NREL Transforming ENERGY

BETO 2021 Peer Review 1.3.5.270 Rewiring Algal Carbon Energetics for Renewables (RACER)

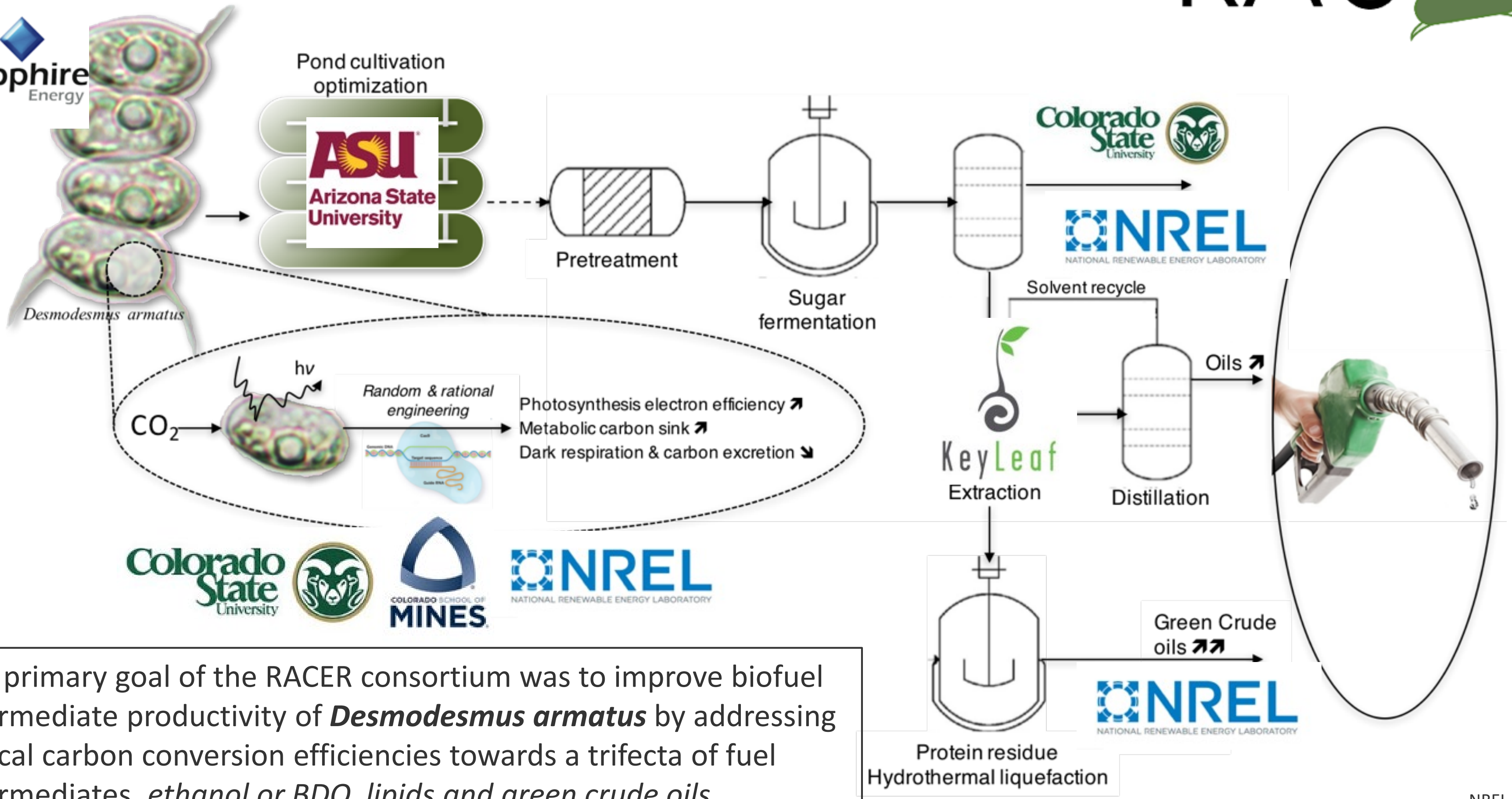
March 10, 2021

Advanced Algal Systems

Lieve M. Laurens
National Renewable Energy Laboratory

Project Overview

RACER



The primary goal of the RACER consortium was to improve biofuel intermediate productivity of ***Desmodesmus armatus*** by addressing critical carbon conversion efficiencies towards a trifecta of fuel intermediates, *ethanol or BDO, lipids and green crude oils*

Project Overview

Goals: Improve the overall carbon-to-fuel intermediate productivity for a biorefinery using *D. armatus* as a production species to reach at least 3,700 gal $acre^{-1}$ yr^{-1}

1. Improvements in **photosynthetic carbon conversion efficiency** through random mutagenesis and targeted engineering
2. **Cultivation management** advances through implementation of informed permutations of operations and nutrient management
3. **Tailoring and optimizing conversion processes** to extractable lipids, carbohydrate-derived fuel intermediate fermentation, and HTL biocrude from protein residue

1. Photosynthesis mutant selection, carbon sink targeted engineering
2. Operations, e.g. pest management and harvesting conditions
3. Fermentation and pretreatment optimization, add mannose utilization to BDO strain, inhibit growth for yeast EtOH fermentation, optimize HTL extraction and nutrient recycling

Impact: Carbon conversion efficiency improvements by coordinating photosynthesis and carbon sink engineering is the basis of biomass accumulation and biofuels production, core to BETO's AAS program

Outcome: This project has studied a high-impact holistic process integration to demonstrate that improvements in strain engineering, cultivation operations and conversion engineering, can yield productivity improvements and cost reductions

Aim: Accelerate carbon to product pathways
Today: No integrated path to targeting algae improvements with conversion in mind
Importance: Holistic conversion relation to biomass composition sheds light on future algae challenges
Risk: Relying on metabolic phenotype risks cascading effects

Market Trends

Product	Anticipated decrease in gasoline/ethanol demand; diesel demand steady
Product	Increasing demand for aviation and marine fuel
Product	Demand for higher-performance products
Product	Increasing demand for renewable/recyclable materials
Feedstock	Sustained low oil prices
Feedstock	Decreasing cost of renewable electricity
Feedstock	Sustainable waste management
Feedstock	Expanding availability of green H_2
Feedstock	Closing the carbon cycle
Capital	Risk of greenfield investments
Capital	Challenges and costs of biorefinery start-up
Capital	Availability of depreciated and underutilized capital equipment
Social Responsibility	Carbon intensity reduction
Social Responsibility	Access to clean air and water
Social Responsibility	Environmental equity

NREL's Bioenergy Program Is Enabling a Sustainable Energy Future by Responding to Key Market Needs

Value Proposition

- Focus on strain development targeting holistic carbon conversion efficiency (CCE) through processing
- Position for future emphasis on carbon assimilation to products and carbohydrates

Key Differentiators

- Unique integration of upstream strain engineering with downstream biomass conversion
- Connection to full project portfolio and SOT cultivation and conversion

1. Management

- **Four subtasks** aligned with project objectives, with monthly project meetings and quarterly all-hands meetings
- Progress tracked through modular process CCE improvements, related to TEA/LCA
- **Commercialization strategy**: Leverage and integrate with BETO's strategy for sustainable aviation fuels and chemicals and lipid and carbohydrate upgrading
- **Deliverable**: Yield and performance data on fuels and chemicals production available to community/industry

Risk: Focused work on one species constrains integrated approach and risks associated with each conversion step requires flexible engineering customization approach

Advisory board:

Lou Brown (SGI)
Shaun Bailey (SGI)
Rebecca White (Qualitas)
Colin Beal (TEA consultant)
Nancy Dowe (NREL Fermentation)
Olaf Kruse (Bielefeld)
Thomas Sharkey (Michigan State)

1. Strain Development
2. Cultivation
3. Conversion
4. Technoeconomic & Lifecycle Analysis

1. Genetic engineering, mutagenesis, physiology
Eric Knoshaug, Damien Douchi, Graham Peers, Max Ware, Matt Posewitz, Melissa Cano

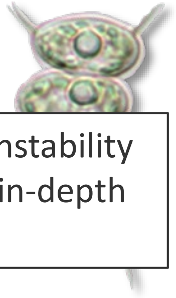
2. Algae cultivation, data analysis
John McGowen, Jessica Forrester

3. Pretreatment Engineering, Fermentation, Extraction
Nick Nagle, Ken Reardon, Yat-Chen Chou, Tao Dong

4. Computational TEA &LCA
Ryan Davis, Jason Quinn

2. Approach

Objective 1: Strain Development



- ✓ 1.1. Metabolic Engineering Tool Development
- ✓ 1.2 Photosynthesis Engineering for Reduced Alternate Electron Transport
- \- 1.3 Metabolic Rearrangement for Inc
- \- 1.4 Engineering for Reduced Dark Re

Missed milestones due to genotype/phenotype instability

- ➢ Rescoping to incorporate new milestones for in-depth comparative genetics

Objective 2: Pond Operational Managem

- ✓ 2.1 Increased productivity through nutrient and pond operational management
- \- 2.2 Submission of TERA Protocols

Objective 3: Conversion to Fuel Intermediates

- ✓ 3.1 Pretreatment for Increased Feedstock Recovery
- ✓ 3.2 Improved fermentation carbon conversion efficiency
- ✓ 3.3 Optimize lipid extraction from fermentation stillage/slurry
- ✓ 3.4 Optimization of HTL conversion of protein-rich residue

Objective 4: Techno-economic and Sustainability Analysis ✓

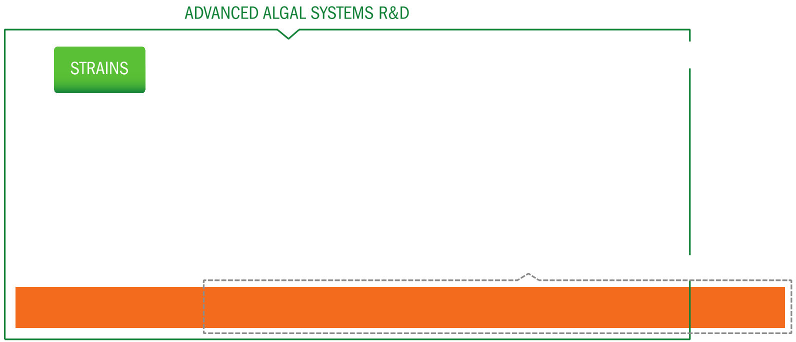
2. Approach: Challenge Identification

Demonstrate improvements in overall productivity and fuel yields for *D. armatus*

Critical Success Factors	Risk	Strategy
Establish **efficient metabolic engineering** tools	Transient unstable expression of transgenes	Build robust strain development toolbox
Demonstrate **physiological phenotype** improvement	Variable gene expression may not lead to measurable improvement in cell carbon physiology	Engineering multiple targets using range of promotors and gene-expression elements and developing strong screening tools for mutagenesis approach as alternative for identifying improved strains
Pretreatment of biomass to supply conversion process with high yielding fractions	Biomass component interactions during acid pretreatment, biomass compositional variability	Optimization of small-to-large scale translation of pretreatment with different biomass material
Demonstrate **carbon conversion** improvements to biofuel streams	Liquors generated vary based on conversion conditions thus yielding different compositional fractions	Use optimized conversion or pretreatment approach at sufficiently large scale from one biomass harvest to supply consistent material to downstream conversion

3. Impact

- Increasing biofuel-intermediate yields by removing key barriers that currently limit overall process carbon efficiency and biomass productivity
- Dissemination of results: 7 publications, 1 patent application, ranging from genetic engineering of non-model algae, nutrient recycling of products after HTL
- Transfer of technologies to other projects in algae projects portfolio:
  - Genetic engineering applied to newly started project
  - Fluazinam cultivation pest control to DISCOVR
  - Conversion and BDO fermentation novel organisms to CPR



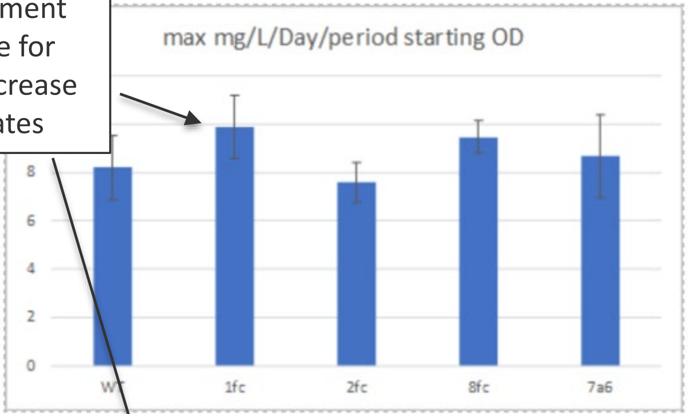
- Summer productivity of *D. armatus* **included in the FY18** productivity SOT assessment
- Pretreatment and BDO fermentation yields included the conversion of sugars, including mannose, was **included in the FY18, FY19 and FY20 SOT**

4. Progress and Outcomes

Objective 1: Strain Development

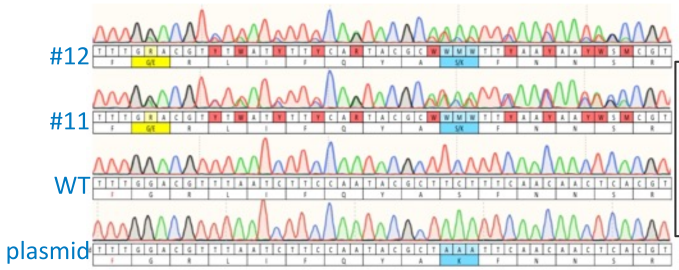
- Developed genetic engineering tools for *D. armatus* a unique and resilient non-model alga
- Selected and engineered mutants with distinct metabolic phenotype (increased carbohydrate and lipid content) and indoor growth rate improvements
- Discovered genetic machinery unique to *D. armatus* phenotype/genotype instability

17% improvement in growth rate for 1FC + 18% increase in carbohydrates



	Carbohydrates	FAME
WT	32.6 ± 1.76	6.3 ± 0.08
1fc	**38.44 ± 2.18**	6.2 ± 0.19
2fc	36.7 ± 1.09	**7.43 ± 0.18**
8fc	38 ± 0.43	6.6 ± 0.45
7A6*	**35.32 ± 1.48**	6.75 ± 0.56

*Calvin cycle overexpressor strain



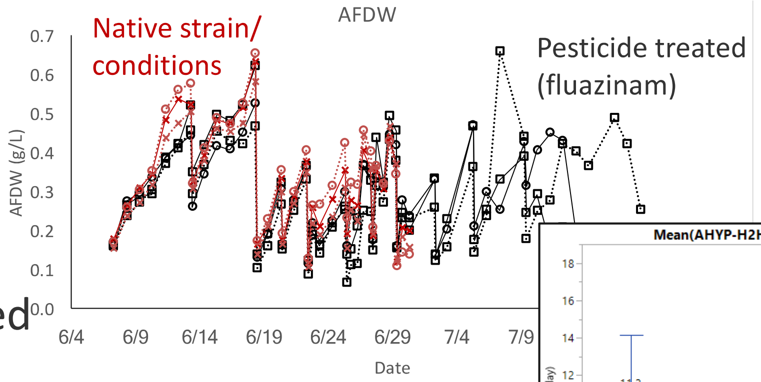
Spontaneous mutations appear in chloroplast genome after introduction of atrazine resistance marker mutation in PsbA

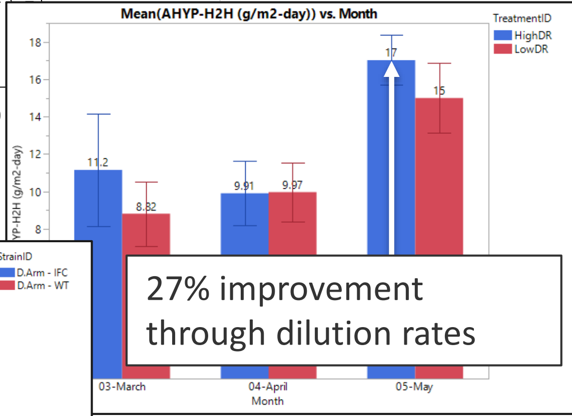
[Knoshaug, E.P., 2019, Microbiol Resour Announc 9:e00896-19. https://doi.org/10.1128/ MRA.00896-19, Ware, M.A. et al. 2020, Plant Phys., 183, 1735-1748 | Ware, M. A., et al., 2020, Algal Research, 51, 102028; Douchi, D., et al., 2021, Algal Research, 53, 102152 | Mosey, M., et al., 2021, Algal Research, in press]

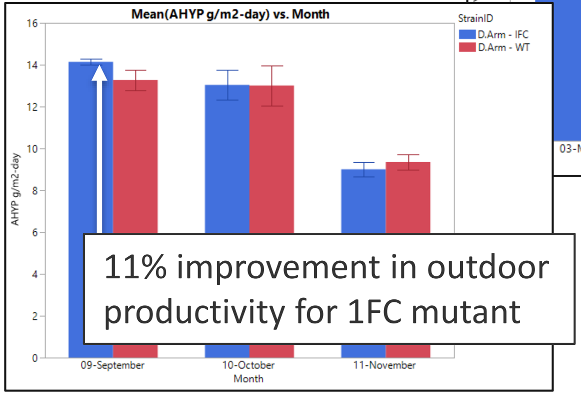
4. Progress and Outcomes

Objective 2: Pond Operational Management

- Pioneered and mainstream implemented the use of Fluazinam pesticide (now routinely applied in DISCOVR consortium SOT trials)
- 27% increased productivity by increasing dilution rate, demonstrated in FY19, relative to FY18 baseline
- 10.9% improvement in productivity for 1FC mutant (September 2019 outdoor growth)
- 50 kg biomass produced for mutant and WT strains for conversion studies





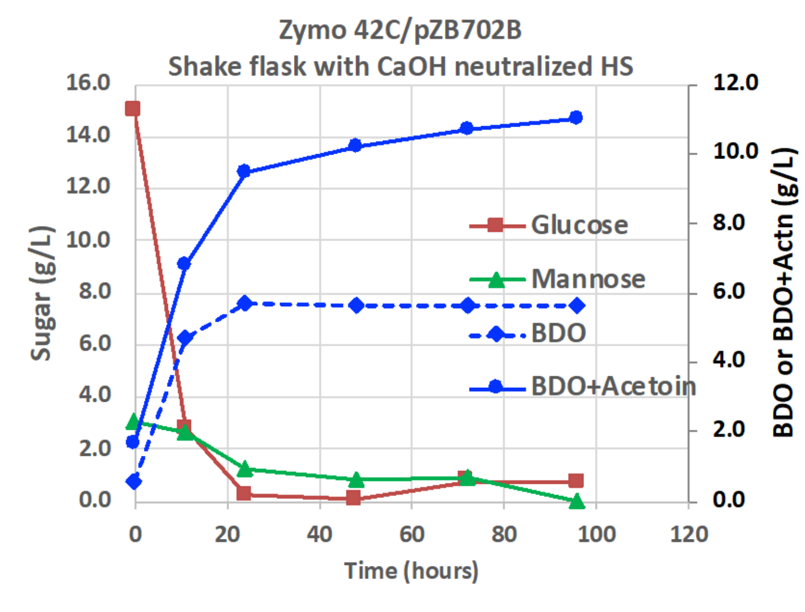


[John McGowen, ASU]

4. Progress and Outcomes

Objective 3: Conversion to Fuels and Products

- Pretreatment with enzymatic hydrolysis pioneered to over 95% glucose release
- Increased ethanol fermentation CCE by growth inhibition (comp P, comp Tp*)
- Showed 100% yield on BDO from algae hydrolysate after Zymomonas mannose utilization engineering
- > 92% lipid extraction efficiency (KeyLeaf) with 20% increased bio-oil yield from HTL (up to 38.8%)
- Recycled N from HTL aqueous phase**, showing a closed loop production-conversion-nutrient recovery study



Engineered mannose utilization in *Zymomonas* BDO strain, **101% yield to 2,3-butanediol and acetoin** based on glucose and mannose in hydrolysate ($Ca(OH)_2$ neutralized)

[Yat-Chen Chou, Min Zhang, NREL]

	DW (mg)	EtOH yield (g/g)
Control	3.6 ± 0.29	0.39 ± 0.01
Comp P (0.2%)	2.57 ± 0.05	0.41 ± 0.01
Comp Tp (0.2%)	1.83 ± 0.09	0.41 ± 0.01

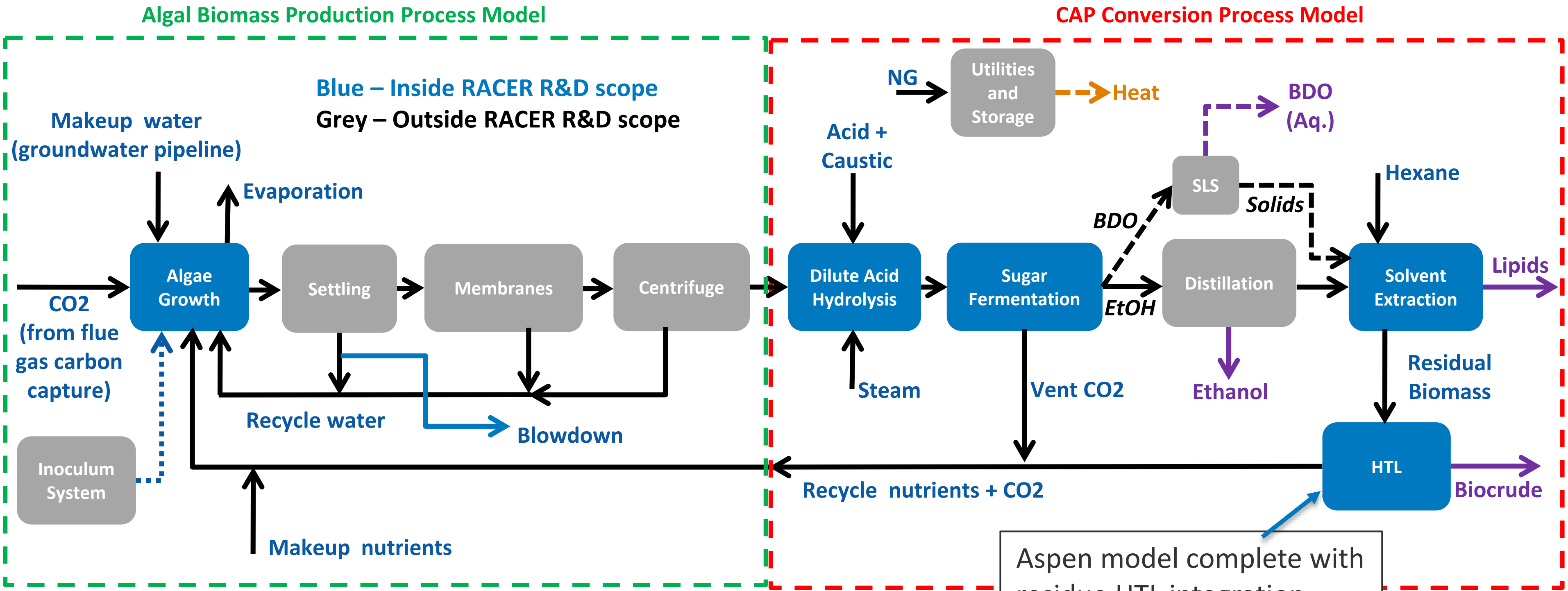
Continuous-flow prod 20% higher than free-cell batch fermentation of *D. armatus* hydrolyzate) →

[*Huang, Reardon, record of invention filed]

[** Chen and Laurens, 2019, Algal Research, 46, 101776]

4. Progress and Outcomes

Objective 4: Technoeconomic and Sustainability Analysis



Aspen model complete with residue HTL integration

4. Progress and Outcomes

	Baseline Update		Intermed. Update		Final Update*	
Parameter	**EtOH**	**BDO**	**EtOH**	**BDO**	**EtOH**	**BDO**
Intermediate fuel yield (GGE/ton AFDW)	76	80	88	88	115	115
Lipid output (MM gal/yr)	1.8	1.8	2.2	2.2	3.0	3.0
Ethanol/BDO output (MM gal/yr)	3.0	2.9	3.2	2.8	8.5	6.9
HTL bio-crude output (MM gal/yr)	4.0	4.1	6.4	6.4	9.6	10.0
Overall intermediate fuel yield (gal/acre-yr)	**1727**	**1719**	**2288**	**2197**	**4086**	**3846**
Integrated conversion CAPEX (TCI, $MM)	$144	$151	$178	$185	$299	$256
MFSP ($/GGE intermediates)	**$15.31**	**$14.97**	**$12.00**	**$12.39**	**$8.34**	**$8.06**

- 22% reduction in MFSP demonstrated (intermediate verification), projected 40% if 17g/m2/day annual average productivity can be achieved
- *Final fuel yield estimated > 3,700 gal/acre-yr (both with BDO and EtOH as fermentation products)

[Ryan Davis, NREL, Jason Quinn, Peter Chen, CSU]

Summary

Holistic process integration demonstrating improvements in strain engineering, cultivation operations and conversion engineering, towards yield increase and cost reduction

Management

- Critical contribution to bioeconomy through integrated objective product demonstration towards cost, value and feasibility demonstration

Approach

- Coordinating photosynthesis engineering with carbohydrate sink manipulation in non-model alga *D. armatus*
- Cultivation management for outdoor productivity improvements of selected cultivars
- Conversion optimization to biofuels and products
- TEA/LCA impact analysis for delivery and integration

NREL's Bioenergy Program Is Enabling a Sustainable Energy Future by Responding to Key Market Needs

Impact

- Carbon conversion efficiency improvements by coordinating photosynthesis and carbon sink engineering for sustainable fuel production
- Improve basis of biomass accumulation and biofuels production, with TEA/LCA impact assessment
- Disseminate findings across collaborative portfolio

Progress & Outcomes

- Discovery and documentation of genome instability of non-model alga, *D. armatus*
- Outdoor growth improvements >27% increased productivity, with and 11% with mutant deployment
- Improved carbon conversion to ethanol and 2,3 BDO as platform chemical and fuel intermediate
- Projected > 20% decrease in MFSP after integration of project deliverables

Quad Chart Overview

Timeline
- 10/1/2017
- 3-year competitive award
- 95% complete

	FY20	Active Project
DOE Funding	$292K	$2.3M
Project Cost Share		$315K

Project Partners*
- Arizona State University (16%)
- Colorado State University (26%)
- KeyLeaf (2%), School of Mines (5%)
- Sapphire Energy (cost share partner)

Project Goal
Improve biofuel intermediate productivity of the commercially-relevant ***Desmodesmus armatus*** by addressing critical carbon conversion efficiencies towards a trifecta of fuel intermediates; ethanol or butane diol, lipids, HTL green crude oils

End of Project Milestone
Achieve overall biofuel intermediate yield of over 3900 gal acre^{-1} yr^{-1} based on both carbon assimilation and conversion improvements (FY20 MYP goal)

Funding Mechanism
FY17 ABY2 FOA, funded in FY18.

Barriers Addressed
Aft-A. Biomass Availability and Cost, Aft-C. Biomass Genetics and Development, Aft-E. Algal Biomass Characterization, Quality, and Monitoring, Aft-I. Algal Feedstock On-Farm Preprocessing Aft-J, Resource Recapture and Recycle

NREL
Eric Knoshaug
Damien Douchi
Bo Wang
Nick Nagle
Tao Dong
Yat-Chen Chou
Min Zhang
Ambarish Nag
Stefanie Van Wychen
Andy Politis
Steven Rowland
Ryan Herold
Megan Mosey
Ryan Davis
Jenifer Markham
Philip Pienkos

CSU
Ken Reardon
Xingfeng Huang
Graham Peers
Max Ware
Laura Hantzis
Jason Quinn
Peter Chen
Juan Venegas

ASU
John McGowen
Jessica Forrester
Henri Gerken

CSM
Matthew Posewitz
Melissa Cano
Amy Ashford

KeyLeaf
Rick Green
Udaya Wanasundara

Sapphire
Craig Behnke
Chris Yohn

U.S. DEPARTMENT OF ENERGY

Thank You

www.nrel.gov
www.nrel.gov/bioenergy/algal-biofuels.html

Lieve.Laurens@nrel.gov

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Additional Slides

Publications, Patents, Presentations, Awards, and Commercialization

1. Chen, P. H., Venegas, J. L., Rowland, S. M., Quinn, J. C., Laurens, L. ML. (2020) Algae Nutrient Recycle from Hydrothermal Liquefaction Aqueous Phase through a Novel Selective Remediation Approach" Algal Research, 46, 101776
2. Knoshaug, E.P., Nag, A., Laurens, L. ML., 2019, Draft genome and chloroplast sequences of the biofuel-relevant microalga *Desmodesmus armatus*, Microbiology Resource Announcement, 9:e00896-19. https://doi.org/10.1128/ MRA.00896-19.
3. Maxwell A. Ware; Darcy Hunstiger; Michael B. Cantrell; Graham Peers, "A Chlorophyte alga utilizes alternative electron transport for primary photoprotection", 2020, Plant Physiology, 183, 1735-1748
4. Ware, M. A., Kendrick, J. M., Hantzis, L. J., Peers, G. A fluorescence-based approach to screen for productive chemically mutagenized strains of Desmodesmus armatus, 2020, Algal Research, 51, 102028
5. Douchi, D., Mosey, M., Astling, D. P., Knoshaug, E. P., Nag, A., McGowen, J., Laurens, L. ML., Nuclear and chloroplast genome engineering of a productive non-model alga *Desmodesmus armatus*: Insights into unusual and selective acquisition mechanisms for foreign DNA, 2021, Algal Research, 53, 102152
6. Mosey, M., Douchi, D., Knoshaug, E. P., Laurens, L. ML., Current Review of the Successes and Hurdles of Genetic Engineering of Non-model Algae, 2021, Algal Research, in press
7. Yeast quorum-sensing molecules reduce the cell growth of Saccharomyces cerevisiae and improve ethanol yield during fermentation (In preparation)
8. Provisional patent filed on quorum sensing molecules to improve fermentation CCE (CSU)

Responses to Previous Reviewers' Comments

Weakness: • They note in the presentation on slide 8 that they are going to increase productivity through nutrient and pond operational management, and later on slide 12 that they did a baseline outdoor cultivation in the summer of 2018. Were any lessons gleaned from this cultivation that could be used for the future cultivations with the genetically improved strains?	The goals of the outdoor cultivation baseline productivity experiments were to establish a seasonal average productivity for D. armatus at the AzCATI testbed location. This was expected to be different from the Las Cruces, NM testsite location, where the strain was grown for multiple consecutive years. In terms of lessons learned, the cultivation of D. armatus outdoors appeared to be robust in the first year of this project, but also prone to contamination with chytrids. This has promted us to implement a procedure for pond crash mitigation by pesticide application, which has yielded promising results. Until the improved strains become available, we are testing the impact of different cultivation conditions, e.g. semi-continuous cultivation at different densities, on the biomass productivity and composition.
Weakness: It was unclear what characteristics would be considered 'desireable' from a TEA standpoint. For example, mutagenesis work identified a mutant with increased lipid production, but it was unclear if the TEA would benefit from such a mutant.	While prior NREL TEA work has identified lipid content as a strong cost driver, particularly for fuel-focused conversion pathways included in the RACER project as a whole, in the context of this entire project, changes to biomass composition towards carbohydrate or lipid content increases are of interest to the overall biofuel intermediate yields. Work is ongoing both within the RACER project and under core BETO Platform efforts to specifically exercise NREL TEA models to identify cost versus value tradeoffs between biomass growth and composition, and the resultant implications on yields and fuel selling prices.
Weakness: Roll-out plan is focused on the genetic improvements that are being made to improve strains. What process improvements are being utilized to improve biomass productivities at scale? The methods for "increased productivity through nutrient and pond operational management" are not thoroughly clarified and should be seriously considered before large-scale implementation to ensure that ideal conditions are determined to produce optimum biomass, lipids, and carbohydrates in a production timely manner.	We believe that the biggest obstacles to outdoor cultivation improvements are related to creating stable mutant strains that show improvement in (mimicked) outdoor conditions. The ongoing experiments at the AzCATI testbed continue to collect outdoor data by comparing different cultivation conditions, in particular semi-continuous harvesting strategies (harvest frequency, biomass concentration and nutrient delivery permutations). Our primary target for the current outdoor experiments with the wild type strain is maximizing productivity, with continuous measurement of biomass composition. This will ultimately achieve a strong experimental basis to rapidly compare improved cultivars over the summer of FY19.
Weakness: • Growth parameters need to be optimized. • Strain has been reported to have 10% lipid during mid phase but increases to 20% after 13 days of induction. A large-scale production site will not be viable if induction to reach 20% lipids takes 2 weeks. Modes of induction to reach high lipids more rapidly should be considered. • Steps are delineated for how strains are going to be genetically modified for improvements, but no steps are noted for how strains are going to be enhanced through "nutrient and pond operational management" (slide 8). What approaches are being utilized to boost productivity for these strains from an operational standpoint?	We appreciate the comments relating to the cultivation work but wanted to clarify that we are not planning to include a dedicated lipid induction phase during the outdoor cultivation experiments. The targeted metabolic engineering tools that are in the process of being developed focus on targeting central carbon assimilation and carbohydrate storage, so in our targeted effort, it is much more likely that carbohydrates will be accumulated, and lipids are projected to either stay constant or increase slightly as an overflow storage for metabolic energy. The experimental outdoor cultivation approach on nutrient and pond operational management will include different permutations on the harvesting strategy, e.g. keeping the cultures at high cell density but low depth will increase light stress and thus has the potential to rapidly shift the composition, while also maximize biomass productivity, both of which would be highly beneficial in the overall integrated process operations.

Approach

- Go/No-go Decision point (FY19)

Key TechFin Cultivation parameters in put to TEA

Metric	Baseline Update	Intermed. Update	Final Update
Productivity (g/m^2/day AFDW, annual average)	11.0	13.0	17.0
Harvest density (g/L AFDW)	0.4	0.4	0.5
Harvest composition, 100% closure (dry wt%):			
Ash	3.5	2.8	2.8
FAME lipids	7.6	7.4	7.4
Non-FAME lipid + sterols	3.8	3.7	3.7
Fermentable carbs	37.2	43.9	48.3
Non-fermentable carbs	2.5	2.9	2.9
Protein	27.2	23.3	21.1
Biomass	17.0	14.8	12.6
Elemental C/N/P content at harvest (wt% AFDW)	47.3/5.9/1.1	47.7/5.0/1.1	47.7/5.0/1.1

TechFin: Key TEA model outputs

Parameter: EtOH [BDO]	Baseline	Baseline Update	Intermediate	Intermed. Update	Final
Biomass yield (tonne/yr AFDW)	87,338	87,338	102,361	102,361	131,608
Biomass selling price ($/ton AFDW)	**$935/ton**	**$909/ton**	**$810/ton**	**$783/ton**	**$651/ton**
Intermediate fuel yield (GGE/ton AFDW)	102	76 [80]		88 [87]	
Lipid output (MM gal/yr)	4.4	1.8		2.2	
Ethanol/BDO output (MM gal/yr)	3.3	3.0 [2.9]		3.2 [2.8]	
HTL bio-crude output (MM gal/yr)	3.7	4.0	4.4	6.4	6.7
Overall intermediate fuel yield (gal/acre-yr)	**2200**	**1727 [1719]**	**2771**	**2288 [2197]**	
Integrated conversion CAPEX (TCI, $MM)	$121	$144 [$151]		$178 [$185]	
MFSP ($/GGE intermediates)	**$11.63**	**$15.31 [$14.97]**	**$9.67**	**$12.00 [$12.39]**	**$7.07**

Lower net fuel yield: lower lipid content, higher carbs offset by SLS losses + lower ferm yields; higher HTL yields

Higher lipid extraction yield, higher carbs offset by lower prt yields, higher HTL yield

Higher MFSP due to lower overall yields, higher processing costs from model refinements

22% and 17% lower MFSP for EtOH and BDO vs updated baseline – lower MBSP, higher yields

- Final MFSP projected at $7.07/GGE (~40% improvement over original baseline or ~50% over updated baseline on EtOH) – will not achieve “economic viability” by end of project, but this was never expected (not until beyond 2022) – that would require ≥25 g/m^2/day and inclusion of coproducts (at expense of fuel yields)
- **Final target case achieves primary FOA objective: fuel yield target >3700 gal/acre-yr**
- **Updated intermediate case achieves Phase I TEA objective: >10% MFSP improvements over updated (re-cast) baseline (both EtOH and BDO)**

TechFin: Conversion: Key modeling inputs

Metric	Baseline	Baseline Update	Intermediate	Intermed. Update	Final
Pretreatment					
Solids loading (wt%)	20%	20%	20%	18%	20%
Temperature (°C)	150	150	150	170	150
Acid loading (wt% vs feed liquor)	2%	2%	2%	3%	1%
Fermentable sugar release (wt% of initial carbs)	74%	74%		56% glucose, 39% mannose	90%
Fermentation					
Monomeric sugar utilization; EtOH [BDO]	95%	9 % [26%]		✓ 95% [98%]	98%
Metabolic yield (g/g utilized sugars, % theor); EtOH [BDO]	86%	80% [73%]	86%	✓ 88% [90%]	92%
Batch time (days); EtOH [BDO]	1.5	1.5 [3.5]	1.5	✓ 1.5 [2.2]	1.5
Lipid Extraction					
Solvent loading (solvent/dry biomass ratio, g/g)	5.9	5.9	5.9		5.0
Total FAME lipid extraction yield	87%	87%	90%	✓ 92%	95%
HTL Conversion of Residue					
Biocrude yield (wt% of organic feed)	23.3%	23.8%	26.		35.7%
on (% of HTL feed)	63% / 50%	63% / 50%	63% / 50%	63% / 50%	63% / 50%

High recalcitrance with *D. armatus*

CSU free cell fermentation

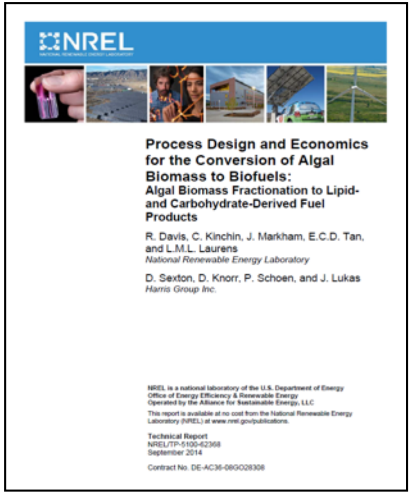
-EtOH: immob. cells
-BDO: *Zymo* improvements, lower toxicity

POS extr. yields w/ hexane

New Keyleaf extraction data

- Baseline: Prior NREL SOT data (HTL assumed)
- Baseline update: Corrected EtOH yield based on CSU *S. cerevisiae* free cell ferm. + NREL HTL baseline data
- Intermediate/Final: Original verification goals
- Intermed. update: RACER Phase 1 demonstrated

NREL CAP Design Report: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Process Design and Economics for the Conversion of Algal Biomass to Biofuels: Algal Biomass Fractionation to Lipid- and Carbohydrate-Derived Fuel Products

R. Davis, C. Kinchin, J. Markham, E.C.D. Tan, and L.M.L. Laurens
National Renewable Energy Laboratory

D. Sexton, D. Knorr, P. Schoen, and J. Lukas
Harris Group Inc.

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http://www.nrel.gov/docs/fy14osti/62368.pdf

RACER

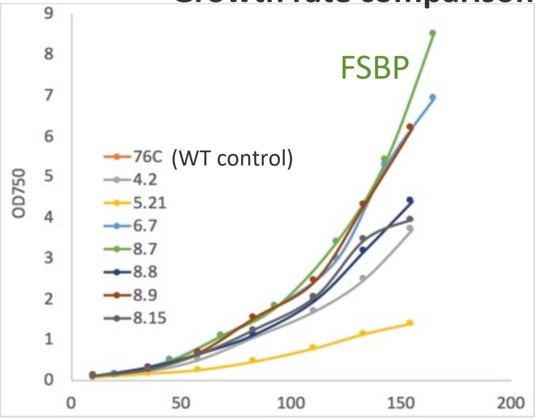
Overexpression targets

#1 = Levansucrase in the vacuole – no transformation yet
#2 = Cellulose synthase – no transformation yet
#3 = Fbp-Sbp (D-fructose 1,6-bisphosphatase class 2/sedoheptulose 1,7-bisphosphatase)
#4 = Levansucrase in the cytoplasm – 2 transformant lines
#5 = PGM (Phosphoglucomutase) – 2 transformant lines
#6 = g7207 - 7 transformant lines
#7 = fbaA (Fructose-bisphosphate aldolase class 2)
#8 = FSBP (Fructose-1,6-/sedoheptulose-1,7-bisphosphatase) – 15 transformant lines
#9 = ADP-glucose pyrophosphorylase – no transformation yet
#10 = Isoamylase – no transformation yet

**Calvin cycle carbon assimilation*
**Storage carbon sink*

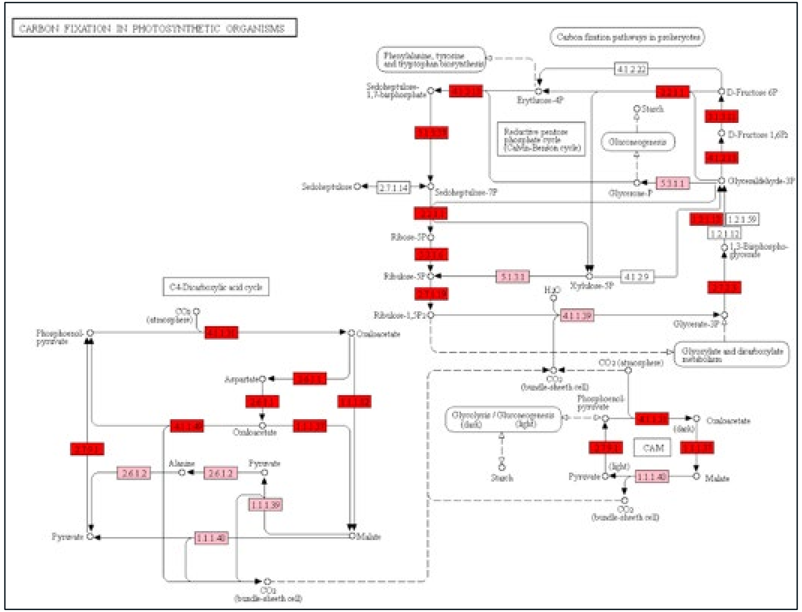
cNAT (nourseothricin acetyl transferase) resistance marker confirmed insertion:

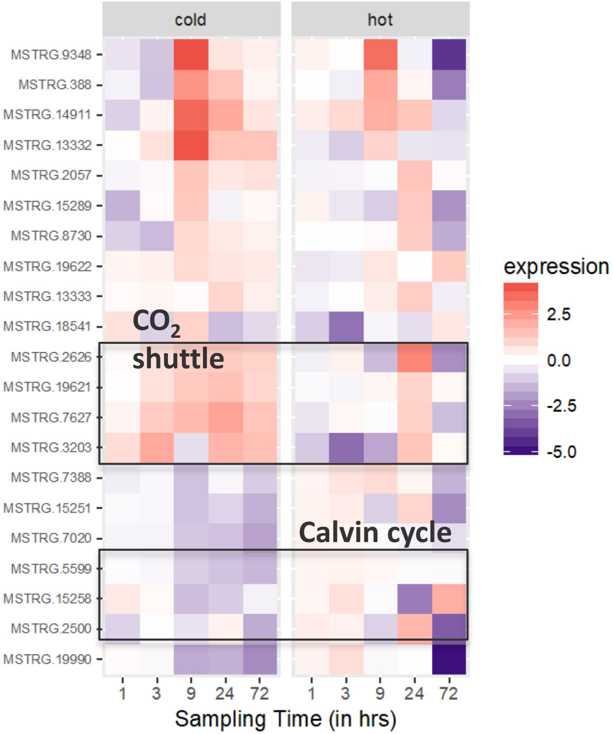
Growth rate comparison:



Multivariate Analysis – 'Omics' Integration

Established RNASeq analysis pipeline for *D. armatus* for transcriptomics of **Carbon Fixation** pathway under abiotic stress

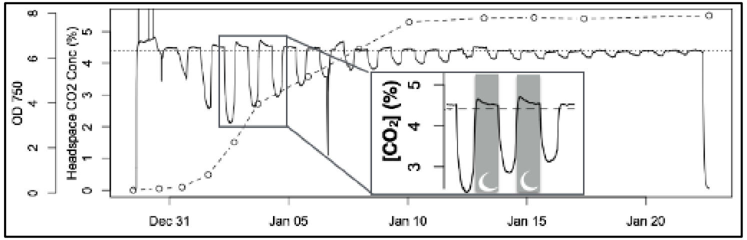




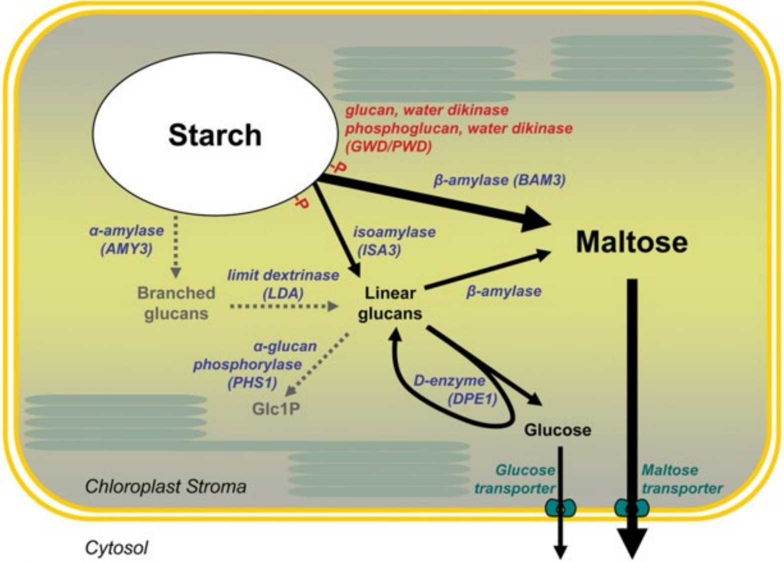
Lee, P., Astling, D., et al. (2018) *in preparation*

Night-time Respiration – Starch Degradation

CO_2 uptake/release in the headspace for three weeks of *Scenedesmus acutus* cultivation in programmable SAGE reactor indicating night-time respiration



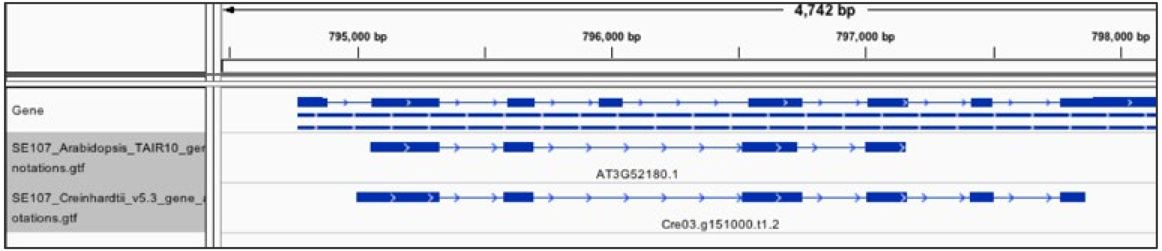
Starch degradation pathway model in *Chlamydomonas* and higher plants indicating starch phosphorylation at the core of regulating degradation flux

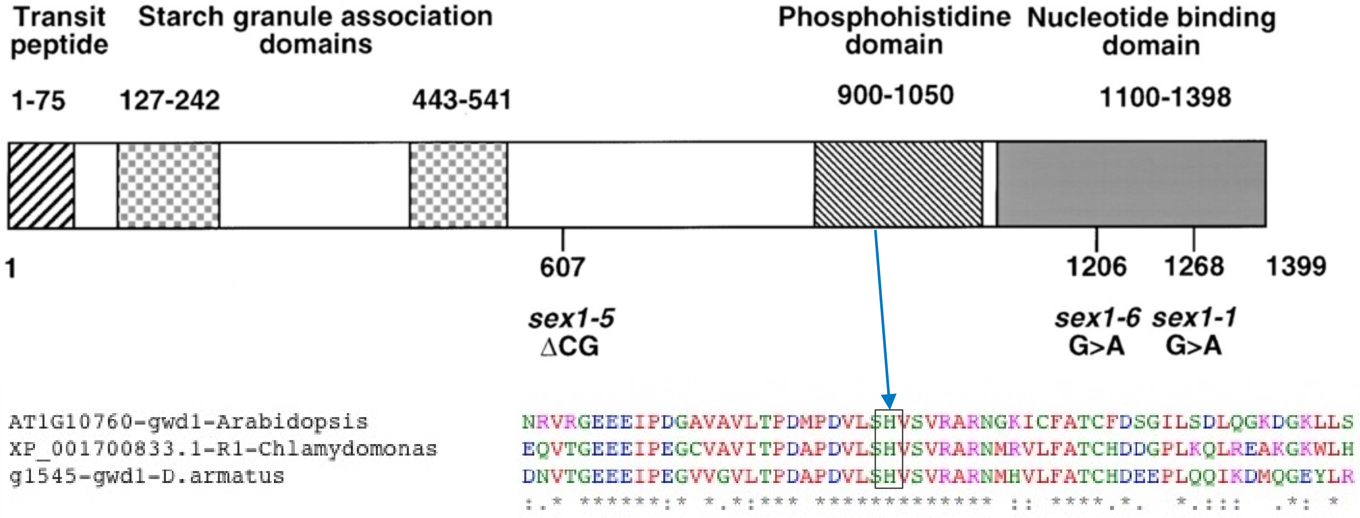


Remains to be demonstrated whether *D. armatus* exhibits this starch metabolism

Glucan Water Dikinase

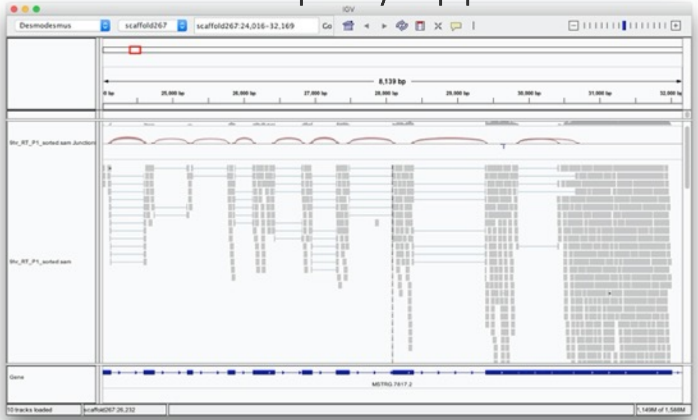
Genome mining of *Desmodesmus armatus* indicates the presence and similar structure of the gene for **Glucan Water Dikinase (GWD)** – elimination causes starch excess phenotype without impacting growth



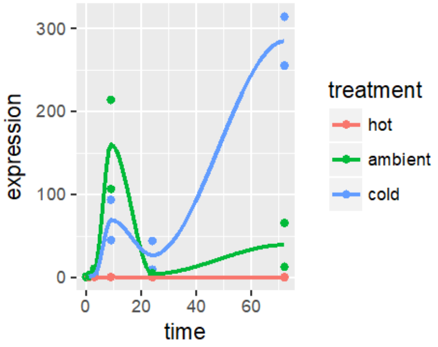


Multivariate Analysis – ‘Omics’ Integration

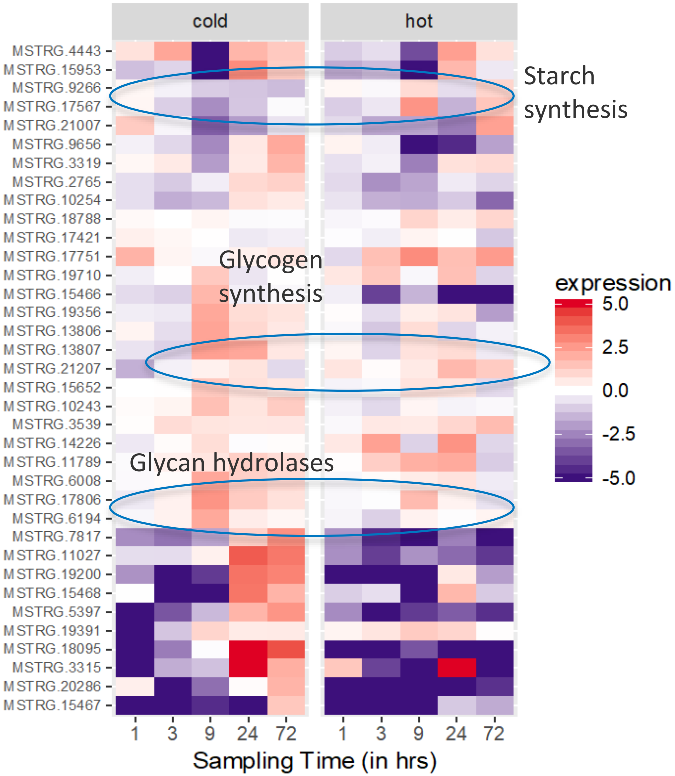
Established RNASeq analysis pipeline:



Endoglucanase expression

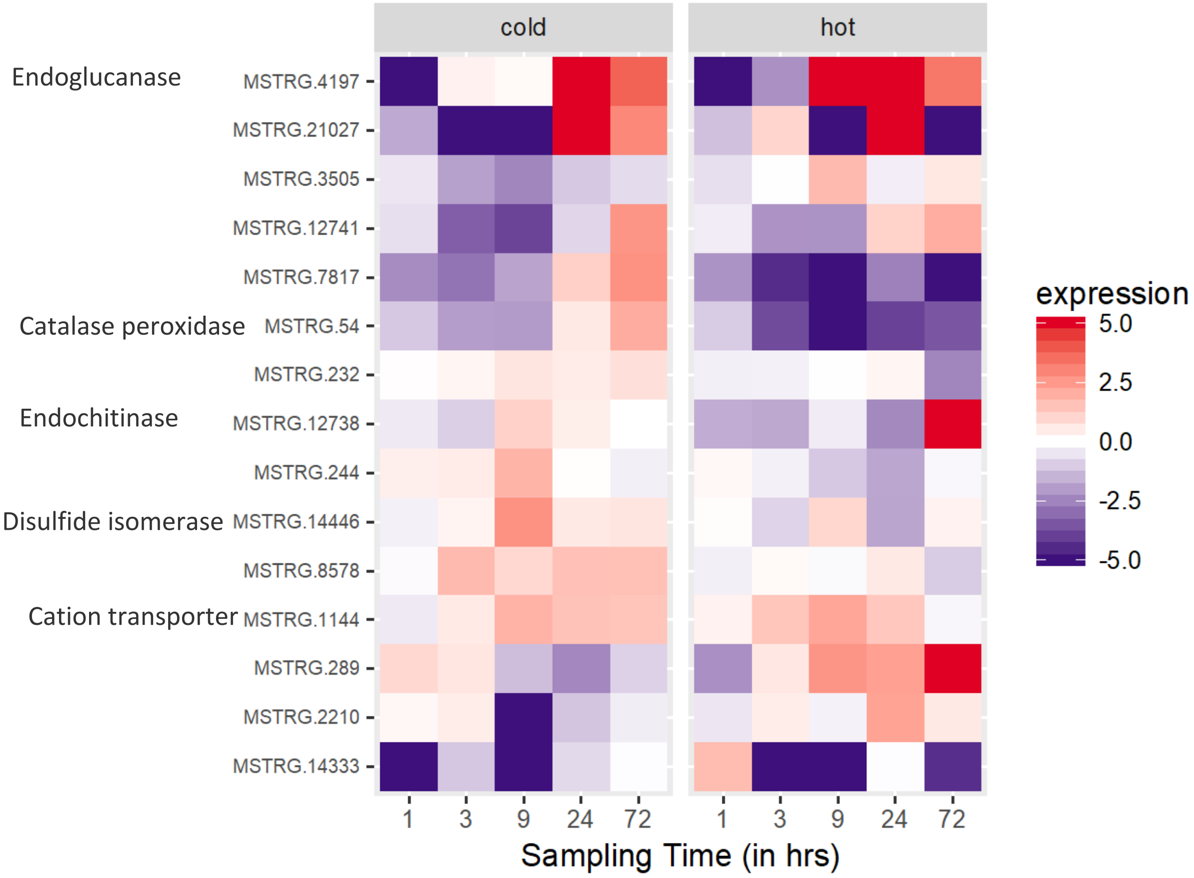


Transcriptomics of Starch and Sucrose Metabolism in *D. armatus*



Lee, Astling, Laurens (2017) in preparation

Extracellular Gene Expression



Engineering Z. *mobilis for* 2,3-BDO production from Algal Sugars

- Introduce mannose utilization pathway in *Z. mobilis* strain
  - Mannose utilization pathway will be introduced into *Z. mobilis* ZM4 (it has previously introduced a different host)
- Introduce 2,3 BDO producing pathway to mannose- utilizing *Z. mobilis* strain
  - 2,3 BDO producing pathway has been engineered into glucose/xylose-utilizing *Z. mobilis* strain.
- Eliminate ethanol formation in the above 2,3 BDO producing/mannose-utilizing *Z. mobilis* strain
- Fermentation testing for 2,3 BDO production from algal biomass sugar streams – comparing yields (g BDO / g sugar) against baseline of pure sugar

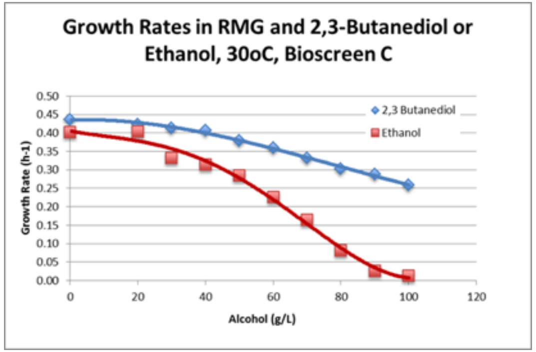
Metabolic Engineering *Zymomonas mobilis for* Producing 2,3-BDO

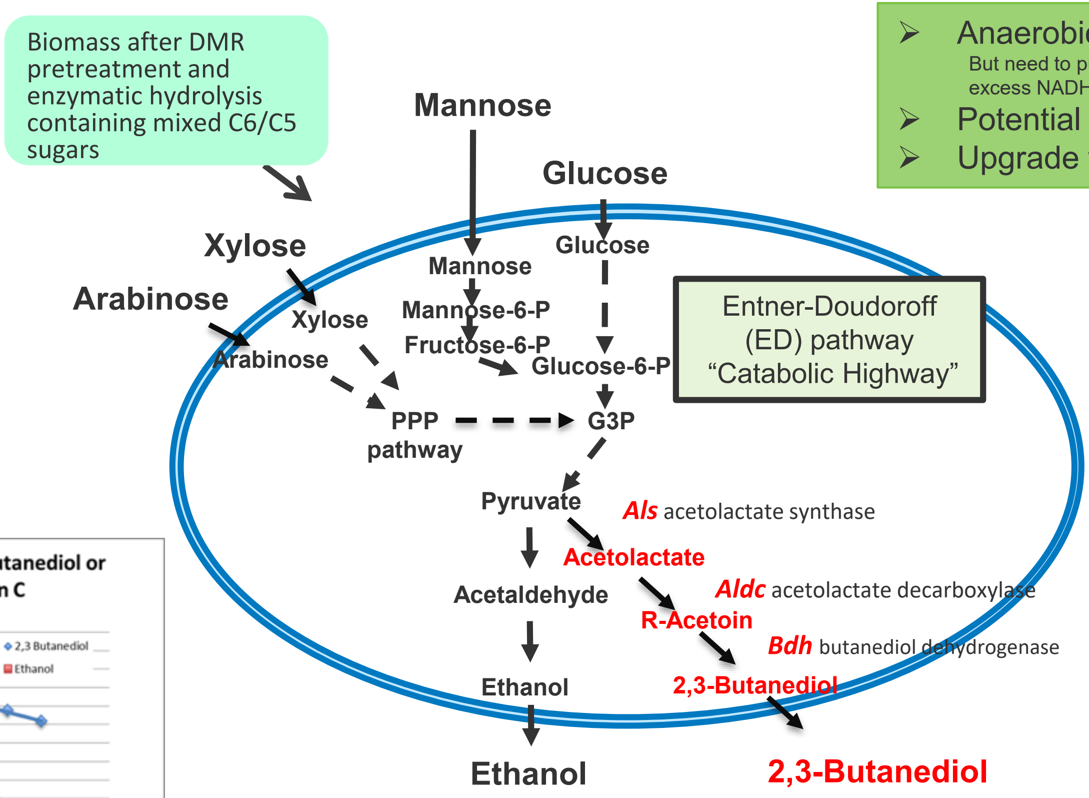
2,3-BDO: versatile chemical building block for producing solvents, chemicals, jet fuels and fuel additives

- Anaerobic process for ethanol
  But need to purge low level of air for oxidizing the excess NADH for BDO
- Potential high TYR
- Upgrade to fuels and chemicals

Addition of phosphomannose isomerase (pmi) conveys mannose utilization

BDO is less toxic





Weisser, Peter, Reinhard Kramer and Georg A. Sprenger. Expression of the Escherichia coli pmi gene, encoding phosphomannose-isomerase in *Zymomonas mobilis*, leads to utilization of mannose as a novel growth substrate, which can be used as a selective marker. Appl. Environ. Microb. 62, 4155-4161. 1996