DOE Bioenergy Technologies Office (BETO) 2023 Project Peer Review

April 4, 2023

Advanced Algal Systems

ASU's DAC polymer-enhanced cyanobacterial bioproductivity (AUDACity)

Project #: DE-EE0009274

DE-FOA-0002203: FY20 Bioenergy Technologies Multi-Topic FOA

Topic Area 3: Algae Bioproducts and CO_2 Direct-Air-Capture Efficiency (ABCDE)

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FOA Goals

Metrics	Unit	Minimum	Stretch
Algal biomass revenue potential	$ per ton harvested algae biomass	25% increase from applicant's baseline*	50% increase
Algal biomass quality for downstream testing	% meeting fuel and product(s) specifications	<10% out of specification	<5% out of specification
Algae areal productivity	$g/m^2/d$	Increase productivity 10% over applicant's baseline with CO_2 from DAC	Increase productivity >10% over applicant's baseline with CO_2 from DAC
DAC CO_2 delivered and utilized by algal system	% of DAC CO_2 delivered and utilized by algal system	20% increase over applicant's baseline	>20% increase over applicant's baseline
Cost** of CO_2 delivered to algal system	$ per volume of CO_2 delivered to the algae system from DAC versus non-DAC	10% decrease in the cost of CO_2 delivered via DAC versus non-DAC CO_2 delivery	>10% decrease in cost of CO_2 delivered via DAC versus non-DAC CO_2 delivery

*Applicant's baseline must include valorization pertinent characteristics such as moisture content; ash content; lipid, protein, carbohydrate content; cultivation media; nutrients; others

**Cost must be presented without incentives or tax credits.

What we are doing

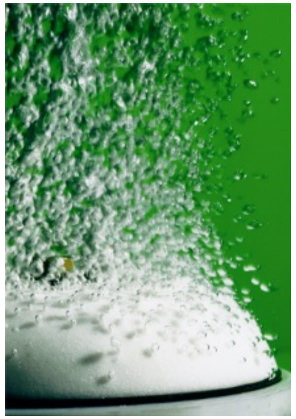
- Increase revenue potential >>25% over baseline (phycocyanin, methyl laurate)
- Increase biomass quality (>10% PC and methyl laurate by dry weight)
- Increase productivity >>10% over baseline (33 to 100 mg/L/d)
- Increase % of DAC CO_2 >>20% over baseline (from ~10% to ~100% DAC CO_2)
- Decrease cost of CO_2 delivered >>10% vs non-DAC CO_2 delivery (reduce DAC cost from $160/tonne to $50/tonne) using water to release captured CO_2

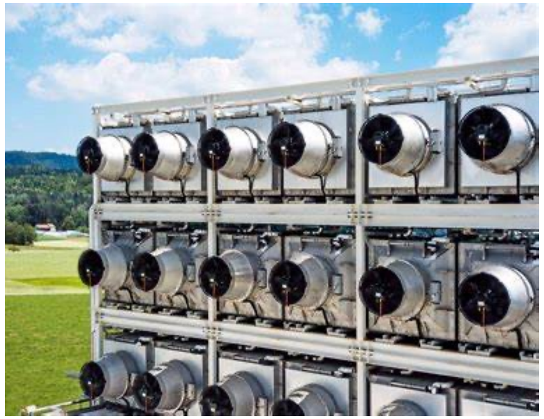
Project Overview

Challenges with existing CO_2 delivery approaches

- High cost and complexity in transporting concentrated CO_2 from industrial waste streams to the algae cultivation site
- 60–80% of CO_2 is lost when delivered by sparging
- CO_2 captured from air directly into ponds requires very high pH and alkalinity not suitable for most algal strains
- Direct air capture (DAC) of CO_2 requires energy intensive thermal or pressure swings to release CO_2 from sorbents

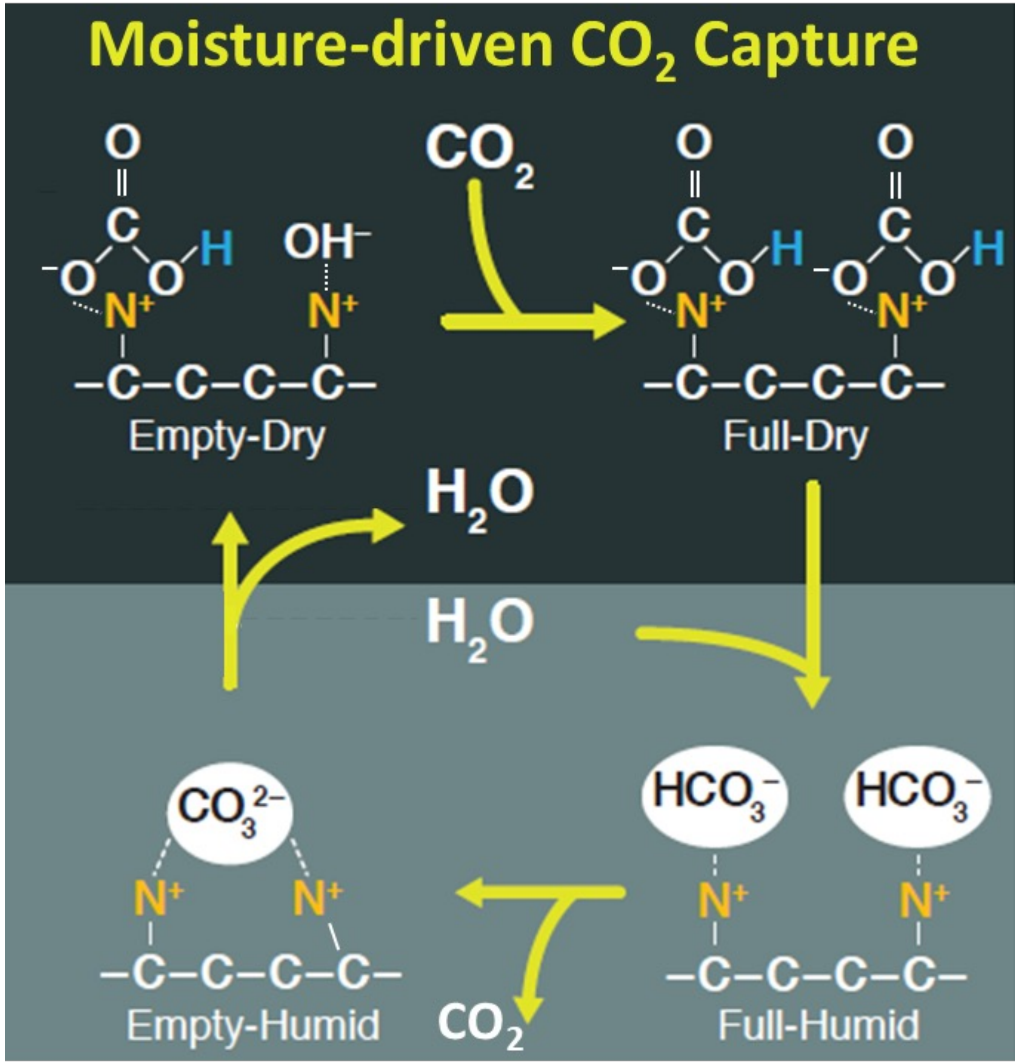
Also: Biomass cultivation and processing costs are too high to support fuel as the sole product





Moisture-driven direct air capture (DAC)

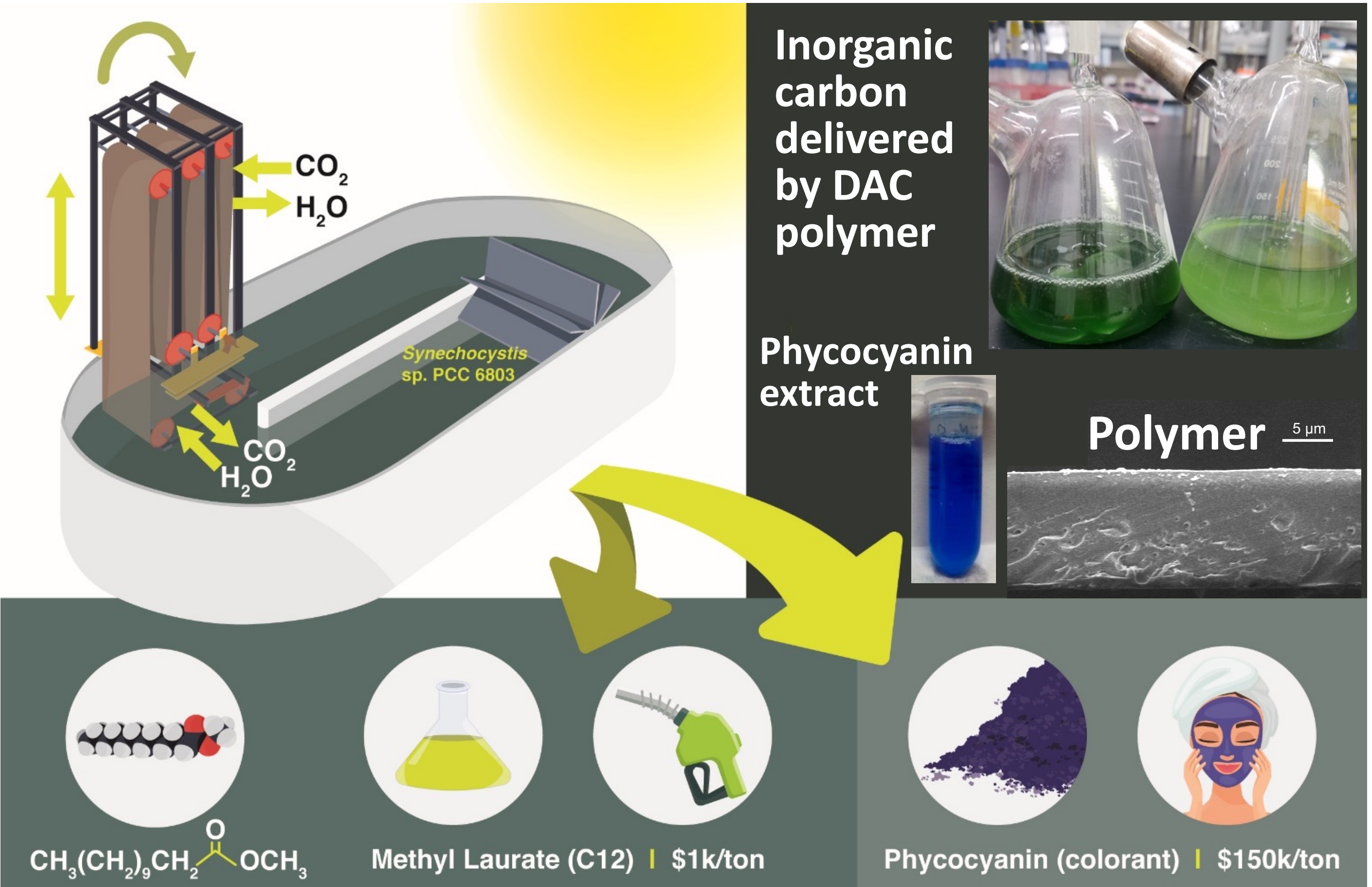
- Anion exchange resins used for water treatment also reversibly capture CO_2 when dry (≤ 50% relative humidity) and release it when wet



Project Overview

Technology Summary

- Use DAC polymers to passively capture CO_2 from air; deliver CO_2 directly into culture medium to grow cyanobacteria
- Engineered cyanobacteria excrete (methyl) laurate (chemical, fuel) and produce phycocyanin (blue colorant)



Advantages

- **Low projected cost** <$50/tonne CO_2 released into the culture medium from low-cost polymers.
- **Carbon-neutral** feedstock with all CO_2 from air to produce sustainable fuels and products.
- **CO_2 harvested onsite** to eliminate transport costs, risks and life cycle challenges.
- **pH 8–10** supports cyanobacterial growth.
- **Salt and fresh water**. Polymer designed to resist salts in fresh, brackish and salt water.
- **Sustainably produced (methyl) laurate** from cyanobacteria instead of oil palm and coconut.
- **Phycocyanin quality.** Phycocyanin is tested for improved pH, light and temperature stability.

Potential Challenges

- Develop an **effective DAC polymer**
- **Biocompatibility** of DAC polymers with microalgae
- **Limited current phycocyanin market size** due to limited thermostability

1. Technical Approach

Workplan and Key Milestones

Month = M

- **Task 1. Develop polymers** with improved CO_2 capture and release kinetics, fouling resistance, decreased inactive anion binding, reduced drying times, and process polymers into flexible, porous capture materials
  - **DAC polymer** captures ≥ 0.4 μmol CO_2 s^{-1} g^{-1} polymer and ≤ 1 h cycle time in lab (M24)
- **Task 2. Test biocompatibility** of DAC polymers and use AUDACity systems to cultivate biofuel- and high-value product-producing cyanobacteria at lab scale
  - **Go/No-Go: AUDACity** used to deliver CO_2 from air at a rate of ≥ 6.4 mg CO_2 L^{-1} h^{-1} to cultivate biofuel-producing *Synechocystis* at lab scale **achieves a total productivity of ≥ 50 mg L^{-1} d^{-1}** with all CO_2 sourced from air (M18)
- **Task 3. Develop AUDACity systems** to screen DAC polymer performance (M6), develop a continuous belt design (M12), and collect and deliver CO_2 to cultivate *Synechocystis* in the lab (M15) and outdoors (M33)
- **Task 4. Cultivate cyanobacteria outdoors** in 4 m^2 covered ponds using AUDACity to deliver CO_2 and assess productivity and biomass composition (M27, M33).
- **Task 5. Develop techno-economic and life cycle assessments** to guide research (M21, M36).

Project Success Criteria

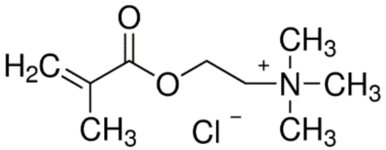
- Pathway to ~$50/tonne CO_2 delivered by AUDACity
- Higher efficiency than sparging; 100% of CO_2 from air
- ≥ 10% biomass productivity from increased C_i
- ≥ 25% revenue potential from (methyl) laurate, phycocyanin

2. Progress and Outcomes

DAC Polymer Preparation

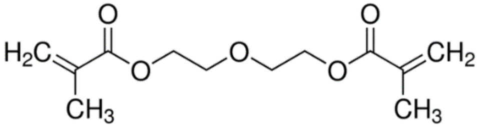
Methacrylate-based DAC polymer synthesis

CO_2 capture



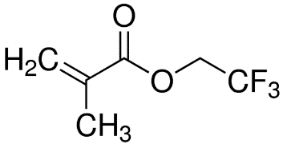
[2-(methacryloyloxy)ethyl] trimethylammonium chloride solution (TMAEMA-Cl)

Crosslinker



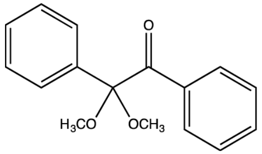
di(ethylene glycol) dimethacrylate (DEGDMA)

Hydrophobicity



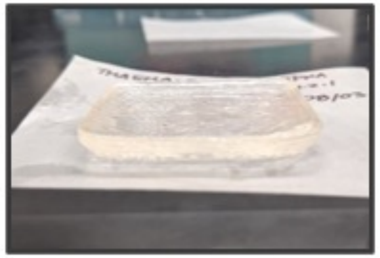
2,2,2-trifluoroethyl methacrylate (TFMA)

Photo-initiator



2,2-dimethoxy-2-phenylacetophenone (DMPA)

Cured with UV (365 nm, 2 mW) light for 6 min



Solid membrane (slow kinetics)

Soak in liquid N_2
Grind into powder

or

Exchange Cl^- (inactive) for HCO_3^- (active)

1 M $KHCO_3$, 24 h

Sorbent powder (faster kinetics)

Load into 25-µm-mesh bag

3 cm x 4 cm

Attach to adhesive sheet

DAC Polymer Performance

Deliverable 1.3.4: sorbent captures ≥ 0.4 μmol CO_2 s^{-1} g^{-1} sorbent and ≤ 1 h cycle time in outdoor environment (M24).

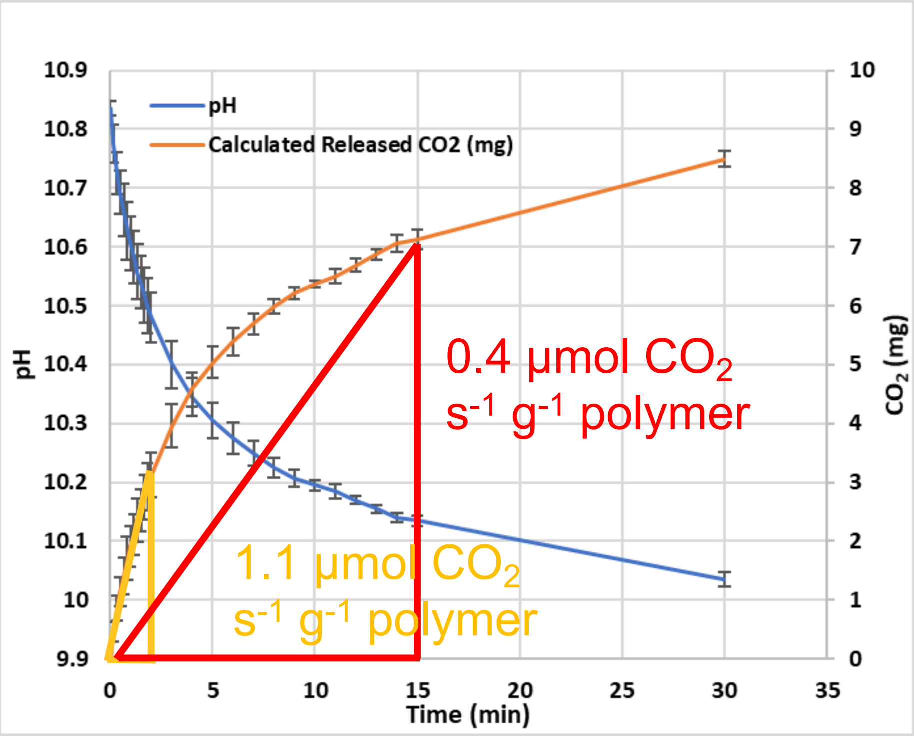
Sorbent CO_2 release into a 10 mM K_2CO_3 solution

- 0.5 g of sorbent particles loaded in a 25-μm-mesh bag and submerged into 50 mL of 10 mM K_2CO_3
- Sorbent was previously used to support *Synechocystis* growth

Sorbent drying and CO_2 loading in the wind tunnel

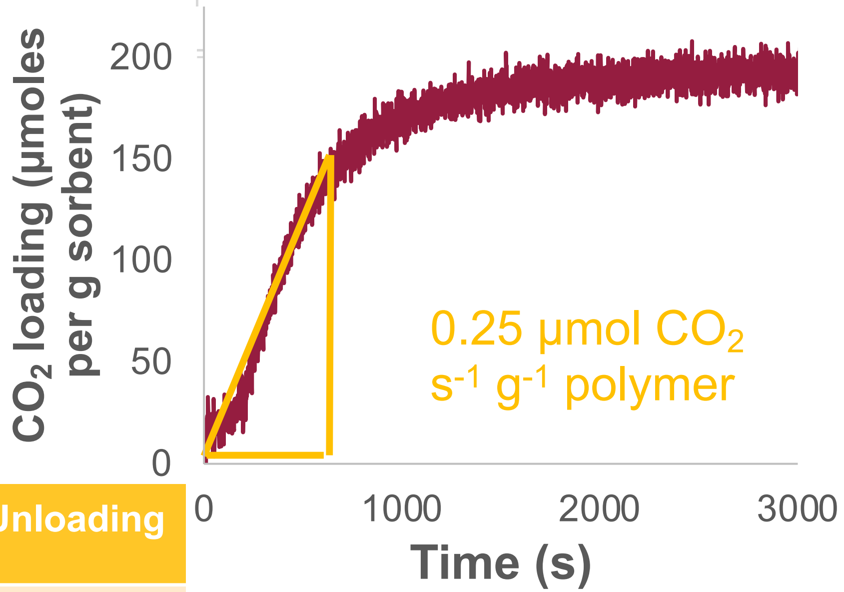
- 1.6 g sorbent particles attached to adhesive sheets
- Submerged in water for 30 minutes to fully unload
- Sorbent drying/loading measured at 2.5 m/s, 22 °C

CO_2 release



0.4 μmol CO_2 s^{-1} g^{-1} polymer

1.1 μmol CO_2 s^{-1} g^{-1} polymer



0.25 μmol CO_2 s^{-1} g^{-1} polymer

CO_2 binding

Capacity	Loading	Unloading
50%	7 min	4 min
80%	12 min	13 min
90%	**18 min**	**20 min**
100%	60 min	30 min

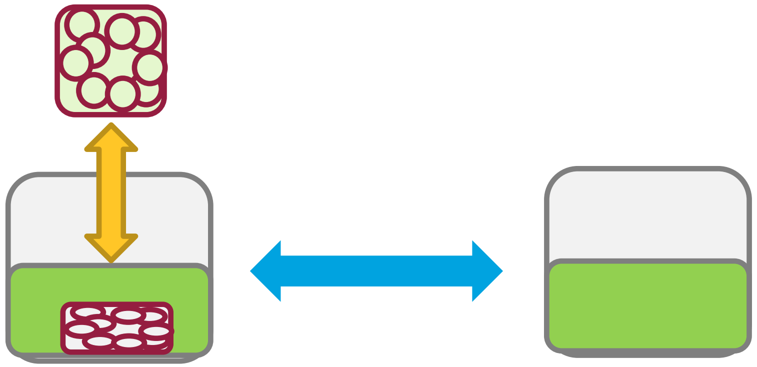
Cultivation with DAC polymers

Milestone 2.2.1 [Go/No-Go]:
AUDACity is used to deliver CO_2 from air at a rate of ≥ 6.4 mg CO_2 L^{-1} h^{-1} to cultivate biofuel-producing *Synechocystis* at lab scale with a total productivity of ≥ 50 mg L^{-1} d^{-1} with all CO_2 sourced from air (M18).

Use methacrylate-based powdered sorbent in 25-µm-mesh bags or three Excellion sheets

1. Add polymer for 30 min
Wash and dry overnight

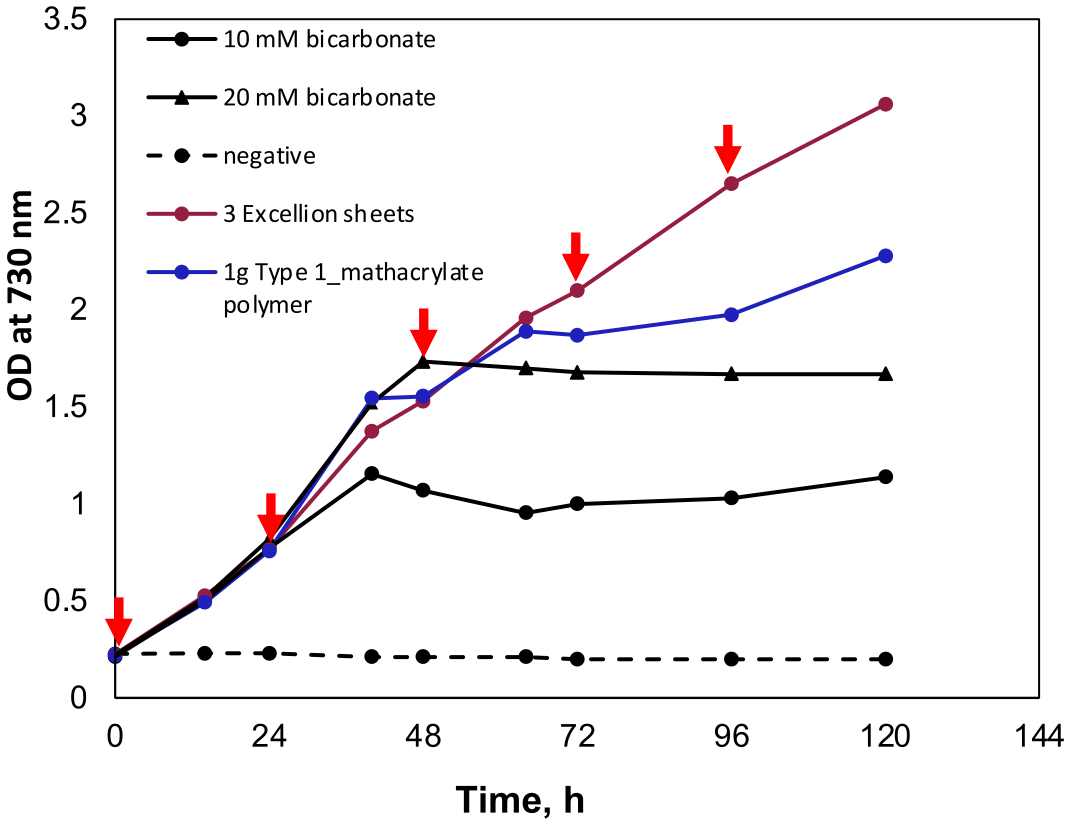
2. Cultivate for 24 h



Culture with CO_2 provided by polymer

Cultivation: 70-80 µmole photons m^{-2} s^{-1} shaking at 100 rpm, 30 °C

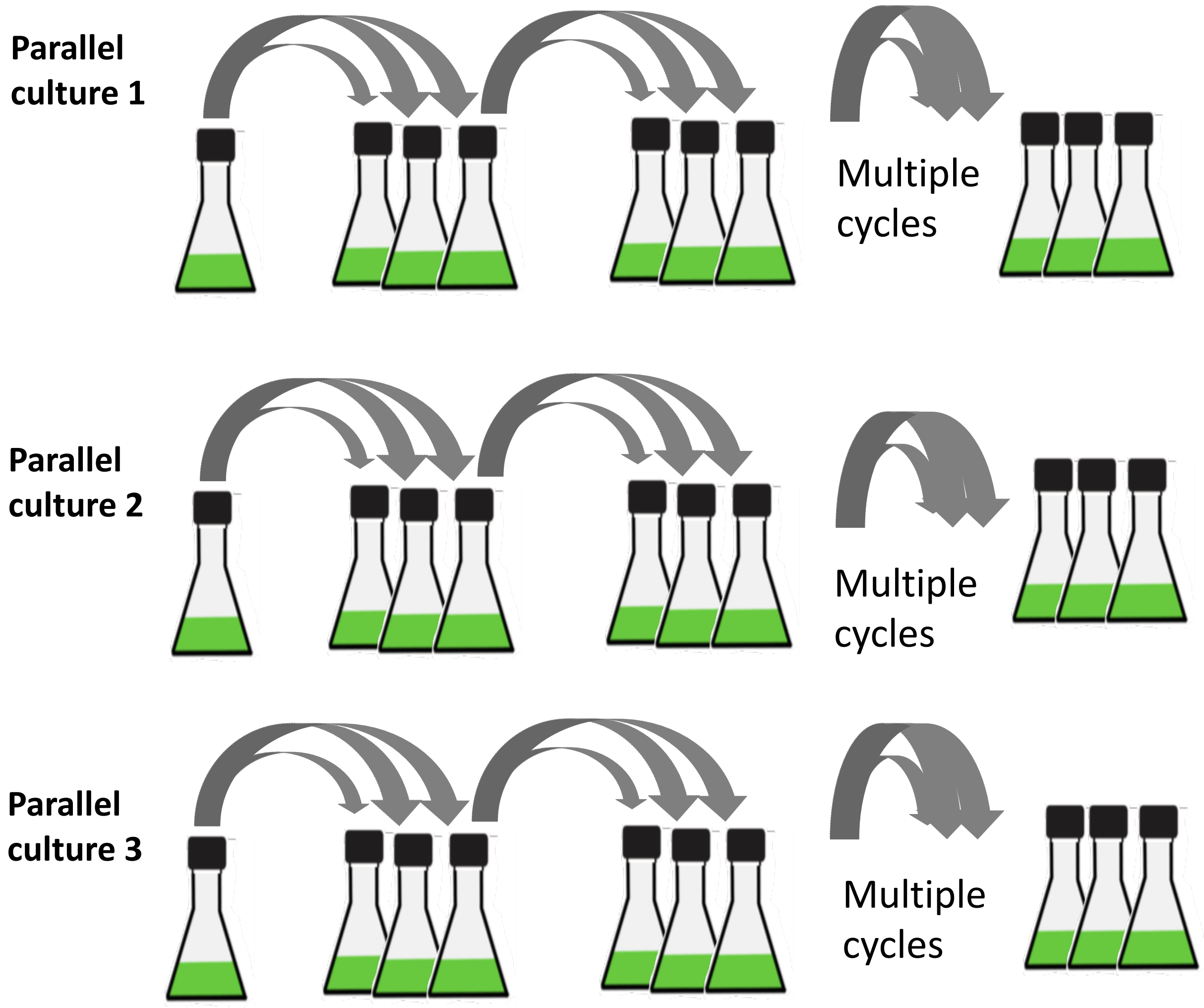
Powdered sorbent: 120 mg L^{-1} d^{-1} (3 d)
Excellion sheets: 115 mg L^{-1} d^{-1} (5 d)



↓: dry (CO_2-loaded) polymer provided for 30 min

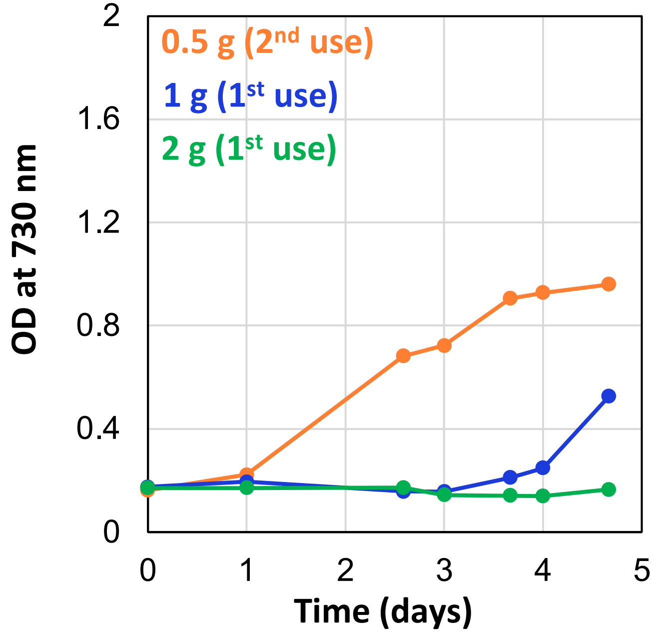
Adaptive laboratory evolution (ALE)

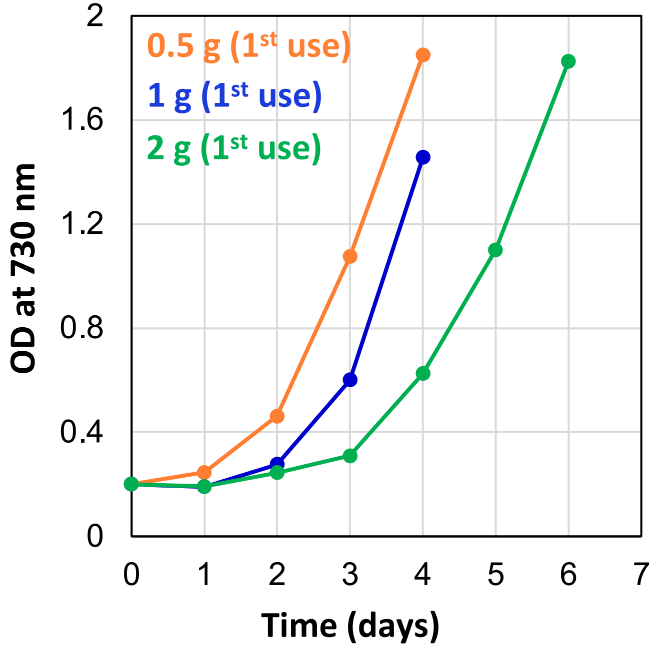
Evolving a polymer-tolerant *Synechocystis* strain

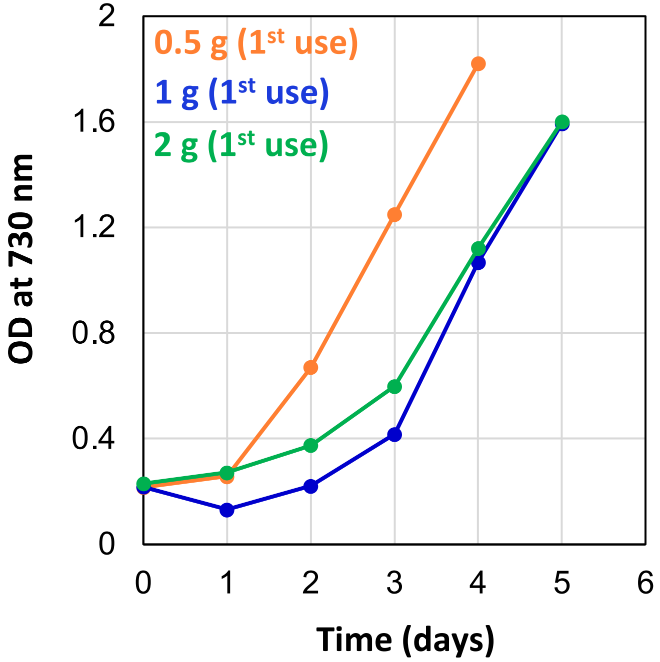


- Cultivate three *Synechocystis* cultures in parallel with increasing DAC polymer amount (0.5, 0.75, 1.0 g) and exposure time (10, 20, 30, 40 min)
- Add 20 mM $KHCO_3$ every 2 days to focus selection on polymer tolerance
- Sub-culture in triplicate after two doublings.
- Maintain three separate parallel cultures to determine common genetic changes.
- Polymer most toxic during first use

Adaptive laboratory evolution (ALE)


1st run, 10 min exposure


11th run, 20 min exposure

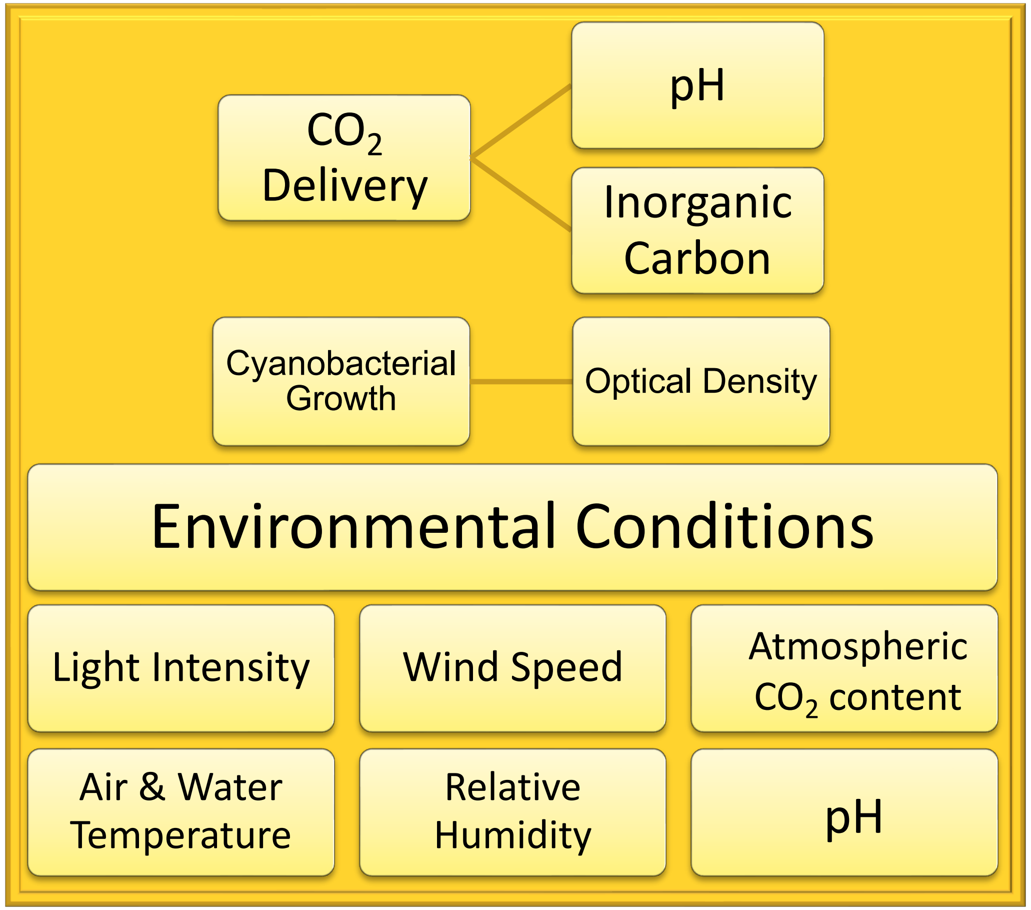

14th run, 40 min exposure

Key findings are that ALE-derived strains:

- Have a reduced lag phase
- Grow to higher densities
- Tolerate longer exposures to the polymer

AUDACity 1g system

Validate system using commercial Excellion strips



CO_2 Delivery

pH

Inorganic Carbon

Cyanobacterial Growth

Optical Density

Environmental Conditions

Light Intensity

Wind Speed

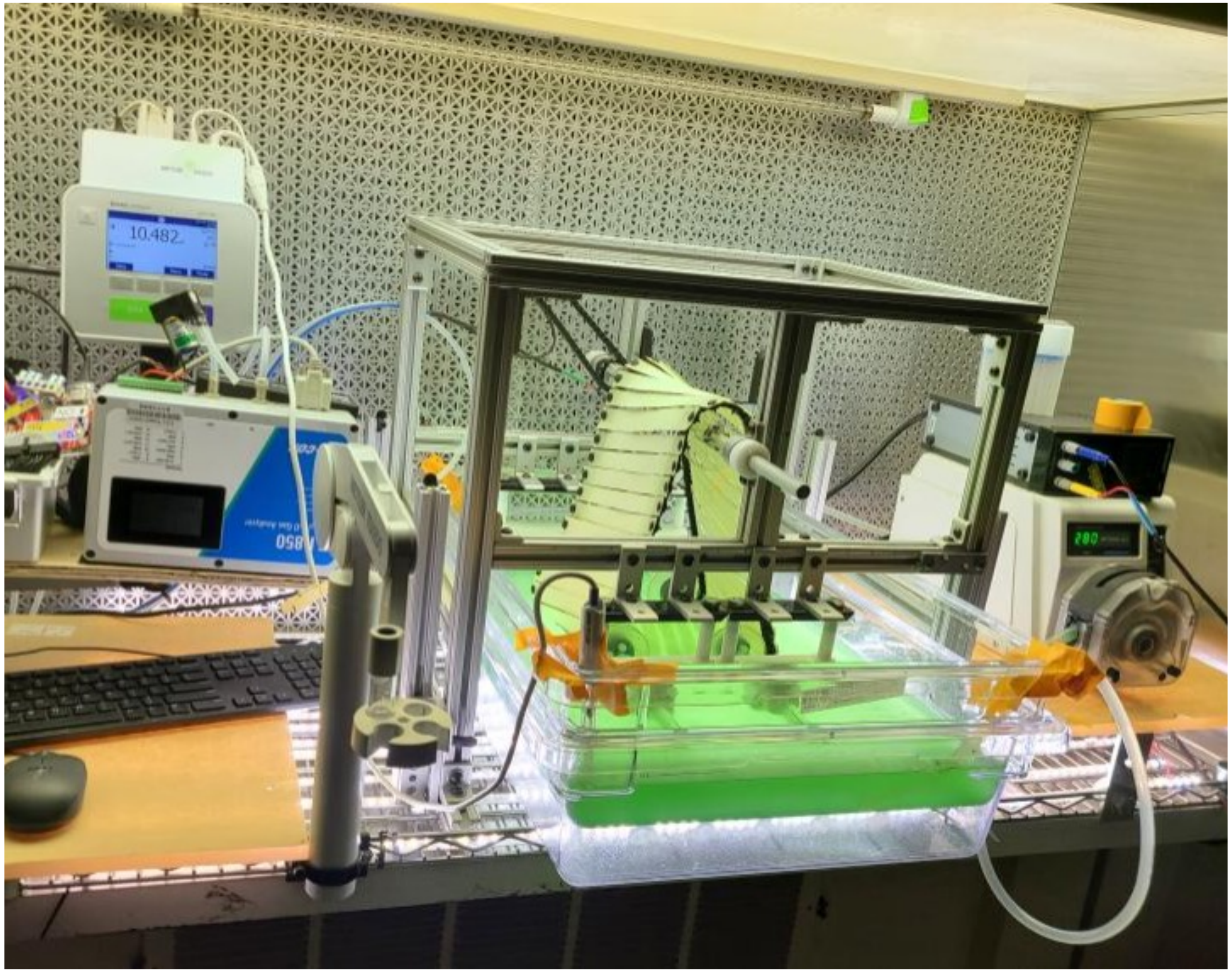
Atmospheric CO_2 content

Air & Water Temperature

Relative Humidity

pH

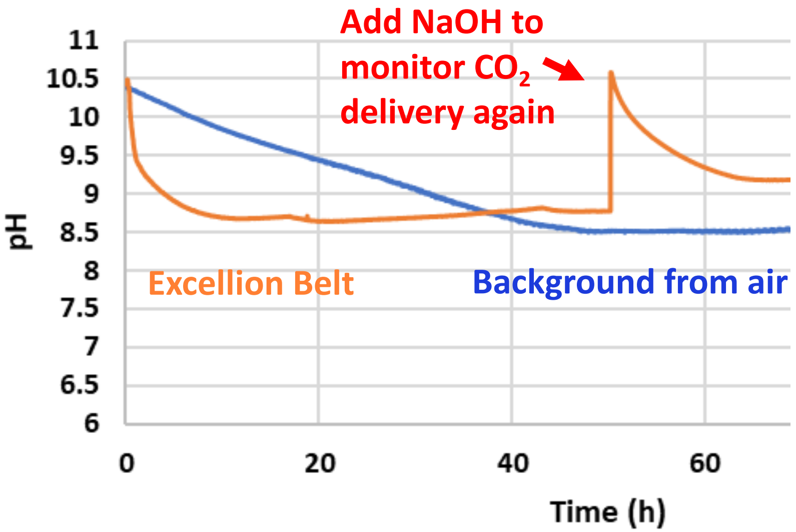
Automated data collection facilitates longer-duration tests for better material lifetime and performance stability projections.



1 g System Excellion Test Run

CO_2 release kinetics in 1 mM $KHCO_3$ (no cells)

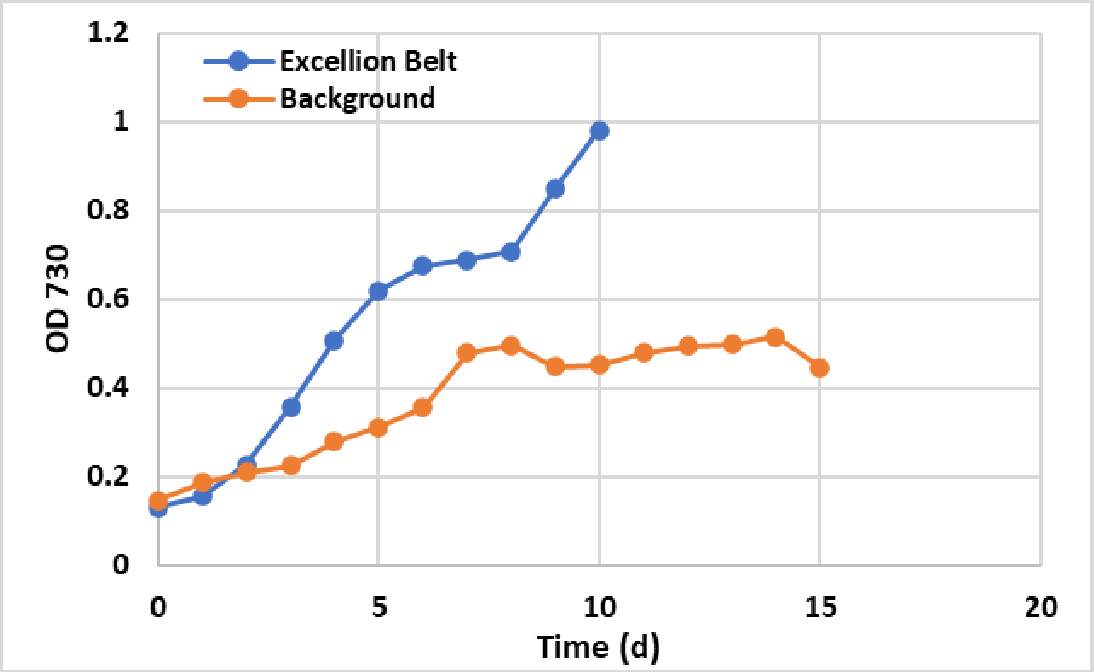
- ~21 min per full rotation of Excellion belt
- ~95% of expected initial performance


Add NaOH to monitor CO_2 delivery again

Slower delivery of CO_2 the second time around: insufficient time to dry Excellion belt. We have increased belt size to improve drying between immersions into culture medium.

Cultivation:

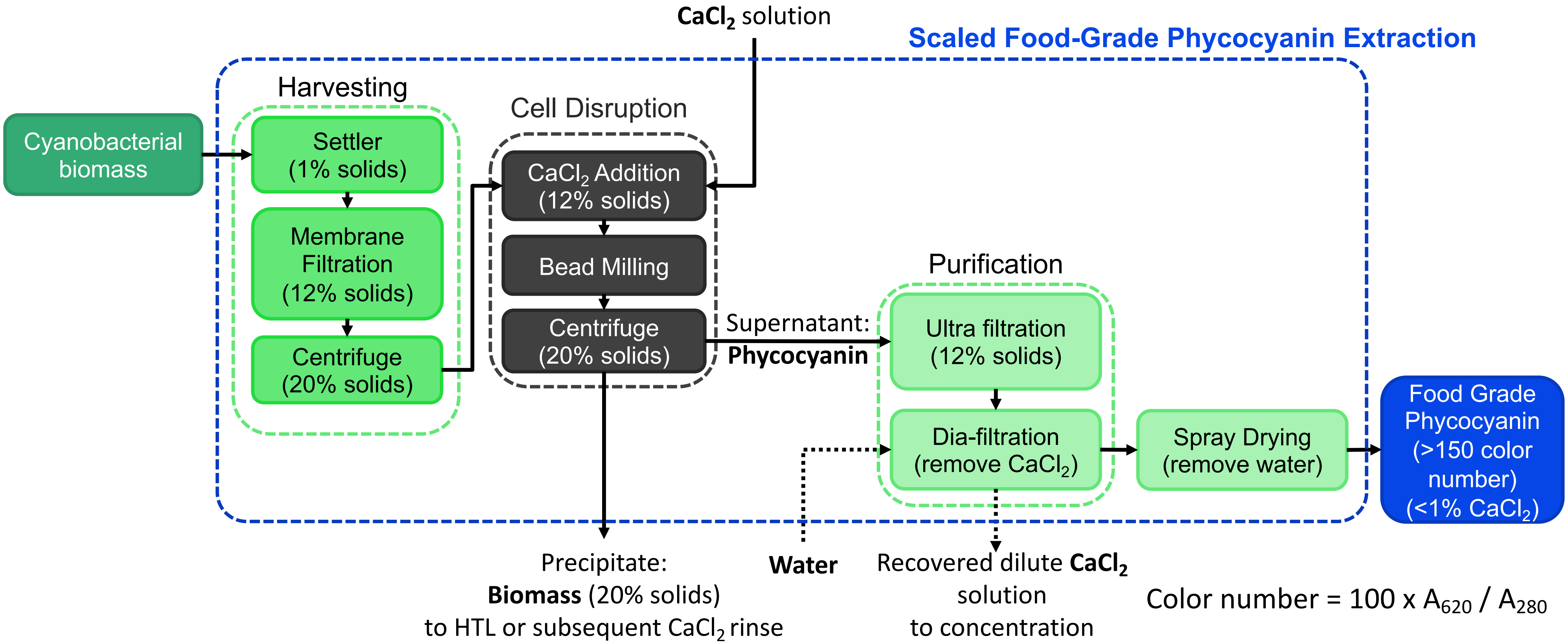
- CO_2 delivery from Excellion reduced over time due to exchange of HCO_3^- for NO_3^- in BG11
- Excellion performance restored after soak in $KHCO_3$
- Some background CO_2 delivery observed (not from belt); this is being remedied with a partial cover



Phycocyanin Co-Product Extraction

- **Up to 20% of dry biomass** in cyanobacteria; value from \$1-200/kg depending on purity
- Natural **alternative to chemical dyes** in food, beverage and cosmetics
- **Low thermal stability:** 60% loss after 1 min at 100 °C or 30 min at 80 °C

Martelli, G. et al. Thermal stability improvement of blue colorant C-phycocyanin from *Spirulina platensis* for food industry applications. *Process Biochemistry* **2014,** *49*, 154-159



$CaCl_2$ solution

Scaled Food-Grade Phycocyanin Extraction

Cyanobacterial biomass

Harvesting

Settler (1% solids)

Membrane Filtration (12% solids)

Centrifuge (20% solids)

Cell Disruption

$CaCl_2$ Addition (12% solids)

Bead Milling

Centrifuge (20% solids)

Supernatant: **Phycocyanin**

Purification

Ultra filtration (12% solids)

Dia-filtration (remove $CaCl_2$)

Spray Drying (remove water)

Food Grade Phycocyanin (>150 color number) (<1% $CaCl_2$)

Precipitate:
Biomass (20% solids)
to HTL or subsequent $CaCl_2$ rinse

Water

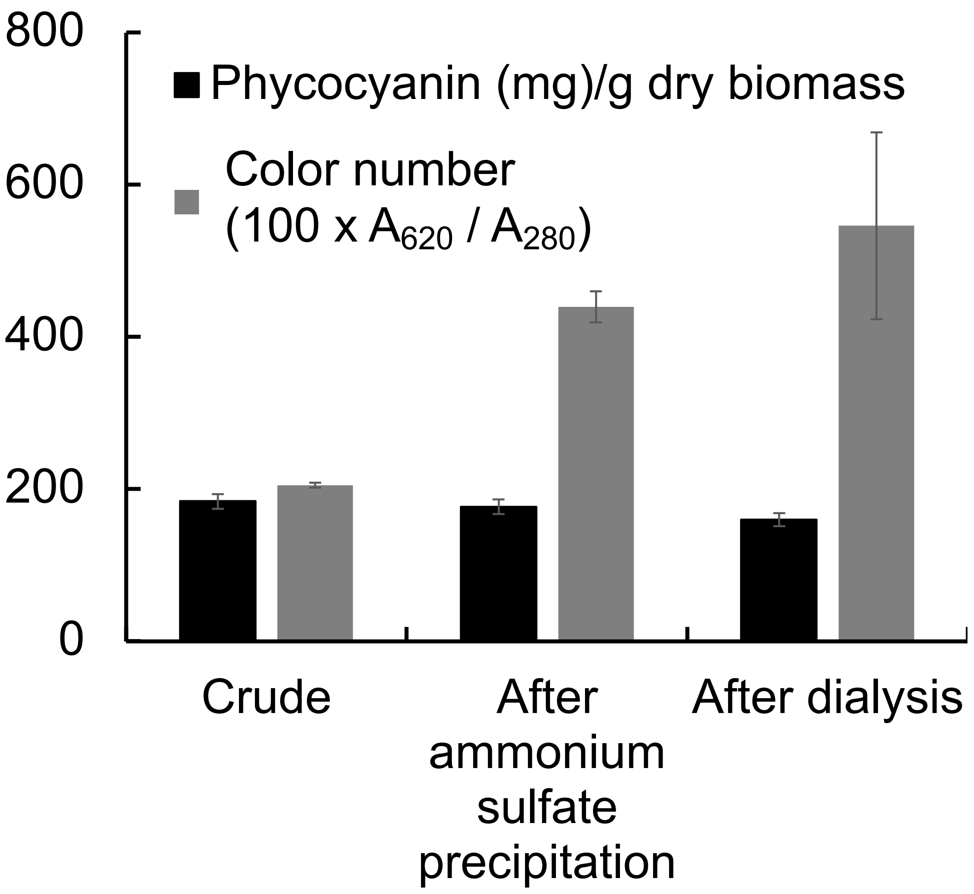
Recovered dilute **$CaCl_2$**
solution
to concentration

Color number = 100 x A_{620} / A_{280}

Phycocyanin (PC) Purity and Thermostability

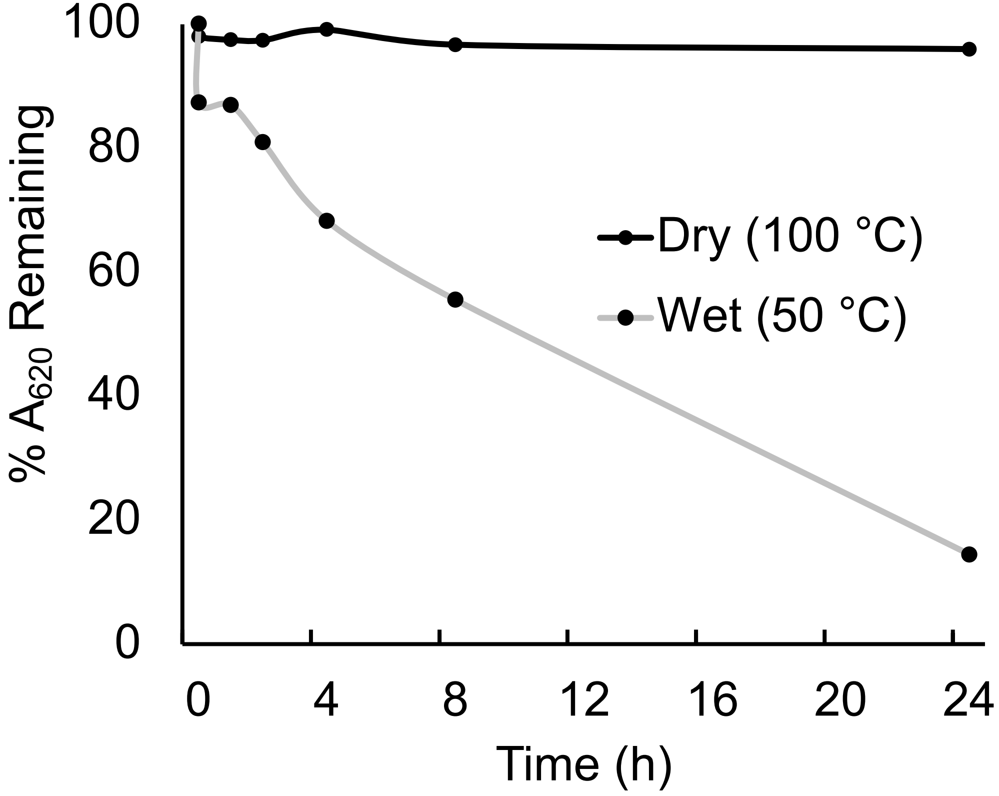
PC yield and purity during extraction

Synechocystis sp. PCC 6803



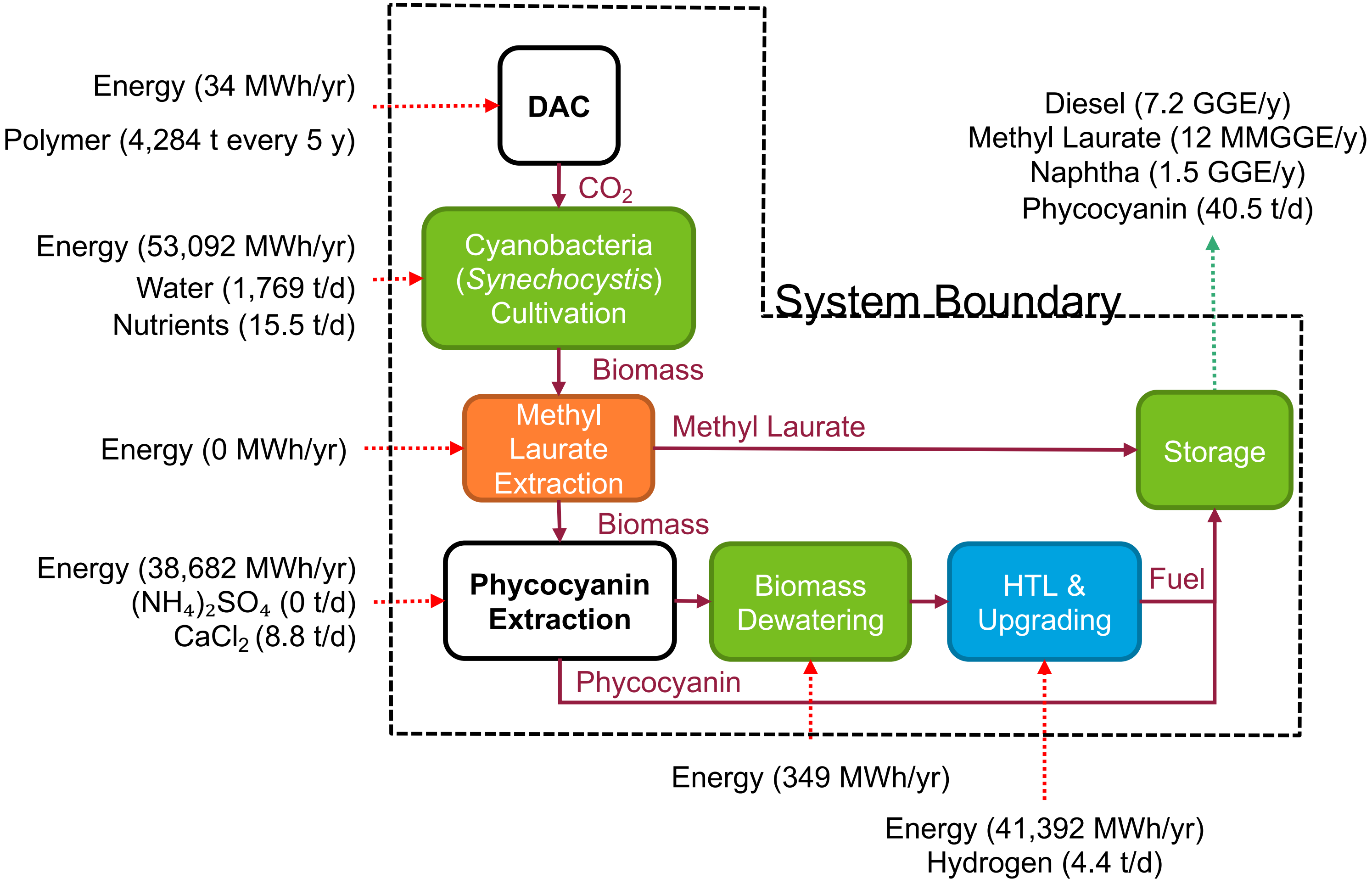
PC thermostability: dry vs wet

Synechocystis sp. PCC 6803



We are developing strategies to encapsulate PC to keep it dry and maintain color upon heating

Techno-Economic and Lifecycle Analysis



Energy (34 MWh/yr)
Polymer (4,284 t every 5 y)

DAC

CO_2

Energy (53,092 MWh/yr)
Water (1,769 t/d)
Nutrients (15.5 t/d)

Cyanobacteria (*Synechocystis*) Cultivation

Biomass

Energy (0 MWh/yr)

Methyl Laurate Extraction

Methyl Laurate

Storage

Diesel (7.2 GGE/y)
Methyl Laurate (12 MMGGE/y)
Naphtha (1.5 GGE/y)
Phycocyanin (40.5 t/d)

System Boundary

Biomass

Energy (38,682 MWh/yr)
$(NH_4)_2SO_4$ (0 t/d)
$CaCl_2$ (8.8 t/d)

Phycocyanin Extraction

Biomass Dewatering

HTL & Upgrading

Fuel

Phycocyanin

Energy (349 MWh/yr)

Energy (41,392 MWh/yr)
Hydrogen (4.4 t/d)

Deliverable 5.1.2: Engineering process model accurately captures the energy and mass of the biorefinery concept (M21).

Cited Models

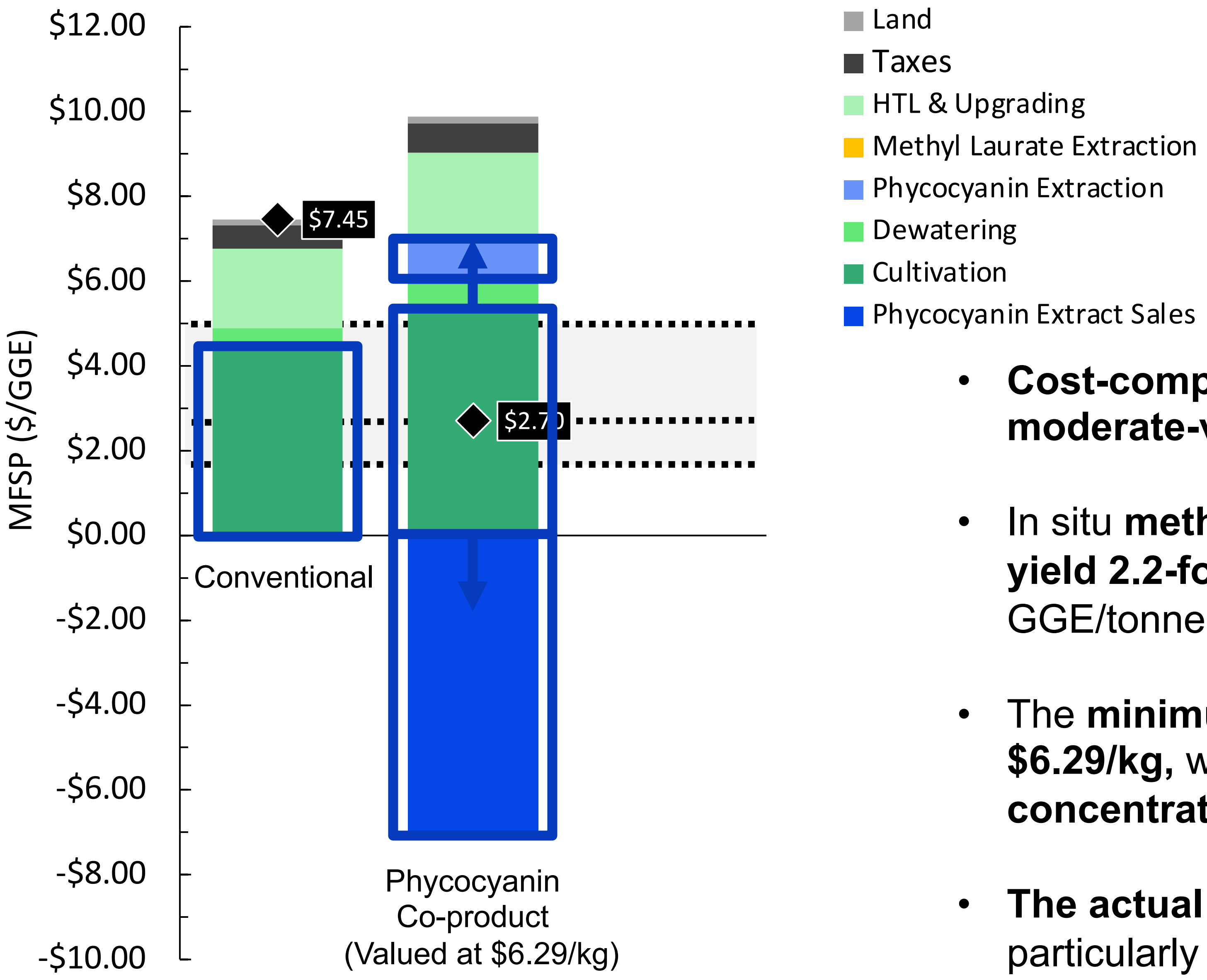
1. HTL Model (Chen et al.,2021)
2. Cultivation Model (Davis et al.,2016)
3. Methyl Laurate Extraction Model (Beattie et al., 2022)

New Models

- DAC Model
- Phycocyanin Extraction Model

1. Chen, P. H., & Quinn, J. C. (2021). Microalgae to biofuels through hydrothermal liquefaction: Open-source techno-economic analysis and life cycle assessment. *Applied Energy*, 289, 116613.
2. Davis, R., Markham, J., Kinchin, C., Grundl, N., Tan, E. C., & Humbird, D. (2016). *Process design and economics for the production of algal biomass: algal biomass production in open pond systems and processing through dewatering for downstream conversion* (No. NREL/TP-5100-64772). National Renewable Energy Lab.(NREL), Golden, CO (United States).
3. Beattie, A., Vermaas, W., Darzins, A., Holland, S. C., Li, S., McGowen, J., ... & Quinn, J. C. (2021). A probabilistic economic and environmental impact assessment of a cyanobacteria-based biorefinery. *Algal Research*, *59*, 102454.

Techno-Economic and Lifecycle Analysis



- **Cost-competitive fuels are possible with moderate-value co-products**
- In situ **methyl laurate harvesting improves fuel yield 2.2-fold** over green algae (from 113 to 259 GGE/tonne).
- The **minimum co-product selling price is $6.29/kg,** which is equivalent to **whey protein concentrate**
- **The actual value of phycocyanin is much higher,** particularly as it becomes much more thermotolerant

Risk Analysis

Demonstrated or Perceived Risk	Mitigation Strategy
1. Biological and inorganic (salt) fouling of the DAC polymers	**1.** Incorporate zwitterions within or apply hydrophobic coatings to surface of the DAC polymer
2. DAC polymer toxicity to cyanobacteria	**2.** Change polymer chemistry and generate tolerant strains
3. Methyl laurate sticks to the hydrophobic DAC polymer	**3(a).** Use perfluorinated or silicone functionality that repels both water and oil. **3(b).** Run DAC polymer through organic solvent to harvest methyl laurate continuously
4. DAC polymer has insufficient CO_2 transport rates	**4.** Increase DAC polymer porosity to increase the surface area for CO_2 capture and to quickly release water to accelerate drying
5. Failure to meet economic and environmental targets	**5.** TEA/LCA to identify performance targets and integration requirements to ensure the system meets targets

3. Impact

Impact on the State-of-Technology if successful

- **Reduce cost of CO_2 delivered to microalgae** (projected <$50/tonne CO_2) without pipelines or fossil inputs
- **Reduce carbon intensity** of algae fuels and products with all CO_2 sourced from air
- **Support cultivating a wide range of algae and cyanobacteria** by operating at somewhat alkaline pH and in saltwater or freshwater medium
- **Establish biorefinery model with cost-competitive fuel product** subsidized by high- and medium-value co-products (phycocyanin, protein, methyl laurate)
- **Reduce cost and increase stability/value of phycocyanin** to enable larger food and beverage markets
- **Enable continuous harvest** of excreted fuel/chemicals (methyl laurate) to reduce harvesting costs

Summary

- **Synthesized and commercial DAC polymers** deliver 90% of their CO_2 captured from air into cultivation medium within 15 minutes and load again with CO_2 from air to 90% of their capacity within 20 minutes without any external energy inputs.

- **Lab-scale cultivation with AUDACity** at a biomass productivity of 115–120 mg L^{-1} d^{-1} using synthesized DAC polymer powders contained in mesh and commercial anion exchange resin sheets.

- **DAC polymer toxicity is significantly reduced** after soaking in water and after 10–15 rounds of adaptive laboratory evolution

- **Phycocyanin** **1)** can be efficiently extracted at high purity, and **2)** has very high thermal stability when dry (>100 ^{o}C), thus providing an avenue to increased phycocyanin use/value.

- **Cost-competitive fuels** are possible with a modest-value phycocyanin co-product ($6.29/kg).

- **Methyl laurate harvesting** improves fuel yield 2.2-fold (259 GGE/tonne) vs green algae.

Quad Chart Overview – ASU Vermaas AUDACity

Timeline

- *Project start date: Oct 1, 2020*
- *Project end date: Sept. 30, 2023*

	FY22 Costed	Total Award
DOE Funding	$622,040	$1,999,051
Project Cost Share	$96,761	$506,343

TRL at Project Start: **2**
TRL at Project End: **5**

Project Goal

Develop an AUDACity system that efficiently delivers direct air captured (DAC) CO_2 directly to photosynthetic microbes using moisture-driven sorbents and demonstrate it in outdoor raceway ponds.

End of Project Milestone

Operation of AUDACity delivering CO_2 from air to Synechocystis with a productivity ≥ 10% over baseline without sparging CO_2

Funding Mechanism

DE-FOA-0002203: FY20 Bioenergy Technologies Multi-Topic FOA

Topic Area 3: Algae Bioproducts and CO_2 Direct-Air-Capture Efficiency (ABCDE)

Project Partners

- Arizona State University (ASU)
- Sustainability Science LLC

Supplemental slide

Feedback from Intermediate Verification (Apr. 2021)

- **Spend more time at lab scale (1 g/day) before scaling to 100 g/day system**; the team has made significant improvements to the 1 g/day system
- **Spend more time investigating DAC polymer toxicity**; the team found the synthesized DAC polymers were less stable under alkaline conditions
- **Start evaluating outdoor cultivation of cyanobacteria ASAP**; the team has been working with AzCATI throughout the project to identify conditions for scaling cyanobacteria outdoors
- **Cultivate at pH above 8 to increase carbon utilization efficiency (CUE)**; the team is evaluating more alkaline pH to improve CUE and mitigate potential contamination