DOE Bioenergy Technologies Office (BETO) 2015 Project Peer Review

Multi-Scale Characterization of Improved Algae Strains

March 23, 2015

Algae Technology Area Review

Dr. Taraka Dale

Los Alamos National Laboratory

This presentation does not contain any proprietary, confidential, or otherwise restricted information

LA-UR-15-21927 Operated by Los Alamos National Security, LLC for the U.S. Department of Energy's NNSA

Goal Statement

The overall goal of this project is to develop a streamlined process for improving algae strains and characterizing their performance at multiple scales, from the bench to outdoors.

Quad Chart Overview

Timeline

- 10/1/2012
- 9/30/15
- Percent complete: 80%

Barriers Addressed

- Aft-C Feedstock Development
- Aft-B Sustainable Production
- Aft-H Integration & Scale-up

Budget

	Total Costs FY 10 –FY 12	FY 13 Costs	FY 14 Costs	Total Planned Funding (FY 15-Project End Date
DOE Funded	0	225k	250k	350k
Project Cost Share (Comp.)*	None	None	None	None

Partners

- Intrafunding Partners
  - ATP3 testbed facility, service contract
- Interfunding Partners
  - PNNL (M. Huesemann, M. Wigmosta), separate AOP funds

1 – Project Overview

- The problem: Laboratory-developed algae strains often do not perform as well or as predictably in outdoor conditions

- This leads to 2 questions:

How do we predict which strains will perform best outside?

How do we effectively transition strains from the lab to the pond?

2 – Technical Approach: Three Objectives

1. Establish a pipeline for evaluating improved strains under conditions that directly simulate outdoor climate conditions

2. Generate additional improved algae strains using flow cytometry, adaptive evolution, and transcriptome analyses

3. Transition strains to outdoor ponds for testing

2 – Management Approach

- Regular (daily to weekly) conversations within the LANL team on current experiments, approach, and progress

- As-needed discussions with PNNL and ATP3 regarding collaborative work

2 – Technical Approach: Three Objectives

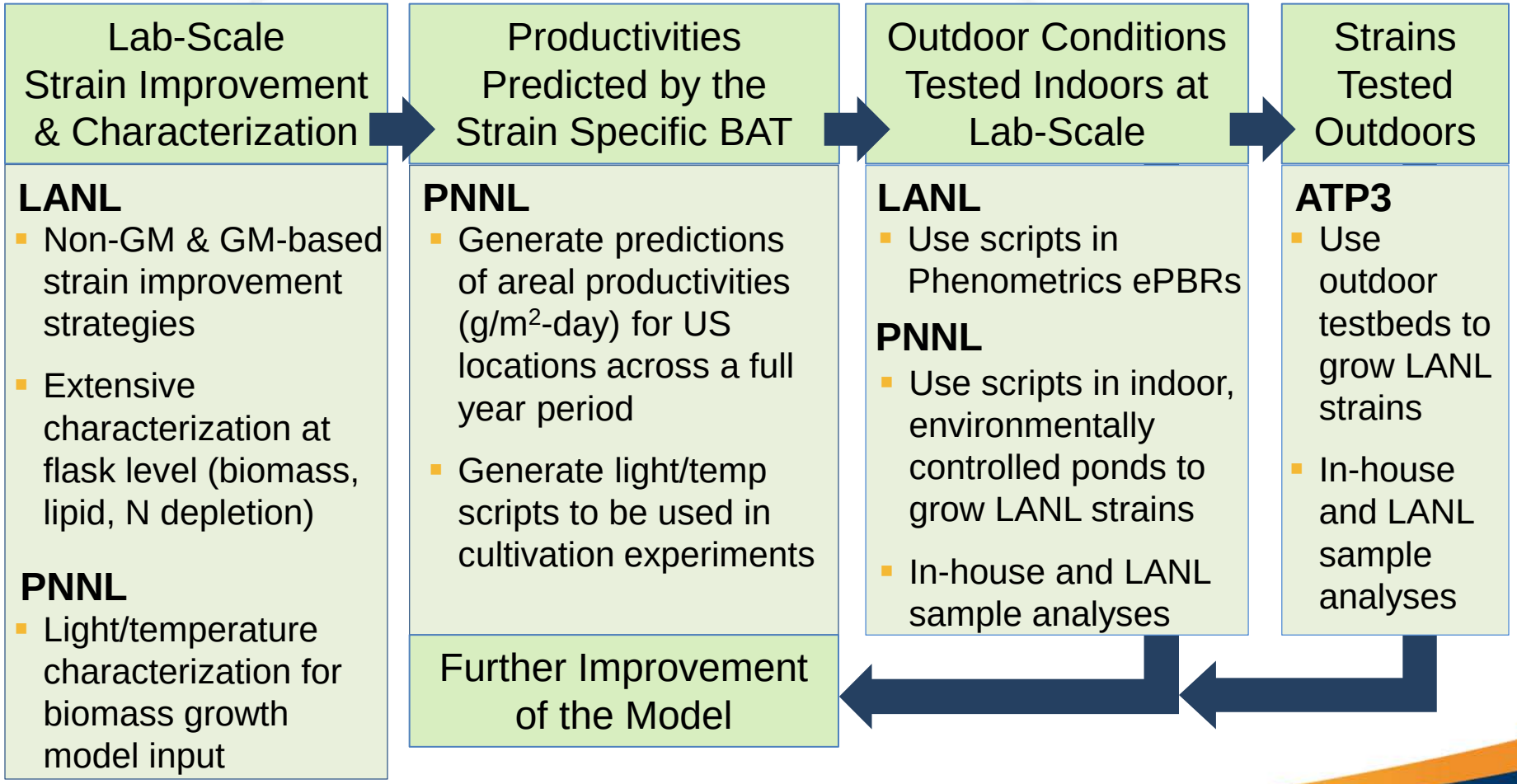
1. Establish a pipeline for evaluating improved strains under conditions that directly simulate outdoor climate conditions

2. Generate additional improved algae strains using flow cytometry, adaptive evolution, and transcriptome analyses

3. Transition strains to outdoor ponds for testing

3 – Accomplishments, Objective 1:

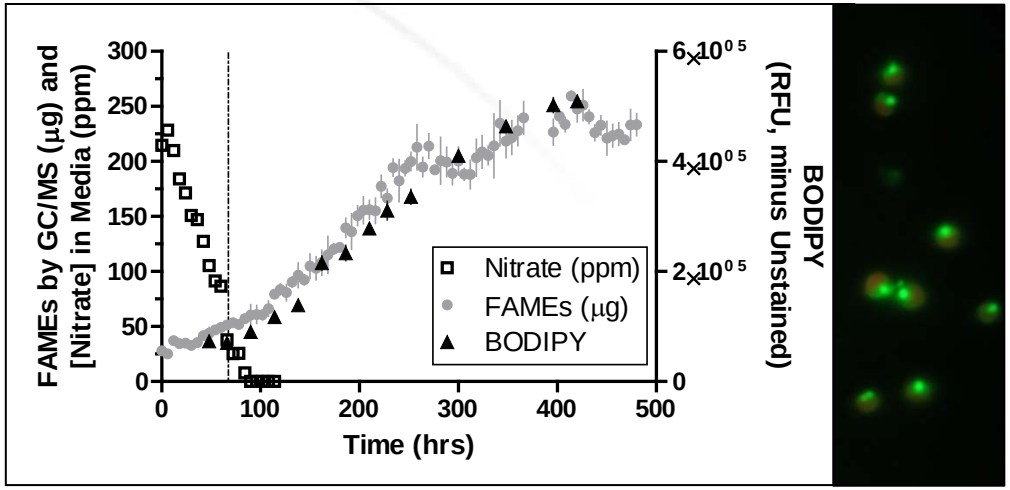
Established a pipeline for characterizing algae strains under outdoor light & temperature conditions

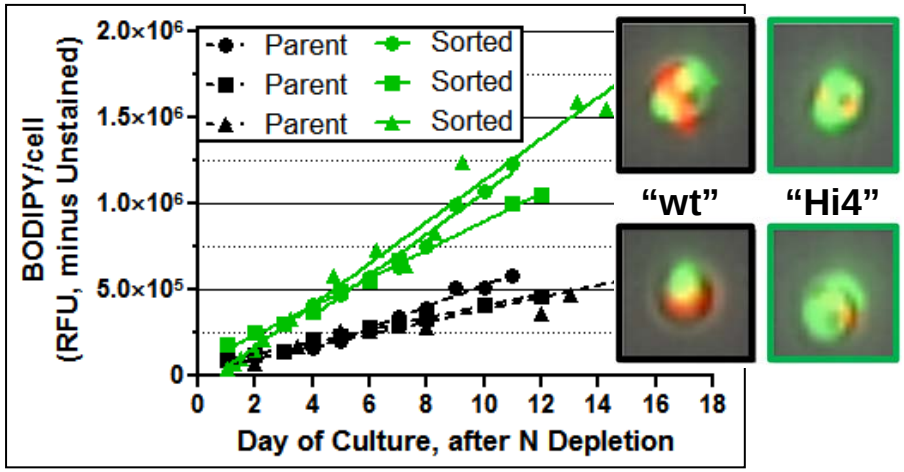


3 – Accomplishments, Objective 1:

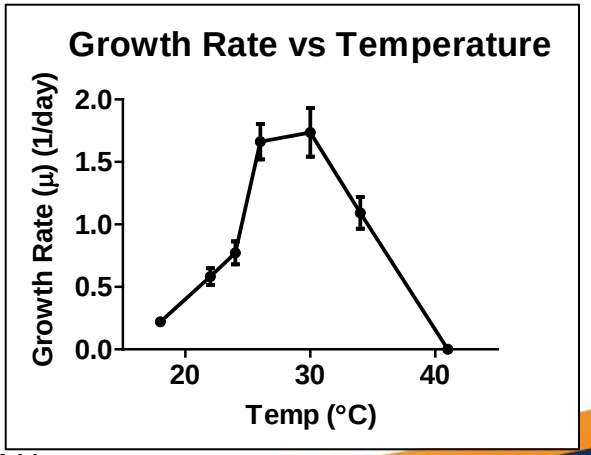
Used Picochlorum *sp. as an example strain for pipeline, strain characterization*

- *Picochlorum* has been identified as a promising biofuel production strain
- Extensive lab-scale characterization at LANL (biomass, lipid, N depletion)
- Within NAABB, *Picochlorum* sp. was improved by 2.8x in lipid accumulation using FACS, & GM tools were developed





- Additional parameters were measured at PNNL for the Strain Specific Biomass Assessment Tool (BAT)

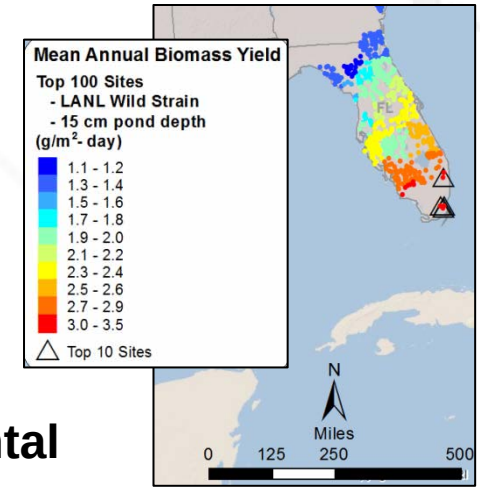


M Huesemann

3 – Accomplishments, Objective 1:

Used Picochlorum *sp. as an example strain for the pipeline, growth prediction and script generation*

- The BAT predicted that the highest areal productivity for *Picocholorum* would be in south Florida during the month of May
- A script using 30-yr average light/temp data for this month/location was generated and used for cultivation at PNNL and LANL



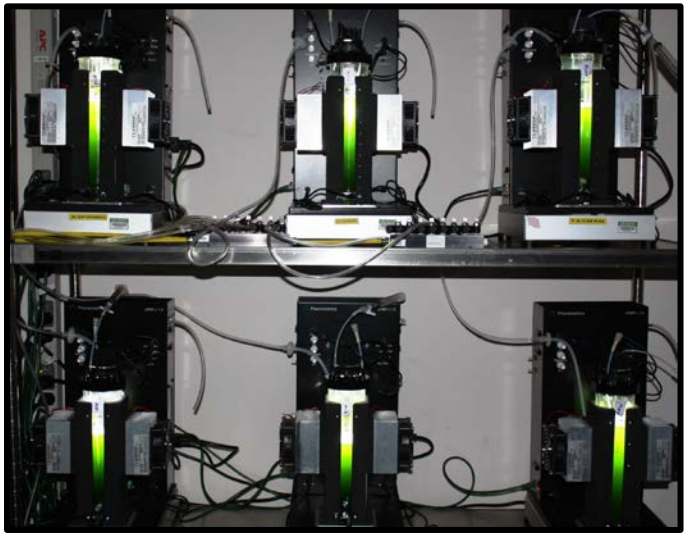
M. Wigmosta

PNNL Environmentally Regulated Ponds

M Huesemann

- Light/temperature/pH control
- Full sunlight intensity
- Duplicate experiments, 800L

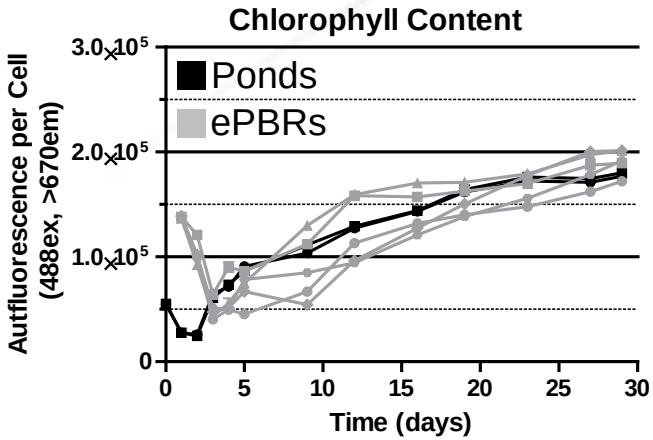
LANL ePBR Matrix Phenometrics Environmental Photobioreactors

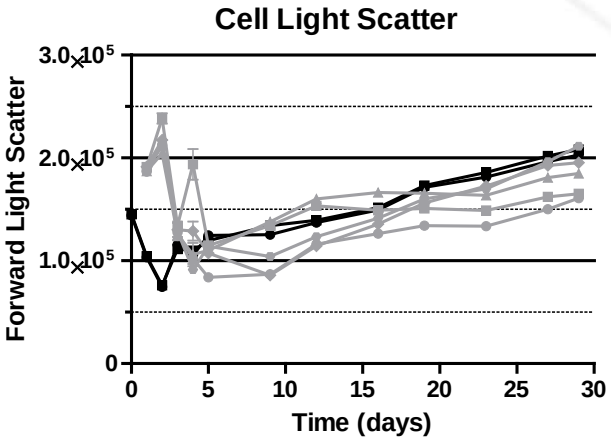


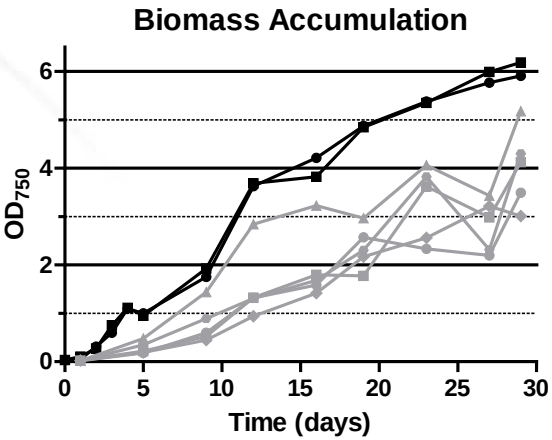
- Light/temperature /pH control
- Full sunlight intensity, light shines from above
- 33 ePBRs (high replicate potential)
- <0.63L

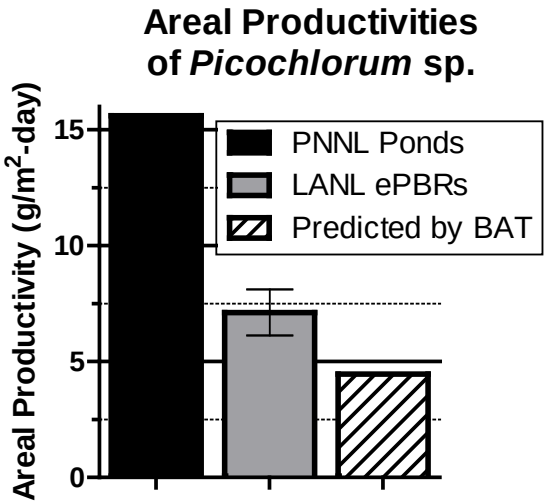
3 – Accomplishments, Objective 1:

Used Picochlorum *sp. as an example strain for the pipeline, culture characterization in PNNL Ponds & ePBRs*









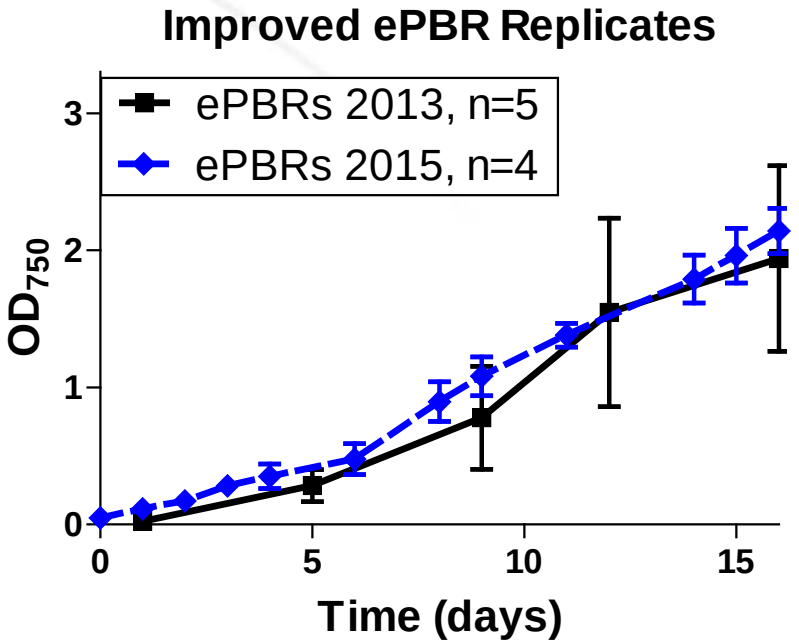
- Single cell characteristics were similar between ponds and ePBRs, but overall growth was quite different
- ePBRs showed just ~50% of the areal productivity of the ponds
- The BAT underpredicted both Ponds and ePBRs: *Picochlorum* grows at 15.6 g/m^2-day, much better than predicted and as productive as the best NAABB strain

3 – Accomplishments, Objective 1:

Modified ePBRs considerably, improved reproducibility

- **ePBR hardware and script modifications, round 1**
  - CO_2 delivery
  - Gas diffusion improved
  - pH control
  - Rapid yet sterile sampling
  - Replenishment of evaporative loss

- **ePBR hardware and script modifications, round 2**
  - Flowmeters to improve consistency of CO_2 delivery
  - 2 gas diffuser stones tested
  - Finer pH control
  - New vortex stir bars

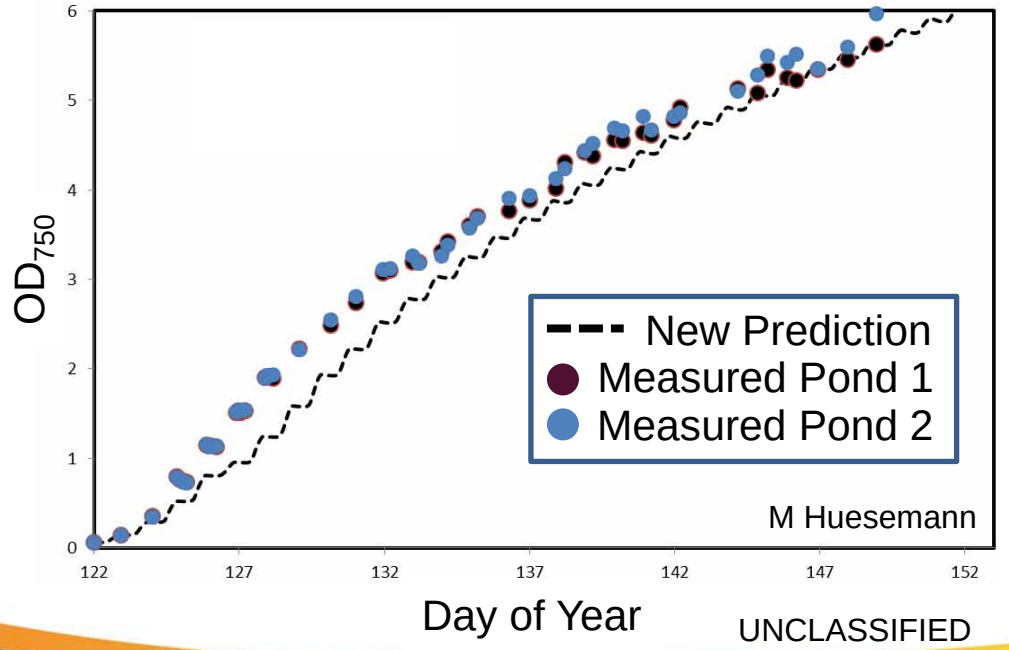


- More work in this area in the AB AOP
- Adding red light supplement → 40% increase in growth for *N. salina*
- Some strain dependencies

3 – Accomplishments, Objective 1:

Used Picochlorum *sp. as an example strain for the pipeline, model improvement*

- Previous strains (*N. salina, C. sorokiniana*) showed good agreement between the PNNL ponds, the BAT prediction, and directly measured outdoor data
- What is different about *Picochlorum*? → Increased light scattering of the culture
- This parameter was measured for *Picochlorum* and added to the model, leading to an improved biomass growth prediction



This result demonstrates the value of adding more strains to the biomass assessment tool, which was initially developed based on a "generic" algae.

2 – Technical Approach: Three Objectives

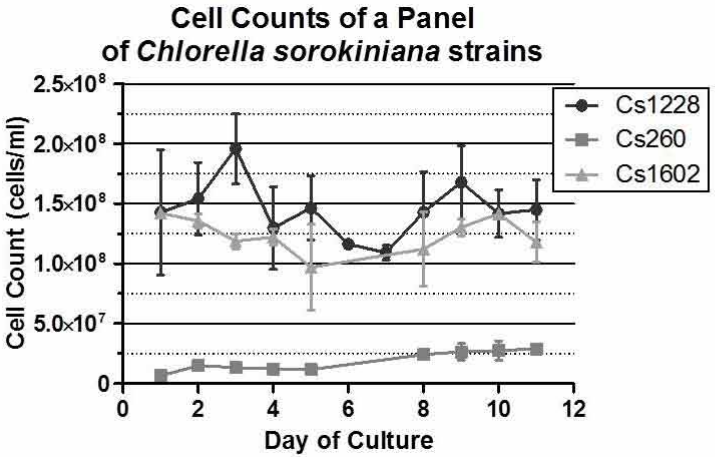
1. Establish a pipeline for evaluating improved strains under conditions that directly simulate outdoor climate conditions

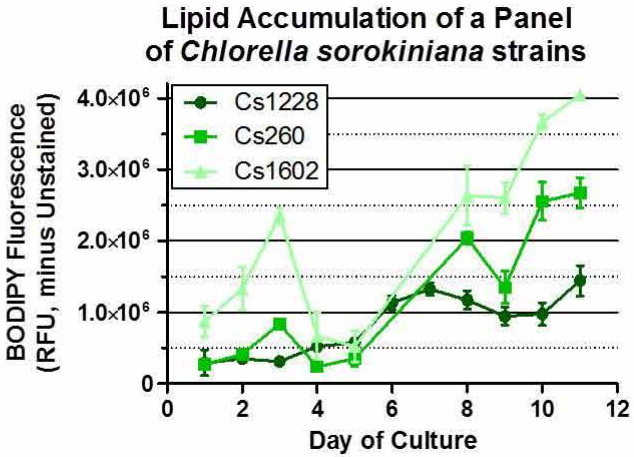
2. Generate additional improved algae strains using flow cytometry, adaptive evolution, and transcriptome analyses

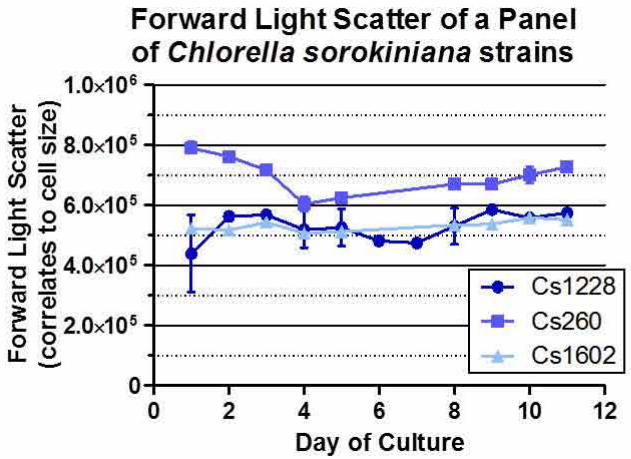
3. Transition strains to outdoor ponds for testing

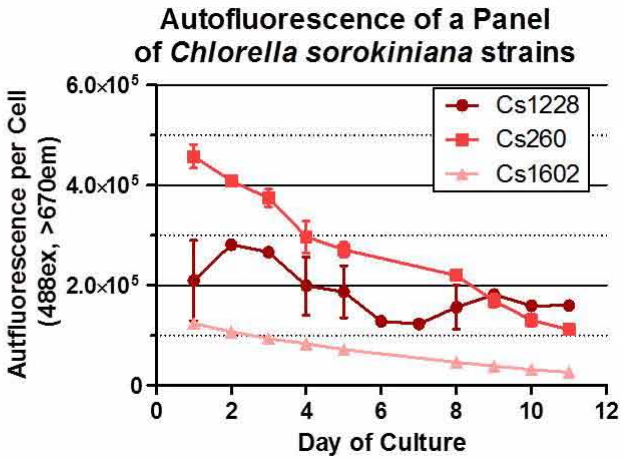
3 – Accomplishments, Objective 2:
Characterization of C.sorokiniana *panel*

- Adding a freshwater strain to our repertoire was desired
- *Chlorella sorokiniana* has been shown in NAABB to be a top performer
- A panel of three additional *C. sorokiniana* strains were characterized during N-depletion
- Cs260 grew poorly
- Cs1228 & Cs1602 were selected



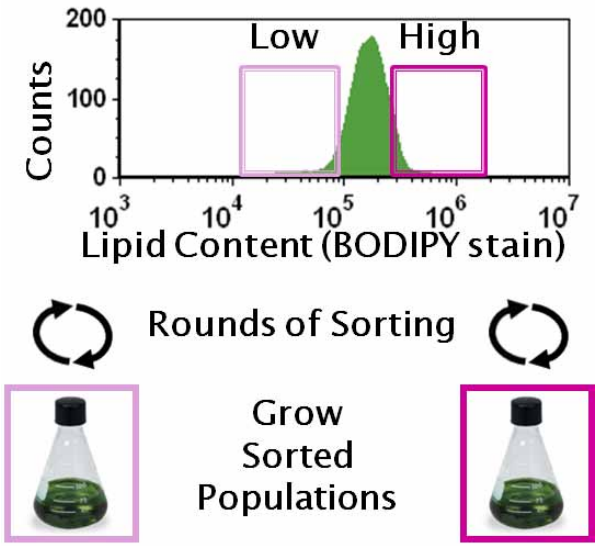






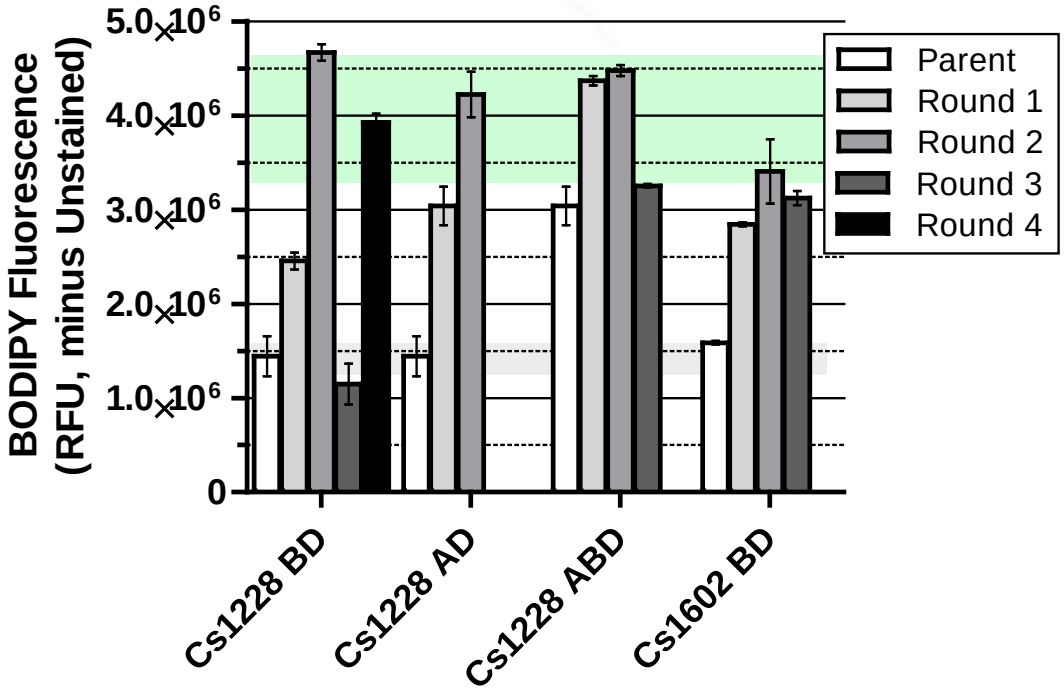
3 – Accomplishments, Objective 2:

Improved the lipid content of two strains of C. sorokiniana



- Fluorescence-activated cell sorting used to isolate subpopulations with improved performance (Non-GMO)
- Our team has demonstrated success with cell sorting of *Picochlorum* (NAABB project) and *N. salina* (AB AOP)

Up to 3x Improvement in *C. sorokiniana* Lipid Content by Cell Sorting

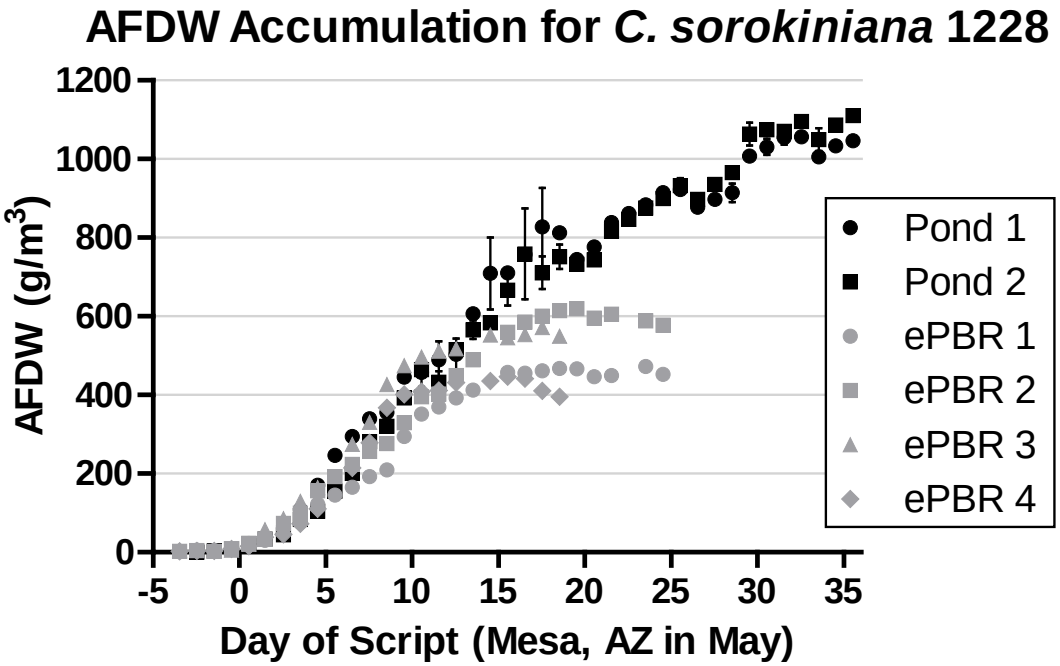


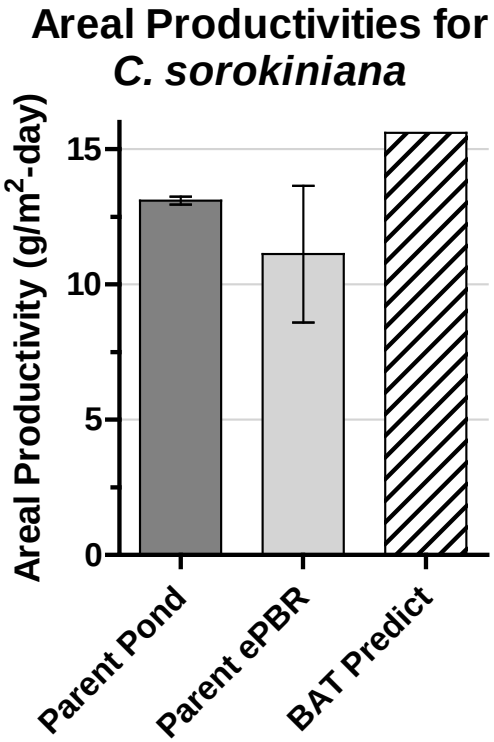
- Similar maximal lipid accumulation observed with several sorting approaches

3 – Accomplishments, Objectives 2, 1:

Submitted C. sorokiniana *1228 to the pipeline; ePBRs track much better with PNNL Ponds*

- Used Cs1412 input parameters for BAT predictions
- Predicted productivities for May at the ATP3 testbed location (Mesa, AZ), used that script

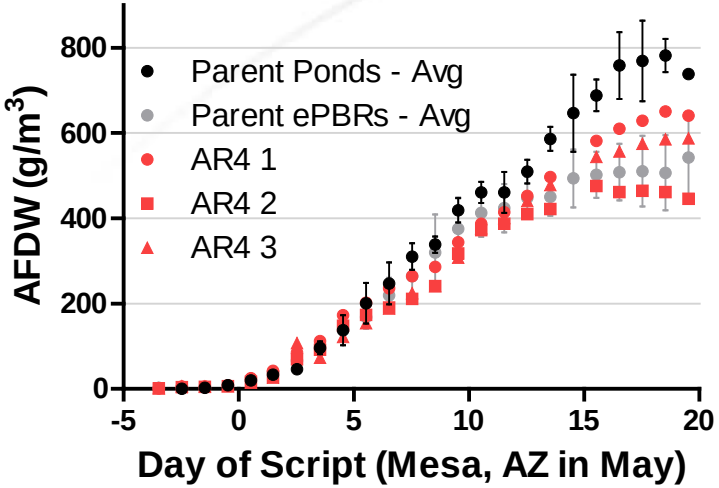


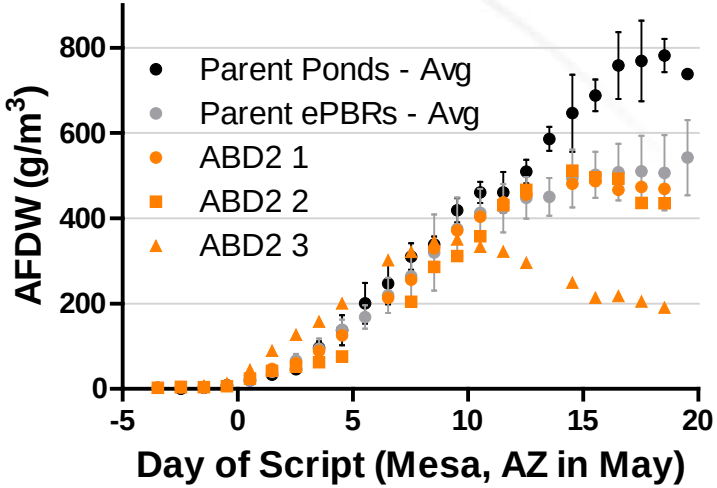


- *C. sorokiniana* ePBRs follow ponds much more closely during linear phase, though they do saturate earlier

3 – Accomplishments, Objectives 2, 1:

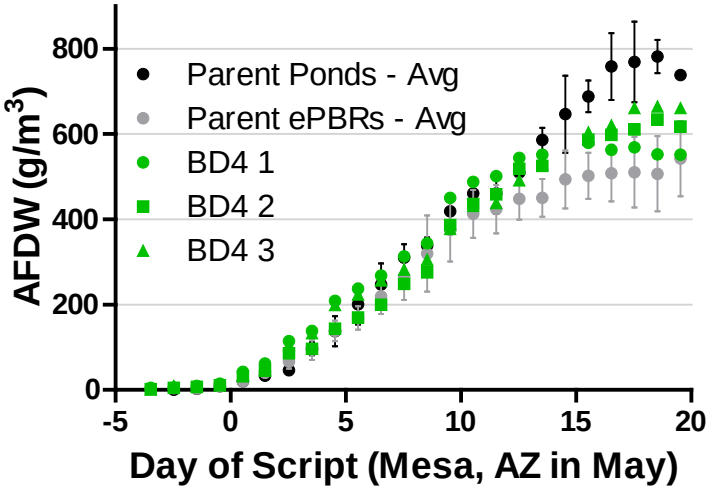
Submitted C. sorokiniana *1228 to the pipeline, biomass accumulation of sorted strains was measured*

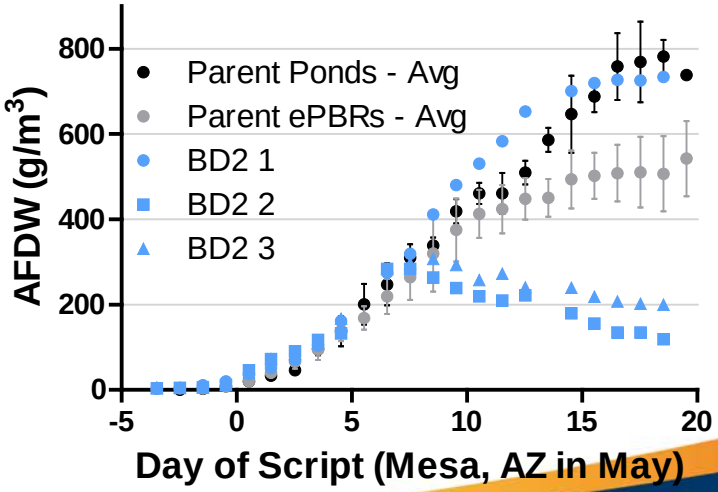




- *ABD2 and BD2 had unexpected crashes in some replicates*

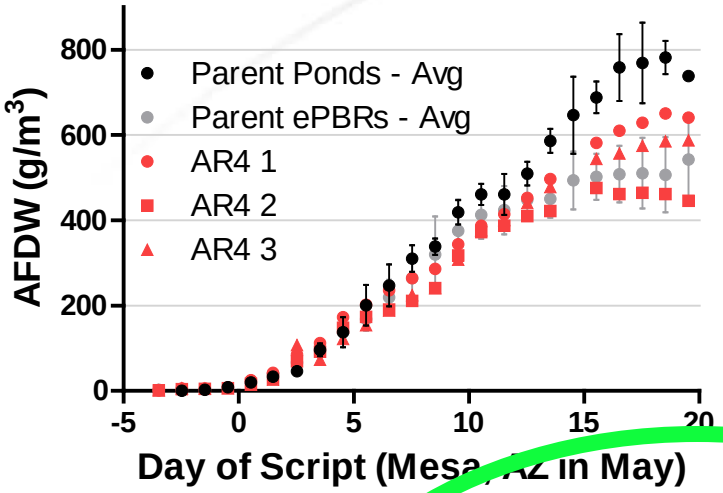
- *AR4 and BD4 accumulated similar biomass levels as the parent cultures*

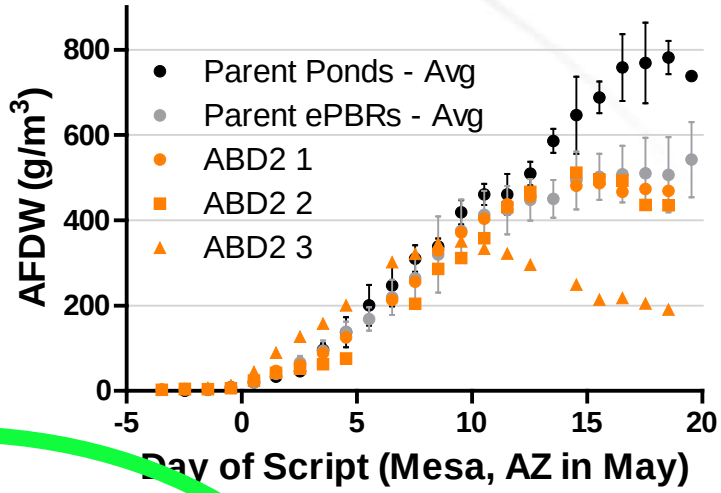




3 – Accomplishments, Objectives 2, 1:

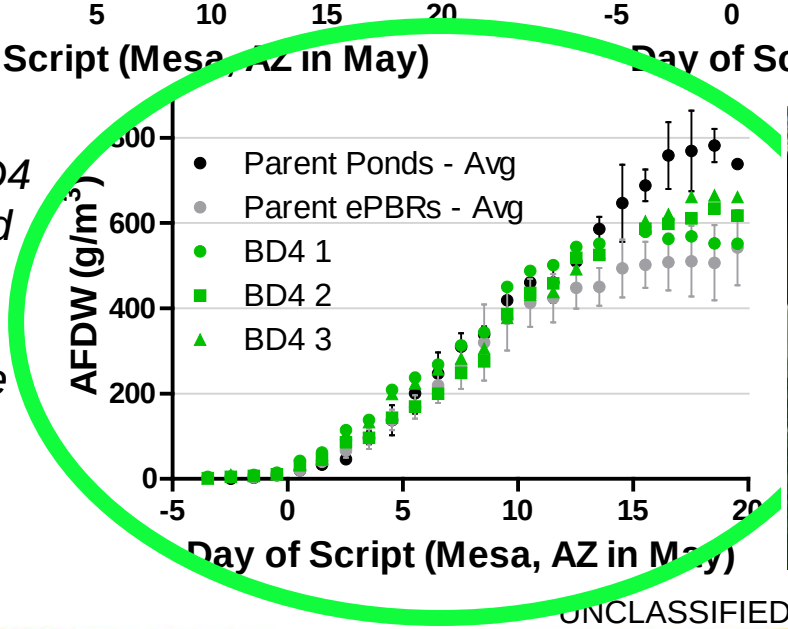
Submitted C. sorokiniana *1228 to the pipeline, biomass accumulation of sorted strains was measured*

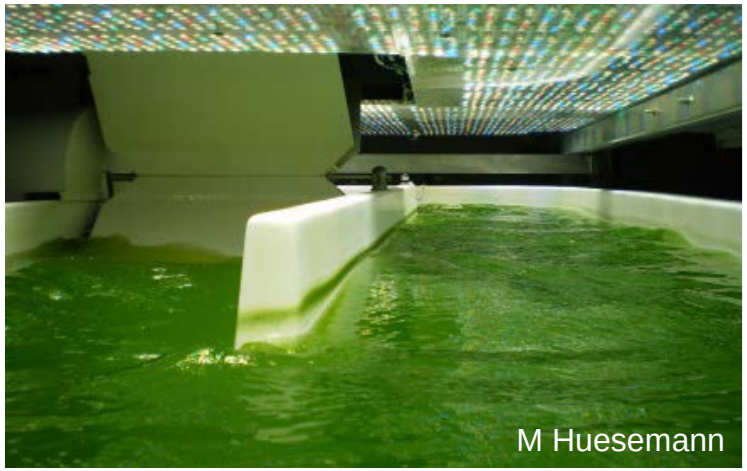




- *ABD2 and BD2 had unexpected crashes in some replicates*

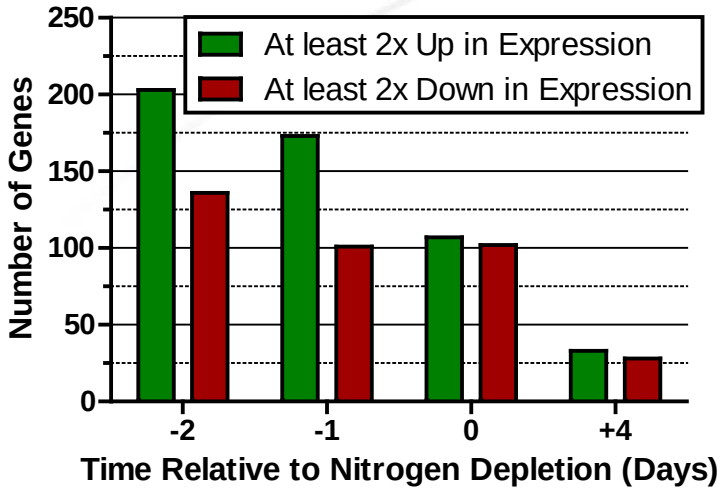
- *AR4 and BD4 accumulated similar biomass levels as the parent cultures*



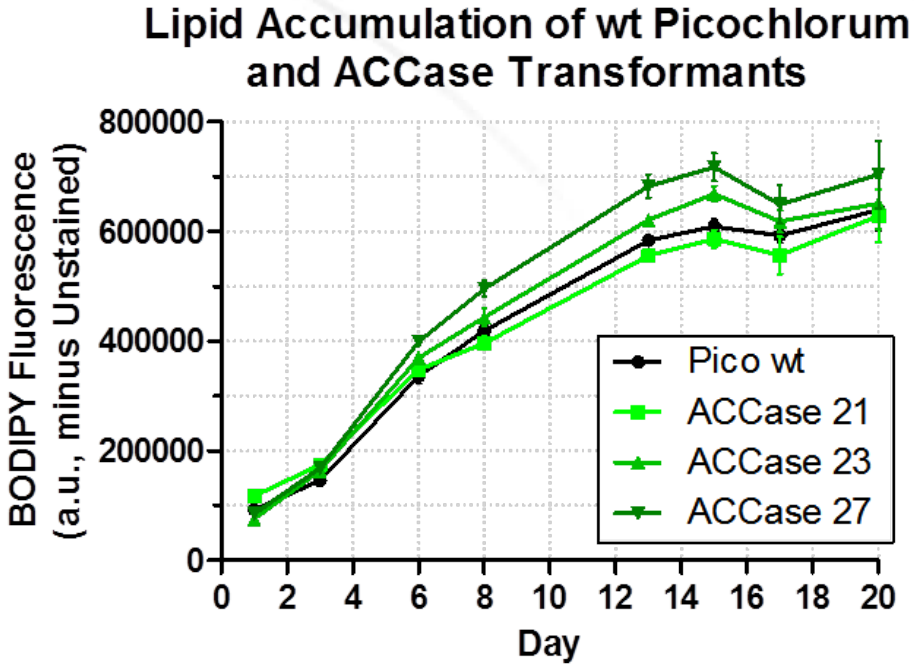


3 – Accomplishments, Objective 2:

Improving strains using transcriptomic data & GM methods



- Differential expression levels were calculated between the Hi4 sorted *Picochlorum* and the parent population, over 4 time points
- Most of the large differences in expression are just prior to nitrogen depletion, when lipid accumulation begins

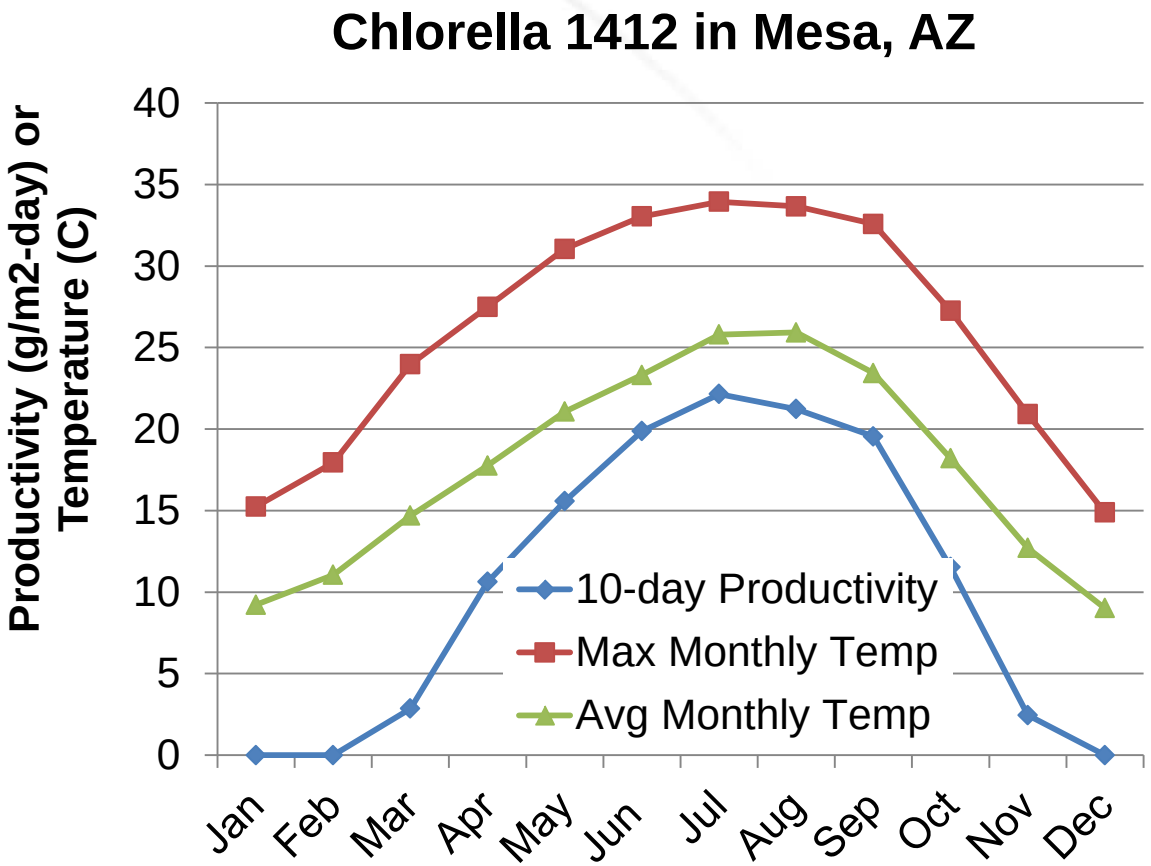


- Up to an 18% increase in lipid accumulation was observed with Acetyl-CoA Carboxylase
- Several other genes have been identified as candidates and are at various stages of transformation

3 – Accomplishments, Objective 2:

Improving strains using adaptive evolution

- Even warm weather sites like ATP3 suffer from reduced productivity in colder months
- We are using adaptive evolution methods in conjunction with the ePBRs, to customize *Chlorella sorokiniana* for improved growth in the colder months in Mesa, AZ
- We aim to increase annual areal productivities
- This method can be integrated with cell sorting and transcriptomics



M Huesemann

2 – Technical Approach: Three Objectives

1. Establish a pipeline for evaluating improved strains under conditions that directly simulate outdoor climate conditions

2. Generate additional improved algae strains using flow cytometry, adaptive evolution, and transcriptome analyses

3. Transition strains to outdoor ponds for testing

3 – Accomplishments, Objective 3:

Going outside, a collaboration with ATP3

- ATP3 is the Algae Testbed Public-Private Partnership, a testbed facility funded by DOE
- Multiple types of outdoor cultivation facilities.
- Our main goal currently is open ponds similar to those used for the PNNL indoor ponds (800-1000L)

J McGowen

3 – Accomplishments, Objective 3:

Plan for moving parent and improved strains outside

Picochlorum sp. Parent & Hi4 Sorted

- Slated to be sent to ATP3 as soon as MTA is signed
- Will grow in open ponds, light/temp data collected
- Light/temp data from growth will be used in the BAT to predict *Picochlorum* performance and compare to measured outdoor data
- Parent and sorted strains will be compared to flask and fermenter data already collected at LANL

In all cases samples will be sent to LANL for characterization in addition to ATP3 analyses

Chlorella sorokiniana 1228 Parent & BD4 Sorted

- Slated to be sent to ATP3 in March/April
- Will grow in open ponds in the month of May, the month the ponds and ePBRs have simulated
- Actual light/temp data will also be used in the BAT for predictions, if considerably different from the 30yr averages used.

Chlorella sorokiniana 1228 Cold Adapted Strains

- Cold adaptation is being designed for fall AZ weather, so the aim is to test these strains in fall 2015.

4 – Relevance to R&D and the Industry

- We are generating actual areal productivity values at lab scale (this is not typical at lab scale but an important BETO metric).
- Soon we will better understand how these productivities translate to outdoor productivities.
- Improvements in the model should permit better predictions and testing of outdoor conditions, inside.
- We can:
  - test a desired season/month of interest at any time
  - simulate locations where testbeds are not available
  - simulate testbed environmental conditions to downselect strains
  - improve strains under more outdoor-relevant conditions (high light, sinusoidal light, varying temperature, etc.)
- This process provides a pathway for transitioning or downselecting strains in a fashion that can be fully coordinated with their move to relevant outdoor testbed facilities or industrial partners.

4 – Relevance to BETO and the MYPP

- Current analyses indicate that “the highest cost to the system is biomass production; key sensitivities are productivity and lipid content” (MYPP Nov 2104)
- Improved lipid contents are important for both MYPP priority pathways.
  - More lipids leads directly to more biofuel in the algal lipid upgrading process
  - A higher lipid content leads to a higher quantity & quality of fuel in HTL
- Increasing the environmental robustness of strains will improve their annual productivity values, critical for increasing overall productivity
- Developing tools/strategies for strain improvement is called for in the MYPP
- Transcriptomics methods can aid in the identification of metabolic pathways relevant to lipid accumulation and biomass accumulation. (“explore & identify underlying biological phenomena & traits in algae” – MYPP).

5 – Future Work, Remainder of Project

- Continue strain improvement work
  - Submit a strain with improved environmental robustness to the pipeline
  - Continue with the GM efforts based on the transcriptomic data

- Execute plan to move strains outside this spring
  - Complete data analysis and work with PNNL for model improvement as needed

5 – Future Work, Outyears:

Coordinated 3-part, multi-lab effort

Algae Biotechnology

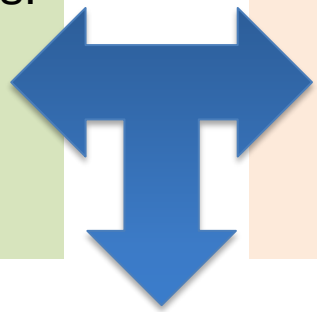
Strategies for strain improvement

- Lab scale development
- Strain improvement tools:
  - GMO
  - Flow sorting
  - Breeding
  - Adaptive evolution

Flask2Farm

Strategies for strain down-selection & transition to outdoors

- Multi-scale development
- Cultivation with environmental challenges
  - Indoor PBRs and ePBRs with BAT scripts
  - Environmental ponds
  - Greenhouse
  - Outdoors/interface with regional testbeds



Data Management System

Data integration at all levels

"Greenhouse" Website and Database

- Omics integration
- Bioinformatics tools
- Cultivation performance at multi-scales
- Metadata

Summary: Progress Toward Goal & Objectives

Overall Goal: Develop a streamlined process for improving algae strains and characterizing their performance at multiple scales, from the bench to outdoors.

- ✓ Establish a pipeline for evaluating improved strains under conditions that directly simulate outdoor climate conditions
- ✓ Generate additional improved algae strains using flow cytometry, adaptive evolution, and transcriptome analyses
-  Transition strains to outdoor ponds for testing

Acknowledgements

- Dr. S. Twary
- Dr. A. Barry
- C. Sanders
- H. Teshima
- R. Yoshida
- Dr. B. Marrone
- Dr. M. Huesemann (PNNL)
- Dr. M. Wigmosta (PNNL)
- Dr. J. McGowen (ASU)
- National Alliance for Advanced Biofuels & Bioproducts
- Annual Operating Program

Additional Slides - Summary

- Established a pipeline for predicting and testing the performance of strains developed in the lab
- Demonstrated that the pipeline can be used to improve the biomass growth model
- Improved replicate data in the ePBRs
- Generated 4 improved algae populations across 2 strains of *Chlorella sorokinana*
- Actively pursuing additional strategies for strain improvement
- Established a partnership with ATP3 in preparation for moving strains outdoors this year.

Responses to Previous Reviewer's Comments

- *Reviewers' Comment*: The project should be focused on a strain with higher productivity
- *PI Response*: The above comment stems from the initial predicted areal productivity of 4.5 g/m^2-day for *Picochlorum* by the BAT. Since the last Peer Review, we have measured the areal productivity at 15.6 g/m^2-day. This led to a modification and improvement in the prediction model. 15.6 g/m^2-day is among the highest productivities observed in open ponds, comparable to the top-performing *Chlorella sorokiniana* 1412. This data, along with observations of strain robustness, relevant levels of lipid accumulation, and published literature on related *Picochlorum* strains, indicate that *Picochlorum* is indeed a promising biofuels production strain. We also expanded our project in FY14 to include additional strains of *Chlorella sorokiniana*, since it was always our intention to continue to add strains to the pipeline.

Responses to Previous Reviewer's Comments

- *Reviewers' Comment*: The development of a simulation tool for predicting productivity potential of strains has value. A more rigorous plan for validation of the model appears to be needed… To have confidence in the model this needs to be a big effort--multiple strains by multiple locations with replications sufficient to provide statistical power
- *PI Response*: We agree that a much larger effort could be made of this project and aim to expand it in outyears, given BETO support. As intended for these first couple of years, we have established a pipeline for moving strains outdoors, improved the model based on new strain input, generated improved strains that can be submitted to the pipeline, and this spring will be sending parent and improved strains outdoors. Thus far, in a handful of trials, the strain specific biomass assessment tool and the PNNL environmental ponds have shown good agreement with outdoor data. Iteration between lab and outdoor experiments for more strains and conditions will be key to improving the power of the model.

Responses to Previous Reviewer's Comments

- *Reviewers' Comment*: The project needs to take advantage of the improved procedures that have been developed by other groups that are doing flow cytometry strain development work
- *PI Response*: It is true that other groups are using flow cytometry for strain development. Our group, however, was one of the first to present this type of methodology for algae strain development at an international meeting (in 2011). LANL has a suite of state-of-the-art equipment, accompanied by extensive expertise in this area, and we have been characterizing algae by flow cytometry since 2009. Other groups have since attained similar results to what we have observed: up to a 3-fold increase in neutral lipid content during nutrient depletion. To date, we have demonstrated success in this area of strain improvement for 4 strains of algae. We will continue to apply our expertise and push the boundaries of using flow cytometry for strain development in this project.

GO/NO Review

- We had a go/no review in FY14 for using the ePBRs to predict absolute areal productivities. After making many modifications to the ePBRs without bringing the *Picochlorum* areal productivities to within 15% of the productivity observed in the PNNL ponds, we decided on a no-go.
- We are still, however, using the ePBRs for comparative and downselect purposes, as they still provide an environment more similar to pond conditions than flasks do and are currently the best way to screen multiple improved strains at one time, under environmental conditions mimicking the outdoors.

Publications/Patents/Presentations/Awards/ Commercialization

- **Publications**
  - M. Huesemann, B. Crowe, A. Chavis, D. Valentine, T. Piers, M. Wigmosta, T. Dale, S.N. Twary, A.N. Barry and R.C. Yoshida. (In preparation) Simulation of outdoor pond cultures using indoor LED-lighted and temperature-controlled raceway ponds and Phenometrics photobioreactors.
  - Dale T, Twary SN, Fearey B, Sanders CK, Teshima H, Marrone BL (in preparation). Multi-parameter flow cytometry analysis of lipid and biomass accumulation in *Picochlorum s*p.
  - Olivares J, Unkefer CJ, Sayre RT, Magnuson JK, Anderson DB, Baxter I, Blaby IK, Brown JK, Carleton M, Cattolico R, Dale T, et al. (submitted). Review of the algal biology program within the national alliance for advance biofuels and bioproducts. *Algal Research*.
- **Presentations**
  - T. Dale, S.N. Twary, A. Barry, M. Huesemann, A. Chavis, P. Chen, J. McGowen. Multi-scale Characterization of Improved Algae Strains. 5th International Conference on Algal Biomass, Biofuels, and Bioproducts, 2015, accepted for oral presentation.
  - M Huesemann, B Crowe, A Chavis, D Valentine, T Dale, S.N. Twary, ANBarry and RC Yoshida. Simulation of Outdoor Pond Cultures using Indoor LED-lighted and Temperature-Controlled Raceway Ponds and Phenometrics Photobioreactors (ePBRs). 4th International Conference on Algal Biomass, Biofuels, and Bioproducts, 2014, oral presentation.
  - T Dale T, SN Twary, SR Starkenburg, CJ Unkefer, BL Marrone. Isolation and Characterization of *Picochlorum* sp. with Increased Lipid Accumulation. 4th International Conference on Algal Biomass, Biofuels, and Bioproducts, 2014, oral presentation.