

Cultivation-Ready Improved Algae Strains (CRIAS) for Increased Carbon Utilization Efficiency

DE-FE0032189

Juergen Polle, Chief Scientist
MicroBio Engineering Inc.

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

Project Overview

Funding and Project Dates

DE-FE0032189

| | Federal | Cost Share | Total Costs | Cost Share % | Duration |
|------------------------|--------------|------------|--------------|--------------|--------------------------------------|
| Budget Period 1 | \$ 972,179 | \$ 269,116 | \$ 1,241,295 | 21.68 | 02/01/2023-04/30/2024 (15 months) |
| Budget Period 2 | \$ 1,027,411 | \$ 239,291 | \$ 1,266,702 | 18.89 | 05/01/2024 - 07/31/2025 (15 months) |
| Total | \$ 1,999,590 | \$ 508,407 | \$ 2,507,997 | 20.27 | 02/01/2023 to 07/31/2025 (30 months) |

Overall Project Objectives

1. Exceed the Carbon Utilization Efficiency (CUE) of 50%
 - Minimize CO₂ Outgassing During Algae Cultivation.
 - Efficient Flue Gas CO₂ Transfer into Ponds.
2. Algae Strain Improvement (increase biomass productivity).
3. *S. obliquus* Genomics for CUE and Productivity.
4. Product Testing in Greenhouse with Crops.
5. TEA/LCA Studies and Model Development.
6. Diversity/Equity Inclusion (DEI).

Project Overview -Project Participants

Dr. Benemann
MicroBio Engineering Inc. (MBE)

PI
Awardee



Task 1, Task 2, Task 4, Task 7

Dr. Pablo Cornejo

Task 7

Task 2

Dr. Trygve Lundquist
California Polytechnic State University (Cal Poly)



Subawardee

Dr. Juergen Polle

Task 2
Task 4

Task 3

Dr. Holguin & Dr. Nirmalakhandan
New Mexico State University (NMSU)



Subawardee

Task 8

Dr. Starkenburg
Los Alamos National Laboratory (LANL)



Subawardee

Task 5

Mr. Luke Cizek
Heliae Development Corp. (Heliae)



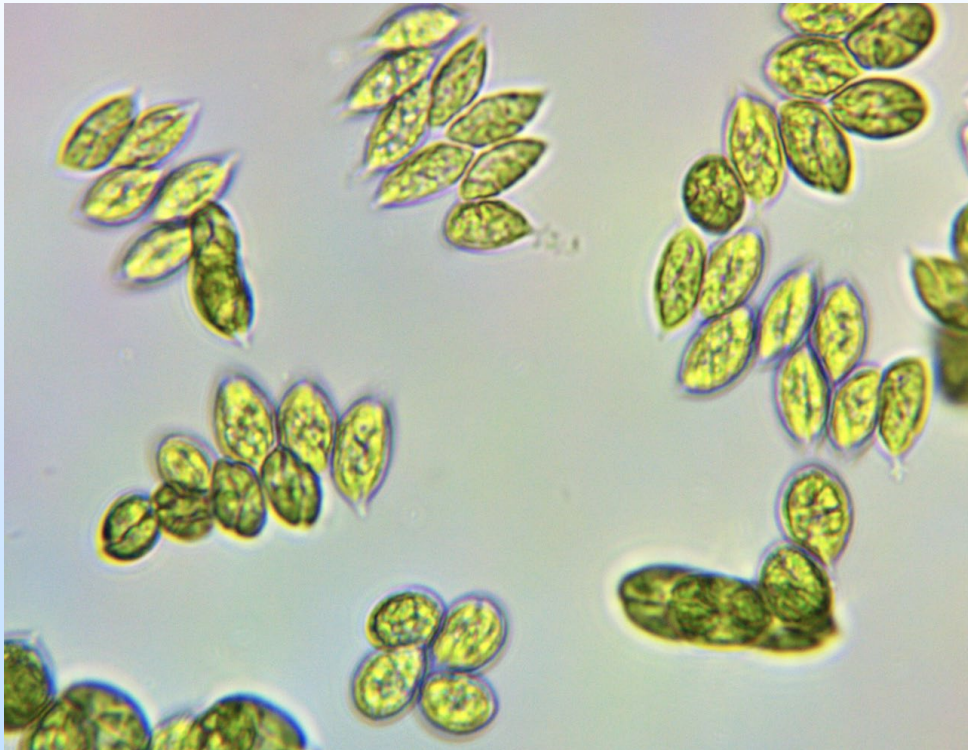
Subawardee

Task 6

- Task 1 - Strain Development
- Task 2 - Strain testing
- Task 3 - Outdoor CUE
- Task 4 - Strain Development
- Task 5 - CUE Genomics
- Task 6 - Regenerative Agriculture
- Task 7 - TEA/LCA
- Task 8 - DEI

Technology Background

Use the green alga *Scenedesmus obliquus*.

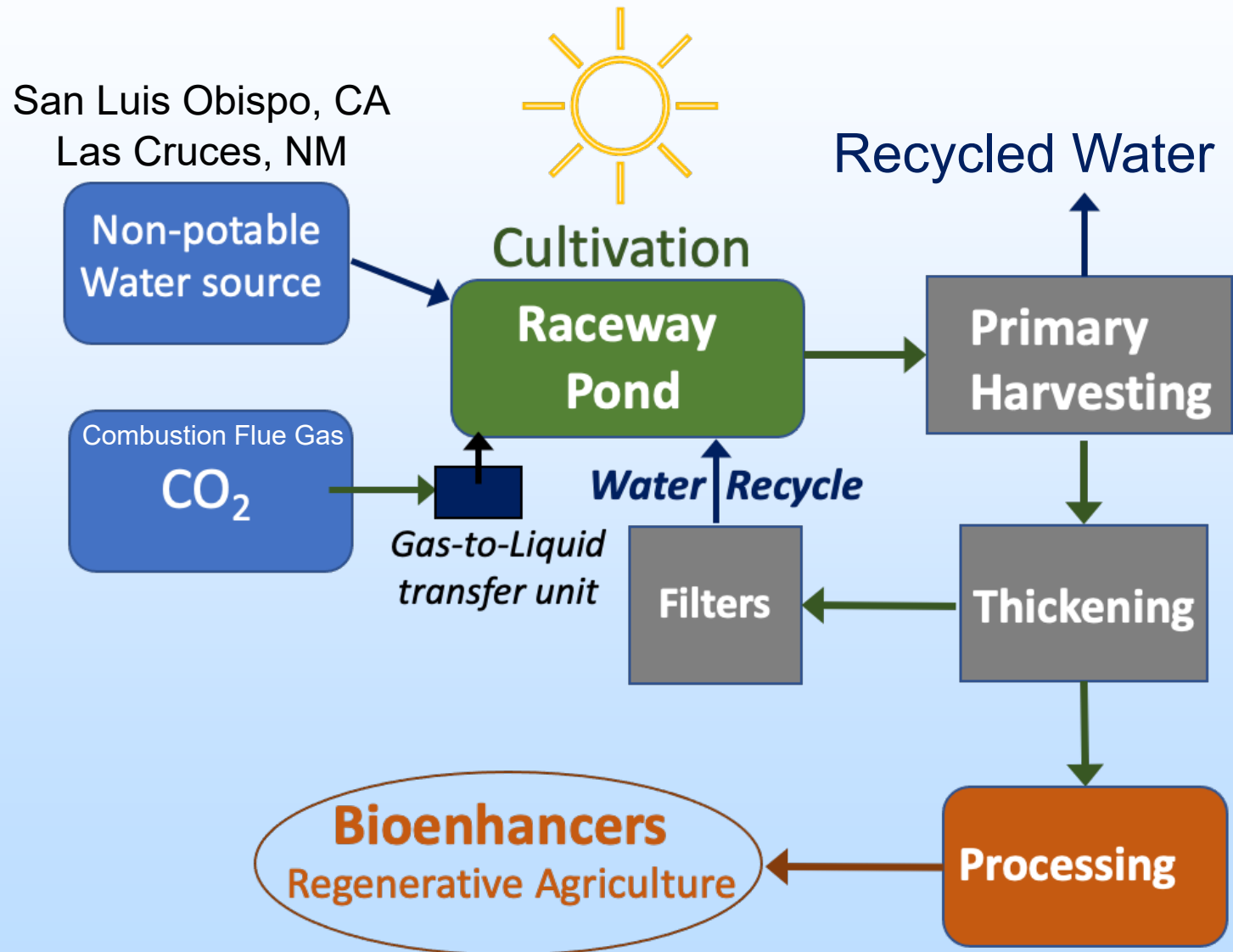


Strain MBE501-12 is improved from a platform strain, which was isolated in the Polle lab in Brooklyn, NY during the NAABB project funded by DOE.

Platform strain: Improved strain MBE501-12 (diploid).

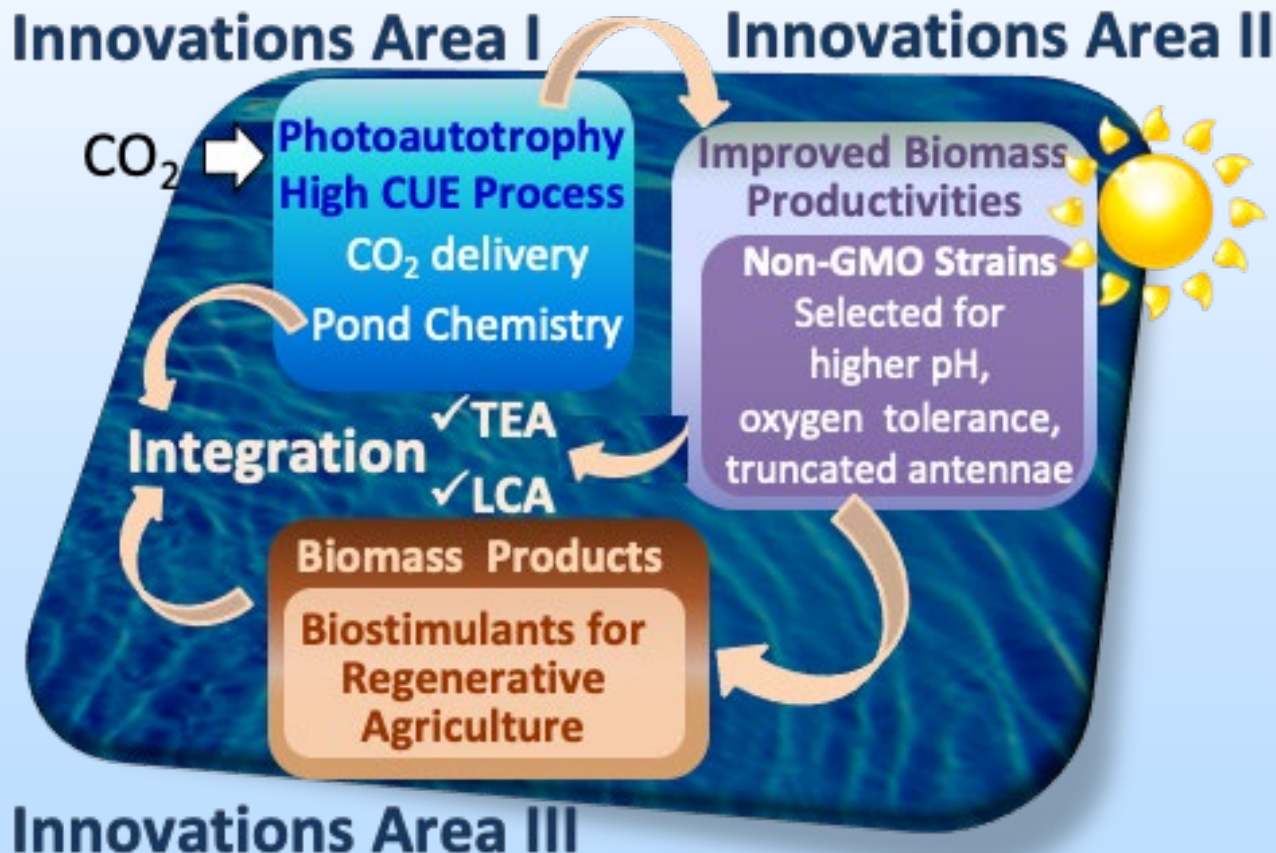
Mitigation strategy - Strain UTEX393 (haploid)

Technology Background



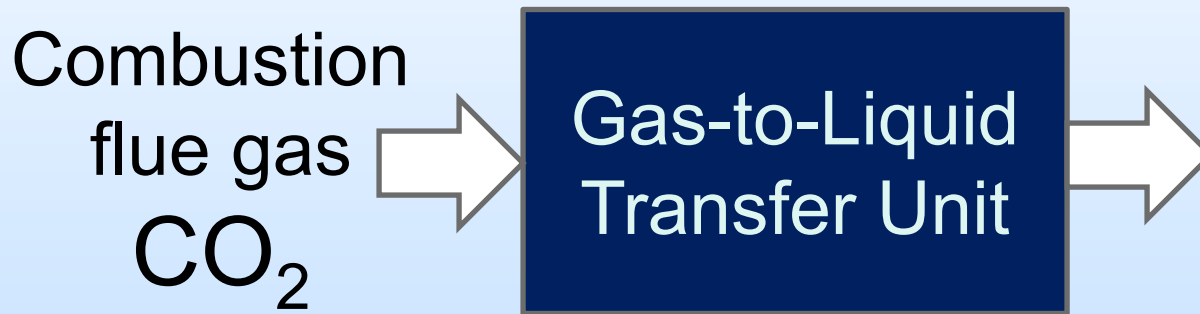
Technology Background

Multiple innovations in CO₂ transfer and utilization technologies combined with game-changing high productivity algae strains, maximize CO₂ utilization and advance commercial applications.



Technical Approach

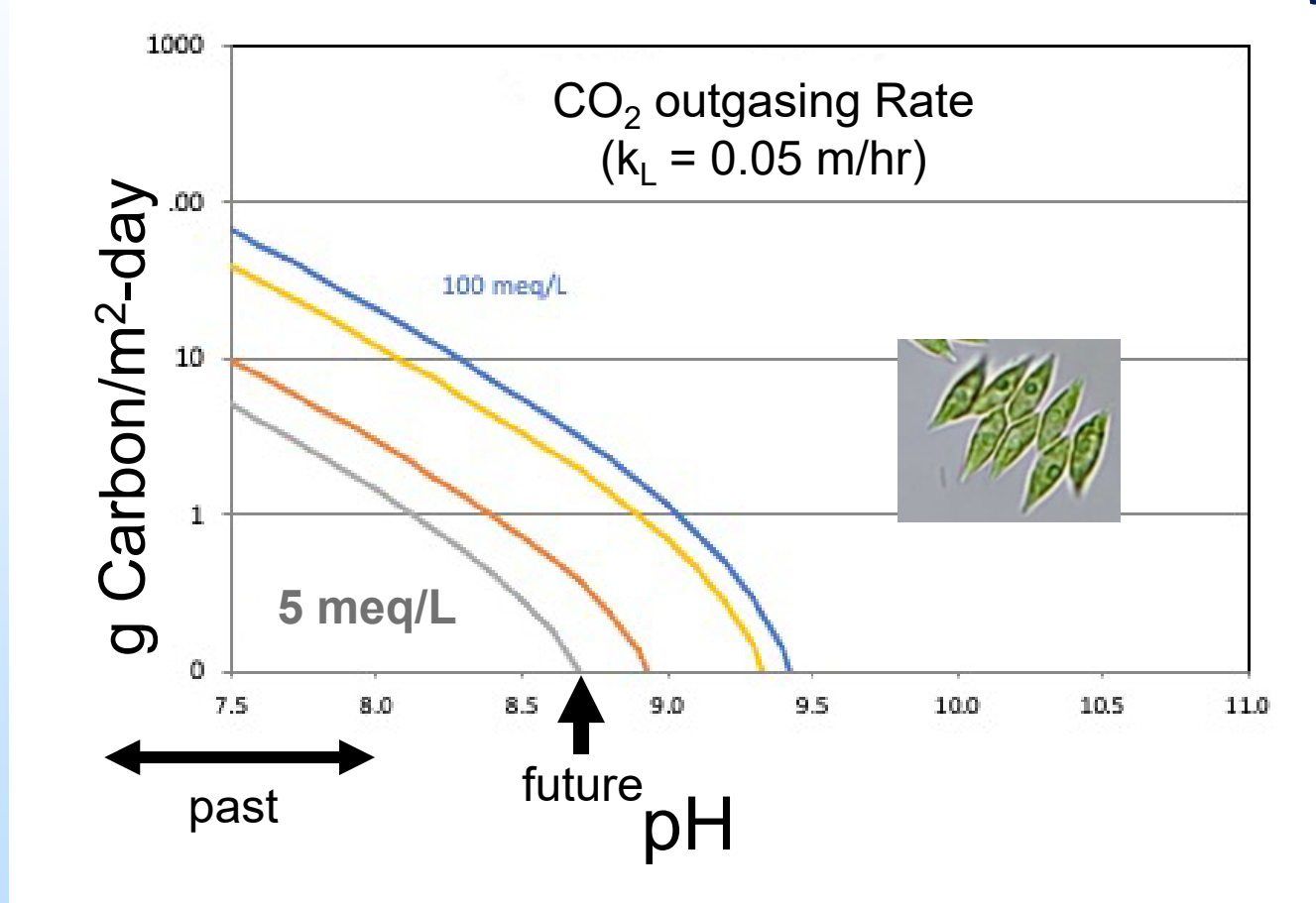
Innovations Area I - CO₂ delivery system



Increase the CO₂ transfer efficiency.

Technical Approach

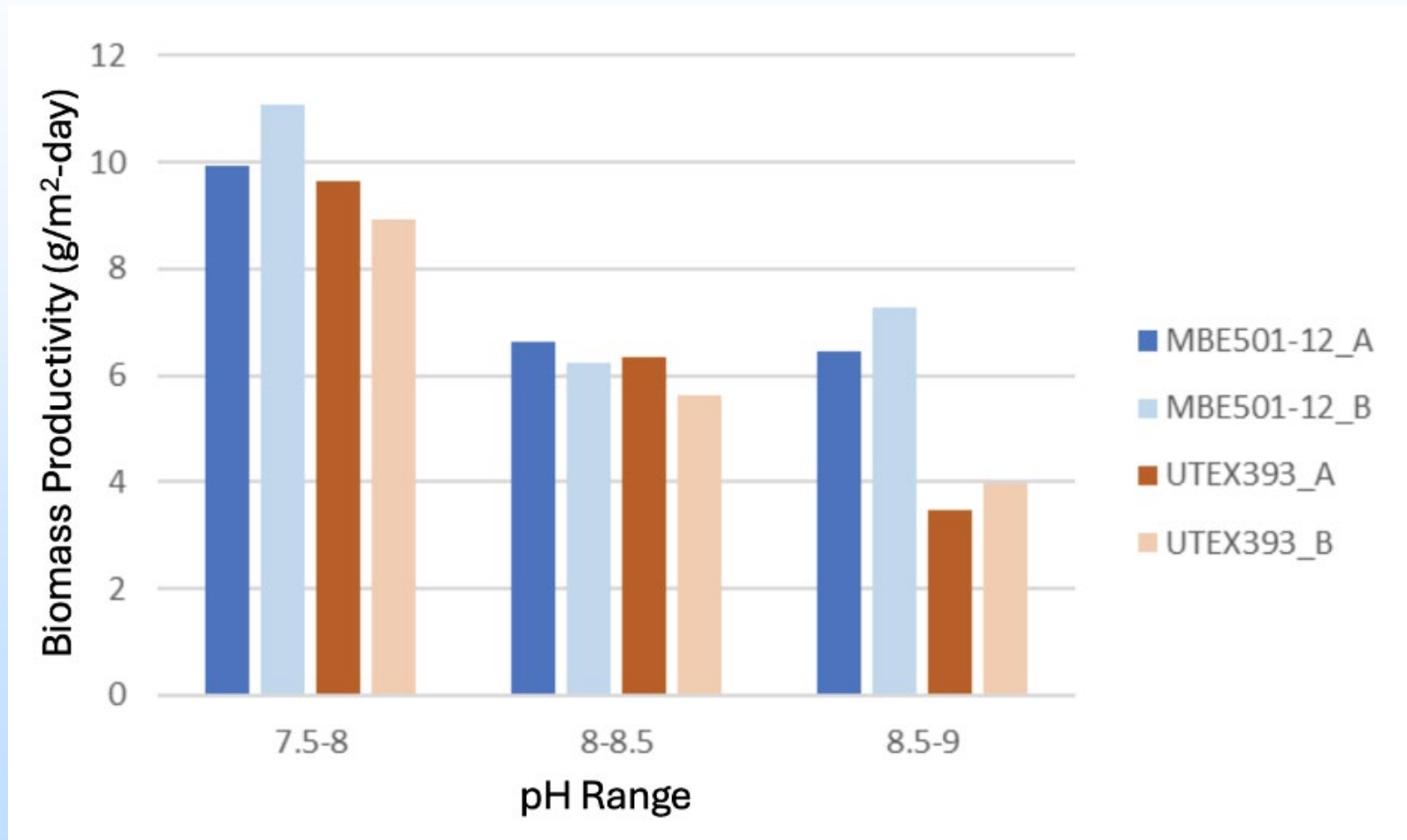
Innovations Area I - Pond Chemistry



Increasing pH and increasing alkalinity will greatly reduce CO₂ outgassing.

Technical Approach

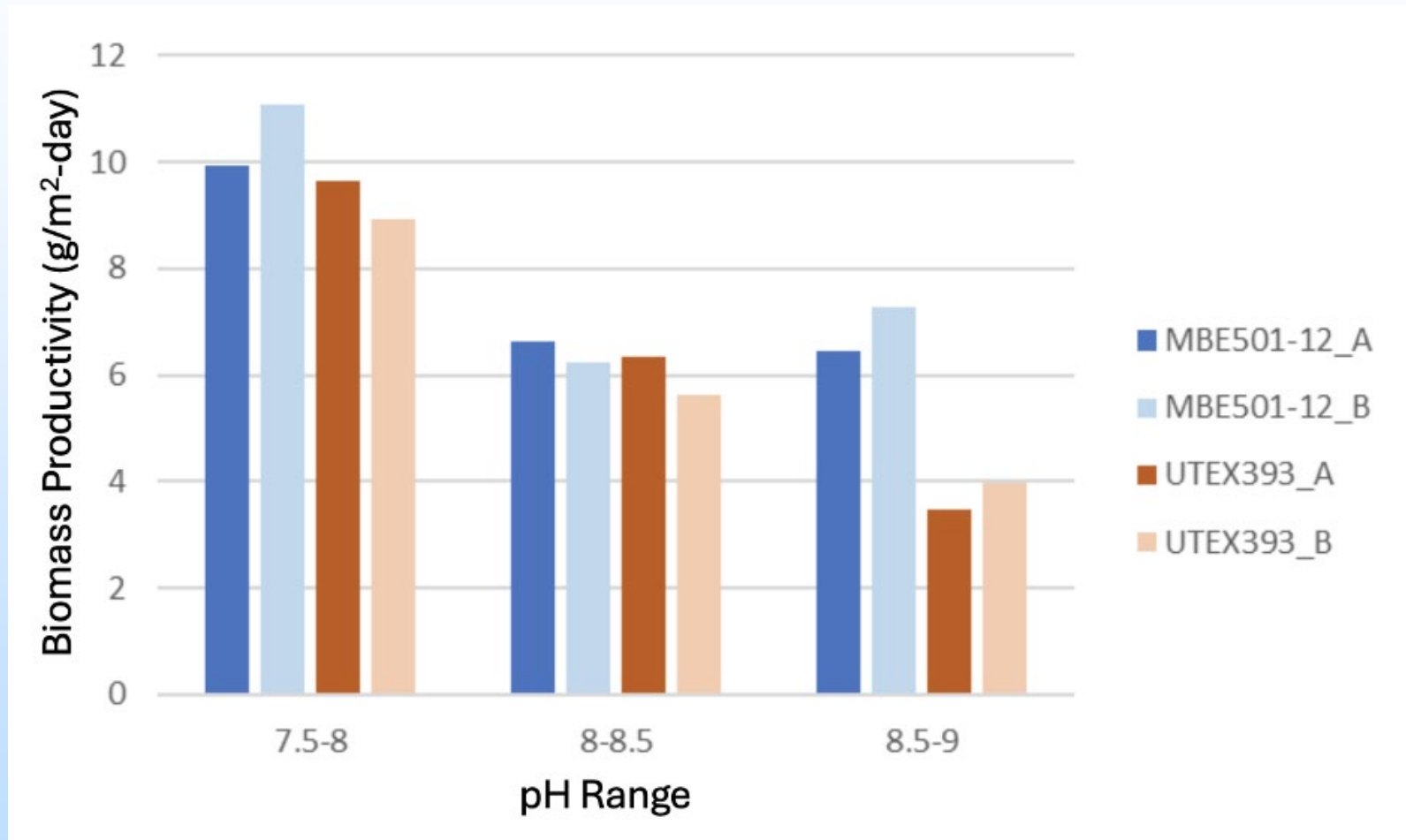
Innovations Area II - Algal Biology



Test in November 2023 in San Luis Obispo, CA

Technical Approach

Innovations Area II - Algal Biology



Improve biomass productivity at pH above 8 for higher CUE

Technical Approach

Innovations Area III - Application



Regenerative Agriculture – Improve Product Quality

Technical Approach

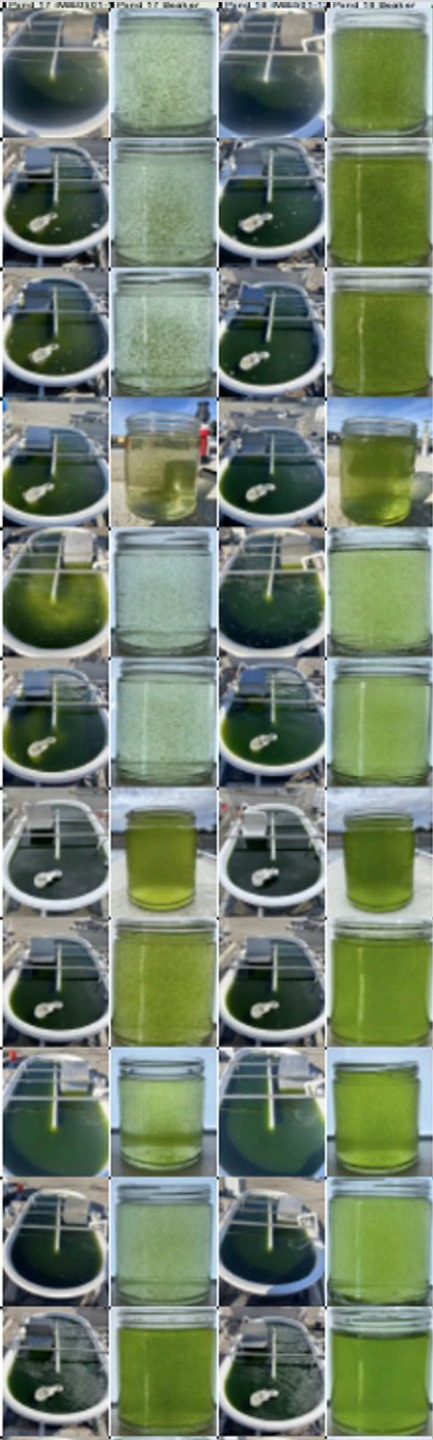
Advantages of the CRIAS technology.

1. Proven platform strains of the species *S. obliquus*.
Strain UTEX 3031-MBE501-12 (and UTEX 393) were cultivated in ponds on reclaimed water previously.
2. Non-GMO algal strain improvement.
Laboratory Adaptive Evolution was used successfully in the DOE funded ABY2 project to create the already improved variety UTEX3031 501-12. Now used as a platform strain.
3. Product for regenerative agriculture.
Collaboration with Heliae Development LLC., the leader in creation of the PhycoTerra algal derived product line.

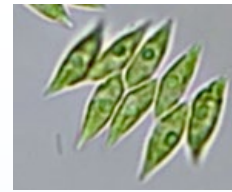
Technical Approach

Success Criteria

Demonstrated stability and measured CUE for platform strain in >30-day trial.



Progress and Current Status of the Project



| Milestone | | Due | Completed |
|-------------------------------|---|-----------------|-------------------|
| 1.1 | <i>Project Management Plan</i> | <i>3/31/23</i> | <i>03/31/2023</i> |
| 1.2 | <i>Project startup completed, and subcontracts executed.</i> | <i>8/31/23</i> | <i>08/31/2023</i> |
| 1.3 | <i>Productivities vs. pCO₂aq/pCO₂air determined for strains in the lab.</i> | <i>8/31/23</i> | <i>08/31/2023</i> |
| 1.4 | <i>Site setup at wastewater treatment plant completed.</i> | <i>9/30/23</i> | <i>09/30/2023</i> |
| 1.5 | <i>Genomes of platform strains sequenced.</i> | <i>10/31/23</i> | <i>10/31/2023</i> |
| 1.6 | <i>First improved cultivar at elevated pH/alkalinity in lab.</i> | <i>1/31/24</i> | <i>01/31/2024</i> |
| 1.7 | <i>Recruiting & mentoring students accomplished.</i> | <i>4/30/24</i> | <i>03/31/2024</i> |
| 1.8 GNG Decision Point | <i>Demonstrated stability & measured CUE for platform strain in >30-day trial.</i> | <i>4/30/24</i> | <i>04/30/2024</i> |

Go/No-Go Decision was April 30th 2024.

Progress and Current Status

1. Strain Improvement

Green alga *Scenedesmus obliquus* (Kuetzing)

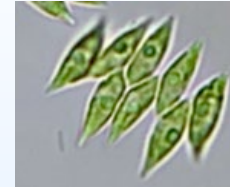


2. Set baseline productivities and CUE with platform strains in San Luis Obispo, CA and Las Cruces, NM.
3. Testing of Novel Strains Outdoors in San Luis Obispo, CA
4. Outdoor Cultivation Trial with Novel Strains.
5. Testing of biomass for use in regenerative agriculture.
6. Technoeconomic Analysis (TEA) and Life Cycle Assessment (LCA).
7. Diversity, Equity, and Inclusion.

Progress and Current Status

Strain Improvement

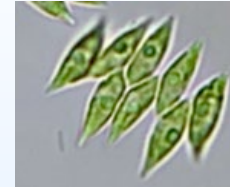
UV-Mutagenesis 



Progress and Current Status

Strain Improvement

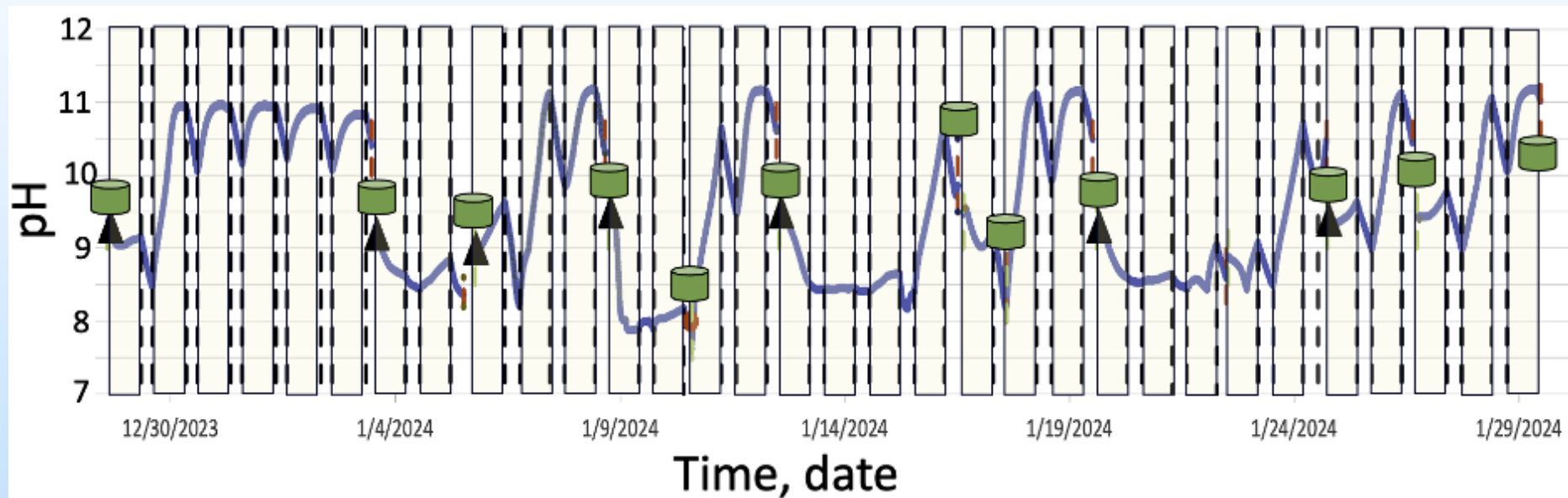
UV-Mutagenesis 



Progress and Current Status

Strain Improvement

Reactor run under diurnal light/dark and temperature cycles.



= dilution times of the culture

Run for about three months with >80 generations.

Progress and Current Status

Strain Testing in Outdoor Ponds

Traditional pond setup
(San Luis Obispo, CA)

Cultivar ALE01

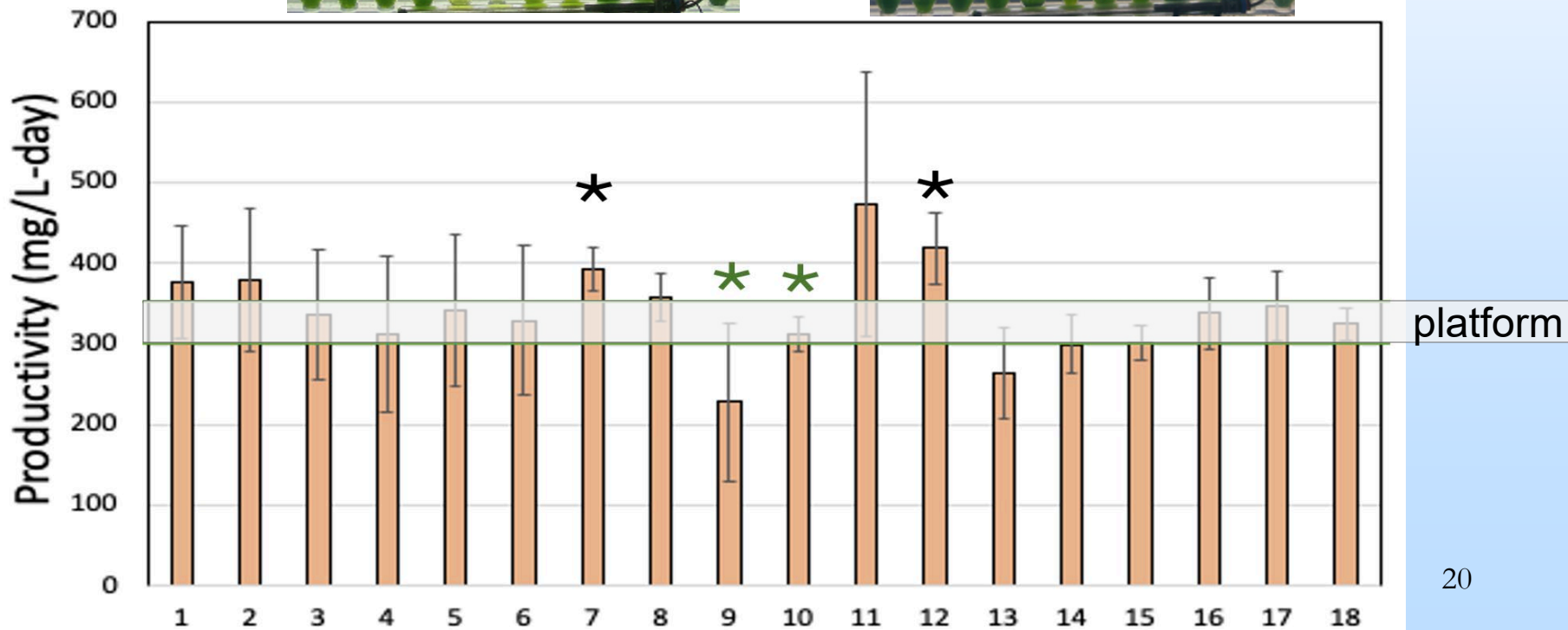
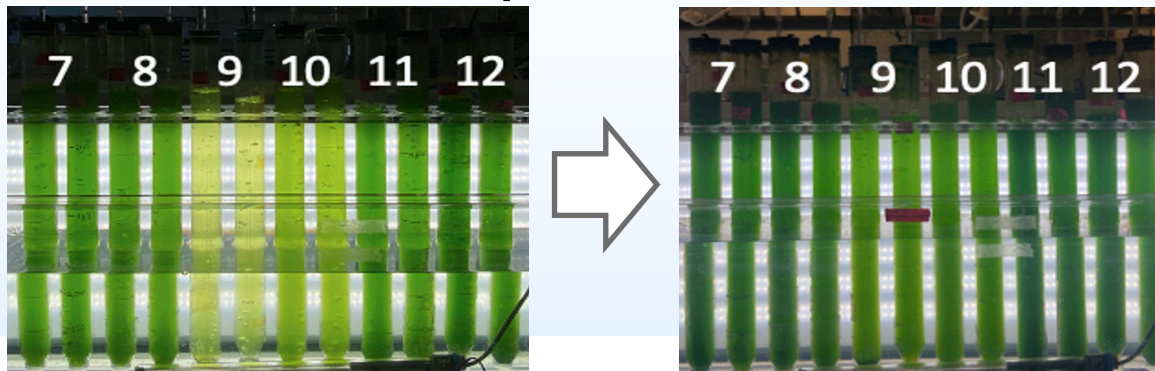
Outcome: Similar productivity to platform strain.

But,.....

Progress and Current Status

Strain Improvement

Laboratory
Testing



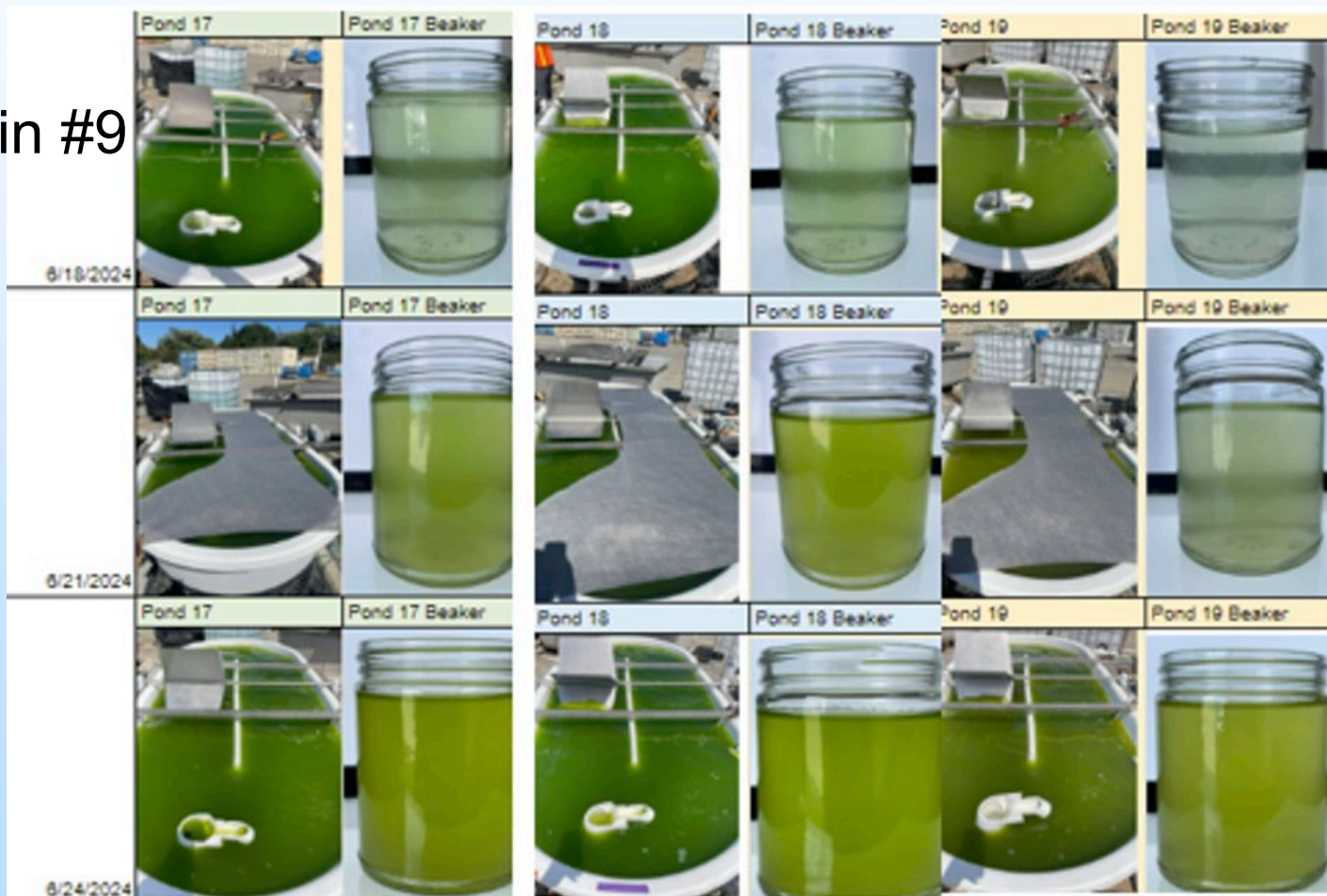
Progress and Current Status

Strain Testing in Outdoor Ponds

Traditional pond setup (San Luis Obispo, CA)

Strain #9

MBE501-12

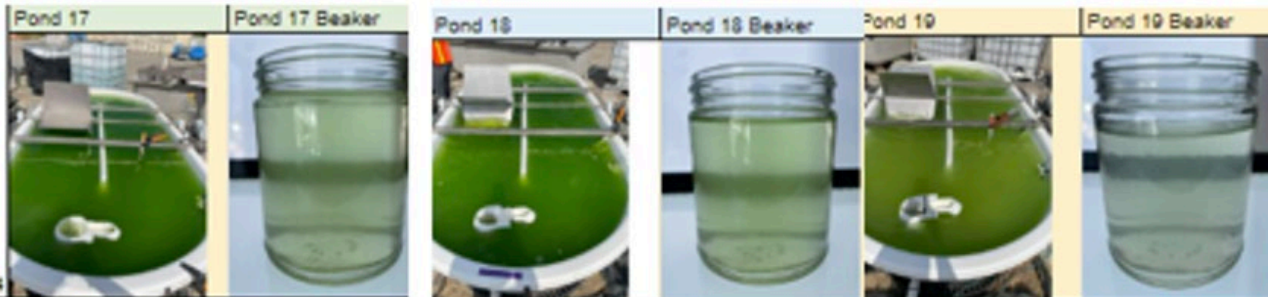


Progress and Current Status

Strain Testing in Outdoor Ponds (San Luis Obispo, CA)

Strain #9

6/18/2024



MBE501-12

23 g AFDW
/m²*day

6/21/2024



13.5 g AFDW
/m²*day

27 g AFDW
/m²*day

6/24/2024



23 g AFDW
/m²*day

Progress and Current Status

New Testbed in Las Cruces, NM



Progress and Current Status

New Testbed in Las Cruces, NM
(supplied by MicroBio Engineering Inc.)



Progress and Current Status

Strain Testing in Outdoor Ponds

Carbon Utilization Efficiency

MBE Ponds with
traditional diffusers

NMSU Ponds with
novel saturator system

pH 7.5-8.0 <40%

pH 7.5-8.0 not determined

pH 8.5-9.0 **50-55%**

pH 8.5-9.0 60-90%

Progress and Current Status

NMSU Testbed in Las Cruces, NM

Platform strain versus first cultivar.

Left to right: Ponds 1, 2, 3, 4

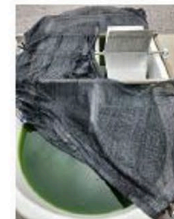
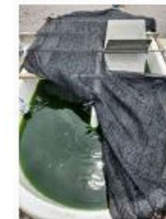
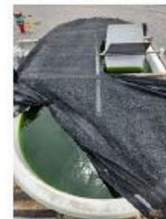
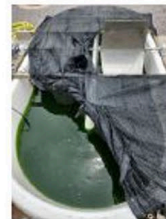
Pond 1

Pond 2

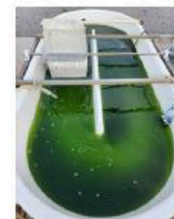
Pond 3

Pond 4

6/4/2024
Day 1



6/14/2024
Day 10
Harvest

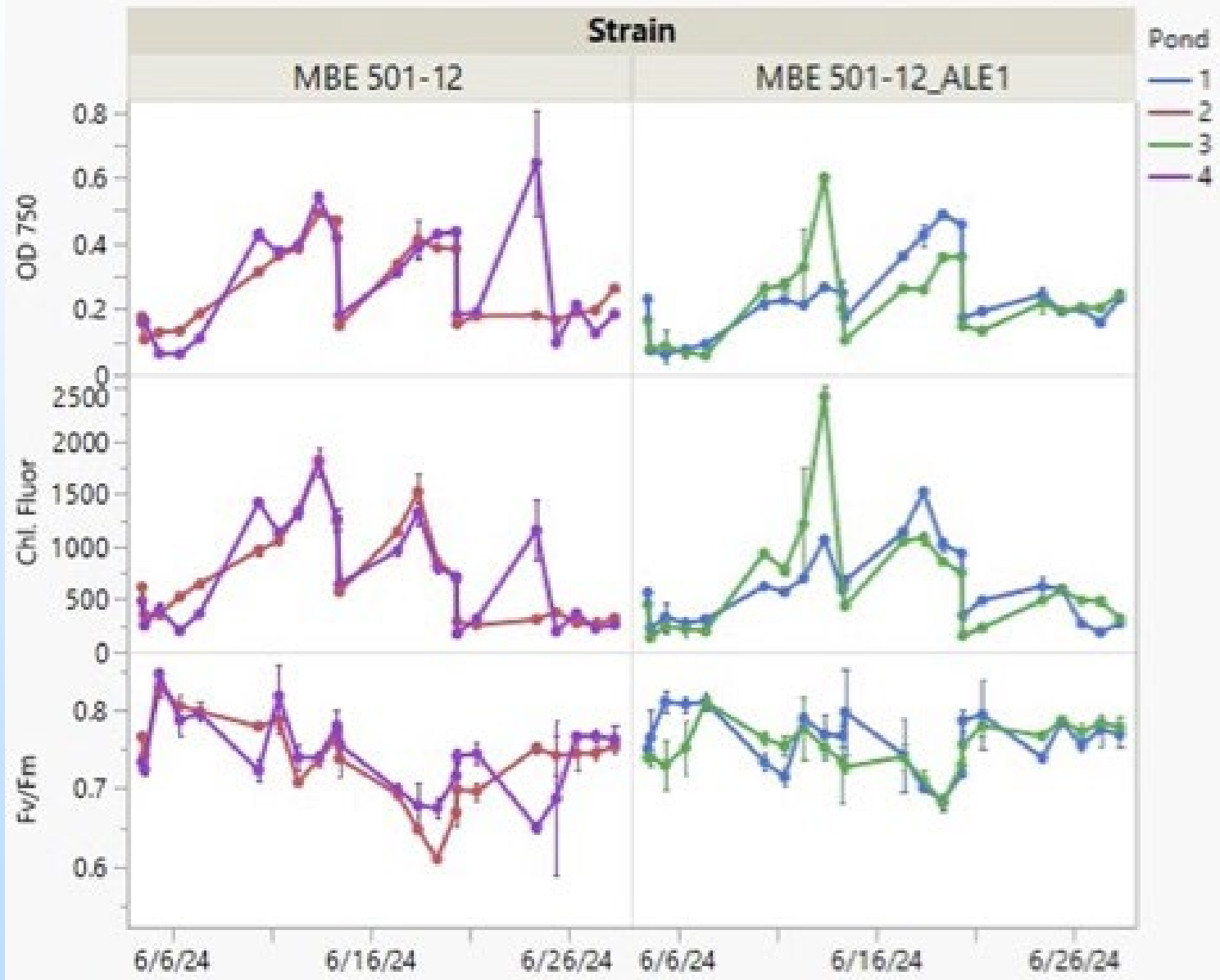


6/20/2024
Day 16
Harvest



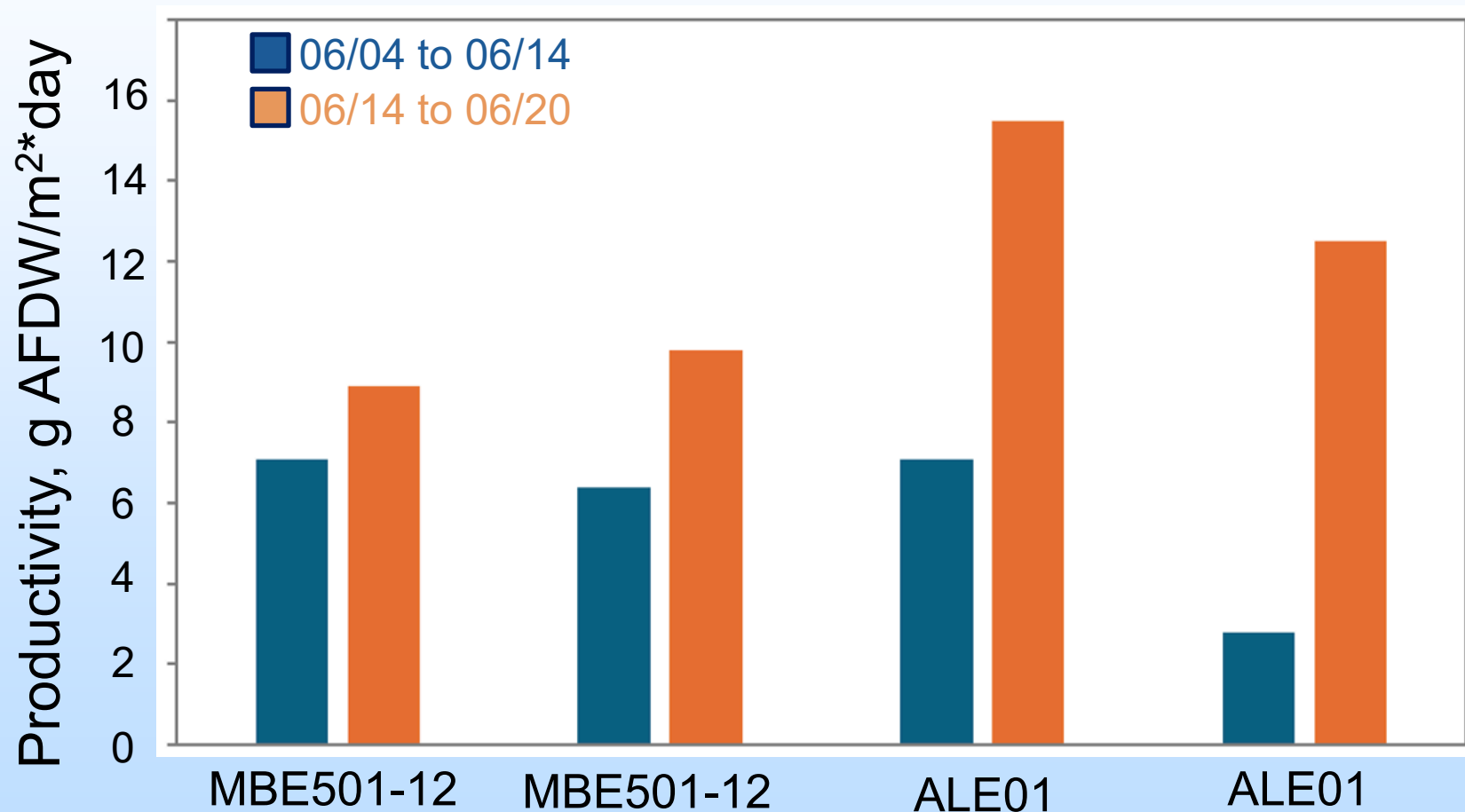
Ponds run between pH 8.5-9.0 in June 2024.

Progress and Current Status



Progress and Current Status

NMSU Testbed in Las Cruces, NM

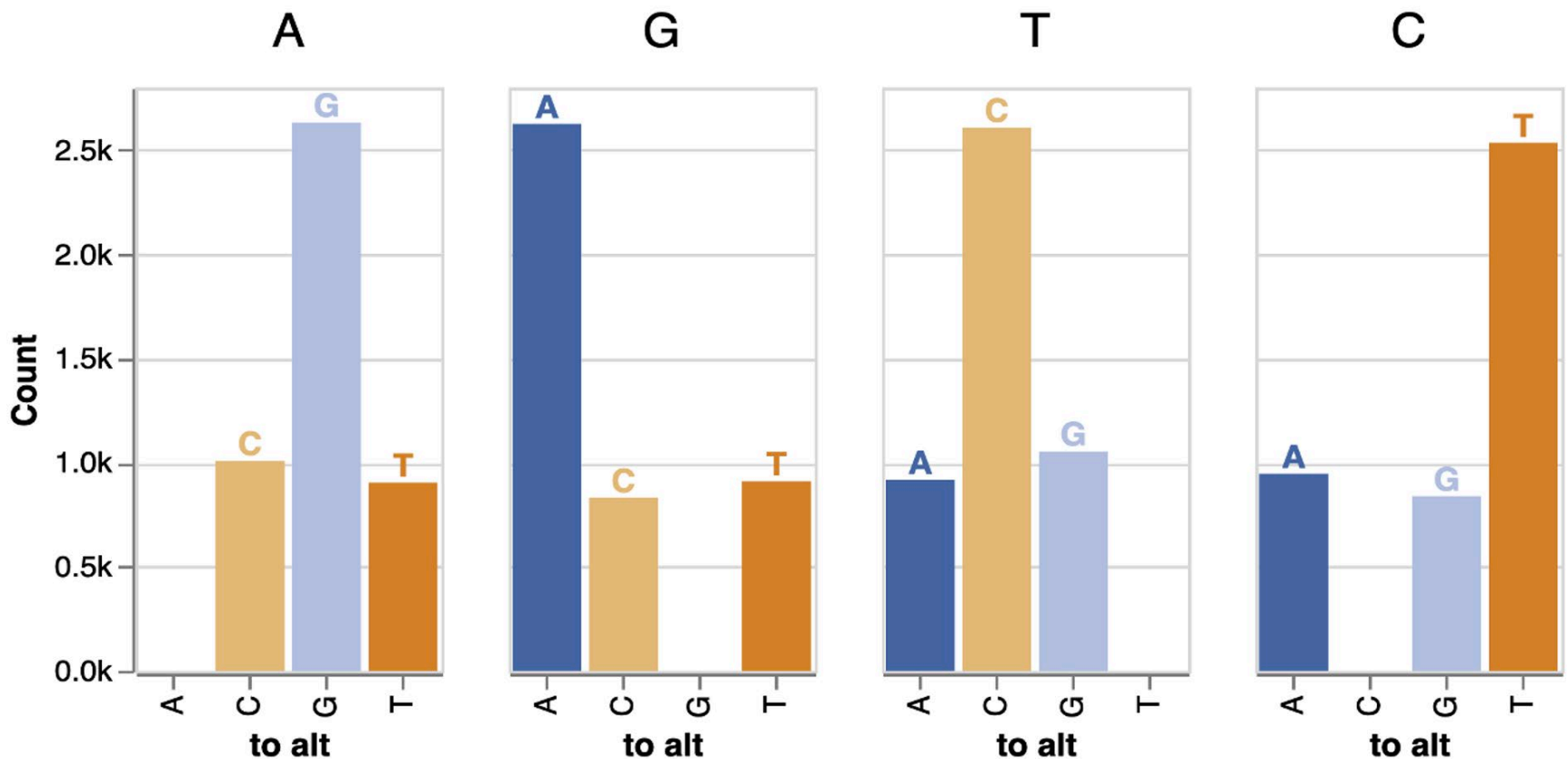


Ponds run between pH 8.5-9.0 in June 2024.

Progress and Current Status

Genomics

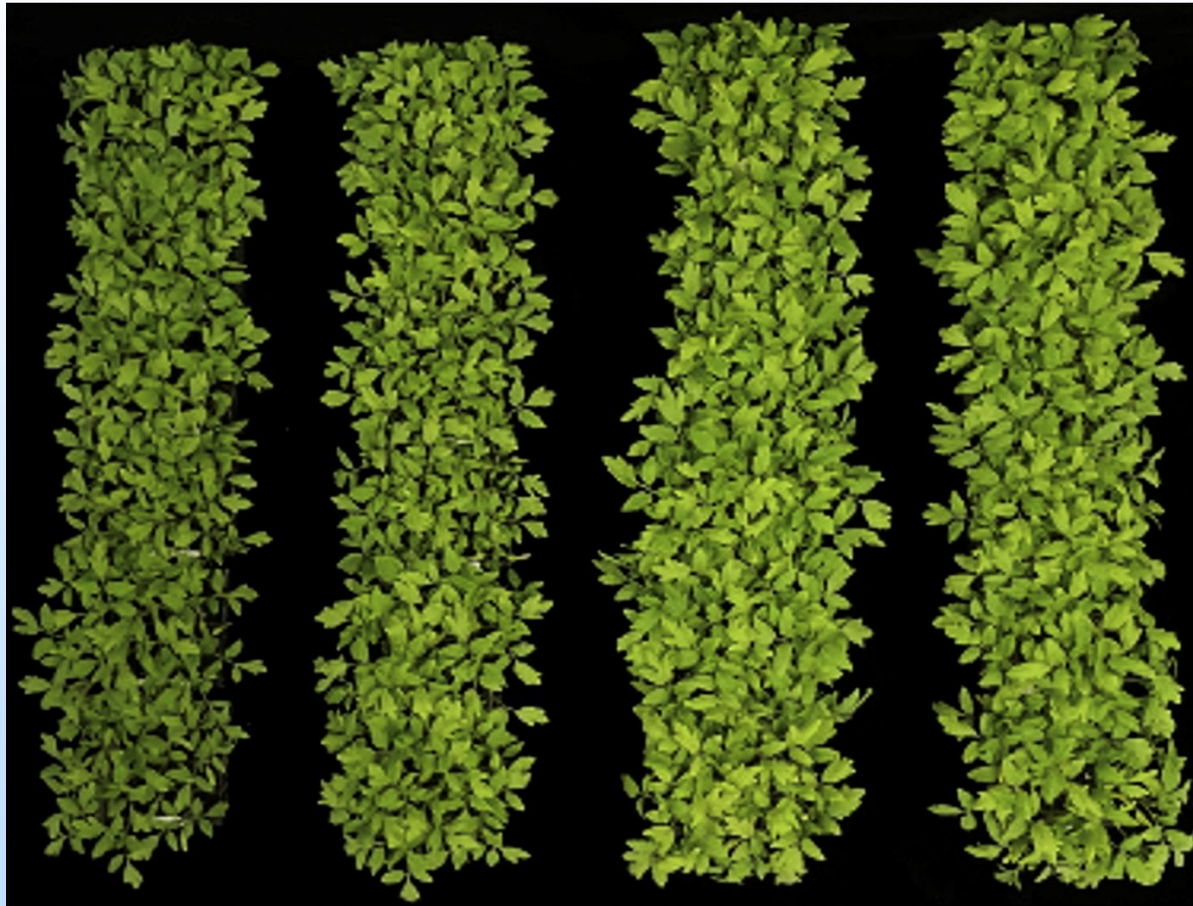
Strain UTEX501-12 was sequenced as a reference strain.



Mutations were mapped and altered genes identified. ²⁹

Progress and Current Status

Regenerative Agriculture



controls

MBE Platform Strains

Progress and Current Status

Technoeconomic Analysis

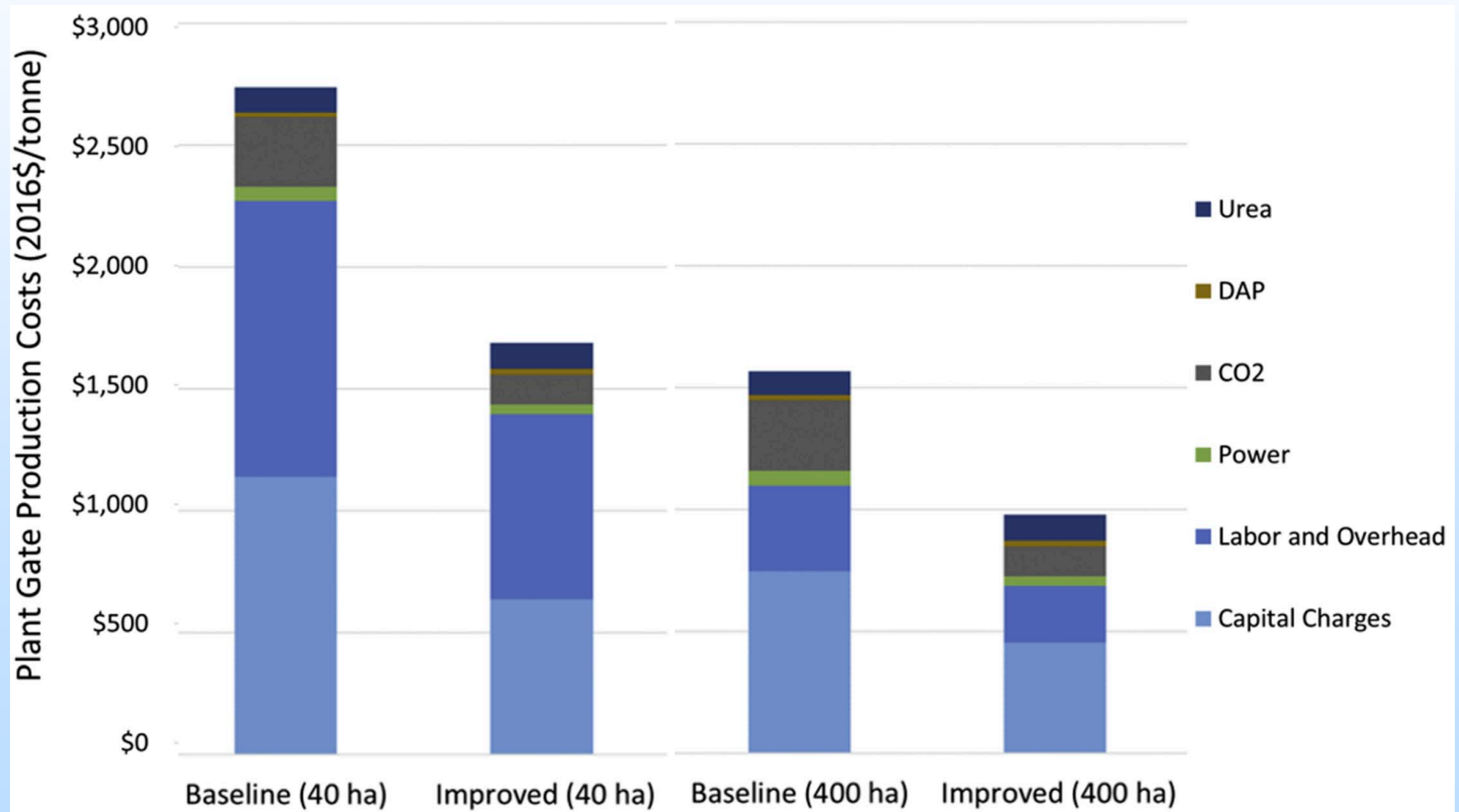
TEA model parameters for the preliminary cost analysis of minimum biomass selling price (MBSP) assuming for photoautotrophic cultivation.

| TEA Parameter | Near-Term Scenario | | Long Term Scenario | |
|---|--------------------|----------|--------------------|----------|
| | Baseline | Improved | Baseline | Improved |
| Plant Scale (ha) | 40 | 40 | 400 | 400 |
| Carbon Utilization Efficiency | 30% | 70% | 30% | 70% |
| Annual Average Productivity (g/m ² /day) | 15 | 25 | 15 | 25 |
| Algae Biomass Production (tonnes/year) | 2,200 | 3,700 | 22,000 | 37,000 |
| HRT (days) | 4 | 4 | 4 | 4 |
| Algae Biomass Density (mg/L) | 200 | 333 | 200 | 333 |

Progress and Current Status

Technoeconomic Analysis

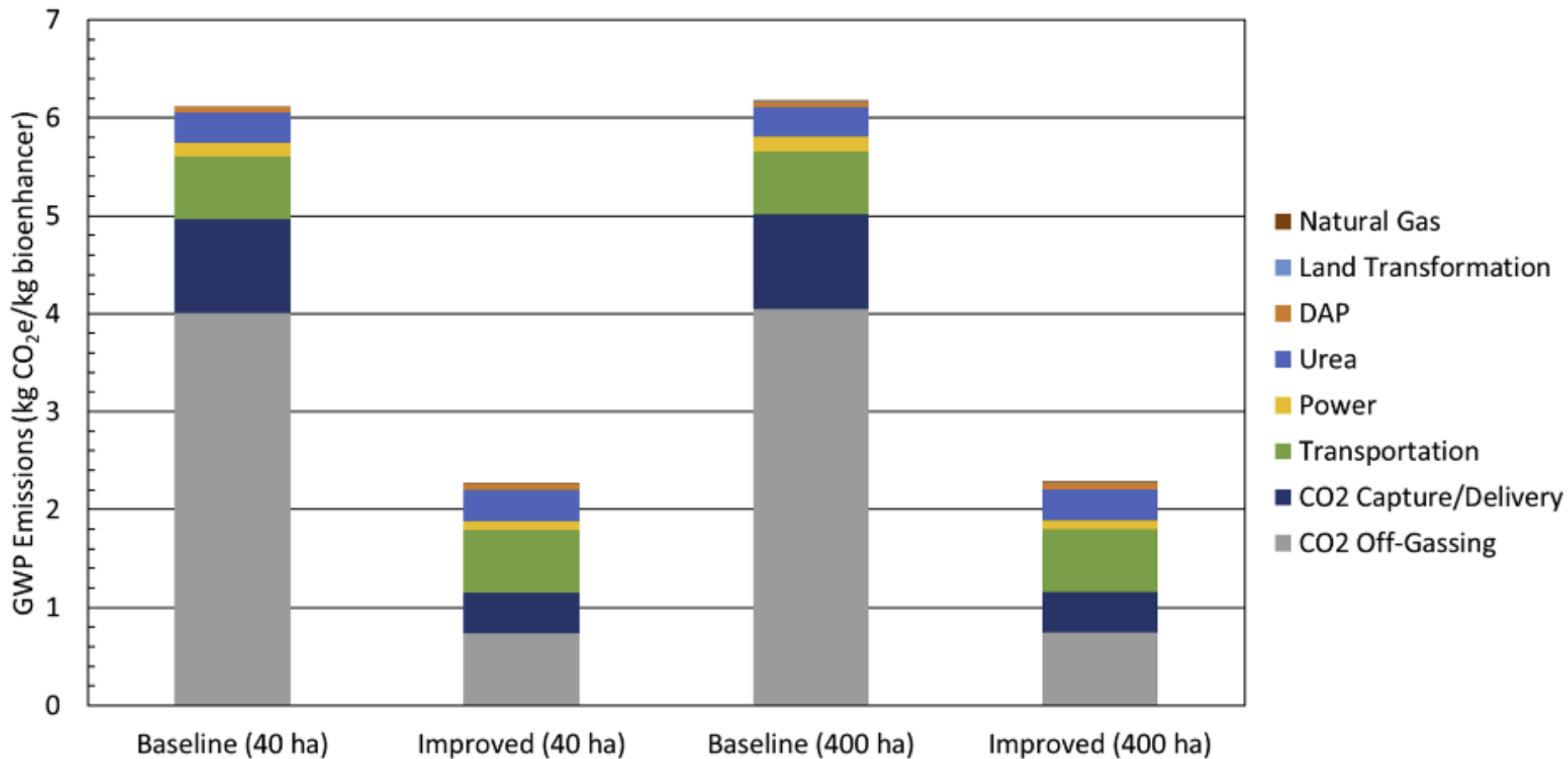
Initial biomass production costs based on prior studies (2016\$/tonne afdw)



Progress and Current Status

Life Cycle Assessment

Preliminary projected lifecycle global warming potential (GWP) emissions.



Progress and Current Status

(MBE - NMSU A. Davis, O. Holguin)

Diversity, Equity, and Inclusion

CRIAS Internship & Research Opportunities

- Recruitment for summer interns at CalPoly and NMSU.
- Collaboration with industry and research partners Heliae and LANL.
- Recruit 2-3 students as summer interns into the companies and to LANL.
- Organize a DEI workshop with students.

Progress and Current Status

Diversity, Equity, and Inclusion

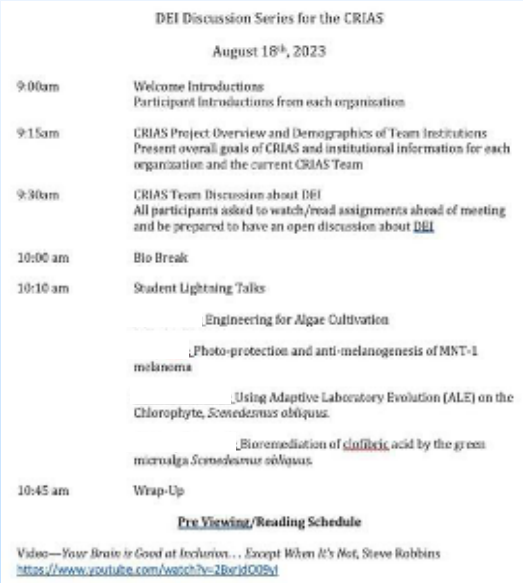
Summer Internships 2023:

- First ever student internships at MBE.
- Two students joined MBE for 6 weeks.
- First workshop held August 18th 2023.

Summer Internships 2024:

- Two students at MBE joined MBE for 6 week summer internships.
- Two students from NMSU at LANL.
- Workshop to be held in mid August 2024.

Workshop Agenda 2023



DEI Discussion Series for the CRIAS
August 18th, 2023

| | |
|----------|---|
| 9:00am | Welcome Introductions Participant Introductions from each organization |
| 9:15am | CRIAS Project Overview and Demographics of Team Institutions Present overall goals of CRIAS and institutional information for each organization and the current CRIAS Team |
| 9:30am | CRIAS Team Discussion about DEI All participants asked to watch/read assignments ahead of meeting and be prepared to have an open discussion about DEI |
| 10:00 am | Bio Break |
| 10:10 am | Student Lightning Talks: Engineering for Algae Cultivation Photo-protection and anti-melanogenesis of MNT-1 melanoma Using Adaptive Laboratory Evolution (ALE) on the Chlorophyte, <i>Scenedesmus obliquus</i>Bioremediation of <u>chlorogenic</u> acid by the green microalga <i>Scenedesmus obliquus</i> . |
| 10:45 am | Wrap-Up |

Pre Viewing/Reading Schedule

Video—Your Brain is Good at Inclusion... Except When It's Not, Steve Robbins
<https://www.youtube.com/watch?v=2Bwjd009dI>

Lessons Learned

Novel Technology

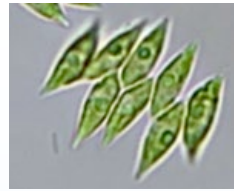
Repeated rounds of Non-GMO Adaptive Laboratory Evolution technology can yield novel strains with improved traits.

Novel Gas-Liquid Transfer Unit and $\text{pH} > 8$ can increase CUE to 90%.

Plans for future testing and development.

Further strain development is in progress.

Modification of novel CO₂ transfer units
for even higher CUE.



Task 1 - PMP delivered.

Task 2 - Strain development continuing.

Task 3 - First 30-day trial period completed.

Task 4 - First novel cultivar/strains developed.

Task 5 - Platform strain genome sequenced.

Task 6 - First round of greenhouse trial completed.

Task 7 - Initial TEA and LCA completed.

Task 8 - DEI student summer internships at MBE and first DEI workshop completed.

Take-Away Messages

1. Improved pond operations and mass transfer could achieve up to 90% CUE.
2. Superior new algal strains to achieve high CUE with high productivity in ponds.
3. Platform strains highly promising as biostimulants in regenerative agriculture.

Questions?

Cultivation-Ready Improved Algae Strains (CRIAS) For Increased Carbon Utilization Efficiency

MicroBio Engineering Inc. (Lead)

Technical Point of Contact: Dr. Juergen Polle (P.I., Chief Scientist)

Business Point of Contact: John Benemann, Ph.D. (CEO)

Participating Scientist: Mr. Braden Crowe (Senior Engineer).

New Mexico State University Las Cruces:

Omar Holguin Ph.D. (PI, Professor),

Khandan Nirmalakhandan Ph.D. (co-PI, Professor)

Los Alamos National Laboratory:

Shawn Starckenburg Ph.D. (PI, Deputy Group Leader)

California Polytechnic State University (Cal Poly):

Aubrey Davis Ph.D. (PI, Senior Scientist)

Heliae Development LLC:

Luke Cizek (PI, VP of Operations)



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