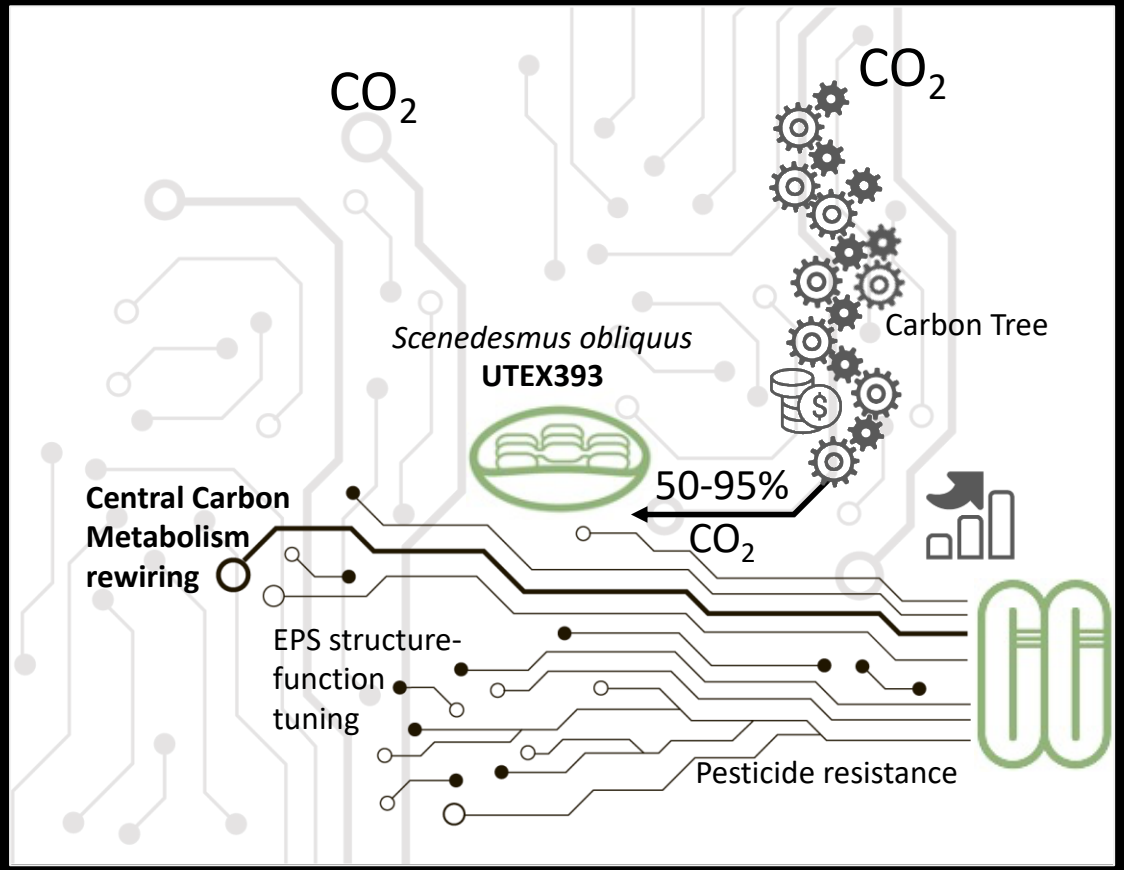
AzCATI

Arizona Center for Algae Technology and Innovation

Direct Air Capture Integration with Algae Carbon Biocatalysis



Award #DE-EE0009674
WBS: 1.3.1.670
BETO Project Peer Review: Advanced Algal Systems
April 4th, 2023

DOE FOA DE EE0002423:
Algae Productivity Exceeding Expectations
Topic 2B: Improvements in Productivity with Direct Air Capture (DAC) of CO_2 from Ambient Air:
Strain AND Cultivation Improvements

ASU Ira A. Fulton Schools of Engineering, Arizona State University

NREL Transforming ENERGY

CARBON COLLECT

Burge Environmental

U.S. DEPARTMENT OF ENERGY

One little cell, a world of possibilities.

Project Overview: Background

- Supplementation of carbon is critical for high productivity cultivation of most microalgae
- Co-location of algae cultivation operations adjacent to waste sources of CO_2 or proximate to existing CO_2 pipelines and water resources may severely limit siting options.[1]
- This project technology uncouples algae production from co-location with point sources of waste CO_2 or proximity to existing CO_2 pipeline infrastructure, opening opportunities for siting near high productivity algae cultivation climate and environmental hotspots or available land.
- This project aims to demonstrate the modeled integration of an innovative passive direct air capture (PDAC™) technology in outdoor algae cultivation with improvements along the CO_2 delivery train, including novel membrane gas-liquid interface solutions
- The challenge is developing technologies, processes, and strategies that utilize carbon efficiently, such that the overall system is sustainable and economical.

1. Somers, M. D. and Quinn, J. C., *Journal of CO2 Utilization*, 2019, **30**, 193-204

Project Overview: Project Objectives and Expected Impact

Project Objectives:

- Deliver a fully modeled integration of Passive-Direct Air Capture (PDAC) with algae cultivation with innovative carbon delivery and enzyme biocatalysis
- Accelerated carbon capture and storage engineering through carbon flux control in *Scenedesmus* UTEX393
- Designed resilient pond ecology for long term outdoor cultivation to improve time to failure and overall productivity

Impacts:

- Uncoupling algae farming from point source CO_2 by relying on novel DAC-sourced carbon, and CO_2 delivery innovations increases national deployment potential
- Metabolic engineering of Calvin cycle control demonstrated in production strain UTEX393 translates broadly to improve photosynthetic carbon assimilation
- Increase carbon capture efficiency through marketable technology development (membrane carbonation)
- Accelerate deployment and commercialization of algae-DAC installations

Project Overview:

Challenges:

1. Carbon supplementation is critical for high productivity cultivation of most microalgae and requires high carbon capture and use efficiency to be sustainable at scale (i.e., current Farm Model assumes 90%) but CUE is highly cultivation system specific and generally much lower.
2. Co-location of algae cultivation to waste sources of CO_2 or proximity to existing CO_2 pipeline infrastructure can severely limit siting options, as the most promising locations possessing both the water resources and ideal climate for high productivity algae cultivation are not near such infrastructure nor have the available land.
3. Though the potential for liquid fuel production with algae is significant, the potential to capture and recycle CO_2 emissions from existing point sources is not. A first-order scalability assessment of available point-source emissions that could meet location, economics, and sustainability metrics for algal biofuels showed carbon to be a limiting nutrient at an algal biorefinery with a national capacity of producing only 360 million gallons per year (see Sommers et al, 2019).
4. In addition, the waste CO_2 streams often proposed, such as power plants, need refinement to remove contaminants and CO_2 sourced on the merchant market can be 3x to orders of magnitude above cost targets for carbon feedstock and there can be significant volatility challenging commodity product economics such as fuel
5. However, with the PDAC™ system we propose, locations where climate and water resources are ideal for algae cultivation can be supplied with a very clean and sustainably sourced CO_2 feedstock increasing the national potential for algae biomass production for biofuels and other bioproducts.
6. Carbon capture utilization efficiency (CCUE) needs to be high (assumed 90% at scale) though few if any systems have demonstrated that, and in fact is usually much lower (<<50%).

Project Overview: DACIACB Team Members

ASU Team

John McGowen (PI)
Taylor Weiss (Co-PI): Task 3 lead
Peter Lammers (Co-PI)
Everett Eustance (Senior Scientist, carbon deliver and efficiency)
Jessica Forrester (Lab Manger),
Prabuddha Laltaprasad Gupta (Post Doctoral Scholar)
Jason Potts (Technician – outdoor cultivation)
Richard Malloy (Greenhouse/Fieldsite Supervisor)
Brendan Wacenske (Grad student)

NREL TEAM:

Lieve Laurens: Co-PI and NREL Lead
Mauro Lua, Andrew Young, engineers, carbon delivery and efficiency improvements
Foteini Davrazou, protein biochemist, carbonic anhydrase
Jessica Loob, molecular biologist, Scenedesmus engineering
Seth Steichen, biologist, bioinformatics & RNAseq
Arnav Deshpande, Steven Rowland, chemist, metabolomics
Bruno Klein, engineer, TEA & LCA

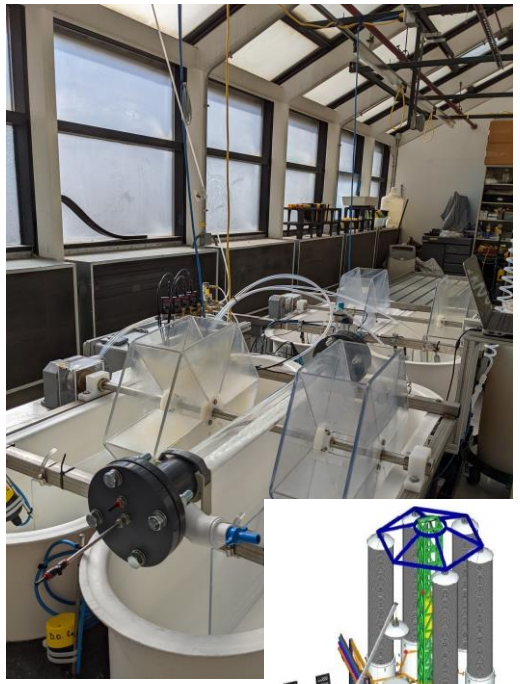
Carbon Collect Team:

Pól Ó Móráin, CEO, Founder and Director
Mike Austell, VP Engineering

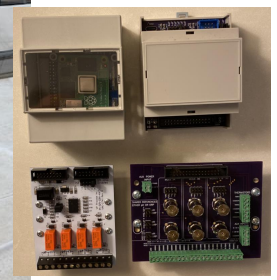
Burge Environmental Team: (vendor on proposal -will be formal sub)

Evan Taylor, Scott Burge, Dave Baker, Brian Ford: pond sensor platform improvements for monitoring and process control, data integration/analysis









Approach: Project Tasks And Team Organization

Task 1: P-DAC Integration Modeling and Testing/Optimization of Carbon Delivery from Commercial Scale Demonstration Unit (Carbon Collect/ASU/NREL/Burge)

- **Subtask 1.1: Baseline Integration Design and Process Modelling of the P-DAC system based on Tempe 1st of a kind full scale Carbon Tree pilot demonstration (CC/NREL)**
- **Subtask 1.2: Carbon Delivery Efficiency Optimization (NREL/ASU)**
- **Subtask 1.3: Accelerating Carbon Capture Rates with CA Biocatalysis (NREL/ASU)**
- **Subtask 1.4: Outdoor testbed cultivation deployment (ASU/Burge/NREL/CC)**

Task 2: Carbon Capture and Storage Improvements in UTEX393 (NREL)

- **Subtask 2.1: Integrated Systems Biology for Dynamic Carbon Flux Mapping**
- **Subtask 2.2: Building Robust Genetic Engineering Toolkit for Scenedesmus sp. Accessing Carbon Assimilation Pathways**

Task 3: Rationally Designed Pond Ecology for Resilient, Year-Round Cultivation (ASU)

- **Subtask 3.1: Selection for Strain Adaptation to Pest Management**
- **Subtask 3.2: Microbial Profiling for Active Crop Management**
- **Subtask 3.3: Biomass Quality vs. Pond Ecology Impact**

Task 4: Technoeconomic and Life Cycle Analysis (NREL/CC)

- **Subtask 4.1: Engineering Process Modeling**
- **Subtask 4.2: TEA and LCA of Fully Integrated Carbon Delivery System**

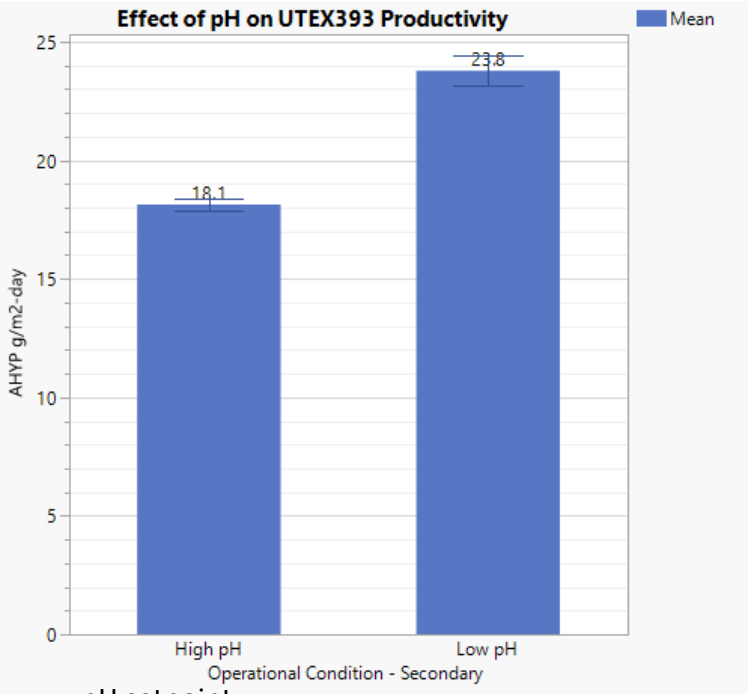
Technical Scope Summary	
Year 1	Process modeling of DAC with interface and conceptual integration with cultivation, selection of CO_2 delivery and enzyme biocatalysis, phenotyping novel UTEX393 cultivars
Year 2	Select technologies to move towards scaled integration and outdoor demonstration
Year 3	Improved operations to demonstrate and collect critical performance metrics for DAC delivery to algae ponds, towards final productivity and biomass quality targets

Approach: Go/No Go Decision Points

Go/No Go Decision Point 2: Demonstrate 10% improvement in productivity (and **achieve 50% improvement**** in carbon utilization efficiency (CUE) at pond interface at the AzCATI testbed site with simulated DAC CO_2 carbon concentration delivery over at least 3 weeks of continuous WT UTEX393 cultivation across at least two seasons, when compared against baseline (SOT) concentrated CO_2 delivery using sparging (Spring 2024)

Note - Milestone M.1.4.1 (Spring/Summer 2023) will establish the CCUE baseline for UTEX393 which is expected to be in the 15-25% range for standard 100% CO_2/sparging (pH setpoint 7). This would mean the intermediate target for the Go/No Go would be a CCUE in the range of minimum 23-38%.

30 % improvement in productivity for low pH, but likely large decrease in CUE



pH setpoint
High pH = 7.9
Low pH = 7.0
May data for ~ 14 days
Media: Modified BG-11 (5 ppt salinity and Ammonium Bicarb as N-source)

End of Project Goal: Assemble critical data around the most carbon-efficient algae-based conceptual biorefinery and demonstrate the end-of-project goal of 100% DAC-derived, dissolved inorganic carbon delivery that achieves a **20% increase in biomass productivity (16.8 g/m2-day) and achieving a carbon capture utilization efficiency (CCUE) at least 75% above baseline** in a conceptual integrated system with UTEX393, while achieving biomass composition quality of **at least 85 GGE/ton fuel intermediate yield**.

Approach: Task 1 Summary

Task 1: P-DAC integration modeling and testing/optimization of CO_2 delivery from commercial scale demo unit.

Subtask 1.1: Baseline Integration Design and Process Modelling of the P-DAC system based on Tempe 1st of a kind full scale Carbon Tree pilot demonstration (CC/NREL)

This task will focus on the testing of a commercial-scale demonstration unit for P-DAC and include ***a conceptual integration*** with the AzCATI testbed operations as well as novel membrane-based CO_2 delivery in combination with an enzymatic biocatalysis unit with the goal of increased carbon capture and utilization efficiency. The conceptual integration is needed because the P-DAC tree is not physically co-located at the AzCATI campus in this first demonstration, and thus algae cultivation will receive a mimic of the CO_2 collected off the Carbon Tree for part of the project:

- **In BP2:** simulated DAC CO_2 based on expected range of composition (CO_2 %) off the PDAC pilot in Tempe, AZ by blending pure CO_2 with air/moisture (target range 50-95% CO_2).
- **In BP3:** actual DAC CO_2 captured and transported (@ 1 atm as target) for at least two separate seasons of cultivation under best case concentration (% CO_2).

Approach: Task 1 Summary

Subtask 1.4: Outdoor testbed cultivation deployment

We will conduct seasonal cultivation field trials using AzCATI's standard scale up pathways for UTEX393 and primarily in 4.2m^2 ORPs, under semi-continuous operation for harvest/resets (i.e., standard SOT protocols). Through an iterative lab to field to lab paradigm, we will verify indoor lab results on CO_2 management informed results from Task 1 on carbon delivery and improving CUE, strains from Task 2 and continue lab to field to lab iterations with Task 3.

In BP2: simulated DAC CO_2 based on expected range of composition (CO_2 %) off the PDAC pilot in Tempe, AZ by blending pure CO_2 with air/moisture (target range 50-95% CO_2).

In BP3: actual DAC CO_2 captured and transported (STP as target) for at least two separate seasons of cultivation.

BP2 Milestones

Milestone 1.4.1: Complete seasonal cultivation trial #1 (summer/fall) and confirm baseline performance of equivalent annual average of at least 14 g/m2-day and establish baseline CCUE under standard sparging/cultivation conditions vs initial improved IC delivery targets identified from Subtask 1.2. (September 2023)

Milestone 1.4.2: Complete seasonal cultivation trial #2 (Fall/Winter) with simulated DAC CO_2 %'s based on Subtask 1.1 design and testbed results, and through CO_2 delivery optimization improve CUE by at least 50% over baseline SOT conditions with sparging (March 2024).

Approach: Task 2

Carbon Capture and Storage Improvement in UTEX393

Goal: Increase growth and productivity by rational and random strain engineering

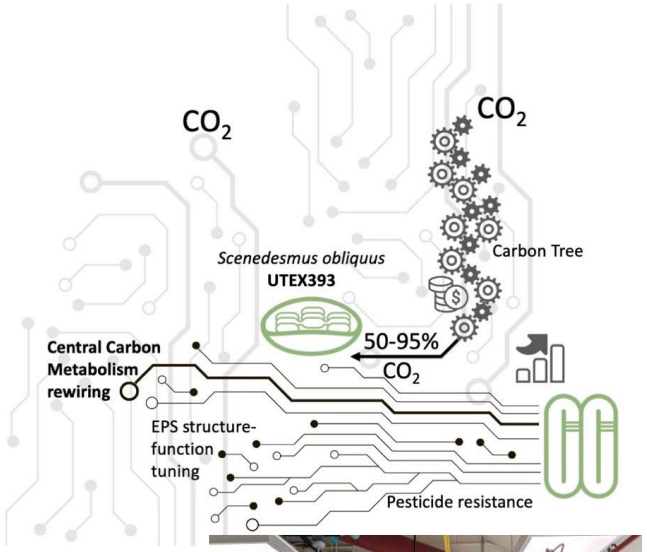
- Comprehensive systems biology (Transcriptomics and metabolomics)
- Metabolic flux under varying CO_2 concentrations
- Carbon storage rates under nutrient limitation
- Random mutagenesis and ALE used as risk mitigation for rational engineering

Subtask 2.1: Integrated Systems Biology for Dynamic Carbon Flux Mapping

- Design simulated physiological testing environment for CO_2 capture and storage calculations (SAGE upgrades complete)
- Elucidate intermediates between inorganic carbon and organic carbon storage, metabolomics
- Genetic and physiological response to changing IC environments, transcriptomics using established pipelines for RNAseq data analysis

Subtask 2.2: Building Robust Engineering Toolkits

- Provide robust rational engineering tools and protocols to be published for UTEX393
- Create at least 5 mutant strains, among which those that exhibit successful integration of the overexpression cassettes and characterized in terms of CBB carbon flux as described in task 2.1



New 18-position reactor for simulated environment cultivation and with 3 dedicated to CUE measurements (2.5 L)

Milestone 2.1.1: Identify flux bottleneck for carbon assimilation in Scenedesmus, as major varying pathway under different CO2 concentrations and including at least one mutant from year 1 screening effort. (9/2023)

Milestone 2.2.1: Select at least one mutant line from top 5 candidates based on growth, CUE or composition improvements, report whether at least one candidate has target gene is inserted and if present initiate TERA application 12/2023

Approach: Task 3 Summary

Task 3: Rationally Designed Pond Ecology for Resilient, Year-Round Cultivation (ASU)

Subtask 3.1: Selection for Strain Adaptation to Pest Management
This subtask will characterize the putative fluazinam resistance mechanism, glutathione detoxification, and select for improved bleach and/or salinity-swing resistance. Identified mechanisms will inform UTEX393 cultivation as well as provide target for genetic engineering in other species.

Subtask 3.2: Microbial Profiling for Active Crop Management
Isolating and leveraging the bacteria which already most successfully fill the niche presented by large scale UTEX393 cultures and examine if occupying this niche with a known bacterial partner prophylactically can diminish the rate of emergence by other random—and possibly pathogenic—microorganisms.

Subtask 3.3: Biomass Quality vs. Pond Ecology Impact
This subtask will focus on the engineered UTEX393 strains which may emit less fixed carbon into their surroundings, disrupting their phycosphere interactions. The presence or absence of consortial members will be observed and the facultative pathogenicity hypothesis tested (i.e., normally co-existing bacteria turn pathogenic when acutely deprived of epiphytic nutrition). (This is a BP3 task)

Micro-encapsulator

Microencapsulation of UTEX-393 beads

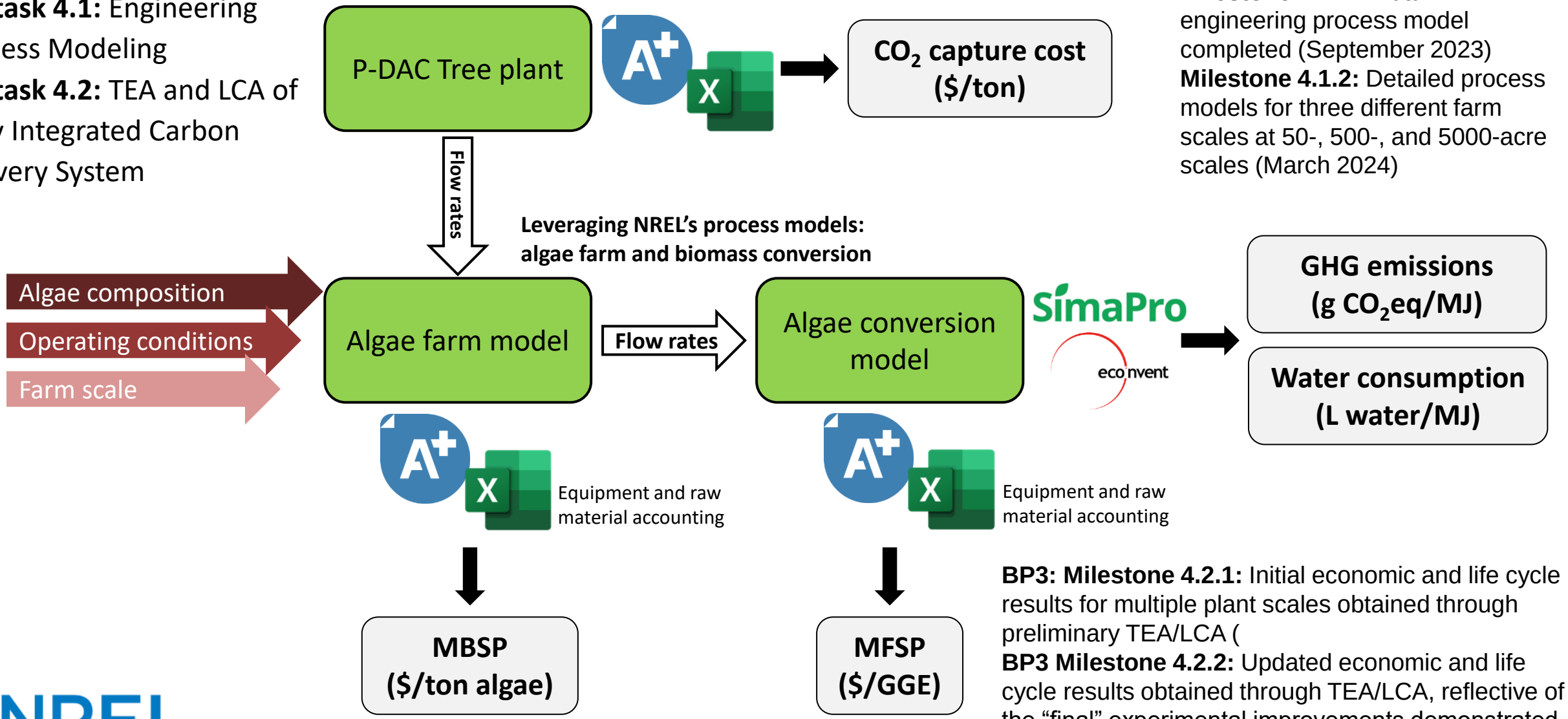
Milestone 3.1.1: Characterization of molecular basis of UTEX393 fluazinam resistance mechanism; best practices temporal application and dosing reported. (September 2023)
Milestone 3.2.1: Prophylactic bacterial consortium open pond testing completed; mean-time to failure enhanced 50% during pathogen challenge. (October 2023)

Approach: Task 4 Summary

Technoeconomic and Life cycle analysis

Subtask 4.1: Engineering Process Modeling

Subtask 4.2: TEA and LCA of Fully Integrated Carbon Delivery System



Milestone 4.1.1: Initial engineering process model completed (September 2023)

Milestone 4.1.2: Detailed process models for three different farm scales at 50-, 500-, and 5000-acre scales (March 2024)

BP3: Milestone 4.2.1: Initial economic and life cycle results for multiple plant scales obtained through preliminary TEA/LCA (

BP3 Milestone 4.2.2: Updated economic and life cycle results obtained through TEA/LCA, reflective of the "final" experimental improvements demonstrated over the course of the project.

Approach: Key Risks

Risk: The key technical risk for the DAC technology will be sub optimal performance of the single tree with respect to daily capture capacity as well as target %CO_2 during regeneration. A key factor for the techno-economic analysis/life cycle analysis (TEA/LCA) is the achievable %CO_2 directly off the tree, at scale (e.g., at multi-ton per day clusters) with full integration in a biorefinery.

- Mitigation 1) By the time this project would start, Carbon Collect would have deployed first commercial scale prototype at ASU and data would be available – this was the case and we now have initial performance data showing the tree is capturing significant quantities of CO_2 and that % CO_2 directly off the tree will be at high end of design target (~95%). We will still test and model down to 50% as there could be energy savings at lower % CO_2.

Risk: Genetic construct maintenance and stability as this has been encountered after successfully engineering other, related species across multiple BETO projects.

- Mitigation: Task 2 is focused on a limited number of metabolic targets. We have built in a contingency plan of working with a mutagenized population of UTEX393 and applying directed laboratory evolution to obtain a non-GM mutant strain that can be transferred for outdoor deployment (without TERA permit).

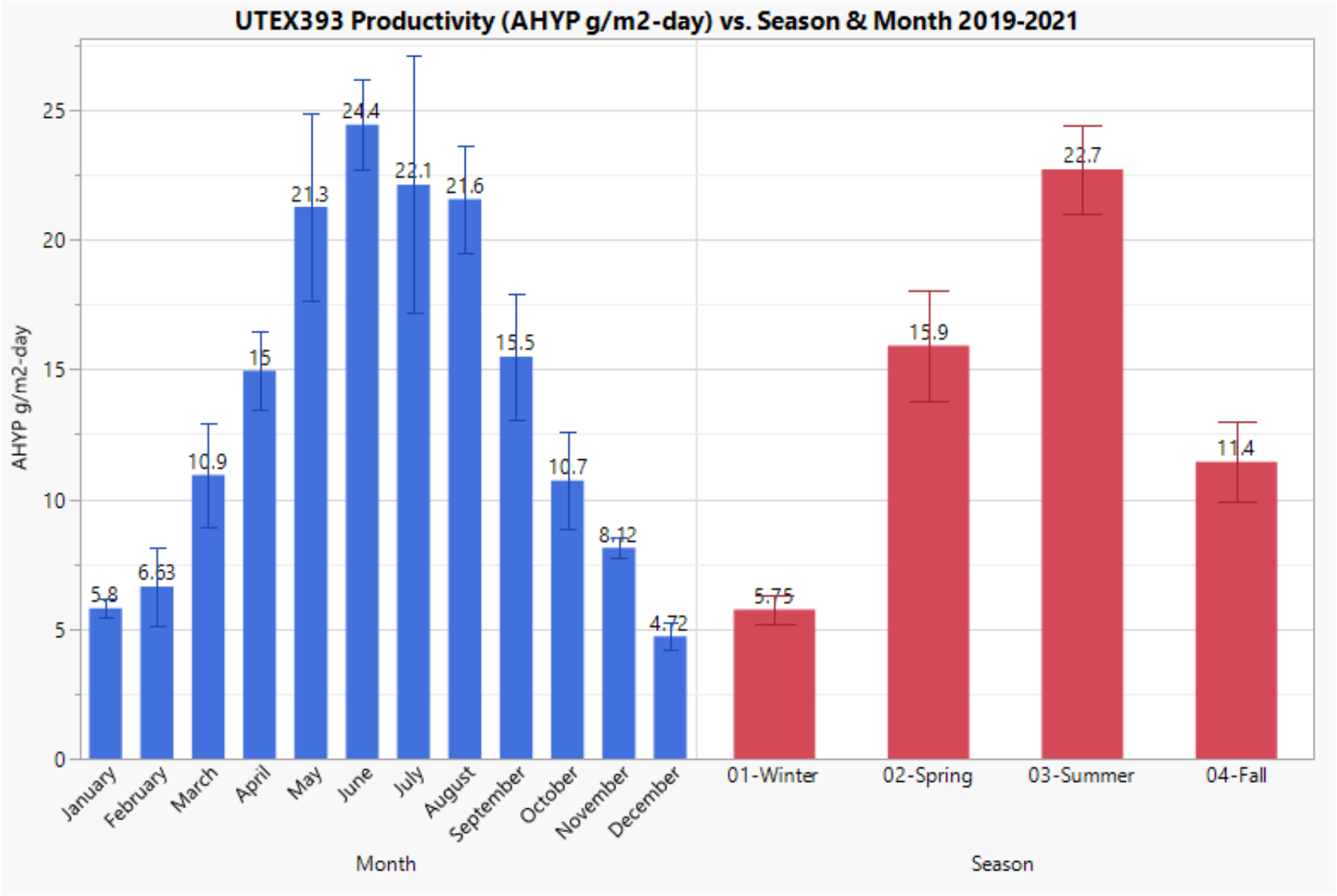
Risk: Predatory bacterial contamination in UTEX393 – HIGH RISK.

- Mitigation: if current traditional crop protection/mitigation strategies and or strain adaptation to proven mitigation steps (e.g., chlorine and or salinity) fail, we likely will not hit productivity and CUE numbers. We could then run other top performing strains for demonstration of carbon efficiency and DAC integration completing the TEA/LCA modeling work based on known good performers at AzCATI from elsewhere in the BETO portfolio.
- NOTE: As of March 2023 an additional, yet traditional, chemical based crop protection methodology for bacterial contamination looks promising in lab even in the absence of strain adaptation. Testing in the field this Spring/Summer with UTEX393 as wrap up on DE-EE0008906

Project Baselines: Cultivation Performance Targets

What's our baseline?

- Standard cultivation format (4.2 m^2 ponds)
- Standard media recipe
  - AzCATI modified BG-11,
  - Ammonium bicarbonate as N-source, dipotassium phosphate as P-source, 16: N:P ratio
  - 5 ppt salinity (*though will explore higher salinity for crop protection/CCUE efficiency improvements)
  - SOPs: same standard methodology as our BETO SOT trials
- Over three years of cultivation data for UTEX393, most with 10+ months within a calendar year (CY)
- We propose a productivity baseline that reflects a three-year average and thus target improvements over that baseline
- With seasonal (and monthly) data available, we can extrapolate to seasonal/yearly improvements with high confidence while running 3-6week trials within a given season.



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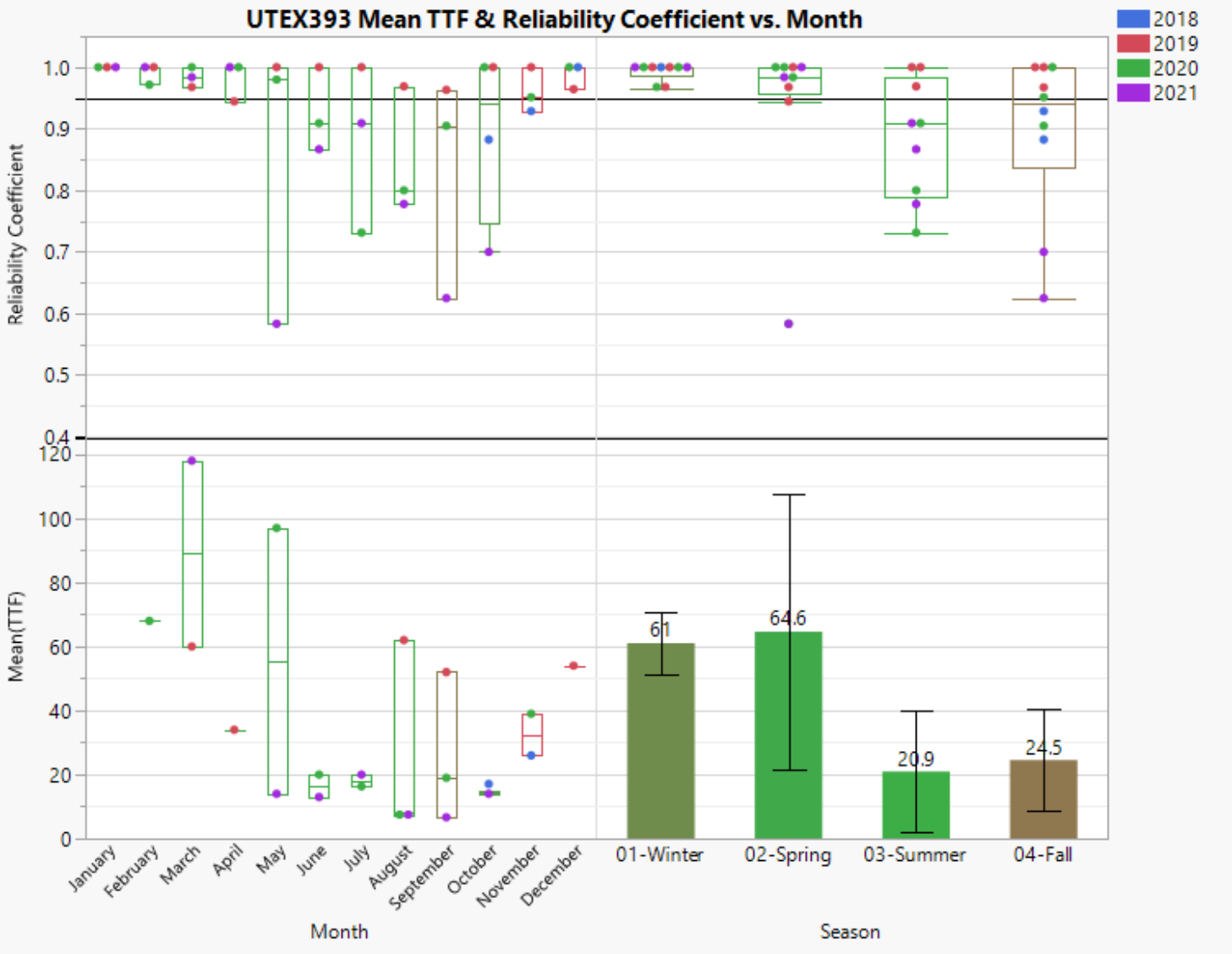
This drop primarily driven by predatory bacterial contaminant in warmer seasons

Season	2018	2019	2020	2021	% change 2019-2021	Baseline (3yr avg.)	Intermediate Target	End of Project Target
Winter	---	4.87	5.93	7.25	49%	6.0	6.6	7.2
Spring	---	16.2	17.6	14.1	-14%	16.0	17.6	19.2
Summer	---	27.1	20.7	20.2	-27%	22.7	24.9	27.2
Fall	10.1	13.9	9.0	11.6	-16%	11.4	12.5	13.7
Annual Avg	N/A	**15.5**	**13.3**	**13.3**	-14%	14.0	15.4	16.8

	Baseline	Interim	Final	
Lipids g/g AF	**0.1205**	**0.1205**	**0.1403**	
Carbohydrates g/g AF	**0.0841**	**0.1487**	**0.3016**	
Protein g/g AF	**0.4585**	**0.3897**	**0.3315**	
Other g/g AF	**0.1128**	**0.1128**	**0.1189**	
GGE/Ton (total)	**51**	**60**	**77**	**CAPS only**
GGE/Ton (total)	**102**	**104**	**108**	**CAPS + Protein to HTL**

Project Baselines: Cultivation Performance Targets

Subtask 1.4 (M4 – M39): Outdoor testbed cultivation deployment



- As with productivity, we also have multiple years of tracking reliability of UTEX393 as a function of cultivation month/season.
- We propose again using a monthly average rolled up to seasonal when we run outdoor trials (in particular for Milestone M.3.2.1 “Prophylactic bacterial consortium open pond testing completed; mean-time to failure enhanced 50% during pathogen challenge.
- We will target months where we know contamination pressure is high for 393 (both aphelid and or bacterial pests)

Project Approach: Synergies with other projects

This proposal leverages prior BETO funding to both ASU, NREL and partners in the areas of carbon delivery, strain improvement, cultivation, conversion and compositional analysis and early PDAC prototyping/small-scale pilot testing and now commercial pilot scale testing, as well as sensor platform development and data management/analytics.

Several aspects of the proposed work extend technical aspects of previously concluded/or ongoing projects including:

- NREL-led Rewiring Algal Carbon Energetics for Renewables (RACER) which is providing the foundational genetically engineering carbon storage components in a promising non-model alga (UTEX393)
- NREL- led DOE-funded completed project leveraged for this work that yielded a suite of robust carbonic anhydrase variants that will be included in the overall carbon delivery and engineering task for this proposal.
- ASU led and subcontract projects:
  - ATP^3 for overall experimental SOT outdoor cultivation framework DE-EE0005996
  - Early PDAC pilot testing and sorbent development along with and membrane carbonation for CCUE improvement (Rittmann/Lackner as ASU PI/Co-PI, respectively) DE-EE0007093 and DE-EE0008517
  - PACE for GM cultivation/TERA protocols
  - DISCOVR and DMSACPE (DE-EE0008906) for baseline cultivation and crop protection work with UTEX393
  - DMSACPE (DE-EE0008906) for improved pond operational monitoring and control and overall data management and analysis integration (pH/CO_2 tracking and control for improved CCUE).
  - Carbon Collect sponsored research with ASU thru ASU's Center for Negative Carbon Emissions (CNCE) including and a concurrently funded DOE (NETL: DE-FE0032097) project and the CNCE PDAC testbed and first commercial pilot in Tempe.

Progress and Outcomes: Task 1.1
Carbon Collect - Key Project Inputs

Data	Data flows and input to support TEA and LCA
Cost Projections	Provide cost projections at scale for different algae farm configurations
Capture & Supply	Run the collector models in Tempe to determine and meet the main performance parameters of interest. Capture time, CO_2 purity, CO_2 storage etc.
Process Engineering Model	Model off current design specifications for configuration options to resolve the asynchronous utilization of CO_2 over diel cycles
Design Targets	CO_2 composition; volume; intermittency; transport, storage medium (low cost), coupling and CO_2 delivery to pond.
Cultivation Trials	Cultivation trials with actual DAC CO_2 for at least 6 weeks in each of two seasons (in BP3, M30 and M39)

Progress and Outcomes: Task 1.1

Carbon Collect - Key Project Inputs and Current Status

MechanicalTree™ in Tempe, AZ extended for CO_2 capture (January 2023). First of kind design in metal. Next iteration will shift to dramatically reduce cost/tree going to plastic composite materials

Why passive direct air capture (P-DAC)

The MechanicalTree™ is unique relative to other DAC technologies in that

(1) passive air delivery by wind avoids the energy penalty of forced convection

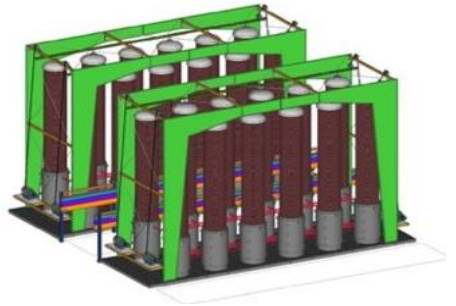
(2) the flexibility to operate hybrid temperature, vacuum, and moisture swing cycles without substantive hardware changes provides commercial access to broad climate geographies

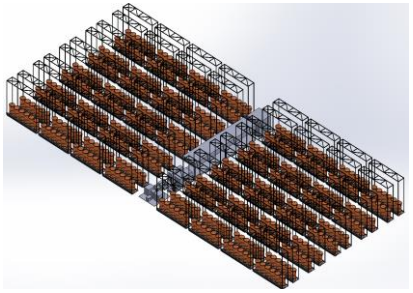
(3) MechanicalTree™ modular commercial design is suited to mass production and solves the scaling limitations and challenges of other DAC solutions, more readily capable to scale up and down depending on the application.

MechanicalTree™ design targets:
Single Tree = 82 kg/d; (batch mode)
Control group (up to 12 capture columns) = 1 ton/d
Control groups form the building blocks for large scale carbon farms producing CO_2 continuously

Current Status

- 1st of kind, commercial scale MechanicalTree™ installed and operational in October 2022
- Initial shakedown complete – optimizing operational hardware/software
- Capture rates hitting kg/cycle targets
- Under current thermal vacuum swing operation – hitting >90% CO_2
  - CC's overall target is <$100/ton (100% liquid CO2), for algae – we avoid backend purification and compression completely which should allow for <$50/ton DOE target to be met





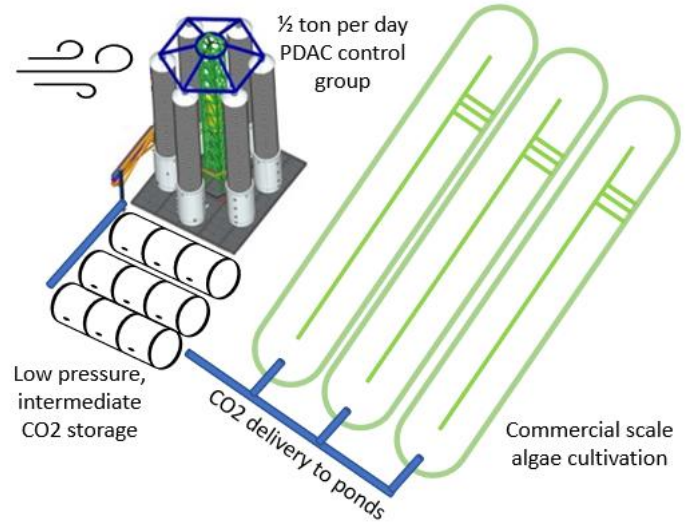
Control group modules – scale to particular application

CARBON COLLECT

Progress and Outcomes: Task 1.1
Carbon Collect - Key Project Inputs and Current Status

Batch Cycle – Single Tree

1) **CAPTURE** – Tree disk stack in full open position
2) Close and collapse disk stack into vessel
3) **PRE-EVACUATION** – rough vacuum to remove most residual air, separate from product withdrawal
4) **REGENERATION** - Temperature-Swing: steam condensation heating under vacuum
5) **POST-EVACUATION** – continue evacuation without steam addition for heat and water recovery
6) Store in ambient bag structure – deliver to algae process
7) Open and raise disk stack

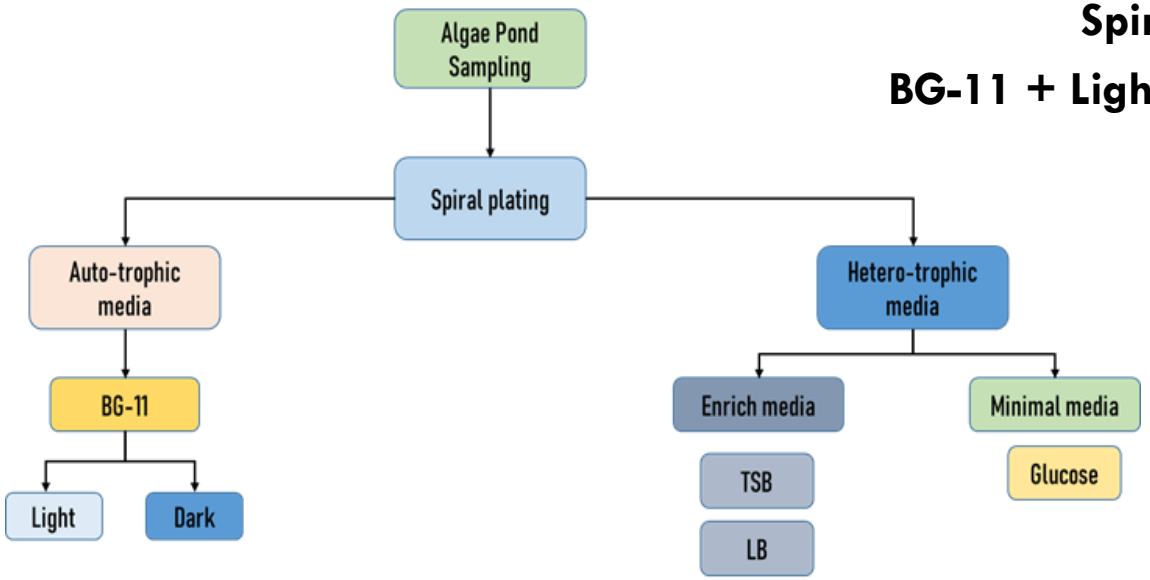


Air to Algae overall concept. We will model small to large scale farms (50-500-5000 acres)

- Intermediate storage: required for algae application due to intermittency (algae growth/CO_2 capture)
- Low-cost, low complexity storage a must: “bag house” approach”
- Stores high purity CO_2 without the requirement for specialist or expensive back-end system (NO COMPRESSION)
- Carbon tree effluent CO_2 will be at 1 bar, saturated with water vapor, and contain 5-10% residual air
- For this project, can be truck mounted to collect and transport the gas over the short distance between the carbon tree pad at ASU Tempe and the location of the algae test facility in Mesa.
- Design for different scale farms for on-site storage part of process engineering modeling in Subtask 1.1 and 4.1

Progress and Outcomes: Subtask 3.2
Microbial Profiling for Active Crop Management

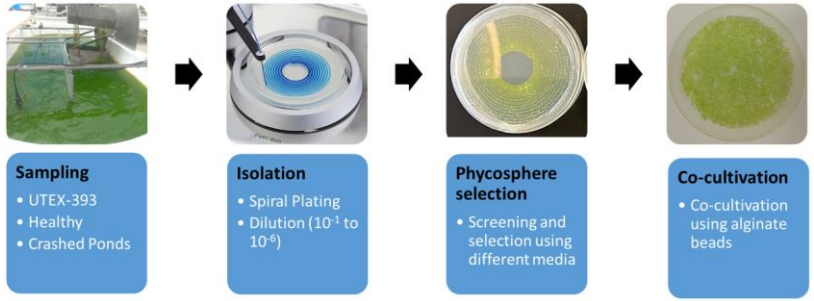
Spiral Plating and Screening

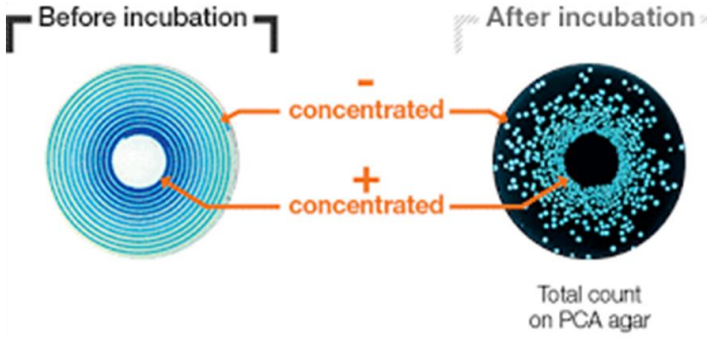


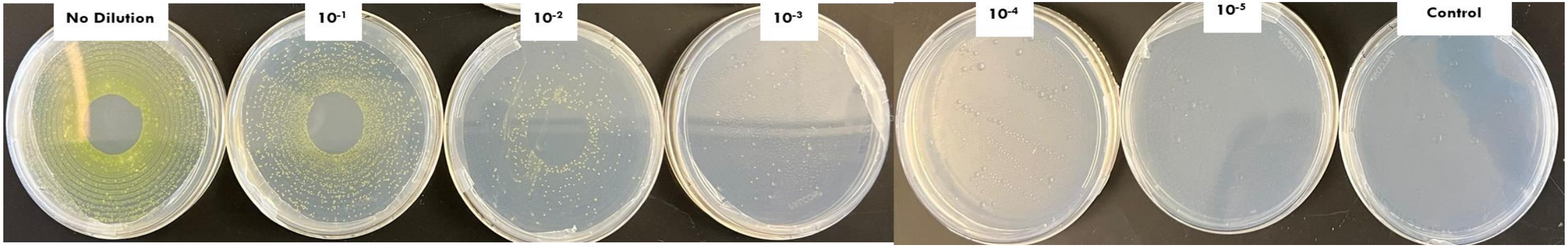
Spiral plating

BG-11 + Light (Incubate at 30°C)

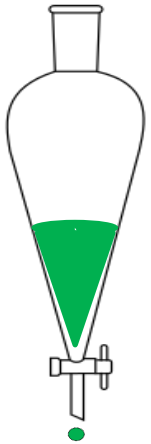
Basic Work-Flow





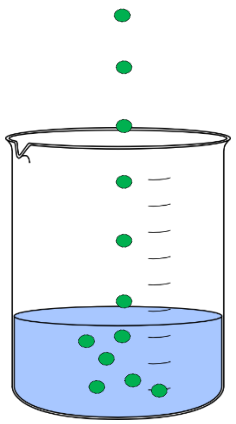


Co-cultivation and Phycosphere Selection

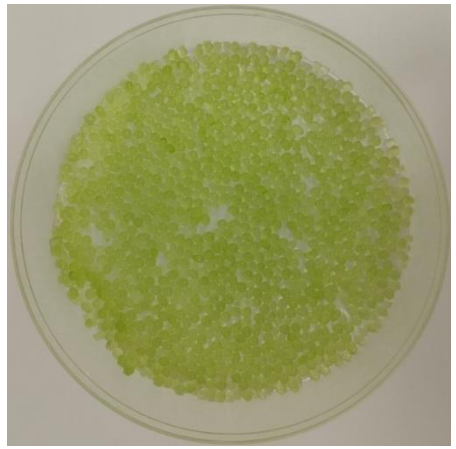


0.1 OD Algae or Bacterial culture + 3% Na-Alginate

Micro-encapsulator

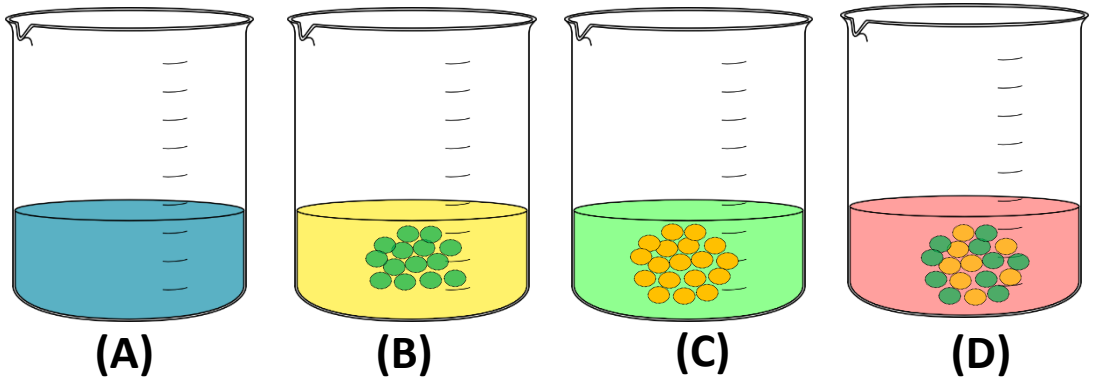


2% $CaCl_2$



Microencapsulation of UTEX-393 beads

Co cultivation methods		
(A) Liquid-Liquid	Bacterial culture (~24 hr Old)	0.1 OD Algae culture
(B) Bead-Liquid	Bacterial culture (~24 hr Old)	Algae Encapsulated Beads
(C) Liquid-Bead	Bacterial Encapsulated beads	0.1 OD Algae culture
(D) Bead-Bead	Bacterial Encapsulated beads	Algae Encapsulated beads



Sampling

50 ml samples were pulled out of ponds and plated on enrichment media to obtain colonies

Sample No	Date of sampling	Pond Number	Strain	Culture Appearance	Sampling	Plating media	Incubation Condition	
							Light/Dark	Temperature
1	4-Jul-22	SPW-26	UTEX-393	Dense Green	Sample pulled before TF	BG-11	Light	30 ℃
2	11-Jul-22	SPW-26	UTEX-393	Dark Green	Sample pulled before TF	TSB Agar	Light	30 ℃
		SPW-23	UTEX-393	Yellowish Brown	AM (No Reset)	TSB Agar		30 ℃
3	18-Jul-22	SPW-26	UTEX-393	Dark Green	Sample pulled before TF	BG-11	Light	30 ℃
						BG-11 + Glucose		
						TSB Agar		
		SPW-26	UTEX-393	Dark Green	Sample pulled before TF	BG-11	Dark	30 ℃
						BG-11 + Glucose		
						TSB Agar		
4	25-Jul-22	SPW-26	UTEX-393	Dark Green	Sample pulled before TF	BG-11 + 1% TSB	Light	30 ℃
						BG-11 + 5% TSB		
						BG-11 + 10%TSB		
5	1-Aug-22	SPW-26	UTEX-393	Dark Green	Sample pulled before TF	BG-11	Light	30 ℃
						TSB Agar		
						LB Agar		

Isolated Consortial Bacteria

- A total of 17 phycosphere bacterial isolates were obtained from different screening media under different condition (light and dark) and sampling time
- These isolates are preserved in Slants/Glycerol stocks and designated as UT1 to UT14
- The most promising bacterial candidates/populations will be DNA sequenced for identification

Isolate name	Date of Sampling	Pond Number	Gram Stain
UT1	4-Jul-22	SPW-26	Gram Negative
UT2	4-Jul-22	SPW-26	Gram Negative
UT3	4-Jul-22	SPW-26	Gram Negative
UT4	4-Jul-22	SPW-26	Gram Negative
UT5	11-Jul-22	SPW-26	Gram Negative
UT6A, UT6B, UT6C	11-Jul-22	SPW-26	Gram Negative
UT7A, UT7B	11-Jul-22	SPW-26	Gram Negative
UT8	11-Jul-22	SPW-23	Gram Negative
UT9	18-Jul-22	SPW-26	Gram Negative
UT10	18-Jul-22	SPW-26	Gram Negative
UT11	25-Jul-22	SPW-26	Gram Negative
UT12	25-Jul-22	SPW-26	Gram Negative
UT13	1-Aug-22	SPW-26	Gram Negative
UT14	1-Aug-22	SPW-26	Gram Negative

Progress and Outcomes: Subtask 3.2
Microbial Profiling for Active Crop Management

Co-cultivation and Phycosphere Selection (Beads, High-Titer)

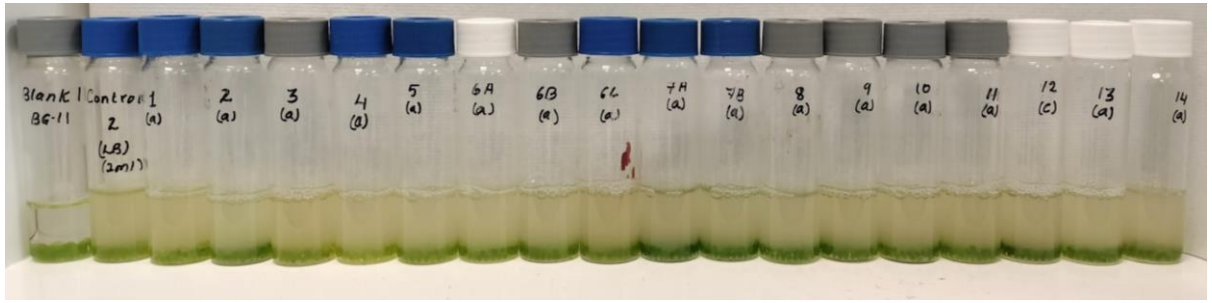
1 gram Immobilized UTEX393 beads (n=3)

10 ml BG 11 (sterile)

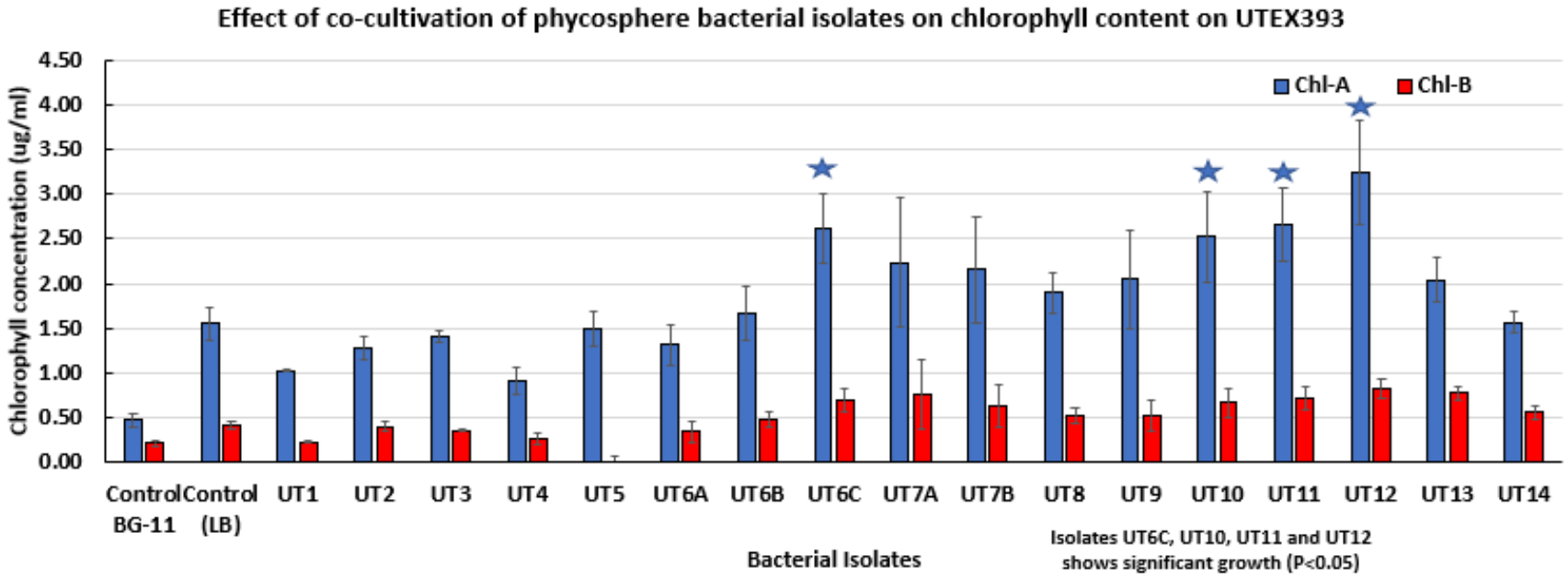
2 ml Bacterial isolates culture (20 hours old)

Incubated at 30°C for 1 weeks continuous shaking in constant light

Growth: Measured by Chlorophyll content (day 0 and day 7)



- Chlorophyll *a*: *Chl-a* (µg/mL) = 16.72 $A_{665.2}$−9.16 $A_{652.4}$
- Chlorophyll *b*: *Chl-b* (µg/mL) = 34.09 $A_{652.4}$−15.28 $A_{665.2}$



AzCATI Arizona Center for Algae Technology and Innovation

Quad Chart Overview

Timeline

- BP1 Initial Verification January 2022
- BP2 Start date delayed due to contracting (October 2022). New BP2 End Date - March 2024 (BP2 18mo).
- BP3 and End of project (September 2025 – BP3 18 mo)

	FY Spend to date Costed	Total Award
DOE Funding	$495,000	$3,200,000
Project Cost Share	$100,000	$800,000

Funding Mechanism

DE-FOA-002 FY21 BETO Feedstock Technologies and Algae FOA Topic Area 2b: Algae Productivity Exceeding Expectations (APEX) with Direct Air Capture

Project Partners*

- National Renewable Energy Laboratory (NREL)
- Burge Environmental
- Carbon Collect LLC

Project Goal: Deliver a fully modeled integration of Passive-Direct Air Capture (PDAC) with algae cultivation with innovative carbon delivery and enzyme biocatalysis and accelerated carbon capture and storage engineering through carbon flux control in *Scenedesmus* UTEX393 designed resilient pond ecology for long term outdoor cultivation to improve time to failure and overall productivity

Mid Project Go/No Go: Demonstrate **10% improvement in productivity (15.4 g/m2-day)** and achieve **50% improvement in CUE** with simulated DAC CO_2 carbon delivery over at least 3 weeks of continuous WT UTEX393 cultivation across at least two seasons, when compared against baseline (SOT) concentrated CO_2 delivery using sparging.

End of Project Goal: Assemble critical data around the most carbon-efficient algae-based conceptual biorefinery and demonstrate the end-of-project goal of 100% DAC-derived, inorganic carbon delivery that achieves a **20% increase in biomass productivity (16.8 g/m2-day)** and achieving a CUE **at least 75% above baseline** in a conceptual integrated system while achieving biomass composition quality of **85 GGE/ton fuel intermediate yield**.

Project Objectives and Expected Impact

Project Objectives:

- Deliver a fully modeled integration of Passive-Direct Air Capture (PDAC) with algae cultivation with innovative carbon delivery and enzyme biocatalysis
- Accelerated carbon capture and storage engineering through carbon flux control in *Scenedesmus* UTEX393
- Designed resilient pond ecology for long term outdoor cultivation to improve time to failure and overall productivity

Impacts:

- Uncoupling algae farming from point source CO_2 by relying on novel DAC-sourced carbon, and CO_2 delivery innovations increases national deployment potential
- Metabolic engineering of Calvin cycle control demonstrated in production strain UTEX393 translates broadly to improve photosynthetic carbon assimilation
- Increase carbon capture efficiency through marketable technology development (membrane carbonation)
- Accelerate deployment and commercialization of algae-DAC installations

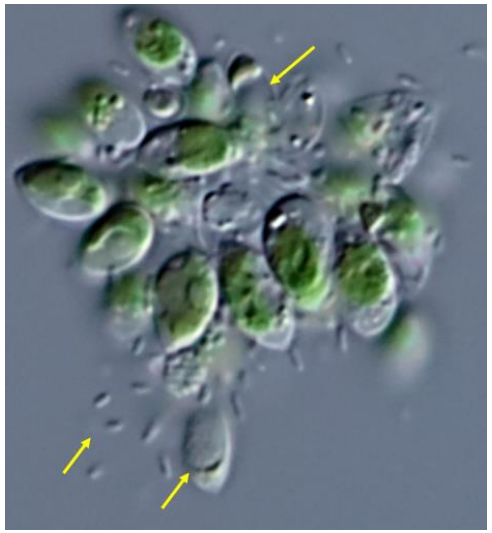
Additional Slides

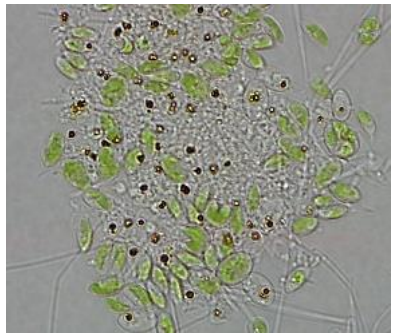
1. Response to previous reviewer's comments: N/A
2. Go/No Go highlights:
   - Passed Initial verification Go/No in January 2022, but project not awarded for BP2 until October 2022, subcontracts through ASU also delayed so BP2 has shifted a 1 quarter (see quad chart). Progress now accelerating.
3. Publications, Patents, Presentations to date: N/A

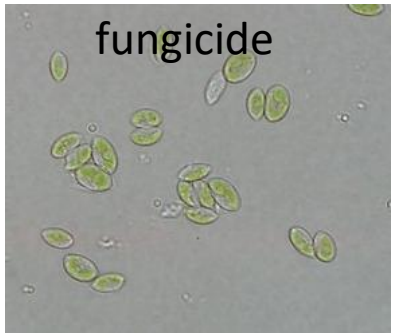
Integrated Pest Management: a continuous cycle

UTEX393 cultivated at AzCATI continuously since Fall 2018

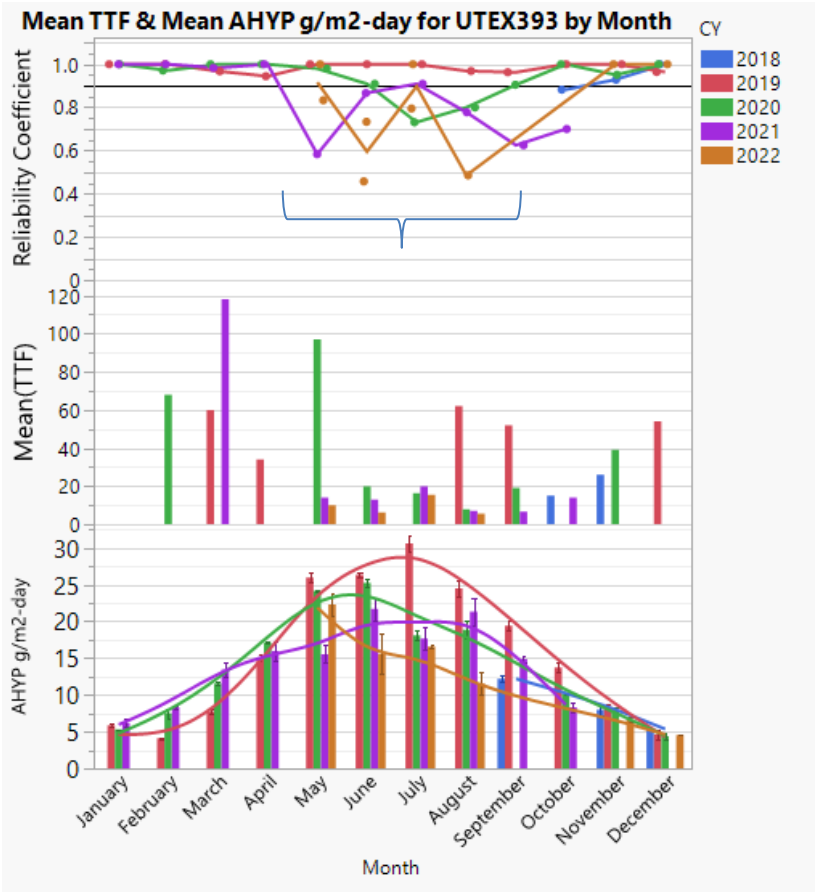
- Rapid culture crashes through Spring 2019
- Fungal parasitoid crash morphology - fungicide mitigation began spring 2019 with success increasing productivity and MTTF
- Highest productivity ever achieved to date with >25 g/m^2-day for 5 months (May-Sept 2019)
  - Increased time to failure (TTF) from 2018 SOT baseline of 30 days to 54 days to ~100 days through Spring 2020
- However, a new contaminant observed in Spring 2020 which looked like a predatory bacterium
- Dropped TTF from over 60 days to **<15 days** and lowered productivity in 2020 and 2021 and 2022



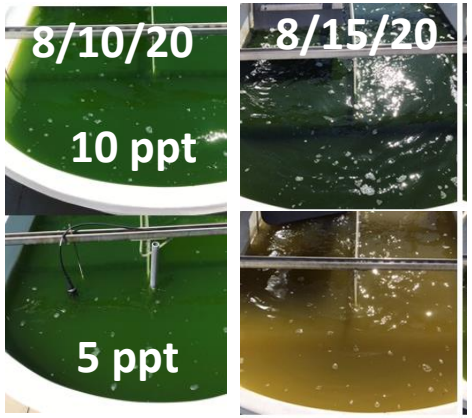




Pre-treatment Post-treatment

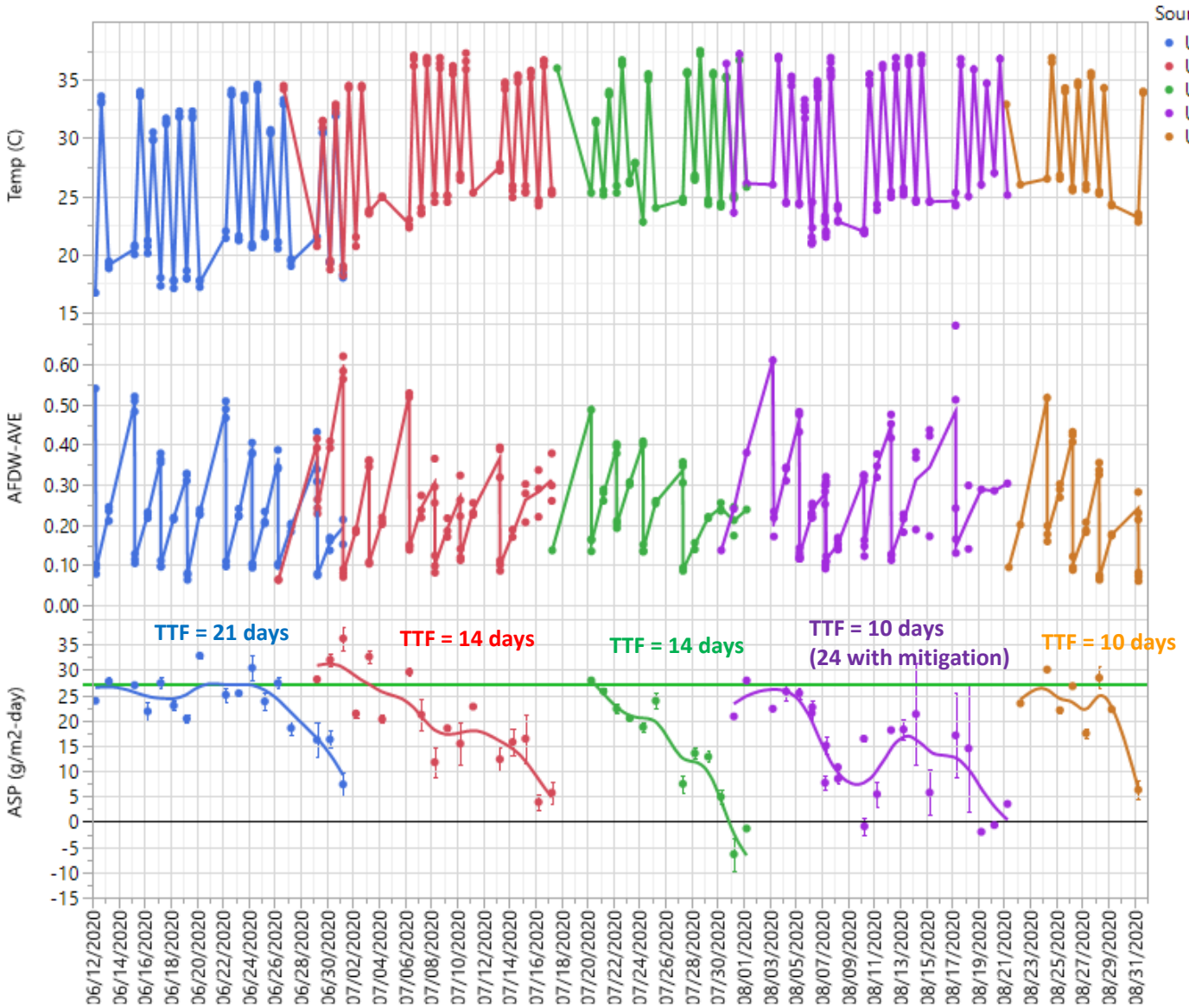


- Mitigation with salinity swings increased TTF from ~ 14 days>24 days but cultures still crashed



- Chlorine intervention showed mixed results but revisiting
- Pest confirmed as bacterial
  - Crash model established
  - Likely identity has been established (at genus level)
  - PCR/qPCR assays developed

Biggest Challenge to UTEX393 an as yet Unidentified Bacterial Pest



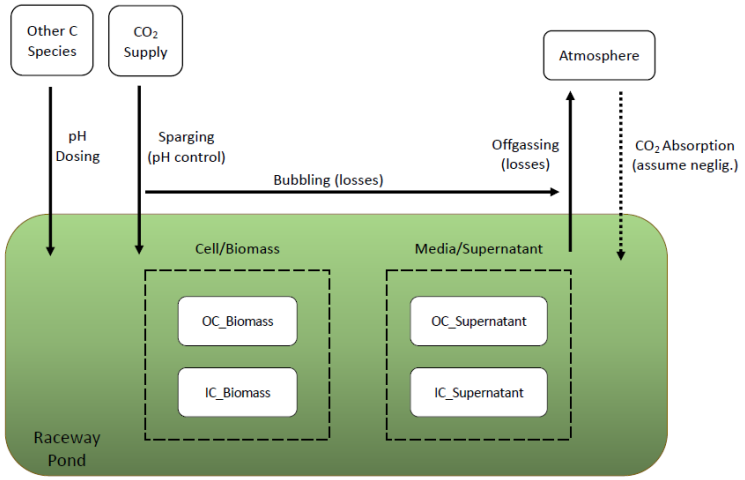
- Spring/Summer/Fall of 2019 successful mitigation of fungal parasitoids
- Achieved highest productivity at AzCATI
- Robust cultivation of UTEX393, experiencing zero culture crashes (did have other algae contamination)
- Run times from a single inoculation event exceeding 90 days – IN THE SUMMER
- However, in 2020 and again in 2021 culture time to failure (TTF) accelerating from 25 days in early June to ~10 days in August
- With aggressive seed train maintenance, we were able to routinely reseed new ponds as older ponds crashing and keep overall productivity from cratering.
- Summer 2020 = 20.8 g/m2-day (Summer 2021 also lower)

Season	2018	2019	2020	2021	% change 2019-2021
Winter	---	4.87	5.93	7.25	48.9%
Spring	---	16.3	17.6	14.0	-14.1%
Summer	---	27.1	20.8	19.7	-27.3%
Fall	10.1	13.8	9.0	11.6	-15.9%

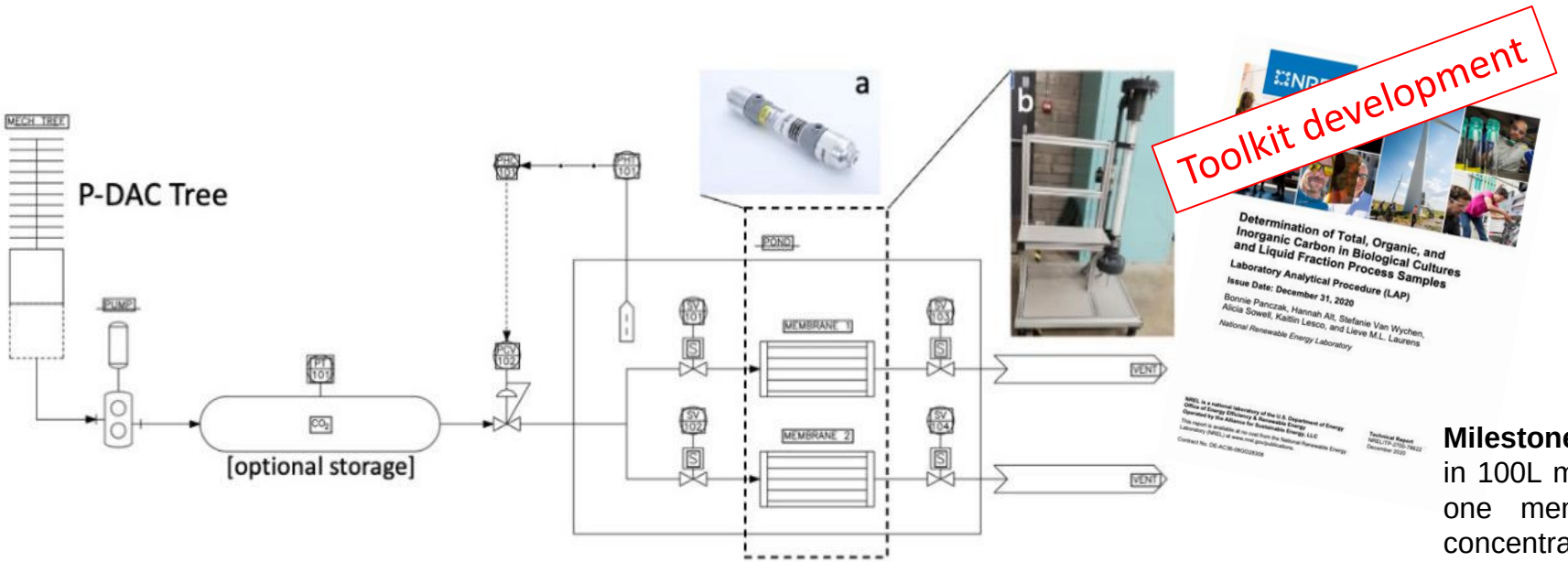
Approach:

Subtask 1.2 – Carbon Delivery Efficiency Optimization

- Integration of commercially-available membrane gas-delivery system and ASU in-house developed MC modules
- Carbon balance accounting development of reference reporting procedure



ASU In-house developed MC modules will be utilized along with commercial for Subtask 1.2 to ensure 100% carbon transfer efficiency into media (CTE) developed under DE-EE0008517.



$$CUE \equiv \text{Carbon Utilization Efficiency}$$

$$CUE = \frac{\text{Carbon assimilated into cells}}{\text{Carbon Input}}$$

$$CUE = \frac{\Delta OC_{biomass}}{\sum(Sparging + pH\ Dosing)} \cdot 100\%$$

$$\%Loss = \frac{Offgassing}{\sum(Sparging + pH\ Dosing)} \cdot 100\%$$

Milestone 1.2.1: Demonstrated baseline CUE improvements in 100L mini-ponds at least 15% over baseline with at least one membrane module installed and integrated with concentrated CO_2 source (September 2023)

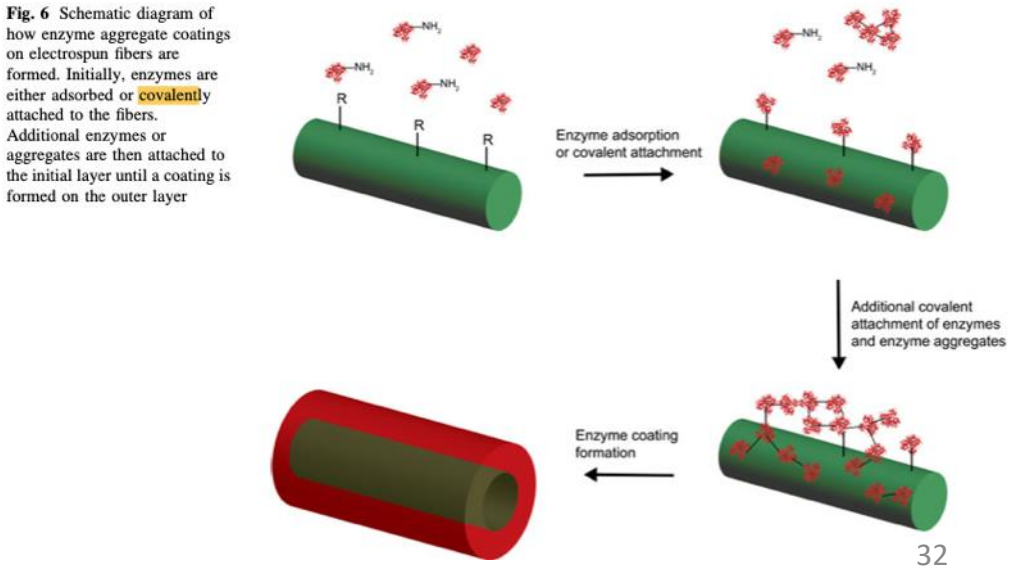
Figure 8: *Overview of process flow diagram of CO2 delivery systems from P-DAC to open ponds based on feedback control and utilizing novel gas-liquid interface membranes, (a) Air Products PRISM PA1010-N1 membrane module, and (b) Dupont OxyMem*

Approach: Subtask 1.3
Accelerating Carbon Capture with CA Biocatalysis

- Improve stability of bicarbonate supply to accelerate growth and increase CUE
- Test and develop CA enzyme variants tolerant to lower pH
- Activity testing with different immobilization methods

Immobilization Strategies
- Decorate hollow fiber membrane module with immobilized CA enzyme, e.g. fiber electrospinning
- Magnetic NanoParticles (MNP) immobilization; place in dialysis bag or attach to electrospun fibers [literature precedent]
- Immobilize enzyme via Histidine residues (or other tag) attached to the C-terminus; place in dialysis bag or attach to electrospun fibers

Parameters for good support material:
- Good CO2 permeability
- Compatible surface activity
- Structural integrity/durability



Milestone 1.3.1: Selection of at least one CA variant demonstrated to have an at least 10x increased IC flux at pH 7.5 in cultivation-relevant media relative to uncatalyzed reaction, present approach for feasibility and activity for at least 2 immobilization strategies for incorporation into carbon delivery system (September 2023)
Milestone 1.3.2: Demonstrated inorganic carbon (IC) delivery by immobilized biocatalysis module interfacing with algae cultivation system, improvement of at least 20% increased IC over baseline direct CO2 delivery at small-scale SAGE reactor (March 2024)

Progress and Outcomes:

Subtask 1.4 Upgrading ponds for CO2 and pH monitoring

Subtask 1.4: Outdoor testbed cultivation deployment – NEW SENSOR/PROCESS CONTROL CAPABILITY

With our partner Burge Environmental, we are leveraging our current DMSACPE project and the Burge sensor and data management platform already established by adding additional upgraded sensor platforms that will enable actual process control for CO_2 delivery/management as well as automate data collection of CO2 flow totalizers, options for additional sensors (e.g., dissolved CO_2 sensors., optical DO, etc. in addition to the Burge microbial sensors, ORP, pH, temp/conductivity). All of this is a single, industrial Rasberry PI/Python based control system.

Additional 6 ponds on top of 6 systems for CCUE tracking deployed under DISCOVR (in place Aril 2023) with the latest hardware configuration/board design.

This will allow for moving the capture of data needed for CCUE analysis into the same platform as the rest of the cultivation data and sensor data,

DIN Enclosure & Industrial Raspberry Pi (Top-Left)
B23T Next-Gen MiProbe Instrument Board (Top-Right)

i2c Relay Control Board (Bottom-Left)
B23T Sensor Connector Board (Bottom-Right)

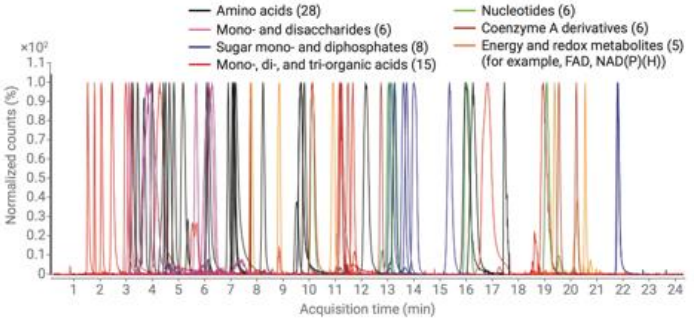
Burge Environmental

Progress and Outcomes:

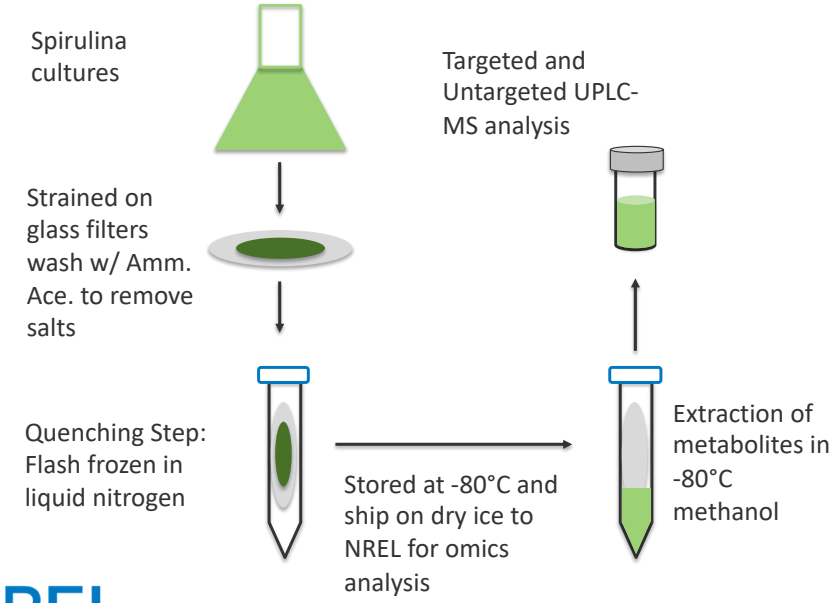
Subtask 2.1 – Integrated Systems Biology for Dynamic Carbon Flux Mapping

- Design simulated physiological testing environment for CO2 capture and storage calculation
- Elucidate intermediates between inorganic carbon and organic carbon storage, metabolomics
- Genetic and physiological response to changing IC environments, transcriptomics using established pipelines for RNAseq data analysis

- ThermoScientific QExactive Orbitrap high-resolution MS installed November 2020, to complement lab analytical suite
- Running HILIC chromatography as generic full metabolite screening panel including energy and redox metabolites



Existing Metabolomics Workflow



Untargeted relative quantitation of products and select pathway mapping

- Central carbon metabolism
- Calvin-Benson Cycle
- C3 cycle
- Others

Milestone 2.1.1: Identify flux bottleneck for carbon assimilation in Scenedesmus, as major varying pathway under different CO2 concentrations and including at least one mutant from year 1 screening effort. (December 2023)

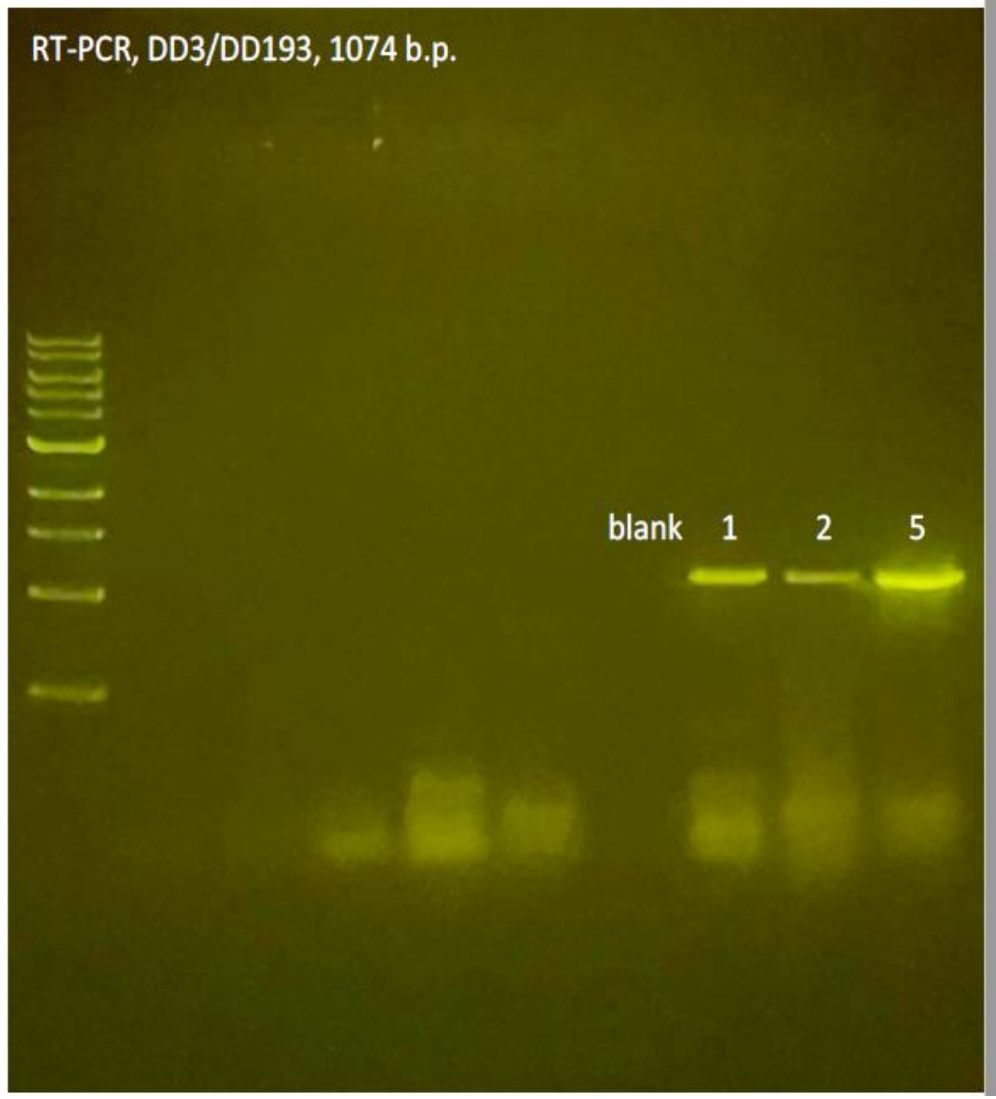
https://www.agilent.com/cs/library/applications/application-discovery-metabolomics-hilic-z-5994-1492en-agilent.pdf

Progress and Outcomes:

Subtask 2.2 - Building Robust Engineering Tools

- Expression of gene confirmed in 3/5 transformants (1, 2, and 5) using RT-PCR in July 2021 (stable since June 2020)
  - Phenol/chloroform RNA extraction followed by RT-PCR
  - Sequencing of product shows mutation

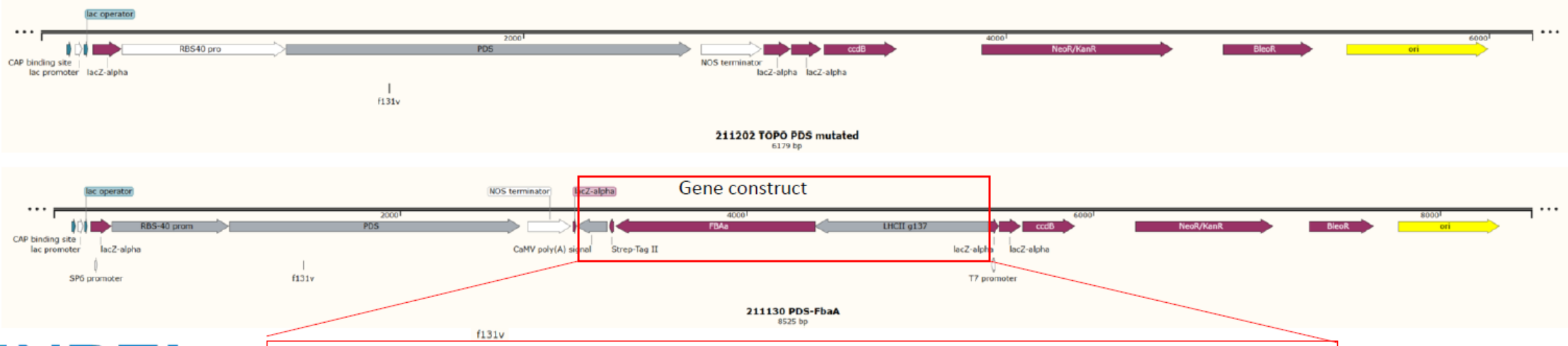
- First 5 lanes of gel from UTEX 393 transformed using Nourseothricin as a resistance marker
  - Lack of gene expression suggests spontaneous resistance to Nourseothricin



Progress and Outcomes:

Subtask 2.2 - Building Robust Engineering Tools

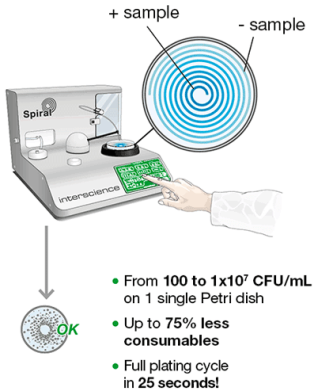
- Modification of the mutated PDS plasmid to include genes of interest has been done with fructose bisphosphate aldolase (FbaA) and can be done with other genes
  - Electroporation with this PDS-FbaA plasmid in addition to a cNAT-FbaA (conferring resistance to Nourseothricin) were unsuccessful in transforming UTEX 393
- PDS-FbaA plasmid below made using In-Fusion cloning with the mutated PDS plasmid as the vector
- New personnel on project from NREL, spinning up on protocols

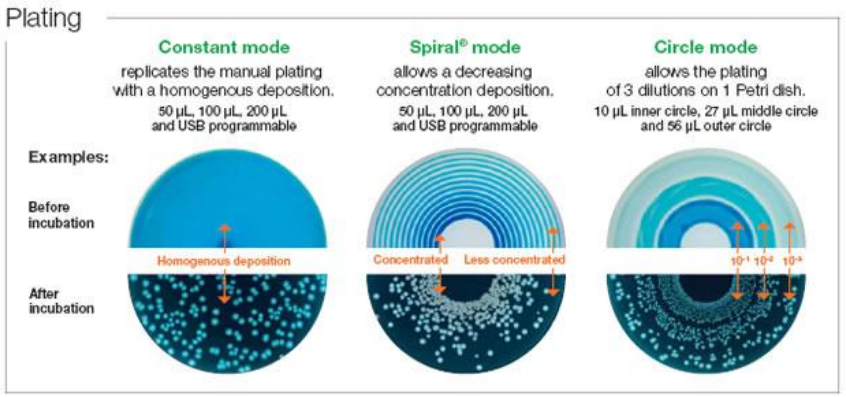


New promotor selected from public RNAseq datasets: EF1 (jgi|Sobl393_1|1284|SZX77192.1)
Native UTEX393 FbaA gene will be amplified and expressed behind EF1 promotor in new construct design

Progress and Outcomes: Subtask 3.2
Microbial Profiling for Active Crop Management

- Algae excrete/shed organic material providing nutrition to contaminating bacteria/fungi
- Algae cultures are inevitably randomly populated by contaminating bacteria/fungi
- Isolating and then prophylactically dosing a numerically dominant, positive/neutral-interacting bacteria will create a most consistent starting point and likely impede further intrusion
- Numerous methods for positive/negative selection available, but spiral plating will expedite isolation and encapsulation directly from operating ponds and across nominal/subpar conditions
- Pathogen resistance will be established in lab, but MTTF reduction tested in scaled ponds





FEMS Microbiology Reviews, fux029, 41, 2017, 880–899
doi: 10.1093/femsre/fux029
Advance Access Publication Date: 22 August 2017
Review Article

REVIEW ARTICLE

Strategies and ecological roles of algicidal bacteria

Nils Meyer[1,#], Arite Bigalke[1,#], Anett Kaulfuß[1] and Georg Pohnert[1,2,*]

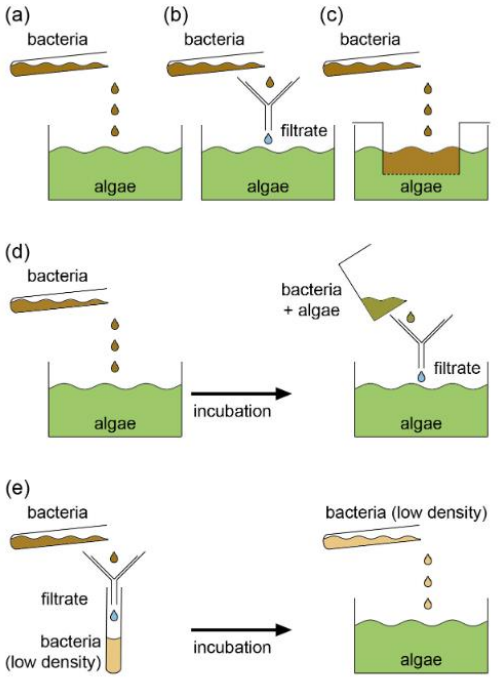


Figure 3. Methods for the elucidation of algicidal mechanisms and their regulation. (a) Cultivation with direct contact allows identification of algicidal bacteria independent of their mechanism of activity. (b) If cell-free spent medium (i.e. filtrate) is active, the mechanism involves excreted algicides. (c) To test whether cross-kingdom signalling is necessary for algicide production, non-contact co-culturing with semipermeable membranes facilitating passage of algicide but not of cells can be used. (d) Cross-kingdom signalling can be monitored after a period of cultivation with direct contact followed by testing the spent medium (i.e. filtrate) of bacteria-killed algae for algicidal activity. The latter approach has the disadvantage not to distinguish between bacterial algicides and toxic algal waste products. (e) To provide initial evidence for a QS-type mechanism algicide, production is stimulated in below quorum density cultures by addition of filtrate from a high-density culture. Algicidal activity of the low-density culture can be tested afterwards with one of the methods described (a–d). Care has to be taken regarding the material of filters (b, d, e) and membranes (c) in order to minimise loss of algicide by adhesion or inhibition of algicide passage through the membranes.

Co-Cultivation and Phycosphere Selection (Planktonic, Low-Titer)

1 ml UTEX393 culture (n=3)

↓

9 ml BG 11 (sterile)

↓

200 µL Bacterial isolates culture (20 hour old)

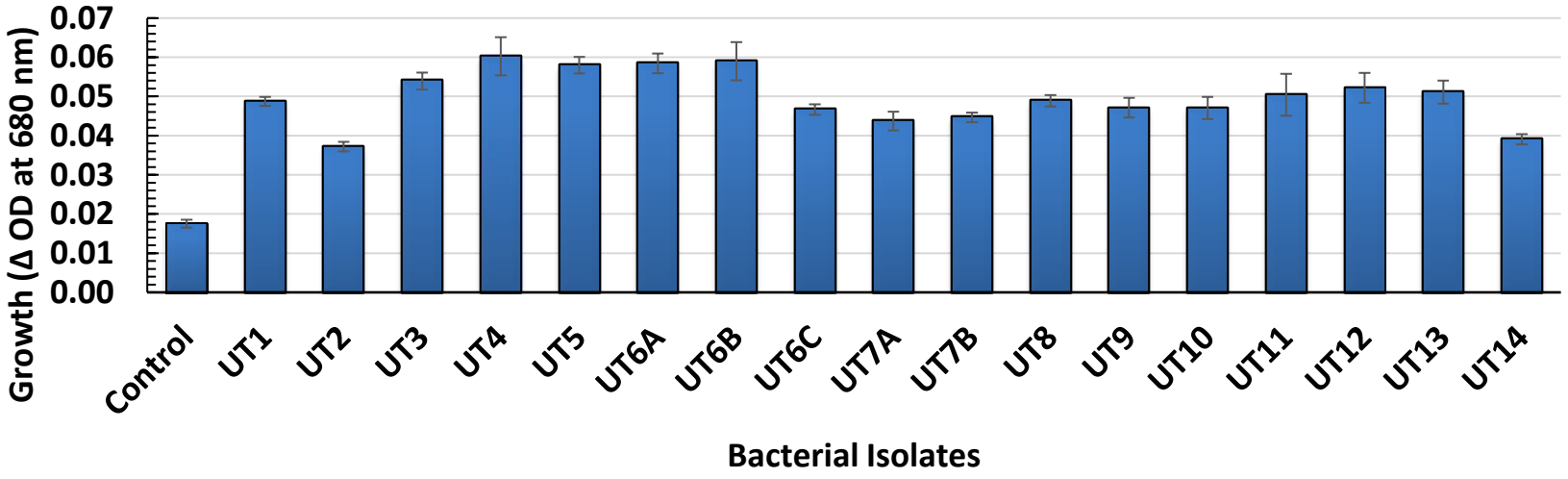
↓

Incubated at 30°C for 1 weeks in culture tube in constant light

↓

Growth: Measured by OD at 680 nm

Effect of co-cultivation of phycosphere bacterial isolates on UTEX393



All the 17 isolates where showing significance results (P<0.05)

Approach: Subtask 4.1
Engineering Process Modeling

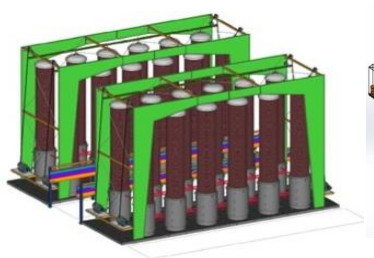
Modification #1: CO_2 source

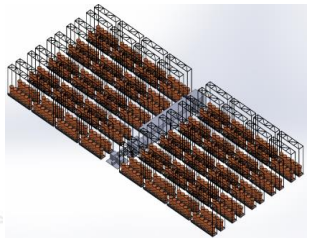
Benefiting from a decentralized CO_2 collection plant

Expanding the number of feasible sites for algae production: proximity to a stationary point CO_2 source is no longer a constraint

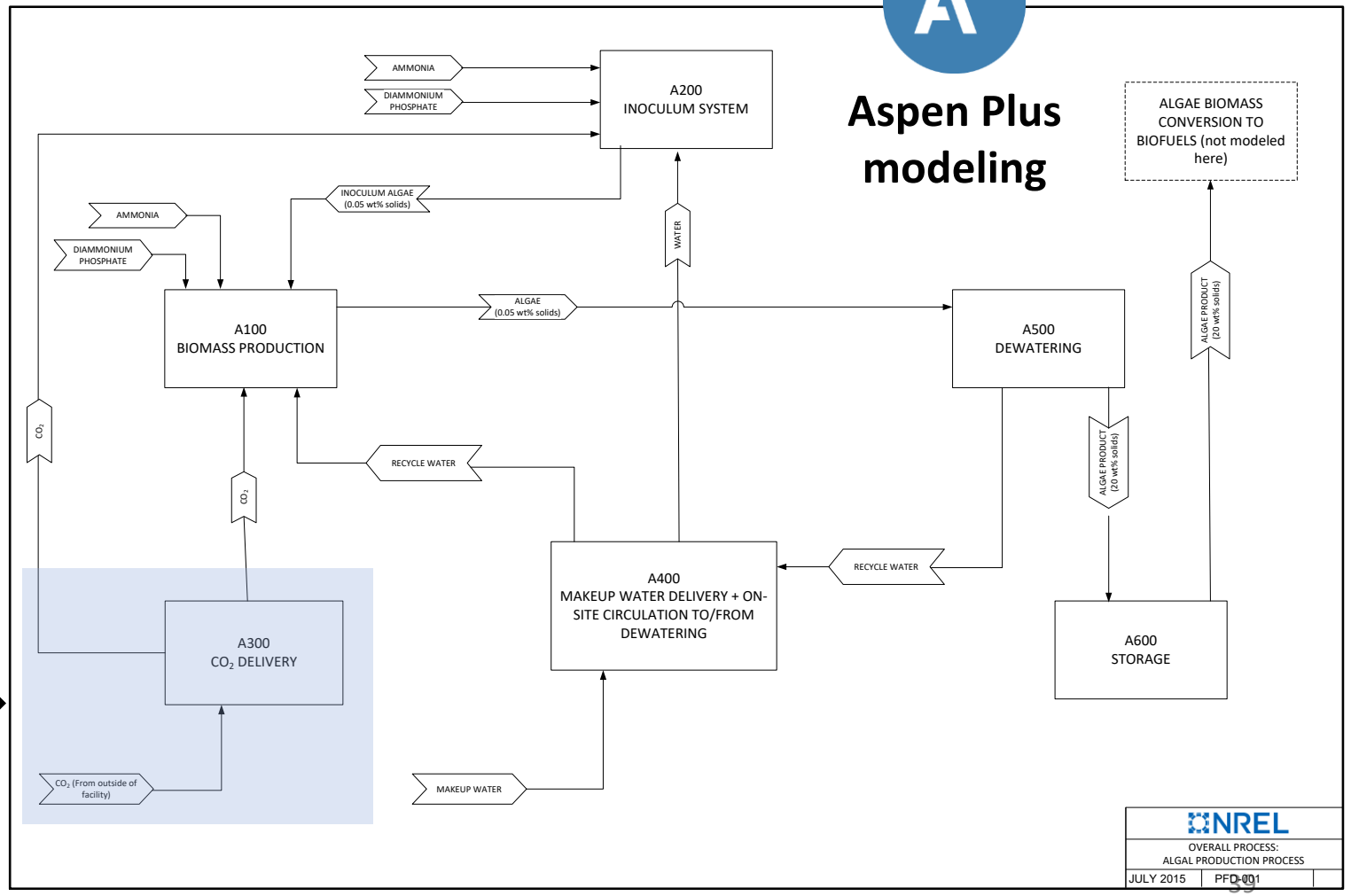
Process model for DAC developed for the operating conditions and setup of the P-DAC Carbon Tree system

Infrastructure requirements and operational costs





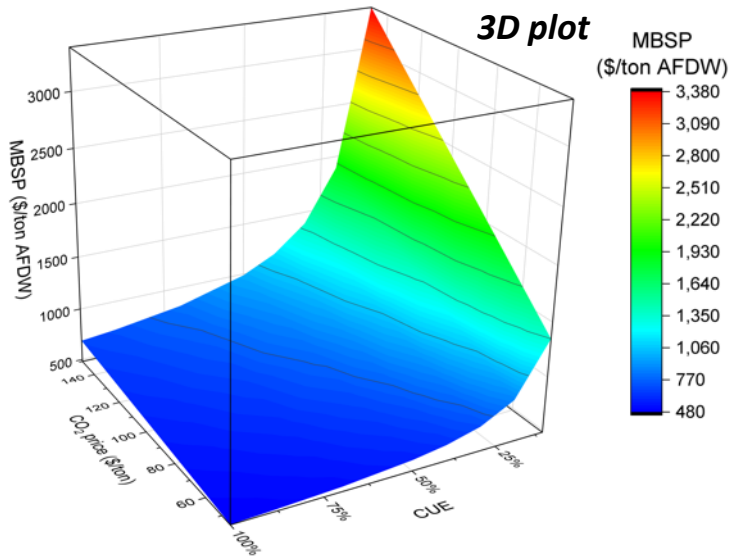
Aspen Plus modeling



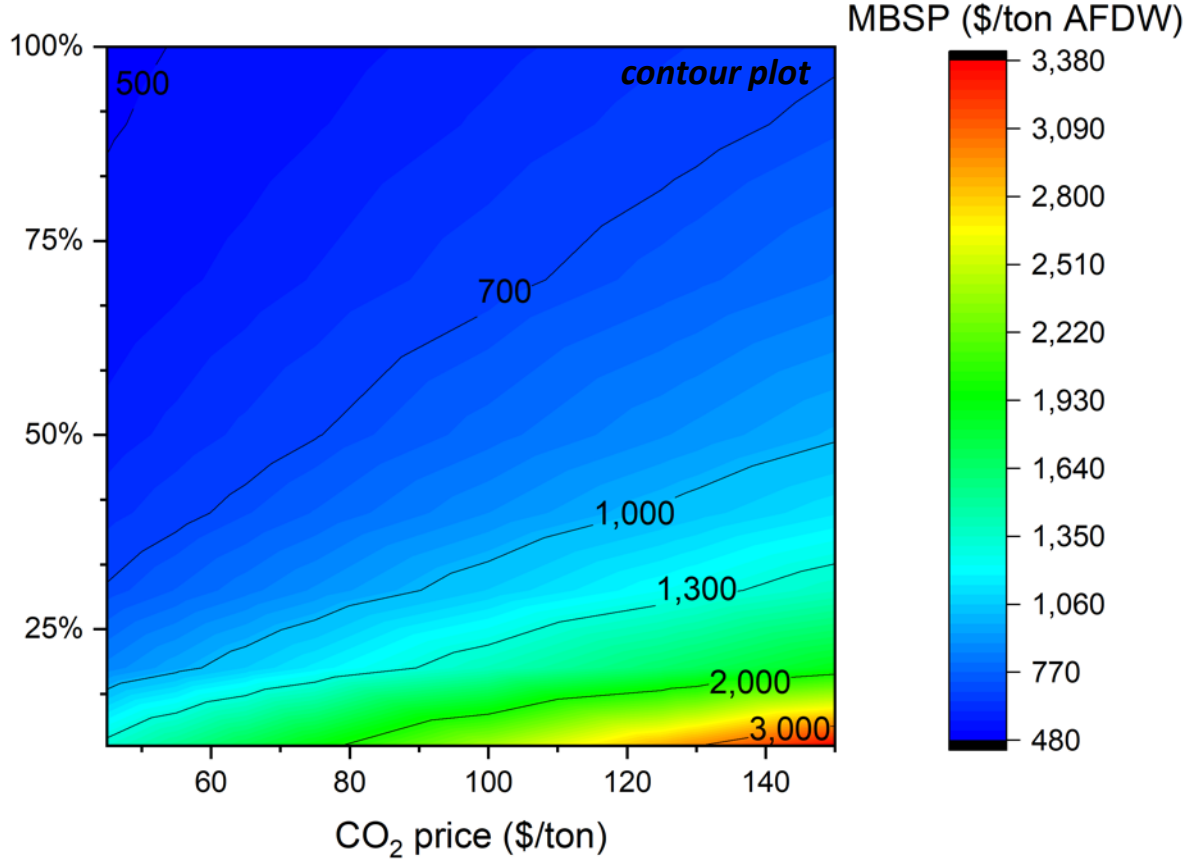
Approach: Subtask 4.1
Engineering Process Modeling

Goal: Developing a process engineering model of a CO_2 capture and compression system

Exploring the impact of:
* Plant scale
* CO_2 price and CUE tradeoffs
* Feeding CO_2 concentration < 100% to algae ponds



Effect of CO_2 price and CUE over MBSP*



** Open algae ponds with "conventional" CO_2 sourcing/supply*

Approach: Subtask 4.1
Engineering Process Modeling

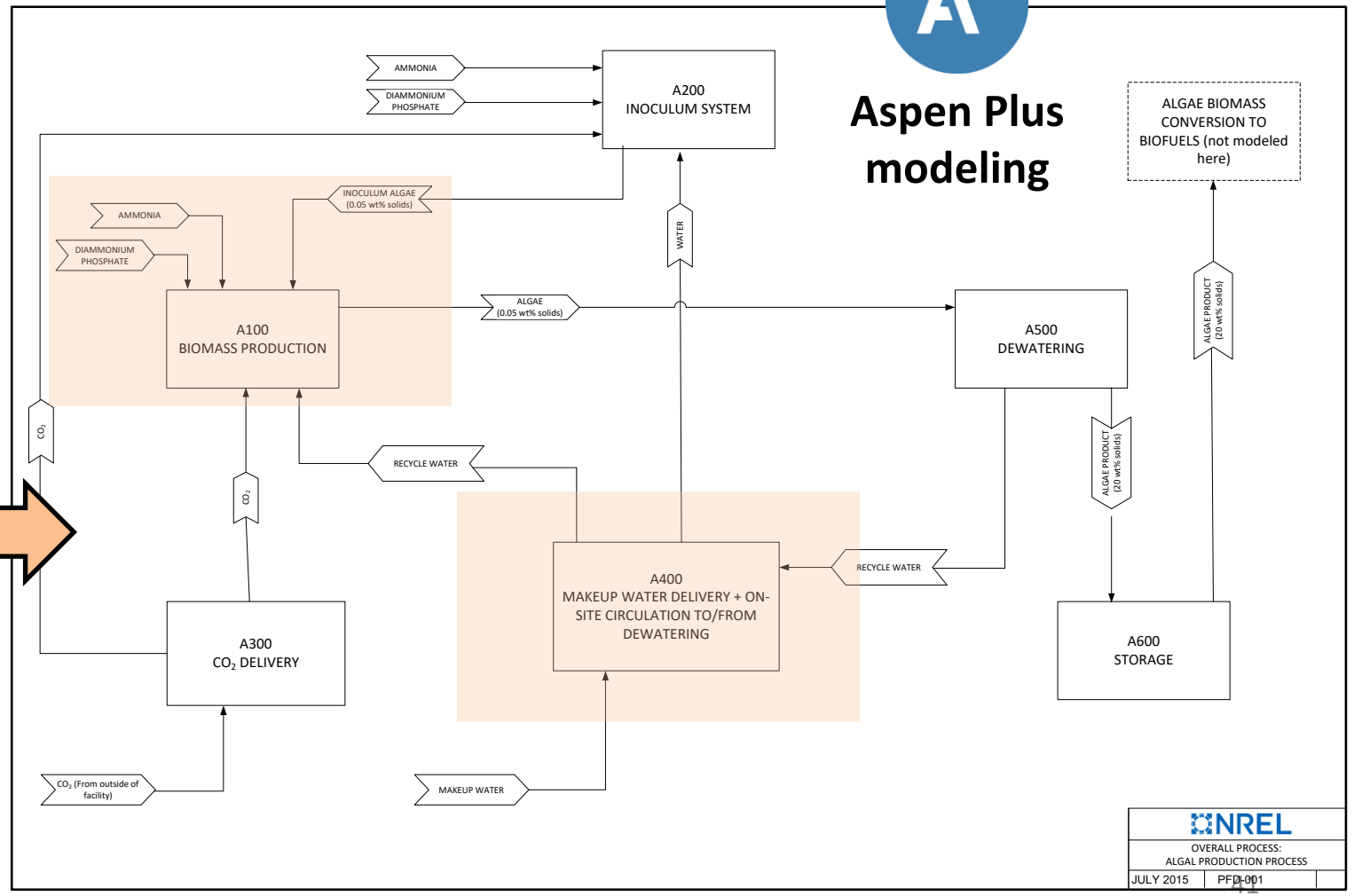
Modification #2: cultivation strategy, farm scales, and associated changes

Using experimental data to inform process models

Adjusting pH and water recirculation strategy

Achieving high carbon uptake efficiency is crucial

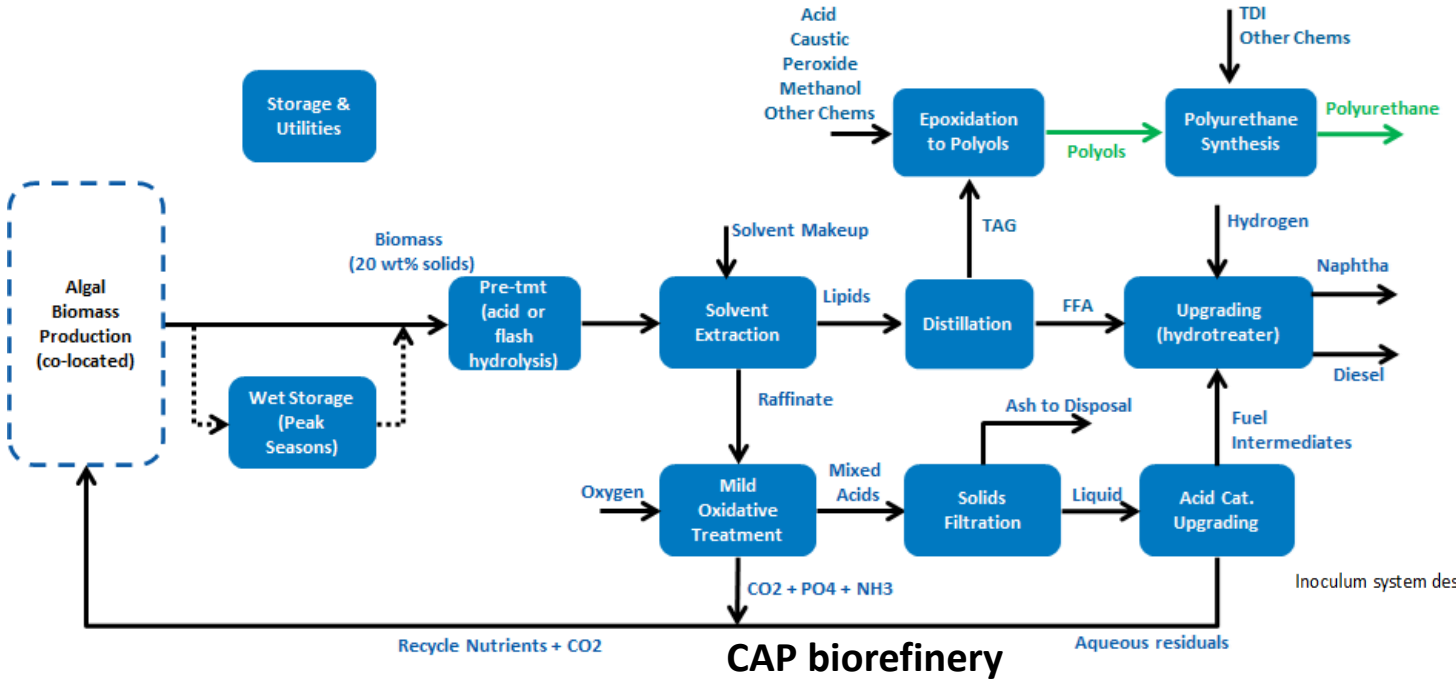
Aspen Plus modeling



Approach: Subtask 4.2
TEA and LCA of Fully Integrated Carbon Delivery System

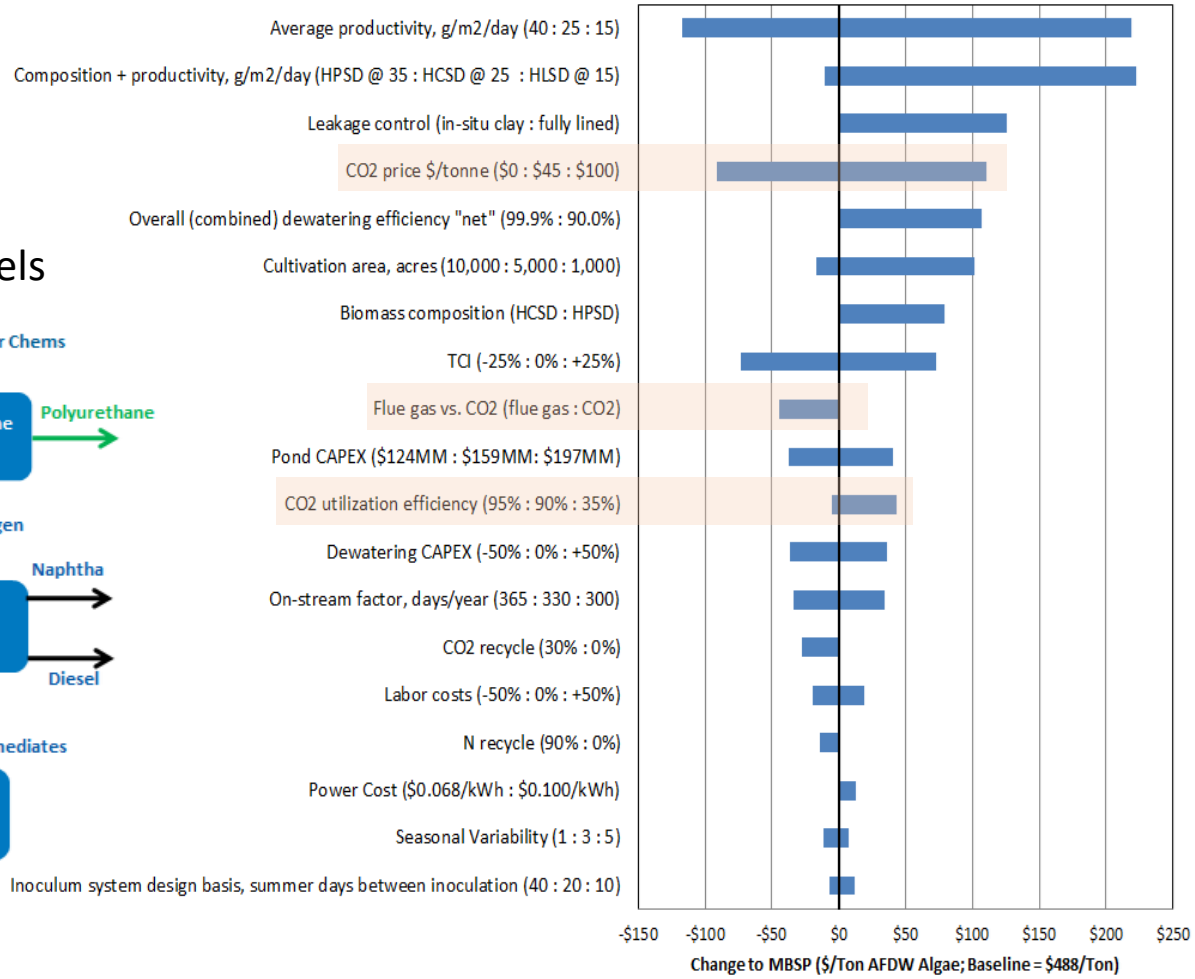
Leveraging NREL's process models: algae farm and biomass conversion

- Algae farm: open raceways
- Biomass conversion: Combined Algal Processing
  - Hydrocarbon fuels: diesel and naphtha
    - *Sustainable aviation fuel (SAF) is an option*
  - Obtaining coproducts is essential to support liquid biofuels



CAP biorefinery

Primary cost drivers (algae farm)*



** Open algae ponds with "conventional" CO_2 sourcing/supply*