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

DE-EE0008499

DOE Bioenergy Technologies Office (BETO) 2021 Project Peer Review

Date

BioEnergy Engineering for Products Synthesis (BEEPS)

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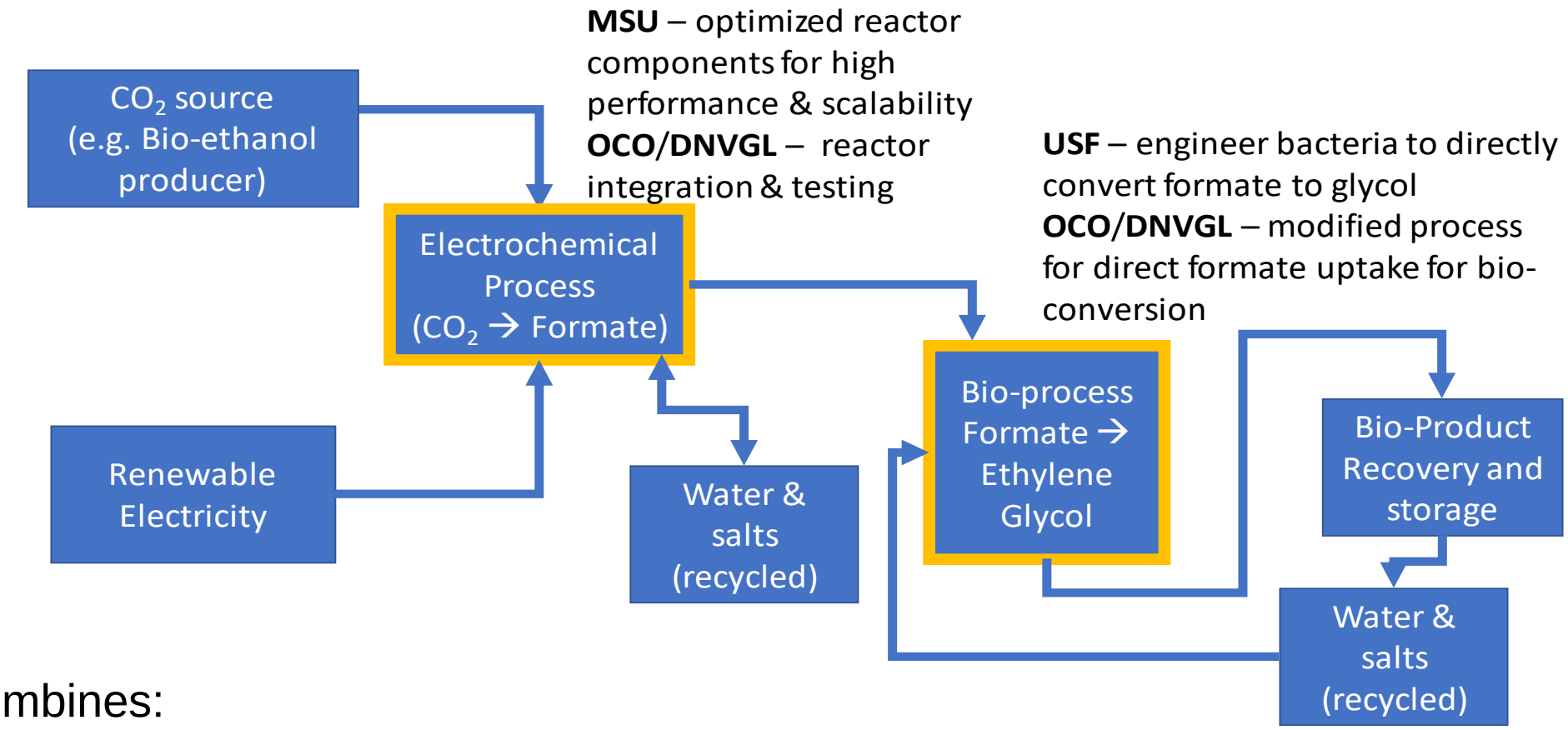
Project Overview

Response to FOA 0001916 BioEnergy Engineering for Products Synthesis (BEEPS) Topic area 5: Rewiring Carbon Utilization: 1) Use catalytic methods to reduce CO2 to single carbon intermediates; 2) Follow with biological upconversion to multi-carbon compounds

- *Improve and scale up an electrochemical reactor for reduction of CO_2 to formate*
  - *Develop graded porosity gas diffusors to address main cause of performance reduction with scale up*
  - *Determine performance / process fluid trade offs*
- *Engineer bacteria for direct upconversion of formate to ethylene glycol*
  - *Identify KCl tolerant host strains*
  - *Identify enzyme variants for the best candidate for each reaction step.*
- *Success would demonstrate new hybrid pathway to products from CO_2*

Block Flow Diagram



Project Combines:

- State-of-the-art electrochemical reactor technology for CO_2 reduction to formate
- Newly patented technology for graded porosity gas diffusion layers for scaling planar reactors
- Novel enzyme that can utilize C1 substrates for upconversion that is not part of the microorganism central metabolism

1 – Management

Project has Project Director supported by an Admin Professional at MSU, and co-PIs at OCO/DNVGL, USF and MSU.

Key Task areas - Responsible parties

Reactor construction, performance evaluation, electrolyte tuning – OCO/ DNVGL

Development of laterally graded porosity gas diffusion elements (and method to cast them) – MSU

Biological upgrading of Format to Ethylene Glycol - USF

Techno-economic analysis – OCO

Project Risks

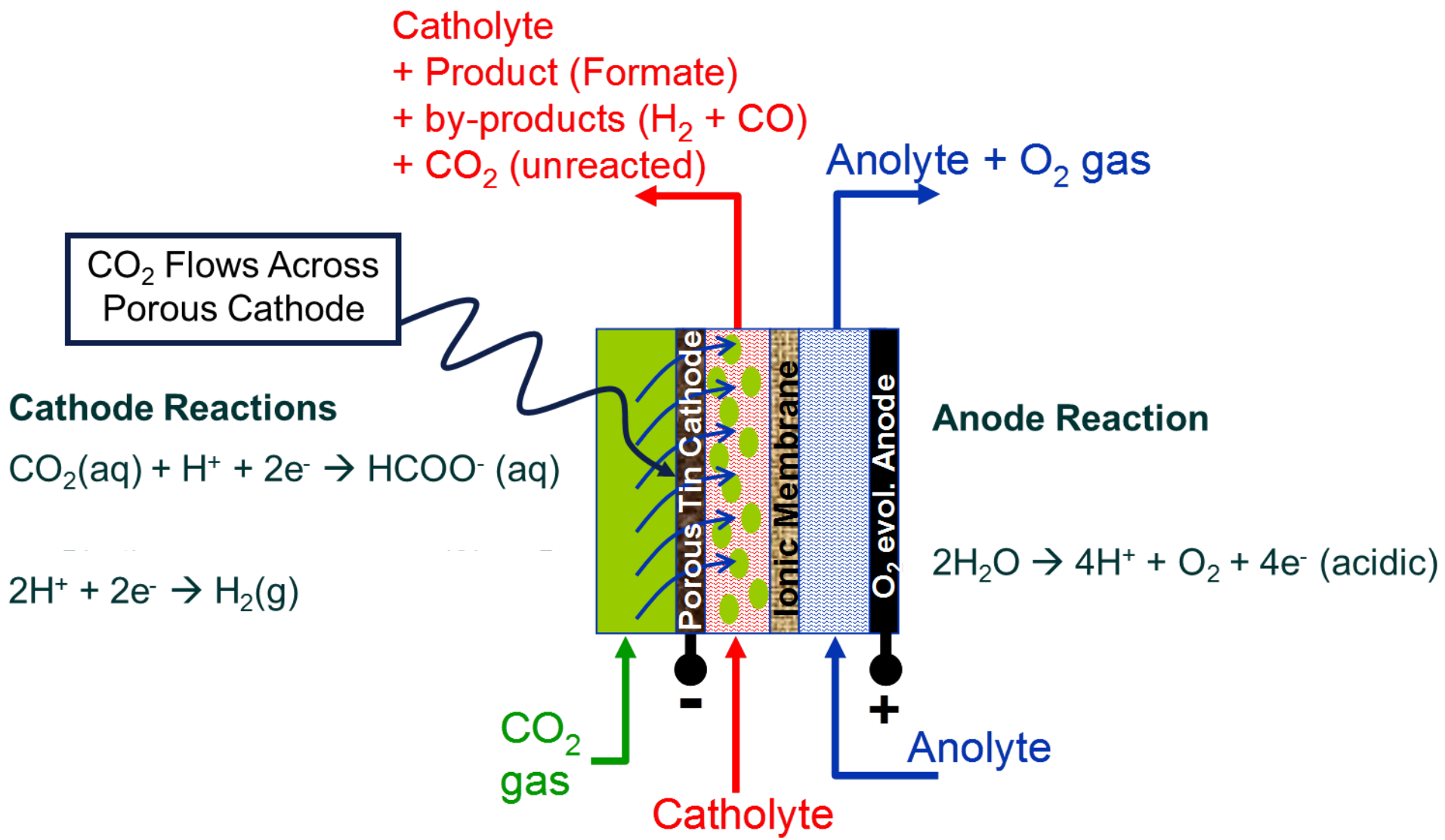
Scope creep & schedule creep – Quarterly virtual meetings with progress updates and checks against milestones and deliverables

Missed "hand-offs" – Also checked on quarterly meetings. Relevant bilateral interactions needed are identified.

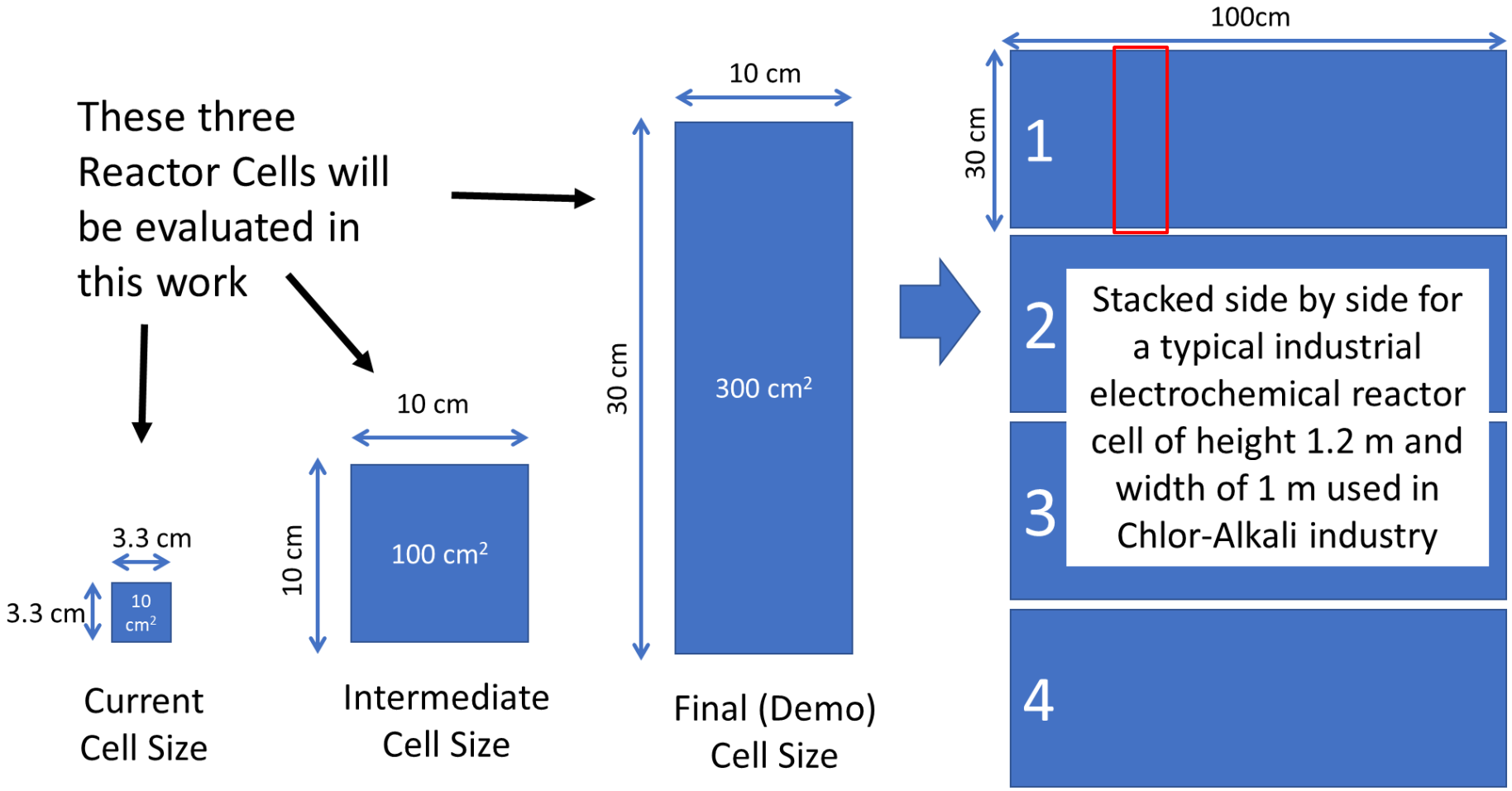
Technical risks – Milestones, SMARTs and Go / No Gos set for significant technical risk components

(Examples – Proof of ability to continuously grade porosity structures; Identification of host strains and enzymes; performance retention in electrochemical reactor scale-up)

2 – Approach: Electrochemical Reactor to Produce Formate



2 – Approach: Electrochemical Reactor Performance / Scaling



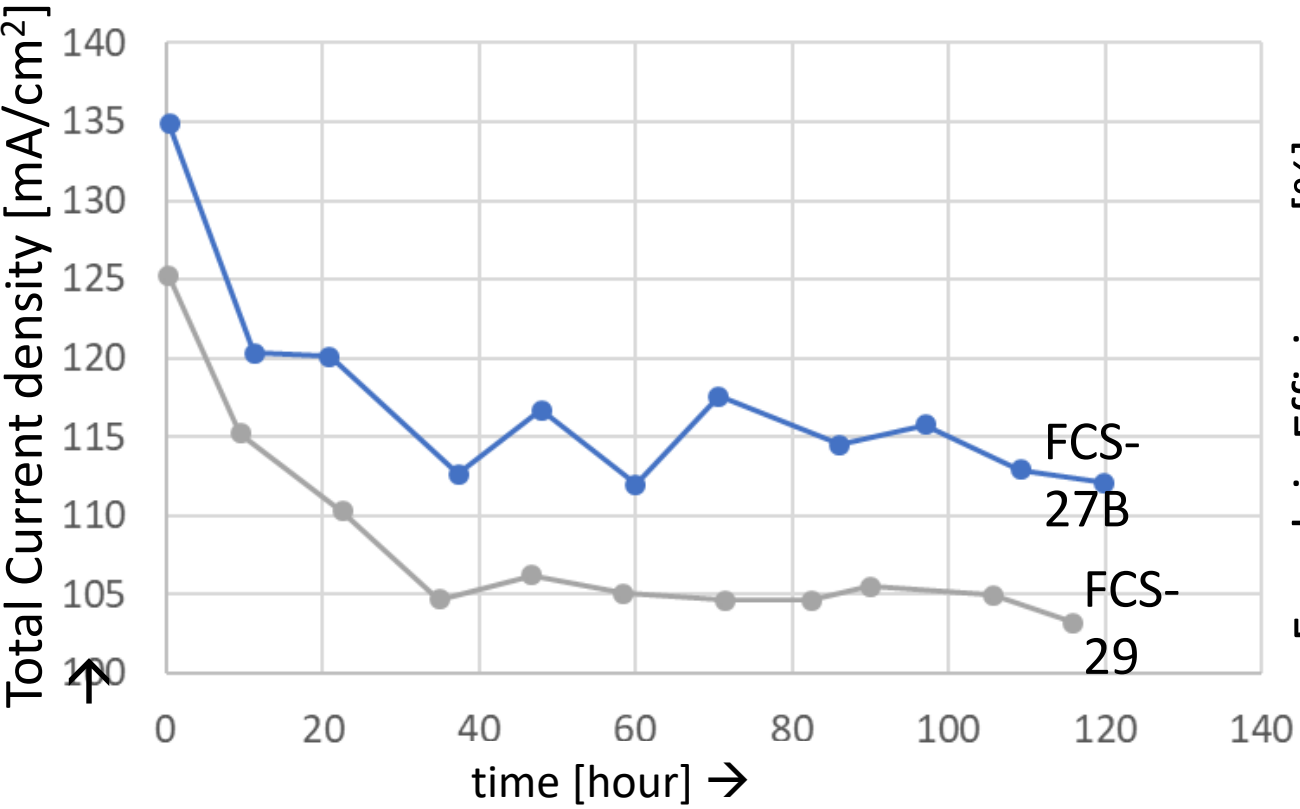
- **Determine optimal operating parameters**
- **Process fluids for bio conversion**

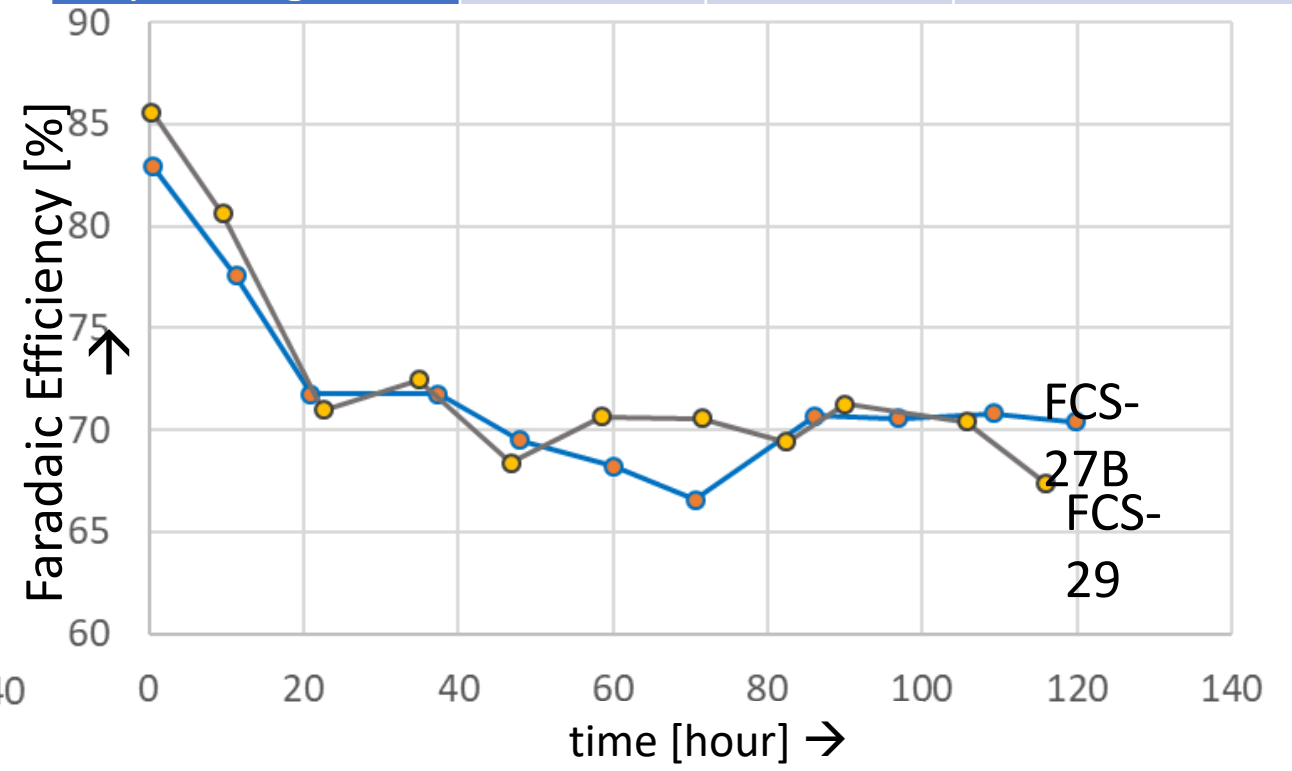
- **Test laterally graded porous structures**
- **Inform scaling process**

Q2-Task 2: Established Optimal Electrochemical Performance with 10 cm2 cell (Milestone 2.1 met) – results within 10% of proposed targets

FCS-27B = 1.85 mg/cm2 of 30 wt% Sn/C (=0.54 mg/cm2 Sn)
FCS-29 = 2.54 mg/cm2 of 52 wt% Sn/C (=1.26 mg/cm2 Sn)

Parameters	units	Proposed	Achieved
Vcell	V	4.2	4.2
Total Current Density	mA/cm2	125	110 (120 hr) 130 (1 hour)
FE	%	75	70
Operating time	hours	120	120





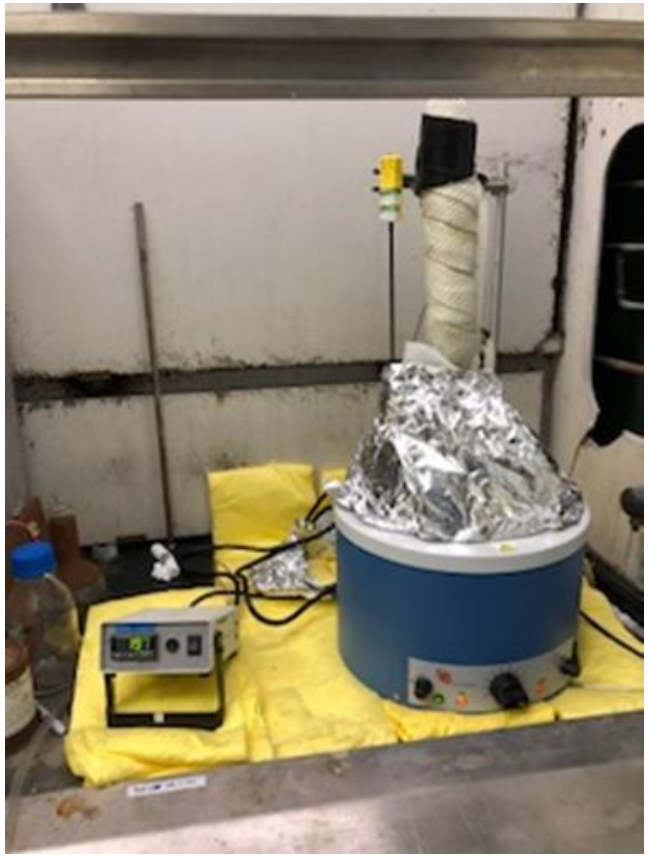
Task 2: Established Optimal Electrochemical Performance with 10 cm2 cell (Milestone 2.1 met) – results within 10% of proposed targets

Optimization of Electrochemical Process resulted from:

- Potentiostat/Booster, connections issues resolved
- Better coated electrodes
  - Optimized catalyst & binder loading
    - Higher Sn/C loading (thicker electrodes) have FE < 60%
    - Lower binder (<3%) causes higher current loss over time
- Optimal catalyst powders prepared:
  - 30 wt% Sn on Sn/C performs better than 50 wt% - more stable current & FE
- **Optimal Electrode = 1.85 mg/cm2 of 30 wt% Sn on Sn/C, 4wt% Nafion binder**

Task 2: Electrochemical Testing: Catalyst Scaleup for 100 cm2 (and BP3 – 300cm2) electrodes

- 10x scale up of Sn/C nano catalyst preparation in the lab (from ~0.25 g to ~2.50 g)
- required for making larger (100 cm^2 (for BP 2) and 300 cm^2 electrodes (for BP 3)).
- Performance matches to previous smaller batches (wt% of Sn in deposited Carbon powder is ~30%, same as that for smaller batch catalyst under same conditions).
- Further verification with electrochemical tests with 100 cm2 reactors in the upcoming quarter



Setup with the 5L heating/stirring mantle used for catalyst preparation, a significant scale up from 1 L previously used batches.

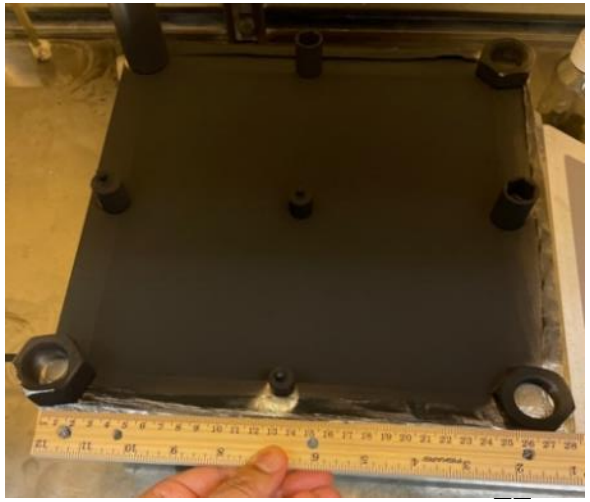
Q3-Task 2: Electrochemical Testing: 100 cm2 reactor cell work:

a) Procurement of 100 cm^2 reactor parts and setup for electrochemical testing, including making the cathode holder and obtaining anodes and Nafion membranes was completed:

b) Full cell assembly, electrode holder and initial electrode coating and incorporation with no-leak – testing completed

Task 2: Electrochemical Testing - Electrode Scale-up

	Electrode coating method	Electrode size	Vcell	Total Current Density (mA/cm2)	Faradaic Efficiency	Time tested for	Observed Catalyst Degradation
1	Air brush (10 tests) **Nov 2020**	100 cm^2	4.2 V	60 to 70	55% to 60% at 1 hr, drops to 15% in 16 hrs	up to 16 hrs	significant initial loss of catalyst particles
2	optimized - preval spray coating (6 tests) **Dec 2020**	100 cm^2	4.2 V	90 to 105	stays consistently 45% to 55% over 50 hrs	up to 50 hrs	after first 15 mins, no loss in catalyst particles

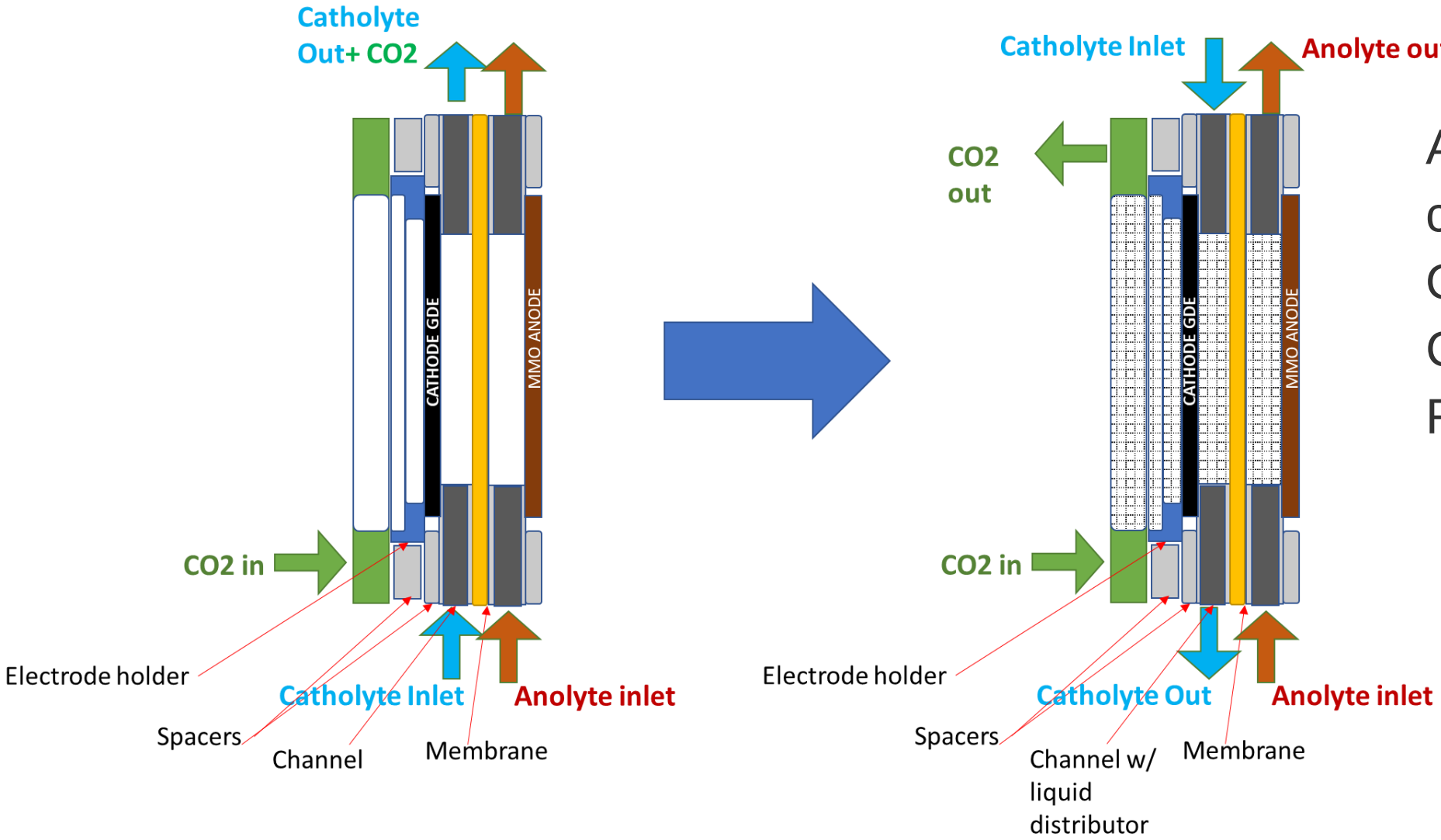


- method was scaled for faster turnaround (4, 100 cm^2 electrodes in 12 hours)
- Up to 625 cm2 electrode (works for BP3, 300cm2 electrodes)

Flooding in GDE

- carbon paper based GDE cathode undergoes ‘flooding’ or wetting over short times (few hrs with 100 cm2 cell vs. > 120 hr with 10cm2 cell)
- flooding reduces ability to generate a three phase CO2 gas-liquid electrolyte-catalyst contact that is needed for high CO2 dissolution and reaction
- Along with application of mesh, adjusting flowrates, improving design to reduce/eliminate flooding is critical

T2-Plans for Next Quarter: Electrolyzer Design Changes to Manage Flooding, optimize performance



Apply design concepts from Oxygen Depolarized Cathode (Chlor Alkali Reactors)

Task 2: Electrochemical Testing – 100 cm2 reactor

		Cell Voltage	Current Density	FE	Time of operation
	Target (achieved with 10cm2)	4.2 V	110 mA/cm2	70%	120 hr
	100 cm2 testing, N117	4.2 V	90-105 mA/cm2	40%-55%	Up to 50 hr

- Current density is 10-15% lower
  - The top portion not wetting even at high flowrates could account for the 10-15% lower current overall
  - Better wetting should account for this reduction

- FE is significantly lower (by 20-30%)
  - Lower FE has to be with less optimal CO2-Liq contact
  - Reduction over time could be due to flooding
  - Despite use of CO2 pre-saturated catholyte used

Task 4. Completed Formate Generation for Bio Processing (DNVGL)

Planned Activities

DNVGL has completed Task 4.

Actual Accomplishments

DNVGL has collected several batches of samples from electrochemical tests during the third quarter of 2020 with long term, 120 hour tests providing optimal electrochemical performance. These samples are in the process of being delivered to USF.

Plans for Next Quarter

Samples will be collected from new electrochemical tests and provided to USF for bio testing, with the modified process chemistry (low or no chloride that could significantly reduce any chloride based inhibition on bacteria growth).

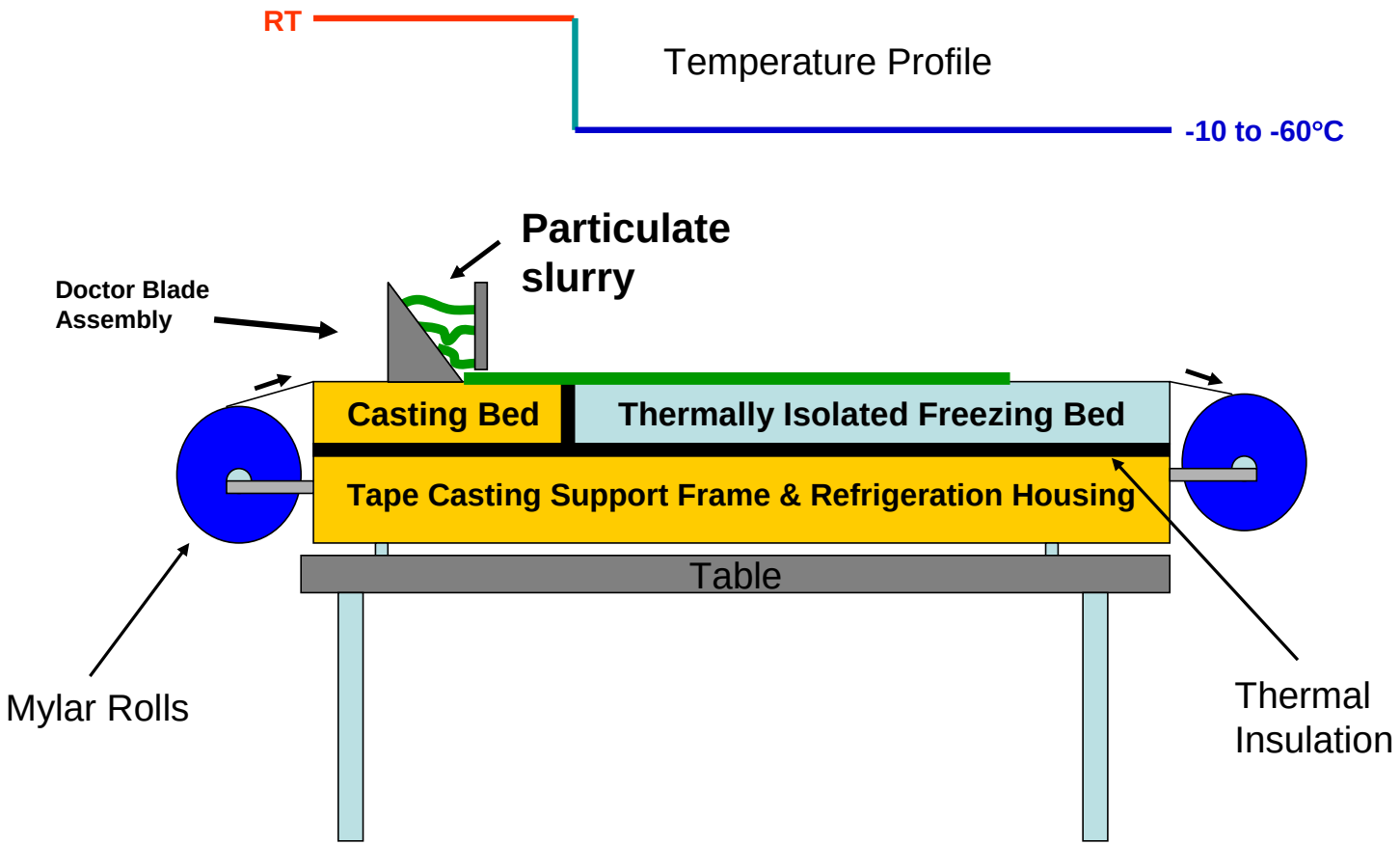
2 – Approach: Reactor Scaling, Graded Porosity Gas Distributer

Traditional pore forming through the utilization of thermal fugitives has limitations to the extent at which porosity can be tailored by size, morphology, cost, and processing complications related to their addition.

Functional grading of pores through traditional means involves several repeated processing steps and thermal decomposition of polymeric additives.

Aqueous freeze based processing provides a unique avenue to manipulate porosity, with ultra-low tortuosity.

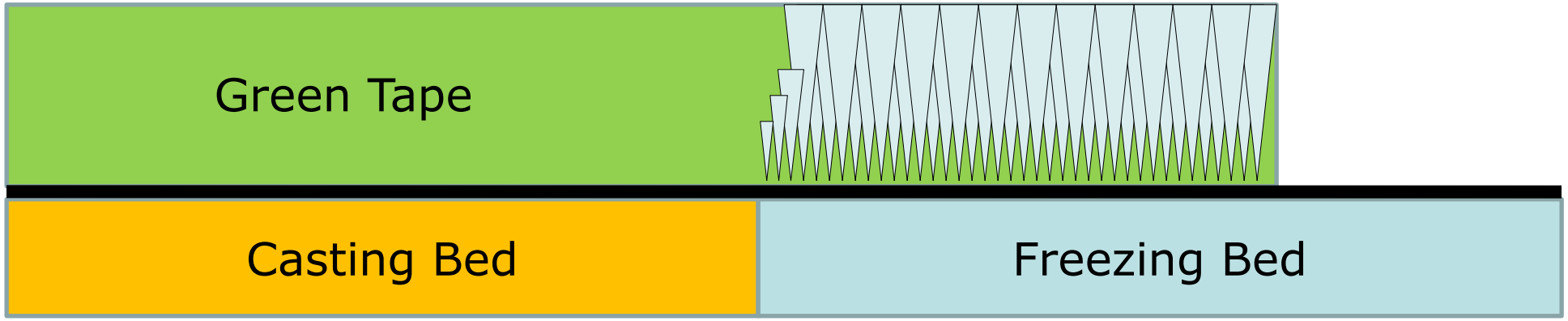
Freeze Based Processing (Freeze Tape Casting)

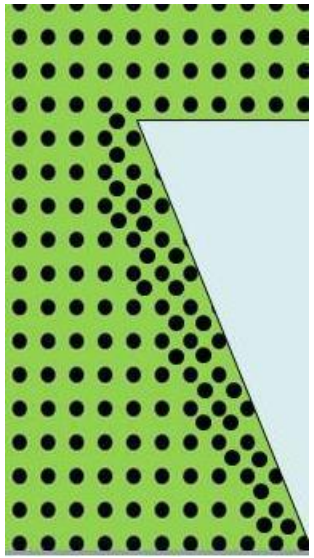


2 – Approach: Reactor Scaling, Graded Porosity Gas Distributer

Ice Templating - Pore Development

Ice crystals ➔ pores

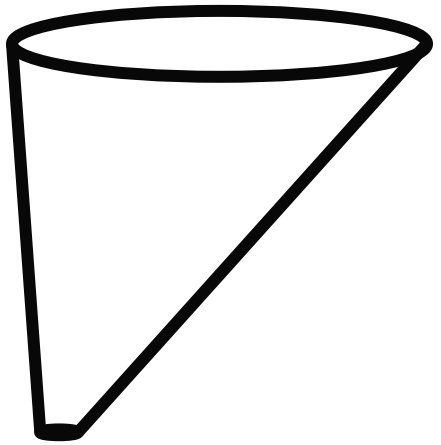




Particle Rejection Phenomenon

- Solidifying water rejects (metal, ceramic, polymer) normal to growth direction
- Regions in 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)

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



2 – Approach: Reactor Scaling, Graded Porosity Gas Distributer

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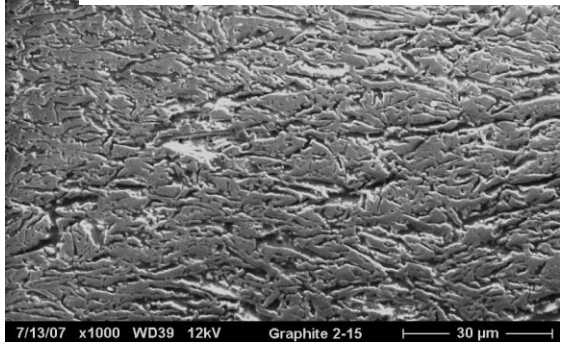
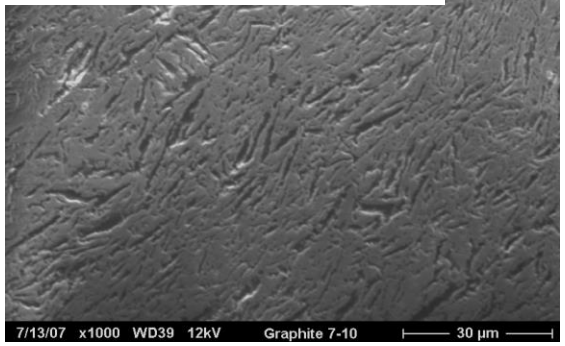
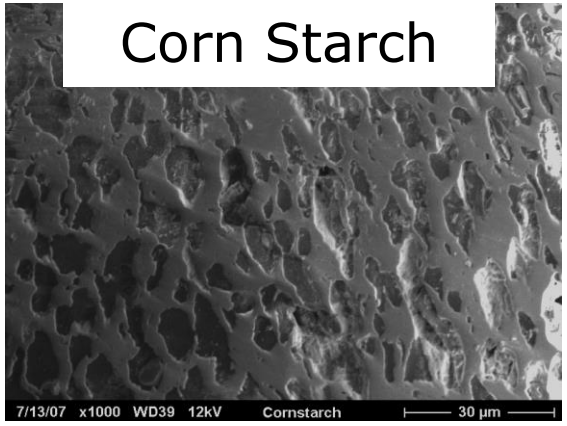
Traditional Pore Forming

Flake Graphite Pore Formers



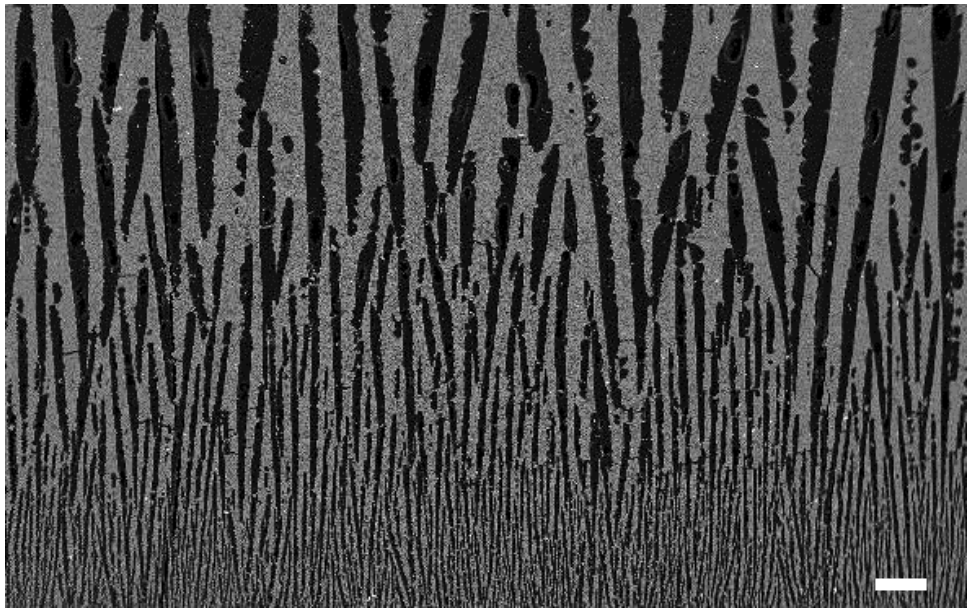


Corn Starch

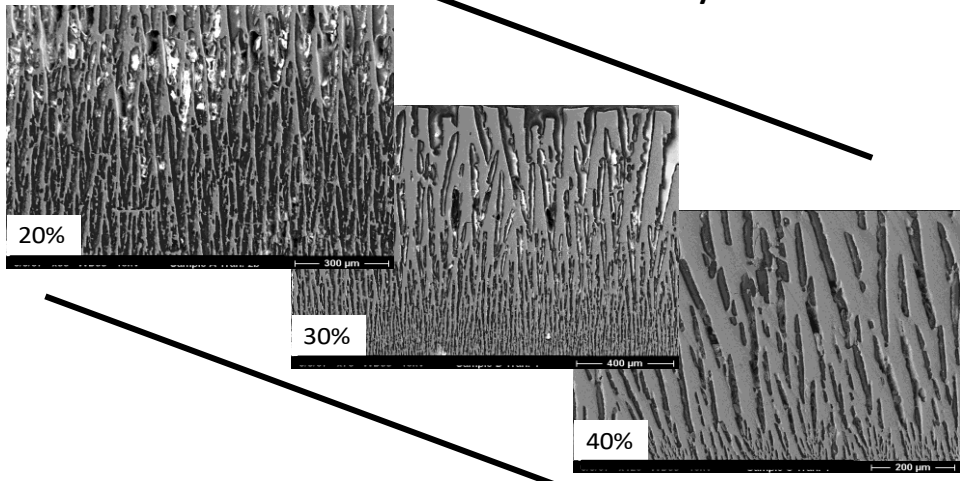


Glassy Carbon

Freeze Tape Casting



Porosity: 55 – 75%



Solids Loading: 20 – 40%

Task 3: Modify existing freeze casting platform to perform lateral pore grading

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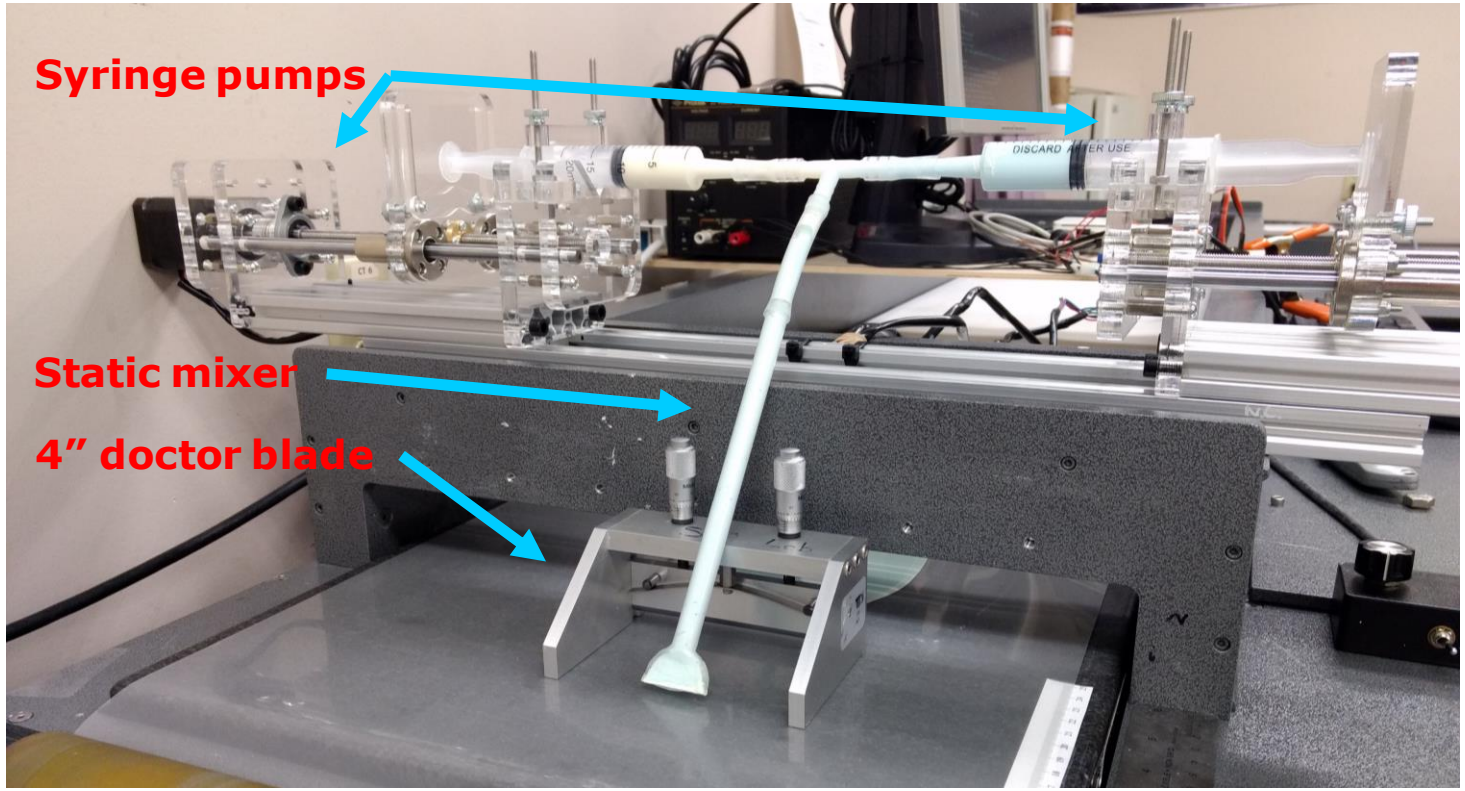
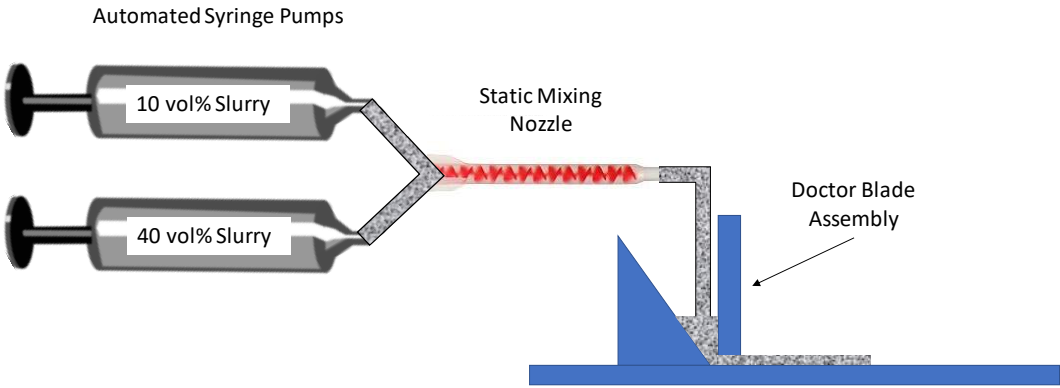
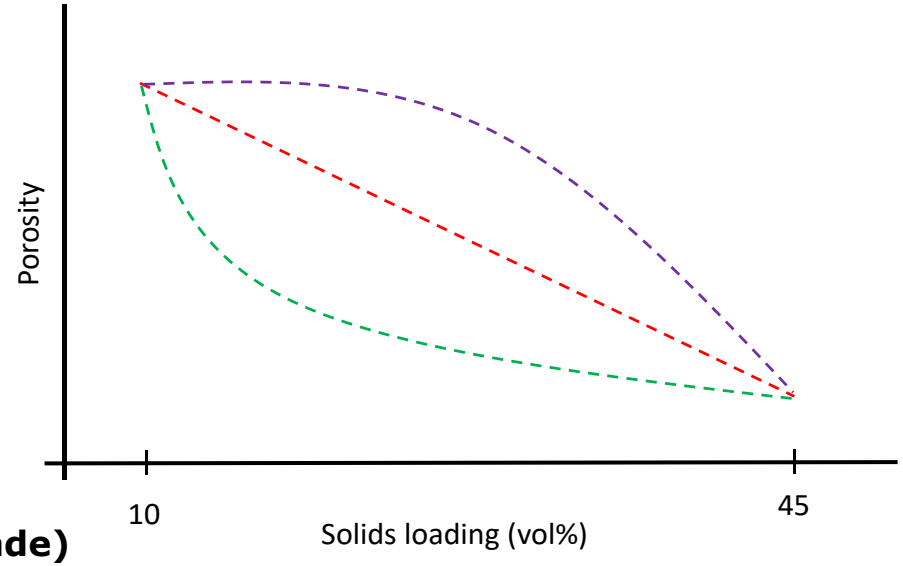
Complete







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)

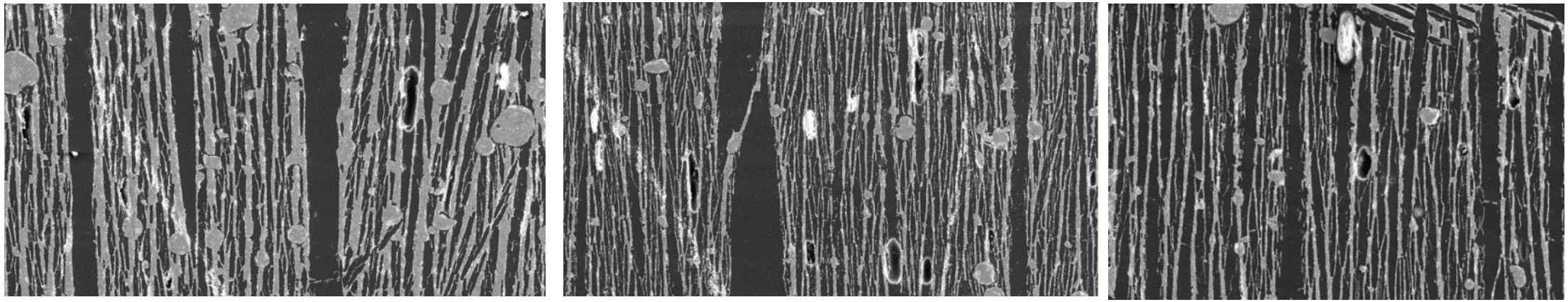
Variable casting rates possible, integrated within existing FTC platform

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

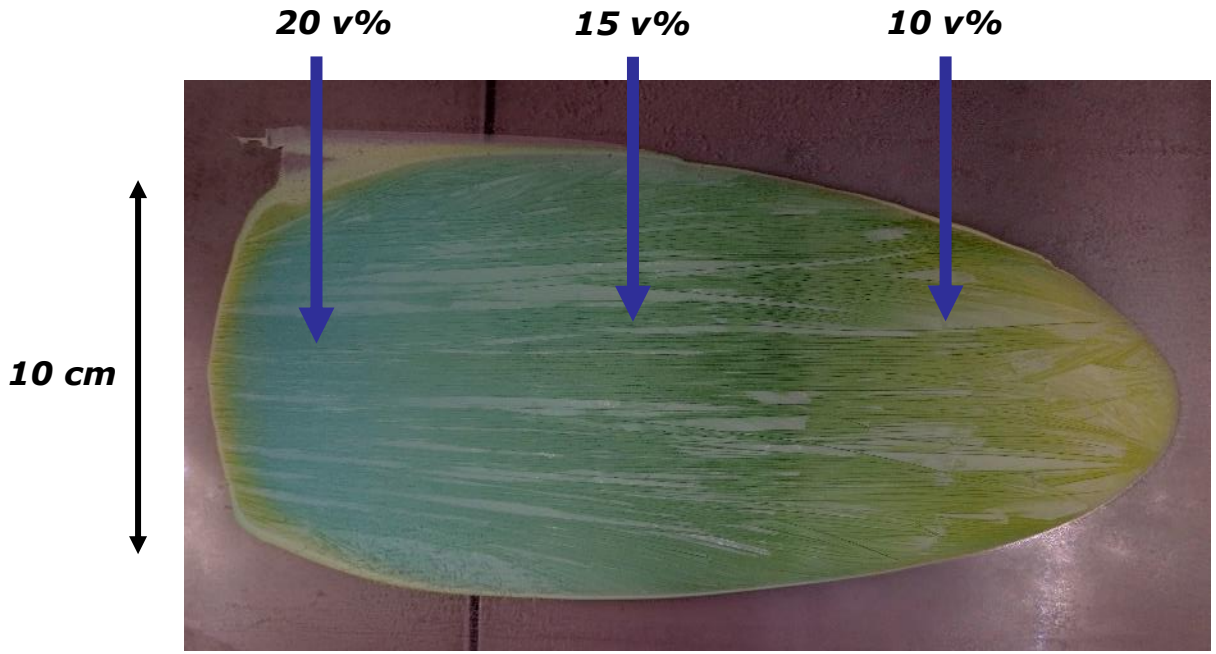
Milestone 3.1 **(Go/No-Go)** Demonstrate lateral pore gradients in cast tapes 2mm thick at >3cm in length

Complete

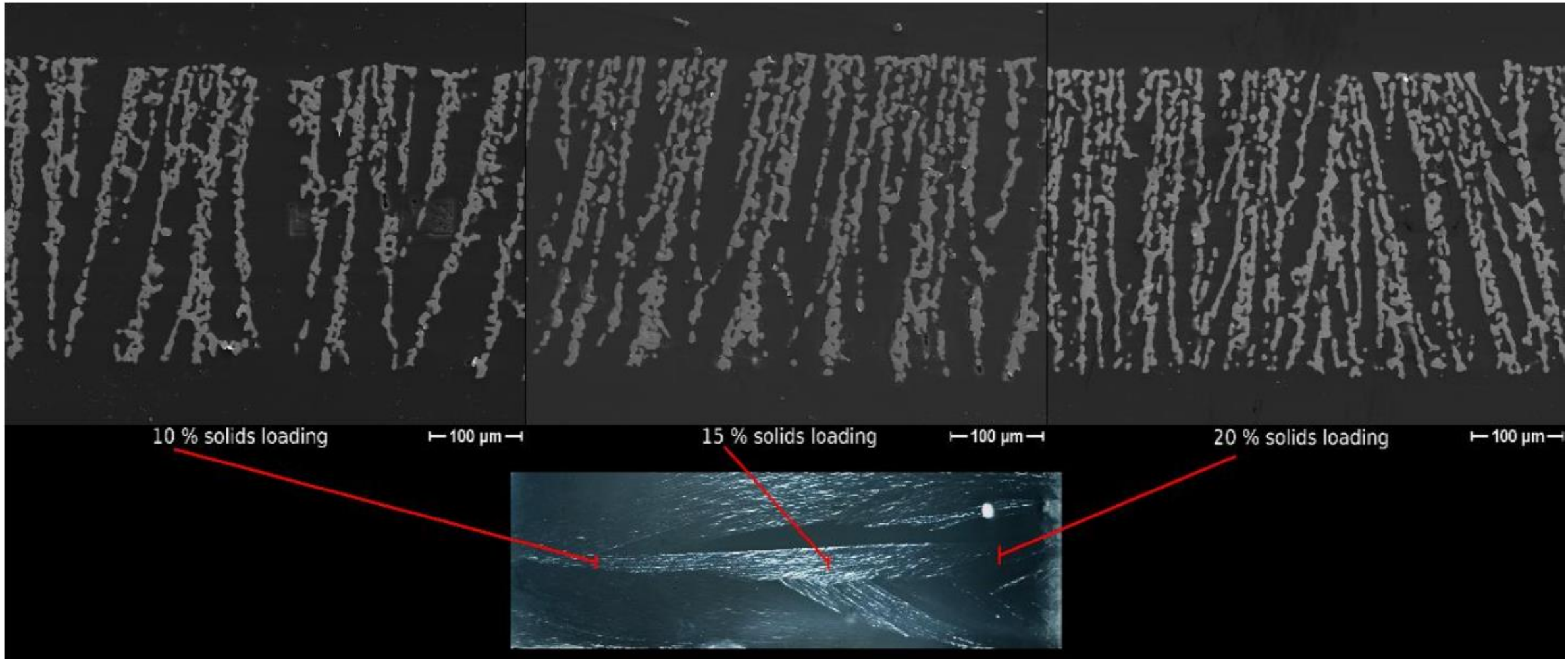
Ceramic Materials: Al2O3 and YSZ (utilized in the grading studies given the substantially improved toughness)



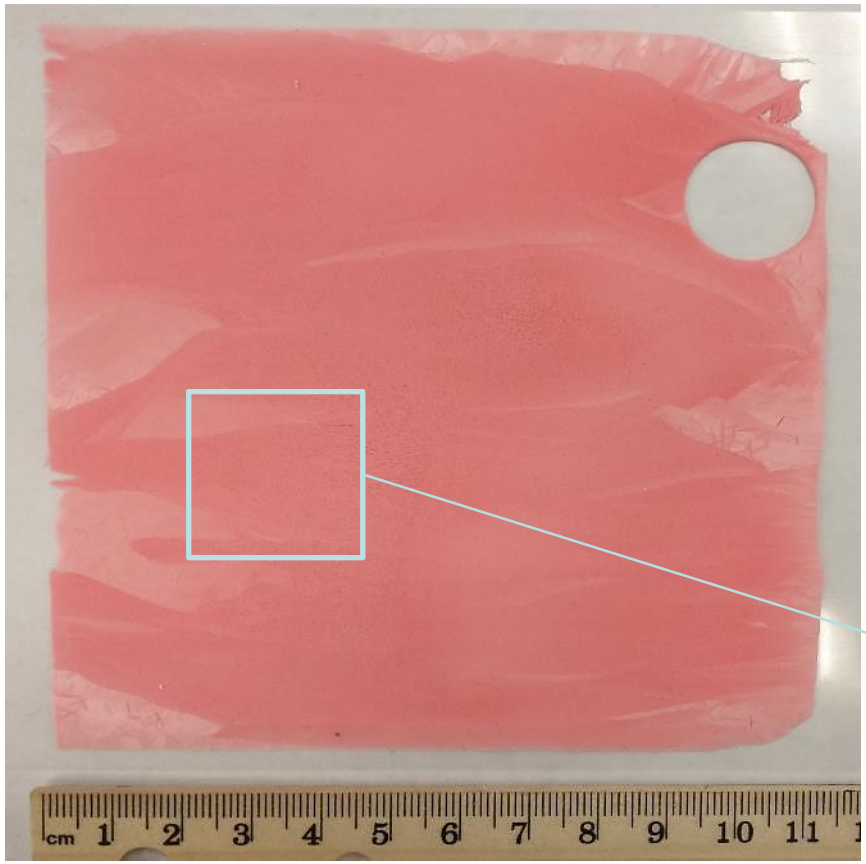
300 microns



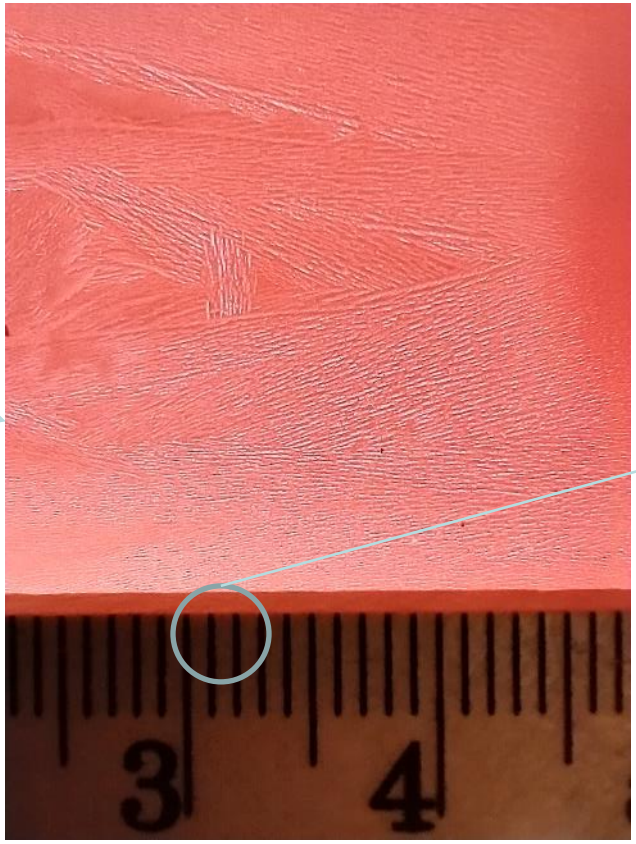
Continuously graded YSZ freeze tape cast
- Yellow - 10 volume percent solids
- Blue - 20 volume percent solids



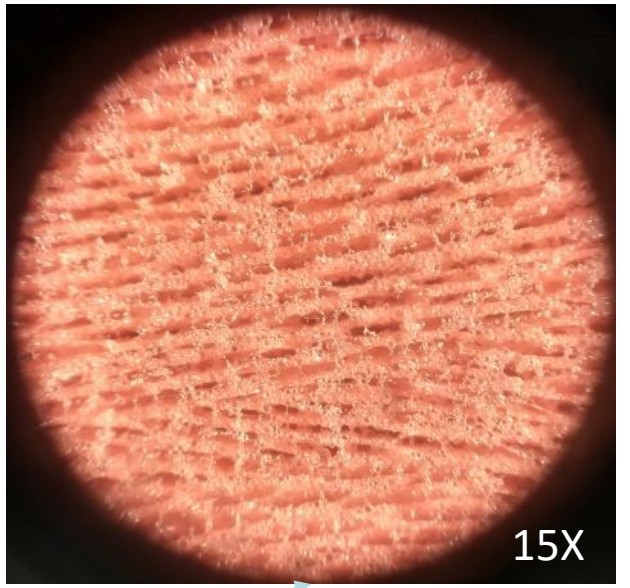
Ag_2O sintered at 300°C (enthalpy of reduction drives sintering)
-Superb microstructure, conductivity, and scalability



Acrylic - ~10x10cm cast



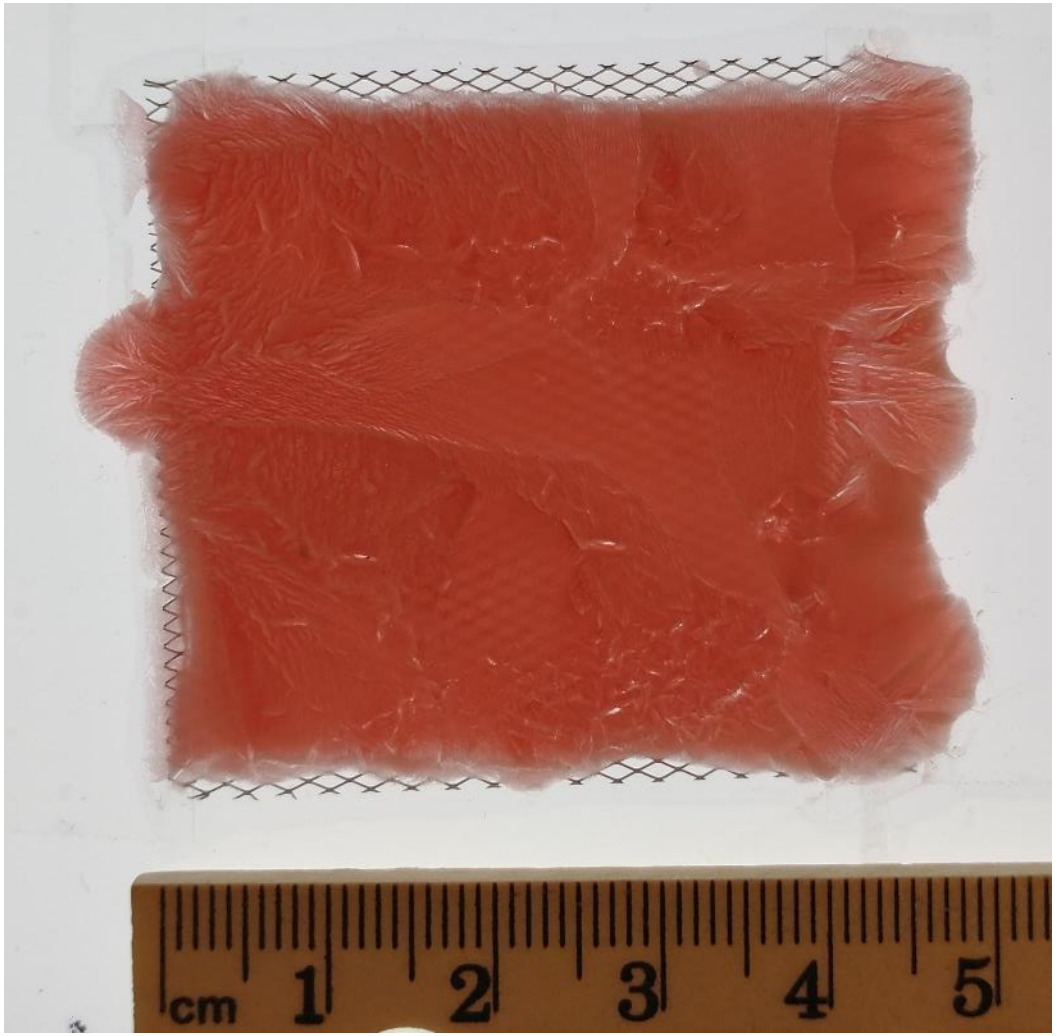
Microstructure



Acetone cold vapor soak, no thermal sintering, excellent flexibility, limited strength

Based on discussion with DNV-GL, the idea to incorporate a supporting and electrically conductive metal mesh within the FTC process was brainstormed.

This approach yields some extraneous nucleation, but yields good FTC structure that can be laterally graded



Hybrid FTC acrylic with embedded nickel mesh

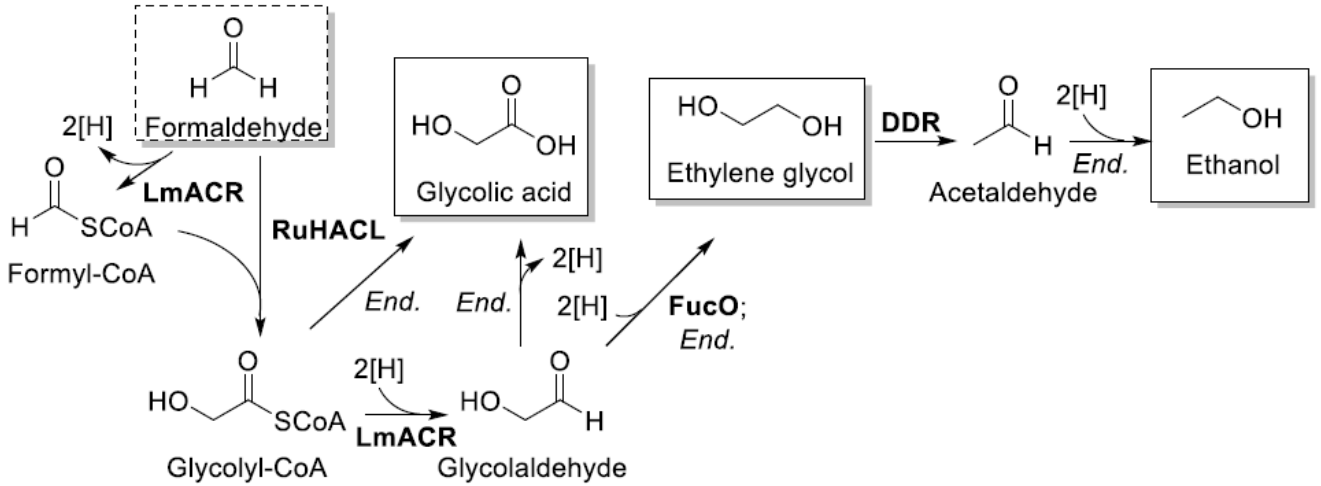
Next Year Go/No Go: Demonstrate100cm2 cell meeting specified performance targets

Progress to date:

- 10x scale up of Sn/C nano catalyst preparation accomplished
- Reactor parts procured
- Electrode Coating optimization underway
- Initial performance testing complete – design improvements underway
- Casting of graded Ceramics, Metals, and Polymers demonstrated at 10 x 10 cm scale for device implementation
- Hydrological testing initiated – inform grading of gas distributer

Biological Upconversion of Formate to Ethylene Glycol

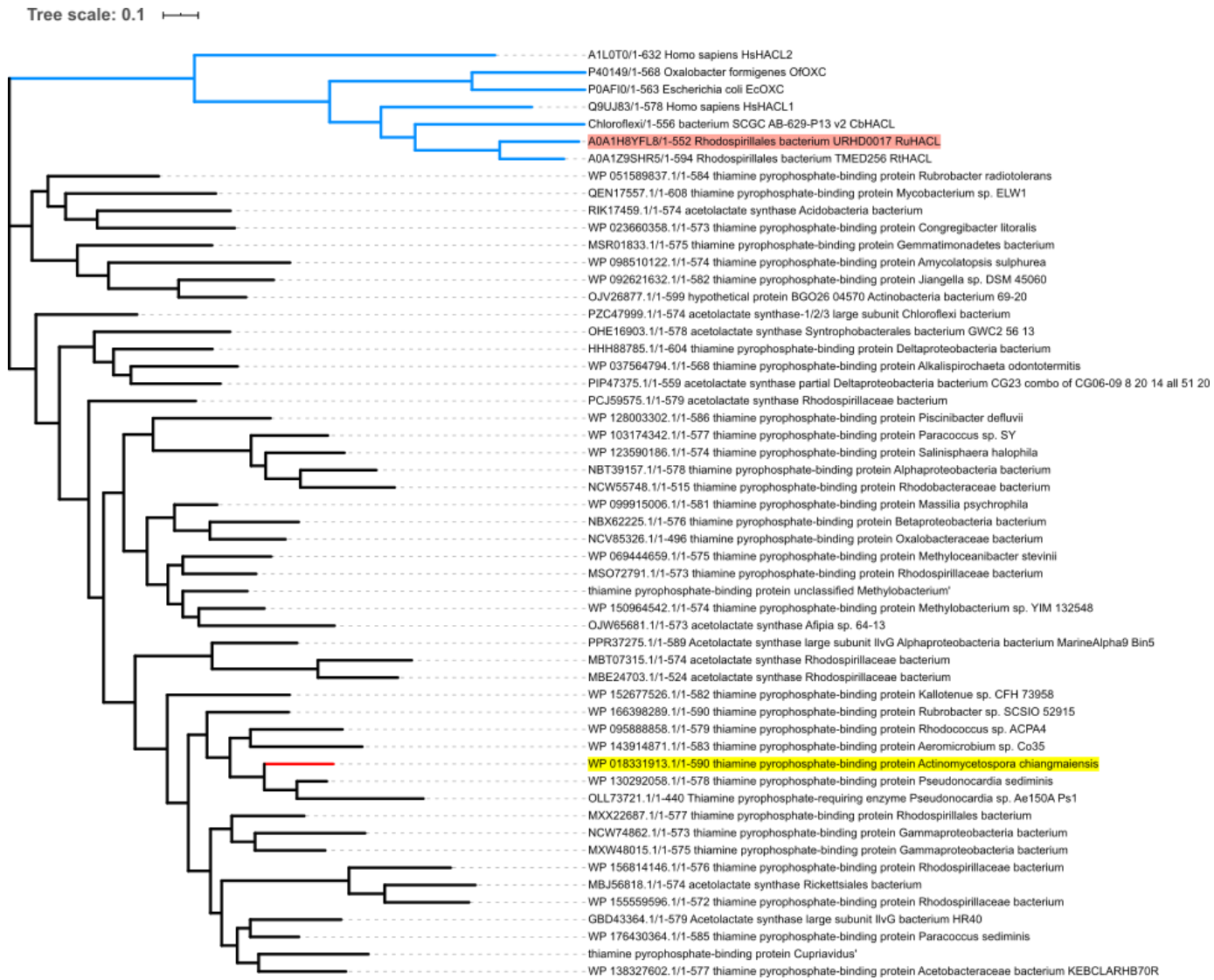
- Ramon Gonzalaz lab discovered C-C bond forming reaction that uses C1 substrates catalyzed by the enzyme 2-hydroxyacyl-CoA lyase (HACL)
- Can condense the C1 unit formyl-CoA in an iterative fashion with varying chain length aldehydes
- This first-of-its-kind pathway circumvents central metabolism to enable the production of industrially relevant chemicals with greater simplicity than alternative approaches. The proposed design requires fewer enzymes and reaction steps, and is complete orthogonal to the central metabolism.



Task 5: Identification of enzyme candidates

Complete

- Recent reports have provided evidence of additional candidate enzyme variants of interest:
  - Burgener et al. (doi:10.1002/anie.201915155) reports a variant of oxalyl-CoA decarboxylase that catalyzes the condensation of aldehydes with formyl-CoA.
  - Rohwerder et al. (doi:10.3389/fmicb.2020.00691) reports an enzyme participating in 2-hydroxyisobutyric acid degradation that serves as a 2-hydroxyacyl-CoA lyase.
    - These enzymes are dissimilar to the previously identified enzymes and are a potential source of yet unexplored variants.



Identification of enzymes similar to *Actinomycetospora chiangmaiensis* DSM 45062 2-hydroxyacyl-CoA lyase (yellow) and phylogenetic tree including HACL/OXC variants previously tested (grouped by blue lines). The newly identified enzymes form a distinct family to the previously tested HACL/OXC variants based on phylogenetic analysis.

Task 6: Characterize bacterial host strains capable of tolerating process conditions

Complete

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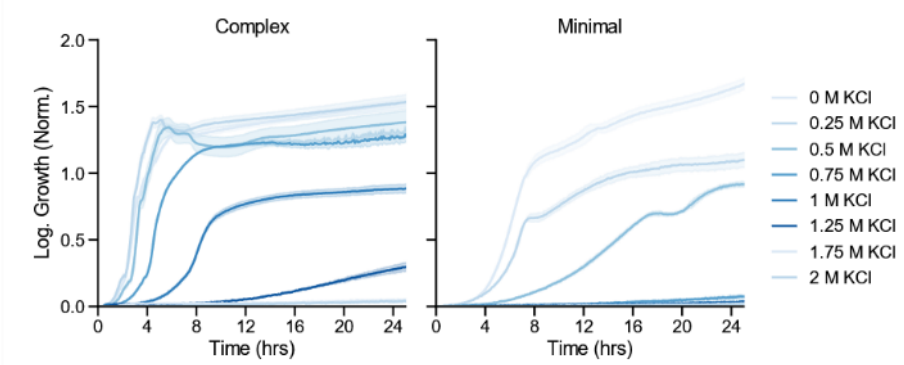
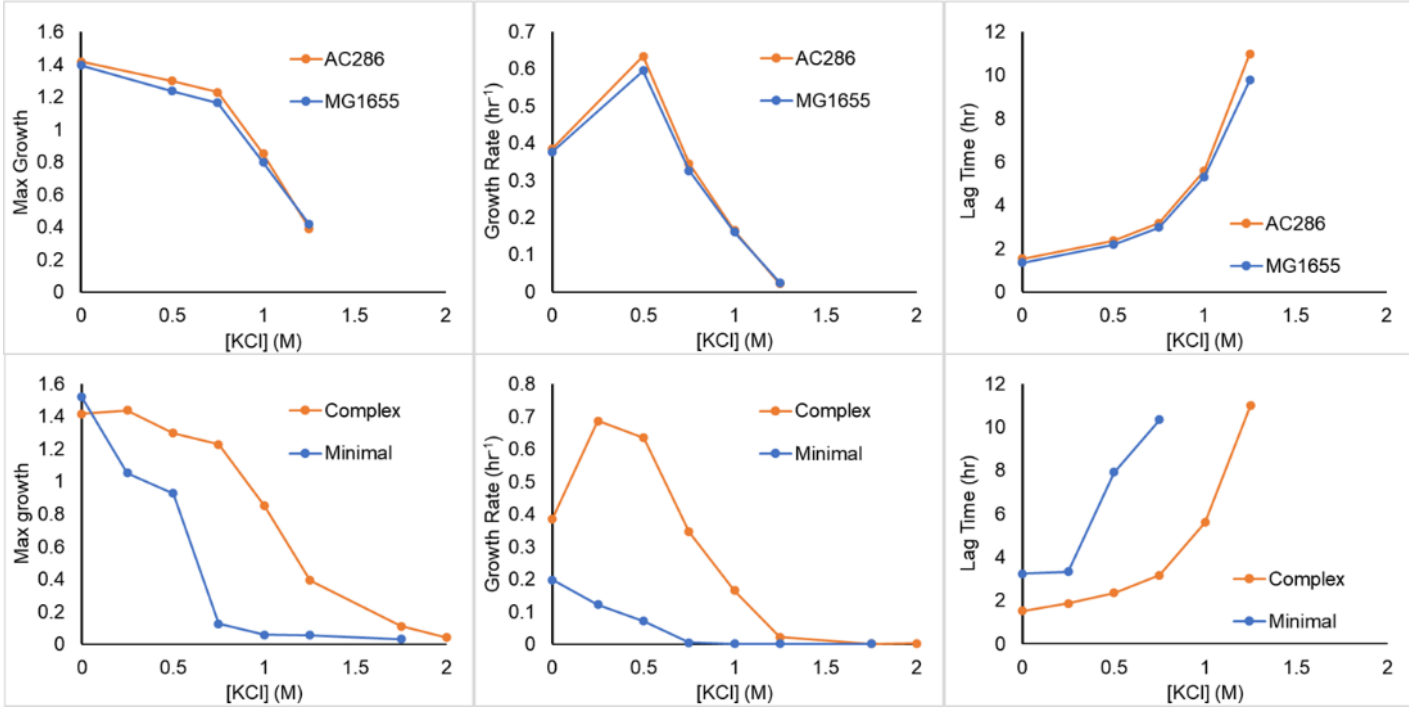
Genotype of *E. coli* strain AC286: MG1655(λDE3) Δ*frmA* Δ*fdhF* Δ*fdhO* Δ*fdhN*

Genotype	Description
MG1655	A common laboratory strain of wild-type *E. coli* K-12.
λDE3	A genetic modification enabling the control of heterologous gene expression.
Δ*frmA*	Deletion of a gene involved in formaldehyde dissimilation.
Δ*fdhF* Δ*fdhO* Δ*fdhN*	Deletion of genes involved in formate dissimilation.

***E. coli* KCl tolerance in complex and defined media**

Complex		Minimal	
Component	**Concentration**	**Component**	**Concentration**
Yeast Extract	5 g/L	Glucose	10 g/L
Tryptone	10 g/L	$NH_4H_2PO_4$	22 mM
KCl	Variable	Na_2HPO_4	48 mM
		$MgSO_4$	1 mM
		$CaCl_2$	0.1 mM
		$(NH_4)_6(MO_7)_{24}$	$3*10^{-6}$ mM
		H_3BO_3	$4*10^{-4}$ mM
		$CoCl_2$	$3*10^{-5}$ mM
		$CuSO_4$	$1*10^{-5}$ mM
		$MnCl_2$	$8*10^{-5}$ mM
		$ZnSO_4$	$1*10^{-5}$ mM
		KCl	Variable





- Wild-type *E. coli* could tolerate high KCl concentrations (greater than 1 M) with complex nutrients and in the absence of additional NaCl.
- A designed strain of *E. coli* was evaluated for KCl tolerance. Similar to the wild type strain, the strain could grow with up to 1.25 M KCl in complex media.
- A minimal defined media was developed to enable *E. coli* growth with high KCl concentration. Growth was observed with up to 0.75 M KCl.
- There is room to improve both the host strain and the media composition through rational and non-rational approaches.

Task 10: Prototyping the conversion pathway of formate to ethylene glycol

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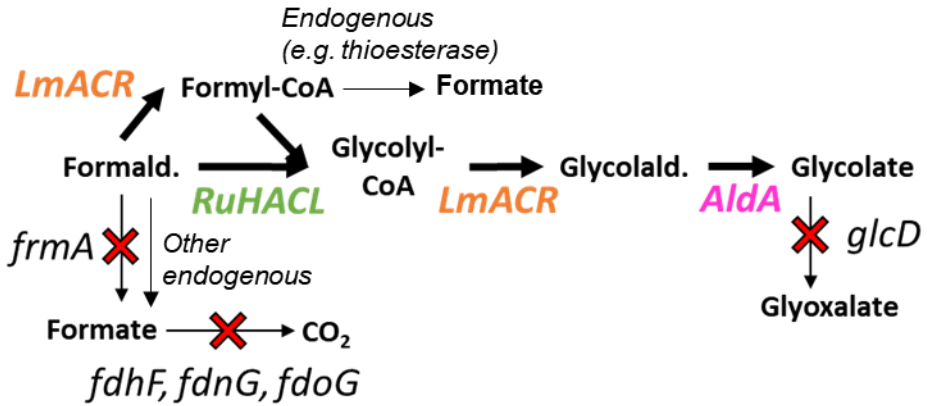
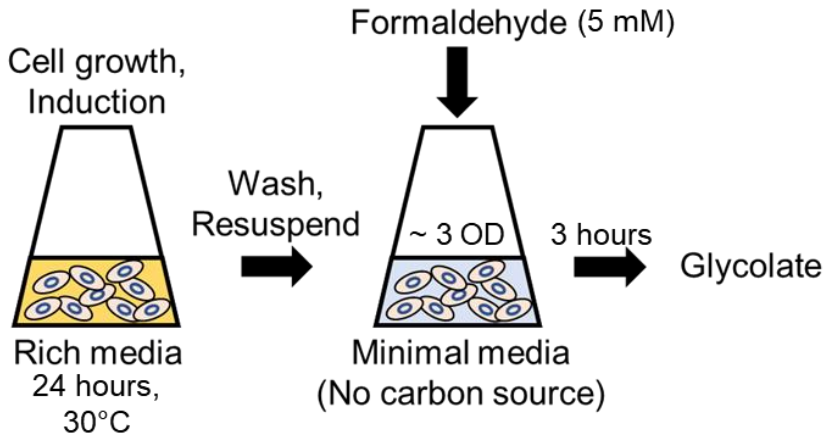
Characterization of 2-hydroxyacyl-CoA lyase/oxalyl-CoA decarboxylase enzymes for efficient C-C bond formation from C1 units

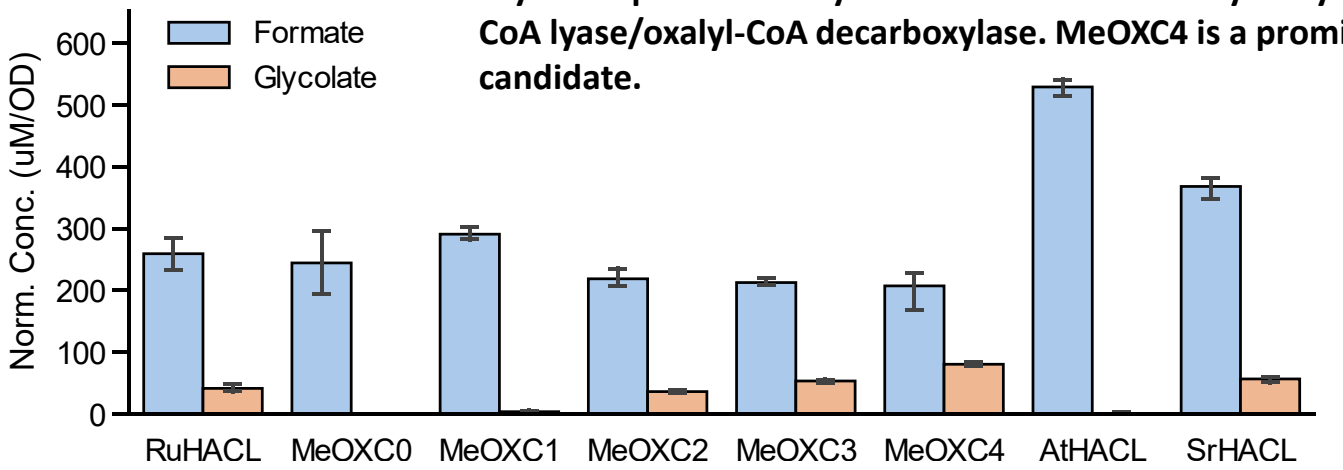
Pathway design to assess 2-hydroxyacyl-CoA lyase/oxalyl-CoA decarboxylase activity.



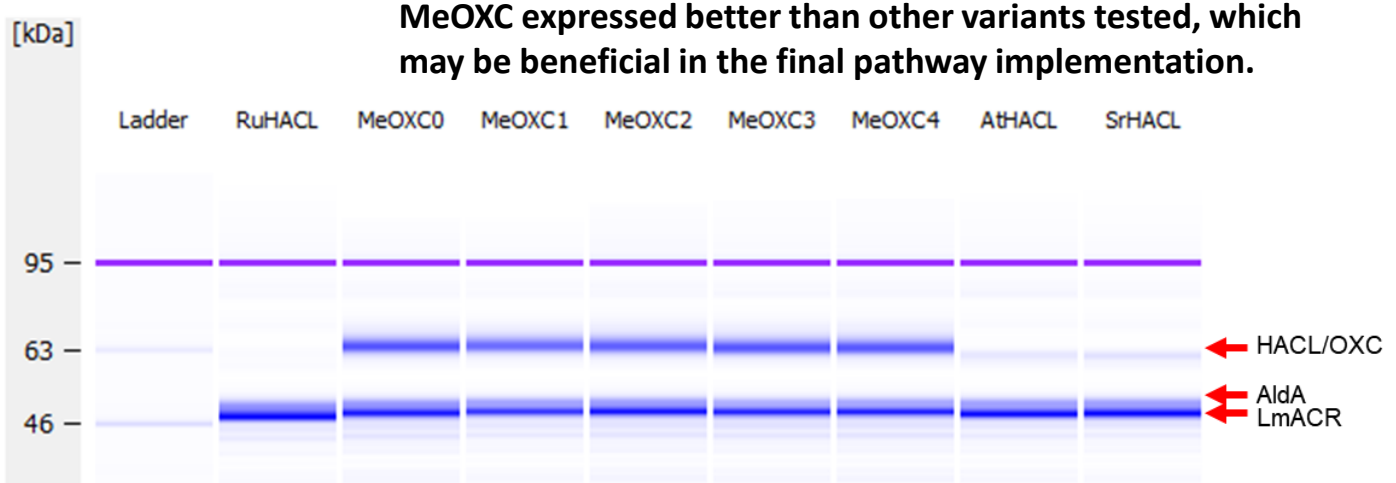
Experimental design to screen 2-hydroxyacyl-CoA lyase/oxalyl-CoA decarboxylase activity.



Glycolate production by screened variants of 2-hydroxyacyl-CoA lyase/oxalyl-CoA decarboxylase. MeOXC4 is a promising candidate.



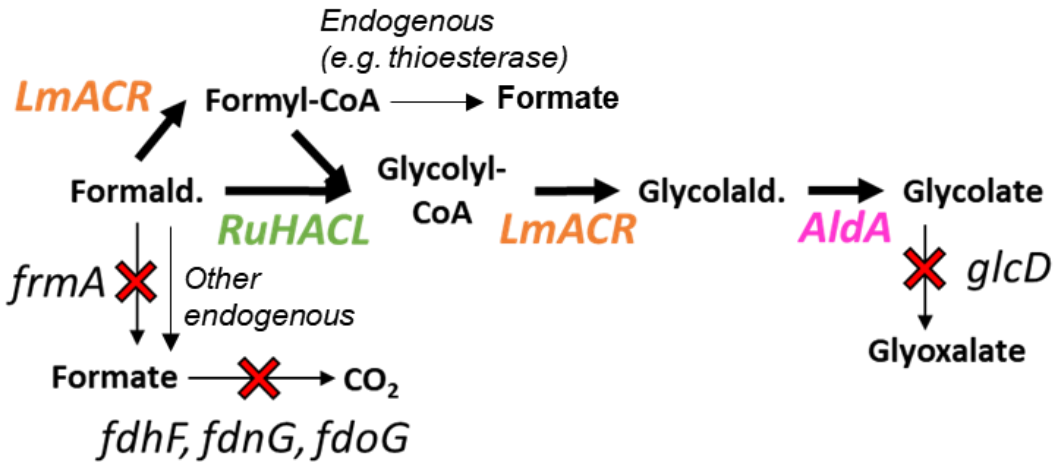
MeOXC expressed better than other variants tested, which may be beneficial in the final pathway implementation.

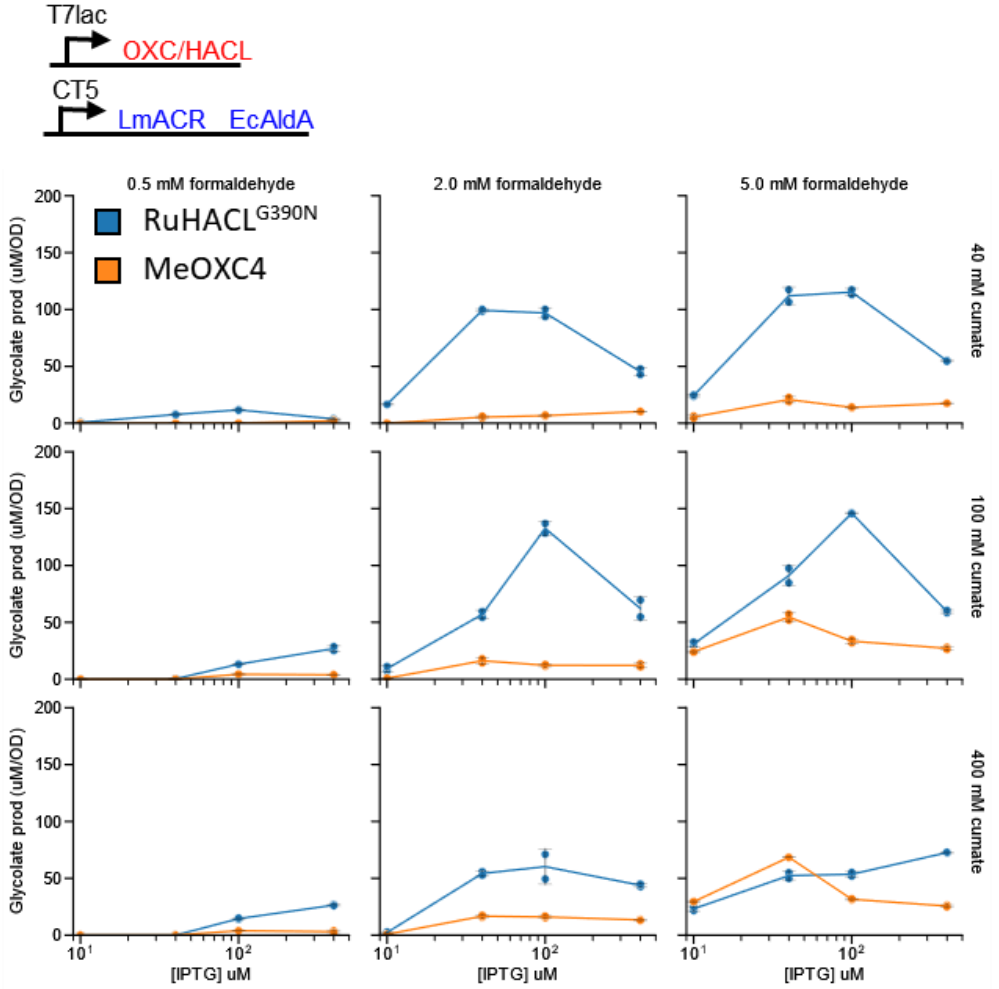


Next steps: Continue to evaluate enzyme variants for this and other pathway steps. Conduct more detailed analysis on promising candidates.

Task 10: Prototyping the conversion pathway of formate to ethylene glycol

Characterization of 2-hydroxyacyl-CoA lyase/oxalyl-CoA decarboxylase enzymes for efficient C-C bond formation from C1 units



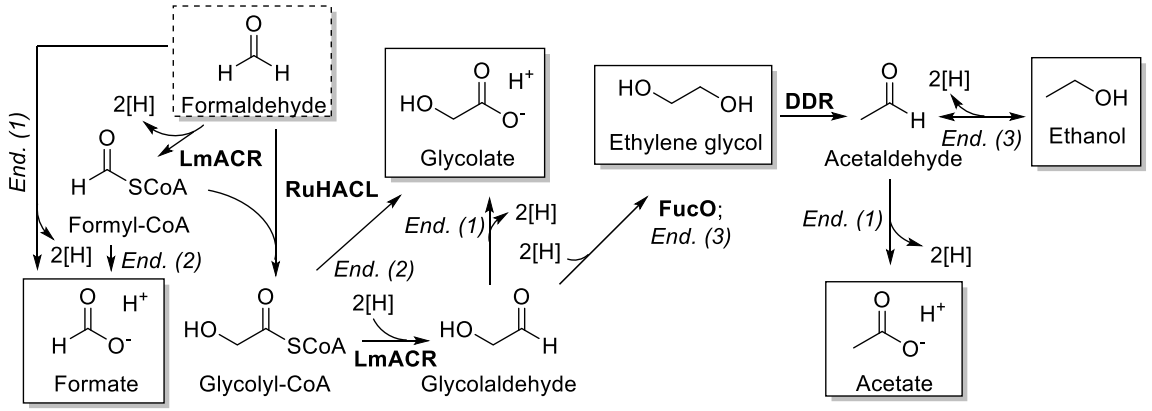


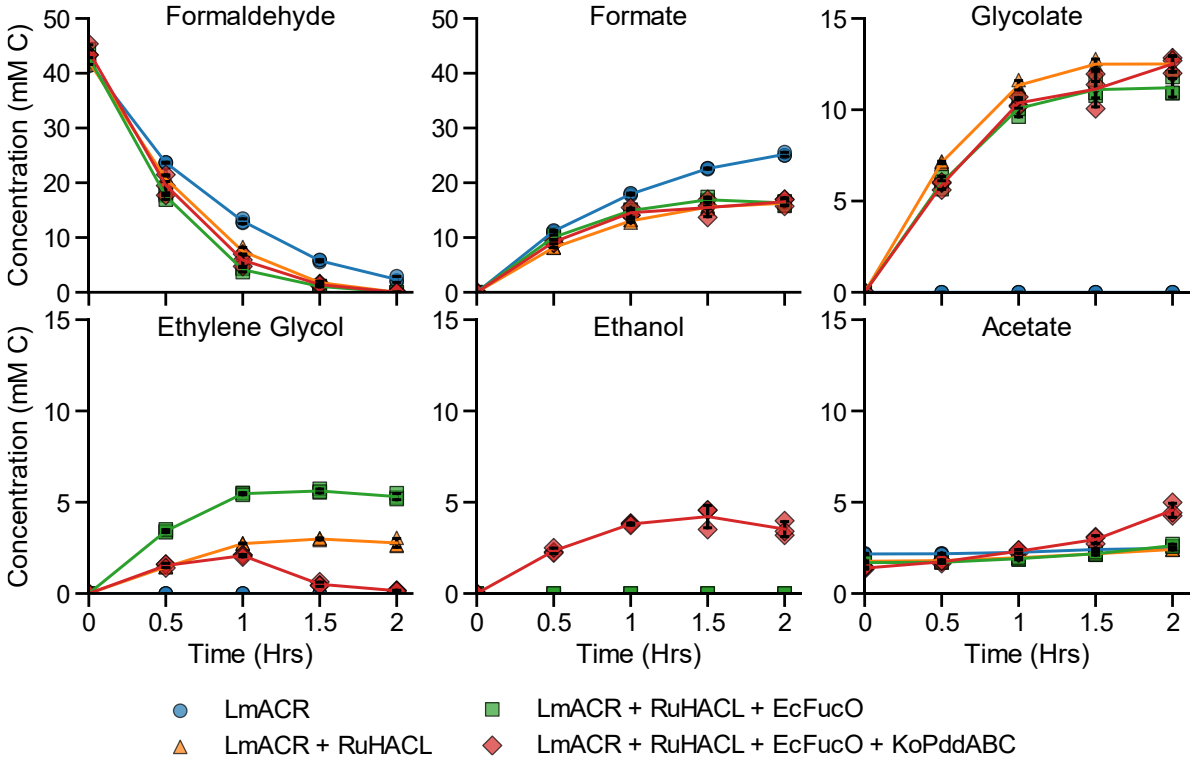
In-depth analysis of condensation enzyme candidate MeOXC4 revealed that performance matching that of the current state-of-the-art, RuHACL^{G390N} could not be reached under optimized (varying inducer concentrations) or preferred (low formaldehyde concentration) conditions.

Next steps: Evaluation of additional variants of the key condensation enzyme is ongoing to find a better performing candidate.

Task 10: Prototyping the conversion pathway of formate to ethylene glycol

Prototyping the downstream product synthesis pathway to ethylene glycol in cell-free extracts





Product synthesis from cell-free reactions including indicated enzymes

Cell-free pathway prototyping demonstrated ethylene glycol production from a single C1 source (formaldehyde)

Control over C2 product synthesis enabled by selection of enzymes included in reaction system

Next steps: Improving ethylene glycol production through minimizing by-product glycolate formation (from glycolyl-CoA/glycolaldehyde nodes); Extending upstream pathway to demonstrate production from formate

Task 10: Prototyping the conversion pathway of formate to ethylene glycol

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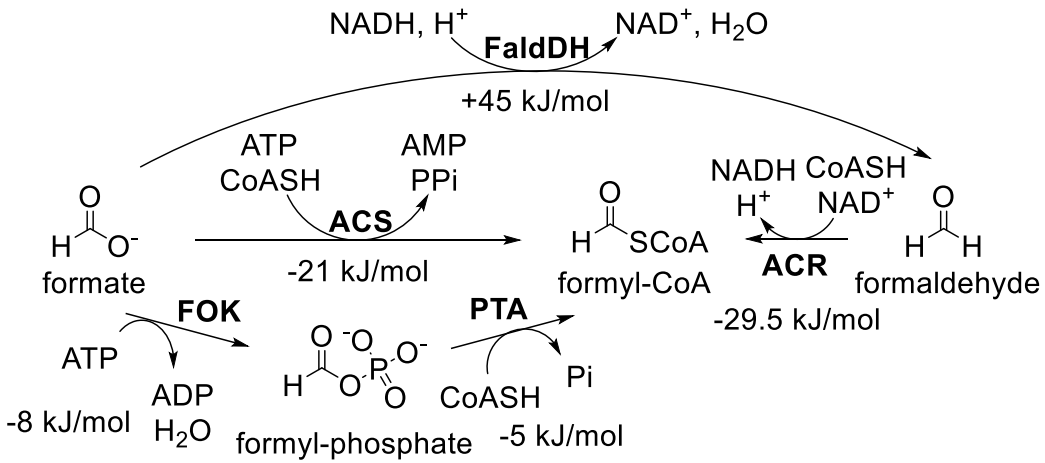
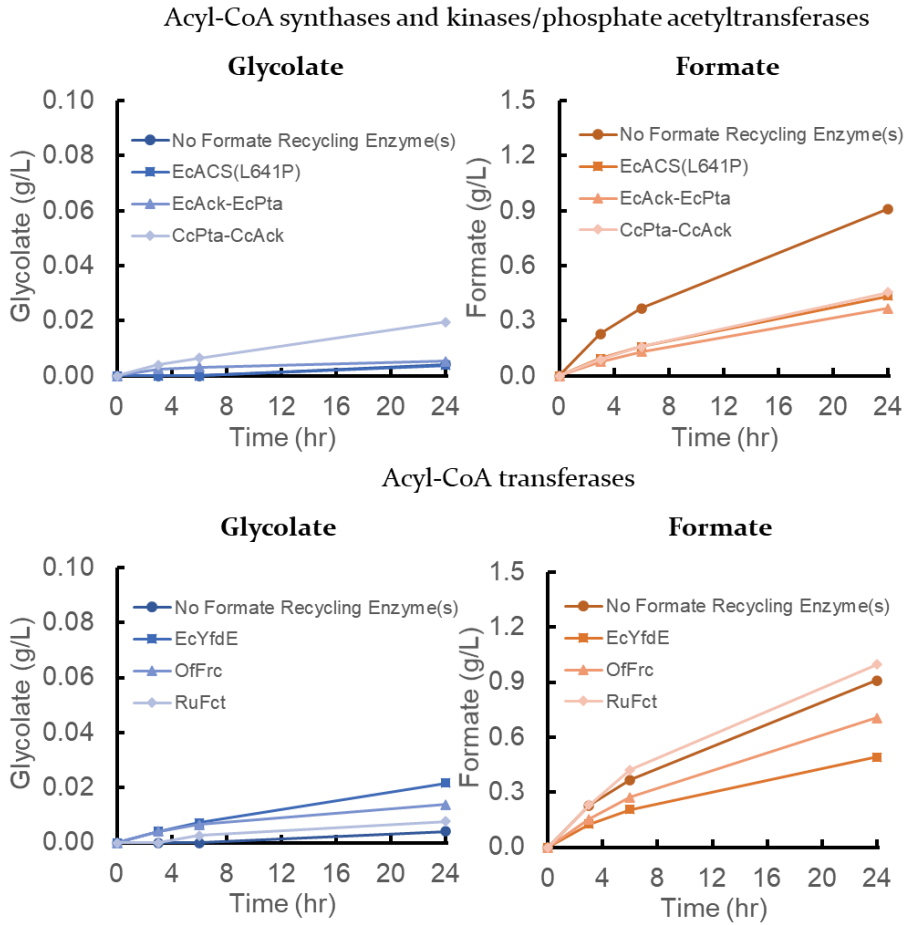
Extending pathway operation to formate



Formate activation enzymes were tested and found to be capable of supporting formyl-CoA production using methanol as a proxy substrate and glycolate as a proxy product.

Next steps: Conduct additional experiments to better understand formate activation (such as using purified enzymes) and to demonstrate the use of formate for product synthesis (i.e. ethylene glycol).

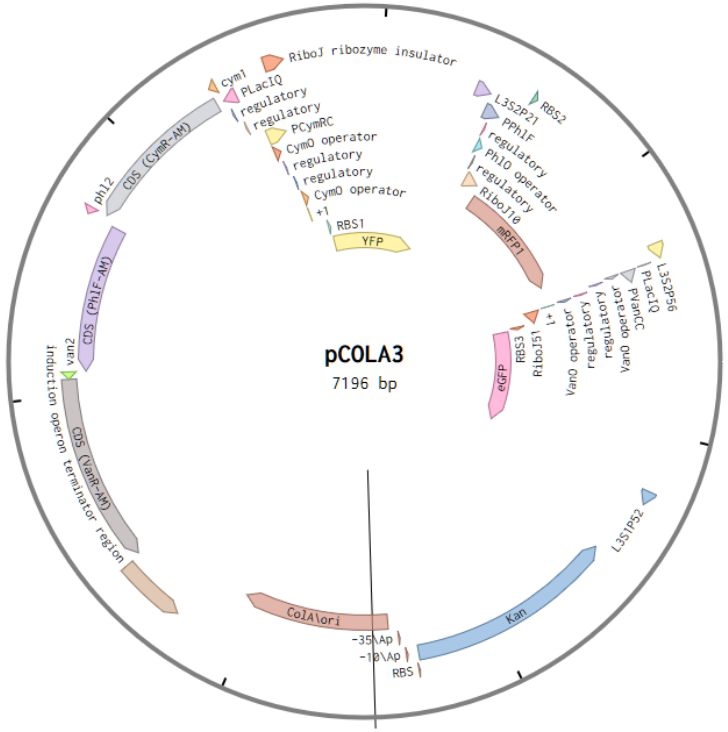


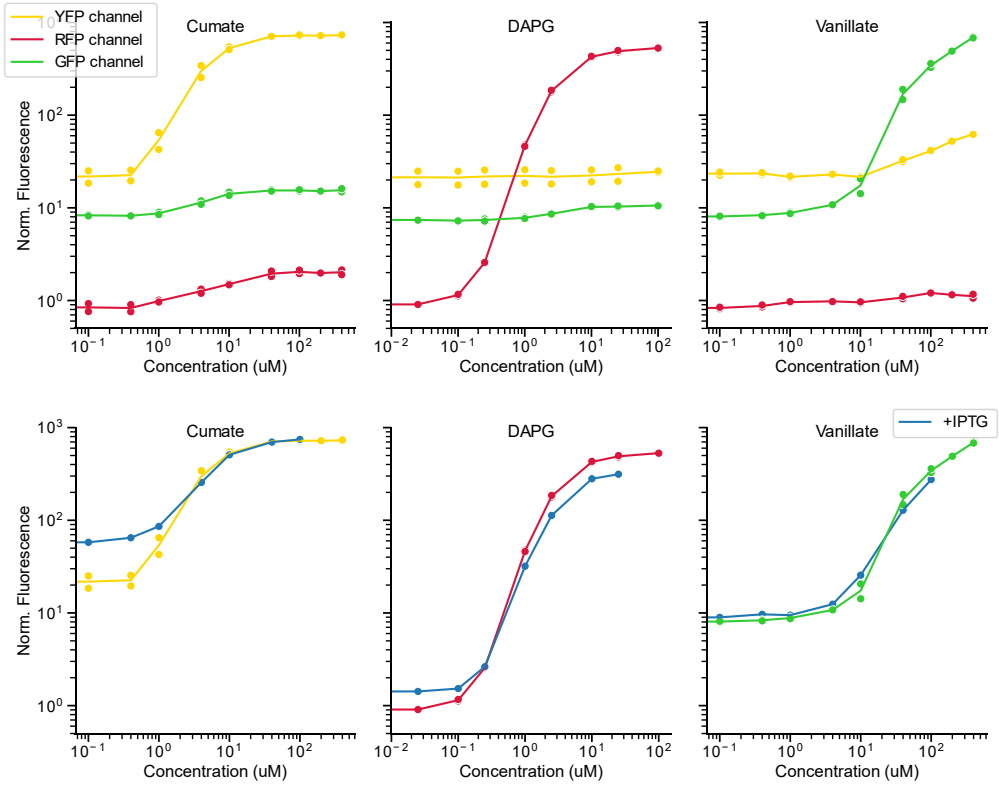
Product synthesis from systems generating formate and NADH from methanol upon expression of RuHACL and indicated formate activation enzyme(s)

Task 11: Engineer microorganisms for formate to ethylene glycol conversion

Multi-enzyme pathway with different requirements and kinetics at each step and with a limit to total enzyme expression necessitates a more sophisticated strategy to control expression.

Developed a vector system with multiple inducible transcription units.
Implementation of Golden-Gate Assembly enables rapid combinatorial assembly.





Independent induction of fluorescent proteins is possible in a graded manner, which should enable fine control over pathway enzyme expression.

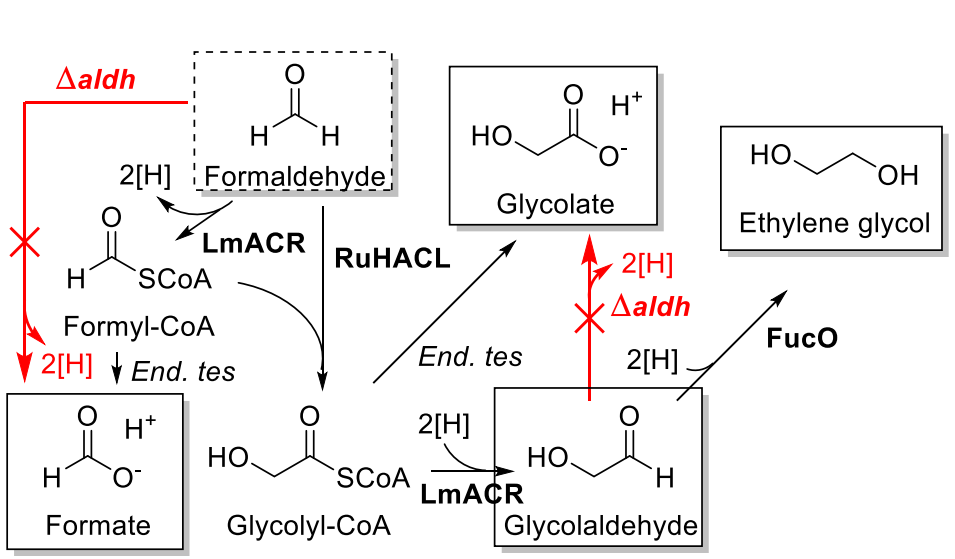
<u>Next steps:</u>

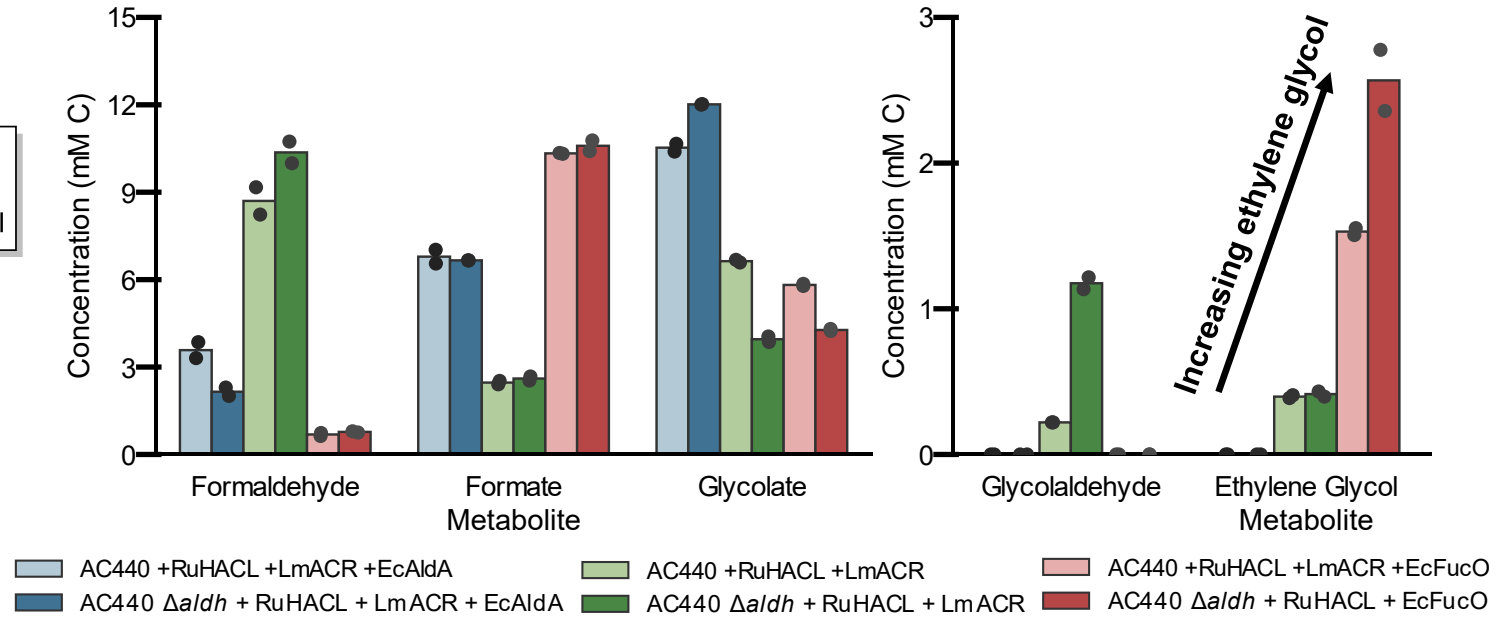
Implement the pathway and test performance with independent control over different enzymes.

Task 11: Engineer microorganisms for formate to ethylene glycol conversion

UNIVERSITY OF SOUTH FLORIDA

Demonstrating and improving ethylene glycol production in whole cell biotransformations





Combination of targeted gene deletions (to aldehyde dehydrogenases) and expression of glycolaldehyde reductase enzyme (FucO) with upstream pathway enzymes yields improved ethylene glycol production

Next steps:

Continue to improve ethylene glycol production by minimizing glycolate production and improving C1-C1 condensation

Implement upstream formate activation enzymes to enable production from formate

Task 11: Engineer microorganisms for formate to ethylene glycol conversion

USF UNIVERSITY OF SOUTH FLORIDA

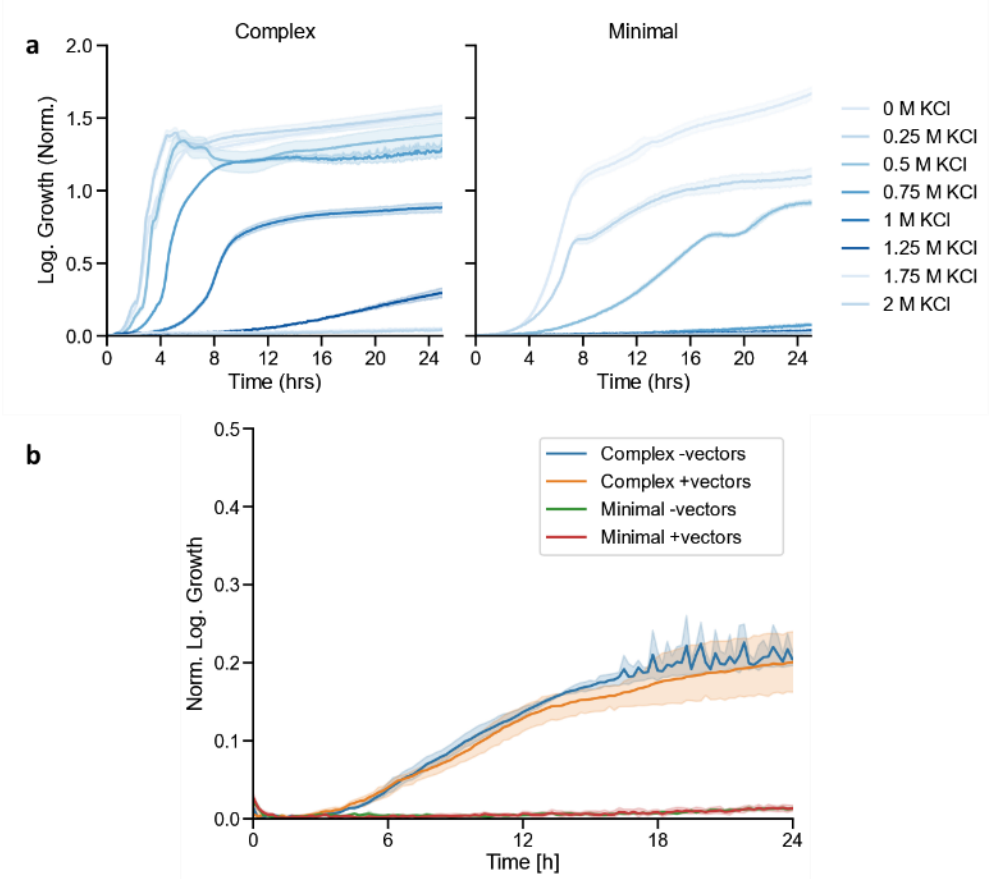
Testing the ability of engineered *E. coli* to tolerate the electrochemical process fluid

Growth of engineered *E. coli* (with multiple gene deletions and harboring plasmids expressing pathway genes) evaluated using actual process fluid obtained from DNVGL.

Growth and vector maintenance was demonstrated with the inclusion of complex nutrients to a 50% solution of the process fluid (Milestone 11.1).

Growth was lower than expected compared to previous results using simulated process conditions, indicating a need for better understanding and iteration to improve performance.

Next steps: USF will work with OCO/DNVGL to better understand the composition of the process fluid and further iterate to optimize the composition of the bioconversion media. Engineering and adaptation of E. coli to the desired conditions is ongoing.



Techno-Economic Analysis

- USF research continues on optimizing pathways for a genetically modified e.coli to a one step conversion of formate to ethylene glycol. Energy for conversion will use the energy inherent in the formate. For TEA we are using a baseline stoichiometric conversion of 4-5mols of formate to make a mol of ethylene glycol.
- Optimal USF inorganic/ organic enzymatic cocktail media additions to facilitate e.coli conversions are still underway. We anticipate updated micronutrients for better mass balance calculations.
- We plan to use ASPEN for the initial process flowsheets, energy and mass balance and Capex /Opex.
- Last quarter we incorporated the need for a flexible waste-water process to convert any and all residuals via state-of-the-art technology. We are targeting PNNL's (Elliot et al) CHG (catalytic hydrothermal gasification) process. Inorganics /organics to CO2/CH4 with inorganics in sterile water. Although beyond the scope of this project, there is a CHG pilot unit in Richland which could facilitate rapid proof of concept and conversion metrics.
- Separations core is ambient condition driven reverse osmosis and/or MEV used to concentrate EG and other co-organics (formaldehyde residual formate, etc.) followed by distillation. CO2/CH4 could be separated or burned for process EG heat. In either option CO2 will be recycled.
- Zea 2—with their bioprocessing pilot and production equipment, proof of concept and scale-up possible quickly.
- Next Qtr. USF e.coli enzymatic process details will be clarified flowed by improved flowsheet.

Techno-Economic Analysis

- Energy /Other Process Options. The TEA OCO project team now has a more complete picture of the USF formate to EG process. We will finalize in 2021 process details and get benchmark economics. Although beyond the scope of the current project, we have identified enabling and significant support technologies and partners. The following highlights these exceptional resources (no priority intended) identified by OCO and the DOE-MSU-USF-DNV project team.

  a) Zea-2 has bio-processing facilities---bench, pilot and production equipment existing in Boardman, OR.

  b) The CHG process (developed by PNNL) can be used for recycling organics to CO2/CH4 and producing a sterile water with inorganic nutrients. Further there are existing lab and pilot facilities that in turn could save $ millions in costs and be demonstrated in literally months .

  c) USF is expert in not only formate to EG development but their one-step-one-bug process can be modified for other organics like formaldehyde. USF leading experts in this bio-space. In short, the envisioned process can make more than one product. Product diversity minimizes risk.

  We will continue to identify synergies.

3 – Impact

- Through 1) development of a first-of-its-kind biocatalyst for formate utilization and 2) integration of this biocatalyst with the electrochemical CO2 reduction process product as the feed (formate salt) for the bio-chemical process, we will demonstrate the production of ethylene glycol from CO2 and electricity at TRL4.
- This project will develop the first *de novo* designed synthetic organism-specific for the conversion of electrochemically produced formate to industrial chemicals and will advance the state-of-the-art in both integrating organisms with electrochemical systems and in engineered formatotrophy.
- The project will be the first is developing a fully optimized 100 cm2 and 300 cm2 electrolyzers for making formate salt from CO2, as most researchers have been unable to go beyond 10 cm2 size due to complexities in GDE flooding, electrode preparation, gas-liquid distribution hydrodynamics at such size scales.
- The engineered porous materials advances have potential for solving reactant distribution problems that occur in scale-up of a wide variety of planar electrochemical devices. Examples include: Solid oxide fuel cells (SOFC) which are subject to damage inducing temperature gradients; polymer electrolyte fuel cells (PEM) where graded porosity is recognized as key solution for water management; and other electrochemical devices where efficiency is often limited by the single pass utilization of fuel.
- A private sector partner is involved in TEA and the Market Transformation Plan.
- Early stages, so no publications yet, but manuscripts in preparation.

Summary

- *Summarize the key points you wish the audience and reviewers to take away from your presentation*
- *The CO_2 – formate small scale reactor met performance targets*
- *Scale-up to intermediate size is well underway*
- *Freeze Tape Casting system has been modified to allow programmable grading of membrane porosity*
- *Ability to scale gas diffusion layer size has been demonstrated*
- *Multiple enzyme variants for formate – ethylene glycol upconversion have been identified and are being evaluated*
- *Promising host strains with some salt tolerance are being evaluated*
- *Tests of reactor performance as a function of process fluid composition and recycle are underway with results feedback to the biological upconversion effort*

Quad Chart Overview (Competitive Project)

Timeline

- Project start date: 10/1/2018
- Project end date: 9/30/2022

	FY20 Costed	Total Award
DOE Funding	(10/01/2019 – 9/30/2020) $348,731	(negotiated total federal share) $1,483,983
Project Cost Share	$95,131	$371,282

Project Partners*

- OCO
- DJNVGL
- USF

*Only fill out if applicable.

Project Goal

Scale an electrochemical reactor to a commercially relevant scale for CO_2 to formate conversion and engineer microrganisms to take the resulting process fluid and perform upconversion of the formate to ethylene glycol

End of Project Milestone

Demonstration of a 300 cm^2 electrochemical reactor meeting performance targets

Funding Mechanism

DE-FOA-0001916 (5/3/2018), BioEnergy Engineering for Products Synthesis, Topic Area 5, Rewiring Carbon Utilization