversion to multi-carbon compounds*

Approach

- ***MSU will develop electrochemical reactor components with laterally graded pore structure to aid in reaction distribution when the reactor is scaled-up. This will be done in stages, first at a 100 cm^2 size, then a 300 cm^2 size.***

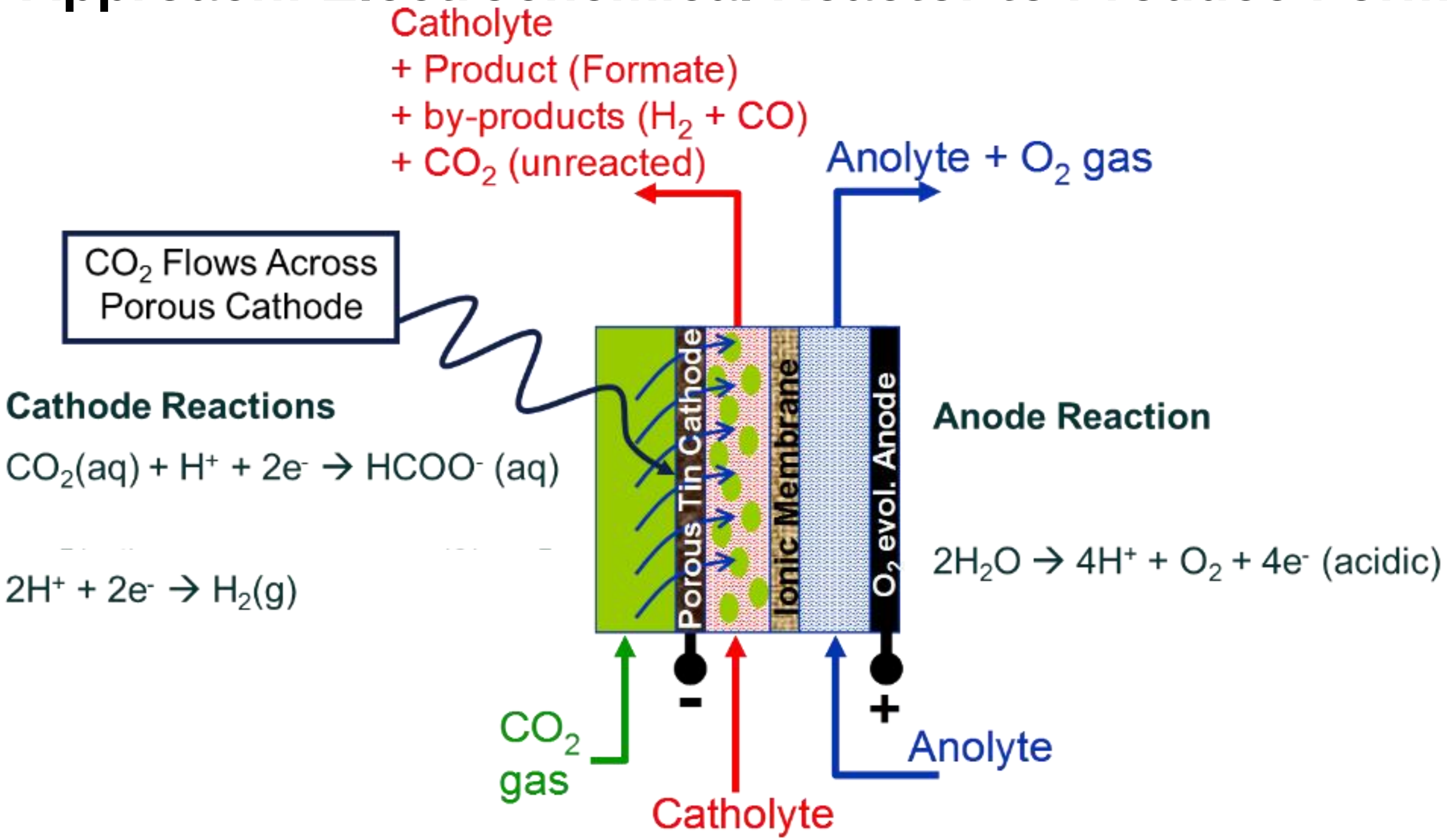
- ***OCO/DNVGL will integrate these components into the reactor and determine performance. The testing, starting at 10 cm2 reactor size will be scaled up 30x, in stages, to 300 cm2***

- ***OCO/DNVGL will also test performance in starting solutions more benign to bacteria to try to eliminate costly separation steps for use of reactor formate solution output in the biological upconversion.***

- ***USF will develop C1 pathway using their recent discovery of a novel C-C bond forming reaction that is orthogonal to central metabolism and requires fewer enzymes and reaction steps (Figure 3)***

- ***Top potential challenges are ability to control lateral pore grading over larger areas and ability to bioengineer bacteria to upconvert in process fluids***

1 – Approach: Electrochemical Reactor to Produce Formate



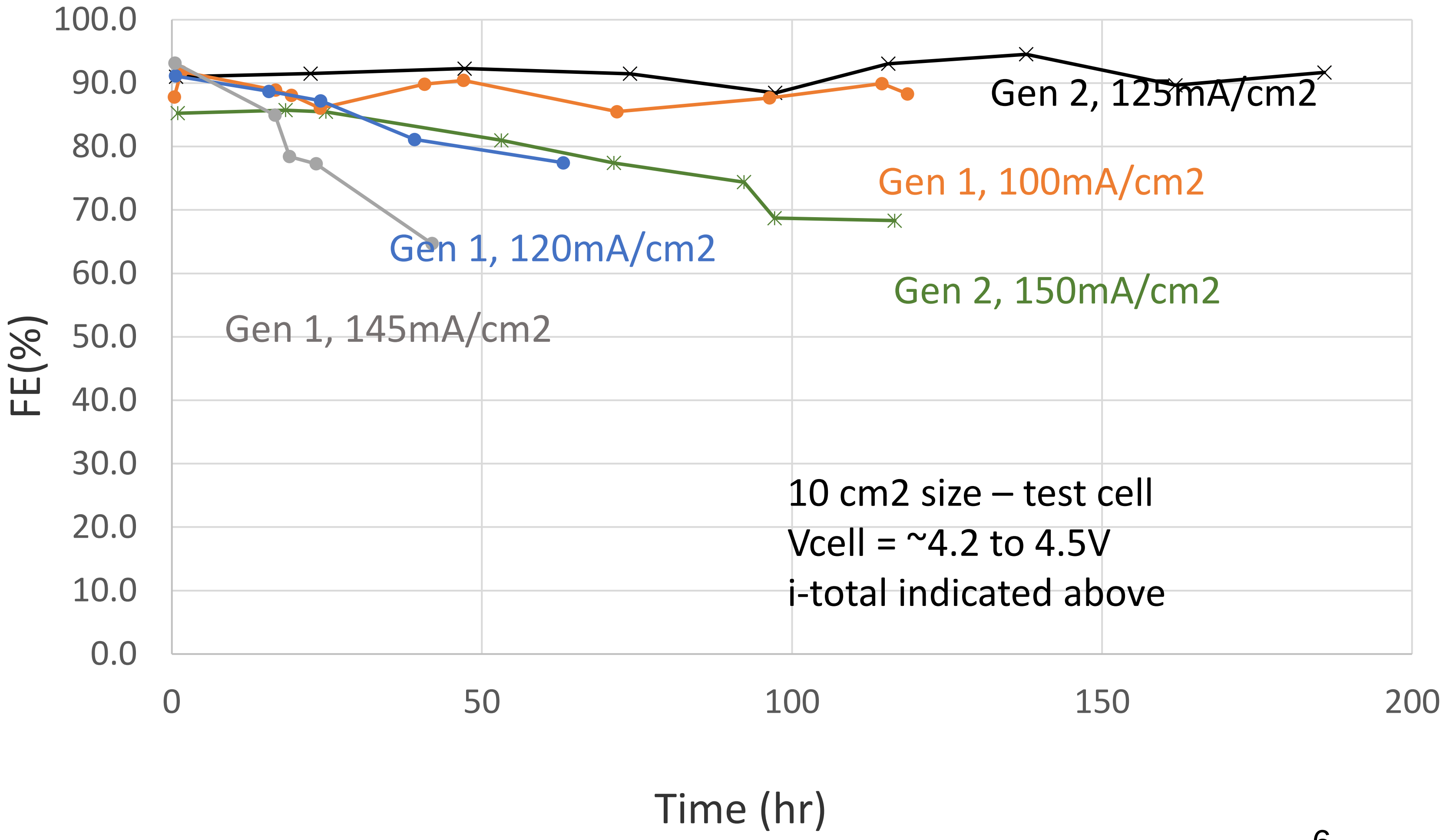
1 – Approach – Electrochemical CO_2 reduction to formate

- Scale-up of electrochemical reactors from academically employed 5-10cm2 to 300cm2 is one of the key objectives of this DOE project.
- CO_2 and liquid electrolyte are flown at opposite sides of the gas diffusion electrode (GDE), the CO_2 gas enters the gas diffusion layer and is available at the wetted catalyst layer applied to the side of the GDE that contacts the electrolyte layer, thus creating a three phase gas-liquid-solid interface required for CO_2 electro-reduction to formate ion.
- For commercial application in industrial height (~1.2m) electrolyzers, GDE design and optimization is key to ensure favorable hydrodynamics and pressure regulation so that the GDE is not flooded with the electrolyte and CO_2 gas is available to freely move across the GDE to react at the wetted catalyst surface, in absence of which mass transport limitations of CO_2 availability will not allow for a feasible selectivity and efficiency of CO_2 conversion to formate
- Approach is to resolve the following technical challenges via electrochemical process development –
  - GDE and its components optimization (gas diffusion layer, hydrophobic coatings and catalyst layer composition and content, including binders, for sustained three phase reaction system
  - Reactor design, operation and process operation – to increase the size of reactor from 10 cm2 or 3.3cm height (BP1, achieved 2019), to 100 cm2 or 10cm height (BP2-achieved, 2022), to 300cm2 or 30cm height (BP3-in progress, target is 2023)
  - At scaled up reactor sizes, achieve/demonstrate optimal electrochemical performance for CO_2 to formate (125mA/cm2, >75% faradaic efficiency, for 120hrs)

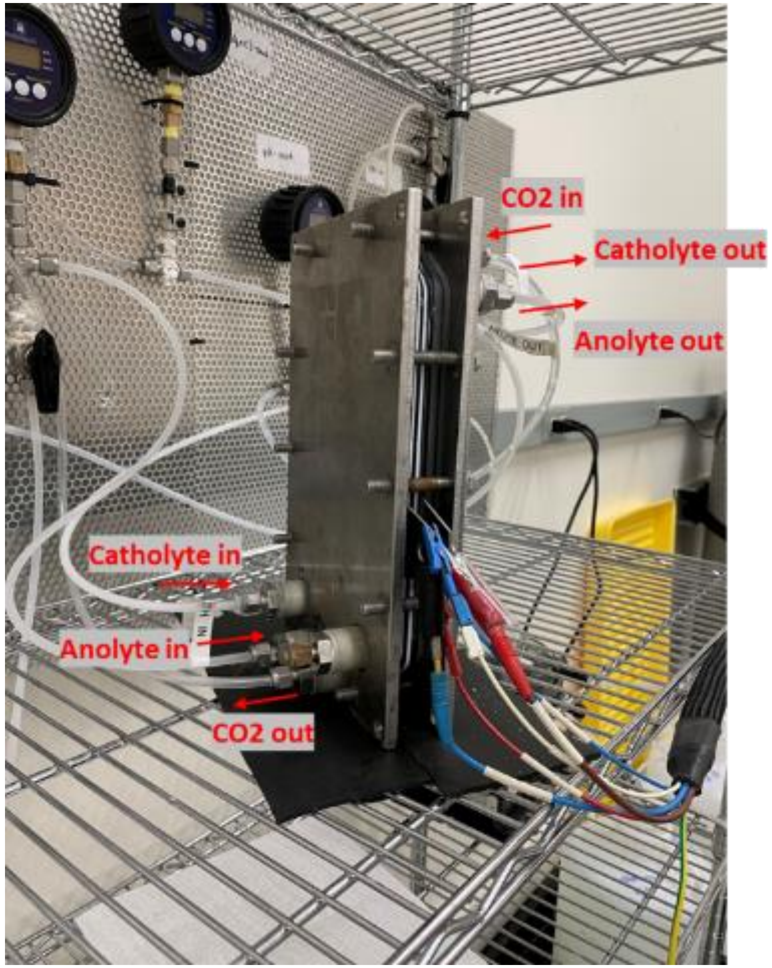
2 – Progress and Outcomes – Electrochemical CO2 reduction to formate

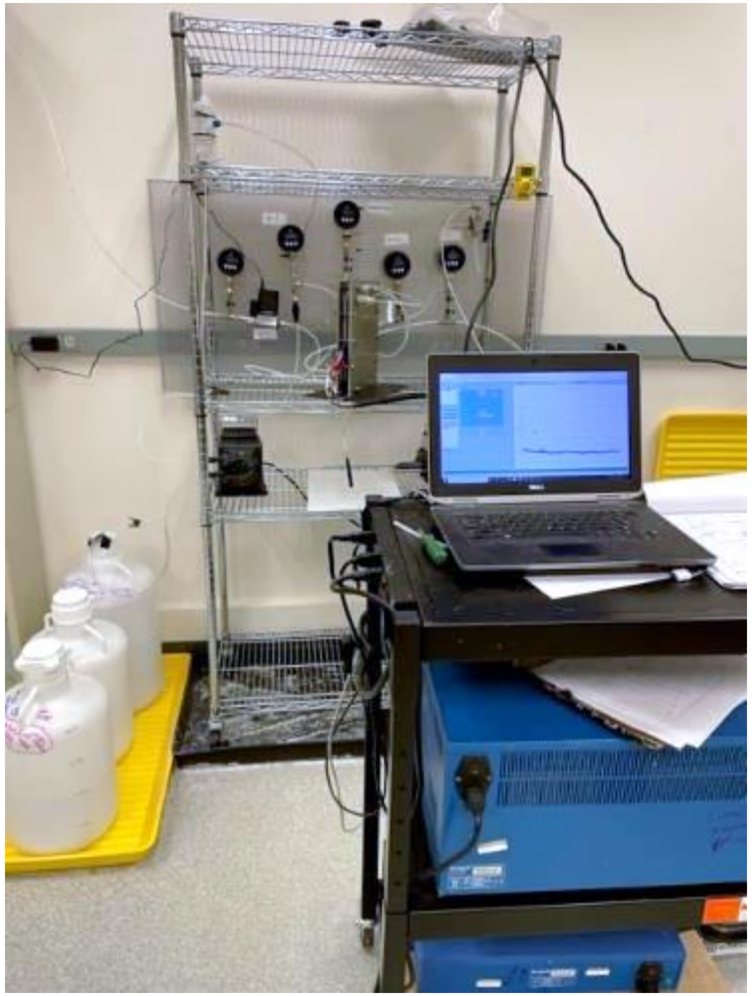
OCO's GDE optimization and testing

- GDE optimization and testing
- Gen 1 GDE has 1 binder
- Gen 2 GDE modified with secondary binder
- Gen 2 GDE indicated better FE values with higher stability at 25% higher current than Gen 1
- **Gen 1: 100 mA/cm2 at ≥85% FE for 100hrs**
- **Gen 2: 125 mA/cm2 at ≥90% FE for 150hrs**
- OCO's Gen 2 GDE enhanced performance, eliminated flooding, met current density target of 125mA/cm2 at FE >70% target



Long Term 100cm2 Reactor Test

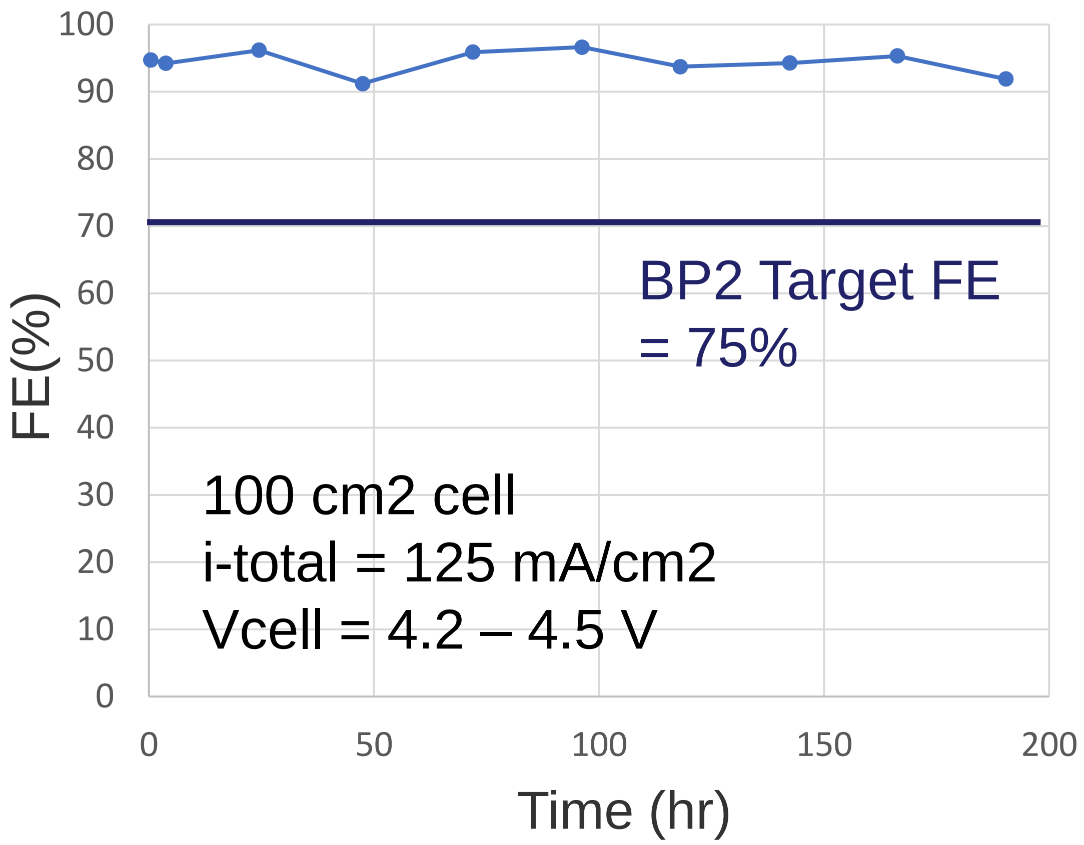




- 100 cm2 cell, galvanostatic at 125 mA/cm2 with Gen 2 GDE
- Freshly cut N117 membrane cation exchange membrane
- Flow channels modified to leave the electrode area open
- Used silicone gel to seal every layer
- Used less torque to tighten the cell @ 40 lbf-ft

Long Term 100cm2 Reactor Test

- Modified GDE (Gen 2) applied in the 100cm2 electrochemical reactors
- Galvanostatic current applied 125 mA/cm2 (12.5 A total current to the 100cm2 reactor)
- FE > 80% target value for long duration (265 hrs),
- Total cell voltage is in the range of 4.2-4.4 V



Reaction Conditions: constant i = 125mA/cm2, N117 membrane, Catholyte = 2M KCl, CO2 satd., pH=3.8 (~100ml/min), Anolyte = 0.5M H2SO4 + 0.5M K2SO4, 140ml/min, pH=0-0.3, CO2 = 0.6 l/min (~14% CO2 gas consumed), Gen 2 GDE: air sprayed with catalyst, ~1 - 4 mg/cm2 catalyst loading, binder (~5 - 50 wt%), GDL (5 to 50wt% PTFE coated carbon paper)

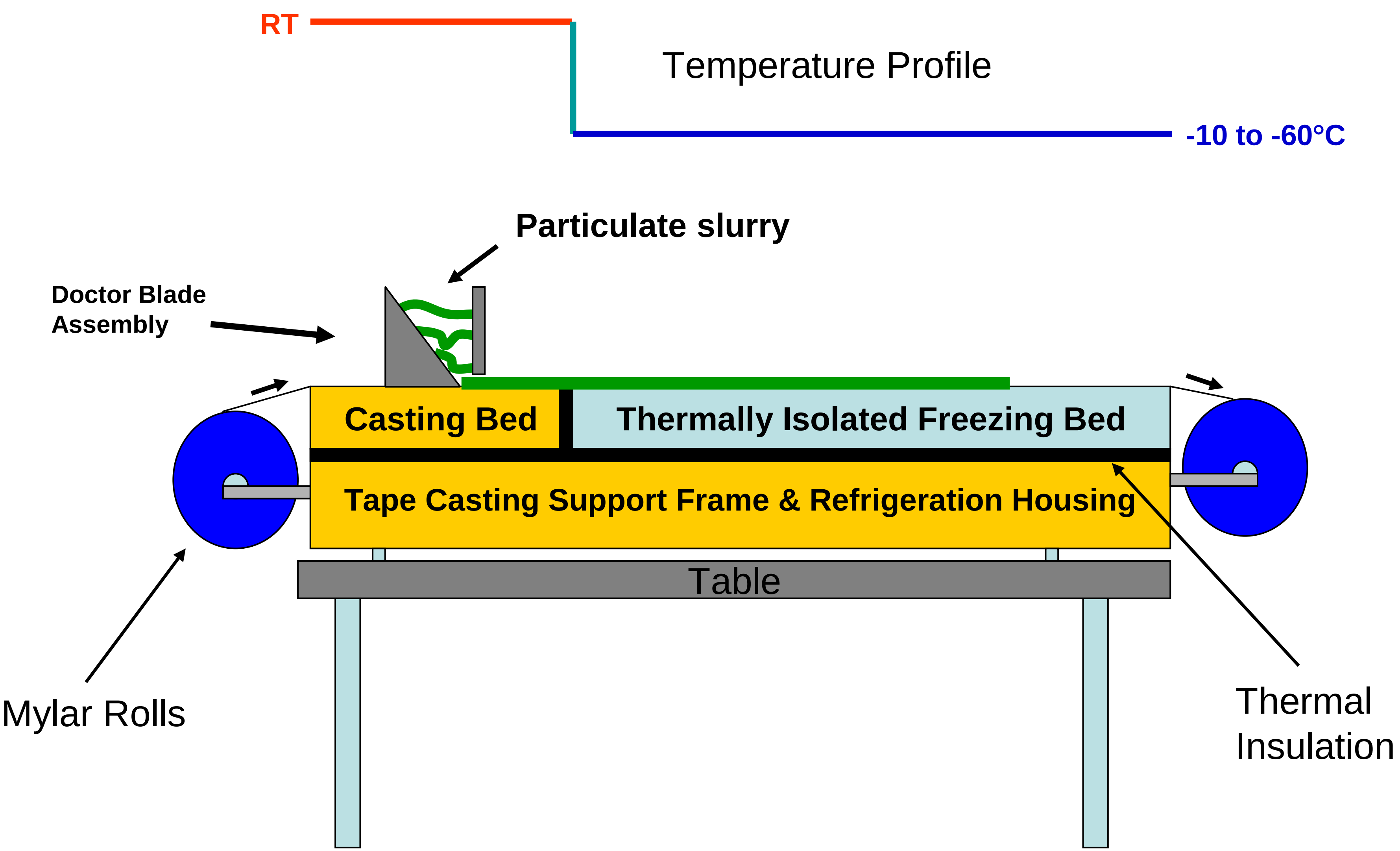
OCO has met the BP2 Electrochemical Performance Targets and Task 2 is completed

Electrochemical Performance Metric	Intermediate BP2 Target	Present achieved/ demonstrated	% of Target
Electrode Size	100cm2	100cm2	Meets target
Applied Voltage	4.2 V	4.5 V	95%
Total Current Density	125 mA/cm2	125 mA/cm2	100% (meets target)
Faradaic Efficiency (FE)	70%	>>70% (avg 85% over 190 hrs)	>100% (exceeds target)
Electrical Energy Demand (kWh/ton-CO2)	7000	<7000 (~6310)	>100% (exceeds target)
Operating Time (hr)	120 hr	>120 hr (190hrs)	>100% (exceeds target)
Major Highlights	Issues with GDE flooding and catalyst activity over time have been resolved by OCO's significant development in areas of: • Gen 2 carbon paper-based gas diffusion electrodes • Modified electrochemical reactor's process operating conditions		

Ideal Gas Diffusion Membrane / Electrode

- Self-supporting, mechanically robust
- Conductive
- Not catalyze competing reactions (tin) Tin mp 231° C
- Have appropriate permeability (potentially graded because of hydrostatic pressure gradient)
- Incorporate the needed catalyst
- Incorporate hydrophobic coating

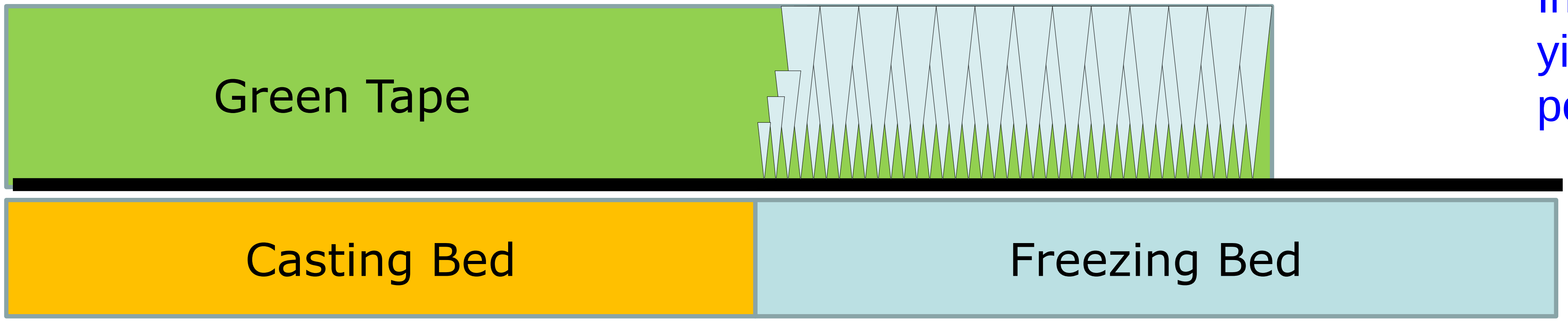
1 – Approach – Freeze Tape Casting of Gas Diffusion Membranes



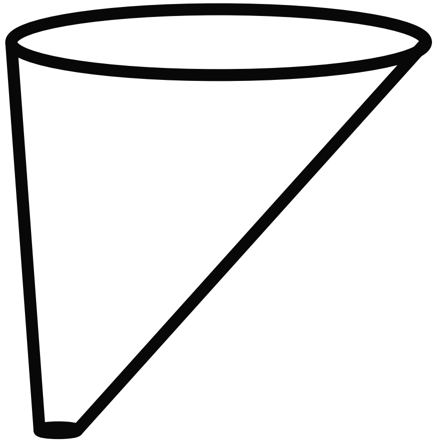
1 – Approach – Freeze Tape Casting of Gas Diffusion Membranes

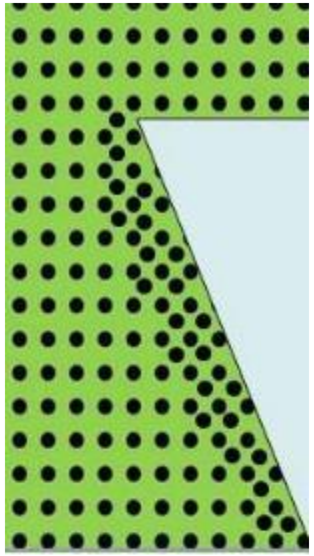
Ice Templating - Pore Development

Ice crystals ➔ pores



c-axis of ice (hexagonal, Ih) grows more rapidly yielding conical, acicular pores

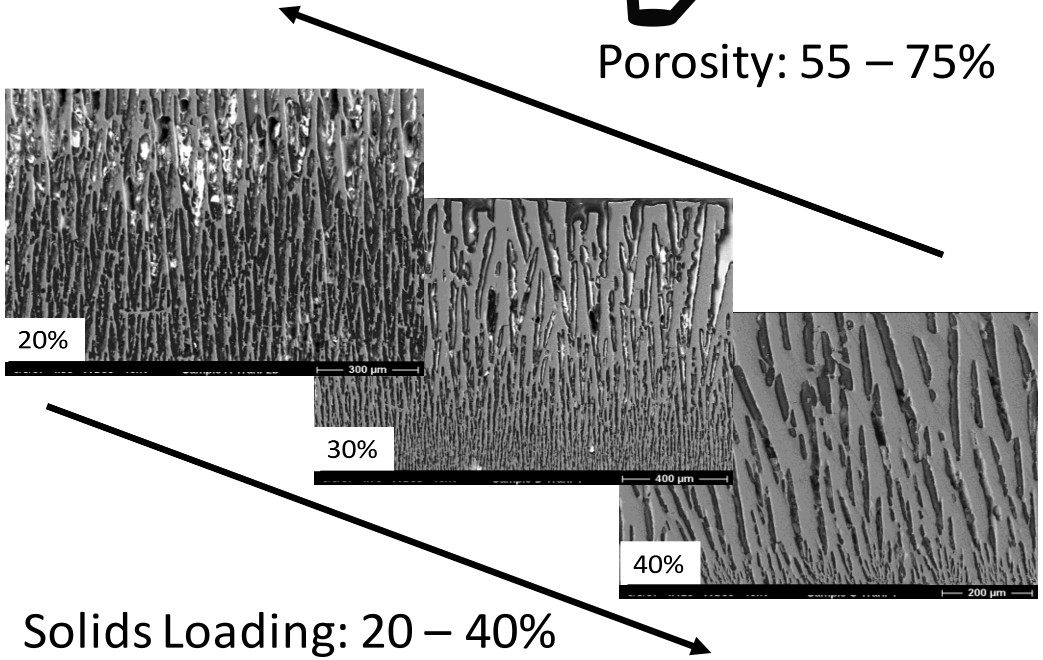




Particle Rejection Phenomenon

- Solidifying water rejects (metal, ceramic, polymer) normal to growth direction
- Regions between growing ice crystals have higher particle packing than the bulk suspension
- Pores diverge as growth continues through cross-section
- Ice is removed by sublimation, leaving porosity in the green state (unique from traditional pore forming)

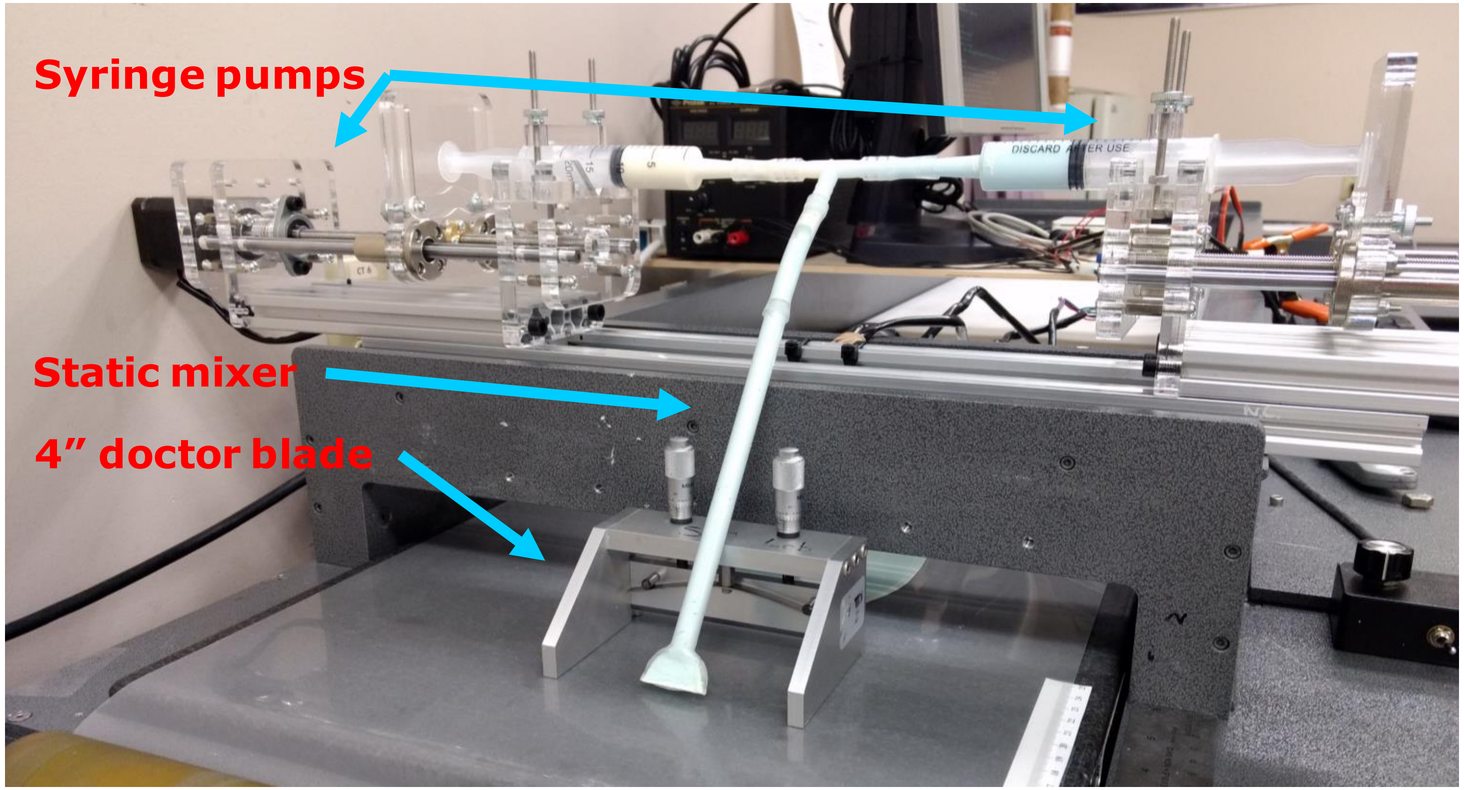
Porosity: 55 – 75%

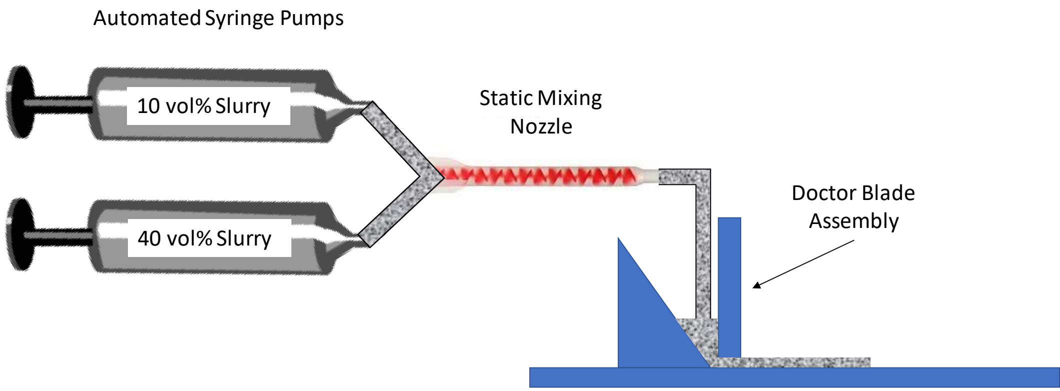


Solids Loading: 20 – 40%

2 – Progress and Outcomes- Freeze Tape Casting of Gas Diffusion Membranes

Development of a variable solids loading FTC system to create laterally graded porosity structures



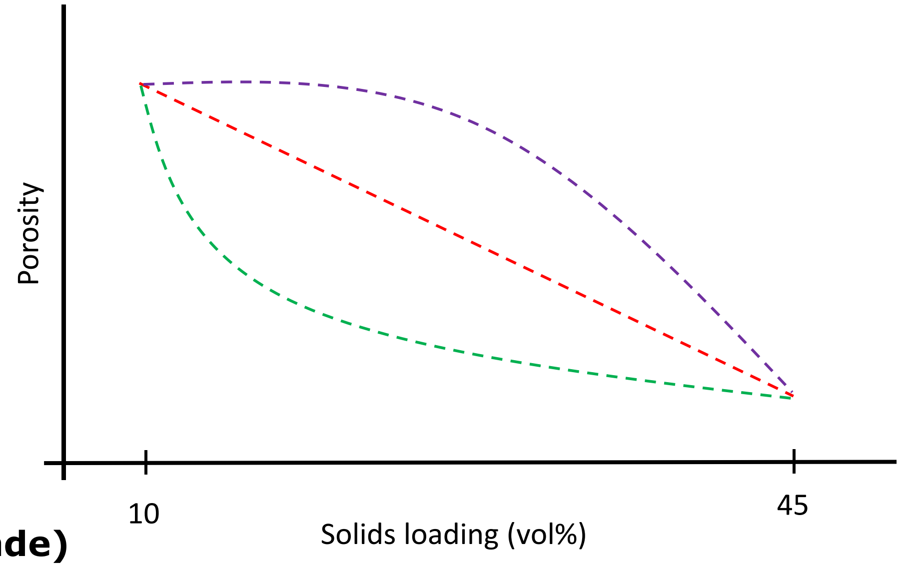


Arduino controlled syringe pump system capable of continuously grading between any arbitrary initial and final solids loading (Linear & Non-linear gradients)

Slurries homogenized without moving parts (statically mixed – ie. epoxy)

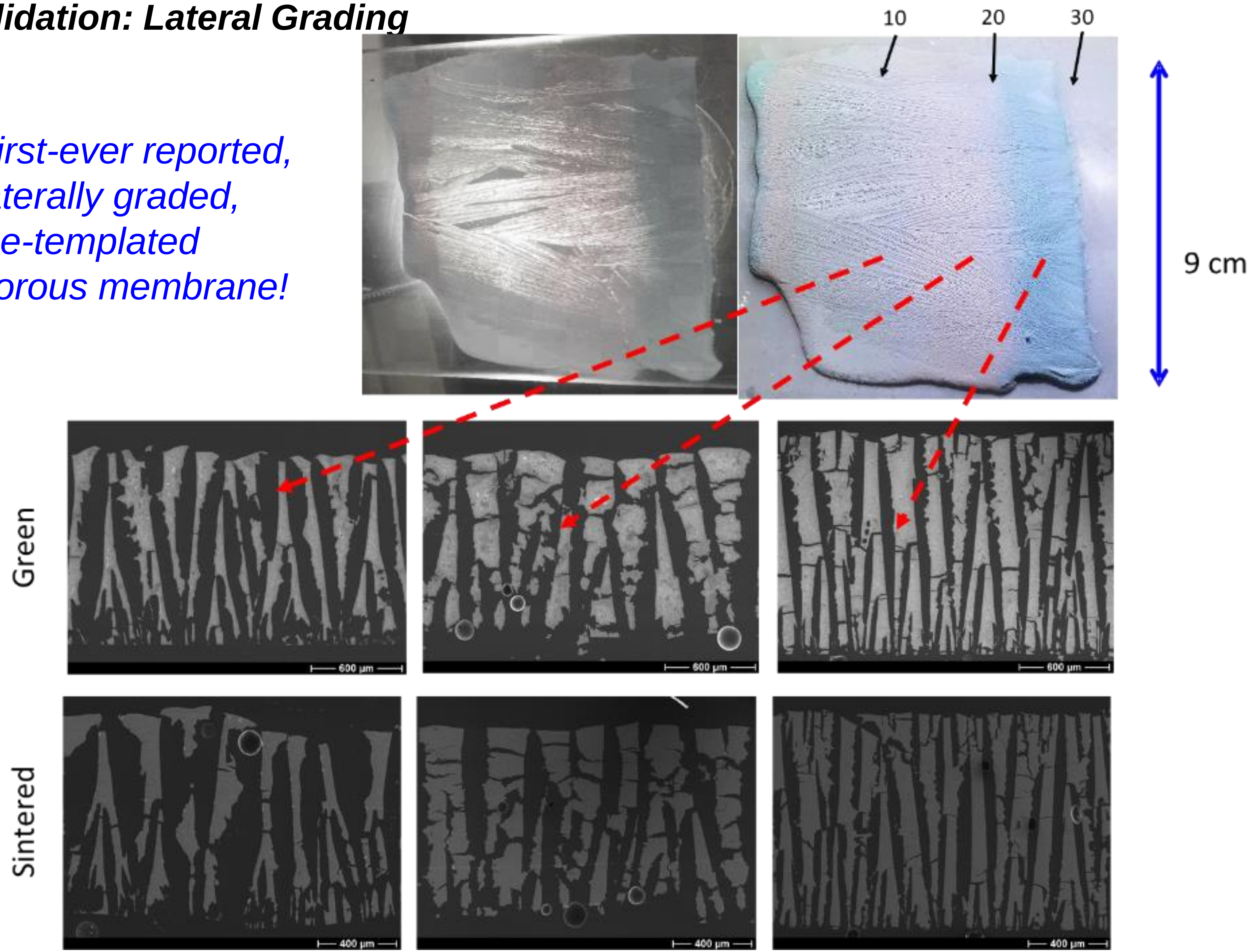
Variable casting rates possible, integrated within existing FTC platform

Variable tape dimensions possible (4" Dr. Blade shown, but scalable to 14" Dr. Blade)

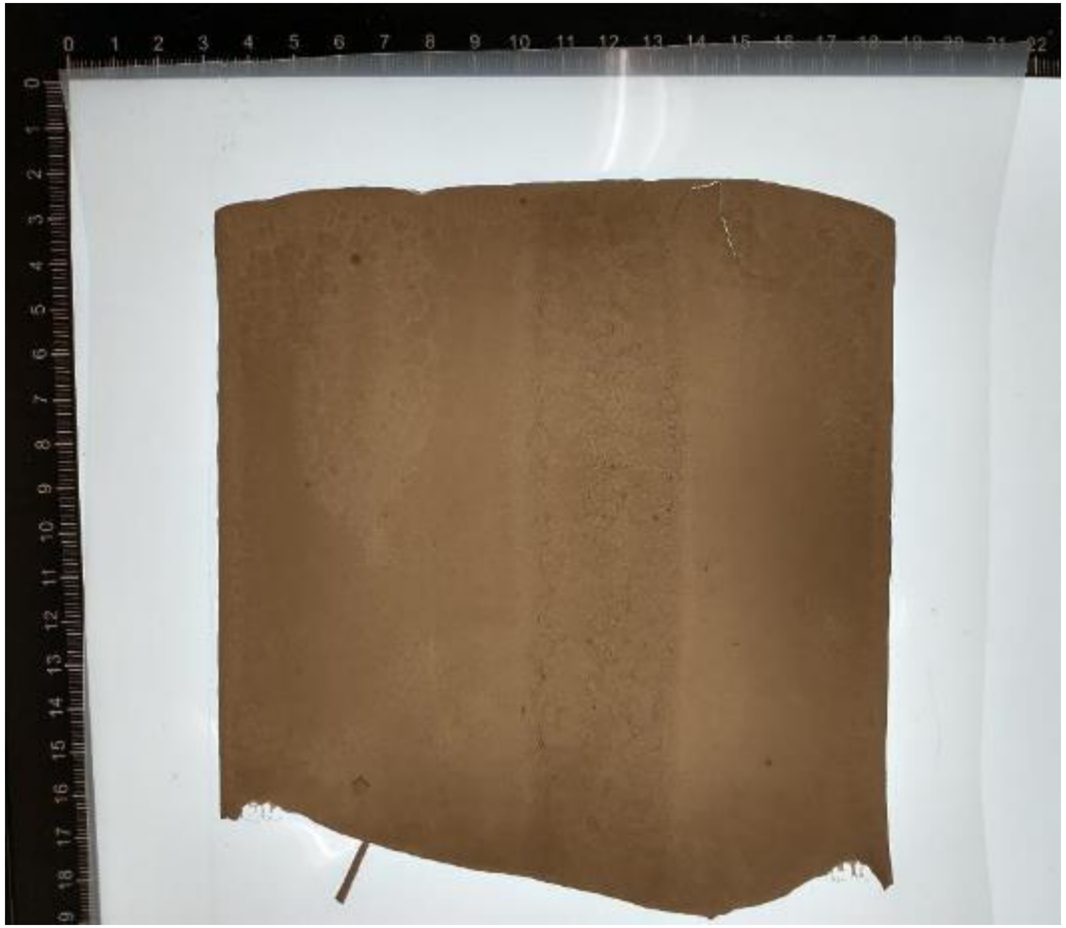


Validation: Lateral Grading

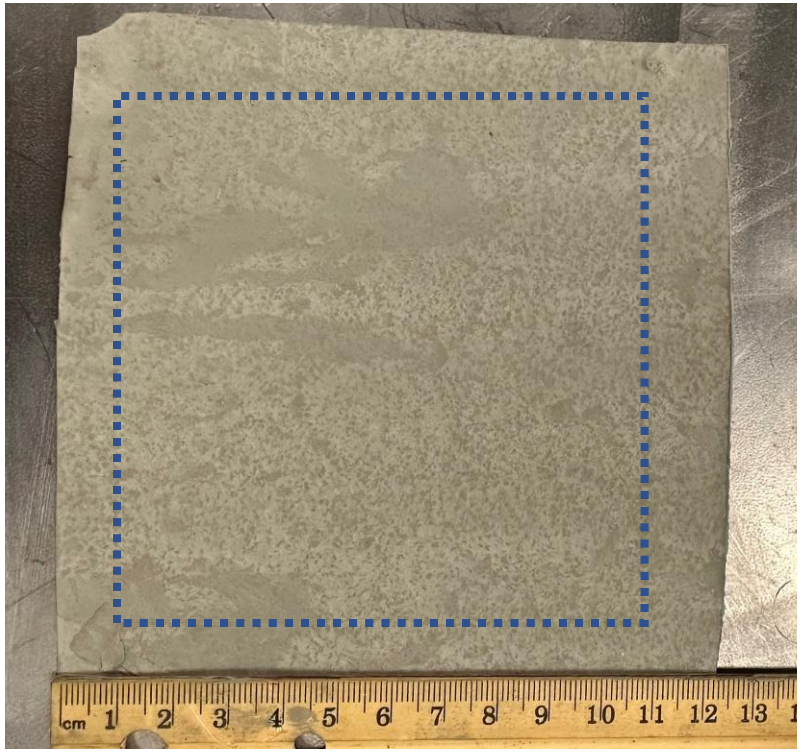
First-ever reported, laterally graded, ice-templated porous membrane!



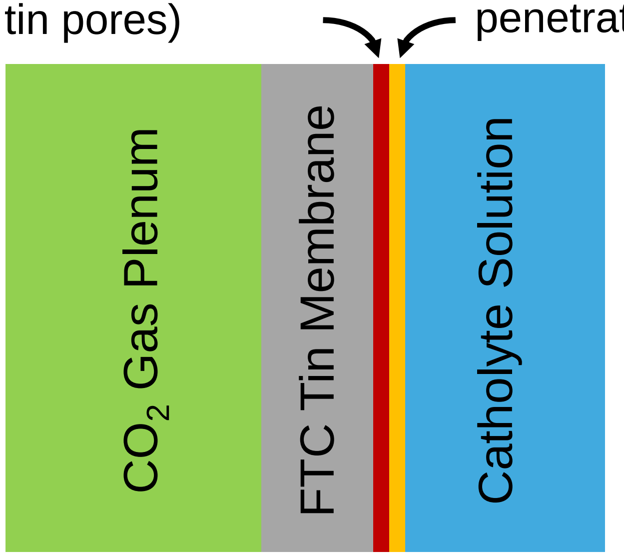
Ability to Freeze Tape Cast at Proposed Scale

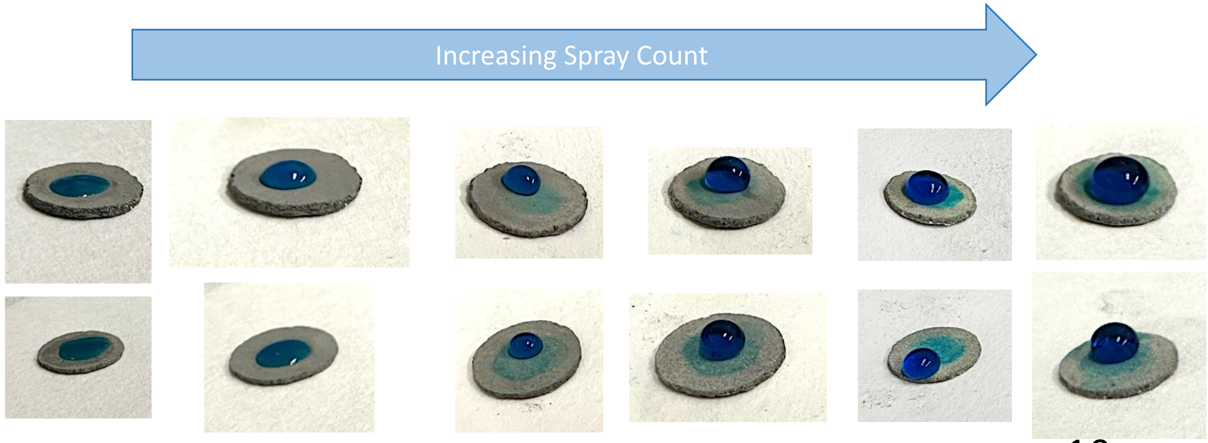


Prior FTC acrylic cast at ~ 300 cm^2 (left) contrasted with FTC tin (right) both shown under light illumination. The non-optical behavior of tin particles limits light transmission, however, the tin shows pore alignment anticipated wit the FTC approach.

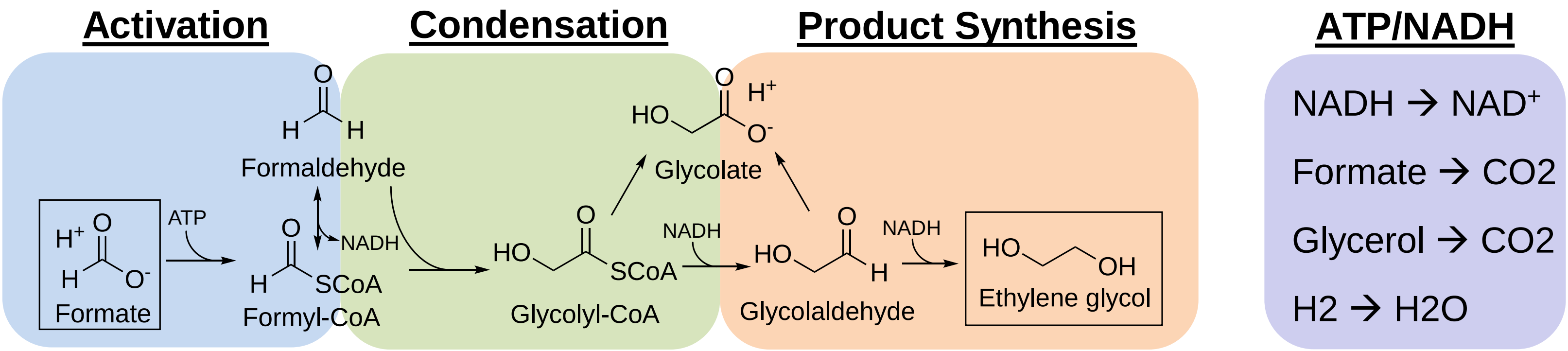


FTC tin, self-supporting 1 mm thick porous membrane sintered at 12 cm x 12 cm with the blue area indicating the 10 cm x 10 cm electrochemically active area.





1 – Approach – Biological Upconversion

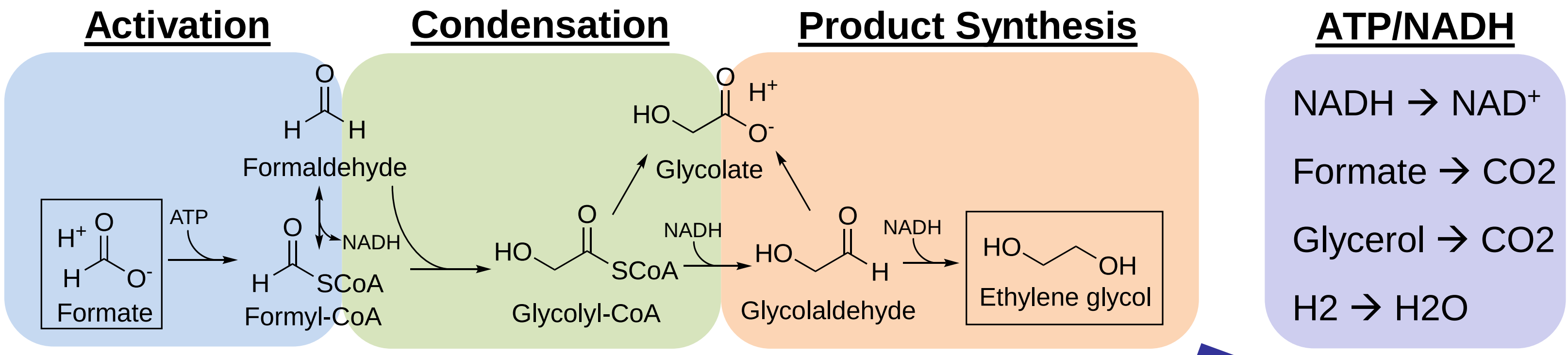


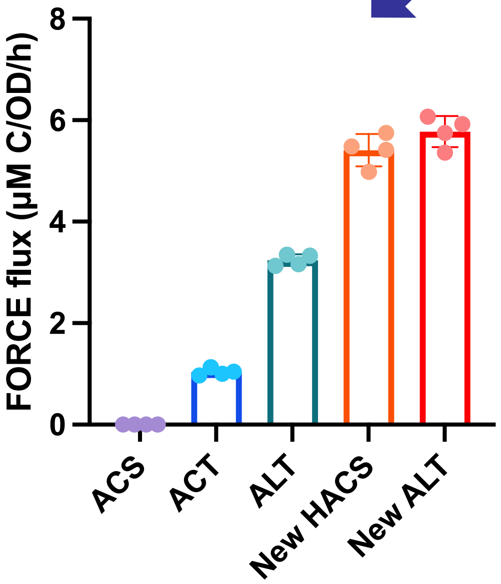
5 $CH_2O_{2(l)}$ → $C_2H_6O_{2(l)}$ + 3 $CO_{2(g)}$ + 2 $H_2O_{(l)}$ ΔG^o = -154.9 kJ/mol Maximum yield: 0.2 mol/mol (0.4 C/C), 0.27 g/g

Specific improvements needed to develop formate to ethylene glycol biocatalyst

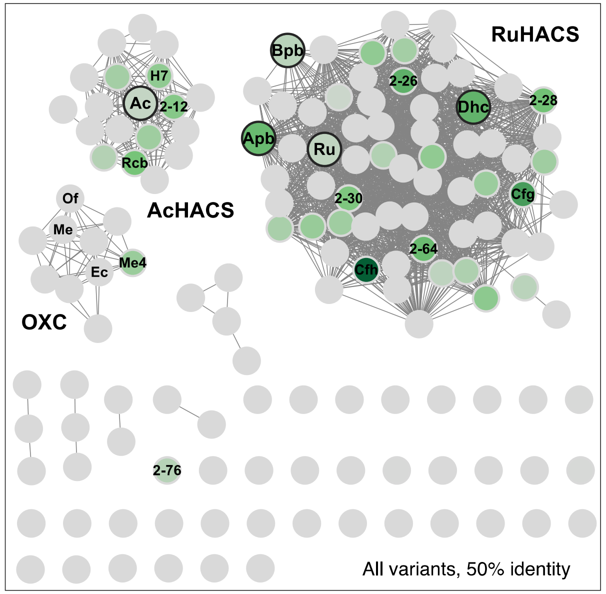
- Activation: enzymes for various formate activation routes, balance generation of formaldehyde/formyl-CoA required for condensation
- Condensation: condensation enzymes with high catalytic efficiency and high soluble expression in *E. coli*
- Product Synthesis: conversion of glycolyl-CoA to ethylene glycol at high flux with minimal by-product formation
- ATP/NADH: generation from different energy source(s) and modulate expression in the context of overall pathway expression and activity
- Bacterial host: proper chassis to maximize pathway activity (functional and balanced expression of pathway enzymes) and minimize formate oxidation & by-product formation; capable of tolerating process conditions, including high KCl concentration in formate-containing media

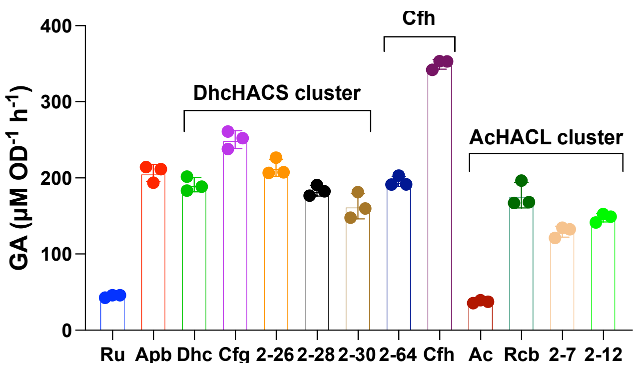
2 – Progress and Outcomes- Biological Upconversion



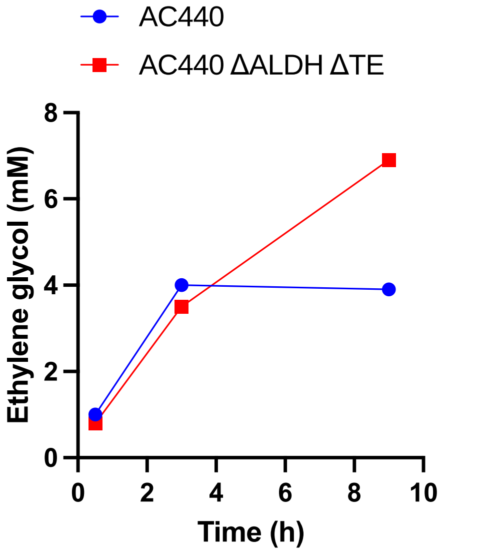


Screened different formate activation routes both *in vitro* and *in vivo*



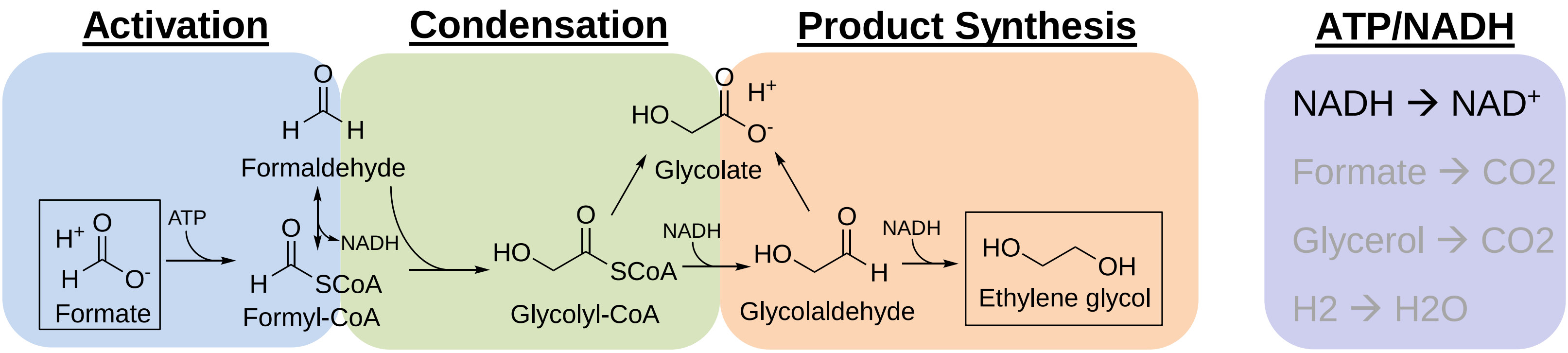


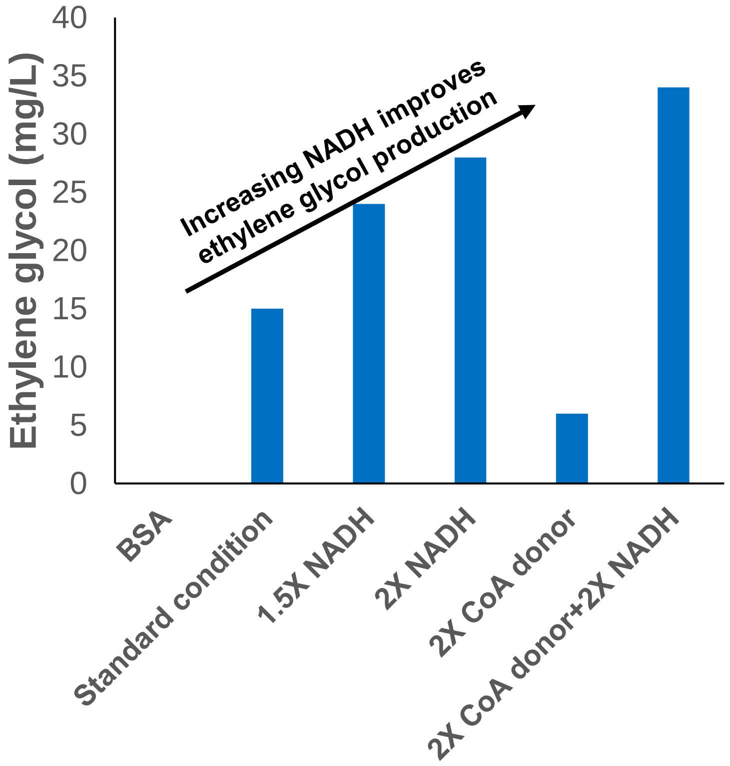
Screened 130 HACS variants for core condensation

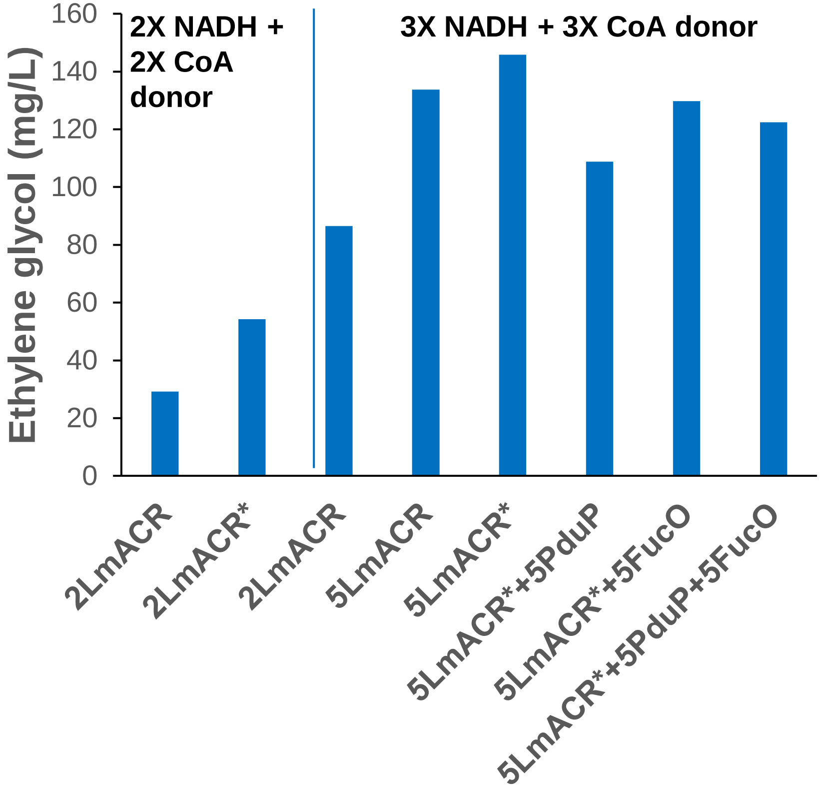


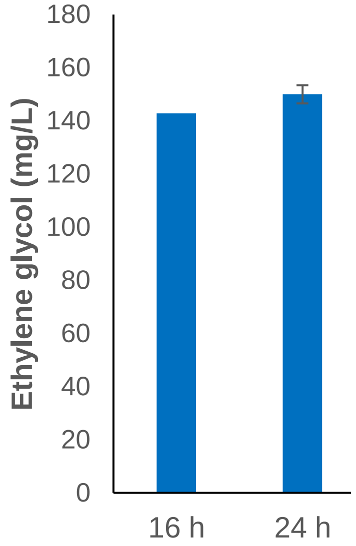
Pathway prototyping and host engineering for EG production

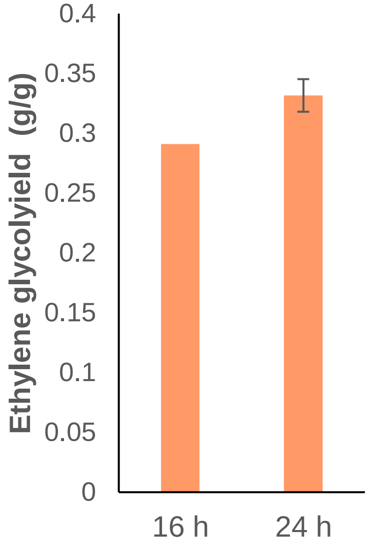
Production of ethylene glycol from formate in cell-free system







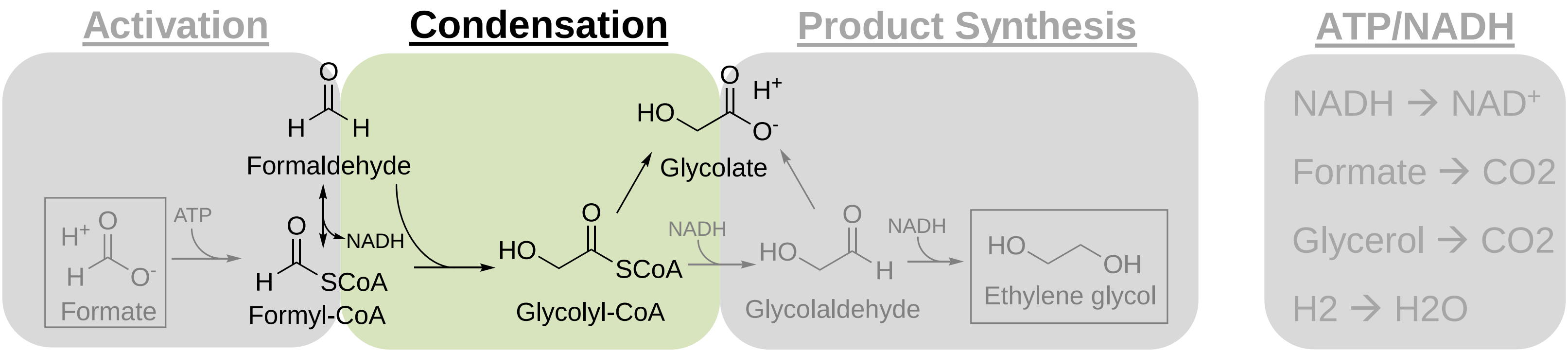


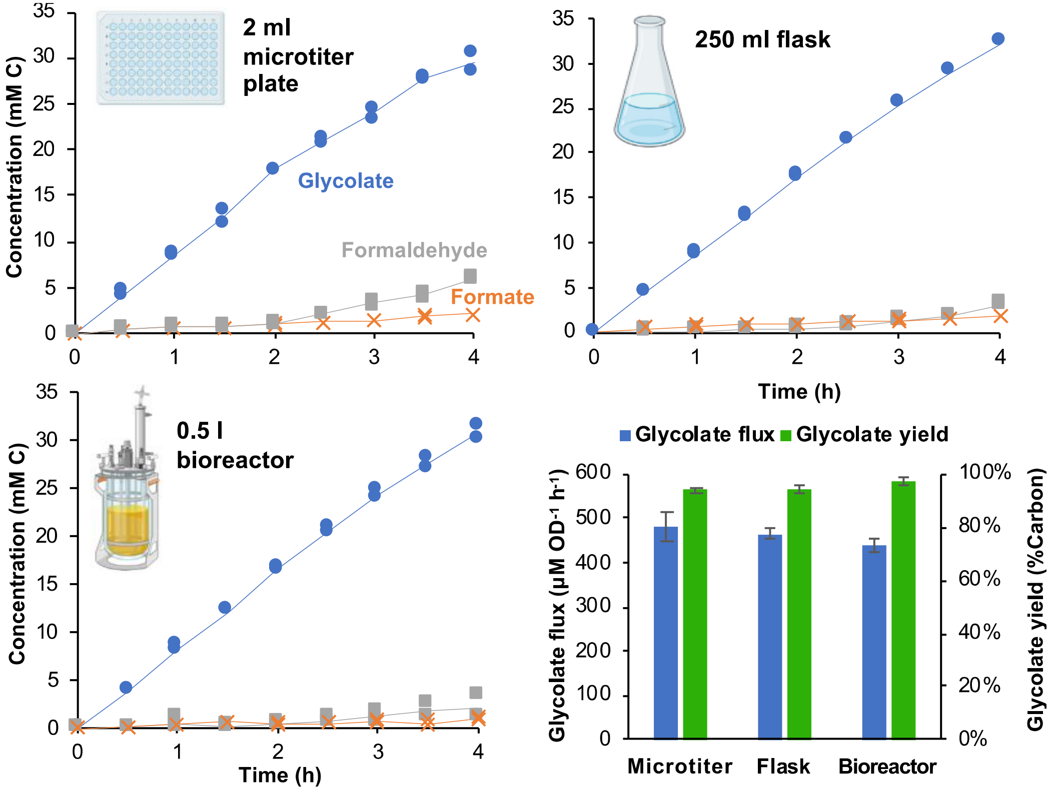


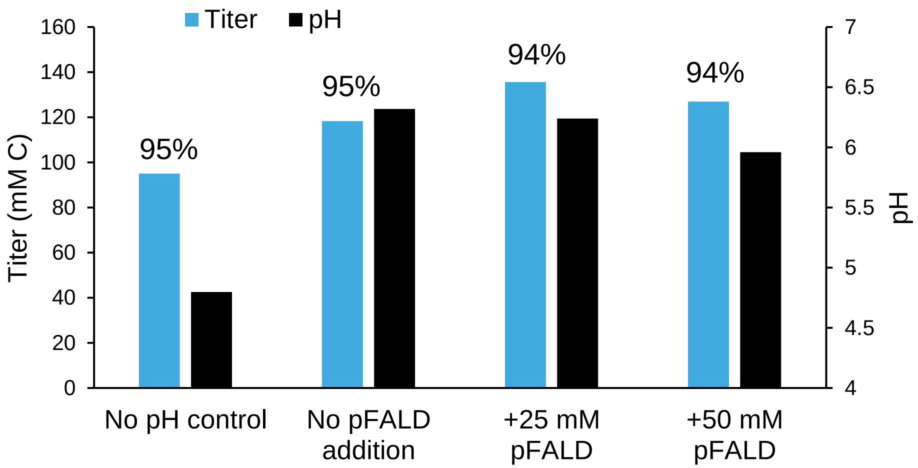
- The cell-free system was successfully scaled up to 15 mL tubes
- The titer reached 143 mg/L after 16 h incubation with a yield of 0.29 g/g

Met the milestone

Demonstration of core condensation pathway at TRL4

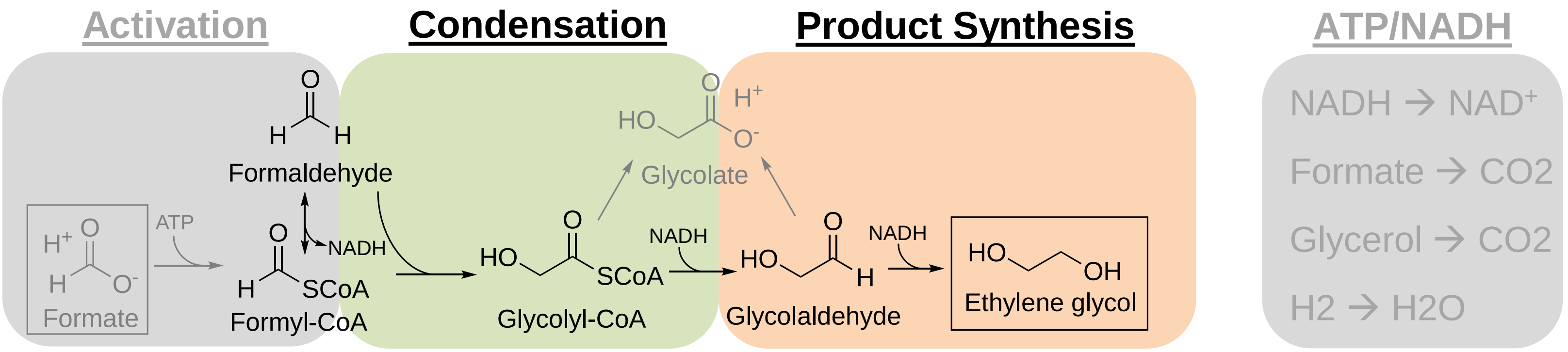


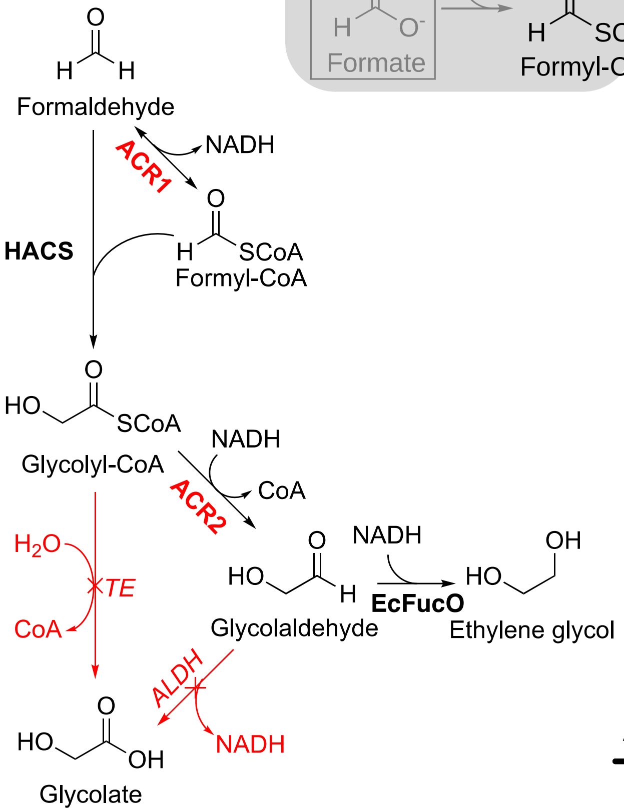


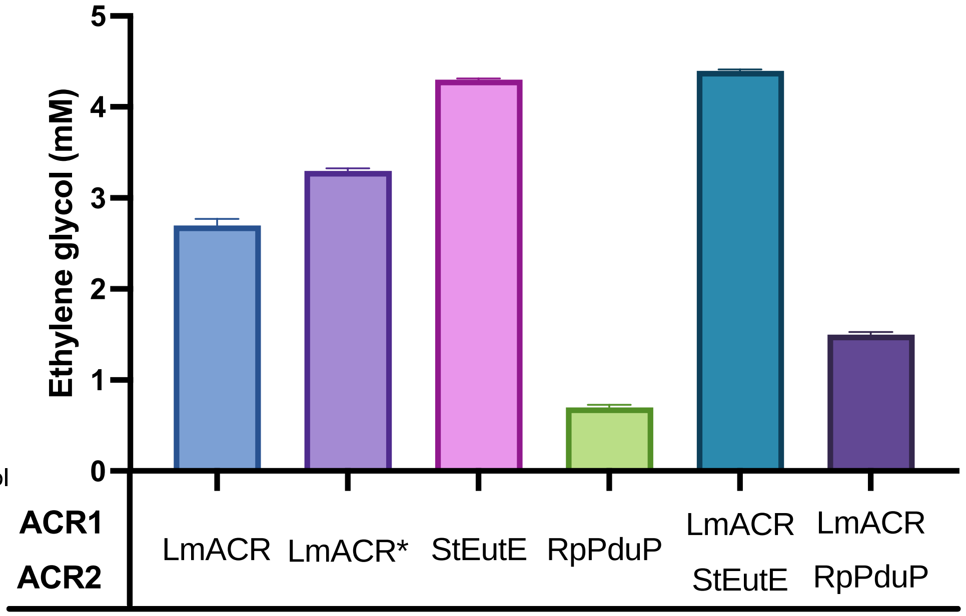


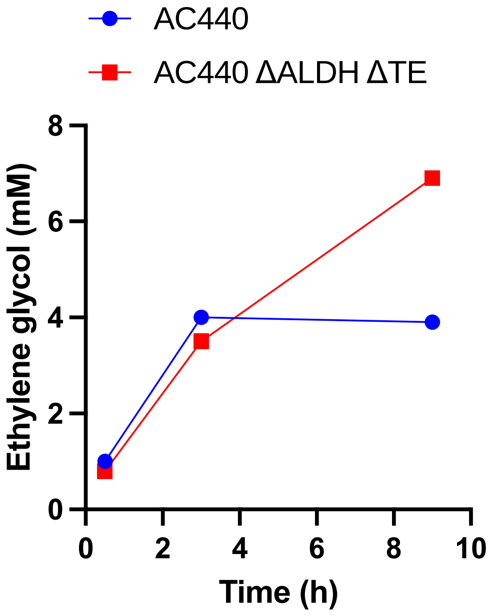
- Core condensation pathway (formaldehyde to glycolate) was demonstrated with remarkable scalability across production platforms and scales (2 ml microtiter plates to 0.5 L bioreactors)
- Industrially relevant product titer, rate and yield of 5.2 g/L (67.8 mM), 0.22 g/L/h and 94% carbon yield, respectively.

Optimization of product synthesis pathways



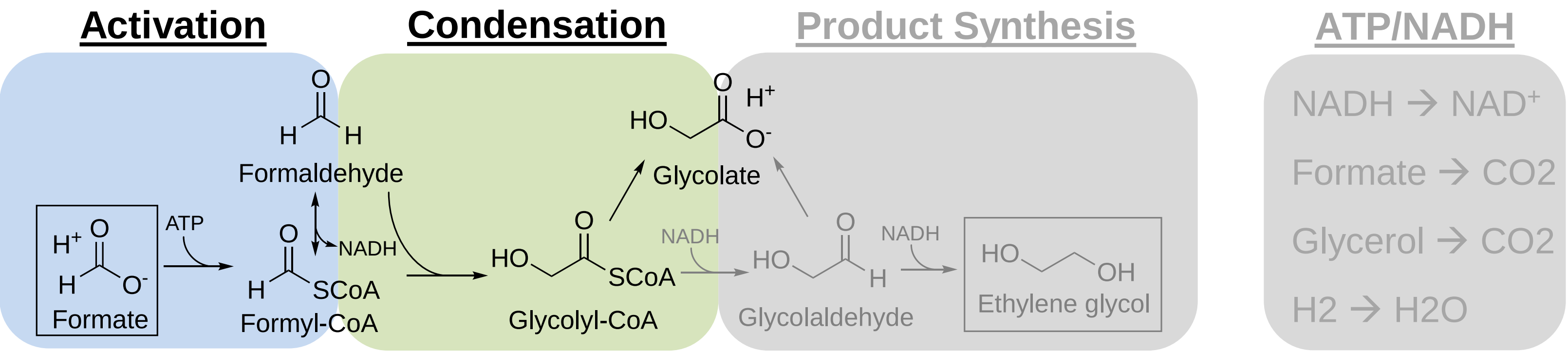


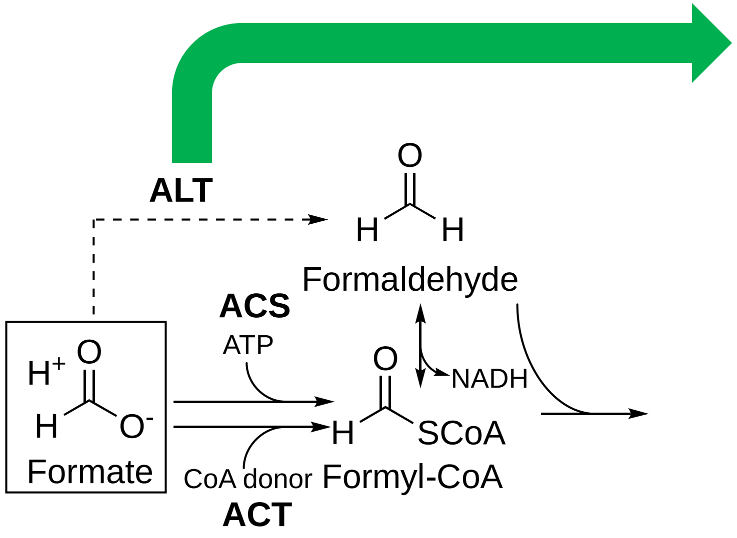


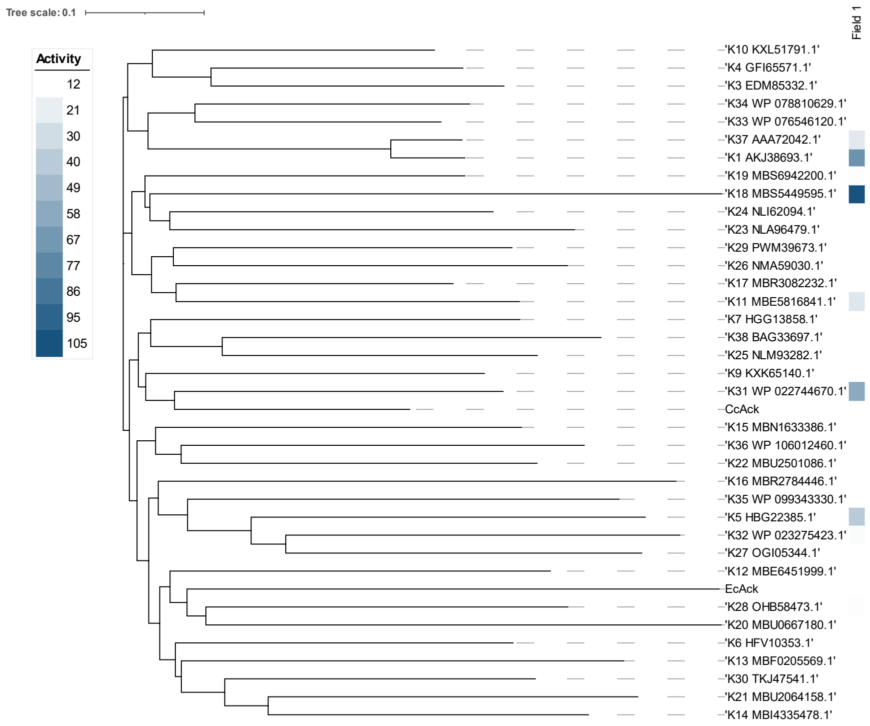


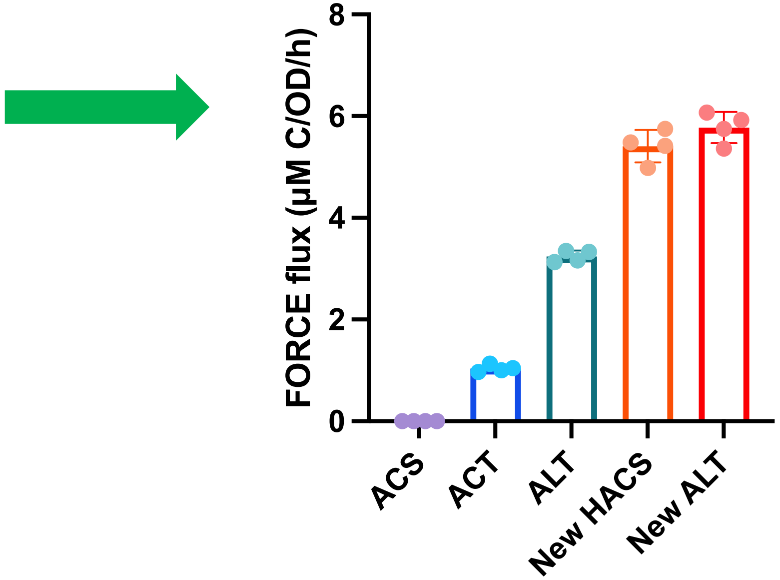
Pathway optimization by screening different combination of ACR1 & 2 and host strain engineering resulted in up to 7 mM (0.43 g/L) ethylene glycol from formaldehyde within 9 hours

Exploring formate activation pathways



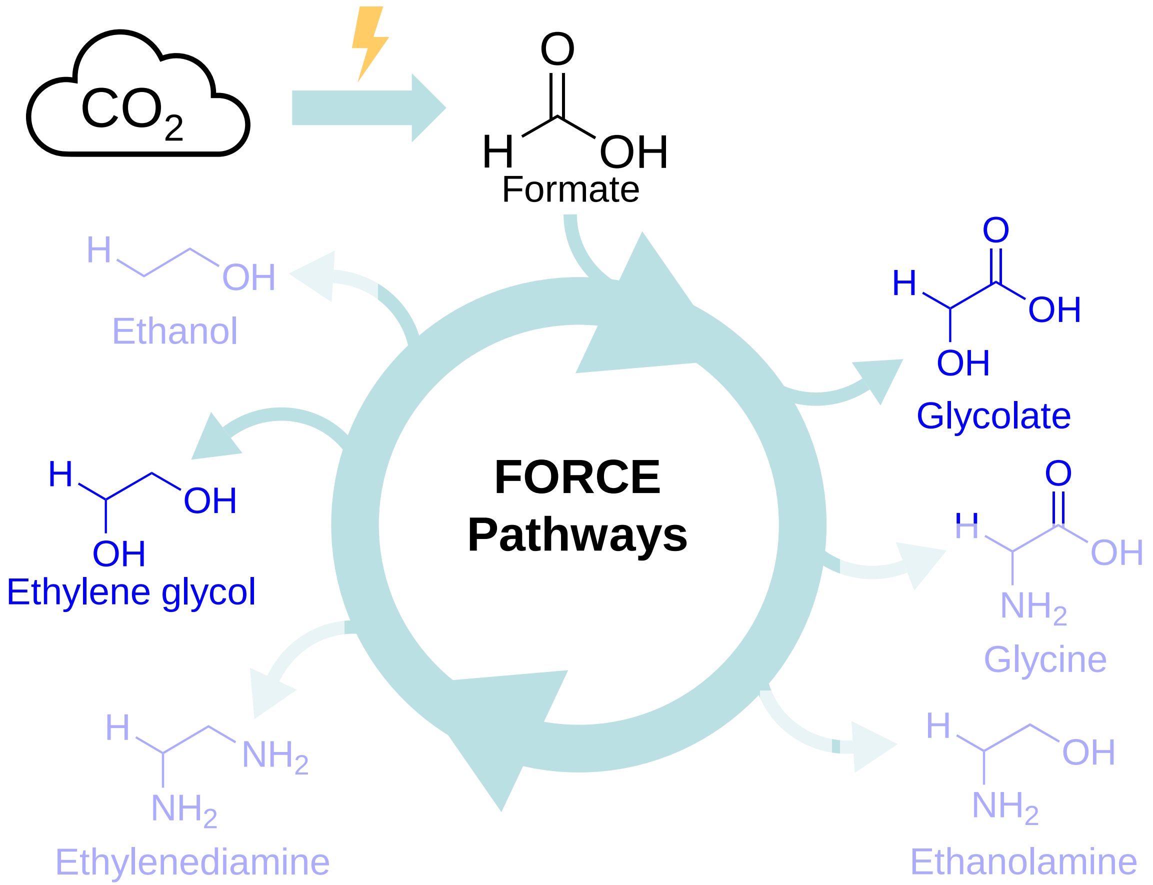






Identification of alternative route (ALT) for formate activation followed by bioprospecting resulted in 6-fold improvement in FORCE flux from formate

3 – Impact – Biological Upconversion



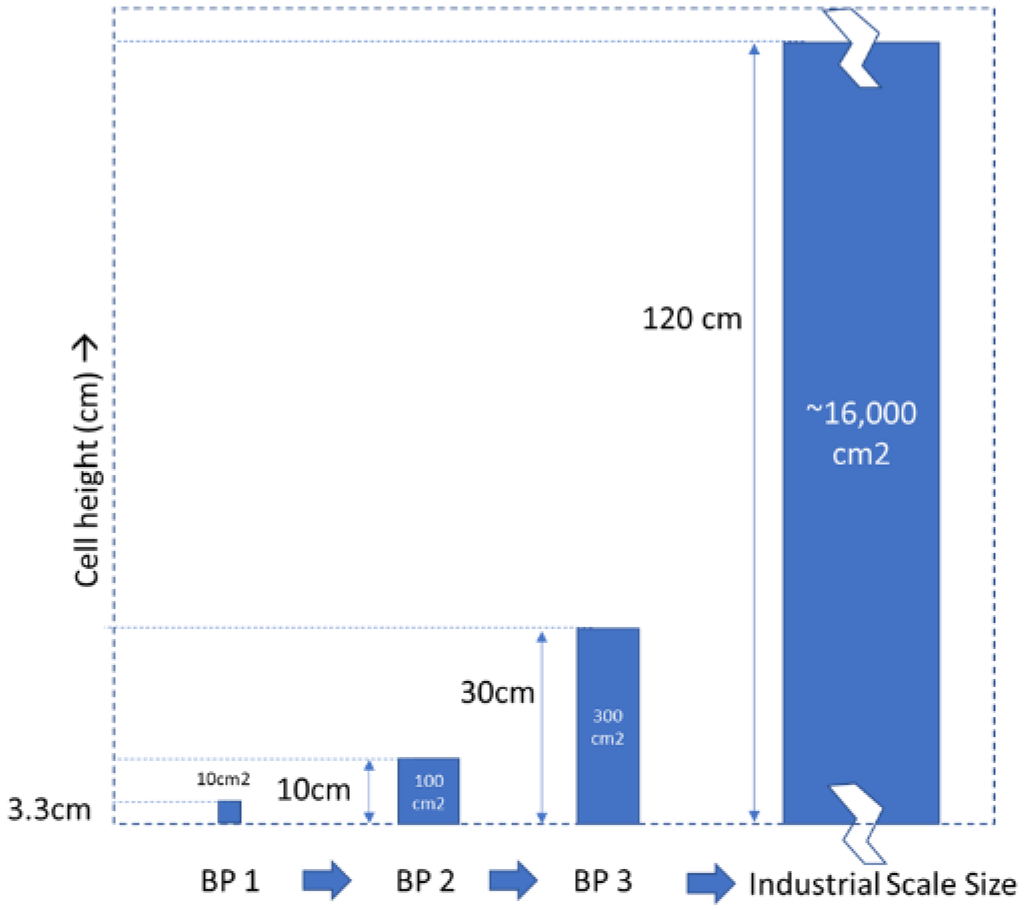
- First demonstration of biological production of a key chemical precursor, ethylene glycol, from CO2-derived formate as sole carbon source
- Industrially relevant titer (>5g/L), rate (>0.2g/L/h) and yield (>90%) and remarkable scalability across different format was demonstrated for the core condensation pathway, which can be further extended for formate to ethylene glycol conversion
- FORCE pathways, the core platform for biological upconversion, allow variety of other value-added product synthesis.

Publications: ***Nature Metabolism 2021, 3:1385; Nat Chem Biol 2019, 15:900***

Patents: PCT/US2021/047765; PCT/US2022/036856

3 – Impact – Electrochemical CO_2 reduction to formate

- Impact of this work is in the scale-up of electrochemical reactors
- Typically, academically employed cells are only 5-10cm2 (about 3cm in height, industrial reactor being ~120cm in height) and hence unable to study the impact of hydrodynamics, mass transport limitations, electrode/GDE contact electrical losses etc., which will become apparent only at sizes of 100cm2 or higher (i.e., 10cm height or higher)
- Through the support provided by DOE, OCOchem and team are able to develop and optimize GDE design that provide favorable hydrodynamics enabling negligible CO2 mass transport limitations and GDE flooding, thereby making the technology progressively feasible for commercial application to industrial scales.
- OCO Presented its formate process at the World Petroleum Conference December 6-9, 2021 in Houston, TX, at the Conoco Phillips Innovation Zone, winning 4th place out of 32 finalists.



3 – Impact - Freeze Tape Casting of Gas Diffusion Membranes

- Developed method of reducing SnO_2 surface layer on tin particles to allow sintering. Allows creation of porous tin membranes that can be sintered at low temperatures compared to other metals.
- Demonstrated ability to incorporate catalyst and hydrophobic treatment into membrane.
- First ever laterally graded porosity structures by tape casting. Has potential for controlling fluid flow distribution in planar reactor systems (electrolytic cells, fuel cells, etc.)
- Sofie, H; Halvoersen, T, Marotta, M and Sofie. S, “Thermal Consolidation of Freeze Tape Cast (FTC) Tin Particulate using Ammonium Chloride, “ in final preparation for submission.

Milestone Summary Table									
	Recipient Name:	Montana State University							
	Project Title:	Development of a scalable, robust electrocatalytic technology for conversion of CO_2 to formic acid via microstructured materials and subsequent upconversion to ethylene glycol							
Task Number	**Task Title**	**Milestone Type**	**Milestone Number***	**Milestone Description (Go/No-Go Decision Criteria)**	**Milestone Verification Process**	**Month**	**Quarter**	**Completion Date**	
1	**Initial Verification**	**Milestone Go/No-Go**		**Initial Verification by DOE**	**performed by DOE**	**10**	**4**	**7/24/2019**	
2	Electrochemical testing	Milestone	2.1	Establish optimal performance indicators	Test data recorded, documented	18	6	6/30/2020	
3	Modify existing freeze casting platform…	Milestone	3.1	Demonstrate lateral pore gradients in cast tapes 2mm thick at >3cm in length SMART (Go/No-Go)	SEM analysis	21	7	9/30/2020	
5	Identification of enzyme candidates	Milestone	5.1	Defined set of enzymes with ≥5 candidates for each bioconversion step	Data recorded and reported to DOE	15	5	3/31/2020	
7	Reactor integration of porosity components	Milestone	7.1	Demonstrate100cm2 cell meeting specified performance targets SMART (Go/No-Go)	Data recorded and reported to DOE	35	12	8/31/2021	
8	Scaling of lateral pore gradient membranes	Milestone	8.1	Demonstration of freeze tape casting scalability and empirical validation of graded porosity flow performance.	Data recorded and reported to DOE	35	12	8/31/2021	
10	Prototyping the bio-conversion pathway…	Milestone	10.1	Demonstrate of ethylene glycol production from formate in vitro	Data recorded and reported to DOE	30	10	3/30/2021	
11	Engineer microorganisms…	Milestone	11.1	Demonstrate growth and genetic manipulation of an organism in process media…SMART	Data recorded and reported to DOE	24	8	12/30/2020	
	Project Intermediate Verification	**Milestone, Go/No-Go**		**Project Intermediate Verification by DOE**	**performed by DOE**	**35**	**12**	**8/31/2021**	
15	Engineer microorganisms…	Milestone	15.1	Engineered organisms having a suitable genetic background and expressing the pathway…	Data recorded and reported to DOE	38	13	11/30/2021	
12	Optimize 300 cm2 graded membranes	Milestone	12.1	Optimize pore gradient distribution for device prototyping and testing	Data recorded and reported to DOE	41	14		
13	Scale reactor to 300 cm2	Milestone,	13.1	300cm2 cell meeting specified electrochemical performance targets SMART (Go/No-Go)	Data recorded and reported to DOE	47	16		
14	Optimize formate generation chemistry	Milestone	14.1	Provision of final optimized chemistry based formate for direct bio consumption SMART	Data recorded and reported to DOE	44	15		
15	Develop bio-conversion process…	Milestone	15.2	Production of ethylene glycol from formate in process media at TRL4	Data recorded and reported to DOE	48	16		

Summary

- *Electrochemical reactor has been scaled to 100 cm^2 and exceeds performance targets*
- *Freeze Tape casting on graded porosity membranes has been achieved and scaled. A method has been developed to sinter tin membranes at relatively low temperatures.*
- *An optimized pathway for formate activation to product synthesis has been devised.*

Quad Chart Overview

Timeline

- *10/01/2018*
- *6/30/2023*

	FY22 Costed	Total Award
DOE Funding	*(10/01/2021 – 9/30/2022)* *$168,464*	*(negotiated total federal share)* *$1,483,982*
Project Cost Share *	$100,977	$378,976

TRL at Project Start: TRL1

TRL at Project End: TRL5 (Electrochemical) 4 (Upconversion)

Project Goal

Include a concise, clear project goal statement

The goal of this project is to develop a scalable electrochemical reduction of CO_2 to formate combined with a biochemical upconversion of formate to ethylene glycol or other commercially relevant multiicarbon compound

End of Project Milestone

300 cm^2 cell meeting electrochemical performance requirements and production of ethylene glycol from formate in process media

Funding Mechanism

- *FOA 0001916 BioEnergy Engineering for Products Synthesis (BEEPS) Topic area 5: Rewiring Carbon Utilization*

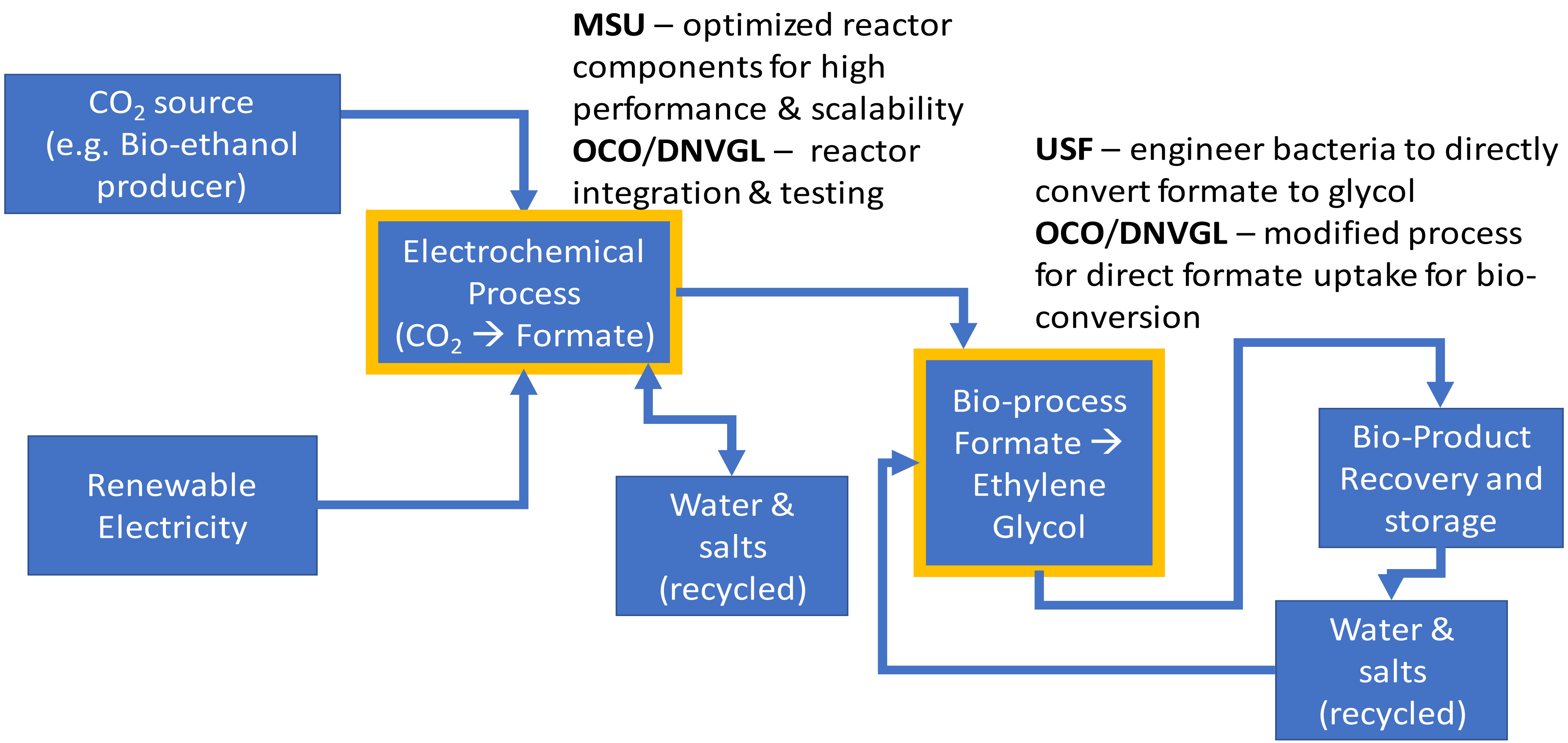
Project Partners*

- University of South Florida
- OCO

*Only fill out if applicable.

Additional Slides

Block Flow Diagram



(Not a template slide – for information purposes only)

- *The following slides are to be included in your submission for evaluation purposes, but will **not** be part of your oral presentation –*
- *You may refer to them during the Q&A period if they are helpful to you in explaining certain points.*

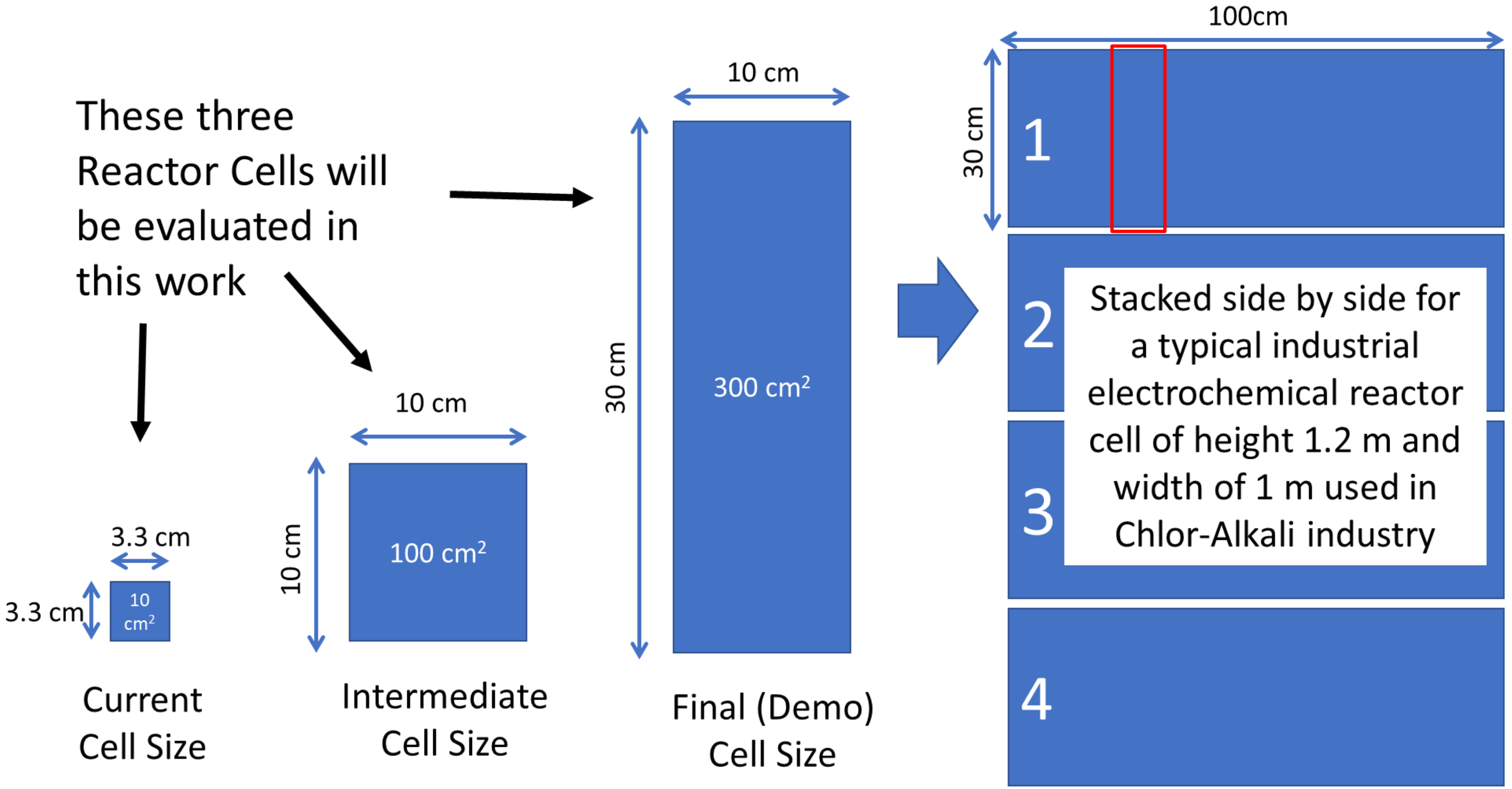
Responses to Previous Reviewers' Comments

- *Comment:* The performance of the larger (100 cm2) electrolyzers is significantly worse than the smaller (10 cm2) electrolyzers and there is a large gap between the current results and the end-of-project goal.
  - *Response*: The performance targets for the 100 cm^2 electrolyzer has now been met and exceeded
- *Comment:* There is not yet any demonstration of microbial ethylene glycol synthesis from a formate feedstock, the use of formaldehyde is not a reasonable substitute for formate.
  - *Response*: Have now demonstrated use of formate as sole carbon source for glycolate production

Publications, Patents, Presentations, Awards, and Commercialization

- Chou, A., Lee, S.H., Zhu, F., Clomburg, J.M., and Gonzalez, R. (2021). An orthogonal metabolic framework for one-carbon utilization. Nature Metabolism 3 (DOI: 10.1038/s42255-021-00453-0).
- OCO Presented its formate process at the World Petroleum Conference December 6-9, 2021 in Houston, TX, at the Conoco Phillips Innovation Zone, winning 4th place out of 32 finalists.
- Gonzalez, Ramon, Metabolic engineering for the biomanufacturing of small organic molecules. Arizona State University, BioDesign Institute, January 12, 2022.
- Sofie, H; Halvoersen, T, Marotta, M and Sofie. S, “Thermal Consolidation of Freeze Tape Cast (FTC) Tin Particulate using Ammonium Chloride, “ in final preparation for submission.

1 – Approach: Electrochemical Reactor Performance / Scaling



- **Determine optimal operating parameters**
- **Process fluids for bio conversion**

- **Test laterally graded porous structures**
- **Inform scaling process**

2 – Progress and Outcomes

Number of Task, Milestone	Tasks, , Milestones, Deliverables	Approved Completion Da	Actual Completion Date	Completion Criteria method of measurement
1	Initial Verification			
1.GN.1	Project initiation verification by DOE		24-Jun-19	Initial Verificatoin Visit and Report
2	Electrochemical testing			
2.ML.1	Establish optimal performance indicators		30-Jun-20	Optimal performace indicators identified.
3	Modify existing freeze casting platform to perform lateral pore grading in membranes			
3.GN.1	Demonstrate lateral pore gradients in cast tapes 2mm thick at >3cm in length		30-Sep-20	2mm thick at >3cm in length
4	Formate generation for bio processing			
5	Identification of enzyme candidates			
5.ML.1	Defined set of enzymes with ≥5 candidates for each bioconversion step		31-Mar-20	Identify ≥5 enzyme candidates
6	Characterize bacterial host strains capable of tolerating process condition			
7	Reactor integration of porosity components	31-Aug-21		
7.GN.1	Demonstrate 100 cm2 cell meeting performance targets	31-Aug-21	31-Aug-21	100 cm2 cell
8	Scaling of lateral pore gradient membranes	31-Aug-21		
8.ML.1	Demonstration of freeze tape casting scalability and empirical validation of graded porosity flow performance	31-Aug-21	31-Aug-21	Data showing improved flow distribution
9	Electrochemical performance at lower chloride concentrations			
10	Prototyping the conversion pathway of formate to ethylene glycol	31-Aug-21		
10.ML.1	Demonstration of ethylene glycol production from formate in vitro		30-Mar-21	Ethylene glycol produced from formate in vitro.
11	Engineer microorganisms for formate to ethylene glycol conversion	31-Aug-21		
11.ML.1	Demonstrate at least one doubling of cell density and the ability to maintain at least one synthetic DNA construct by an organism in process media with a multi-carbon source (e.g. glucose). SMART		30-Dec-20	At least one doubling of cell density and the ability to maintain at least one synthetic DNA construct by an organism in process media with a multi-carbon source (e.g. glucose). SMART
BP2 GN	Project Intermediate Verification by DOE	31-Aug-21	31-Aug-21	Intermediate verification and report
12	Optimize 300 cm2 graded membranes	30-Jun-23		

12.ML.1	Optimize pore gradient distribution for prototype & testing	28-Feb-23		Porous membrane showing flow distribution similar to modeled optimum distribution
13	Scale reactor to 300 cm2	30-Jun-23		
13.GN.1	300cm2 cell meeting specified electrochemical performance targets SMART	30-Jun-23		Obtain the target values set by Task 2
14	Optimize formate generation chemistry for direct bio consumption	30-Jun-23		
14.ML.1	Provision of final optimized chemistry based formate for direct bio consumption SMART	31-May-23		The lowest concentration of chloride where both; least loss in formate productivity (kWh/ton) and highest biocompatibility with USF bacteria for product, will become optimized chemistry for generation of the intermediate potassium formate.
15	Develop bioconversion process using genetically engineered microorganisms	30-Jun-23		
15.ML.1	Engineered organisms having a suitable genetic background and expressing the pathway enzymes		30-Nov-21	Engineered organisms havea suitable genetic background
15.ML.2	Production of ethylene glycol from formate in process media at TRL4	30-Jun-23		TRL4
16	Economic Evaluation	30-Jun-23		
17	Technology to Market	30-Jun-23		