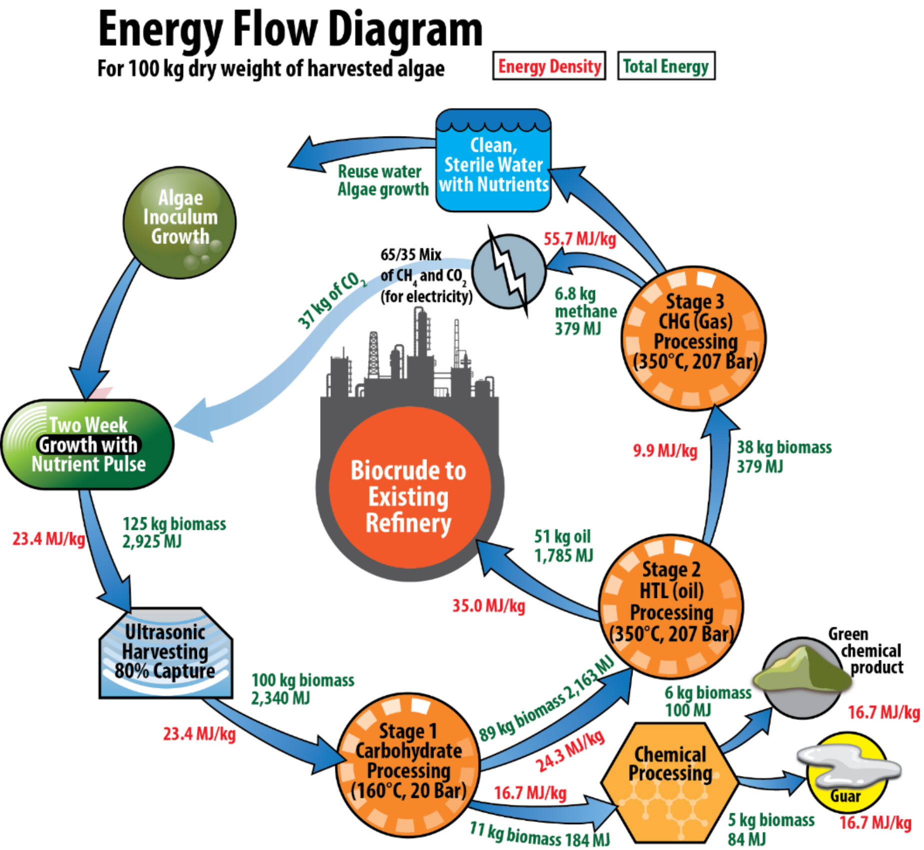
DOE Bioenergy Technologies Office (BETO) 2019 Project Peer Review

PACE: Producing Algae for Coproducts and Energy

March 2019
Advanced Algal Systems

Matthew Posewitz
Colorado School of Mines

Goal Statement



Overarching Project Goals:

- Increase algal biomass productivity to >25 grams dry weight per meter squared per day ($gdw/m^2/day$) using robust engineered algal strains to reduce costs >2×.
- Produce and harvest high-value, high market demand coproducts compatible with continuous flow, sequential HTP technology to further reduce biofuel costs.
- Develop and improve process/engineering systems to achieve overall energy return on investment (EROI) >3, carbon index <55 grams carbon dioxide per megajoule (g CO_2/mJ).

Project Outcome:

- Combined integration of cultivation, harvesting, processing and genetic engineering improvements to increase areal productivity resulting in an increased EROI and lower fuel costs.

Relevance to the Biofuels Industry

- Deployment of engineered strains in algal cultivation testbeds to assess productivity improvements in large scale outdoor facilities.
- Highly integrated process incorporating strain/cultivation improvements through final algal crude production that approaches a barrel of algal crude per day.

Quad Chart Overview

Timeline

- Project start date: April 1, 2016
- Project end date: March 31, 2019 plus 1 year no-cost extension
- Percent complete: 85%

	Total Costs Pre FY17** $555,353	FY 17 Costs $6,092,727	FY 18 Costs $5,724,344	Total Planned Funding (FY 19-Project End Date) $12,372,425
DOE Funded	$299K (54%) $132K LANL (24%) $10K PNNL (2%)	$2,307K (38%) $1,432K LANL (24%) $390K PNNL (6 %)	$2,847K (50%) $1,484,K LANL (26%) $100K PNNL (2%)	$5,452,187 CSM (44%) $3,047,812 LANL (25%) $500,000.00 PNNL (4%)
Project Cost Share*	$114,991 (21%)	$1,963,563 (32%)	$1,293,871 (23%)	$3,372,425 (27%)

Barriers addressed

The PACE project specifically addresses BETO challenges in algal **Biomass Availability/Cost** (Aft-A) and **Biomass Genetics and Development** (Aft-C).

Objective

Improve areal biomass productivities and enable the production of high-value co-products using strain engineering. Integrate cultivation/processing advances to lower the cost of algal derived biofuels.

End of Project Goal

- Engineered algal strains with enhanced biomass yields, the ability to synthesize high-value co-products and/or improved crop stability that attain yields of 25 g/m^2/d and are able to accumulate high-value co-products to offset production costs.
- Improve cultivation, harvesting, and processing technologies to improve the economics of converting algal biomass to fuels using hydrothermal processing technologies.

Quad Chart Overview

PACE Partner	DOE $$	Cost Share
Subcontract: Washington State University	$ 360,760	$ 90,190
Subcontract: Arizona State University	$ 914,141	$ 210,798
Subcontract: Colorado State University	$ 236,640	$ -
Subcontract: New Mexico Consortium	$ 1,261,948	$ 262,984
Subcontract: Pan Pacific	$ 979,892	$ 345,284
Subcontract: Genifuel	$ 173,959	$ 4,868
Subcontract: Reliance	$ 250,000	$ 2,087,581
Subcontract: Sonosep	$ 411,534	$ 102,884
CO Energy Research Collaboratory (CERC)	$ -	$ 248,491
TOTAL	$ 4,588,874.00	$ 3,353,080.00

1 - Project Overview

Project leverages strain improvement technologies from NAABB and state-of-the-art outdoor culturing and processing facilities at Reliance Industries

Improve Biomass Yield:

- Measure lipid/carbohydrate accumulation in relevant cultures to select for downstream characterization and optimize profitability.
- Improve CO_2 utilization to increase yield 30%.
- Increase sink strength to increase yield 20%.
- Develop crop-protection technologies to reduce possible pond crashes.
- Stack value-added traits to give 50% yield increase.

Produce Co-products: Produce and harvest higher-value co-products compatible with continuous flow, sequential HTP technology to reduce biofuel costs.

- Develop GM algae to produce guar gum.
- Produce 2-phenylethanol (PEA) glucoside (PEA Glc).

Improve and Optimize Integrated Process: Develop and improve process integration and engineering systems to achieve overall EROI >3, carbon index <55 g CO_2/mJ, and a nearly 2-fold reduction in fuel cost to ≤$5.00 gallon of gasoline equivalent (gge).

- Comparing and contrasting large-scale outdoor cultivation systems.
- Develop and evaluate ultrasonic harvester.
- Develop and evaluate sequential, continuous flow HTP.
- Evaluate outdoor-relevant media options
- Integrated testing at commercial pilot scale.
- Co-product, biocrude, feedstock and fuel evaluation.
- Perform life cycle assessment (LCA) and techno-economic analysis (TEA).

2 – PACE Management

Matthew Posewitz
Acting-Director
Norman Lewis
Associate Director

David Bruce
LANL
Financial Director

Management Team
Matthew Posewitz, Norman Lewis, Jim Oyler, Taraka Dale, Eric Dunlop, Ramesh Bhujade

Norman Lewis
Scientific Director

Jim Oyler
Engineering Director

Eric Dunlop
TEA/LCA Director

Taraka Dale
Cultiv./Harvest Director

Ramesh Bhujade
Process Scaling Director

Shawn Starkenburg
Team Lead – Biomass and co-products
Posewitz
Twary
Lewis
Negi

Taraka Dale
Team Lead – Cultivation and Harvesting
Oyler
McGowen
Chelliah
Coons
Trampler

Anthony Marchese
Team Lead - Process Improvement and Integration
McGowen
Oyler
Bhujade
Anderson
Dunlop
Chelliah

Partners

- Colorado School Mines
- LANL
- Reliance Industries Ltd
- Arizona St. Univ.
- Colorado St. Univ.
- Genifuel
- Washington St. Univ.
- Pan Pacific
- PNNL
- Sonosep

2 – Approach (Management)

- Monthly PACE ZOOM meeting on research highlights and progress, and management issues.
- Monthly specialized meetings to address unique challenges.
  - Algae transformation group meetings
  - Cultivation group meetings
  - Processing group meetings
- Quarterly survey and reporting on progress towards meeting all milestones and deliverables.
- Annual all hands meeting with review and feedback from BETO.
- Management Team Oversight.

PACE PRODUCING ALGAE FOR COPRODUCTS AND ENERGY

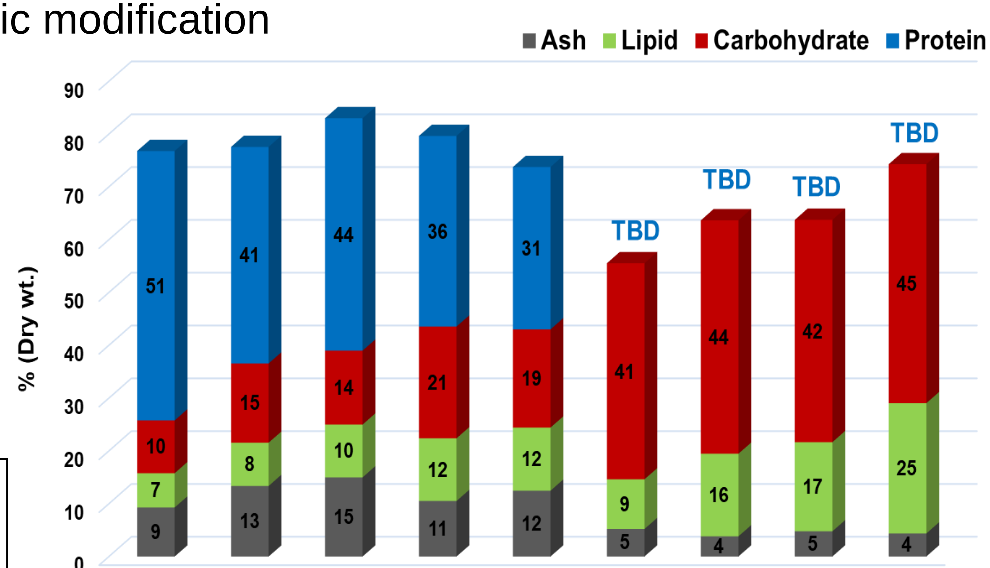
2 – Approach (Technical)

- **Major Challenges:**
  - Developing genetic transformation systems and stacking gene traits for *Chlorella sorokiniana* and other “industrial algae”.
  - Producing and sequentially extracting coproducts (guar and 2-phenylethanol glucoside) from biocrude to offset the cost of biofuel production.
  - Improving cultivation and protection strategies to increase biomass yields.
  - Developing efficient algal harvesting and processing to biofuel technologies.
- **Major Success Indicators:**
  - Overall EROI > 3.
  - Carbon index <55 g CO_2/mJ.
  - Increase in areal biomass production to 25 g/m^2/d.

3 – Technical Accomplishments/ Progress/Results: Shifting Biochemical Composition in *C. sorokiniana*

Shift carbon storage in *C. sorokiniana* in greenhouse mini-ponds

- Nutrient depletion, salinity adaptation, genetic modification
- Proximate analysis using NREL/ATP[3] methods

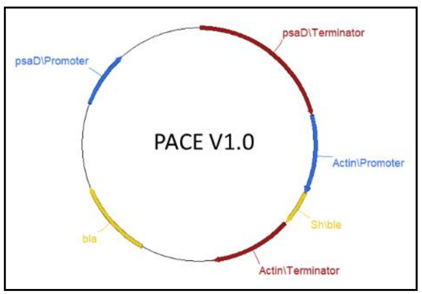


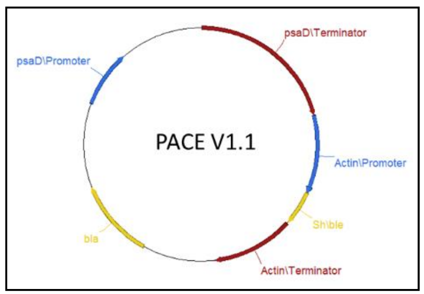
Biochemical composition is important for reaching BETO's biofuel intermediate targets and for meeting the PACE project goals

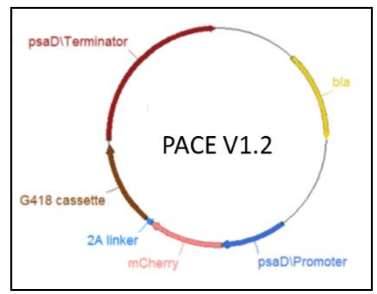
- Generate cultures with varying biochemical composition
- **Outcome:** Increased carbon storage observed in salt adapted and GM *C. sorokiniana*
- *Ongoing*: Develop model for selecting strains with lowest cost/gge based on productivity and biochemical composition

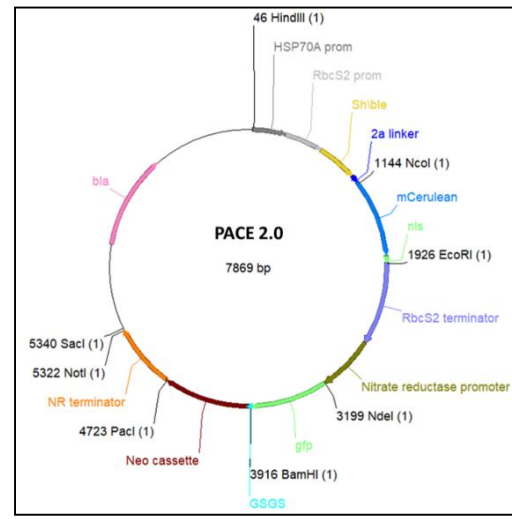
3 – Technical Accomplishments/ Progress/Results: Enhanced CO_2 Levels

- **Subtask: Enhance Chloroplast CO_2 Levels**
  - Design, construct, and transform improved *Chlorella sorokiniana* (or *Nannochloropsis*) genetic cassettes for coordinated gene expression of multiple selection reporters and genes of interest. Validate genomic integration and gene expression after optimized transformation.

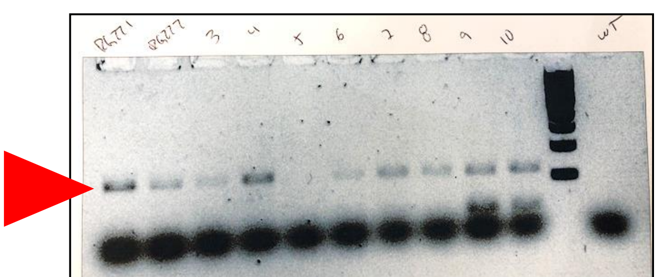








- ➢ We have generated five different backbone vectors for overexpression in *Chlorella* and three for *Nannochloropsis* (various promoters, linkers, antibiotic cassettes).



- ➢ Using this molecular toolbox, we have generated independent transgenics for five CCM genes in *Chlorella* and one in *Nannochloropsis.*

3 – Technical Accomplishments/ Progress/Results: Improve Photosynthesis/Biomass

Subtask: Improve Photosynthetic Efficiency and Biomass Production Rate

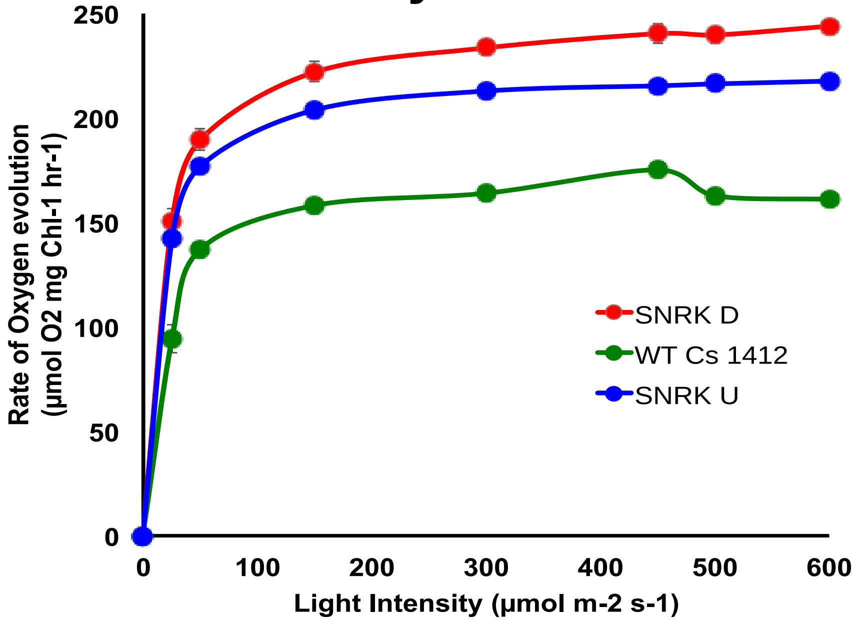
Strategy: Overexpress KIN10/11 (SNRK) gene to increase photosynthesis and increase overall biomass yield

Accomplishments:

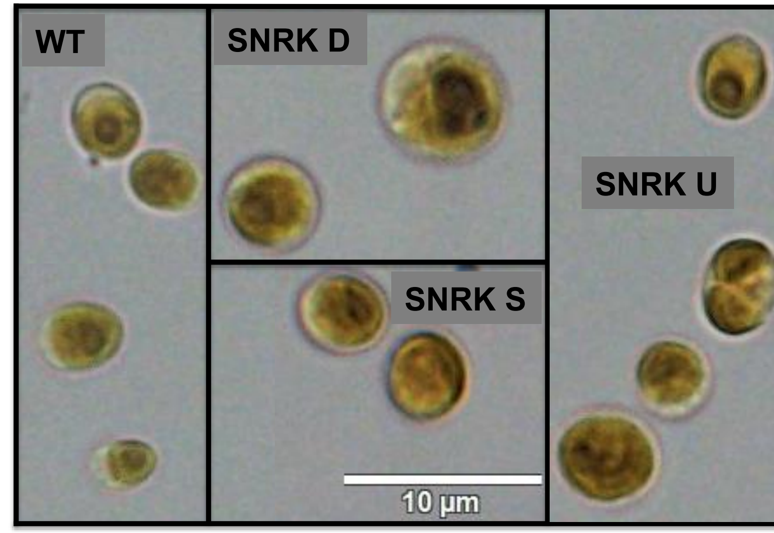
- Successful SNRK gene expression.
- SNRK OE (overexpression lines) line have higher photosynthetic efficiency compared to wild type.
- SNRK OE have higher accumulation of starch.
- SNRK OE lines demonstrate improved growth compared to wild type in semi-continuous harvesting experiment.
- Stable lines for > 2 years.

Improved Biomass Phenotypes from *Chlorella sorokiniana* SNRK GM

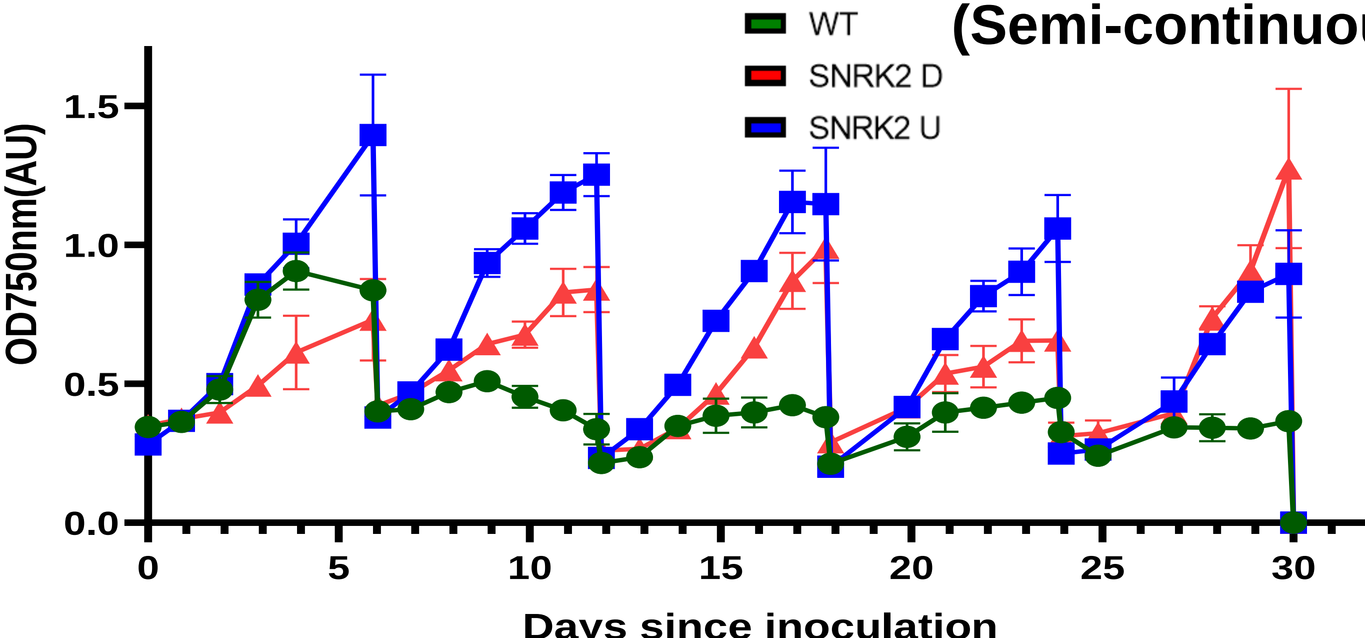
Photosynthesis

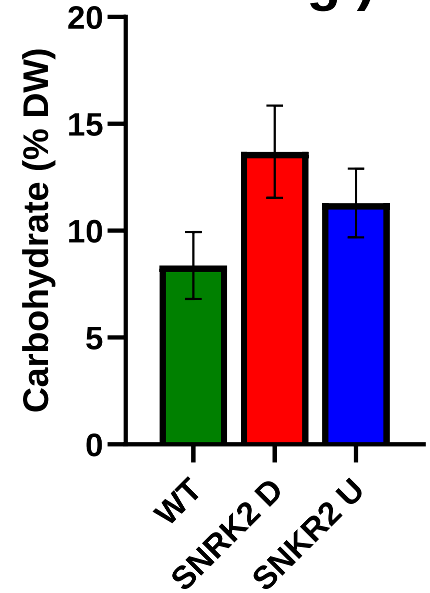


Starch accumulation



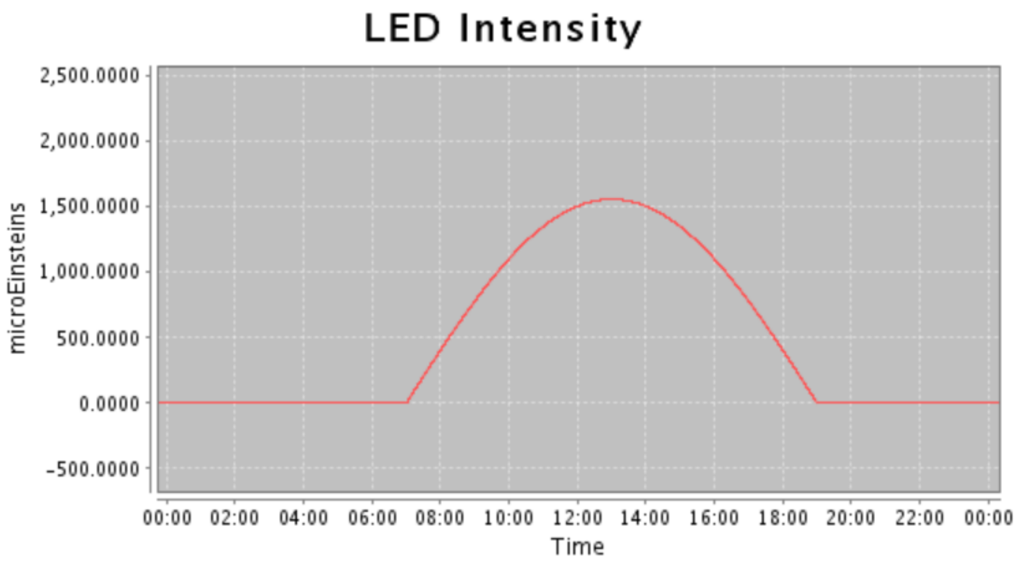
Growth in ePBRs
(Semi-continuous harvesting)

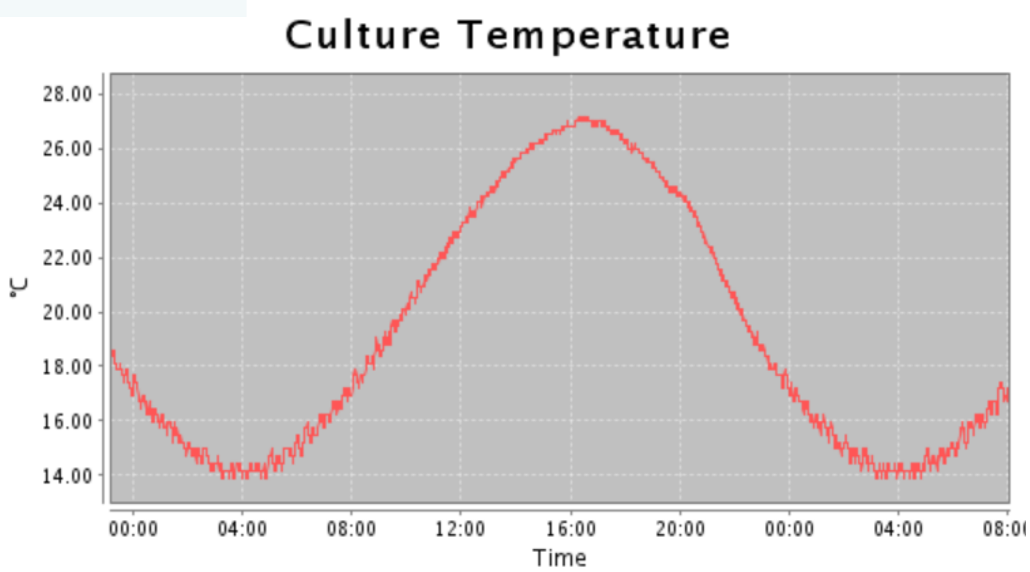




Experimental details for the semi-continuous harvesting growth experiment

	Software
Algal command verson used	2.4
Script used	N/A
Light intensity	1559
Peak intensity	13:00/1PM
Day length	12hours
Light delivery	sinusiodal
Temperature set point(°C)	21
Temperature amplitude	6
Temperature peak	16:00/4PM
Temperature delivery	sinusiodal
pH set point	7.9
tolerence	0.05
CO2 delivery	pH controlled
Spin bar RPM	200



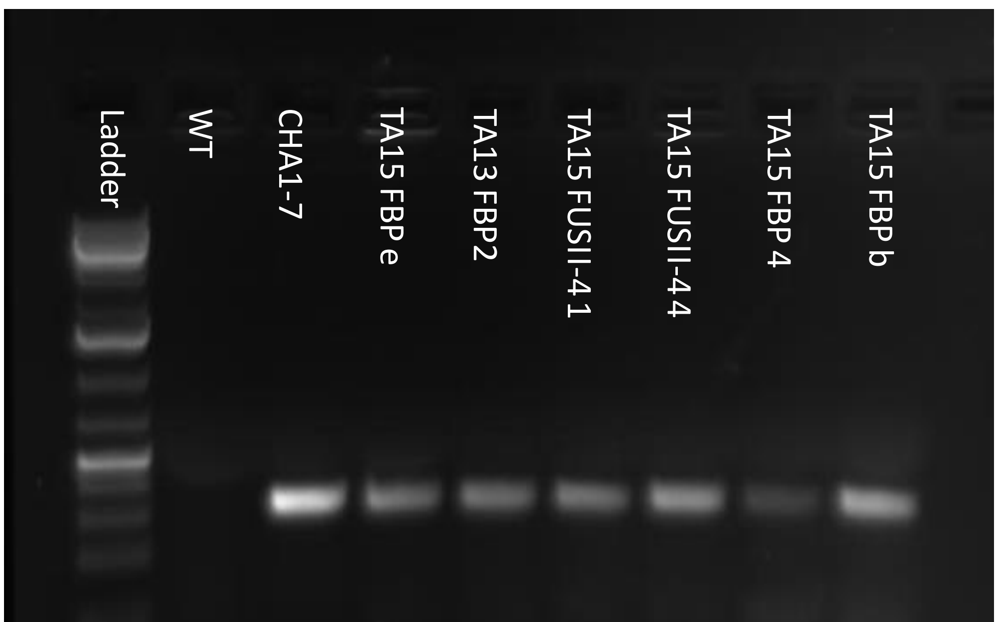


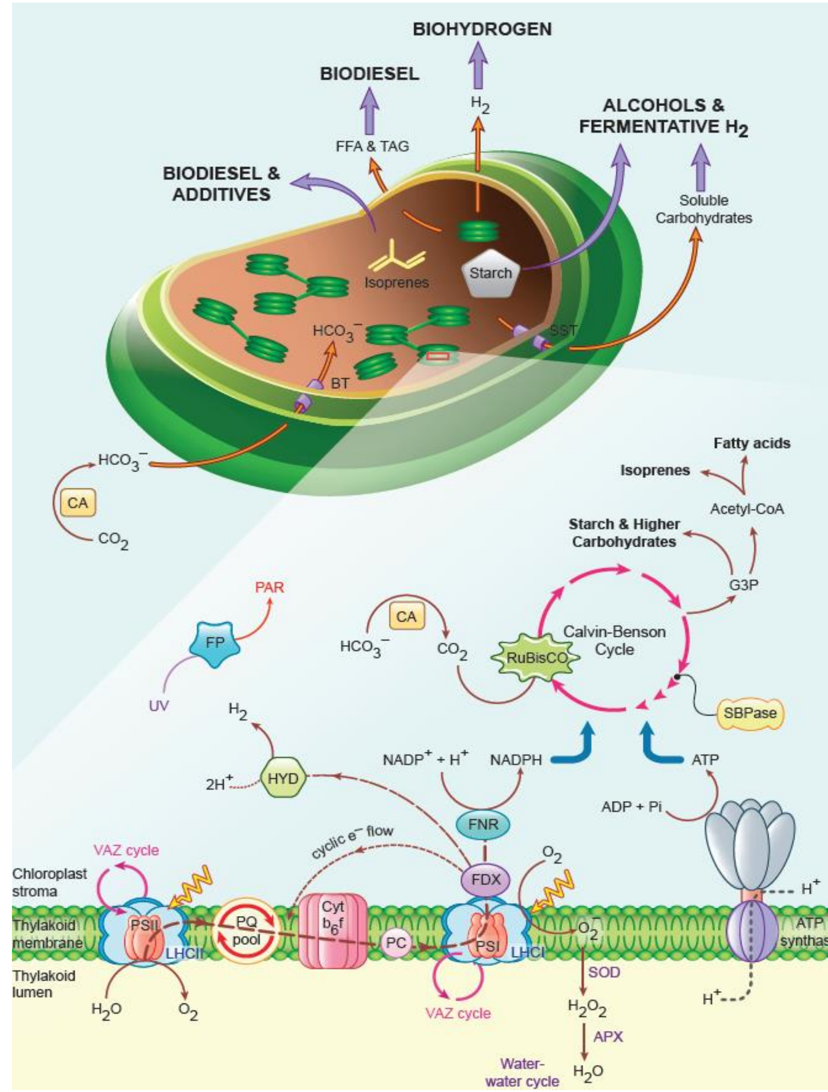
Early October AzCATI script

3 – Technical Accomplishments/ Progress/Results: Enhanced Sink Strength

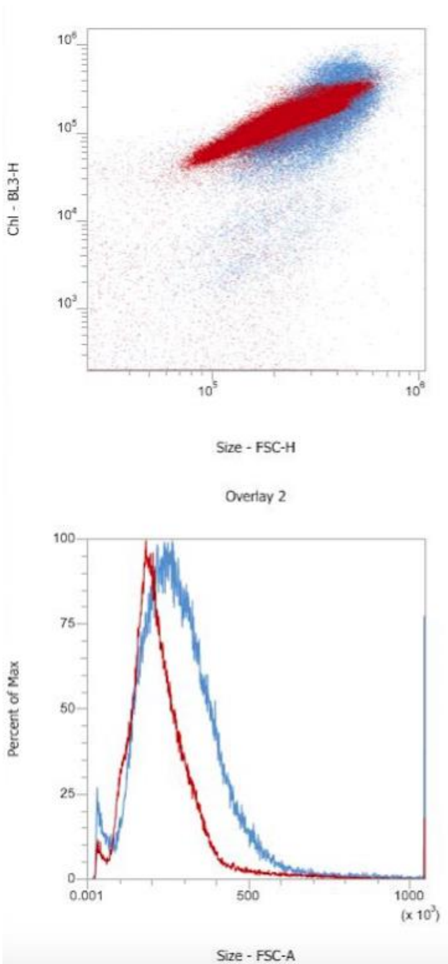
Increase Biomass Sink Strength to Enhance Yields

Overexpression constructs transformed into *Nannochloropsis gaditana* for SBPase/FBPase/fused chimera and SBPase transformed into *C. sorokiniana* to enhance photosynthetic sink strength.

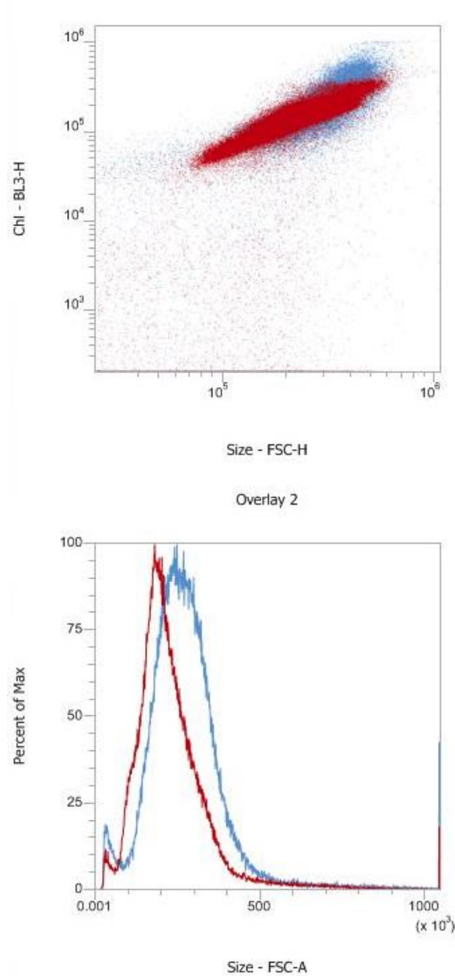




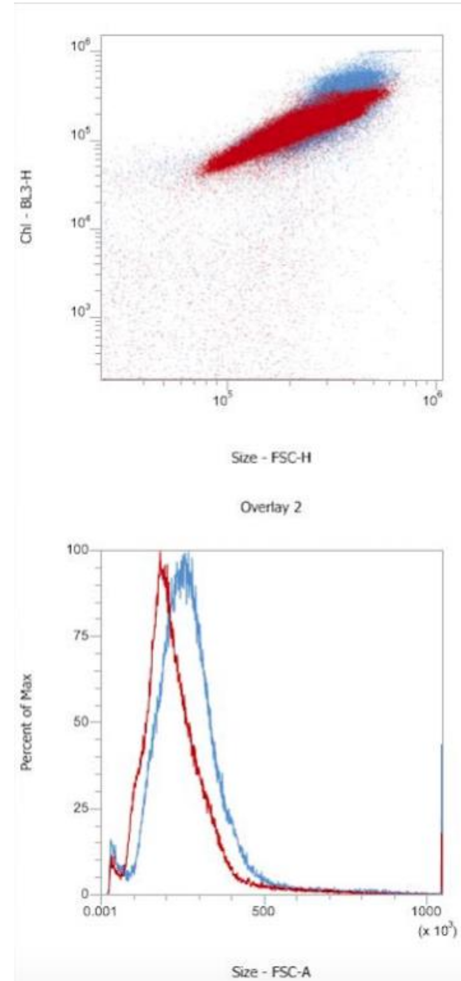
3 – Technical Accomplishments/ Progress/Results: Enhanced Sink Strength



WT and FBP 14



WT and FUS 4

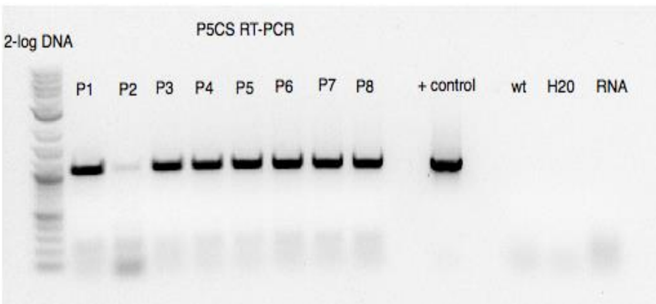


WT and FUSII 4

Larger cells when supplemented with CO_2

3 – Technical Accomplishments/ Progress/Results: Crop Fitness

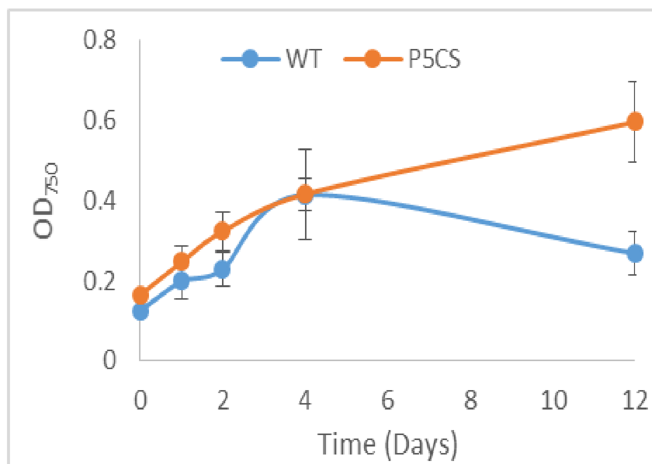
- **Subtask: Develop Crop Fitness Strategies**
  - Determine whether P5CS gene is expressed.



➢ Eight *Chlorella* P5CS mutant lines have been confirmed to express the transgene.

  - Test P5CS line(s) ability to grow under abiotic heat stress compared to wild type at lab scale.

24 h light; 42 °C



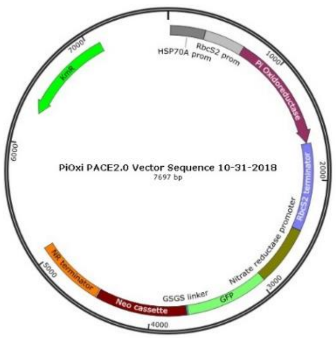
➢ P5CS overexpression (and the resulting proline accumulation) aids in heat stress tolerance when cells are grown in constant light.

3 – Technical Accomplishments/ Progress/Results: Crop Protection

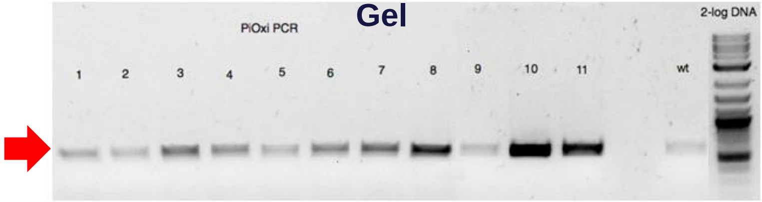
Goal: Express Phosphite Oxidoreductase (*ptxD*) Gene

- Orthophosphate (PO_4^{3-}) is the only chemical form assimilated and utilized by autotrophs
- Phosphite has pesticide potential due to its capacity to limit the growth of competing microorganisms
- Genetically modified CS1412 to be able to oxidize phosphite into phosphate through expression of the codon-optimized *ptxD* from *Pseudomonas* sp.

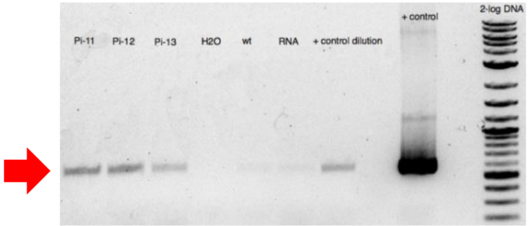
Transformation Vector

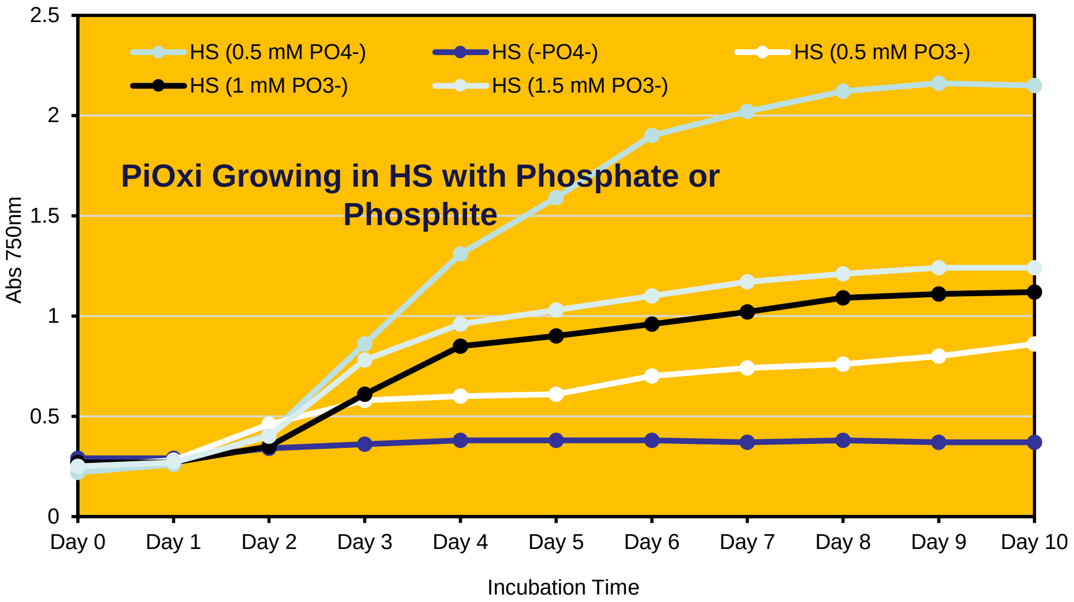


Gene Integration Gel



PiOxi Expression





Growth of PiOxi Mutant for 120h in HS Media

Flask 1: HS Medium (No P)
Flask 2: HS Medium (0.5 mM PO4-)
Flask 3: HS +0.5 mM PO3-
Flask 4: HS +1 mM PO3-
Flask 5: HS +1.5 mM PO3-
(Left to right)

3 – Technical Accomplishments/ Progress/Results: Trait Stacking

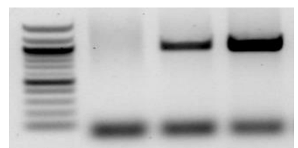
- **Subtask: Stack two value-added traits**
  - Determination of gene expression for both of the stacked traits.

1st generation

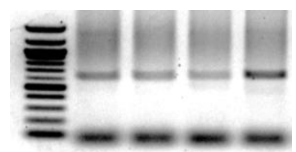
P5CS#4
(g418 resistant)

SNRK "D"
(zeocin resistant)

2nd generation
(blasticidin resistant)



SNRK_P6



P5CS_S9

➢ New SNRK and P5CS backgrounds that are blasticidin resistant have been generated, which allows for trait stacking with prior strains.

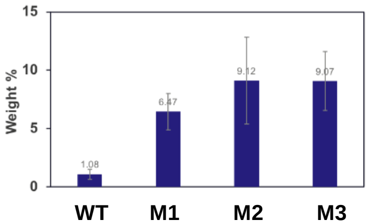
- Measured photosynthetic, growth rates, and biomass productivity of stacked phenotypes at lab scale.

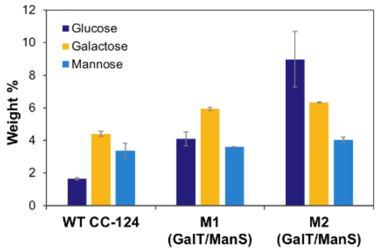
➢ *Trait stacking in progress*

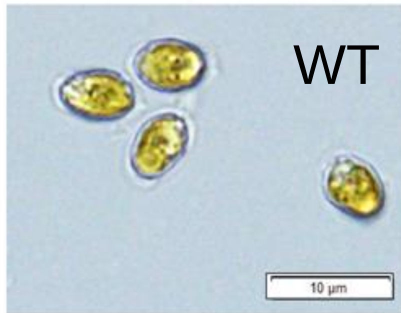
3 – Technical Accomplishments/ Progress/ Results: Coproduct Production

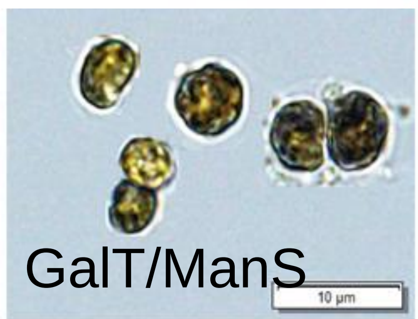
Goal: Genetically modify microalgae to produce guar gum >5% total biomass

Chlamydomonas

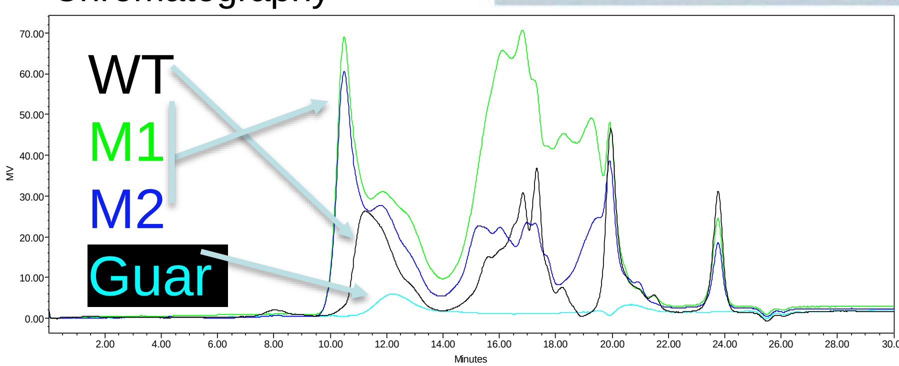




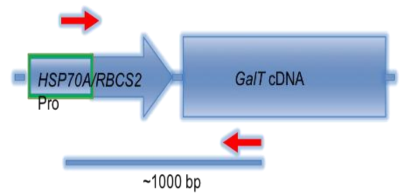


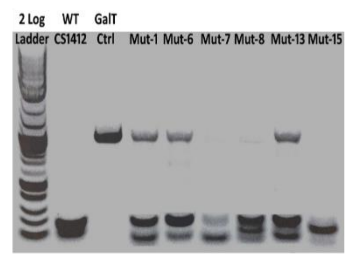


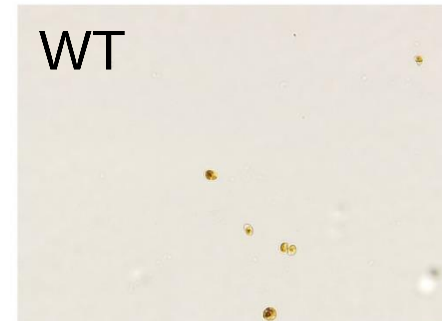
Size Exclusion Chromatography

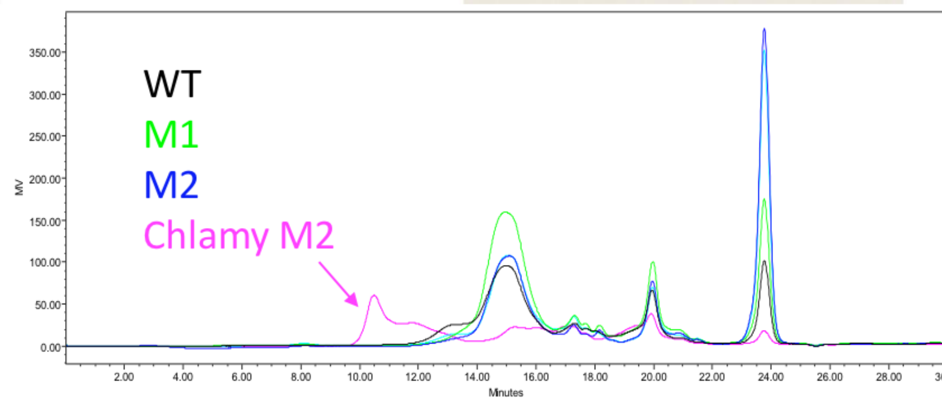


Chlorella







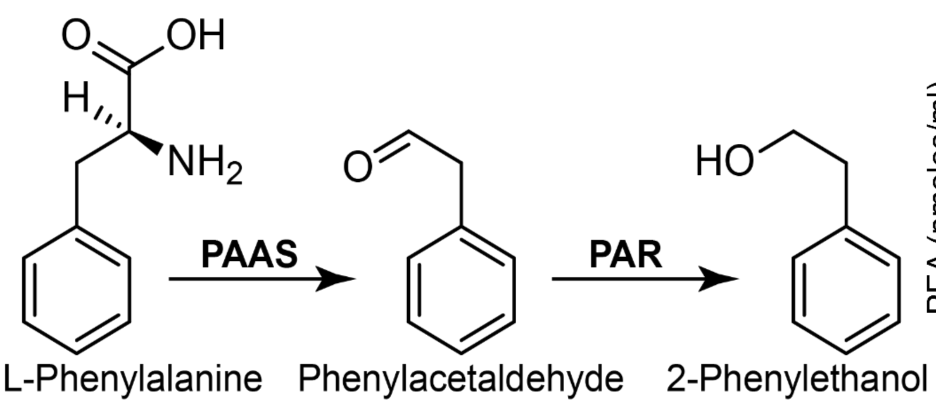


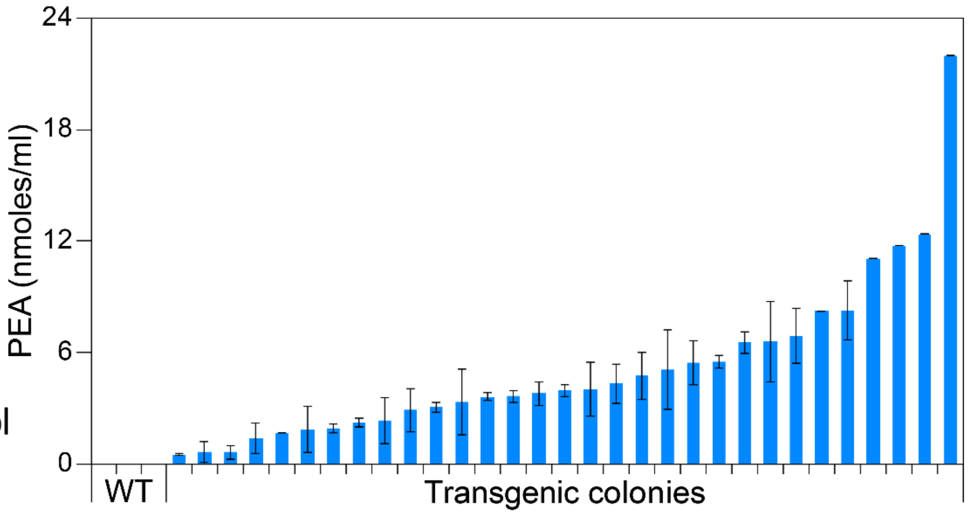
Guar synthesis genes successfully transformed

3 – Technical Accomplishments/ Progress/ Results: Coproduct Production

Task: 2-phenylethanol (PEA, small market with potential of significant expansion, high value; Norman Lewis, WSU)

- *PAAS, PAR* and *UGT* engineered into PACE vectors, with genes transformed into *C. sorokiniana.*
- PCR confirmation of gene integration.
- PCR confirmation of mRNA expression.
- Demonstrated PEA synthesis in transgenic *Chlorella.*

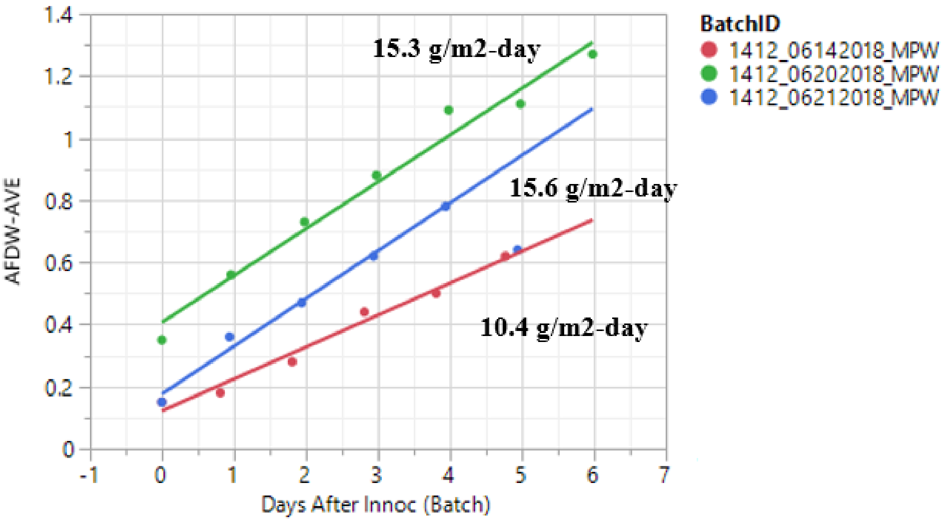




3 – Technical Accomplishments/ Progress/ Results: Integrated Processes

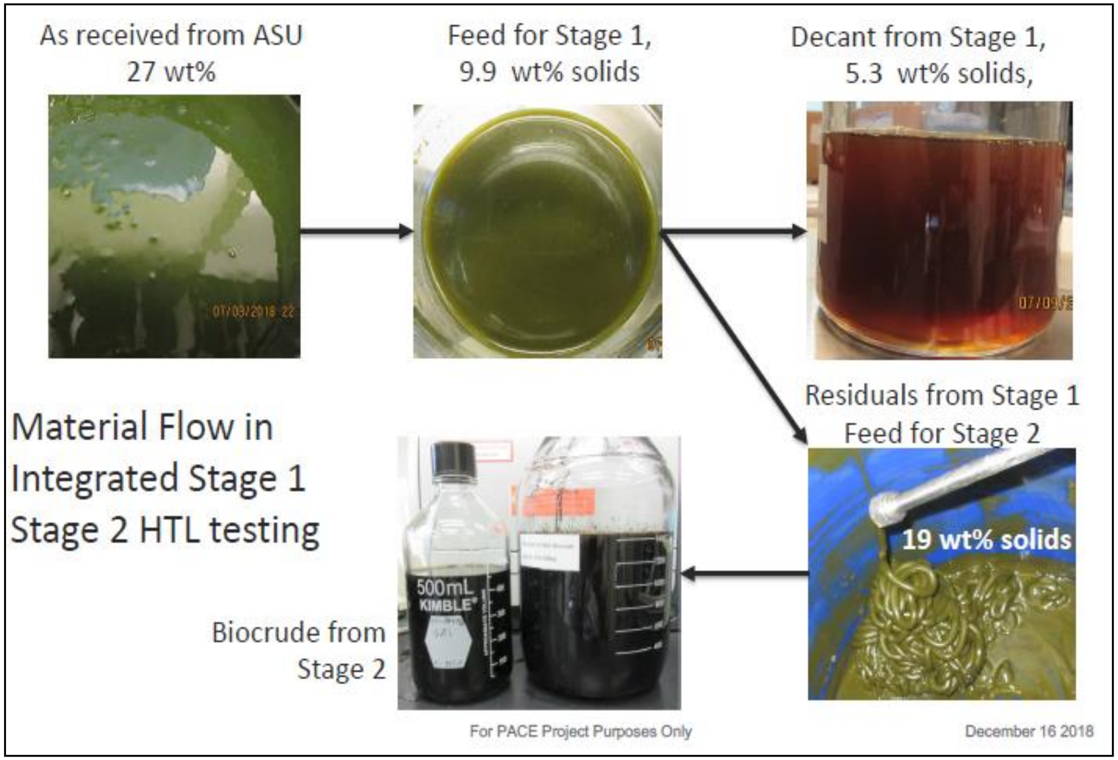
Outdoor Cultivation (AzCATI)

- Cultivate WT at scales of 4.2 m^2 (1000 L) to 60 m^2 (15,000 L) at AzCATI.
- Produced biomass for downstream testing and HTP at PNNL.
- Obtained EPA approval for TSCA Environmental Release Application (TERA) for open release of GM algae field-trial.
- Cultivate *Nannochloropsis sp.* strains to establish baseline WT performance across 3 seasons.



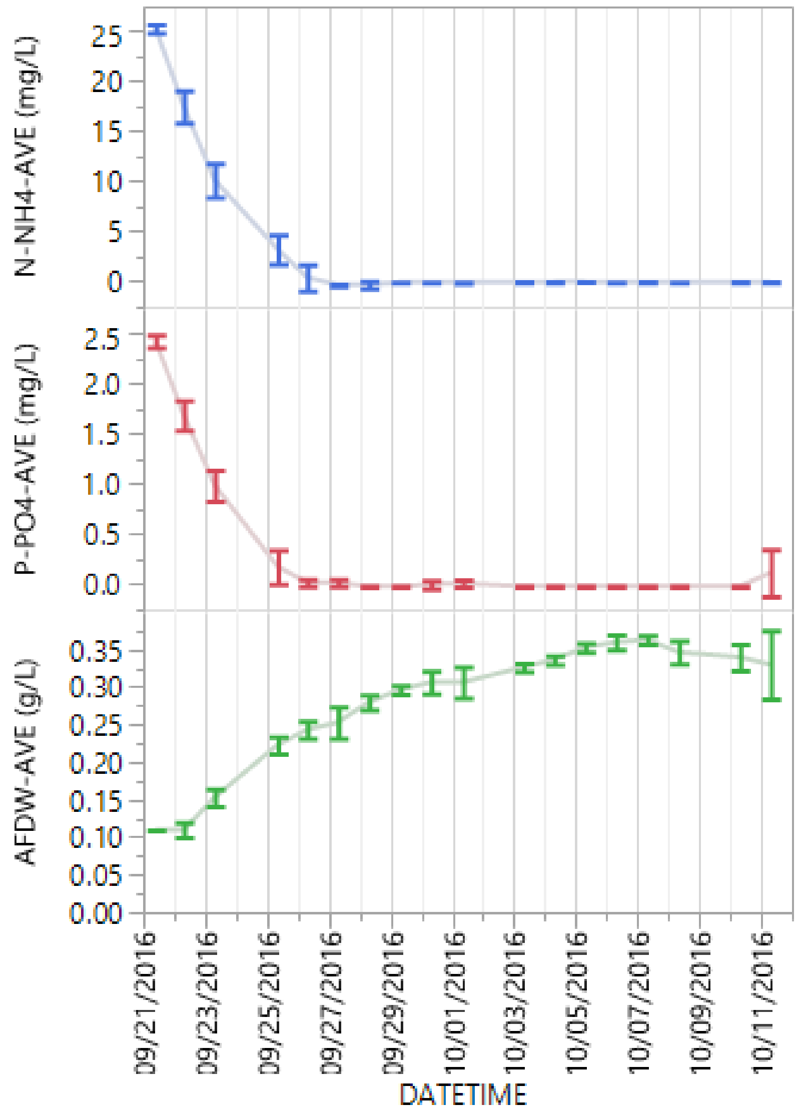
3 – Technical Accomplishments/ Progress/ Results: Two-Stage HTP

- PNNL processed algae from AzCATI via two-stage HTP
  - **Stage 1 recovered 54% of total carbohydrates at 180°C**
  - **Stage 2 converted residual to biocrude oil at 350°C**
- Residual solids after carb removal can be processed at ~20% solids vs. ~10% before carb removal
  - **Higher solids allows smaller HTP systems and improves biocrude**



3 – Technical Accomplishments/ Progress/Results: Integrated Processes

- Scale-up of WT 1230 and 1412, media optimization, salt adaptation and 10’s kg biomass provided to partners, including PNNL for HTP.
- Analytical method alignment was established across partners for proximate analysis.
- Baseline productivity established for both 1230 and 1412.
- Two (2) TERA applications submitted and approved
  - 1st TERA approved for P5CS (1228) in September 2017
  - 2nd TERA approved for SNRK2 (1412) in December
  - SNRK2 deployment planned during NCE.
- Screening runs to establish baseline seasonal productivity of WT *Nannochloropsis sp.* with developed genetic tools underway for Winter and planned for Spring and Summer 2019.



3 – Technical Accomplishments/ Progress/ Results: Integrated Processes

Reliance Industries Growth Facility

- **RIL providing process information using their proprietary strain for TEA/LCA analysis. Salt water based >20 g/m^2 d annual productivities**
- **Adapting *C. sorokiniana* from LANL at the Gagwa facility.**
- **Evaluating membrane filtration unit for harvesting.**
- **Providing algal biocrude for upgrading to fuel and engine testing.**

Image of RIL Gagwa facility. Longer ponds on left are over 1 km long. Includes harvesting, HTL, media mixing, central control room. Refinery nearby.

3 – Technical Accomplishments/ Progress/ Results: Integrated Processes

Reliance Industries

Summary of Tasks:

- Establish the technical feasibility of integrating the various upstream & downstream processes involved in making biofuels from algae through field demonstration studies.
- The data generated from this integrated testing of technologies along with the key energy, chemical and resource consumption figures will form basis for LCA/TEA analyses

Milestones:

- ❑ **20kg Bio-oil was dispatched to DOE-PACE, USA**
- ❑ **Salt adapted PACE *C. sorokiniana* strain was inoculated and cultivated in outdoor pond for more than month (Total Operational Days:- 37) with RIL in house treatment to overcome clumping and settling issue.**
- ❑ **PACE *C. sorokiniana* strain (total 30 batches) was grown in Gagwa fresh water as well as sea water at 1 m^2 scale in outdoor ponds.**
- ❑ **25 m^3/hour PACE harvesting system is successfully commissioned and the optimization of the unit with RIL strains.**
- ❑ **Strain *C. sorokiniana* has been cultivated by using RIL media to overcome contamination and clumping issue of the strains.**

3 – Technical Accomplishments/ Progress/ Results: Integrated Processes

- **RIL supplied 20 L of biocrude to PACE team in 2018.**

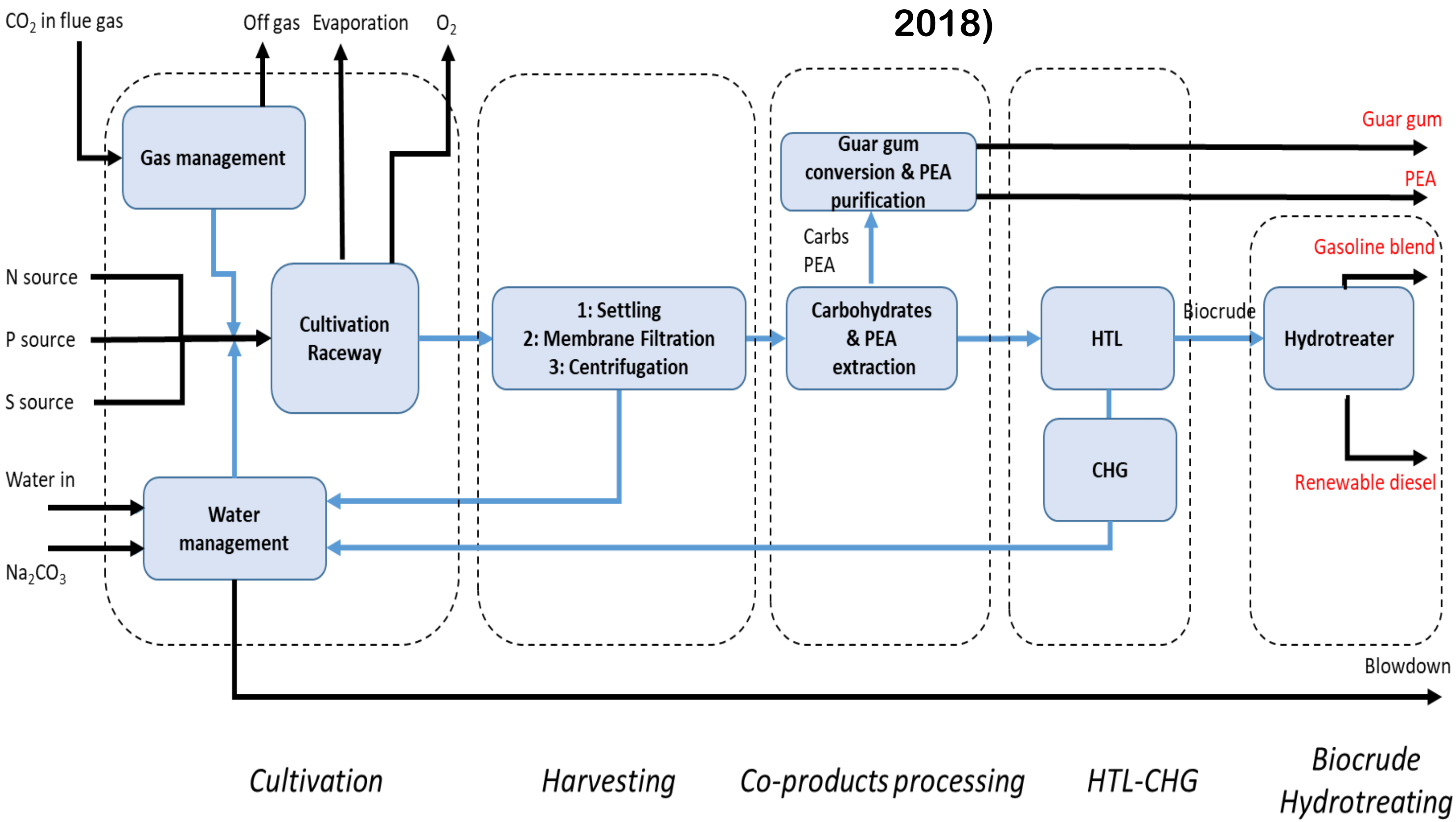
  - RIL facility can produce ~1 barrel/day

- **PNNL upgrading via hydrotreating and testing several paths to final fuels**
  - **Coprocessing with petroleum in refineries**
  - **Direct blending with diesel for final fuel**
- **PNNL/NREL testing coprocessing options and alternate insertion points in refinery**
- **CSU will lead engine tests on final products.**

3 – Technical Accomplishments
Major Accomplishments

- Successful development of end-to-end PACE Aspen model for fully integrated TEA/LCA analysis including mass and energy balances, nutrient flows, energy assessments, capex and opex, cost contribution of individual components, cash flow, and cost summaries with sensitivity analysis, EROI.
- Continual updates of Aspen model as new data available to identify gaps in data and optimize process design.

PACE Aspen Model (December 2018)



3 – Technical Accomplishments

Major Accomplishments

- Successful development of end-to-end PACE Aspen model for fully integrated TEA/LCA analysis including mass and energy balances, nutrient flows, energy assessments through advanced pinch analysis, capex and opex, cost contribution of individual components, cash flow, and cost summaries with sensitivity analysis, EROI.
- Continual updates of Aspen model as new data available to identify gaps in data and optimize process design.

PACE Aspen Model (December 2018)

Aspen Model Inputs (Assumptions)

Cultivation → *Harvesting* → *Co-products processing* → *HTL-CHG* → *Biocrude Hydrotreating*

Algal productivity for each site and season

Solid concentration
In pond: 0.3- 0,5 g/L
1st stage: 10 g/L (Settling)
2nd stage: 50 g/L (Membrane Filtration)
3rd stage: 100 g/L (Centrifugation)

Co-products yield of harvested biomass
PEA:
Extraction yield: 1 %wt.
Purification loss: 5 %wt.
Assumed price: $50/kg
Guar gum:
Extracted Carbs: 10 %wt. Conversion yield of carbs to guar gum: 80 %wt.
Assumed price: $3/kg

Reaction conditions and products yield of treated biomass*
HTL: 350 °C, 207 bar
Biocrude: 57 %wt.
CHG: 350 °C, 207 bar
CH_4: 8 %wt.
CO_2: 20 %wt.
Aqua products: 15 %wt.

H_2 consumption
0.043 wt/wt of biocrude
Products yield of biocrude
Renewable diesel: 69 %wt.
Gasoline blend: 14 %wt.
Gas: 7 %wt.
Aqueous products: 10 %wt.

3 – Technical Accomplishments

Major Accomplishments: Modelling

1. Reactor operations

Relevance: Overall integration and setup

Aim: Analysis of dilution rates for different sites and operational procedures to increase growth rate & yield

(i) Batch (ii) Draw and fill

(iii) Semi-continuous/continuous (turbidostat)

Conclusion: Dilution vs Productivity rates analysis strongly suggests turbidostat is preferable. Experiments to test in progress.

2. Reactor design optimization: Comsol models

Relevance: Overall integration and setup

Aim: Develop COMSOL models for process optimization.

In progress: Modeling of PBR hydrodynamics, kinetics, mass transfer, light assimilation, ultrasonic harvesting.

3. CO_2 utilization

Relevance: Sustainable Algae Production

Aim: Model and design of CO_2 absorption system for sustainable cultivation and cost reduction.

In Progress: Optimization in process.

4. Commercialization

Relevance: Scale-up and commercialization

Aim: Develop TechFin analogue for co-product commercialization and analysis including:

(i) Value Chain: Who makes the money?

(ii) Price Elasticity of Demand: What happens to the price when more product comes on the market?

(iii) Porter's Five Forces: What do we do about it all? Where is our place? Barriers to entry

In progress: Updating TEA to assess results/targets.

5. Beyond Aspen: Hydrotreating (HySys) model

Relevance: Next generation pinch analysis and EROI optimization

Aim: Develop new model for biocrude processing using HysSys software.

In progress: Allows critical analysis of co-products for technological process design and economics.

3 – Technical Accomplishments/ Progress/Results Summary

- *Multiple targets are now successfully transformed into* Chlorella sorokiniana. *Major successes in developing genetic tools for this alga*.
- *Salt water adaptation of* Chlorella sorokiniana *achieved and several mechanisms to shift biomass compositions developed.*
- *Successful coproduct accumulation achieved to offset biofuel costs.*
- *SNRK transformants have promising improved biomass phenotypes and are in the outdoor testing pipeline.*
- *TERA application with EPA was successful and the SNRK lines will be tested at AzCATI in 2019.*
- *Kilograms of algal biomass generated and used to improve HTP.*
- *Reliance facility allowing complete TEA/LCA access to investigate a large integrated process from cultivation through algal oil.*
- *Several liters of phototrophic algal biocrude is in hand and will be processed into fuels for engine testing.*

4 – Relevance

- **Goals**
  - **Increase algal biomass productivity to >25 gdw/m^2/day.**
  - **Produce and harvest high-value, high market demand coproducts compatible with continuous flow, sequential HTP technology to further reduce biofuel costs.**
  - **Develop and improve process/engineering systems**

- **Algal productivities and process integration remain significant hurdles in realizing the cost-effective production of biofuels from algae.**
- **The PACE project is exploring multiple mechanisms to enhance yields and valuable co-product accumulation through strain engineering. Close to most targets.**
- **Engineered strains are being tested outdoors – a critical step in evaluating the stability and production phenotypes of engineered strains.**
- **The ability to attain large scale (barrels of algal crude) is important in process evaluation at scale.**
- **Rigorous TEA/LCA analysis is important for further advances.**
- **Directly addresses BETO goals of improving the cost of biomass feedstock.**

5 – Future Work

- *Complete first outdoor GM strains with approved TERAs in 4.2 m^2 1000 L raceways in 2019.*
- *Evaluate growth productivities of parental strains of Nannochloropsis species that have been widely engineered.*
- *Process Reliance Industries algal biocrude to fuel and perform engine testing.*
- *Continue to stack genetic traits and determine phenotypes of strains relative to the single transformants.*
- *Finalize phenotype testing of transformed lines in the laboratory.*
- *Finalize and optimize co-product engineering.*
- *Finalize TEA/LCA data analyses.*

Summary

- Increasing algal biomass yields and improving process integration are required to lower algal biofuel costs.
- The PACE project has successfully transformed multiple genes hypothesized to increase biomass yields or enable valuable coproduct synthesis into algae.
- SNRK lines have promising preliminary phenotypes and PEA accumulation demonstrated.
- Importantly, TERA application approved to determine whether biomass yields are enhanced outdoors in an SNRK transformant.
- Successfully extracted starch from algae and processed residual via HTP with higher biocrude yields
- TEA/LCA analysis being done using data from one of the world's largest production facilities.
- Multiple efforts underway to further improve biomass yields and inform process integration improvements.
- Approaching most key target values.

Responses to Previous Reviewers' Comments

- Primary reviewer suggestions from 2017 review
  - Expand focus beyond *C. sorokiniana*
  - Focus strain engineering efforts
  - Ultrasonic harvesting challenges
- Major PACE modifications since 2017
  - Expanded efforts to marine algae beyond *C. sorokiniana*
  - Terminated several strain engineering efforts, primarily in crop protection to better focus resources
  - Finalized ultrasonic harvesting research

Representative Publications, Patents, and Presentations

Publications:

- Banerjee A., Banerjee C., Negi S., Chang JS., Shukla P. (2018) Improvements in algal lipid production: a systems biology and gene editing approach. *Critical reviews in biotechnology,* 38, 369-385.
- Aligata, A., Tryner, J., Quinn, J. and Marchese A. J. (2018). Effect of Microalgae Cell Composition and Size on Responsiveness to Ultrasonic Harvesting. *Journal of Applied Phycology*. https://doi.org/10.1007/s10811-018-1682-0.
- Hincapie, E., Tryner, J., Aligata, A., Quinn, J. and Marchese, A. J. (2018). Measurement of Acoustic Properties of Microalgae and Implications on the Performance of Ultrasonic Harvesting Systems. *Algal Research*. **31**, p. 77-86.
- Vogler, B.W., Brannum, J., Chung, J.W., Seger, M., and Posewitz, M.C. (2018) Characterization of the *Nannochloropsis gaditana* storage carbohydrate: a 1,3-beta glucan with limited 1,6-branching. *Algal Research*, **36**, 152-158. Msanne J., Holguin O., Sudasinghe N., Barry A.N., Dale T., and Starkenburg S.R. (2019). Engineering *Chlamydomonas reinhardtii* to produce galactomannan polysaccharides. (In preparation).
- Msanne J., Goncalves E.C., Holguin O., Sayre R., and Starkenburg S.R. (2019). Genetical engineering of *Chlorella sorokiniana* 1412 to support growth on phosphite. (In preparation).
- Britton T., Gonzalez R., Carr K., Pittman K., Sudasinghe N., Dale T., Starkenburg S., Barry A, Hanson D., Twary S., Sayre R., and Negi S. (2019) Overexpression of *SNRK2* gene from *Picochlorum soleocismus* leads to improved photosynthesis and growth in *Chlorella sorokiniana*. In preparation).

Presentations:

- Dunlop, E.H. An Integrated Framework for Process Design and techno-Economic Analysis for Algal Processes. Regional Workshop on Production of Biofuel and Value-added Products from Microalgae. Kuwait Institute for Scientific Research (KISR), Kuwait City, November 12-13, 2018.
- Sudasinghe, N., Sanders, C.K., Kwon, I., Cirigliano, E., Wright, K.T., McGowen, J., Dale, T. Saline adaptation of freshwater microalga Chlorella sorokiniana for sustainable biofuel production, 12th Annual Algae Biomass Summit, The Woodlands, TX, October 2018.
- Cirigliano, E., Sanders, C.K., Dale, T. Adaptation of freshwater microalgae Chlorella sorokiniana to higher salinities for the production of biofuel. LANL Student Symposium, Los Alamos, NM, August 2018.

Representative Publications, Patents, Presentations, and Awards Cont.

Presentations Cont.

- Sudasinghe, N., Dale, T., Kempkes, S., Chinn, S., Van Wychen, S., Sayre, R. Establishment and harmonization of algal biomass characterization methods for reducing the cost of advanced algae biofuels, 7th International Conference on Algal Biomass, Biofuels and Bioproducts, Miami, Florida, June 2017.
- Tryner, J., Albrecht, K., Billing, J., Hallen, R. T. and Marchese, A. J. (2018). Performance of a Compression-Ignition Engine Fueled with Hydrothermal Liquefaction-Derived Algae Biofuel and other Renewable Diesel Blends. *8th International Conference on Algal Biomass, Biofuels and Bioproducts*. Seattle, WA, June 2018.
- Tryner, J., Albrecht, K., Billing, J., Hallen, R. T. and Marchese, A. J. (2017). Characterization of Fuel Properties and Engine Performance of Renewable Diesel Produced from Hydrothermal Liquefaction of Microalgae and Wood Feedstocks. *11th Annual Algae Biomass Summit.* Salt Lake City, UT, October 2017.
- Negi S. Blue Light Mediated Regulation of Microalgal Life Cycle” ATP3 Spring 2016 workshop, Los Alamos, NM, 16-20th May, 2016
- Negi S. Phototropin: A master regulatory control gene that controls photosynthesis and growth processes in *Chlamydomonas*. 5th Pan Pacific Conference Plants and Bioenergy , Santa Fe, NM, USA, 4-7th August, 2016
- Negi S. Engineering light and sugar signaling pathway regulators lead to improved photosynthesis and growth in microalgae SIMB (Society for Industrial Microbiology and Biotechnology) Annual Meeting, Chicago IL, 12-16 August, 2018
- Msanne, J. Engineering microalgae to produce galactomannan polysaccharides (guar gum). The 12th Annual Algal Biomass Summit, Houston, TX, October 14-17, 2018.
- Msanne, J. Engineering microalgae to produce galactomannan polysaccharides (guar gum). The 8th International Conference on Algal Biomass, Biofuels, and Bioproducts, Seattle, WA, June 11-13, 2018.

Patents:

- PCT/US16/36077 For: Improved Productivity and Bioproduct Formation In Phototropin Knock/Out Mutants in Microalgae. Inventors: Sangeeta Negi, Richard Sayre, Shawn Starkenburg.
- Acoustic Manipulation of Fluids based on Eigenfrequency, filed January 2017. James Coons.
- Ultra Low Power Acoustic Separation, filed June 2017. James Coons.