DOE Bioenergy Technologies Office (BETO)
2021 Project Peer Review

Enhanced Algal Production of CA for Improved Atmospheric Delivery of CO_2 to Ponds

March 23, 2021
Advanced Algal Systems

Andrew E. Allen
J. Craig Venter Institute

Project Overview

- *Project establishes partnership between Allen lab at JCVI and GAI* ***to combine strengths*** *(molecular physiology and genetic engineering with optimization of productivity and cultivation at scale)*
- ***Goal:*** *Develop an* ***integrated*** *system for productivity of 12 g/m2d on atmospheric carbon dioxide alone, and reduce costs of a renewable advanced biofuel*

1 – Management

Overview of Project Structure & Task Delineation

JCVI (Project Lead)

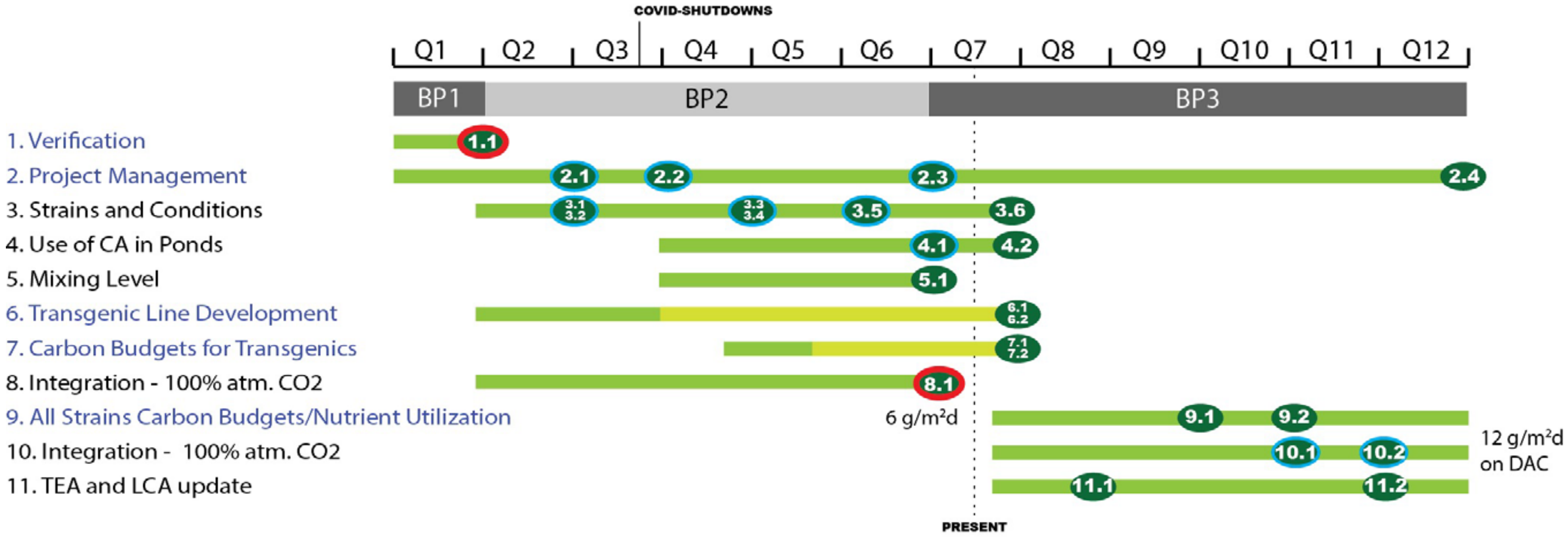
Lead PI: Andrew E. Allen

Project Manager: Sarah R. Smith

Postdoc: Tyler Coale

PhD student: Mark Moosburner

Cal Poly SLO: Emily Bockmon



GAI

Lead PI: Dave Hazlebeck

Jesse Traller

Rod Corpuz

Aga Pinowska

Bill Rickman (subcontractor)

Key Task Personnel

Task 2.0 – Allen, Smith
Task 6.0 – Allen, Smith, Moosburner, Bockmon, Rickman
Task 7.0 – Allen, Smith

Task 3.0 – Hazlebeck, Traller, Pinowska, Rickman
Task 4.0 – Hazlebeck, Traller, Corpuz, Rickman, Bockmon
Task 5.0 – Hazlebeck, Traller, Corpuz
Task 8.0 – GAI team

1 – Management

Project Risks	Mitigation Strategies
• Unsuccessful integration of objectives/results between partners	• Regular communication between project partners (details below)

• Transgenic lines fail
  • Inherent risks of genetic engineering
  • Covid-closures, reduced time in the lab
	• Strategies to test the impact of CA on algal growth that don't rely on engineering
	• Incorporation of bioinformatics-driven analyses
	  • Annotation of the CCM in GAI-293 (Nitzschia hildebrandi)
	  • Comparative model of CCM in algal taxa

• Strategies to improve DAC at-scale are unsuccessful	• Several different approaches to maximize probability of success

Project Communication Management

Internal Team

- Biweekly (at minimum) calls between JCVI and GAI (Smith and Traller)
- Biweekly (at minimum) calls between JCVI and CalPoly (Smith and Bockmon)
- Regular calls between Cal Poly (Bockmon) and Rickman re: carbonate chemistry (as needed)
- Quarterly full team Zoom meetings (at minimum) with more regular meetings planned for BP3
- File sharing of confidential information via email or box.com (secure)

DOE

- High-level project management (Andy Allen)
- Quarterly Reporting and Biweekly Calls with Christy Sterner (Sarah Smith)

Initial Verification In person
June 27, 2019

Kickoff Meeting Zoom
March 20, 2020

Intermediate Verification Zoom
October 13, 2020

BP3 Regular Meetings Zoom
Jan 29, 2020

2 – Approach

Goal: *Develop an* ***integrated*** *system for productivity of 12 g/m2d on atmospheric carbon dioxide alone, and reduce costs of a renewable advanced biofuel*

JCVI

- Generate transgenic lines to use algae as a platform to produce carbonic anhydrase (CA)
  - Genetically tractable *P. tricornutum* as a proof-of-concept
  - Would reduce cost associated with commercial CA
- Test the effect of added CA and algal-derived CA on atm. CO_2 absorption and productivity
  - *P. tricornutum* and production strain(s)
- Evaluate efficacy and systems biology of other cultivation-relevant parameters that result from media optimization tests
  - *P. tricornutum* and production strains(s)
- BONUS: Evaluate the CCM of a production strain bioinformatically to inform genetic engineering approaches

GAI

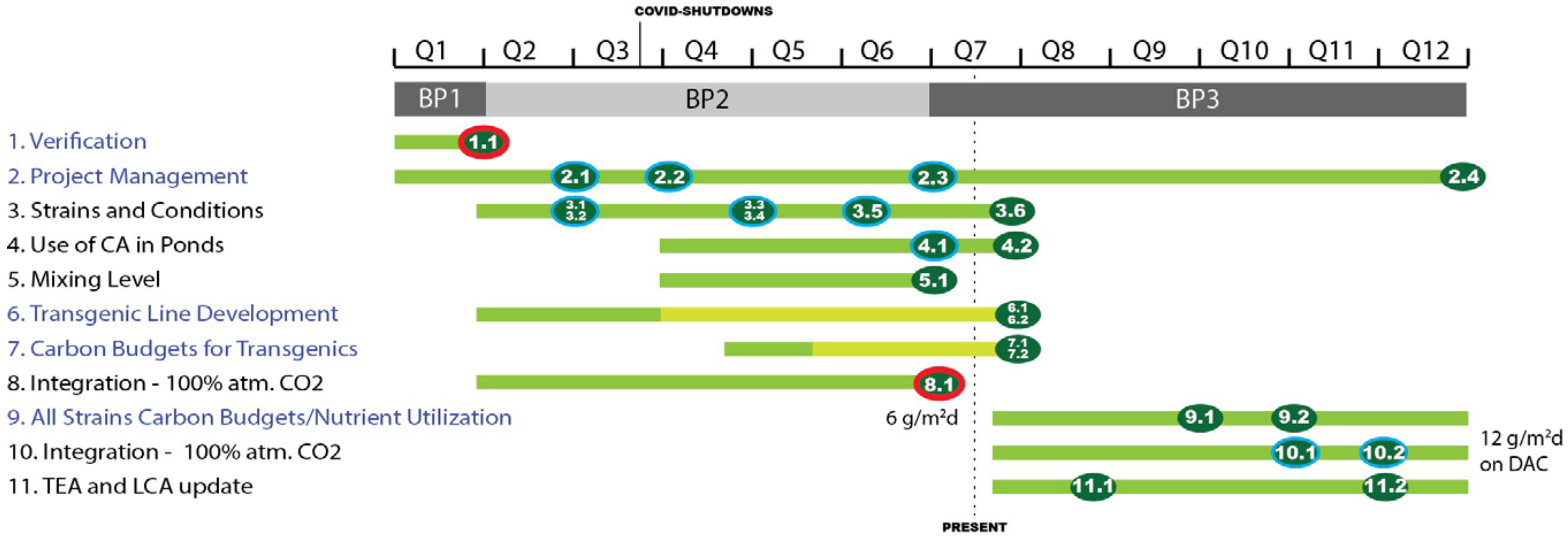
- Develop small raceways to mimic conditions of large ponds
- Optimize media and test new strains for max productivity and ability to accelerate atm. CO_2 absorption
- Test use of commercially available CA to accelerate atm. CO_2 absorption
- Test mixing level affect on atm. CO_2 absorption
- Demonstrate productivity with DAC alone

Factor demonstrated improvements and proof-of concept improvements into updated TEA & LCA

Have met (and exceeded goal)

Work continues testing concepts that will further improve productivity and economics of DAC

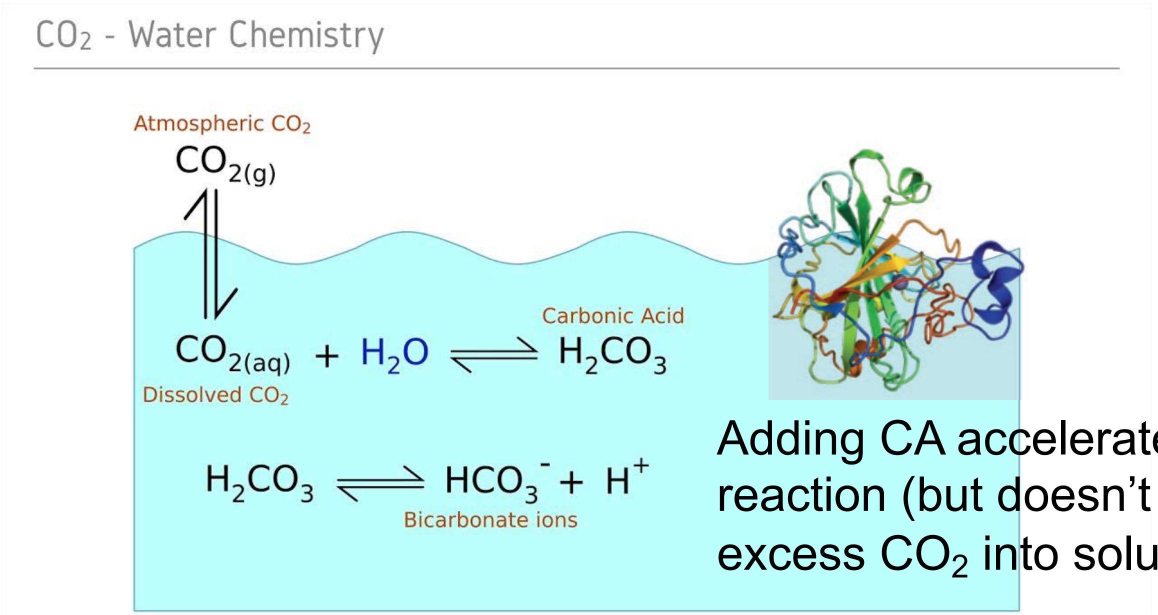
2 – Approach cont.



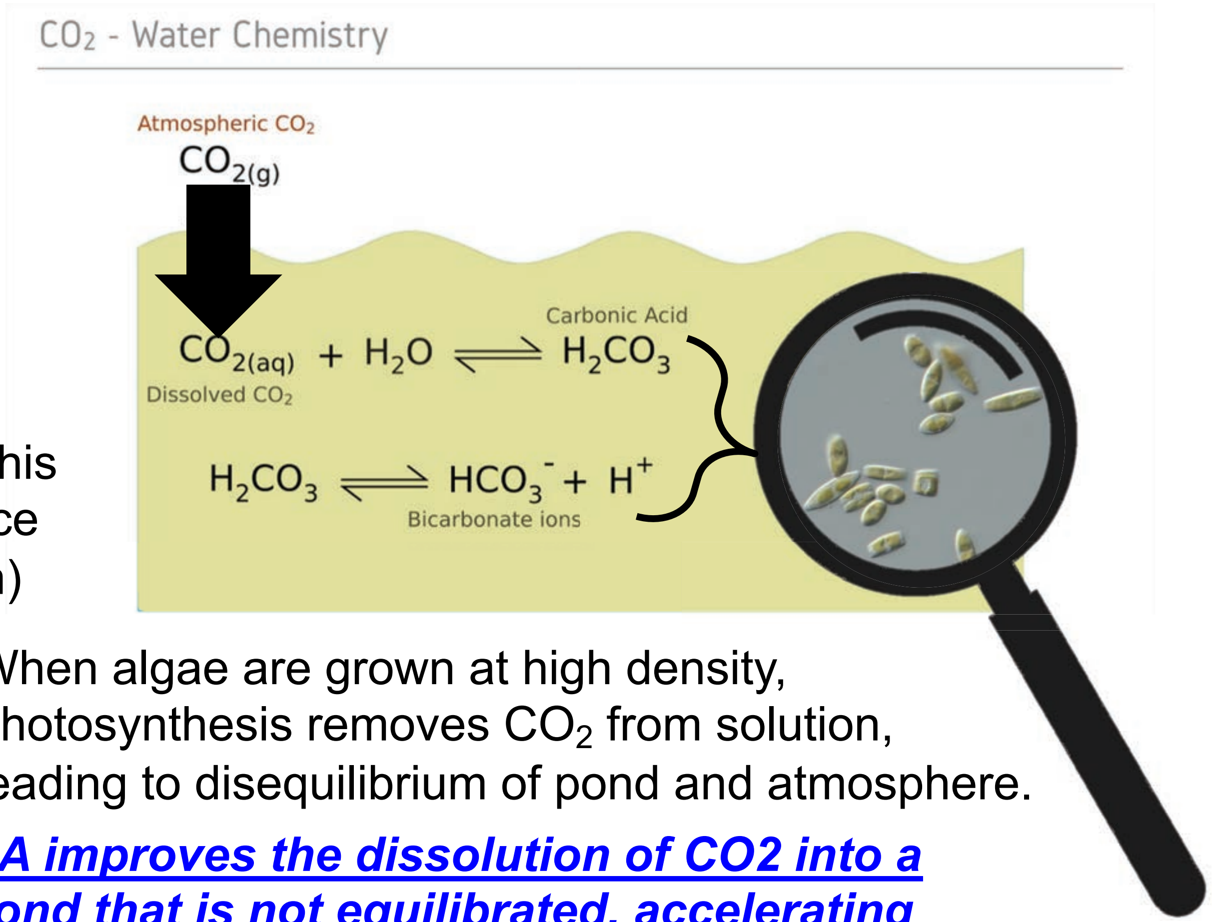
- This project aims to achieve high productivity with direct air capture, using a multi-pronged approach to optimize strains, conditions, mixing level in ponds, and through use of **carbonic anhydrase**
- Cost associated with CA purchased commercially, concerns with stability
  - Can algae be engineered to produce the CA? Does it work to improve DAC?

What CA does,

and why it should help pond productivity



Adding CA accelerates this reaction (but doesn't force excess CO_2 into solution)



- As CO_2 enters solutions (seawater, media) it forms carbonic acid and bicarbonate but this is SLOW
- Driving force for CO_2 to enter solution depends on equilibrium state

- When algae are grown at high density, photosynthesis removes CO_2 from solution, leading to disequilibrium of pond and atmosphere.
- ***CA improves the dissolution of CO2 into a pond that is not equilibrated, accelerating rate of absorption of CO2 from the atmosphere and supporting high productivity***

Figure from: scienceprimer.com

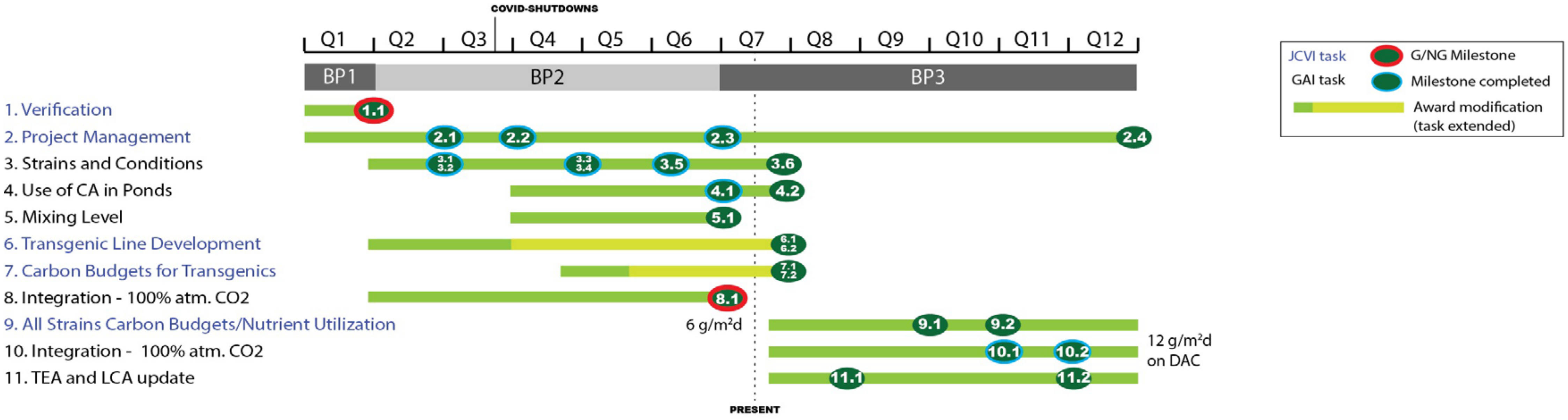
3 – Impact

- Successful direct air capture in large scale algal systems for the first time.
- Availability of land near CO_2 sources is currently the limiting factor in quantity of algal biofuels that could be produced. Decoupling algae facility location from CO_2 sources will enable siting plants anywhere, which greatly increases the quantity algae biofuels that could be produced.
- Technology being developed for use in system with a fully-integrated projected cost of production for algal biomass of less than $500/mt and into the life cycle assessment model to support projected production of an “advanced biofuel” that would meet the definition of the Energy Investment and Security Act of 2007

Disseminating results:

- GAI: patent;
- GAI partners with many other organizations on R&D
- GAI currently working on scale-up and plans on using a licensing/franchise model for widespread application in the algae industry
- Publication on the CCM in *Nitzschia hildebrandi* (JCVI)
- JCVI developing promoters and tools for algal production of CA (publication of results anticipated)

4 – Progress and Outcomes



Project largely on-track

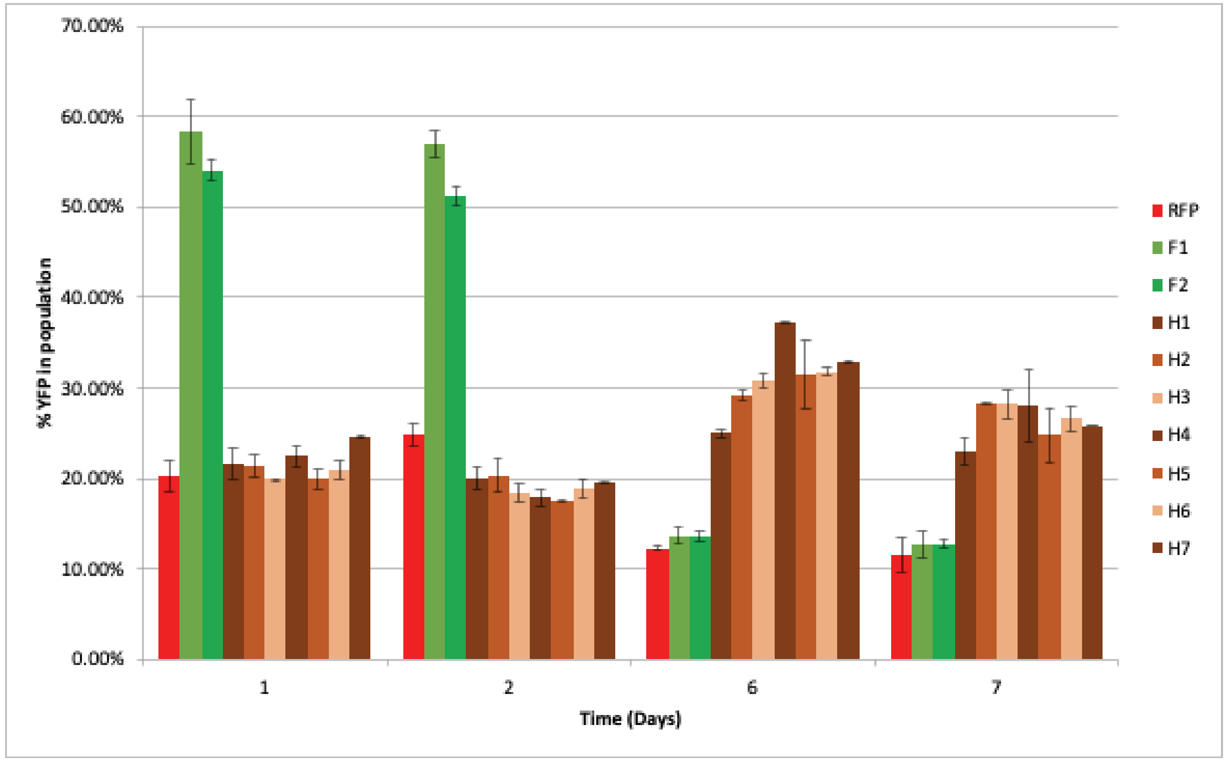
Achieved Key milestones

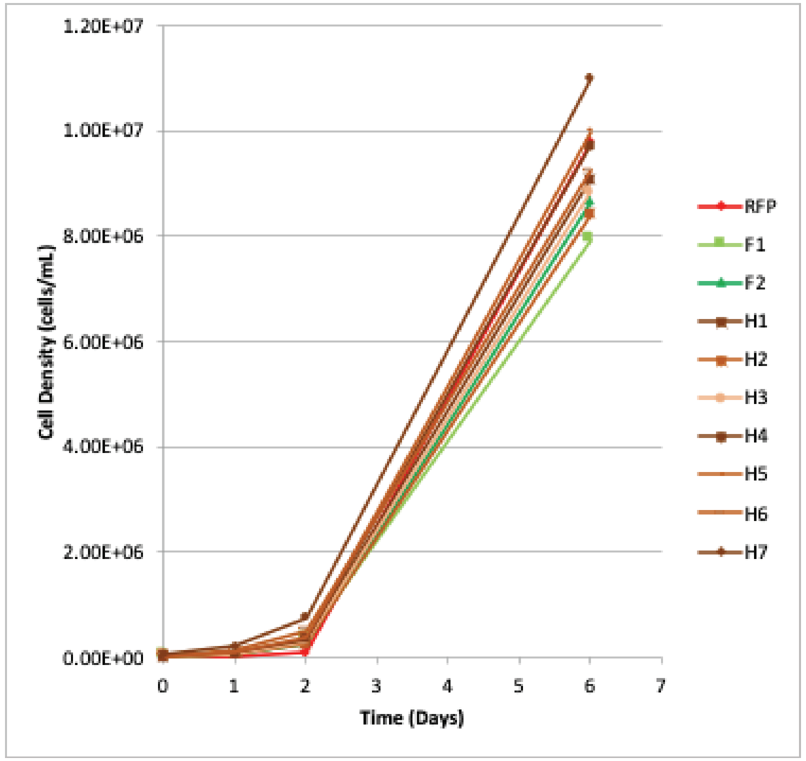
- M8.1 – 6g/m^{2}d ✓
- M10.1 – 9g/m^{2}d ✓
- M10.2 – 12g/m^{2}d ✓

Award Modification to extend Task 6 and 7 milestones into BP3 to accommodate Covid-related delays

M6.1 eCA transgenic lines created
M6.2 Characterization of eCA lines
M7.1 Carbon uptake experiments using pHOS
M7.2 Demonstration of enhanced atmospheric CO2 absorption in algae with eCA

Demonstrated "proof of concept" that diatoms will express a target CA

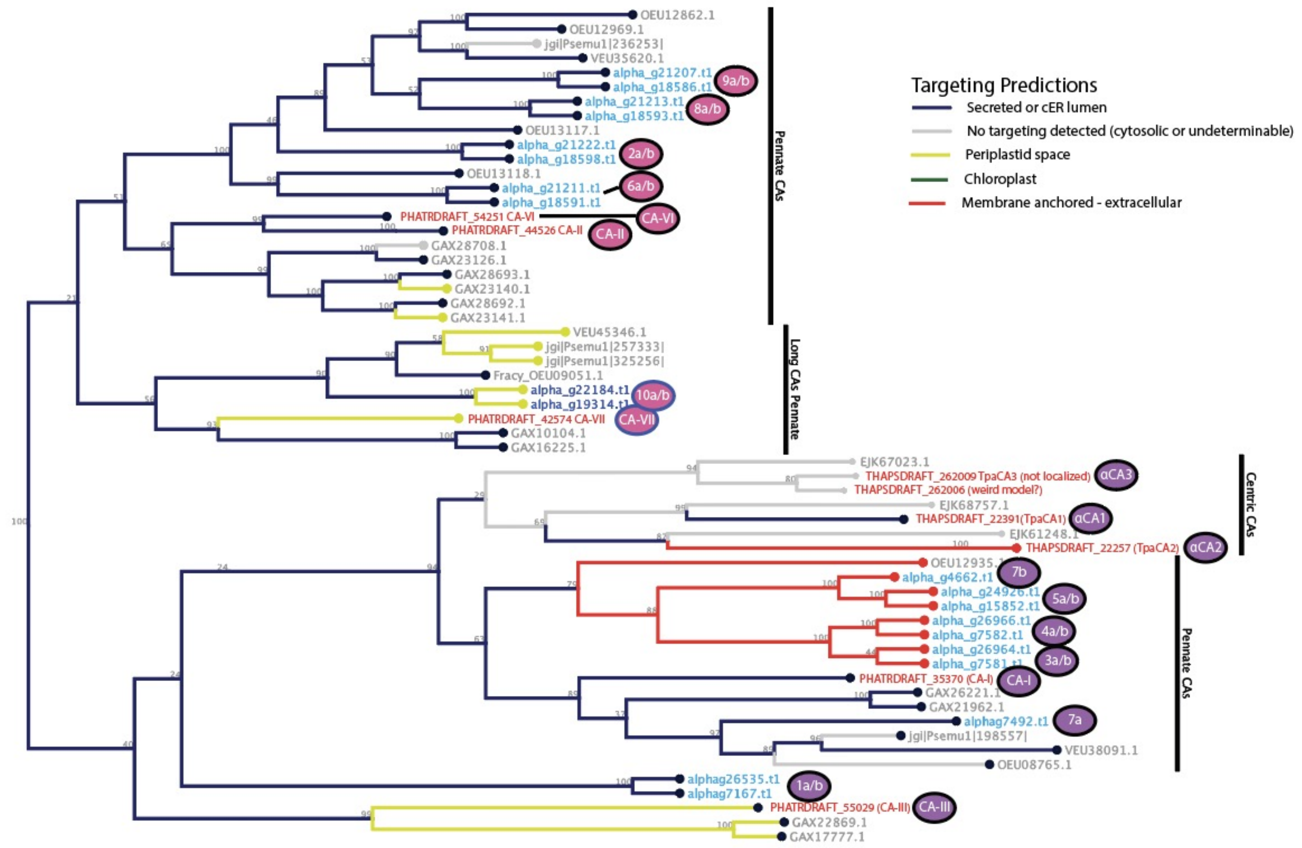


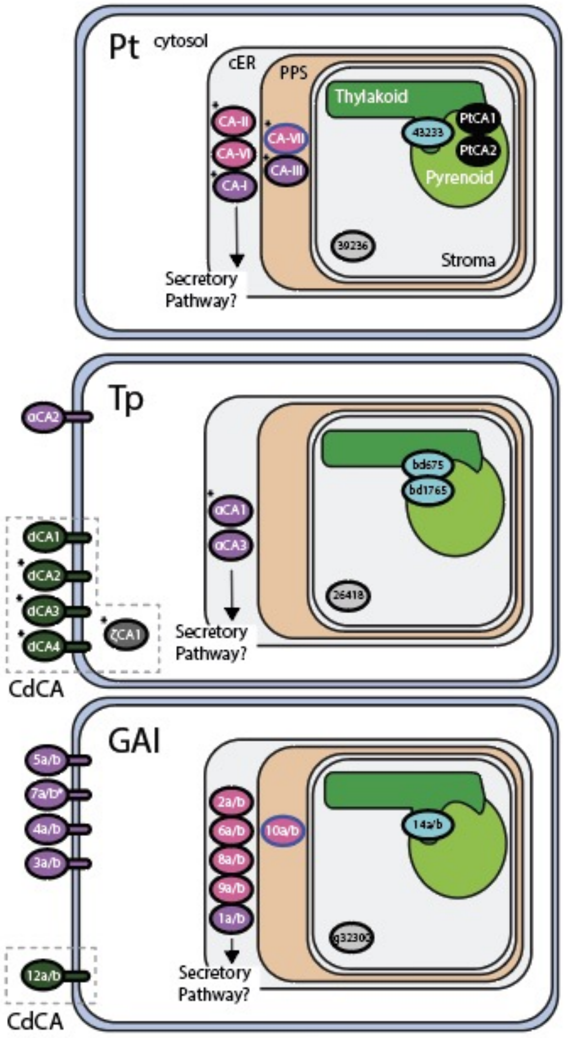


Increased expression of CA-YFP in transgenic lines at stationary phase, which is expected from the promoter used

No compromised growth in transgenic lines

Evaluating the CCM from GAI293





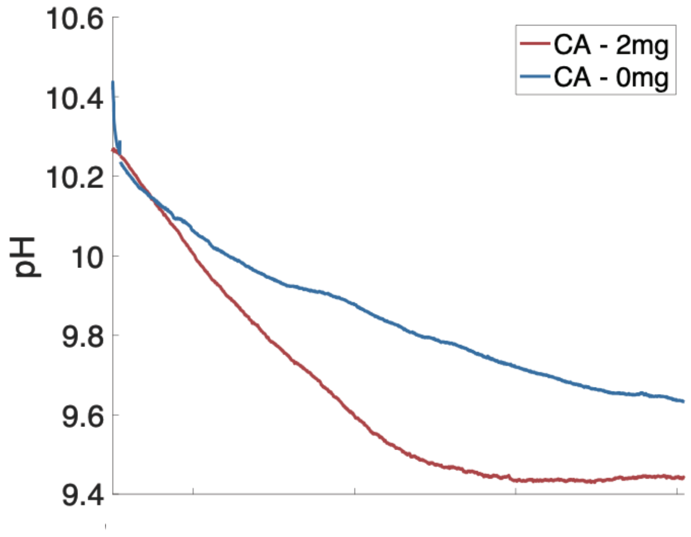
DE-EE0008121 (GAI, UCSD, JCVI)
DE-EE0008245 (Colorado School of Mines)

Oliver, Podell, et al. (*In* Review) Diploid genomic architecture of *Nitzschia hildebrandi*, an elite biomass production diatom

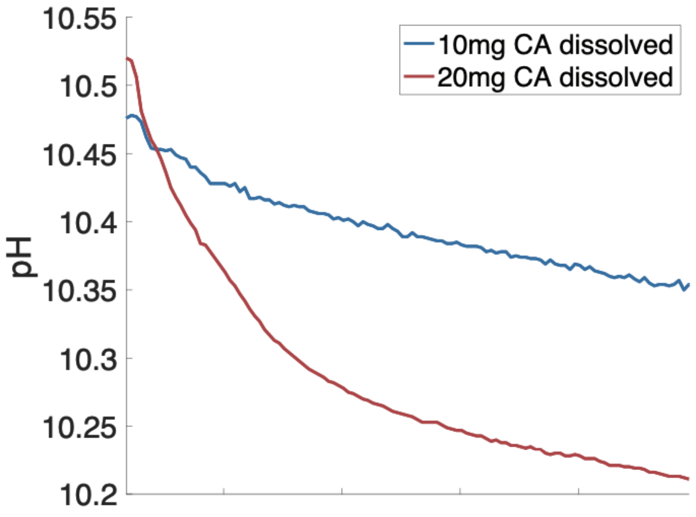
CAL POLY

Assessment of passive CO_2 uptake rates

Evaluation of dependency of passive CO_2 uptake on carbonic anhydrase concentrations and on media composition



- Increased rate of passive uptake of CO_2 into media (0.01 M Na^+) even at low concentration of carbonic anhydrase (~0.02 mg/ml; red)

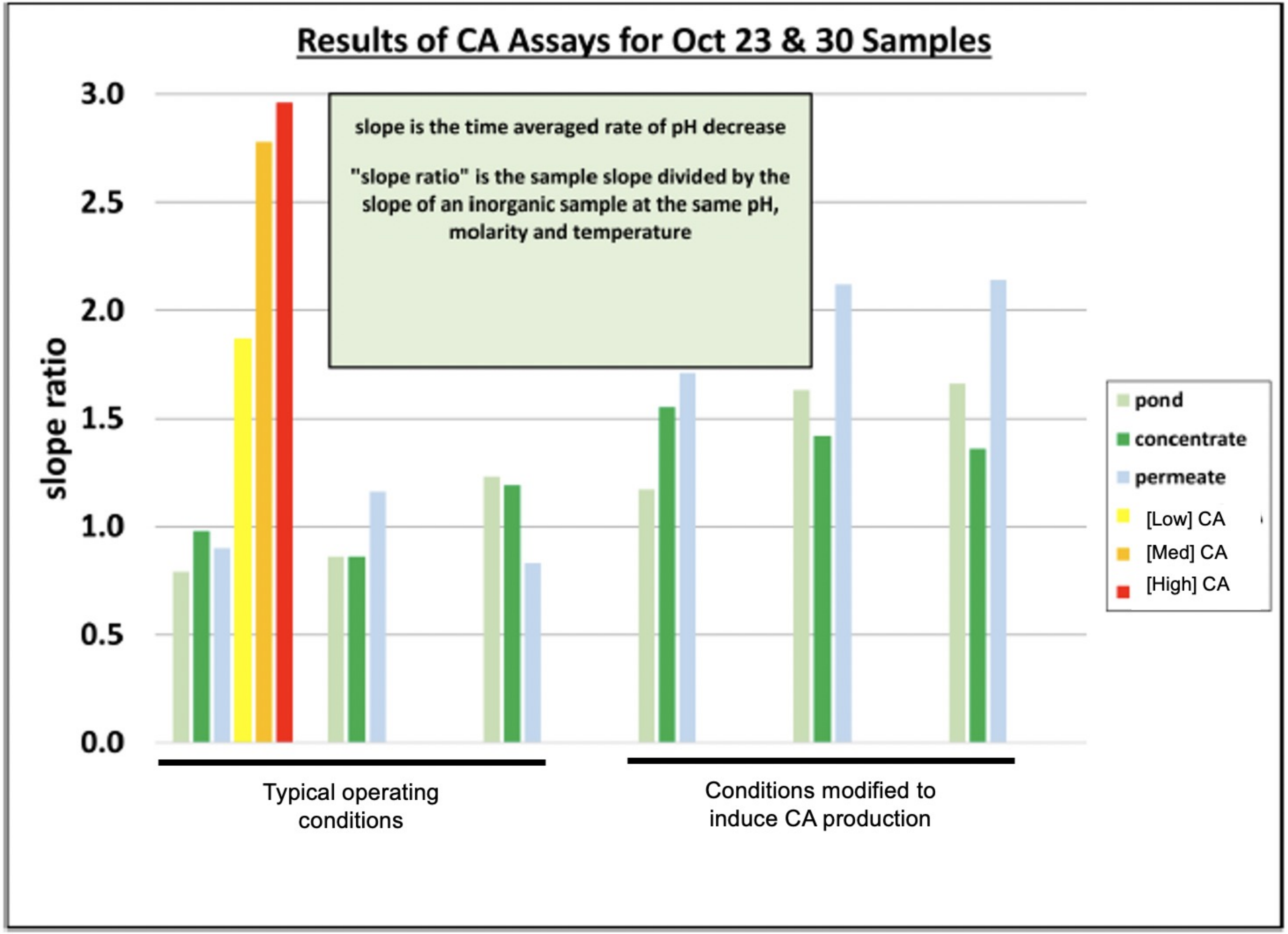


- Increasing rate of CO_2 uptake with increasing carbonic anhydrase concentrations (0.1 mg/ml (blue) & 0.2 mg/ml (red))

Emily Bockmon

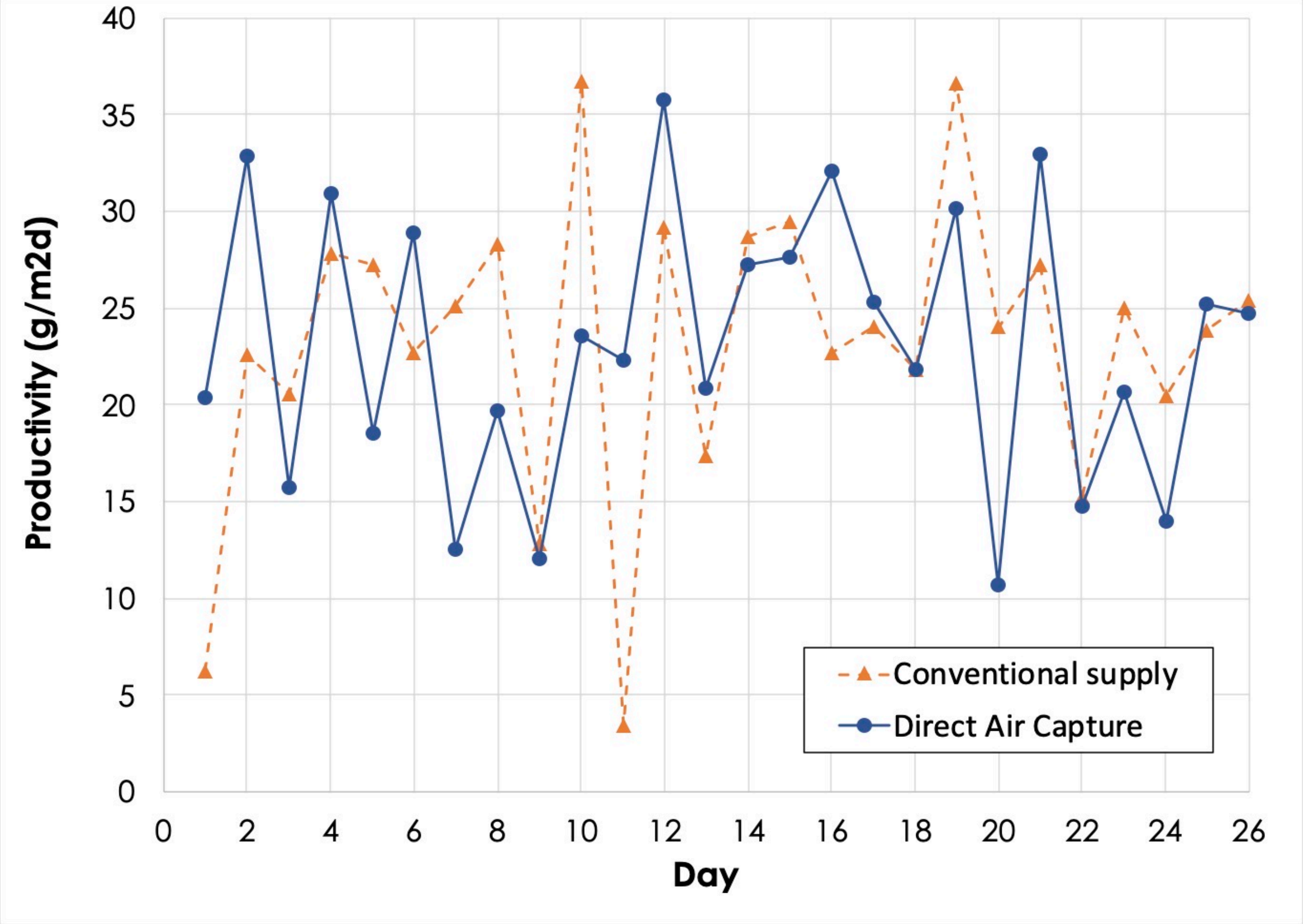
Insights:

- Stability of CA is important
- Accelerates CO_2 absorption of medium in unequilibrated solutions



Developed an assay to show efficacy of CA for CO_2 absorption at different media formulations

Achieve same productivity with direct air capture or externally supplied CO_2



GLOBAL ALGAE INNOVATIONS

Summary

- ***Goal Exceeded:*** *Develop an **integrated** system for productivity of 23 g/m2d (target was 12 g/m2d) on atmospheric carbon dioxide alone, and reduce costs of a renewable advanced biofuel*
- Target productivity on direct air capture (DAC) alone achieved through media optimization, strain selection, tests with CA, and cultivation system engineering improvements
- Successful generation of transgenic lines of algae expression carbonic anhydrase, further optimization underway
- Since target productivity achieved, updated end-of-project goal will be to further improve and optimize the operating range, TEA, and LCA for low cost, advanced algal biofuels with DAC CO_2 supply.

*Established successful partnership between Allen lab at JCVI and GAI **to combine strengths** towards this ultimate goal*

Acknowledgements

Sarah Smith (JCVI)

Mark Moosburner (JCVI)

Tyler Coale (JCVI)

David Hazelbeck (GAI)

Rodney Corpuz (GAI)

Aga Pinowska (GAI)

Emily Bockmon (Cal Poly)

Bill Rickman (TSD)

Office of Energy Efficiency & Renewable Energy

DE-EE0008639

Quad Chart Overview

Timeline
- June 1, 2019
- June 30, 2022

	FY20 Costed	Total Award
DOE Funding	$1,281,458	*$1,999,562*
Project Cost Share	$326,647	$500,615

Project Partners*
- J. Craig Venter Institute
- Global Algae Innovations

Project Goal
Develop an integrated system capable of obtaining high rates of algal productivity (biomass production) using 100% atmospheric carbon dioxide (direct air capture)

Achieved the overall project goal.

End of Project Milestone
Obtain 12 g/m2d productivity with direct air capture

Exceeded the end of project milestone with a productivity of 22 g/m2 productivity solely on CO_2 from direct air capture

Funding Mechanism
FOA: De-FOA-0001908 (due June 27, 2018)

Topic Area 1: CO_2 Utilization within Algae Cultivation Systems

*Only fill out if applicable.