DOE Bioenergy Technologies Office (BETO) 2019 Project Peer Review

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

03/05/2019

Advanced Algal Systems

Sridhar Viamajala

University of Toledo

Goal Statement

- *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 dissolved inorganic carbon (DIC) concentrations for growth.
- *Outcome:* High biomass and biofuel-precursor productivities in outdoor open ponds using **atmospheric CO_2 alone**.
- *Relevance:*
  - Our project seeks to **eliminate** the cost and site-location constraints posed by supply of **concentrated CO_2** to microalgae farms while simultaneously achieving **high** seasonal **productivities**.
  - Our project will contribute to the development of diverse molecular biology **toolkits** for use by the algal research community
    - Algae community analysis/dynamics – *To assess the development and structure of stable microbial communities that contribute to productivity*
    - Transcriptomic and metabolomic analysis – *To map and ultimately control the responses of microalgae cultures*
    - Metabolic network model – *To predict genome editing targets in-silico*
    - CRISPR/Cas9-based genome editing – *To improve carbon flow to biofuel and bioproduct precursors*

Quad Chart Overview

Timeline

- Start date: 09/30/2017
- End date: 09/29/2021
- Percent complete - **5%**

	FY 18 Costs	Total Planned Funding (FY 19-Project End Date)
DOE Funded	$ 30,400	$ 2,866,276
Project Cost Share*	$ 2,100	$ 496,878

•**Partners:** **Montana State University (44%); University of North Carolina at Chapel Hill (13%)**

Barriers addressed

Aft-A. Biomass Availability and Cost
Aft-B. Sustainable Algae Production
Aft-C. Biomass Genetics and Development

Objective

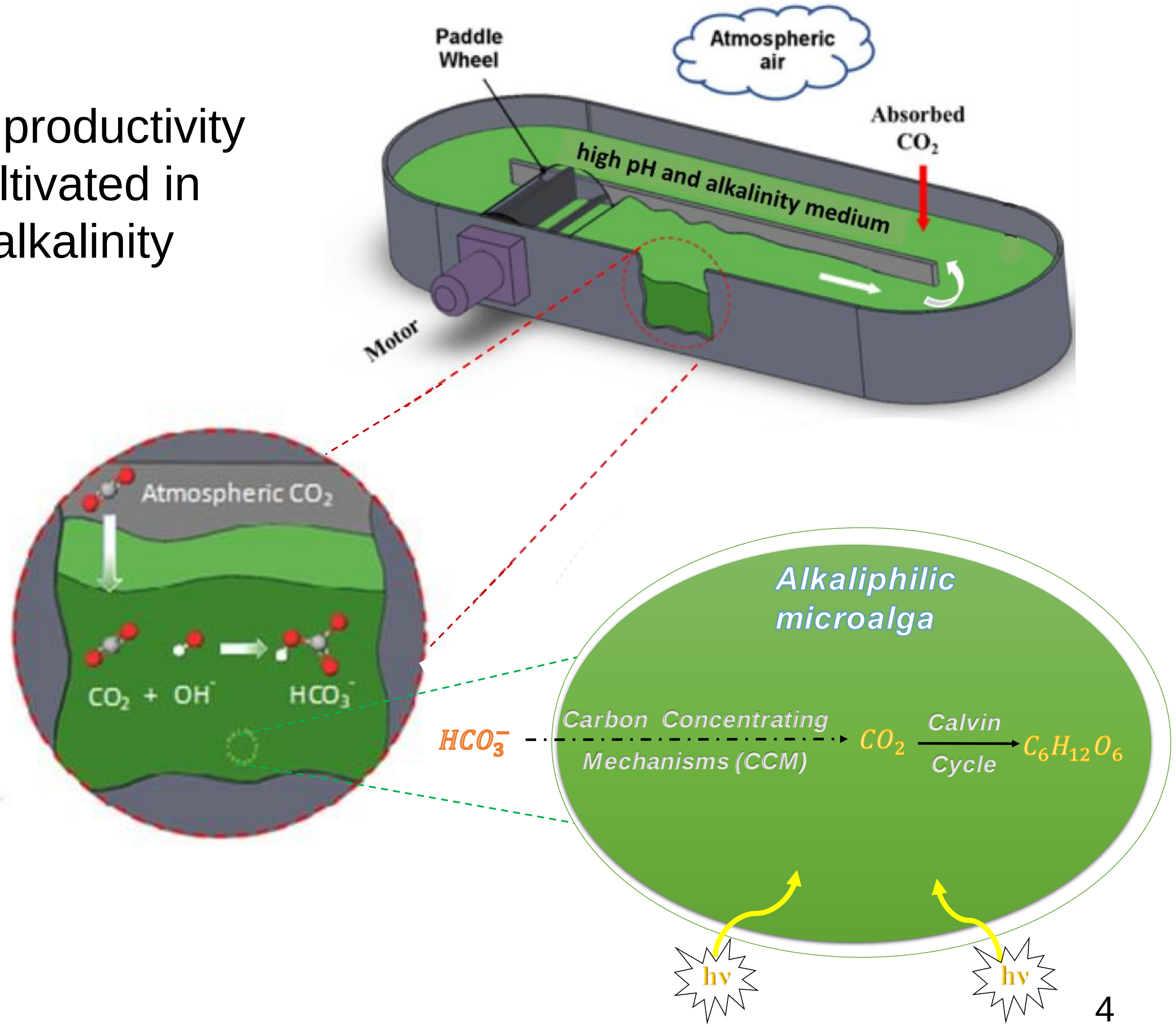
Develop high productivity algal biofuel systems that are not constrained by CO_2 costs or availability of concentrated CO_2

End of Project Goal

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.

Project Overview/Objectives

1. Improve scale and productivity of algal cultures cultivated in high-pH and high-alkalinity media.
2. Improve biomass composition for improved biofuel productivity
3. Develop molecular biology toolkits



Advantages

- <u>Advantage 1</u>: Harsh pH conditions (pH>10) can mitigate detrimental microbial contamination and predator populations
  - *e.g.* Daphnia (zooplankton) egg and neonate viability is low

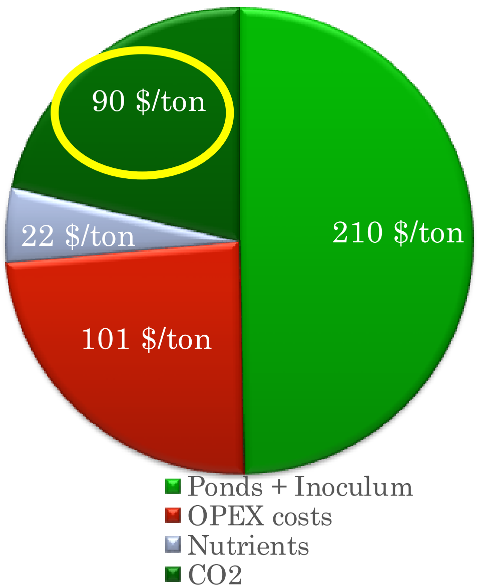
pH = 8.5

pH = 10.2

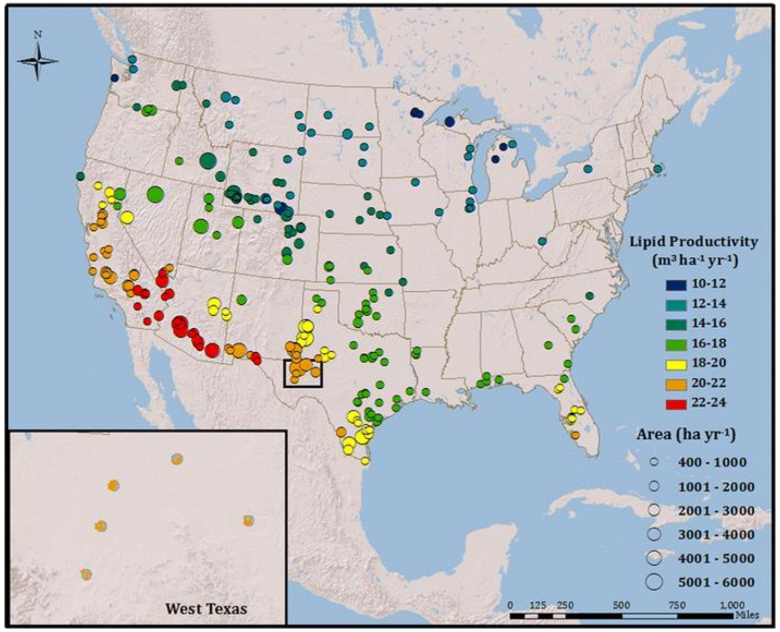
Vijverberg, J. et al. (1996). Decrease in Daphnia egg viability at elevated pH. *Limnology and Oceanography, 41: 789-794*.
Vadlamani, A. et al. (2017). *ACS Sustainable Chemistry & Engineering, **5**: 7284-7294.* **DOI**: 10.1021/acssuschemeng.7b01534

Advantages

- <u>Advantage 2</u>: Alkaline solutions scavenge CO_2 from the atmosphere at rapid rates.
  - Costs and geographical constraints associated with CO_2 supply can be mitigated (or eliminated)



Cost components for microalgae cultivation



Geographical constraints based on simultaneous CO_2 and land availability

Max. biofuel production with CO_2 supply constraints

= 44 million barrels per year

EISA mandate for non-cellulosic advanced biofuel

= 100 million barrels per year

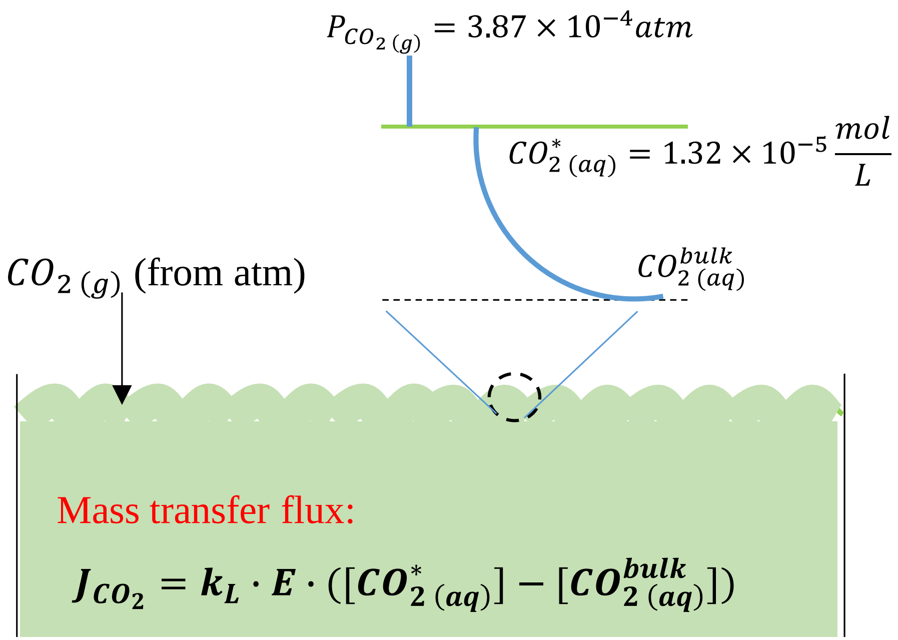
(1) Davis, R., et al. (2016) *Technical Report NREL/TP-5100-64772;* Huntley, M.E., et al. (2015) *Algal Research*, **10**: 249-265; (3) Quinn, J. C., et al. (2012). *BioEnergy Res.* ***6****: 591-600;* (4) Bracmort, K. (2014). Congressional Research Service Report 7-5700. https://www.fas.org/sgp/crs/misc/R42122.pdf

2 - Approach (Management)

- Team
  - Sridhar Viamajala: Biochemical engineering - Cultivation and scale-up
  - Sasidhar Varanasi: Chemical engineering – Mass transfer modeling
  - Robin Gerlach: Biochemical engineering – Cultivation and nutrient management
  - Ross Carlson – Chemical Engineering – Metabolic flux modeling
  - Brent Peyton – Biochemical engineer – Cultivation and scale-up
  - Matthew Fields – Microbiology – Microbial ecology
  - Blake Wiedenheft – Molecular biology – Gene editing
  - Greg Characklis – Environmental Engineering - Resource management, Economics
  - Jordan Kern – Environmental Engineering - Sustainability and Life Cycle Assessment
- History
  - Ongoing collaborations for >10 years
  - Builds on recently concluded DOE ASAP project – resulted in 21 journal publications (~10 more in preparation); 9 patents (8 awarded and 1 pending); numerous presentations
- Interactions
  - PIs, students and postdocs participate in biweekly team conference calls – milestone discussions and research updates
  - Annual team meetings at ABS
  - Student exchange, PI visits, numerous phone/email conversations

2 - Approach (Technical)

Developing a mathematical framework



Danckwerts, P.V., (1970) *Gas-liquid reactions*. McGraw-Hill Book Co.
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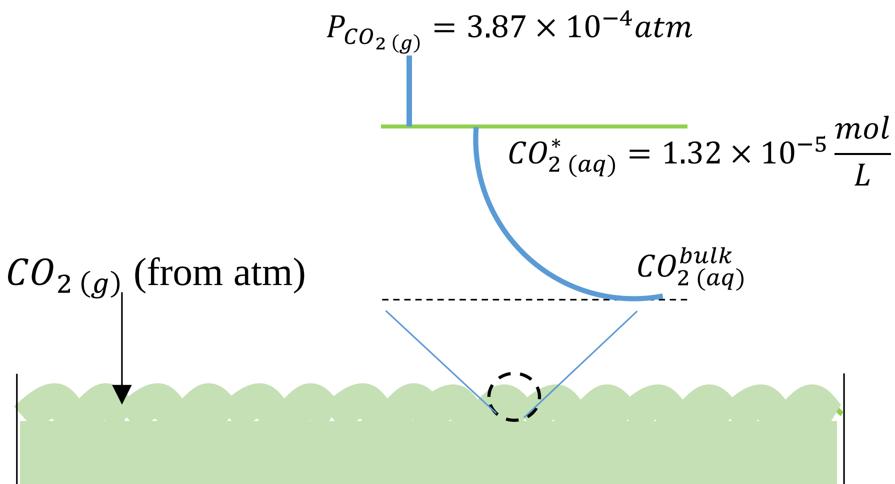

Mass transfer flux:

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

$\boldsymbol{J_{CO_2}}$ = CO_2 transfer flux (mol/m^2/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

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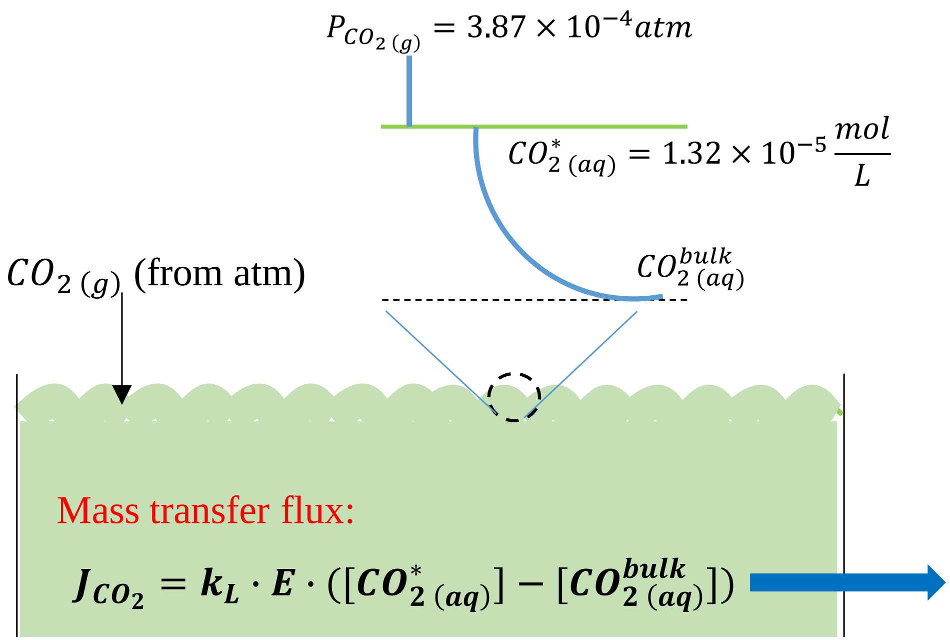

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Abiotic reactions:

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

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

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

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

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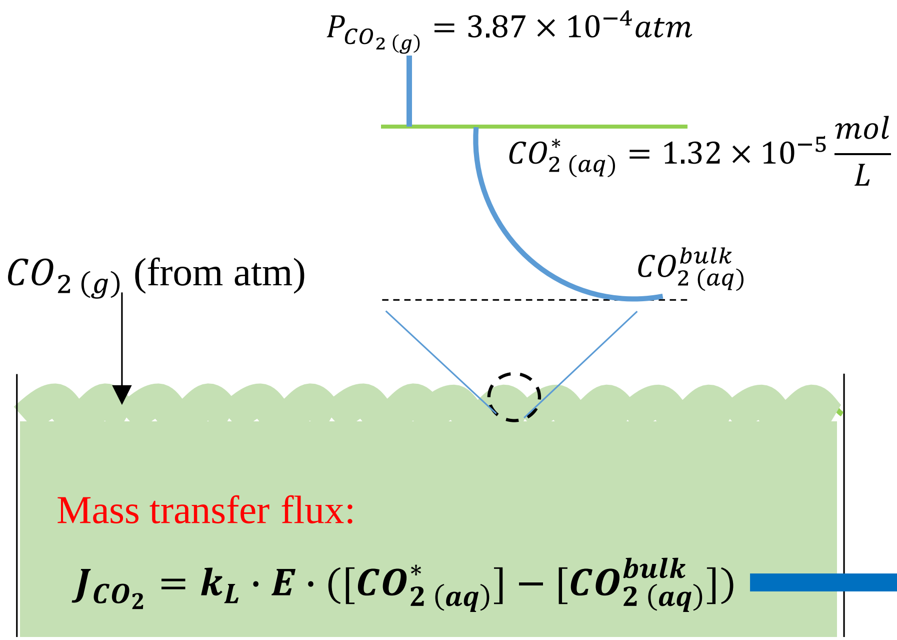

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= 0.1 m/h for 20 cm ponds mixed at 30 cm/s

$\boldsymbol{E}$ = 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

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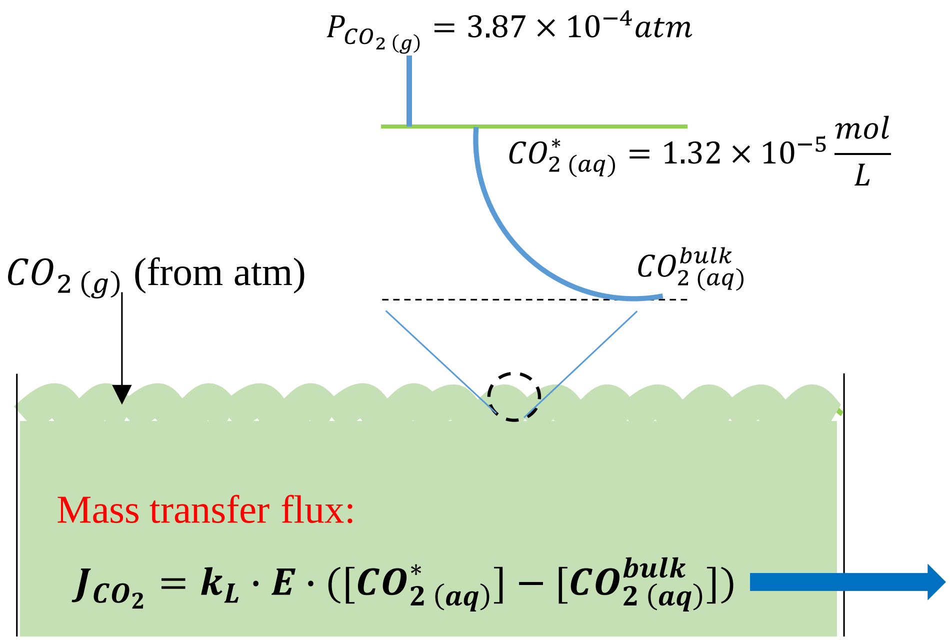

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Biotic reaction:

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

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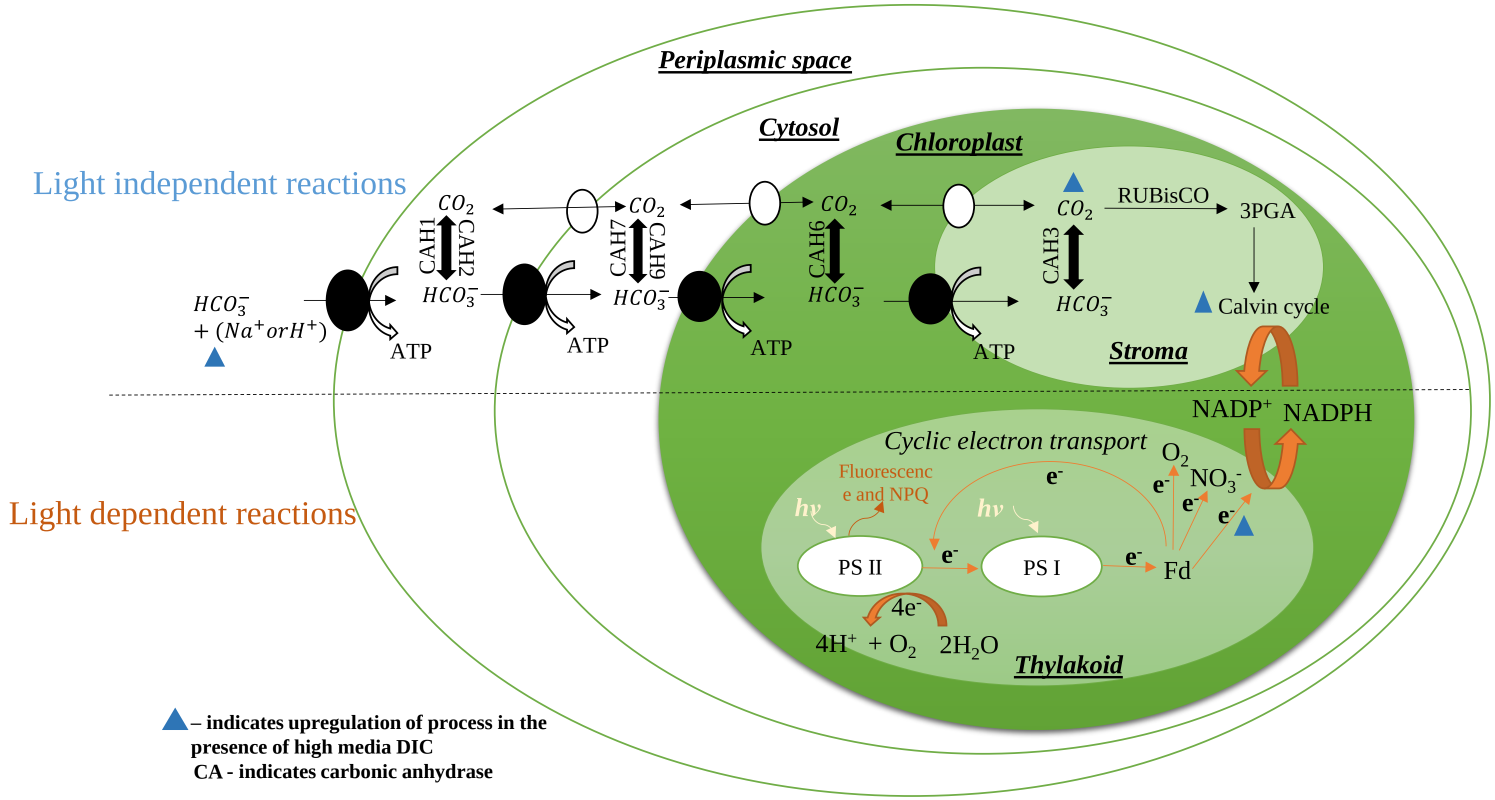
Weissman, J.C., et al. (1988). Photobioreactor design: Mixing, carbon utilization, and oxygen accumulation. *Biotech. Bioeng.* ***31****: 336-344.*

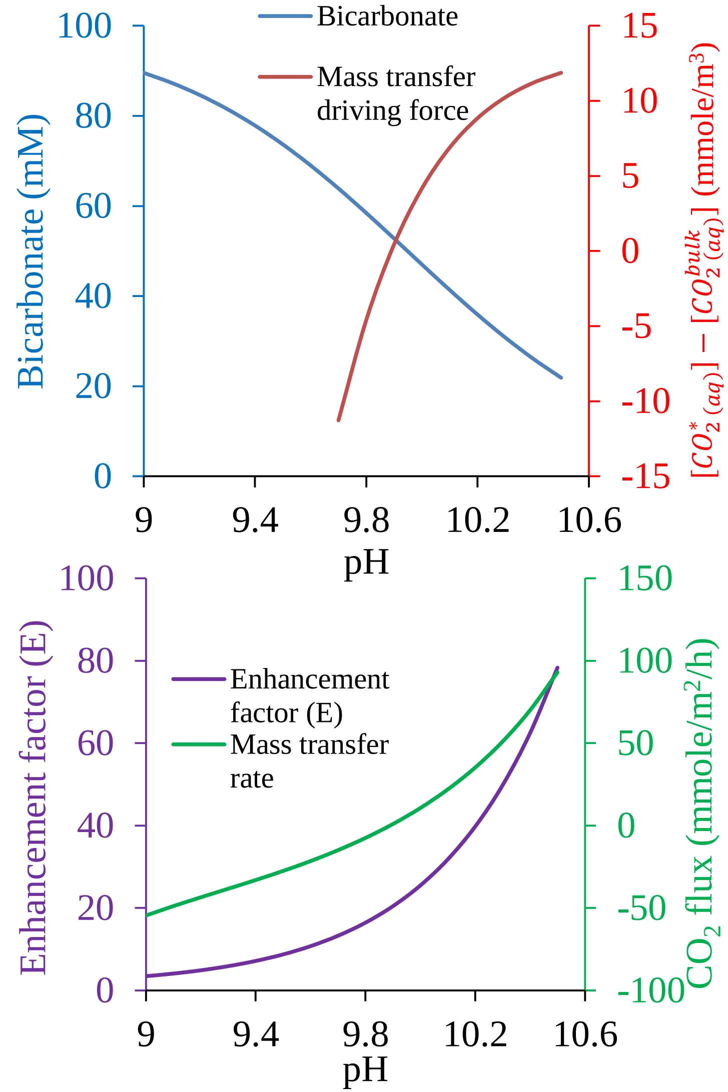
High media alkalinity increases availability of HCO_3^-



- 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

CO_2 transfer from the atmosphere into alkaline media



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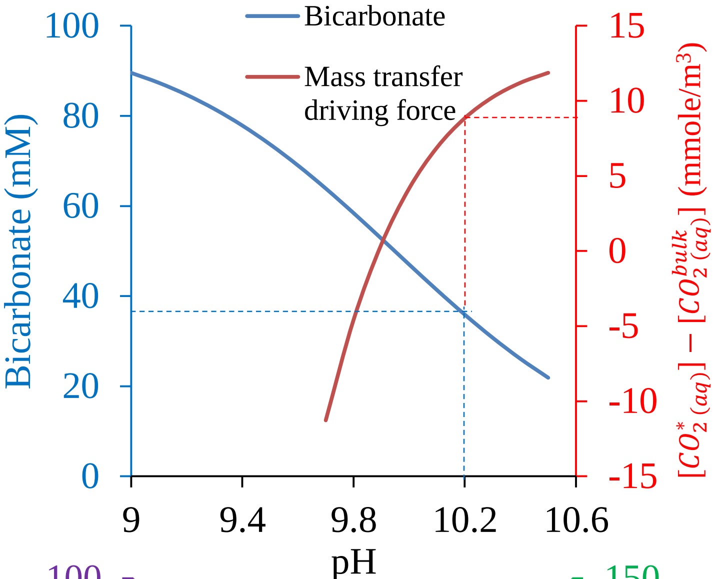
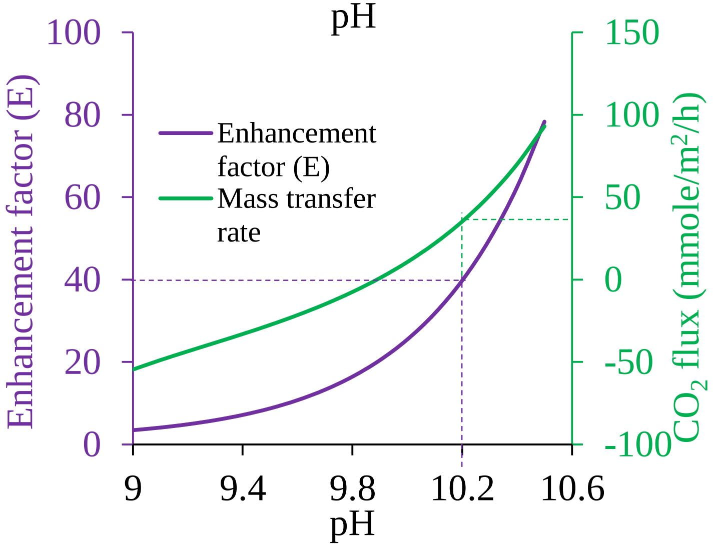
CO_2 transfer from the atmosphere into alkaline media

To maintain high atmospheric CO_2 flux and allow growth without concentrated CO_2 input

- Maximize mass transfer driving force ($[CO^*_{2\,(aq)}] - [CO^{bulk}_{2\,(aq)}]$)
- High media alkalinity to maintain high HCO_3^- concentrations in the medium for photosynthesis to occur without inorganic carbon limitations
- Maximize enhancement factor (E) by maintaining high pH; E~40 at pH 10.2
  - E indicates improvement in CO_2 dissolution rate due to acid-base reaction between CO_2 and OH-
- 40mmole/m²/h = 11.5 g-C/m²/d
  = 25 g-biomass/m²/d
  (45% carbon content)





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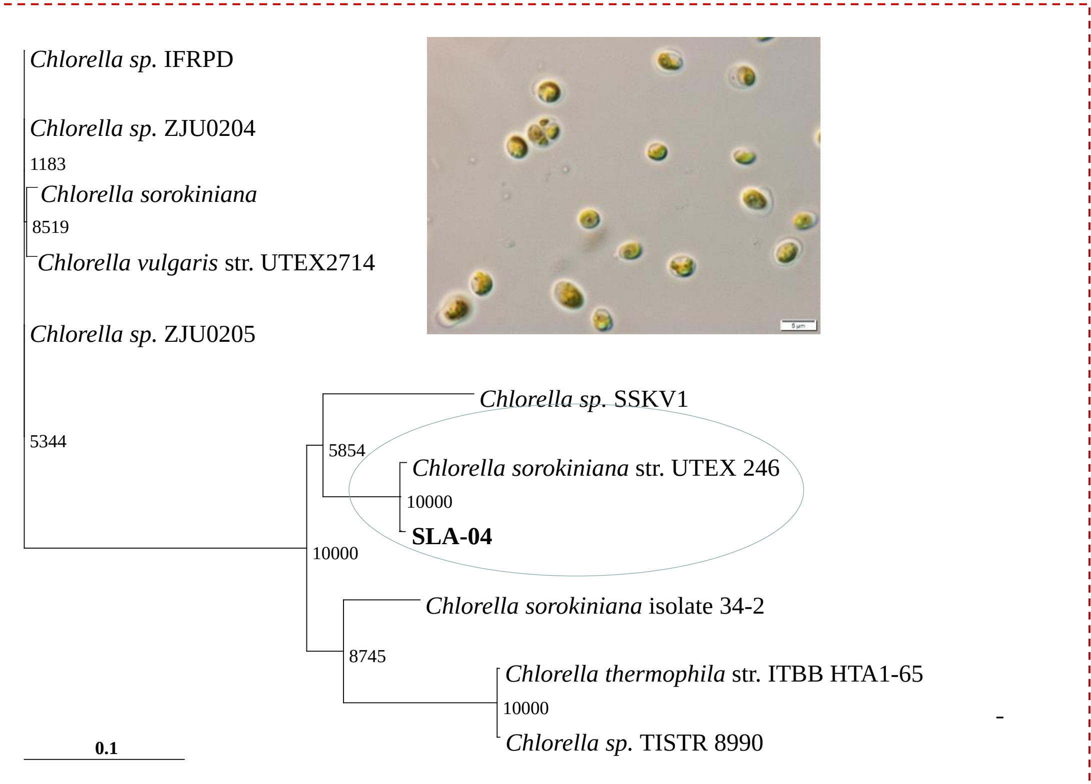
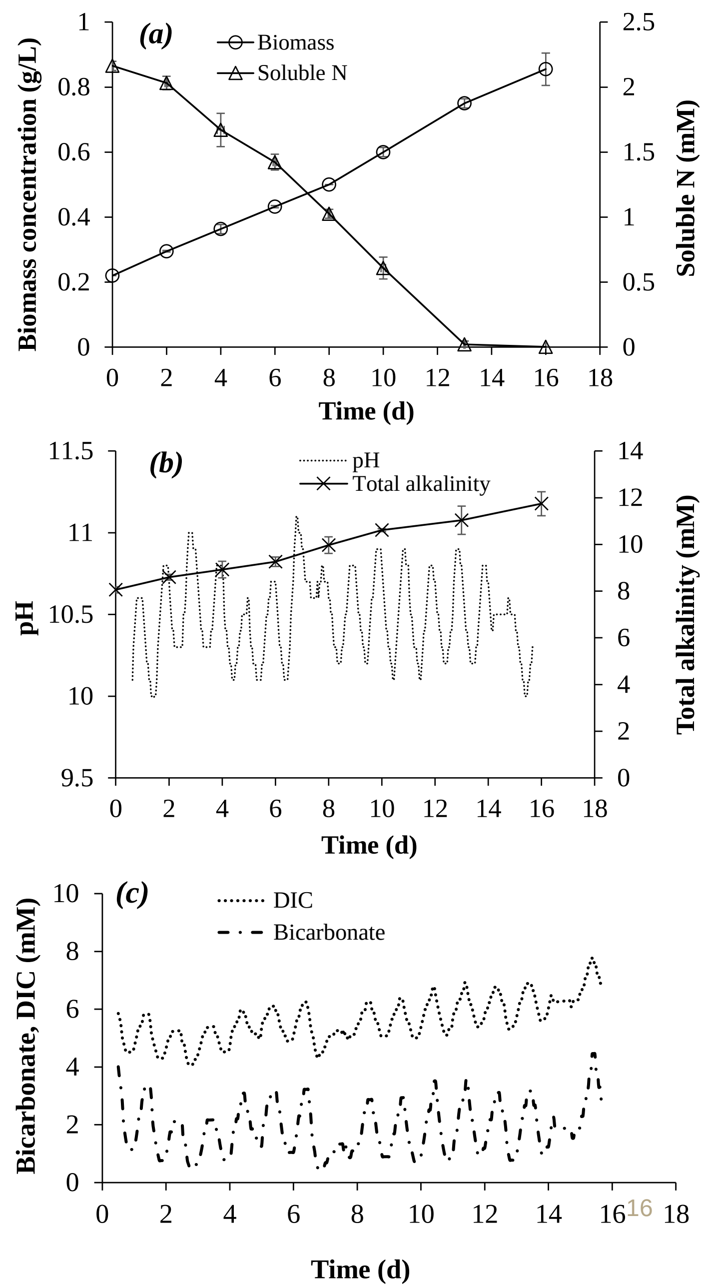
Isolation, identification and initial cultivation of strain SLA-04

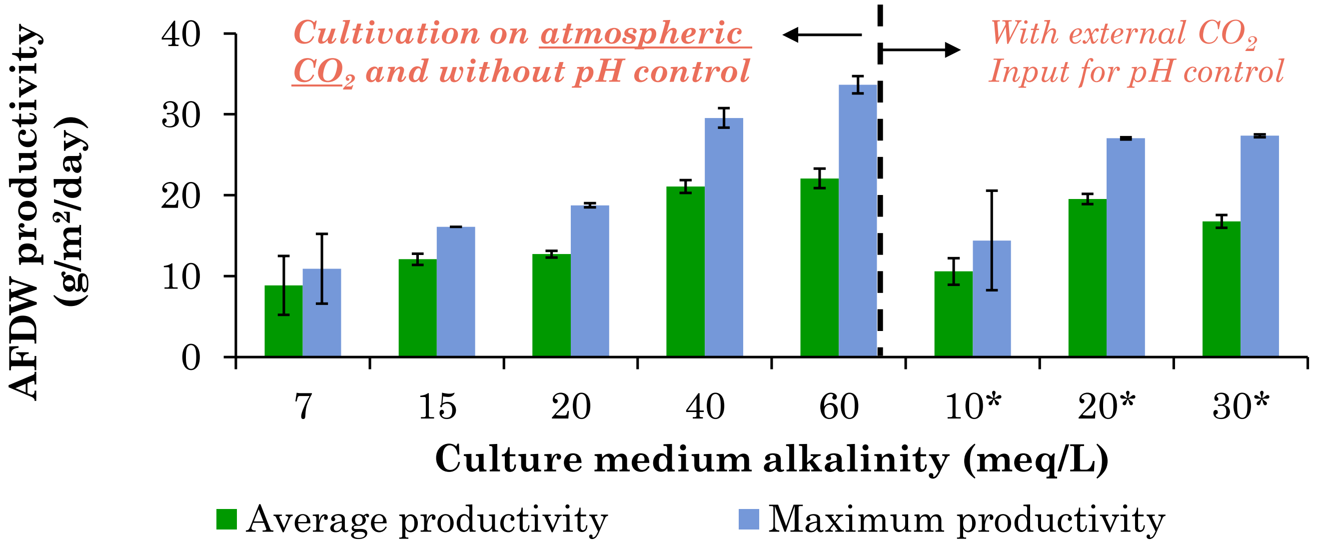




- Isolated from Soap Lake, WA
- Initial raceway pond cultivation (30 L, 0.18 m^2) in high pH, but low alkalinity media resulted in low productivity (6-8 g-AFDW/m^2/d)
- Low HCO_3^- concentrations were suspected to be the reason for low productivity

Vadlamani, A. et al. (2017). Cultivation of Microalgae at Extreme Alkaline pH Conditions: A Novel Approach for Biofuel Production. *ACS Sustainable Chemistry & Engineering, **5**: 7284-7294.* **DOI**: 10.1021/acssuschemeng.7b01534

Cultivation in high pH and high alkalinity media – 0.18 m^2 raceway ponds



- Experiments were performed in 0.18 m^2 (30 L) raceway ponds – July and August
- Without concentrated CO_2 inputs in high alkalinity media (40-60 meq/L),
  - Average areal productivities were 22 g-AFDW/m^2/d
  - Maximum productivity of 32 g-AFDW/m^2/d was measured.
- Average productivities of cultures grown without concentrated CO_2 inputs were similar to productivities of cultures grown with concentrated CO_2 input (pH maintained at 8.5).

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Cultivation in high pH and high alkalinity media - 30 L raceway ponds

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

- Cultures growing at pH>10 and in the presence of high media HCO_3^- show high ETR_{max}, Y(II), and α values.
  - Better utilization of incident light for photosynthetic carbon fixation
- Cultures growing in low HCO_3^- media (pH>10) show high dissipation of electrons (cyclic electron transport)
  - Electron generation is inhibited due to low availability of cellular DIC.
- Maximum quantum yield (F_v/F_m) was not affected by HCO_3^- concentrations

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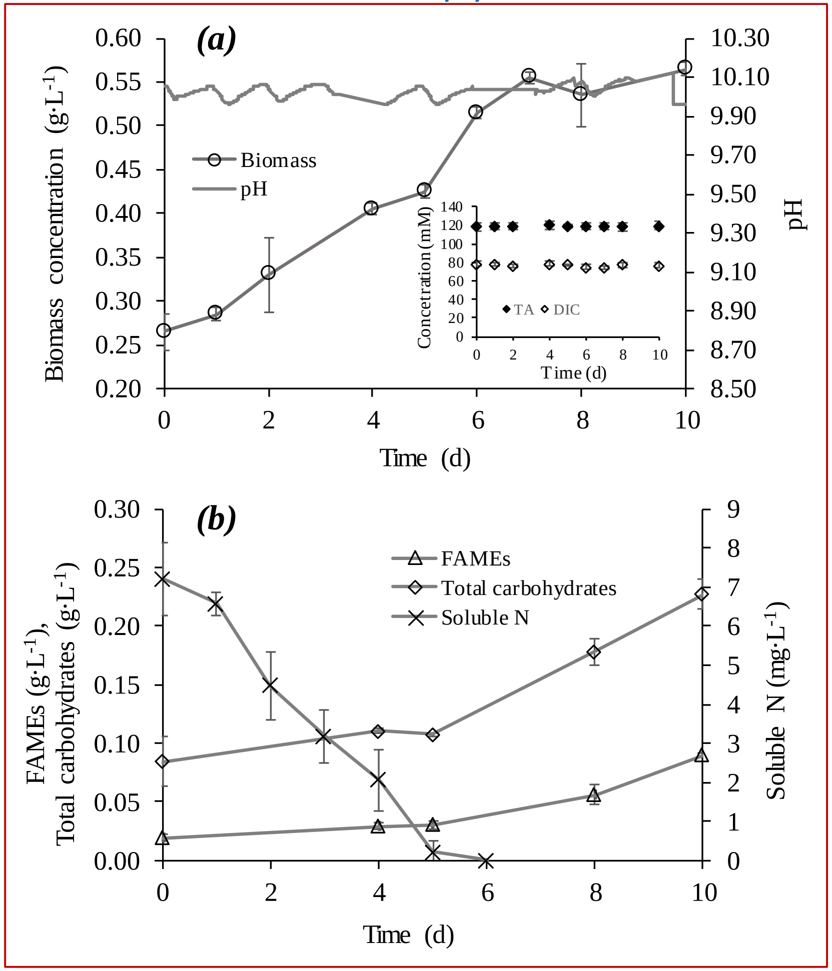
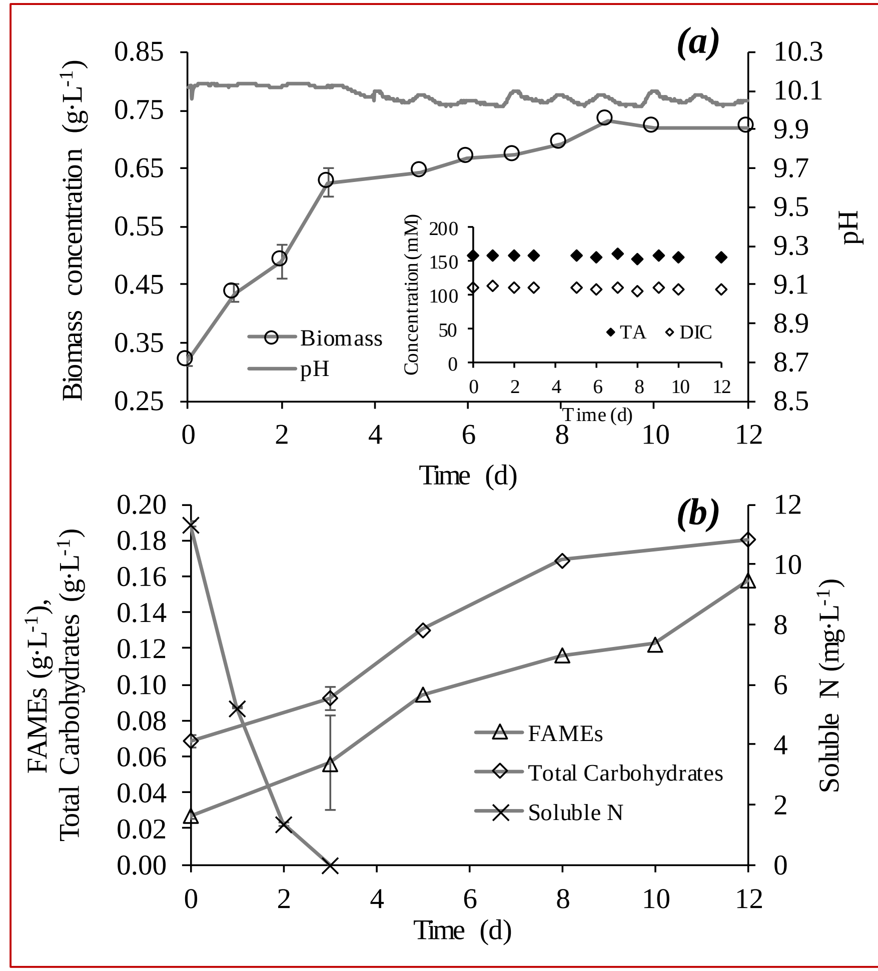
Raceway pond cultivation in 4.2 m^2 ponds

10-inch deep ponds



7-inch deep ponds



- Biomass productivity (until N depletion)
  - 18 g-AFDW/m^2/day (7″ ponds)
  - 10.4 g-AFDW/m^2/day (10″ ponds)
- Lipid productivity (overall) = 1.7 to 2 g/m^2/day
- Carbohydrate productivity (overall) = 1.6 to 3.4 g/m^2/day

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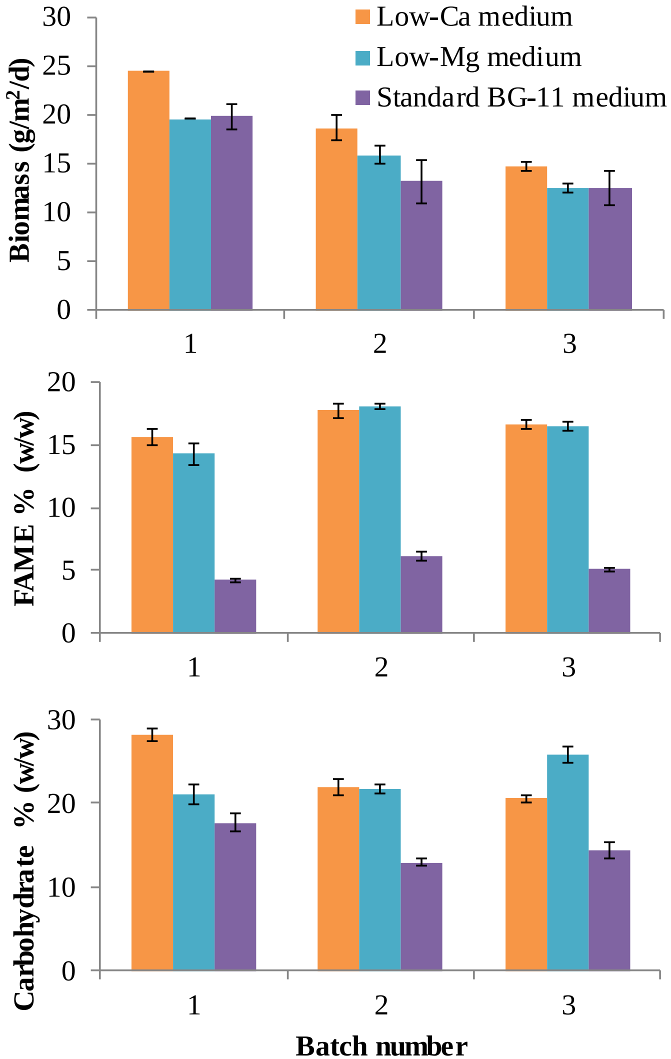
3 – Technical Accomplishments/ Progress/Results

- "Go" decision from "DOE verification" into BP2.
  - BP2 started in October 2018
  - Subcontract awards made in Nov-Dec 2018
  - Personnel hiring partially complete – graduate students hired; post doc interviews are in-progress
- Task 1 – Productivity and composition improvements through improvements in cultivation methods
  - Multi-season experiments started
- Task 2 – Modeling CO_2 mass transfer in high-pH/alkalinity media
  - Initial model developed; Experiments for enhancement of mass transfer with borate as "rate-promoter" are in-progress.

Raceway pond experiments in low-Ca and low-Mg media (0.18 m^2, 20 L raceway ponds).



Biomass, FAME and carbohydrate productivities are higher in low-Ca and low-Mg media

3 – Technical Accomplishments/ Progress/Results

- Task 3 - Algal community dynamics
  - Evaluated methods to separate tightly and loosely associated prokaryotic community members from SLA-04 cells; Biomass collected for DNA extraction.
- Task 4 - Transcriptomics/metabolomics
  - Antibiotic cocktail being tested to obtain axenic SLA-04 cultures for sequencing; ongoing discussions with the Greenhouse leadership at LANL regarding DNA extraction, preparation and sequencing
- Task 5 - Metabolic flux modeling
  - Modeling efforts initiated based on previous MSU co-PI Ross Carlson’s work with *P. tricornutum*
- Task 6 - CRISPR/Cas9-based genome editing
  - Potential gene editing targets identified - 1. AMP kinase (AMPK), 2. Lactate dehydrogenase, 3. Acetate kinase, and 4. Phosphotransacetylase
  - Additionally, nitrate reductase identified for proof-of-principle study based on the publicly available genome information of UTEX 395
  - Guide RNAs were designed using a combination of tools necessary for Cas9 binding.
  - Guides were evaluated for predicted activity and crosschecked for their potential for off-target cleavage.
- Task 7 - Process economics and LCA
  - A time-dynamic, stochastic weather component is being developed for integration into existing TEA/LCA model. The meteorological model has been calibrated with historical air temperature, windspeed, relative humidity and solar loss data.
  - Model will forecast algae production and project revenues due to seasonal and year-to-year changes in biomass productivity

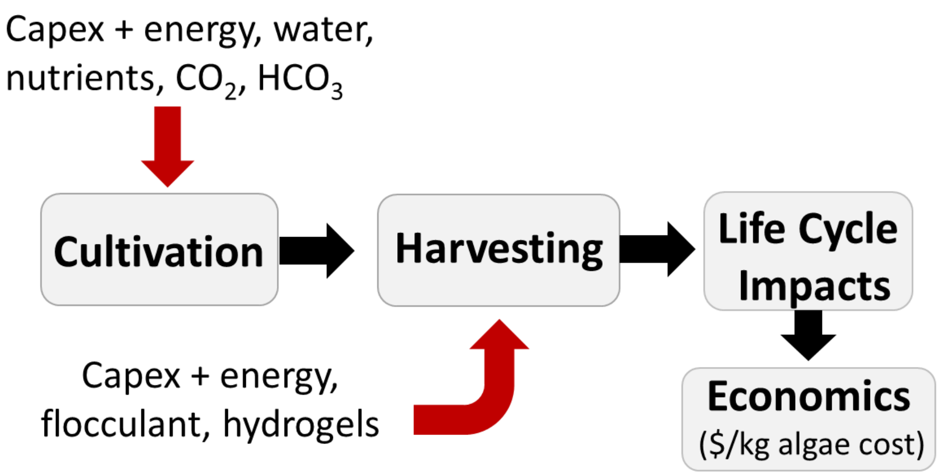
4 – Relevance

- _Goal:_ The goal of this project is to 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 dissolved inorganic carbon (DIC) concentrations for growth.
- 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 toolkits for broad use by the microalgae community
- 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.
- Reduction in biofuel costs are driven by
  - Reduction in cost of CO_2 supply
  - Improved culture stability through lower susceptibility to microbial contamination and predator attacks
  - Higher productivity through strain improvements
- Utility patent application US/15/498,621 filed 04-27-17.

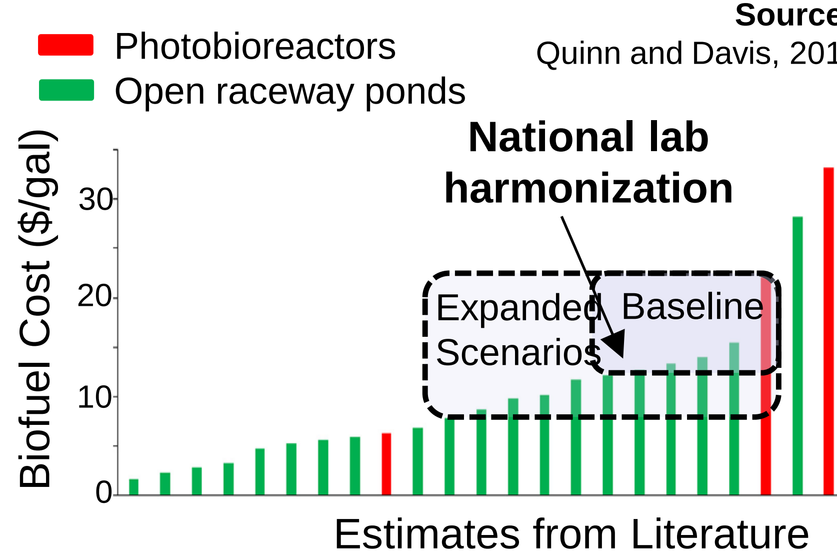
5 - Future Work

1. Improve scale and productivity of algal cultures cultivated in alkaline media.
   a) Without concentrated CO_2 inputs
   b) Multi scale experiments across seasons – 500 mL e-PBRs, 30 L raceway ponds, 1000 L raceway ponds
   c) Productivity enhancements through
      - media optimization
      - targeted genetic improvements based on genome, transcriptome analysis and metabolic flux modeling
      - Understanding and ultimately controlling microbial ecology
2. Improve biomass composition for improved biofuel productivity
   a) Control of media conditions
   b) Additional strategies will be guided by microbial ecology and -omics data
3. Toolkit development
   a) quantification of microbial interactions and enrichment of productive communities
   b) publication of a well-annotated genome of a highly productive algal strain
   c) insights into regulatory mechanisms (transcript response) of algal cells grown at high alkalinities
   d) development of a metabolic network model to inform genome editing approaches for strain improvement
   e) development of genome editing approaches based on the CRISPR-Cas9 technology

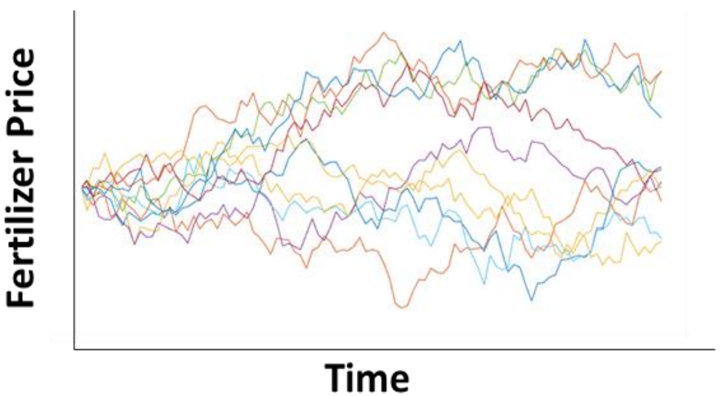
LCA/TEA Modeling in Support of PEAK

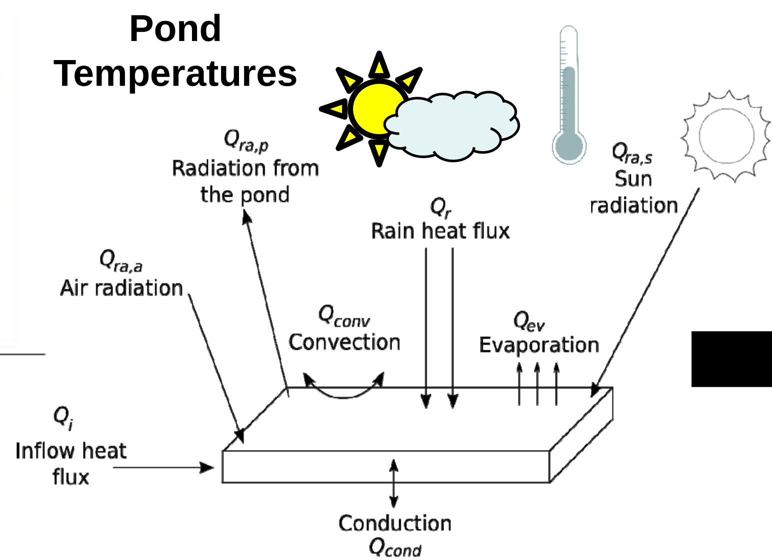


Source: Quinn and Davis, 2015

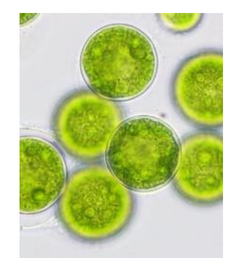


Special Features: Dynamic Economic and Weather Inputs



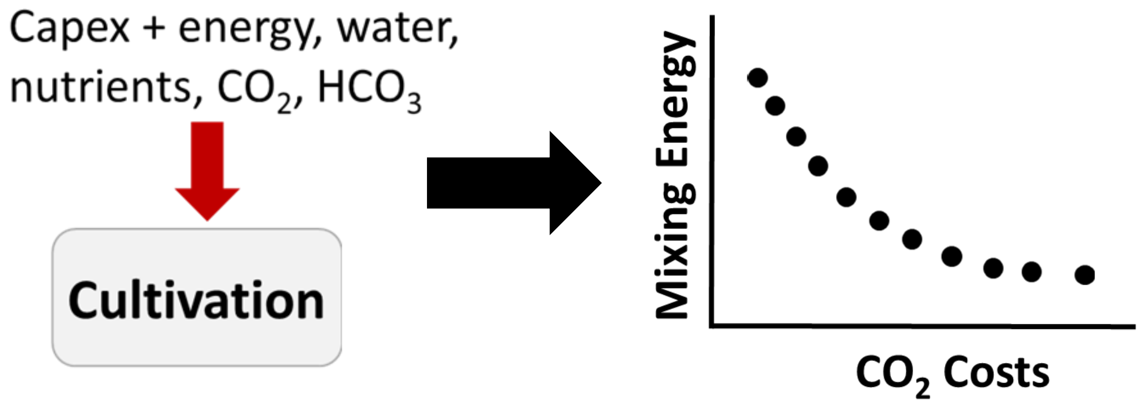


Biophysical Model



THE UNIVERSITY *of* NORTH CAROLINA *at* CHAPEL HILL

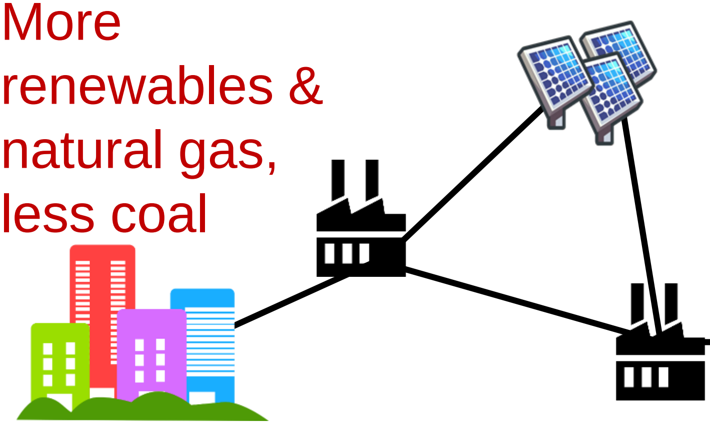
LCA/TEA Modeling in Support of PEAK

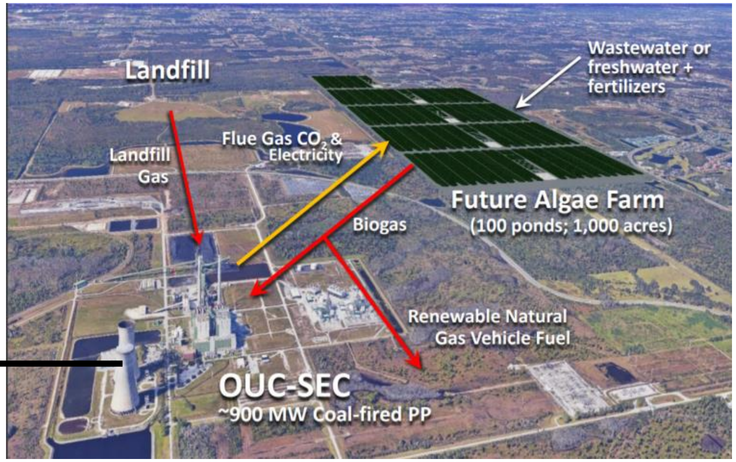


Quantifying tradeoffs between mixing energy requirements and CO_2 costs in high pH, high alkalinity systems

Benefits of air capture of CO_2 vs. risks of co-locating with power plants under regulatory and technological change

More renewables & natural gas, less coal





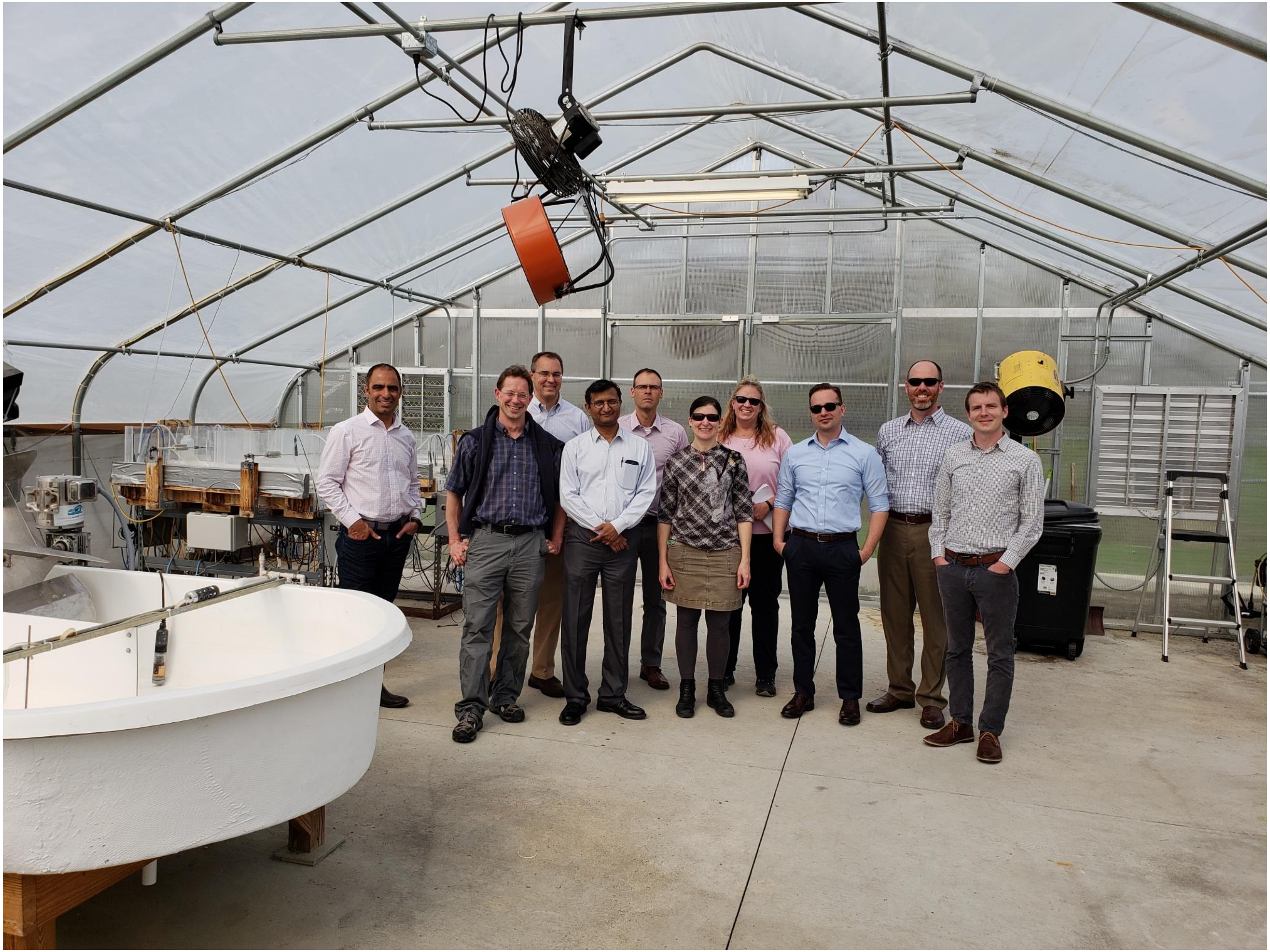
Major milestones

- <u>Milestone 2.2.1</u>: Develop and validate comprehensive CO_2 mass transfer model in alkaline media for non-isothermal conditions. (***Q6***)
- <u>Milestone 7.2.1</u>: Identify and evaluate risk management approaches under uncertainty related to price of competitive fuels, subsidies and physical or natural inputs. (***Q7***)
- <u>**Go/no-go**</u>: Demonstrate the potential for production of >1200 GGE/acre/year. (***Q7***)
- <u>Milestone 5.1.1</u>: In silico reconstruction of SLA-04 metabolic potential with partitioning of activity between cytosol, mitochondria and plastids. (***Q8***)
- <u>Milestone 3.2.1</u>: Determine active microbial populations that develop in the outdoor SLA-04 cultures. (***Q9***)
- <u>Milestone 4.2.1</u>: Elucidate expressed genes unique to enriched pool of high-productivity populations.(***Q10***)
- <u>Milestone 6.3</u>: Isolate one or more isogenic gene-edited mutants and test for novel phenotypes. (***Q11***)
- <u>Milestone 1.3.1</u>: Demonstrate a biofuel intermediate productivity >1500 GGE/acre/year. (***Q12***)
- <u>Milestone 3.2.2</u>: Correlate microbial community structure to SLA-04 culture productivity. (***Q13***)

Summary

- High media pH (>10) drives rapid transfer of CO_2 from the atmosphere to growth media
- High DIC concentrations "buffer" the media and allow high media concentration of HCO_3^-
  - Improves "electron transfer rates" – Likely due to higher rate of delivery of CO_2 to RuBisCO
- Under high-pH AND high-alkalinity conditions, cultures achieve high productivity *even in the absence of* concentrated CO_2 inputs.
- In cultivation experiments over 2 years, we haven't observed a "culture crash"
- Biomass composition can be improved by "adjusting" nutrient composition without significantly compromising biomass productivity

Additional Slides



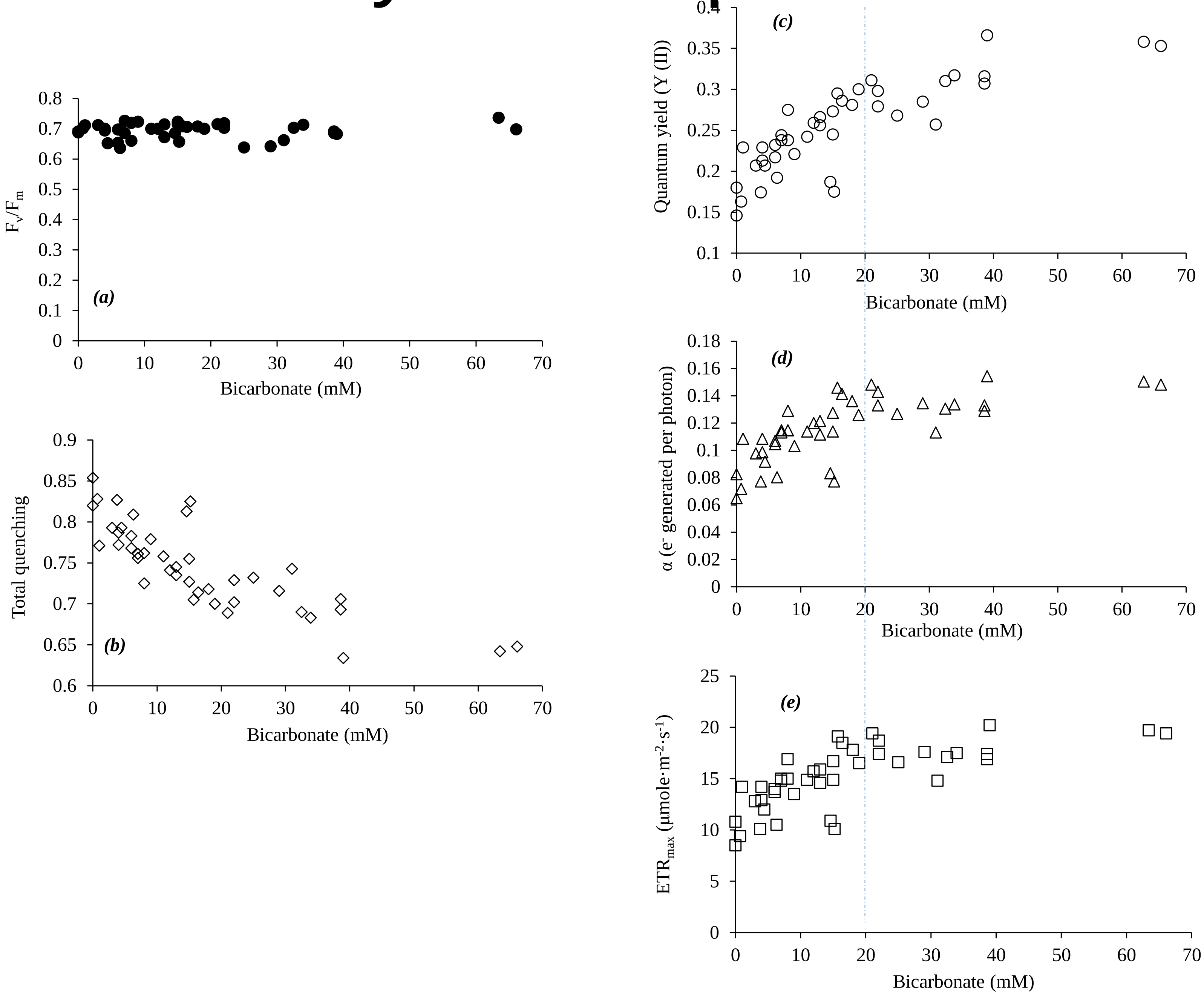
Composition analysis – Mass balance closure

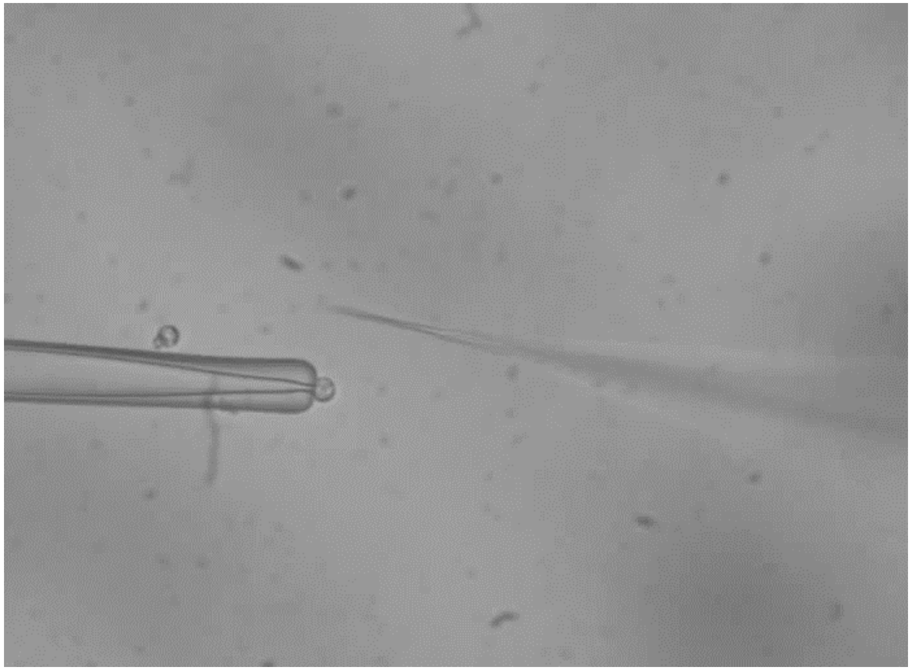
Experiment	Day	FAMEs (%(w·w^{-1}))	Total carbohydrate (%(w·w^{-1}))	Protein* (%(w·w^{-1}))	Nucleic acids** (%(w·w^{-1}))	ASH content (%(w·w^{-1}))	Total
4.2 m^2, 10″ depth	Day 0	7.8 ± 0.6	33.7 ± 2	17.2	5	18.1 ± 0.5	81.8 ± 3.3
	Day 10	17 ± 0.15	42.9 ± 1	14.6 ± 0.02		7.5 ± 0.5	87.0 ± 1.2
4.2 m^2, 7″ depth	Day 0	7.1	20.3	36.7	5	18.7	87.8
	Day 5	14.6	20.1	32.1		9.5	81.3
	Day 12	21.8	25	27.5		8.8	88.1

*Protein content was estimated using a conversion factor of 5.04.

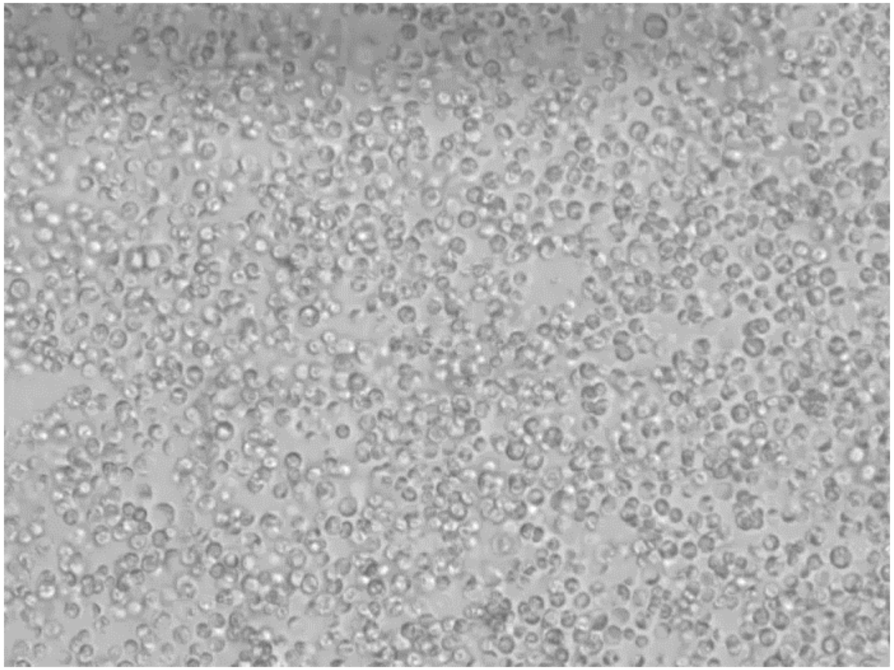
**Nucleic acid content was obtained from literature [9].

Photosynthesis parameters





Algae moves with injection



Holding pipette