DISCOVR

Development of Integrated Screening, Cultivar Optimization, and Verification Research

Michael Huesemann, Scott Edmundson, Song Gao
Pacific Northwest National Laboratory
Taraka Dale, Sangeeta Negi
Los Alamos National Laboratory
Lieve Laurens, Eric Knoshaug, Ryan Davis, Bruno Klein
National Renewable Energy Laboratory
Todd Lane, Jeri Timlin, Tom Reichardt, Chuck Smallwood, Ryan W. Davis
Sandia National Laboratories
Xavier Mayali
Lawrence Livermore National Laboratory
Bradley Wahlen
Idaho National Laboratory
John McGowen
AzCATI, Arizona State University
Matt Posewitz
Colorado School of Mines

Bioenergy Technology Office (BETO) - Peer Review Presentation, Advanced Algal Systems Technology Area (April 4, 2023)

Objective of the DISCOVR Consortium Project (FY19-FY22 3Y Cycle)

Reduce biofuel costs by increasing biomass productivity

Challenge

- A **major driver of algae biofuel costs is productivity**, including culture **resilience** and biochemical **composition**

Project Goals

- **Reduce total microalgae biofuels production costs** by
  - applying an **integrated screening platform** for the **identification of high productivity strains with cellular composition suitable for biofuels and bioproducts for resilient, year-round outdoor cultivation**
  - testing **new concepts** to **increase annual State of Technology (SOT) productivities and reduce the MBSP**, such as
    - improving **biomass productivity (≥20%)**
    - enhancing **biomass intrinsic value** and **value productivity (≥20%)**
    - increasing **crop protection** and **culture stability (≥25% increase** in **mean time between failures)**
  - Use **Techno-Economic Analyses (TEAs) to screen economic feasibility of new concepts** prior to pond trials
  - Evaluate **new strains** and **optimized conditions** in **year-round seasonal SOT trials** at the BETO testbed at AzCATI

Expected Outcomes

- **Increases in annual SOT productivity with concomitant decreases in Minimum Biomass Selling Price (MBSP) to reach BETO 2030 target of $488/T years ahead of schedule**

DISCOVR Project Framework and Roles

Identify & evaluate new high productivity strains & innovative approaches to move "SOT" needle

TAB

Technical Advisory Board

DISCOVR Strain Pipeline

TIER I: Characterize Strains in Flask Cultures (PNNL, LANL, SNL)

TIER II: Quantify Strain Performance in Climate-Simulation PBRs (PNNL, NREL, LANL)

TIER III: Test Strains in Pilot-Scale Indoor and Outdoor Ponds (SNL, PNNL)

TEA

TEA Operational Baseline (NREL)

Testing New Concepts to Move SOT Needle

Improve Biomass Productivity

- Evaluate Growth Promoting Molecules (LANL)
- Reduce Impact of Oxygen Inhibition (PNNL)
- Optimize Pond Operational Conditions (LANL, PNNL)
- Enhance Growth using Plant Substrates (SNL)
- Identify High Productivity *Picochlorum* Enrichments (CSM)

Enhance Biomass Intrinsic Value

- Measure Compositional Shift Dynamics (NREL)

Increase Crop Protection and Culture Stability

- Develop Integrated Pest Management Strategies (SNL)
- Evaluate Companion Cultures (PNNL)
- Implement Spectroradiometric Monitoring (SNL)

Outdoor SOT Trials to Verify Performance of Strains and Optimized Operational Conditions

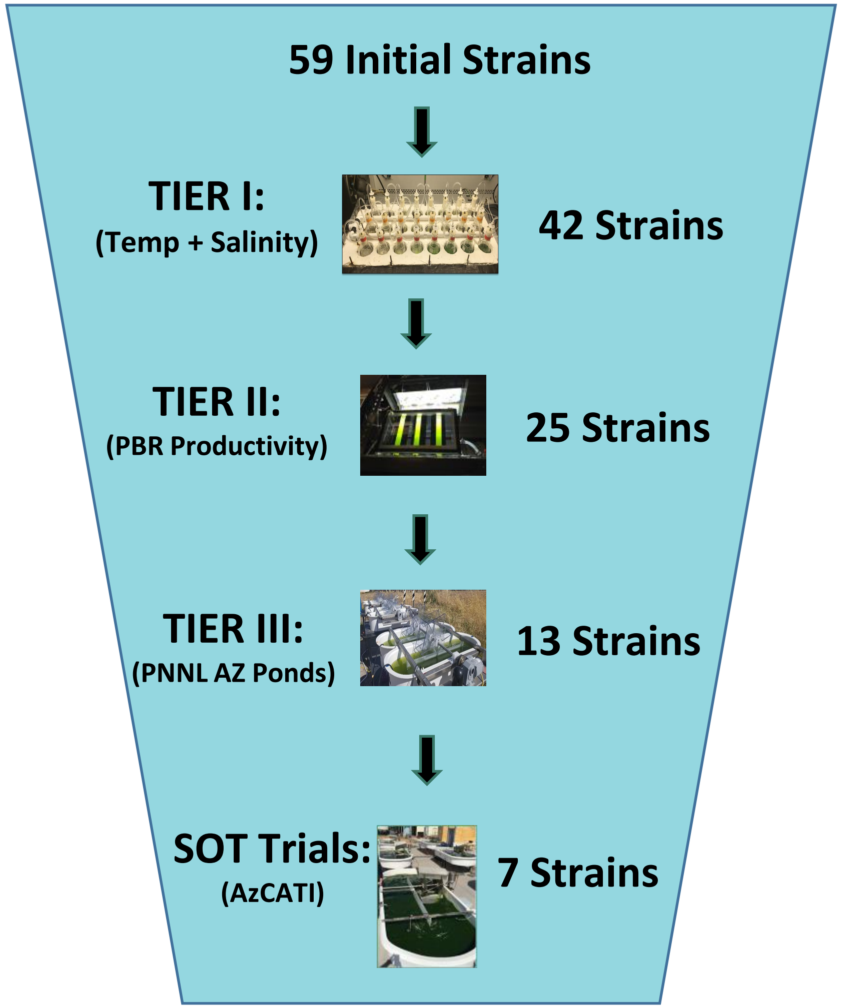
- Outdoor pond SOT Trials (AzCATI)
- Compositional Analysis (NREL)
- SOT Documentation/Data Analytics (NREL)
- Annual TEA SOT assessment (NREL)

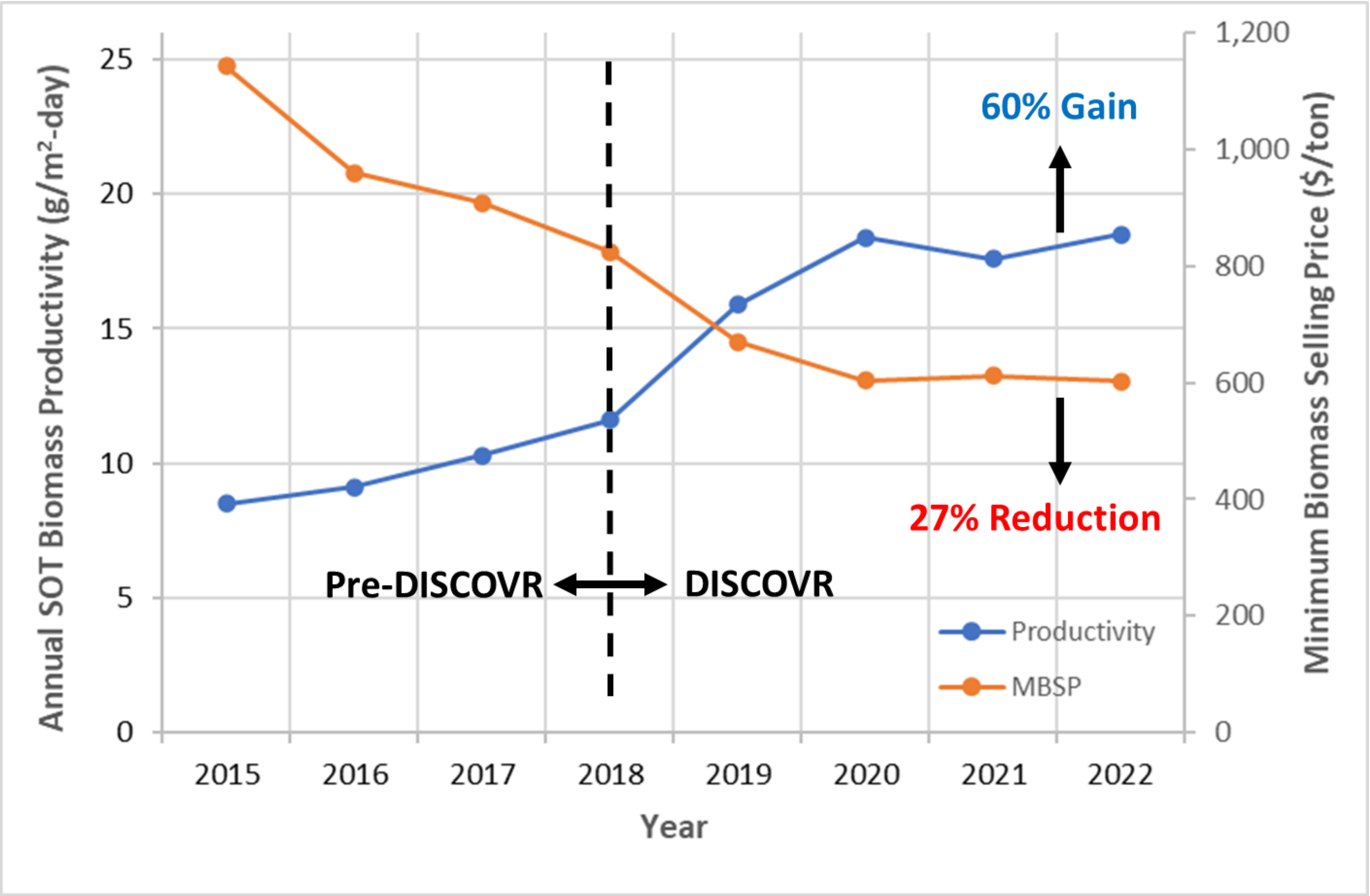
Other Projects in AAS Portfolio or Call for Collaboration

DISCOVR Success Preview: Strain Pipeline & SOT Annual Productivities

Use of top DISCOVR strains resulted in 60% increase in SOT productivity and 27% decrease in MBSP

DISCOVR Strain Pipeline





Relation to BETO Renewable Carbon Resources Portfolio

Data and new strains are delivered to other projects and community

Strains:
- Culture Collections
- Algae Industry/Academia
- Other BETO Projects

Core Capabilities:
- Environmental Simulation
- Strain Characterization
- Biomass Quality Measurements
- Performance Improvement
- Algae:Microbe Interactions
- Pond Crashes & Signatures
- Flask-to-field Expertise
- Testbeds
- Biomass Storage

DISCOVR

Downstream Projects:
- Genome Sequencing
- Multi-Omics Analyses
- Molecular Toolboxes
- Biomass Conversion
- Nutrient Recycling

Data:
- Strain Characterization
- Innovative Improvements
- State of Technology

DISCOVR Management Team and Activities (FY19-FY22 3Y Cycle)

The cohesive DISCOVR team successfully executes tasks within effective management framework

Michael Huesemann (PNNL)

Lieve Laurens (NREL)

Taraka Dale (LANL)

Todd Lane (SNL)

John McGowen (AzCATI)

Matt Posewitz (CSM)

Meetings

- Weekly teleconferences with DISCOVR & SOT teams
- Quarterly planning sessions for seasonal SOT trials
- Quarterly meetings with the Technical Advisory Board
- Annual face to face meetings (pre-COVID)

Quarterly Reports and Tracking of Milestones

- Data flows through the PI
- PI tracks milestones and generates all reports with input from technical leads (TLs)
- Technical leads are responsible for achieving task milestones
- Synthesis of results into publication and solutions tracked and mediated by PI

Decision Making

- Decision making is through consensus of PI and TLs
- PI retains ultimate decision-making authority

DISCOVR Management

Critical success factors and risk mitigation strategies

Critical Success Factors

- **Unique state-of-the-art** technical **capabilities** are employed for execution of DISCOVR tasks.
- **Complementary core competencies** of the consortium labs and SOT testbed are **applied together** to make progress towards BETO's **targets**.
- **Effective communication** and **cohesive decision-making across** DISCOVR team.
- **Strong partnership** with outdoor testbed.
- **Cooperation** with **industrial partners** for enhanced impact.

Risk Mitigation Strategies

Risk	Mitigation Strategy
Unable to identify high productivity strains in first round of screening (PNNL).	Identify additional strains from culture collections, industrial partners, and academia.
Strategies for shifting biomass composition do not result in rapid increased compositional quality due to decrease in biomass productivity (NREL).	Evaluate additional strategies for rapidly shifting the composition in culture and report on response of tested top DISCOVR strains.
Growth promoting molecules (GPMs) are not broadly effective for increasing biomass productivity of DISCOVR/SOT stains (LANL).	Add hormone producing bacteria for GPM delivery as a co-culture strategy for improved biomass productivity of DISCOVR/SOT strains
New crash agents arise rapidly and dominate SOT testbed, decreasing productivity (SNL)	Carry out regular monitoring of pond community, rapid identification of crash agents, countermeasures development and real time pond monitoring.
Novel isolates are now enriched using new selective pressures (CSM).	Pivot to high-productivity isolates from collections or in house strains.

DISCOVR Management: 18 Month Go/No-Go and Year 3 Milestones

All DISCOVR Go/No-Go and Year 3 milestones were successfully met or exceeded

Go/No-Go Milestones (18 Month):

- Show that for at least 2 out of the 5 DISCOVR new concepts being tested, as well as new strains going through the pipeline, the following improvements under laboratory conditions are achieved:
  - **20% increase in productivity** (g/L/day or g/m^2/day) relative to control cultures or benchmark strains (***GNG-1***)
  - **20% increase in value productivity** (\$/m^2/day) (i.e., composition-based intrinsic value (\$/g) times biomass productivity (g/m^2/day)) for the top DISCOVR strains (***GNG-2***)
  - or **25% increase in mean time between failure** (MTBF) for crop protection strategies compared to standard crash test (***GNG-3***)
- **Correctly identify crash agents** through anomaly detection tool Pond Sentry for SOT cultivation runs and/or spectroradiometric monitoring **with mean early warning of 72 hrs or more before a deleterious event for 75% of cultivation runs** (***GNG-4***).

End of 3 Year Cycle Joint Milestone:

- Employ **new strains** and **novel concepts** that were successfully demonstrated in the laboratory to **achieve at least 10% year over year (YoY) annual productivity improvement (g/m^2/day) at SOT testbed**, relative to the pre-DISCOVR 2018 annual SOT productivity of 11.7 g/m^2/day, and normalized for climate anomalies (PNNL, LANL, NREL, SNL, AzCATI). **Outcome: 2022 annual SOT productivity was 18.5 g/m^2/day or 12.5% YoY improvement.**

DISCOVR Management: Technical Advisory Board

Meetings restructured to address primary risk factors: Focus on single topic

- DISCOVR core team **discusses immediate highest impact topic** to TAB using WebEx on quarterly basis with BETO staff in attendance.
- Presentations are designed to **spark discussion and elicit dialogue** on DISCOVR **critical path elements** and help prioritize research or identify research gaps
  - **Data management**
  - **Crop Protection**
  - **Harvesting Operations**
- Include **10 guiding questions,** open discussion on levers and strategies to test and implement
- TAB recommendations and responses from the DISCOVR team how to specifically follow TAB guidance are included in each quarterly report.

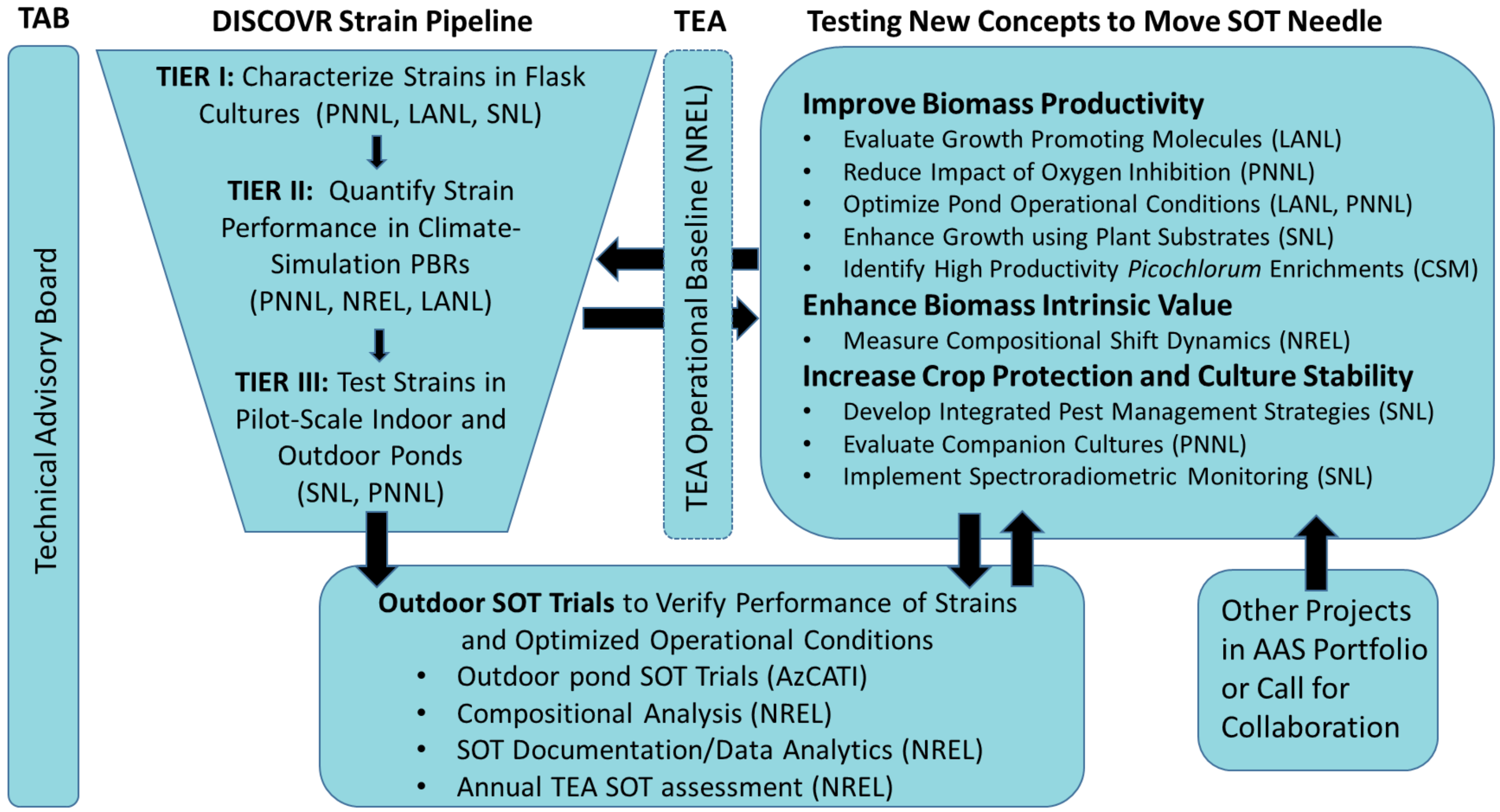
- **2019-2021 TAB members**
  - Rebecca White, Qualitas Health*
  - Lou Brown, Synthetic Genomics
  - John Benemann, MicroBio Engineering
  - Valerie Harmon, Harmon Consulting
  - Juergen Polle, Brooklyn College
  - Craig Behnke, Lumen Biosciences
  - Philip Pienkos, Polaris Renewables*
  - Matt Posewitz, CSM*

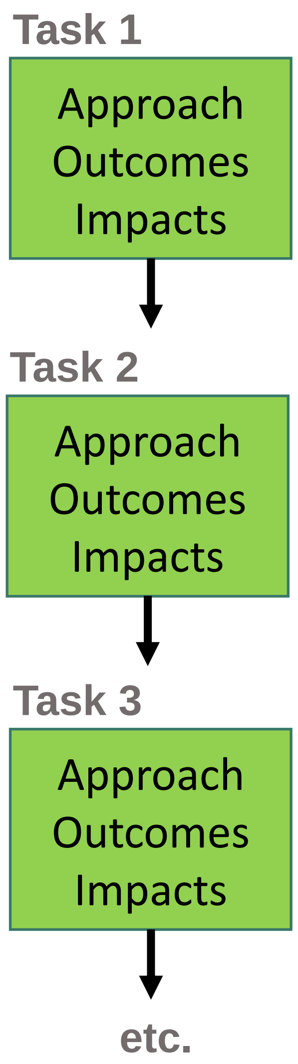
- **2022 TAB composition changed to reflect emphasis on scaling technologies**
  - Beth Zotter, Umaro Foods
  - Michael Kanyi, Imperial Valley College
  - Joseph Weissman, ExxonMobil
  - Jakob Nalley, iwiLife

* Retired TAB members

Organization of Presentation on Task Approaches, Outcomes, Impacts

For each DISCOVR task, the respective approach, outcomes, and impacts are presented as a unit





- **Note:** To facilitate the reviewer rating of the different sections (management/overview, approach, outcomes, and impacts), all slides are also organized by section, rather than by task, in a separate PDF file entitled: "**1.3.1.501-DISCOVR-Slides-Organized-by-Sections-for-Scoring.pdf**".

DISCOVR Pacific Northwest NATIONAL LABORATORY Los Alamos NATIONAL LABORATORY EST.1943 NREL Transforming ENERGY Sandia National Laboratories INL Idaho National Laboratory AzCATI Arizona Center for Algae Technology and Innovation COLORADO SCHOOL OF MINES

DISCOVR Strain Pipeline Tasks

Approach – Progress & Outcomes - Impacts

TAB

Technical Advisory Board

DISCOVR Strain Pipeline

TIER I: Characterize Strains in Flask Cultures (PNNL, LANL, SNL)

TIER II: Quantify Strain Performance in Climate-Simulation PBRs (PNNL, NREL, LANL)

TIER III: Test Strains in Pilot-Scale Indoor and Outdoor Ponds (SNL, PNNL)

TEA

TEA Operational Baseline (NREL)

Testing New Concepts to Move SOT Needle

Improve Biomass Productivity

- Evaluate Growth Promoting Molecules (LANL)
- Reduce Impact of Oxygen Inhibition (PNNL)
- Optimize Pond Operational Conditions (LANL, PNNL)
- Enhance Growth using Plant Substrates (SNL)
- Identify High Productivity *Picochlorum* Enrichments (CSM)

Enhance Biomass Intrinsic Value

- Measure Compositional Shift Dynamics (NREL)

Increase Crop Protection and Culture Stability

- Develop Integrated Pest Management Strategies (SNL)
- Evaluate Companion Cultures (PNNL)
- Implement Spectroradiometric Monitoring (SNL)

Outdoor SOT Trials to Verify Performance of Strains and Optimized Operational Conditions

- Outdoor pond SOT Trials (AzCATI)
- Compositional Analysis (NREL)
- SOT Documentation/Data Analytics (NREL)
- Annual TEA SOT assessment (NREL)

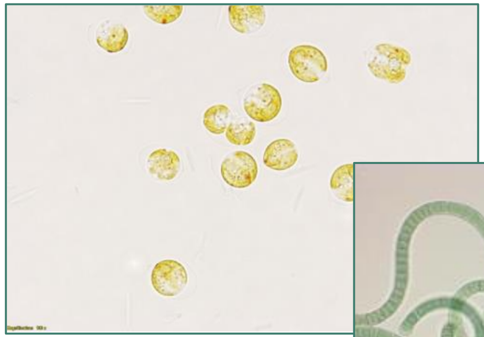
Other Projects in AAS Portfolio or Call for Collaboration

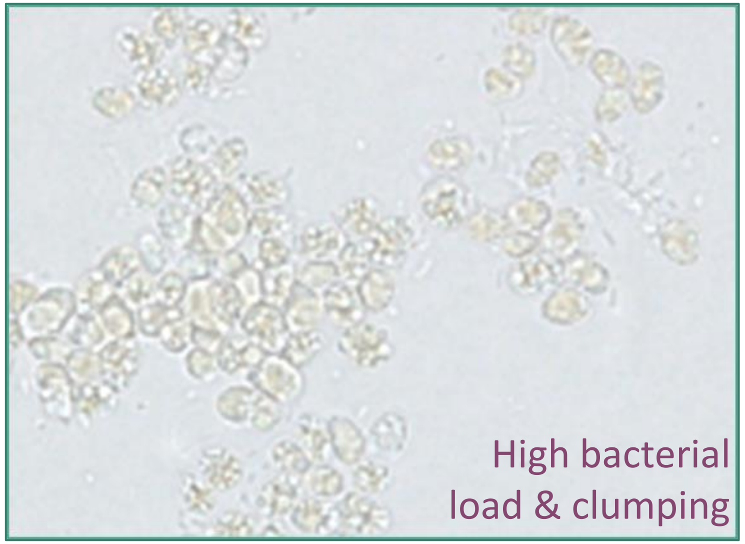
Approach: Strain Identification and Confirmation

Pipeline

Ensuring a known starting point for DISCOVR strains

- **Background/history:** Ongoing effort as part of Tier I Characterization
  - *Algae cultivation expertise used to revive cultures and adapt to DISCOVR media*
  - *Initial flask characterization provides insight into strain ease of handling*
  - *16S(bacterial) and 18S(algae) data informs DISCOVR team on bacterial load and strain identity*
- **Objective:** Revive strains, evaluate bacterial load, confirm strain identity, adapt to DISCOVR media and perform initial characterization, and deliver to PNNL
- **Challenges:**
  1. Strains do not always revive well or grow in DISCOVR media
  2. Some strains have high bacterial load or are a different strain than listed by the culture collection
- **Economic/Technical Metrics:** 16S/18S sequencing of >40 DISCOVR strains.





Outcomes: Strain Identification and Confirmation

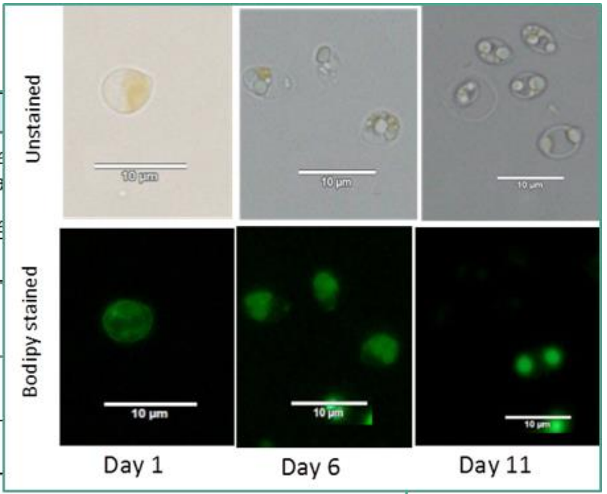
Revived/evaluated 28 strains, sequence checked 41, and delivered 22 to PNNL for screening

Over the course of the project:

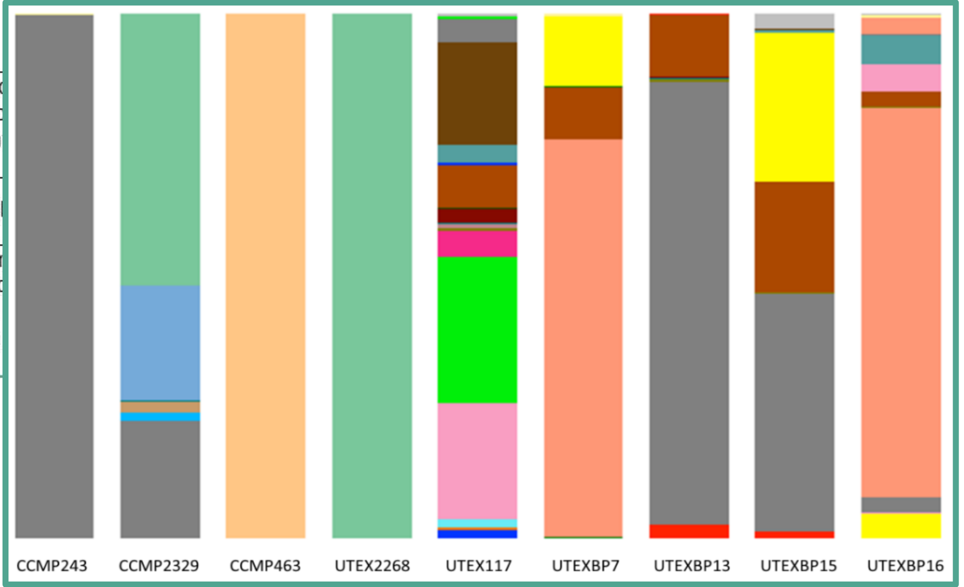
- Identify, order, and revive (**n = 28**)
- Initial growth curves & morphology
- Adapt to media (**26**)
- 16S and 18S sequencing (**41**)
- Clean-up cultures as needed
- Deliver to PNNL (**22**)

Example of a strain tracking sheet

Culture Collection Name	UTEX BP13
Proposed species	*Chlorella sorokiniana* DOE1044 (a green a
Species Identification by 18S	*Chlorella sorokiniana*
Sent to PNNL	Yes
PNNL Screening Status	In progress
Tier I	Yes
Tier II	TBD
Tier III-V	TBD
Media	Grows well in BG11 DISCOVR media
Microscopy	Complete
Growth curves in CO2 chamber	Complete
DNA Isolation	Complete
16S Analysis	9365 sequence counts (1236 were bacterial of the bacterial fraction was from a single bac chloroplast fraction of the counts is consisten identifcation.
18S Analysis	18S is consistent with the culture collection s *C. sorokiniana*.
Basic N depletion and BODIPY staining for flow cytometry	Clear carbon storage upon N depletion, amen cytometry. Lipid bodies interestingly polarized depletion and distribution of staining is broad depletion (11d). Early depletion (6d) shows a straightforward shift in BODIPY stain.



16S data showing that some cultures were free of bacteria (solid bar) and some had a variety and heavy fraction of bacteria in the culture (many colors in one bar)



DISCOVR | Pacific Northwest National Laboratory | Los Alamos National Laboratory EST. 1943 | NREL Transforming ENERGY | Sandia National Laboratories | INL Idaho National Laboratory | AzCATI Arizona Center for Algae Technology and Innovation | Colorado School of Mines

Impact: Strain Identification and Confirmation

Pipeline

Reducing risk associated with using culture collection strains in DISCOVR

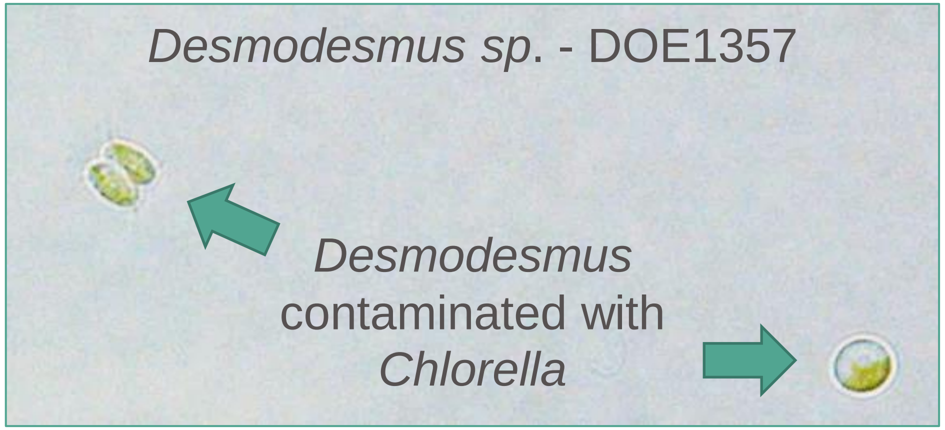
Culture collection strains may have limited characterization or identification data available

- Strains sometimes classified in culture collection by morphology, rather than sequence data
- Bacterial ID & load usually lacking
- Growth on DISCOVR media unknown

Impact

Early strain characterization reduces risk by:

- **Eliminating non-unialgal cultures**
- **Providing information on bacterial load and identity**
- **Establishing growth in DISCOVR media before intensive characterization begins**
- **Important info for future users of strains**



Publication

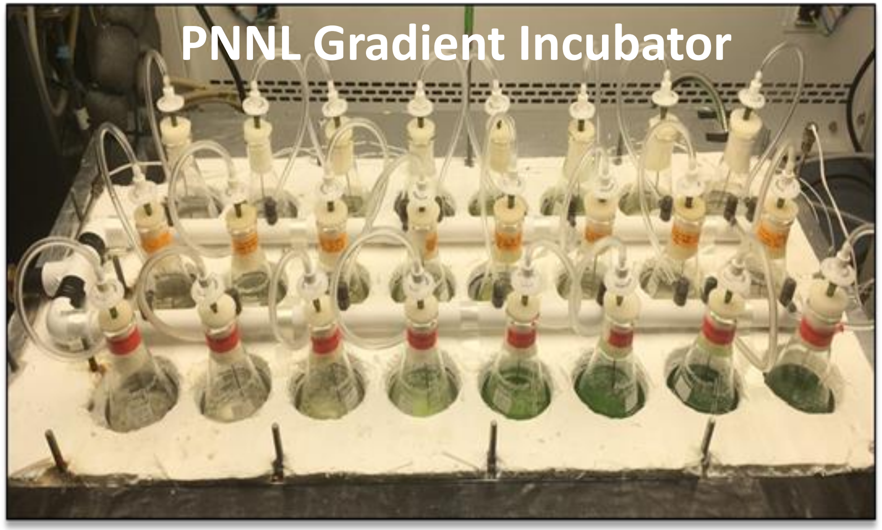
- Huesemann, Edmundson, Gao, Negi, Dale, et al. (2023), "DISCOVR Strain Pipeline Tier I Screening: Maximum Specific Growth Rate as a Function of Temperature and Salinity for 38 Candidate Microalgae Strains for Biofuels Production", *Algal Research* – Special Issue DISCOVR, https://doi.org/10.1016/j.algal.2023.102996

DISCOVR | Pacific Northwest NATIONAL LABORATORY | Los Alamos NATIONAL LABORATORY EST. 1943 | NREL Transforming ENERGY | Sandia National Laboratories | INL Idaho National Laboratory | AzCATI Arizona Center for Algae Technology and Innovation | COLORADO SCHOOL OF MINES

Approach: TIER I Strain Characterization

Temperature and salinity tolerance of each strain is measured in gradient incubators

- **Background/history:** Strain characterization using temperature and salinity gradient incubators
  - *In 2011, as part of the NAABB consortium project, PNNL designed and build a thermal gradient incubator to measure maximum specific growth rates as a function of temperature, for input into the PNNL growth model*



- **Challenges:**
  - Identify the **optimum** medium **salinity** and suitable **growing season** for all candidate DISCOVR strains.
  - Quantify **maximum specific growth rate data** for down-selection to LEAPS (Laboratory Environmental Algae Pond Simulator) testing.

- **Experimental Conditions:**
  - Screening strains in **Salinity Gradient Incubator** at 5, 15, and 35 ppt salinity (25 °C)
  - Testing strains in **Thermal Gradient Incubator** at saturating light intensity from 4 to 45 °C.

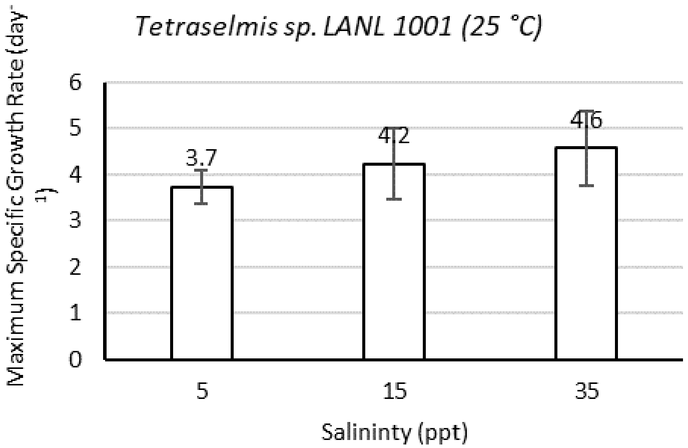
- **Economic/Technical Metrics:** Identify optimum medium salinity and suitable growing season. Down-select strains with highest max specific growth rates for subsequent (Tier II) testing in LEAPS.

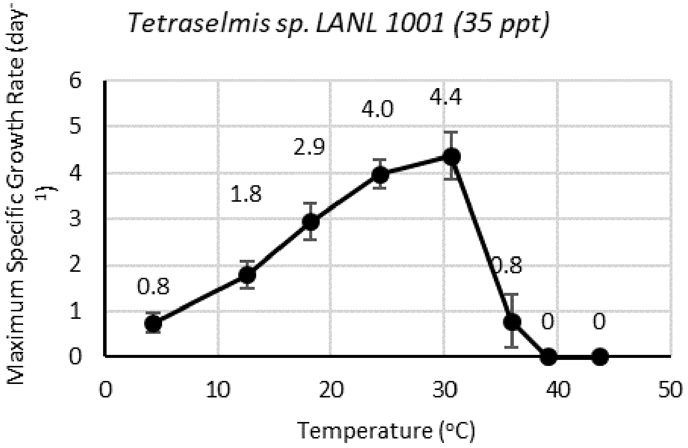
Outcomes: TIER I Strain Characterization

Each strain (42 tested so far) has a unique temperature and salinity tolerance range

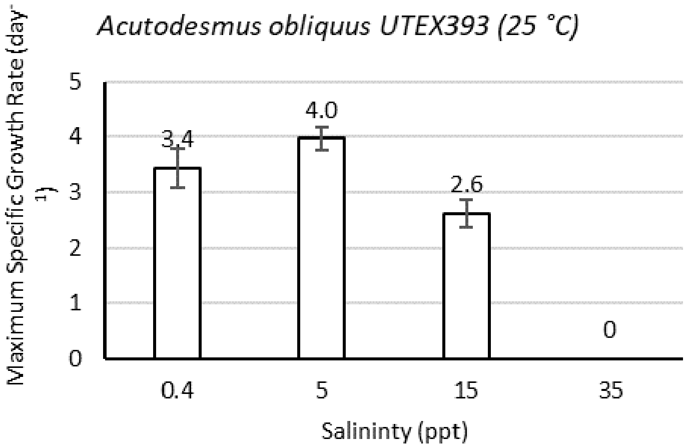
Pipeline

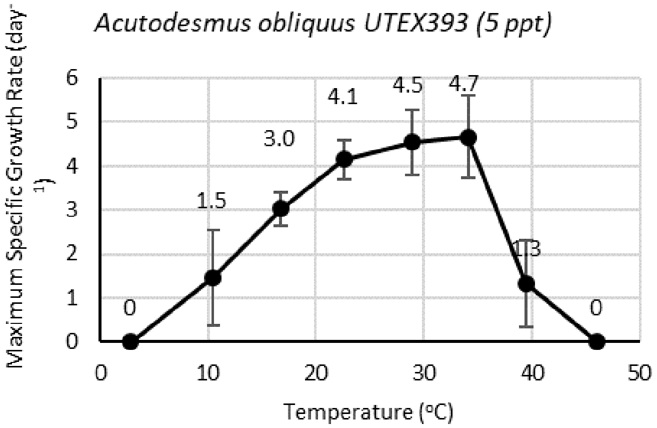
Example #1: Cold Season Strain (Marine)

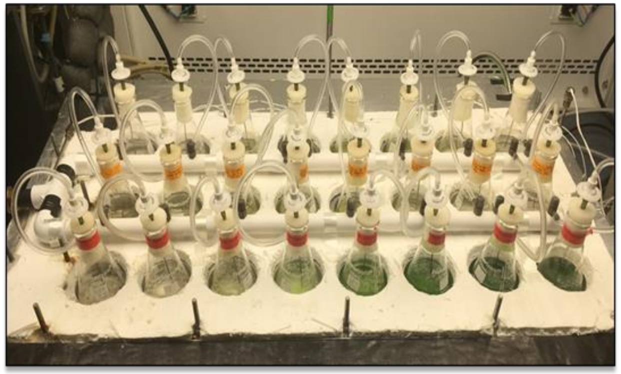




Example #2: Warm Season Strain (Brackish)

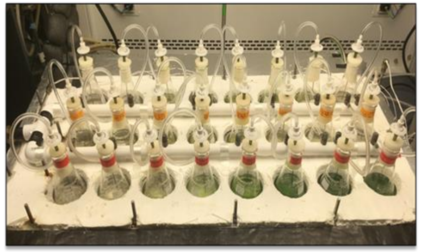






Impact: TIER I Strain Characterization

Identify optimum salinity and suitable growing season, down-select for subsequent LEAPS testing



42 Tier I strains, including three industrial strains, were cultured in PNNL gradient incubators to determine their respective maximum specific growth rate as a function of:
- Temperature
- Salinity

Impact

MicroBio Engineering

ExxonMobil

ALGENOL BIOFUELS

TIER I strain characterization resulted in:
- Finding the optimum medium salinity
- Identifying the most suitable growing season
- Down-selecting 25 strains for subsequent TIER II testing in LEAPS PBRs

3 Publications
- Krishan et al. (2021), "*Picochlorum celeri* as a model system for robust outdoor algal growth in seawater", *Scientific Reports*, **11** (No: 11649), https://doi.org/10.1038/s41598-021-91106-5
- Huesemann et al. (2023), "DISCOVR Strain Pipeline Tier I Screening: Maximum Specific Growth Rate as a Function of Temperature and Salinity for 38 Candidate Microalgae Strains for Biofuels Production", *Algal Research* – Special Issue DISCOVR, https://doi.org/10.1016/j.algal.2023.102996
- Beirne, Edmundson, Gao, Freeman, Huesemann et al., (2023), "A Streamlined Approach to Characterize Microalgae Strains for Biomass Productivity under Dynamic Climate Simulation, *Algal Research* – Special Issue DISCOVR, under review.

Approach: TIER II Strain Culturing in LEAPS PBRs

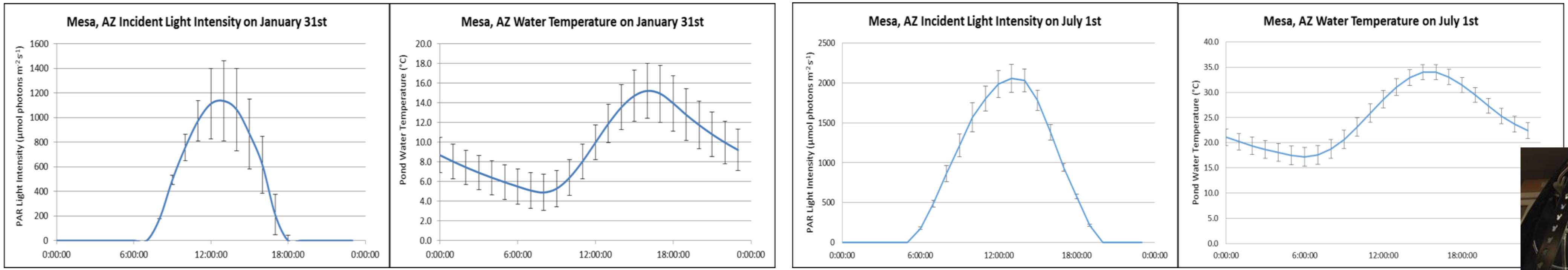
Pipeline

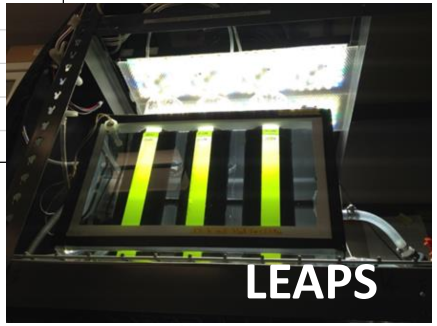
Use unique pond simulator PBR to measure AZ winter and summer season productivity (25 strains)

- **Background/history:** PNNL **Laboratory Environmental Algal Pond Simulator (LEAPS)** PBR
  - *In 2015, PNNL designed the LEAPS photobioreactors which **accurately simulate growth in outdoor ponds** (Huesemann et al., 2017).*
  - *The LEAPS allows **objective comparison** of growth/composition of strains using similar light/temp scripts*

- **Challenge:** Quantify Arizona (AzCATI) winter and summer season biomass productivity **under identical climate-simulated culture conditions** and **identify best** (highest productivity) **strains**.

- **AZ Winter and Summer Season Light and Temperature Scripts used in the LEAPS:**





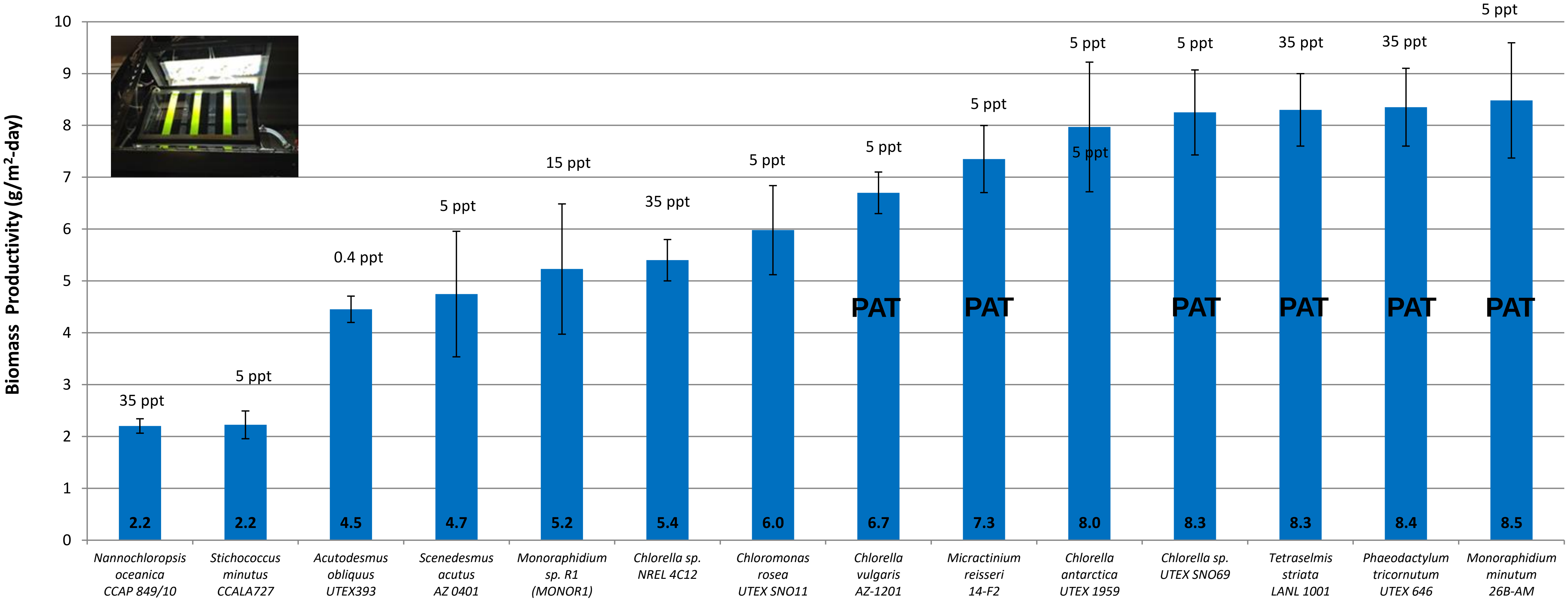
- **Economic/Technical Metrics:** Identify highest productivity winter and summer season DISCOVR strains under identical culture and script conditions.

DISCOVR Pacific Northwest NATIONAL LABORATORY Los Alamos NATIONAL LABORATORY EST. 1943 NREL Transforming ENERGY Sandia National Laboratories INL Idaho National Laboratory AzCATI Arizona Center for Algae Technology and Innovation COLORADO SCHOOL OF MINES

Outcomes: TIER II Winter Strain Culturing in LEAPS PBRs

Pipeline

Winter strains with the highest productivity were tested at the PNNL Algae Testbed (PAT) in AZ



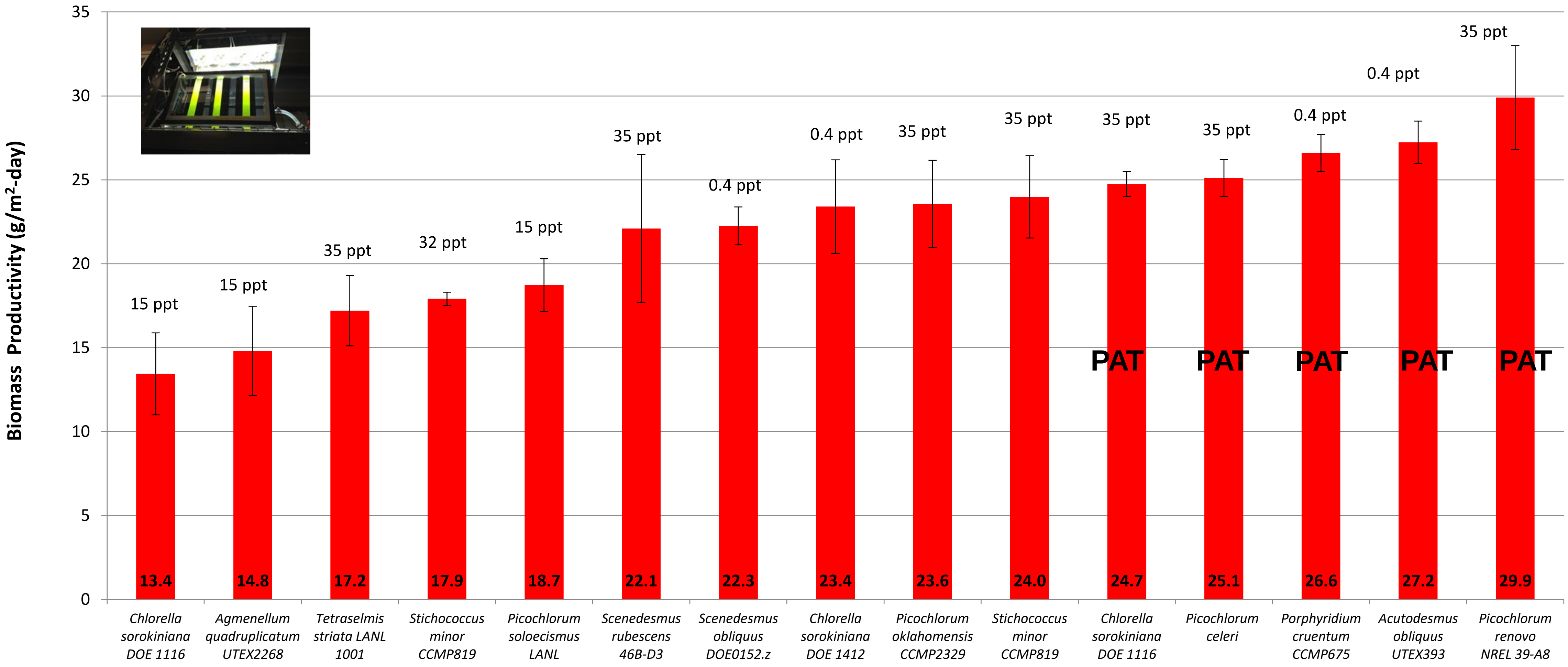
Salinity in parts per thousand (ppt). Error bars are one stdev (n=4).

PAT = Tested at the PNNL Algae Testbed

Outcomes: TIER II Summer Strain Culturing in LEAPS PBRs

Pipeline

Summer strains with the highest productivity were tested at the PNNL Algae Testbed (PAT) in AZ



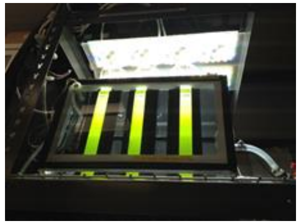
Error bars are one stdev (n=4, with the exception of *Picochlorum renovo*, n=20).

PAT = Tested at the PNNL Algae Testbed

Impact: TIER II Strain Culturing in LEAPS PBRs

Pipeline

Down-select high productivity strains for subsequent testing at the PNNL Algae Testbed in AZ



Top Tier I strains are cultured in LEAPS using identical AZ winter and summer season temperature scripts to comparatively determine:

- Areal biomass productivity
- Biomass compositional shifts upon N-depletion

ExxonMobil

Impact

The highest productivity TIER II strains, including *P. celeri* from ExxonMobil, were down-selected for subsequent testing in AZ outdoor ponds at the PNNL Algae Testbed.

- **2 publications:**
  - Huesemann, Edmundson, Gao, Laurens, et al. (2023), “DISCOVR Strain Pipeline Tier II Screening: Winter and Summer Season Areal Productivities and Biomass Compositional Shifts in Climate-Simulation Photobioreactor Cultures”, *Algal Research* – Special Issue DISCOVR, **102948**, https://doi.org/10.1016/j.algal.2022.102948
  - Beirne, Edmundson, Gao, Freeman, Huesemann et al., (2023), “A Streamlined Approach to Characterize Microalgae Strains for Biomass Productivity under Dynamic Climate Simulation, *Algal Research* – Special Issue DISCOVR, under review.

DISCOVR Pacific Northwest NATIONAL LABORATORY Los Alamos NATIONAL LABORATORY EST.1943 NREL Transforming ENERGY Sandia National Laboratories INL Idaho National Laboratory AzCATI Arizona Center for Algae Technology and Innovation COLORADO SCHOOL OF MINES

Approach: TIER III Strain Outdoor Pond Culturing

Pipeline

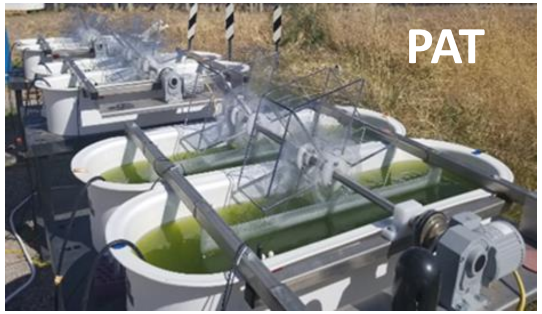
Top strains are tested at the PNNL Algae Tested (PAT) in Arizona

- **Background/history:** Evaluation of new strains in open outdoor ponds in Arizona
  - *In 2012, PNNL established an outdoor testbed in Arizona consisting of 100 L and 800 L raceway ponds*
  - *The growth performance and stability of strains were evaluated in different BETO funded projects, i.e., the NAABB and RAFT consortium projects, and the AlgaeAirFix Incubator project.*

- **Objectives:** Quantify areal biomass productivity and determine culture stability of TIER III DISCOVR strains, evaluate harvestability via centrifuge, and ship biomass to NREL for compositional analyses

- **Challenges:**
  1. Compared to LEAPS cultures, outdoor pond cultures are exposed to more stressors:
  - *More extreme light and temperature fluctuations (including freezing) due to constant weather changes*
  - *Presence of predators (e.g., rotifers), infectious agents (e.g., chytrids) and competitors (e.g., diatoms).*

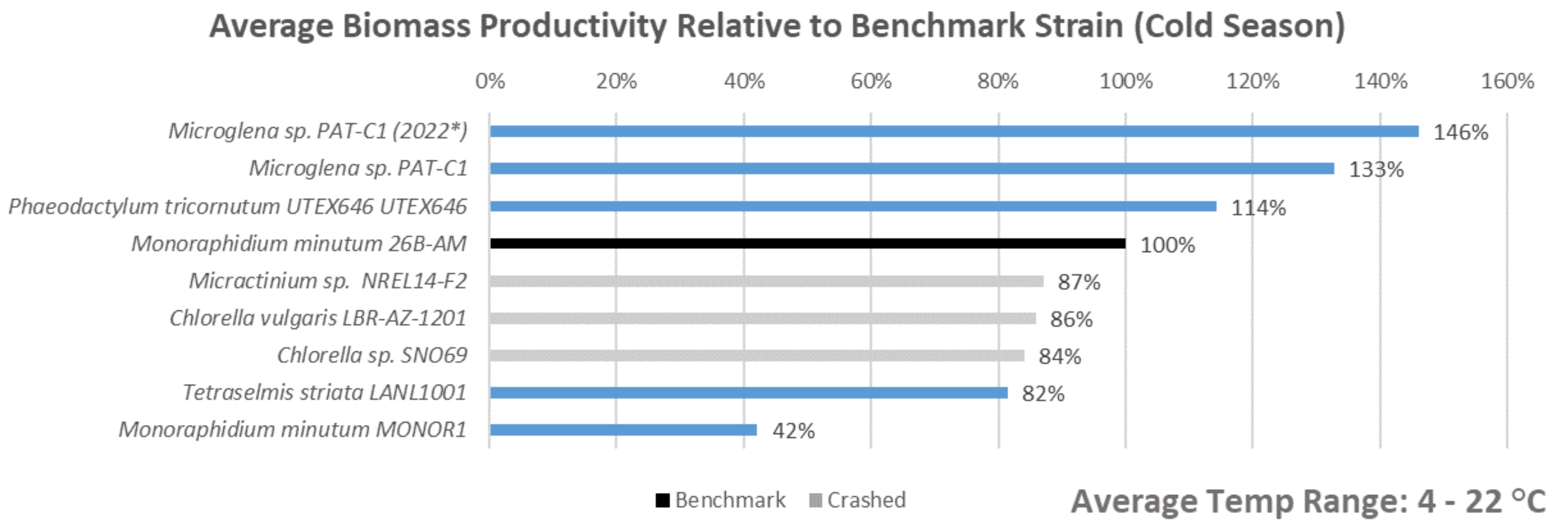
- **Economic/Technical Metrics:** Down-select highest productivity most stable strains, relative to benchmark strains, for subsequent evaluation at SOT testbed at AzCATI.



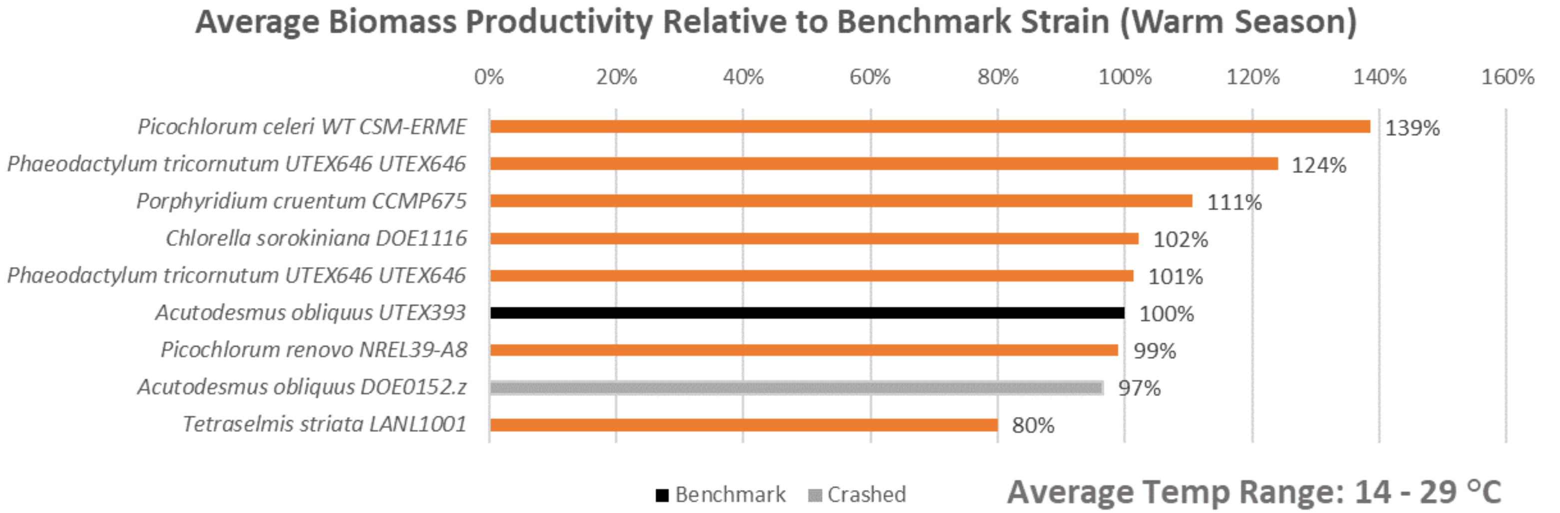
DISCOVR Pacific Northwest NATIONAL LABORATORY Los Alamos NATIONAL LABORATORY EST. 1943 NREL Transforming ENERGY Sandia National Laboratories INL Idaho National Laboratory AzCATI Arizona Center for Algae Technology and Innovation COLORADO SCHOOL OF MINES

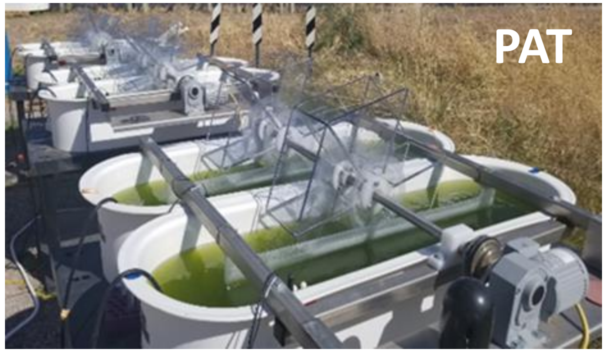
Outcomes: TIER III Strain Outdoor Pond Culturing

Top 13 strains were tested in 42 duplicate pond runs at the PNNL Algae Tested (PAT) in Arizona



* *Tetraselmis striata* became new benchmark in 2022





Strains that had stable growth performance (no crashes) and greater or comparable biomass productivity relative to benchmarks "graduated" for subsequent testing at the BETO SOT testbed at AzCATI.

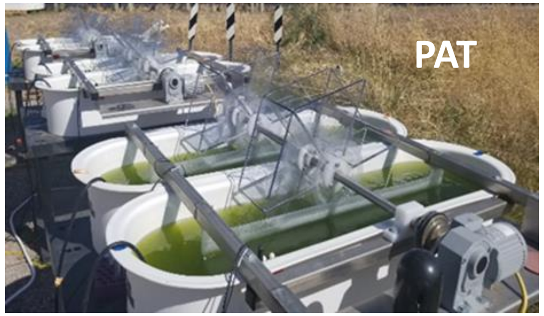
DISCOVR · Pacific Northwest National Laboratory · Los Alamos National Laboratory EST.1943 · NREL Transforming ENERGY · Sandia National Laboratories · INL Idaho National Laboratory · AzCATI Arizona Center for Algae Technology and Innovation · Colorado School of Mines

Impact: TIER III Strain Outdoor Pond Culturing

Down-select stable and high productivity strains for subsequent testing in SOT trials at AzCATI

Strains with high biomass productivity in the LEAPS PBRs need to be tested under realistic outdoor pond culture conditions to determine their respective

- Areal biomass productivity relative to benchmark
- Stability for sustainable biomass production
- Harvestability
- Biomass composition



Impact

Seven DISCOVR strains, including the ExxonMobil strain *P. celeri*, "graduated" at the PAT for subsequent testing in SOT trials at AzCATI (see slide 53 for strain names**).**

The GNG-1 Milestone "Improve Biomass Productivity by 20% Relative to Control" was met for 4 strains tested at the PAT.

Publication

Gao, Edmundson, Huesemann, et al. (2023), DISCOVR Strain Pipeline Tier I Screening: Strain Evaluation in Outdoor Raceway Ponds", *Algal Research* – Special Issue DISCOVR, https://doi.org/10.1016/j.algal.2023.102990

Approach: Isolate Novel *Picochlorum* Enrichments/Strains

Pipeline

Enrich rapidly growing Picochlorum strains/consortia to test relative to current benchmarks

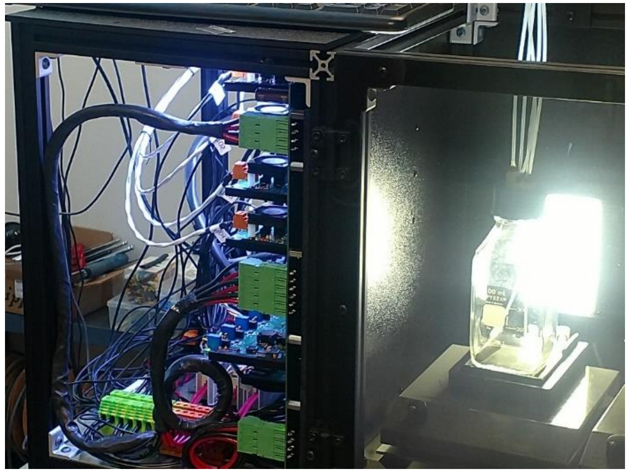
- **Background/history:** To date, the majority of outdoor *Picochlorum* studies have focused on *Picochlorum celeri* TG2 - one of the few *Picochlorum* isolates tested outside. Due to the limited strain sampling to date, additional *Picochlorum* isolates and consortia were pursued to test whether higher productivity strains/consortia could be attained relative to the current benchmark.

- **Objective:** 1) Enrich *Picochlorum* consortia in bioreactors using pond mimicking summer light/temperature scripts. Test whether Gulf of Mexico consortium diversity is a productivity/stability asset relative to monoclonal *Picochlorum celeri* TG2. 2) If necessary, isolate and test single clones from consortia to probe whether "heavy lifter" and "cheater" cultivars may exist within enrichments from natural waters and isolate best strains.

- **Challenges:**
  1. Extensive biodiversity can be attained from natural isolates/enrichments that needs to be managed. Timely down-selection to attain promising strains.
  2. Enrichments often generate biofilms that hinder planktonic strain enrichment and rapid dilution regimes are necessary.

- **Economic/Technical Metrics:** Technical metrics focused on productivities (g/m^2/d).

Sampling off of oil platforms in the Gulf of Mexico near the Mississippi River



Bioreactors for strain enrichment with selective pressures

DISCOVR — Pacific Northwest National Laboratory — Los Alamos National Laboratory EST. 1943 — NREL Transforming ENERGY — Sandia National Laboratories — INL Idaho National Laboratory — AzCATI Arizona Center for Algae Technology and Innovation — Colorado School of Mines

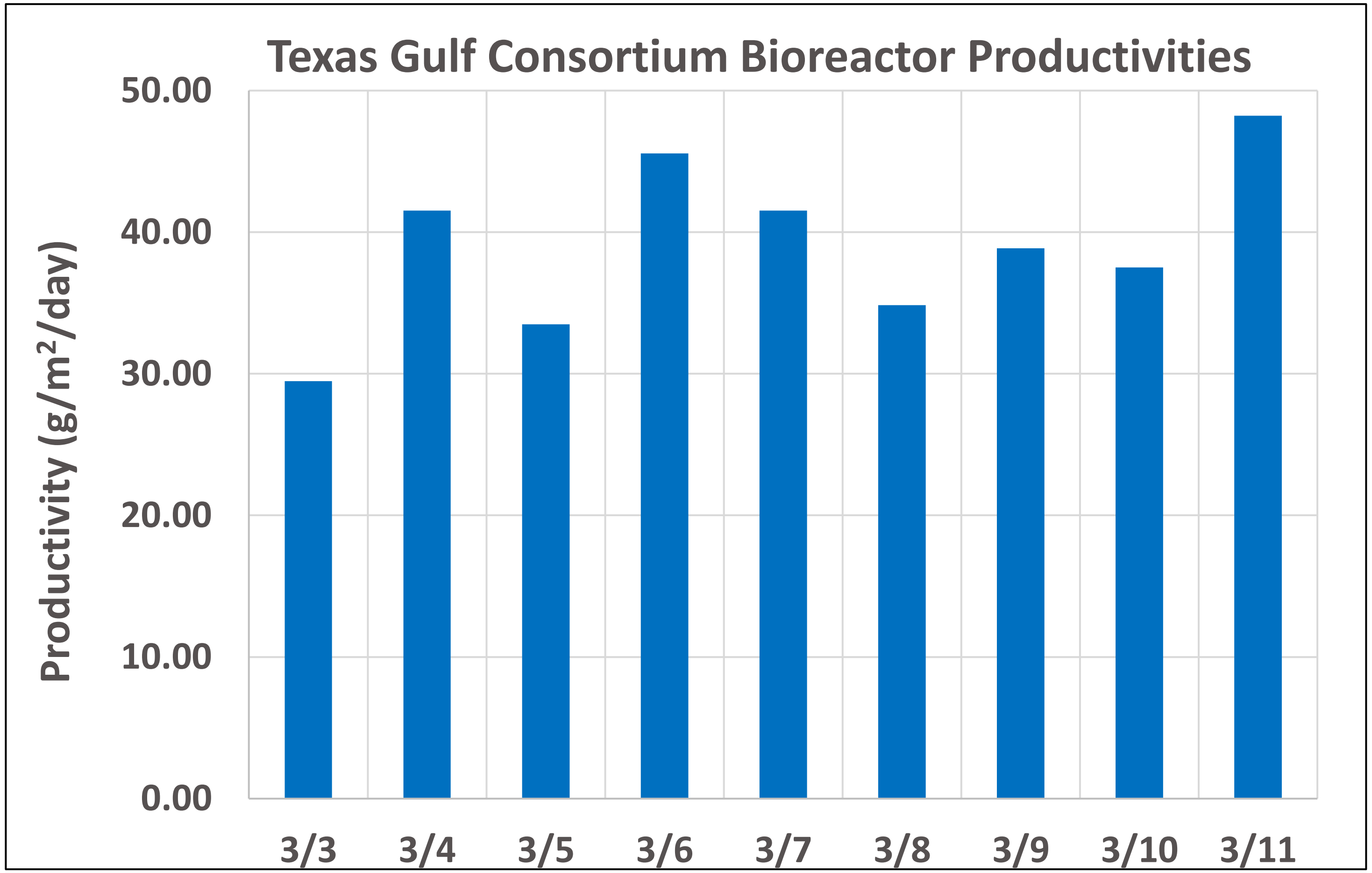
Outcomes: Isolate Novel *Picochlorum* Enrichments/Strains

Pipeline

High-productivity enrichments attained from Gulf of Mexico sampling

Gulf of Mexico *Picochlorum* Consortia

- Enriched *Picochlorum* consortia from Texas coast and Louisiana coast
- High bioreactor productivities attained from both consortia (~40 g/m^2/d) for Texas Gulf, which was slightly higher relative to Louisiana Gulf (~36 g/m^2/d)
- TG2 productivities are ~40 g/m^2/d using same bioreactor script



Daily Productivities of Texas Coast Picochlorum Consortium

DISCOVR

Pacific Northwest National Laboratory · Los Alamos National Laboratory · NREL Transforming ENERGY · Sandia National Laboratories · Idaho National Laboratory · AzCATI Arizona Center for Algae Technology and Innovation · Colorado School of Mines

Impact: Isolate novel *Picochlorum* enrichments/strains

Pipeline

Additional high-productivity Picochlorum enrichments were attained

- Picochlorum enrichments from both Texas and Louisiana coasts were attained using selective pressures
- Exemplary bioreactor productivities attained
- Outdoor July 2022 productivities ~29 g/m^2/d wrt ~24 g/m^2/d for TG2 – more testing necessary over other timeframes/conditions needed

Picochlorum growth campaigns at AzCATI testbed

Impact

- **Additional high-productivity *Picochlorum* isolates attained that are stable in sustained outdoor AzCATI growth campaign (Texas Gulf consortium)**
- **Testing underway to determine if diversity within a consortium is an asset relative clonal isolate**

Invited Speaker at 2022 Algae Biomass Summit (DISCOVR Special Session)

Posewitz et al. (2022), "*Picochlorum*: Model systems for robust outdoor growth in marine media".

DISCOVR · Pacific Northwest National Laboratory · Los Alamos National Laboratory EST. 1943 · NREL Transforming ENERGY · Sandia National Laboratories · INL Idaho National Laboratory · AzCATI Arizona Center for Algae Technology and Innovation · Colorado School of Mines

DISCOVR Tasks of Testing New Concepts to Move SOT Needle

Approach – Progress & Outcomes - Impacts

New Concepts

TAB

Technical Advisory Board

DISCOVR Strain Pipeline

TIER I: Characterize Strains in Flask Cultures (PNNL, LANL, SNL)

TIER II: Quantify Strain Performance in Climate-Simulation PBRs (PNNL, NREL, LANL)

TIER III: Test Strains in Pilot-Scale Indoor and Outdoor Ponds (SNL, PNNL)

TEA

TEA Operational Baseline (NREL)

Testing New Concepts to Move SOT Needle

Improve Biomass Productivity

- Evaluate Growth Promoting Molecules (LANL)
- Reduce Impact of Oxygen Inhibition (PNNL)
- Optimize Pond Operational Conditions (LANL, PNNL)
- Enhance Growth using Plant Substrates (SNL)
- Identify High Productivity *Picochlorum* Enrichments (CSM)

Enhance Biomass Intrinsic Value

- Measure Compositional Shift Dynamics (NREL)

Increase Crop Protection and Culture Stability

- Develop Integrated Pest Management Strategies (SNL)
- Evaluate Companion Cultures (PNNL)
- Implement Spectroradiometric Monitoring (SNL)

Outdoor SOT Trials to Verify Performance of Strains and Optimized Operational Conditions

- Outdoor pond SOT Trials (AzCATI)
- Compositional Analysis (NREL)
- SOT Documentation/Data Analytics (NREL)
- Annual TEA SOT assessment (NREL)

Other Projects in AAS Portfolio or Call for Collaboration

DISCOVR | Pacific Northwest National Laboratory | Los Alamos National Laboratory Est. 1943 | NREL Transforming ENERGY | Sandia National Laboratories | INL Idaho National Laboratory | AzCATI Arizona Center for Algae Technology and Innovation | Colorado School of Mines

Approach: Growth promoting Molecules to Boost Productivity

Increasing biomass productivity using phytohormone treatment

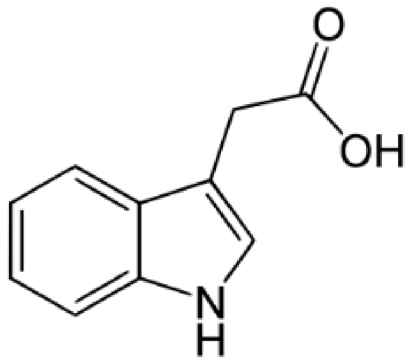
New Concepts

- **Background/history:** Growth Promoting molecules (GPMs) have the potential to boost growth and carbon storage
  - GPMs regularly used in agriculture to improve yield
  - Algal literature showed GPMs can increase growth, but
    - DISCOVR strains had not been tested
    - Outdoor-relevant conditions had not been tested

- **Objective:** Examine growth of DISCOVR algae strains in presence of known plant growth promoting molecules, under outdoor-relevant conditions (media, CO_2, light, temperature)

- **Challenges:**
  1. Cost of GPMs may outweigh benefits to algae productivity
     - *Collaborated with NREL analysis team to identify "tipping points" for costs vs productivity increases*
  2. GPM uptake may influence overall increase in productivity

- **Economic/Technical Metrics:** May be able to boost algae growth and/or biochemical storage using a relatively inexpensive additive



Indole-3-acetic acid (IAA)

ePBR

Flask

DISCOVR Pacific Northwest NATIONAL LABORATORY · Los Alamos NATIONAL LABORATORY EST.1943 · NREL Transforming ENERGY · Sandia National Laboratories · INL Idaho National Laboratory · AzCATI Arizona Center for Algae Technology and Innovation · COLORADO SCHOOL OF MINES

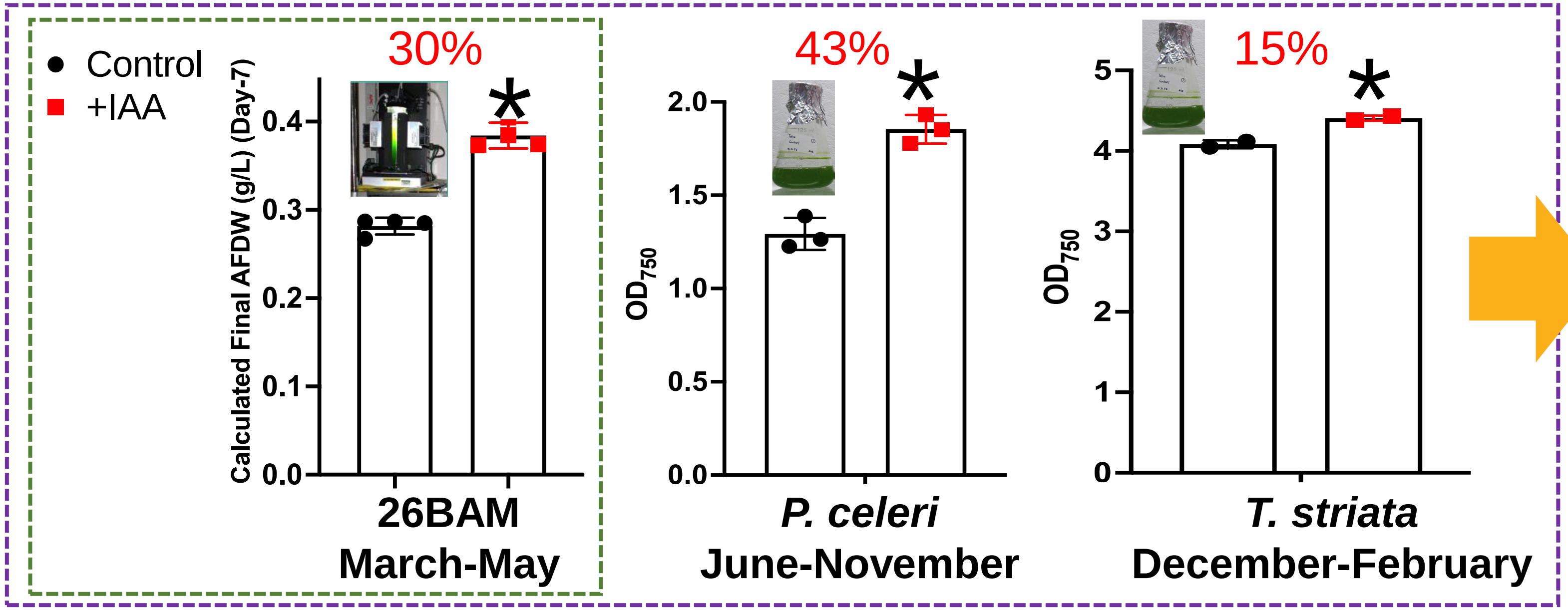
Outcomes: Using GPMs to Boost Productivity

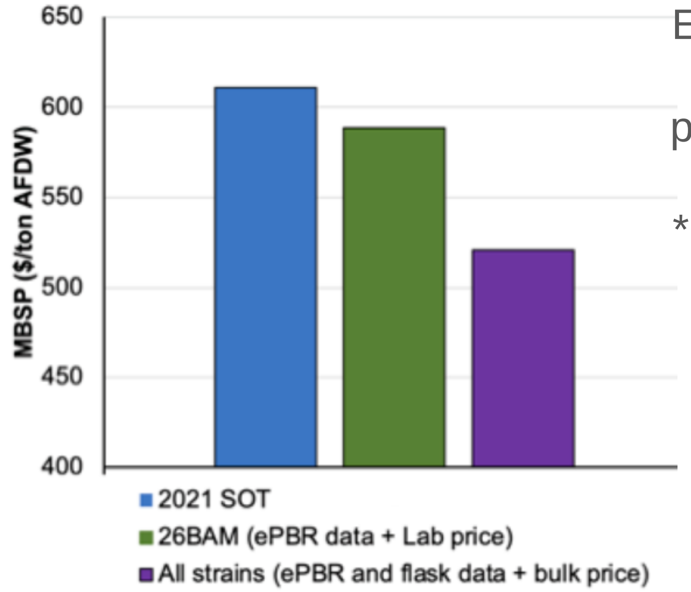
GPMs increases biomass productivity by 30% in winter under outdoor relevant conditions

New Concepts

Effects of GPMs tested in 4 DISCOVR SOT strains

- Strains tested: *M. minutum* 26BAM, *Tetraselmis striata, Picochlorum celeri, Chlorella sorokiniana*
- *M. minutum* 26BAM, tested in ePBRs +/- 1μM IAA using outdoor relevant winter conditions (under AzCATI retrospective February script). Final AFDW show 30% increase with IAA treatment.
- Flask experiments using IAA showed improved growth (OD_{750}) in *P. celeri (43%)* and *T. striata (15%)*
- Benchmark Economics showed GPM supplementation to DISCOVR SOT strain cultivations can significantly reduce Algae MBSP year-round





Experiments were performed in triplicate.
* Represents significant difference (p-values <0.01)

Impact: Using GPMs to Boost Productivity

Increasing biomass productivity with phytohormones can reduce year-round MBSP

New Concepts

New approaches/ideas tested to determine feasibility of application in outdoor ponds

- DISCOVR SOT strains in SOT media tested under flask and outdoor relevant conditions
- Year-round Benchmark Economics performed to show impact of GPM supplementation on MBSP

Impact

Demonstrated that GPM treatment on SOT strains:

- **Improved growth both in flasks and ePBRs (including outdoor-relevant media, CO_2, light, temperature)**
- **Covered entire year of outdoor cultivation with improved growth**
- **Updated TEA analysis suggest significant reduction in MBSP with 1μM IAA**

Invited Speaker at 2022 Algae Biomass Summit (DISCOVR Special Session)

Negi, Carr, Daughton, Dale et al. (2022), "Effect of plant growth-promoting molecules on improving biomass productivity in DISCOVR production strains".

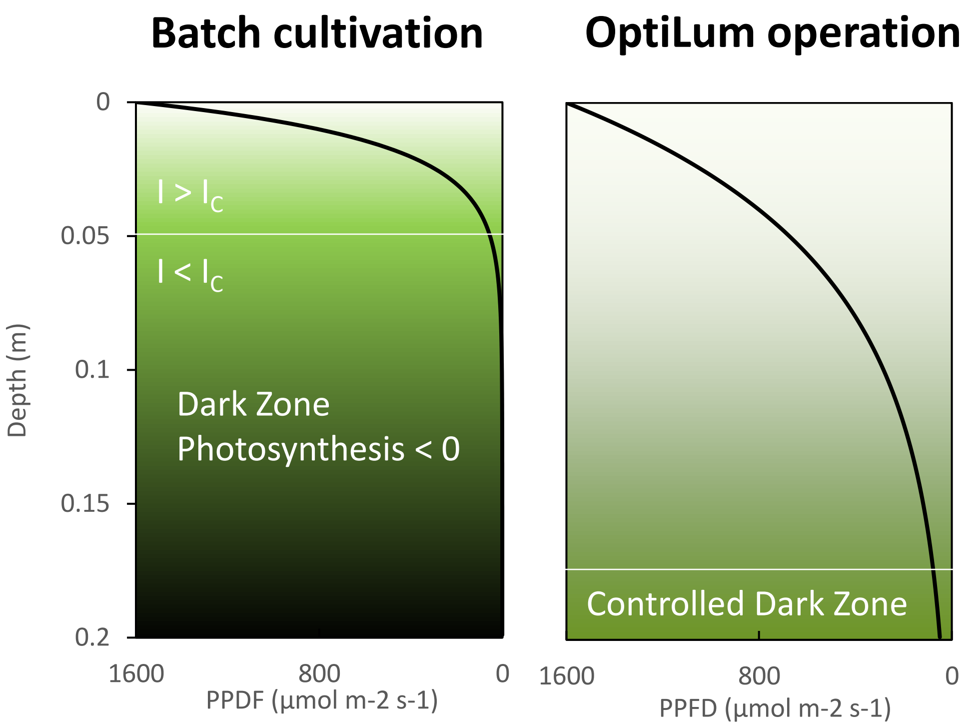
Publication: Negi, Daughton, Carr, Klein, Davis, Banerjee, Dale (2022), Effect of plant growth-promoting molecules on improving biomass productivity in DISCOVR production strains. *Algal Research* Special Issue DISCOVR, under review.

Approach: OptiLum Operations

Optimize light distribution in the culture to improve biomass productivity + lower MBSP

New Concepts

- **Background/history:**
  - Light intensity attenuates exponentially with depth and is a primary limiting factor in the culture.
  - Dark zone develops in the culture and results in non-ideal lighting conditions and respiratory biomass loss.
- **Objective:**
  - Evaluate automation based on an Optimized Luminance (OptiLum) operation under variable weather conditions.
  - Provide information for TEA to determine effect on MBSP.
- **Challenges:**
  1. Low harvest biomass density.
  2. Large amount of water movements.
- **Economic/Technical Metrics:** Biomass productivity and MBSP



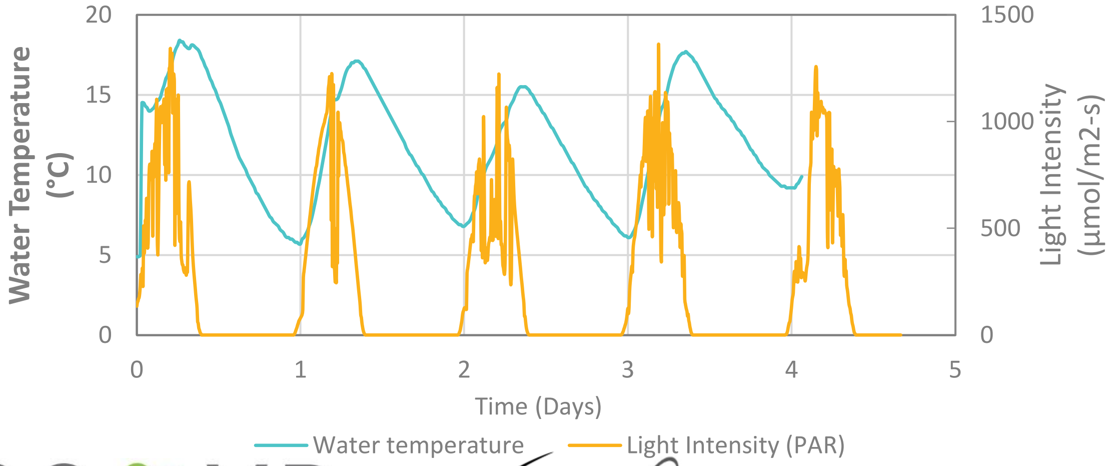
Dark zone is controlled by OptiLum operation to maximize biomass productivity by minimizing respiratory loss and photoinhibition

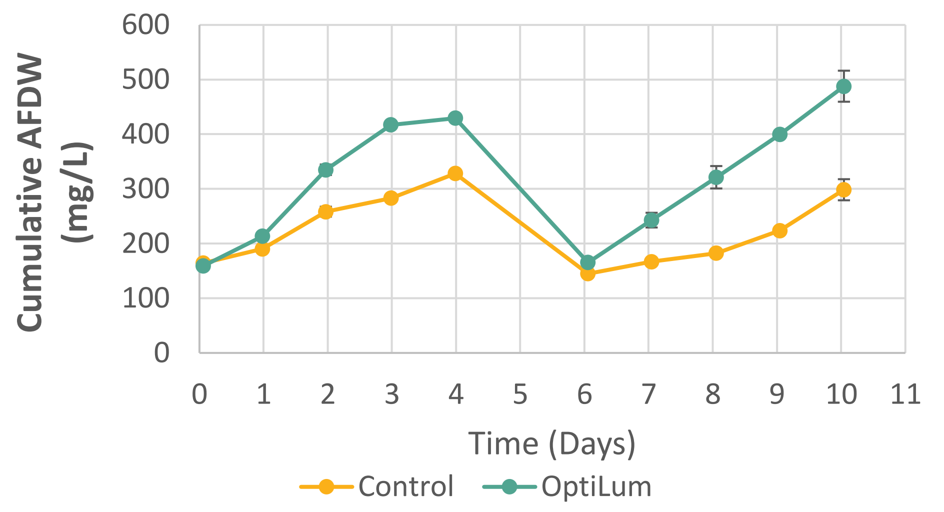
Outcomes: OptiLum Operations

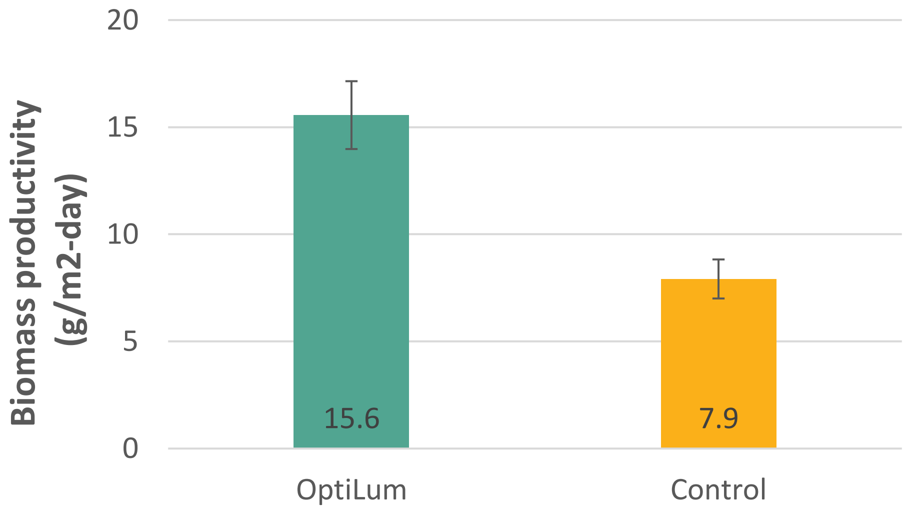
>90% improvement in productivity by OptiLum operations vs. batch culture baseline

New Concepts

- Strain: *Tetraselmis striata* LANL1001
- Automated dilution control: Light intensity at the bottom ≥ Compensation light intensity (minimized dark zone)
- Conditions: Nov 28 – Dec 2 2022, AzCATI
- Biomass productivities:
  - Control: 7.9 g m^{-2} day^{-1}
  - OptiLum: 15.6 g m^{-2} day^{-1}
  - Improvement: 97%







DISCOVR | Pacific Northwest National Laboratory | Los Alamos National Laboratory EST. 1943 | NREL Transforming ENERGY | Sandia National Laboratories | Idaho National Laboratory | AzCATI Arizona Center for Algae Technology and Innovation | Colorado School of Mines

Impact: OptiLum Operations

New Concepts

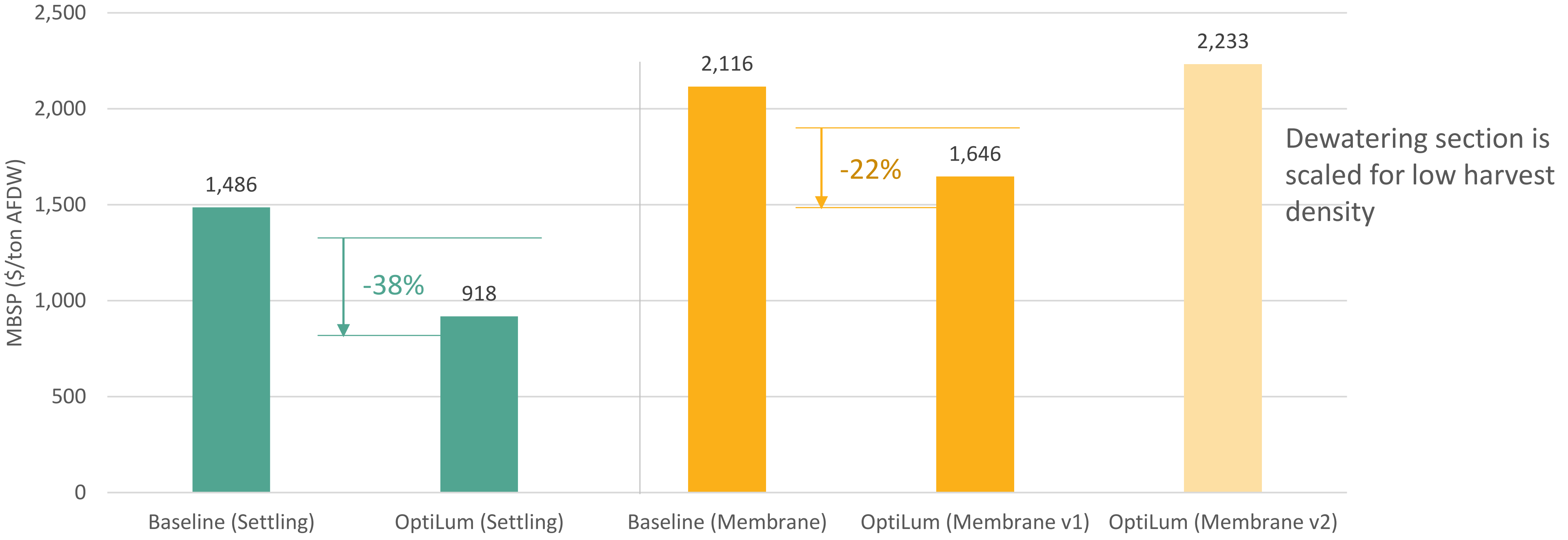
MBSP significantly reduced by OptiLum operations vs. batch culture baseline

- Biomass productivity was almost doubled by OptiLum
- Ca. 2 times more water movement
- MBSP was reduced based on TEA depending on harvesting methods

Impact

- **GNG-1 Milestone achieved (>20% productivity improvement)**
- **Significant reduction in MBSP, particularly for strains that gravity-settle**

NREL TEA results for MBSP in OptiLum and batch culture baseline for gravity settling, membrane filtration (v1 and v2), see notes for additional details.

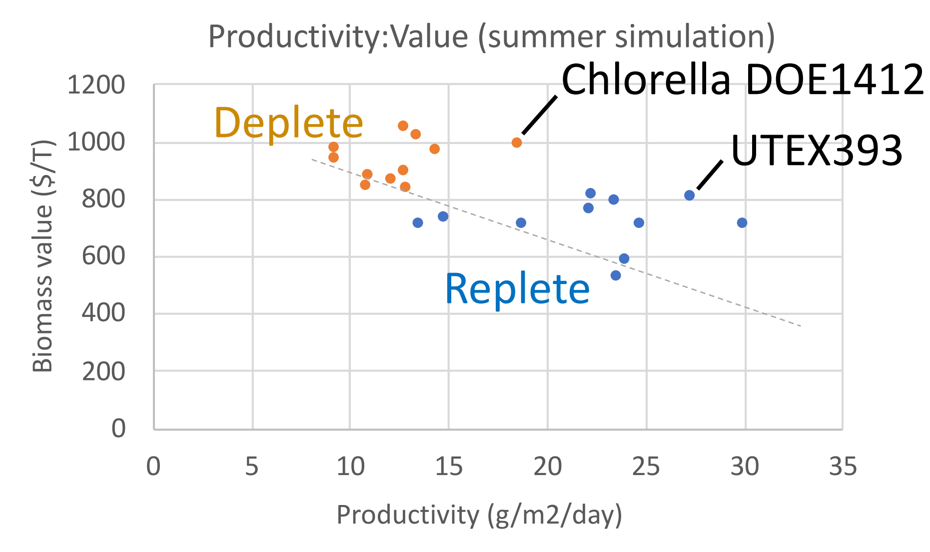


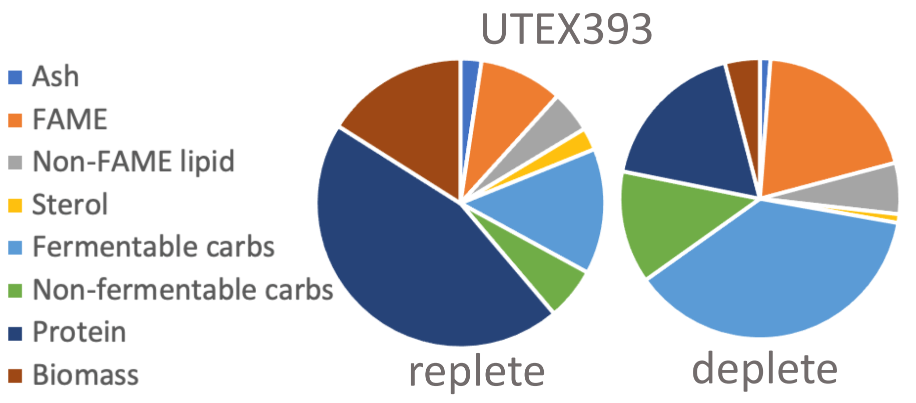
Approach: Manipulating Biomass Composition

New Concepts

Improving biomass value

- **Background/history:** Different algae species have distinct capacity and rates to shift biomass composition in response to environmental stimuli
  - Biomass composition and shift upon nutrient depletion was used for ranking strains [**Value Productivity ($/m²/day)**]
- **Objective:** Improve biomass value productivity by shifting biomass composition to carbon storage products, for valorizing in biomass conversion process
- **Risk:**
  - Simulated conditions cultivation reactors often poorly mimic biomass physiology, limiting translation of reactor results
  - Composition shift frequently happens with reduction in growth rates, thereby increasing biomass cost
- **Approach:**
  - Implement a cultivation simulation that matches growth and composition of select algae species with outdoor cultivation
  - Develop approach to shift composition to higher storage carbon with minimal productivity reduction
- **Economic/Technical Metrics:** Achieve economic profitability by demonstrating that biomass value exceeds biomass production cost.





*Laurens, 2014, Anal. Biochem, 452:86-95

DISCOVR | Pacific Northwest National Laboratory | Los Alamos National Laboratory | NREL | Sandia National Laboratories | Idaho National Laboratory | AzCATI | Colorado School of Mines

Outcomes: Manipulating Biomass Composition

Composition rapidly shifts under validated simulated conditions of nutrient stress

New Concepts

- **Progress:**
  1. Matched outdoor growth rates (**within 2 to 10% deviation**) in simulated indoor conditions for Fall and Summer season cultivation
  2. Matched composition under simulated conditions to within 10% (protein, lipid), and 25% (carbs)
  3. Transfer to low NP conditions, shifts biomass to high carbon-storage composition, **over 70% increase in carbohydrates and 50% higher lipids** within 4 days, with **less than 5% drop in productivity**
- **Outcomes:**
  - **Simulated conditions allow for small scale cultivation and low-cost testing** of physiological drivers of biomass composition and quality
  - Rapid (<4 days) induction phase shifts composition without growth impact
  - Pathway to significant intrinsic value increase for biorefinery optimization

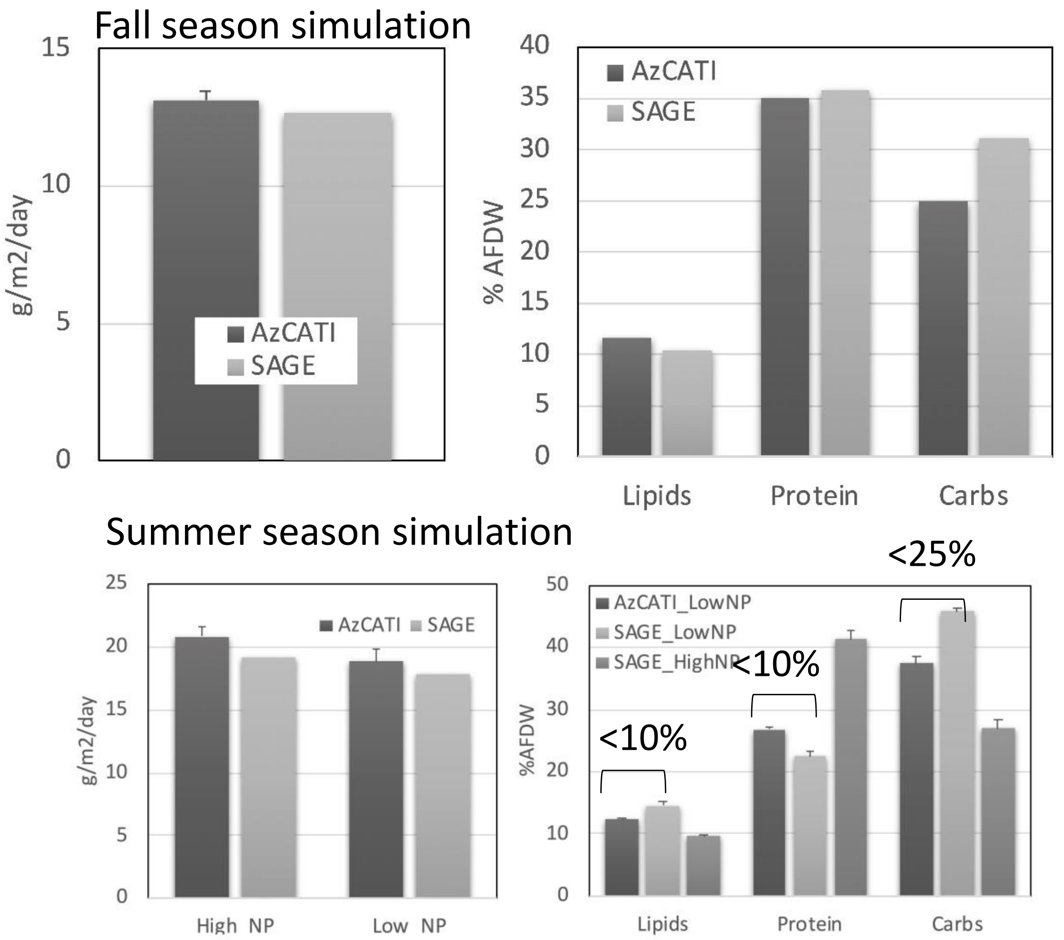


Figure 1: *Illustration of agreement between outdoor and simulated environment Scenedesmus UTEX393 productivity and composition for two seasons, for summer season, 4-day low-nutrient induction shifts composition to fuel-ready biomass with no growth impact*

DISCOVR · Pacific Northwest National Laboratory · Los Alamos National Laboratory · NREL Transforming ENERGY · Sandia National Laboratories · Idaho National Laboratory · AzCATI Arizona Center for Algae Technology and Innovation · Colorado School of Mines

Impact: Manipulating Biomass Composition

New Concepts

Algal biomass composition shift to high carbon storage benefits TEA of fuels

- Biomass value and conversion process technical and economic viability directly related to composition
  - Outdoor-simulated environment cultivation allows for rapid and cost-effective testing of strategies
  - Rapid induction of carbohydrate and lipid accumulation with no productivity drop shows route to high carbon-storage algae farming
- A quantified tradeoff between compositional value vs production cost for **one biomass-derived product portfolio** suggests increase in biomass value with shifting towards higher storage carbohydrates benefits a fuel-conversion pathway

Impact

- Validated simulated cultivation allows for testing outdoor-implementable physiological strategies to increase carbon storage for sustainable aviation fuels applications
- Demonstrated approach to achieve higher biomass value (increased carbohydrate and lipid content) for equivalent production costs

$$\frac{\$}{T_{biomass}} = \sum a[Lipid_{ash-free}] + b[Carbohydrate_{ash-free}] + c[Protein_{ash-free}]$$

***Eq. 1:** Value of the biomass linearly correlated to the respective value assigned to the components in their final application after conversion*

Publication

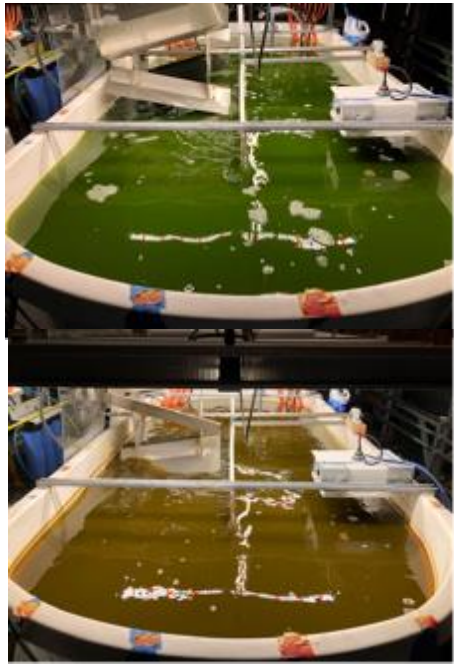
Klein, Davis, Laurens, et al., 2023 “Quantifying the Intrinsic Value of Algal Biomass Based on a Multi-Product Biorefining Strategy” *Algal Research* - Special Issue DISCOVR, under review.

Approach: Increase Crop Protection and Culture Stability

Develop integrated pest management strategies

New Concepts

- **Background/history:** SNL's capabilities in pond monitoring, pest identification and countermeasures were developed over a series of BETO funded projects (Pond Crash Forensics, ATP3, TABB, PEAK)
  - Developed and demonstrated methods for detection and genetic identification of pond crash agents.
  - Developed strategies for control of pest species that are cost free and independent of chemical treatment.
- **Objective:** Develop an integrated pest management strategy to control grazers and parasites, through a combination of spectroradiometric monitoring, agent identification and characterization, and development of crop protection technologies and strategies, and to deploy crop monitoring and protection strategies at the SOT testbed to improve seasonal productivity.
- **Challenges:**
  1. Diversity of pond crash agents
     - A wide variety of agents can cause crashes and the dominant agent can change rapidly (whack-a-mole).
     - Broader spectrum countermeasures need to be developed against entire classes of agents.
  2. Identification of agents
     - Novel agents (especially bacteria) are difficult to identify requiring a combination of microbiome analyses and classical microbiology
     - Pond symptoms can be general and mimic environmental stress



1000 L pond crashes on demand at the SNL testbed: ***Top:*** *control algal culture.* ***Bottom:*** *amoeboaphelid infected algal culture.*

Outcomes: Industrial Partnership Evaluating Novel Countermeasures

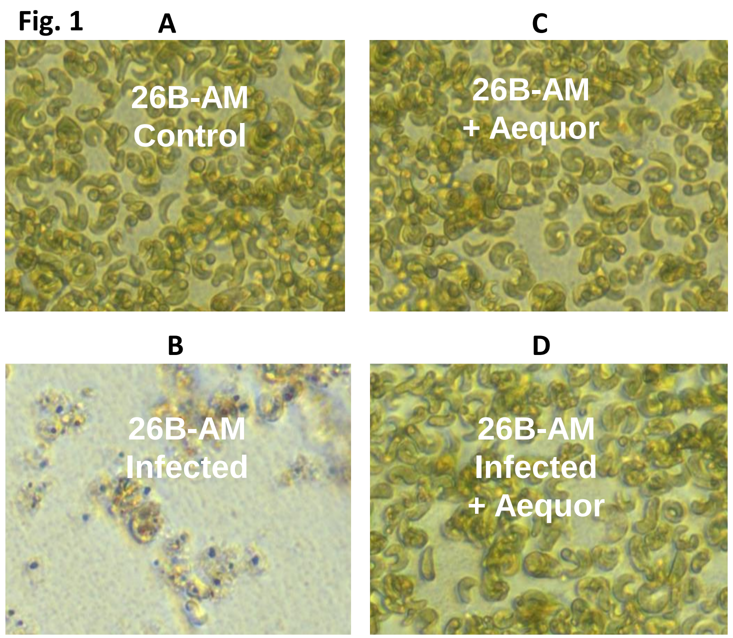
Proprietary chemicals from Aequor successfully protect against fungal infection

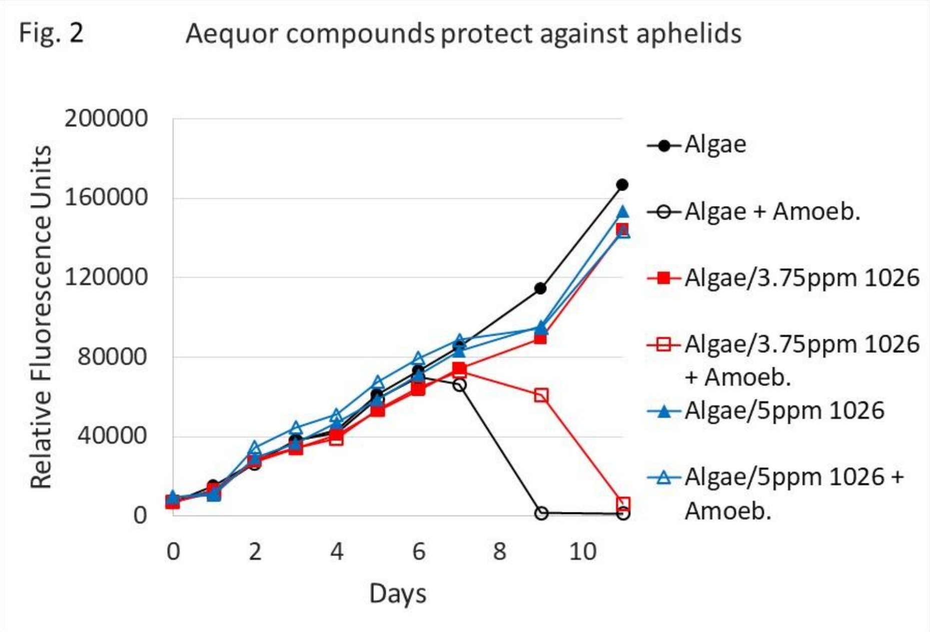
- Sub-project developed via the DISCOVR Call for Collaborations: Aequor Inc.
  - Developed a protocol for the evaluation of Aequor proprietary compounds for protecting algal cultures from amoeboaphelid infections in standard laboratory infection assays.
  - Demonstrated that 2 compounds demonstrated high levels of protection against amoeboaphelid infection at concentrations as low as 5 ppm.
  - Compounds displayed little or no effect on algal growth rate at protective concentrations.

Ability of Aequor compound to protect against fungal infection in *Monoraphidium minutum* 26B-AM

Fig. 1A *Monraphidium minutum* 26B-AM
1B: *M. minutum* 26B-AM infected with *Amoeboaphelidium sp.*
1C*:* *M. minutum* 26B-AM grown with 5 ppm Aequor compound 1026
1D: same 1C but infected with *Amoeboaphelidium sp.*

Fig. 2: Standard growth/infection assays demonstrating inhibition of *Amoeboaphelidum sp.* infection by addition of Aequor compound 1026





Outcomes: Development of Novel Biological Countermeasures

Pond-derived, chitinolytic isolates delay crashes in amoeboaphelid infections

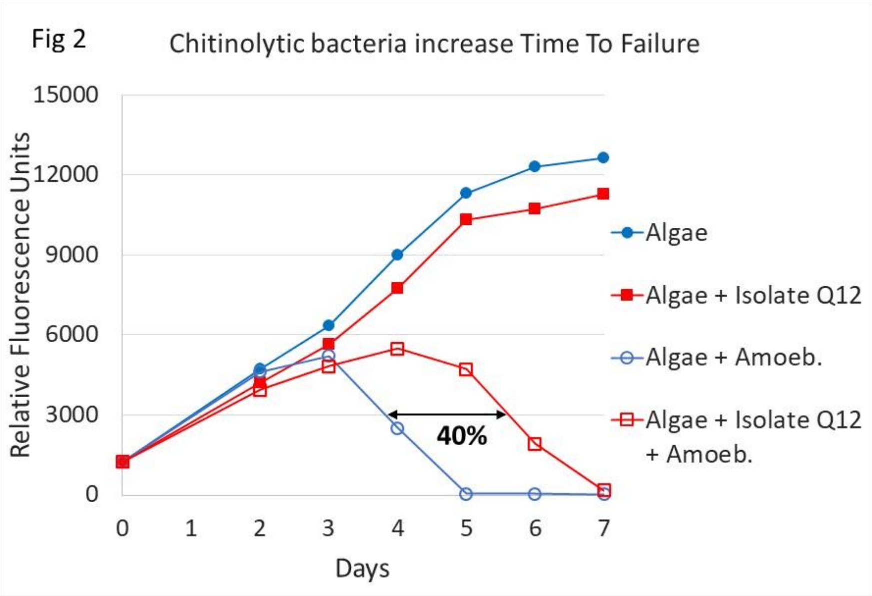
New Concepts

- Tested a diversity of pond derived microbial consortia for chitinolytic activity and ability to inhibit amoeboaphelid infections
  - Protection was strongly correlated to chitinolytic activity
- Isolated chitinolytic bacteria from protective consortia and cultivated in co-culture with algal production strains
  - Selects for isolates capable of growth on chitin as “sole” carbon source
  - Clearing of colloidal chitin provides additional evidence of chitinase activity
  - Chitinolytic isolates increased the time to failure of amoeboaphelid-infected cultures by 40%.
  - Protection persisted in algal bacterial co-cultures for >30days.

Fig. 1

Fig. 1: *Streak of chitinolytic bacterial consortium on chitin medium demonstrating clearing of colloidal chitin and growth of one or more isolates using chitin as sole carbon source*

Fig. 2 *Infection assay of Monoraphidium minutum 26B-AM with Amoeboaphelidium sp. demonstrating a 40% increase in time to failure of the infected culture by co culture with chitinolytic bacteria*



DISCOVR · Pacific Northwest National Laboratory · Los Alamos National Laboratory EST.1943 · NREL Transforming ENERGY · Sandia National Laboratories · INL Idaho National Laboratory · AzCATI Arizona Center for Algae Technology and Innovation · Colorado School of Mines

Impact: Leadership in the Field of Detection and Countermeasures

Development of novel stand alone monitoring and prophylactic treatment

Annualized production is limited, in part, by pond failure due to biocontaminants

- Treatment options are often limited and can increase the cost of production.
- Because of expense, current crop protection strategies are often “treat upon detection”.
- Improved strategies, including prophylaxis or innate stability, would be integral to the production system and prevent crashes

Impact

We have advanced toward a fully integrated pest management strategy by:

- **Developing automated monitoring systems**
- **Developing low or zero cost methods for crop protection**
- **Creating a “defense-in-depth” approach that can compensate for the failure of one or more strategies**

Publications

- Atencio, L., Maes, D., Hipple, T., and Timlin, J.A. “Susceptibility of Two Saltwater Strains of *Chlorella sorokiniana* to *Vampirovibrio chlorellavorus*”. *J. Appl. Phycol*., 2022, 34, 81-87, https://doi.org/10.1007/s10811-021-02602-0
- Lane, T.W., 2022, “Barriers to microalgal mass cultivation” *Curr. Opin. Biotechnol.* 20273:323-328.
- Lane et al., 2022, “Resistance of DISCOVR algae strains to deleterious species”, *Algal Res.* – Special Issue DISCOVR, 66: 102793, https://doi.org/10.1016/j.algal.2022.102793
- Poorey et al., 2022, “Characterizing industrial pond ecology timeline in DISCOVR cultivation trials for early detection of pond crashes”, *Algal Research* – Special Issue DISCOVR, under review.

Outdoor State of Technology (SOT) Trials

Approach – Progress & Outcomes - Impacts

TAB

Technical Advisory Board

DISCOVR Strain Pipeline

TIER I: Characterize Strains in Flask Cultures (PNNL, LANL, SNL)

TIER II: Quantify Strain Performance in Climate-Simulation PBRs (PNNL, NREL, LANL)

TIER III: Test Strains in Pilot-Scale Indoor and Outdoor Ponds (SNL, PNNL)

TEA

TEA Operational Baseline (NREL)

Testing New Concepts to Move SOT Needle

Improve Biomass Productivity

- Evaluate Growth Promoting Molecules (LANL)
- Reduce Impact of Oxygen Inhibition (PNNL)
- Optimize Pond Operational Conditions (LANL, PNNL)
- Enhance Growth using Plant Substrates (SNL)
- Identify High Productivity *Picochlorum* Enrichments (CSM)

Enhance Biomass Intrinsic Value

- Measure Compositional Shift Dynamics (NREL)

Increase Crop Protection and Culture Stability

- Develop Integrated Pest Management Strategies (SNL)
- Evaluate Companion Cultures (PNNL)
- Implement Spectroradiometric Monitoring (SNL)

Outdoor SOT Trials to Verify Performance of Strains and Optimized Operational Conditions

- Outdoor pond SOT Trials (AzCATI)
- Compositional Analysis (NREL)
- SOT Documentation/Data Analytics (NREL)
- Annual TEA SOT assessment (NREL)

Other Projects in AAS Portfolio or Call for Collaboration

Approach: State of Technology Cultivation Trials

SOT Trials

Robust experimental framework for conducting trials based on > 9 years of experience

- **Background/history:** SOT cultivation framework established under ATP[3] (2013-2018) and continued under DISCOVR (2018-present).
- **Objective:** Conduct year-round, outdoor cultivation trials to determine monthly/seasonal/annual biomass productivity.
- **Challenges:** Implementing effective and robust crop protection and integrated pest management strategies remains the most significant challenge to achieving AND maintaining high productivity.
- **Economic/Technical Metrics:** The SOT cultivation trials are THE main source of data for the determination of current MBSP and MFSP allowing for AAS to measure progress towards the 2030 goals of achieving **25 g/m²-day** annual average productivity and **<$2.5 GGE**.

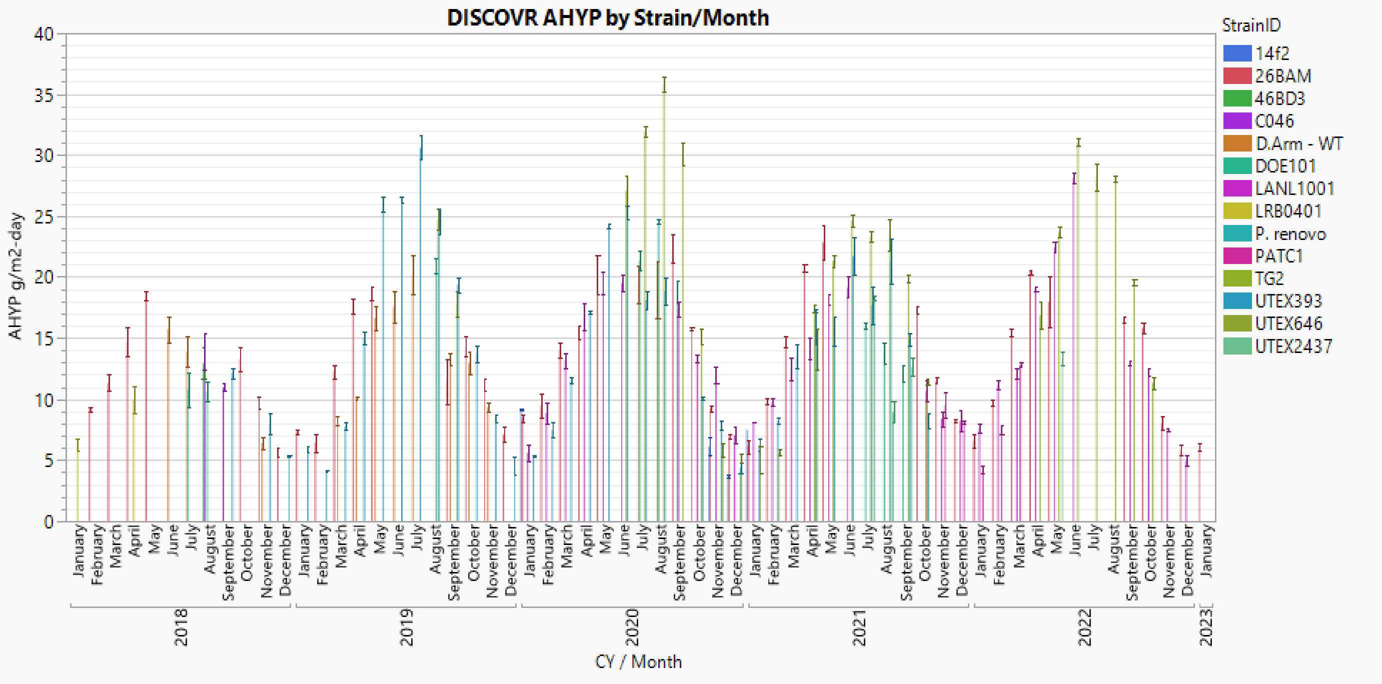
Standard 4.2 m² open raceway ponds (ORP) for SOT cultivation trials at AzCATI. Twelve (12) ORP's dedicated to DISCOVR seasonal cultivation trials allowing up to four experimental conditions to be run simultaneously in triplicate.

Outcomes: SOT Cultivation Trials

Outdoor verification of best strains/approaches with primary focus on improving productivity

SOT Trials

- **Under DISCOVR – a 60% improvement from 2018 to 2022**
  - >16% for just completed 3 yr AOP cycle
  - 117% improvement in annual average productivity since inception of SOT analysis in 2015
- **A drop in annual average productivity was observed in 2021, primarily driven by lower summer and early fall productivity**
- **Averaged >10% per year since 2015, but only 5.5% for last 3 years**
  - Have we reached a plateau in productivity gains?
  - Can this be overcome with the current cultivars and biotic and abiotic conditions at the AzCATI site?
- **BETO target remains 25 g/m^2-day annual average by 2030**
  - requires ~ 5% year over year improvement



Season	2015	2016	2017	2018	2019	2020	2021	2022
Fall	6.8	7	8.5	9	11.4	15.0	19.1	16.2
Winter	5	5	5.5	7.7	6.5	8.3	8.3	9.0
Spring	11.4	11.1	13.2	14.8	18.6	18.5	19.4	19.9
Summer	10.9	13.3	14.1	14.9	27.1	31.6	23.8	28.9
Average	**8.5**	**9.1**	**10.3**	**11.6**	**15.9**	**18.4**	**17.6**	**18.5**
Year over year (YOY) Improvement	N/A	7%	13%	12%	37.0%	15.4%	-3.9%	5.0%
SOT Improvement since 2015		7%	21%	36%	87%	115%	107%	117%

DISCOVR

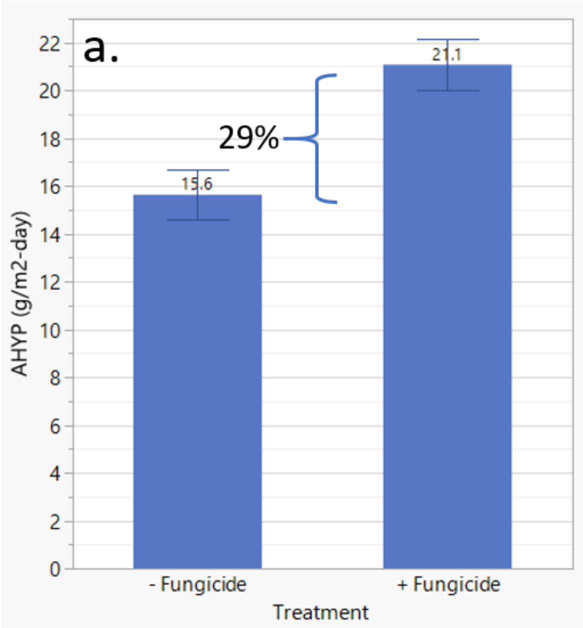
Pacific Northwest NATIONAL LABORATORY · Los Alamos NATIONAL LABORATORY EST.1943 · NREL Transforming ENERGY · Sandia National Laboratories · AzCATI Arizona Center for Algae Technology and Innovation · MINES

Impact: SOT Cultivation Trials

SOT Trials

Outdoor verification of best strains/approaches with primary focus on improving productivity

- Implementing effective strain rotation and robust crop protection and integrated pest management strategies remains the most significant challenge to achieving AND maintaining high productivity.



a) Fungicide treatment with 26BAM increased productivity ~29% and 2x increase in time to failure

Impact

- **Algae strain specific fungicide dosing regime reduced contamination pressure and increased productivity**
- **Seasonally (crop rotation) and salinity selected algae strains increased productivity**
- **Over 60% increase in productivity since 2018**
- **Multi-year algae cultivation agronomic data publicly available**

Publication

McGowen et al., 2023, “Outdoor annual algae productivity improvements at the pre-pilot scale through crop rotation and pond operational management strategies”, *Algal Research* – Special Issue DISCOVR, https://doi.org/10.1016/j.algal.2023.102995

DISCOVR Techno-Economic Analysis (TEA) Modeling

TEA

Approach – Progress & Outcomes - Impacts

TAB

Technical Advisory Board

DISCOVR Strain Pipeline

TIER I: Characterize Strains in Flask Cultures (PNNL, LANL, SNL)

TIER II: Quantify Strain Performance in Climate-Simulation PBRs (PNNL, NREL, LANL)

TIER III: Test Strains in Pilot-Scale Indoor and Outdoor Ponds (SNL, PNNL)

TEA

TEA Operational Baseline (NREL)

Testing New Concepts to Move SOT Needle

Improve Biomass Productivity

- Evaluate Growth Promoting Molecules (LANL)
- Reduce Impact of Oxygen Inhibition (PNNL)
- Optimize Pond Operational Conditions (LANL, PNNL)
- Enhance Growth using Plant Substrates (SNL)
- Identify High Productivity *Picochlorum* Enrichments (CSM)

Enhance Biomass Intrinsic Value

- Measure Compositional Shift Dynamics (NREL)

Increase Crop Protection and Culture Stability

- Develop Integrated Pest Management Strategies (SNL)
- Evaluate Companion Cultures (PNNL)
- Implement Spectroradiometric Monitoring (SNL)

Outdoor SOT Trials to Verify Performance of Strains and Optimized Operational Conditions

- Outdoor pond SOT Trials (AzCATI)
- Compositional Analysis (NREL)
- SOT Documentation/Data Analytics (NREL)
- Annual TEA SOT assessment (NREL)

Other Projects in AAS Portfolio or Call for Collaboration

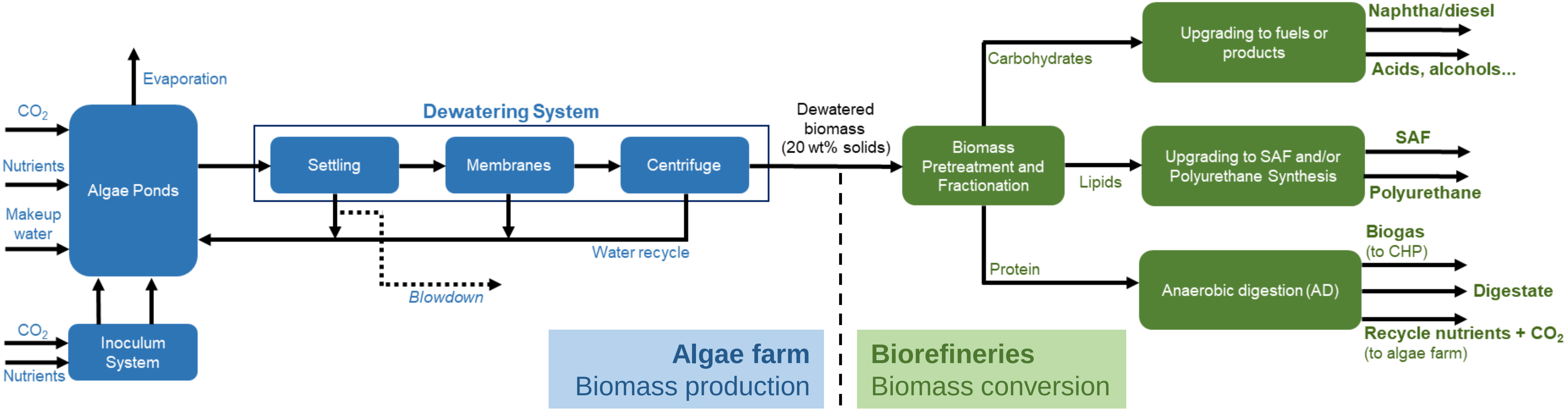
Approach: Techno-Economic Assessment (TEA)

TEA

Supporting the definition of research priorities from an economic standpoint

- **Background/history:** Process simulations and techno-economic assessment of algae biomass production in algae farms and conversion in biorefineries (diagram below)
- **Objective:** Inform the DISCOVR team on the impact of experimental achievements by translating technical performance to economics
- **Risks:** Experimental data not being relayed on time (mitigated by *what-if* sensitivity analyses)
- **Main metric:** Minimum biomass selling price (**$/dry ton AFDW)**

Simulation and TEA



DISCOVR · Pacific Northwest National Laboratory · Los Alamos National Laboratory · NREL Transforming ENERGY · Sandia National Laboratories · Idaho National Laboratory · AzCATI Arizona Center for Algae Technology and Innovation · Colorado School of Mines

Outcomes: Techno-Economic Assessment (TEA)

TEA

Biomass value increases when moving from nutrient-replete to nutrient-deplete cultivations

Quantifying the intrinsic value of algal biomass based on a multi-product biorefining strategy

- **Goal:** determine the economic potential that can be derived from microalgae biomass based on tradeoffs between productivity *vs.* composition
- Need to assess a large pool of strains and compositional shift data from LEAPS experiments
  - Methodology developed to pre-screen and downselect strains for in-depth TEA
  - Development of the "intrinsic biomass value" metric: revenue obtained from the conversion of algae biomass to a portfolio of products
- Compositions with best results are as enriched as possible in FAME and carbohydrates (Figure 1)
- Analysis set future performance targets for strains to achieve economic feasibility

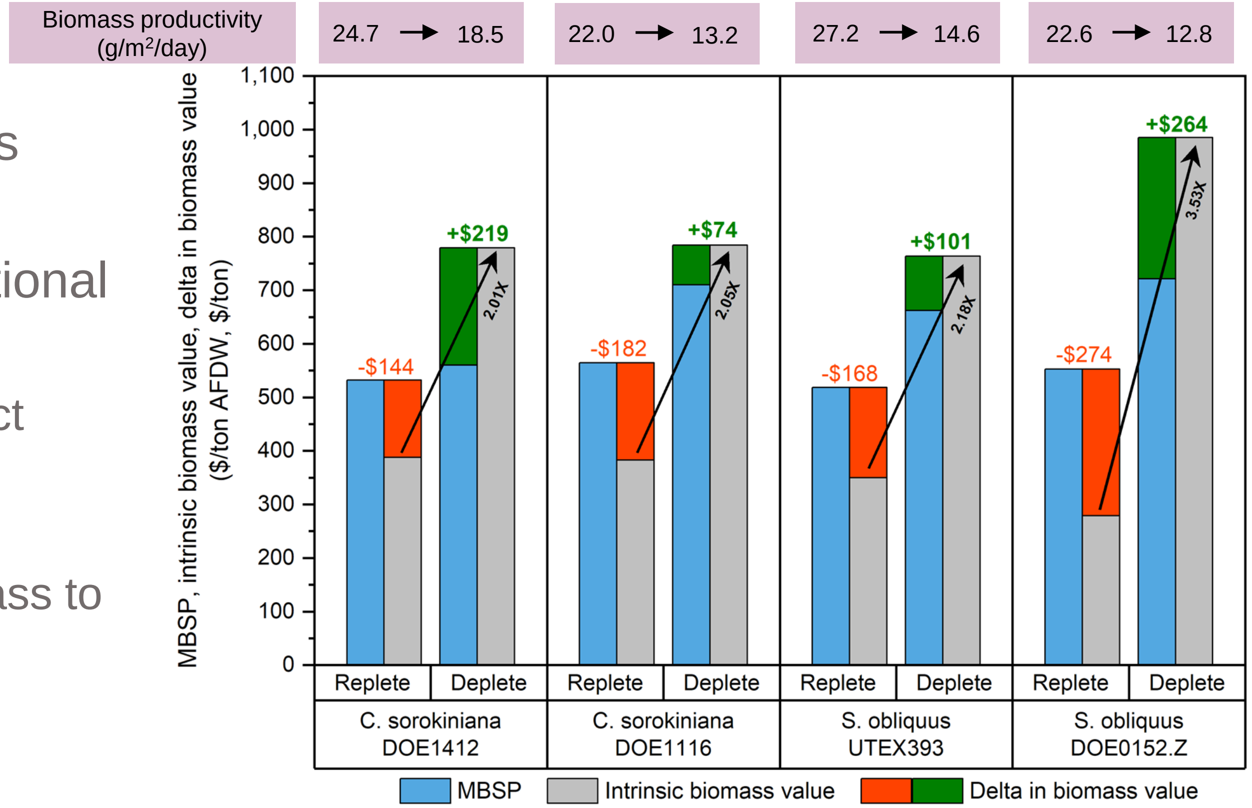


Figure 1. *Visual representation of the economic impact of moving to deplete harvesting: assessed strains present an increase of at least **twofold** in intrinsic biomass value after the compositional shift.*

Study under review in *Algal Research* (Klein et al., 2023)

DISCOVR · Pacific Northwest National Laboratory · Los Alamos National Laboratory · NREL Transforming ENERGY · Sandia National Laboratories · Idaho National Laboratory · AzCATI Arizona Center for Algae Technology and Innovation · Colorado School of Mines

Impact: Techno-Economic Assessment (TEA)

TEA

TEA as a cost-effective tool to help guiding experimental research in DISCOVR

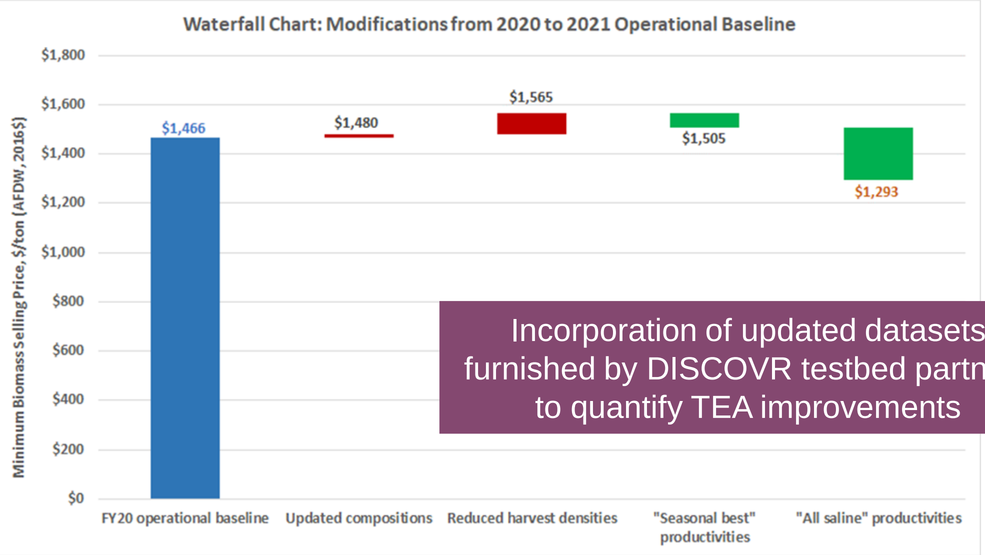
Understanding critical factors in large-scale algae biomass cultivations:

- Deployment of crop protection strategies
- Addition of growth promoting molecules
- Radiospectrometric monitoring of ponds
- Mixotrophic growth of algae with acetic acid

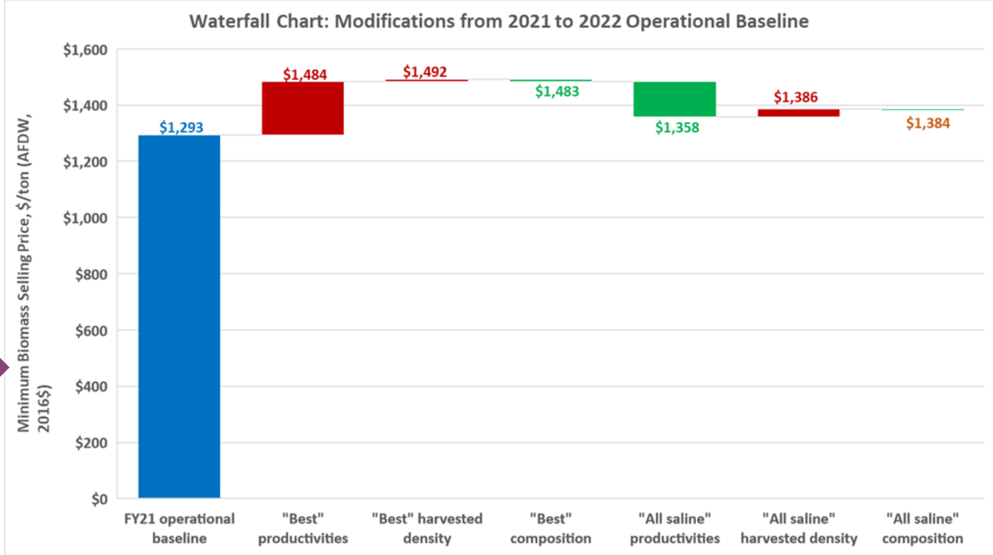
Impact

Defining DISCOVR's future experimental directions based on TEA guidance:

- **Maximum price/dosing/uptake of compounds**
- **Improvements in time-to-failure metrics**
- **Increases in biomass productivity**
- **Updating the "DISCOVR Operational Baseline"**



Incorporation of updated datasets furnished by DISCOVR testbed partners to quantify TEA improvements



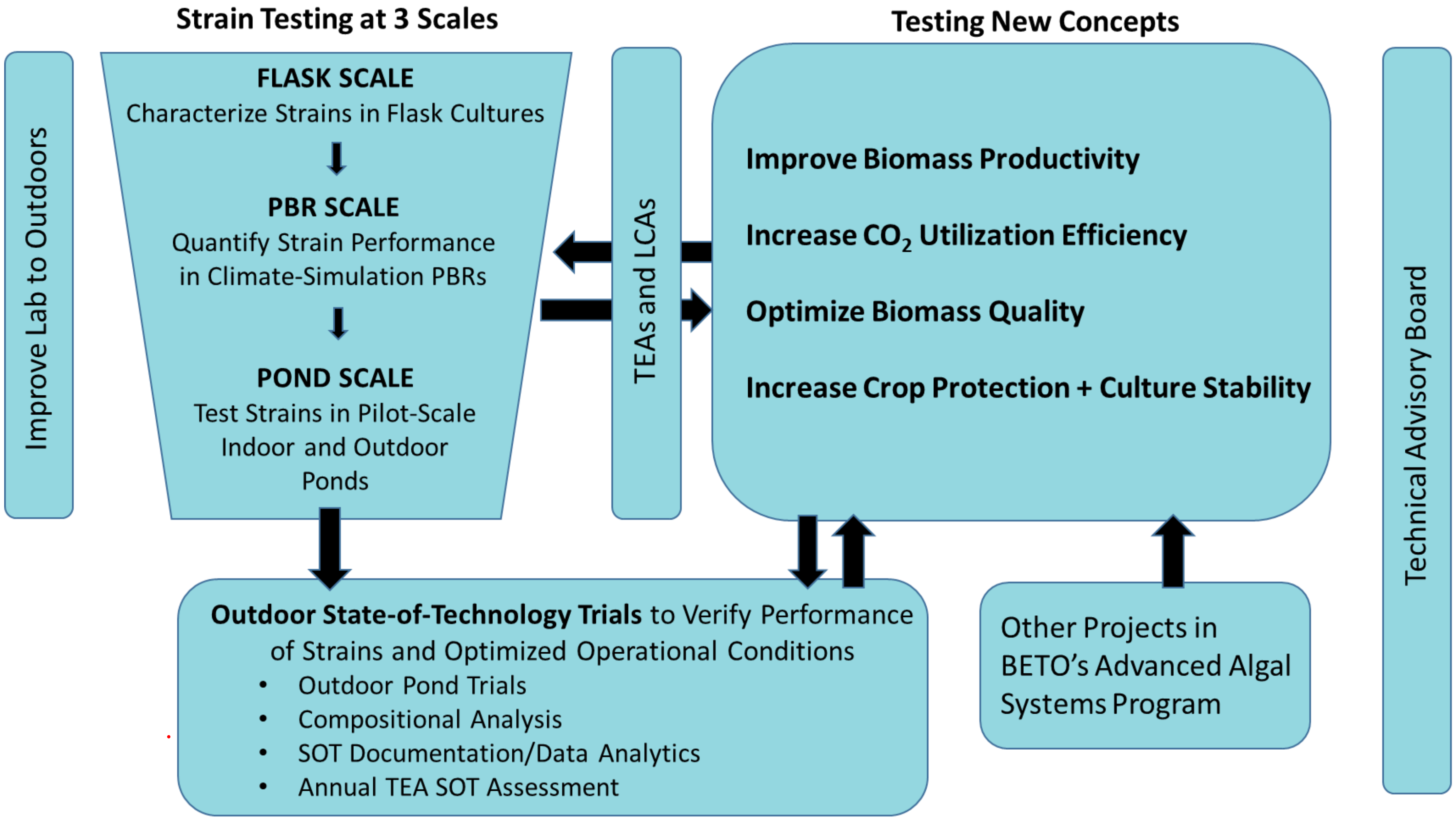
Figures 1 and 2. *Evolution of the "DISCOVR Operational Baseline" after the 2021 BETO Peer Review.*

DISCOVR · Pacific Northwest National Laboratory · Los Alamos National Laboratory EST. 1943 · NREL Transforming ENERGY · Sandia National Laboratories · INL Idaho National Laboratory · AzCATI Arizona Center for Algae Technology and Innovation · Colorado School of Mines

DISCOVR Project Framework for the Next 3-Year Cycle (FY23-FY25)

Testing new concepts at three cultivation scales, guided by TEA/LCA and Tech Advisory Board

2 additional Labs joined the DISCOVR Consortium: Idaho National Laboratory and Lawrence Livermore National Laboratory



DISCOVR · Pacific Northwest NATIONAL LABORATORY · Los Alamos NATIONAL LABORATORY EST. 1943 · NREL Transforming ENERGY · Sandia National Laboratories · INL Idaho National Laboratory · AzCATI Arizona Center for Algae Technology and Innovation · COLORADO SCHOOL OF MINES

DISCOVR Objectives and Milestones for the Next 3-Year Cycle

Objectives aligned with BETO targets of Ann Prod ≥25 g/m^2-day, MBSP<$488/ton, GHG ≤ 27g C_{eq}/MJ

Objectives:

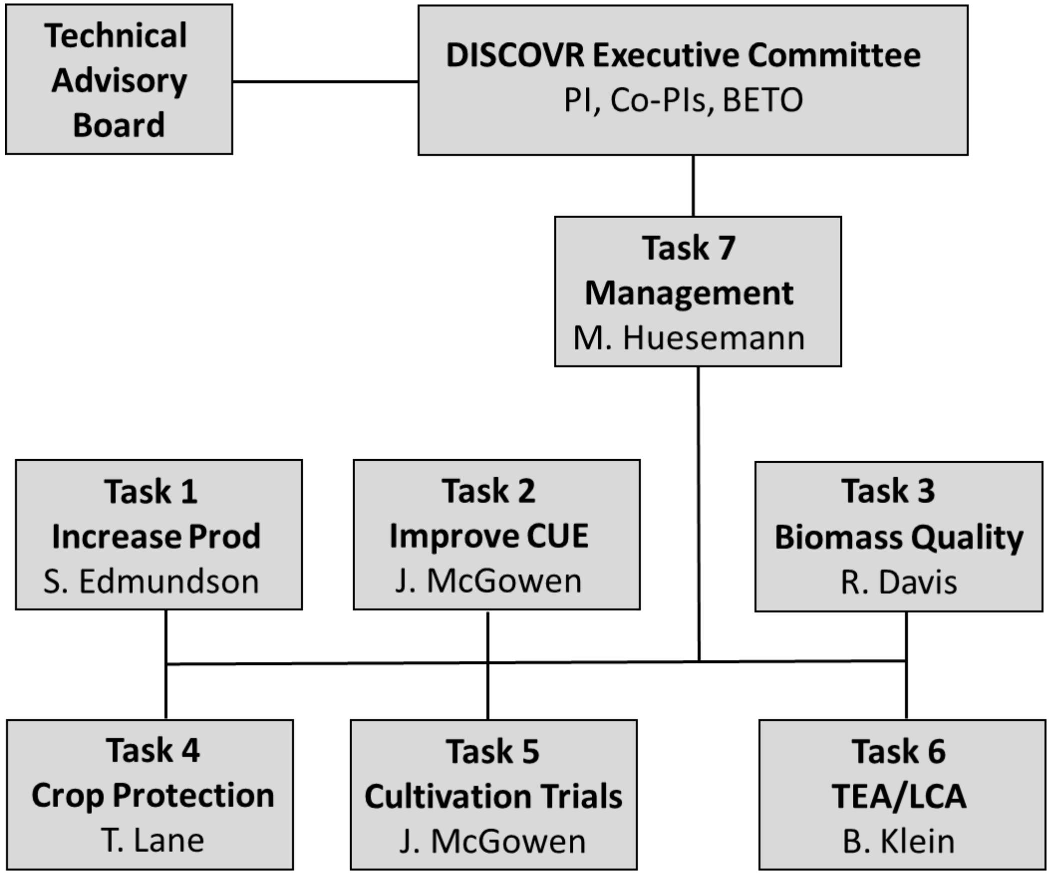
- Demonstrate ≥ 70% CO_2 utilization efficiency (CUE) in pond cultures
- Achieve Mean Time Before Failure (MTBF) target of ≥ 20 days
- Demonstrate annual biomass productivity of ≥ 20 g/m^2-day

End of 3 Year Cycle Milestones (4QFY25):

- Demonstrate at least 2 improvements in outdoor cultivation trials (4.2 and/or 100 m^2)
  - CUE ≥ 70%
  - MTBF ≥ 20 days
  - Annual biomass productivity ≥ 20 g/m^2-day
- Demonstrate at least one concept (of the 5 evaluated) for improving biomass productivity in outdoor ponds without increasing the MBSP.
- Demonstrate a net biomass value improvement of at least $100/T, relative to CAP-AD baseline.

DISCOVR Task and Management Structure for the Next 3-Year Cycle

The cohesive DISCOVR team successfully executes tasks within effective management framework



Details about the scope of each task and their respective subtasks are given in Supplemental Slides (94 to 113)

DISCOVR Executive Committee (ExCom)

- Composed of representatives of all 8 institutions + BETO
- Provides strategic direction and oversight
- Supports coordination of research between institutions
- Tracks progress towards meeting deliverables
- Makes decisions by consensus, otherwise BETO guidance

Task Management

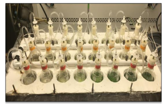
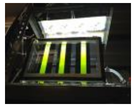
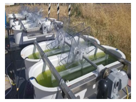
- Task leads are responsible for coordinating the respective subtasks and ensuring that task milestones are met.
- Task leads submit their respective task quarterly report inputs to the PI who submits the final report to BETO.
- Task Leads provide regular updates to the ExCom.

Meetings

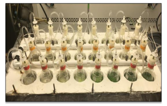
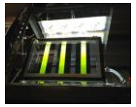
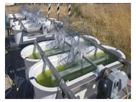
- ExCom and All-Hands meetings every 4 weeks (2 wks offset)
- Task planning meetings every 4 weeks (or as needed)
- Technical Advisory Board meeting every 3 months
- Annual face-to-face meeting for reviewing + planning research

Summary I: Status of Strain Pipeline

DISCOVR strain pipeline has been successful in down-selecting top strain for SOT trials

Strain	Temperature + Salinity Tolerance + 16s/18s ID (42 Strains)	LEAPS Photo-Bioreactor Testing (25 Strains)	Outdoor Pond Testing (PAT) (13 Strains)	Outdoor Pond Testing at BETO SOT Testbed (AzCATI) (7 Strains)
Cyanobacterium *AB1* (from Algenol)				
Agmenellum quadruplicatum UTEX2268				
Anabaena sp. ATCC 3308.1				
Arthrospira platensis UTEX3086				
Arthrospira fusiformis UTEX2721				
Chlorella antarctica UTEX 1959				
Chlorella autorophica CCMP243				
Chlorella sorokiniana DOE1044				
Chlorella sorokiniana DOE1116				
Chlorella sorokiniana DOE1412				
Chlorella vulgaris NREL 4-C12				
Chlorella vulgaris LRB-AZ-1201				
Chlorella sp. *UTEX SNO 69*				
Chlorococcum littorale UTEX 117				
Chlorococcum sp. UTEX-B P7				
Chloromonas reticulata CCALA870				
Chloromonas UTEX SNO11				
Coelastrella sp. DOE0202				
Micractinium reisseri NREL 14-F2				
Microchloropsis gaditana CCMP1894				

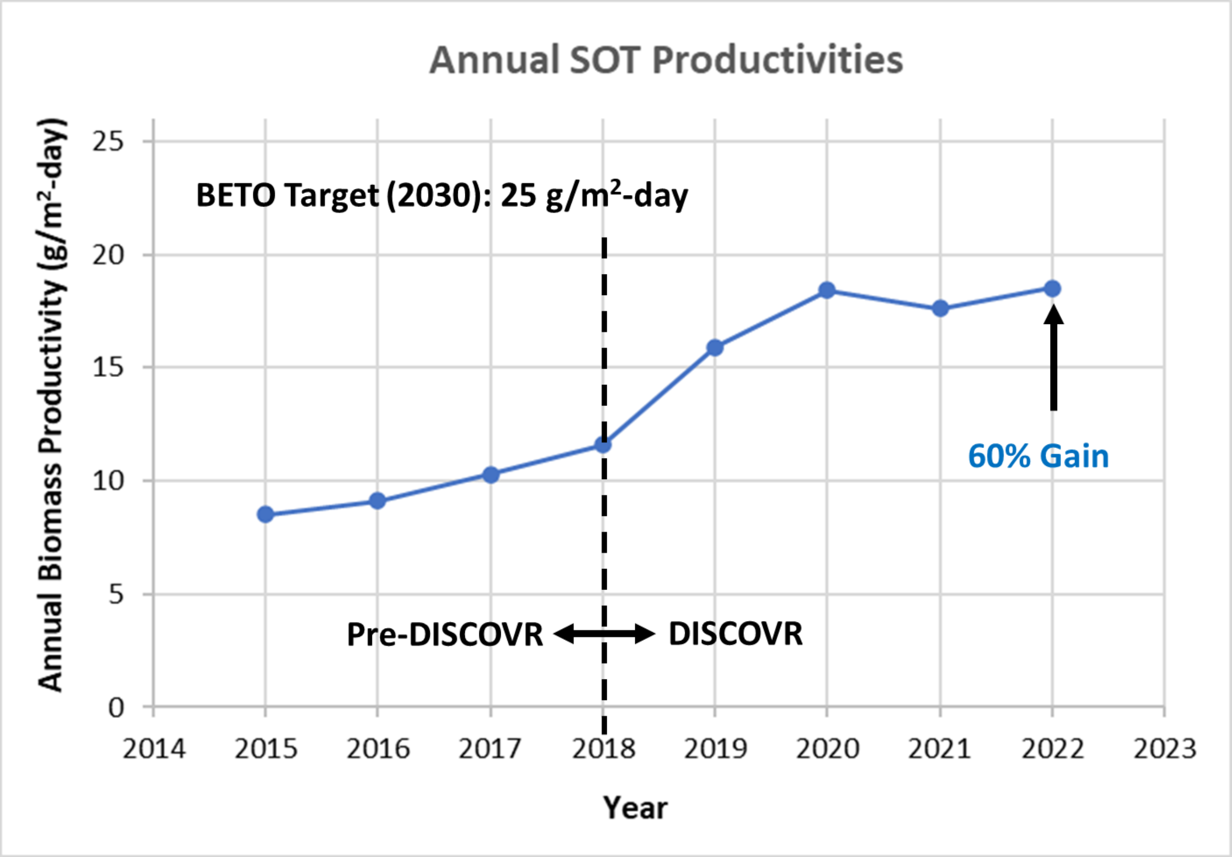
   

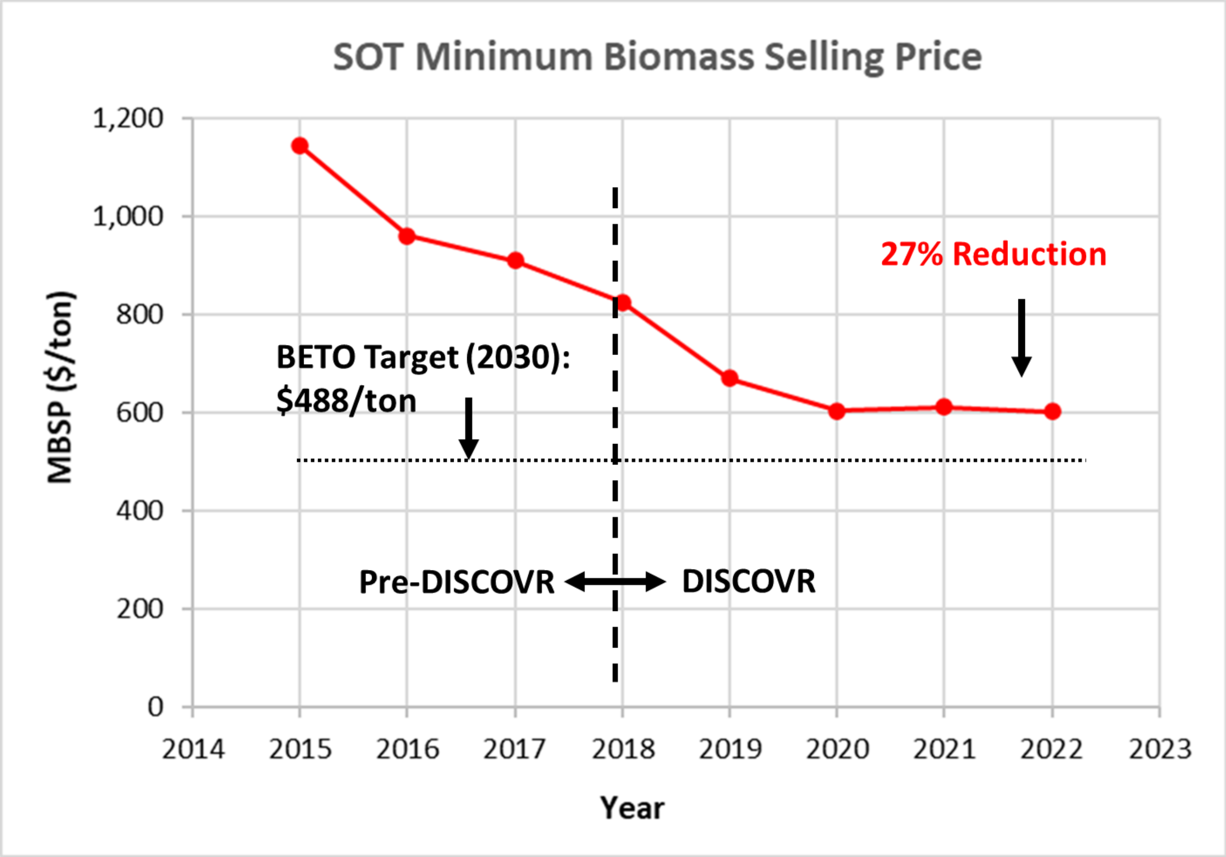
Strain	Temperature + Salinity Tolerance + 16s/18s ID (42 Strains)	LEAPS Photo-Bioreactor Testing (25 Strains)	Outdoor Pond Testing (PAT) (13 Strains)	Outdoor Pond Testing at BETO SOT Testbed (AzCATI) (7 Strains)
Microchloropsis salina CCMP1776				
Monoraphidium sp. MONOR1				
Monoraphidium minutum 26B-AM				
Nannochloropsis oceanica CCAP 849/10				
Oscillatoria cf. priestleyi CCMEE5020.1-1				
Phaeodactylum tricorutum UTEX 646				
Picochlorum renovo NREL39-A8				
Picochlorum celeri TG2-WT-CSM/EMRE				
Picochlorum oklahomensis CCMP2329				
Picochlorum soleocismus DOE101				
Porphyridium cruentum CCMP675				
Scenedesmus acutus LRB-AZ-0401				
Scenedesmus rubescens 46B-D3				
Scenedesmus obliquus DOE0152.z				
Scenedesmus obliquus UTEX393				
Scenedesmus sp. IITRIND2				
Stichococcus minor CCMP819				
Stichococcus minutus CCALA727				
Synechococcus elongatus UTEX2973.1				
Tetraselmis striata LANL 1001				
Tisochrysis lutea CCMP1324				
Tribonema minus UTEXB3156				

DISCOVR Pacific Northwest NATIONAL LABORATORY Los Alamos NATIONAL LABORATORY EST.1943 NREL Transforming ENERGY Sandia National Laboratories INL Idaho National Laboratory AzCATI Arizona Center for Algae Technology and Innovation COLORADO SCHOOL OF MINES

Summary II: Gains in SOT Productivity (2018-2022)

Use of DISCOVR strains resulted in 60% increase in SOT productivity and 27% decrease in MBSP





Summary III: Success Highlights

DISCOVR is BETO's flagship consortium project to rapidly meet productivity and cost targets

- Identified **stable high productivity winter & summer season strains** for deployment at **AzCATI**
- Attained **high productivity *Picochlorum* enrichments** from Gulf of Mexico sampling
- Showed **~100% improvement** in **biomass productivity** using **OptiLum** vs. batch culture baseline
- Demonstrated successful **simulation** of **outdoor compositional shift** in **indoor reactors**
- **Developed countermeasures to parasitic fungi** increasing mean time to failure in crash assays
- The **plant hormone IAA increased** total biomass accumulation **of *P. celeri* by 43%**
- Demonstrated **year-over-year improvements** in AzCATI outdoor algae cultivation **productivity annual average metric,** reaching **18.5 g/m^2-day, exceeding** end of cycle target of **17.1 g/m^2-day**
- Used **TEA modeling to identify key cost drivers** and **guide** future **DISCOVR research priorities**
- **Published 11 papers in *Algal Research* Special Issue DISCOVR + 13 conference presentations**

*Success highlights for 9 additional tasks are shown in the supplemental section

Supplemental Slides Section

Acknowledgements

DISCOVR is a highly collaborative effort with many contributors

- **BETO ALGAE TEAM**
  - Dan Fishman
  - Christy Sterner
  - Phil Lee
- **PNNL**
  - Nathan Beirne
  - Jacob Freeman
  - William Wixson
  - Ray Cabreriza
- **NREL**
  - Stefanie Van Wychen
  - Seth Steichen
  - Foteini Davrazou
- **LANL**
  - Kay Carr
  - Bridget Daughton
- **SNL**
  - Chris Katinas
  - Cameron Kunstadt
  - Pamela Lane
  - Deanna Curtis
  - Elise Wilbourn
  - Wittney Mays
  - Morgan Mackenzie
  - Brooke Davis
  - Jenna Schambach
  - Amanda Barry
- **AzCATI**
  - Henri Gerken
  - Jessica Forrester
  - Everett Eustance
  - Taylor Weiss
  - Jason Potts
  - Emilie Smith
  - Aaron Geels
  - Raafay Jafri
  - Pedro Caballero
  - Richard Malloy
  - AzCATI Undergrads
- **CSM**
  - Galen Dennis

DISCOVR Management: Quad Chart

Budgets, project partners, TRLs, project goals, and end of project milestone

Timeline

- October 1, 2016
- September 30, 2025 (3^{rd} 3 Year Cycle)

	FY22	Total Award
DOE Funding	**$3,275K**	**$9,400K** (FY20 – FY22)

Project Partners (FY22 Funding %)

PNNL: 24%
NREL: 22%
SNL: 20%
LANL: 12%
AzCATI: 17 %
CSM: 4%

TRL at Project Start: 2
TRL at Project End: 4

Project Goals

- Use the DISCOVR Strain Pipeline to deliver **new high-productivity strains** for robust annual outdoor cultivation.
- Test **new concepts** using DISCOVR Strain Pipeline to move the SOT needle via **improvements in biomass productivity, biomass value**, **crop protection and culture stability.**
- Verify performance of strains in outdoor **SOT Trials** at AzCATI.

End of Project Milestone

Employ new strains and novel concepts that were successfully demonstrated in the laboratory to **achieve at least 10% year over year annual productivity improvement (g/m^2/day) at SOT testbed**, relative to the pre-DISCOVR 2018 annual SOT productivity of 11.7 g/m^2/day, and normalized for climate anomalies (**PNNL, LANL, NREL, SNL, AzCATI**).

Funding Mechanism

AOP funding to each respective Lab.
NREL subcontract to ASU. PNNL subcontract to CSM.

DISCOVR Pacific Northwest NATIONAL LABORATORY Los Alamos NATIONAL LABORATORY EST.1943 NREL Transforming ENERGY Sandia National Laboratories INL Idaho National Laboratory AzCATI Arizona Center for Algae Technology and Innovation COLORADO SCHOOL OF MINES

Responses to 2021 Peer Review Comments

Potential media biases were evaluated by measuring growth of top DISCOVR strains in different media

Concerns:

- “.. Bias [in choice of DISCOVR medium] may favor performance of some species over others [in strain pipeline].”
- “Larger productivity gains will likely be necessary to justify the addition of the growth promoting molecules.”
- “Consideration should be given the maximum practical extent to enhance the DISCOVR platform into being able to test with GM strains in open pond environments.”
- “The funneled down selection of strains seems well planned and logical, but it also seems redundant to the NAB consortium effort that launched the BETO AAS program.”

Responses:

- **Unfortunately, the potential for biases in any screening effort is unavoidable. The screening medium was chosen with input and feedback from the Technical Advisory Board as a reasonable approximation for an industrially-relevant cultivation medium. So far, we have explored variations in medium composition on the growth performance of top DISCOVR strains, e.g., *Scenedesmus obliquus* UTEX393, *Picochlorum celeri* TG2-WT-CSM/EMRE, and *Phaeodactylum tricornutum UTEX646*.**
- **Assuming a 30% increase in outdoor productivity in response to 1 µM IAA addition (as was observed in our ePBR experiments) for all 12 months of the year, the overall reduction in MBSP would be 16% and thus more than make up for the additional cost of adding GPMs.**
- **So far, there have been only limited reports of GM strain candidates exhibiting higher biomass productivity with sufficient long-term genomic stability, and therefore pursuit of outdoor deployment has not been an immediate priority for DISCOVR.**
- **Relative to the NAABB project, two innovative strain characterizations are carried out in the DISCOVR project to objectively down-select strains: Tier I screening in gradient incubators for temperature and salinity tolerance, and Tier II screening for seasonal productivities in climate-simulation PBRs.**

Publications (2021-2022)

Results from DISCOVR were widely communicated to algae community

1. Atencio et al., 2022, “Susceptibility of Two Saltwater Strains of *Chlorella sorokiniana* to *Vampirovibrio chlorellavorus*”, *Journal of Applied Phycology*, **34:**81-87, https://doi.org/10.1007/s10811-021-02602-0
2. Beirne et al., 2022, “A streamlined approach for the identification and characterization of promising season-specific microalgae strains”, *Algal Research* – Special Issue DISCOVR, under review.
3. Gao et al., 2022, “DISCOVR Strain Pipeline Tier III Screening: Strain Evaluation in Outdoor Raceway Ponds”, *Algal Research* – Special Issue DISCOVR, https://doi.org/10.1016/j.algal.2023.102990
4. Gao et al., 2022, “Oxygen stress mitigation for microalgal biomass productivity improvement in outdoor raceway system”, *Algal Research* – Special Issue DISCOVR, https://doi.org/10.1016/j.algal.2022.102901
5. Huesemann et al., 2022, “Development of Integrated Screening, Cultivar Optimization, and Verification Research (DISCOVR): A Coordinated Research-Driven Approach to Improve Microalgal Productivity, Composition, and Stability for Commercially Viable Biofuels Production”, *Algal Research* – Special Issue DISCOVR, https://doi.org/10.1016/j.algal.2022.102961
6. Huesemann et al., 2022, “DISCOVR Strain Pipeline Tier I Screening: Maximum Specific Growth Rate as a Function of Temperature and Salinity for 38 Candidate Microalgae Strains for Biofuels Production”, *Algal Research* – Special Issue DISCOVR, https://doi.org/10.1016/j.algal.2023.102996
7. Huesemann et al., 2022, “DISCOVR Strain Pipeline Tier II Screening: Winter and Summer Season Areal Productivities and Biomass Compositional Shifts in Climate-Simulation Photobioreactor Cultures”, *Algal Research* – Special Issue DISCOVR, **102948,** https://doi.org/10.1016/j.algal.2022.102948
8. Klein et al., 2022, “Quantifying the intrinsic value of algal biomass on a multi-product biorefining strategy”, *Algal Research* – Special Issue DISCOVR, under review.
9. Krishnan A., et al. 2021, "*Picochlorum celeri* as a model system for robust outdoor algal growth in seawater", *Scientific Reports*, **11** (No: 11649), https://doi.org/10.1038/s41598-021-91106-5
10. Lane, T.W., 2022, “Barriers to microalgal mass cultivation” *Current Opinion in Biotechnology*,**20273:**323-328.
11. Lane et al., 2022, “Resistance of DISCOVR algae strains to deleterious species”, *Algal Research* – Special Issue DISCOVR, 66: 102793, https://doi.org/10.1016/j.algal.2022.102793
12. McGowen et al., 2022, “Outdoor annual algae productivity improvements at the pre-pilot scale through crop rotation and pond operational management strategies”, *Algal Research* – Special Issue DISCOVR, https://doi.org/10.1016/j.algal.2023.102995
13. Negi et al., 2022, “Effect of plant growth promoting molecules on improving biomass productivities in DISCOVR production strains”, *Algal Research* – Special Issue DISCOVR, under review.
14. Poorey et al., 2022, “Characterizing industrial pond ecology timeline in DISCOVR cultivation trials for early detection of pond crashes”, *Algal Research* – Special Issue DISCOVR, under review.

Conference Presentations (2021-2022)

Results from DISCOVR were widely communicated to the algae community

1. Edmundson, S.J., N. Beirne, S. Gao, J Freeman, and M.H. Huesemann, et al. “Characterizing Microalgae Strains for Biomass Productivity Under Climate Simulation Conditions”, Algae Biomass Virtual Summit, October 2022.
2. Huesemann M.H., S.J. Edmundson, S. Gao, T. Dale, S. Negi, C.K. Carr, and L. Laurens, et al. "Development of Integrated Screening, Cultivar Optimization, and Verification Research (DISCOVR): A Coordinated Research-Driven Approach to Improve Microalgal Productivity, Composition, and Stability for Commercially Viable Biofuels Production." Invited presentation by M.H. Huesemann at Algal Biomass, Biofuels, and Bioproducts, Online Conference, June 2021.
3. Huesemann, M.H., S.J. Edmundson, S. Gao, T. Dale, S. Negi, C. Carr, L. Laurens, P. Pienkos, E. Knoshaug, R. Davis, B. Klein, T. Lane, J. Timlin, K. Poorey, T. Reichardt, A. Barry, C. Smallwood, J. McGowen, T. Weiss, H. Gerken, J. Forrester, and M. Gonzales, "DISCOVR: Development of Integrated Screening, Cultivar Optimization, and Verification Research." Algae Biomass Virtual Summit, October 2021.
4. Huesemann, M.H., S.J. Edmundson, S. Gao, T. Dale, S. Negi, C. Carr, L. Laurens, P. Pienkos, E. Knoshaug, R. Davis, B. Klein, T. Lane, J. Timlin, K. Poorey, T. Reichardt, A. Barry, C. Smallwood, J. McGowen, T. Weiss, H. Gerken, J. Forrester, M. Gonzales, "DISCOVR: Development of Integrated Screening, Cultivar Optimization, and Verification Research." AIChE 3rd Bioenergy Sustainability Online Conference, December 2021.
5. Huesemann, M.H., S.J. Edmundson, S. Gao, T. Dale, S. Negi, L. Laurens, E. Knoshaug, R. Davis, B. Klein, T. Lane, J. Timlin, C. Smallwood, J. McGowen, and M. Posewitz, "DISCOVR: Development of Integrated Screening, Cultivar Optimization, and Verification Research." Algae Biomass Virtual Summit, October 2022.
6. Lane, T.W., J. Timlin, T. Reichardt, C.L. Fisher, K. Reese, M. Moorman, and P.D. Lane “Towards integrated pest management for algal production systems” Algae Biomass Virtual Summit, October 2022.
7. McGowen, J. et al., “Algae Cultivation at the Arizona Center for Algae Technology and Innovation – Generating Data to Support Algal TEA/LCA and the Broader Modeling Community” Algae Biomass Summit, October 2022.
8. McGowen, J. et al., “Multi-Year Cultivation Trials to Inform the State of Technology (SOT) for DISCOVR” Algae Biomass Summit, October 2022
9. McGowen, J. et al., “Advancing the State of Technology in Algae R&D” Algal Biomass, Biofuels and Bioproducts, June 2021.
10. Negi, S., Carr C. K., Daughton, B., McGowen, J., Klein B. C., Davis, R. and T. Dale, “Effect of phytohormones on improving biomass productivities in algal production strains”, Algal Biomass, Biofuels, and Bioproducts, Online Conference, June 2021.
11. Negi, S., Carr C. K., Daughton, B., Klein B. C., Davis, R., Banerjee., S., and T. Dale, “Effect of phytohormones on improving biomass productivities in algal production strains”, Algae Biomass Virtual Summit, October 2022.
12. Posewitz, M. et al. “*Picochlorum*: model systems for robust outdoor growth in marine media” Algae Biomass Virtual Summit, October 2022.
13. Wixson, W.C., S.J. Edmundson, and M.H. Huesemann. “Extraction of Protein from *Picochlorum celeri* and other algal feedstocks”, Algae Biomass Virtual Summit, October 2022.

DISCOVR Diversity, Equity, and Inclusion (FY22 Activities)

A wide range of DEI accomplishments were achieved in FY22

PNNL Accomplishments

We hired two SULI summer interns for workforce development. One intern was a Hispanic and a science teacher at a high school in Florida, serving primarily minorities. After return to Florida, our intern is volunteering to serve as a PNNL STEM ambassador to attract students from his minority serving high school to pursue science and engineering careers. Michael Huesemann served as a judge for reviewing proposals submitted by students, including many from minority serving institutions, to the BETO Algae Prize competition. Scott Edmundson participated as a panelist discussing careers in science during a workshop for educational professionals, a total of nine teachers representing 5 school districts across the Olympic Peninsula were present. Scott's talk was a personal story of his own unique career path and how to inspire K-12 students to consider a career in science, technology, engineering, and mathematics (STEM). Scott Edmundson also presented PNNL's algal research to a group of local community members, including local tribal representatives: Chairman W. Ron Allen, Chairman, Jamestown S'Klallam Tribe, Chairman Timothy Greene, Sr., Chairman, Makah Tribe. In addition, Scott Edmundson gave an interactive classroom lecture on algal and marine biology to the fifth-grade class at the Sunfield Farm and School in Port Hadlock, Washington. The enthusiastic class was eager to learn about some amazing algae, draw algal cells, and ponder the many different materials that can be made with algal biomass (shoes, cements, plastics, fuels, etc.). Students also admired and were excited by the incredible biological diversity of the algae. The report from the class was the next day many students had decided to become marine biologists.

LANL Accomplishments

This year, we participated in the Joint Science and Technology Institute West (JSTI), a summer camp for high school students that inspires and encourages them to pursue careers in science. During this summer camp, Taraka Dale and Sangeeta Negi gave talks and tours of related algae work displayed at the Bradbury Museum (Los Alamos) to engage students in current and future applications of microalgal biofuels and biomaterials. We also participated in a teaching class for the students to identify several microalgal strains using microscopy. This year we also hired a female student for our DISCOVR team to increase LANL's female and underrepresented R&D population.

NREL Accomplishments

The data dissemination completion delivers on the NREL DEI milestone. When the final data sets for FY22 are available, including the biomass composition, the online repository will be completed and become a resource for a data mining community.

SNL Accomplishments

We hired a minority summer student intern from a HBCU. In addition, we hired a female summer student intern and anticipate re-hiring next calendar year as a technologist during her "gap year" prior to attending grad school. This will serve to increase the gender diversity at Sandia and extend workforce development opportunities.

Tie-In BETO Projects

WBS #s of projects that tie into the inputs and outputs of DISCOVR

Tie-Ins	WBS #s
Resource Assessment & Environmental Simulation	1.3.4.101
Biomass Characterization	1.3.2.001
Productivity Improvement	1.3.1.120, 1.3.2.610, 1.3.2.103
Pond Crashes & Signatures	1.3.2.631, 1.3.1.103
Testbeds	1.3.5.101
Genome Sequencing	1.3.1.600, 1.3.1.111, 1.3.2.110
Molecular Toolboxes	1.3.1.112, 1.3.1.100, 1.3.1.001
Biomass Storage	1.3.3.100
Biomass Conversion	1.3.4.201, 1.3.5.202
Nutrient Recycling	1.3.5.203
State of Technology	1.3.5.200, 1.3.1.200
CO_2 Utilization	1.3.2.601
Integrated Process Improvements	1.3.5.302, 1.3.5.270, 1.3.4.288

Approach: Assess *Picochlorum* Consortium Productivities

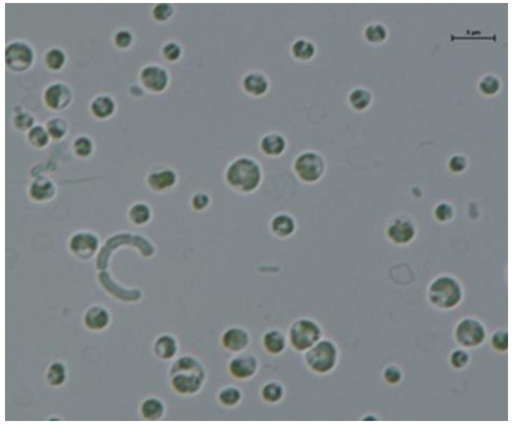
Enrichment of Picochlorum consortium from Gulf of Mexico

- **Background/history:** *Picochlorum celeri* TG2 gave maximal productivities during the summer 2020 AzCATI outdoor campaigns. *Picochlorum celeri* TG2 productivities were lower during the 2021 growth campaigns. A possible explanation is that weather/conditions were more adverse during 2021. One hypothesis is that a consortium of several *Picochlorum* cultivars may be more competitive to dynamic outdoor conditions relative to a single clonal monoculture.

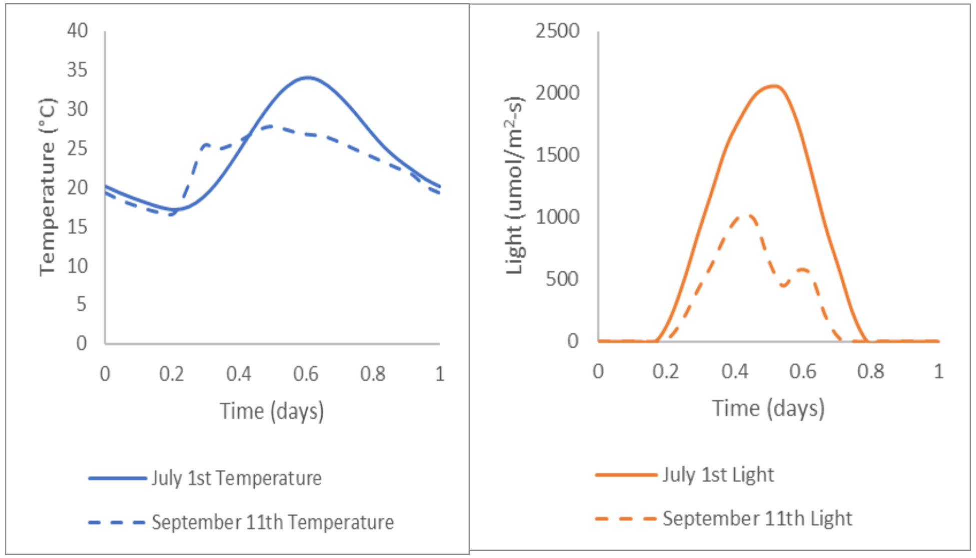
- **Objective:** Enrich *Picochlorum* consortia in bioreactors using pond scripts. Use dynamic “weather front” scripts (July 1 followed Sept 11) in PNNL LEAPS bioreactors to test whether Gulf of Mexico consortium diversity is a productivity/stability asset relative to monoclonal *Picochlorum celeri* TG2.

- **Challenges:**
  1. *Picochlorum* isolates to date are very similar. PCR primers were developed to specifically distinguish TG2 from other *Picochlorum* isolates.
  2. Establishing light/temperature scripts that relate to outdoor challenges.

- **Economic/Technical Metrics:** Areal biomass productivities ($g/m^2/d$).



Consortium enrichment



“Weather front” LEAPS bioreactor scripts

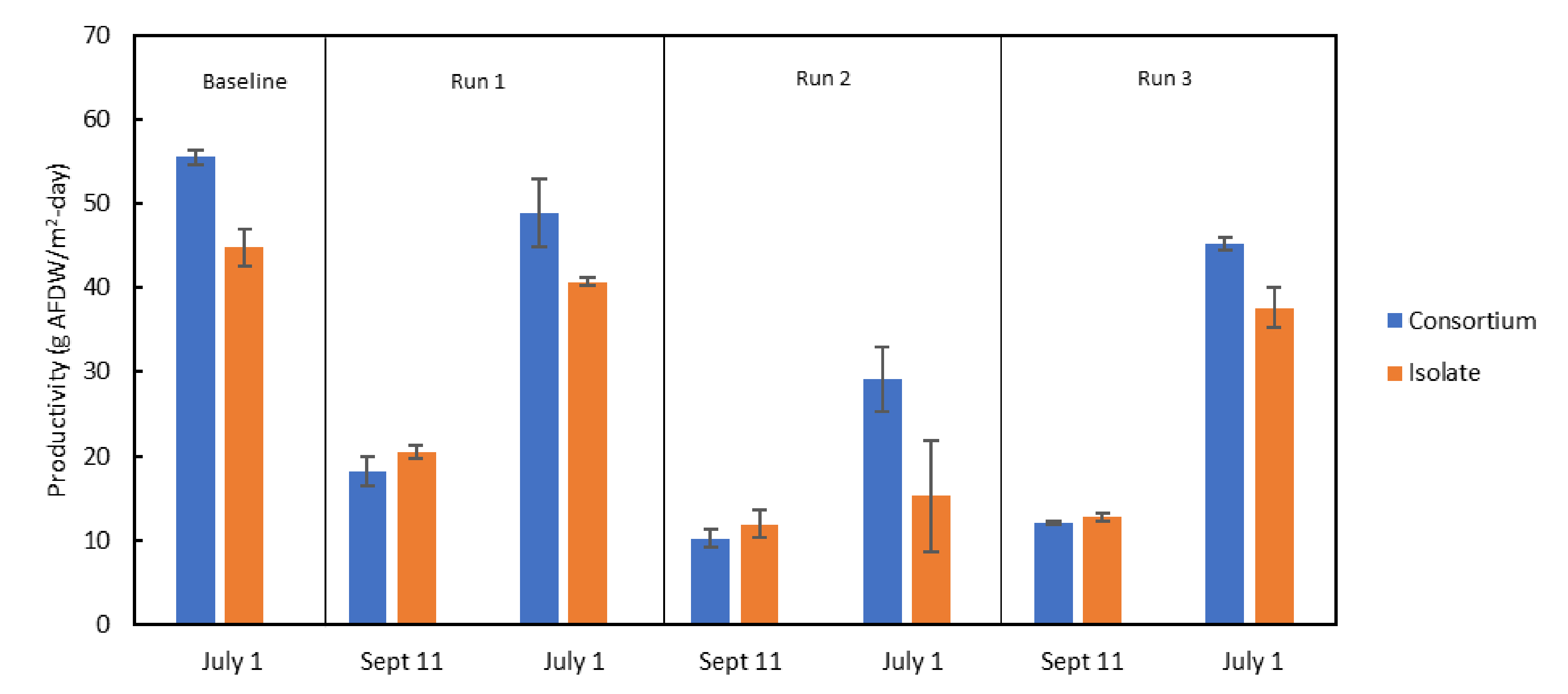
Outcomes: Assess *Picochlorum* Consortium Productivities

Pipeline

***Picochlorum* consortium in LEAPS reactors outperformed *P. celeri* TG2 using dynamic diel days**

Gulf of Mexico *Picochlorum* Consortia (Texas) wrt TG2

- Enriched *Picochlorum* consortia from Texas coast had exemplary productivities ranging 45-55 g m^{-2} d^{-1} on most favorable days
- Consortium productivities “rebounded” to higher productivities (July 1) following adverse diel script (Sept 11)
- Similar productivities under unfavorable diel scripts (Sept 11)



Productivities Cycling Between Favorable/Unfavorable Diel Scripts

Impact: Assess *Picochlorum* Consortium Productivities

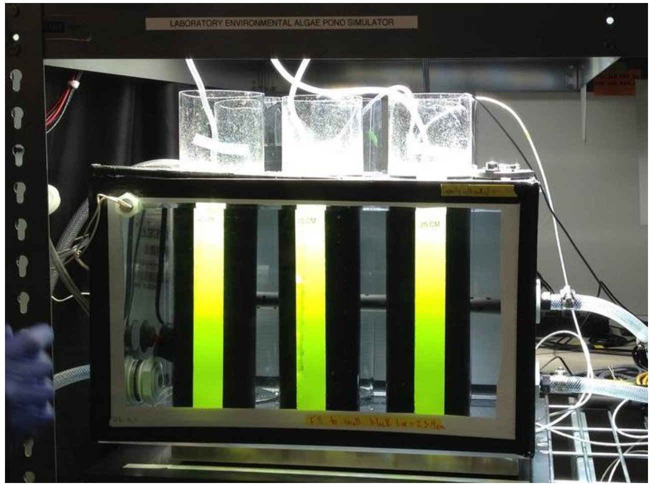
Pipeline

Picochlorum consortium in LEAPS reactors outperformed P. celeri TG2 using dynamic diel days

- *Picochlorum* consortium enrichment from Texas coast was highly productive in LEAPS bioreactors
- Relative to TG2, consortia recovered to higher July 1 productivities following suboptimal Sept 11 scripts

Impact

- **High-productivity *Picochlorum* consortium attained and tested in LEAPS growth campaign (Texas Gulf consortium)**
- **Testing underway to determine if diversity within a consortium is an asset relative clonal isolate**



PNNL LEAPS Photobioreactor

Invited Speaker at 2022 Algae Biomass Summit (DISCOVR Special Session)

Posewitz et al. (2022), "*Picochlorum*: Model systems for robust outdoor growth in marine media".

DISCOVR · Pacific Northwest National Laboratory · Los Alamos National Laboratory EST. 1943 · NREL Transforming ENERGY · Sandia National Laboratories · Idaho National Laboratory · AzCATI Arizona Center for Algae Technology and Innovation · Colorado School of Mines

Approach: Binary Cultivation of DISCOVR strains

Moving algae cultivation beyond monocropping to improve biomass productivity

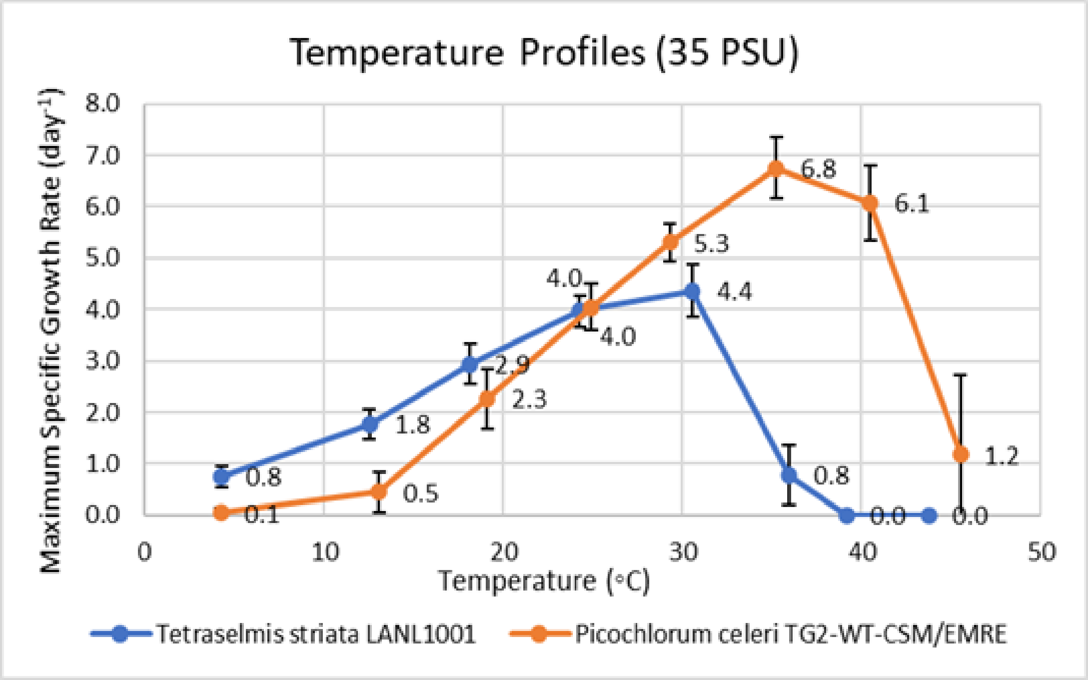
New Concepts

- **Background/history:**
  - DISCOVR algal strains passing Tiers I, II, and III were considered as strains of interest for binary/companion cultivation.
  - Strains with complimentary temperature tolerance profiles increase the cultures functional diversity, which may allow for greater resilience and productivity across seasons (see temperature tolerance figure).

- **Objective:** Assess binary cultivation of top-performing DISCOVR strains for allelopathic effects.

- **Challenges:** Strain stability: binary/companion cultures must thrive in the same medium (e.g., salinity) and be non-allelopathic.

- **Economic/Technical Metrics:** Biomass productivity



Temperature tolerance profiles of *Tetraselmis striata LANL1001 and Picochlorum celeri TG2*

DISCOVR Pacific Northwest National Laboratory Los Alamos National Laboratory EST.1943 NREL Transforming ENERGY Sandia National Laboratories INL Idaho National Laboratory AzCATI Arizona Center for Algae Technology and Innovation Colorado School of Mines MINES

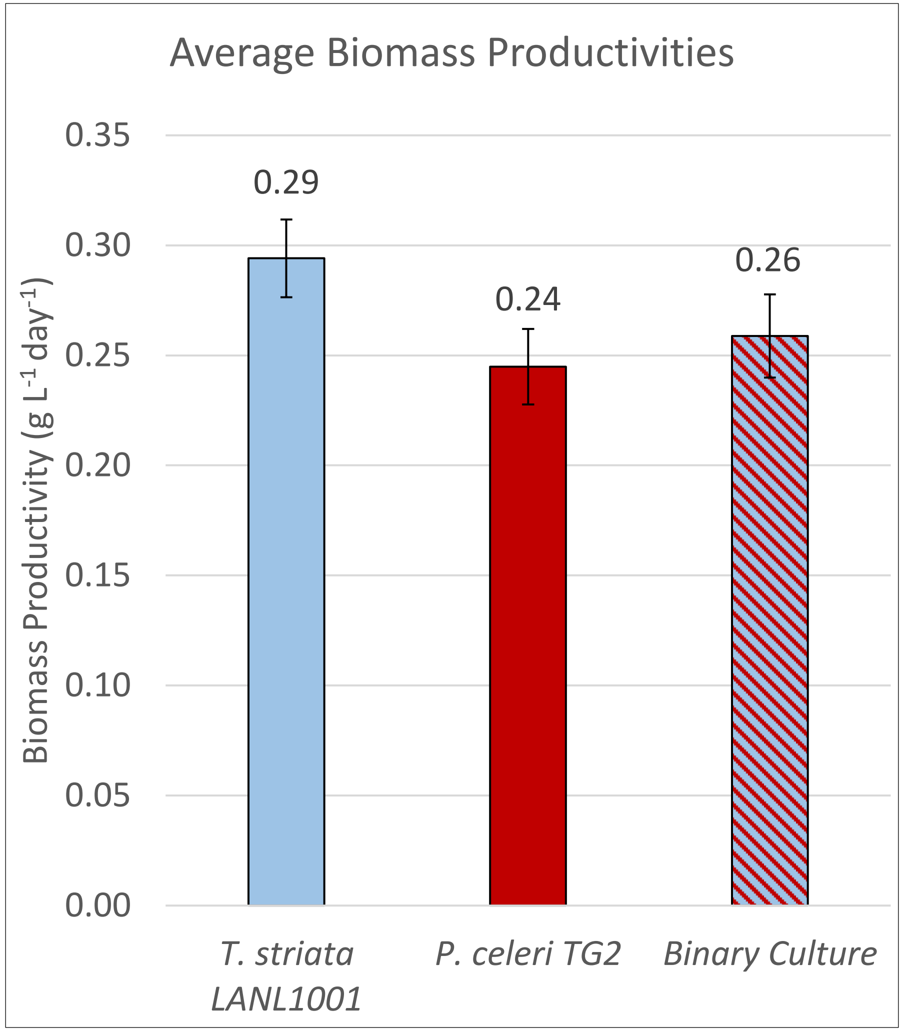
Outcomes: Binary Cultivation of DISCOVR strains

Top DISCOVR marine strains P. celeri and T. striata were compatible in binary culture

New Concepts

- We assessed the compatibility of the top marine winter strain *Tetraselmis striata* LANL1001 and the top marine summer strain *Picochlorum celeri* TG2
  - Binary cultures remained stable after multiple dilution cycles
  - **No indication of allelopathic effects were observed**

Strain of Interest	Salinity tolerance (PPT)	Ideal Temperature Range (ºC)	Outdoor cultivation	Challenges	Strengths
Picochlorum celeri TG2	5-100	25 – 40	Yes	Poor harvestability, Poor growth in winter	Excellent summer biomass productivity
Tetraselmis striata LANL1001	15-75	10 – 30	Yes	Cell aggregation, moderate growth in summer	Excellent cold tolerance



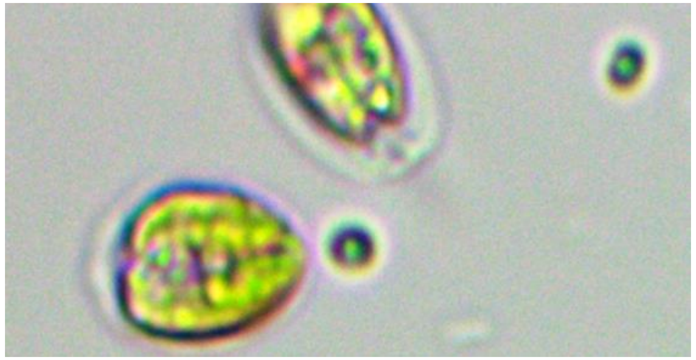
DISCOVR Pacific Northwest NATIONAL LABORATORY Los Alamos NATIONAL LABORATORY EST. 1943 NREL Transforming ENERGY Sandia National Laboratories INL Idaho National Laboratory AzCATI Arizona Center for Algae Technology and Innovation COLORADO SCHOOL OF MINES

Impact: Binary Cultivation of DISCOVR strains

Companion cropping of top DISCOVR marine strains will likely improve annual productivity

New Concepts

- We determined that *Picochlorum* and *Tetraselmis* are likely to make excellent companion crops.



The large, oblong cells of *Tetraselmis striata* LANL1001 and the small, round cells of *Picochlorum celeri* TG2 growing together.

Impact

- Crop rotation is critical to maximizing annual biomass productivities for an algae farm. Crop rotation works best if the strains grow at the same salinity and are not biologically antagonistic.
- A *Picochlorum* and *Tetraselmis* companion crop will likely be more reliable through transition seasons eliminating periods of low productivity or pond downtime for strain transitions, which will improve annual biomass productivity and reduce MBSP.

- **Manuscript in preparation, pending successful follow-on results:**
  - Draft title: "High-productivity Algal Companion Cropping for Sustained Marine Biomass Productivities"

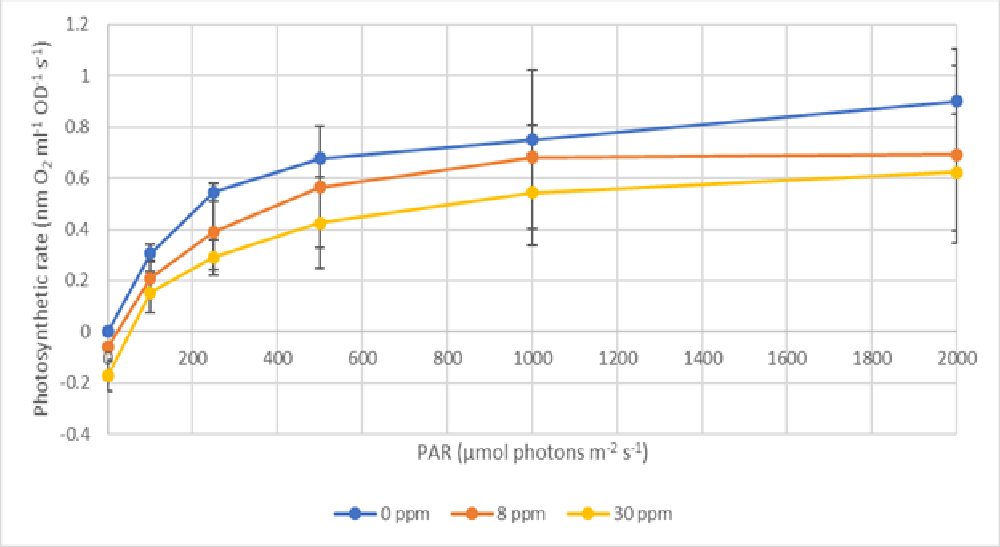
DISCOVR Pacific Northwest NATIONAL LABORATORY Los Alamos NATIONAL LABORATORY EST. 1943 NREL Transforming ENERGY Sandia National Laboratories INL Idaho National Laboratory AzCATI Arizona Center for Algae Technology and Innovation COLORADO SCHOOL OF MINES

Approach: Air Sparging to Remove Inhibitory Dissolved O_2

Increase biomass productivity of UTEX393 by removing inhibitory dissolved oxygen

New Concepts

- **Background/history:** Dissolved O_2 inhibits Photosynthesis of *Acutodesmus obliquus* UTEX393
  - *P-I curve measurements indicated that photosynthetic oxygen production rates for UTEX393 decreased with increasing dissolved oxygen concentration (DO).*
  - *Air-sparging to strip dissolved oxygen from ponds may reduce inhibition and increase biomass productivity.*



- **Objectives:** Sparge raceways (at PAT) from 6 am to 6 pm with air (1%v/v/min) to remove inhibitory dissolved photosynthetic oxygen and increase biomass productivity.

- **Challenges:**
  1. **Air-sparging of large-scale ponds may be cost-prohibitive and increases MBSP.**
  2. **Need to conduct TEA trade-off analysis.**

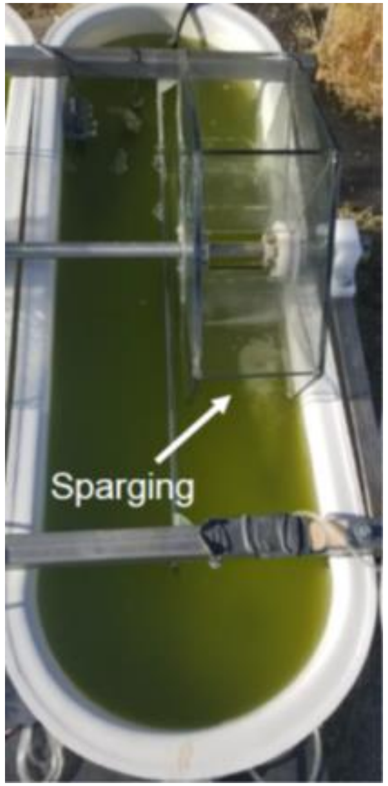
- **Economic/Technical Metrics: Increase biomass productivity** of DISCOVR strains subject to inhibition by photosynthetic O_2 **by ≥20%.** Impact on MBSP needs to be determined by TEA.

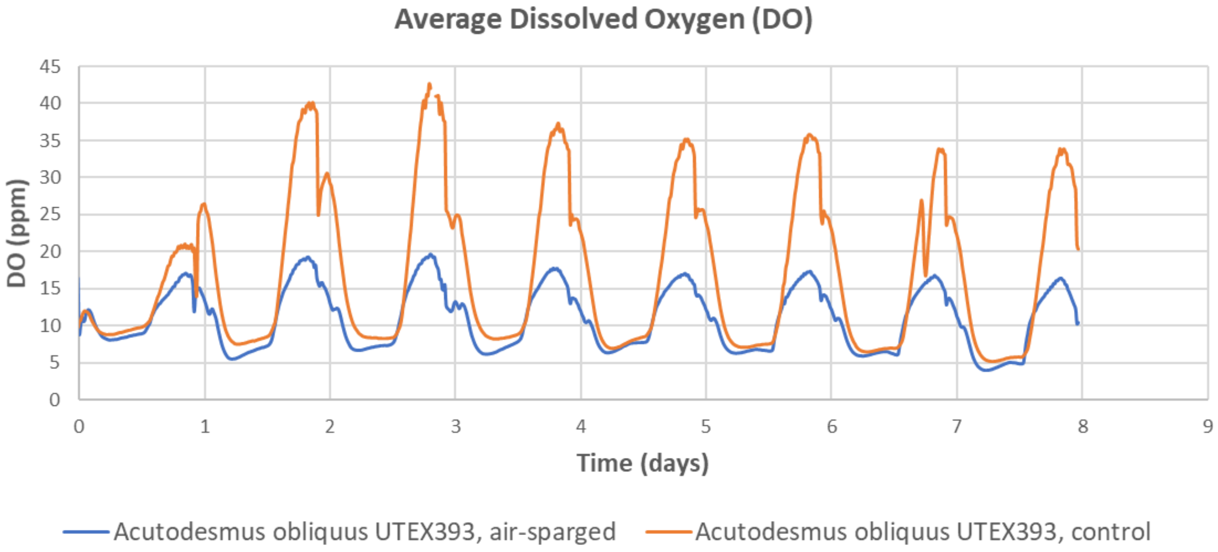
DISCOVR | Pacific Northwest National Laboratory | Los Alamos National Laboratory EST. 1943 | NREL Transforming ENERGY | Sandia National Laboratories | INL Idaho National Laboratory | AzCATI Arizona Center for Algae Technology and Innovation | Colorado School of Mines

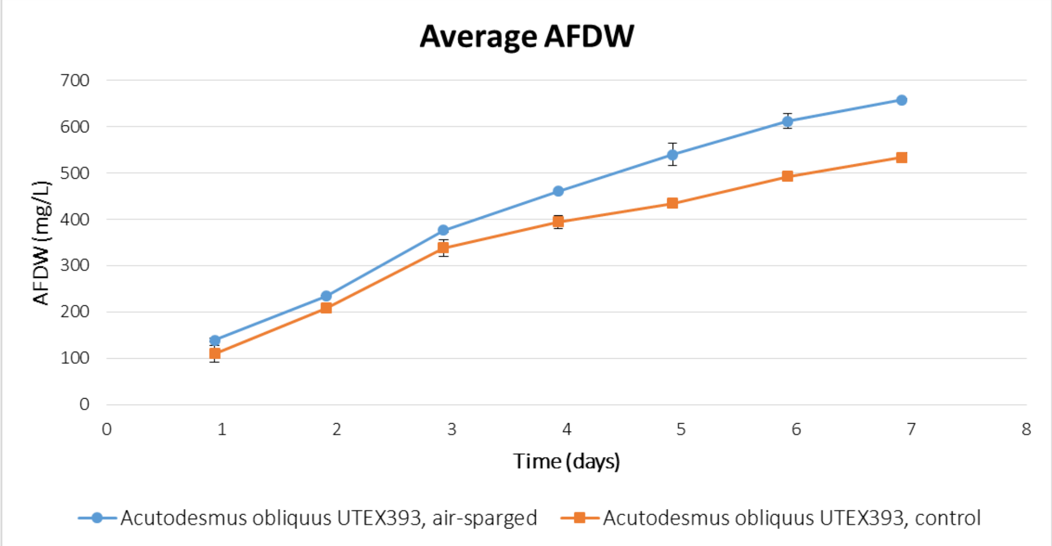
Outcomes: Air Sparging to Remove Inhibitory Dissolved O_2

New Concepts

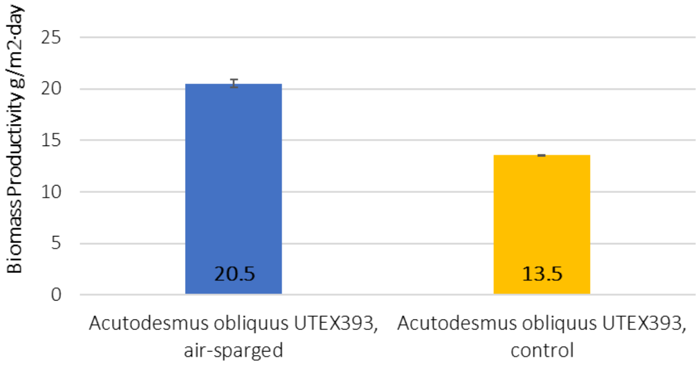
Sparging UTEX393 cultures decreased DO < 20 mg/L + increased productivity by 36%







- Increase in biomass productivity by 36%



Impact: Air Sparging to Remove Inhibitory Dissolved O_2

Sparging UTEX393 cultures increased productivity by 36%, exceeding Go/No-Go

New Concepts

For some DISCOVR strains, inhibition of photosynthesis by elevated dissolved oxygen concentrations can be reduced or avoided by sparging pond cultures with air, stripping excess O_2.

Impact

Air sparging of outdoor pond cultures of *Acutodesmus obliquus* UTEX393 had the following impacts:

- Dissolved oxygen concentrations decreased below inhibitory levels (<20 mg/L)
- Biomass productivity increased by 36% relative to non-sparged controls
- **The GNG-1 Milestone "Improve Biomass Productivity by 20% Relative to Control" was exceeded and therefore met.**

Publication

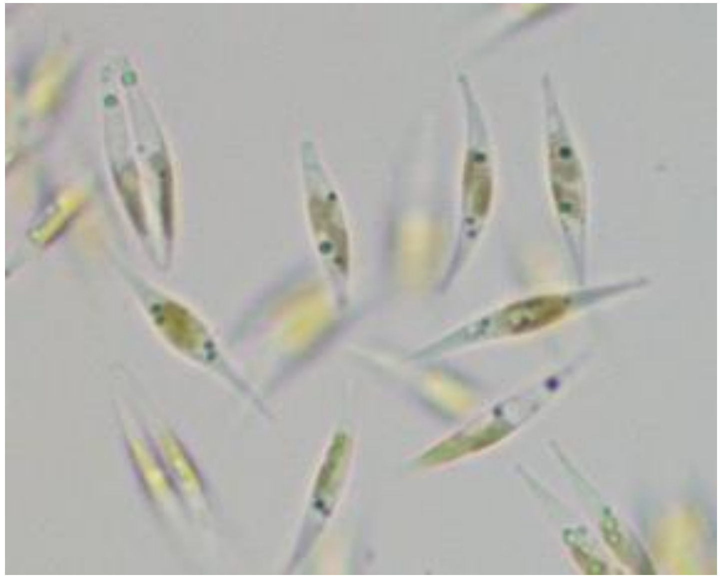
Gao, Edmundson, Huesemann, 2022, "Oxygen stress mitigation for microalgal biomass productivity improvement in outdoor raceway ponds", *Algal Research* – Special Issue DISCOVR, **68**, 102901 https://doi.org/10.1016/j.algal.2022.102901

Approach: Maximizing Algal Growth

Exploring Medium Additions that Sustain Higher Growth Rates

New Concepts

- **Background/history:**
  - *Phaeodactylum tricornutum* UTEX646, which passed the DISCOVR Tiers I, II, and III, is an intriguing strain with salinity and cold tolerance and demonstrated high areal biomass productivity in outdoor field studies.
  - During the screening of this strain, growth was inconsistent. We thus explored the addition of vitamins and found that the addition of vitamins singly or in combination changed the observed maximum specific growth rate.
- **Objective:** Assess vitamin additions on the growth rate of *Phaeodactylum tricornutum* UTEX646.
- **Challenges:** Strain stability: vitamin additions may increase strain growth.
- **Technical Metrics:** Growth rate



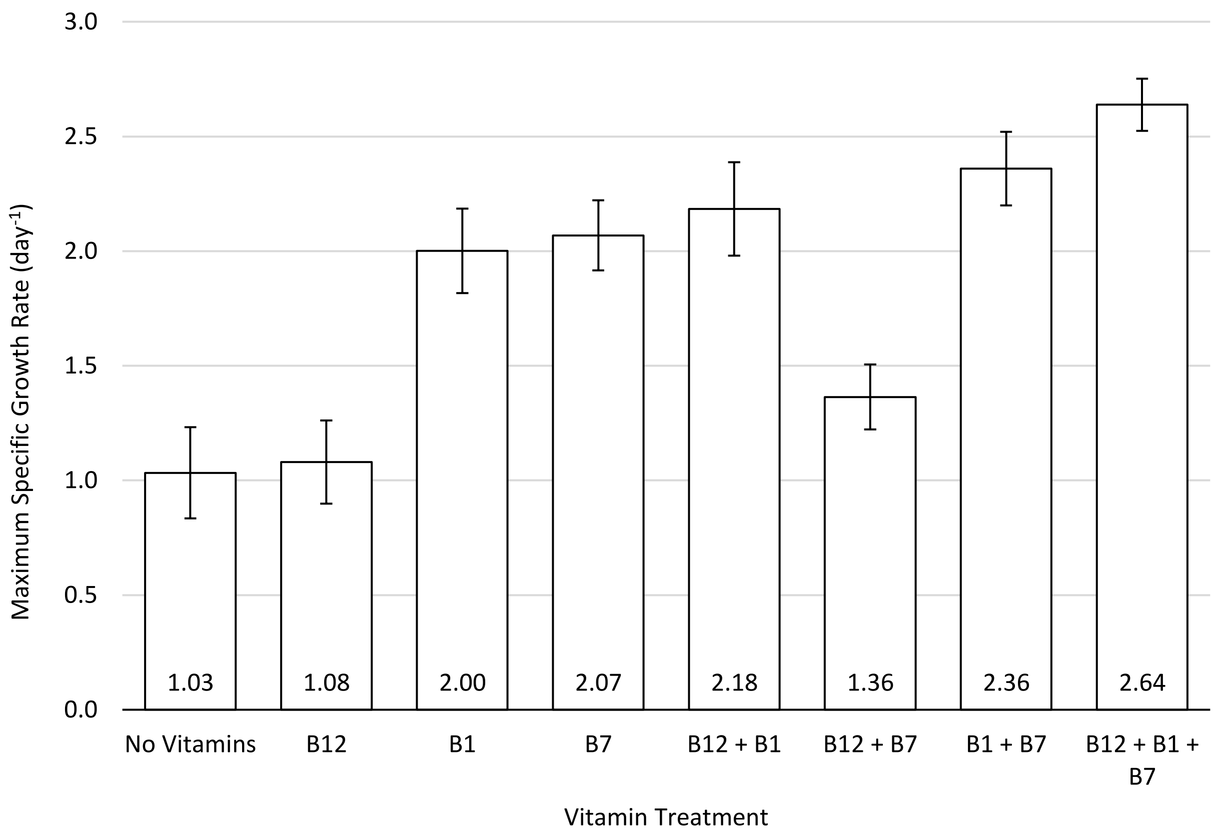
Cells of the diatom *Phaeodactylum tricornutum* UTEX646

Outcomes: Maximizing Algal Growth

Vitamin additions significantly impact the growth rate of a top DISCOVR strain

New Concepts

- We observed stable growth rates with no vitamin additions, indicating that this strain is truly autotrophic.
  - The addition of vitamin B12 alone did not significantly reduce or improve the observed growth rate.
  - The addition of vitamin B1 and vitamin B7 both significantly improved the growth rate of *Phaeodactylum tricornutum* UTEX646.
  - Adding all three vitamins had a synergistic impact that was greater than the expected benefit from single vitamin additions.



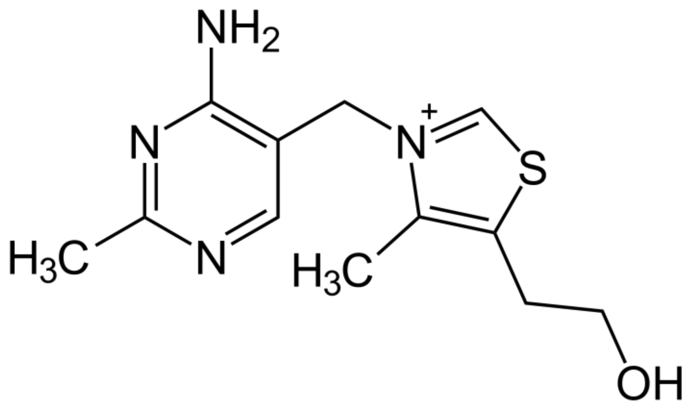
Maximum specific growth rate of Phaeodactylum tricornutum UTEX 646 as a function of different vitamin additions, singly or in combination, plus control (no vitamins).

Impact: Maximizing Algal Growth

Vitamin additions to top DISCOVR strains may significantly improve biomass productivity

New Concepts

- We assessed vitamin additions on the growth rate of *Phaeodactylum tricornutum* UTEX646.
- Vitamin additions can increase the specific growth rate of *Phaeodactylum tricornutum* UTEX646 by greater than **150%**



Thiamine, vitamin B_1, a simple chemical compound with a significant impact on the growth rate of P. tricornutum UTEX646

Impact

This discovery raises interesting scientific questions:

- If vitamin impacts can provide such a significant boost to this "autotrophic" organism, could vitamin additions to other strains previously assumed to not require them improve growth?
- Does this growth improvement translate to productivity improvements?
- Can bacteria that produce these vitamins be co-cultivated?

Manuscript in preparation:

Edmundson, Freeman, et al. (2023), "Beyond Vitamin Requirements for *Phaeodactylum tricornutum* UTEX 646", *Manuscript in preparation.*

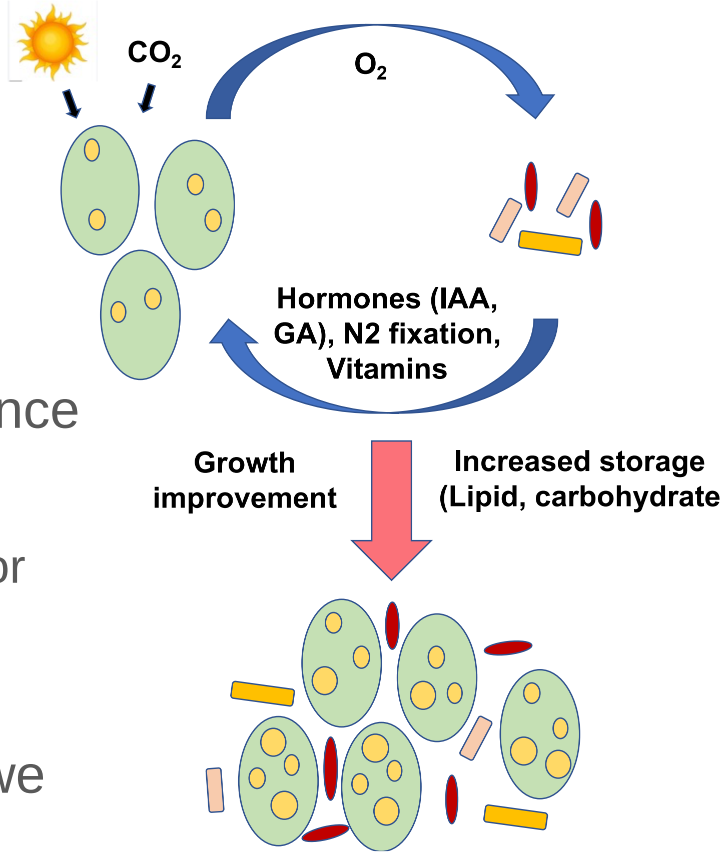
DISCOVR | Pacific Northwest National Laboratory | Los Alamos National Laboratory EST.1943 | NREL Transforming ENERGY | Sandia National Laboratories | INL Idaho National Laboratory | AzCATI Arizona Center for Algae Technology and Innovation | Colorado School of Mines

Approach: Bacteria/hormone interactions with SOT strains

Improving biomass productivity, quality and resilience

New Concepts

- **Background/history:** We see clear biomass productivity improvements with hormone supplementation in the ePBRs. Bacteria that produce Growth Promoting molecules (GPMs) have the potential to boost growth and improve climate resilience
  - Bacteria are shown in plants and algae to improve yield
  - Bacterial application is less expensive, and self replicating



- **Objective:** Examine growth of DISCOVR algae strains in presence of known plant growth promoting bacteria to improve productivity and resilience

- **Challenges:**
  1. Growth effects of bacteria may be influenced by complex environment outdoor (native microbes, pests, grazers)
  2. Maintaining stable bacteria population throughout the cultivation

- **Economic/Technical Metrics:** Realize the 20% increase in productivity we have seen indoors, or at least a 15% increase in carbon storage/biomass quality or 20% increase in salinity tolerance

DISCOVR | Pacific Northwest National Laboratory | Los Alamos National Laboratory | NREL Transforming ENERGY | Sandia National Laboratories | INL Idaho National Laboratory | AzCATI Arizona Center for Algae Technology and Innovation | Colorado School of Mines

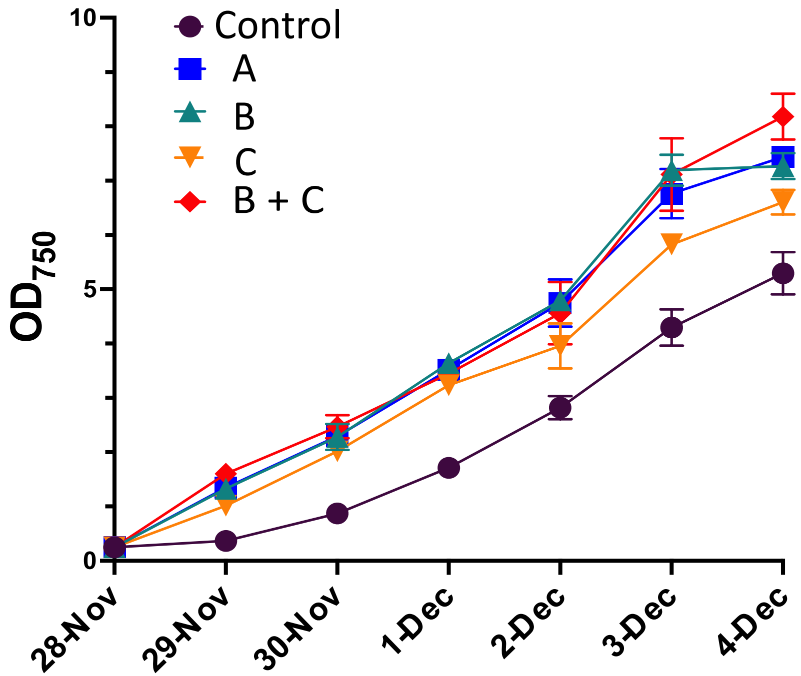
Outcomes: Using Beneficial bacteria to Boost Productivity

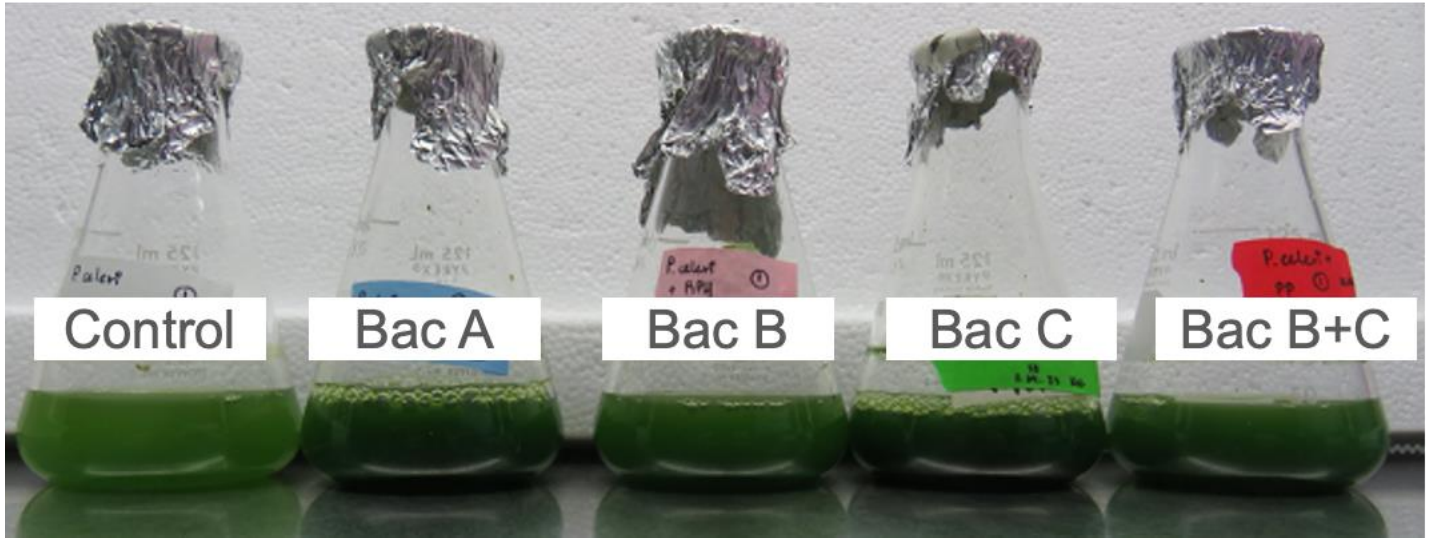
We are screening beneficial bacteria to improve growth

New Concepts

Effects of selected bacteria tested in 2 DISCOVR SOT strains

- Algae SOT strains tested: *T. striata* and *P. celeri* using SOT media
- 6 bacterial strains screened for growth promotion under 1% CO_2 supplementation conditions
- Identified 3 beneficial bacterial strains that improve growth in two DISCOVR SOT strains





Culture density at the end of the experiment

DISCOVR | Pacific Northwest National Laboratory | Los Alamos National Laboratory | NREL Transforming ENERGY | Sandia National Laboratories | INL Idaho National Laboratory | AzCATI Arizona Center for Algae Technology and Innovation | Colorado School of Mines

Impact: Using Beneficial bacteria to Boost Productivity

New Concepts

Beneficial bacteria have the potential for improving productivity

New approaches/ideas need to be tested to determine feasibility of application in outdoor ponds

- DISCOVR SOT strains tested with selected hormone producing beneficial bacteria for the first time

Impact

Successful cultivation of beneficial bacteria with DISCOVR strain has the potential to:

- **Increase productivity**
- **Increase algae carbon storage (lipids, carbohydrates)**
- **Increased resilience (salinity and pH tolerance)**

Next: Bacterial populations will be tracked (using 16S sequencing) at the end of the experiment, and outdoor inoculum from AzCATI ponds will be used to evaluate the impact of native microbes on the efficiency of beneficial bacteria on DISCOVR strains. Promising results could be further tested under more outdoor relevant conditions in outyears.

Approach: Continuous/Automated Cultivation

Increasing biomass productivity using Turbidostat mode in ePBRs

New Concepts

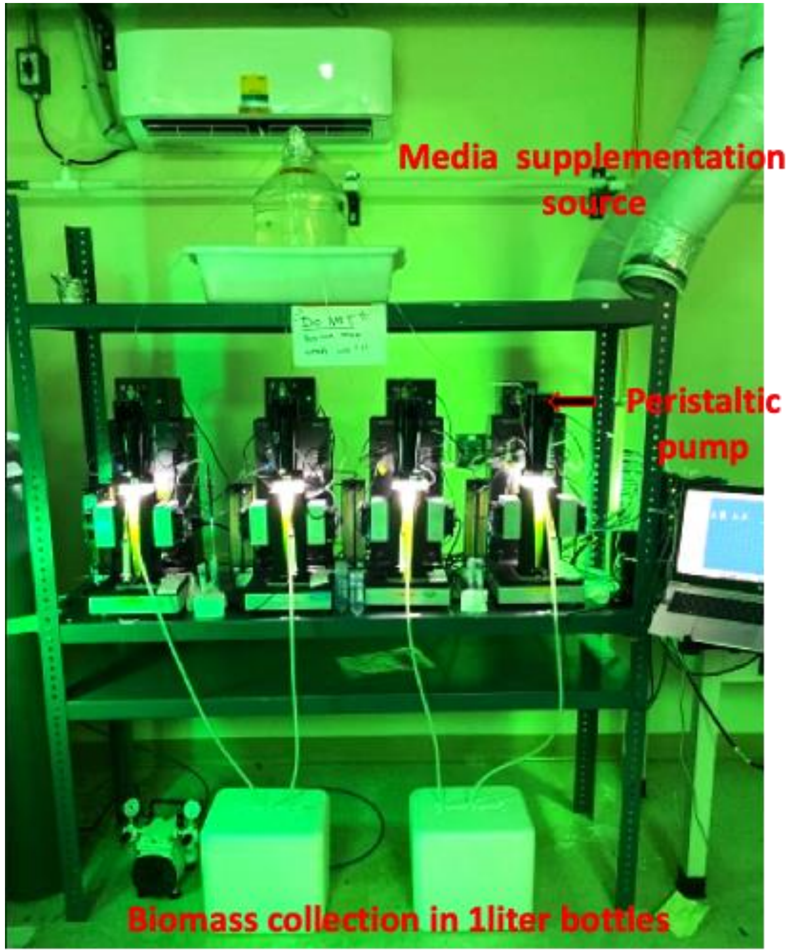
- **Background/history:** Continuous (or automated) harvesting can better tune productivity based on weather and algal strain requirements, and the level of standing biomass may be tuned to limit light stress or to increase carbon shifting.

- **Objective:** Assist in the prioritization of outdoor testing conditions by evaluating the effect of changing operational conditions across high performing strains, with an emphasis in *P. celeri* and *T. striata*.

- **Approach:**
  1. Setup and demonstrate the feasibility of continuous or automated cultivation using ePBRs.
  2. Demonstrate at least 10 days of growth of *P. celeri* under turbidostat-mode in ePBRs using AzCATI outdoor cultivation script.

- **Economic/Technical Metrics:** Aim to increase biomass productivity due to increased operational control.

Experimental setup: automated harvesting based on culture turbidity

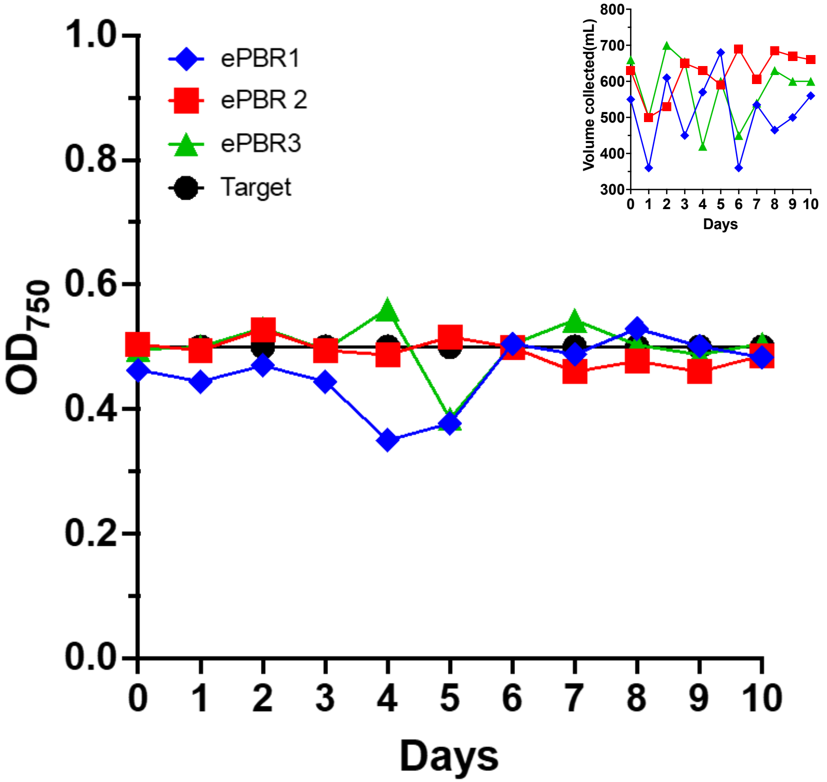


Outcomes: Continuous/Automated Cultivation

Validated Turbidostat mode in ePBRs

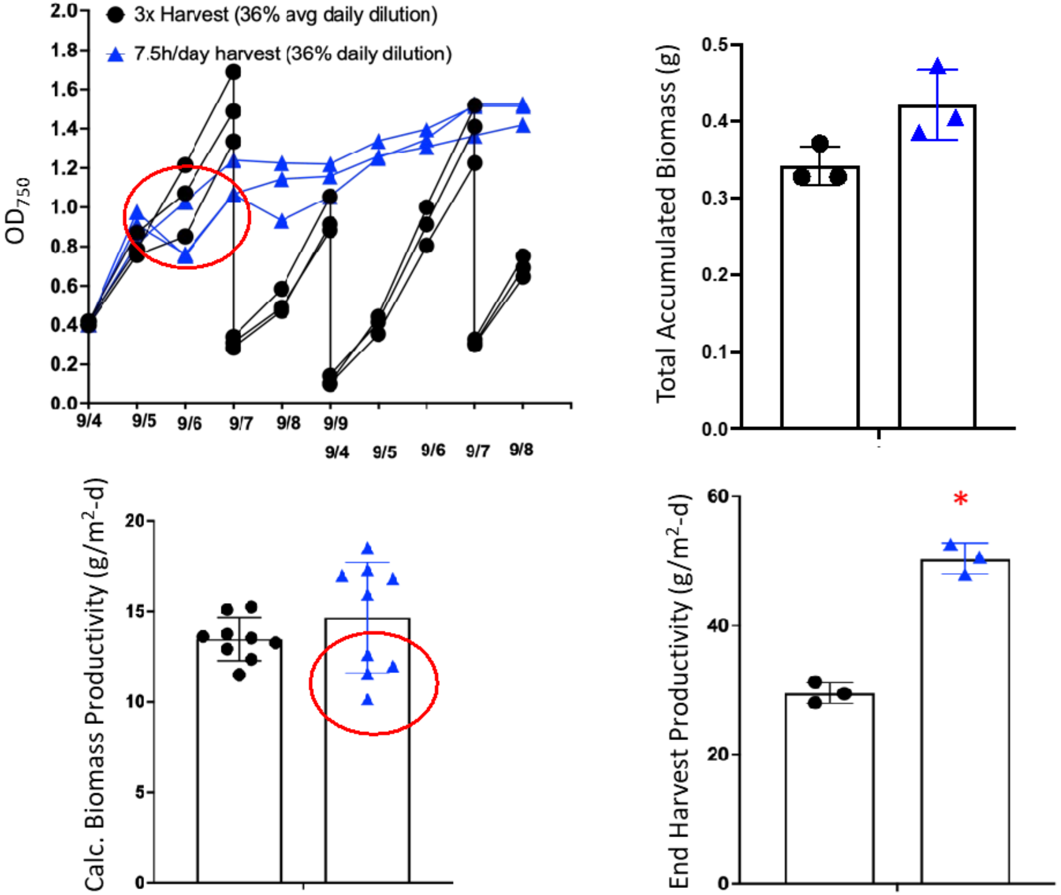
New Concepts

Turbidostat/automated dilution experiments performed on *P. celeri* using an Aug 2020 AzCATI script



Relatively flat OD values show the system is maintaining a constant cell concentration by turbidity, while collecting at least the equivalent of the full culture volume each day

Preliminary comparison of 3x/week harvest vs automated harvesting 7.5h/day, using looped Sept 2020 AzCATI script



In the daily dilution (36% per day over 7.5h each day) experiment, the P. celeri cultures showed fast recovery from the dilutions and slowly accumulated a higher standing biomass concentration over time.

More frequent harvesting may increase harvested biomass and productivities

Outcomes: Optimize Pond Operational Control

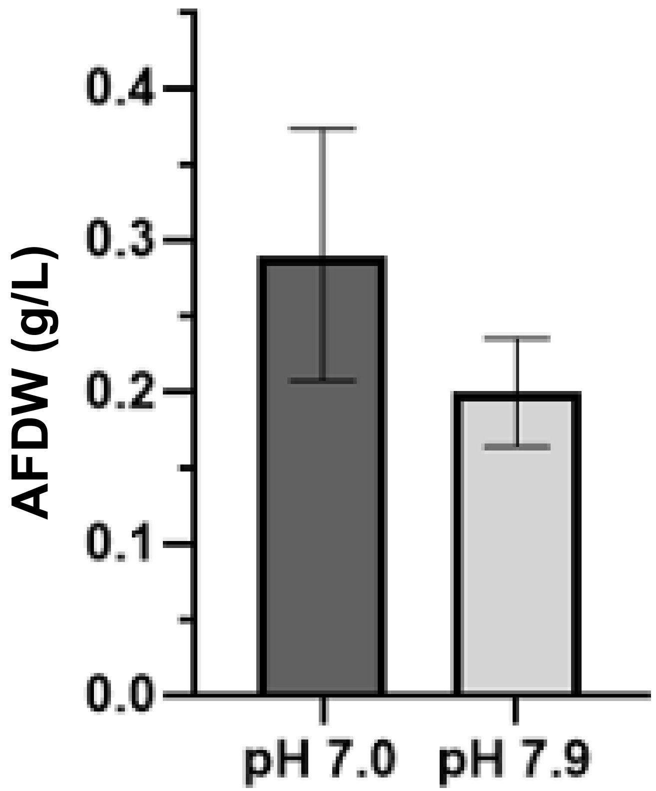
Understand pH modulation on algal growth under outdoor conditions

New Concepts

- **Objective:** Assist in the prioritization of outdoor testing conditions by evaluating the effect of changing operational conditions across high performing strains.

- **Approach:** Test hypotheses for changing operational conditions by using retrospective scripts from SOT trials and altering operations in ePBRs.
  - Strain tested: *T. striata*
  - Comparison of *T. striata* growth using SOT media with pH 7.0 and 7.9

- **Impacts:**
  - Higher pH negatively impacts *T. striata* growth
  - 38% increase in AFDW of *T. striata* growth at pH 7.0 compared to at pH 7.9 grown cells. Consistent with outdoor pond results.



Impacts: Continuous/Automated Cultivation

Automated cultivation has potential to increase biomass productivity

New Concepts

New approaches/ideas need to be tested to determine feasibility of application in outdoor ponds

- DISCOVR SOT strain *P. celeri* tested with Turbidostat/automated dilution experiments using an Aug 2020 AzCATI script.
- Performed a comparative analysis of 3x/week harvest vs automated harvesting 7.5h/day, using looped Sept 2020 AzCATI script for *T. striata.*

Impact

Successful automated cultivation of DISCOVR strains has the potential to:

- **Increase productivity**
- **Improved biomass quality**
- **Operational conditions can be customized for strain requirements to increase annual biomass productivity**

Approach: Spectroradiometric Monitoring of Pond Health

New Concepts

Models predict algal health and functional pest presence in the field

- **Background/history:** Spectroradiometric monitoring has **demonstrated ability** to provide real-time biomass measurements and detect changes in algal cultures before physical observation.
  - Initial technology development (10.1016/j.algal.2011.12.001)
  - Demonstrated in outdoor ponds (10.1364/AO.53.000F31)
  - Demonstrated early warning potential (10.1016/j.algal.2020.102020)
- **Objective:** Monitor the reflectivity AzCATI ponds to demonstrate the potential of spectroradiometric monitoring over traditional off-line pond measurements.
- **Challenges:**
  1. Operational: need near real-time communication & info from AzCATI
  2. Technical: defining optimal alert conditions difficult due to environmental factors (fouling, flocculation, etc.)
  3. Sensors are not monitoring the DISCOVR SOT ponds
- **Economic/Technical Metrics:** Monitoring >75% of the month, 85% prediction accuracy

Spectroradiometric sensor (yellow arrow) monitoring pond at AzCATI (photo taken June 2022)

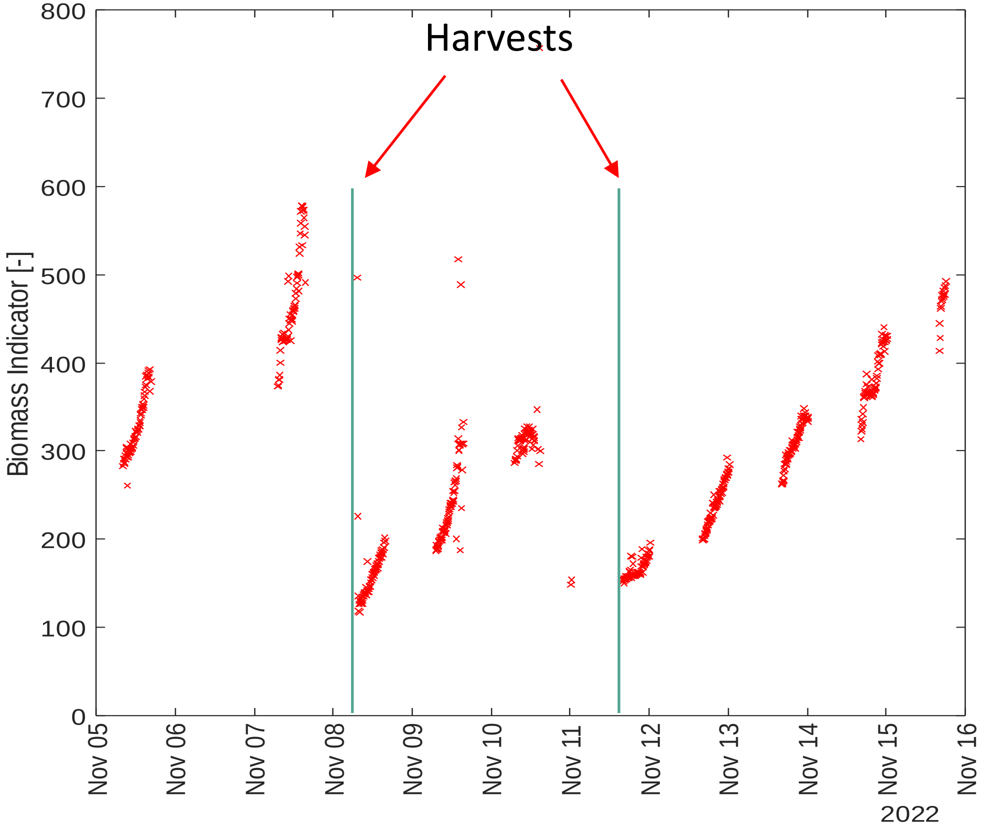
Outcomes: Spectroradiometric Monitoring of Pond Health

Advances in hardware and software have enabled improved operation

New Concepts

Since last review we have made the following advances towards a **fielded** integrated early detection system:

- Upgraded outdated, unsupported spectrometers (Apr 2022)
- Upgraded analysis software to support new spectrometers and changing needs of DISCOVR Feb 2021- current)
- Redeployed these units at AzCATI with update site and fiber infrastructure (May – Jun 2022)
- Extended and validated the methods with newest DISCOVR strains (26-BAM, *P. celeri*, and T. *striata* data shown in Figure) (Oct 2022-current)



Prediction of biomass (relative) from spectroradiometric data for T. striata in AzCATI ponds from Nov 2022. Note: Absolute biomass values can be predicted once data is received from the AzCATI team

DISCOVR

Impact: Spectroradiometric Monitoring of Pond Health

New Concepts

Improvements need to be made to fully realize impact

Improvements to the AzCATI site network and power upgrades have addressed prior challenges reducing downtime, but still fall short of our goal of monitor 75% of the month.

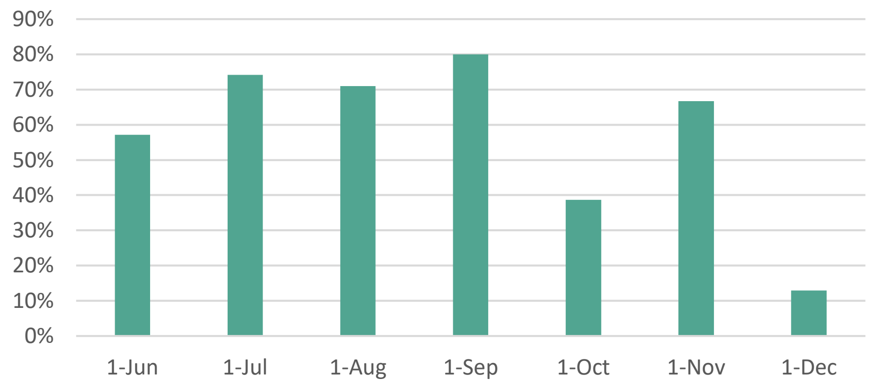
Impact

Spectroradiometric monitoring can serve as an integrated early detection system

- **Identifying declining culture conditions**
- **Reducing MTBF**

Operational/communication improvements are needed to collected sufficient data to demonstrate this.

% Time Spectroradiometric Monitoring was Operational Jun – Dec 2022



Percent of time per month that spectroradiometric monitoring was active and functional. Note: Start is June 17, 2022 following hardware updates, installation and calibration.

Publication

Atencio, L., Maes, D., Hipple, T., and Timlin, J.A. "Susceptibility of Two Saltwater Strains of *Chlorella sorokiniana* to *Vampirovibrio chlorellavorus". Journal of Applied Phycology, 2022, 34, 81-87. DOI: 10.1007/s10811-021-02602-0*

Publication highlights how we used our expertise in host/pathogen detection to assist DISCOVR broadly.

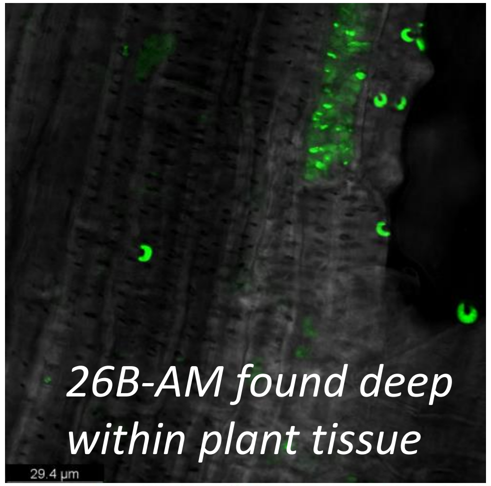
DISCOVR · Pacific Northwest National Laboratory · Los Alamos National Laboratory EST.1943 · NREL Transforming ENERGY · Sandia National Laboratories · INL Idaho National Laboratory · AzCATI Arizona Center for Algae Technology and Innovation · Colorado School of Mines

Approach: Improve Biomass Productivity with Plant Substrate

New Concepts

Plant substrate additions may supplement carbon during dark respiration

- **Background/history:** FY21 results of DISCOVR strain *Monoraphidium minutum 26B-AM* at flask scale revealed increased biomass accumulation with plant substrate additions. Results from *Picochlorum celeri* and *Tetraselmis striata LANL1001* were inconclusive and required additional testing with plant substrates.
- **Objectives:**
    - Test 26B-AM performance on plant substrate utilization at 50 L mini-ponds scale in the SNL greenhouse.
    - Continued flask screening of *P. celeri* and *T. striata LANL1001* with switchgrass and corn stover plant substrate additions.
- **Challenges:**
    1. 26B-AM adheres to the surface and found inside plant substrates could lead to an underestimation of cell density and convoluting impact of plant substrate impacts on overall growth of algal biomass.
    2. The heterogeneity of the plant substrates presented challenges in determining the benefit of plant substrate to algal biomass increases.
- **Economic/Technical Metrics:** Plant substrate additions may provide carbon supplement during dark respiration improving overall growth of algal biomass.



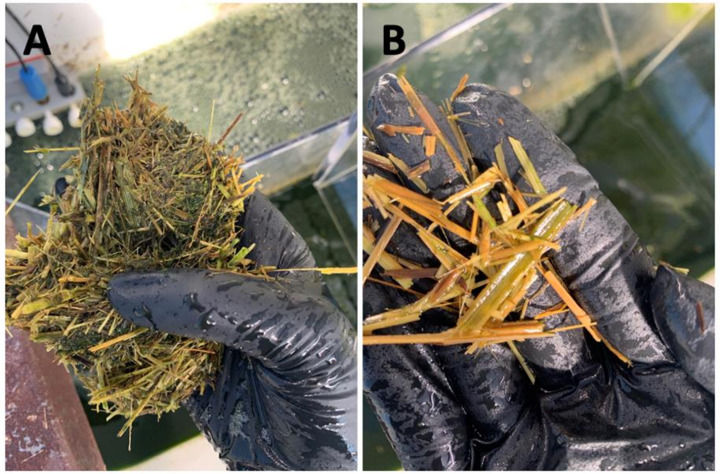


Photo of switchgrass pulled from bottom of pond before thorough mixing. Algae settles in and on the plant substrate (Panel A). Photo of switchgrass pulled from pond and gently rinsed with milli-q water (Panel B).

DISCOVR, Pacific Northwest National Laboratory, Los Alamos National Laboratory EST. 1943, NREL Transforming ENERGY, Sandia National Laboratories, INL Idaho National Laboratory, AzCATI Arizona Center for Algae Technology and Innovation, Colorado School of Mines

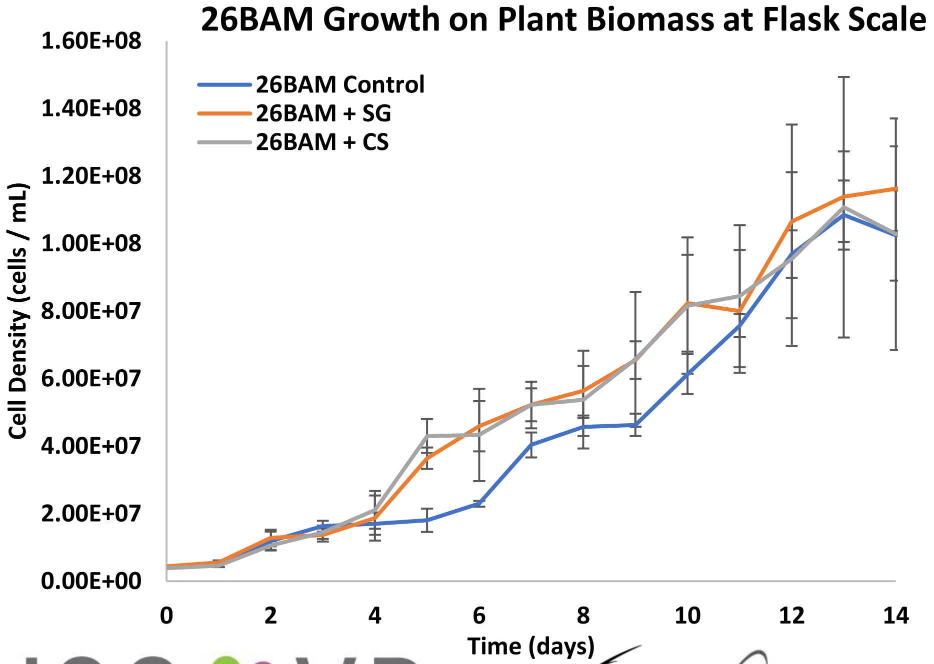
Outcomes: Improve Biomass Productivity with Plant Substrate

26B-AM growth shows variable results with switchgrass

New Concepts

Results of DISCOVR Strains on Plant Substrate at Flask Scale

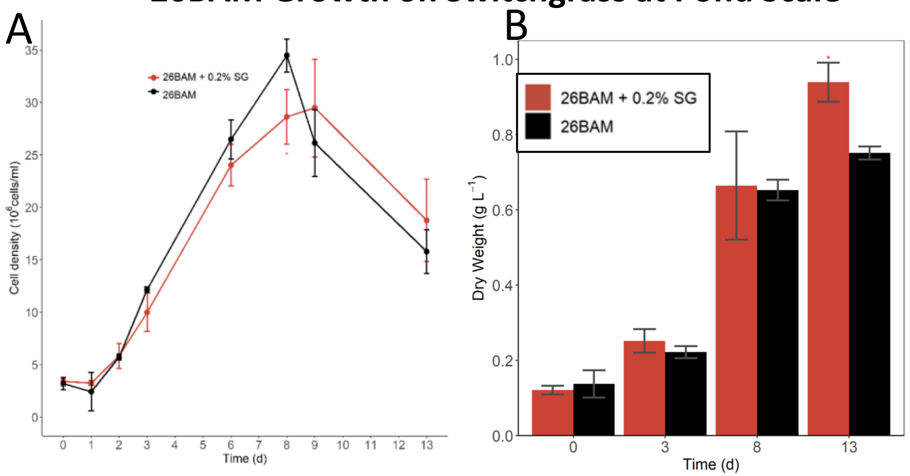
- *26B-AM*+SG cell counts displayed some algal growth increases at flask scale.
- *P. celeri* was negatively impacted by both plant substrate additions indicating a lack of tolerance.
- *T. striata appears* cell density and chlorophyll greening indicated growth on plant substrate is at least beneficial at flask scale after 8 days.



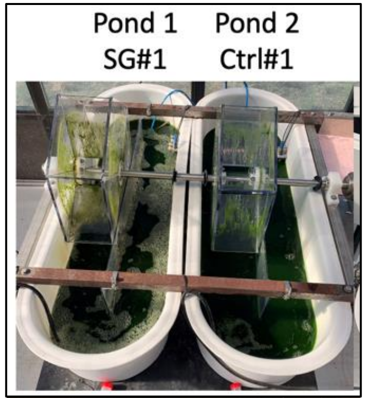
Results of 26B-AM on Switchgrass Pond Scale

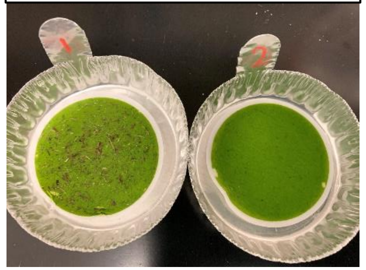
- We observed no noticeable difference in 26B-AM color or cell density between controls and plant substrate ponds until Day8.
- 26B-AM+SG in the ponds had faster than normal uptake of CO_2 and resulted in no CO_2 supplementation after Day8.
- Dry weights show an improvement for the 26B-AM with switchgrass after depletion of CO_2.
- Cell size increases were observed by flow cytometry in nitrogen replete conditions.

26BAM Growth on Switchgrass at Pond Scale



Cell density and dry weight results from raceway pond trial. Cell density (A) and dry weight (B) for 26BAM cultivated in mini raceway ponds with and without 0.2% (w/v) switchgrass addition.





DISCOVR · Pacific Northwest National Laboratory · Los Alamos National Laboratory · NREL Transforming ENERGY · Sandia National Laboratories · INL Idaho National Laboratory · AzCATI Arizona Center for Algae Technology and Innovation · Colorado School of Mines

Impact: Improve Biomass Productivity with Plant Substrate

Only specific DISCOVR strains can utilize plant substrate under certain conditions

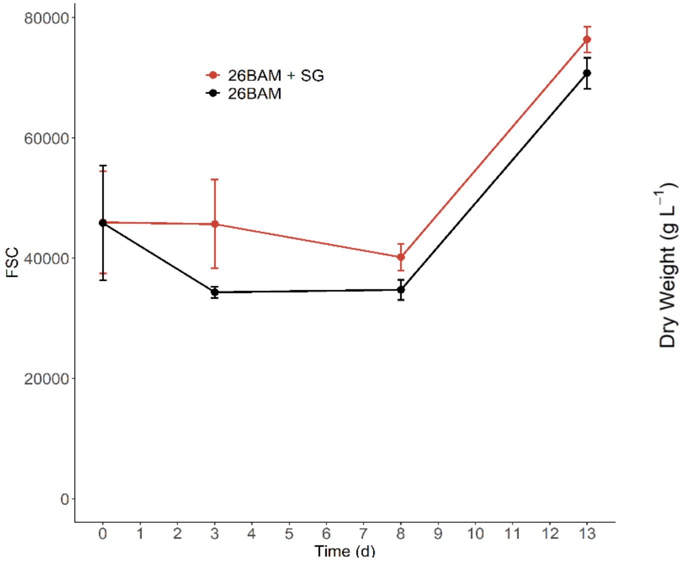
New Concepts

Challenges determining benefits of plant substrate:

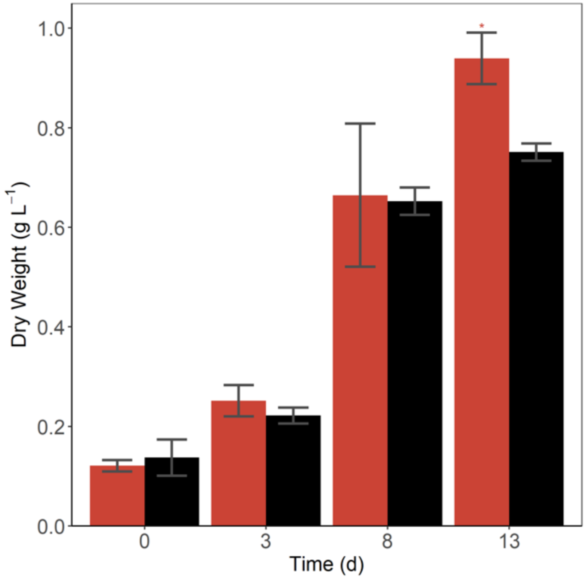
- Plant material complicates accurate algal biomass assessments.
- 26B-AM found in plant tissue.
- CO_2 supplementation confounds plant substrate utilization by algae in raceway ponds.

Impact

- **Only certain DISCOVR strains can utilize plant substrate under certain conditions.**
- **26B-AM dry weight and cell size increases with switchgrass additions in 50L ponds after CO_2 depletion.**



Cell size increases confirmed by FACS. Relative mea forward scatter measured using BDFACs Aria Fusion for 26BAM cultivated in mini raceway ponds with and without 0.2% (w/v) switchgrass addition.



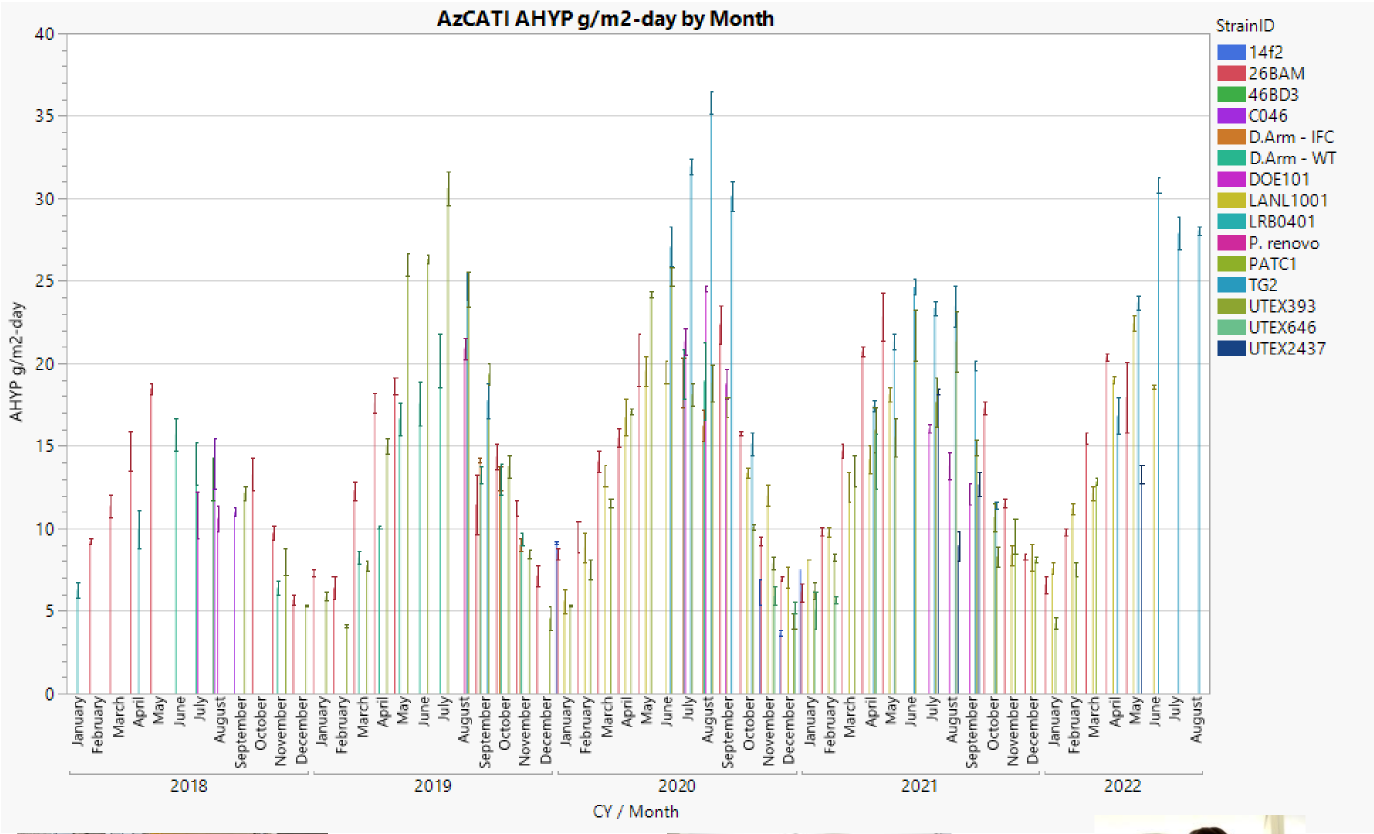
Dry weight results from raceway pond trial. Dry weight for 26BAM cultivated in mini raceway ponds with and without 0.2% (w/v) switchgrass addition.

DISCOVR | Pacific Northwest National Laboratory | Los Alamos National Laboratory EST. 1943 | NREL Transforming ENERGY | Sandia National Laboratories | Idaho National Laboratory | AzCATI Arizona Center for Algae Technology and Innovation | Colorado School of Mines

Outcomes: State of Technology Trials

SOT Trials

Outdoor verification of best strains/approaches with primary focus on improving productivity



Between 2018 and 2022

- Cultivation experiments 365 days a year
- 14 strains tested under DISCOVR for at least 30 days
- Over 27,000 individual grab samples:
  - AFDW, OD, nutrients, microscopy
- Over 5900 individual measurements of harvest yield productivity
- Biomass and pond samples supplied to DISCOVR and BETO AOP projects
  - ~500kg of algae paste collected from >100 experiments
- Mature operations with experienced faculty, staff and student researchers

Standard AzCATI
4.2 m^2 raceway
24 ponds on-site

DISCOVR

Pacific Northwest NATIONAL LABORATORY

Los Alamos NATIONAL LABORATORY EST.1943

NREL Transforming ENERGY

Sandia National Laboratories

INL Idaho National Laboratory

AzCATI Arizona Center for Algae Technology and Innovation

COLORADO SCHOOL OF MINES

Approach: State of Technology Data Management

SOT Trial

Centralized, accessible data to enable analysis, discussion, and planning; NREL

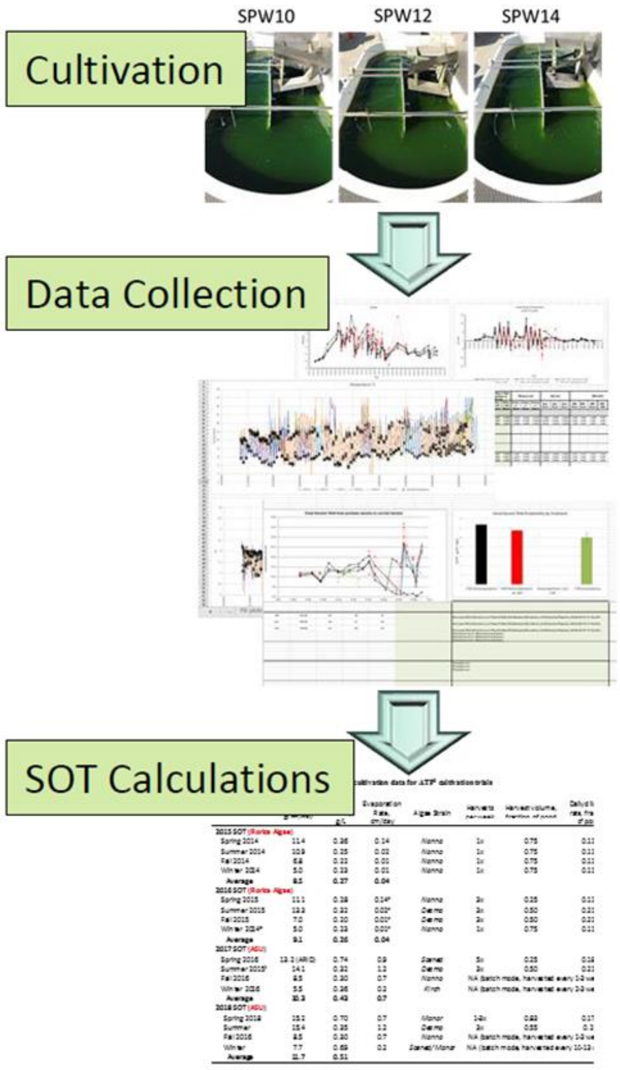
- **Background/history:** Expanded on previous data capture methods to collaboratively develop comprehensive data collection spreadsheets. Applied lessons-learned from previous data collection efforts under ATP3.

- **Objective:** Capture cultivation and composition data from year-round, outdoor cultivation trials in a format that facilitates rapid analysis and allows up-to-date understanding of biomass productivity and enables planning for further improvements.

- **Challenges:**

  1. Tracking multiple experiments across spreadsheets
  2. Delay between end of experiment and completion of analyses

- **Economic/Technical Metrics:** Provides accessible data to measure progress towards Program goals. Facilitates experimental design by providing a central repository for the underlying data.

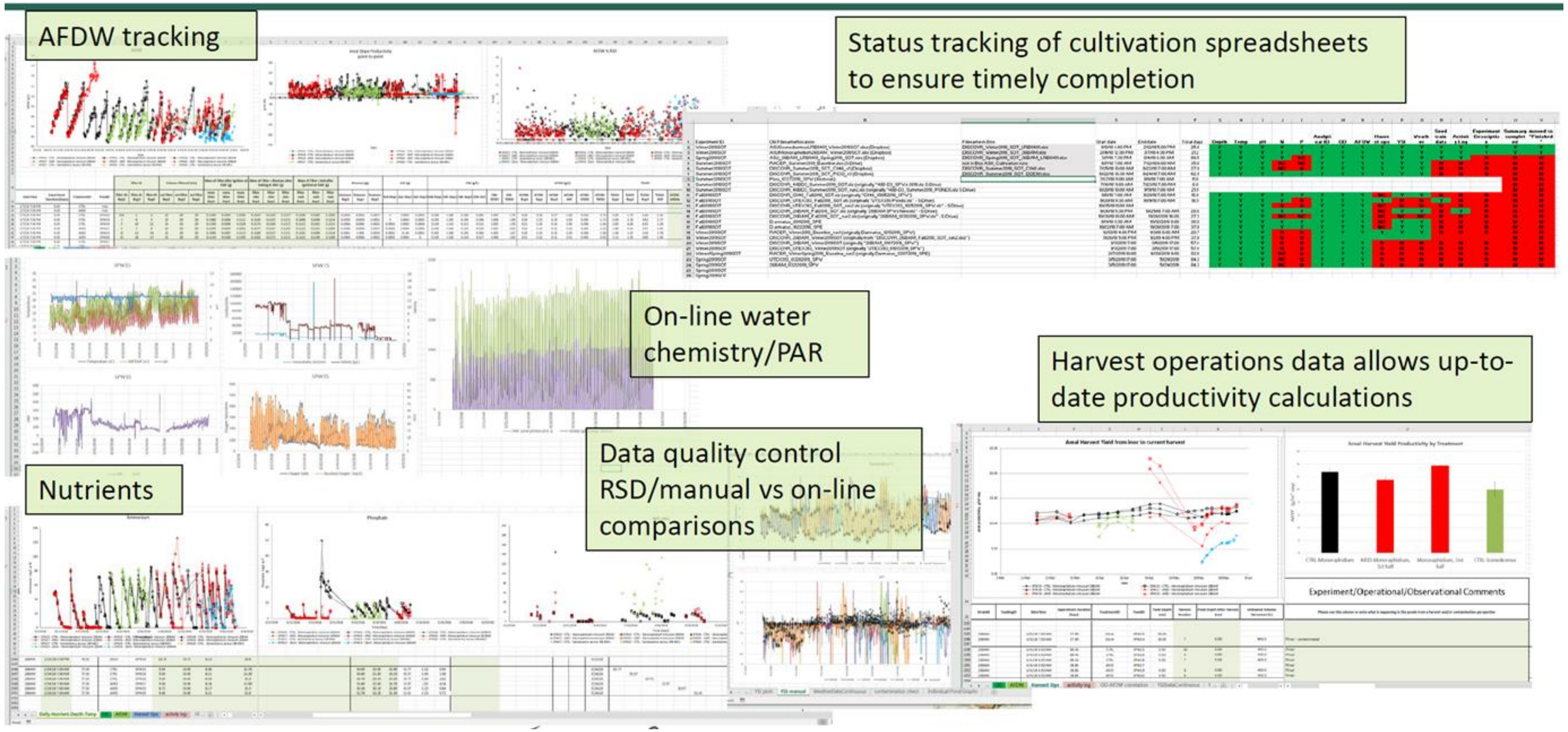


Outcomes: State of Technology Data Management

Data collection format allows data interpretation for experimental planning; NREL

SOT Trial

- Currently 4 years of data (2018-2021)
- Data spreadsheets contain >15,000 cultivation time points encompassing 53 variables
- Data for 6 species of algae
- Freely available at https://apps.openei.org/DISCOVR/



DISCOVR | Pacific Northwest National Laboratory | Los Alamos National Laboratory EST.1943 | NREL Transforming ENERGY | Sandia National Laboratories | INL Idaho National Laboratory | AzCATI Arizona Center for Algae Technology and Innovation | Colorado School of Mines

Impact: State of Technology Data Management

Centralized, accessible data to enable analysis, discussion, and planning; NREL

SOT Trial

Comprehensive spreadsheets collaboratively developed to capture critical metrics of algae cultivation

- Active graphs at top of each tab for facile data visualization
- Includes measured metrics and up-to-date calculations
- Includes checks on data quality
- Includes tab specifically for pond operator observations
- Critical to understanding cultivation in the event of a pond failure
- Allows easy visualization of underlying data leading to current improvements such as the 57% increase in productivity for 2021 over the last 2 years

Impact

Our data management strategy facilitates data analysis and discussion of current experiments during biweekly meetings and facilitates discussion to develop a favorable path forward

Publication:

McGowen et al., 2023, "Outdoor annual algae productivity improvements at the pre-pilot scale through crop rotation and pond operational management strategies", *Algal Research* – Special Issue DISCOVR, https://doi.org/10.1016/j.algal.2023.102995

Future Work Slides

Future Work: Task 1 - Improving Biomass Productivity

Task 1.1 Maximize biomass productivity using OptiLum operations (PNNL)

- **Objective:** Evaluate the feasibility of the OptiLum operation strategy under outdoor pond relevant conditions using the top DISCOVR winter strain

- **Approach:**
  - Use stable outdoor pond culture from AzCATI as inoculum to minimize microbial difference between indoor and outdoor cultures.
  - Evaluate OptiLum operation under light and temperature conditions measured in outdoor ponds to provide more realistic data for TEA.

- **Expected Outcomes:**
  - Show >20% improvement in biomass productivity relative to the current semi-continuous batch cultivation strategy.
  - Demonstrate reduced MBSP under outdoor measured pond conditions.
  - Identify solutions to possible challenges in outdoor application.

- **Impacts:** Reduced MBSP

Indoor bench-scale LEAPS reactor

Outdoor large-scale raceway ponds

Future Work: Task 1 - Improving Biomass Productivity

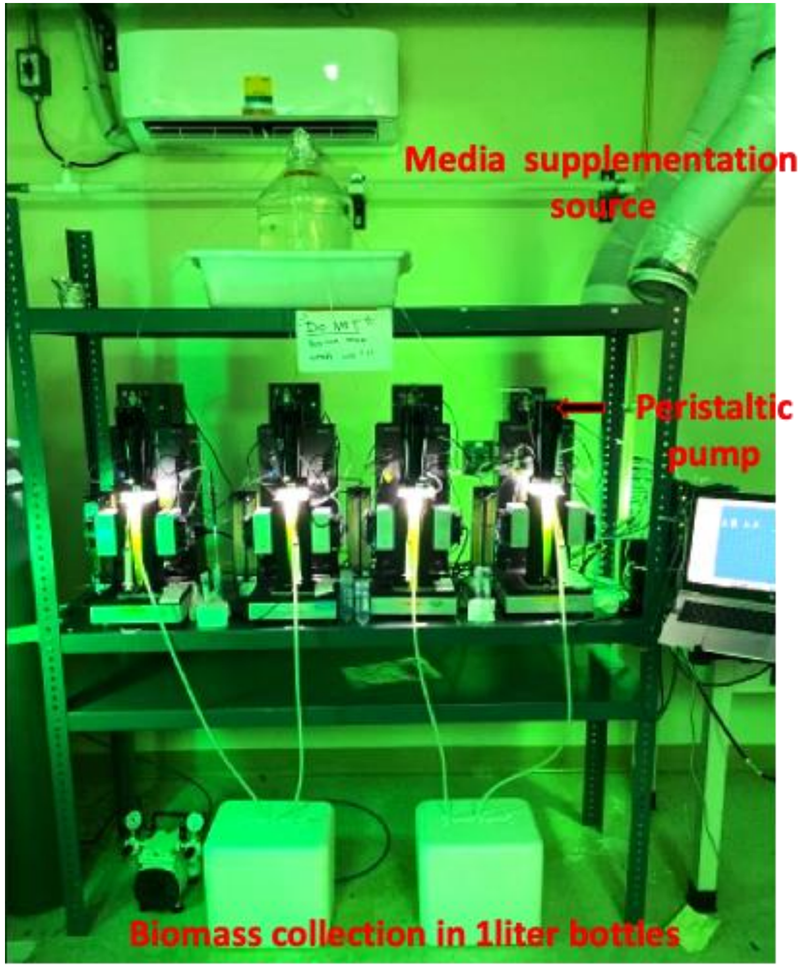
Task 1.2 Increase biomass productivities using Turbidostat mode in ePBRs (LANL)

- **Objective:** Assist in the prioritization of outdoor testing conditions by evaluating the effect of changing operational conditions across high performing strains, with an emphasis in *P. celeri* and *T. striata*.

- **Approach :**
  - Continue using the set-up established in FY22.
  - Top DISCOVR strains will be grown in the ePBRs under simulated outdoor pond conditions. The turbidostat operation will adjust dilution automatically according to the measured turbidity of the culture (i.e., biomass density).
  - The automated dilution is controlled by the a through-culture turbidity detection system. Dilution occurs if the biomass density exceeds the set point.

- **Expected Outcome:**
  - Increase in productivity by 20% due to increased operational control

- **Impacts:** Improved annual biomass productivity due to tailored operational conditions.



Experimental setup: automated harvesting based on culture turbidity

Future Work: Task 1 - Improving Biomass Productivity

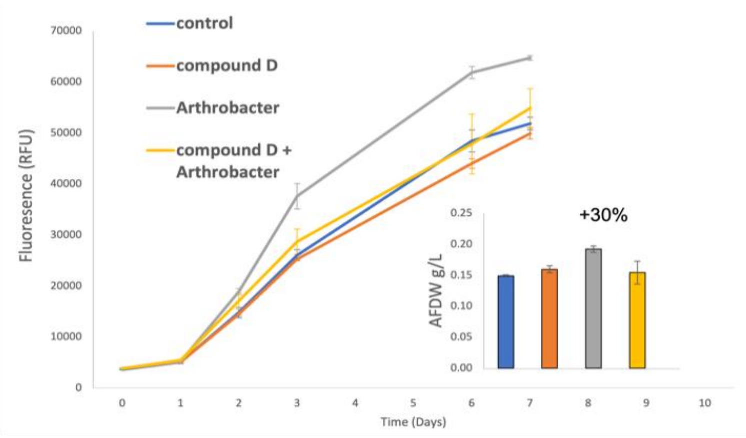
Task 1.3 Additions of a growth-promoting bacterium (GPB) (LLNL)

- **Objective:** Test application of a novel *Arthrobacter* GPB with added hormone precursor to DISCOVR strains to increase productivities

- **Approach:** Screen *P. celeri* & *T. striata* to optimize GPB & hormone combinations
  - Scale up from 3 mL, 75 mL, 10L, 100L outdoors if lab-scale is successful
  - Go/no-go summer 2023 (lab scale >25% improvement)
  - NREL TEA identifies level of improvement needed for MBSP improvement

- **Expected Outcomes:**
  - Compare impact on axenic vs cultures collected from outdoor ponds
  - Preliminary data suggest *T. striata* responds positively to hormone & GPB
  - Identify if GPB can survive long term or requires multiple additions

- **Impacts:** Increased productivities of known high biomass strains with minimal effort through co-cultivation



Preliminary data showing Arthrobacter additions increase algal AFDW

axenic *Arthrobacter co-culture*

Future Work: Task 1 - Improving Biomass Productivity

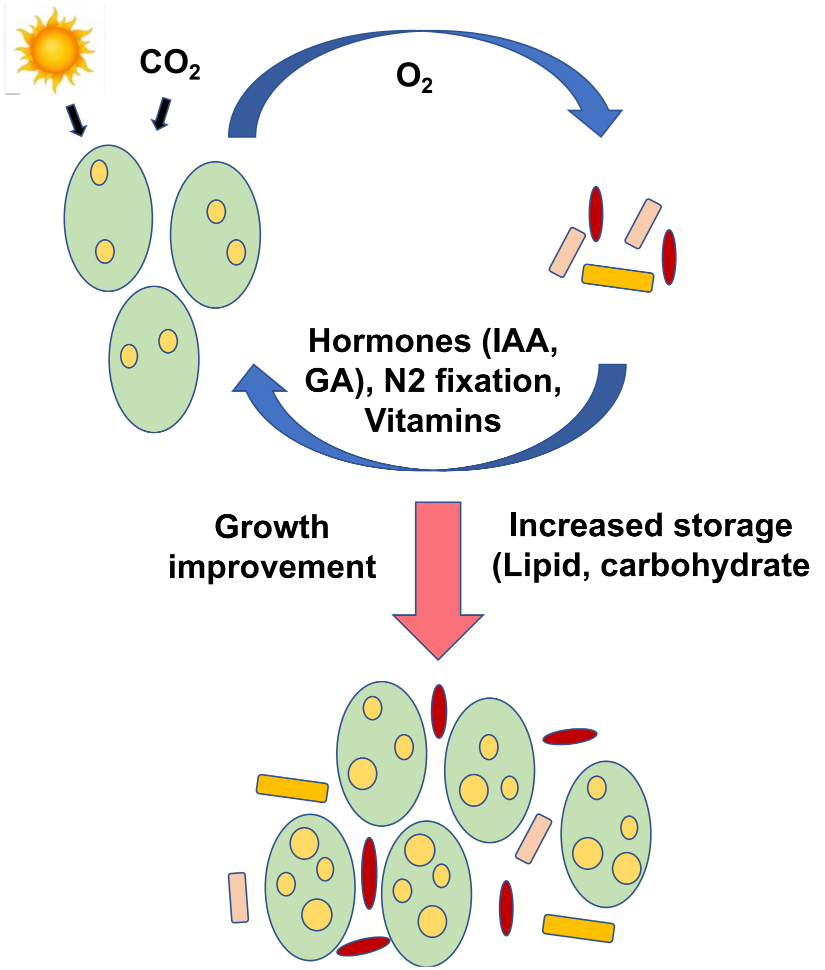
Task 1.4 Bacteria-delivered hormone treatment (LANL)

- **Objective:** Examine growth of DISCOVR algae strains in presence of known plant growth promoting bacteria to improve productivity and resilience.

- **Approach*:*** This is follow-on work for our current task in DISCOVR.
  - Continue testing a suite of bacteria (9 bacterial strains) with two SOT strains, *T. striata* and *P. celeri*
  - Evaluate growth (ODs and cell count), salinity and pH tolerance.
  - If "Go" then perform experiments in ePBRs using AzCATI scripts.

- **Expected Outcomes:**
  - Realize the 20% increase in productivity or
  - Increase carbon storage/biomass quality (at least 15%).

- **Impacts:** Improved annual biomass productivity and reduced MBSP.

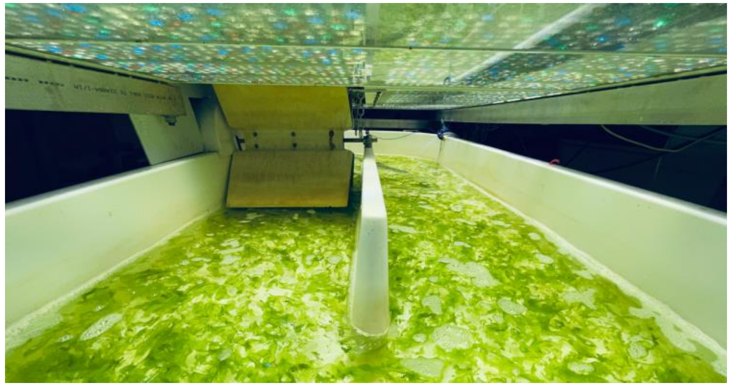


Future Work: Task 1 - Improving Biomass Productivity

Task 1.5 Macroalgal cultivar evaluation under climate-simulated conditions (PNNL)

- **Objective:** Measure the areal biomass productivity of macroalgae in raceway ponds under Arizona climate-simulated conditions and compare to DISCOVR benchmark strains.
- **Approach:** Conduct a comparative biomass productivity assessment.
  - High productivity seaweed, such as the green macroalga *Ulva expansa* will be cultivated in PNNL's indoor climate-simulation ponds supplied with seawater growth medium, using Arizona seasonal light and temperature scripts.
- **Expected Outcomes:**
  - Biomass productivities directly comparable to the benchmark seasonal strains (*Monoraphidium minutum* 26B-AM and *Tetraselmis striata* LANL1001).
  - Evaluation of challenges and benefits of assuming *Ulva expansa* as a production organism in the NREL Farm Model.
- **Impacts:** Improve annual biomass productivities, reduce harvest costs.

Ulva expansa PNNL-PC3 a promising macroalgal cultivar compatible with microalgal cultivation formats (i.e., raceway ponds).



Raceway pond cultivation of Ulva expansa

DISCOVR Pacific Northwest National Laboratory Los Alamos National Laboratory NREL Transforming ENERGY Sandia National Laboratories INL Idaho National Laboratory AzCATI Arizona Center for Algae Technology and Innovation Colorado School of Mines

Future Work: Task 2 - Improve Carbon Utilization Efficiency

Task 2.1 Determine biomass productivity at high pH (PNNL)

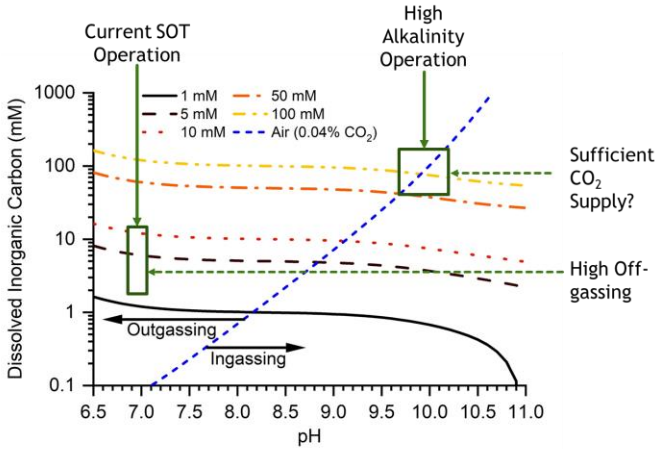
- **Objective:** Provide TEA with areal biomass productivities at high pH for the top DISCOVR strains and potentially new strains in LEAPS photobioreactors to inform whether high pH cultivation reduces MBSP.

- **Approach:**
  - Top DISCOVR strains will be cultured in the LEAPS at higher medium pH (air-CO_2 equilibrium level), using respective Arizona winter/summer season scripts.
  - Biomass productivity data will be feed into NREL TEA model to estimate MBSP for high pH cultivation.

- **Expected Outcomes:**
  - Demonstrate improved CUE and reduced MBSP under outdoor pond relevant conditions.
  - Establish protocol for evaluating new promising strains.

- **Impacts:** Improved CUE and reduced MBSP.

Indoor bench-scale LEAPS reactor



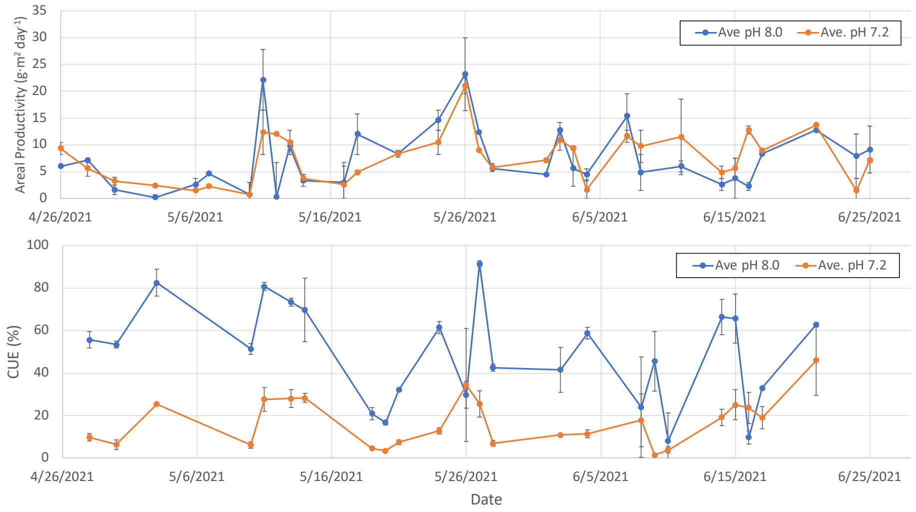
Equilibrium pH as a function of medium pH and alkalinity

DISCOVR · Pacific Northwest National Laboratory · Los Alamos National Laboratory EST.1943 · NREL Transforming ENERGY · Sandia National Laboratories · Idaho National Laboratory · AzCATI Arizona Center for Algae Technology and Innovation · Colorado School of Mines

Future Work: Task 2 - Improve Carbon Utilization Efficiency

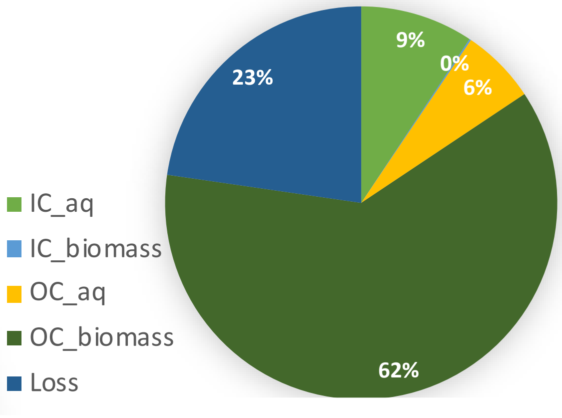
Task 2.2 CUE Improvement by Water Chemistry Optimization (NREL)

- **Objective:** Leverage strong impact of water chemistry, pH, and nutrient source on carbon utilization efficiency (CUE)
- **Hypothesis:** Operating at the optimal equilibrium alkalinity-pH of a given water chemistry formulation can deliver close to max (100%) CUE, with a practical and production-relevant N:P formulation
- **Approach:**
  - Develop and test tools that enable and accurately describe CUE for select strains under high-productivity conditions
  - Optimize media formulation and operational conditions to maximize CUE for DISCOVR strains
- **Expected Outcome:** Systematically and consistently report CUE under translatable conditions, with tested or theoretical framework in place, with outdoor-implementable cultivation strategy
- **Impact:** High CUE will reduce CO_2 costs and improve LCA metrics for process carbon intensity



[NREL 2021, UTEX393, NH4HCO3]

Allocating inorganic carbon for carbon storage in biomass can lead to 62% CUE in organic carbon by changing alkalinity and N source.



Future Work: Task 2 - Improve Carbon Utilization Efficiency

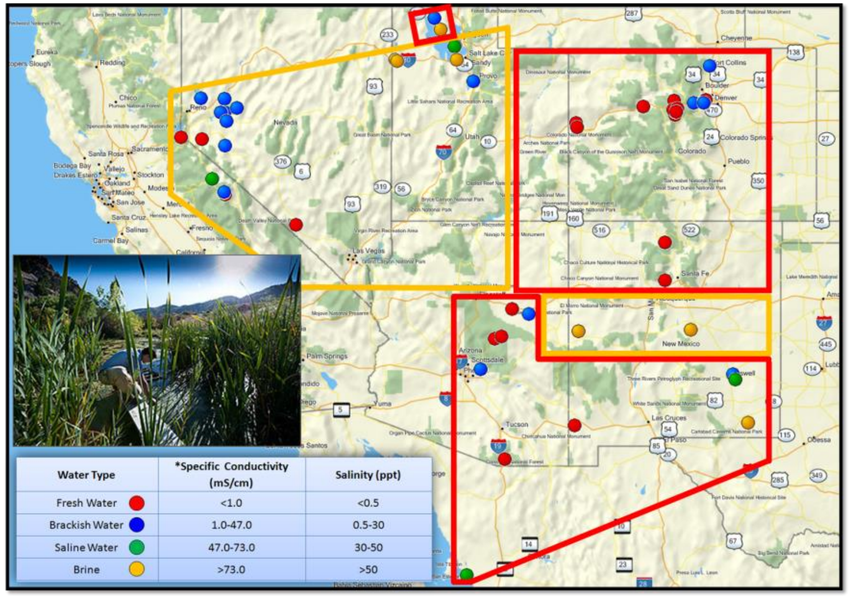
Task 2.3 Isolation of high-lipid alkali-tolerant strains for outdoor cultivation (CSM)

- **Objective:** To improve CO_2 utilization efficiency (CUE), stains that remain productive at higher pH may be required. We propose to use bioprospecting to enrich strains from natural waters using high pH (≥ 8.2) selective pressures.

- **Approach:** Strain selection at high pH
  - Collect water samples from diverse ecosystems including high-pH alkaline waters
  - Enrich strains at high pH in photobioreactors
  - Test diel productivity outcomes using DISCOVR pipeline and outdoor testing for most productive strains

- **Expected Outcomes:**
  - Isolation of strains with superior growth metrics at high pH
  - Isolation of strains with favorable CO_2 utilization efficiencies
  - Isolation of high pH strain with high lipid content (diatom focus)

- **Impacts:** Improve CUE in high-productivity strains

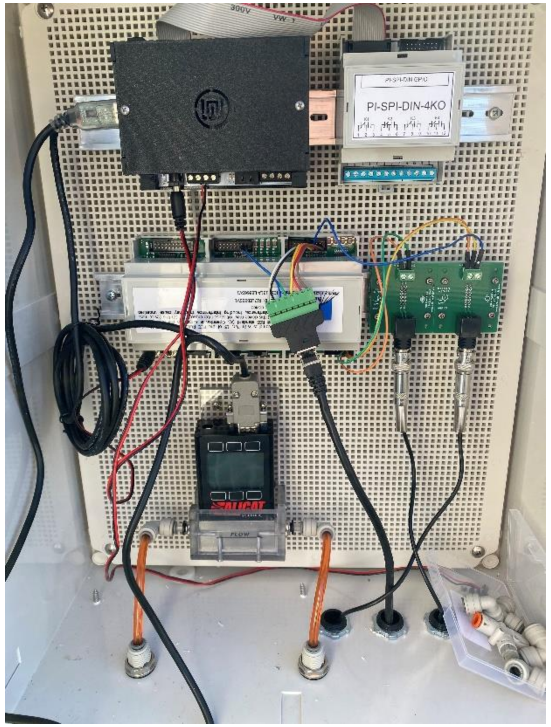


Potential geographic locations for sampling that include soda lakes for bioprospecting

Future Work: Task 2 - Improve Carbon Utilization Efficiency

Task 2.4 CUE optimization in larger outdoor ponds (AzCATI)

- **Objective:**
  - Verify outdoor CUE performance of DISCOVR strains at more relevant pond scales and aspect ratios.
- **Rational:**
  - small-scale equipment comes with limitations for extrapolating to larger, commercially-relevant scales
  - May contribute to misunderstanding key performance indicators CUE and productivity.
- **Approach:**
  - Implement routine measurements of CTE/CUE
  - Baseline current performance of DISCOVR strains
  - Verify key findings from Subtasks 2.1, 2.2, and 2.3.
  - Utilize 100 m^2 ponds with mixing profiles (i.e., aspect ratios) and paddlewheel to surface area ratios that more closely resemble large scale ponds
- **Expected Outcomes:**
  - Demonstrate CUE ≥70% (End of Project Goal) while identifying key parameters and control strategies for optimized CUE outdoors.
- **Impacts:** CUE is a critical TEA.LCA driver and needs to be high to allow for successful commercialization of algae biofuels

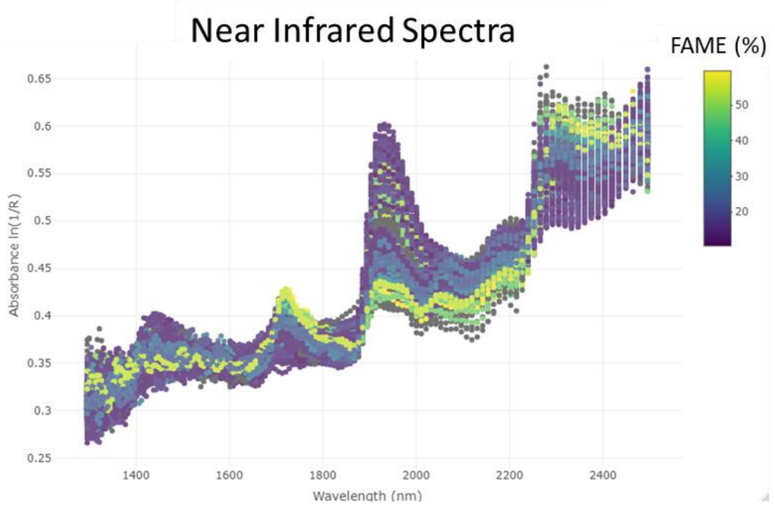


Burge Environmental monitoring and control system with integrated Alicat Flow Totalizer for tracking CTE/CUE

Future Work: Task 3 - Biomass Quality Tracking and Optimization

Task 3.1 High throughput biomass compositional analysis (NREL)

- **Objective:** Leverage infrared spectroscopy and machine learning to build high-throughput algae compositional analysis
- **Approach:**
  - Train machine learning models on primary compositional analysis samples and data
  - Test and define uncertainty of predictions, establish outlier detection algorithms
  - Implement user-friendly interface and deploy cost-effective spectrometer at testbed
- **Expected Outcome:** Implementable interface and (low-cost) instrument infrastructure for rapid compositional analysis based on validated, trained models
- **Impact:** cost-effective and fast-turn-around compositional analysis that can be applied in the field



Example of prior work at NREL on 96-well plate infrared spectroscopy screening and application to rapid compositional analysis

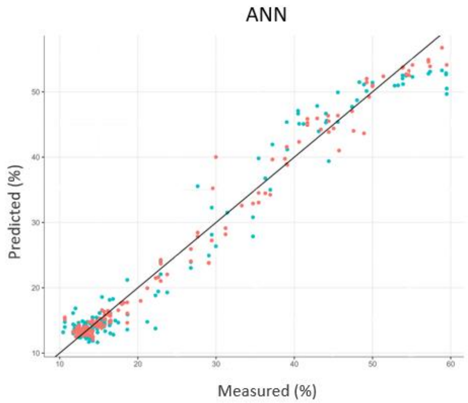
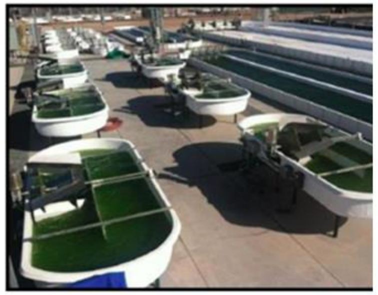
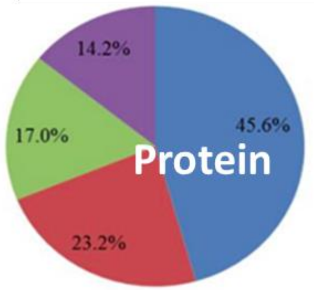


Illustration of machine learning model feasibility prediction of algae composition with <7% error

Future Work: Task 3 - Biomass Quality Tracking and Optimization

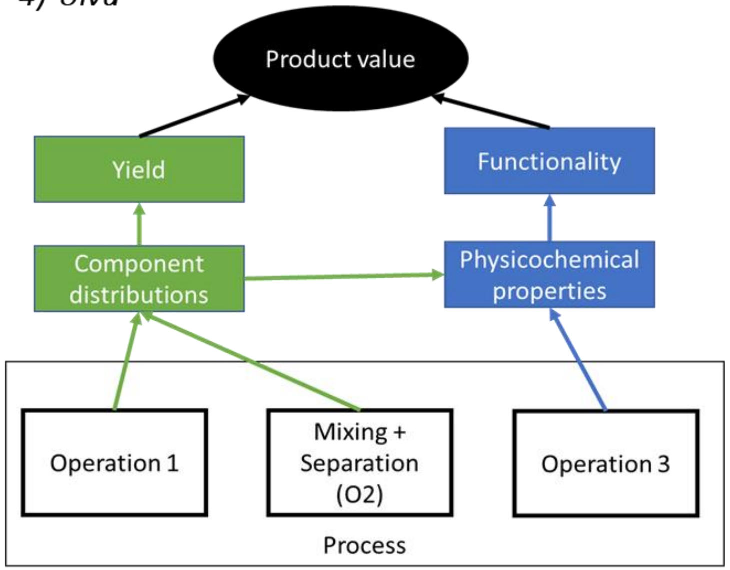
Task 3.2 Valorization of protein (SNL & PNNL)





1) Tetraselmis
2) Picochlorum celeri
3) Monoraphidium
4) Ulva

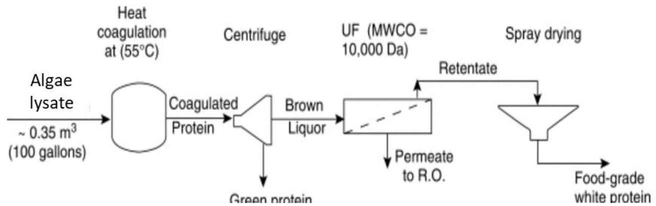
Representative composition of DISCOVR strains



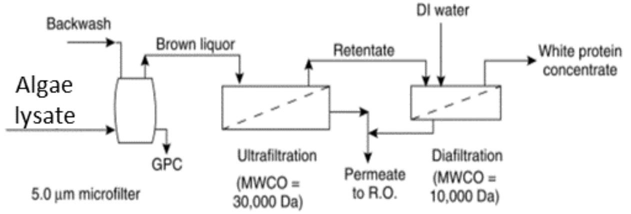
Operation (parameter) selection influences product value

- **Objective:** Need for strain-agnostic, composition-tailored processing
  - High productivity correlates to high protein
  - Protein has intrinsic value (food, feed) and decarbonization potential
  - Reduction in N-content of biomass necessary for fuels conversion
- **Approach:** Evaluate technology for extraction of high value proteins or amino acids for **food & feed**, respectively.
- **Expected outcomes:** Demonstrate process for efficient recovery of at least 50% of soluble protein, with preservation of residues for fuels conversion.
- **Impact:** Improve algae biomass value and decarbonization potential by generation of complete protein isolate (direct replacement for whey protein isolate).

Heat coagulation Approach:



Ultra-filtration Approach:



DISCOVR | Pacific Northwest National Laboratory | Los Alamos National Laboratory EST.1943 | NREL Transforming ENERGY | Sandia National Laboratories | INL Idaho National Laboratory | AzCATI Arizona Center for Algae Technology and Innovation | Colorado School of Mines

Future Work: Task 3 - Biomass Quality Tracking and Optimization

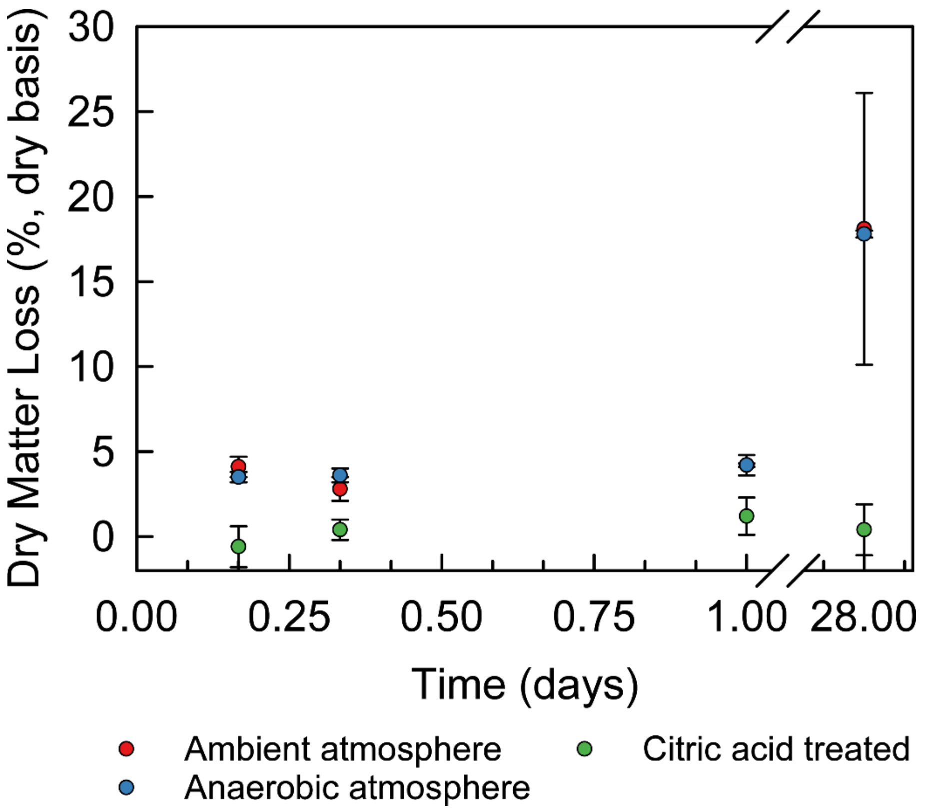
Subtask 3.3 Preserve biomass intrinsic value (INL)

- **Objective:** Establish the risk for degradation of DISCOVR strains during queueing, up to 72 hours post-harvest, and develop handling approaches to actively manage post-harvest algae biomass during the period between harvest and conversion

- **Approach:** Conduct quarterly stability studies of DISCOVR strains at AzCATI
  - Assess post-harvest stability of each strain
  - Determine impacts on biomass quality and composition
  - Evaluate low-cost stabilization strategies

- **Expected Outcomes:**
  - Maintain quality and quantity of post-harvest algae biomass

- **Impacts:** Prevent loss of fixed carbon in algae biomass and preserve biomass for efficient conversion.



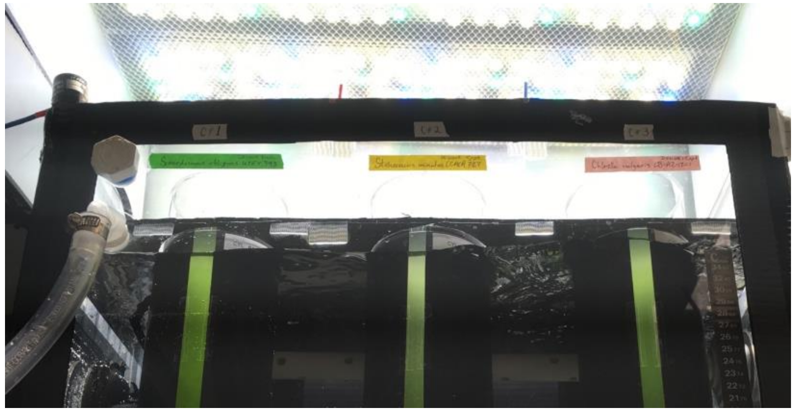
Algae stability in queueing and long-term storage. At 4 hrs post-harvest UTEX 393 had lost 4% biomass, representing a significant risk to algae commercialization

DISCOVR

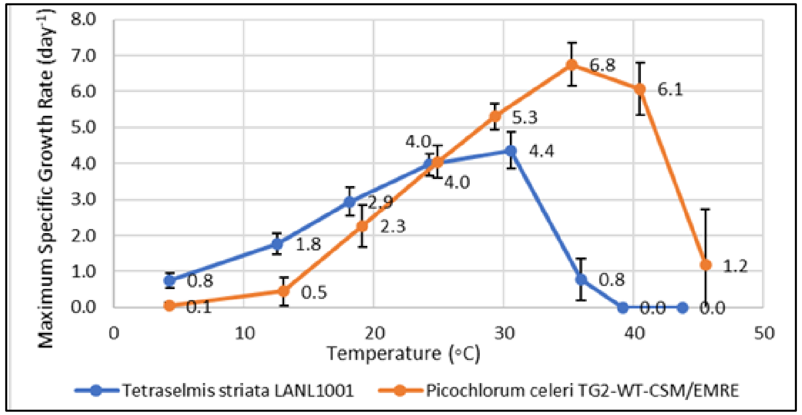
Future Work: Task 4 – Increase Crop Protection and Culture Stability

Task 4.1 Measure biomass productivity of companion cultures (PNNL)

- **Objective:** Reduce periods of low productivity and susceptibility to crashing, compared to monocultures, by cultivating "companion crops" composed of two or more compatible algae with divergent trait characteristics.
- **Approach:** Compatible DISCOVR strains will be co-cultivated in the LEAPS to assess areal biomass productivity and stability.
  - *P. celeri* and *T. striata* will be cultivated in the LEAPS as companion cultures and monocultures, using light and temperature scripts for transition seasons. Productivity will be measured for the companion cultures and respective monocultures.
- **Expected Outcomes:**
  - More resilient productivity in variable weather
  - Reduced pond downtime
  - Progress towards more stable crop rotation
- **Impacts:** Improvement in the annual biomass productivity by reducing periods of low productivity during seasonal transitions.



The Laboratory Environmental Algae Pond Simulator - LEAPS



Temperature Tolerance Profiles of *Tetraselmis striata* LANL1001 and *Picochlorum celeri* TG2

DISCOVR | Pacific Northwest National Laboratory | Los Alamos National Laboratory | NREL Transforming ENERGY | Sandia National Laboratories | INL Idaho National Laboratory | AzCATI Arizona Center for Algae Technology and Innovation | Colorado School of Mines

Future Work: Task 4 – Increase Crop Protection and Culture Stability

Task 4.2 Robust early detection of culture decline and impact on productivity *(SNL)*

- **Objective:** Monitor the reflectivity of *P. celeri* (summer) and *T. striata* (winter) ponds to demonstrate the potential of spectroradiometric monitoring over traditional off-line pond measurements.

- **Approach:** We will monitor ponds cultivating *P. celeri* and *T. striata* with spectroradiometric technology and
  - Compare results to observations, traditional measurements & decisions
  - Optimize algorithms to identify indicators of culture decline
  - Quantify the prediction accuracy of spectroradiometric monitoring

- **Expected Outcomes:**
  - Algal biomass predictions in near real-time (5 min)
  - Pest presence prior to pond crash (1-3 days)
  - Impact of early warning quantified

- **Impacts:** Early indicators of pond productivity declines and/or pest presence for early intervention and mitigation, thereby improving MTBF.

Future Work: Task 4 – Increase Crop Protection and Culture Stability

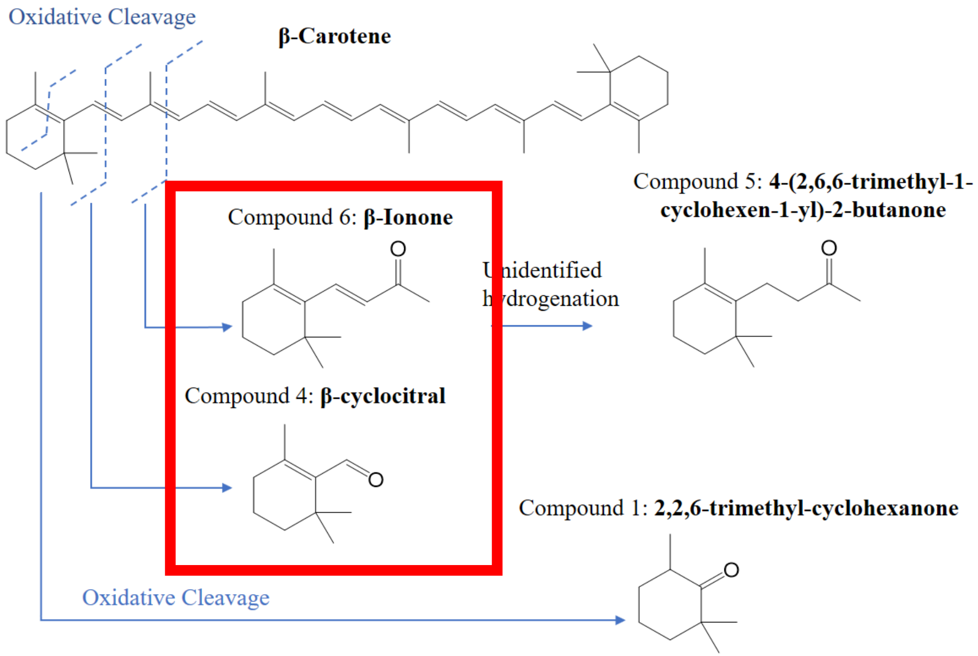
Task 4.3 Improve annualized production through assay deployment (SNL)

- **Objective:** The objective of this task is to produce inexpensive field deployable assays of pond health based on biomarkers.

- **Approach:** We will extend our published work on the identification of biomarkers of crop damage by creating field-deployable antibody-based assays based on previously identified compounds.

- **Expected Outcomes:** We will develop and deploy, in the field, immunoassays against the carotenoid breakdown products trans β ionone and β cyclocitral allowing for early detection of these biomarkers of grazing and cell damage.

- **Impacts:** Successful deployment of a biomarker based early detection system would decrease production costs by reducing the requirement for prophylactic treatments. It would also increase annualized productivity by decreasing the incidence of pond crashes.

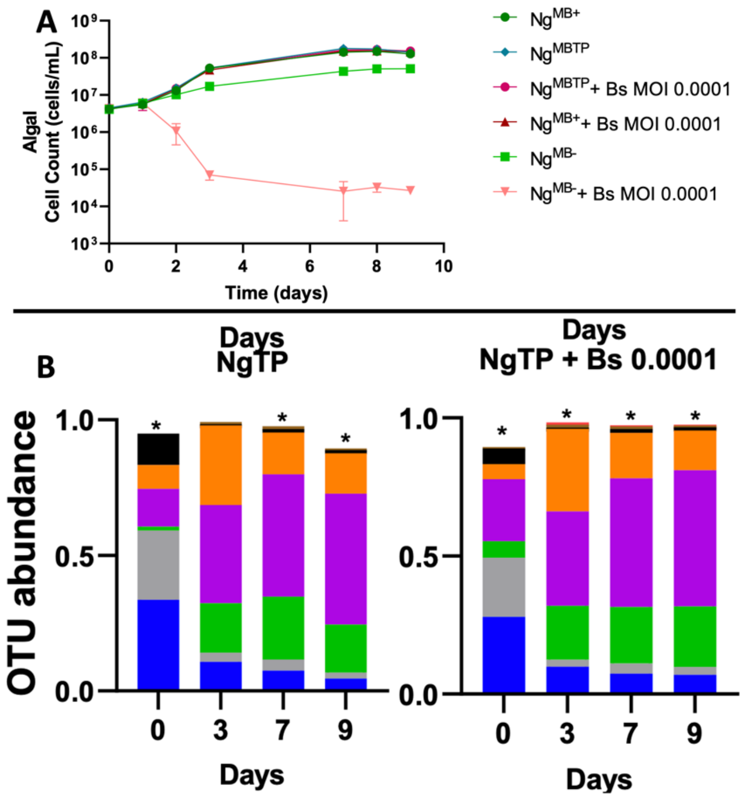


Carotenoid breakdown products (boxed in red) determined to be biomarkers of grazing or algal cell damage

Future Work: Task 4 – Increase Crop Protection and Culture Stability

Task 4.4: Improve MTBF through biotic agent characterization (SNL)

- **Objective:** Provide rapid crash agent identification and countermeasure development to support outdoor cultivation efforts.
- **Approach:** Monitoring algae ponds will facilitate countermeasure development and identify ways to modulate the composition of the microbiome using native bacterial members.
  - screen composition of the microbiome using native bacterial members;
  - transplant existing beneficial microbes to DISCOVR high performance strains and monitor responses;
  - optimize and probe bacterial roles at multiple scales for DISCOVR strains;
- **Expected Outcomes:**
  - Resilient pond cultivations improving SOT.
  - Identification of growth promoting consortia.
  - Screen AzCATI ponds for potential pathogens.
  - Develop phage countermeasures against discovered pathogens.
- **Impacts:** Identification of biotic factors to stabilize pond cultivations at AzCATI to increase productivity.



The protective role of a transplanted microbiome. ***A)*** *Growth profile of N. gaditana (Ng) cultures were infected with and without Bacillis species (Bs) at the indicated MOI (ratio of bacteria: algae). Ng^{MB+} = N. gaditana with intact native microbiome. Ng^{MB-} = N. gaditana axenic culture. Ng^{MBTP}= transplant microbiome from Ng^{MB+} to Ng^{MB-} using a cell sorter. B) Relative OTUs (operational taxonomic units) for bacterial 16S rRNA measured on the indicated days were identical despite infection.*

DISCOVR Pacific Northwest NATIONAL LABORATORY Los Alamos NATIONAL LABORATORY EST.1943 NREL Transforming ENERGY Sandia National Laboratories INL Idaho National Laboratory AzCATI Arizona Center for Algae Technology and Innovation COLORADO SCHOOL OF MINES

Future Work: Task 5 - Outdoor Cultivation Trials

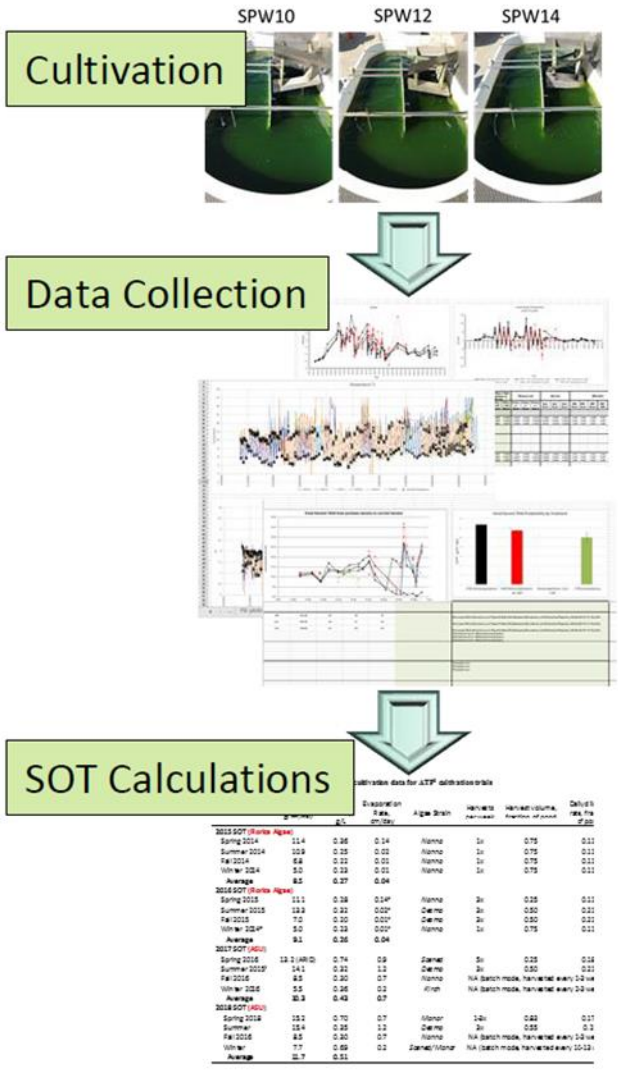
Task 5.1 Conduct outdoor cultivation trials (AzCATI)

- **Objective:** AzCATI will conduct rigorous, standardized, cultivation trials outdoors under real-world conditions in support of Tasks 1-4 with delivery of final curated dataset for public dissemination and as input to Task 6.
- **Rational:**
  - Operating in a standardized, experimental, real-world format, now backed up by over 5 years of near continuous annual production data allows for greater confidence in estimating what may be achievable at scale and identify additional gaps in knowledge that need to be addressed.
- **Approach:**
  - Utilize an outdoor experimental cultivation framework in use by DISCOVR since summer of 2018.
  - Verification of strategies identified in other tasks
- **Expected Outcomes:**
  - Seasonal and annual productivities for top-performing DISCOVR strains and verification of operational conditions identified thru Tasks 1-4 that close the gap with expected operations at scale with curated data deposited for public use.
  - Supply of samples to partners: biomass composition, outdoor-indoor biotic vs. abiotic experimentation, characterization of whole pond culture samples and pest and or novel strain isolations
- **Impacts:** High quality, public data to inform the state of technology

Future Work: Task 5 - Outdoor Cultivation Trials

Task 5.2 Data management and compositional analysis (AzCATI)

- **Objective:**
  - Data curation and dissemination in open access form that is easily accessible to the community and maintain and update primary data collection spreadsheets as required to reflect current operational strategies blic by posting on OpenEI.org.
- **Rational:**
  - Thorough and effective data collection and management is critical to capture primary cultivation and composition data as well as meta-data (pond observations, operational parameters) efficiently and completely to facilitate ongoing experimental design and guiding future strategy.
- **Approach:**
  - Supply samples for proximate analysis to NREL
  - AzCATI to maintain Excel-based primary data collection spreadsheets
  - Capture current operational strategy and facilitate data reporting on monthly, seasonal, and yearly time frames.
- **Expected Outcomes:**
  - Direct data-driven experimental design and TEA through the delivery of final cultivation and composition data to the DISCOVR team as well as well as public facing data catalog.
- **Impacts:** High quality, public data to inform the state of technology



Future Work: Task 6 - TEA/LCA Modeling

Subtask 6.1: Perform TEA Modeling in Support of Experimental Research Efforts

- **Objective:** Use process modeling and techno-economic assessment capabilities to understand critical factors in algae biomass cultivation and provide benchmarks to better guide experimental efforts under the consortium.
- **Approach:**
  - Connecting with researchers to gather information prior to the assessment
  - Carrying out TEA through the development and/or modification of existing tools to accommodate the assessment of the technology in question
  - Providing feedback to researchers and iterating if any updates are needed
- **Expected Outcomes:** Several approaches have been assessed in previous years in support of experimental researchers under DISCOVR; future efforts will focus on updating existing work and assisting new tasks from a TEA standpoint.
- **Impacts:**
  - Cost-effective manner of prioritizing research and guiding the overall direction of the DISCOVR consortium.
  - Solidifying the expertise of researchers in multiple areas through the addition of a TEA layer to existing experimental results.

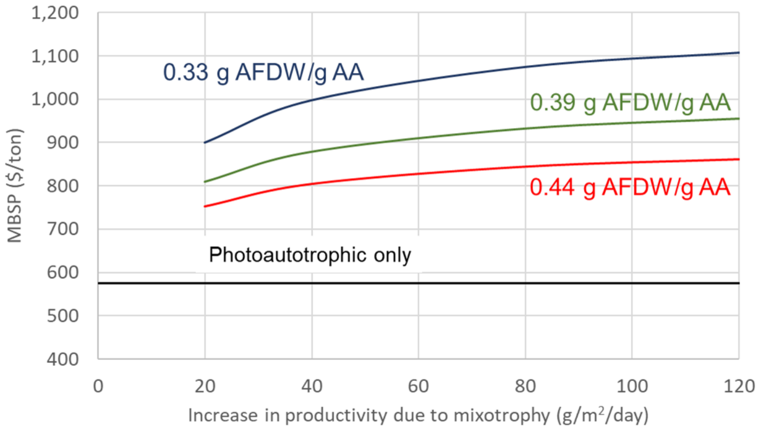


Figure 1. *MBSP of mixotrophic cultivations with acetic acid (AA).*

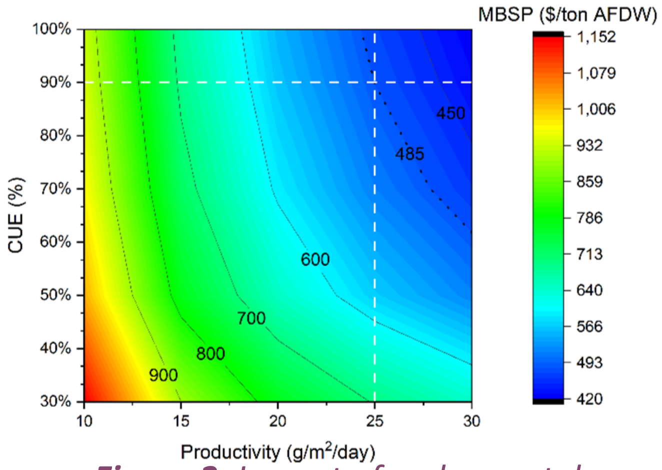


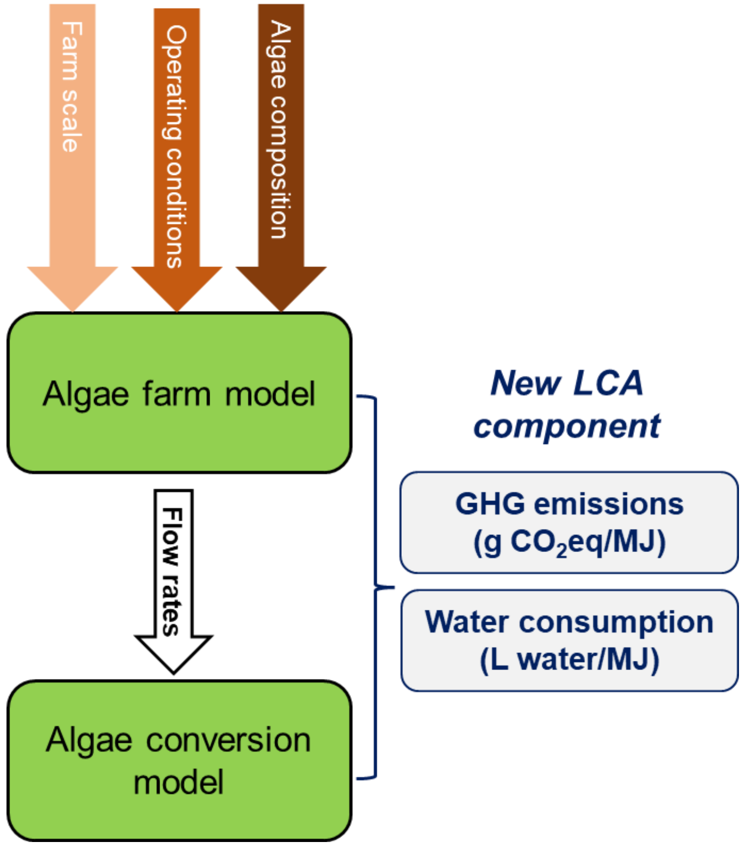
Figure 2. *Impact of carbon uptake efficiency and CO_2 price on MBSP.*

DISCOVR | Pacific Northwest National Laboratory | Los Alamos National Laboratory EST. 1943 | NREL Transforming ENERGY | Sandia National Laboratories | INL Idaho National Laboratory | AzCATI Arizona Center for Algae Technology and Innovation | Colorado School of Mines

Future Work: Task 6 - TEA/LCA Modeling

Subtask 6.2: Perform LCA Modeling in Support of Experimental Research Efforts

- **Objective:** Leverage process models from Subtask 6.1 to identify critical factors in algae biomass cultivation from an environmental impacts standpoint and provide benchmarks to better guide experimental efforts under the consortium, as LCA will be done concurrently with TEA.
- **Approach:** Employing life cycle inventories derived from process models in appropriate LCA tools to accommodate the assessment of the technology in question.
- **Expected Outcomes:** Use LCA as a tool to highlight trends and identify optimization points for reductions in GHG emissions, from which point DISCOVR will coordinate with the (NREL) algae TEA project and the (ANL) LCA project as appropriate to incorporate any insights into future SOT/design case iterations more formally in the BETO algae platform.
- **Impacts:**
  - Provide process-related guidelines to the experimental teams so the consortium can achieve the desired outcomes from a sustainability perspective
  - Diversifying the types of results derived from process simulation efforts in the consortium
  - Aiding the DISCOVR consortium in reaching BETO's goals tied to the decarbonization of bioprocesses and algae biofuels



Overall framework of process simulation and LCA

DISCOVR Pacific Northwest NATIONAL LABORATORY Los Alamos NATIONAL LABORATORY EST.1943 NREL Transforming ENERGY Sandia National Laboratories INL Idaho National Laboratory AzCATI Arizona Center for Algae Technology and Innovation COLORADO SCHOOL OF MINES