DOE Bioenergy Technologies Office (BETO) 2021 Project Peer Review

A comprehensive strategy for stable, high productivity cultivation of microalgae with controllable biomass composition

03/22/2021
Advanced Algal Systems

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University of Toledo

THE UNIVERSITY OF TOLEDO

MONTANA STATE UNIVERSITY
Mountains & Minds

NC STATE UNIVERSITY

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

Project Overview

Context: This project builds upon previous collaborative work of project participants for isolating and characterizing high productivity alkaliphilic algal strains.

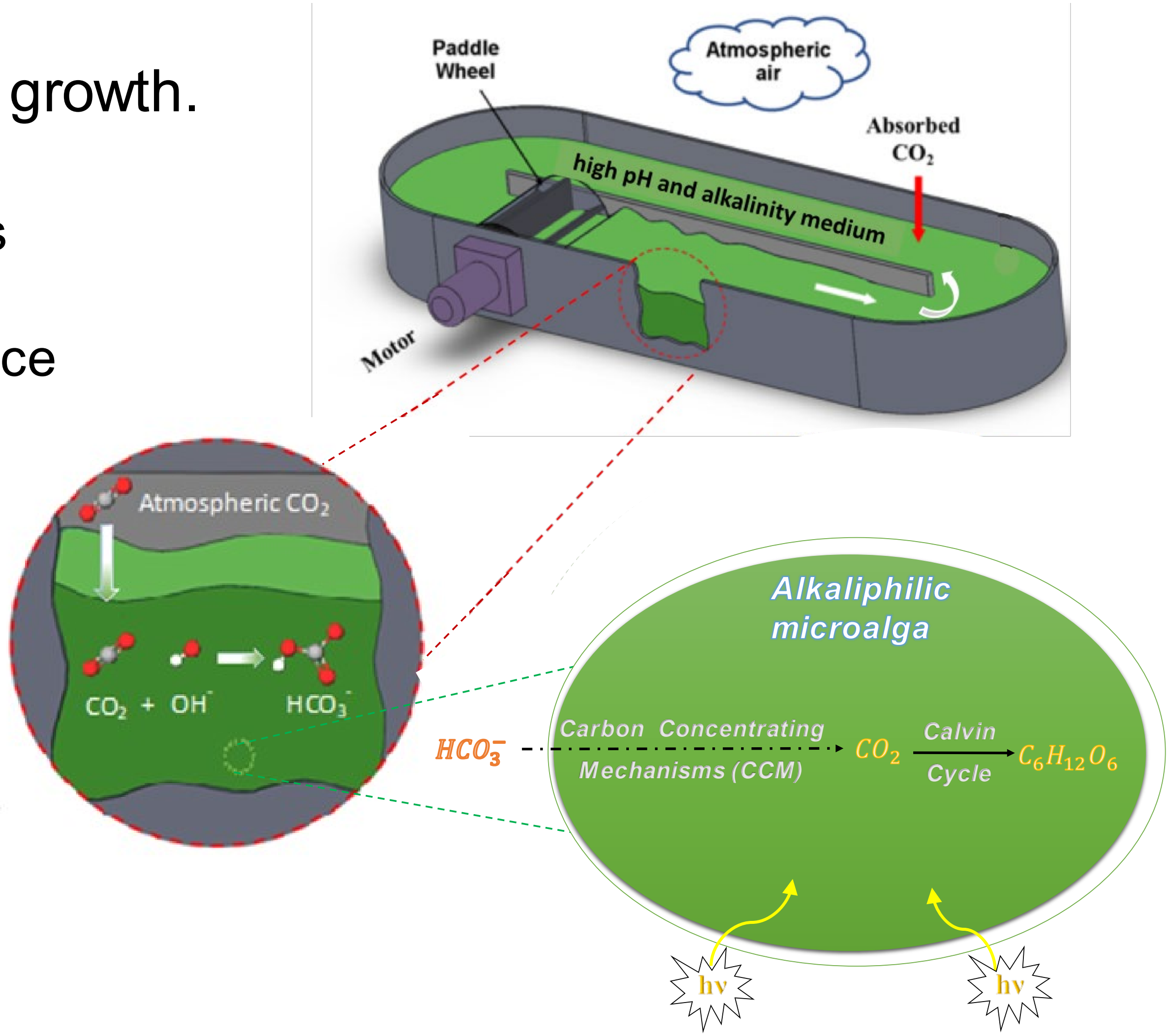
Overall Goal: Develop cultivation approaches that use high-pH and high-alkalinity media for

(1) high rates of atmospheric CO_2 capture and

(2) providing non-limiting HCO_3^- concentrations for growth.

Specific Objectives:

1. Improve scale and productivity of alkaliphilic cultures cultivated in high-pH and high-alkalinity media.
   - Establish seasonal productivities and the influence of scale-up
2. Improve biomass composition
   - Media and cultivation conditions optimization for higher carbohydrate and lipid content
3. Develop molecular biology toolkits
   - Novel gene editing, microbial ecology and metabolic flux modeling tools
   - Not yet available for diverse microalgae
   - Technically risky, but have the potential to significantly improve culture performance



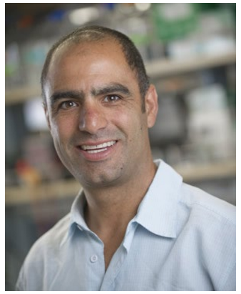
1 - Management

- Team

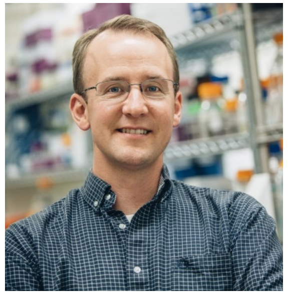
Sridhar Viamajala
Cultivation and scale-up

Robin Gerlach
C and nutrient management

Matthew Fields
Microbial ecology



Blake Wiedenheft
Gene editing



Ross Carlson
Metabolic flux modeling

Brent Peyton
Cultivation and scale-up

Greg Characklis
TEA and LCA

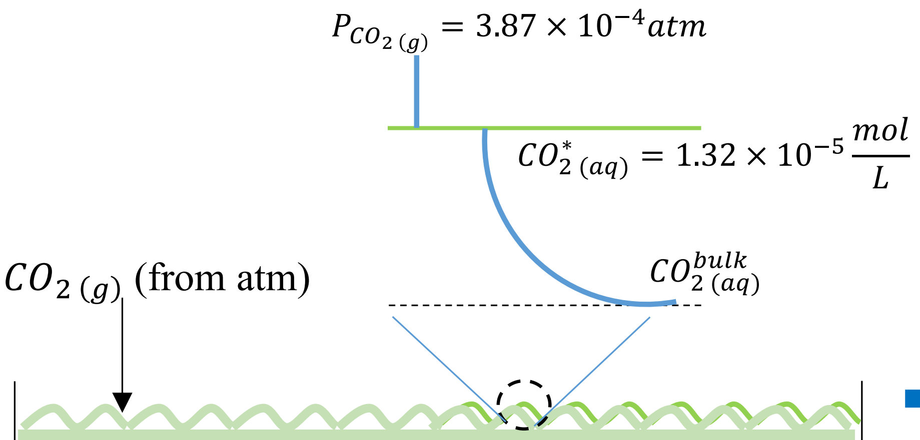
Jordan Kern
TEA and LCA

- Risk identification/mitigation via team interactions and external collaborations
  - Biweekly team conference calls, annual team meetings at ABS, numerous phone/email conversations
  - Cross-disciplinary discussions on technical challenges and potential solution approaches
  - Sample analyses and culture(s) maintenance at multiple locations
  - Critical experiments repeated at multiple locations
  - Team is well connected with the algal biofuels community
    - Numerous ad hoc conversations with industry and other algal researchers on specific technical issues
- Formal external collaborations
  - PNNL-EMSL via Facilities Integrating Collaborations for User Science (FICUS) project
  - Joint Genome Institute (JGI) via the Community Sequencing Program (CSP)
  - LANL (Shawn Starkenburg) through CSP supplemental funding

~6 month-delays experienced due to CoVID-19 pandemic

2 - Approach

Facilitating high rates of atmospheric CO_2 capture



Mass transfer flux:

$$\boldsymbol{J_{CO_2} = k_L \cdot E \cdot ([CO^*_{2\,(aq)}] - [CO^{bulk}_{2\,(aq)}])}$$

Abiotic reactions:

(1) $CO_{2,\,dissolved} + OH^- \rightleftharpoons HCO^-_{3,\,media}$;

$$K_{Eq,1} = 4.5 \times 10^7 \frac{L}{mol}$$

(2) $HCO^-_{3,\,media} + OH^- \rightleftharpoons CO^{2-}_{3,\,media} + H_2O$;

$$K_{Eq,2} = 4.9 \times 10^3 \frac{L}{mol}$$

Biotic reaction:

(3) $HCO_3^- \rightarrow CO_{2\,fixed} + OH^-$

$\boldsymbol{J_{CO_2}}$ = CO_2 transfer flux (mol/m²/h)

$\boldsymbol{[CO^*_{2(aq)}]}$ = Dissolved CO_2 concentration in equilibrium with the atmosphere; calculated from Henry's constant.

$\boldsymbol{[CO^{bulk}_{2(aq)}]}$ = Aqueous CO_2 concentration; determined by the equilibrium established with HCO_3^-, OH^- and CO_3^{2-} in the medium (Eq. 1 & 2)

$$= \frac{K_2}{K_1} \times \frac{[HCO_3^-]^2}{[CO_3^{2-}]}$$

$\boldsymbol{k_L}$ = Mass transfer coefficient; governed by mixing rates and pond depth

= 0.1 m/h for 20 cm ponds mixed at 30 cm/s

$\boldsymbol{E}$ = Enhancement factor for mass transfer due to chemical reaction;

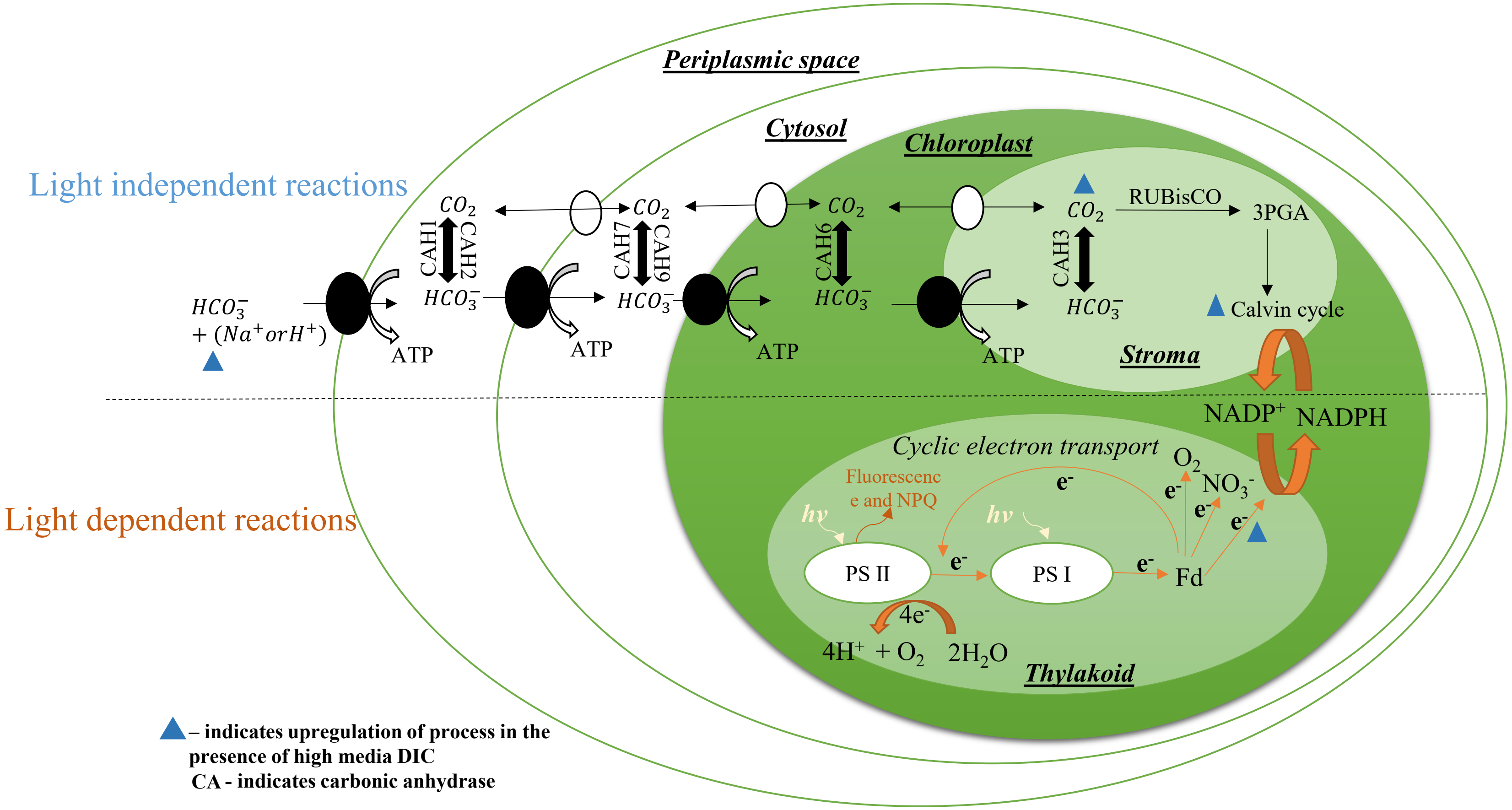
$$= 1 + \frac{\mathcal{D}_{OH^-} \cdot \mathcal{D}_{HCO_3^-} \cdot K_1 \cdot [OH^-]}{\mathcal{D}_{CO_2}(K_1 \cdot [CO^*_{2\,(aq)}] \cdot \mathcal{D}_{HCO_3^-} + \mathcal{D}_{OH^-})}$$

where, the subscripted $\mathcal{D}$'s represent diffusion coefficients of the various dissolved species

Danckwerts, P.V., (1970) *Gas-liquid reactions*. McGraw-Hill Book Co.
Weissman, J.C., et al. (1988). Photobioreactor design: Mixing, carbon utilization, and oxygen accumulation. *Biotech. Bioeng.* ***31****: 336-344.*

2 - Approach

Maintaining non-limiting HCO_3^- concentrations in media for photosynthesis



High media alkalinity increases availability of HCO_3^-

- Under highly alkaline conditions, DIC is transported by CCMs
- High media DIC increases rate of cellular DIC transport
- Simultaneously, the high cellular DIC flux allows light dependent reactions towards higher production of NADPH for use in carbon fixation.

Moroney, J. V. and Ynalvez, R. A. (2007) Proposed CO_2 concentrating mechanism *in Chlamydomonas reinhardtii. Eukaryotic Cell.* 6: 1251-1259.
Vadlamani, A. et al. (2019). High Productivity Cultivation of Microalgae without Concentrated CO_2 Input. *ACS Sustainable Chemistry & Engineering,* **7**: *1933-1943.* **DOI**: 10.1021/acssuschemeng.8b04094

2 - Approach

- Project approach is guided by TEA and LCA
  - Algae production costs can be lowered by ~25% if the approach is successful
  - Prevention of culture crashes using our approach improves average annual productivity
  - Our approach allows sustainable use of low-quality land and water
- Successful application of molecular toolkits could further improve productivity, composition and robustness, but is technically challenging
- Metrics
  - GGE/acre/yr – biofuel productivity by CAP method
    - Fall season –

      (i) Baseline – 1100, (ii) Intermediate/Current – 1500, End of project – 1900
  - GGE/ton – biomass quality by CAP method
    - Fall season –

      (i) Baseline – 53, (ii) Current – 65, End of project – 80
- Go/No-Go decision points
  - GNG 2: Demonstrate the potential for production of >1200 GGE/acre/year (Q7)
    - Successfully completed milestone verification

3 – Impact

- When successful, the project will
  - De-couple microalgae biofuels production from CO_2 sources and significantly expand possible geographical locations for cultivation
  - Decrease the cost of microalgae cultivation
  - Develop diverse toolkits for broad use by the microalgae community
    - Algae community analysis/dynamics – *To assess and control the structure of stable microbial communities that contribute to productivity*
    - Transcriptomic and metabolomic analysis – *To map and ultimately control the responses of microalgae cultures to cultivation process inputs*
    - Metabolic network model – *To predict genome editing targets in-silico*
    - CRISPR/Cas9-based genome editing – *To improve carbon flow to biofuel and bioproduct precursors*
- Directly supports BETO's goals
  - ***Increase the mature modeled value of cultivated algal biomass*** by 30% over the 2015 SOT baseline.
  - Develop ***strain improvement toolkits*** that enable algae biomass compositions in environmental simulation cultivation conditions that represent an energy content and convertibility of 80 GGE of advanced biofuel per AFDW ton of algae biomass.
- US 10,457,909 B2 awarded October 29, 2019.
- 2 peer-reviewed articles published, several others in various stages of preparation.

4 – Progress and Outcomes

Task 1 - Productivity and composition improvements through improvements in cultivation methods

Milestone	1.1.1	Establish baseline cultivation productivity.	Q3
Go/No-go decision point	Go/No-Go Decision Point #2	Demonstrate the potential for production of >1200 GGE/acre/year	Q7
Milestone	1.1.2	Obtain multi season phototrophic cultivation productivity data (ongoing)	Q8

Season	Vol.	Area, m^2	AFDW prod., $g/m^2/d$	Protein, wt%	Carb, wt%	Lipids, wt%	GGE/ac/yr, CAP	GGE per ton	Days
Fall	218 L	0.91	17.1	42.7	23.1	10.3	1400	60	32
	1100 L	4.2	9.62	15.8	46.4	18.4	1430	100	10
	1100 L	4.2	16.4	30.1	27.4	23.9	2370	100	12

Season	AFDW prod., $g/m^2/day$
Fall	18.9
	15.4
	14.6
Winter	4.6
	8.8
	7.9
Spring	13.6
Summer	34.7
	35.9
	22.6

Productivities in 0.2 m^2 raceway cultivations

4 – Progress and Outcomes

Task 2 - Development of a process model describing inorganic carbon mass transfer in high alkalinity cultivation media

Milestone	2.1.1	Develop and validate baseline isothermal model for CO_2 mass transfer in alkaline media	Q2
Milestone	2.2.1	Develop and validate comprehensive CO_2 mass transfer model for non-isothermal conditions in the presence of a rate promoter (i.e. borate)	Q6

- Use the total alkalinity concentration to solve $[HCO_3^-]$ and $[CO_3^{2-}]$ in terms of [OH-]

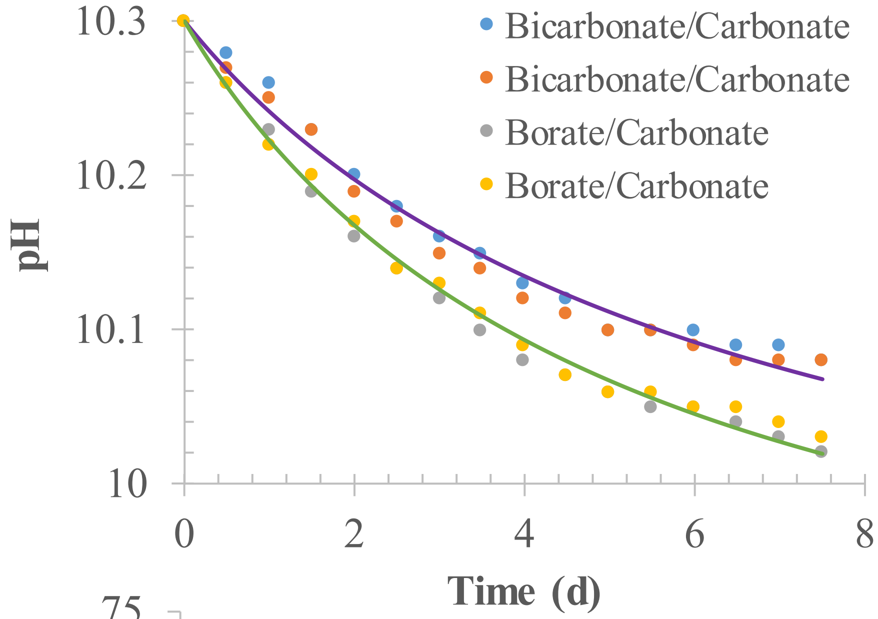
$$TA = [HCO_3^-] + 2[CO_3^{2-}] + [OH^-] - [H^+]$$

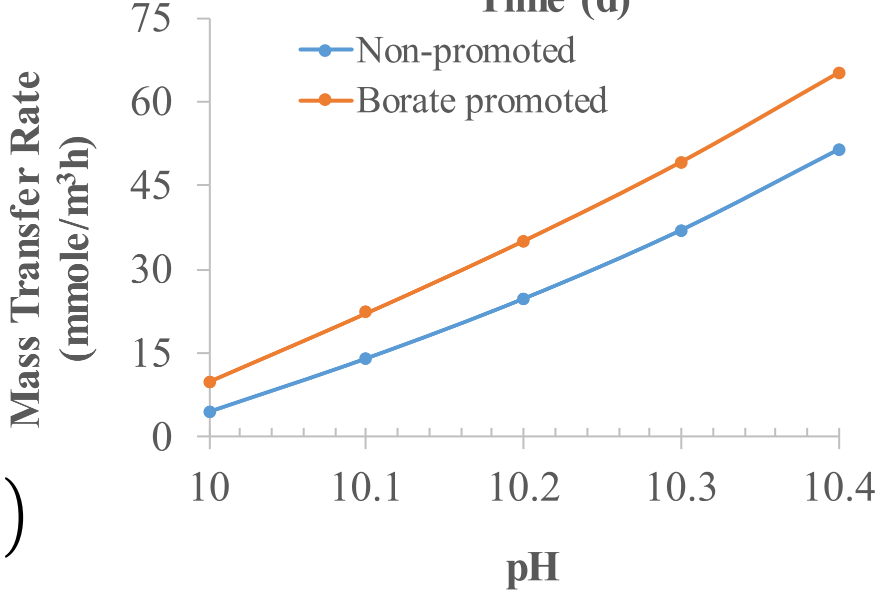
- Substitute into DIC equation

$$DIC = [HCO_3^-] + [CO_3^{2-}] + [CO_2]$$

- Differentiate DIC with respect to time; assume all DIC change comes from CO_2 uptake; Set equal to CO_2 mass transfer equation

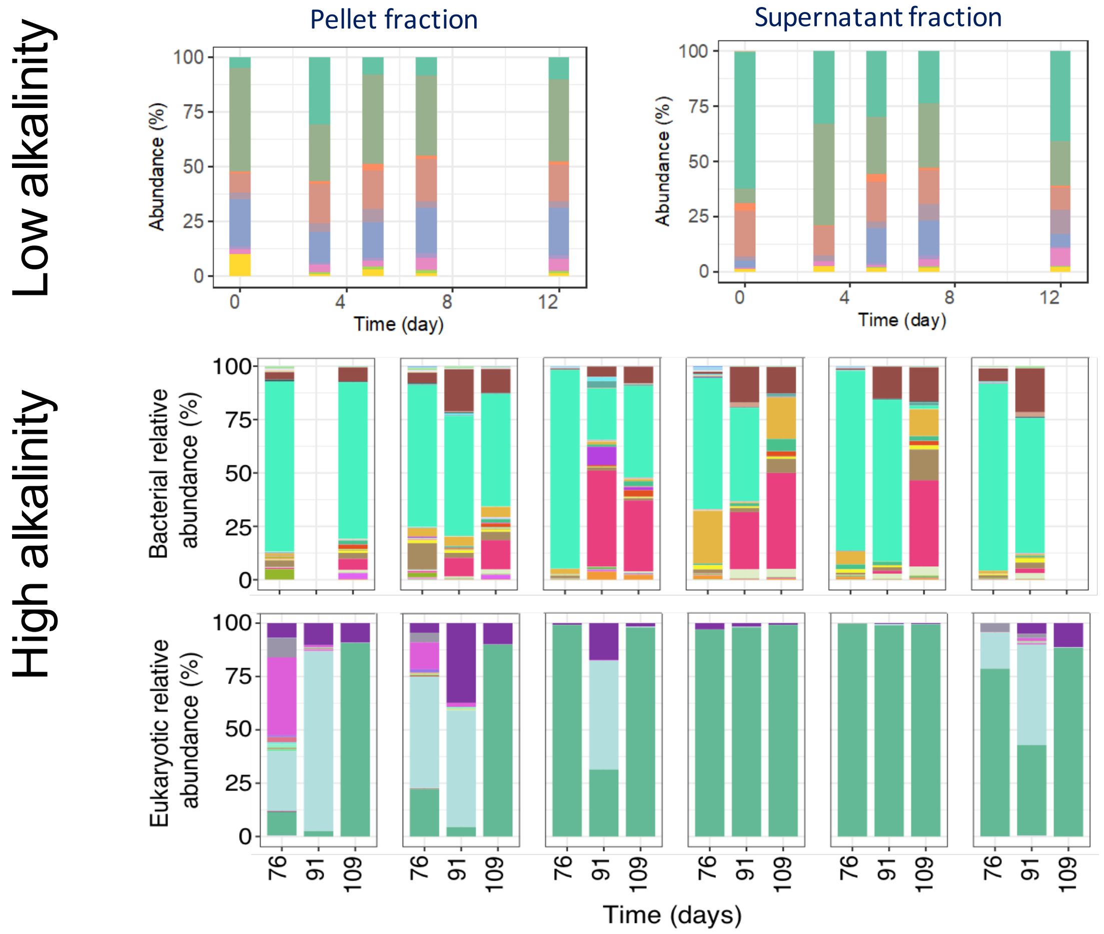
$$\frac{d[CO_2]}{dt} = \frac{dDIC}{dt} = \frac{d\,([HCO_3^-] + [CO_3^{2-}])}{dt} = k_L a \cdot E_1 \cdot \left([CO^*_{2\,(aq)}] - [CO_{2\ bulk\ (aq)}]\right)$$





4 – Progress and Outcomes

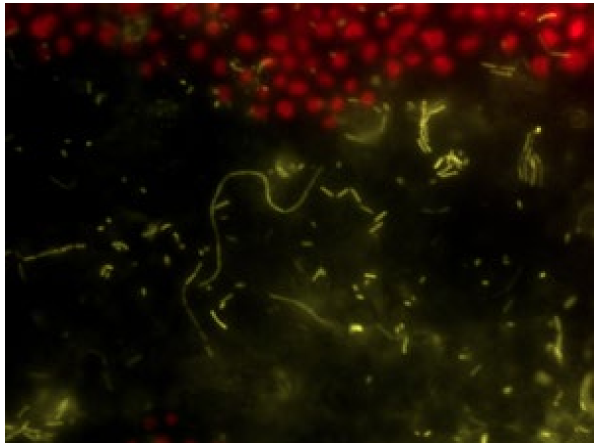
Task 3 - Understanding algal community dynamics for increased culture stability, productivity, and enhanced biomass collection in alkaliphilic algal production systems



Milestone 3.1.1 Identify microbial populations in SLA-04 cultures – Q6

Milestone 3.2.1: Determine active microbial populations that develop in the outdoor SLA-04 cultures. – Q9

Labeling of active bacterial members using BONCAT

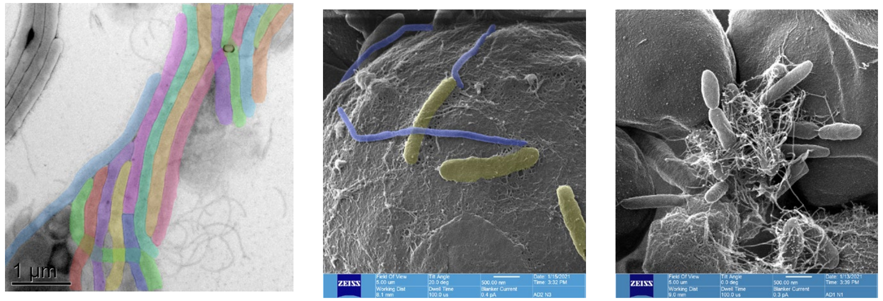


Challenges:

- Algal cells have low permeability for the BONCAT dye.
- Chlorophyll autofluorescence interferes with detection of Raman signal.

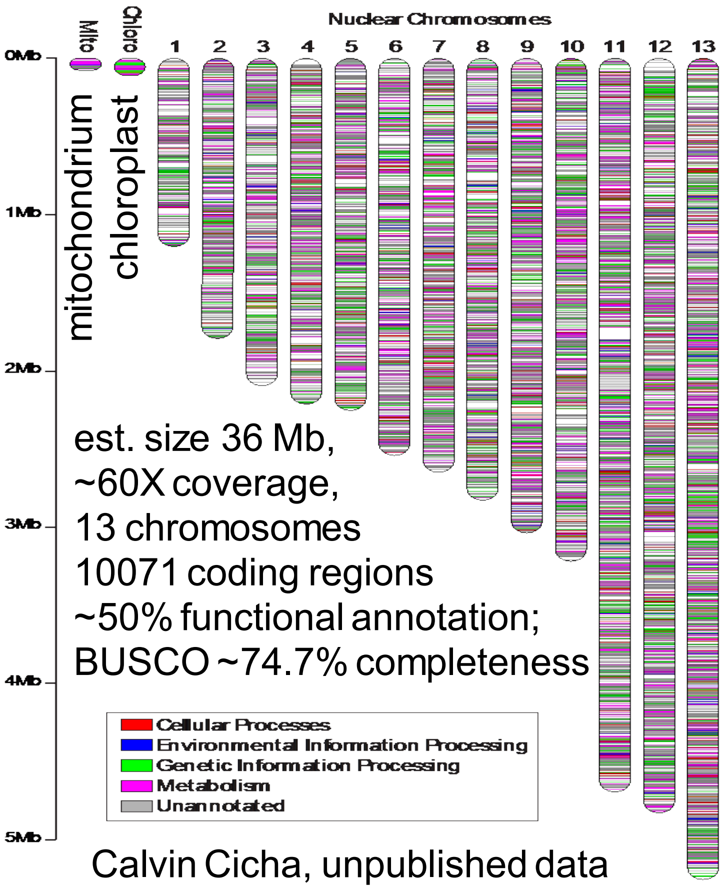
Have > 19 phylogenetically characterized, bacterial isolates from SLA-04 cultures.

Community and activity data will support metabolic model development (Task 5)



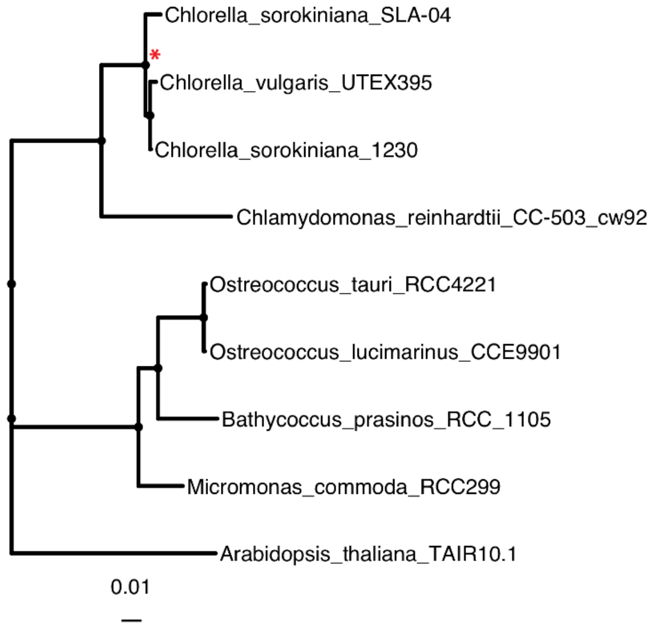
4 – Progress and Outcomes

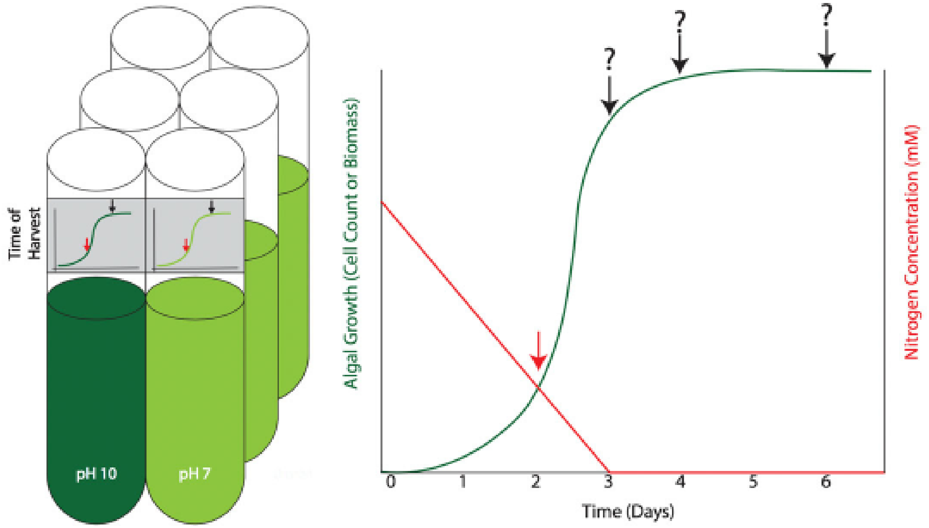
Task 4 - Transcriptomic and metabolomic analysis to map and ultimately control the response of alkaliphilic cultures



est. size 36 Mb,
~60X coverage,
13 chromosomes
10071 coding regions
~50% functional annotation;
BUSCO ~74.7% completeness

Calvin Cicha, unpublished data



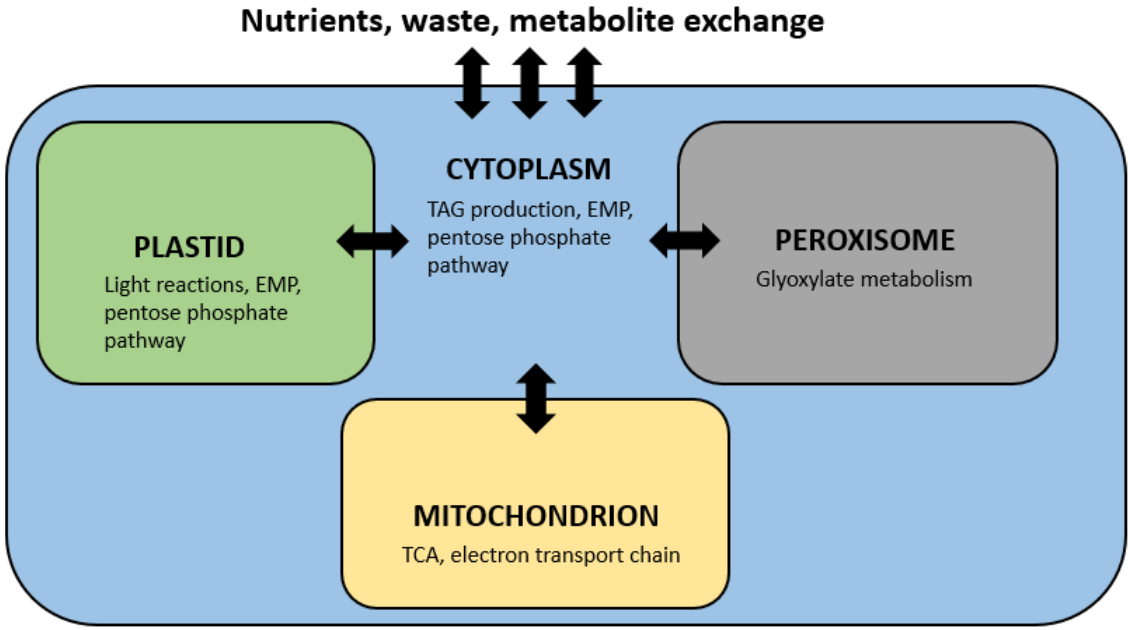


Milestone 4.1.1. & 4.1.2: Sequenced, annotated and improved (through transcriptomic work) SLA-04 genome assembly. – Q9

Working towards M 4.2.1 (Q10): Genes expressed in high-productivity populations & M 4.2.2 (Q12): Transcriptome at optimal conditions.
Additional transcriptome data will improve metabolic model (Task 5) and will predict genome editing targets (Task 6)

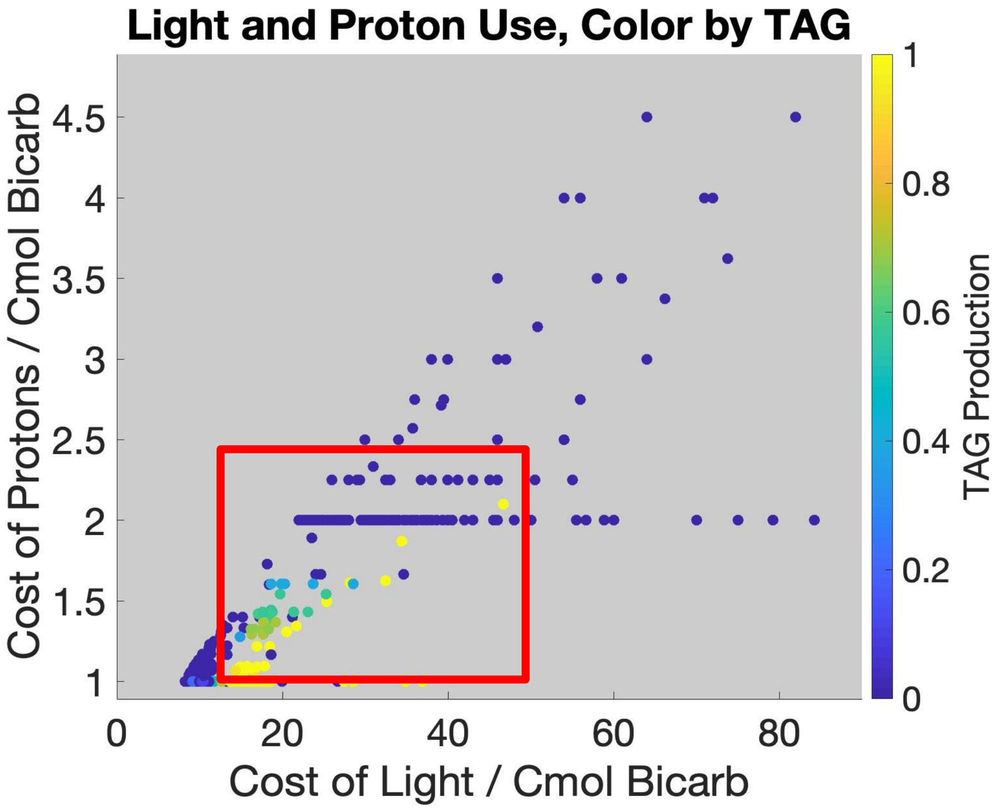
4 – Progress and Outcomes

Task 5 - Developing a metabolic network model for SLA-04 to predict cultivation improvements and genome editing targets in-silico



1 succinate_m + 1 oxQ_m = 1 fumarate_m + 1 redQ_m

1 a-D-glucose-6-phosphate_c = 1 D-fructose-6-phosphate_c



Milestone 5.1.1: In silico reconstruction of SLA-04 metabolic potential with partitioning of activity between cytosol, mitochondria and plastids. - Q8

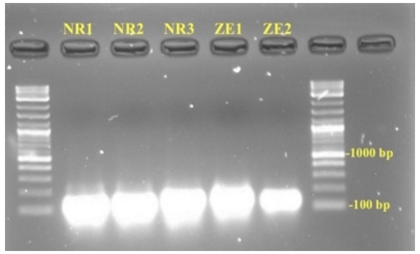
Each reaction is assigned to a compartment using subscripts. The subscript "m" denotes the mitochondria; the subscript "c" denotes the cytosol.

Elementary flux mode analysis is being used to predict preferred electron sinks under combined light and pH stress.

Here, the model predicts that TAGs are preferably produced at high pH (low cost of protons) and low (cost of) light

4 – Progress and Outcomes

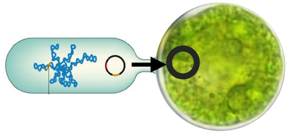
Task 6 - CRISPR/Cas9-based genome editing of strain SLA-04 for productivity enhancement (highest risk task of project)



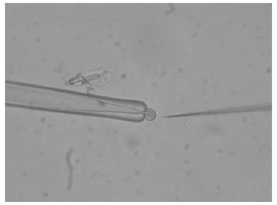
Milestone 6.1: Identify conserved gene sequences in *Chlorella*, and design RNA guides for targeted mutations using Cas9 – Q5

Milestone 6.2: Express and purify Cas9 proteins bound to RNA guides. Optimize transformation of *Chlorella sorokiniana* strain SLA-04

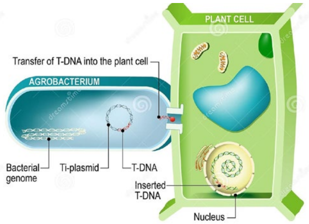
Transformation of SLA-04 is challenging (confirmed by Starkenburg Lab at LANL)



E. coli conjugation



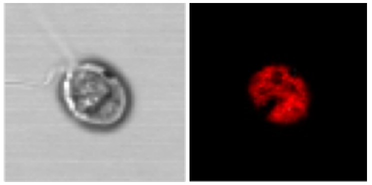
Direct injection



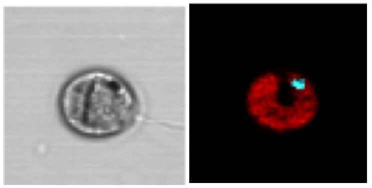
Agrobacterium infection

Electroporation

Success in nucleus transformation of *Chlamydomonas reinhardtii*



C. reinhardtii



C. reinhardtii + plasmid

In Progress: Genome editing targets being predicted through transcriptomic/metabolomic work (Task 4) and metabolic modeling (Task 5)

End of Project Goal: Isolate one or more isogenic mutants and test for novel phenotypes.

4 – Progress and Outcomes

Task 7 - Process economics and LCA – feedstock production through biofuel intermediate

Increased carbon capture

(milestone 7.1.1—in progress)

- Evaluated enhanced carbon uptake technology scenarios via LCA/TEA

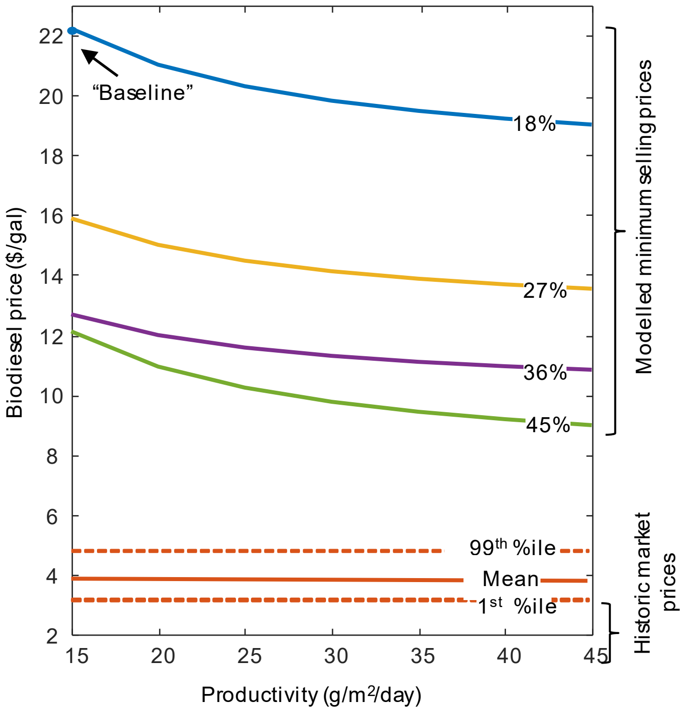


Figure 1 *The effect of increasing mixing energy to boost productivity on minimum biodiesel selling price; the lipid contents at 18% (baseline), 27%, 36%, and 45% (very optimistic) are shown, as are the historic biodiesel market prices at the mean and 1st/99th percentiles*

Biorefinery Financial Risk Management

- Modelled the financial risk for an algal biorefinery site
- Developed an index-insurance contract to manage both weather- and market-related revenue variability
- Found the lowest cost risk management strategy using both reserves and index-insurance (*Figure 2*)
- Evaluated the effectiveness of the various risk management strategies (*Figure 3*)

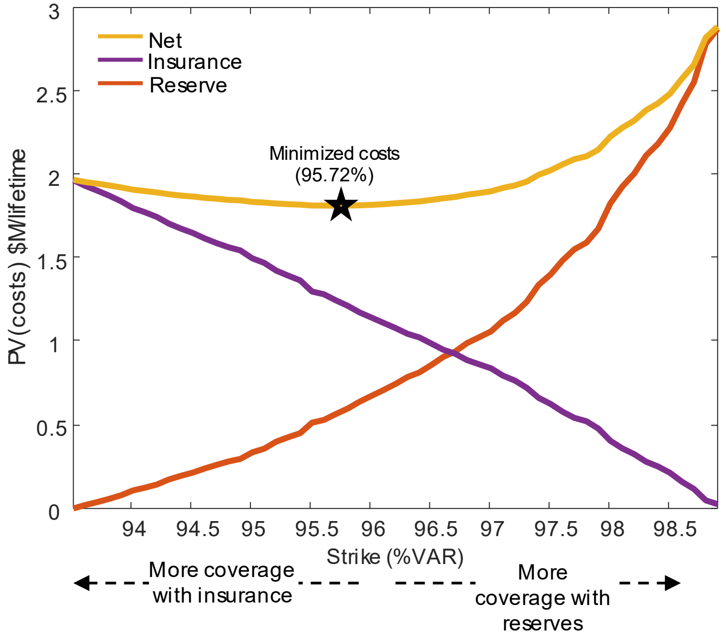


Figure 2 *Optimization of the present value of risk management costs across various strike values, where the lowest net cost, which corresponds to the 95.72% VAR, is starred.*

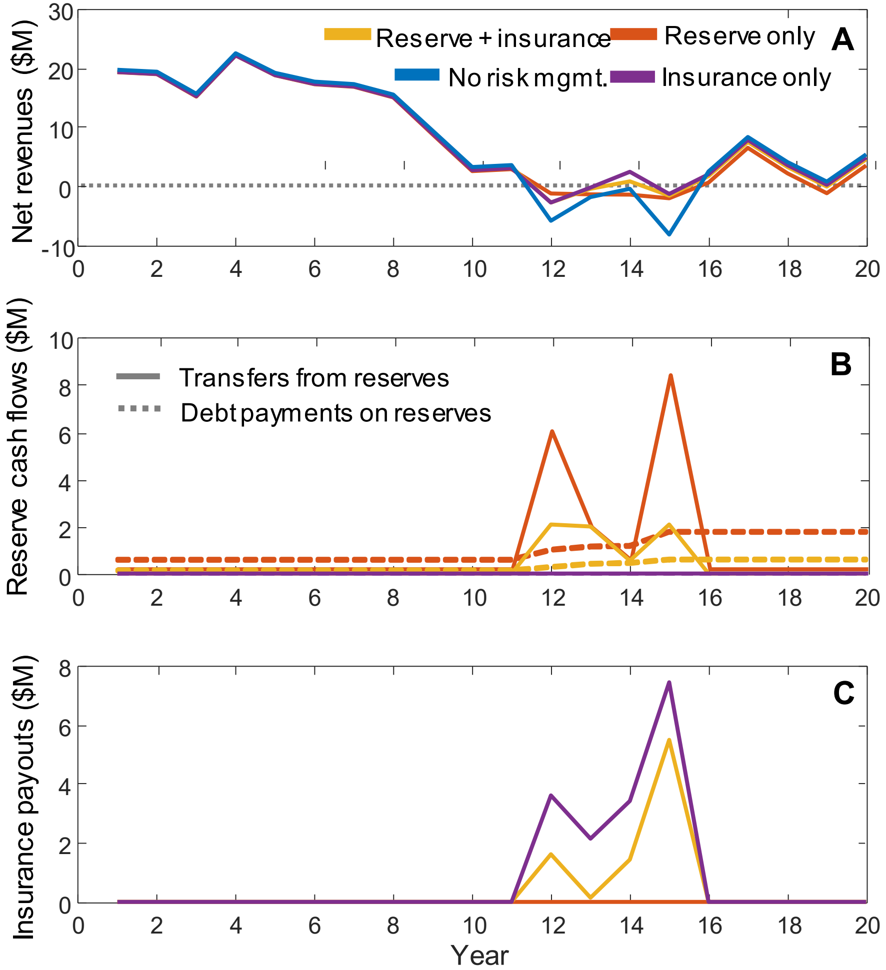


Figure 3 *Simulated yearly (A) net revenues, (B) reserves cash flows, and (C) insurance payouts for a single model realization with low net revenues*

Kleiman, R. M., Characklis, G. W., Kern, J. D., & Gerlach, R. (in revision). "Characterizing Weather-Related Biophysical and Financial Risk in Algal Biofuel Production," Applied *Energy (in revision)*

Kleiman, R. M., Characklis, G. W., & Kern, J. D. "Managing Weather-Related Financial Risk in Algal Biofuel Production," *Bioresource Technology* (in preparation)

Summary

- High media pH (>10) drives rapid transfer of CO_2 from the atmosphere to growth media.
- Borate is an effective rate promoter for CO_2 mass transfer from the atmosphere into alkaline media.
- Under high-pH AND high-alkalinity conditions, cultures achieve high productivity even in the absence of concentrated CO_2 inputs.
- Biomass composition can be improved by “adjusting” nutrient composition without significantly compromising biomass productivity
- Increasing mixing rates for higher mass transfer may not negatively impact fuel price under high productivity scenarios
- Sequenced, annotated and improved (through transcriptomic work) SLA-04 genome assembly
- In silico reconstruction of SLA-04 metabolic pathways is in progress and is being used to predict gene editing targets in conjunction with transcriptomic/metabolomic work.
- BONCAT labeling is being developed to identify active populations in the microbiome under various growth conditions

Quad Chart Overview

Timeline
- 09/30/2017
- 09/29/2021

	FY20 Costed	Total Award
DOE Funding	(10/01/2019 – 9/30/2020) *$ 304,599*	(negotiated total federal share) *$ 2,397,698*
Project Cost Share	$ 58,733	$ 498,978

Project Partners*
- Montana State University
- University of North Carolina at Chapel Hill
- North Carolina State University

*Only fill out if applicable.

Project Goal
Develop high productivity algal biofuel systems that are not constrained by CO_2 costs or availability of concentrated CO_2

End of Project Milestone
18 g/m^2/d AFDW over a 4 week cultivation period in 4.2 m^2 outdoor ponds without CO_2 sparging or pH control.

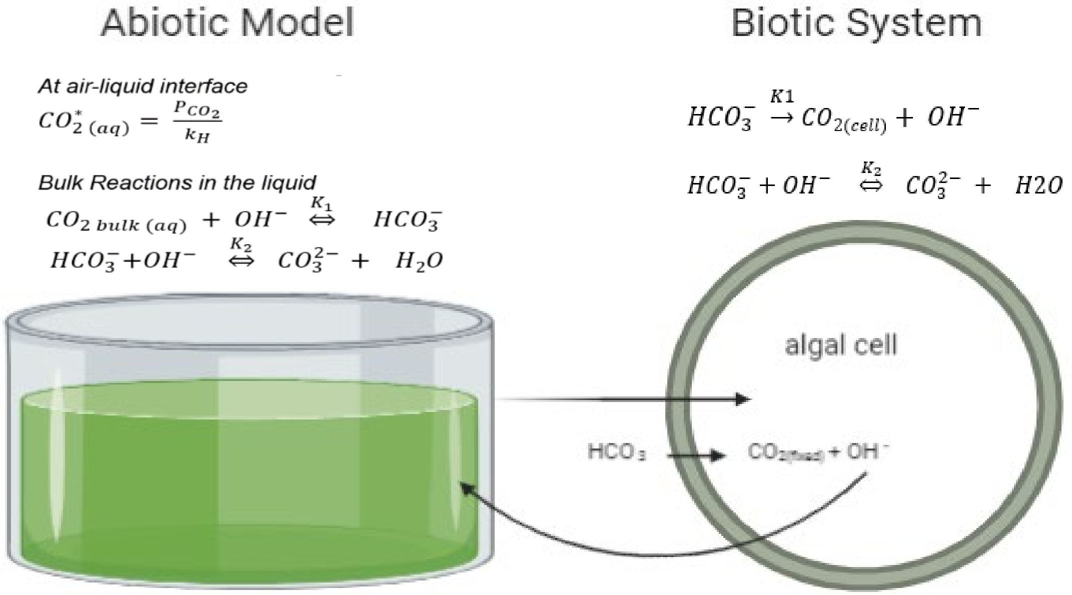
Funding Mechanism
DE-FOA-0001628 – Performance Enhanced Algae Toolkits (PEAK)

Additional Slides

Responses to Previous Reviewers' Comments

- <u>Comment 1</u>: *"… cultivation experiments are tightly connected to the tool development …"*
  - <u>Response</u>: We are actively using microbial ecology toolkits to assess culture microbiome composition from cultivation experiments

- <u>Comment 2</u>: "*It is critical that the biology impact is considered when refining models for CO_2 exchange with the pond media.*"
  - <u>Response</u>: We have developed a first-principles mathematical model of the mass transfer process and correlated model predictions with experimental data to estimate mass transfer rates. We are currently integrating the model with algae growth models to describe mass transfer during cultivation. We are using a close-coupled approach to simultaneously solve the biotic and abiotic models as shown below.



Publications, Patents, Presentations, and Commercialization

Commercialization: Synergia Biotech Inc. is commercializing a phycocyanin product from algae using the high pH/high alkalinity approach developed by our team.

Patents

- Pendyala B, Vadlamani A, Viamajala S, Varanasi S. 2016. High yield algal biomass production without concentrated CO2 supply under open pond conditions. US 10,457,909 B2 awarded October 29, 2019.

Publications:

- Corredor L, Barnhart EP, Parker AE, Gerlach R, Fields MW. 2021. "Effect of temperature, nitrate concentration, pH and bicarbonate addition on biomass and lipid accumulation in the sporulating green alga PW95", Algal Research, 53: 102148. DOI: 10.1016/j.algal.2020.102148
- Pendyala B, Hanifzadeh M, Abel GA, Viamajala S, Varanasi S. 2020. "Production of Organic Acids via Autofermentation of Microalgae: A Promising Approach for Sustainable Algal Biorefineries", Industrial & Engineering Chemistry Research, 59:1772-1780. DOI: 10.1021/acs.iecr.9b05493.
- Kleiman, R., Characklis, G. W., Kern, J. D. and R. Gerlach. "Characterizing Weather-related Biophysical and Financial Risk in Algal Biofuel Production," *Applied Energy* (in revision)
- Kleiman, R., Characklis, G. W. and J. D. Kern, J. D. "Managing Weather-related Financial Risk in Algal Biofuel Production," *Bioresource Technology* (in preparation)
- Bui, Huyen; Miller, Isaac; Fields, Matthew W.; Gerlach, Robin. Ecological Engineering of Industrial Algal Cultures. Algal Research. In preparation
- Lu, Shipeng; Bui, Huyen; Moll, Karen; Gardner, Robert D.; Fields, Matthew W.; Gerlach, Robin. Transcriptional changes underlining metabolic switches in Chlorella vulgaris UTEX 395 cultivated under nitrogen depletion with bicarbonate amendment. Algal Research. In preparation

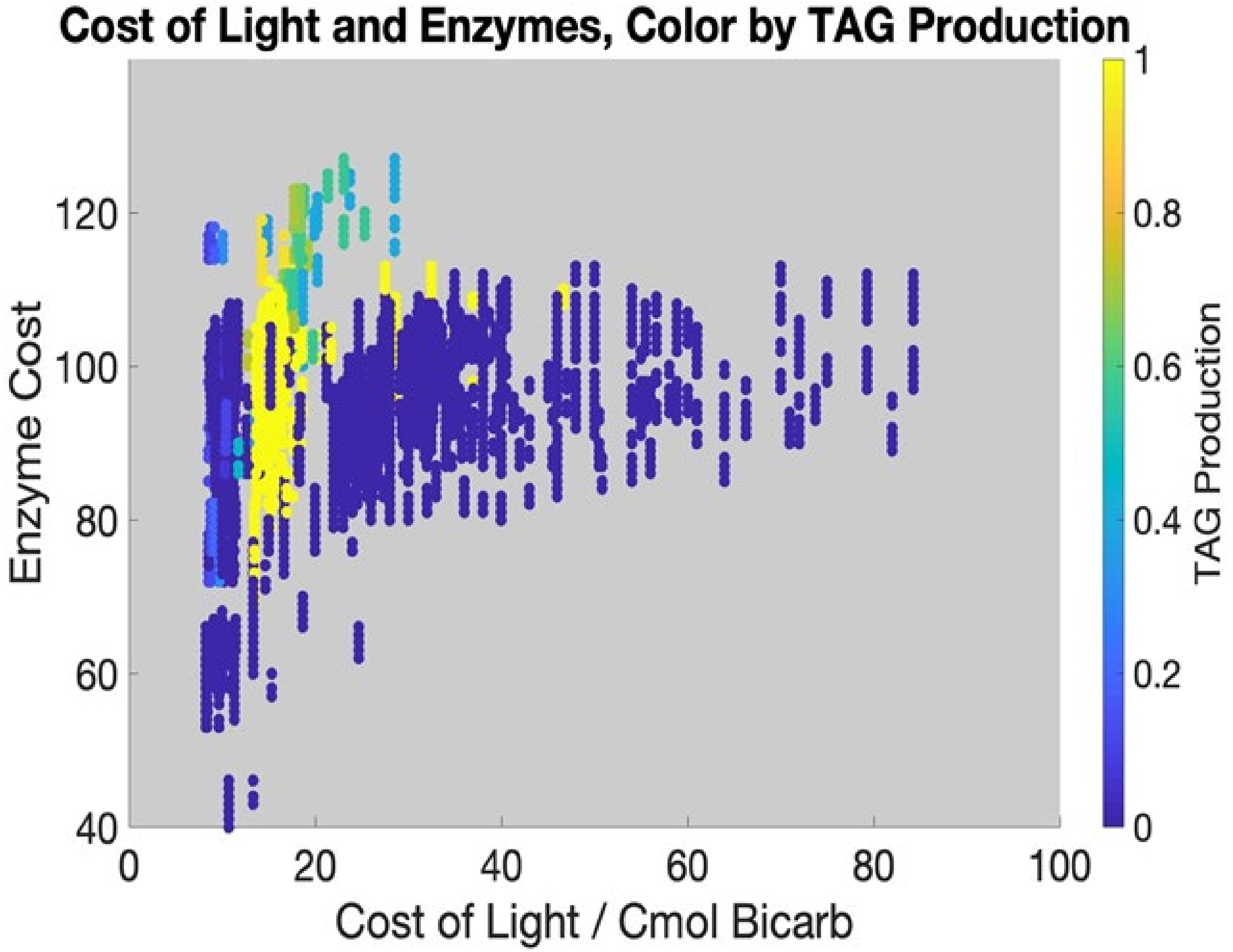
Presentations:

- Kleiman, R., Kern, J. D., Gerlach, R. and G. W. Characklis. "Managing Weather- and Market-Related Financial Risk in Algae Production for Biofuel and Co-products," Fall Meeting of the American Geophysical Union, New Orleans, LA, virtual, December 2020.
- Nowzaridalini N, Maddi B, Viamajala S. "Phosphorus Utilization in Phototrophic Cultivation of the Alkaliphilic Chlorella sorokiniana SLA 04." Poster presentation at the 2020 Algae Biomass Summit, Virtual Conference (September 8 – October 2, 2020).
- Kleiman R, Characklis G, Kern J, Gerlach R, Viamajala S. "Characterizing and managing weather-based uncertainty to algal biofuel production." Platform presentation at the 2019 Algae Biomass Summit, Orlando, FL (September 16-19, 2019).
- Vadlamani A, Kolapalli J, Viamajala S, Avila N, Gerlach R. "Development and experimental verification of a mass transfer model to predict dissolution of atmospheric CO2 into alkaline media." Platform presentation at the 2019 Algae Biomass Summit, Orlando, FL (September 16-19, 2019).

4 – Progress and Outcomes

<u>Task 5 Supplemental Info:</u>

- Roughly 300 reactions
  - ~115 cytosol
  - ~50 mitochondria
  - ~80 chloroplast



Elementary flux mode analysis can also be used to analyze the relationship between enzyme use and light stress. Here, it is shown that TAG production is possible with a moderate number of enzymes under a low light cost.

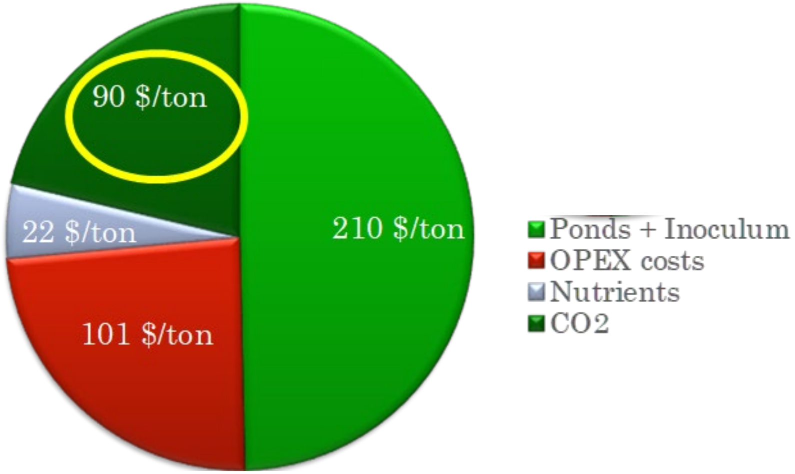
Technology Background

Advantages of our technology

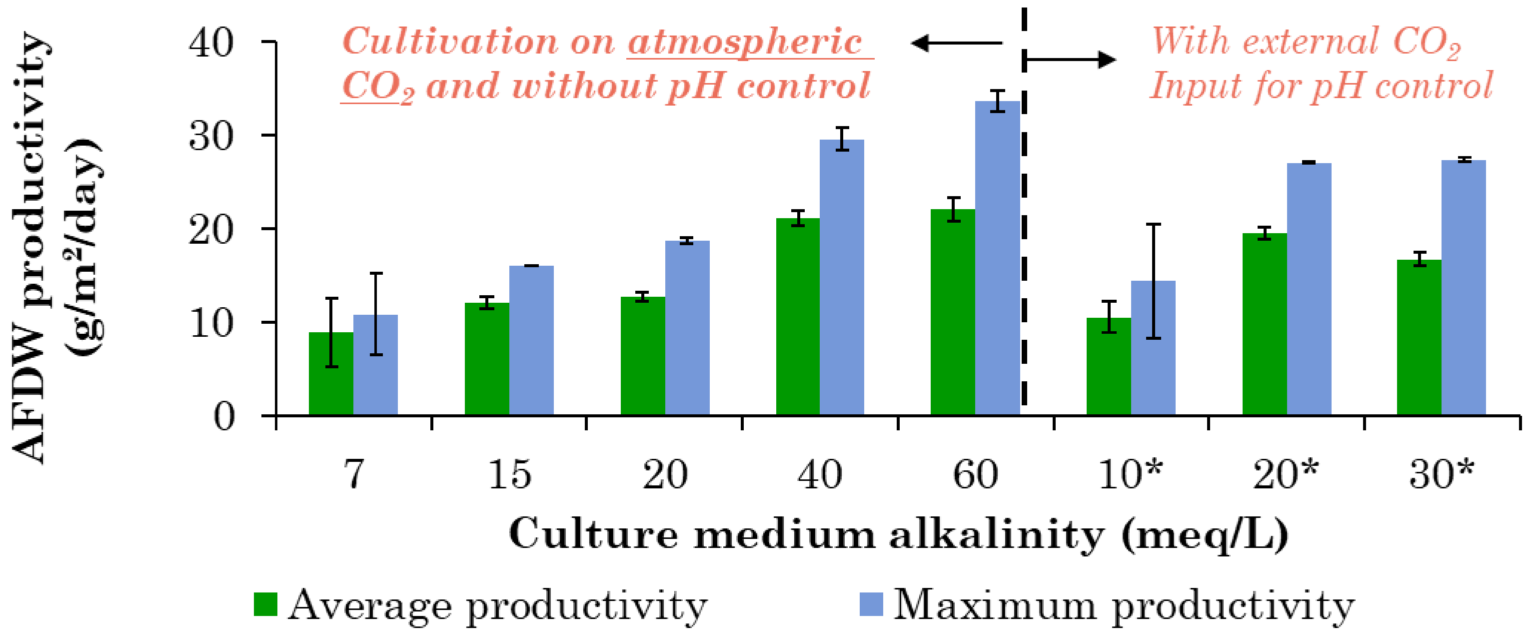
1. Harsh pH conditions (pH>10) can mitigate detrimental microbial contamination and predator populations
2. Alkaline solutions scavenge CO_2 from the atmosphere at rapid rates. Thus, costs and geographical constraints associated with CO_2 supply can be mitigated (or eliminated)

pH = 8.5

pH = 10.2



Technology Background



High media alkalinity improves CO_2 fixation and biomass growth rates due to higher availability of bicarbonate

Energy flow	Description	Notation	High HCO_3^- (65 mM)	Low HCO_3^- (7 mM)
Towards carbon fixation	Effective PS II quantum yield *(photons utilized per incident photons)*	Y(II)	0.37	0.23
	Photosynthetic efficiency *(electrons per photon)*	α	0.16	0.10
	Maximum electron transfer rate *(μmole/m²/s)*	ETR_{max}	20	15
Dissipation	Total regulated + unregulated dissipation *(photons dissipated per incident photon)*	Y(NPQ) + Y(NO)	0.65	0.78
	Maximum quantum yield	F_v/F_m	0.7	0.7

Experimental design and work plan