Efficient CO_2 Use for Robust Marine Microalgae Biomass Yields (MASS)

DE-EE0010292

Matthew Posewitz
Colorado School of Mines

2024 FECM/NETL Carbon Management Research Project Review Meeting
August 5 – 9, 2024

Overview: Efficient CO_2 Use for Robust Marine Microalgae Biomass Yields (MASS)

DE-EE0010292

Technology Summary

- **Combine high-productivity (high carbon uptake) alga with advanced diffuser/pond designs that are based on seminal designs first successfully implemented for efficient CO_2 utilization at the Roswell NM ponds. Use this combination to achieve 70% CUE and 20 gAFDW m^{-2} d^{-1} productivities.**
- **Use membrane filtration/pH-based cell flocculation for media clarification and medium recycle (residual carbon capture).**
- **Use cell mutagenesis and strain selection/screening to isolate strains with improved growth at elevated pH (~8.0) and for strains with higher lipid levels.**

High density Picochlorum

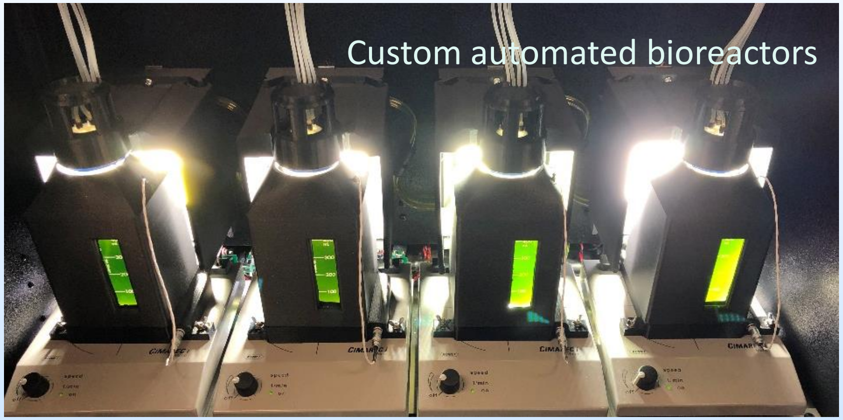
Key Personnel

Matthew Posewitz (Mines), Joseph Weissman, Arthur Grossman (Carnegie Institution for Science), Jason Quinn (Colorado State University), Braden Crowe (MicroBio Engineering), Michael Guarnieri (NREL)

Program Summary

Period of performance: 36 months

Federal funds: $3,000,000
Cost-share:$750,000
Total budget:$3,750,000



Custom automated bioreactors

Technology Impact

- **Provide industrially-relevant *Picochlorum* strains and pH cycling pond operations that achieve DOE BETO targets of at least 70% CUE and 20 gAFDW m^{-2} d^{-1} productivities in two summer campaigns. Publish advances for the algal biotechnology community.**
- **Develop *Picochlorum* strains with increased carbon use at pH 7.8 to 8.0; and with higher lipid content for conversion to SAF.**

High CUE/productivity using efficient CO_2 injection coupled with rapid carbon fixation

U.S. DEPARTMENT OF ENERGY

Overview: Efficient CO_2 Use for Robust Marine Microalgae Biomass Yields (MASS)

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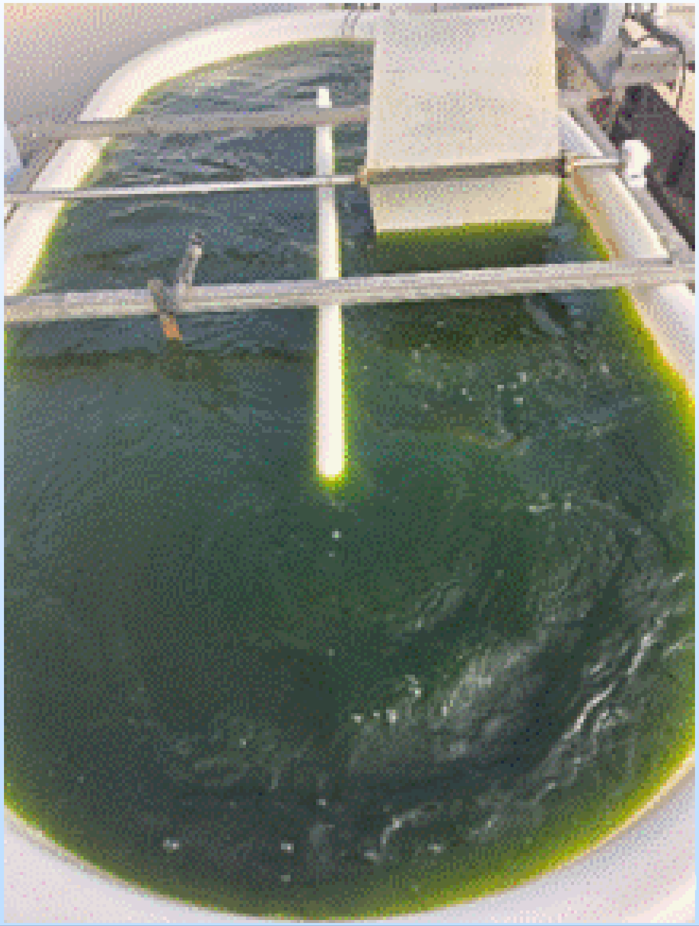
Project Dates

- BP1: 7/1/2023-9/30/2023
- BP2: 10/1/2023-3/31/2025
- BP3: 4/1/2025-9/30/2026



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Technology Summary: We propose to combine high-productivity (high carbon uptake) algae with advanced CO_2 transfer systems and pond operations based on innovations beyond the seminal designs for efficient CO_2 utilization, first demonstrated by the DOE- Aquatic Species Program at the Roswell, NM project. These efforts, combined with medium recycle and innovative strain improvements, will enable exceeding the FOA targets of 70% CUE (Carbon Use Efficiency) at 20 gAFDW m^{-2} d^{-1} productivity. CO_2 will be provided by DAC.

Description of the Technology's Impact: Develop and demonstrate in bioreactors and ponds (40 m^2) strains of *Picochlorum* with increased carbon use at optimized pH cycling regimes and with higher lipid yields. Target SAFs (sustainable aviation fuels) as biofuel products, with high value nutritional co-products.

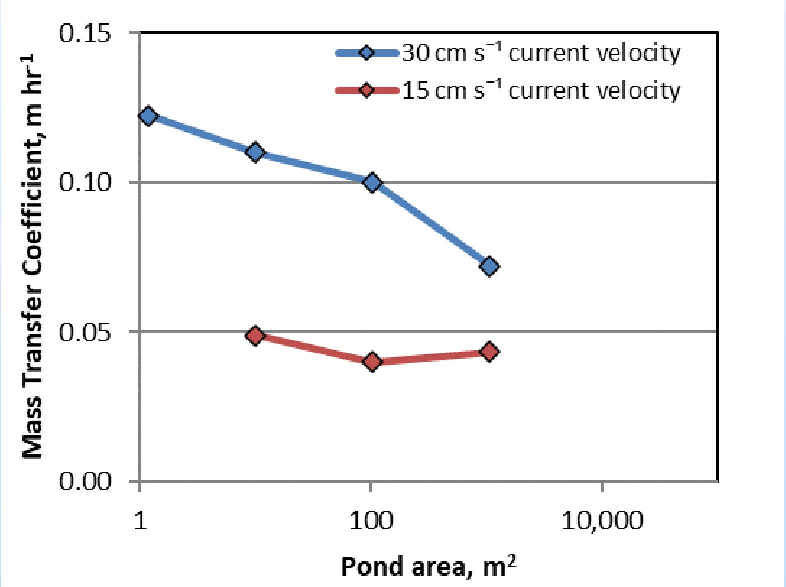
High CUE/productivity using efficient CO_2 injection coupled with rapid carbon fixation

U.S. DEPARTMENT OF ENERGY

Technology Background

Technological Advantages

- ***Fast growing marine alga***
- ***Extensive outdoor pond growth experience***
- ***Sump and CO_2 delivery expertise***
- ***Extensive strain development capabilities***

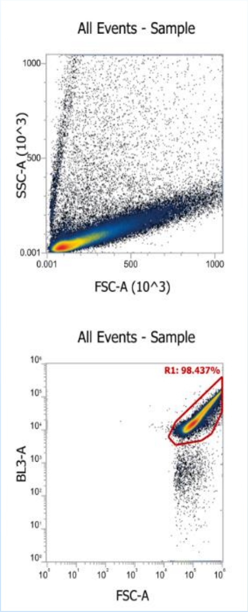


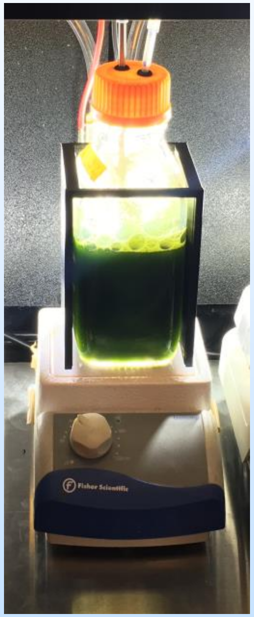
Asadollahzadeh, et al., (2014) *Korean Journal of Chemical Engineering* **31**, 1425-1432.
Weissman, et al., (1988) *Biotechnology and Bioengineering* **31**, 336-344.
Weissman, et al., (1989) SERI/STR-232-3569 SERI report.

Technology Background

Back to nature for new strains

Field → Selection → Isolation → Evaluation





Sampling from Natural Environments

Selective Growth Pressure and Enrichments

Flow Cytometry Isolation

Evaluate Productivities

Joseph Weissman

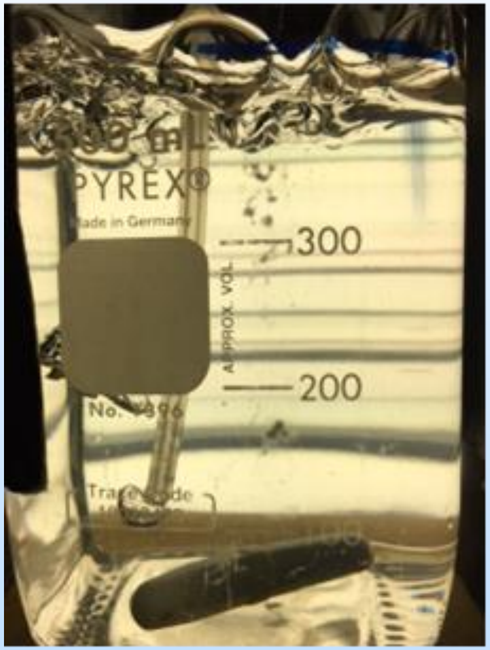
Thrive in high light, high salt, high temperature

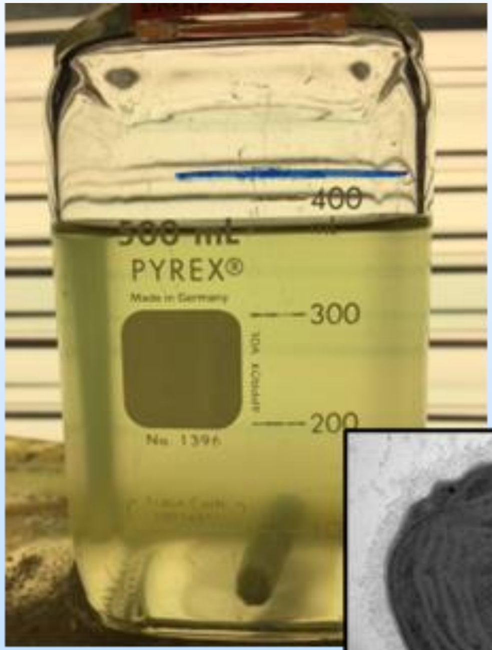
Technology Background

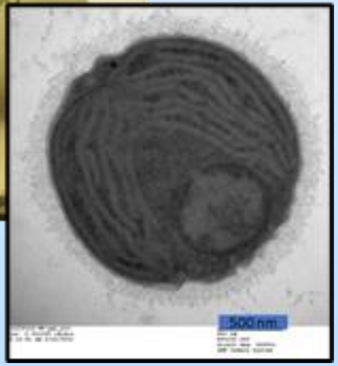
Back to nature for new strains

Field → Selection → Isolation → Evaluation

Joseph Weissman



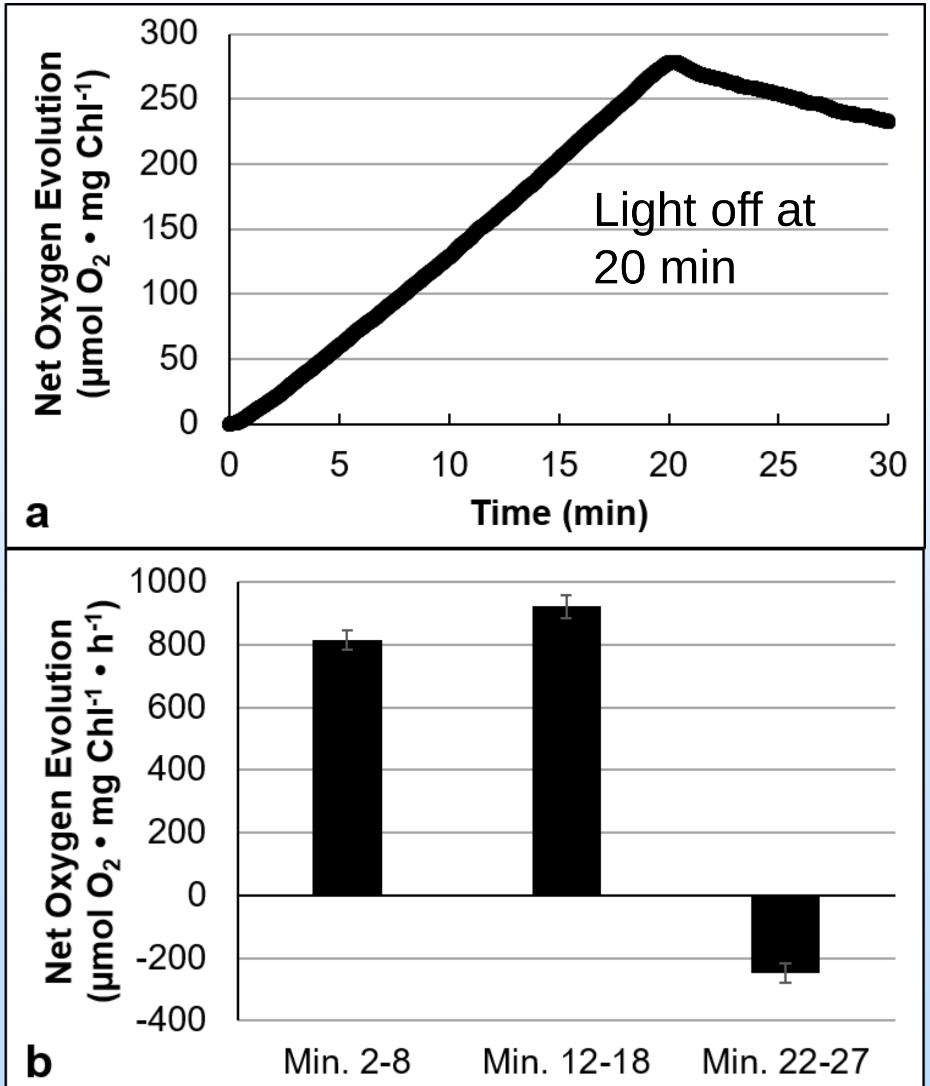




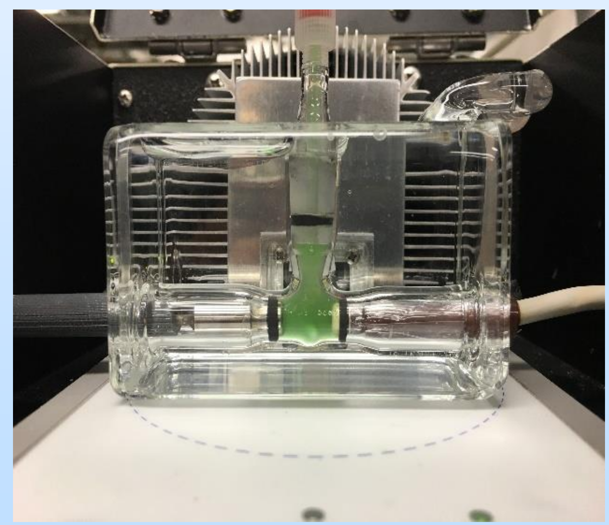
- **Enriched under high light (1000 μmol PAR $m^{-2}s^{-1}$ in seawater medium)**
- **Enriched *Picochlorum celeri***
- **Doubling time 2h**

Technology Background

Picochlorum celeri: high-light tolerant – rapid growth



P. celeri Isolation Vessel, Name	μ, h^{-1}	SD (n)	τ, h	$24 \cdot (\tau^{-1})$
E1	0.28	0.012 (5)	2.5	9.7
FACS Sorted, TG1	0.22	0.014 (9)	3.2	7.5
FACS Sorted, TG1 axenic	0.21	0.005 (3)	3.4	7.1
#2	0.29	0.013 (6)	2.4	10.0
#3, TG2	0.33	0.009 (14)	2.1	11.5
#3 replicate, TG2	0.33	0.003 (6)	2.1	11.5
#3, TG2 axenic	0.34	0.026 (3)	2.0	12.0
#6	0.30	0.010 (8)	2.3	10.4



Weissman et. al., *Algal Research* (2018) **36**, 17-28

Technology Background

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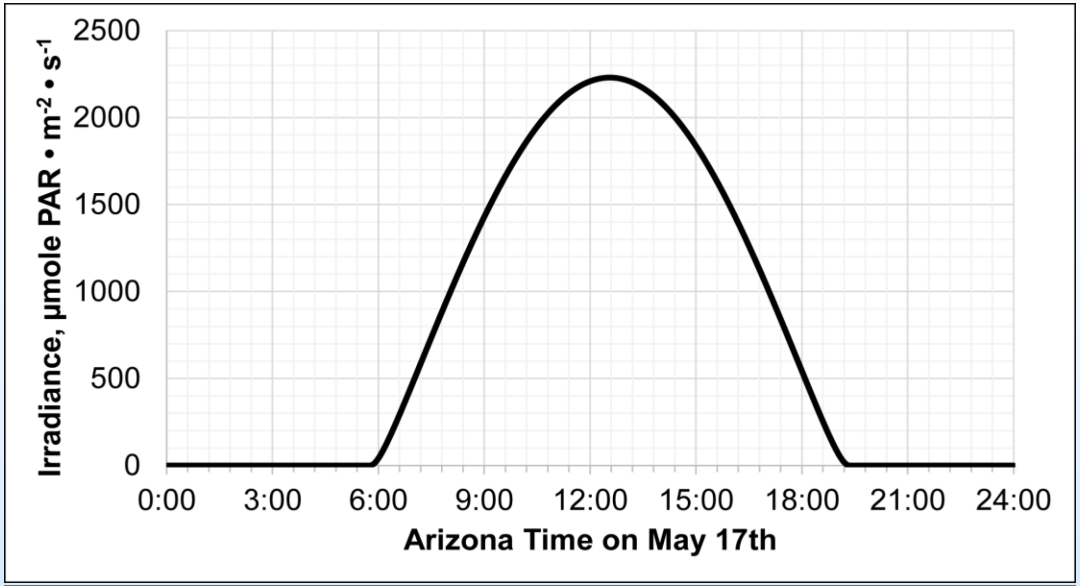
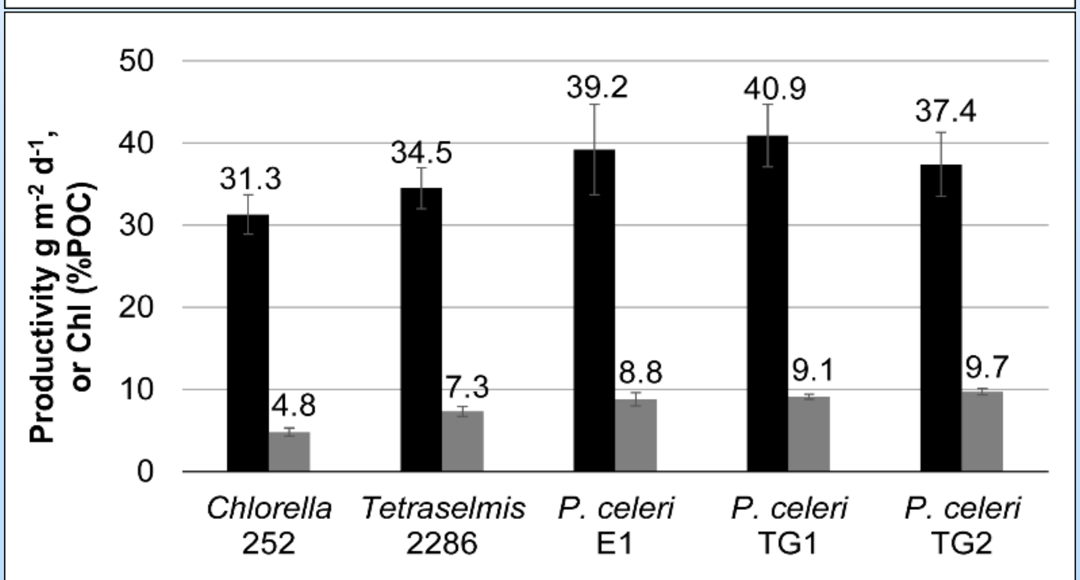
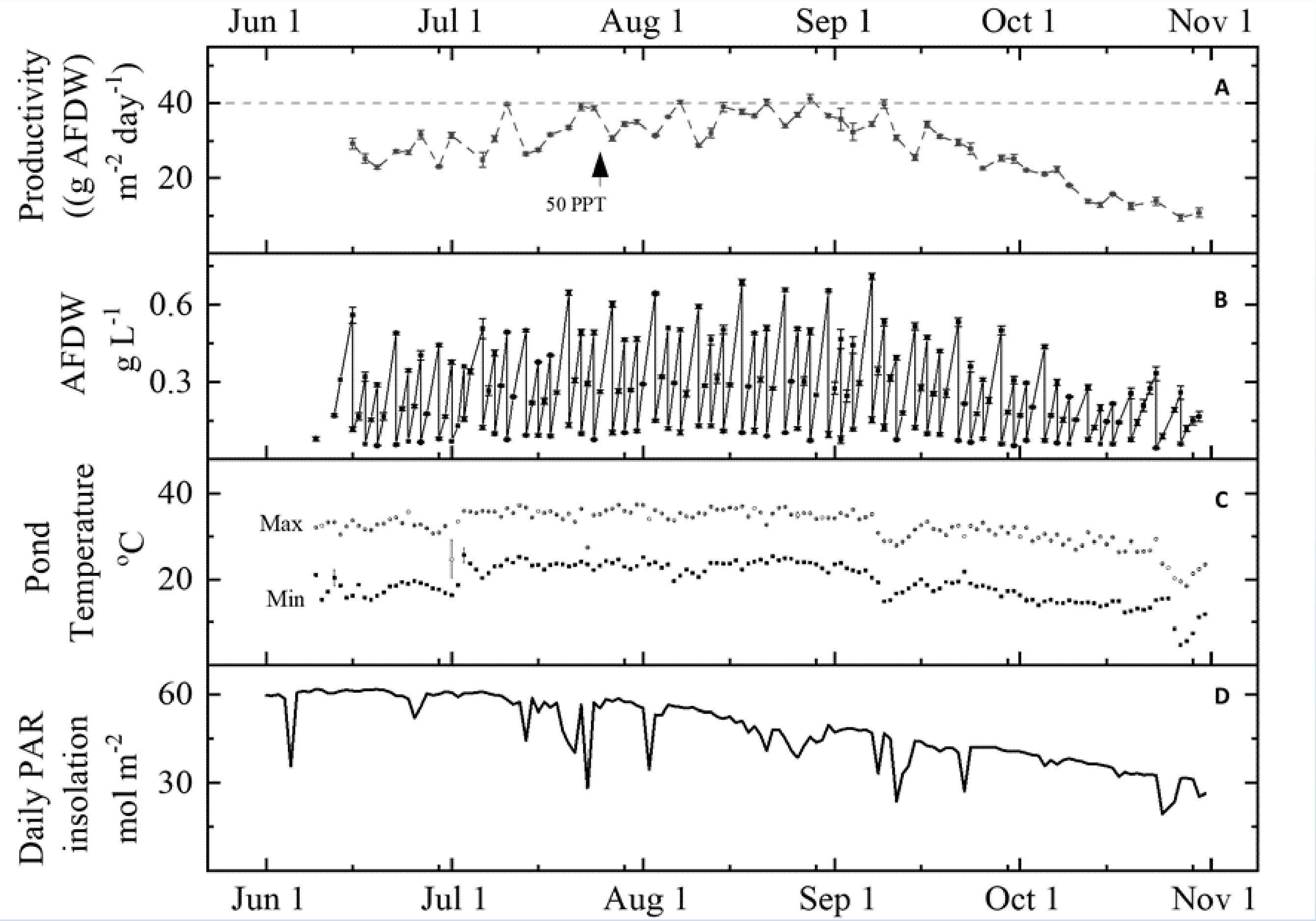


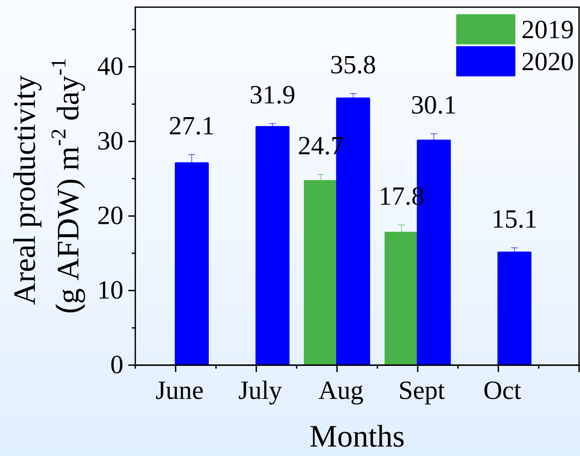


Weissman et. al., *Algal Research* (2018) **36**, 17-28

Technology Background

exemplary outdoor biomass yields in seawater





Krishnan et al. *Scientific Reports* (2021) **11**, 11649

-John McGowen AzCATI

Technical Approach/Project Scope
Key Milestones

- **Characterization of changes in C_i as a function of pH to determine CO_2 utilization [Q2]**
- **Optimize urea content in high-biomass media to minimize C loss [Q3]**
- **Experimentally characterize CO_2 injection efficiencies and outgassing to minimize C_i loss [Q4/Q5/Q6]**
- **Determine biomass productivities using CO_2 delivery on demand for increasing pH and selecting for higher CUE strains [Q7/Q8/Q9]**
- **Isolate high lipid *P. celeri* cells [Q10/Q11]**
- **Develop Custom Membrane Harvesting Unit and Quantify flocculation using high pH [Q4/Q7]**
- **Outdoor growth campaigns of *P. celeri* cells to determine productivities and CUEs [Q12/13]**
- **Techno-Economic and Life-Cycle Analyses [Q13]**
- **Diversity, equity and inclusion**

Technical Approach/Project Scope

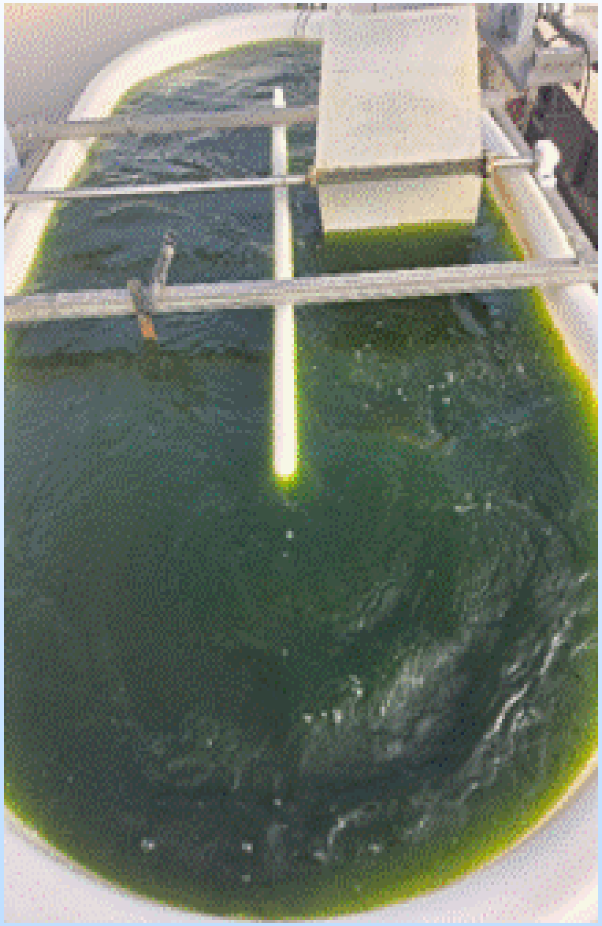
project success criteria and risks

Project Goals:

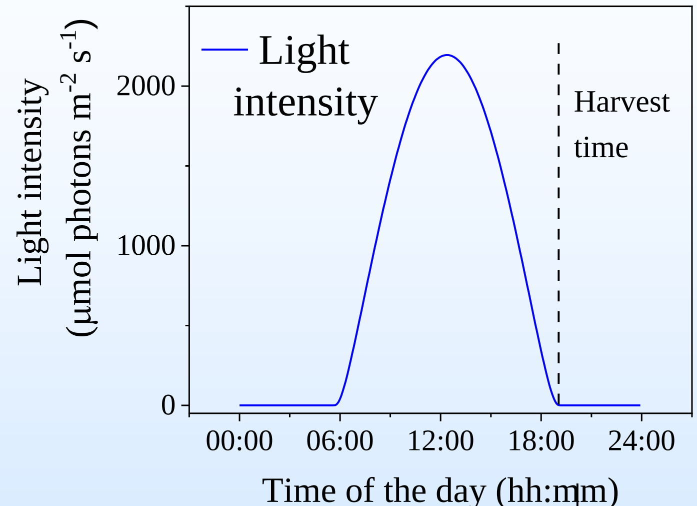
- Demonstrate the ability to achieve 20 g m^{-2} d^{-1} in *P. celeri* biomass productivity at 70% CUE in two 30-day summer campaigns outdoors.
- Improve carbon use efficiencies under high-productivity growth.

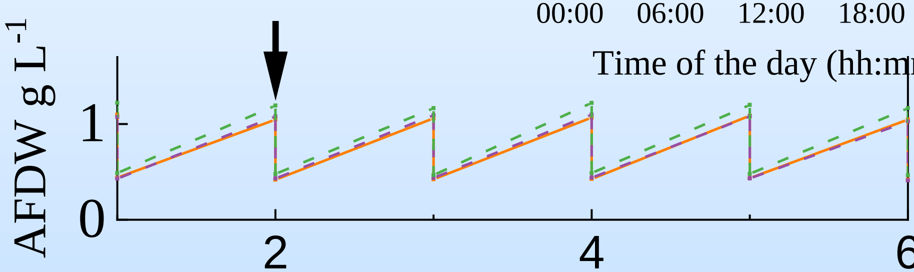
Project risks/mitigation strategies:

- Decreased carbon severely attenuates productivity/strain improvements, pond operation, new cultivars.
- High oxygen reduces biomass productivities/use selective pressures to attain higher O_2-tolerant strains.
- Harvesting and media recycling are inefficient/distinct harvesting mechanisms being investigated.



Progress and Current Status
testing MBE-site water





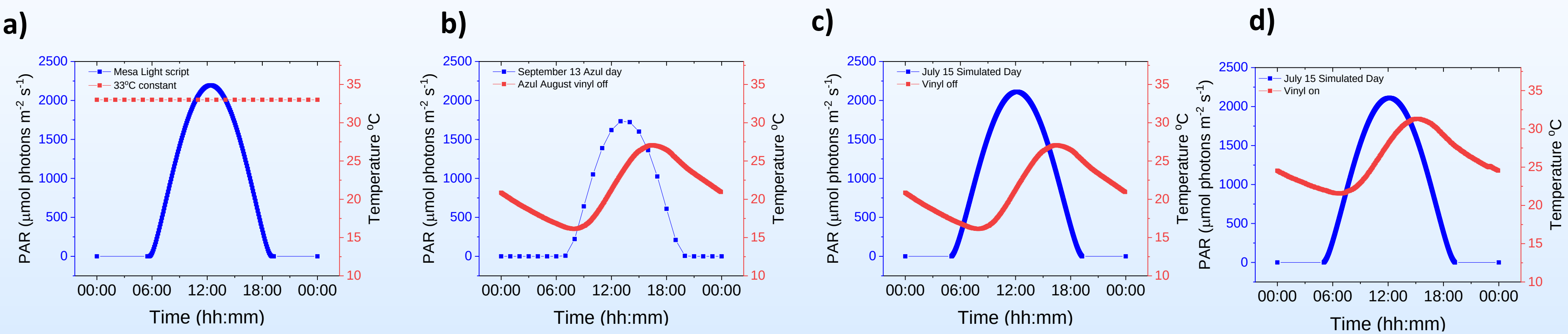
Media: Well water ~ pH 8.0, 0.5ml HCl was used to neutralize
Media composition: 100N:10P:3Fe (Proline)
Media was not filtered
Light script: Mesa Arizona day
Temperature: 33°C constant
Harvest time: 7 pm (dark)

AFDW (g L^{-1})	0.97 (0.01)
Areal Productivity (g m^{-2} d^{-1})	39.4 (0.8)

Typical 40 g/m^2/d

Progress and Current Status

Picochlorum celeri *growth at variable temperatures*

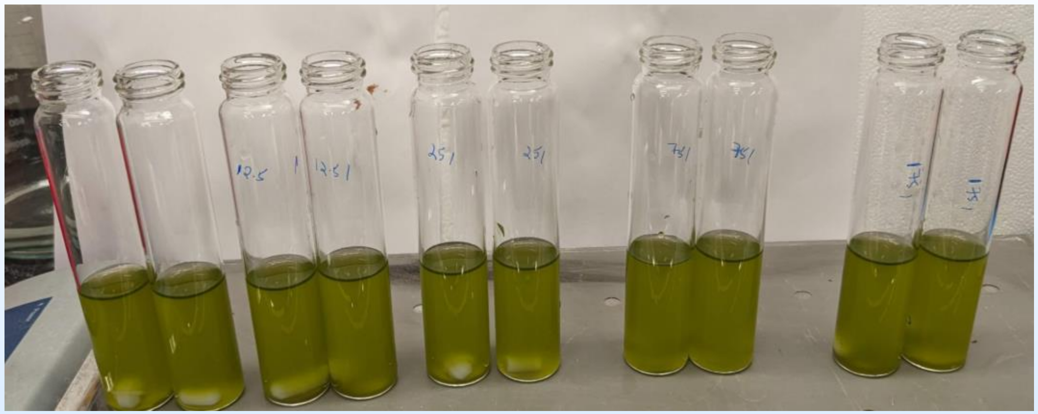


Light	Mesa light script (Control) PAR (62 mol photons/m²)	September 13 Azul day (CIMIS data) PAR (47 mol photons/m²)	July 15 Simulated Day PAR (64 mol photons/m²)	July 15 Simulated Day PAR (64 mol photons/m²)
Temp	33°C constant	Vinyl off (August)	Vinyl off (August)	Vinyl on (September)
Dilution	60%	35%	45%	55%
Producitivty	40.1 ± 2.4 g/m²/d	20.4 ± 1.1 g/m2/d	30.4 ± 0.5 g/m2/d	37.9 ± 0.3 g/m²/d

Progress and Current Status

Picochlorum celeri flocculation

Time 0



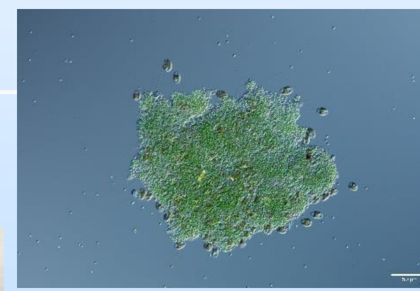
Ctrl

12.5 ul 1M NaOH

25 ul 1M NaOH

75 ul 1M NaOH

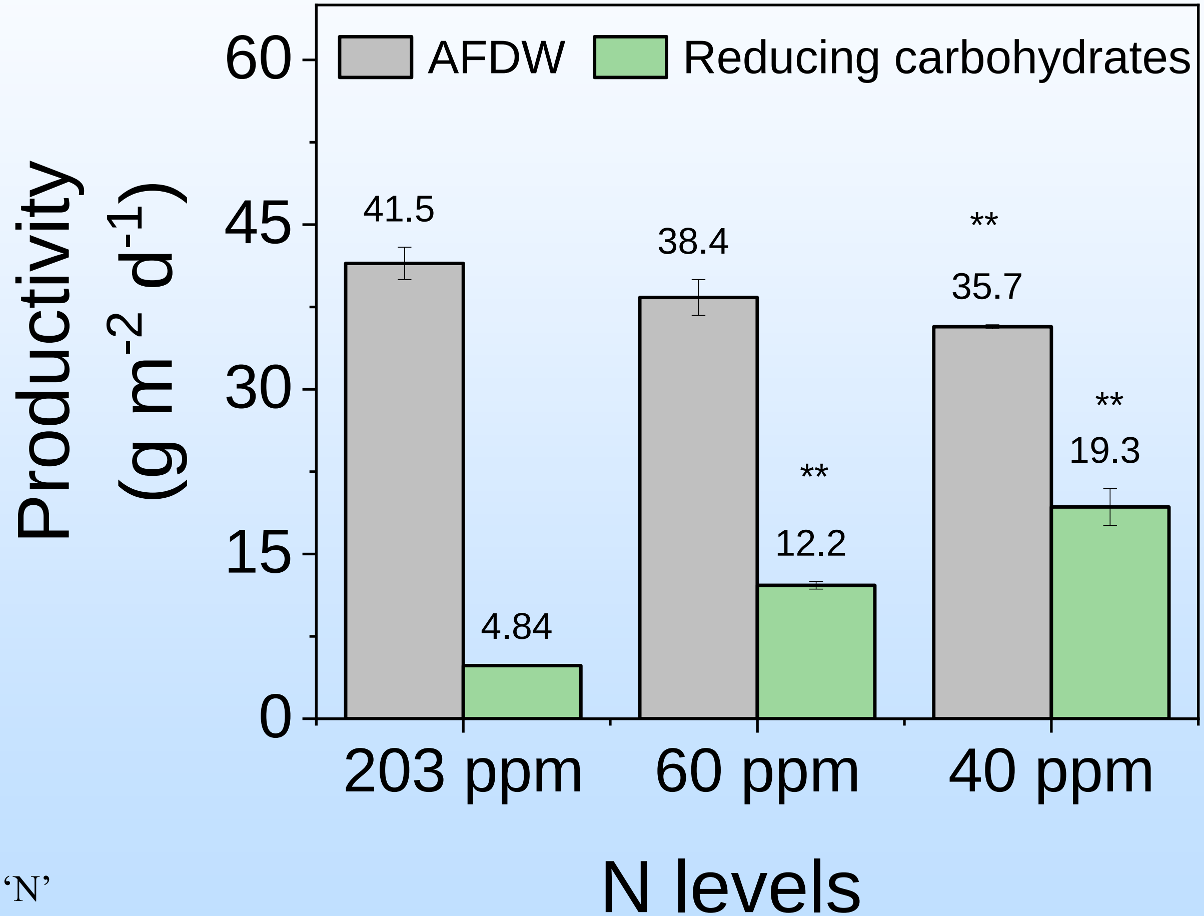
175 ul 1M NaOH



Overnight incubation

Progress and Current Status

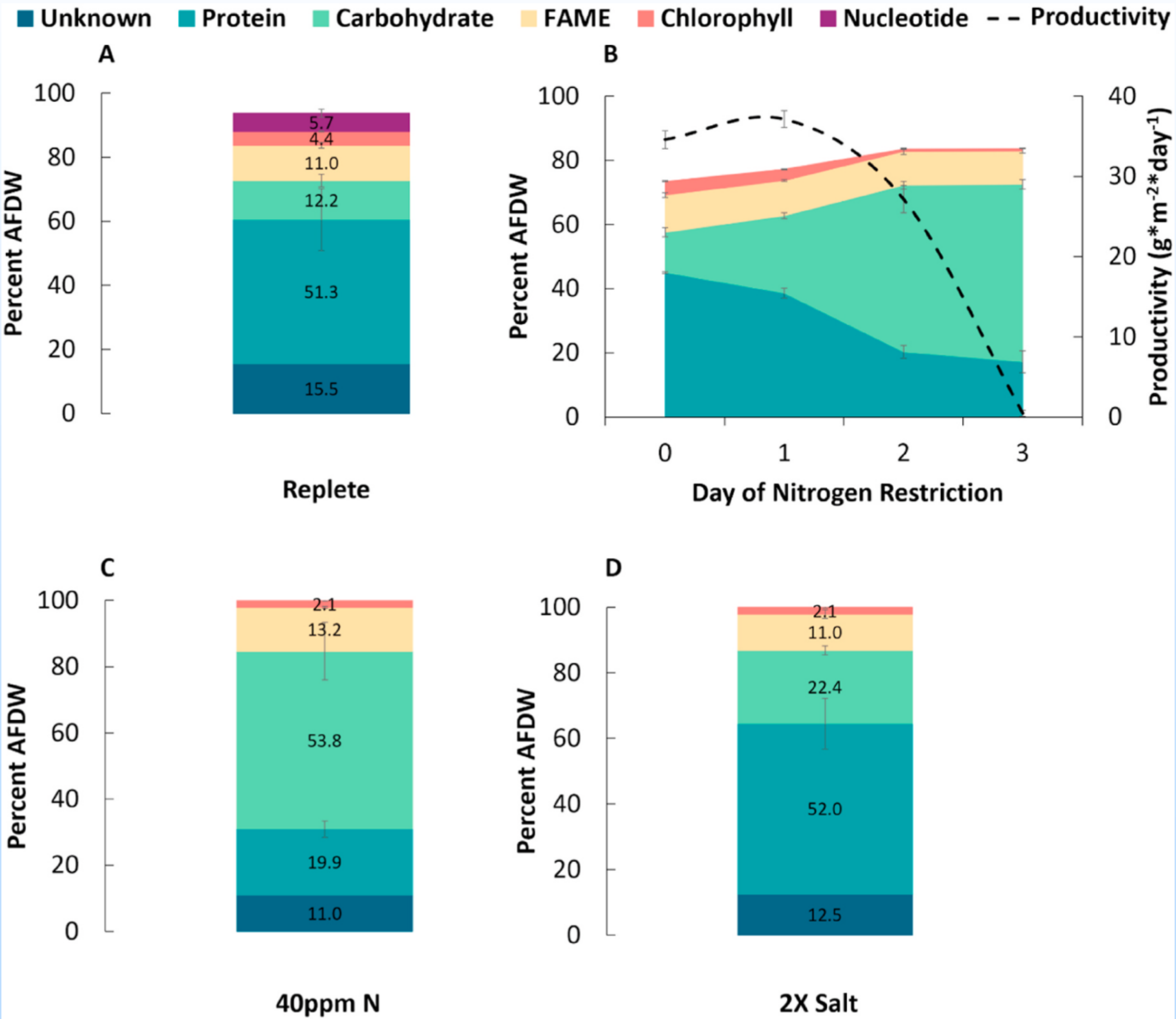
Picochlorum celeri *productivities at distinct urea levels*



1ppm N =1 mg/L 'N'
203 ppm N = 436 mg/L urea (Dense culture medium)
60 ppm N = 128.6 mg/L urea
40 ppm N = 85.7 mg/L urea

Progress and Current Status

Picochlorum celeri *productivities at distinct urea levels*

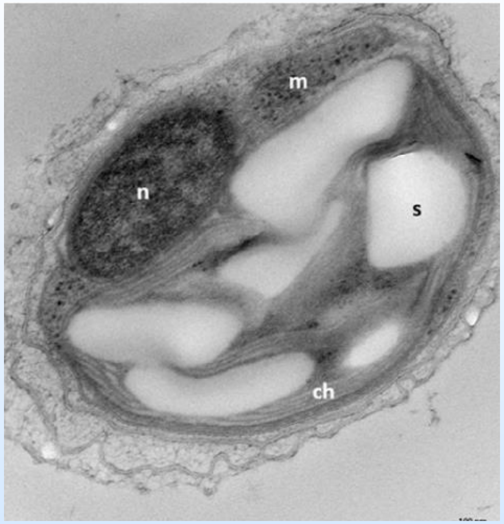


Progress and Current Status

Picochlorum celeri *productivities at distinct urea levels*

	203 ppm N	60 ppm N	40 ppm N
AFDW (g L^{-1})	1.04 (0.03)	0.98 (0.03)	0.89 (0.01)**
Chlorophyll (mg L^{-1})	48.0 (1.5)	31.6 (1.4)***	18.7 (1.1)***
Chl a/b	4.6 (0.2)	5.1 (0.4)	4.3 (0.3)
Carbohydrate content (mg L^{-1})	122.7 (1.9)	308.3 (10.5)***	475.7 (39.7)***
[a]Carbohydrate fraction (%)	11.7 (0.5)	31.9 (1.8)***	53.8 (4.3)***

Each data point is average and standard error for 4 biological replicates. * indicates statistical significance

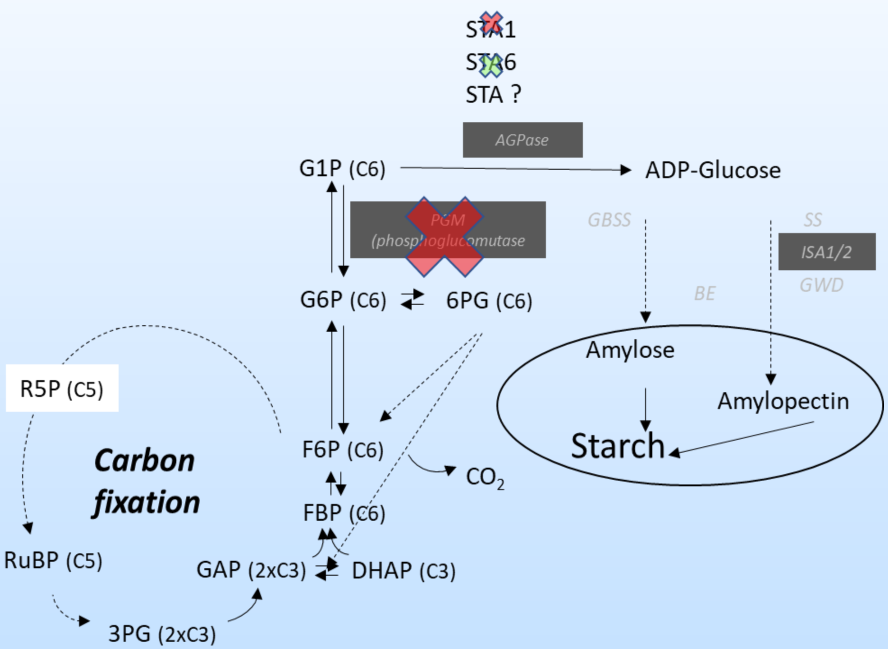


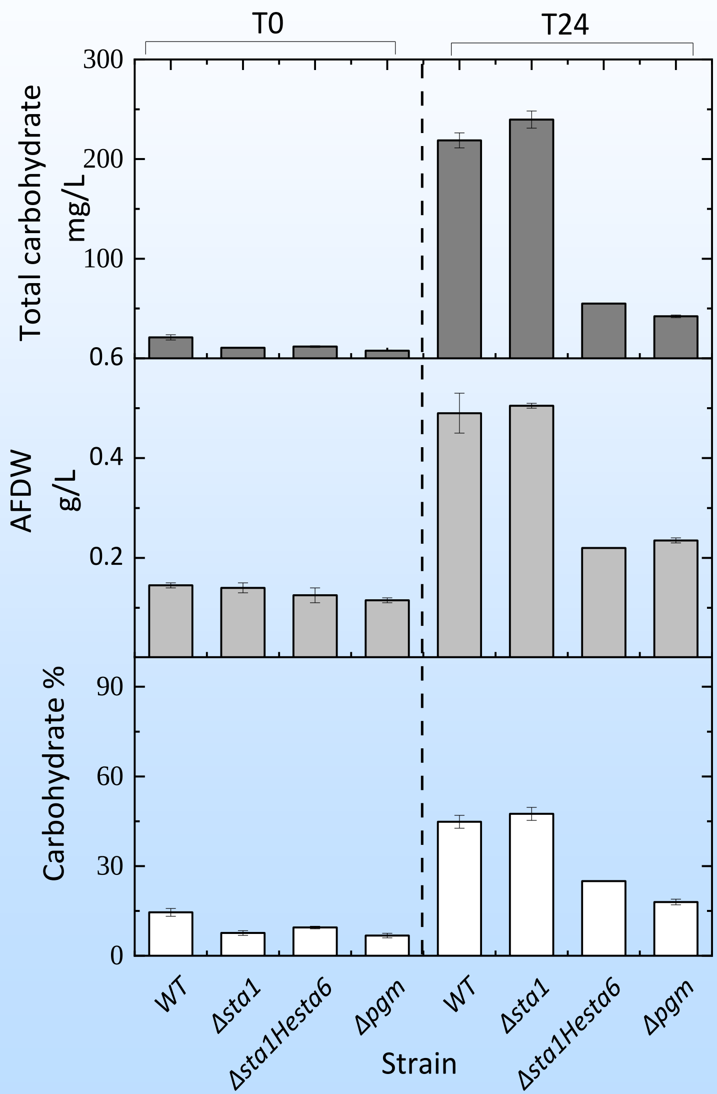
Starch rich cells with thylakoid membranes

- Increases carbohydrate productivity by simply adjusting media composition
- Model system to understand carbon remodeling in *P. celeri*

Progress and Current Status

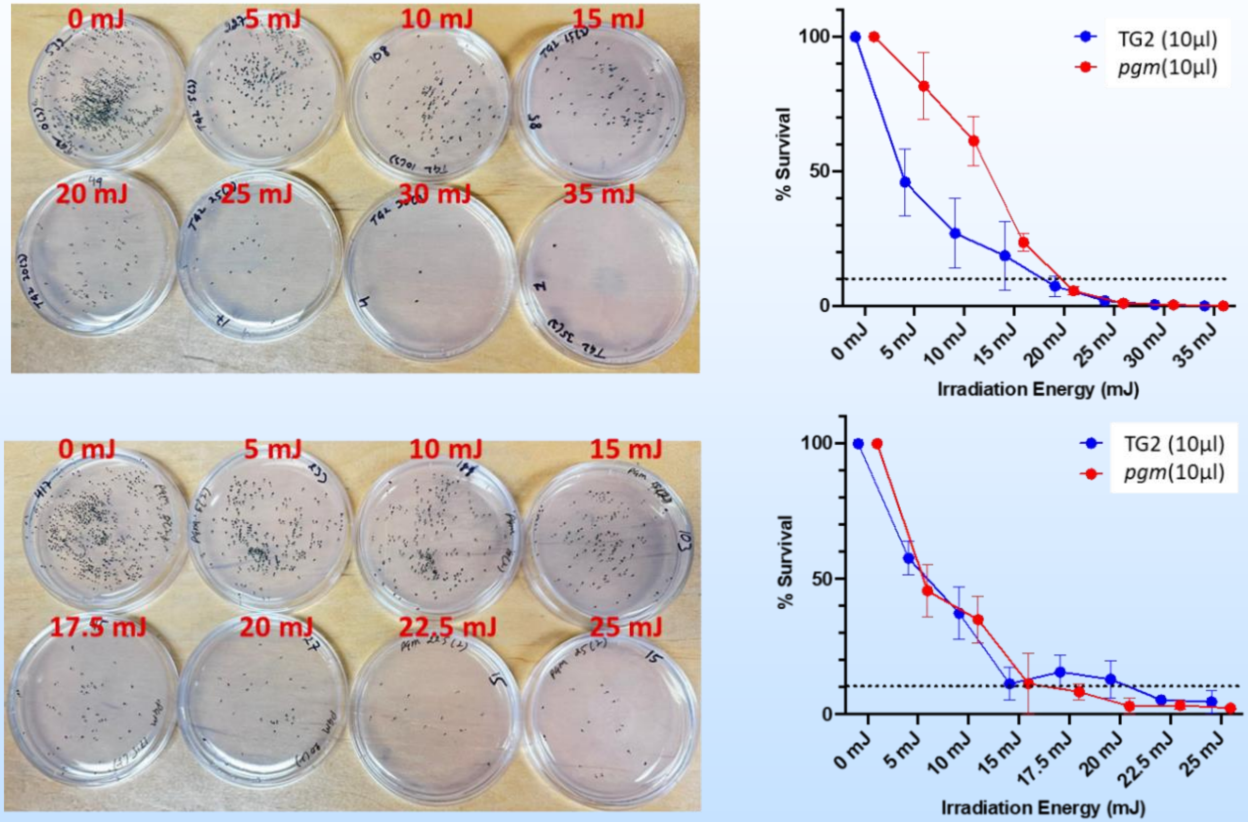
low carbohydrate strains





Progress and Current Status

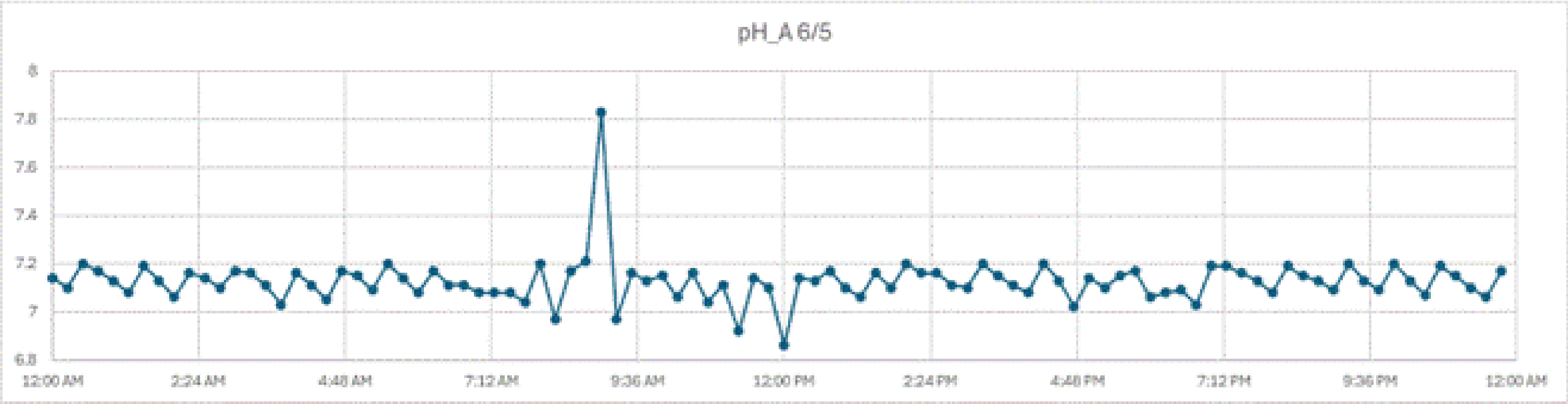
mutant libraries

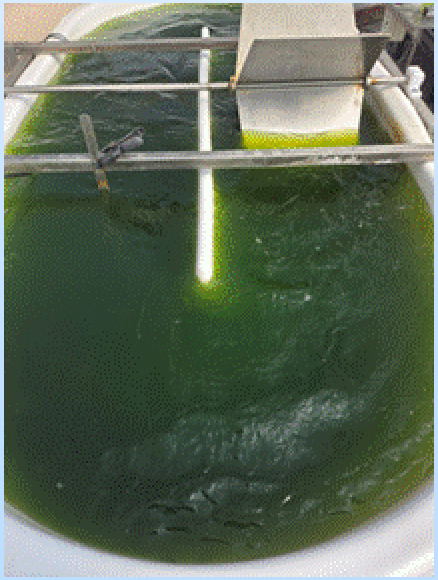


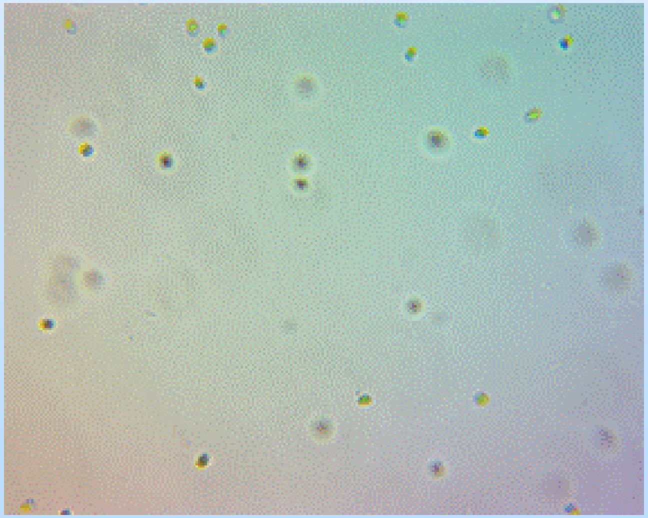
Kill curves following exposure of the *P. celeri* cells to different levels of UV-C. Top left: Survival of TG2 cells following exposure to different UV-C energy levels. Bottom left: Survival of *pgm* cells following exposure to different UV-C energy levels. Top right and bottom right: Plots showing the percent survival of the cells after exposure to the different radiation energies. For the mutagenesis experiments, the cells were grown at 160 µmol photons $m^{-2}s^{-1}$ at 34 ˚C, with a constant supply of 2% CO_2.

Progress and Current Status

MBE pond growth



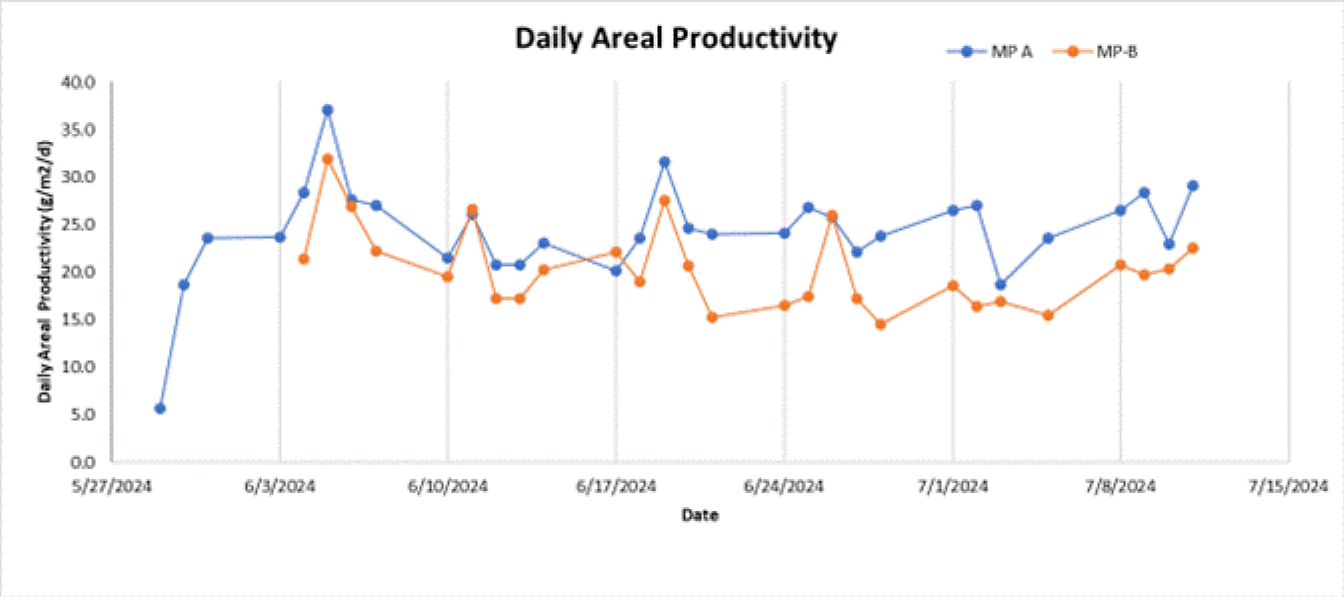


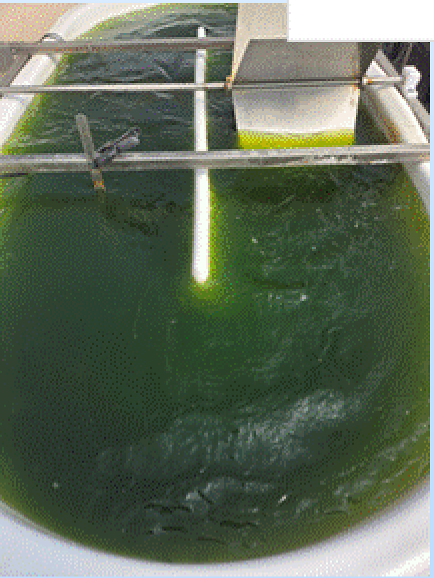


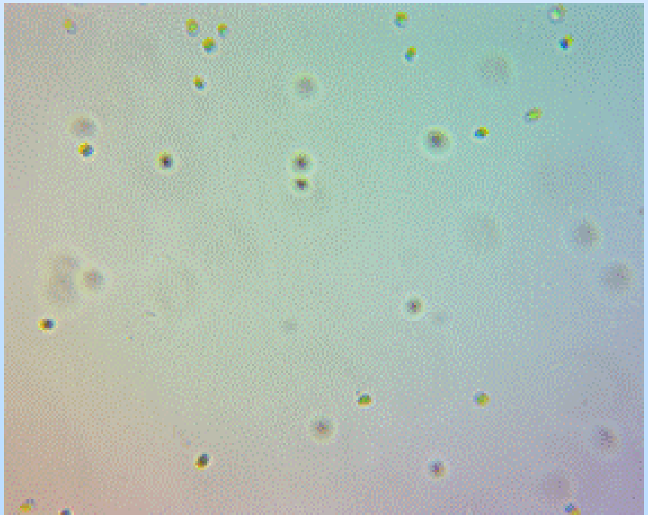
Stable "farm" production for six weeks at ~27 g/m^2/d

Progress and Current Status

MBE pond growth





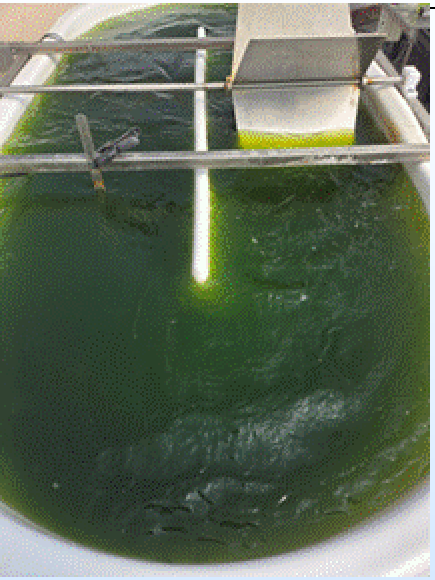


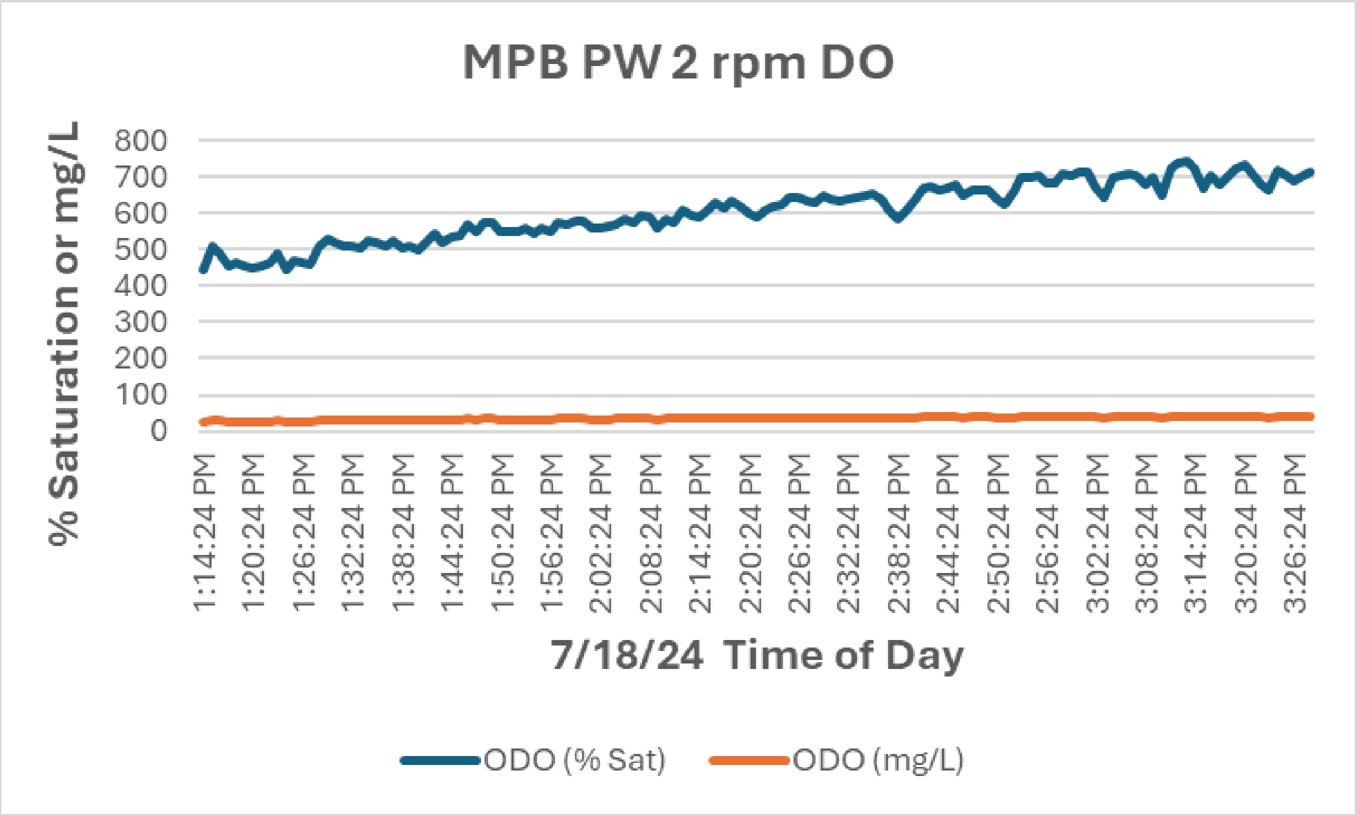
Stable "farm" production for six weeks at ~27 g/m^2/d

Modest productivity reductions as pH/O_2 change

Progress and Current Status

MBE pond growth

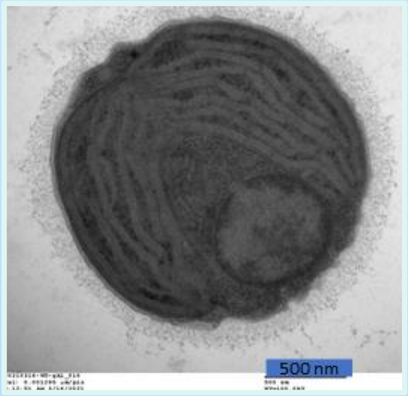


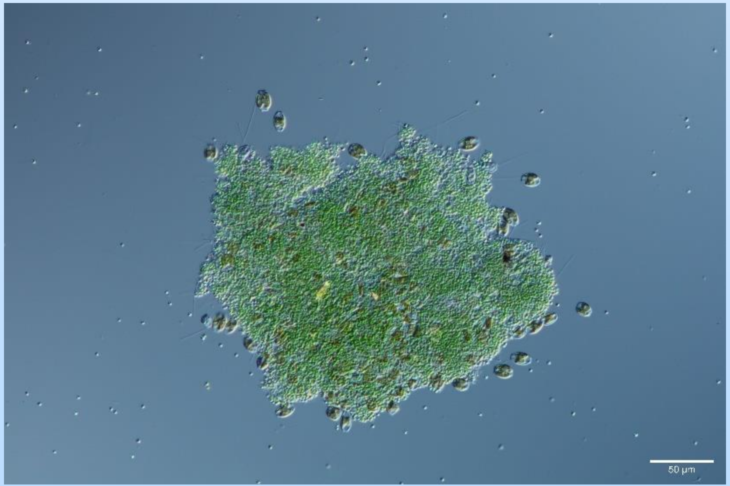


High O_2 burden with only modest reduction in productivity

Lessons Learned

- *Picochlorum celeri* exhibiting robust stable growth.
- Oxygen sensitivity and CO_2 delivery require further investigation to improve CUE.
- Mutant libraries prepared.
- Urea level will contribute to CUE.
- Carbohydrate mutants are in hand.
- Preliminary pond studies are promising for attaining end of project goals.



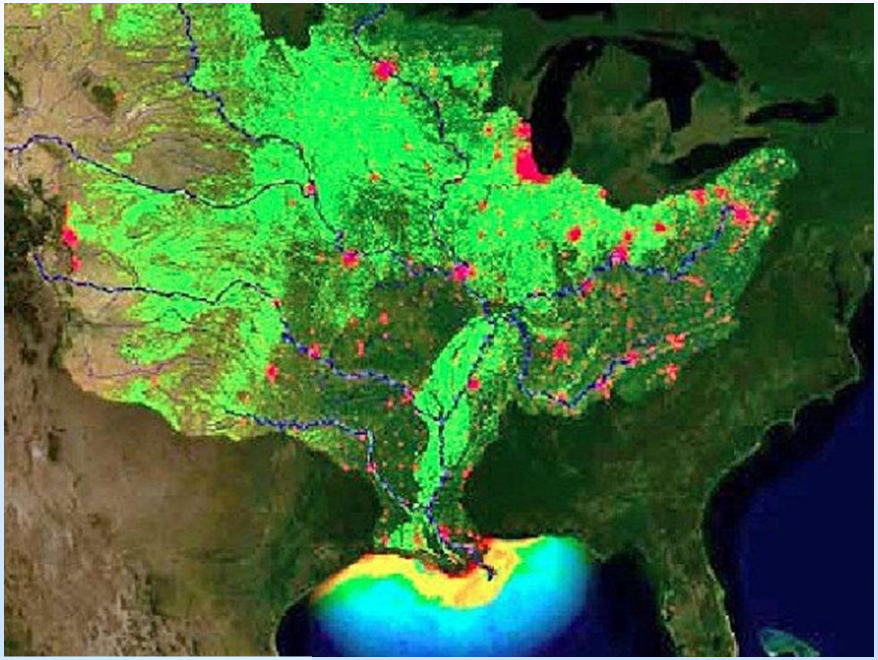


Plans for future testing/development/ commercialization

a. In this project scale-up will be demonstrated at MBE.
b. After this project larger scales will ultimately be proposed at appropriate testbeds.
c. Ultimately, marine sites in the Gulf of Mexico are envisioned near CO_2 sources.

Plans for future testing/development/ commercialization

Gulf of Mexico "Deadzone" ~6500 Square Miles in 2021

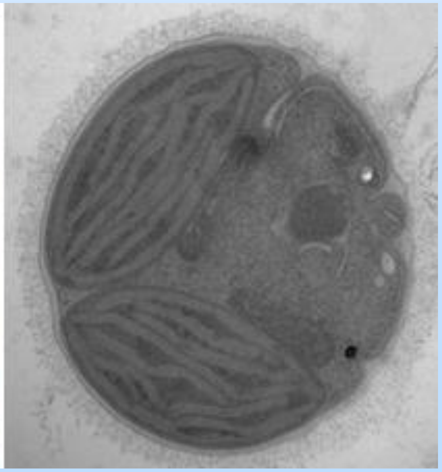


NOAA

Summary Slide

a. The green alga *Picochlorum celeri* is a rapidly (>25 g/m^2/d) growing photoautotroph that effectively competes with CO_2 off-gassing.
b. Farm preparations are underway for scaled testing.
c. Strain improvement screening underway.
d. Preliminary growth campaigns are promising for realizing end of product goals.
e. Hosted DEI SWEET high-school workshop in collaboration with NREL. 15 teachers (1,000+ students).



Organization Chart

- Colorado School of Mines is responsible for using bioreactor experiments to quantify cellular productivities under conditions designed to enable more efficient pond operations.
- Carnegie Institute for Science is responsible for generating *P. celeri* mutants and screen for high lipid and high pH strains.
- MicroBio Engineering is responsible for outdoor growth campaigns, cell harvesting and final CUE calculations.
- NREL is responsible for strain development for harvesting and high lipid.
- Colorado State University is responsible for using TEA/LCA to inform process improvement.

Gantt Chart

Task; Subtask; Milestone; Go/No-Go	Project Quarter (Q)											
Task 1: Project Verification	1	2	3	4	5	6	7	8	9	10	11	12
Subtask 1.1: Complete DOE verification												
Milestone 1.1.1: Complete DOE verification												
Go/No-Go #1: Successfully pass verification												
Subtask 1.2: Project Management												
Task 2: Characterize C_i as a function of pH												
Subtask 2.1: Measure C_i and pH at constant %CO_2 in gas phase												
Milestone 2.1.1: Determine C_i in high biomass density medium												
Task 3: Optimize urea to minimize C loss												
Subtask 3.1: Optimize urea content in high biomass density medium												
SMART Milestone 3.1.1: Quantify optimal urea levels												
Task 4: Characterize CO_2 injection efficiencies and outgassing												
Subtask 4.1: Adjust mixing speeds to probe CO_2 outgassing												
Milestone 4.4.1: Determine mixing speeds for K_L of 0.5 and 0.1 h^{-1}												
Milestone 4.1.2: Minimize nighttime C loss from respiration												
Milestone 4.1.3: Determine CO_2 injection efficiencies in 40 m^2 ponds												
Task 5: Establish on demand pH productivities/improved CUE strains												
Subtask 5.1: Determine pH regimes to attain 20 g m^{-2} d^{-1}												
SMART Milestone 5.1.1: Attain >20 g m^{-2} d^{-1} cycling pH 7.0-7.8												
Go/No-Go #2: Attain >20 g m^{-2} d^{-1} cycling pH 7.0-7.8 in bioreactors												
Milestone 5.1.2: Determine 3.4 m^2 pond productivities - cycling pH												
Subtask 5.2: Generate *P. celeri* random mutant library												
Milestone 5.2.1: Generate random mutant libraries												
Milestone 5.2.2: Select strains for improved high pH growth												
Task 6: Isolate high lipid *P. celeri* strains												
Subtask 6.1: Isolate high lipid *P. celeri* strains from mutant library												
Milestone 6.1.1: Select random mutants with increased lipid												
Milestone 6.1.2: Use gene editing to knockout starch synthesis												
Task 7: Develop Membrane harvesting unit - flocculation												
Subtask 7.1: Design/test membrane harvesting unit												
Milestone 7.1.1: Demonstrate ability to harvest 8,000 L d^{-1} at MBE												
Subtask 7.2: Quantify flocculation at high pH												
Milestone 7.2.1: Quantify flocculation and clarification at high pH												
Task 8: Outdoor *Picochlorum* growth campaigns												
Subtask 8.1: Determine CUE/productivities in 30-day campaigns												
Milestone 8.1.1: Membrane capture with 90% media recycle												
SMART Milestone 8.1.2: Run two 30-day outdoor campaigns												
Task 9: TEA/LCA												
Subtask 9.1: LCA/TEA												
Milestone 9.1.1: Develop TEA/LCA for integrated process												