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

DE-FE0032189

John Benemann, CEO

MicroBio Engineering Inc.

2023 Carbon Management Research Project Review Meeting
Pittsburgh, PA, August 28 – September 1, 2023

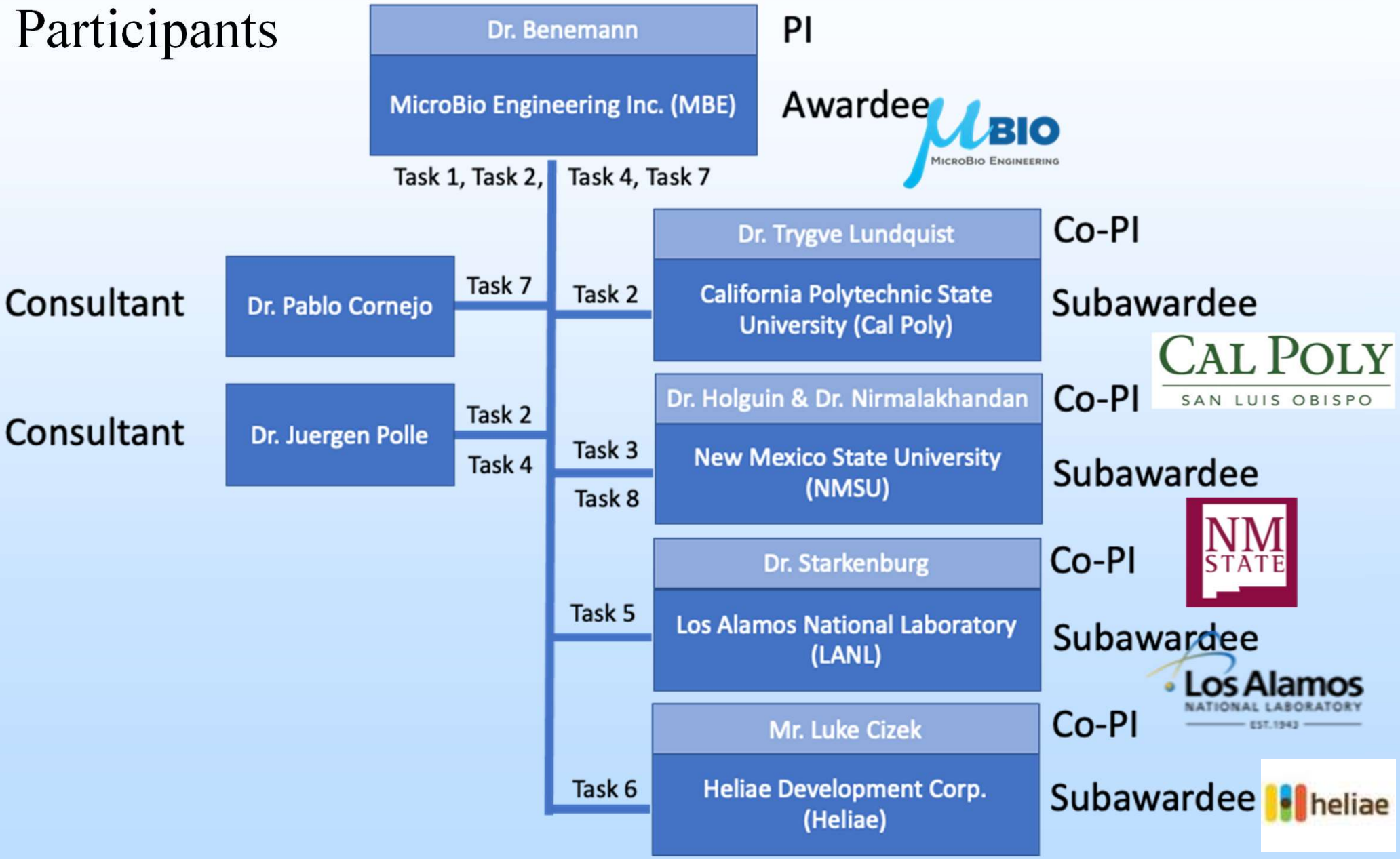
Project Outcomes

The main project outcomes are to use the green alga *Scenedesmus obliquus* to:

1. Demonstrate innovative technologies for efficient Carbon Dioxide (CO_2) gas-to-liquid transfer and reduced CO2 losses, to greatly exceed the Carbon Utilization Efficiency (CUE) of 50%; and
2. Perform strain development to achieve breakthrough biomass productivities that exceed >20g Ash-Free-Dry-Weight (AFDW)/m2-day under pond operating conditions allowing for high CUE.

Project Overview

Participants



Technical Scope Tasks Summary

Task 1 - Project Management and Planning. (MBE)

Task 2 - Minimize CO2 Outgassing During Algae Cultivation (MBE, Cal Poly).

Task 3 - Efficient Flue Gas CO2 Transfer into Ponds (NMSU).

Task 4 - Algae Strain Improvement (MBE).

Task 5 - *S. obliquus* Genomics for CUE and Productivity (LANL).

Task 6 - Product Testing in Greenhouse with Crops (Heliae).

Task 7 - TEA/LCA Studies and Model Development (MBE)

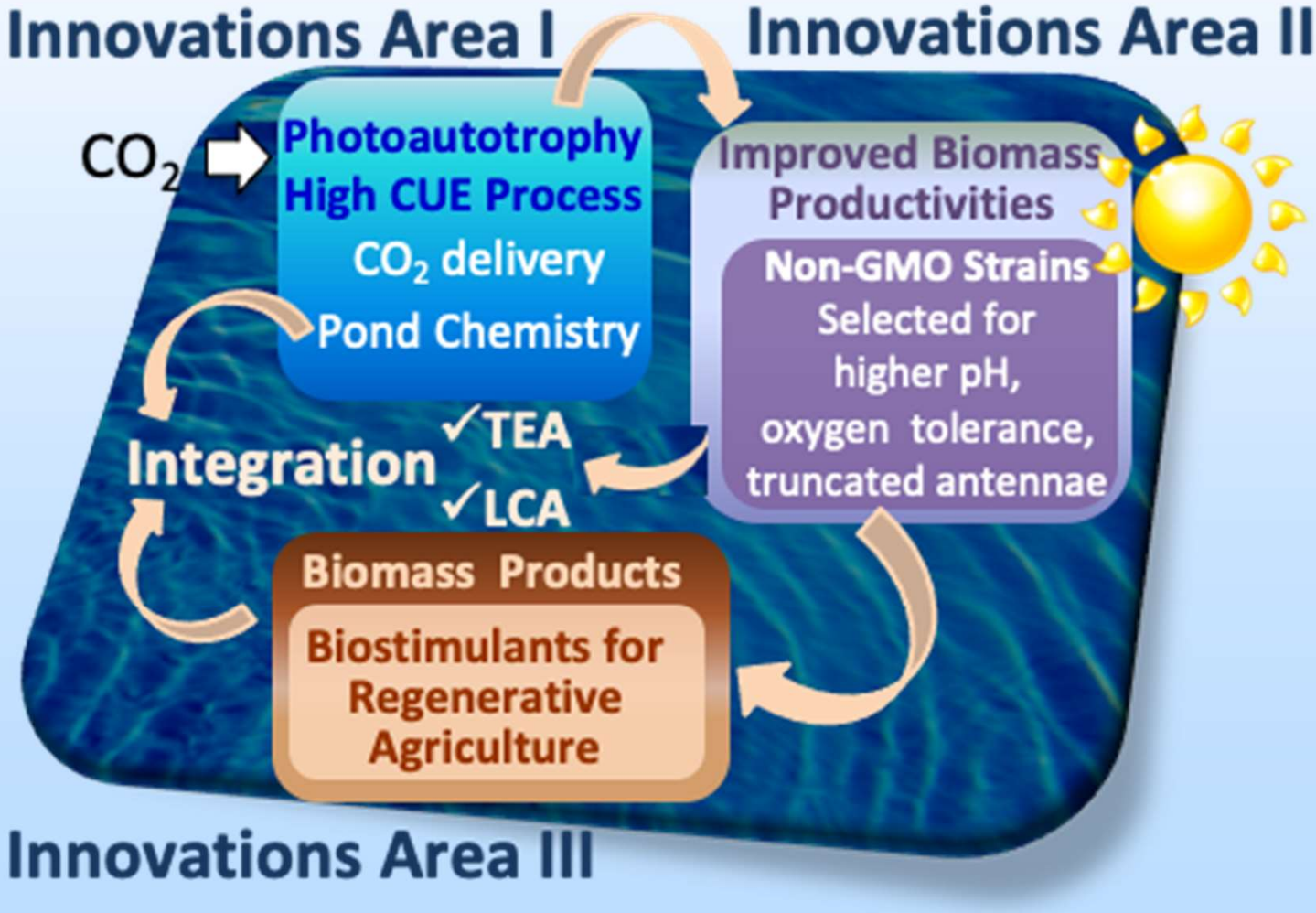
Task 8 - Diversity/Equity Inclusion (DEI) (NMSU).

Funding and Period of Performance

	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)

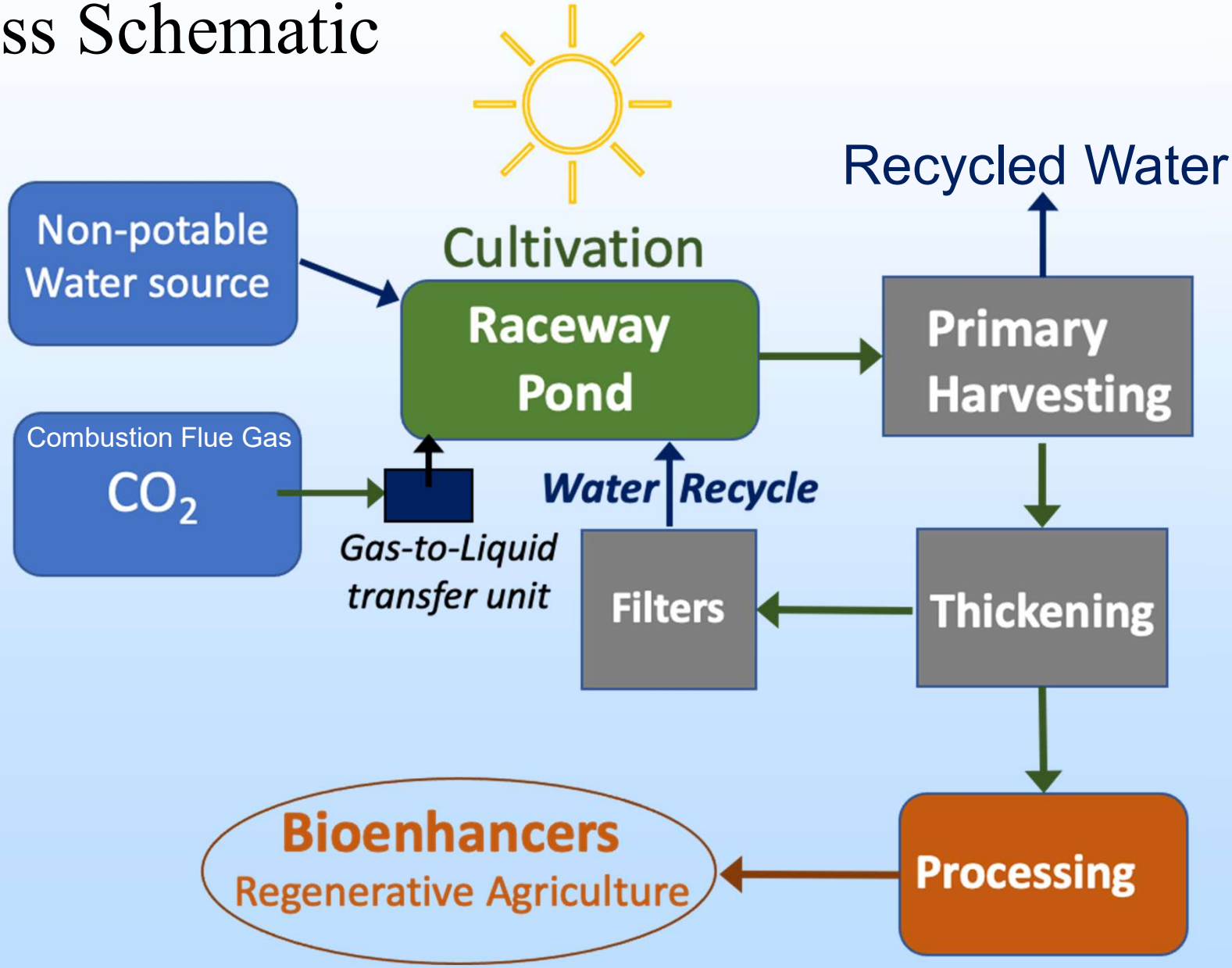
‘CRIAS’ Technology Background

Multiple innovations in CO_2 transfer and utilization technologies combined with game-changing high productivity algae strains, will maximize CO_2 utilization and advance commercial applications.

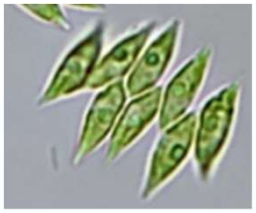


Technology Background

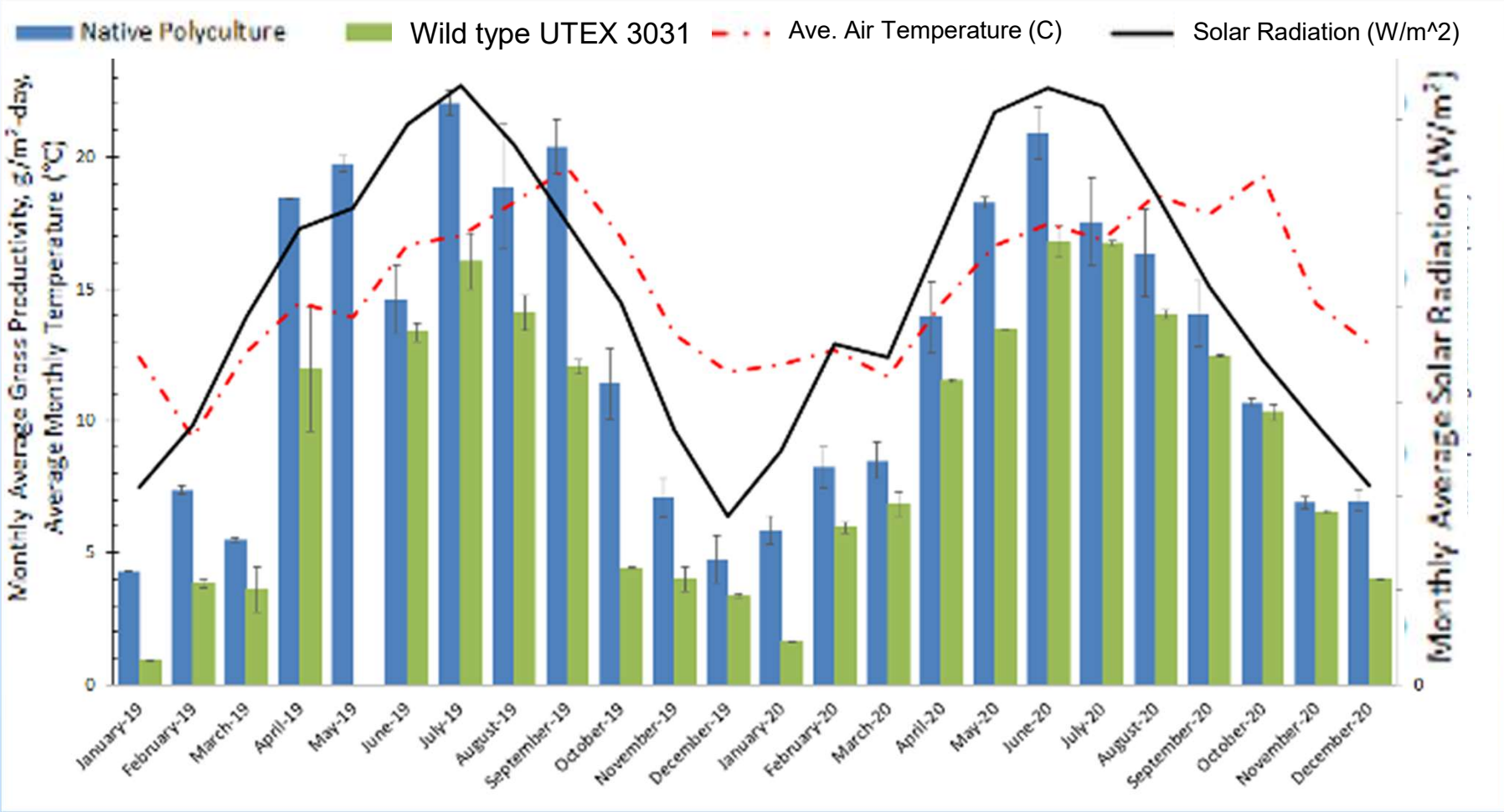
Process Schematic



Technology Background



Organism – The green alga *Scenedesmus obliquus*.

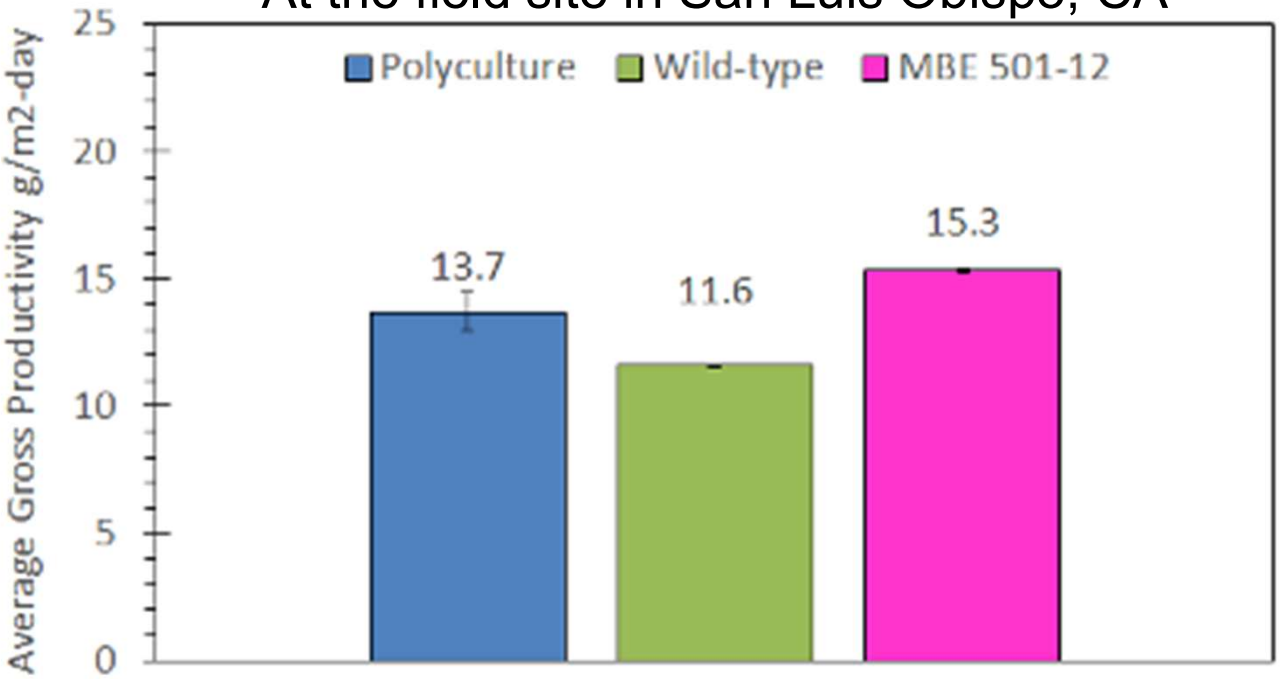


Monthly average biomass productivity of *Scenedesmus obliquus* in ponds at the MBE/Cal Poly field site in San Luis Obispo, CA. DOE funded ABY2 project.

Technology Background

Previous Achievements with *Scenedesmus obliquus*.

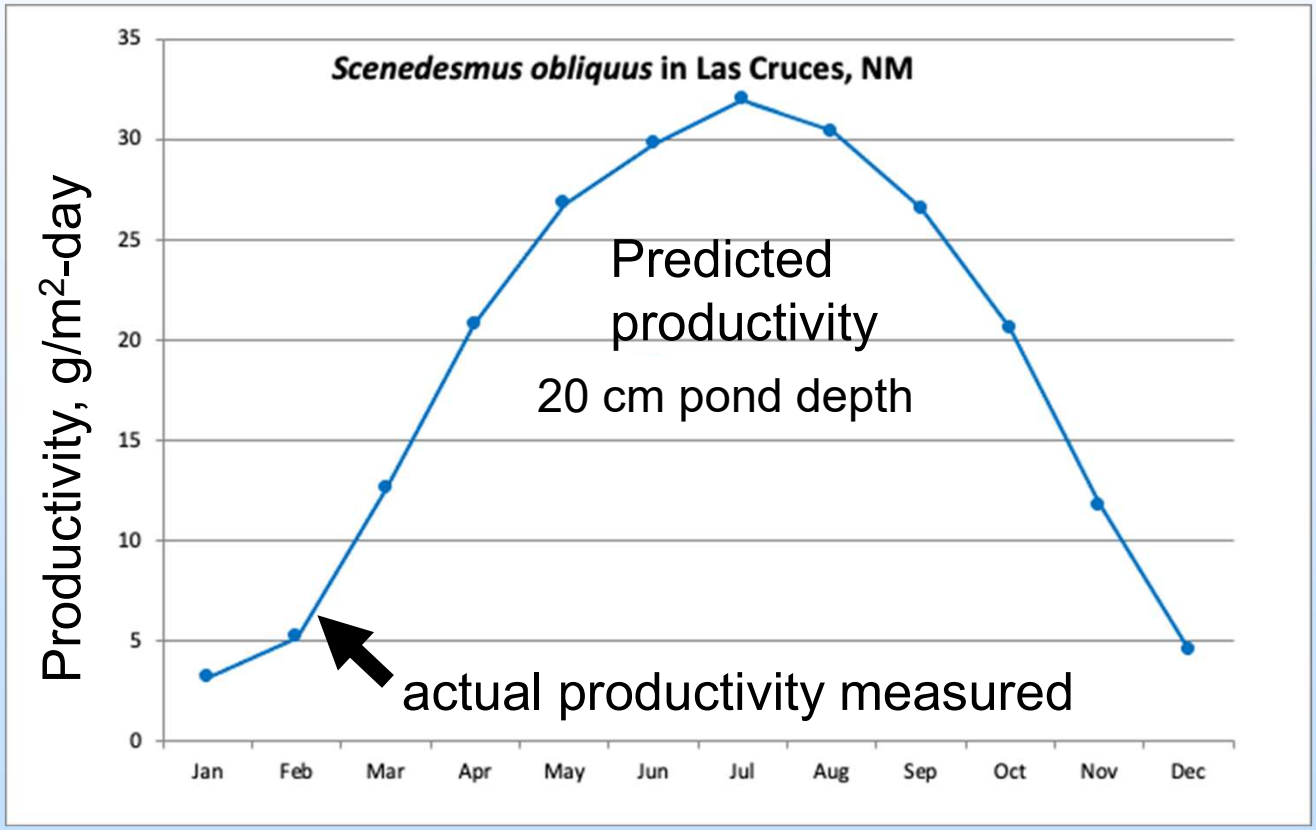
At the field site in San Luis Obispo, CA



Adaptive Laboratory Evolution resulted in improved strain 501-12.
Used for cultivation in raceway ponds in reclaimed water.

Technology Background

Predicted and Prior Cultivation of *S. obliquus* at NMSU



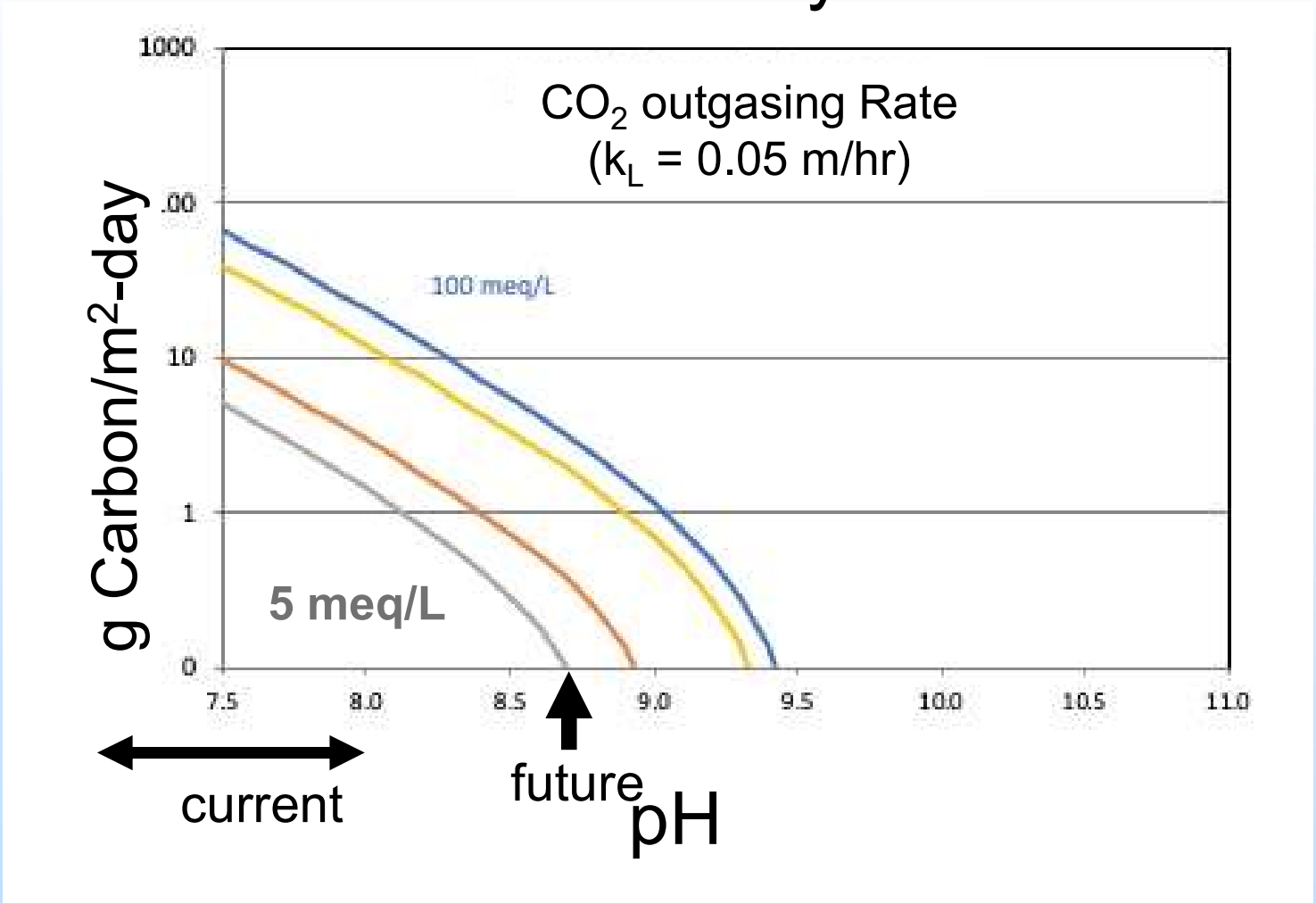
February/March 2016

Strain UTEX 3031

Predicted productivity based on production model (Huesemann et al., PNNL)

Technology Background

Pond Chemistry and CUE



Increasing pH and increasing alkalinity greatly reduces CO_2 outgassing.

Technology Background

Regenerative Agriculture – Microalgae Products

heliae AGRICULTURE

PhycoTerra® ST - Seed Treatment

PhycoTerra® - Soil Microbial Food

PhycoTerra® Organic - Soil Microbial Food

PhycoTerra® FX - Foliar Microbial Food

Technology Background

Advantages of the CRIAS technology.

1. Proven platform strains of the species *S. obliquus*.

   Strains UTEX 393 and UTEX3031 501-12 were previously cultivated in ponds on reclaimed water.

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.

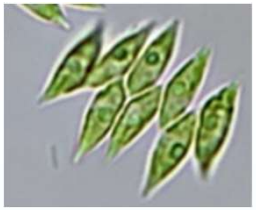
   Collaboration with Heliae Development LLC., the leader in creation of the PhycoTerra algal derived product line.

Technology Background

Technical and Economic Challenges

1. Generate strains with improved productivity.
2. Robustness of cultures in reclaimed water.
3. Efficient CO_2 transfer into cultures.
4. Efficacy of the product in agriculture.

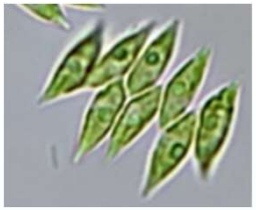
Progress and Current Status of Project



Project start date February 1st 2023.

BP	Mo	Q	Major Budget Period Objectives
1	1-15	1-5	1. Setup and initiate operations of the outdoor 1 m^3 algal pond cultivation testbeds at the Las Cruces, NM and San Luis Obispo, CA wastewater treatment facilities. 2. Establish optimal pond chemistry productivity parameters in laboratory experiments and demonstrate these in outdoor pond cultivation with the initial platform algal strains in repeated 30-day cultivation trials. 3. Carry out complete carbon mass balances for inorganic and organic carbon. 4. Initiate bioenhancer trials using wild type biomass. 5. Initiate strain improvement work and Techno-Economic Analysis and Life-Cycle Assessment. 6. Complete baseline genomic analysis.

Progress and Current Status of Project



Milestone BP1		Due	Completed
1.1	Project Management Plan	3/31/23	03/31/2023
1.2	Project startup completed, and subcontracts executed.	8/30/23	in progress
1.3	Productivities vs. pCO_2aq/pCO_2air determined for strains in the lab.	08/30/23	08/30/2023
1.4	Site setup at wastewater treatment plant (Las Cruces, NM) completed.	10/31/23	in progress
1.5	Genomes of platform strains sequenced.	10/31/23	
1.6	First improved cultivar at elevated pH/alkalinity in lab.	1/31/24	
1.7	Recruiting & mentoring students accomplished.	4/30/24	
1.8 GNG Decision Point	Demonstrated stability & measured CUE for platform strain in >30-day trial.	4/30/24	

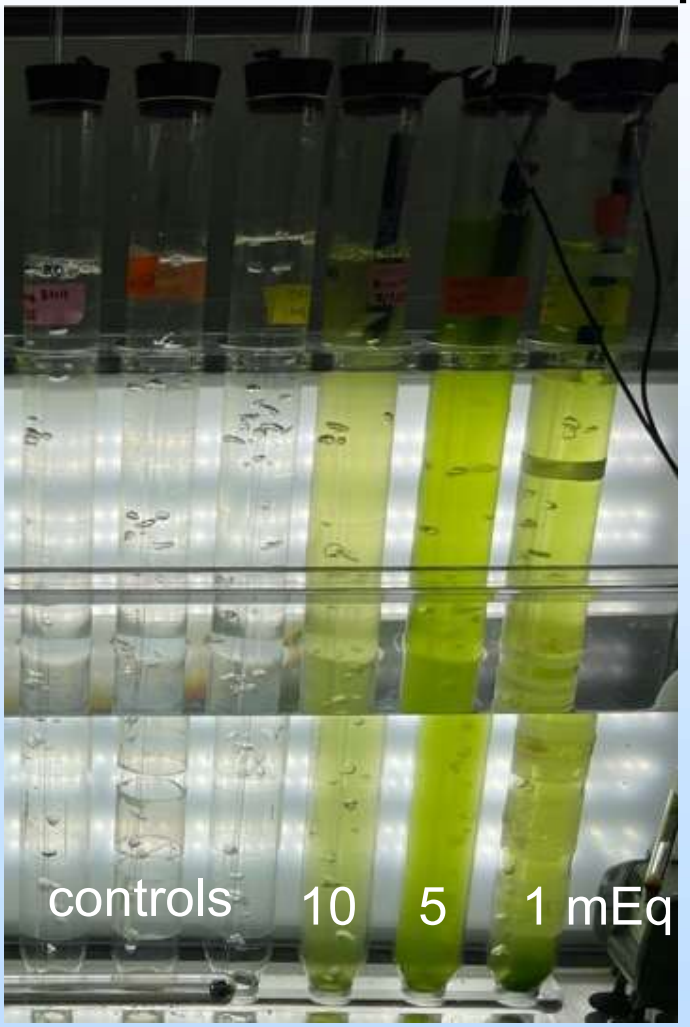
Progress and Current Status of Project

Task 2 – Setup and initiate operations with wild type strains.

- Set of four CRIAS ponds at the SURE! field site in San Luis Obispo, CA refurbished.
- UTEX 393 (haploid) and 501-12 (diploid)
- Startup of pond cultivation accomplished.
- Produced about 2.5 Kg of biomass and forwarded to Heliae Development LLC.

Progress and Current Status of the Project

Task 2 - Established optimal pond chemistry parameters.



- Set of bubbling columns with *S. obliquus* provided with air.
- Strain UTEX 393 nor strain 501-12 tolerated alkalinity of higher than 10 mEq.

Progress and Current Status of the Project

Task 3 - Initiated strain development work.

- Kill curves for UV mutagenesis established.

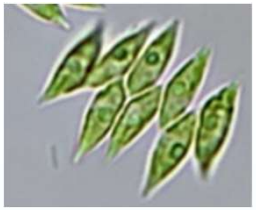
UTEX 393 (Haploid)

50% reduction at 10 seconds exposure

501-12 (Diploid)

50% reduction at 20 seconds exposure

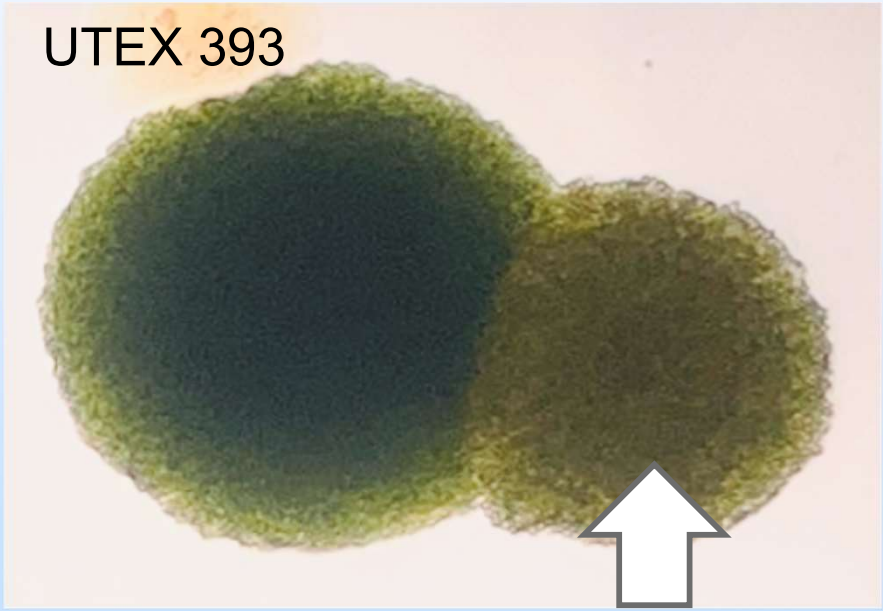
Progress and Current Status of the Project



Task 3 - Initiated strain development work.

- First pigment mutants identified for both strains.

Algae Strain	Number of Mutants
UTEX 393	26
MBE 501-12	24



wild type pigmentation

Pigment Mutant less chlorophyll

Diversity Equity & Inclusion

- DEI efforts planned:

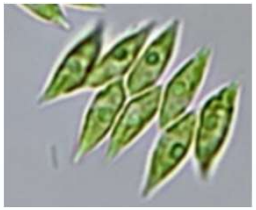
  Recruit 2-3 students as summer interns into the companies and to LANL and organize a DEI workshop with students.

- Accomplished for Summer 2023:

  - Two DEI students from Cal Poly at MBE worked on this project.

  - DEI Workshop completed on 18th of August in online format (ZOOM).

Summary of Progress



Task 1 - PMP delivered.

Task 2 - Pond cultivation initiated.

Task 3 - Strain Development in progress.

Task 4 - Pond preparation in progress.

Task 5 - Samples sent for genome sequencing.

Task 6 - Biomass sent for processing.

Task 7 - TEA and LCA to be initiated later this year.

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

Plans for future research.

Strain development is in progress.

Setup of the novel CO_2 transfer unit from flue gas into algal ponds is in progress.

Take-away Message

Improved biomass productivity achieved with superior new strains will be crucial to meeting goals of high CUE in ponds for CO2 utilization for microalgae biomass prouction.

Cultivation-Ready Improved Algae Strains (CRIAS) for Increased Carbon Utilization Efficiency DE-FE0032189

THANK YOU

John Benemann, CEO, MicroBio Engineering Inc
Tryg Lundquist, Cal Poly
Shawn Starkenburg, Los Alamos National Laboratory
Omar Holguin and Nirmal Khandan, New Mexico State U.
Luke Cizek, Heliae

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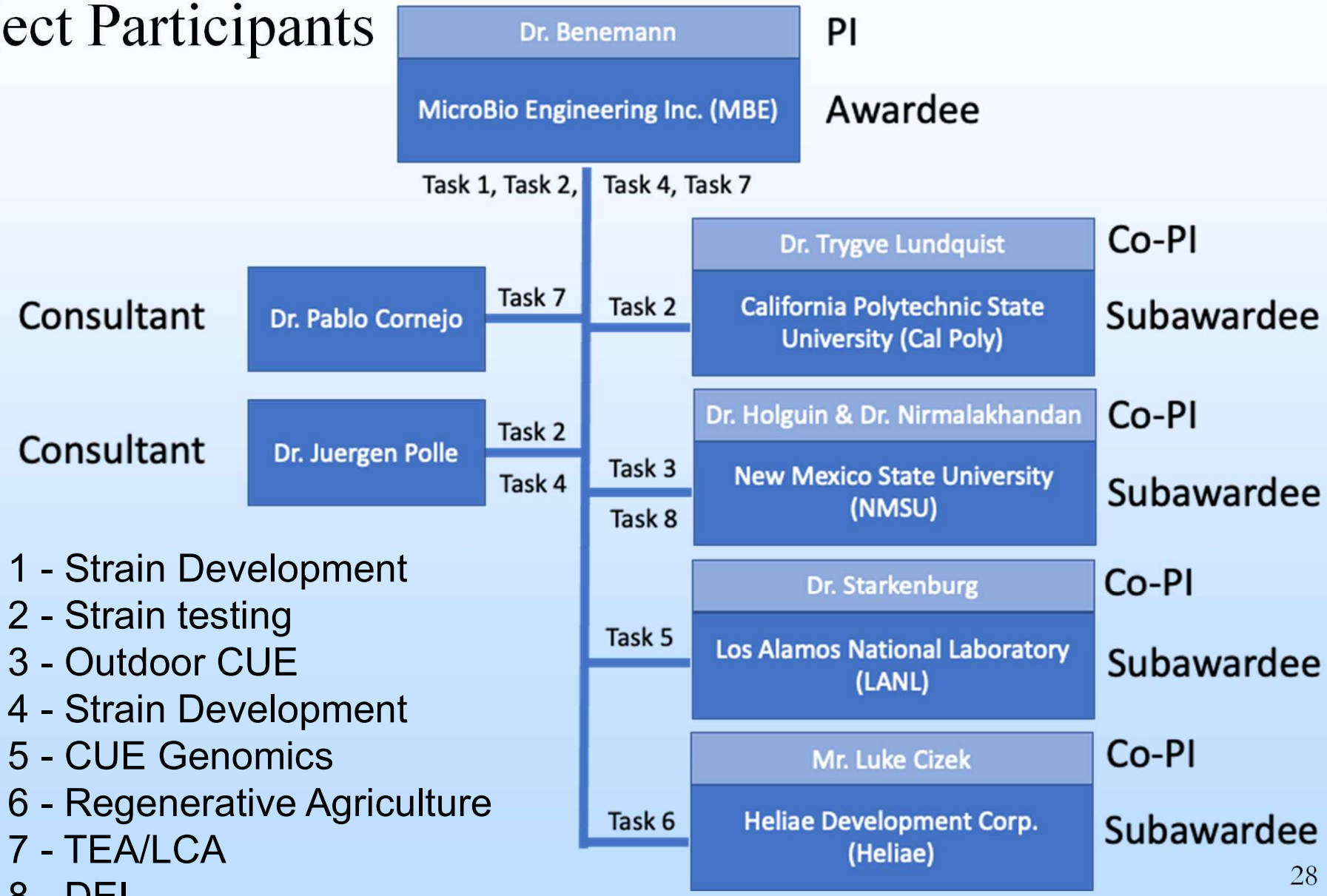
Appendix

- Organization Chart
- Gantt Chart
- Milestones
- Success Criteria

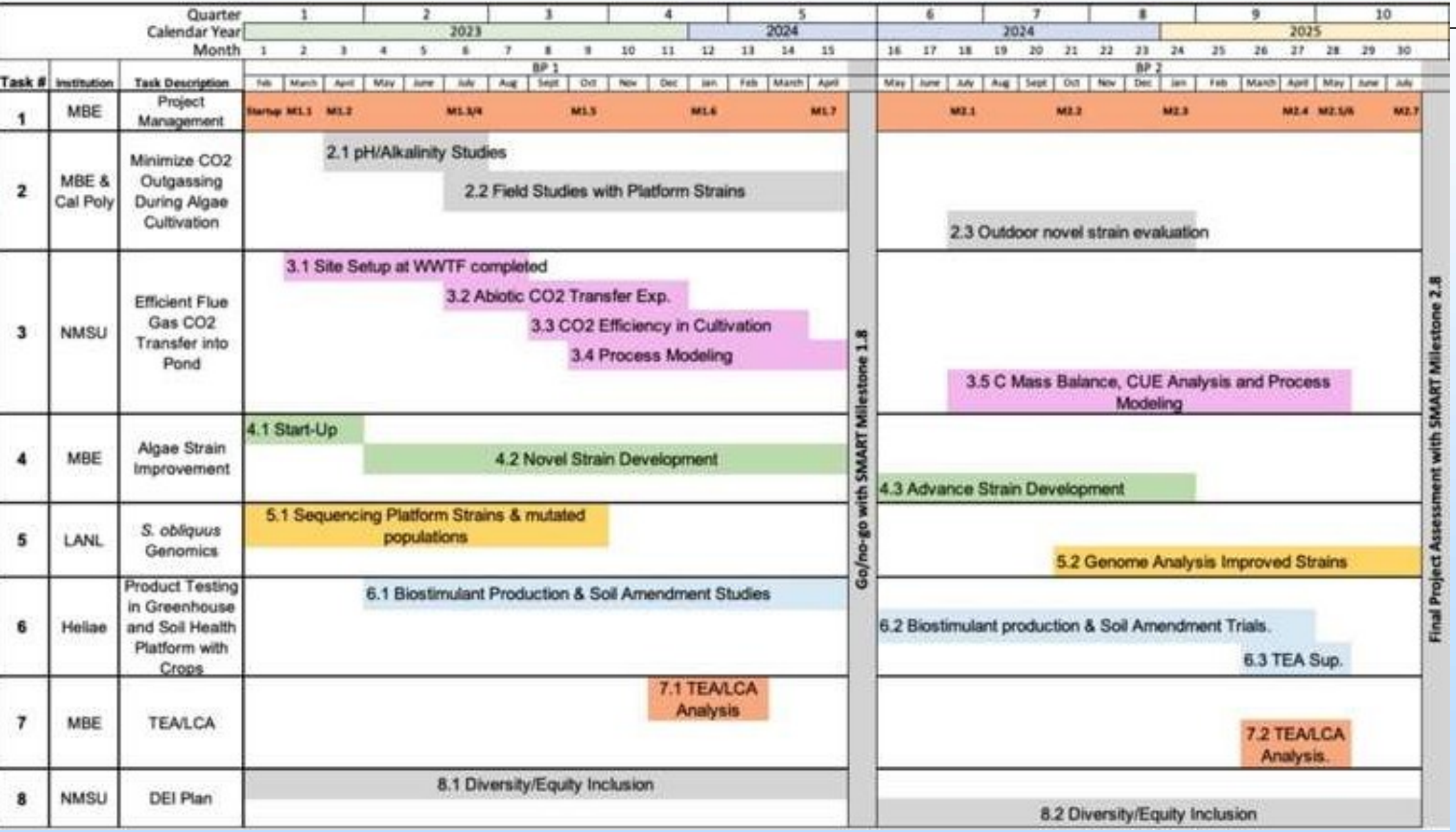
Organization Chart

Project Participants



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

Gantt Chart


Quarter
Calendar Year
Month
BP 1
BP 2
Task #
Institution
Task Description
1
MBE
Project Management
Startup M1.1 M1.2 M1.3/4 M1.5 M1.6 M1.7
M2.1 M2.2 M2.3 M2.4 M2.5/6 M2.7
2
MBE & Cal Poly
Minimize CO2 Outgassing During Algae Cultivation
2.1 pH/Alkalinity Studies
2.2 Field Studies with Platform Strains
2.3 Outdoor novel strain evaluation
3
NMSU
Efficient Flue Gas CO2 Transfer into Pond
3.1 Site Setup at WWTF completed
3.2 Abiotic CO2 Transfer Exp.
3.3 CO2 Efficiency in Cultivation
3.4 Process Modeling
3.5 C Mass Balance, CUE Analysis and Process Modeling
4
MBE
Algae Strain Improvement
4.1 Start-Up
4.2 Novel Strain Development
4.3 Advance Strain Development
5
LANL
S. obliquus Genomics
5.1 Sequencing Platform Strains & mutated populations
5.2 Genome Analysis Improved Strains
6
Heliae
Product Testing in Greenhouse and Soil Health Platform with Crops
6.1 Biostimulant Production & Soil Amendment Studies
6.2 Biostimulant production & Soil Amendment Trials.
6.3 TEA Sup.
7
MBE
TEA/LCA
7.1 TEA/LCA Analysis
7.2 TEA/LCA Analysis.
8
NMSU
DEI Plan
8.1 Diversity/Equity Inclusion
8.2 Diversity/Equity Inclusion
Go/no-go with SMART Milestone 1.8
Final Project Assessment with SMART Milestone 2.8

Task/ Subtask	Milestone Number	Description **Milestones**	Planned Completion Date	Verification Method
1.1	1.1	Project Management Plan.	3/31/2023	PMP File
1.0	1.2	Project startup completed, and subcontracts executed.	04/30/2023	Quarterly Report
2.1	1.3	Productivities vs. pCO_2aq/pCO_2air determined for *S. obliquus* strains MBE501-12 and UTEX393 in the lab.	07/31/2023	Quarterly Report
3.1	1.4	Site setup at wastewater treatment plant completed.	07/31/2023	Quarterly Report
5.1	1.5	Genomes of platform strains sequenced	10/31/2023	Quarterly Report
4.2	1.6	First improved cultivar at elevated pH/alkalinity in lab.	01/31/2024	Quarterly Report
8.1	1.7	Recruiting & mentoring students accomplished.	04/30/2024	BP1 report
Go/No-Go Deci. Point	1.8	Demonstrated stability & measured CUE for platform strain in >30-day trial.	04/30/2024	BP1 report
4.3	2.1	A second improved strain demonstrated in the lab.	07/31/2024	Quarterly Report
2.3	2.2	At least one improved strain demonstrated in ponds.	10/31/2024	Quarterly Report
2.3	2.3	A second improved strain demonstrated in ponds.	01/31/2025	Quarterly Report
3.5	2.4	Completed pond test run with improved strain.	04/30/2025	Quarterly Report
7.2	2.5	TEA	05/31/2025	TEA Report
7.2	2.6	LCA	05/31/2025	LCA Report
8.2	2.7	Mentored at least 5 students in BP2.	07/31/2025	Quarterly Report
	End of Project Goal 2.8	Demonstrated at least one improved strain in >30 day outdoor trial with near 70% CUE and 25 g AFDW/m2-d.	07/31/2025	Final report

Success Criteria

Decision Point	Date	Success Criteria
Completion of BP1	04/30/2024	Demonstrated stability and measured CUE for platform strain in >30-day trial. Trial run completed, CUE and biomass productivity determined for one platform strain outdoors.
End of Project	07/31/2025	Demonstrated at least one improved strain in >30 day outdoor trial with near 70% CUE and productivity of 25 g AFDW/m2-d. Trial run completed in outdoor 1,000 liter pond, CUE and productivity targets met.