Decision-Model Supported Algal Cultivation Process Enhancement

Award #DE-EE0008906
WBS: 1.3.5.287
BETO Project Peer Review: Advanced Algal Systems
March 11, 2021 1:05 pm

Dr. John McGowen, PI

One little cell, a world of possibilities.

Project Overview: The challenges

For commodities (e.g., biofuels), it is assumed large-scale, (semi) continuous cultivation is required

- Cultivation footprint is largely composed of low-cost, open raceway ponds (ORPs)
- Seed-inoculum scale-up consists of successive culture transfer between ORPs of increasing size and represent <5% of the overall areal production footprint
- However, at very large scales this strategy amplifies the economic risk of culture failures due to very long scale-up recovery times if major crop loss experienced
- Mitigating this risk requires significant operational knowledge and CAPEX/OPEX investment in crop protection and pest-management strategies as a necessary hedge against failures

Alternatively

- Larger investment in intensified photobioreactor (PBR) seed-inoculum scale-up capacity (>>10% of cultivation footprint) and…
- Complete batch-mode ORP harvests can manage cultivation failure risk via an avoidance mechanism
    - **minimizing** algal seed culture exposure time in ORP
    - **maximizing** post-failure recovery speed

The precise advantages and feasibility of either approach is difficult to disambiguate, let alone confidently implement broadly, as cultivation risks are likely to show significant strain-, location-, and seasonal-dependencies.

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Project Overview: The challenges

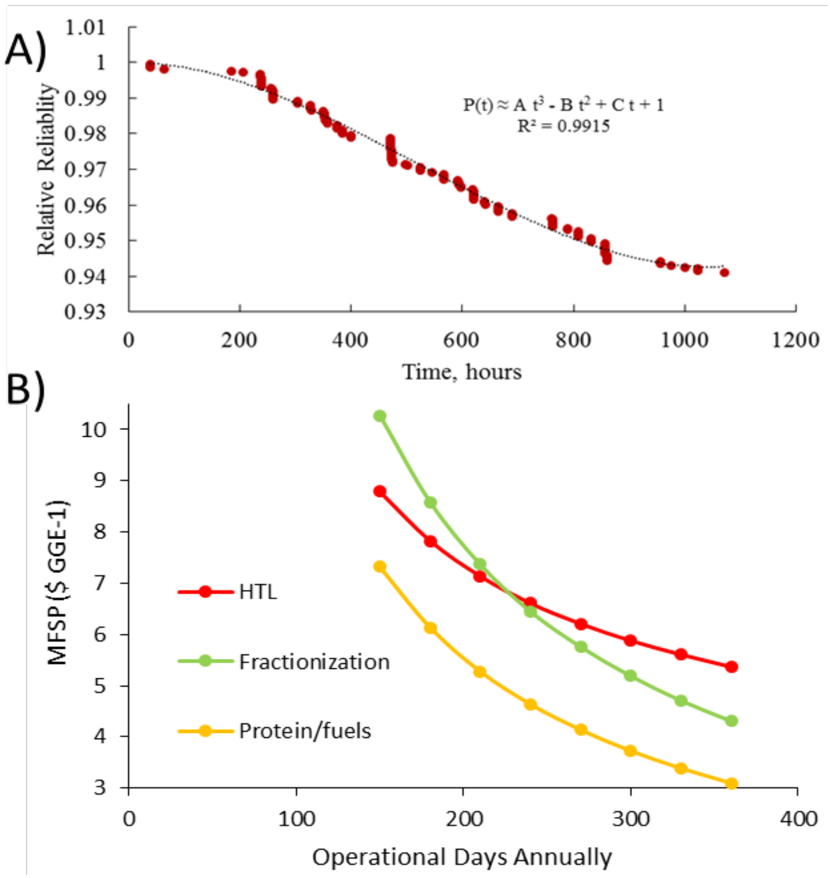
Current decision-support models (TEA, LCA, and growth/productivity) for large-scale algae cultivation systems lack critically important, quantitative culture-failure risk data

- (Semi)continuous versus full-batch cultivation present different risk profiles
  - For semi-continuous/continuous operation
    - lower cost structure but
    - potentially higher risk with respect to the consequences of culture failures
  - Full-batch operations require
    - larger investments in seed-train systems but potentially lower financial risks associated with culture failure
      - *Speed up the restart/recovery process*
      - *Minimizing risk of contamination in first place*
      - *May offer routes to enhanced biomass quality control*
- The unknowns around failure rates associated with semi-continuous versus full-batch operations constitute that must be closed to guide major investments in commercial algal biofuel production, while also creating the foundational data necessary to enable crop insurance

Project Overview: The challenges

- The productivity of algae cultivation systems represents a critical sustainability parameter and yet there are two important aspects that are not regularly considered in sustainability assessments e.g.,
    1) the impact of culture failure
    2) the impact of operational days
- Limited published work of long-term experimental trials, quantifying the impact of culture failure on
    1) biomass loss and a delay in further operation, due to re-scaling the seed train and
    2) diminished operational days negatively impacts the economics of the system dramatically
- The current state of sustainability assessment, however, has not quantified the importance of culture reliability as most nth-plant TEA/LCA simply assume a certain amount of up-time and any consideration for culture failure/restarts are buried within a single value for modeled overall facility downtime

For this project **we will generate empirically derived culture-failure risk data** for concurrent TEA/LCA modeling and **quantify the risks associated with culture failure**, and the corresponding impacts on sustainability, will be assessed through **sensitivity and scenario analyses**



A) Pond reliability based on data from ATP[3].B) Economic impact of reduced operational days for three different production pathways with an assumed baseline productivity of 25 g m^{-2} d^{-1}. Adapted from Cruce and Quinn (2019).

Project Overview: The challenges

Regardless of operational scenario, algal cultivation at scales that can support commodity-product prices requires

- Robust cultivars with high productivity and robustness
- Comprehensive monitoring programs to determine and maintain optimal growth conditions
- Defined agronomic best-practices—including integrated pest-management
- *Supporting tools and data-management structures* that are both implementable and effective ($) at enabling data-driven decision-support models for large-scale production

Current state of the art for algae cultivation water quality monitoring are sensors adopted from aquaculture

- YSI, Neptune, etc.
- Continuous measurement of temperature/conductivity, pH, DO, and ORP (redox) is typical
- Sensors are expensive and require frequent maintenance/replacement but are critical, in particular pH, for feedback control
- Dissolved CO_2 probes are available but expensive
- Real-time measurements of other critical parameters like OD or nutrients is limited

For this project, we will **develop and deploy two novel sensor platforms** that show promise for **real-time measurements** of nutrients and other water quality monitoring (e.g., ORP/DO), but also potentially measure biomass concentration indirectly and may serve as early warning to deleterious culture perturbations including pond crashes. We will **integrate our discrete and continuous data** to establish a comprehensive platform for **data-driven decision-support model development**

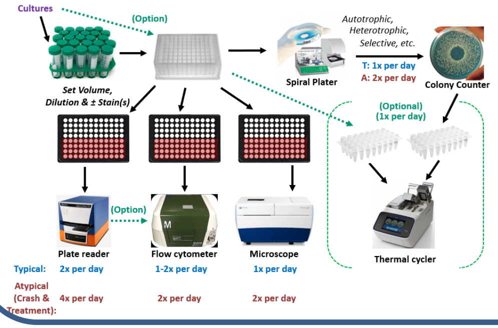
Management and Team Roles and Responsibilities

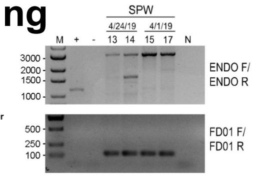
ASU Ira A. Fulton Schools of Engineering
Arizona State University

Project lead

Outdoor cultivation

integrated pest management and culture health monitoring

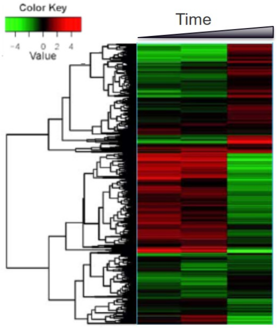




Indoor pond simulation

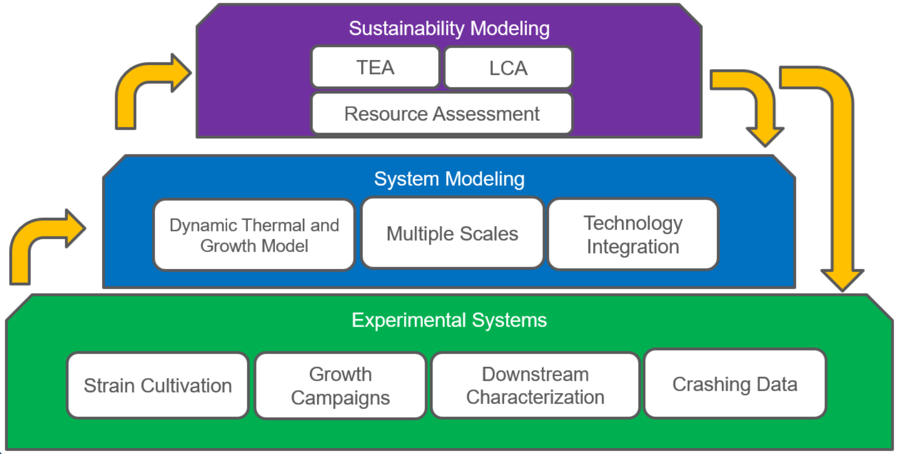
Los Alamos NATIONAL LABORATORY EST. 1943

'omics'

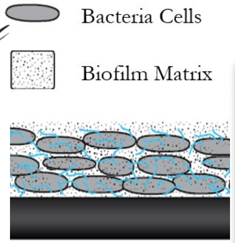


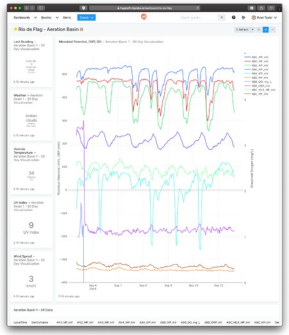
Single cell phenotyping for culture health

ENERGY INSTITUTE COLORADO STATE UNIVERSITY



Burge Environmental

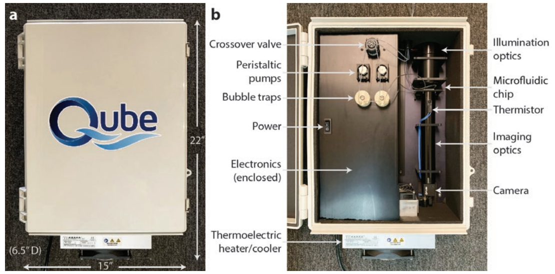




Novel microbial sensors/ data integration/analysis

QBI Quantitative BioSciences, Inc.

Novel microbial sensors for real-time nutrient analysis



DIVERSIFIED TECHNOLOGIES, INC.

Fieldsite deployed PEF

untreated

treated

Pulsed Electric Field (PEF) for crop protection

Technical Approach

Task 1: Addressing culture failure risks and quantifying impacts (ASU/DTI)

- Multi season cultivation trials comparing (semi)continuous vs. batch operation
- Crop protection through integrated pest management
  - Chemical and physical/mechanical means for crop protection
  - Regulatory aspects/barriers to deployment

Task 2: Integrated Lab to Field to Lab (LFL) to optimize cultivation performance (ASU/LANL)

- Whole culture and single cell phenotyping
  - Developing 96-well plate diagnostics workflow including flow cytometry assays for monitoring culture health
- Environmental simulation with ePBR's based on retrospective scripts
  - Iterative indoor/outdoor flow and 'omics' approach to track and understand culture health/stress as a function of key operational variables (e.g., seed train/culture age and abiotic/biotic crash events)

Task 3: Optimized process monitoring for improving performance (QBI/Burge/ASU)

- Novel sensor development for continuous, real-time monitoring of key cultivation parameters including water quality and nutrients
- Data integration platform to support decision-supported cultivation improvements
  - Goal to develop AI/machine learning ready data sets with cloud based, open access database and analysis platform

Task 4: Sustainability assessment - coupled TEA and LCA (CSU)

- Concurrent TEA/LCA/resource assessment
- Dynamic thermal and growth model development integrated with crash model

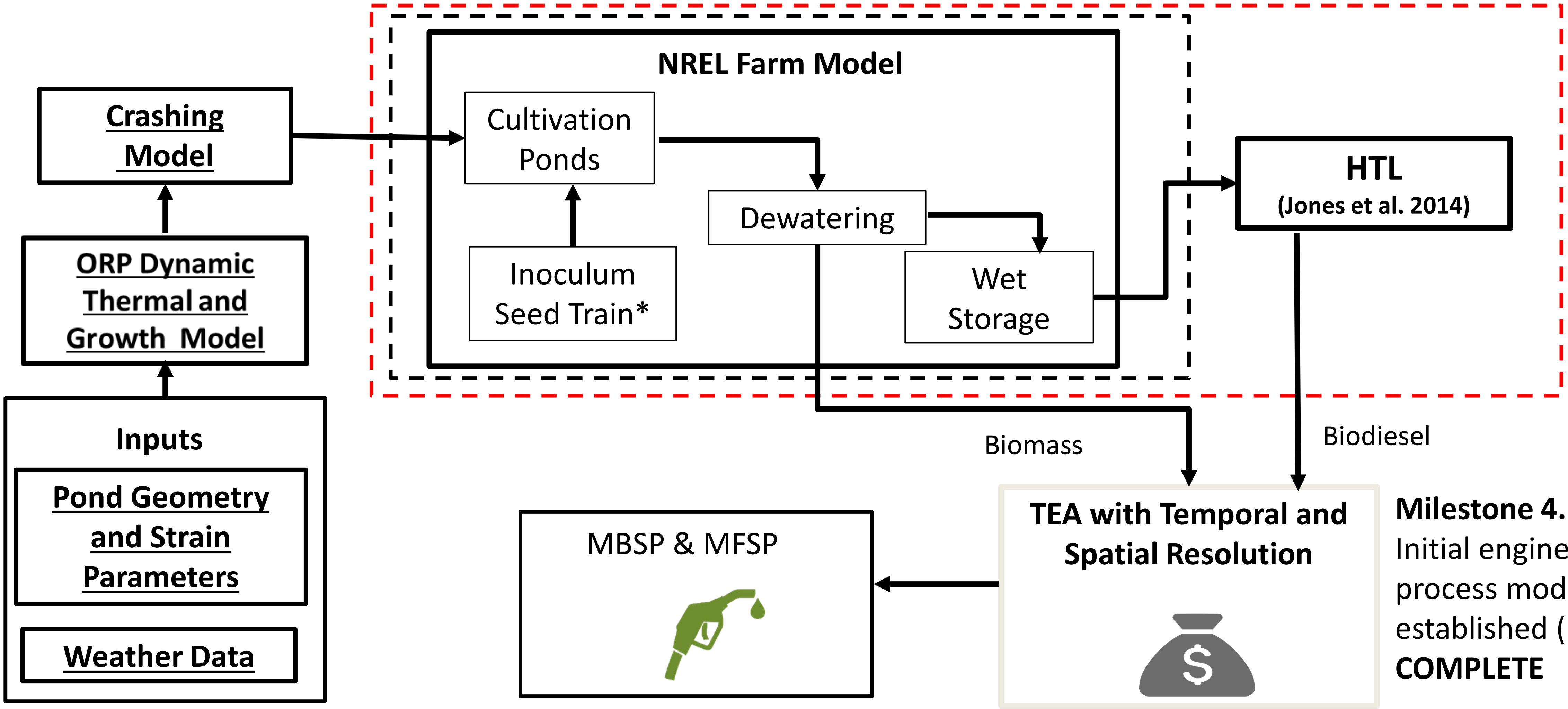
Impact

- We will **quantify the economic and technical risks** associated with different cultivation strategies and crop protection approaches through **an integrated program of indoor lab studies, cultivation optimization and simulation, multi-scale ‘omics, and robust outdoor cultivation campaigns**
- Through the **development and deployment of a suite of novel real-time sensors** for nutrient and water quality monitoring, **gain better process control** though novel insights, plus the ability to **optimize productivity, robustness, and biomass quality** of our selected high-performance strains.
- **Robust TEA, LCA, and biomass productivity modeling** will be utilized to: a) assess progress towards performance targets b) identify critical research and development priorities; and c) evaluate the impact of sub-system technologies at a systems level, allowing for more rapid advancement of those strategies that generate scalable best practices.
- **Variability and sensitivity analysis through Monte Carlo modeling** will be used to understand the **risks** associated with **culture failures** and the **sustainability impact** of **avoidance and mitigation** strategies.
- Produce a more **integrated and realistic assessment of risks**, the current state of technology, and pathways to BETO’s target of **$3.00 GGE^{-1} and trajectory to $2.50 GGE^{-1}**.

Progress and Outcomes: Task 4

Sustainability assessment - coupled TEA/LCA

Methods – Dynamic Growth Model with Crashing Incorporated and Seed Train Economics

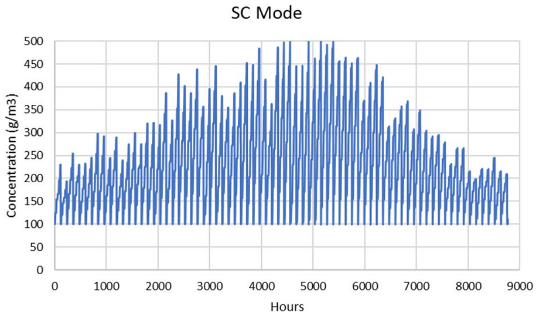


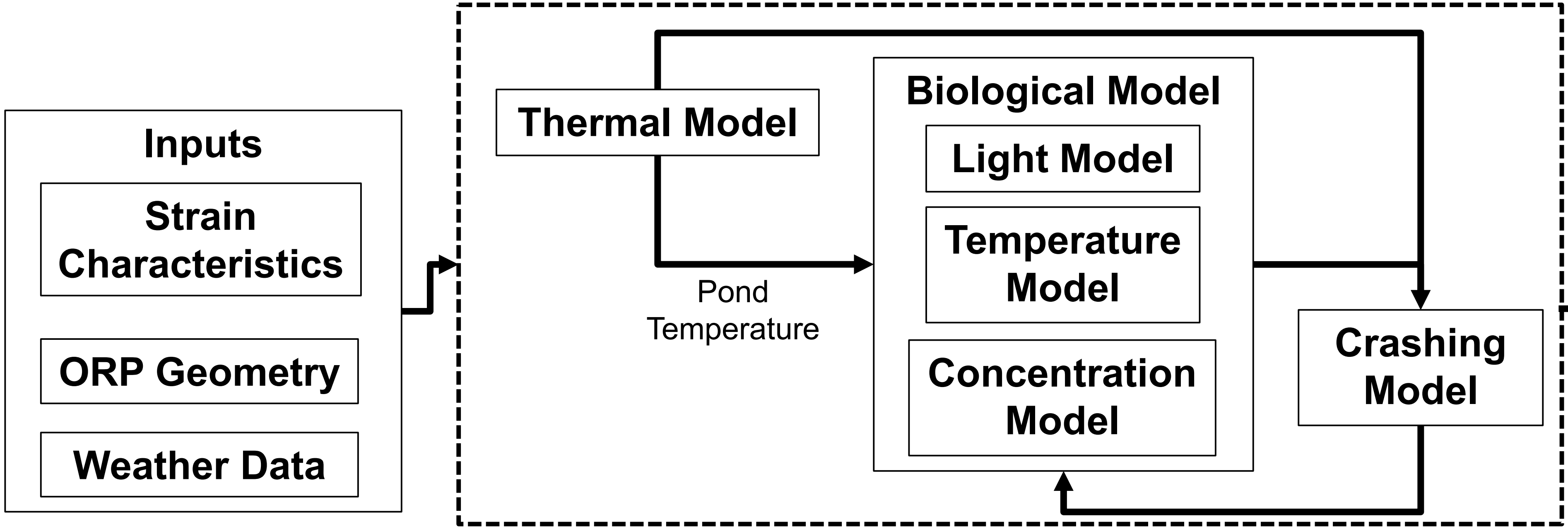
Milestone 4.1.1: Initial engineering process model established (M12) - **COMPLETE**

Preliminary work presented at 2020 ABO: "Impact of ORP Reliability on Seed Train Economics" D. Quiroz, et al.

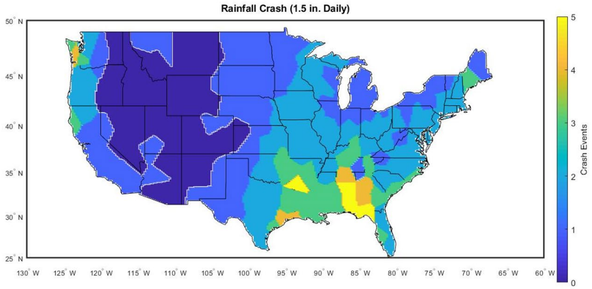
Progress and Outcomes: Task 4
Sustainability assessment - coupled TEA/LCA

Methods-Dynamic Growth Model





Outputs
- Areal Productivity
- CO2 consumption
- Average concentration (g/L)
- Evaporation rate (cm/day)
- Pond temperature



$$\frac{dCx}{dt} = \frac{\emptyset_{photon} P \varphi_L \varphi_C \varphi_T}{V} - \frac{\mathrm{D}}{\mathrm{V}}$$

- **Thermal and crash model currently validated with ATP³ data (seasonal average of all strains and locations).**
- **Additional data sets available/in process**
  - **more spatial and strain resolution with >3 years of DISCOVR data (26BAM and UTEX393) and**
  - **Initial seed train batch/semi cultivation trials from Task 1 ready**
- **More detail in additional slides**

Preliminary work presented at 2020 ABO: "Impact of ORP Reliability on Seed Train Economics" D. Quiroz, et al.

Progress and Outcomes: Task 1

Subtask1.1 Batch vs semi-cont. cultivation trials

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Task 1: Addressing culture failure risks and quantifying impacts (ASU/DTI)

NREL Farm Model Inoculum Stage

PBR – 25 g/m²/day → **Covered Pond – 25 g/m²/day** → **Lined Pond – 25 g/m²/day** → To production ponds

production ponds

100 % (semi) continuous

DMSACPE Inoculum Stage

HELIX PBR – 0.7 g/L/day → **LGEM PBR – 0.7 g/L/day** → **ORPs – 15.9 g/m²/day** → To production ponds

Y% cont.

X% Batch

X + Y = 100%

Milestone 1.1.1 Establish protocols for managing semi-continuous seed train in PBR's based on initial engineering baseline model. (M10) - **COMPLETE**

Reactor	Start Date	Harvest/ Reset Date	T0 (g/L)	Tf (g/L)	Vol. Prod. (g/l-day)	Prod. R^2	Vol.% Reset	Days to Reset	Comment
HLX	11/5/2020	11/10/2020	0.19	2.3	0.406	0.95	51%	5	
HLX	11/10/2020	11/12/2020	1.12	2.15	0.615	0.99	46%	2	
HLX	11/12/2020	11/17/2020	1.16	4.29	0.637	0.96	71%	5	Used to launch LGEM
HLX	11/17/2020	11/24/2020	1.24	5.85	0.702	0.96		7	
LGEM	11/13/2020	11/16/2020	0.38	1.95	0.52	0.99	25%	3	
LGEM	11/16/2020	11/19/2020	1.46	3.09	0.55	0.99	61%	3	
LGEM	11/19/2020	11/23/2020	1.22	3.92	0.697	0.92	76%	4	
LGEM	11/23/2020	11/30/2020	0.93	3.62	0.397	0.99	73%	7	Contamination
LGEM	11/30/2020	12/4/2020	0.97	2.41	0.34	0.90	73%	4	Contamination
LGEM	12/4/2020	12/14/2020	0.66	3.56	0.244	0.90		10	Crashing

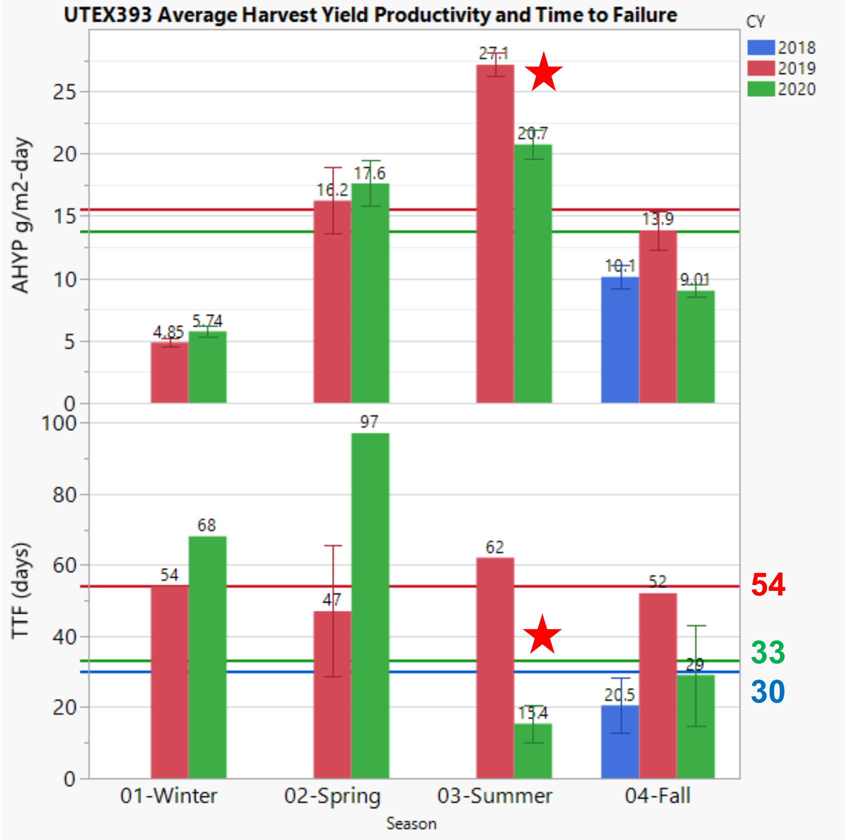
Milestone 1.1.2 Complete at least three (3) seasonal cultivation trials sixty (60) days in length (target is summer/fall, fall/winter and winter/spring for maximum seasonal transition/contamination pressure) and deliver productivity and crash rate data to Task 4 (M25) – **In progress with 26BAM/UTEX393**

Key outputs: productivity and reliability (i.e. time to failure)

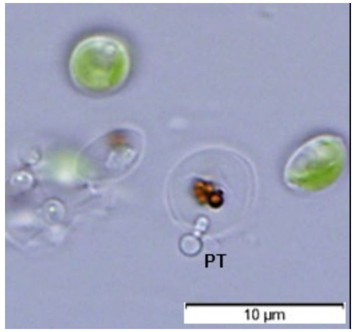
Season 1 completed (Fall/Winter Nov-Dec 2020) and baseline PBR productivities for 26BAM established and initial batch vs semi-cont. cultivation comparison completed (see additional slides).

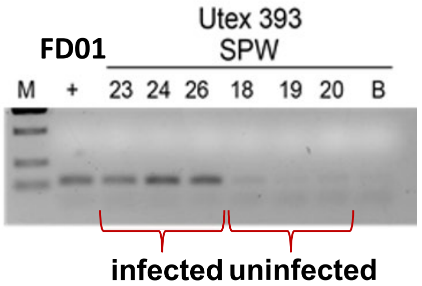
Progress and Outcomes: Task 1

Subtask1.2 Crop protection through integrated pest management



- UTEX393 cultivated at AzCATI **continuously since Fall 2018**
- Rapid infection and culture crashes through Spring 2019
- Infection by fungal parasitoids common with multiple host/parasite infections



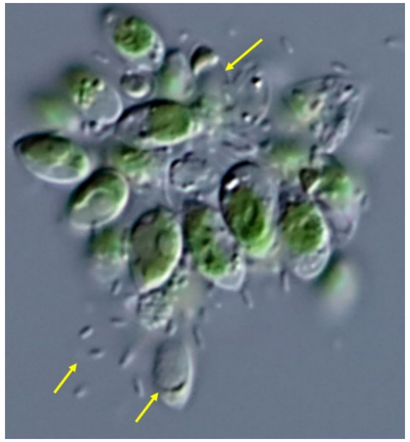


- A fungicide (Fluazinam) showed great results (both prevention and recovery)
- Year over year performance peaked in 2019 significant increase in productivity and time to failure for UTEX393 relative to the 2018 SOT (**54 vs. 30 days)**

- Under DISCOVR, confirmed presence of *A. occidentale* (isolate FD01), an aphelid parasitoid with amoeboid zoospores in both UTEX393 and 26BAM.
- Present year-round, most active in warmer seasons

- Fluazinam provides robust mitigation for UTEX393 (and 26BAM)
- However, dramatic drop in productivity and time to failure (TTF) Spring/Summer 2020)

- Bacterial pest confirmed (but not yet ID)
- Early mitigation steps have proven marginally effective (salinity/pH swings)



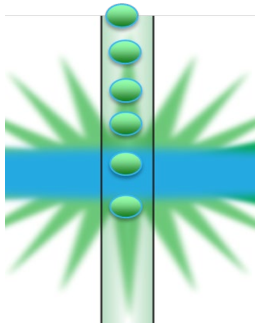
- Indoor crash models established for both pest types
- Bacterial pest ideal system to test in spring/summer as temps rise (evidence for year-round presence)
- Hypothesis: batch cultivation maintains higher overall productivity without any mitigation relative to semi-continuous w/wo mitigation
- Additional mitigation strategies to be tested indoors

Progress and Outcomes: Task 2

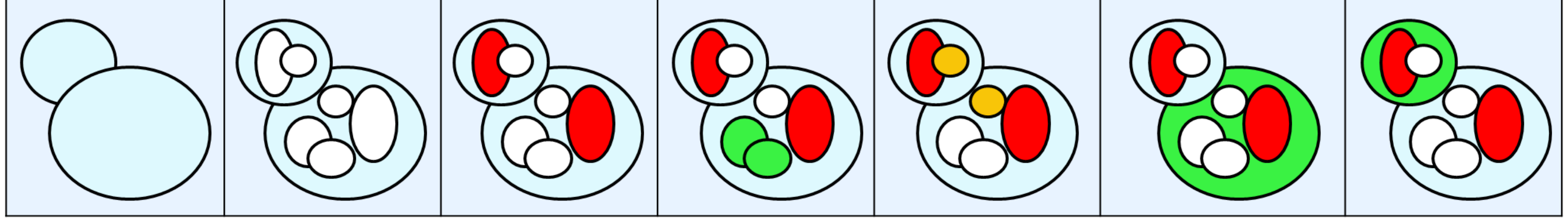
Integrated Lab to Field to Lab (LFL)

Subtask 2.1: Whole culture and single cell phenotyping

- Track various macromolecules and organelles, as well as specific cellular activities/function
- Can be applied to laboratory cultures (ePBR) as well as to samples taken from outdoor



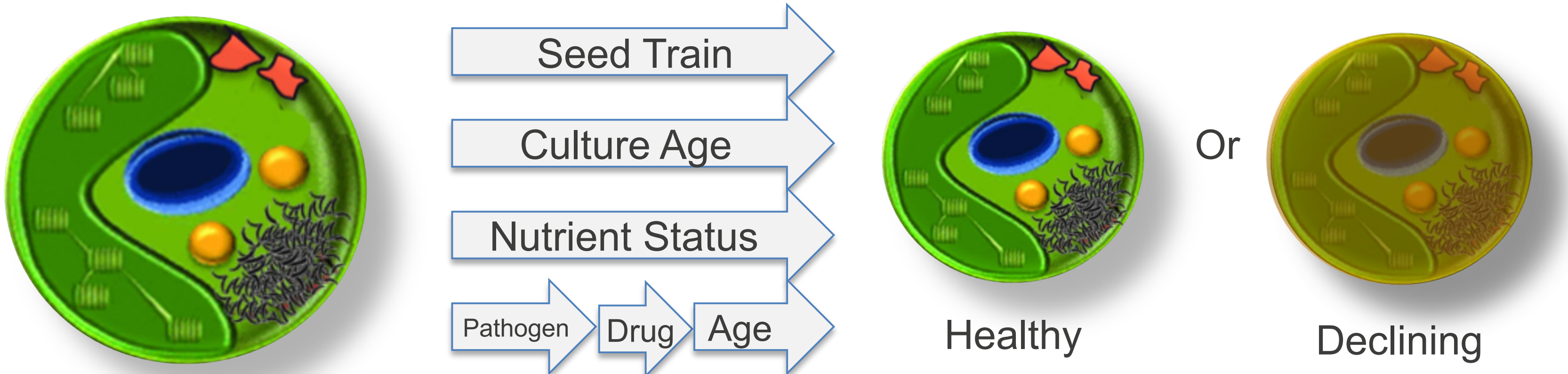
Forward Light Scatter	Side Light Scatter	Red Fluorescence	Green Fluorescence	Orange Fluorescence	Green Fluorescence	Green Fluorescence
Size	Granularity	Chlorophyll	Lipids (when stained)	DNA (when stained)	Metabolic Activity (when stained)	pH (when stained)



Takeaway: We hypothesize that changes in metabolic activity and reactive oxygen species will be early indicators (signatures) of abiotic and/or biotic stress

Subtask 2.2: Environmental Simulation with ePBR's and Transcriptomics

- Can we use flow cytometry to identify early(ier) signatures of culture decline?
- Can we layer in gene expression (transcriptome) or metabolomics changes?
  - Are these signatures identifiable in the lab ***and*** outdoors (**and are they the same**)?
- Can we connect those signatures to the whole culture measurements (2.1.2)?
- Can we identify risks in operation and inform mitigation in near real-time (same day)?
  - Can we deconvolute (even roughly) abiotic from biotic stressors?



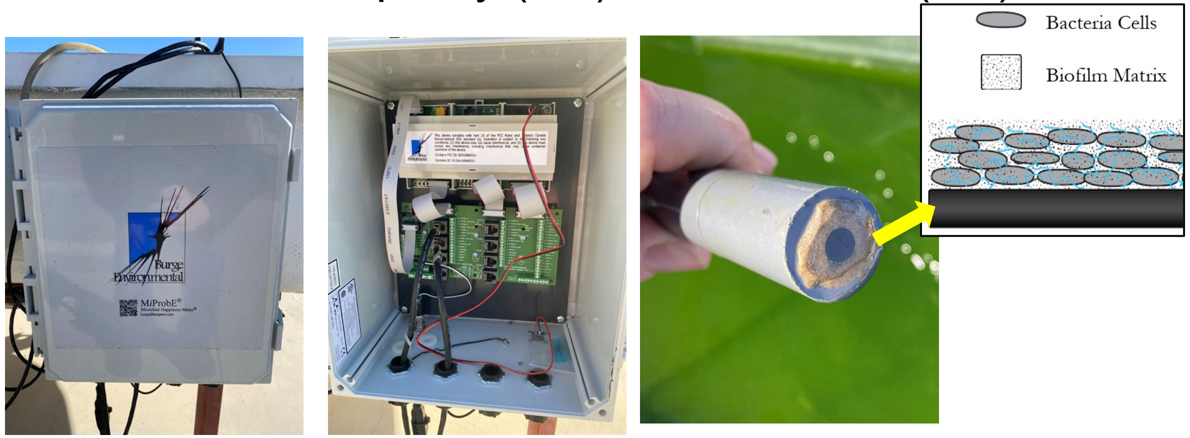
Takeaway: we are in an excellent position to ask these questions, which should help us better understand algae growth and stability.

Progress and Outcomes: Task 3

Optimized process monitoring for improving performance

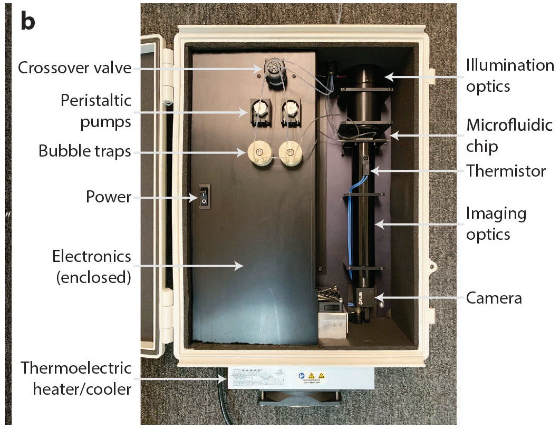
Subtask 3.1 and 3.2

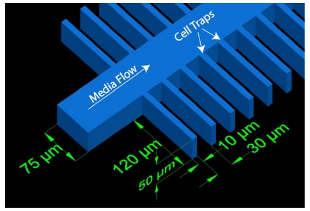
- Novel sensor development for continuous, real-time monitoring of key cultivation parameters including water/culture quality (3.1) and nutrients (3.2)

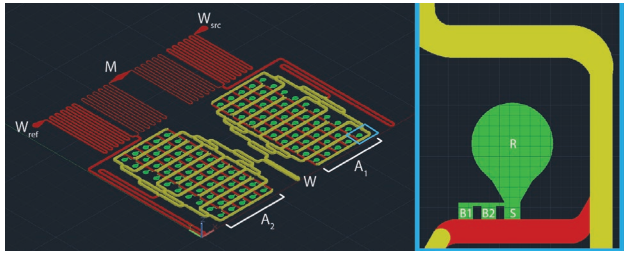


MiProbe system and example of current sensor deployed

- Novel, continuous operation, real-time data/results
- MiProbe measures electron potential on electrode surface populated with a biofilm made up of endemic species of microbes.
- ‘Microbial Potential’ responds to changes in the environment from the perspective of the biofilm.
- Redox changes, photosynthesis, biomass (e.g. Ash Free Dry Weight / MLSS / BOD / COD), nutrient loading, presence of biocidal compounds/events can be monitored in real-time.
- Very LOW-COST sensors, easy to deploy remotely (low power – solar battery typical)







- Novel, continuous operation, real-time data/results
- GFP based microbial microfluidic sensor genetically engineered to respond to different analytes (e.g., heavy metals, nitrate/ammonia/phosphate)
- Housed in environmental enclosure with optics, hardware, software all integrated to sustain cell growth and perform image acq./processing

Milestone 3.1.1: Deliver and install MiProbe systems at AzCATI and LANL. **COMPLETE for AzCATI –** in progress for LANL (expected Q2FY21)

- 12 ponds operational with multiple sensors/sensor arrays
- Custom sensor deployment for LANL ePBR’s (designed/prototype complete)

Milestone 3.1.2: Operational protocols for deploying and maintaining MiProbes systems with initial demonstration of >/= 85% correlation to one or more production metrics. (M15) – **COMPLETE – see next slide**

Milestone 3.2.1: Qube systems deployed at AzCATI (M18 – Q2FY21) on track

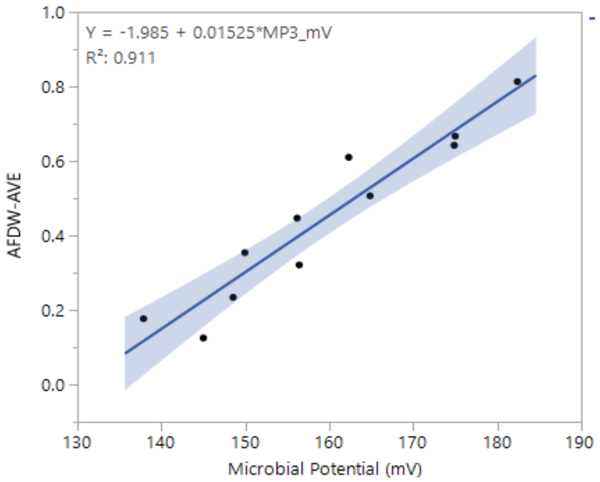
Progress and Outcomes: Task 3

Optimized process monitoring for improving performance

Subtask 3.3: Data Integration Tool Development.

- Leverage Burge's MiProbe Cloud platform for all project data integration
  - MiProbe Cloud developed to integrate with machine learning, AI, and other advanced **open-source analysis** tools (Jupyter Notebooks, Julia, Redash, Plot.ly, etc.).
- Synchronized data structures
  - e.g., all sensor data and lab (grab sample) analyses)
- Custom APIs and Web-Front-end tools to automate data integration.
- All data integration code to be provided as open-source software for ongoing research use beyond the scope of this project.
- Main outcome of task 3.3 will be initial automated heuristics that will function as an alerting tool for non-scheduled sample collection, disruptive event categorization, and analysis while logging information that can later be utilized in advanced machine learning applications.

Operational Dashboard showing both real-time MiProbe Sentry data and integrated local weather station data.



Validation of MiProbe microbial potential correlation to key process measurements. Demonstrated correlation for 2 strains already (marine and brackish). Two additional strains in progress

Microbial Potential vs. AFDW. Over 11 days of an automated pilot ORP deployment, Microbial Potential strongly correlated to average AFDW measurements, but was labor-free.

Milestone 3.3.1: Data architecture and integration defined. (M9) - **COMPLETE**
Milestone 3.3.2: Automated data integration tool and analysis platform deployed and in-use across project with correlation to key process measurements (e.g., OD/AFDW) demonstrated (>85% correlation). (M15) - **COMPLETE**
Milestone 3.3.3: Initial feasibility assessment report of integrated discrete and continuous data across project with identification of initial automated heuristics (M24)

Quad Chart Overview

Timeline
- May 2020-December 2021 (BP2 target start/end)
  - Formal contracting not completed until October 2020
  - Limited at-risk spending in FY20
- January 2022-March 2023 (BP3 start/end)

	FY20 Costed	Total Award
DOE Funding	$0 FY20 (~$350K FY21 to date)	$3,500,000
Project Cost Share	$0 FY20 (~85K FY21 to date)	$875,000

Funding Mechanism
DE-FOA-002029 FY19 BETO Multitopic FOA
Topic Area of Interest 1: Cultivation Intensification Processes for Algae

Project Partners*
- Los Alamos National Laboratory
- Colorado State University (TEA/LCA/process modeling)
- Burge Environmental (novel sensor development)
- Quantitative Biosciences Inc. (novel sensor development)
- Diversified Technology (crop protection)

Project Goal:
Assess the cost-benefit tradeoff of enhanced "crash-recovery" routes and their impact on biomass productivity and quality and thus economic impact on biomass and biofuel production costs.

Mid Project Go/No Go:
Increase mean time to failure from 2018 baseline of 30 days to **>/= 45 days**, while meeting or exceeding 2019 SOT productivity baseline for annual average of **15.9 $g{\cdot}m^{-2}{\cdot}d^{-1}$** (November 2021)

End of Project Goal:
Demonstrate increase in mean time to failure to **>/= 60 days** (100% improvement over 2018 baseline of 30 days) while achieving an annual average productivity of at least **17.6 $g{\cdot}m^{-2}{\cdot}d^{-1}$** (50% improvement over 2018 baseline value of 11.7 $g{\cdot}m^{-2}{\cdot}d^{-1}$) and at least **20% improvement** in composition to achieve at least **80 GGE** (March 2023).

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Summary

- We will **quantify the economic and technical risks** associated with different cultivation strategies and crop protection approaches through **an integrated program of indoor lab studies, cultivation optimization and simulation, multi-scale ‘omics, and robust outdoor cultivation campaigns** generating novel, high-quality cultivation datasets that leverages over 8 years of standardized outdoor cultivation experience and a team with years of experience collaborating on BETO lab and competitive awards.
- Additionally, through the development and deployment of a suite of **novel real-time sensors** for **nutrient and water quality** monitoring with significant, and already demonstrated commercial potential, we will gain better **process control** though novel insights to optimize **productivity, robustness, and biomass quality** of our selected high-performance strains.
- Finally, guiding our overall R&D throughout the project will be our **concurrent and integrated TEA, LCA, and biomass productivity modeling** allowing for:

  a) assessing progress towards performance targets

  b) identifying critical research and development priorities

  c) evaluating the impact of sub-system technologies at a systems level, allowing for more rapid advancement of those strategies that generate scalable best practices and

  d) variability and sensitivity analysis through Monte Carlo modeling **to quantify the risks associated with culture failures and the sustainability impact of avoidance and mitigation strategies**.

DMSACPE Team Members

Task 1 ASU Team
John McGowen (PI), Peter Lammers (Co-PI),
Jessica Forrester, Jason Potts, Clara Missum, Richard Malloy

Task 1 DTI
Mike Kempkes

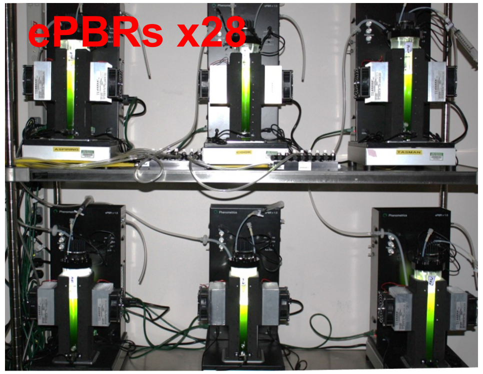
Task 2 ASU Team
Taylor Weiss (Co-PI),
Henri Gerken, Mauricio Gonzalez, Aaron Geels

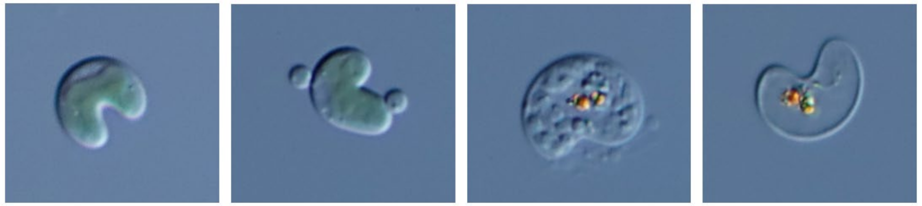
Task 2 LANL Team
Taraka Dale (Co-PI)
Claire Sanders, Shawn Starkenburg, Carol “Kay” Carr

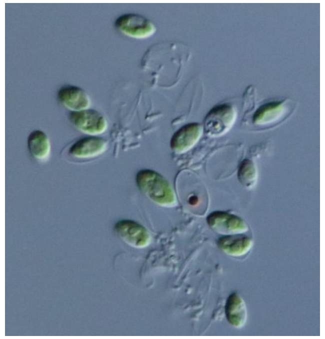
Task 3 Burge Env.
Evan Taylor (Co-PI), Brian Ford, Dave Baker, Chad Ripley, Scott Burge

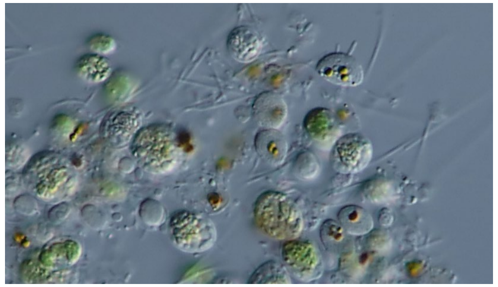
Task 3 QBI
Natalie Cookson (Co-PI), Michael Ferry, Scott Cookson

Task 4 CSU
David Quiroz, Jason Quinn (Co-PI)







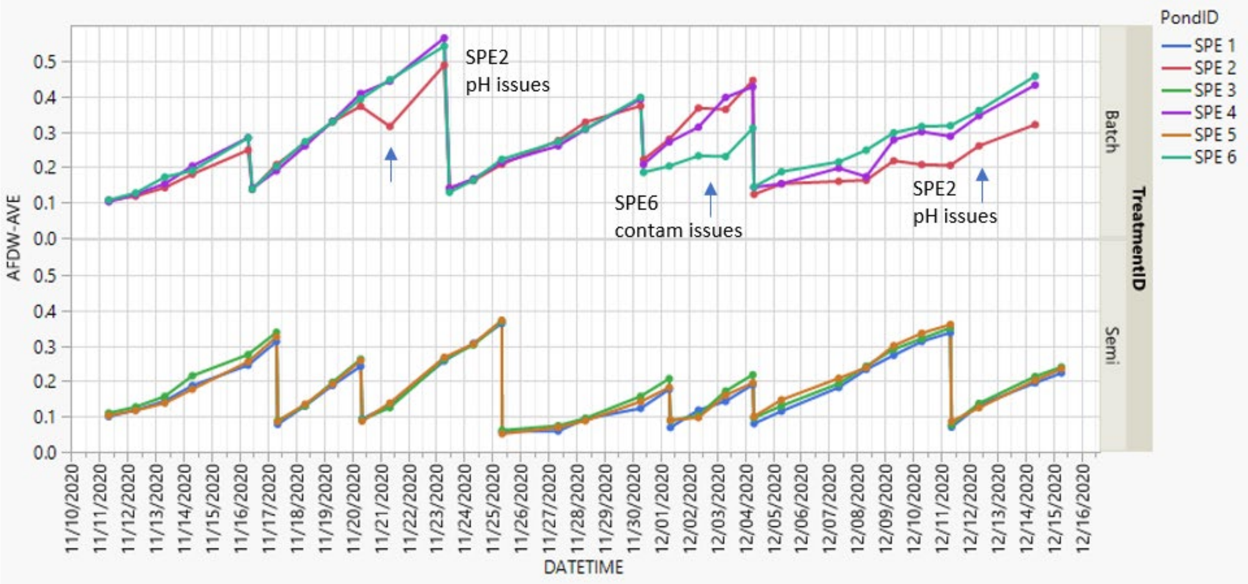


Additional Slides

- Responses to Previous Reviewers’ Comments: N/A
- Passed Initial verification Go/No Go in March 2020 and had a target start date of May 2020, but contracting with DOE not completed until October 2020 (for prime- ASU), and December 2020 for all subs. ASU/Burge/CSU operated at risk with reduced capacity until Q1 FY21 (federal).
- Publications, Presentations, Patents
  – *“Impact of ORP Reliability on Seed Train Economics” D. Quiroz, et al. ABO September 2020*
  – “*Cultivation reliability and its impact on the economics and sustainability for algae-based products. What data is needed?” J. McGowen, et al, presented at ABO September 2020*

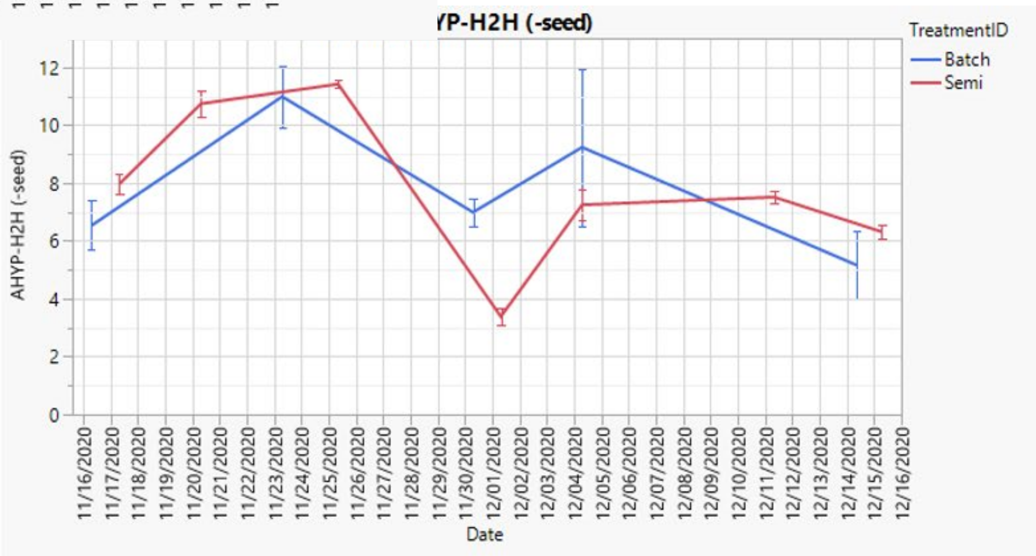
Progress and Outcomes: Task 1

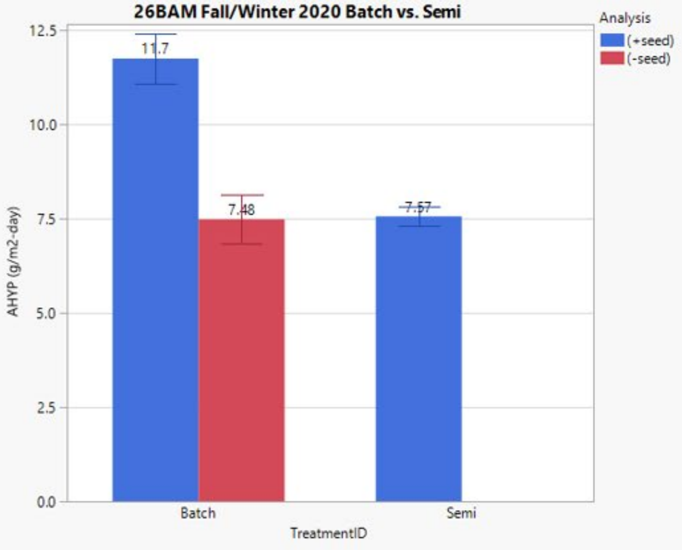
Subtask1.1 Batch vs semi-cont. cultivation trials



AFDW for each TreatmentID (Batch and Semi) for ORP's. SPE1,3,5 were operated semi-continuously and SPE2-4-6 were operated in full batch mode. SPE2 had pH control issues due to fault pH probes during the 2nd and 5th growth cycles, and SPE6 had significantly more contamination than SPE2-4 in the 4th growth cycle.

AHYP-H2H comparisons for batch versus semi over the course of the cultivation trial (Left graph) and the summary value for the full 33 days of cultivation (right graph) with (+seed) and without (-seed) contribution of the biomass coming from the PBR. A refined process model assumption is in progress to account for the seed train productivity contribution as it is ultimately biomass contributing to overall yield.





Similar to past results at AzCATI, when contamination pressure is relatively low as in late fall/winter, limited benefit to batch vs. semi-continuous for ORP productivity. We expect this to shift as contamination pressure increases for Winter/Spring and Spring Summer runs later in 2021.

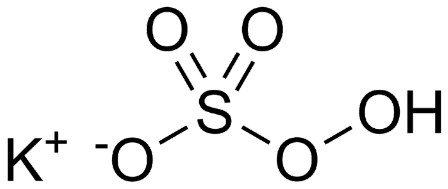
Progress and Outcomes: Task 1

Subtask1.2.1 Chemical treatments and media optimization to control pests

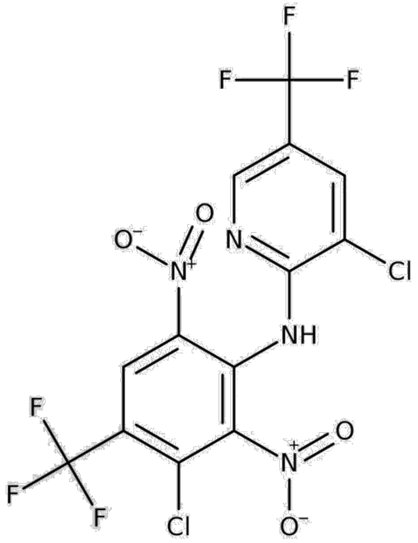
Control Agents: Batch vs. Semi-Continuous

- Sterilizing biocides essentially kill microorganisms equally, may offer greater utility in batch-modes of operation, and are **not** regulated as pesticides
- Pesticides which essentially target specific organisms, offer greater utility in semi-continuous modes of operation, and are **strictly** regulated

Compounds	Virkon®	Secure®
Category	biocide	fungicide
Active Ingredient	"Oxone"; pentapotassium bis(peroxymonosulphate) bis(sulphate)	Fluazinam; 3-chloro-N-(3-chloro-2,6-dinitro-4-trifluoromethylphenyl)-5-trifluoromethyl-2-pyridinamine
Mechanism of Action	Peroxy compound (i.e., oxidizes)	Uncoupler of oxidative phosphorylation
Global Usage	Global	Banned: Norway
Restrictions	Not approved for outdoor or aquatic use (yet)	Not approved for aquatic applications
Degradation	Hydrolysis, photolysis; very sensitive to heat and salinity	Hydrolysis, photolysis
Aquatic DT_{50} (abiotic)	pH 4 = 800 h pH 7 = 145 h pH 9 = 2.8 h *20 ºC, pH 8 seawater = 5.5 hours* *20 ºC, pH 8 freshwater = 215 hours*	pH 7 = 42 d pH 9 = 6 days
Aquatic DT_{50} (biotic)	Tests are not required, due to rapid abiotic decomposition	~8 hours aerobic and anaerobic
Assay	Colorimetric peroxide assay	LC/MS/MS (EPA MERID No. 48635802)



Oxone



Fluazinam

Progress and Outcomes: Task 1

Subtask1.2.1 Chemical treatments and media optimization to control pests

Milestone 1.2.1.1: Literature review and initial risk assessment of potential pest control agents and top candidates identified for indoor crash assays (M9) **Complete**.

Milestone 1.2.1.3 Data-supported risk assessment of aquatic algal pest control agents and regulated implementation scenarios, including economic and environmental impacts (M30)

Toxicological risk assessment = hazard identification, dose-response assessment, exposure assessment, and risk characterization

Two reports will be generated with different impacts intended, but assessing the externality of **feasibility** is a common criteria

The intent of these reports is **not** to advocate for the use of pesticides, but rather detail what commercial use would actually entail

Toxicological Risk Assessment[2]

Report #1 (M9) - **COMPLETE**

- Primarily for internal consumption, establishing the parameters of further inquiry
- Summary of primarily public information (e.g., literature)
- Will contain a general framework for feasibility concerns facing any algal pesticide (e.g., fungicide) use
- Will contain a specific framework around fluazinam as a highly relevant pesticide example
- Will contain recommendation(s) for further pesticide examples which should be included in laboratory research, with special attention to provisioning pesticide-mechanism rotation
- Results will help guide laboratory studies toward highest impact activities, including protocol developments that will generate regulatory-relevant data

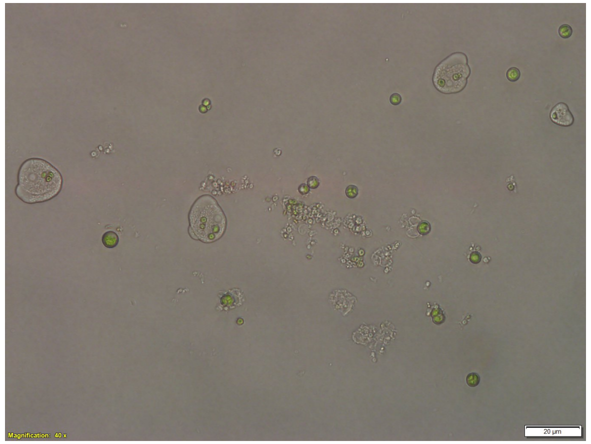
Report #2 (M30)

- Primarily for external consumption, establishing specific contents for further engagement (DOE, EPA, manufacturers, etc.)
- Combination of public information and new research
- Will specifically incorporate the results of LCA and TEA modeling specific to the value and impacts of pesticides
- Will contain the broader framework for algal pesticide use within the project as a documented example, following both established and likely guideline parameters
- Intended to outline the necessary details of applying any pesticide to commercial algal practice

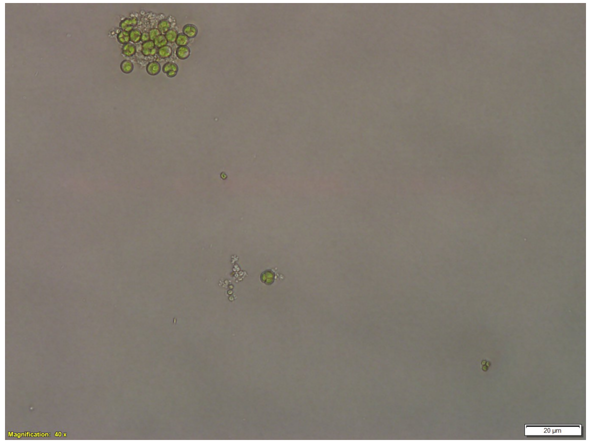
Progress and Outcomes: Task 1

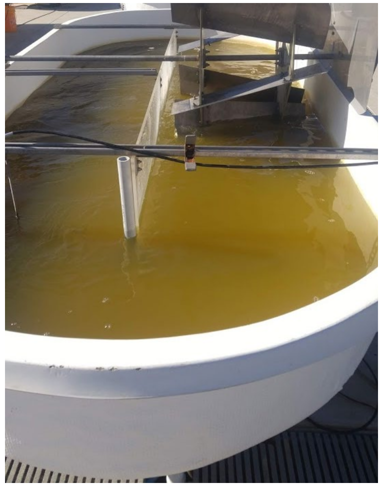
Subtask 1.2.2: Physical treatments for algal pest control - Pulsed Electric Field (PEF)

Untreated (SPW12)



PEF treatment 9 kV
20 µsec TT (SPW10)

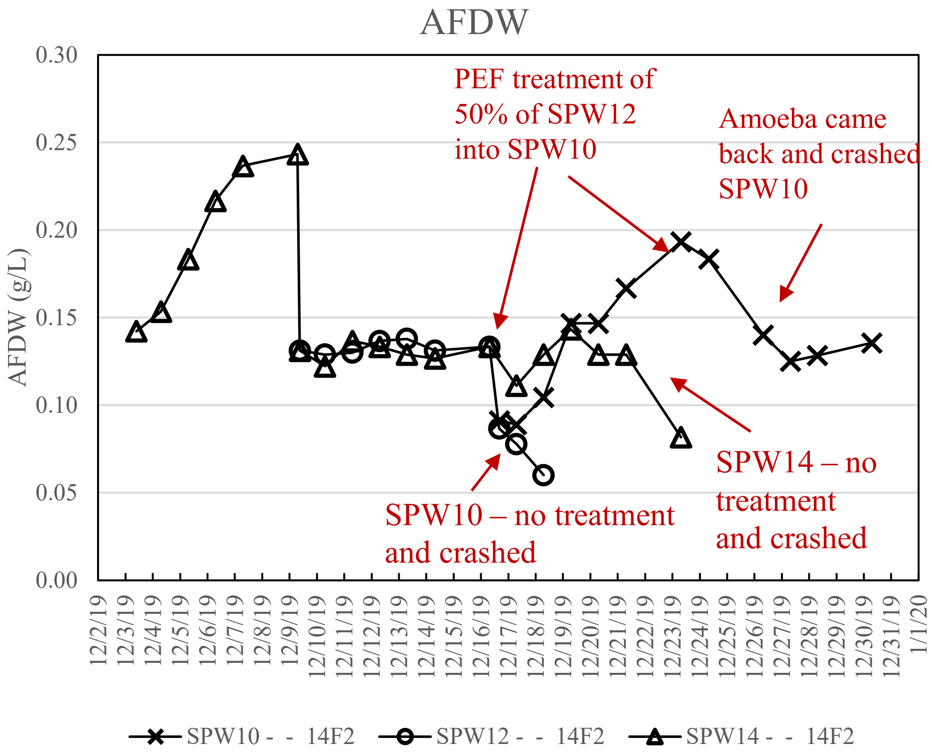




By Day 2 post PEF the treated pond looked green and healthy while the untreated pond had crashed. Note – culture was at full salinity – limits range of voltages that can be applied – but was still effective!

Note – PEF ***has been shown*** to ***NOT*** be effective on fungal parasitoid contamination

12/16/19 *Micractinium sp* 14-F2 ponds crashing due to amoeba infection. PEF treated ½ of SPW12 (500L) and transferred into new pond (SPW10). Volumed up both ponds with fresh media. Single pass treatment.



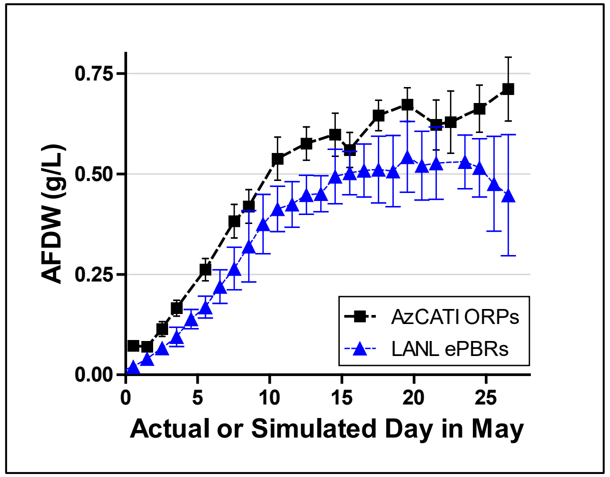
Milestone 1.2.2.1: Baseline OPEX costs established for PEF treatment based on actual outdoor testing. (M18)

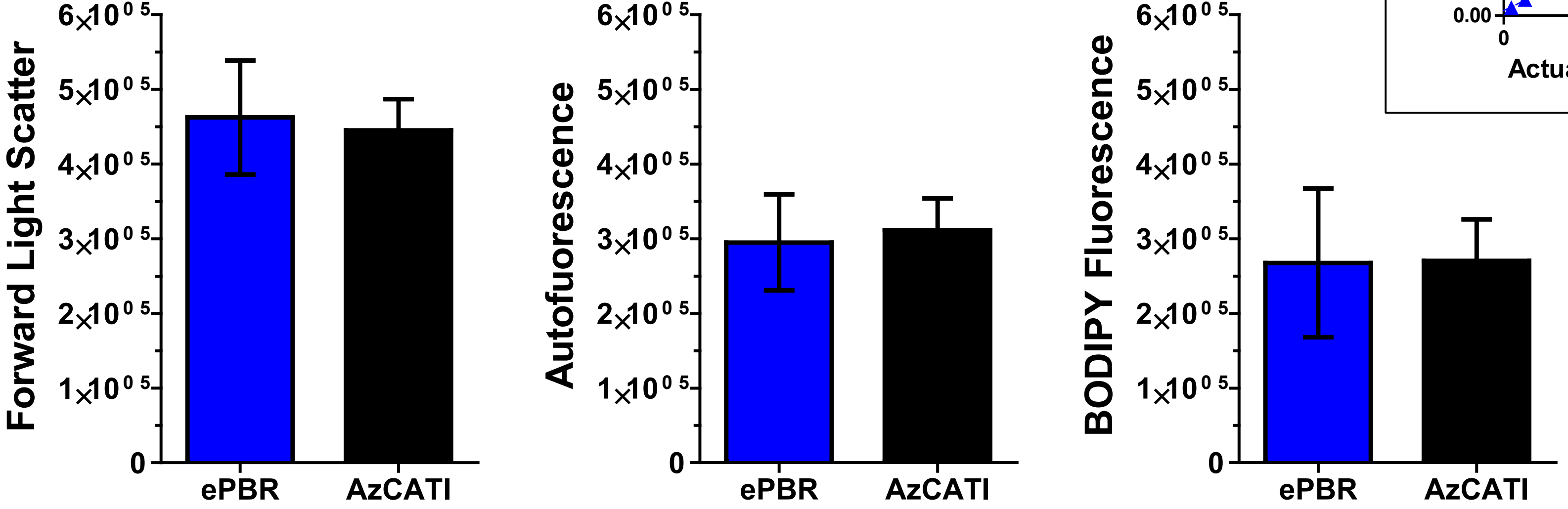
Additional experiments currently in progress in Q2FY21

Task 2 Background

Flow cytometry data: ePBRs vs ORPs

- More data from batch *Chlorella* experiment (inset, right)
- Replete conditions: Average cell morphology, autofluorescence, and lipids are similar in both systems.
- ePBRs = 30 day light/temp avg for May at AzCATI….. AzCATI = May 2015.





- **Takehome:** Similar to bulk biomass productivities, single cell data from ePBRs and outdoor ponds indicate that cell properties are similar in the two systems

Task 2 Background

Subtask2.2: ePBRs and “omics” proposed workflow

Outdoor experiment conducted at AzCATI
- Whole culture & flow assays
- Transcriptome sampling
- Send samples to LANL for comparative flow

ePBR experiment conducted at LANL
- Retrospective scripts (no crash)
- Try to ‘subtract’ abiotic from biotic
- Same sampling plan

Initial Data Workup
- Growth, biochemical on harvested biomass
- Flow data
- Compare across sites, select ‘omics smpls

Transcriptome Data
- Growth
- Biochemical on harvested biomass
- Comparison across sites

Correlate infection with culture health signatures

- Possible variations
  - Induced crash
  - Treatment times/regimes/where in seed train
  - Culture memory
- Stretch
  - Bring treated cultures indoors and use as seed (longer question about culture memory)

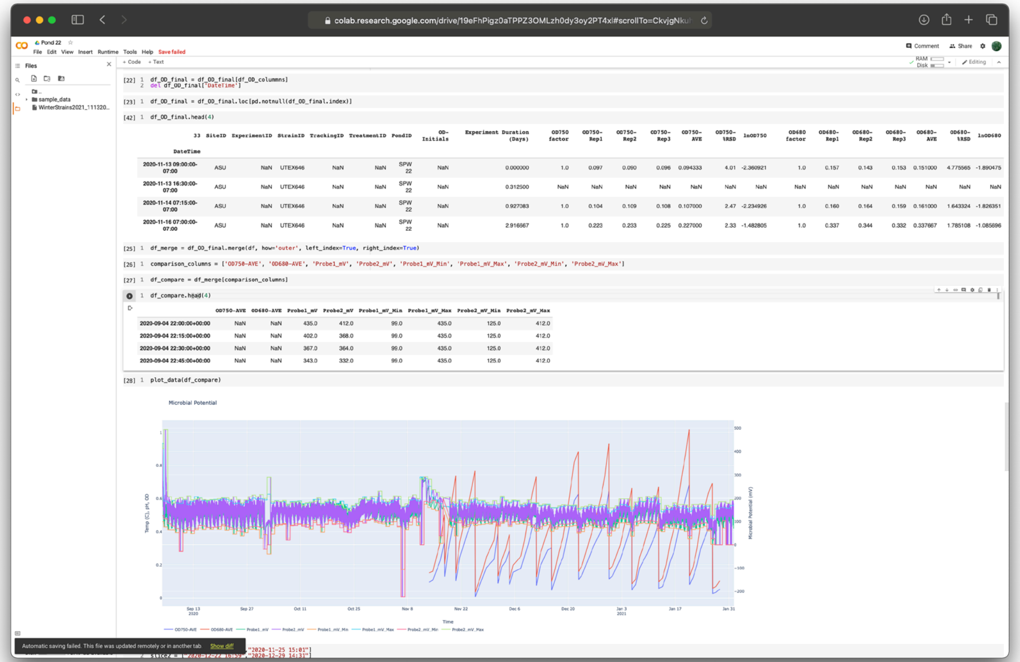
AzCATI
Arizona Center for Algae Technology and Innovation

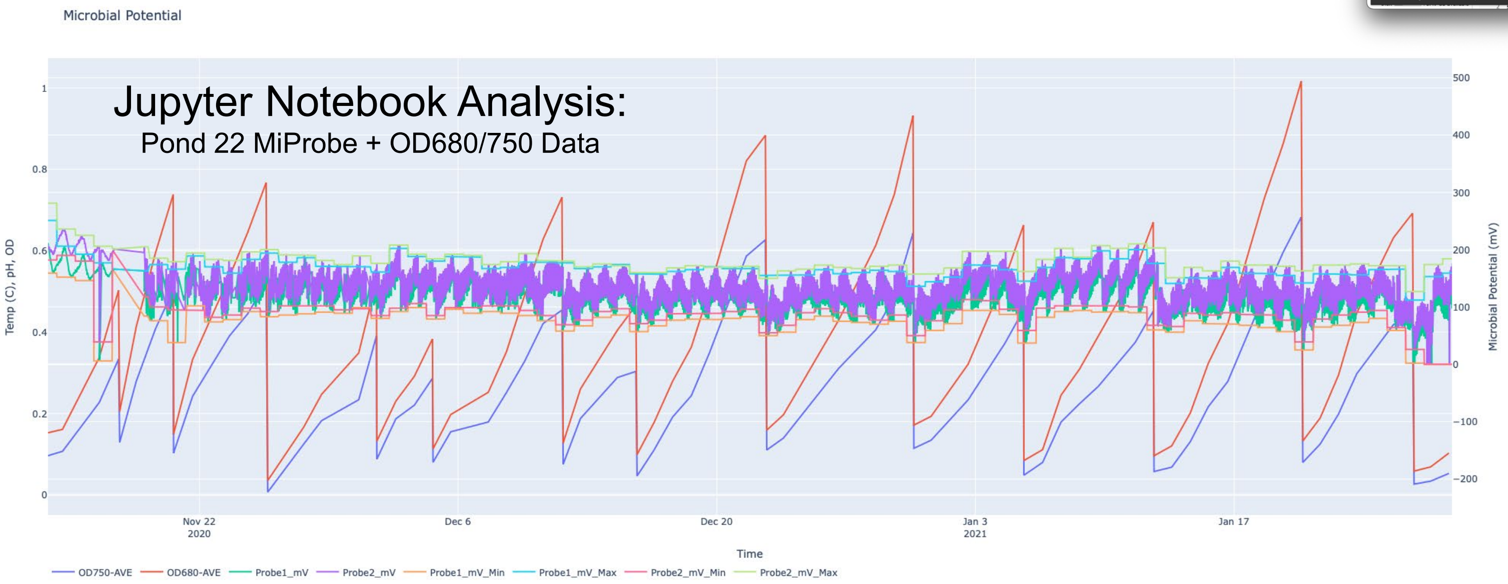
Progress and Outcomes: Task 3

Optimized process monitoring for improving performance

Subtask 3.3: Data Integration Tool Development.

- Using Google Colab, real-time sensor data and lab sample report data is integrated allowing interactive timeseries visualizations and custom analyses.
- Advanced Data Science tools, better analysis traceability, and improved collaboration are possible using this tool.
- Simplified data APIs will be developed to deliver completely combined datasets to enable improved analysis for researchers.

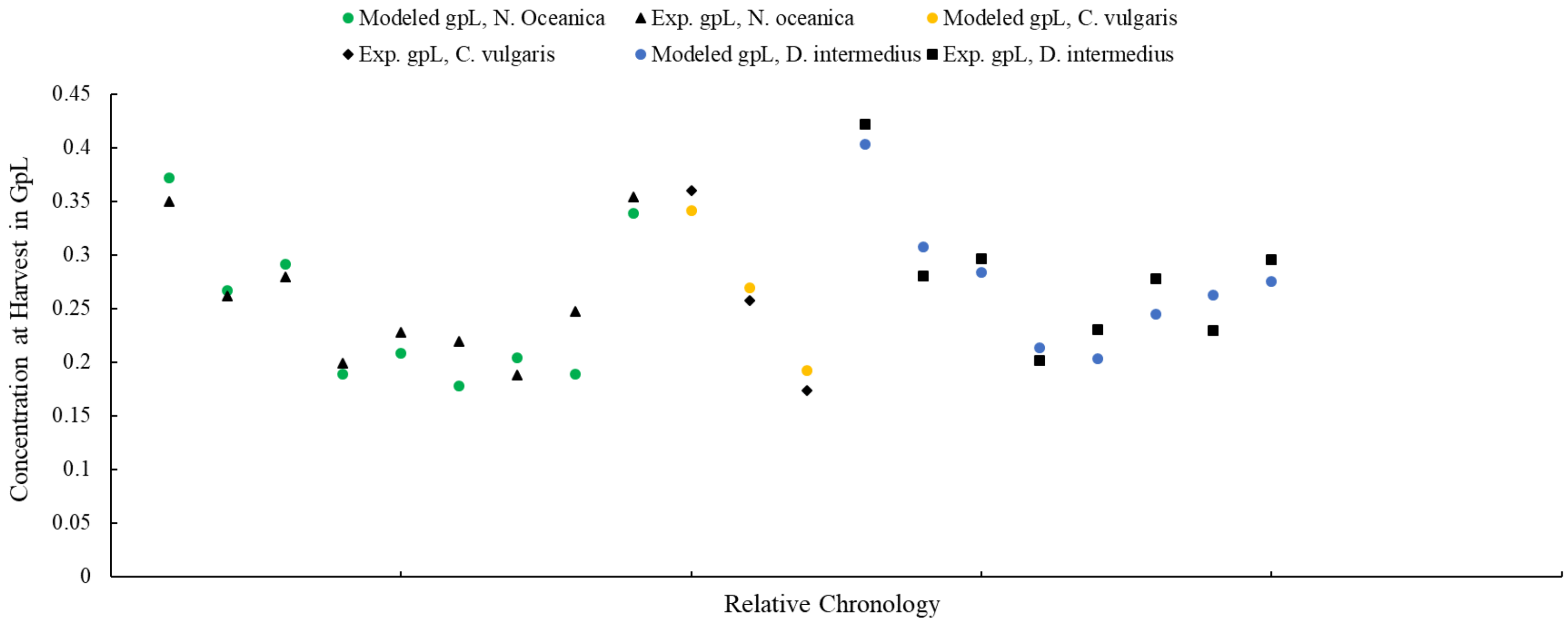




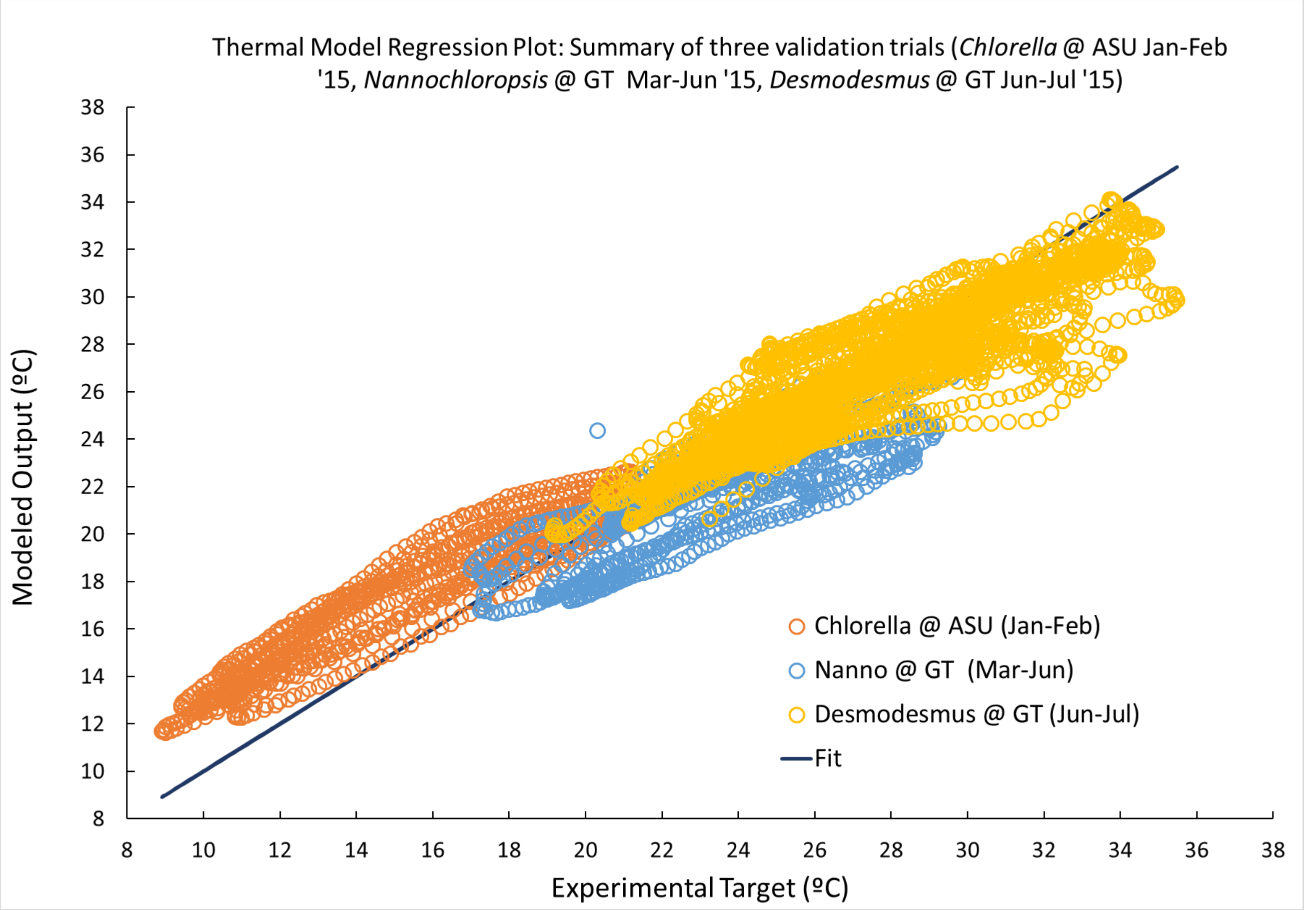
	OD680-AVE	OD750-AVE	Probe1_mV_Max	Probe2_mV_Max
OD680-AVE	1.000000	0.997861	0.977625	0.999966
OD750-AVE	0.997861	1.000000	0.974227	0.999973
Probe1_mV_Max	0.977625	0.974227	1.000000	0.942286
Probe2_mV_Max	0.999966	0.999973	0.942286	1.000000

Comparison of Daily Max Values of 5-minute real-time MiProbe Data with Daily OD680/750 Measurements results in a 0.8 to 0.99 correlation between these values across three separate grow/harvest cycles (one shown above).

Growth and Thermal Model Validation



Growth and Thermal Model Validation



Progress and Outcomes: Task 4

Sustainability assessment - coupled TEA/LCA

Crash Model

Crash Model

Temperature Failure Function

- Checks exposure of culture to maximum tolerance temperature over 1, 2, or 3 continuous hours.

Rainfall Failure Function

- Checks exposure of culture to daily rainfall data.

Culture Crash Function

- Checks exposure of culture to invasive species, based on ATP3 data

Crashed ?

Yes

Restart ponds after a period of 24 hours.

No

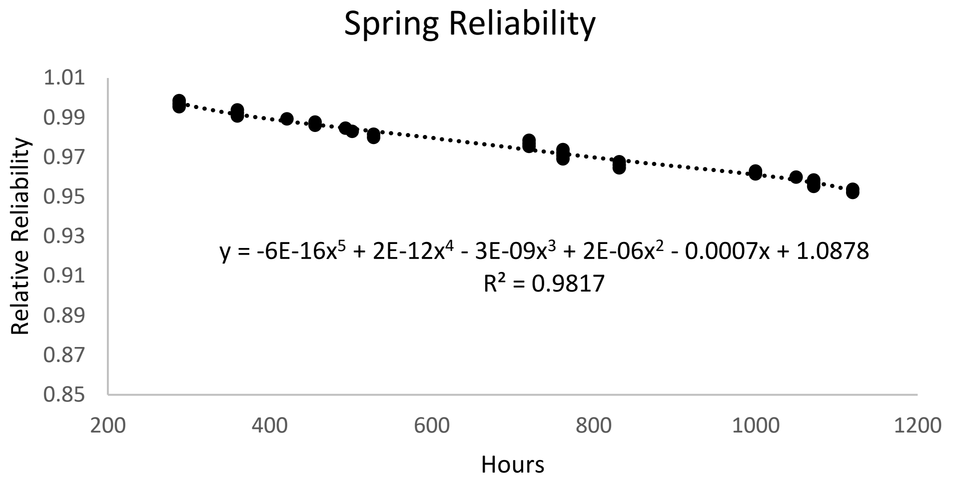
Dynamic Growth Model

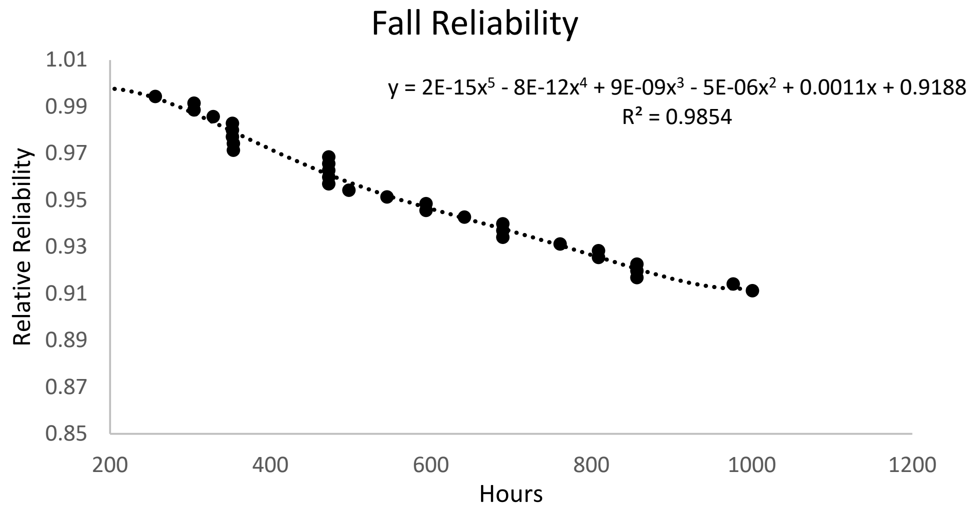
Outputs

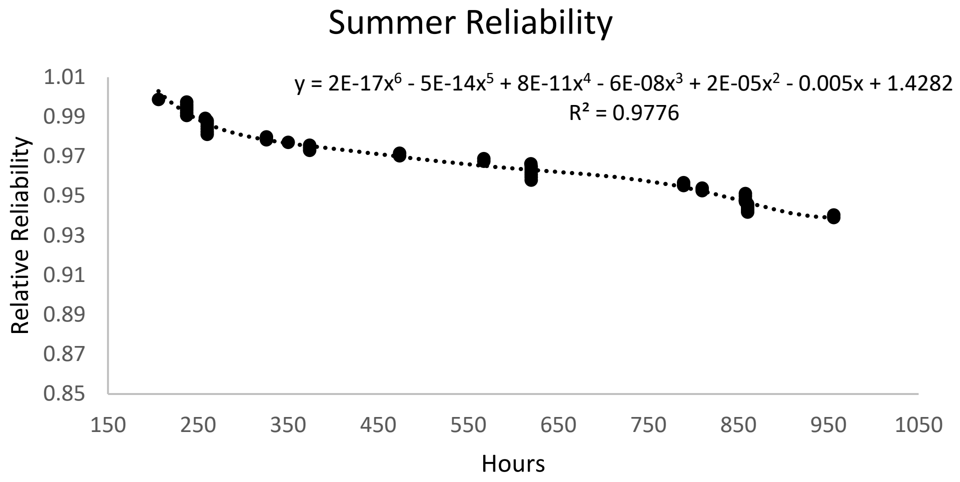
- Areal Productivity
- Operating days
- Number of harvests
- Number of crashes
- Mean Time to Failure

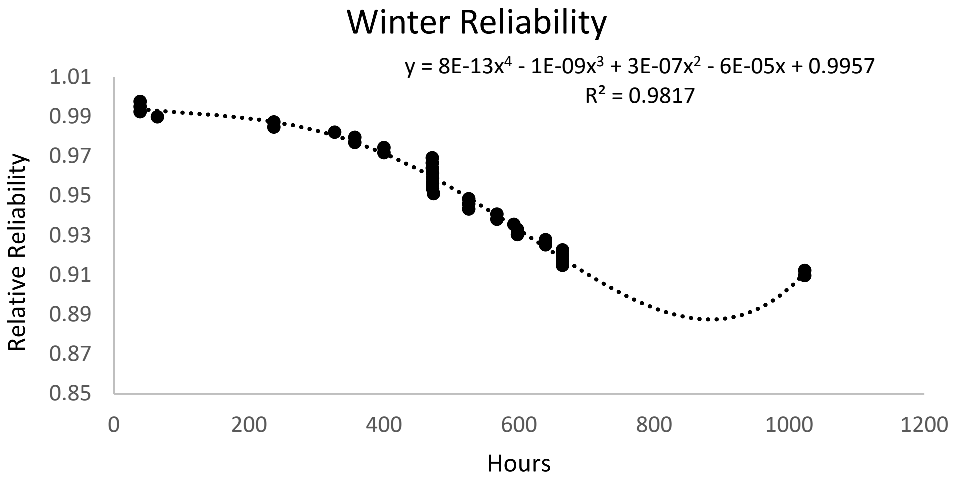
Progress and Outcomes: Task 4
Sustainability assessment - coupled TEA/LCA

Crash Model

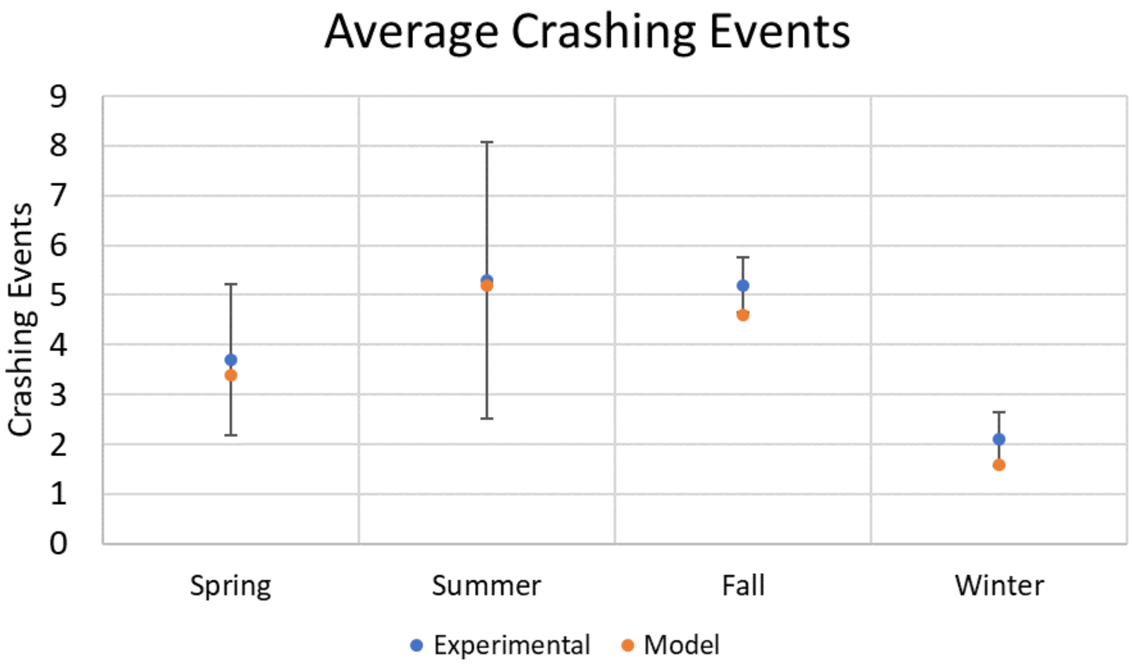


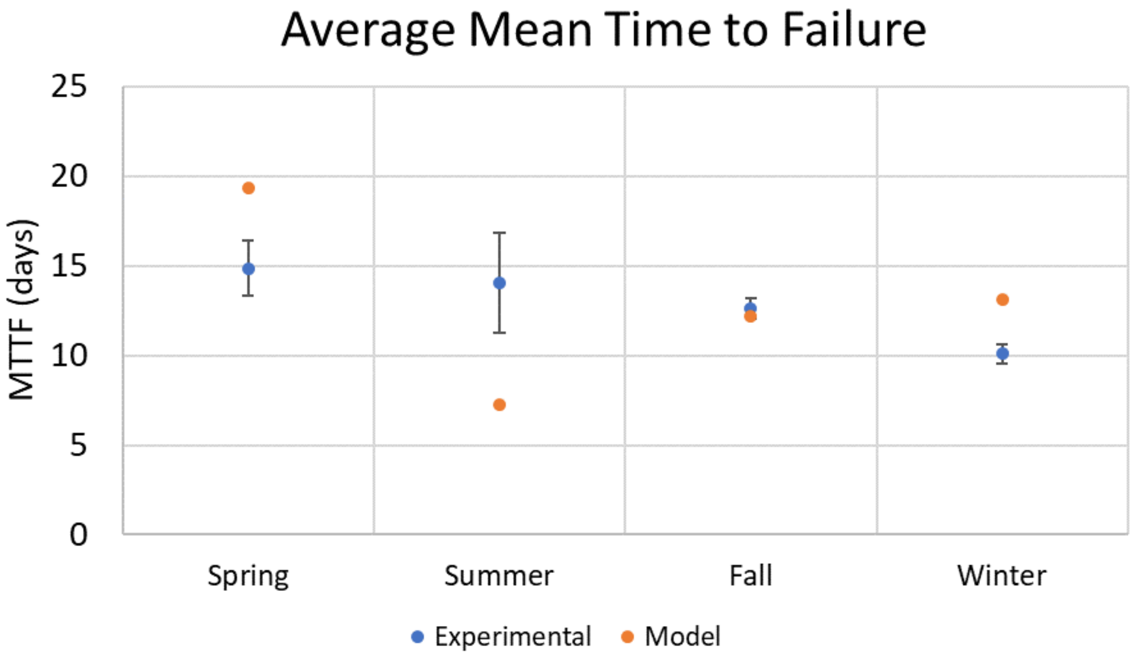






Crash Model Validation





Crash model can be calibrated to any data set, but currently lacks spatial resolution. Validated with ATP3 data, seasonal average of all strains and locations. Mean time to failure is defined as the mean time from inoculation to failure.