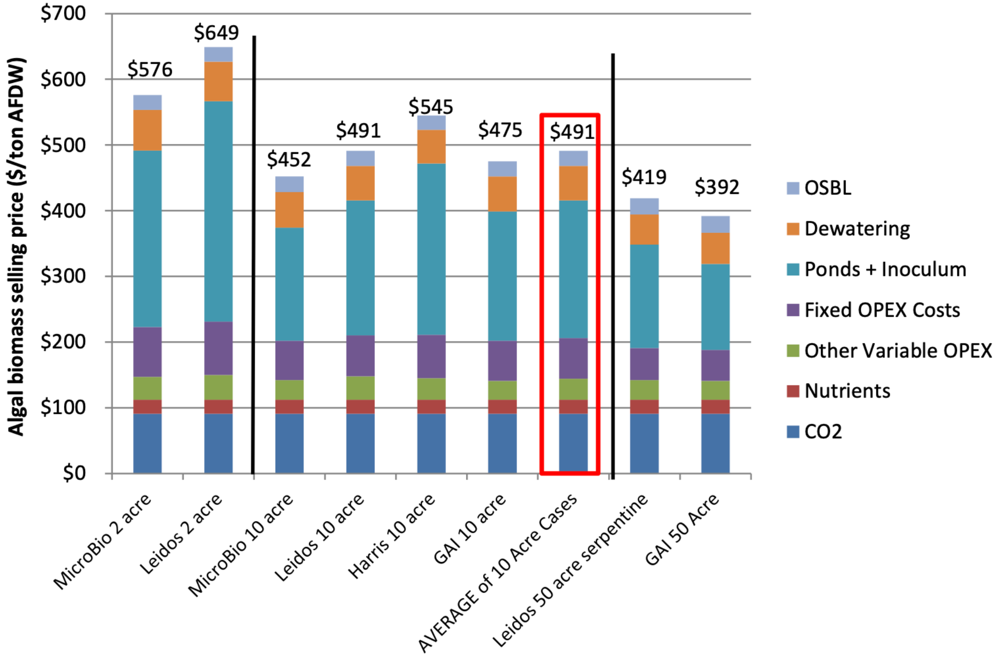
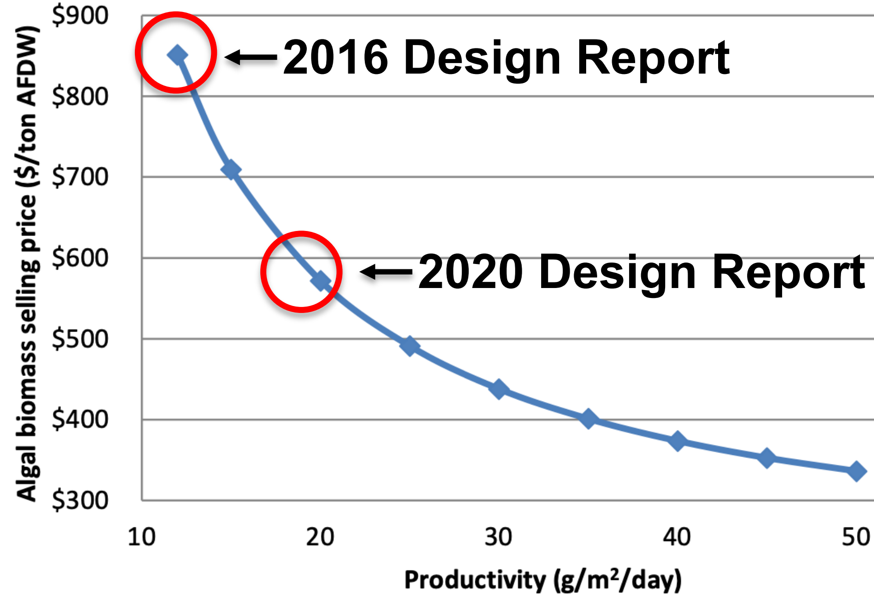
Biomolecular Films for Direct Air Capture of CO_2

BETO Peer Review
April 3, 2023 | Denver, CO

Karsten Zengler, Juan Tibocha-Bonilla, Martin Gross, Drew Greene, Ashton Zeller, Luke Dahlin, Matt Wiatrowski, Ryan Davis, **Mike Guarnieri**

Background: CO_2 Is A Key Cost Driver in Algal Biofuels





NREL/TP-5100-64772

- CO_2 accounts for ~20% of total costs of Algal Biomass Selling Price (ABSP).
- ABSP is a function of productivity.
- Technologies that enable direct air capture (DAC) of atmospheric CO_2 to decouple algae cultivation from CO_2 point sources and enhance productivity present an opportunity to improve the economics and resource potential of algal biomass.

FY20 Bioenergy Technologies Multi-Topic FOA

- **DE-FOA-0002203 Topic Area 3:** Algae Bioproducts and CO2 Direct-Air-Capture Efficiency (ABCDE)
- **FOA Issue Date:** 1/23/2020
- **Award Start Date:** 7/31/2020
- **Initial Verification:** 12/1/2020

Topic Area 3 Metrics:

The application must propose to meet all the minimum targets in the table below by the end of the project.

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

Project Overview

Objective: We propose to integrate recent advances in computational metabolic modeling, algal genetic engineering, algal cultivation, and algal biomass upgrading to enable secretion of carbonic anhydrase for enhanced CO_2 capture and conversion in immobilized algal biofilms.

End-Project Goal: Achieve a 25% increase in algal biomass revenue potential, algal biomass quality <10% out of specification for downstream testing, a 10% increase in productivity, a 20% increase in CO_2 obtained from ambient air, and a 10% decrease in CO_2 costs via bio-based air capture technology.

Approach: Team and Task Structure

UC San Diego

Genome-scale Metabolic Model, Engineering Designs
Task Lead: Karsten Zengler

NREL Transforming ENERGY

Enzyme and Strain Engineering | Task Lead: Mike Guarnieri
TEA/LCA | Task Leads: Ryan Davis and Matt Wiatrowski

GWT GROSS-WEN TECHNOLOGIES

Revolving Algal Biofilm (RAB) System Deployment
Task Lead: Martin Gross

ALGIX®

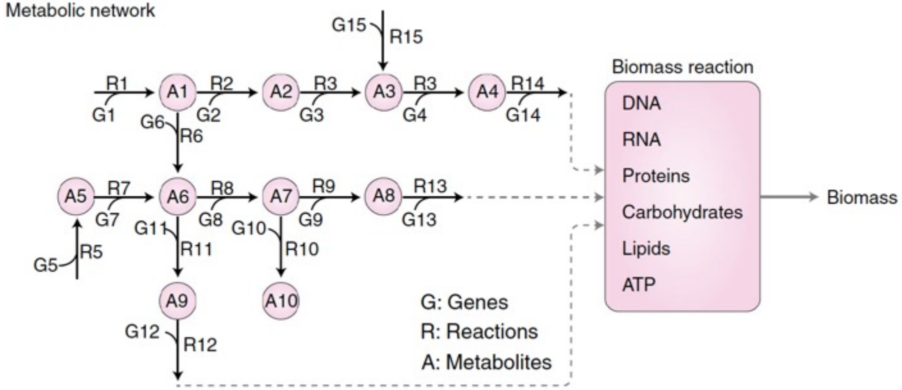
Product Development | Task Lead: Ashton Zeller

Approach: Task 2 - Computational Modeling

Task 2 will reconstruct a metabolic model of *Picochlorum renovo* and deploy this model for the design of improved protein production and secretion to achieve maximal productivity in this microalga.

- **Sub-task 2.1:** Genome-scale metabolic model reconstruction.
- **Subtask 2.2:** Model Refinement and Rational Strain Engineering Design

UC San Diego



Milestone 2.1.1 (M6): Reconstruction of a genome-scale metabolic model of *P. renovo*.

Milestone 2.2.1 (M18): GEM Refinement: integrate phenotypic and omic data and all possible secretion and carbonic anhydrase (CA) sequences into the model and predict optimized secretion without loss in productivity.

Approach: Task 3 - CA Production and Algal Strain Engineering

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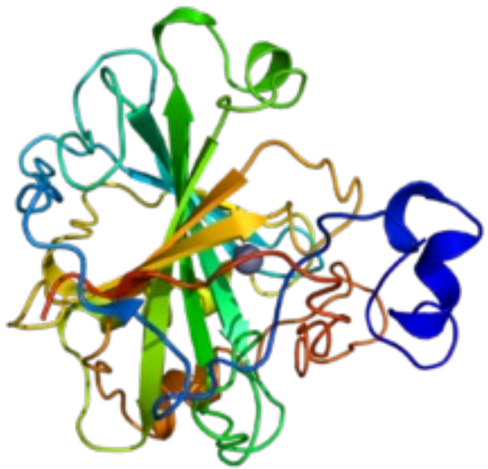


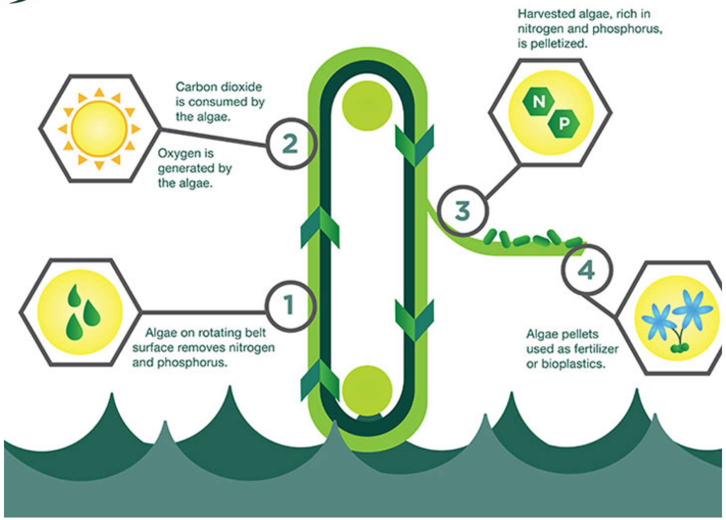
Task 3 will target expression and down-selection of candidate CA variants. Top-candidate CA will be incorporated into *P. renovo* to enable photoproduction and secretion. This work will ultimately deliver i) CA variants suitable for cultivation supplementation and ii) algal biocatalysts with *in situ* CA photoproduction capacity.

- **Sub-task 3.1:** CA Production
- **Sub-task 3.2:** Algal Strain Engineering

Milestone 3.1.1 (M15): Down-select and produce >10g of two top-candidate CA in *P. pastoris* for delivery to GWT for assessment in RAB systems.

Milestone: 3.2.1 (M30): Demonstrate functional extracellular secretion of a heterologous CA. SDS-PAGE, Western Blot, and/or densitometric analyses will be employed to quantify protein in the culture secretome.

Approach: Task 4 – RAB Deployment & Compositional Analysis



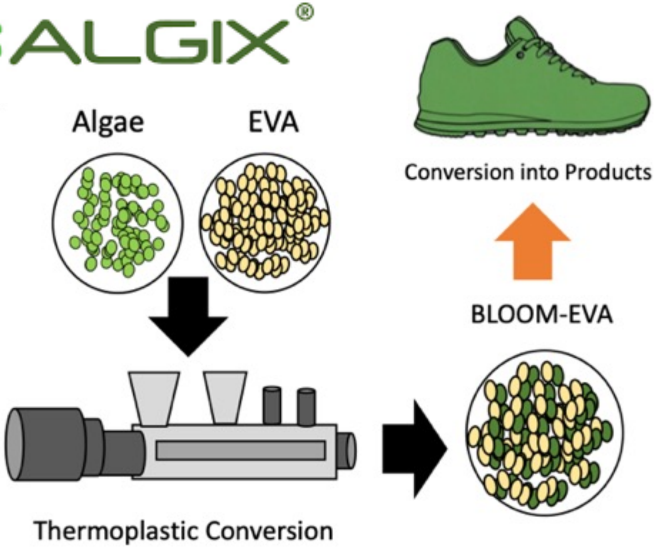
Task 4 will assess algal biomass yield and productivity increases as a function of CA supplementation and/or algal secretion to further inform CA down-selection, define CA concentration requirements, and assess process enhancement metrics.

Milestone 4.1 (M9): Establish a productivity baseline, reporting g/m^2/day ash-free dry weight, and composition for *P. renovo* in a lab-scale RAB system.

Milestone 4.2 (M21): Deploy top-candidate exogenously supplemented CA (2) in >100L RAB systems to establish algal productivity and conversion yield enhancement relative to baseline, reporting biomass productivity and AFDW composition.

Milestone 4.2 (M33): Deploy engineered algal CA secretion strains at 800L scale, with semi-continuous harvest, reporting biomass productivity and AFDW composition.

Approach: Task 5 - Product Development



Task 5 will focus on the development of a novel thermoplastic composite using RAB-derived algae produced in Task 4.

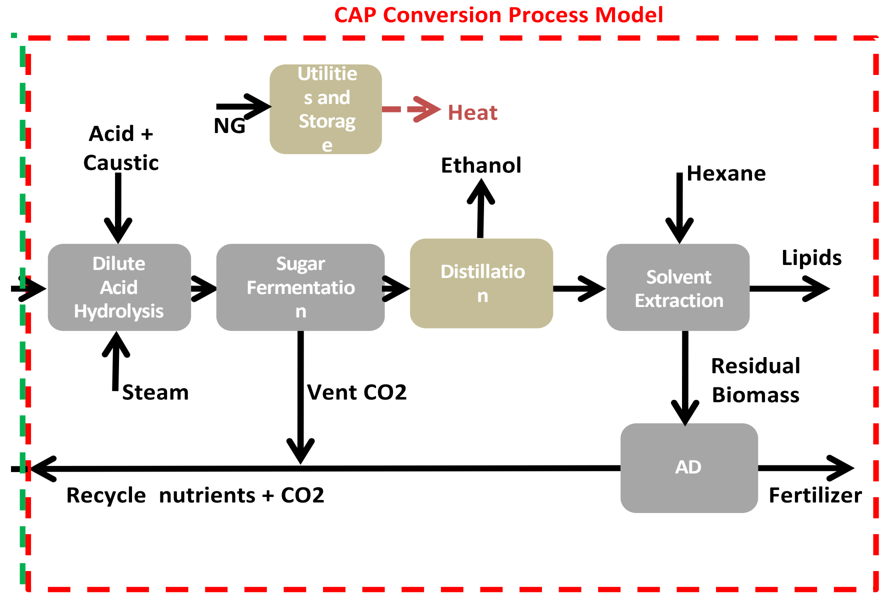
Milestone 5.1 (M12): Compositional Analysis for Bioplastic Conversion. RAB biomass compositional analysis from Task 4 will be evaluated to determine expected performance in bioplastic conversion.

Milestone 5.2 (M27): Formula Development and Compounding. Develop a formula and compound it into a 45% algae masterbatch suitable for injection molding test parts.

Milestone 5.3 (M35): Injection Molding and Material Property Testing. Injection mold parts containing 20% algae and then test them for material properties such as MFI, moisture, tensile, flexural, and other characteristics.

Approach - Task 6: TEA/LCA

Task 6 will serve to assess mass and energy balances in the proposed CA-enhanced deployment process, ultimately defining an economically-viable and sustainable path to commercialization via iterative TEA/LCA model establishment and refinement.



Milestone 6.1 (M24): Modification of the established CAP TEA model with modeled carbon utilization efficiency enhancements. Establish LCA model for utilization of atmospheric CO_2 enabled by CA supplementation and/or secretion.

Milestone 6.3 (M36): Refine models with BP2 inputs and deliver techno-economic path to commercialization.

Progress and Outcomes

Mathematical model of *Picochlorum renovo*

*i*CZ1179-Picre → 6,374 reactions, 3,587 metabolites, and 1,179 genes

Mathematical model of *Picochlorum renovo*

*i*CZ1179-Picre → 6,374 reactions, 3,587 metabolites, and 1,179 genes

Carbon source	Dark or Light	Experimental	Simulation	Conclusion
glycerol	Dark	Non-Growth	Non-Growth	TN
arabinose	Dark	Non-Growth	Non-Growth	TN
acetate	Dark	Non-Growth	Non-Growth	TN
galactose	Dark	Non-Growth	Non-Growth	TN
formate	Dark	Non-Growth	Non-Growth	TN
pyruvate	Dark	Non-Growth	Non-Growth	TN
glucose	Dark	Non-Growth	Non-Growth	TN
glutamine	Dark	Non-Growth	Non-Growth	TN
uridine	Dark	Non-Growth	Non-Growth	TN
fumarate	Dark	Non-Growth	Non-Growth	TN
succinate	Dark	Non-Growth	Non-Growth	TN
sorbitol	Dark	Non-Growth	Non-Growth	TN
lactate	Dark	Non-Growth	Non-Growth	TN
ribose	Dark	Non-Growth	Non-Growth	TN
glycerol	Light	Non-Growth	Non-Growth	TN
arabinose	Light	Non-Growth	Non-Growth	TN
acetate	Light	Growth	Growth	TP
galactose	Light	Non-Growth	Non-Growth	TN
formate	Light	Non-Growth	Non-Growth	TN
pyruvate	Light	Growth	Growth	TP
glucose	Light	Non-Growth	Non-Growth	TN
glutamine	Light	Growth	Growth	TP
uridine	Light	Growth	Growth	TP
fumarate	Light	Non-Growth	Non-Growth	TN
succinate	Light	Non-Growth	Non-Growth	TN
sorbitol	Light	Non-Growth	Non-Growth	TN
lactate	Light	Non-Growth	Non-Growth	TN
ribose	Light	Growth	Growth	TP

True Positives	5
True Negatives	23
False Positives	0
False Negatives	0

Accuracy	100%
Sensitivity	100%
Specificity	100%

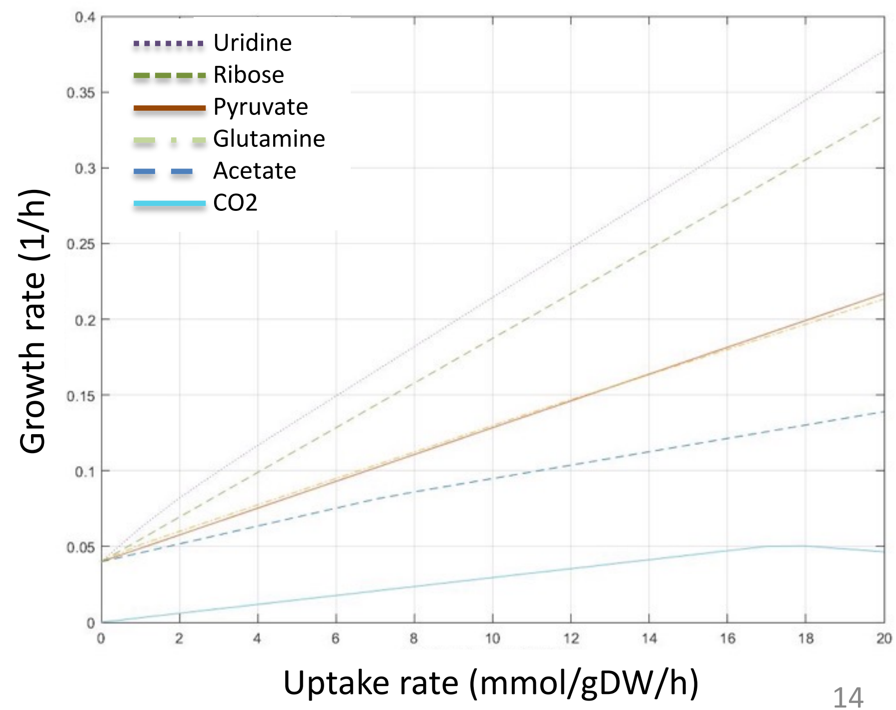
Mathematical model of *Picochlorum renovo*

*i*CZ1179-Picre → 6,374 reactions, 3,587 metabolites, and 1,179 genes

Growth under light conditions

Using NH_4 as nitrogen source

- Acetate
- Pyruvate
- Glutamine
- D-Ribose
- Uridine

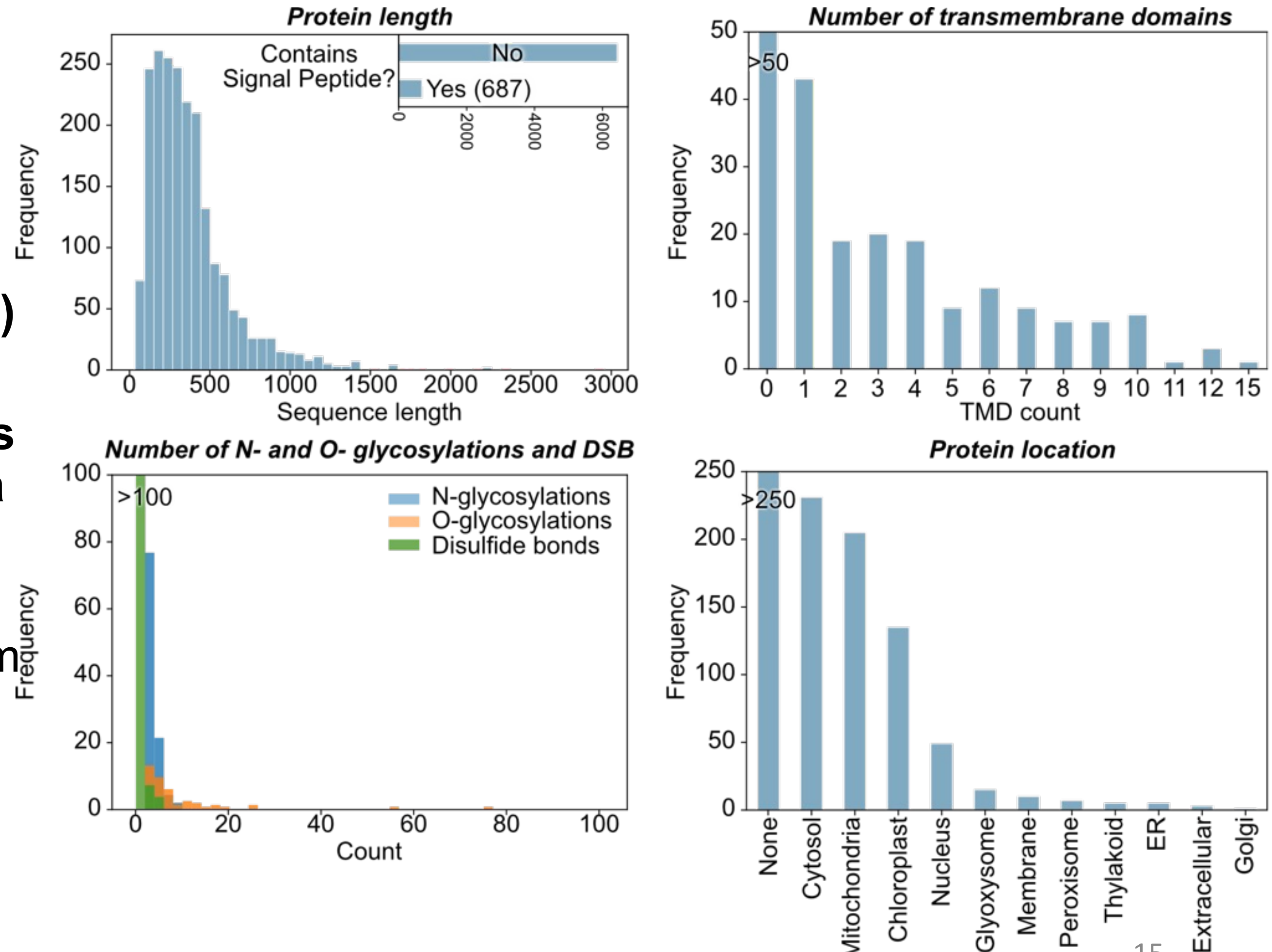


Protein Secretion Network

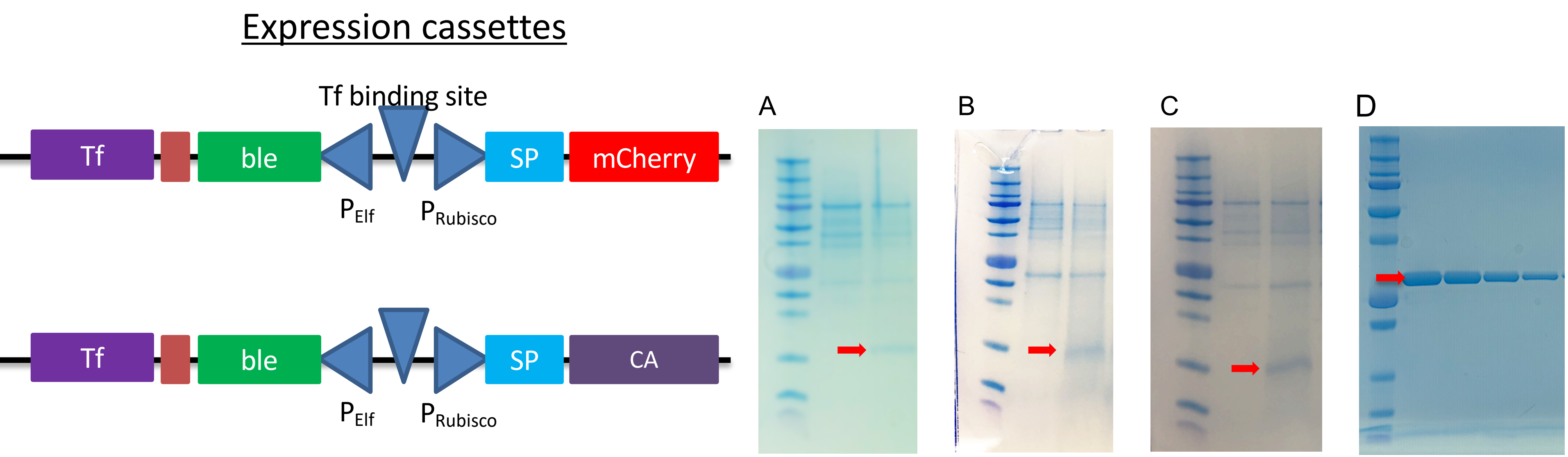
- We have successfully finalized the main requirement to build a secretory model: **The Protein-Specific Information Matrix (PSIM)**

- **687 (out of 2071) proteins** were identified to contain a **Signal Peptide**

- PSIM was constructed from homology with ***Chlamydomonas reinhardtii*** and from available information in **UniProt database**

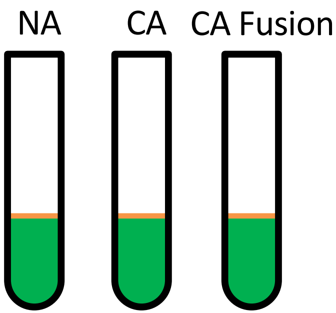


Heterologous Expression of CA

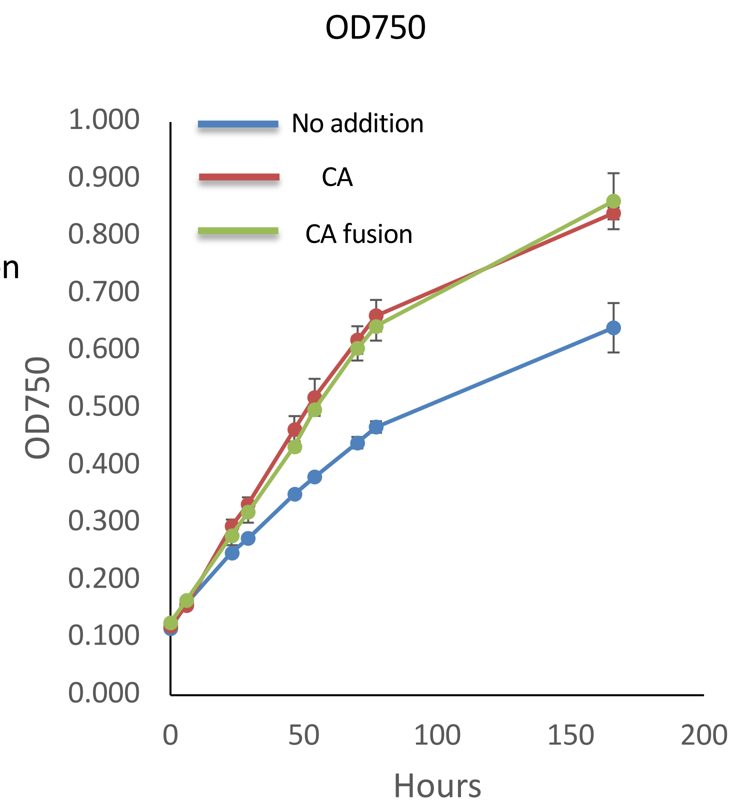


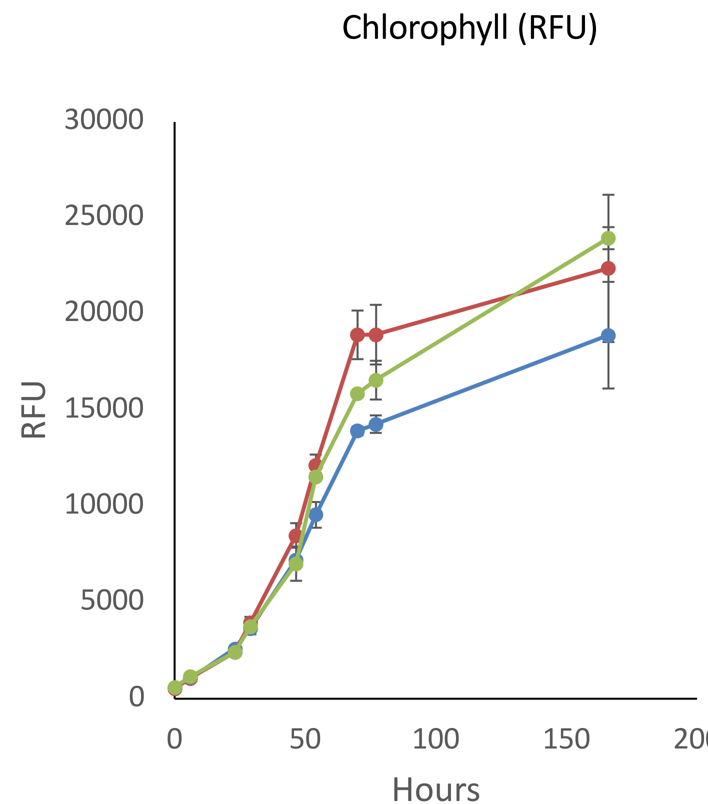
- Successful CA expression and secretion achieved in *P. pastoris*.
- Over 12 unique CA were evaluated for secretion potential and down-selected based upon solubility, stability, and CA activity in *P. renovo* cultivation media.

CA Supplementation Enhances Growth



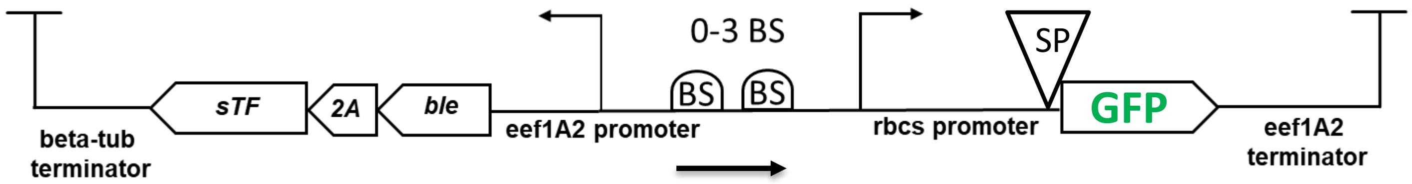
f/2 media
3X NH_4Cl
0.2 % CO_2
300 RPM
33C
350 mU enzyme



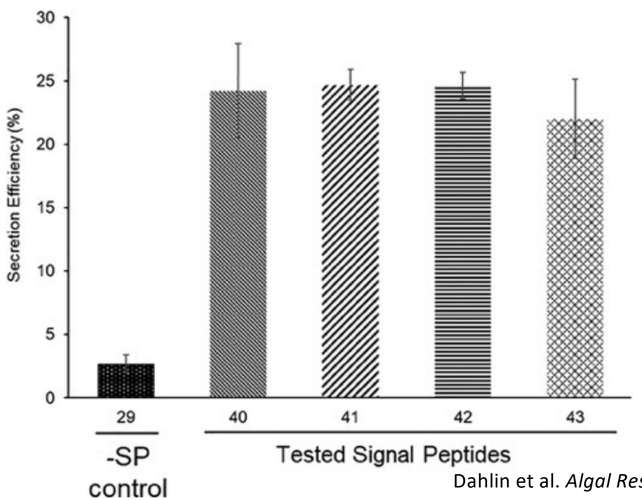


Enhanced Secretion Achieved in *P. renovo*

Establishment of a functional synthetic transcription factor in P. renovo

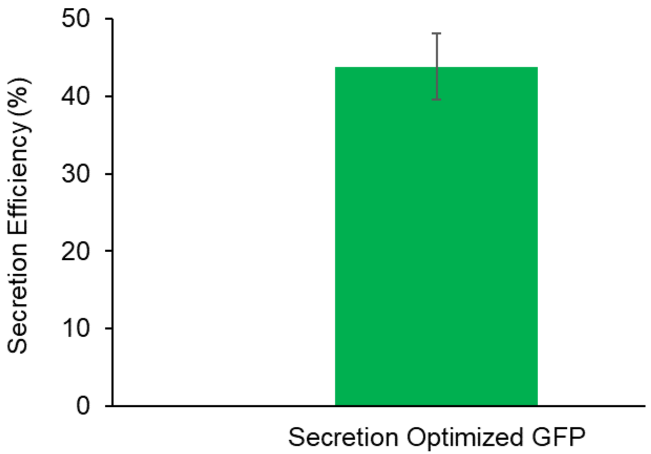


Original cassette



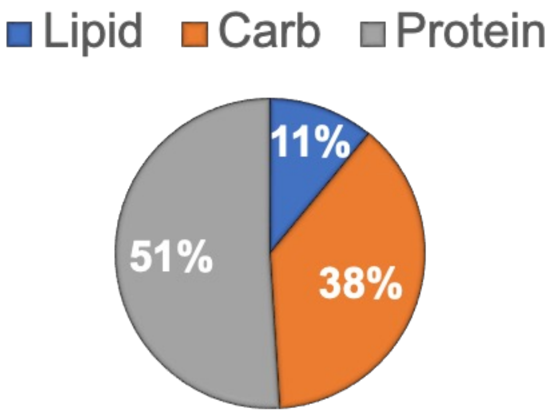
Dahlin et al. *Algal Research* 2021

New cassette

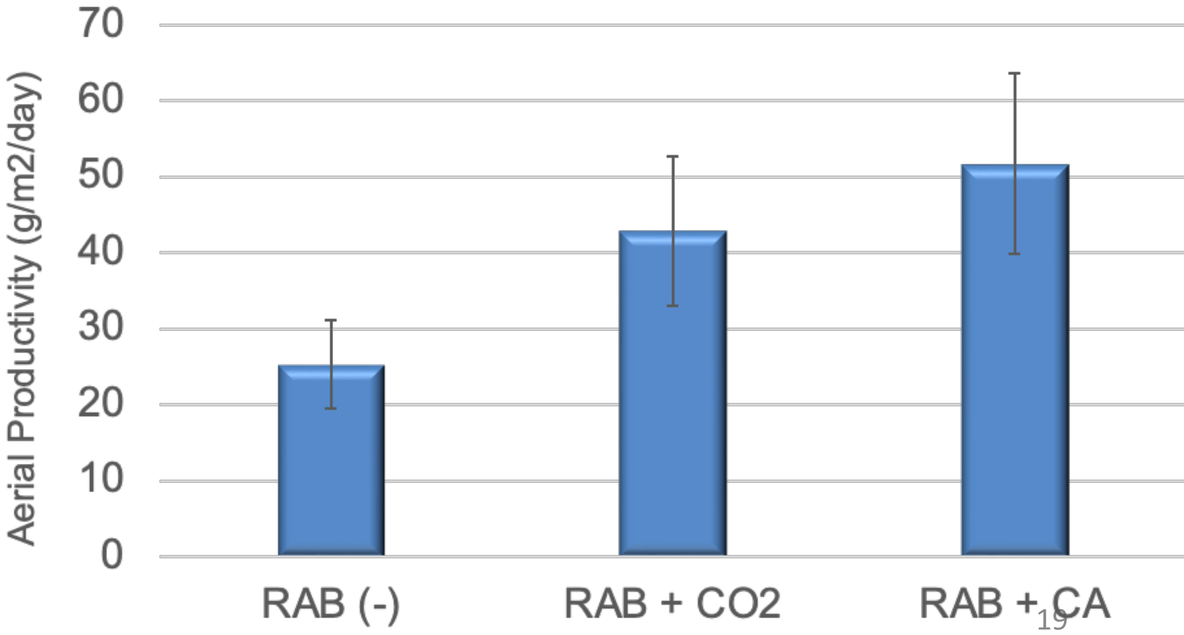


~2X secretion efficiency achieved

P. renovo lab-scale RAB trial results



- > 25g/m²/day productivity in RAB configuration under ambient CO_2
- > 50g/m²/day productivity in RAB configuration under ambient CO_2 with CA supplementation
- RAB biomass composition has minimal differential relative to pond cultivated biomass



Plasticization Trials Reflect Favorable Biomass Composition

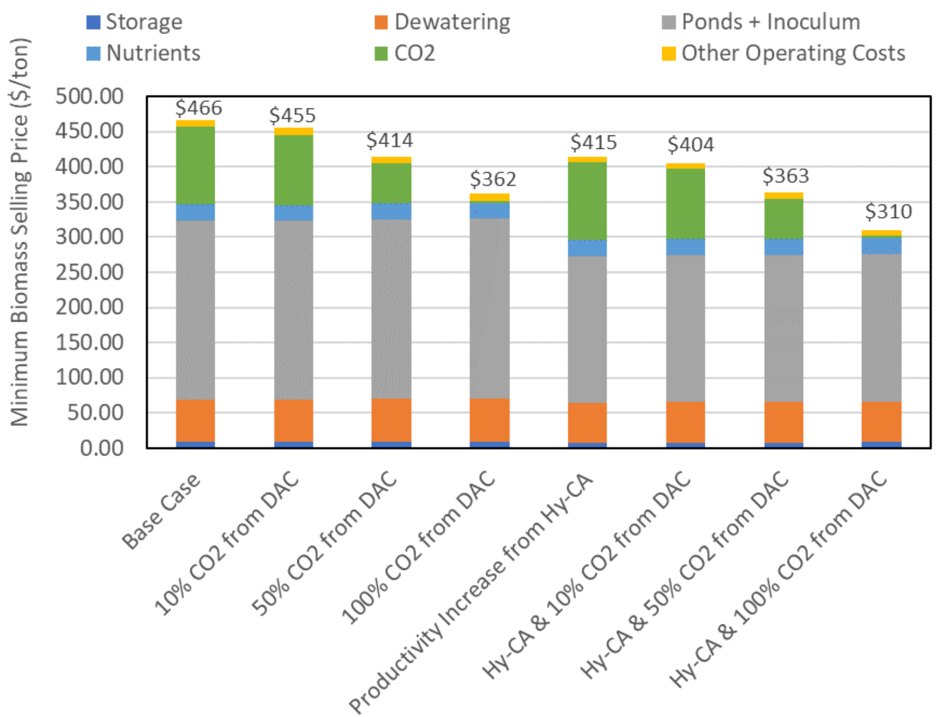
- Algix conducted compositional testing and showed that RAB biomass is suitable for plastic composite production.
- The algae contained only 6 compounds associated with degradation in the odor testing.
  - This is below current thresholds for deeming algae biomass significantly degraded and qualifies the RAB algae material for use in commercial applications.
- Protein, ash, and water contents met minimum requirements.

Initial Plasticization

Modified Plasticization

Progress Towards Interim Go/No-Go

(Go/No-Go): Year 2 Q7 – Interim Validation Period. Demonstrate a 10% increase in algal biomass revenue potential, algal biomass quality <15% out of specification for downstream testing, a 5% increase in productivity, a 10% increase in CO_2 obtained from ambient air, and a 5% decrease in CO_2 costs via enhanced air capture.



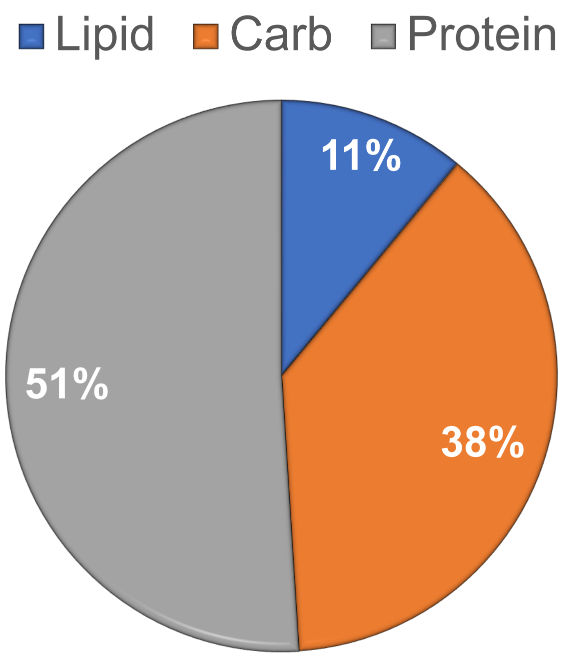
Preliminary results (RAB data extrapolated to open ponds)

- CA productivity increase:
  - Revenue +20%
  - MFSP -10%
  - *25.3 → 51.7 $g/m^2/day$
- CO_2 from Ambient capture:
  - 18% MFSP decrease
- Fuel Yield: 54 → 71 GGE/ton
- Solid coproduct
  - Revenue up to +27%
- Further improvements possible with integration of RAB system (in progress)

Progress Towards Interim Go/No-Go

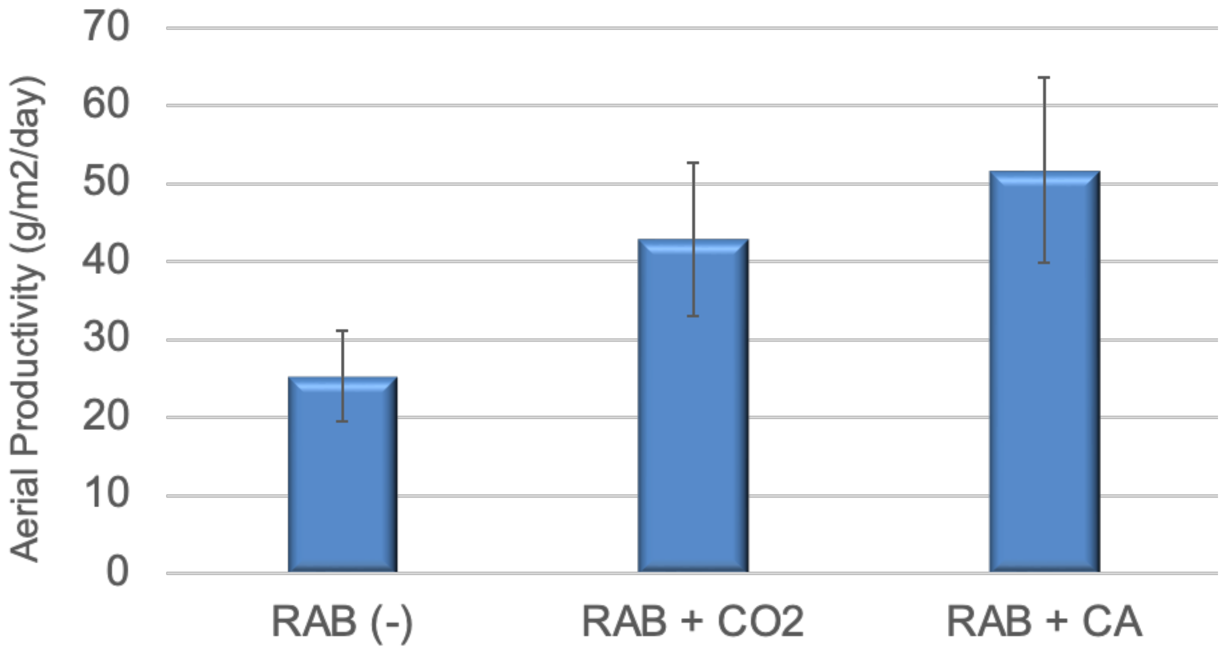
(Go/No-Go): Year 2 Q7 – Interim Validation Period. Evaluate Hy-CA pond supplementation at 100mL outdoor simulation scale and >100L RAB scale, demonstrating a 10% increase in algal biomass revenue potential, algal biomass quality <15% out of specification for downstream testing, a 5% increase in productivity, a 10% increase in CO_2 obtained from ambient air, and a 5% decrease in CO_2 costs via enhanced air capture.

Target Protein Content: *53.5% ± 15%*
Achieved Content: *51% ± 11%*



Progress Towards Interim Go/No-Go

(Go/No-Go): Year 2 Q7 – Interim Validation Period. Evaluate CA pond supplementation at 100mL outdoor simulation scale and >100L RAB scale, demonstrating a 10% increase in algal biomass revenue potential, algal biomass quality <15% out of specification for downstream testing, a 5% increase in productivity, a 10% increase in CO_2 obtained from ambient air, and a 5% decrease in CO_2 costs via enhanced air capture.



Progress Towards Interim Go/No-Go

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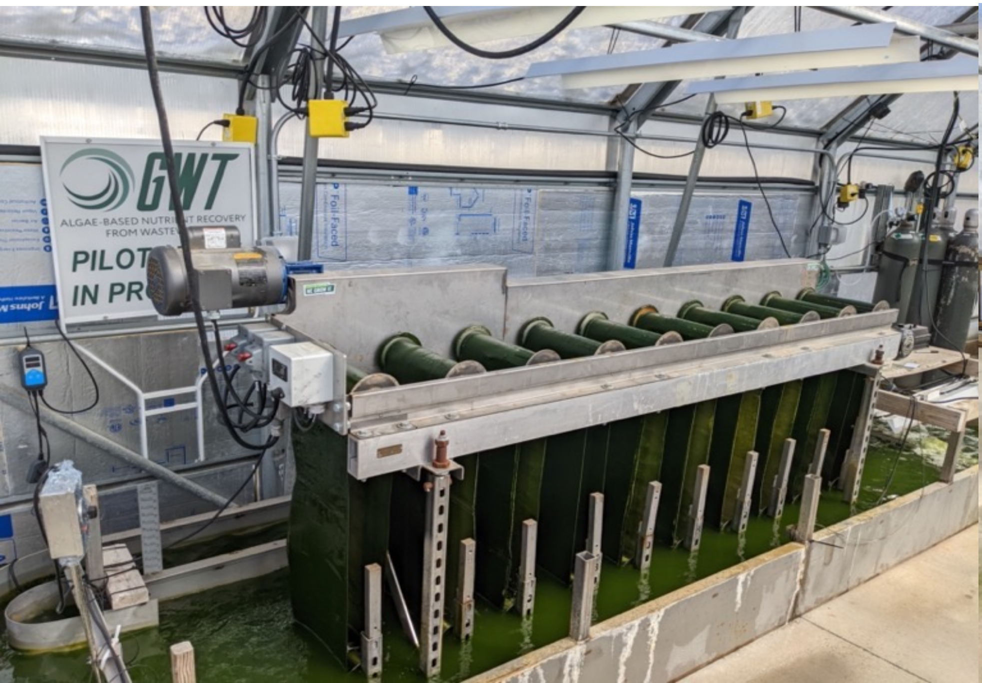

Progress Towards Interim Go/No-Go

(Go/No-Go): Year 2 Q7 – Interim Validation Period. Evaluate CA pond supplementation at 100mL outdoor simulation scale and >100L RAB scale, demonstrating a 10% increase in algal biomass revenue potential, algal biomass quality <15% out of specification for downstream testing, a 5% increase in productivity, a 10% increase in CO_2 obtained from ambient air, and a 5% decrease in CO_2 costs via enhanced air capture.

- **All Go criteria successfully achieved.**
- **BP2 Completed in October, 2022**
- **BP3 Initiated in January, 2023**

- BP3 will focus upon:
  - GEM optimization using multi-omic and physiological inputs
  - Secretion optimization using GEM-informed strain engineering designs
  - Scale-up to 800L RAB system
  - CAP processing of RAB biomass and conduct injection molding
  - Refinement of TEA/LCA models

Impact

- Moving the needle on algae productivity: achieved **>50g/m^2/day areal productivity *from atmospheric CO_2.***
- Enhances algal biomass revenue potential via reduction (elimination) of CO_2 sparging requirements, improved bioproductivity, and high-value co-production.
- Established comprehensive *Picochlorum* genome-scale model for basic and applied research pursuits.
- RAB cultivation enables in-line harvesting with dramatic reduction in dewatering requirements.
- 30% fuel yield improvement and nearly 20% MFSP improvement relative to baseline.
- Carbon intensity reduction achieved via improved carbon utilization efficiency and green biopolymer production.
- Bio-based DAC has a number of advantages over abiotic DAC technologies including minimal CAPEX/OPEX relative to current and emerging abiotic CO_2 capture and delivery systems, which require substantial water and energy inputs.

Summary

- **Problem:** CO_2 accounts for nearly 20% of ABSP
- **Solution:** We are pursuing and integrated computational modeling, strain engineering, cultivation engineering, and biomass valorization strategy to enable high-productivity biomass with dramatically reduced CO_2 requirements.
- **Progress to Date**
  - Established a complete genome-scale metabolic model for the high productivity microalga*, P. renovo*.
  - Successfully engineered *P. renovo*, for secretion of carbonic anhydrase.
  - Deployed *P. renovo* in a RAB configuration, achieving > 50g/m^2/day biomass productivity on atmospheric CO_2.
  - Initiated co-production of biopolymer(s) from RAB-derived biomass
  - Generated preliminary TEA/LCA models for RAB-mediated cultivation
- **Impact:** We have established a high-productivity, atmospheric CO_2 cultivation regime for *P. renovo*, enabling enhanced fuel yield and economics, and reduced carbon intensity.

Acknowledgements

U.S. DEPARTMENT OF ENERGY | Energy Efficiency & Renewable Energy

GWT GROSS-WEN TECHNOLOGIES

Drew Greene
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Martin Gross

ALGIX®

Ashton Zeller

UC San Diego

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Euihyun Kim
Sophia Alonso
Cristal Zuniga
Karsten Zengler (PI)

NREL
NATIONAL RENEWABLE ENERGY LABORATORY

Marcus Bray
Lukas Dahlin
Ryan Davis
Jeff Linger
Kim Rosenbach
Matt Wiatrowski
Mike Guarnieri

Additional Slides

Quad Chart Overview

UCSD has partnered with the Gross-Wen Technologies (GWT), Algix, and the National Renewable Energy Laboratory (NREL) to establish carbon sequestering molecular films for enhanced atmospheric CO_2 capture and increased biomass productivity. The project, entitled "**Biomolecular Films for Direct Air Capture of CO_2**" will integrate core competencies from all partners, including computational modeling, protein and algal strain engineering, mass cultivation, biomass upgrading, and TEA/LCA in order to develop a conversion process demonstrating the production of fuel intermediates and bioplastics from atmospheric CO_2 direct air capture (DAC). Successful implementation of the proposed project offers dramatic economic and sustainability benefits relative to conventional DAC systems.

Enabling bio-based DAC via molecular film technology.

Key Personnel

UCSD: Karsten Zengler (PI) **GWT:** Martin Gross; **Algix:** Ashton Zeller; **NREL:** Mike Guarnieri

Program Summary

Period of performance: 36 months

Federal funds:	$ 2M
Cost-share:	$ 0.5M
Total budget:	$ 2.5M

	Key Milestones & Deliverables
Y1	• Establish baseline for *P. renovo* cultivation in GWT RAB system grown on atmospheric CO_2.
Y2	• Establish baseline for molecular film-mediated enhancement of atmospheric CO_2 capture in open pond systems. • Achieve a 10% increase in algal biomass revenue potential, algal biomass quality <15% out of specification for downstream testing, 5% increase in productivity, 10% increase in CO_2 obtained from ambient air, and a 5% decrease in CO_2 costs via bio-based DAC.
Y3	• Achieve 25% increase in algal biomass revenue potential, algal biomass quality <10% out of specification for downstream conversion to fuel intermediates and bioplastics, a 10% increase in productivity, a 20% increase in CO_2 obtained from air, and a 10% decrease in CO_2 costs via bio-DAC • Demonstrate upgrading of algal protein to bioplastics

Technology Impact

- Our bio-based, photoproduction approach offers a number of advantages over abiotic DAC technologies; notably, little-to-no CAPEX/OPEX expenditures relative to current and emerging abiotic CO_2 capture and delivery systems, which require substantial water and energy inputs.
- Our technology can be seamlessly integrated into conventional algal growth systems that utilize either point source CO_2 streams, or conventional DAC technologies, synergistically reducing costs.

Responses to Previous Reviewers' Comments

N/A – Project was not previously reviewed.

Key Patents, Publications, and Presentations

Publications

- Dahlin LR, Guarnieri MT. (2021) Development of the high-productivity marine microalga, *Picochlorum renovo*, as a photosynthetic protein secretion platform. *Algal Research* 54, 102197

Patent Applications

- Photosynthetic protein secretion platform. US Patent App. 17/666,345

Presentations

- Dahlin LR. International Conference on Algal Biomass Biofuels, & Bioproducts. Waikoloa, HI, 2023.
- Guarnieri MT, et al. SIMB SBFC, Portland, OR, 2023.
- Dahlin LR. Algal Biomass Summit. Virtual. Oral Presentation. October 13th 2021.
- Dahlin L.R and Guarnieri, M.T. International Conference on Algal Biomass, Biofuels, & Bioproducts. Virtual. June 14-16 2021.
- Kim, Euihyun. Et al. Reconstruction of the Genome-scale Metabolic Model of *Picochlorum renovo*. GEAR: Guided Engineering Apprenticeship in Research.

References and Resources

- Algae farm design report: https://www.nrel.gov/docs/fy16osti/64772.pdf
- NREL algae farm TEA Excel tool: https://www.nrel.gov/extranet/biorefinery/aspen-models/ (first set of files)
- NREL 2014 CAP design report: https://www.nrel.gov/docs/fy14osti/62368.pdf
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- NREL 2015 SOT milestone: R. Davis, J. Markham, C. Kinchin, E. Tan, "2015 State of Technology Update." Internal BETO milestone report, Sept 30 2015 (rev3 re-issued Nov 24 2015)
- Dong et al. CAP publication (public reference that reflects key SOT parameters for CAP): T. Dong et al., "Combined algae processing: A novel integrated biorefinery process to produce algal biofuels and bioproducts." *Algal Research* 19 (2016): 316-323.
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- USDA fertilizer pricing data: https://quickstats.nass.usda.gov/