DOE Bioenergy Technologies Office (BETO) 2023 Project Peer Review

Microalgae Commodities Production with a Direct Air Capture Process

DE-FOA-0002203; Topic Area 3: Algae Bioproducts and CO_2 Direct-Air-Capture Efficiency
Award Number: DE-EE0009276; WBS# 1.3.4.006

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Global Thermostat LLC.: Eric Ping, Miles Sakwa-Novak, Stephanie Didas, Zach Foltz

PNNL: Michael Huesemann, Scott Edmundson, Song Gao, Charles Hibbeln, Ray Cabreriza

April 4, 2023
Advanced Algal Systems

This presentation does not contain any proprietary, confidential, or otherwise restricted information

Project Overview

Goal

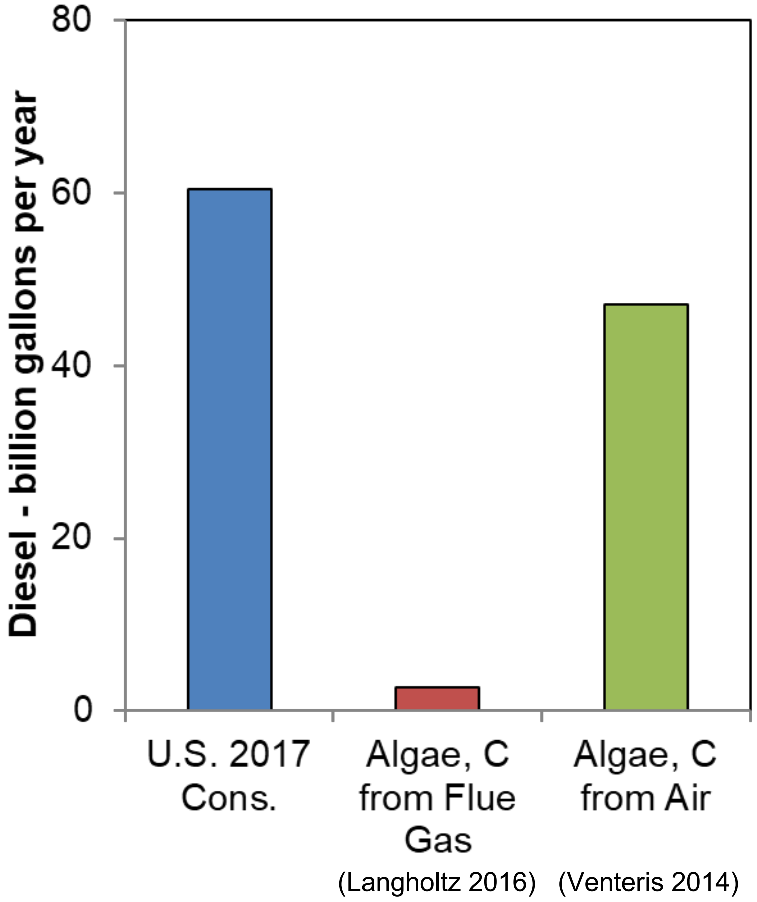
Demonstrate cultivation on air-derived CO_2 as an alternative to merchant and/or fossil-derived CO_2 supply

Motivation

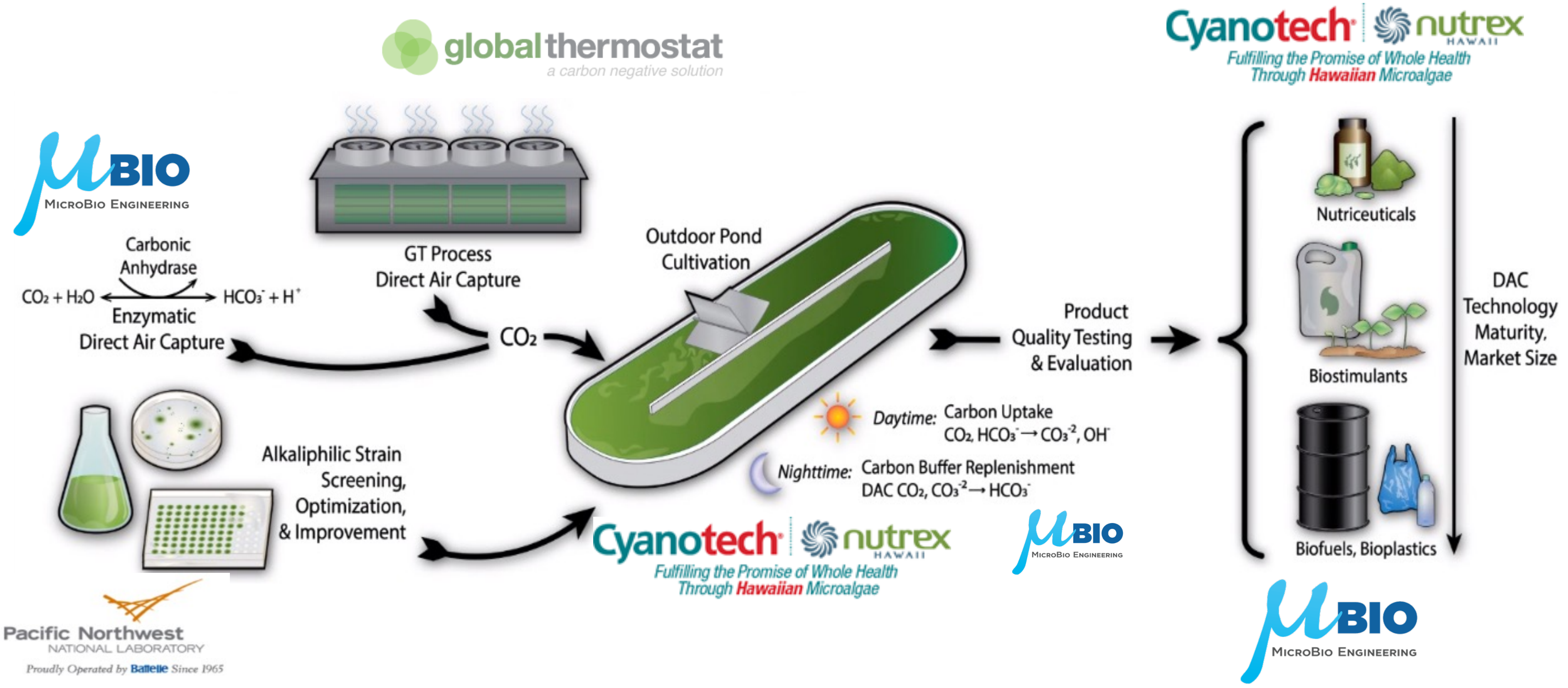
Current domestic algal production is reliant on merchant-sourced CO_2

Flue-gas CO_2 sources limit production of algal derived commodities such as biofuels

Local capture and utilization of air-CO_2 expands resource potential 10-fold



Approach



global thermostat
a carbon negative solution
Cyanotech
nutrex HAWAII
Fulfilling the Promise of Whole Health Through Hawaiian Microalgae
BIO
MicroBio Engineering
GT Process Direct Air Capture
Carbonic Anhydrase
$CO_2 + H_2O \rightleftarrows HCO_3^- + H^+$
Enzymatic Direct Air Capture
CO_2
Outdoor Pond Cultivation
Product Quality Testing & Evaluation
Nutriceuticals
Biostimulants
Biofuels, Bioplastics
DAC Technology Maturity, Market Size
Daytime: Carbon Uptake
$CO_2, HCO_3^- \rightarrow CO_3^{-2}, OH^-$
Nighttime: Carbon Buffer Replenishment
DAC $CO_2, CO_3^{-2} \rightarrow HCO_3^-$
Alkaliphilic Strain Screening, Optimization, & Improvement
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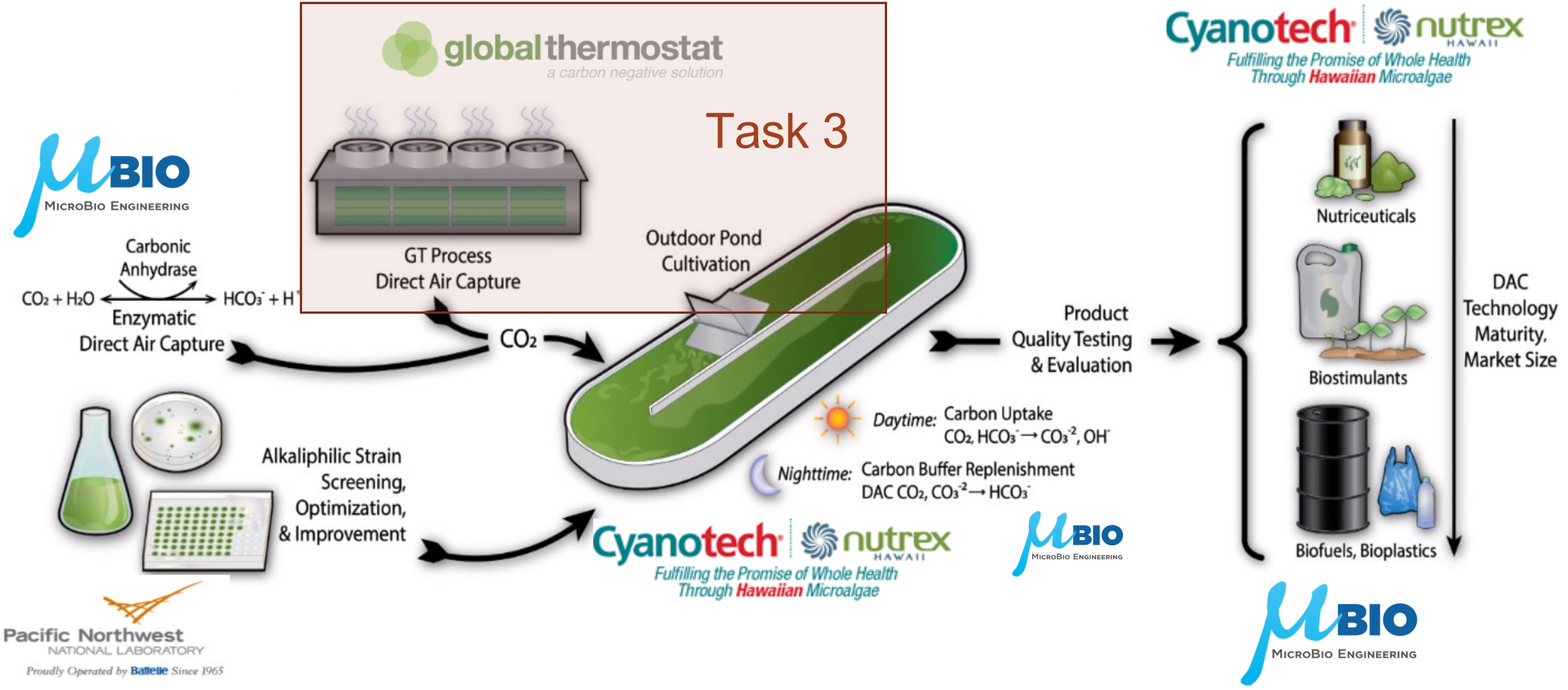
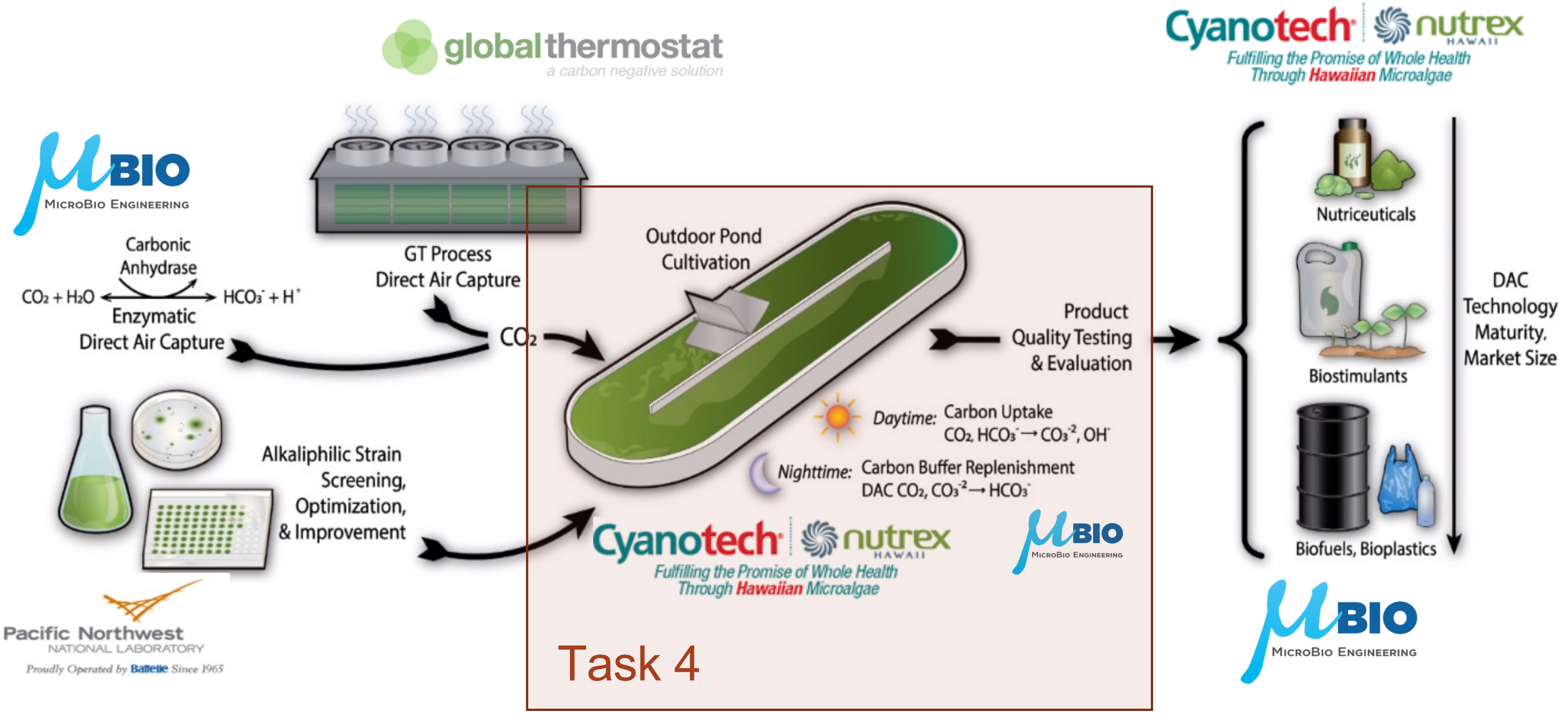
Approach



Task 1 and 2: Verification and Project management

Approach



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Task 4
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Biostimulants
Biofuels, Bioplastics
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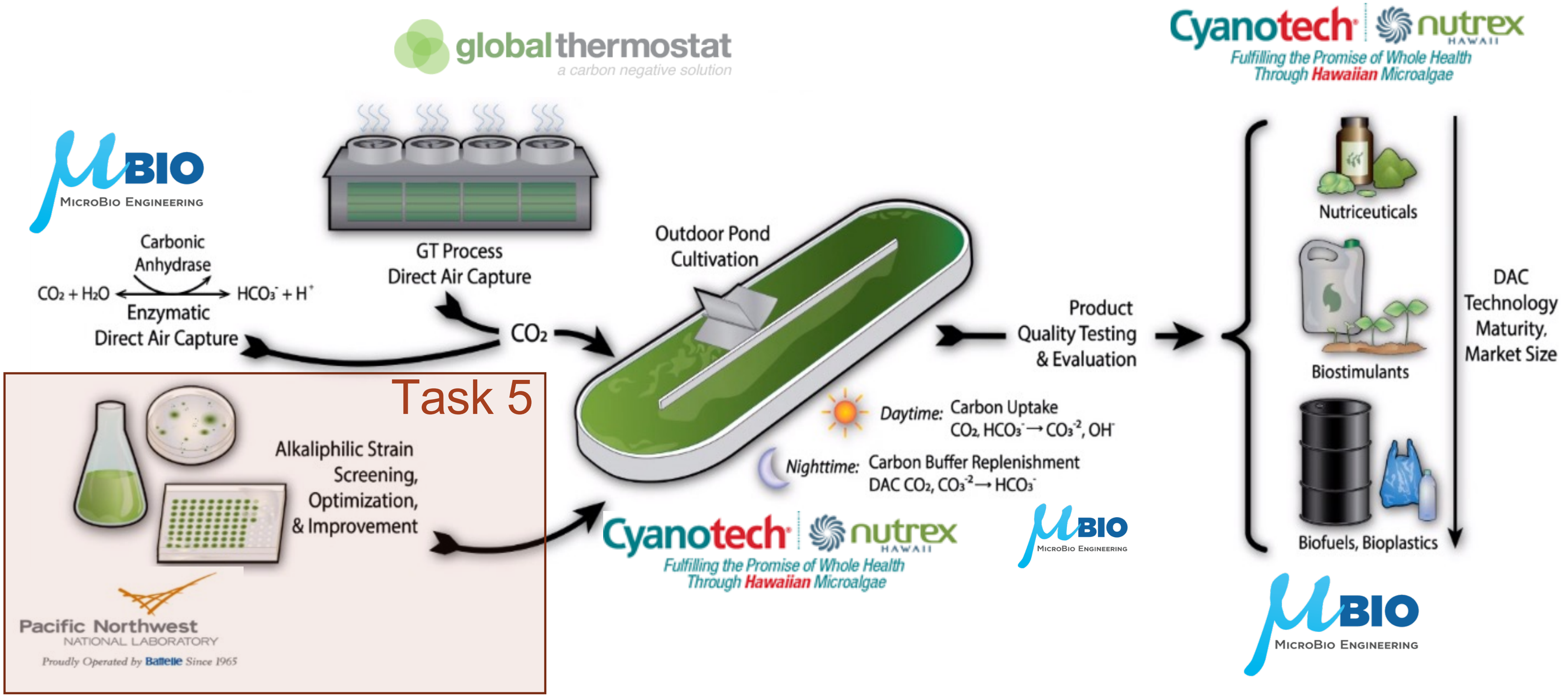
Approach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Task 5
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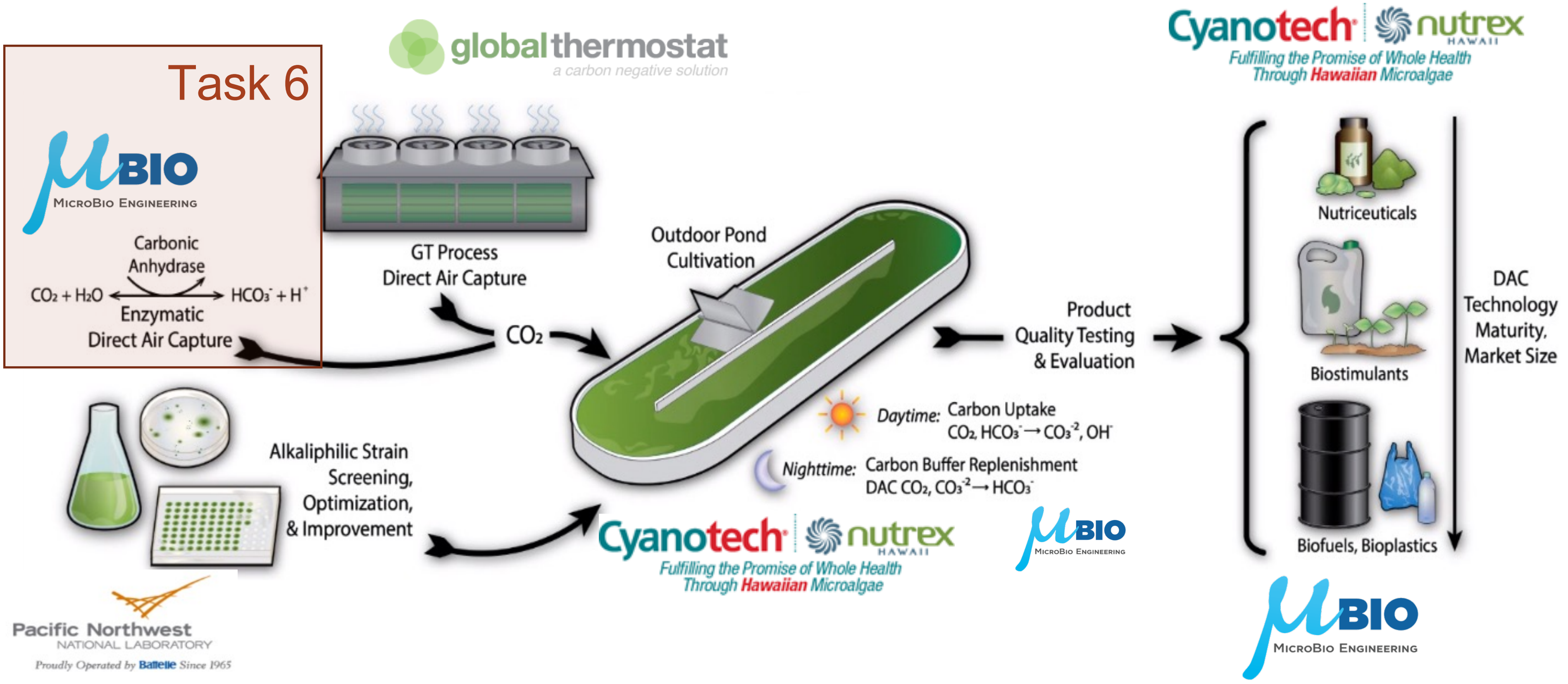
Approach



Task 6
MicroBio Engineering
Carbonic Anhydrase
$CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$
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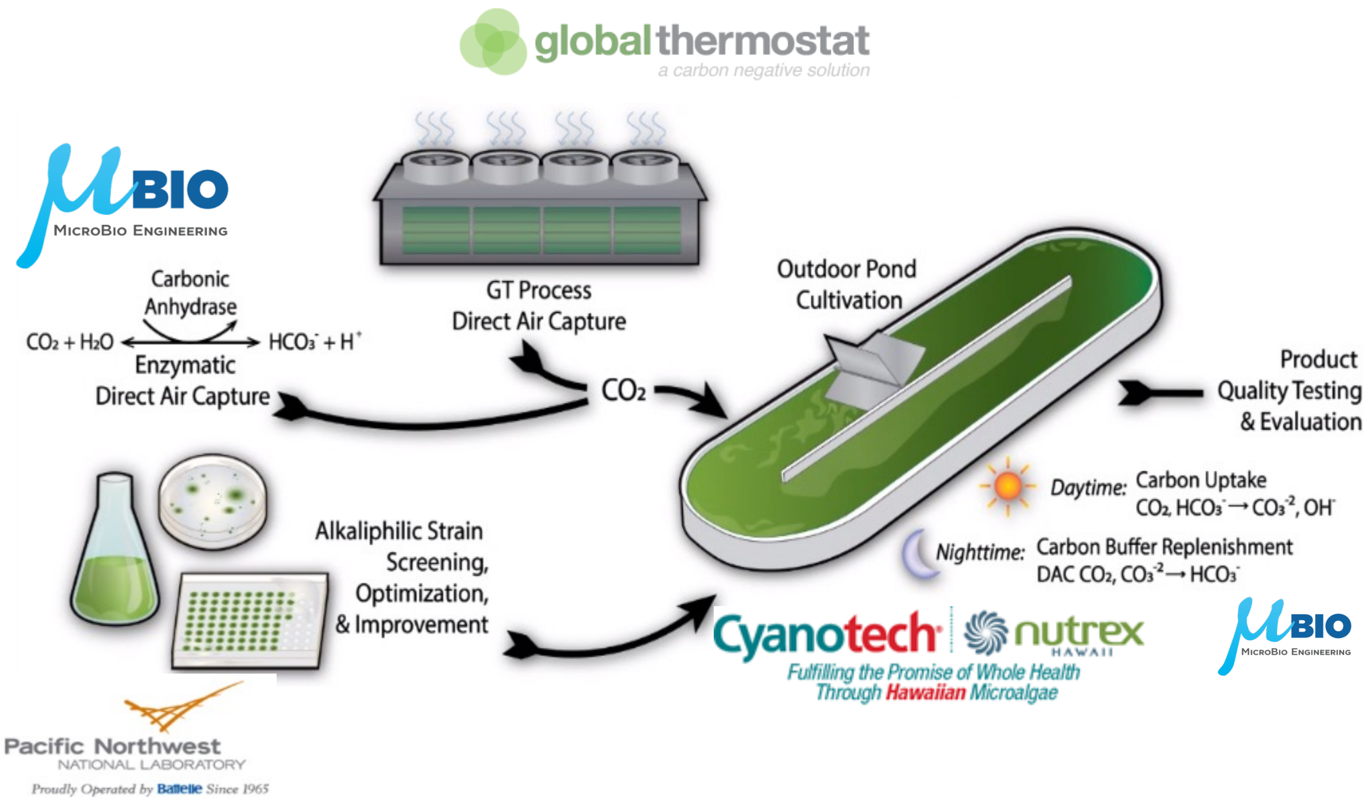
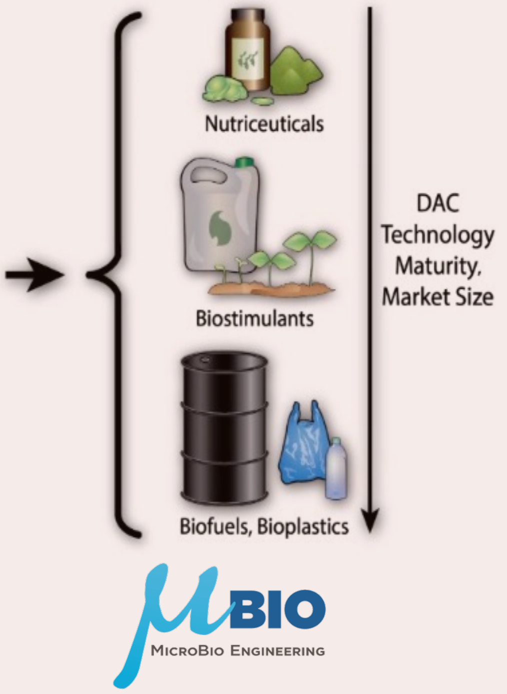
Approach



Task 7

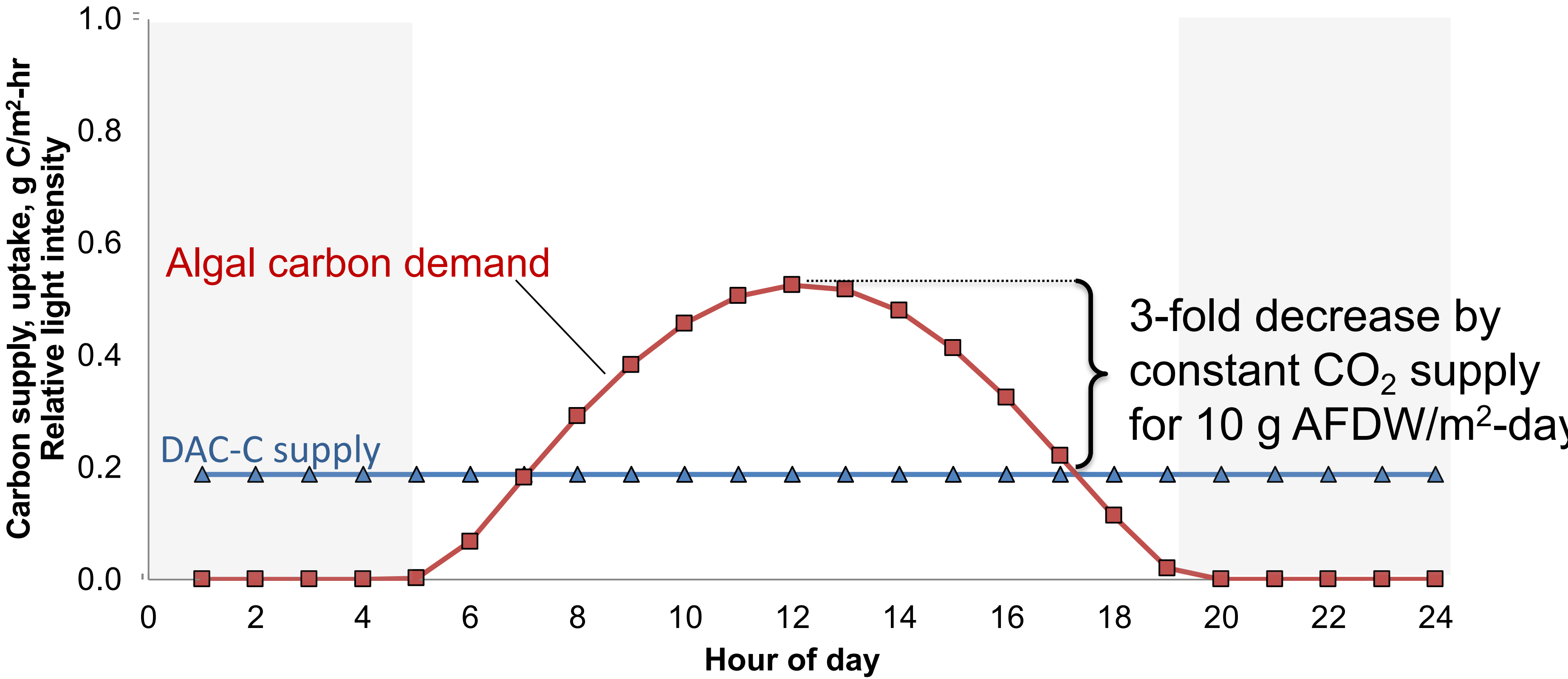
Comparative TEA/LCA



Approach: Advancing the state-of-the-art

Current State of the Art	Project Innovation	BETO Relevance
Merchant CO_2 used in research and industrial cultivation	CO_2 from GT-DAC or enzyme-facilitated in-pond transfer	Expand resource potential 10-fold
Cultivation trials conducted at pH 7-8 with high CO_2 outgassing losses (low CUE)	Develop strain screening framework to understand CUE, pest management, productivity tradeoffs	Reduce gap between research (low CUE) and n^{th} plant TEA/LCA assumptions (>75% CUE)
No domestic *Chlorella* production	Establish domestic *Chlorella* supply	Increase domestic manufacturer; develop pathways to commodities

Approach: Direct air capture (DAC) CO_2 supply scale is based on *daily*, rather than peak hourly, algal CO_2 demand



Approach: In-pond DAC-CO_2 storage requires tradeoffs between growth, carbon utilization efficiency (CUE), and cost

Buffering capacity: Sufficient alkalinity required to store CO_2 injected overnight; high alkalinity increases costs, GHG footprint, and requires operation at elevated pH

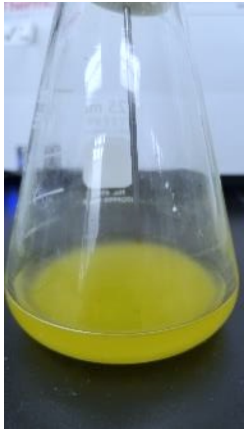
CUE: Operating at a pH below air-equilibrium incurs outgassing losses. High CUE needed to reduce costs and GHG footprint.

Growth: Strain tolerance to alkaline conditions varies; pest management techniques must be compatible with operating conditions

$$J_{CO_2}[=]\frac{g\ C}{m^2-day} = k_L * \left(P_{CO2}^{air} * H - C_{CO2}^{bulk}(x=\delta)\right)$$

When $C_{CO2}^{bulk}(x=\delta) > P_{CO2}^{air} * H$:

- Net CO_2 ‘outgassing’ rate



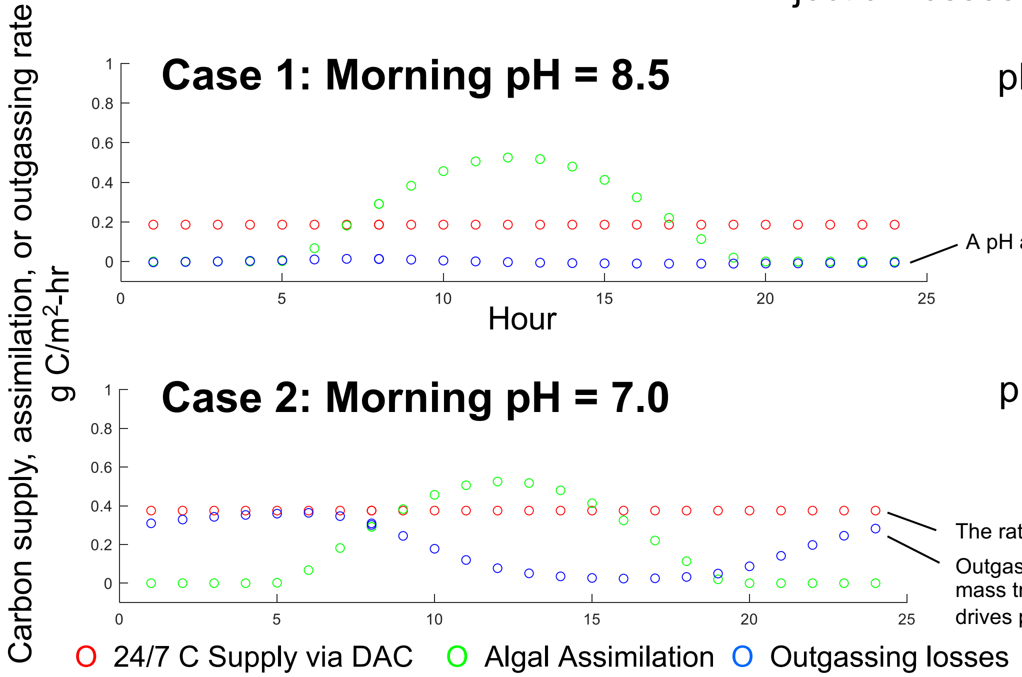
Slow and stressed

Severe clumpiness

Precipitation

Approach: Tradeoffs between pH, alkalinity, productivity and depth explored via a carbon-flux model – **outgassing is key**

Dissolved Inorganic Carbon (@time = i+1) = Initial – Outgassing Losses + DAC-C supplied – Assimilated – Injection losses + C absorbed from atmosphere – Blowdown losses



pH 8.5 – 9.2 diel swing; Low outgassing losses

<u>90% CUE predicted</u>

A pH at or near the air-equilibrium pH keeps CO_2 outgassing losses near zero

pH 7.5 – 8.5 diel swing; High outgassing losses

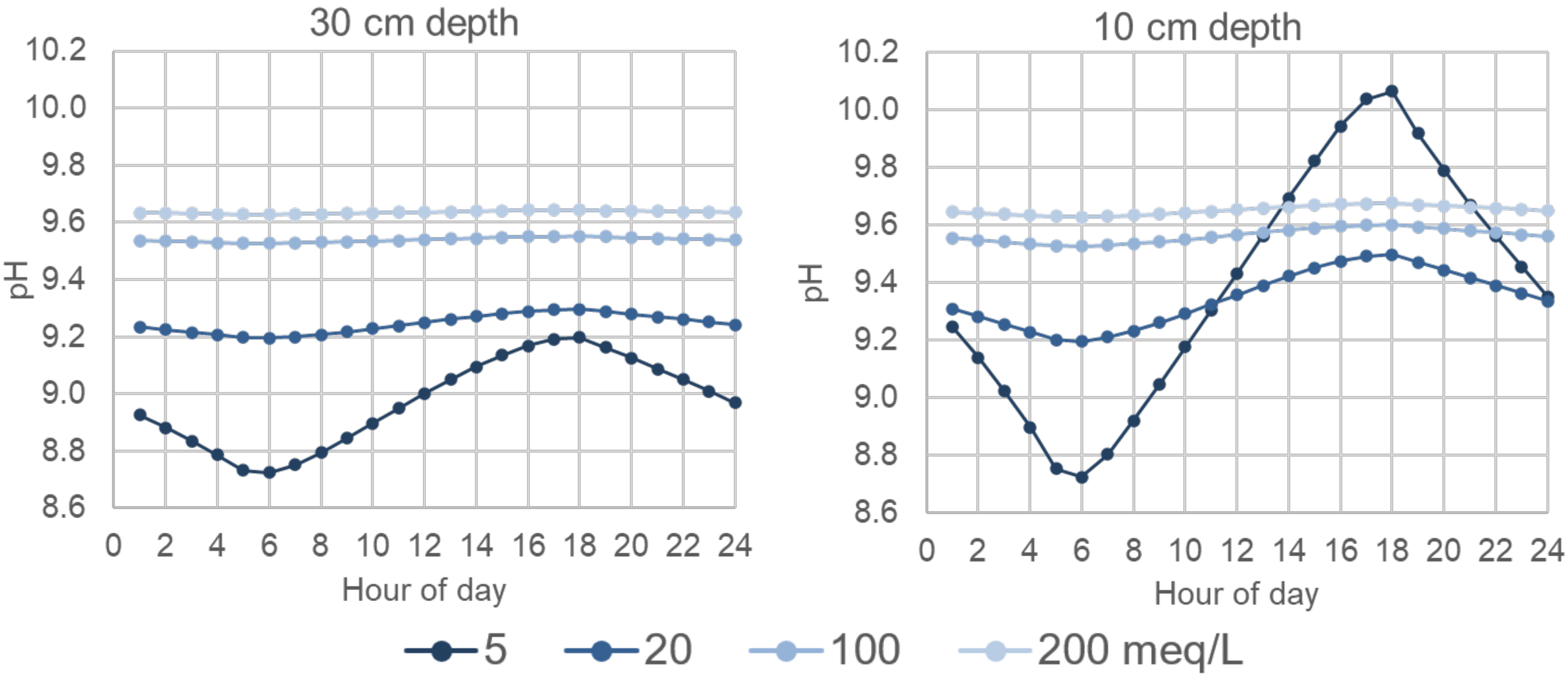
<u>45% CUE predicted</u>

The rate of carbon supply increases to account for outgassing losses

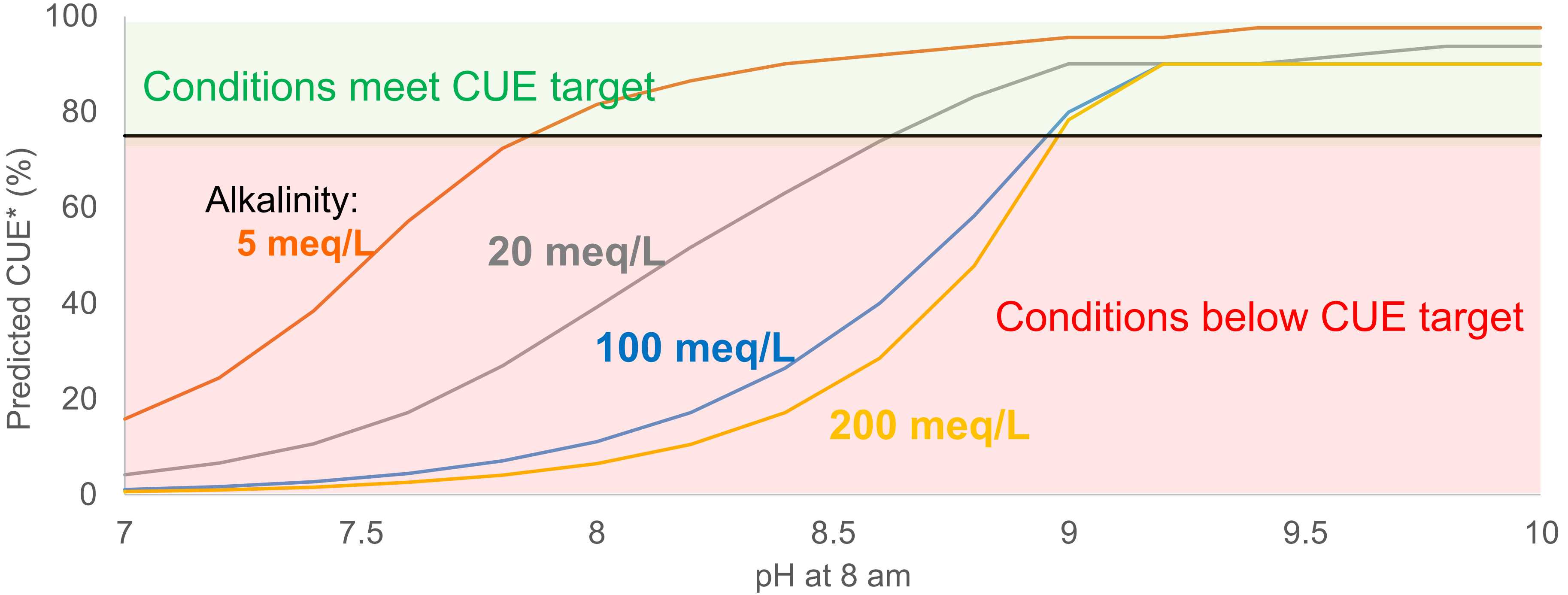
Outgassing rates approach the rate of supply due to the large driving force for mass transfer at pH 7. Outgassing decreases during the day as photosynthesis drives pH up, reducing the mass-transfer driving force

*Productivity of 4.5 g C/m²-day (10 g AFDW/m²-day), 30 cm depth, 5 meq/L alkalinity assumed in each case.

Approach: Pond depth and alkalinity dictate carbon storage capacity and diel pH swing



Approach: Combinations of alkalinity, pH, depth, and productivity represent 'operating points' for constant CO_2 supply by DAC



CUE predictions define conditions for indoor strain screening and pond cultivation to meet target (>75%)

*90% CO_2 injection efficiency assumed; 10 g AFDW/m^2-day productivity level; 30 cm depth, blowdown not included

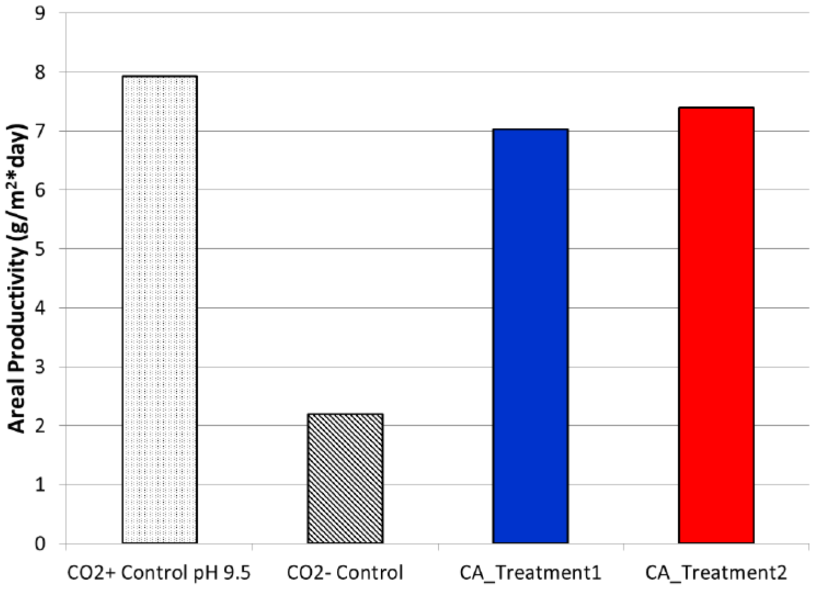
Approach: Enzyme-enhanced transfer explored as alternative to GT DAC

Proof-of concept for increasing air-CO_2 flux via exogenous CA addition demonstrated in previous joint MBE-PNNL AlgaeAirFix project

Chemically enhanced (high pH) air-CO_2 flux explored in MBE-PNNL-Qualitas Health AirCAP project.

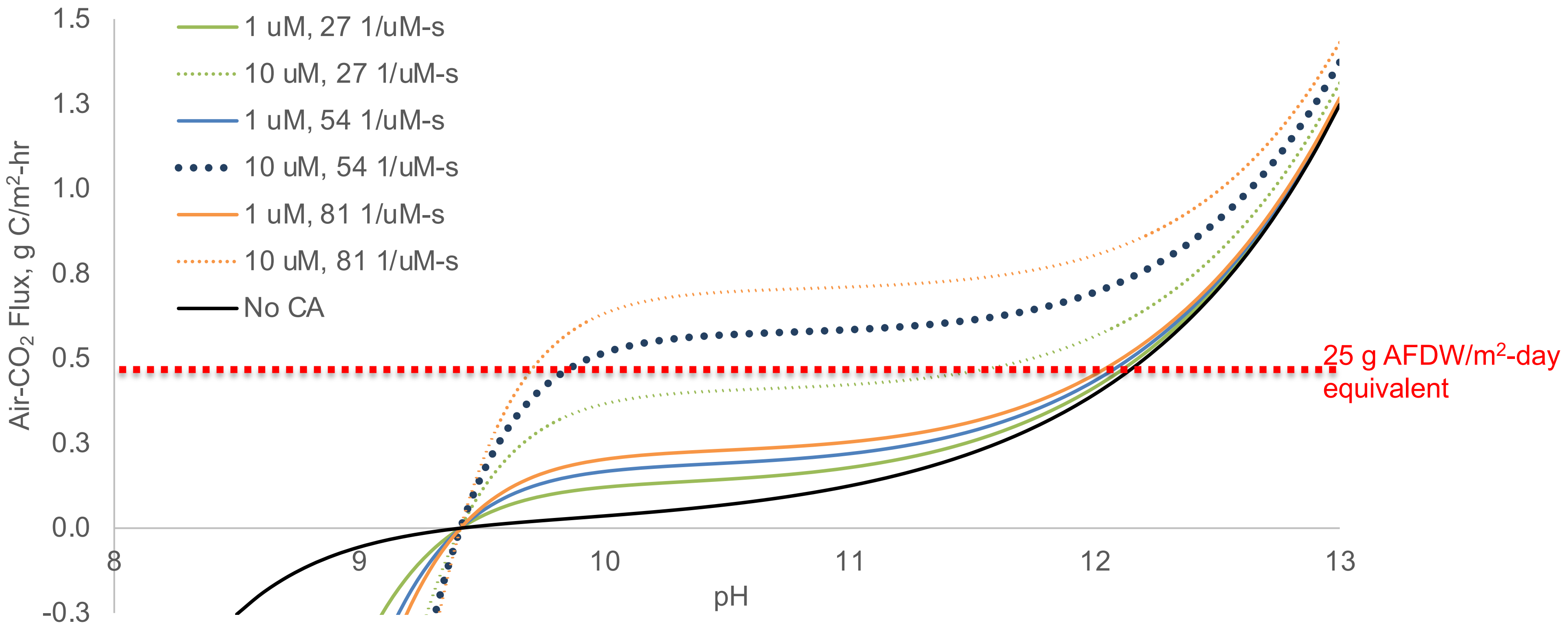
<u>Conclusions</u>:

- pH 11+ is required to meet target rate.
- Increasing turbulence in outdoor ponds also did not increase flux



Endogenous CA production hypothesized to increase air-CO_2 flux in oceanographic literature and is explored herein

Approach: 10 fold flux enhancement needed for 25 g AFDW/m^2-day; 10 µM enzyme at 54/µM-s gets there at pH<10



Approach: Management

μBIO MicroBio Engineering

P.I.: Dr. John Benemann

Mr. Crowe: Tasks Lead

Mr. Ortiz: Administration Lead

Project management, coordination
Collect external feedback, initiate external collaborations. Integrated TEA/LCA, feeding results back.

Global Thermostat

Dr. Eric Ping

Fabrication of a DAC pilot producing 1-2 kg CO_2/hr
2,000 hr continuous trial at Cyanotech
Provide DAC-CO_2 TEA/LCA values for MBE model

Cyanotech — Fulfilling the Promise of Whole Health Through Hawaiian Microalgae
nutrex Hawaii

Dr. Charley O'Kelly

Glenn Jensen:DAC integration

Strain collection, identification, down-select
Outdoor pond trials
Integrated DAC trials;
Evaluate product quality.

Pacific Northwest National Laboratory

Dr. Michael Huesemann

Leverage and adapt an established strain screening pipeline to identify conditions that optimize biomass production, CUE, and product quality

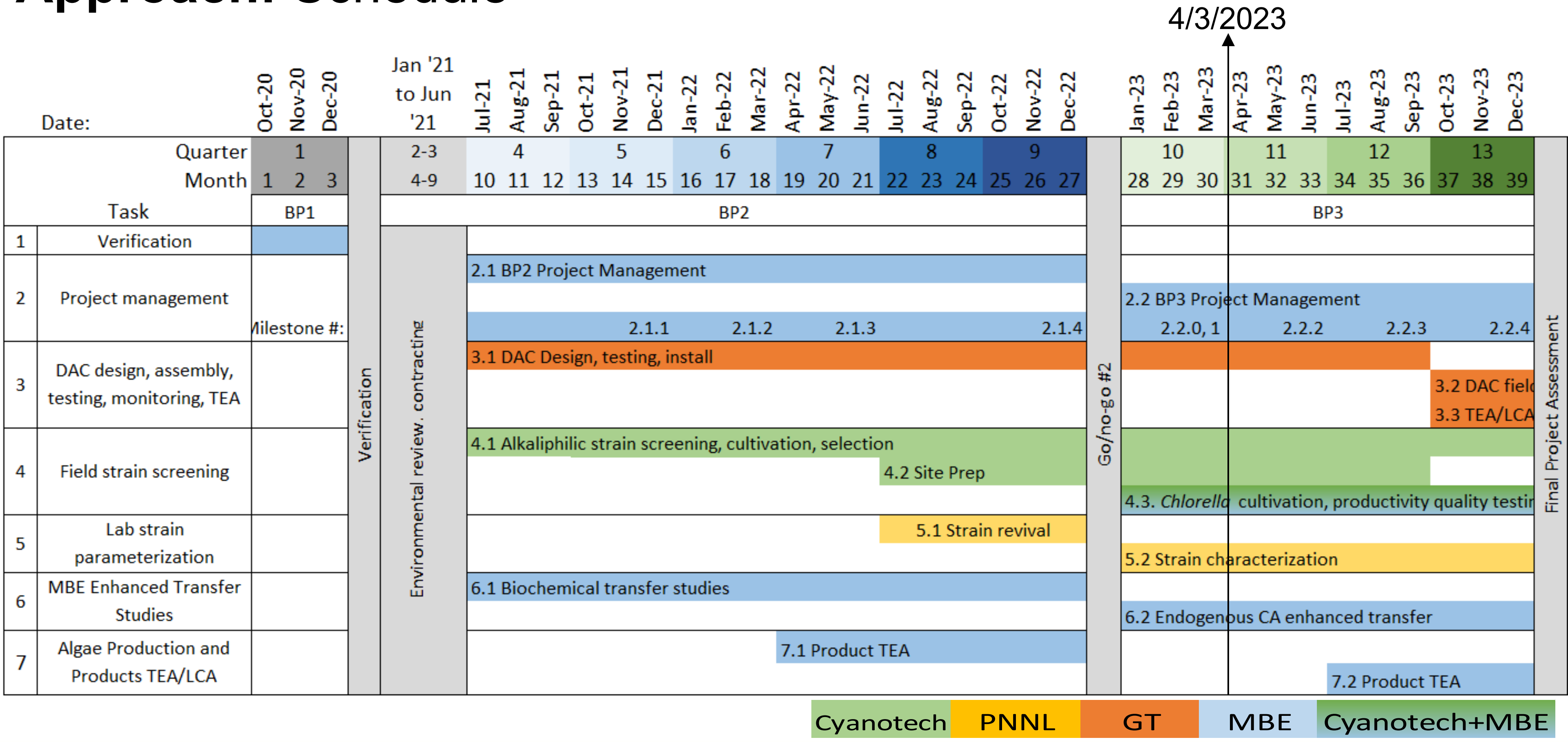
Risks and mitigation strategies

R1: Delay in delivery of GT-DAC to Hawaii (Task 3)
Mitigation: Use merchant-CO_2 in place of GT-DAC provided C

R2: Cyanotech Innovation Facility refurbishment cost exceed budget
Mitigation: Shift DAC-integrated cultivation studies to Cyanotech main farm (Task 4)

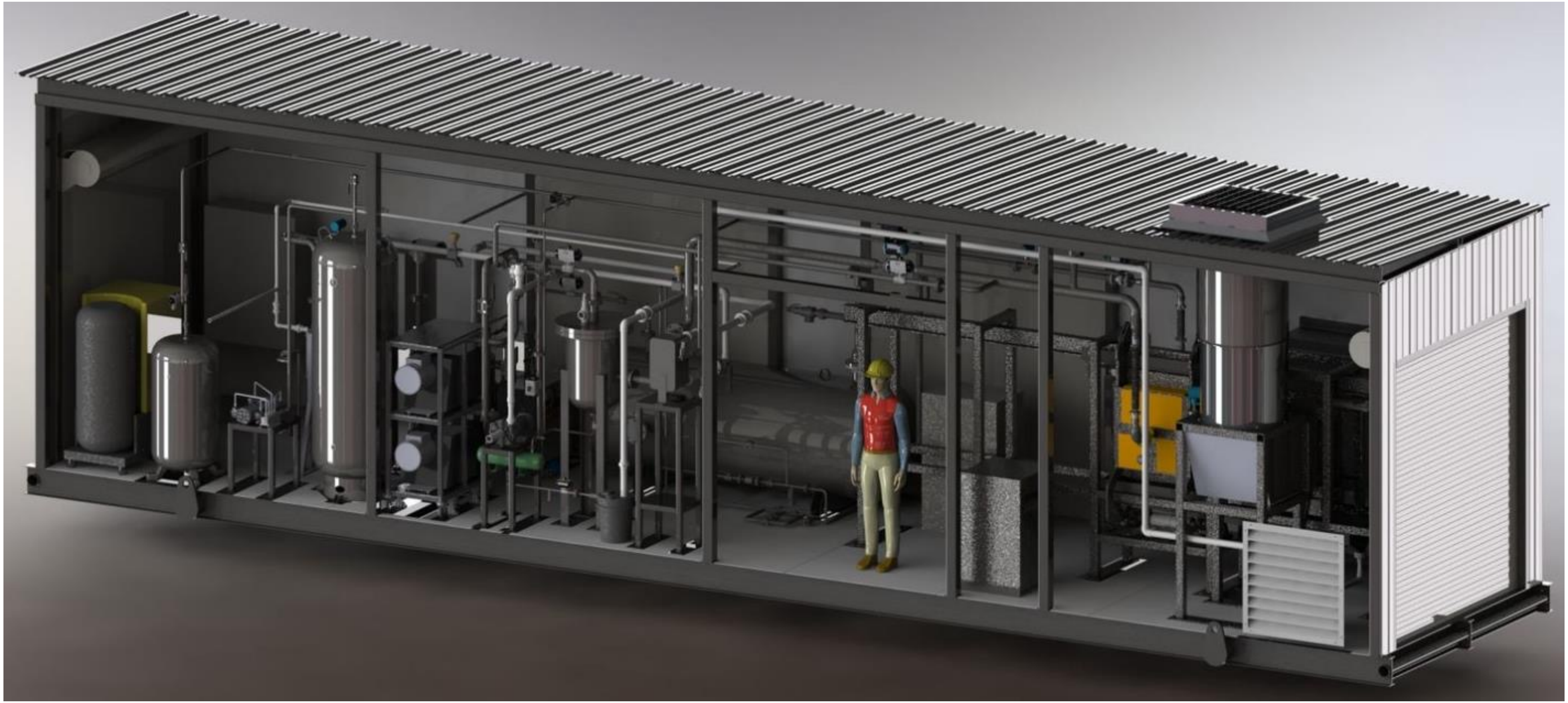
R3: Unable to detect endogenous-CA flux enhancement (Task 6)
Mitigation: Shift to other strains, discontinue task.

Approach: Schedule



Progress and Outcomes

GT-DAC skid design complete

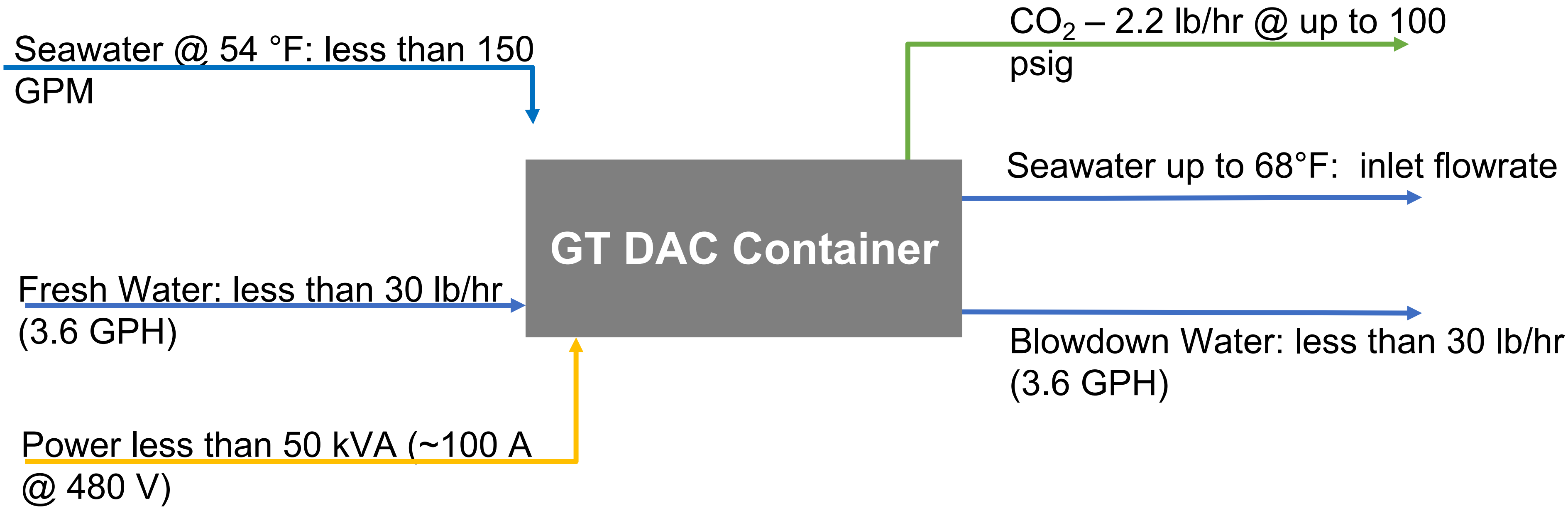


Fabrication underway – Summer 2023 Delivery

Operational data validates that GT-DAC can hit target metrics

10 ton CO_2 per year pilot operating at GT headquarters since July 2021

GT-DAC integration at Cyanotech

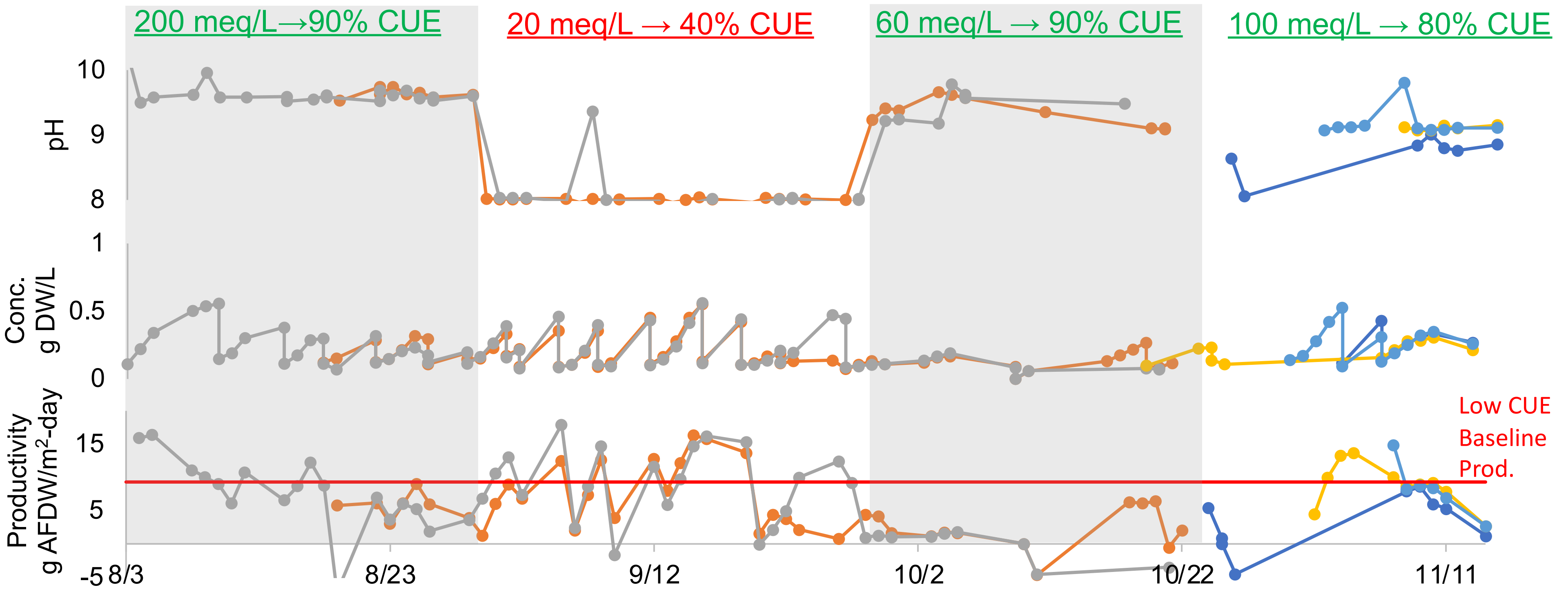


20 strains evaluated in lab, 4 tested in outdoor ponds; *Graeseilla* (CT3072) selected for optimization

Strain ID	Taxonometric group	Sent to PNNL?	Current Status
CT3069	*Graesiella*	Yes, 01/2022	• PNNL-CT MTA in place 12/2021 • Down selected Oct-Dec 2021 due to poor growth relative to CT3074
CT3074	*Parachlorella*	Yes, 01/2022	• Tested at 200 L scale Jan-Apr • Tested at 600L photobioreactor scale Jun • Tested at 1000L minipond scale Jun ○ Downgraded to first alternate status
CT3067	*Graesiella*	Yes, 06/2022	• Flask Scale Feb-Mar • Outdoor Scale Mar-May ○ Down selected due to poor growth in outdoor conditions relative to CT3074
CT3072	*Graesiella*	Yes, 08/2022	• Well plate scale in late June • Outdoor Scale July-current ○ Lead Candidate

Progress and Outcomes

Strain CT3072 matched baseline productivity with CUE > 75% in Summer; improved pest management needed in Fall

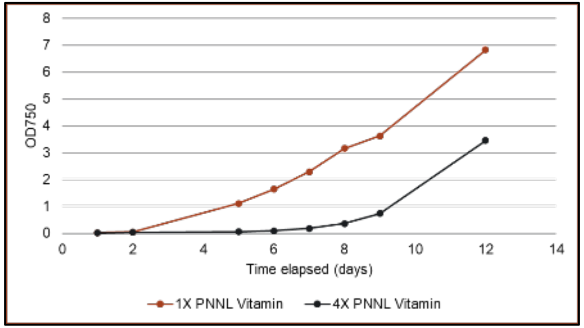


Progress and Outcomes

Top Cyanotech strains revived at PNNL; BP3 trials to optimize growth and product quality under <75% CUE conditions (Task 5)

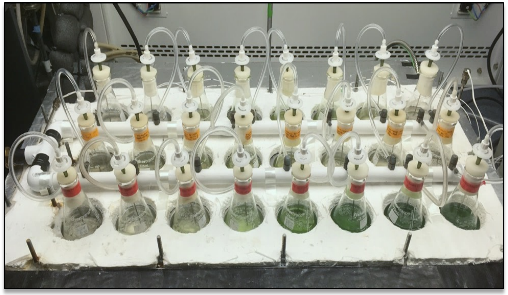
Medium optimization

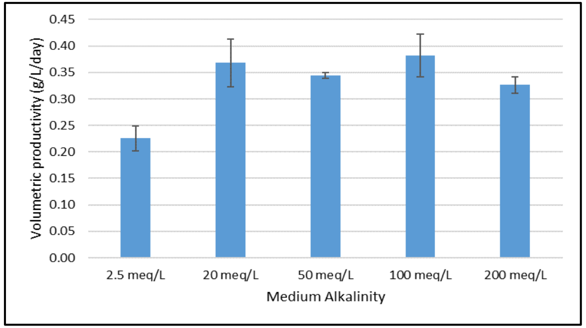
-Lag period reduced after lowering vitamin concentrations



Alkalinity profiling

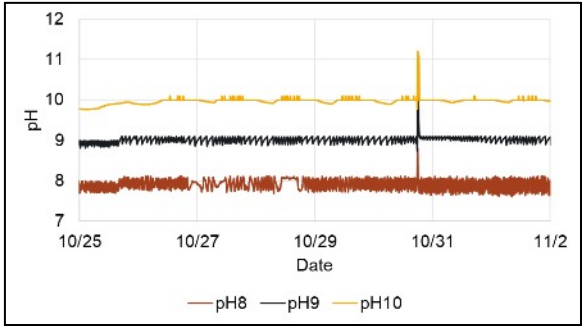
- Tolerance to wide range in initial screening





Future experimental setup

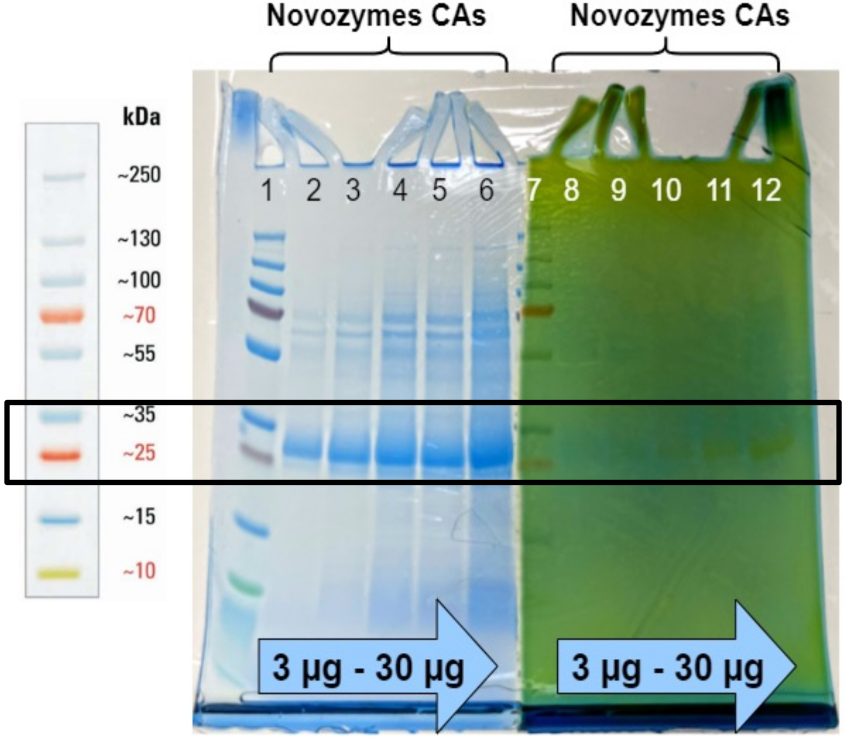
-85 mL automated PBR configured to control pH at different alkalinities



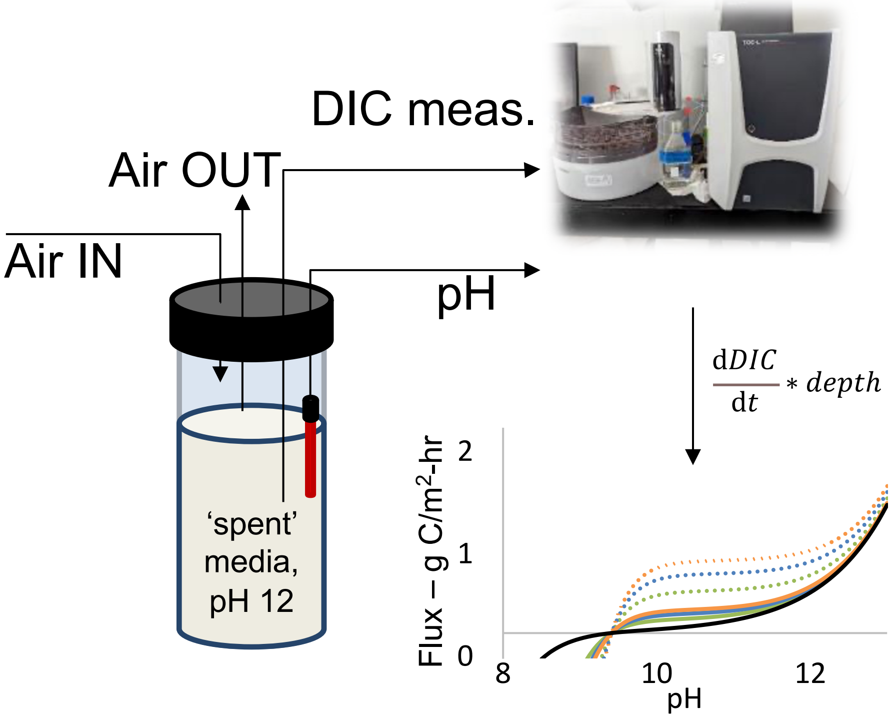
Progress and Outcomes

Assays validated to detect active carbonic anhydrase at levels required to increase air-CO_2 flux to target levels (Task 6)

Assay 1: Identify presence/absence, relative levels



Assay 2: Identify flux ehancment due to CA



Cell wall-deficient *Chlamydomonas* mutant hypothesized to release CA into the culture media and increase air-CO_2 flux. Cultivation and assay application underway.

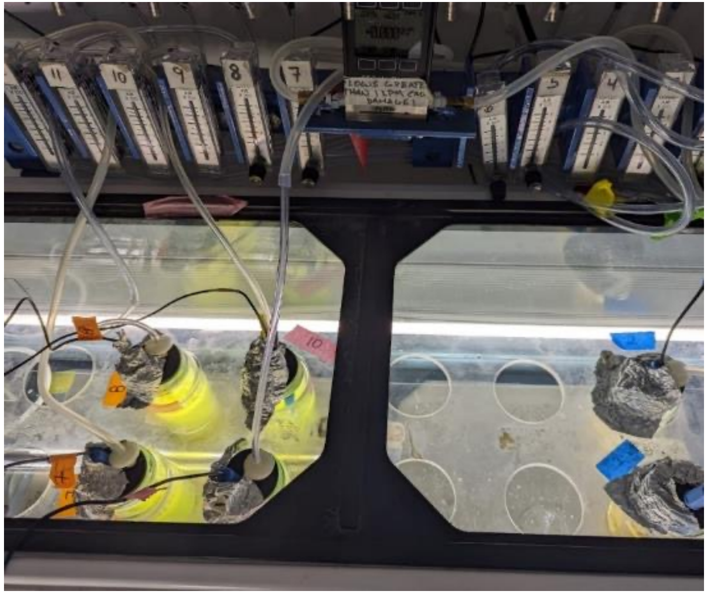
Plant & Cell Physiol. 24(2): 255–259 (1983)

Carbonic Anhydrase in *Chlamydomonas reinhardtii* I. Localization

Donald L. Kimpel[1], Robert K. Togasaki[1] and Shigetoh Miyachi[2]

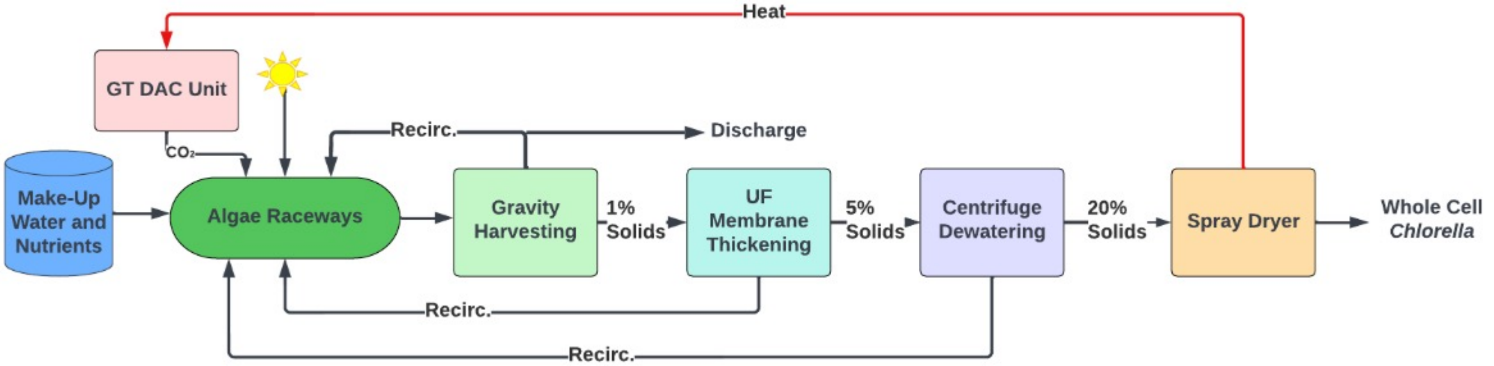
[1] *Department of Biology, Indiana University, Bloomington, Indiana 47405, U.S.A.*
[2] *Institute of Applied Microbiology, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan*

Most of the carbonic anhydrase (CA) activity was released into the growth medium, when a cell wall-deficient mutant strain of *C. reinhardtii* was cultured under low CO_2 conditions. Treatment of wild-type cells carrying high CA activity with a gametic wall-lysing enzyme resulted in the release of the CA activity into the medium. Both data indicate that the majority of CA activity in *C. reinhardtii* is located outside the plasmalemma, either in the periplasmic space or attached to the cell wall.



Progress and Outcomes

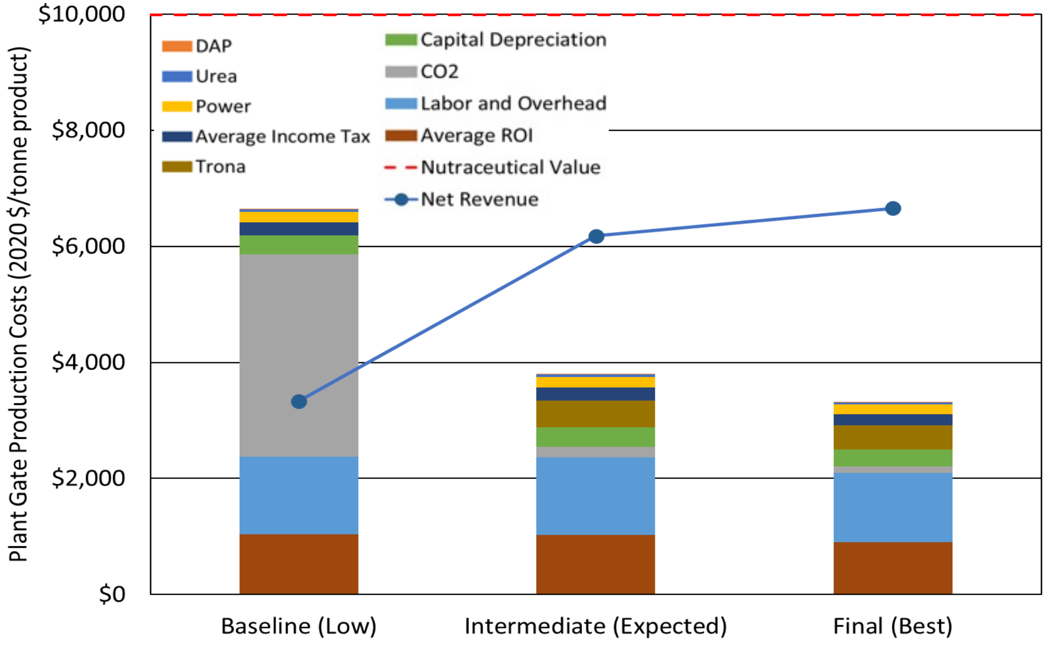
Process model is based on near-term production of a whole cell *Chlorella* neutraceutical product



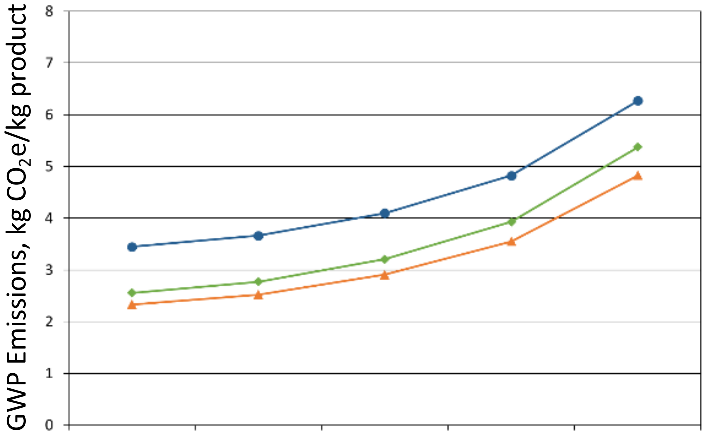
TEA Parameter	Baseline	Intermediate	Final
CO_2 Source	Merchant CO_2	GT DAC	GT DAC
Chlorella productivity (gmd)	9.2	9.2	10.4
CO_2 utilization efficiency	50%	75%	75%
Alkalinity (meq/L)*	5	50	50
CO_2 CAPEX (\$/tonne CO_2)	-	Not Disclosed	
CO_2 OPEX (\$/tonne CO_2)	1,000	Not Disclosed	

*Results are presented for 50 meq/L, but varied from 5, 20, 50, 100, and 200 meq/L in sensitivity analysis)

In locations where CO_2 is expensive, efficient use of DAC-CO_2 halves minimum biomass selling price



Interim LCA



<u>Alkalinity sensitivity</u>: 2-fold increase in greenhouse gas emissions from 5 to 200 meq/L

Significant decreases in biomass production costs – targeted via shifting to more productive strains and reductions in CO_2 cost – is needed for commodities

Impact: Deep industry engagement between leading domestic air-CO_2 supplier (GT) and algal producer (Cyanotech)

<u>Near Term</u>

- Produce high value products in locations where existing CO_2 supply is limited
- Justify DAC scale-up to meet carbon requirements of Cyanotech main farm

<u>Longer Term</u>

- Justify expansion into lower value, larger markets via reductions in DAC-CO_2 costs and increases in biomass productivity
- Apply strain optimization framework to other CO_2 sources

Summary: An integrated, coordinated approach for producing algal commodities from air-CO_2

- **Completed design for GT-DAC pilot** to provide 1-2 kg air-CO_2/hr. Fabrication underway.
- GT-DAC infrastructure requirements coordinated with Cyanotech Engineering Team. <u>Site is ready</u>.
- Established framework to define outdoor pond conditions that **meet CUE targets**.
- Evaluated 20 alkaliphilic *Chlorella* strains in the lab; downselected to 4 for outdoor testing.
- Outdoor productivity **met targets for commercial production** of a high-value whole cell product.
- Consistent growth observed by incorporating dilute bleach additions, but under conditions that did not meet CUE targets; trials underway to evaluate conditions compatible with pest control and high CUE
- Top performing strains from Cyanotech **successfully revived at PNNL**, culture media improvements implemented at Cyanotech. Existing strain screening equipment modified to control pH.
- **Validated an assay to identify presence/absence and relative levels of carbonic anhydrase**; Cultivation of strains hypothesized to release CA underway
- Experimental trials **guided by TEA/LCA**: alkalinity levels above 50 meq/L increase cost, and more significantly, GHG footprint.

MicroBio Engineering

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Timeline

- *BP1 start: 10/2020; BP2: 7/2021 – 12/2022*
- *End: 12/31/2023*

	FY22 Costed	Total Award
DOE Funding	$490,175	$1,999,881
Project Cost Share	$290,870	$528,913

TRL at Project Start: 4
TRL at Project End: 6

Project Partners

- Global Thermostat
- Cyanotech
- Pacific Northwest National Laboratory

Project Goals

1. Demonstration of a path forward for large-scale microalgae production in the US, through provision of air-CO_2 via Direct Air Capture (DAC) with affordable capital and operating cost and favorable environmental footprint for commodities production.
2. Evaluation of processes for providing air-CO_2 directly to algal cultures through their metabolic processes to establish the trade-offs between the costs savings and inevitable reductions in biomass productivity.

End of Project Milestones

Operation of DAC pilot unit over 2,000 hrs; projected DAC-CO_2 costs <$350/Mg CO_2

Alkaliphilic *Chlorella* biomass productivity exceeding 10.5 g AFDW/m^2-day at a CUE >75%

Proof-of-concept for endogenous CA production and flux enhancement

Projection of 15% improvement in biomass productivity under optimized conditions

TEA/LCA outcomes supporting progression to demonstration-scale

Greater than 95% of biomass meeting product quality specifications

Funding Mechanism

DE-FOA-0002203; Topic Area 3: Algae Bioproducts and CO_2 Direct-Air-Capture Efficiency (ABCDE)