DOE Bioenergy Technologies Office (BETO) 2021 Project Peer Review

Advanced Algal Systems

March 23, 2021

Air Carbon for Algae Production – AirCAP

DOE-BETO Award DE-EE008519

μBIO
MicroBio Engineering

Braden Crowe (Presenting), Aubrey Davis, Erik Hoffnagle, Maria Reyna, Cooper Gibson, Ernesto Ortiz, John Benemann (P.I.)

QUALITAS HEALTH

Jakob Nalley, Eneko Ganuza, Bart Reid

Pacific Northwest NATIONAL LABORATORY
Proudly Operated by Battelle Since 1965

Michael Huesemann, Scott Edmundson, Song Gao

U.S. DEPARTMENT OF ENERGY

Dan Fishman (Project Officer)
Evan Mueller (Project Monitor)

Project Overview

Context, History

Efficient Carbon Utilization in Algal Systems (ECUAS) FOA, 2018 Issue Date Direct Air Capture Topic Area

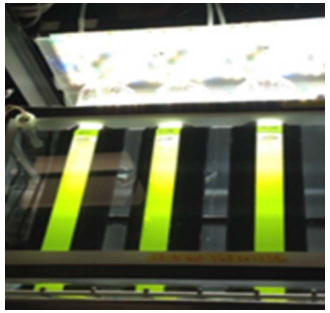
- Microalgae biofuel resource potential is severely constrained by few suitable cultivation locations with nearby sources of enriched CO_2[1].
- Relieving this constraint expands resource potential to levels equal to U.S. diesel[2] needs
- FOA Objective: Provide at least 20% of the required CO_2 from direct air capture

Project Goals – Provide 100% of algal carbon with CO_2 from air to greatly expand algae biomass resource potential and reduce greenhouse gas footprint.

How will it be done?

- Take advantage of chemical enhancement, or the increase in air-CO_2 flux at elevated pH, to achieve high rates of air-CO_2 absorption, providing 100% of CO_2 via direct air capture.
- Develop tools to assess scalabilty to commodity scale ponds (10-acres).
- Find algae that thrive under conditions that achieve the target flux.
- Guide selection of appropriate conditions for techno-economic, life-cycle assessments.

If successful, the project will expand algae potential, create well-paying, rural jobs, lower costs, and improve the greenhouse gas impact



---

[1] Langholtz 2016; [2] Venteris 2014

1. Management Leverage the core competencies of the project participants to direct the research approach and generate high-impact data.

Project Lead

P.I.: Dr. John Benemann

Mr. Crowe: Tasks Lead

Mr. Woertz: Administration Lead

- Develop tools to predict air-CO_2 flux at scale, techno-economic and life-cycle analyses, validate strain performance in pilot ponds, develop analytical approach to track carbon in biotic, abiotic trials.
- Coordinate activities between the project team, stakeholders.
- Identify and mitigate project risks.
- Develop external feedback, collaborate with related projects

  Examples: Exxon Mobil, PNNL-Wigmosta, NREL-Davis, DISCOVR

Task Lead

Dr. Jakob Nalley

- Implement, manage 1-acre pond abiotic trials
- Provide TEA, LCA guidance for operation of large, unlined pond operation, water quality considerations.
- With MBE, PNNL adapt a production strain for growth using 100% air-CO_2

Task Lead

Dr. Michael Huesemann

- Leverage, adapt an established strain screening pipeline to identify strains that thrive at high pH.
- Use MBE, QH findings to inform strain screening criteria, pipeline
- Test top performing strains under climate simulating conditions, outdoors

1. Management Project scope is organized into concurrent, complementary tasks, roughly one per participant per budget period.

- Complex tasks are divided into subtasks in order to organize workflow.
- **Project roles are clearly defined** based on this task structure
- Quarterly milestones **provide a framework to meet aggressive goals.**
- **Key findings are disseminated** via conference presentations, peer reviewed articles

Stakeholder feedback is exchanged via group-teleconference calls, including:

- Weekly MBE, QH coordination of project activities, budgets, and timelines.
- Experimental or modeling results are shared between all project partners during monthly calls, feedback is incorporated into experimental plans.
- Monthly calls with DOE-BETO provides high-level guidance.
- Communication and collaborations with related DOE-funded and other projects

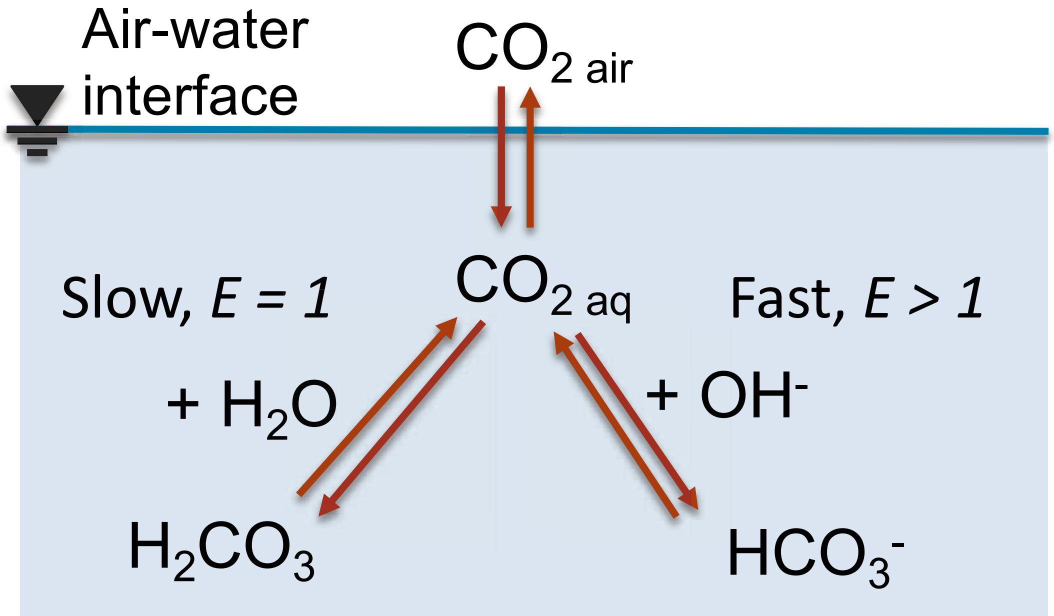
Risks are inherently minimized by this management approach. For example:

- <u>Risk</u>: Screening yields fast growing strains, but under conditions that are not scalable or that require expensive chemical inputs
- <u>Mitigation</u>: Experiments conducted in 1-acre ponds and TEA/LCA conducted early in the project to guide the screening approach; simultaneous experiments in smaller ponds

2. Approach: At elevated pH, the $CO_{2,aq}$ + OH^- reaction pathway appreciably increases air-CO_2 flux, known as chemical enhancement

Without chemical enhancement, $E = 1$, and air-CO_2 flux is given by:

$$J_{CO_2} = k_L * \left(C_{CO2}^{sat} - C_{CO2}^{bulk}\right) * E$$



Where:

C_{CO2}^{sat} = $CO_{2\ aq}$ at the air-water interface, in equilibrium with the gas phase, ~10 μM

C_{CO2}^{bulk} = $CO_{2\ aq}$ in pond 'bulk', ~0 μM at pH > 10

k_L= mass transfer coefficient, ~0.1 m/hr for ponds, a measure of turbulence as it relates to mass transfer

Under non-enhanced ($E = 1$) conditions:

$$J_{CO_2} = 0.1\,\frac{m}{hr}(10 - 0\ \mu M) = 0.3\,\frac{g\ C}{m^2 day} \approx 0.6\,\frac{g\ AFDW}{m^2 day}$$

E must be ~ 30 to support 20 g AFDW/m^2-day!

Depending on the assumed mass-transfer regime, estimates for E range from 2 to 40 at pH 10.5

Hypothesis: *E is* sufficient to support high rates of carbon uptake, at a biologically compatible high pH

2. Approach – Identifying conditions to meet the target air-CO_2 flux

- Measure air-CO_2 exchange rates via abiotic (without algae) trials in 1-acre ponds.
  - k_L is measured in 'outgassing trials', by supersaturating the pond with CO_2, then measuring the rate of <u>pH increase</u>.
  - Air-CO_2 flux, J_{CO_2}, at elevated pH is measured by displacing the pond from air-equilibrium with a strong base, then measuring the rate of return to equilibrium (<u>pH decrease</u>)
- Compare ingassing rates over a wider current velocity range in more easily managed 3.4 m^2 ponds
- Compare experimental results to model predictions to identify the appropriate mass-transfer model

Top: 1-acre ponds at QH, used to measure ingassing rates at a commercially relevant scale. Bottom: MBE 3.4 m^2 used to more fully parameterize air-CO_2 flux.

<u>Challenge:</u> Sensor error at high pH confounds flux measurements; source-water hardness results in solidification of incoming CO_2
Mitigation: Develop comprehensive experimental workplans (Task 2) that employ a variety of approaches to track carbon.

2. Approach - Finding alkaliphilic strains

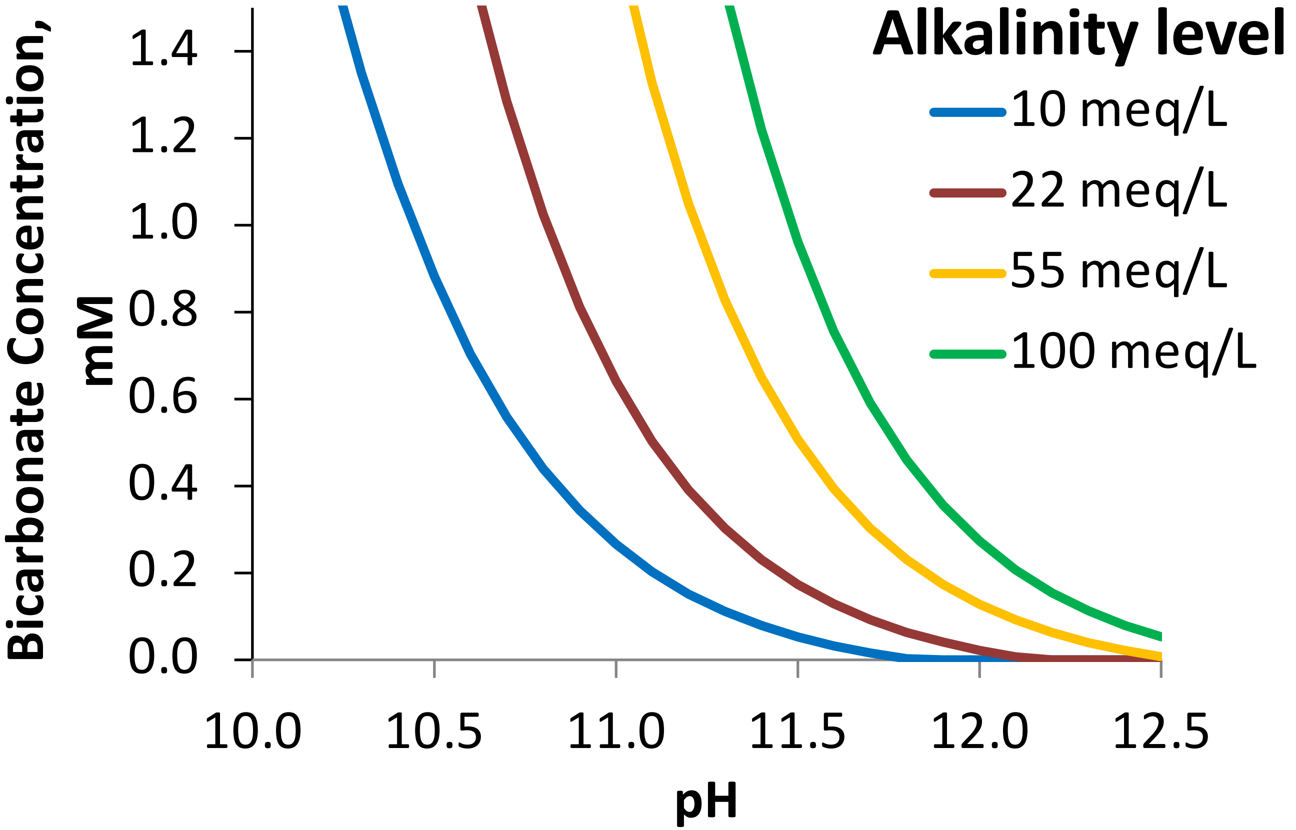
Bioavailable carbon, predominately in the form of bicarbonate, approaches growth-limiting concentrations (0.008 – 1.8 mM)[1] at elevated pH. Adding alkalinity increases cost.

Three strain-selection approaches used to identify alkaliphilic candidates,

(1) Screening of culture collection strains isolated from alkali environments,

(2) Bioprospecting from the natural environment, and

(3) Strain improvement, via adaptive laboratory evolution



Top-performers in the lab will be tested in outdoor ponds.

Challenge: Find strains that thrive at low alkalinity and high pH.

---

[1] Raven and Johnston 1991

2. Approach: Go/No-Go Decisions points, Key Metrics

Budget Period 2 (ended 12/31/2020) Go/No-Go Criteria

Validate air-CO_2 ingassing into ponds to equal or exceed 5 g C/m^2-d at or near pH 12.5, allowing for a projection of 10 g AFDW/m^2-d with air-CO_2 only.

Budget Period 3 (ends 6/30/2022) Go/No-Go Criteria

1. Achievement of 15 g AFDW/m^2-d of productivity from air-CO_2 only with alkaliphilic algae in indoor and outdoor cultures, validated with a mass-transfer model.
2. TEA/LCA projecting that at 15 g/m^2-d, process economics would be similar as with flue gas CO_2, at 20 g/m^2-d, but with a nationwide algal biofuels resource potential an order of magnitude higher than for a flue gas colocation scenario

Key economic and/or technical metrics

15 g AFDW/m^2-day summer biomass productivity chosen to demonstrate incremental progress

- Further productivity increases are required to achieve economic viability
- Current State of Technology trials operated under conditions that result in major CO_2 outgassing losses, and are out of alignment with 'nth plant' TEA/LCA assumptions

3. Impact - Increase U.S. algal biofuels resource potential 10-fold

Global potential increase is even higher.

- Avoids dependency on advances in ‘2nd generation’ carbon capture technologies

State of technology impacts:

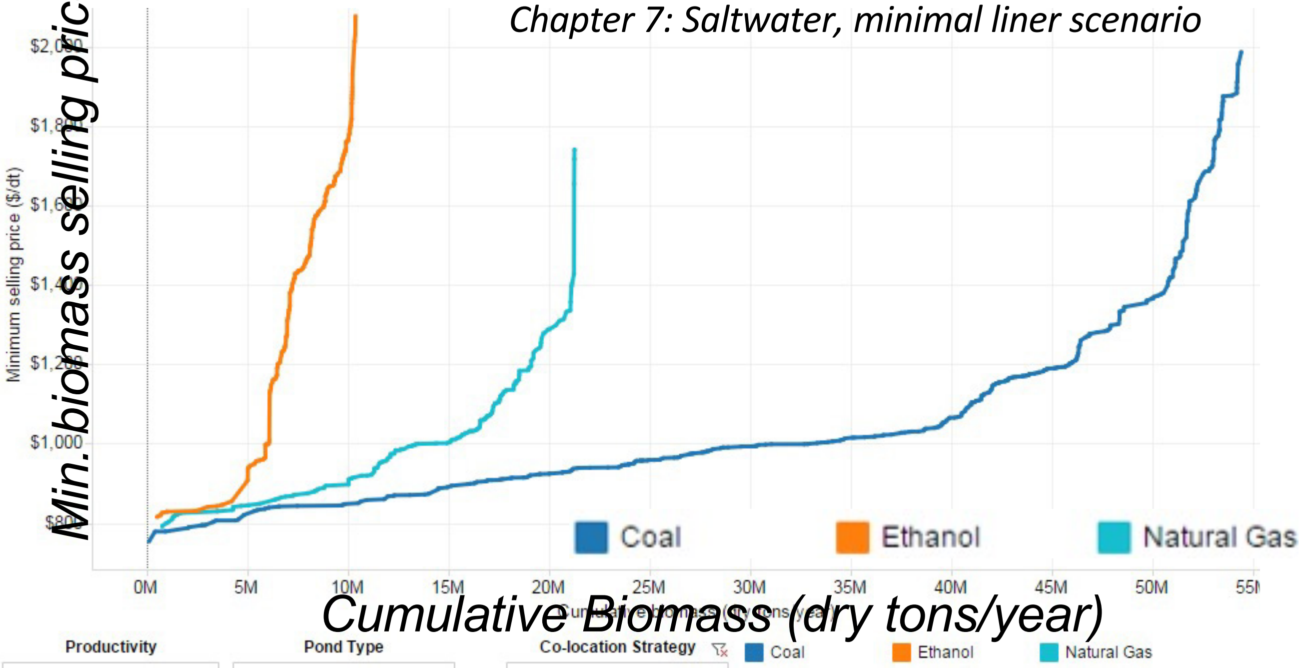
- Establish baseline biomass productivity without CO_2 fertilization
- Identify TEA/LCA implications of any additional inputs required for growth using 100% CO_2 from air

Commercialization potential:

- No capital costs for CO_2 supply.
- Minimal increase in operating costs
- Partnered with US algae producer to guide, inform project approach

Langholtz 2016 Billion-Ton Report

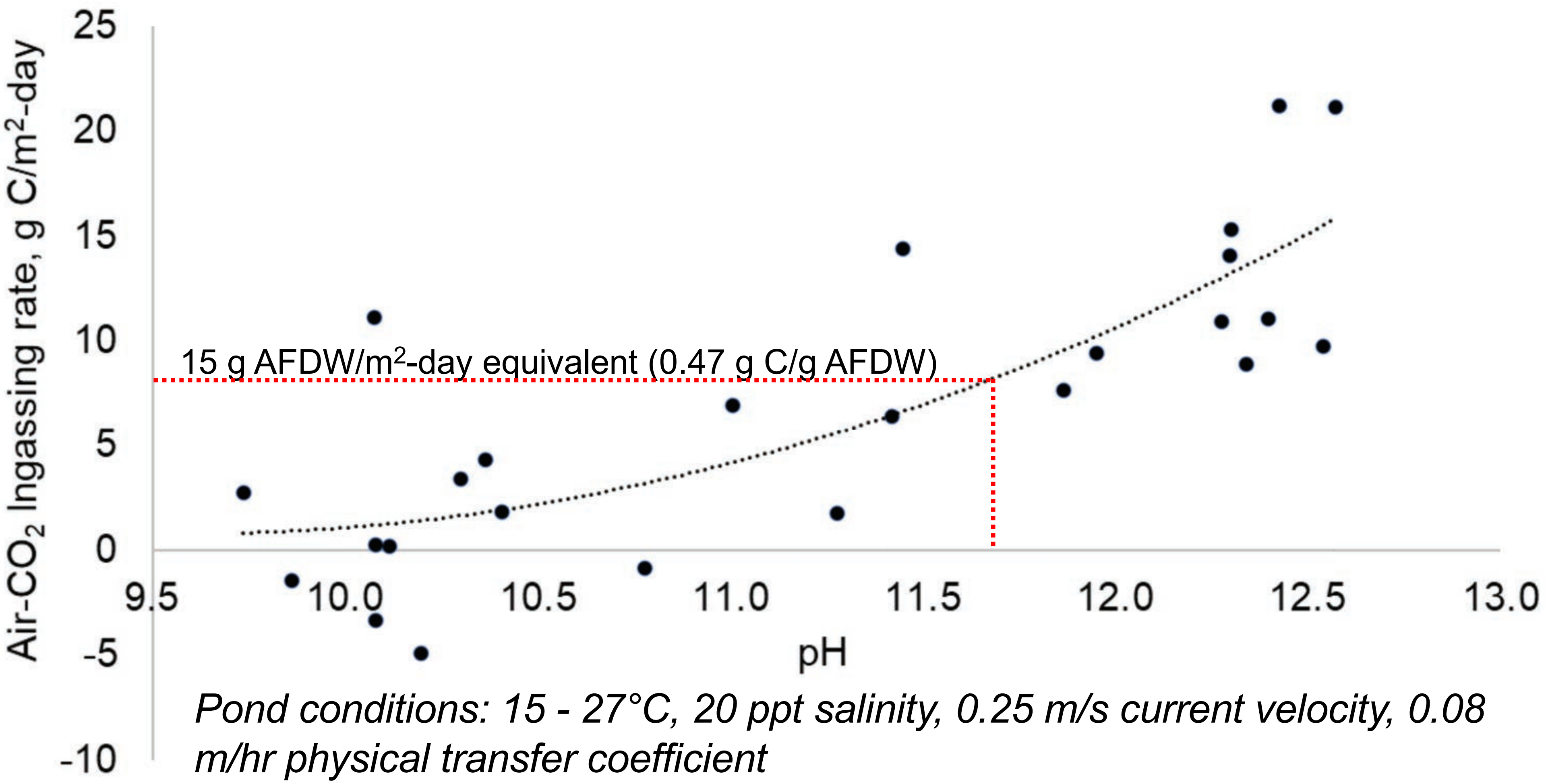
Chapter 7: Saltwater, minimal liner scenario



- Cumulative biomass production cost increases dramatically when enriched CO_2 is required
- Constrains resource potential to ~5 BGY

4. Progress and outcomes

1-acre pond ingassing rates rates increase with pH due to chemical enhancement; pH > 11.7 required to meet the BP3 target (15 g AFDW/m²-day)



Pond conditions: 15 - 27°C, 20 ppt salinity, 0.25 m/s current velocity, 0.08 m/hr physical transfer coefficient

<u>> pH 12 required to support 2025 DOE productivity target</u>

Mass-transfer model predictions of *E*, for two limiting cases:

Second order <u>instantaneous</u> reversible reaction, 'Diffusion Limited' (Olander 1960)

$$J_{CO_2}=k_L * \left(P_{CO2}^{air} * H - C_{CO2}^{*}(x=\delta)\right) * \left[1+\frac{D_{OH^-}.D_{HCO_3^-}\cdot K\cdot [OH^-]}{D_{CO_2}\left(K\cdot CO_{2(aq)}^{*}\cdot D_{HCO_3^-}+D_{OH^-}\right)}\right]$$

$$K=\frac{[HCO_3^-]}{[OH^-][CO_2]}$$

Flux increases proportionally to turbulence

pH dependent, but not influenced by CO_2+OH^- reaction rate

First-order, finite reaction rate, 'Reaction Rate Limited' (Hatta 1932)

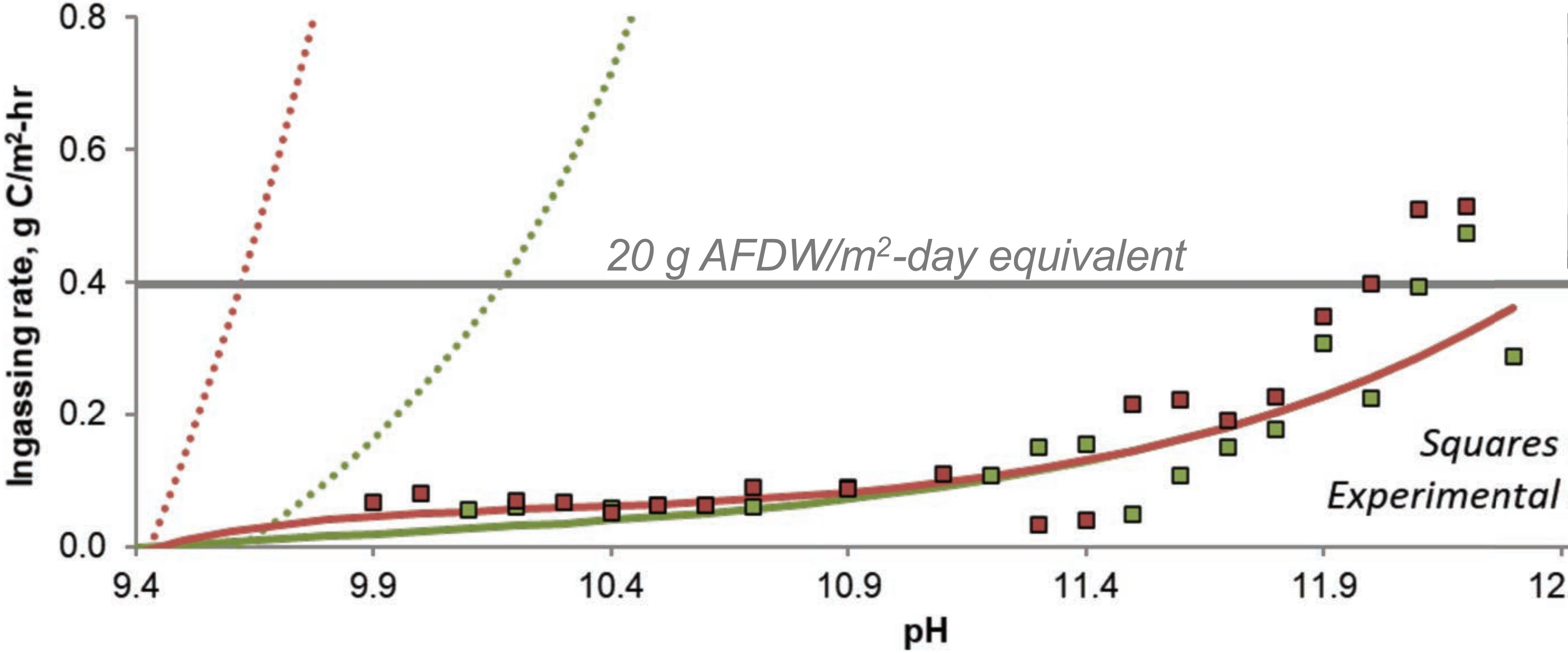
$$J_{CO_2}=\left(P_{CO2}^{air} * H - C_{CO2}^{*}(x=\delta)\right) * \left[\sqrt{\mathcal{D}_{CO2} * k_{tot} * [OH^-]} * \coth\left(\sqrt{\frac{\mathcal{D}_{CO2} * k_{tot}}{(k_L)^2}}\right)\right]$$

Square root reaction rate dependance

Turbulence independent when $\sqrt{\frac{\mathcal{D}_{CO2}*k_{tot}}{(k_L)^2}} < \sim 2$

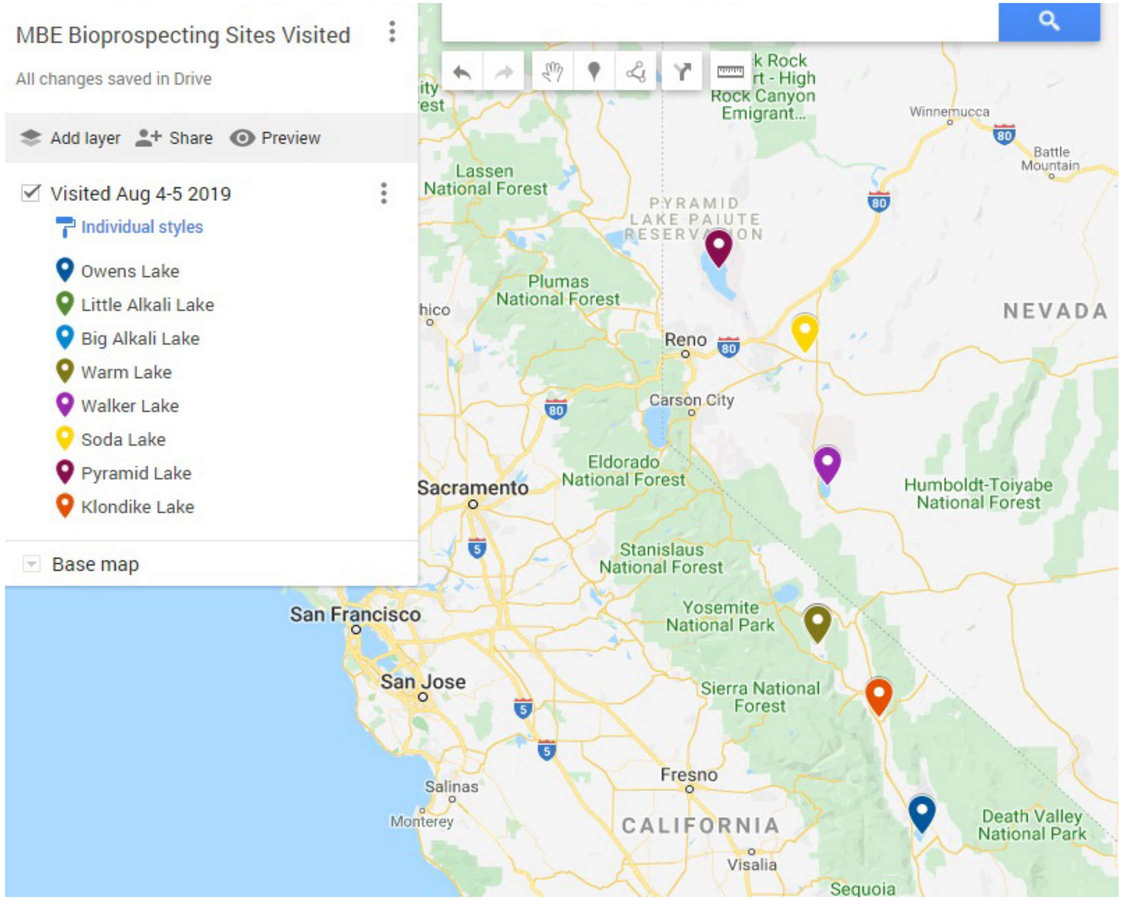
Experimental data validates the reaction-rate limited model; air-CO_2 flux is independent of the mass-transfer coefficient, k_L

Model predictions for 'diffusion' (dotted lines) and 'reaction rate' limited (solid lines) transfer, at low (kL = 0.06 m/hr) and high (kL = 0.36 m/hr) turbulence levels



Conclusion: Decreases in turbulence levels in 10-acre ponds are not expected to influence chemically enhanced air-CO_2 flux.

Two bio-prospected strains delivered to PNNL for further screening



MBE Bioprospecting Sites Visited

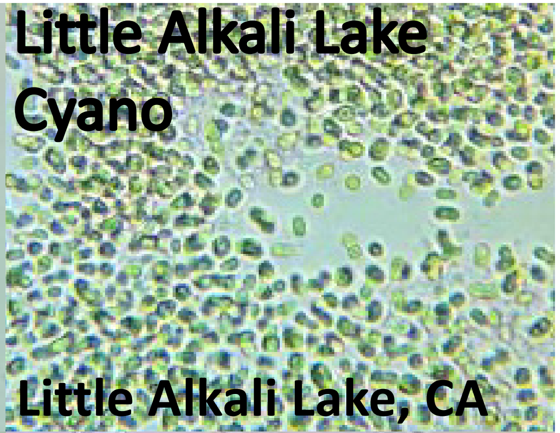
All changes saved in Drive

☑ Visited Aug 4-5 2019

- Owens Lake
- Little Alkali Lake
- Big Alkali Lake
- Warm Lake
- Walker Lake
- Soda Lake
- Pyramid Lake
- Klondike Lake

‘Oblong Cyano’

Soda Lake, NV



Little Alkali Lake Cyano

Little Alkali Lake, CA

Soda Lake Diatom

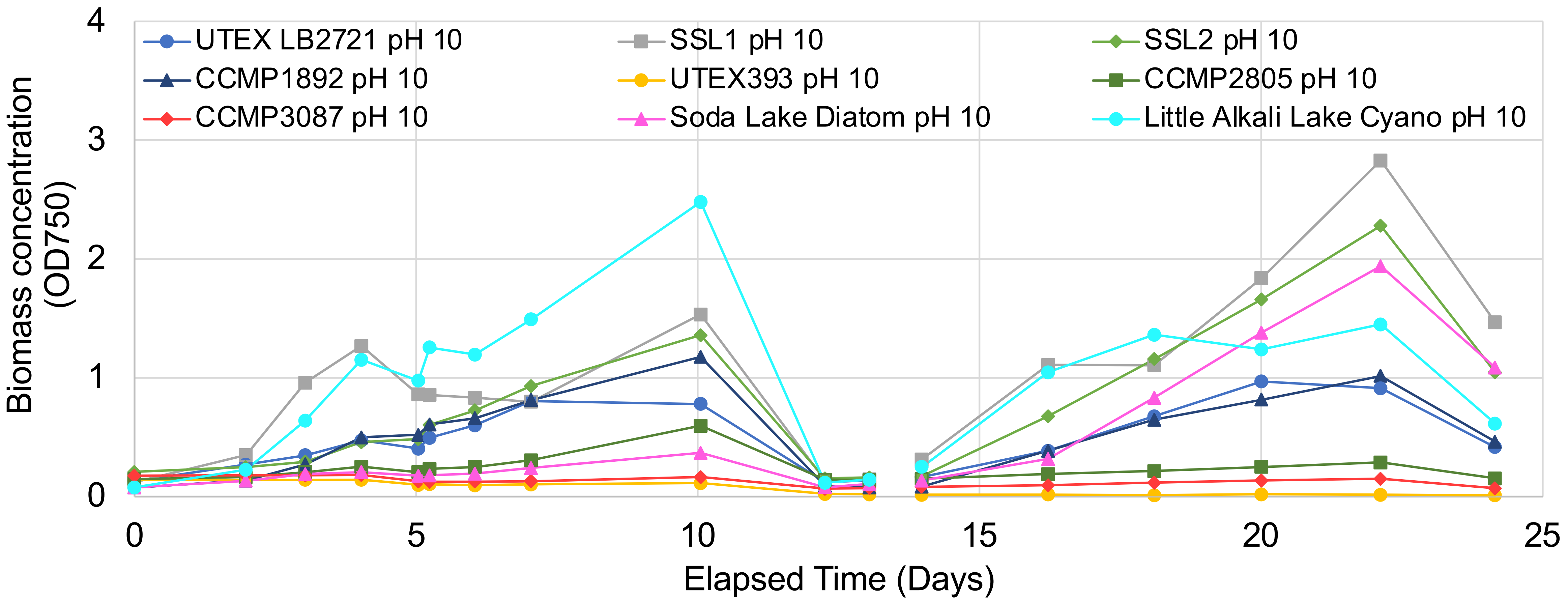
Soda Lake, NV

‘Long Cyano’

Soda Lake, CA

- Partial 16s rDNA sequence suggests Little Alkali Lake Cyano, Oblong Cyano and SSL1 are related.
- Similarity searches against GeneBank reveal these strains to be most similar to an uncultured bacterium sequenced from a photosynthetic biofilm collected from a Mono Lake hot spring.

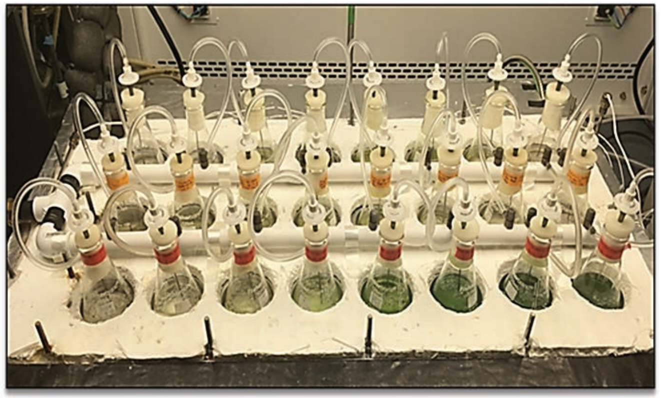
9 strains examined at the 2nd screening level, 10 culture collection strains initially downselected. Top performing strains to be tested in ponds.



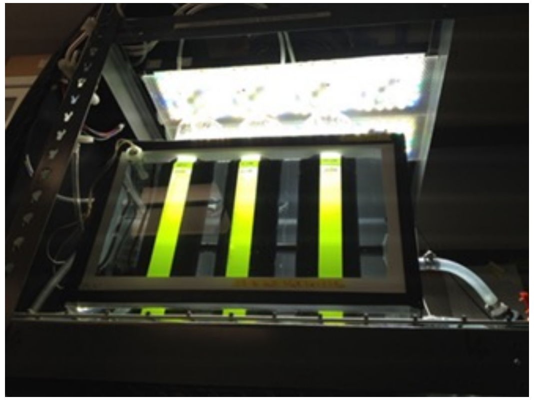
Outcome: SSL1 selected for baseline pond trials using only air-CO_2

NOTE: Some strains with poor initial growth have improved and will be tested in BP3.

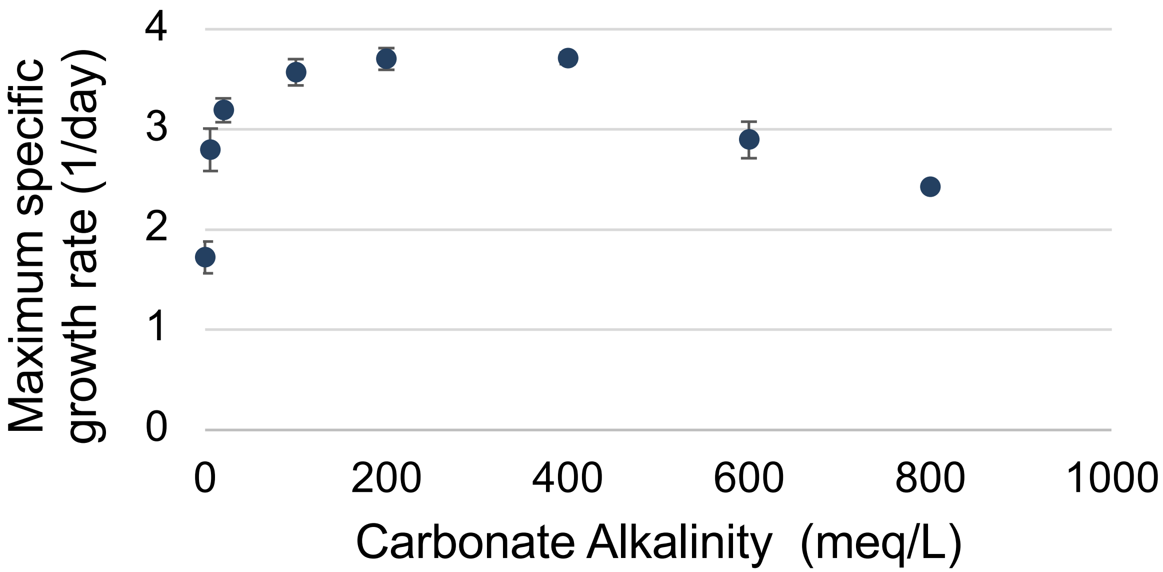
80 – 200 meq/L alkalinity avoids both bicarbonate limitation and sodium stress identified; 'SSL1-Turbo' improved cultivar selected after 200+ days of adaptive evolution

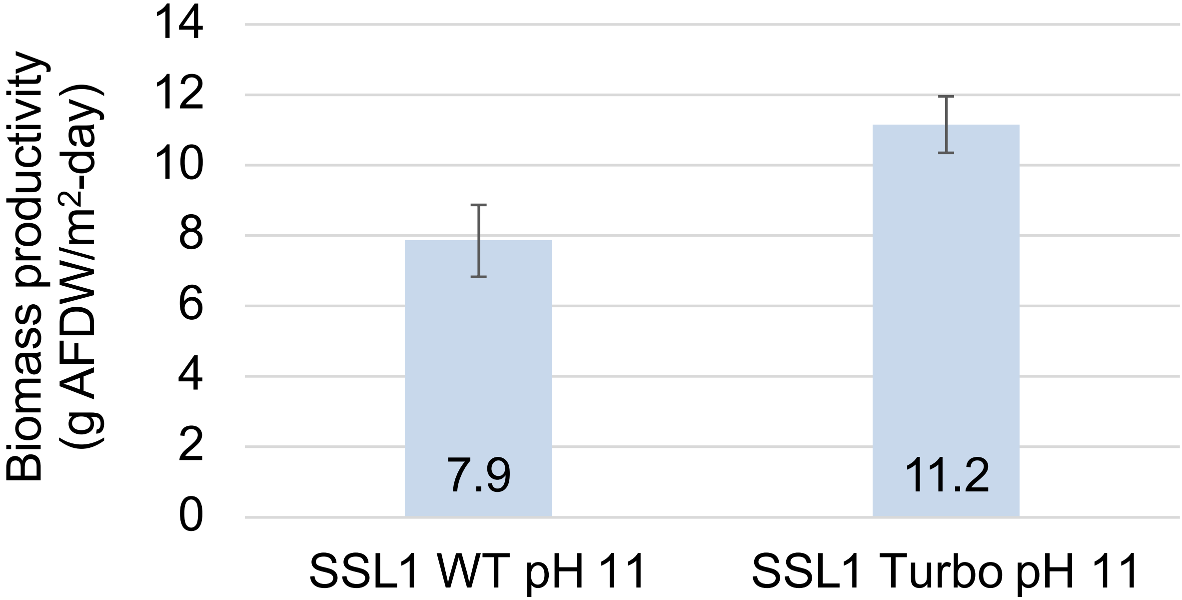


The indoor alkalinity gradient incubator used to quantify the relationship between alkalinity and the specific growth rate.

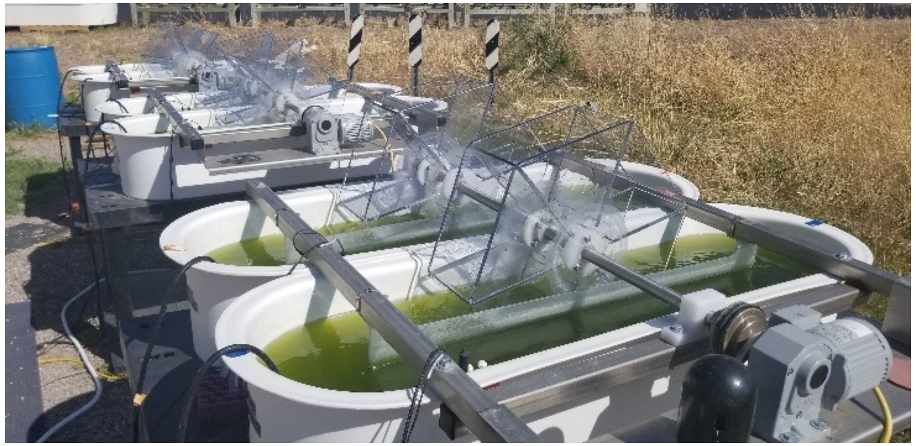


The Laboratory Environmental Algae Pond Simulator (LEAPS) that replicates outdoor pond conditions for simulating outdoor biomass growth indoor.

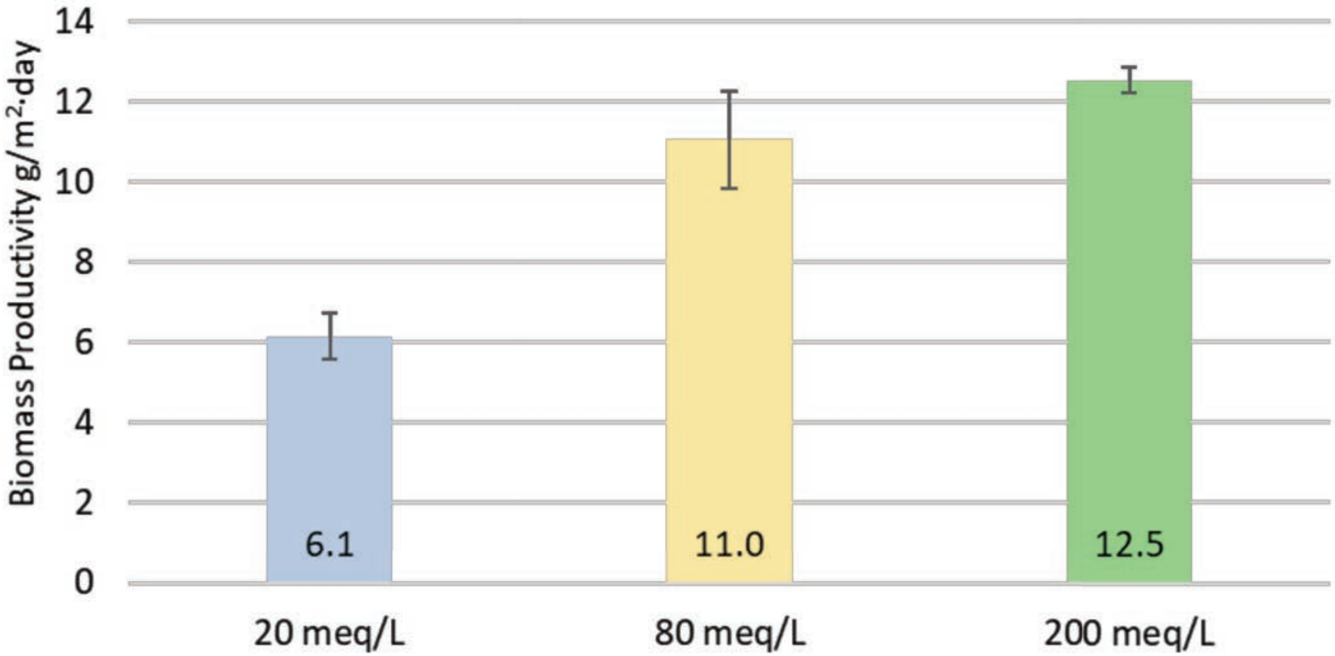


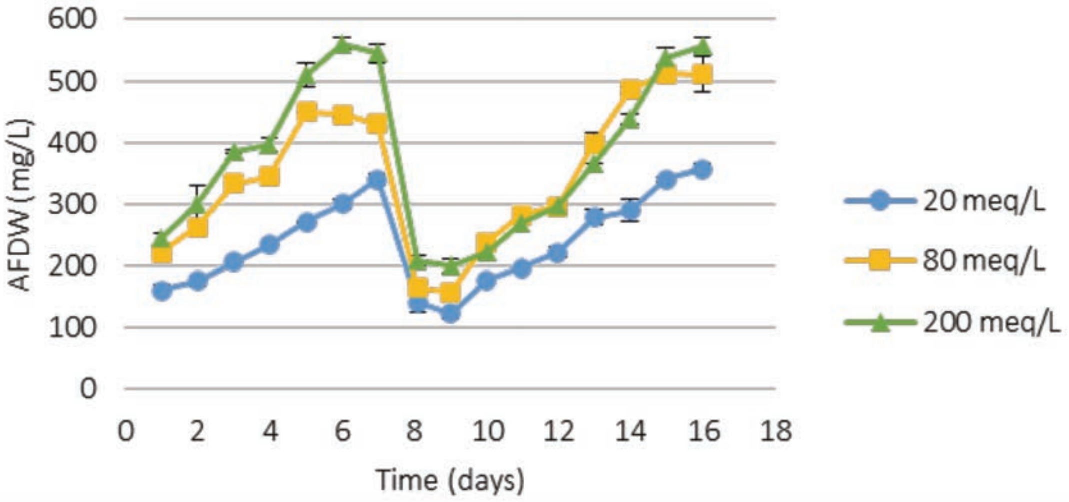


11 – 12.5 g AFDW/m^2-day productivity using only air-CO_2. Elevated alkalinity required to increase bicarbonate concentrations at high pH.



Northern Arizona late-summer conditions
15 – 30°C water temperature, 1,500 µM/m^2-s max. irradiance



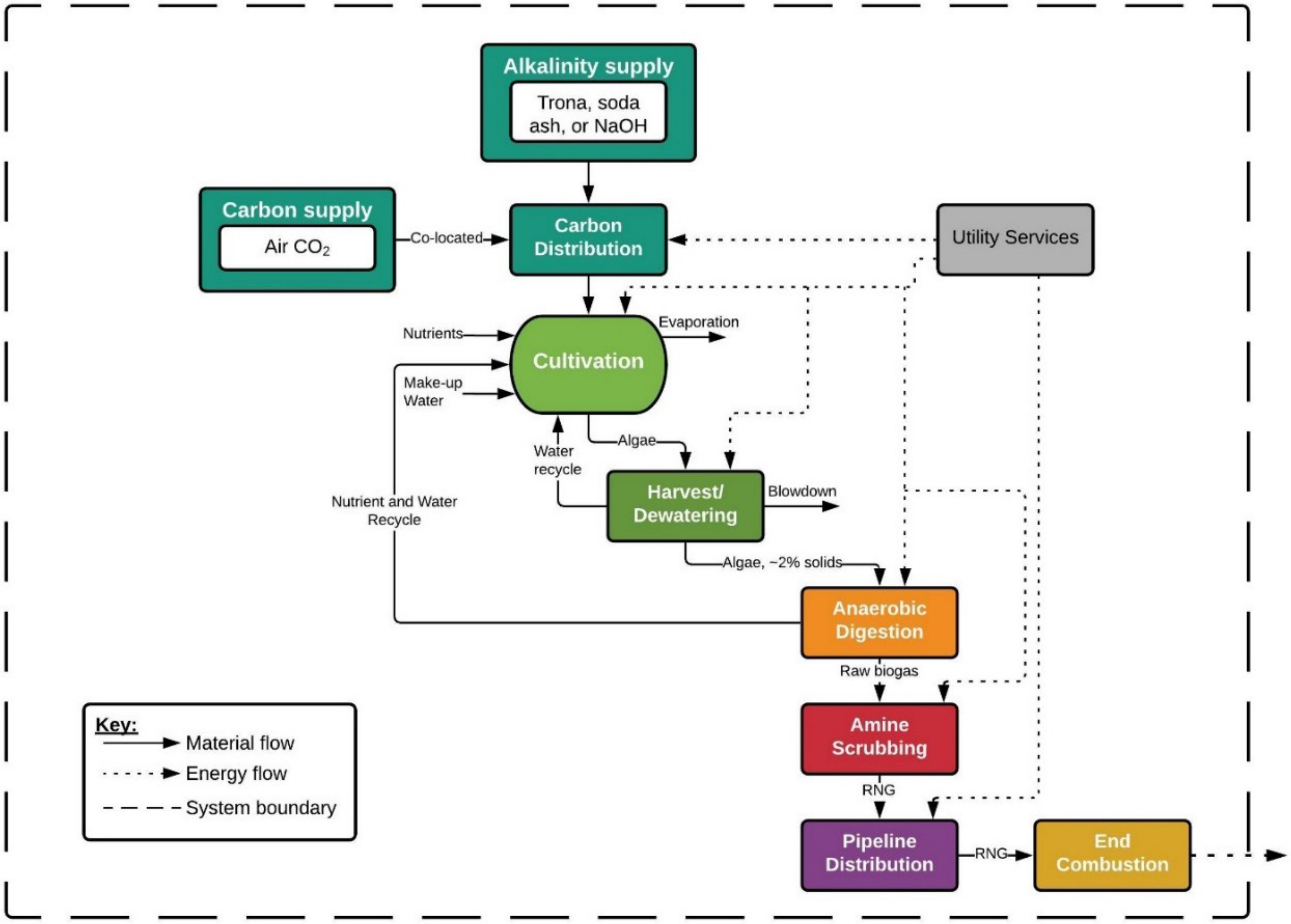


The final dissolved inorganic carbon (DIC) was greater than or equal to the initial DIC at the three alkalinities, indicating 100% air-CO_2 supported biomass growth.

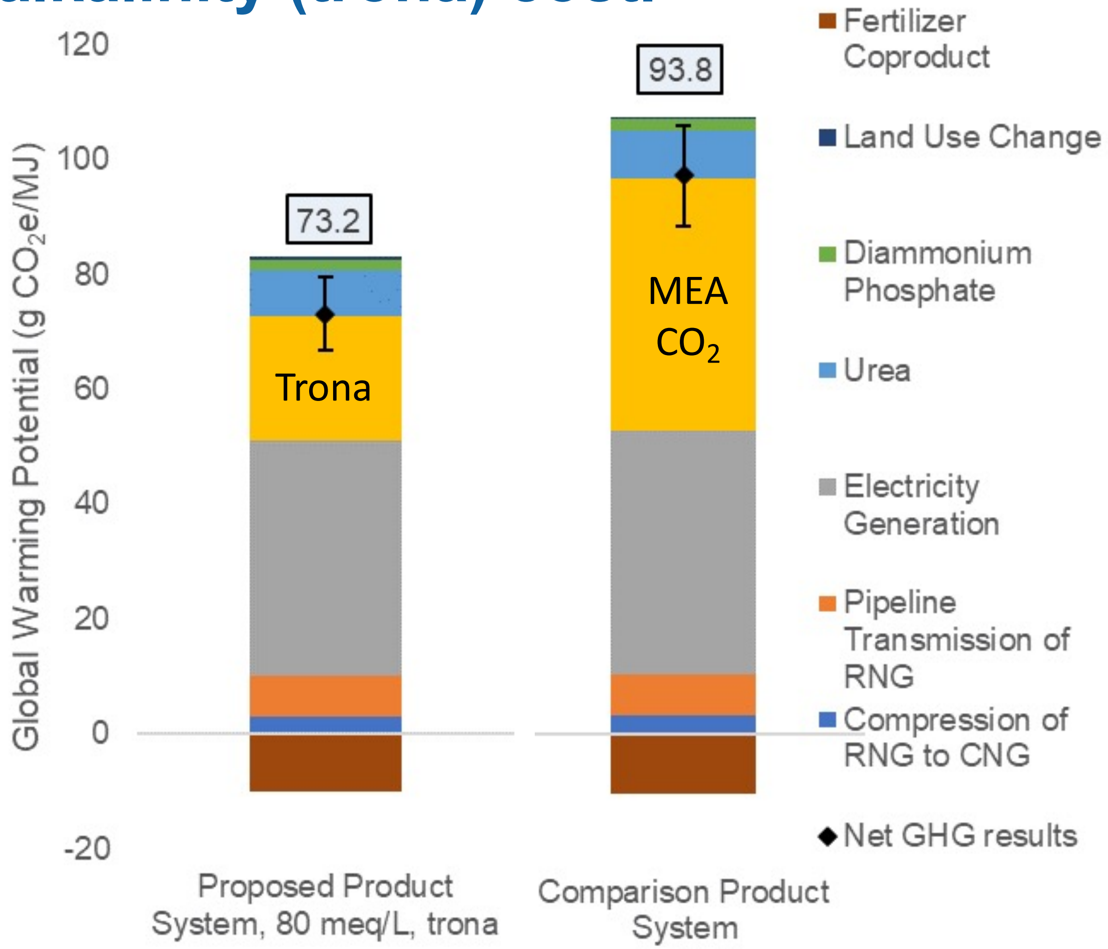
TEA/LCA (cost and life-cycle assessment) for biomass conversion to renewable natural gas

Parameter	Value
Facility size	400 ha
Unit pond size	4 ha
Facility location	Florida
Water type	Seawater
Net evaporation rate	0.04 cm/day
Annual average productivity	10 g/m²-day
CO_2 source	Air
Main product	RNG
Coproducts	Biofertilizer

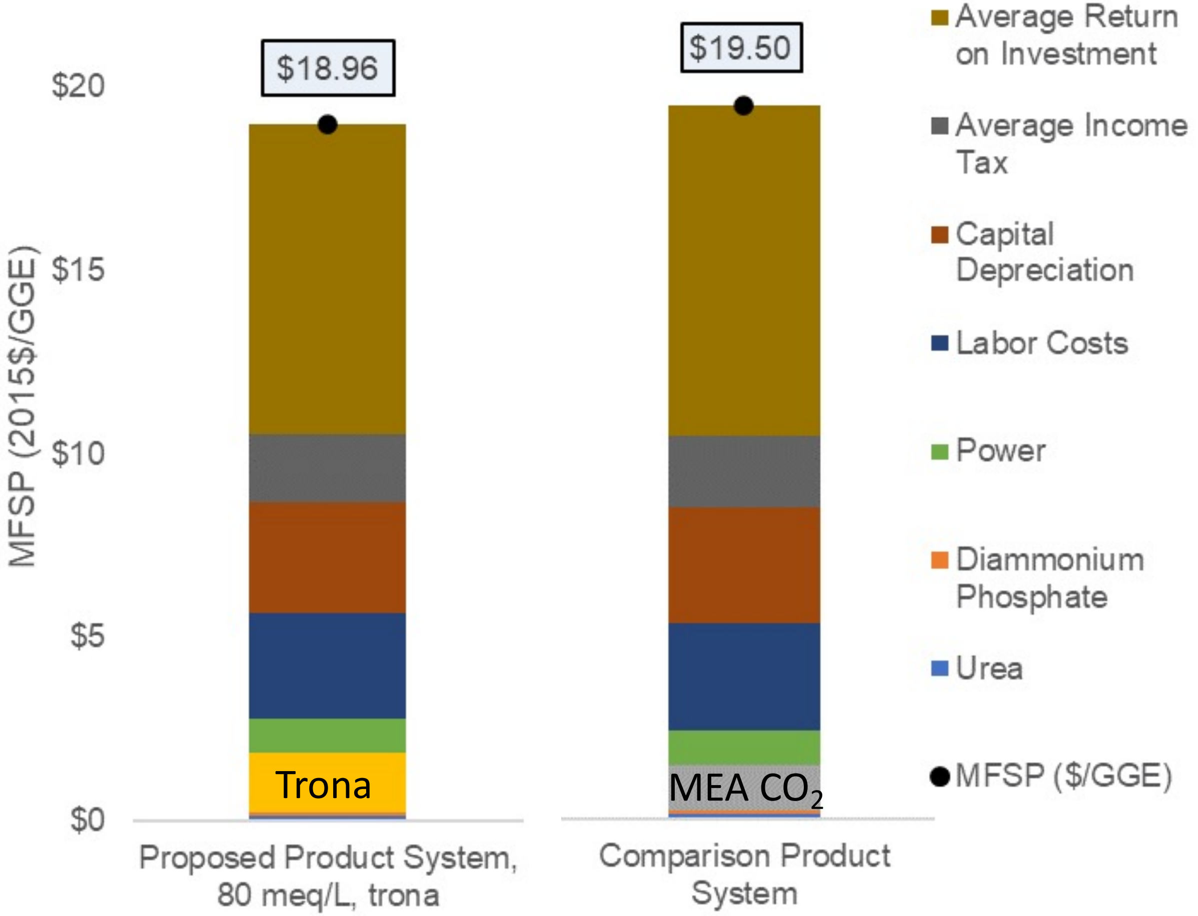
Comparison system: Identical facility layout and scale, but with post-combustion MEA purification of power plant CO_2 and a 20 km downstream delivery to raceways.



Substituting air-CO_2 for '2^nd^ generation' carbon capture technologies yields appreciable LCA benefits. TEA savings are minimal, as savings in CO_2 are offset by alkalinity (trona) cost.

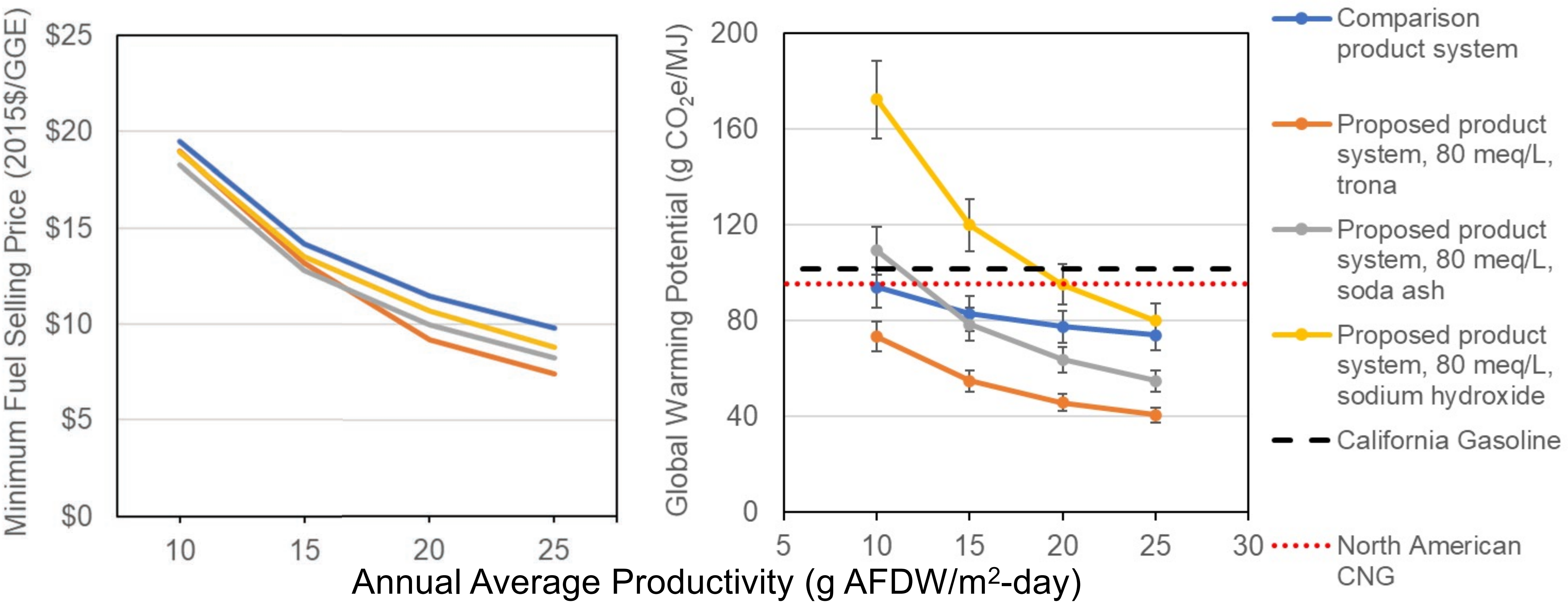


22% reduction in life cycle GHG emissions



3% MFSP reduction compared to supercritical CO_2 transport

Of the alkalinity sources examined, contributions to MFSP are comparable. Trona offers some savings via carbon credits (D5 RINS, LCSF)



MFSP decreases by ~$1 - 1.50/GGE when decreasing the alkalinity requirement to 20 meq/L

Summary

Overview – Increase raceway pond air-CO_2 transfer rates by cultivation at high pH.

Management – Experienced team with expertise in algae cultivation, from mL to acre scale.

Approach – Abiotically, identify pond conditions (pH, mixing speed) that yield ingassing rates to support high biomass productivity. Predict performance at scale (10-acre ponds). Next, identify strains able to thrive under such conditions. Use TEA/LCA outcomes to guide the research approach.

Impact – *10-fold expansion expansion* in algal feedstock resource potential, production of a lower-carbon intensity fuel, with minimal reduction in cost.

Progress and Outcomes – A pH of 12 or higher is needed to achieve air-CO_2 ingassing rates to support 20 g AFDW/m^2-day. Pond size is not a limiting factor for chemically-enhanced air-CO_2 transfer. Laboratory studies optimized and improved cyanobacterium SSL1 growth at elevated pH, achieving a productivity of 11 g AFDW/m^2-day in late-summer northern AZ conditions using only air-CO_2.

Future Work

Further increases in productivity, high value co-products are essential to meet cost targets.

- Test top-performing alkaliphilic candidates in repeated, replicated outdoor pond trials, tracking carbon inputs, outputs, sources, and sinks. Continue indoor screening/optimization to identify additional strains.
- Identify suitable downstream applications based on biochemical composition.
- Identify techno-economic, life-cycle implications of chemical softening
- Evaluate strain tolerance to source-water hardness (Ca, Mg), precipitation at high pH.

Quad Chart Overview (Competitive Project)

Award DE-EE008519: Timeline

- Start Date: 1/1/2019
- End Date: 6/30/2022

	FY20 Costed	Total Award
DOE Funding	$547,543	$1,314,406
Project Cost Share	$176,706	$2,899,179

Project Partners

- Qualitas Health
- Pacific Northwest National Laboratory (PNNL)

Project Goal

The goal of this project is to eliminate the need to co-locate algal cultivation facilities with concentrated sources of CO_2, such as power plant flue gases, by identifying strains that take advantage of the increase in air-CO_2 flux at high pH, thereby increasing the CO_2 resource potential for production of algal fuels by well over ten-fold and reducing the overall feedstock production cost.

End of Project Milestones

1. Achievement of 15 g/m^2-d of algae AFDW productivity from air-CO_2 only with alkaliphilic algae in indoor and outdoor cultures, validated with a model for air CO_2 transfer at scale.
2. TEA/LCA projecting that at 15 g/m^2-d, process economics would be similar as with flue gas CO_2, at 20 g/m^2-d, but with a nationwide algal biofuels resource potential an order of magnitude higher than for a flue gas colocation scenario

Funding Mechanism

Efficient Carbon Utilization in Algal Systems (DE-FOA-0001908)

Topic Area 2: Direct Air Capture Systems (5/3/2018 Issue Date)

Dan Fishman (Project Officer). Evan Mueller (Project Monitor)

Additional Slides

Responses to previous reviewers' comments or Go/No-Go Review

This project was not reviewed during the 2019 DOE BETO Peer Review

No comments have been provided from the Go/No-Go meeting (12/14/2020)

Task Overview

The project approach is organized into ten tasks over two budget periods.

Budget Period 2 (24 months)

- Task 1, Validation: (All)
- Task 2, Experiment design, mass transfer model development, techno-economic, life cycle analyses (MBE)
- Task 3, Hydrodynamic and mass-transfer studies (QH, MBE)
- Task 4, Alkaliphilic strain characterization (PNNL)
- Task 5, Development of carbon mass-balance approach, Bioprospecting (MBE)

Budget Period 3 (18 months)

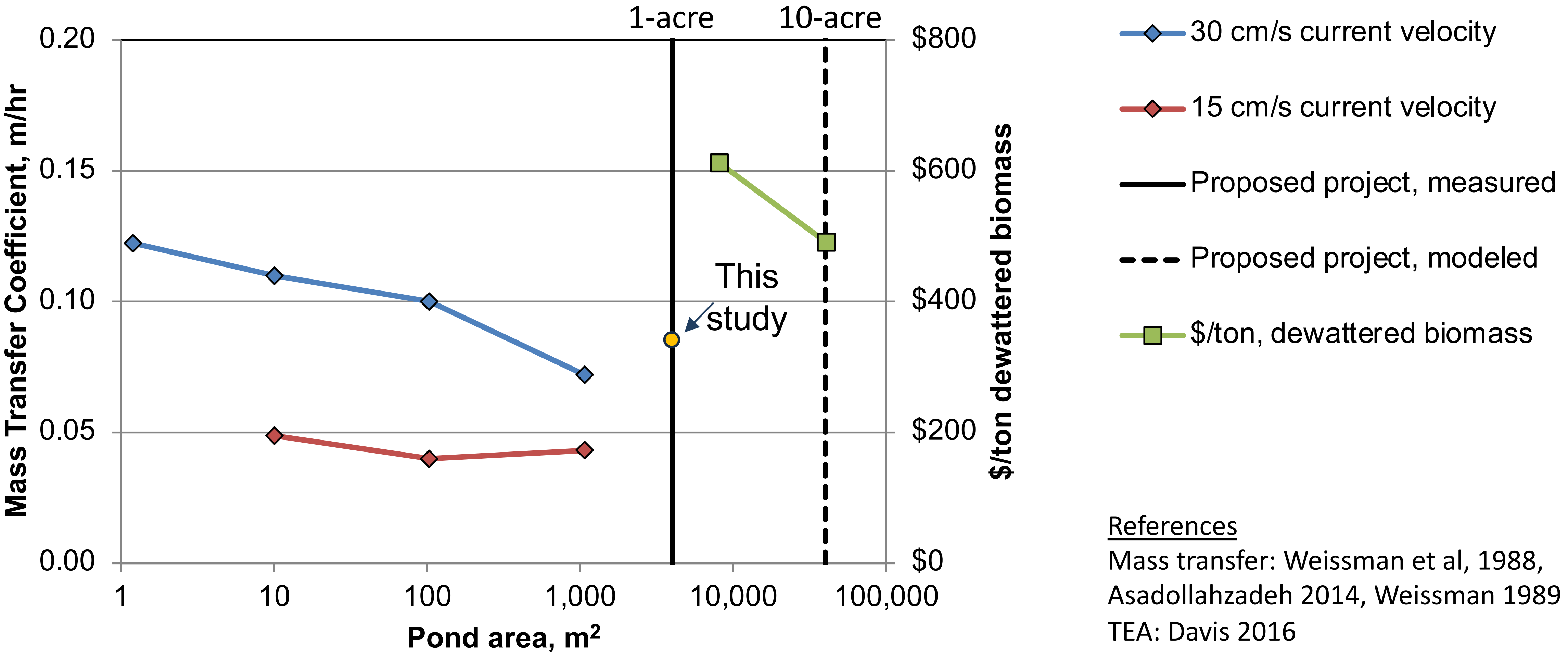
- Task 6, Complete 1-acre pond studies, begin biotic trials (QH, MBE)
- Task 7, Validate alkaliphilic strain performance in ponds (PNNL)
- Task 8, Carbon mass balances for sustained cultivation trials (MBE)
- Task 9, Update mass-transfer, TEA/LCA models (MBE)
- Task 10, Final data analysis, experiments, and report (All)

Jan/2018

Dec/2020

Jun/2022

Increasing pond size can decrease biomass costs ($612 to $491/ton, Davis 2016) As pond size increases, the mass transfer coefficient, k_L, decreases, even when current velocity stays at 30 cm/s. Our data suggest that this should not affect the chemically-enhanced tranfer rate.



References

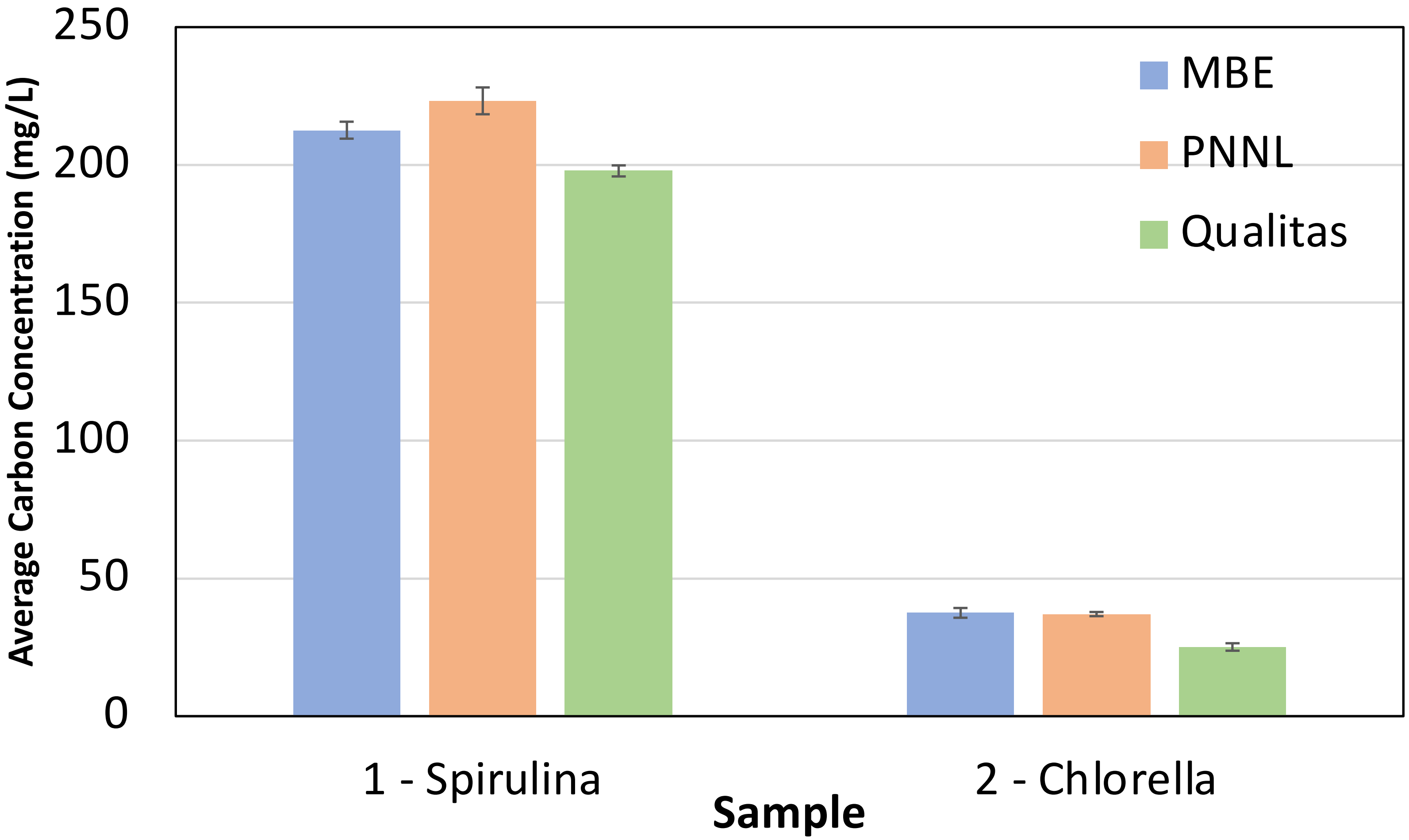
Mass transfer: Weissman et al, 1988, Asadollahzadeh 2014, Weissman 1989

TEA: Davis 2016

An initial round robin aligned analytical methods between project partners to allow accurate and reproducible carbon mass balances

Additional harmonized values:

- pH
- Salinity
- Alkalinity
- Dissolved inorganic carbon
- Dissolved organic carbon
- AFDW



Alkaline Strain Screening Methodology:

~28 Strains

Revive in Alkaline Media

- Qualitative observations
- Alkaline-activated mother cultures stored in library

9 Strains

Cultivate in Alkaline Media

- Comparative growth assessment
- Monitor daily:
  - OD_{750}
  - pH

3 Strains

LEAPS Areal Productivity

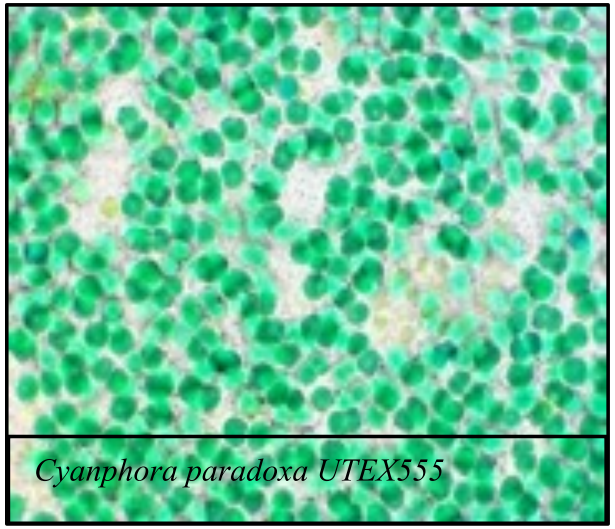
- Climate simulated cultivation

1 Strain

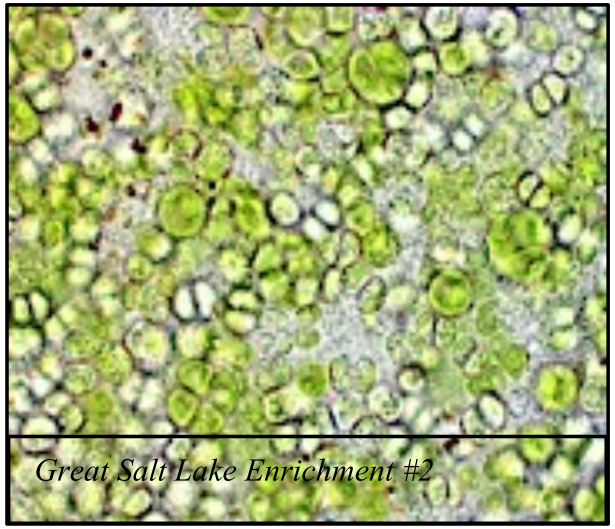
Optimize medium HCO_3^-

- Growth as a function of alkalinity
- Saturating light intensity

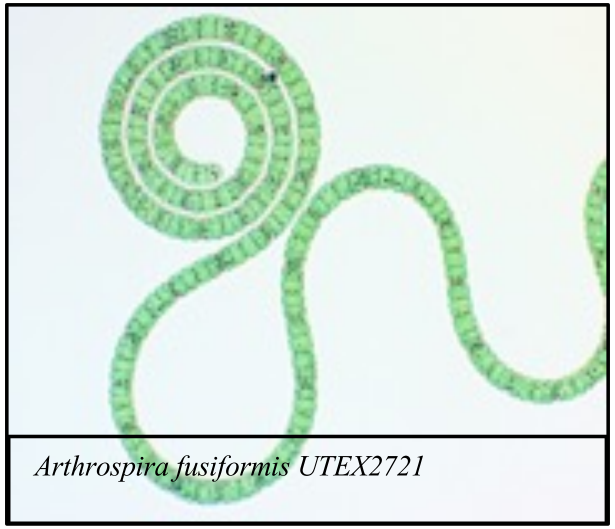
A wide range of genera examined:



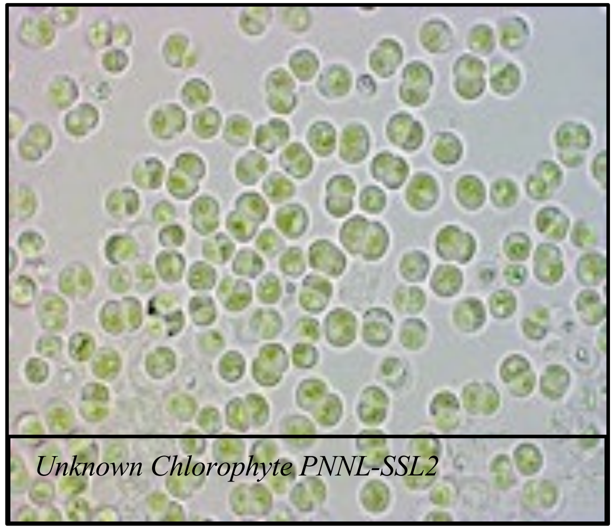
Cyanphora paradoxa UTEX555



Great Salt Lake Enrichment #2

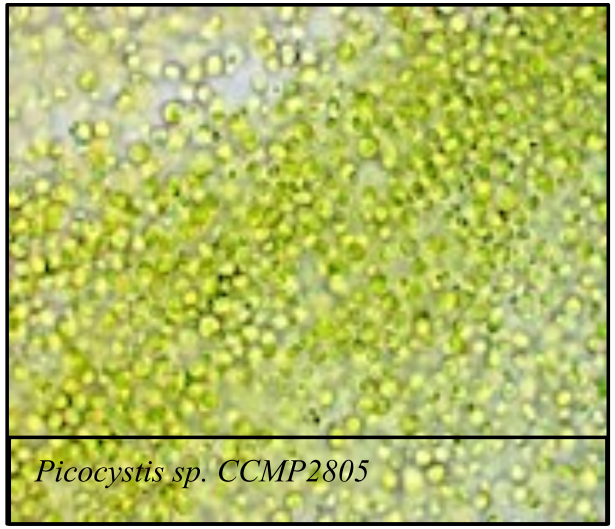


Arthrospira fusiformis UTEX2721

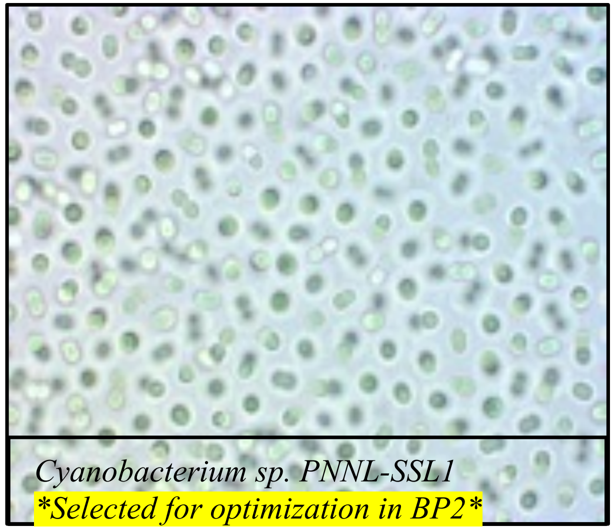


Unknown Chlorophyte PNNL-SSL2

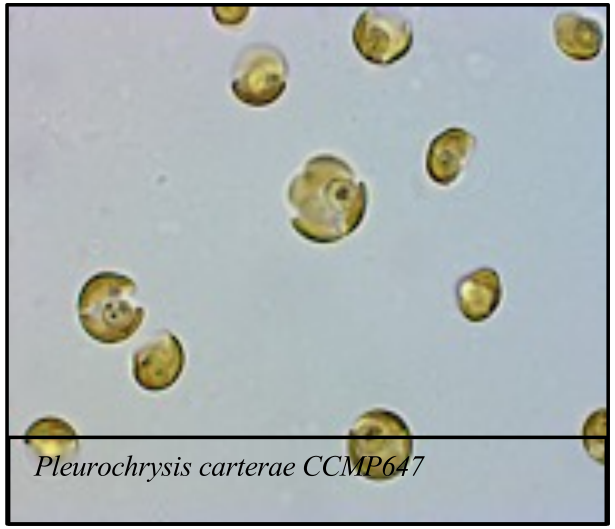
Phaeodactylum tricornutum UTEX555



Picocystis sp. CCMP2805

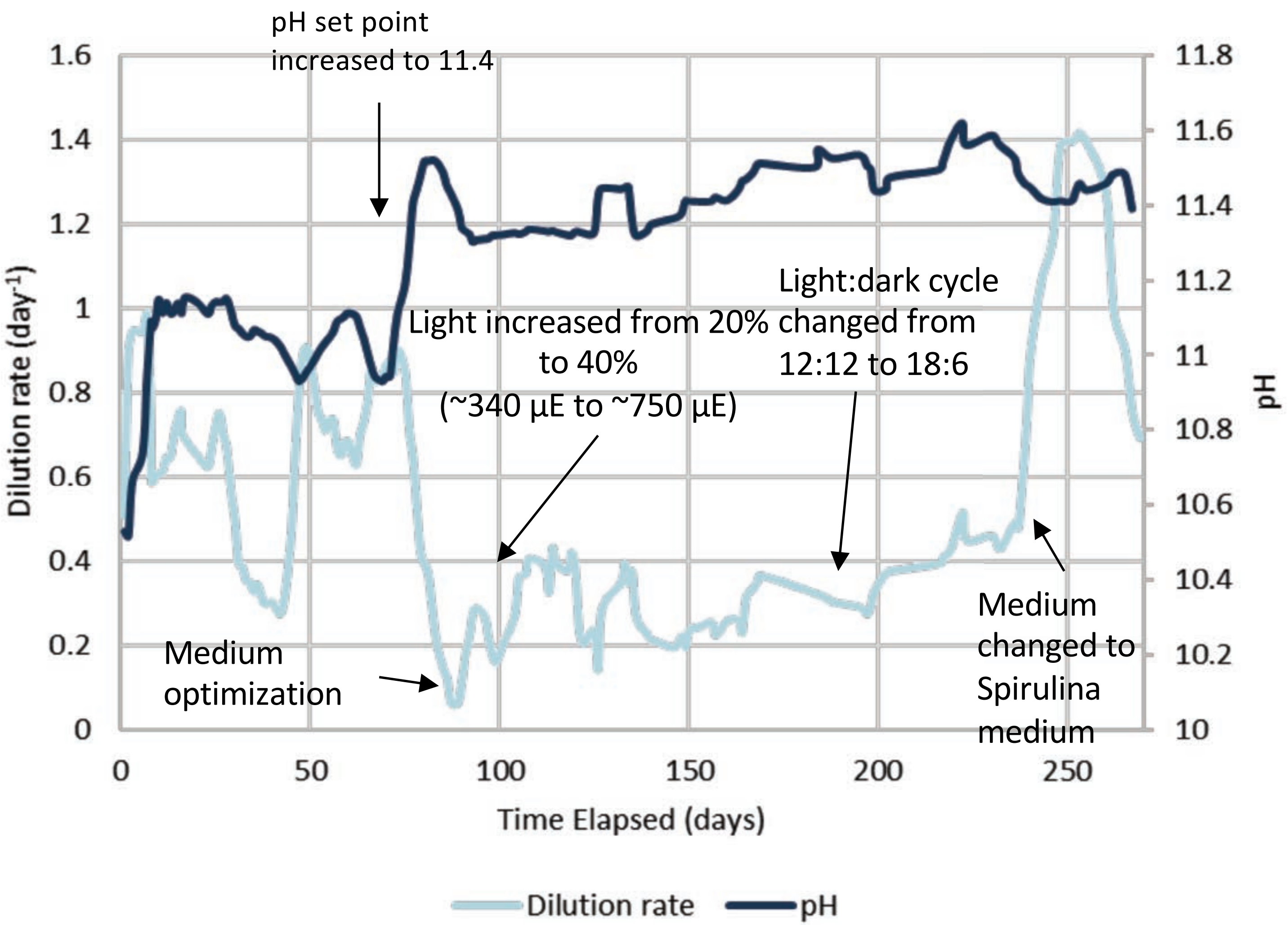


Cyanobacterium sp. PNNL-SSL1
Selected for optimization in BP2



Pleurochrysis carterae CCMP647

SSL1 Turbo Adaptive Laboratory Evolution



pH set point increased to 11.4

Light increased from 20% to 40% (~340 µE to ~750 µE)

Light:dark cycle changed from 12:12 to 18:6

Medium optimization

Medium changed to Spirulina medium

Adaptive laboratory evolution of SSL1 was carried out in a high-pH turbidostat for more than 250 days. The culture conditions changed over time as the strain became more adaptive to high pH and other research work progressed (e.g., medium optimization), and dilution rate changed consequently. The strain was improved and achieved higher biomass productivity at pH 11 than the wild type in the LEAPS.

A carbon balance compares the change in particulate and dissolved organic and inorganic carbon throughout a batch growth cycle to the expected amount ingassed, based on the air-CO_2 mass transfer model. Example dataset:

Growth Cycle	DIC Start	PC Start	NPOC Start	DIC End	PC End	NPOC End	DIC change	PC Change	NPOC Change	Measured C Ingassed	Expected C Ingassed	% Closure
C Replete A												
10/15-10/22	1,579	42.5	5.0	1,485	163	5.0	-94.0	121	0	26.8	16.0	168
10/22-11/5	1,578	29.0	5.0	1,556	196	5.0	-22.4	167	0	144	32.8	440
11/5-11/19	1,568	26.6	5.0	1,617	120	5.0	49.0	93.3	0	142	28.4	502
												> 100% due to CO2 sparging
C Replete B												
10/15-10/22	1,579	41.0	5.0	1,478	157	5.0	-101	116	0	15.3	16.4	93.0
10/22-11/5	1,534	26.4	5.0	1,557	202	5.0	23.8	176	0	200	31.8	628
11/5-11/19	1,576	28.0	5.0	1,592	114	5.0	16.7	86.0	0	103	30.3	339
												> 100% due to CO2 sparging
Air Only A												
10/15-10/22	1,303	42.8	5.0	1,280	131	5.0	-22.9	87.8	0	64.8	38.3	169
10/22-11/5	1,274	29.7	5.0	1,326	118	5.0	52	88.6	0	140	90.3	156
11/5-11/19	1,333	29.3	5.0	1,353	92.5	5.0	20.5	63.3	0	83.8	65.1	129
Air Only B												
10/15-10/22	1,307	38.8	5.0	1,292	123	5.0	-15.1	84.1	0	69.0	34.2	202
10/22-11/5	1,273	30.3	5.0	1,347	107	5.0	74	76.2	0	150	95.2	158
11/5-11/19	1,340	28.6	5.0	1,353	87.3	5.0	12.9	58.8	0	71.7	64.0	112

Goals are to (1) verify that assimilated carbon is derived from air (2) validate the air-CO_2 transfer model, and (3) verify analytical measurement accuracy.

Publications, Patents, Presentations, Awards, and Commercialization

Oral presentation at the International Conference on Algal Biomass, Biofuels and Bioproducts, “Air Carbon for Algae Production (AirCAP) – Expanding algae resource potential via direct (in-pond) air-CO_2 capture”. Presented by Braden Crowe. June 2019.

Oral presentation at the Algae Biomass Summit, “Microalgae Biomass Production for the Utilization of CO_2 and Mitigation of Greenhouse Gas Emissions”. Presented by John Benemann. September 2020.

References

Asadollahzadeh, M. J., Ardjmand, M., Seafkordi, A. A., & Heydarian, S. M. (2014). Efficient storage and utilization of CO 2 in open raceway ponds for cultivation of microalgae. *Korean Journal of Chemical Engineering*, *31*(8), 1425-1432.

Davis, R., Markham, J., Kinchin, C., Grundl, N., Tan, E. C., & Humbird, D. (2016). *Process design and economics for the production of algal biomass: algal biomass production in open pond systems and processing through dewatering for downstream conversion* (No. NREL/TP-5100-64772). National Renewable Energy Lab.(NREL), Golden, CO (United States).

Hatta, S. (1932) *Technical Reports Tohoku Imperial University* 10 (119)

Langholtz, M. H., Stokes, B. J., & Eaton, L. M. (2016). 2016 Billion-ton report: Advancing domestic resources for a thriving bioeconomy, Volume 1: Economic availability of feedstock. *Oak Ridge National Laboratory, Oak Ridge, Tennessee, managed by UT-Battelle, LLC for the US Department of Energy*, *2016*, 1-411.

Olander, D. R. (1960). Simultaneous mass transfer and equilibrium chemical reaction. *AIChE Journal*, *6* (2), 233-239.

Raven, J. A., & Johnston, A. M. (1991). Mechanisms of inorganic-carbon acquisition in marine phytoplankton and their implications for the use of other resources. *Limnology and Oceanography*, *36*(8), 1701-1714.

Venteris, E. R., McBride, R. C., Coleman, A. M., Skaggs, R. L., & Wigmosta, M. S. (2014). Siting algae cultivation facilities for biofuel production in the United States: trade-offs between growth rate, site constructability, water availability, and infrastructure. *Environmental science & technology*, *48*(6), 3559-3566.

Weissman, J. C., Tillett, D. M., & Goebel, R. P. (1989). *Design and operation of an outdoor microalgae test facility* (No. SERI/STR-232-3569). Microbial Products, Inc., Vacaville, CA (USA).

Weissman, J.C., Goebel, R.P., and Benemann, J.R., "Photobioreactor Design: Comparison of Open Ponds and Tubular Reactors", *Bioeng. Biotech., 31*: 336-344 (1988).