Multi-Scale Characterization of Improved Algae Strains

Bioenergy Technologies Office 2021 Peer Review

Taraka Dale

March 9, 2021

Los Alamos NATIONAL LABORATORY

NNSA National Nuclear Security Administration

Managed by Triad National Security, LLC for the U.S. Department of Energy's NNSA

LA-UR-20-27558

Project Overview

Strain improvement tool/approach development and testing at multiple scales

Challenge Productivity needs to be improved to reduce the cost of algal biofuels and bioproducts: Includes growth (productivity) and carbon storage (biomass quality)

Goal **Establish methods for generating improved algae strains/conditions and evaluating their performance at multiple scales, from the bench to outdoors**, by integrating national lab capabilities in algal biology, flow cytometry, and environmental simulation experiments, and by leveraging BETO's investment in algae testbed facilities.

What are we trying to do? Increase algae productivity under outdoor-relevant conditions and translate to outdoors.

How is it done today, limits? Indoor experiments may not sufficiently mimic outdoor conditions; outdoor experiments are resource-intensive and weather-dependent.

Why is it important? Algae productivity is still a key driver for algae biofuels/products costs.

What are the risks? Translation of indoor laboratory studies to outdoor performance is challenging.

1 – Management

Project is organized into 3 primary capabilities

Principal Investigator

T. Dale. Provides expertise, ensures progress and execution, makes final scientific decisions, meets milestones, and complies with reporting requirements

Characterization

Flow Cytometry
C. Sanders

ePBRs
C. Carr

Analytical
N. Sudasinghe

Strain Improvement

Non-GM
C. Sanders

Genetic Modification
R. Gonzalez

Outdoor Testbed

AzCATI
Arizona Center for Algae Technology and Innovation
J. McGowen

- Each subtask is led by a subject matter expert (shown), who guides scientific work and additional staff in using that capability or applying that approach
- Expertise depth is at least 2 people “deep”

1 – Management

Strong communication within team

- Weekly meetings with the project (LANL) team and individual researchers regarding current experiments, approach, and progress
- Weekly meetings with AzCATI
- Monthly calls with BETO Technology & Project Managers
- Regular discussion of risks and mitigating strategies across team

Illustrative Risks	Mitigation
UV mutagenesis fails to result in identifiable mutations attributable to improved phenotypes.	Accept. Either use mutant lines without causal mutations known, or pivot to other strain improvement approaches.
Fitness of selected lab strains does not translate between indoor & outdoor growth systems.	Mitigate. Aim for 20%+ improvement in lab because effect may be muted outdoors. Parallel approaches to strain improvement. Characterization at multiple scales indoors before going outside.
Limited seasons/availability for outdoor testing	Mitigate. Aim to complete ePBR experiments in off season to prepare for upcoming outdoor season. Regular communication with AzCATI for experiment planning. Long-standing partnership reduces risk of miscommunication.

1 – Management

Strong communication & collaboration with related BETO projects

Multi-Scale	Exchange	Communication/Collaboration	Related Project
characterization improvement outdoors		Apply tools, share strains and outdoor cultivation lessons learned	DISCOVR / State of Technology
		Harmonized analytical, cultivation studies and strategies	AzCATI Testbed
		Develop tools, harmonize nutrients, and outdoor cultivation lessons learned	Decision Model Supported Algae Cultivation
		ePBR expertise	OSPREY
		‘Omics data & expertise	Algal Translational Genomics
			IGET
		Nitrogen source analysis	NREL Algae TEA
		Flow cytometry & genetic modification tools	Functional Characterization of Cellular Metabolism
		General strain info and genetic modification tool development	Algae Biotech Partnership
			Genetic Blueprint

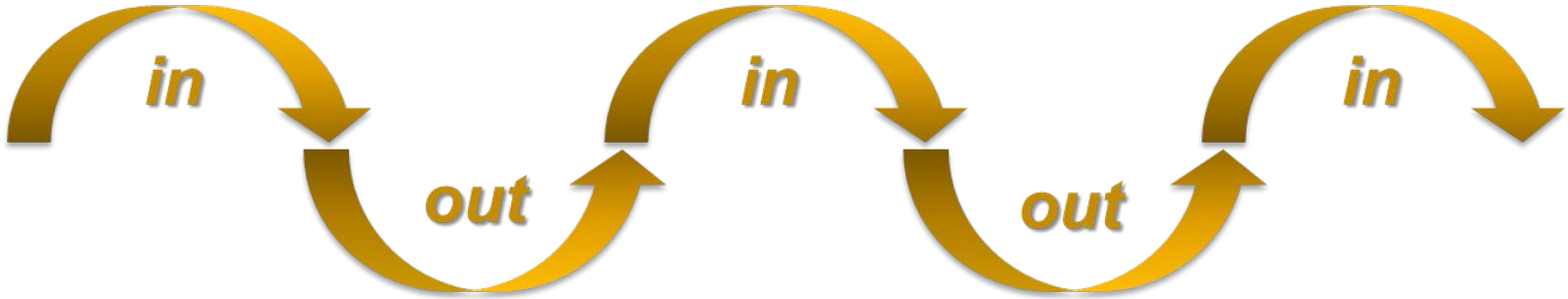
2 – Approach

Iterative approach for indoor-outdoor-indoor work using our 3 capabilities

Strain selection, initial **characterization** & lab understanding of strain

Strain and/or conditions improvement & **characterization**

Next round of **improvements** & **characterization**



Outdoor baseline, lessons learned

Outdoor test, lessons learned

- Different versions of this overall scheme exist, including parallel improvement tracks and a subtask that starts outside rather than inside
- **Outcome:** Process for identifying & testing outdoor-relevant strain/condition improvements

2 – Approach

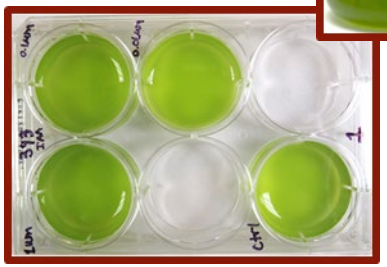
Measurements & methods are harmonized with AzCATI whenever possible

Characterization	Improvement		Outdoor Testbed
Growth – OD, AFDW, Cell Count, [N]	Non-genetic mod.	Carbon Storage – Lipids, Carbs	AzCATI Testbed at ASU, Mesa AZ
Single Cell – Size, Shape, Fluor Dyes	Genetically modify	Biomass Growth – Early, Linear	Batch, Semi-Continuous Runs
Biochemical – FAMEs, Carbs, Protein		Salinity Tolerance – Non-freshwater	

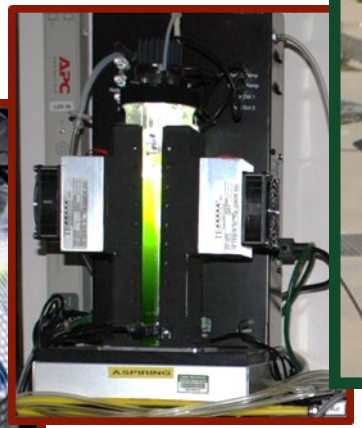
Multiple scales

Shake flask

6 well plate



Spin flask



ePBR

825L, 4.2m^2 Open raceway ponds

2 – Approach

Primary technical metric is highlighted in the end-of-project milestone

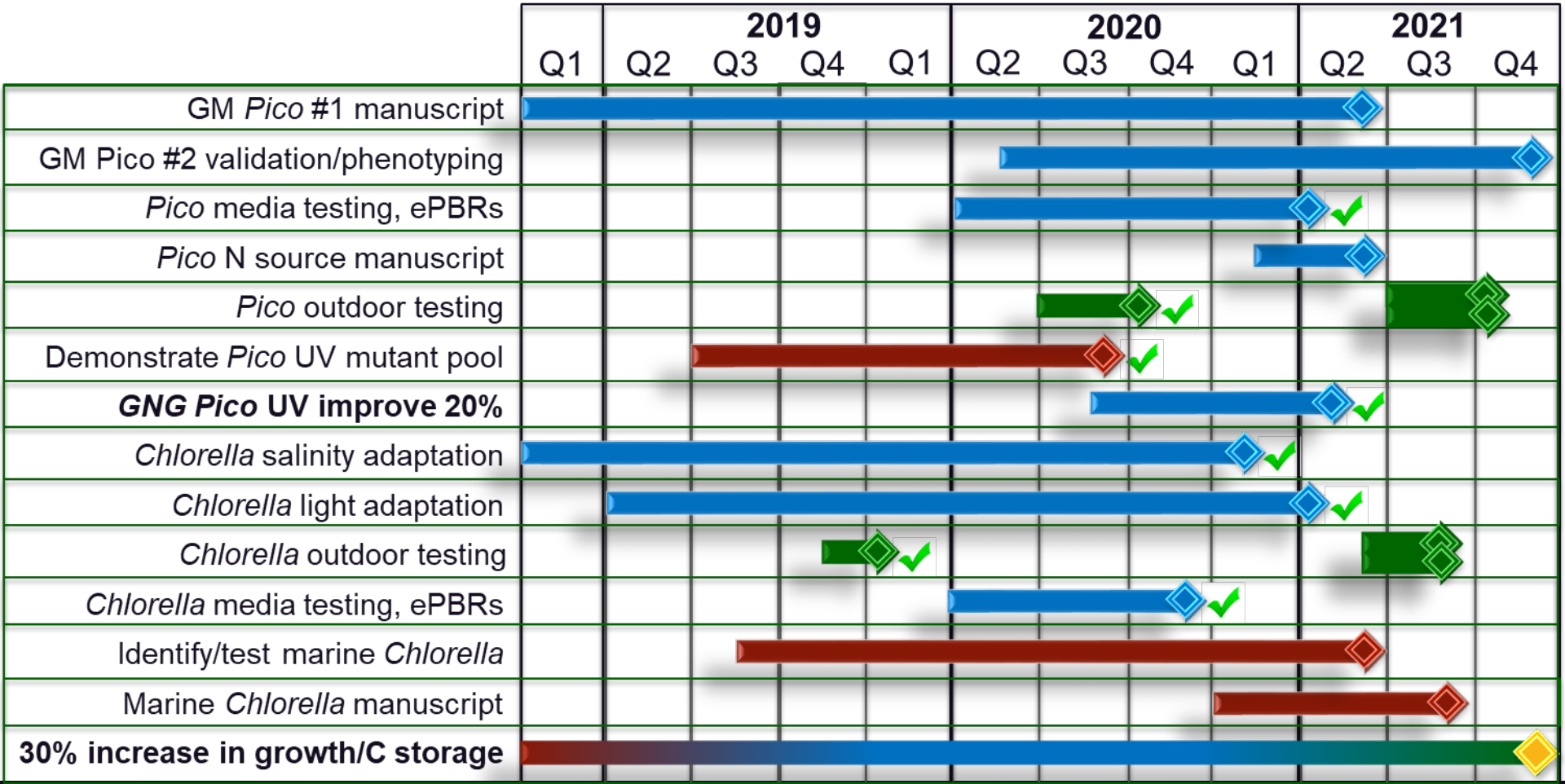
3 Year Goal (FY21Q4 Milestone): Deliver at least two microalgae strains with an outdoor biomass productivity (AFDW) or carbon storage in the form of lipids or carbohydrates of 30% increase over baseline, in a non-freshwater growth medium under a semi-continuous harvesting regime.

Strain baselines:

Metric	*P. soloecismus*	*C. sorokiniana* 1228	Notes
Operation	Semi-continuous	Batch	4.2 m^2 open raceway ponds
Timeframe	July-Aug 2017, 2018	May-June 2015, 2016, 2017	
Productivity (g/m^2-d)	10.0 +/- 1.0	12.8 +/- 1.3	Avg. of 2-3 experiments
FAME	9.9% (2018)	6.7% (2016)	Nutrient replete
Carbohydrate	7.4% (2018)	10.1% (2016)	Nutrient replete
Media	f/2 NO_3^-, pH 7.9	HS or BG-11 NO_3^-, pH 7	

2 – Approach

Experimental timeline is guided by Milestones and Go-NoGo; project is on track



3 – Impact on R&D and Industry

Delivering tools, production-relevant strains, and informing in-out-in approaches

Goal

- Develop tools for strain improvement, test/verify that tools generate strains that are fit outdoors, learn from outdoor experiments to improve tools, deliver strains and tools to algae community

Industry Relevance

- Aim to
  - Work with **relevant strains under outdoor-relevant conditions,** indoors -- media, light cycle/intensity, productivities in g/m^2-d where possible
  - Move **outdoors relatively quickly** – non-GM improvement methods do not require regulatory approval
  - **Adapt laboratory experiments** to better reflect outdoor performance
  - Develop **strain improvement strategies** for general use

Technology Transfer

- Share knowledge gained through presentations (19 posters/talks) and publications (2 this cycle, plus a thesis, with multiple submissions expected this spring)

3 – Impact on R&D and Industry

Using production-relevant strains

- *P. soloecismus* was selected to be part of the summer Algae SOT trials -- high performer in LEAPs and stable in summer at AzCATI. ***Picochlorum* genus is of high interest in the algae community** due to superior outdoor performance
  - Example*: Picochlorum celerei* is the top summer strain for Algae SOT, studied/developed in collaboration with Exxon. Multi-Scale *P. solo.* data helps inform AzCATI SOT Pico experiments.

- *C. sorokiniana* is a known fast grower but crashes at AzCATI in summer; data/strains may be of use at other sites or when crash protection is stronger (forward looking)
  - Example*:* ***Chlorella* genus is commercially relevant for dietary supplements**. Because it's well-studied in the lab, it has served as a good model for salinity adaptation and strain improvement by cell sorting.

3 – Impact on BETO Mission

Informing in-out-in approaches

- Strategies for **strain improvement and relating laboratory to outdoor data (and back again) are needed** to increase productivity and reach BETO cost targets
- **Work closely with AzCATI, DISCOVR, and the SOT** Team to share lessons learned & accelerate implementation of the best strains & practices
  - Salinity adaptation results helped inform DISCOVR salinity results
  - ePBR capability enables work in multiple BETO projects (DISCOVR, Decision Model, OSPREY)
- **Align experiments with SOT cultivation conditions** to enable cross-comparisons and relevance → harmonization broadens datasets

BETO Milestone

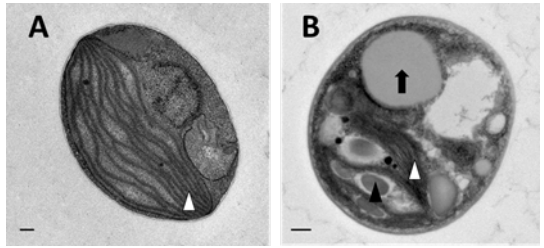
By 2023, develop technologies for **strain development, mass cultivation**, and harvest that, **through laboratory scale experiments**, enable mature modeled algae biomass yields of **30 tons ash-free dry weight algae biomass per acre per year with conversion yields of 80 GGE per ton of biomass**.

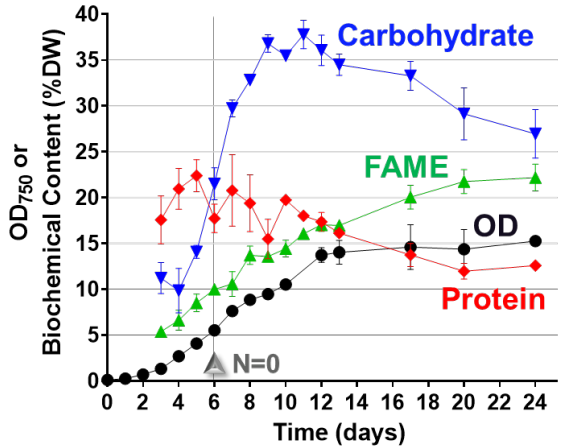
25 g/m^2-d by 2030, also need increases in carbon storage

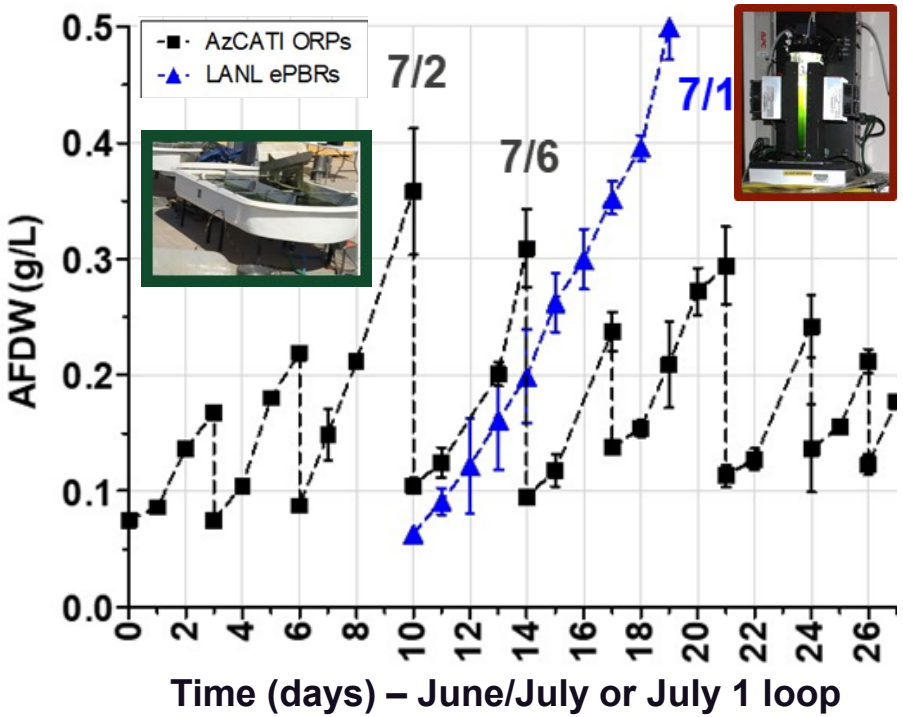
4 – Progress & Outcomes - *Example*

LANL P. solo. *indoor characterization*

Picochlorum soloecismus





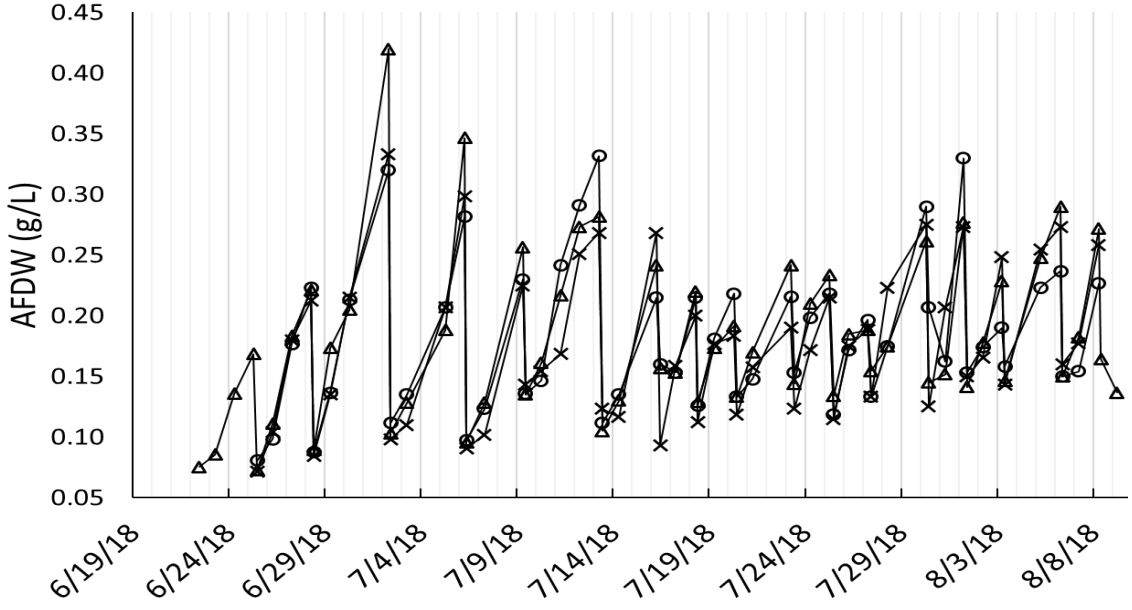


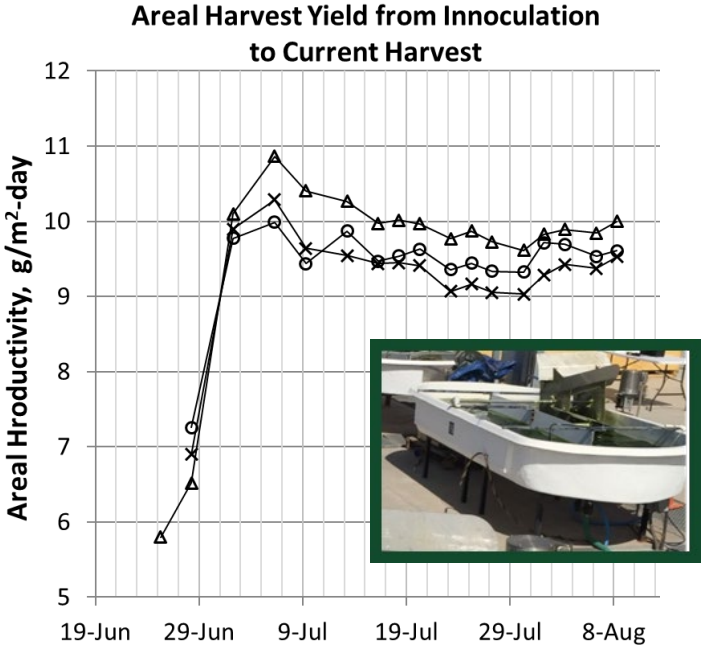
- **Outcome:** *P. solo.* grows quickly with a high salinity tolerance; stores up to 50% DW in carbon; ePBRs simulate *P. solo.* summer productivity at AzCATI well.

4 – Progress & Outcomes - *Example*

P. solo. *outdoor baseline at AzCATI*

- *P. soloecismus* cultivated in triplicate, 1000L open ponds for 40+ days in f/2 in summer





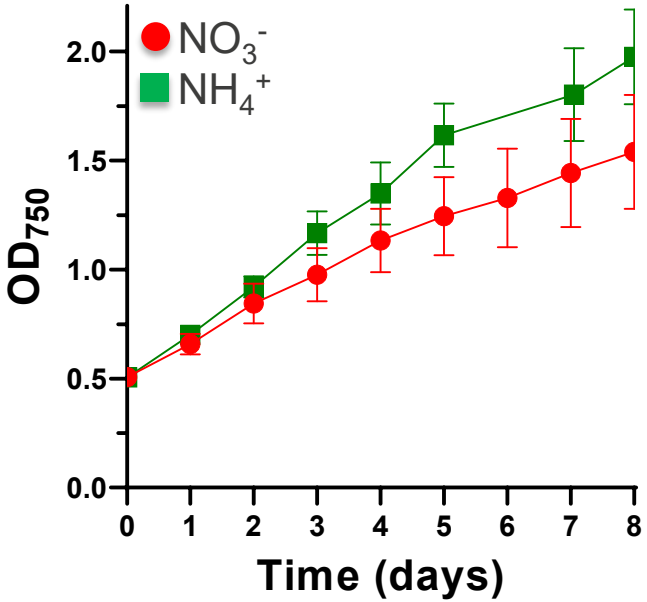
- **Outcome:** *P. solo.* grows slow & steady: 10.7 g/m^2-day in 2018, 17 harvests, ***no crashes***
- [Algae SOT strains were crashing or needing fluazinam treatment during this time]

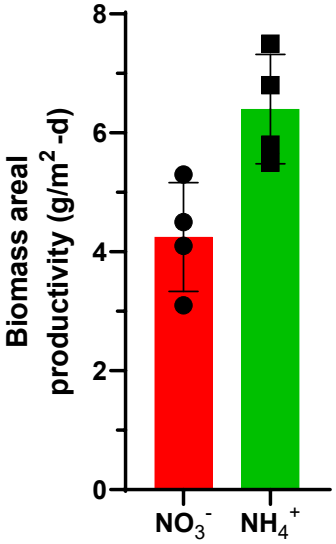
4 – Progress & Outcomes - *Example*

P. solo. *targeted media studies*

- Algae State of Technology (SOT) work is conducted at pH 7, using ammonia as N source

AzCATI May script

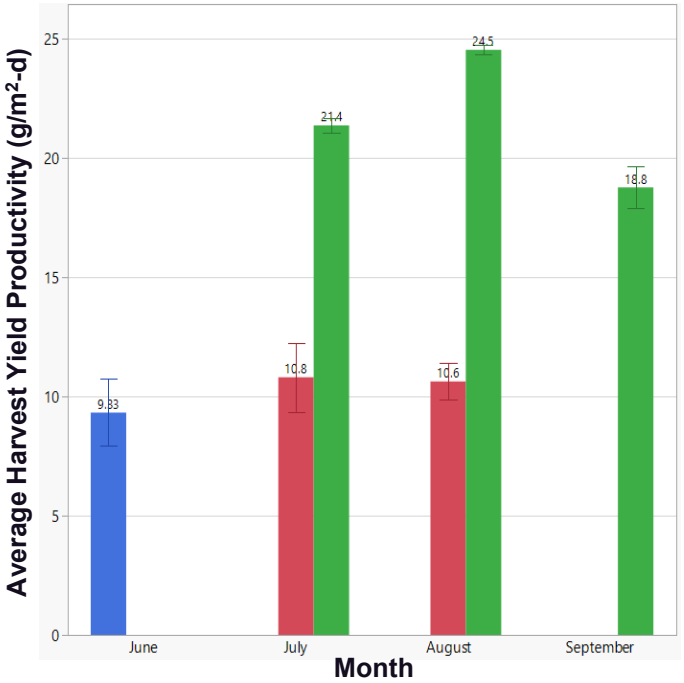


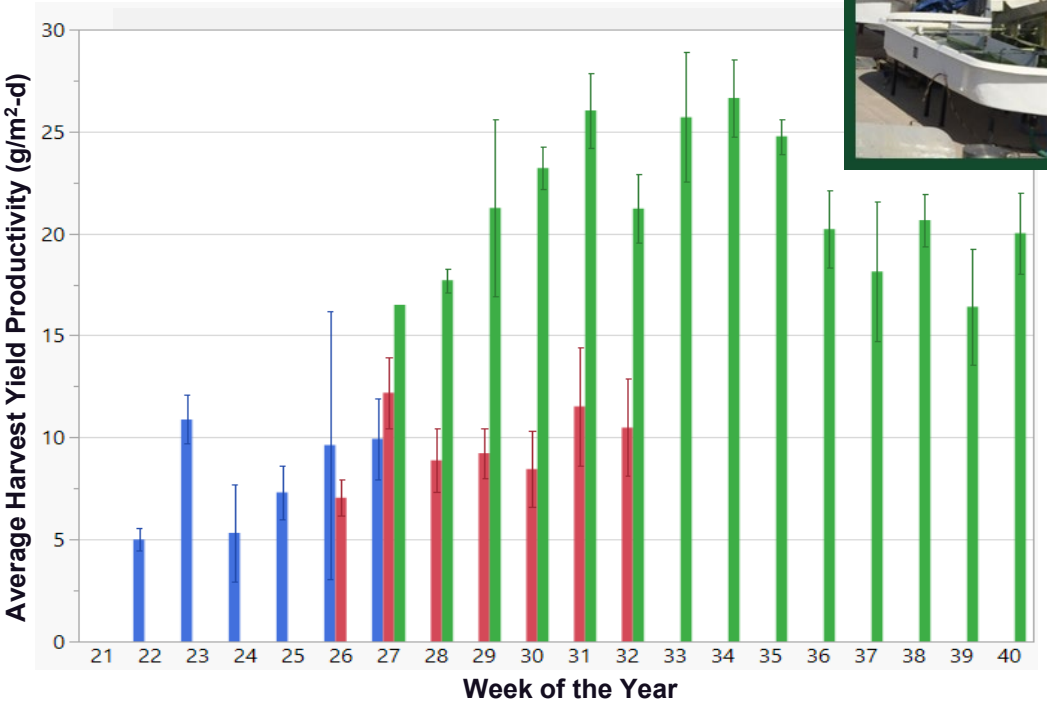


- ODs converted to AFDW and slope productivities calculated; pH also tested (not shown)
- **Outcome:** *P. solo*. grows better (~40%, p=0.03) in ammonia compared to nitrate, and also shows improved growth at pH 7 (not shown)

4 – Progress & Outcomes - *Example*

P. solo. *2nd best strain at AzCATI in 2020, matched Aug 2019 SOT*





- *P. solo.* in 2020: NH_4^+, pH 7, more aggressive harvesting regime, warmer
- **Takehome:** *P. solo*.2020 run showed >2x improvement in productivity over 2018 baseline. Contributes to **FY21Q4 (Final 3-year) milestone**.

4 – Progress & Outcomes: *Picochlorum soloecismus*

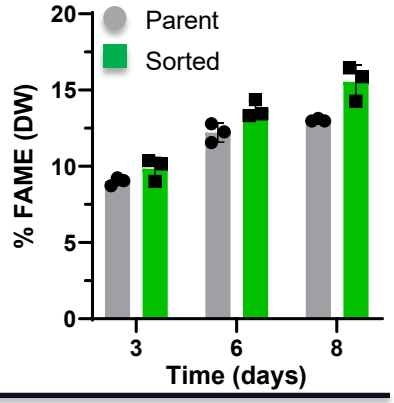
Ongoing: Parallel tracks for strain improvement

Nutrients & Operations

- Complete media studies w/ Aug script
- Test daily dilution rate, starting AFDW
- **FY21Q3 Milestone**: ePBR analysis of 2018 & 2020 AzCATI runs, aim to find contributors to 2020 improvements

Early deplete cell sorting

- Cell sorting by BODIPY early in N depletion
- 20% increase in FAMEs 5 days past depletion (day 8)

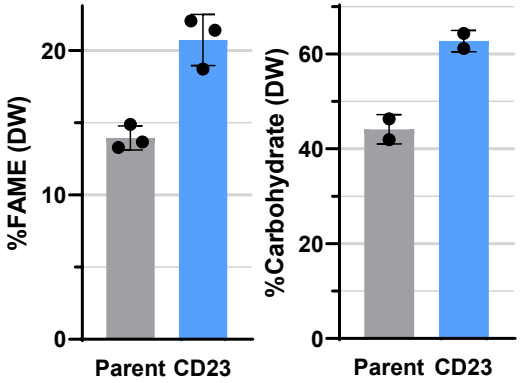


Genetic Modification

- GM strain with 50% increase in Carbohydrates at 3 days past N=0
- Planning AzCATI experiment in outdoor hanging bags (EPA approval not needed)
- Transformants of ≥1 new gene, **FY20Q4 Milestone** ✔

UV mutagenesis

- **FY21Q1 GNG** was NoGo for # of strains (Aim: 3), but did get 1 mutant with increase in FAMEs (50%+) and Carbs (40%) at N=0 +3 ✔



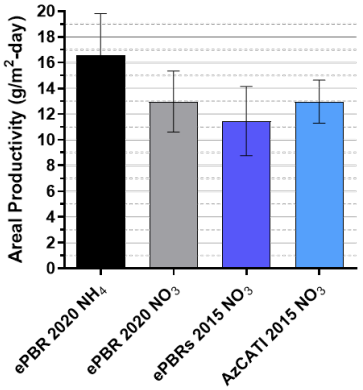
- **Takehome:** Final strains/conditions will be downselected and sent to AzCATI for summer

4 – Progress & Outcomes: *Chlorella sorokiniana*

Similar ongoing approach: baseline, strain improvement, outdoor iteration

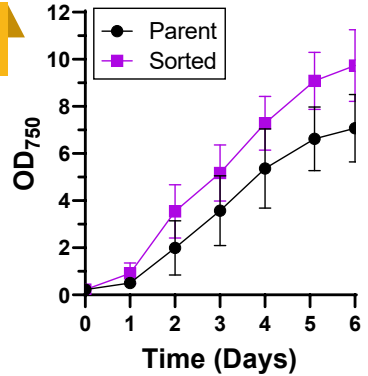
Nutrients & Operations

- NH_4^+ also appears to be beneficial to Cs1228, compared to NO_3^-
- **FY20Q2 milestone** ✔



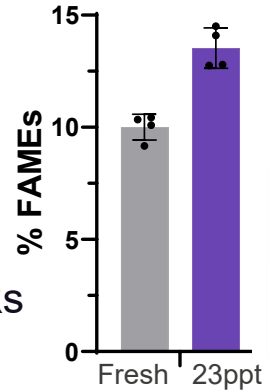
Early deplete cell sorting

- Cell sorting by BODIPY early in N depletion
- Preliminary increase in growth and BODIPY
- Effort ongoing



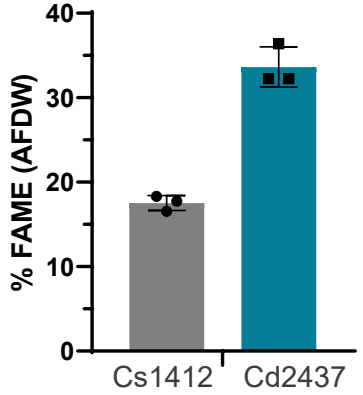
Salinity Adaptation

- Cs1228 adapted to 23ppt increased growth and FAMEs **FY21Q4 milestone** ✔
- Dual salt/light-adapted strain (26ppt) increases growth in ePBRs **FY19Q3 milestone** ✔



New Strains

- Marine "*Chlorella*" strains are salt tolerant & high FAMEs (**FY21Q1 Milestone**)… also are actually *Nannochloris*
- Manuscript draft **FY21Q2**



- **Outcome:** Several promising improved conditions and strains. Final downselect of strains/conditions will take place in the next month, to prepare for AzCATI summer runs.

Summary

1. Overview

We are **establishing methods for generating improved algae strains and evaluating their performance at multiple scales**, from the bench to outdoors, using national lab core expertise

2. Approach

We are using **an iterative, in-out-in process** that incorporates **Strain Characterization, Strain Improvement**, and BETOs' **Outdoor Testbed**

3. Impact

- **Methods for increasing productivity & robustness** using conditions, cell sorting, adaptive evolution, and genetic modification approaches
- ***P. soloecismus*** was the second-best strain in 2020 on AzCATI site. *Picochlorum* is broadly proving to be a highly robust, productive genus
- Learnings from this project **directly feed into and enable DISCOVR, the Algae SOT, and competitive awards**

4. Progress

- **On track to meet/exceed 3-year, 30% improvement goal**. Showed >2x increase in *P. soloecismus* outdoor productivity and 50% increase in *C. sorokiniana* growth and 35% in FAMEs outdoors *via* salinity adaptation.
- Aim to test at least two more strains/conditions this summer at AzCATI

Quad Chart Overview

Timeline

- Start of 3 year cycle: 10/1/2018
- End of 3 year cycle: 9/30/2021

	FY20	Active Project
DOE Funding	$600,000	$1.8M

Project Partners

- Arizona Center for Algae Innovation & Technology (AzCATI); John McGowen

Barriers addressed

Biomass Genetics & Development: The productivity and robustness of algae strains against such factors as temperature, seasonality, predation, and competition could be improved by selection, screening, breeding, mixing cultures, and/or genetic engineering. These approaches require extensive ecological, genetic, and biochemical information. In addition, any genetically modified organisms deployed commercially will also require regulatory approval by the appropriate federal, state, and local government agencies.

Project Goal

Establish methods for generating improved algae strains/conditions and evaluating their performance at multiple scales, from the bench to outdoors.

End of Project Milestone

Deliver at least two microalgae strains with an outdoor biomass productivity (AFDW) or carbon storage in the form of lipids or carbohydrates of 30% increase over baseline, in a non-freshwater growth medium under a semi-continuous harvesting regime.

Funding Mechanism

Annual Operating Plan

Responses to Previous Reviewers' Comments

Reviewer comments	Response
Reviewer(s) stated that the management approach appeared to be poorly defined in the last Peer Review presentation, and that timelines for developing strains and tools were not adequately covered.	We do have a detailed task structure and quarterly SMART milestones. Subject matter experts are responsible for executing on their subtasks. Regular communication ensures that risks are mitigated and deliverables are met. We attempt to describe this more clearly here in 2021.
Reviewer(s) stated that strain stability from cell sorting should be evaluated in liquid culture under field relevant conditions. Retention of a trait on plate passages may not translate to the field.	For the traits we have been able to test (growth, early lipid phenotypes), we have indeed observed a translation of traits from lab to field. Even when we conduct new liquid culture experiments months to (2+) years after our initial improved population is generated, we see consistent stable phenotypes. For the *Chlorella* sorted strains we observed, two years in a row, an improved growth phenotype indoors and at 1000L outdoors. Those cultures did not starve of N, so their lipid phenotype was difficult to assess, and in those populations the largest difference in lipids is in very late deplete phase. So we are working isolating populations with replete/early deplete cultures with increased lipids, to better test that trait outdoors. Also, we had a separate project (same team and methods) wherein we cell sorted *Nannochloropsis* for improved lipid content. We worked with Cellana in their 60,000L ponds in a competitively awarded BETO project to test that strain. In that case, we observed the same improvements in growth (g/L), total FAME (g/L) and lipid profile (increase in EPA fraction) in the 60,000L ponds as we did in the flask experiments indoors (cell sorted relative to parent population). For salinity adaptation we've observed similar growth and similar lipid data in the flasks and in outdoor flat panel reactors.
Reviewer(s) commented that progress on lipid improvements was not described.	Not all subtasks can be described in such a short talk. There has always been active work on improving carbon storage (FAMEs and carbohydrates). We attempt to highlight that more here in 2021.

Full text and status of milestones from slide 9

(Timelines shown in slide 9)

Milestone/Go-NoGo Text	Metric	Status
Draft manuscript competed describing generating, validation, and characterization of *Picochlorum soloecismus* overexpression mutant.	Draft completed	**Complete**
Validate transgenic lines - Validated gene integration and expression of AtPAP/PFK transgenic lines of *P. soloecismus* resulting from the utilization of the molecular toolbox.	Transgenic lines demonstrated	Ongoing
Characterization of at least one new genetically modified *P. soloecismus* mutant line.	Top selected mutant tested for growth, photosynthesis, and biochemical composition.	Ongoing
Completion of performance comparison of *Chlorella sorokiniana* and *Picochlorum* in nitrate and ammonia-based media, to moved towards less expensive nitrogen source.	2 strains tested against 2 nitrogen sources	**Complete**
Draft manuscript prepared describing the effect of different N sources on *Picochlorum soloecismus* growth and FAME content under environmental simulation conditions.	Manuscript draft submitted to BETO.	Ongoing
Demonstration of at least a 20% increase in DNA heterogeneity over the starting population, measured by SNP (point mutation) and/or K-mer analysis of sequenced DNA of mutant pools & control.	1 pool generated	**Complete**
Go-NoGo. UV mutagenesis can be coupled to high throughput screening for an ≥ 20% improvement in FAME content in *P. soloecismus*.	3 independent mutant lines with an ≥ 20% increase in lipid content isolated via cell sorting	**Complete**
Interplay between salt adaptation and response to high light delineated for at least one algae strain.	1 strain demonstrated	**Complete**
Identify panel of *Chlorella* strains that will be used to compare gene expression profiles across salinities.	Panel identified	**Complete**
Draft manuscript prepared describing two marine *Chlorella* strains and their salinity tolerance and relative growth and carbon storage performance.	Manuscript draft submitted to BETO.	Ongoing
End of Project Milestone. Deliver at least two microalgae strains with an outdoor biomass productivity (AFDW) of 30% increase over baseline, in a non-freshwater growth medium under a semi-continuous harvesting regime.	2 strains delivered	Ongoing

Publications, Patents, Presentations, Awards, and Commercialization (project period: FY19-present)

Publications

Wright KT (2020). Characterization of the overexpression of the native H+-pumping pyrophosphatase in the microalga *Picochlorum soloecismus*. Masters thesis, Department of Biology, University of New Mexico.

Gonzalez-Esquer CR, Wright KT, Sudasinghe N, Carr CK, Sanders CK, Turmo A, Kerfeld CA, Twary SN, Dale T* (2019). Demonstration of the potential of *Picochlorum soloecismus* as a microalgal platform for the production of renewable fuels. *Algal Research,* 43, article 101658.

Tyler CRS, **Sanders CK**, Erikson RS, **Dale T, Twary SN**, Marrone BL (2019). Functional and phenotypic flow cytometry characterization of *Picochlorum soloecismus* DOE101 isolates. *Algal Research,* 43, article 101614.

Presentations and Invitations

2021 ≥3 posters/presentations planned for ABBB conference 2021

2020 14th Annual Algae Biomass Summit Biology Track Chair. **Dale T.** Invited co-organizer.

2020 NSF/BETO Workshop 'Rules of Life: Complexity in Algal Systems'. **Dale T.** Invited participant. **Gonzales-Esquer CR, Sanders C**, participants.

2020 University of New Mexico Biology Seminar. **Wright K**. *Engineering Microalgae to Increase Carbon Storage Molecules for Biofuels*. Oral Presentation.

2020 LANL Virtual Student Symposium. **Daughton B.** *Varying nitrogen sources to reduce algae production costs*. Oral presentation.

2020 LANL Virtual Student Symposium. **Ivankovich X.** *Mutagenesis and screening of the green microalga* Picochlorum soloecismus. Oral presentation.

Publications, Patents, Presentations, Awards, and Commercialization (project period: FY19-present)

Presentations and Invitations, *continued*

2020 LANL Virtual Student Symposium. **Ivankovich X.** *Mutagenesis and screening of the green microalga* Picochlorum soloecismus. Oral presentation.

2020 LANL Virtual Student Symposium. **Pacheco S.** *Algae to biofuels research: Chlorella salinity tolerance*. Oral presentation.

2020 University of New Mexicon Masters Thesis Defense. **Wright KT**. *Characterization of the overexpression of the native H+-pumping pyrophosphatase in the microalga* Picochlorum soloecismus. Oral presentation.

2020 Cytometrists of the Western States. **Sanders C**. *Flow cytometry as a tool for biomanufacturing*. Oral presentation.

2019 13th Annual Algae Biomass Summit Biology Track Chair. **Dale T.** Invited co-organizer.

2019 LANL Bioscience Division Seminar. **Sudasinghe N.** *Harnessing algae for bioenergy and co-product development to advance the bioeconomy.* Oral presentation.

2019 BIO World Congress on Industrial Biotechnology. **Dale T.** Panel topic: *Advancements in technologies to produce "green crude" and other products from algae, yeast, cellulosic, and other feedstocks to meet the growing demand for renewable energy.* Talk title: *Altering carbon partitioning using high throughput whole cell screening.* Invited speaker and panelist.

2020 The 9th International Conference on Algal Biomass, Biofuels and Bioproducts. **Wright K.** *Overexpression of the vacuolar proton-pumping pyrophosphatase, AVP1, increases starch accumulation in* Picochlorum soloecismus. Poster presentation.

2019 LANL Bioscience Division Seminar. **Gonzalez-Esquer CR.** *Reconfiguring the metabolism of photosynthetic microbes for their development as biotechnological platforms.* Oral presentation

Publications, Patents, Presentations, Awards, and Commercialization (project period: FY19-present)

Presentations and Invitations, *continued*

2019 41st Symposium on Biotechnology for Fuels and Chemicals. **Dale T**. *Lipid production and processing.* Co-responsible for organizing the session, including inviting speakers. Invited co-chair of the session.

2019 UNM Poster Symposium. **Wright KT**. *Overexpression of the native H+-pumping pyrophosphatase in the microalga Picochlorum soloecismus stimulates the accumulation of carbon storage molecules*. Poster presentation.

2019 3rd annual University of New Mexico STEM Research Symposium. **Wright K.** *Overexpression of the vacuolar proton-pumping pyrophosphatase, AVP1, increases starch accumulation in* Picochlorum soloecismus. Poster presentation.

2019 LANL Bioscience Division Seminar. **Dale T.** *Multi-scale characterization of improved algae strains.* Oral presentation.

2018 University of Georgia. **Dale T.** *Optimizing metabolic flux for improving the productivity of biofuels and bioproducts.* Invited seminar.

2018 University of New Mexico Molecular Techniques Course Lecture. Wright K. Genetic Engineering Microalgae for Biofuels. Oral Presentation.

2018 12th Annual Algae Biomass Summit Biology Track Chair. **Dale T.** Invited co-organizer.

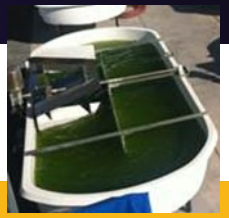
2018 12th Annual Algae Biomass Summit. *Saline adaptation of the freshwater microalga* Chlorella sorokinaina for sustainable biofuel produciton. **Sudasinghe N.** Poster presentation.

2018 12th Annual Algae Biomass Summit. **Wright KT.** *Overexpression of the native H+-pumping pyrophosphatase, AVP1, increases starch accumulation in* Picochlorum soloecismus. Poster presentation.

Cultivation Systems Comparison

Indoor and Outdoor

- Substantial consideration is given for how outdoor experiments are conducted and how indoor experiments can be harmonized with that.
- Use light/dark cycles at relatively high light intensities (at least 800 µmol/m-sec^2) in most systems.
- ePBRs are used with scripts that mimic outdoor sinusoidal light and temperature conditions, with intensities of light matching full sunlight.
- CO_2 delivery on demand by pH control in most systems.
- Media and nutrients are harmonized.
- Use the same analytical methods as AzCATI whenever sufficient biomass is available, and are harmonizing our operational conditions and starting densities when possible.



Variable	Lighted CO_2 Incubator	Spin Flask	Environmental Photobioreactors (ePBRs)	MicroBio Ponds	Outdoor Ponds (AzCATI)
System	Flasks (~30 ml)	Flasks (1-1.6L)	Cylinder ~0.6L	Raceway 50-100 L	Raceway 1000 L
Mixing	Shaker	Stir bar	Bubbled air & stir bar	Paddlewheel	Paddlewheel
Temp	Constant (25C)	Constant (25C)	Sinusoidal light/temp script	Greenhouse	Seasonal temps
Lights (µmo/m^2-s)	300, 16:8 on/off	800, 16:8 on/off	2000, script, sinusoidal	1200, sinusoidal with artificial add	Seasonal daylight
Media	HS (fresh), F/2 (marine)	HS (fresh), F/2 (marine)	HS (fresh), F/2 (marine)	HS (fresh), F/2 (marine)	HS or BG11 (fresh), F/2 (marine)
Operational Conditions	Batch, replete/ deplete, high [biomass]	Batch, replete/ deplete, high [biomass]	Batch or semi-continuous, replete/deplete, outdoor [biomass]	Batch or semi-continuous, replete/deplete, outdoor [biomass]	Batch or semi-continuous, replete/depl, outdoor [biomass]
Productivity	OD_{750} & cell counts time course, AFDW end point	OD_{750} & cell count time course, AFDW end point	OD_{750} & cell count time course, AFDW end, calculated slope g/m^2-day	OD_{750}, cell count, AFDW time course, slope or harvested g/m^2-day	OD_{750}, cell count, AFDW time course, slope or harvested g/m^2-day
Biochemical	End point	End point	Time course	Time course	Time course
CO_2 Delivery	Constant 1% CO_2	pH control by CO_2	pH control by CO_2	pH control by CO_2	pH control by CO_2