Dioxide Materials™
The CO_2 Recycling Company™

Improved Microalgal Carbon Utilization Efficiency via integrated CO_2 Electro-conversion to Formate and Microalgal Sequestration

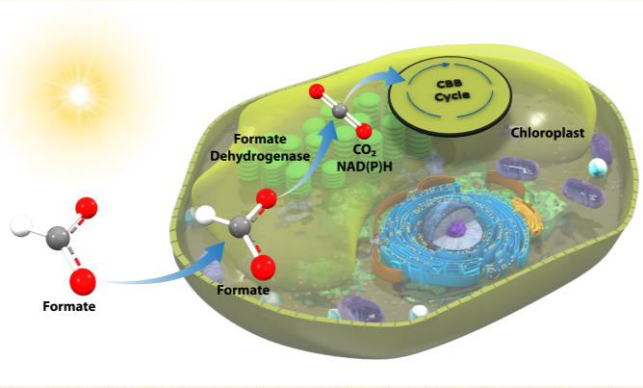
DE-FE0032186

Rich Masel, Dioxide Materials, Inc. (PI)
Isaac ‘Andy'Aurelio (BP1- PM)
Richard Dunst (New PM)

2024 Carbon Management Research Project Review Meeting
August 5 – 8 2024

Objective: Develop Photoformatotrophy

Formate as a carbon source for algae



Properties of formate/formic acid

- Easy to store
- High water solubility
- Enables conversion of electrical energy to cellular energy (i.e., reductant)
- Broadly toxic to many organisms,

Overview

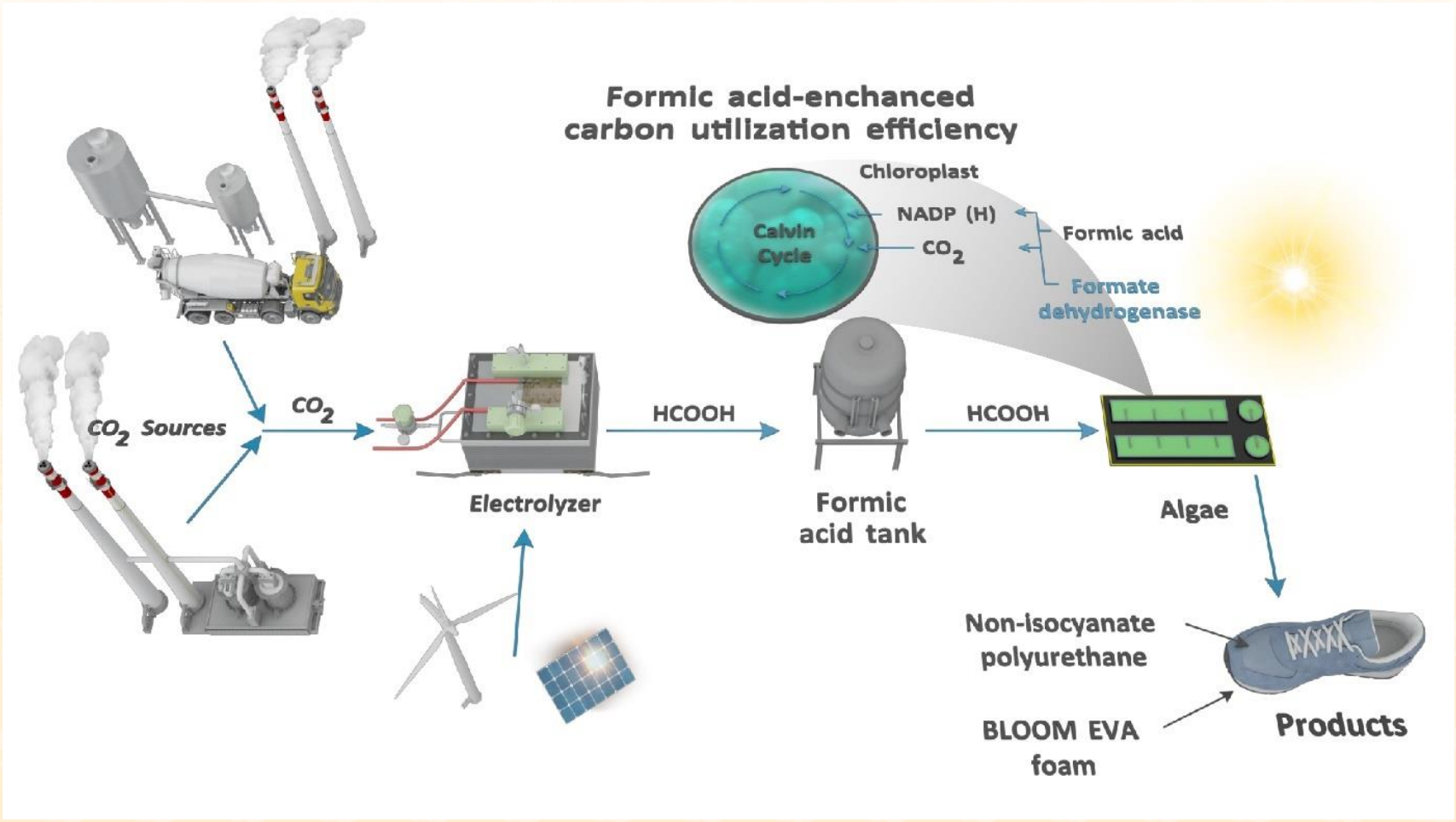
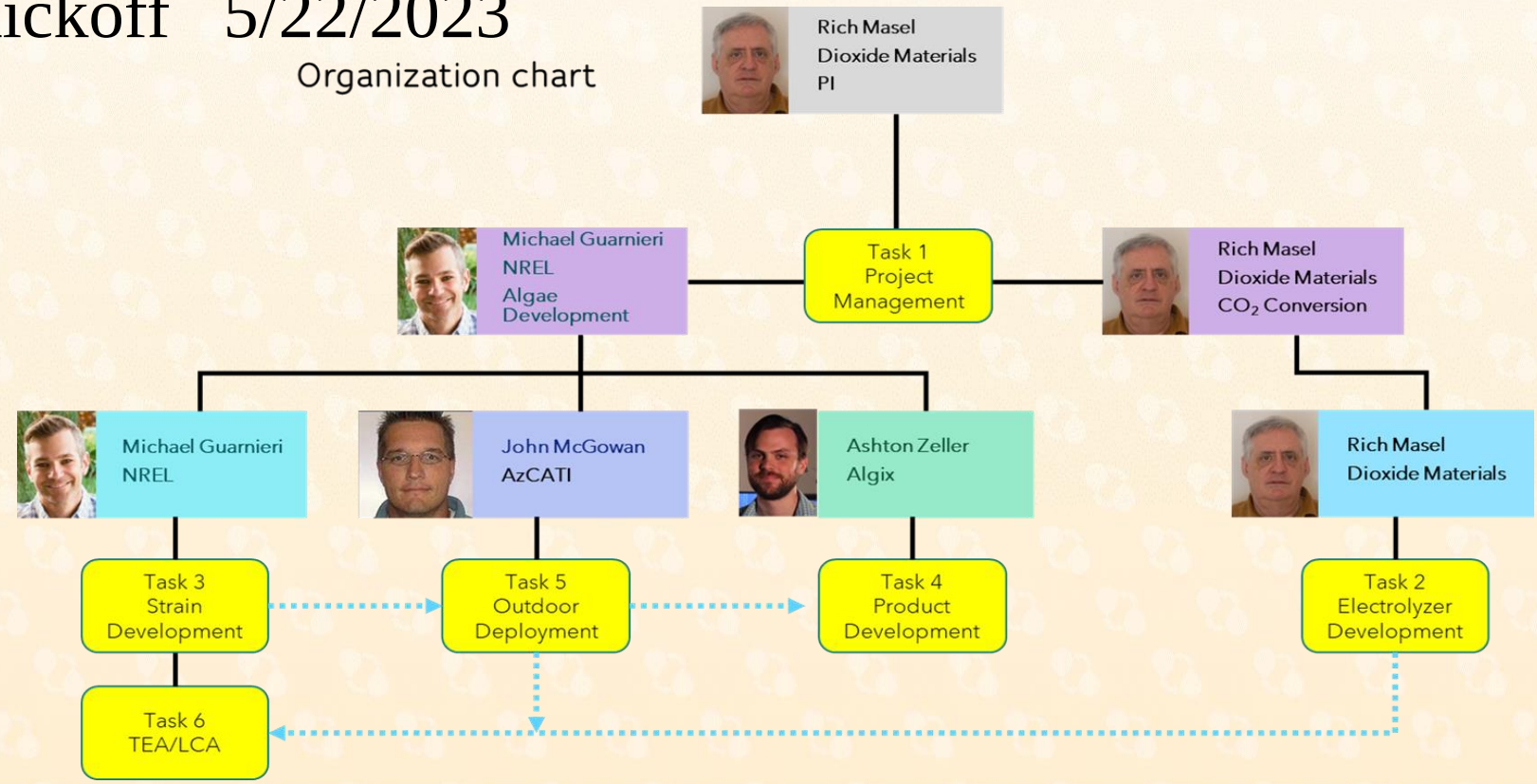


Figure from Josh Bauer and Lukas Dahlin, NREL.

Project Overview

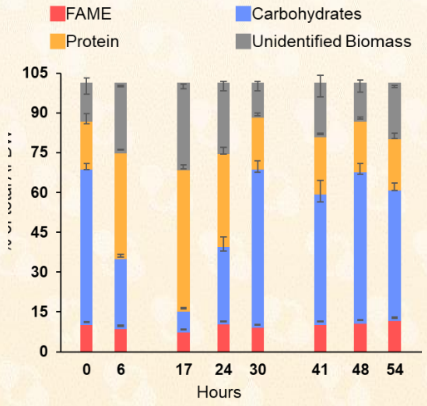
- Funding $2,000,000 (DOE) and ($500,000) Cost Share)
- 2/1/2023-1/31/2026
- Kickoff 5/22/2023

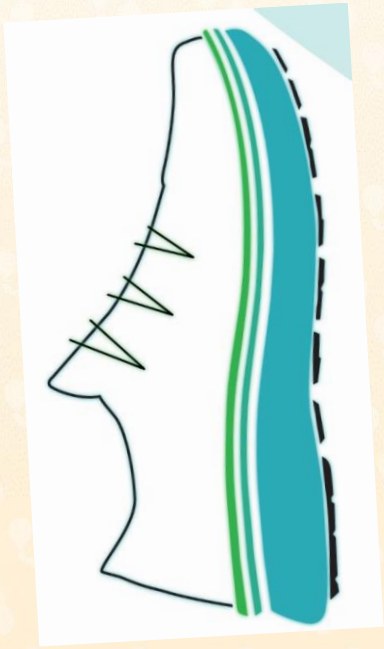
Organization chart



Technology Background

Dioxide Materials	NREL	AzCati	Algix
Formic Acid at a power plant	Algae Strain that can grow in formic acid	Existing Algae Ponds	Incorporate Algae in Polymers





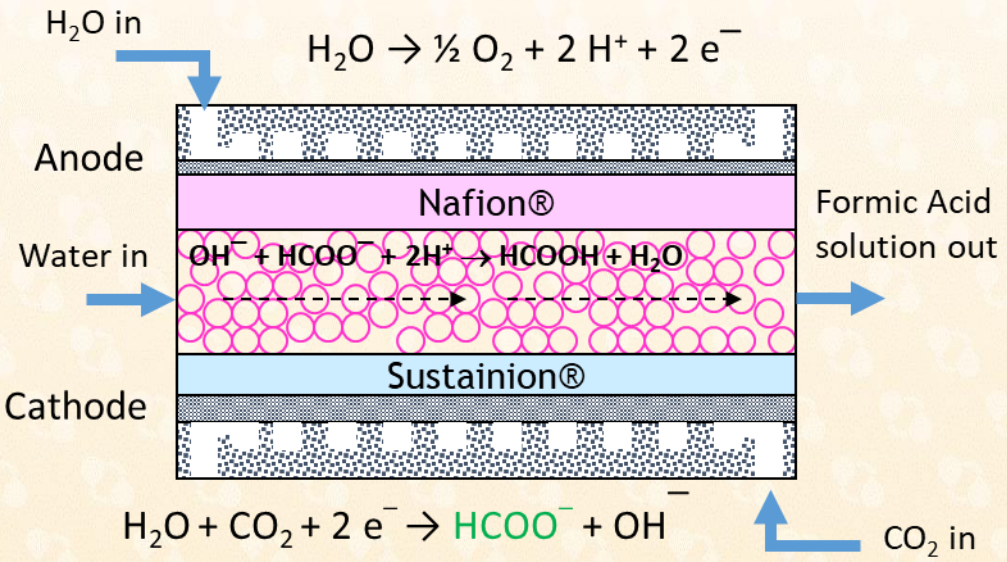
Goals Of The Program

- Electrolyzer 5 cm^2 ⇨1000 cm^2
- Productivity on Formate 1-5 $gm/m^2/day$ ⇨ >20 $gm/m^2/day$
- Pond carbon efficiency 30% ⇨ >50%
- Algae growth 250 mL ⇨ 1000 L pond
- Use products in Bloom EVA

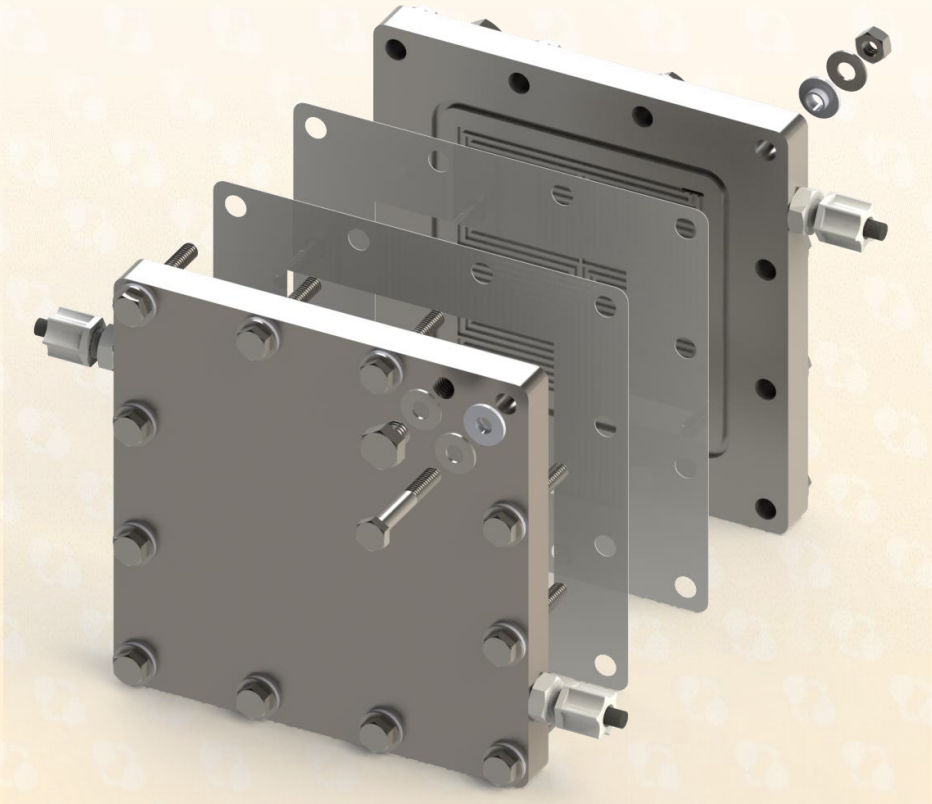
Technical Approach

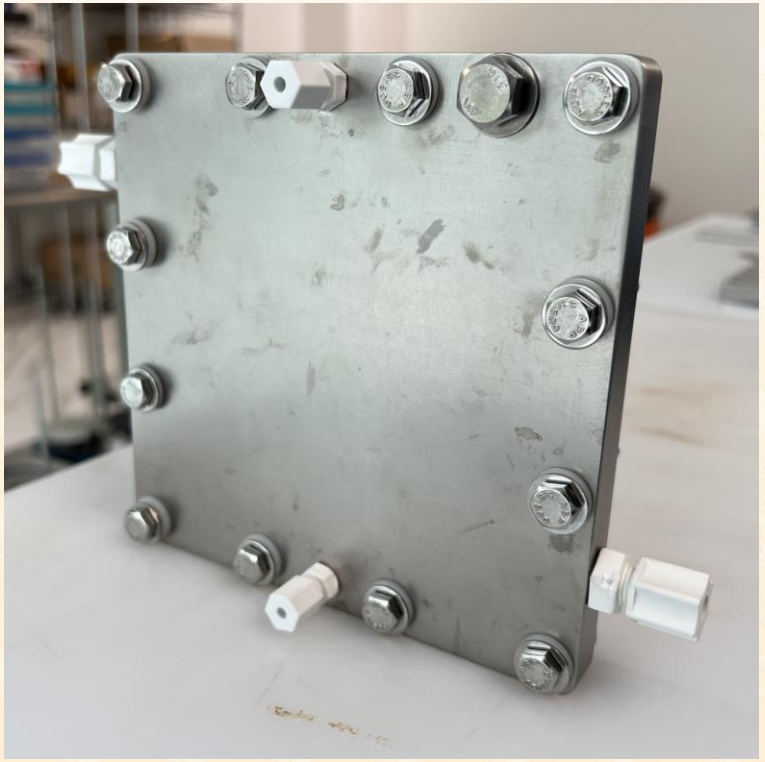
- Formic acid electrolyzer scaling
  - New materials
  - Oxygen control
- Engineer *P. renovo*
  - Incorporate Formate Dehydrogenases
  - Adaptive lab evolution
- Test in indoor photoreactors (BP1) open ponds (BP2)

Background: Electrolyzer Development

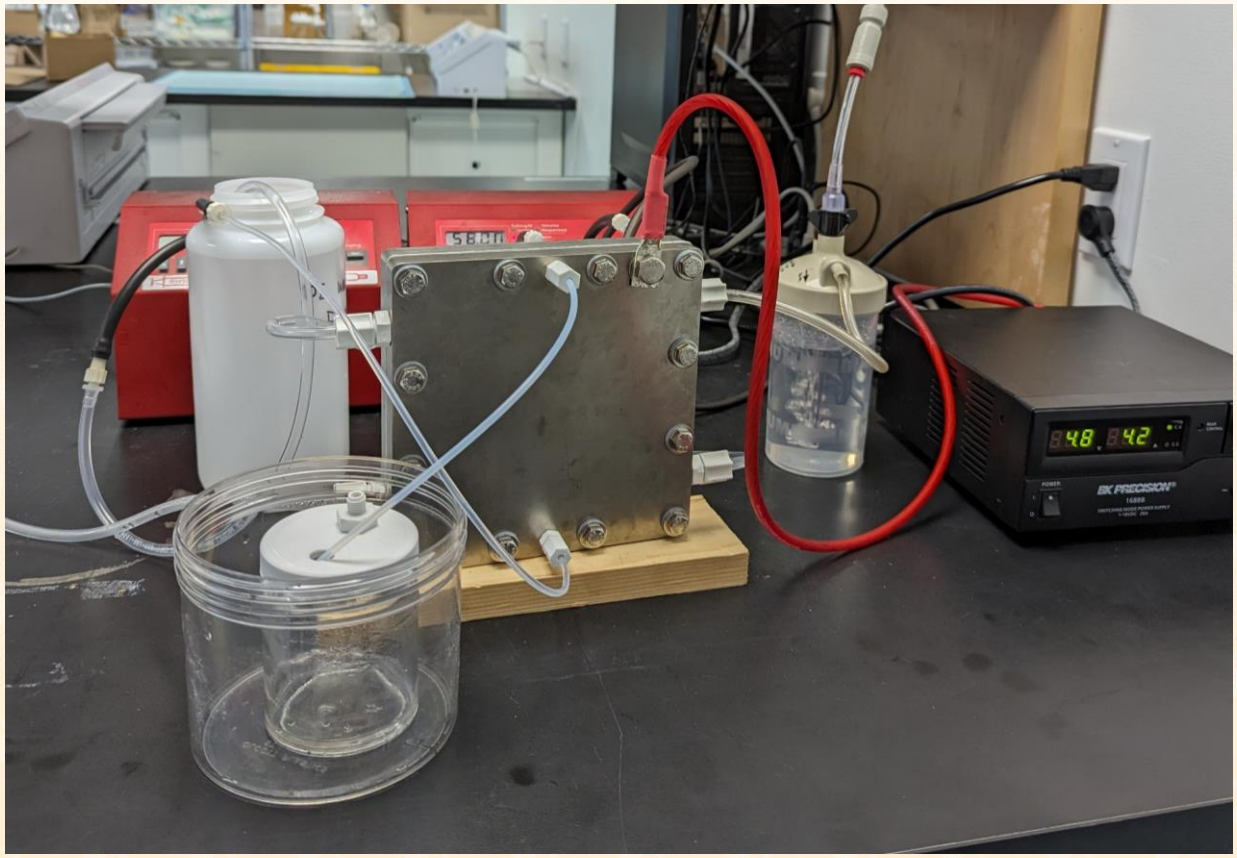


Task 2.1.1: 100 cm^2 Cell Fabricated





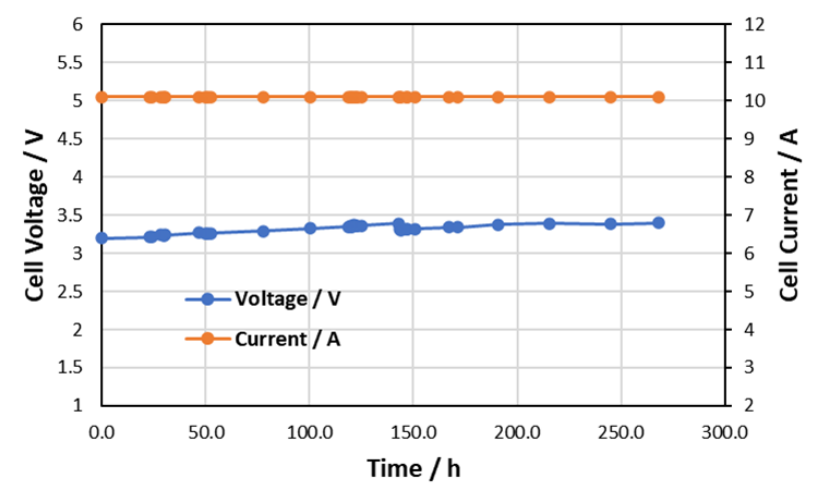
Picture Of Device

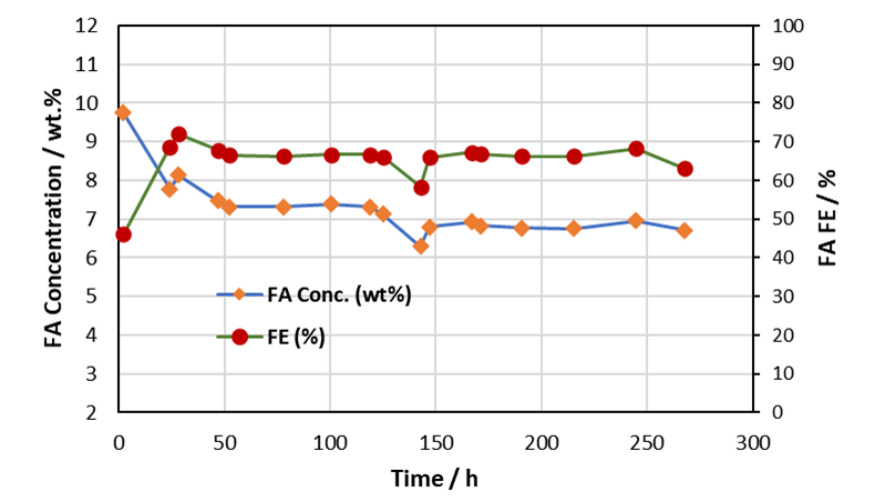


Improved performance via better center compartment & Nafion
Again meeting go/no-go milestone

Improving cell performance

- IR120 beads/sulfonated PEEK IEX
- Nafion 324 membrane

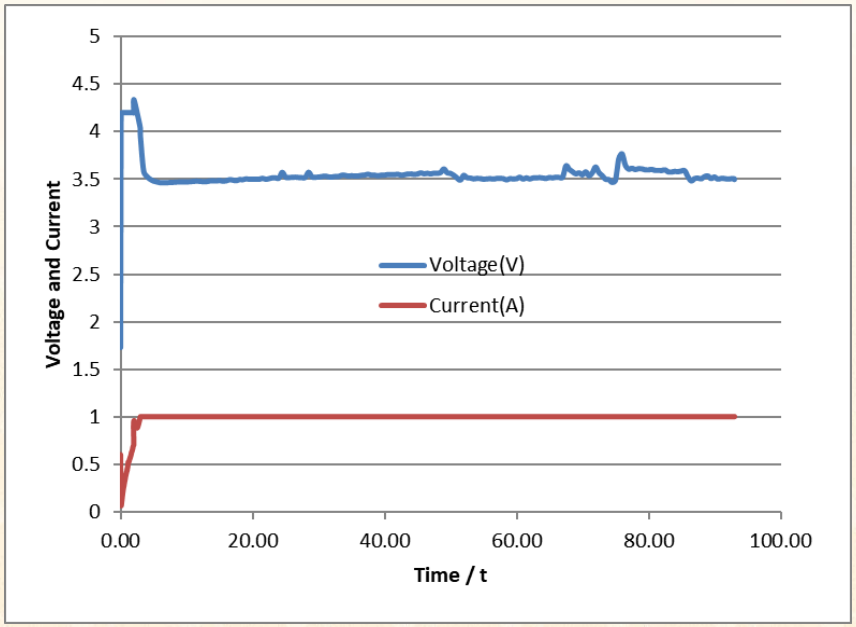




- >5 wt% formic acid
- Formic acid FE (>60% vs. ~35%) and performance stability greatly improved

New membrane to replace nafion

New membrane to replace Nafion (~50um vs. ~200um Nafion)



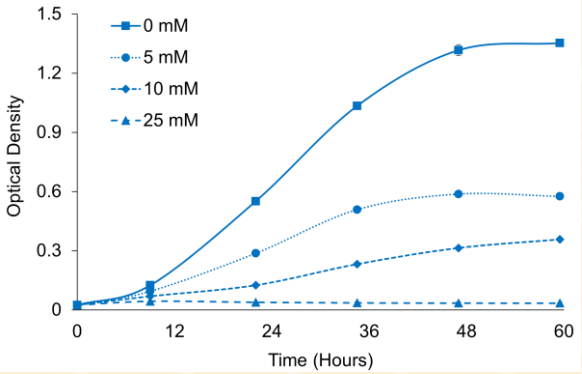
- ~3.5V at 1A current (200 mA/cm^2 current density)
- 8.3wt% FA after start
- 1.6 wt% FA 42%FE after overnight
- FA crossover to anode side (NMR confirmed tiny amount of FA, GC analysis showed CO2 in anode side, ~30% FA transferred to anode side)
- Didn't find pinholes on PEM membrane after disassembled the cell

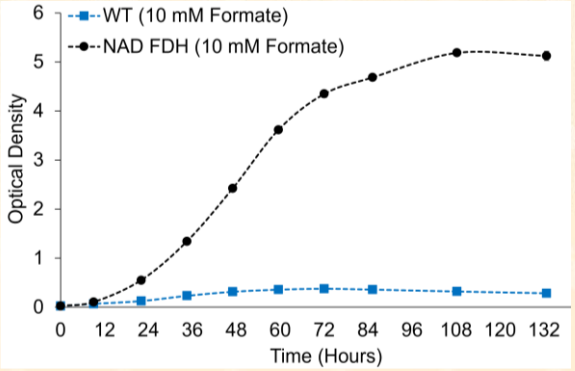
Background: Strain Development

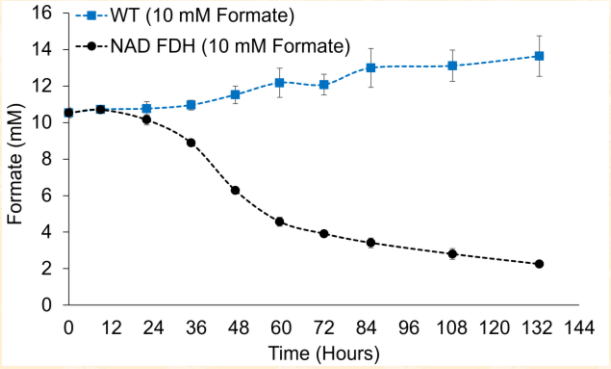
- Wild type *P. renovo* intolerant to formate > 5 milli-molar
  - No growth on formate alone
- Need to express formate dehydrogenase (FDH) to enable growth
  - FDH from *Cupriavidus* or *Candida*

Background - Engineering *P. renovo* for Photoformatotrophic Capacity

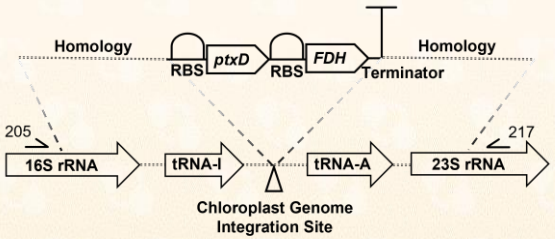
- Formate is toxic to wild-type *P. renovo* at concentrations as low as 5mM.
- Heterologous expression of formate dehydrogenase enables growth in up to 25mM formate.
- **Key Challenge:** growth on formate is currently slower than on concentrated CO_2 hindering economics
  - FDH expression and/or activity limitations
  - Formate transport limitations
  - NAD^+ limitations

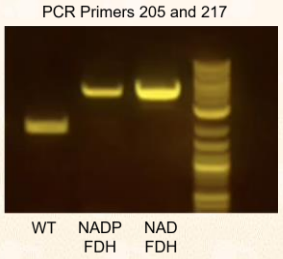




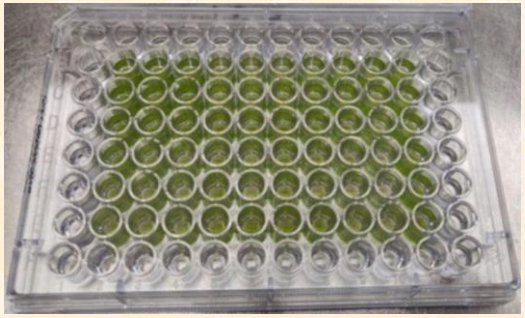


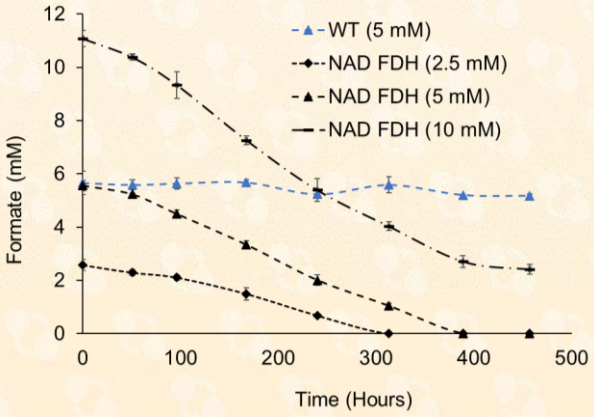
Progress –Targeted Genetic Engineering



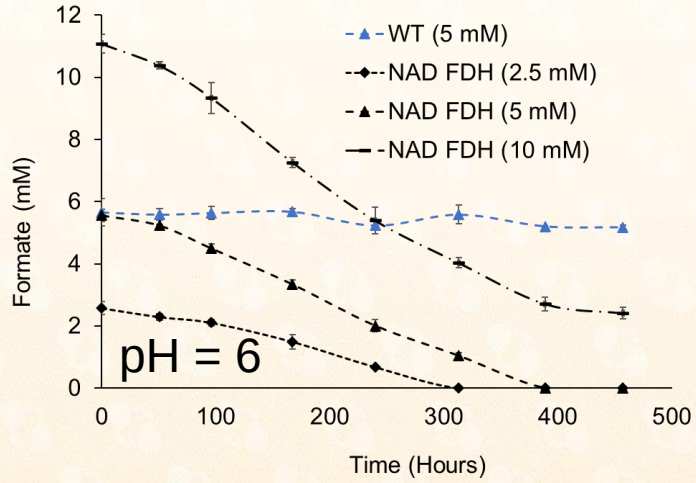


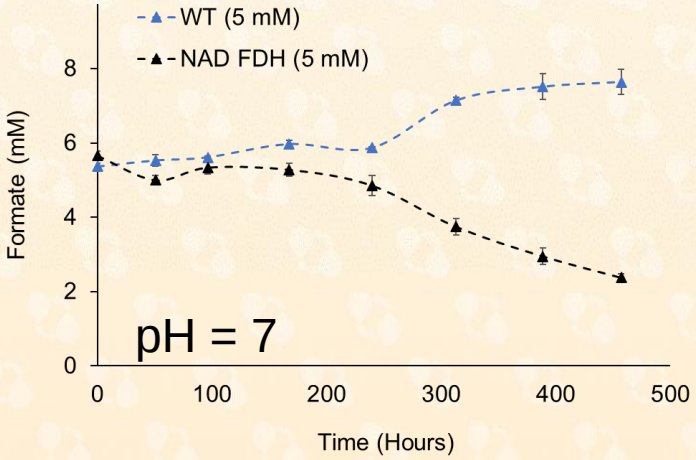
- Successfully designed, synthesized, and assembled FDH integration cassettes from 16 species of bacteria with known formate utilization capacity.
- Cassettes were heterologously incorporated into *P. renovo* and screened for formate utilization capacity.





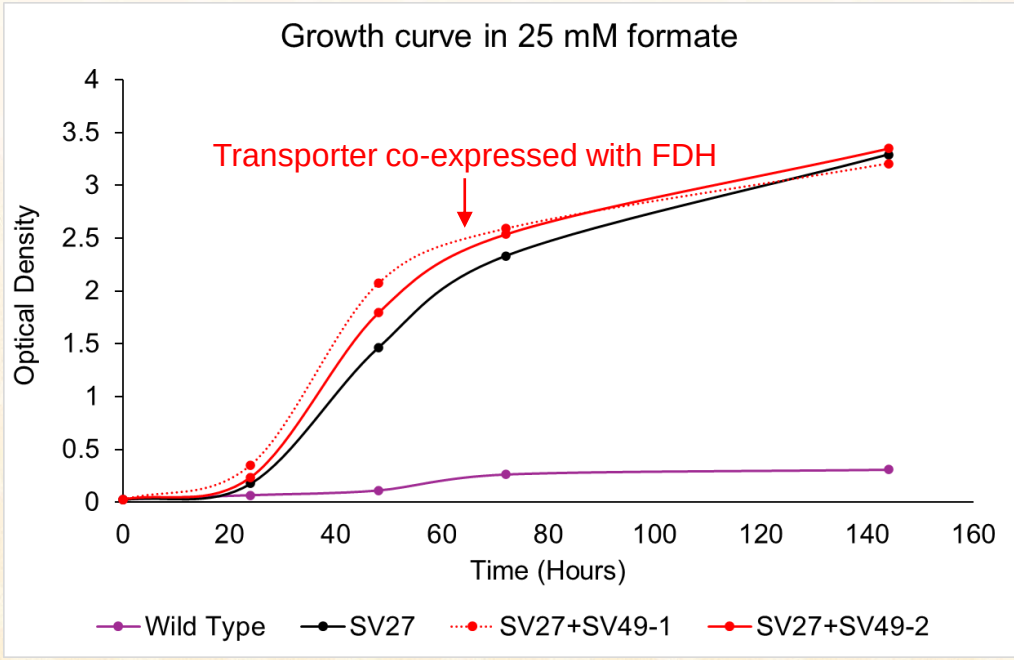
Progress – pH-mediated Optimization of Formate Uptake



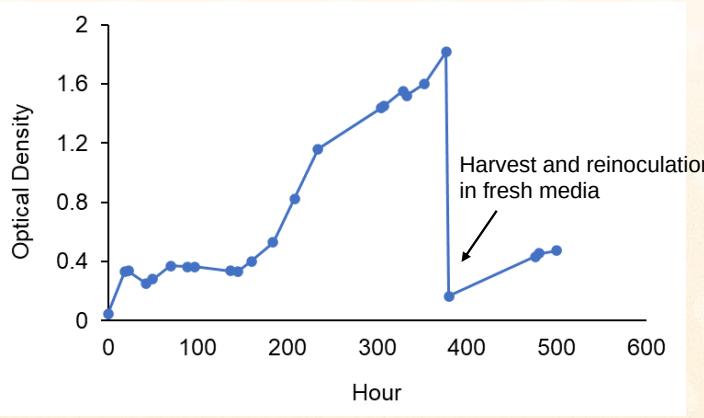


Formate Transporter Expression

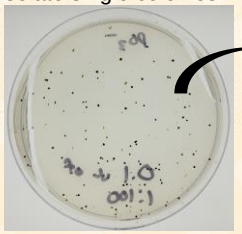
- Over expressed native formate-nitrite transporter from *P. renovo* in top candidate FDH strain (red lines).
- Preliminary results indicate a **putative growth enhancement with formate transporter expression**
- Additional transporter options from formatotrophs are being synthesized for expression and evaluation.



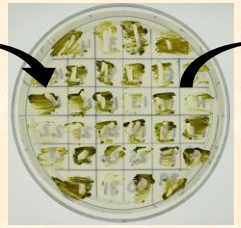
Improving Algal Formate Utilization

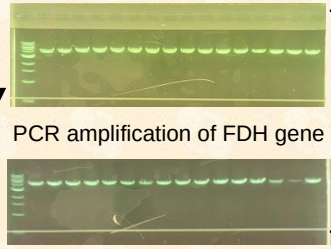


Dilution series plating to isolate single colonies



32 single colonies patched





DNA sequencing to determine which FDH strain(s) grew best and any resultant mutations

A PBR was inoculated with FDH-harboring *Picochlorum* variants to initiate a competition assay. Following 2 weeks of outgrowth, the culture was harvested.

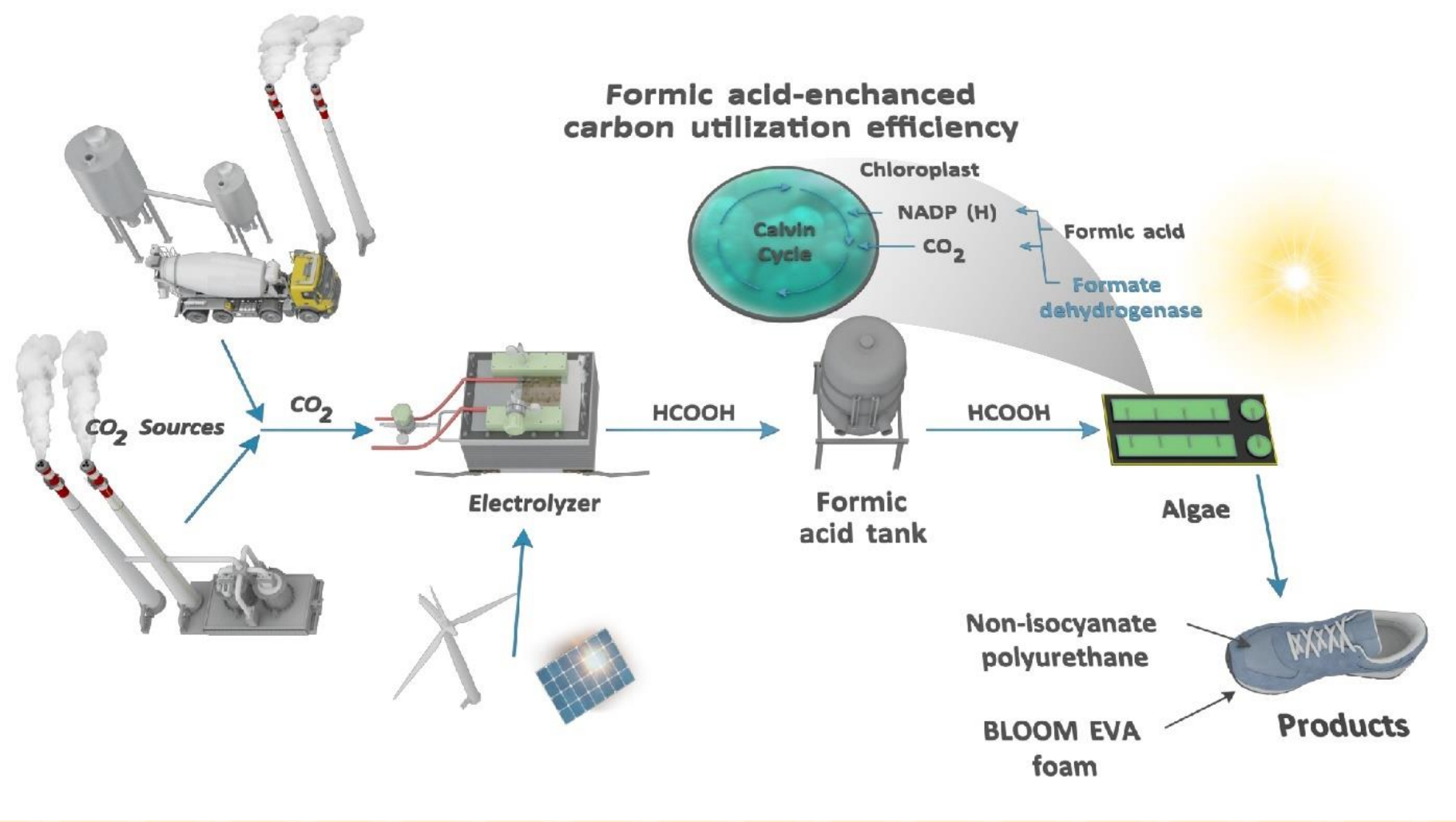
The mixed culture was plated on solid media, single colony isolates were patched onto a new selection plate, and the FDH gene from each strain was sequenced.

Initiated adaptive lab evolution experiments by resetting the PBR with fresh media to identify any FDH variants or mutants as emerged additional top-candidates.

Status Vs BP1 milestones

Item	Date
Revised Management Plan ✓	8-Mar-23 ✓
100 cm^2 cell designed ✓ and parts ordered✓	29-May-23 ✓
100 cm^2 electrolyzer producing 5% formic acid at ≥100 mA/cm^2 from simulated flue gas for ≥24 hrs ✓	04-Oct 23 ✓
100 cm^2 electrolyzer producing 5% formic acid at ≥100 mA/cm^2 from simulated flue gas for ≥100 hrs	04-Jan 24 ✓
Design, synthesize, and transform 5 formate dehydrogenase enzymes into P. renovo. Achieve >50% formate utilization	31-Mar 24 ✓
Acid pretreat biomass and quantify lipid class and fatty acid profile, utilize extant database to predict NIPU performance.	4-May 24 ✓
Generate 0.5 kg of biomass for downstream product testing	31-July 24 ✓
Utilize biomass composition to evaluate expected bioplastic conversion performance	31-July 24 ✓
100 cm^2 electrolyzer producing ≥5% formic acid from simulated flue gas at a current of ≥100 mA/cm^2 for ≥250 hours	31- July 24 ✓

Plans for future testing commercialization



Summary

- Program moving forward
  - Demonstrated scaling of electrolyzer 20x
    - Active area from 5 cm^2 to 100 cm^2 (250 hr)
  - Demonstrated *P. renovo* strains that grow on formate (5x than previous)
    - outperform wild type on atmospheric CO_2, Illumination 280µmol/m^2/sec

Acknowledgement

This work was supported by the department of energy under contract DE-FE0032186