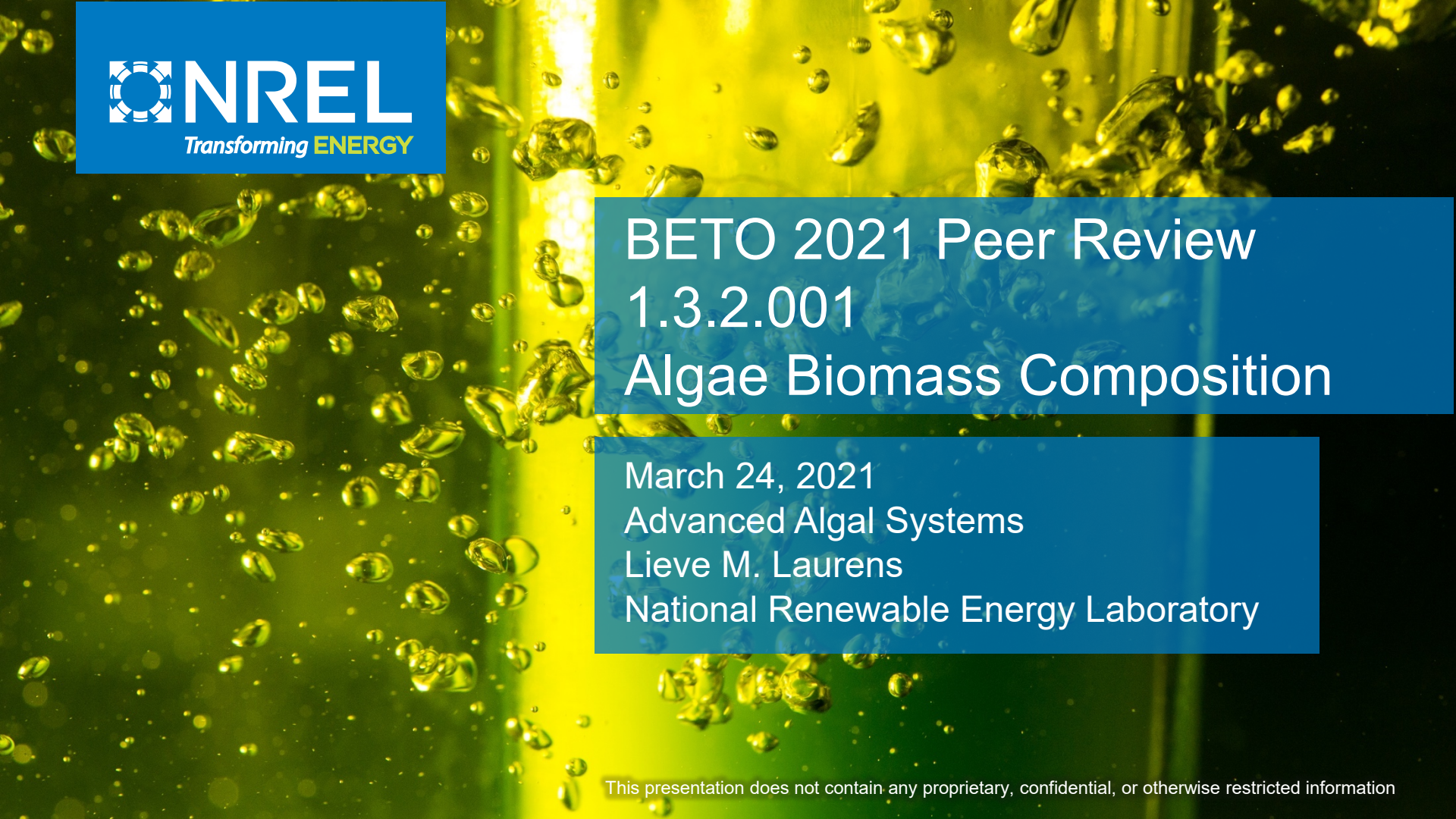


BETO 2021 Peer Review
1.3.2.001
Algae Biomass Composition

March 24, 2021
Advanced Algal Systems
Lieve M. Laurens
National Renewable Energy Laboratory

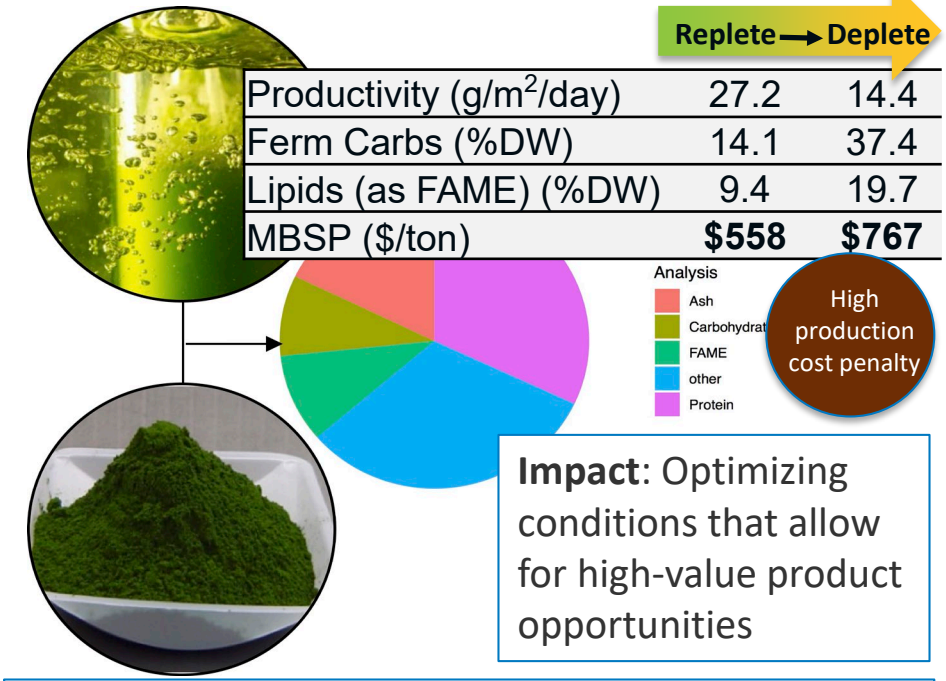
Overview

Identify critical factors for economic development of the algae bioeconomy

- Build quantitative framework of biomass composition, energy, productivity for important model species
- Integrate algae compositional dynamics to identify a co-product portfolio to increase the intrinsic value of biomass beyond the cost of production

Reduce uncertainty around process inputs and outputs

- Establish common language for characterization of a range of species and process configuration
- Solicit input on commercial need for standards and route to adoption and implementation



Impact: Optimizing conditions that allow for high-value product opportunities

Aim: Increase the value and opportunity for algal biomass
Today: No pathway to targeted and consistent biomass value and composition manipulation
Importance: Characterization critical to define bioproduct opportunities and quantify intrinsic value of biomass
Risk: Control over species and environmentally specific biochemistry leads to unpredictable value projections

Energy and Environmental Science (2017) 10, 1716-1738

Market Trends

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

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

Value Proposition

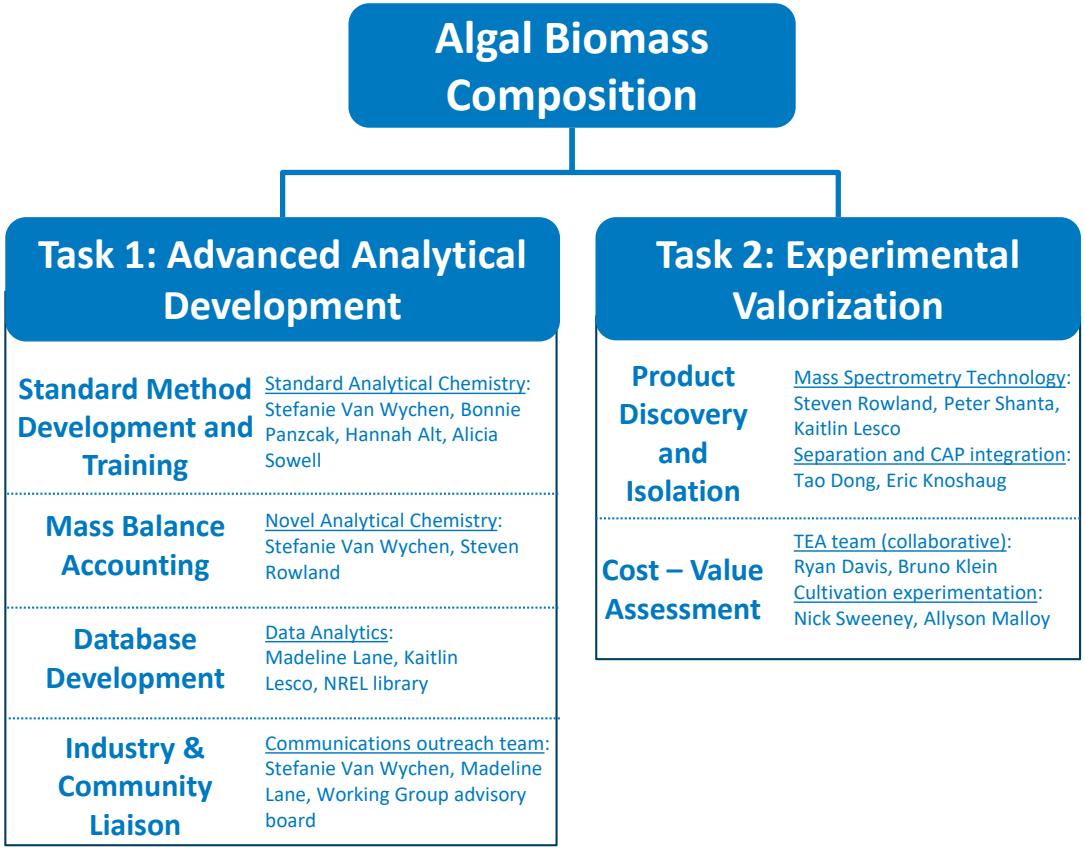
- Optimize value of algae in response to cultivation management and physiological stressors
- Leverage algae *Metabolic Plasticity* to create attractive feedstocks for bioproducts
- Disseminate algae performance data through open access tools and databases for industry use

Key Differentiators

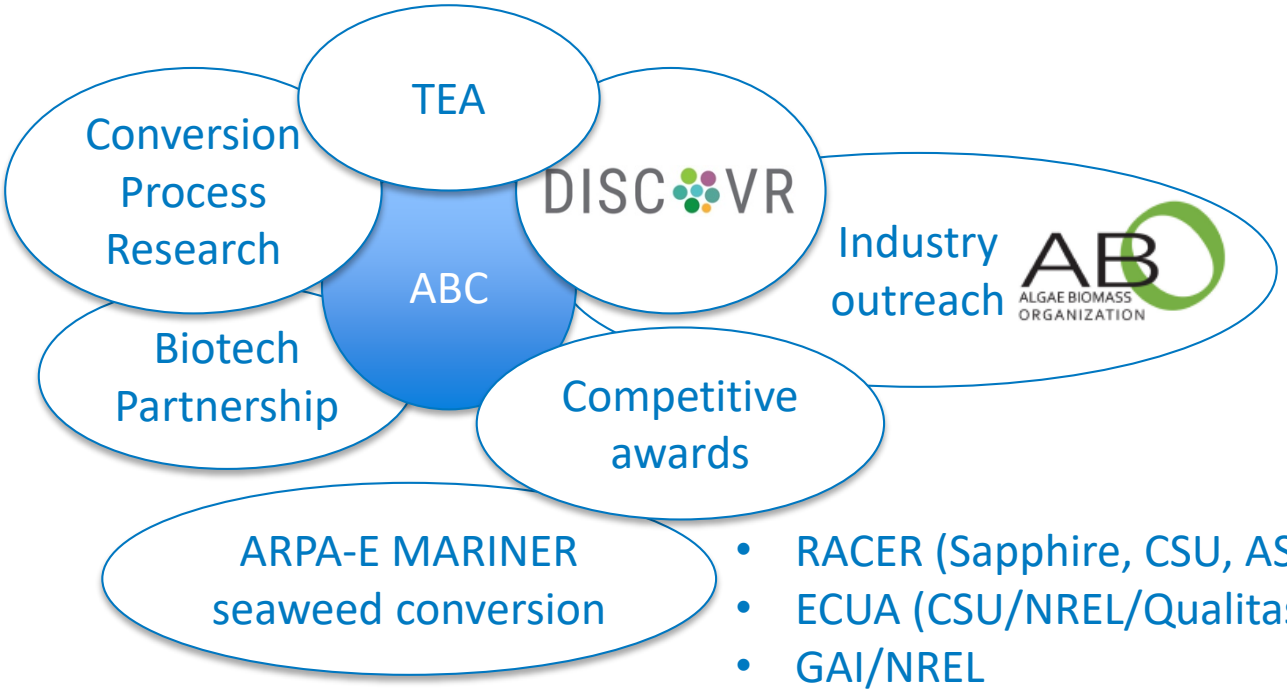
- Enable assessment of strain performance and biomass quality under environmental stressors
- Integrate with cultivation and conversion research to optimize productivity

1. Management Strategy

1. Project progress tracked through milestones surrounding **quantitative compositional analysis and biomass value metrics**, quarterly progress reports and peer-reviewed publications
2. Publish open access **online standard procedures and reports**, tracking access and implementation across AAS platform
3. **Integrate research** with conversion (CPR), cultivation and strain selection (DISCOVR) R&D projects
4. Close **collaboration with TEA** group on value, cost and productivity calculations
5. **Industry outreach** through technical standards working group



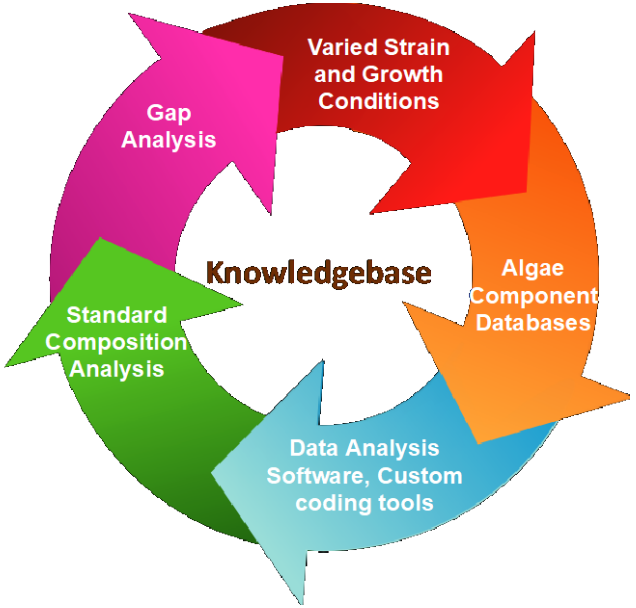
Management: Impact across Research and Industry Community



- RACER (Sapphire, CSU, ASU)
- ECUA (CSU/NREL/Qualitas)
- GAI/NREL
- Lumen Biosciences
- ExxonMobil CRADA

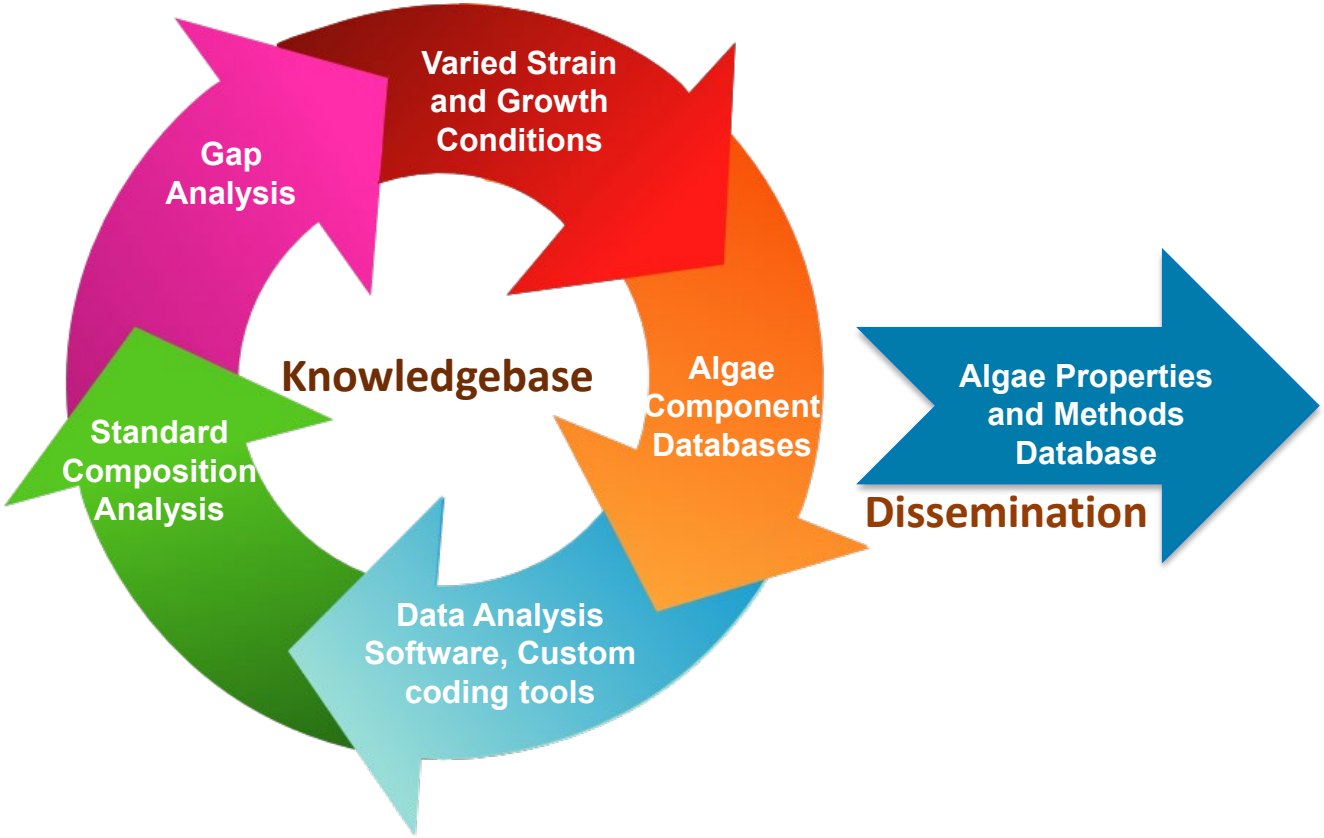
2. Technical Approach

- **Develop and apply advanced compositional analysis** approach coupled with innovative cultivation approaches, covering biochemical diversity of algae phylogeny
- **Implement intrinsic value calculations** for novel components
- **Develop accessible databases** of biomass components for major reference species/biomass of algae
- **Convene Technical Standards working group** with industry-led experts guiding future trade metrics



***Significance**: This is a cross cutting project at the interface of cultivation, conversion and TEA, collaborative across the program*

Approach: Algae-Specific Molecular Diversity Mapping for Products Discovery



Economic Impact

Molecular Identification for Product Development

Strain / Cultivation Improvement & Modeling

Data Mining / Advanced Analytics

Approach: Critical Success Factors and Risks

Critical Success Factor	Risk	Strategy
Experimental demonstration of **integration of co-products** identified with CAP conversion pathway resulting in cost reduction	Co-products are strain and process dependent and may not be applicable across algae value chain	**Species diversification** covers phylogeny and cultivation environment, collaboration with DISCOVR consortium
Demonstration of **tunable biomass composition** in outdoor-relevant conditions	Physiological and environmental dependence of observed compositional shifts	Collaborate closely with DISCOVR for **outdoor composition shift experiments** ponds to test hypothesis that cultivation method impact biomass composition
Analytical procedures **accepted by and implemented in community**, with full accounting of biomass mass balance	Methods are difficult to implement which may increase uncertainty and mass balance accounting with current method set still short of 100%	Develop strong **outreach strategy** for method implementation and verification with standard reference biomass

3. Impact

- Applied technology to DISCOVR consortium strain outdoor deployment to understand **year-round outdoor cultivation manipulation impact on composition**
- New methods developed and made available to algae industry
  - Implement methods with rigorous QC protocol across algae community
  - Outreach for reference material NIST, AAFCO
  - Lead Technical Standards Committee at ABO

ALGAE BIOMASS ORGANIZATION

https://www.nrel.gov/bioenergy/microalgae-analysis.html

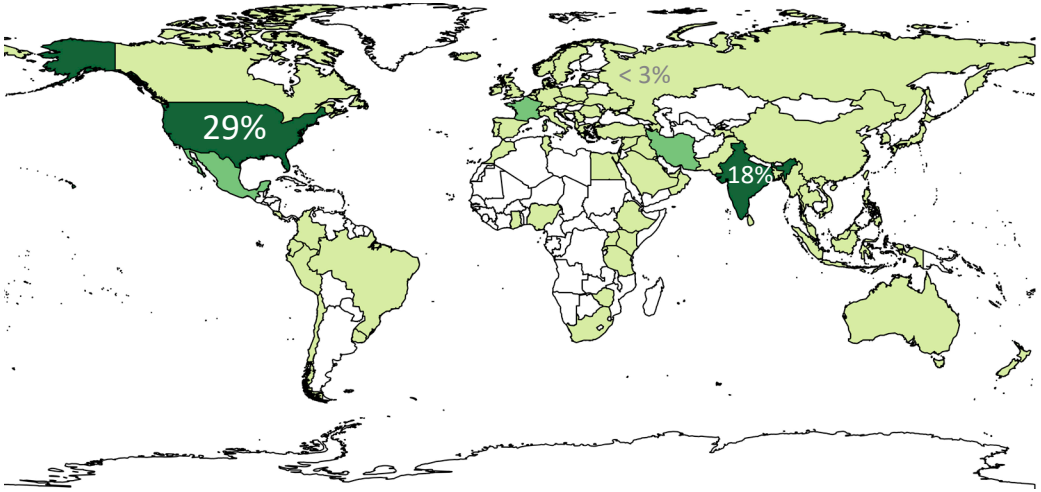
Impact: Analytical Web Page Shows Significant Impact Growth

Web Analytics, total visits (>15,000/yr)

Method downloads (>2,000/yr)

Total Downloads by method (since Sept 2018):

	Total	FY19	FY20	FY21
Summative Mass Analysis of Algal Biomass.	575	236	271	68
Determination of Total Solids and Ash in Algal Biomass	299	131	131	37
Determination of Total Carbohydrates in Algal Biomass	497	224	201	72
Determination of Total Lipids as Fatty Acid Methyl Esters	503	209	227	67
Determination of Total Sterols in Microalgae	213	102	80	31
Determination of Total, Organic, and Inorganic Carbon	16	-	-	16



February 2019:

> 14,000 method access
> 5,000 downloads
(since 2015)

> 900 downloads yr^{-1}
~ 4,000 web visits yr^{-1}

February 2021:

> 45,000 method access
> 9,500 downloads
(since 2015)

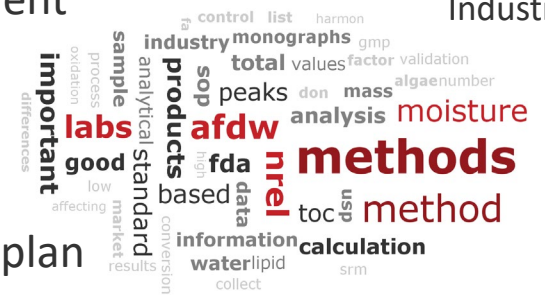
> 2,000 downloads yr^{-1}
> 15,000 web visits yr^{-1}

Impact: Technical Standards Focus Group Identifies Priorities

Convened group of 15 key representatives:

- Support for concerted effort in reference material deployment across industry
- Successful Gap and Priority Analysis
- Extended participation and plan towards guidance document (monograph)

Gap and Priority Analysis
Biomass composition, elemental composition, energy content
Identification methodology, contamination tracking and genetic analysis
Safety, toxins (e.g. mycotoxins), heavy metals
Labeling/reporting requirements



Industry + Contract Laboratories

- Qualitas (Jakob Nalley)
- Corbion (John Carney)
- MicrobioEngineering
- Algae4all (Amha Belay)
- Harmon Consulting (Valerie Harmon)
- Eurofins (Lars Reimann)
- Exact Scientific (Kent Oostra)
- ARPA-e (MARINER) (Marc von Keitz, David Lee)
- BETO (Devinn Lambert, Liz Burrows)

ThinkTank
the platform for collective intelligence

https://www.sciencedirect.com/science/article/pii/S2211926420310092

4. Progress and Outcomes

Growth

Growth conditions provide unique and different lipid composition

Extraction

Extraction of products ensures accurate representation of products

Analysis

Ultra-high resolution mass spectrometry provides unique chemical and molecular diversity

Identification

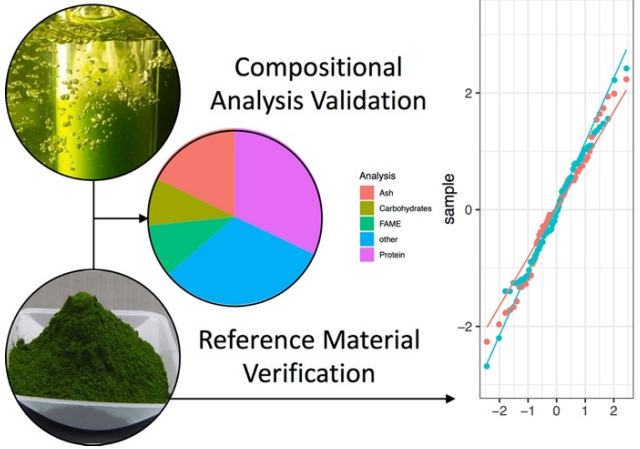
Spectral matching and via software and databases provides compound identification

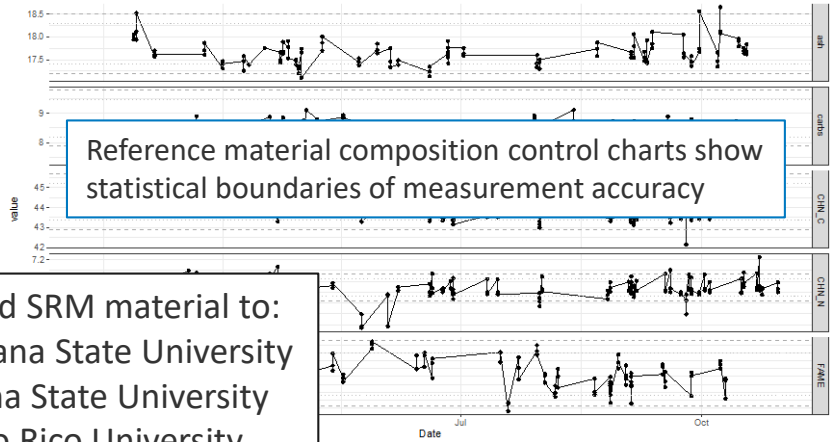
Innovation

Identification of algae-derived products provides insight for future innovation

Task 1: Algae Characterization Streamlining

- First of its kind reference material established for algae, *Nannochloropsis* and *Scenedesmus* biomass (ASU/AzCATI-supplied)
- Rigorous statistical characterization of methodology for characterization of reference material
- Novel reporting on boundary conditions and data reporting acceptance criteria
- Interlaboratory composition data for comparison against commercially-available and accepted methods
- **Discussions with NIST for incorporation microalgae in standard reference material (SRM) portfolio**





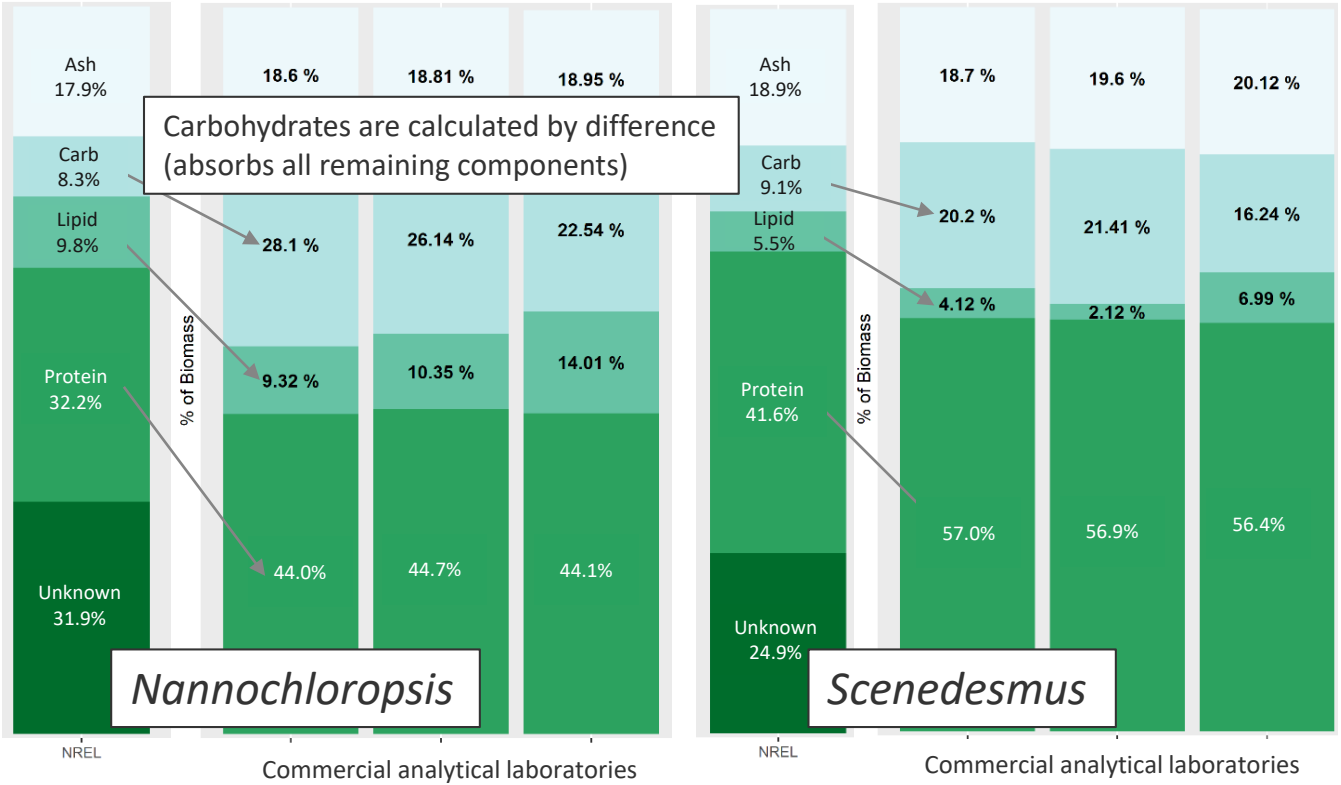
Reference material composition control charts show statistical boundaries of measurement accuracy

Distributed SRM material to:

- Montana State University
- Arizona State University
- Puerto Rico University
- Duke University
- CalPoly
- Los Alamos National Lab

Politis, A., Van Wychen, S., et al., 2021, *submitted*

Reported analytical data can be misleading



Methodology available at Contract Analytical Laboratories directly impacts reported compositional data

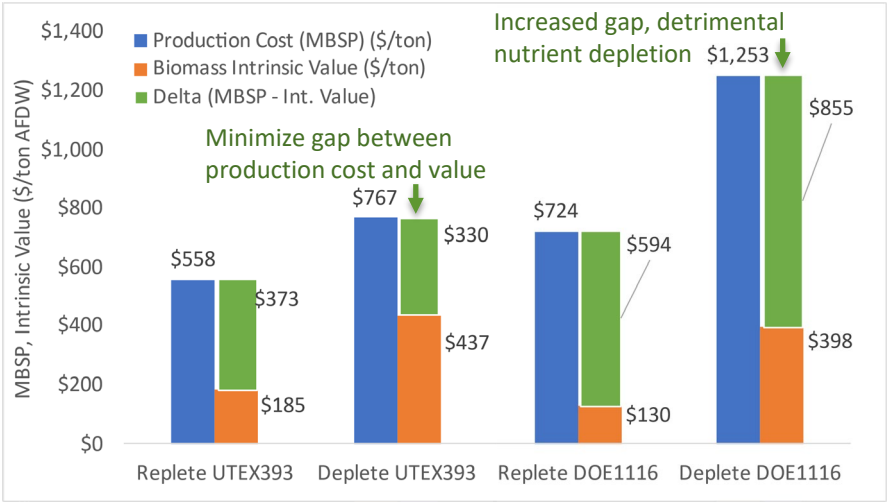
Ash
Cabohydrate
Lipid
Protein
Unidentified

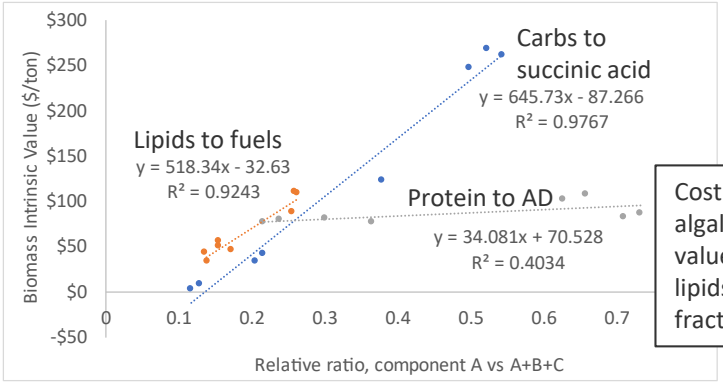
A suggested suite of standard methods available commercially is proposed to support and harmonize the algae producers' data reporting

Lane, M. et al., 2021, Algal Research, 52, 102134 (https://www.sciencedirect.com/science/article/pii/S221192642031002X)

Task 2: Intrinsic Value Opens up Bioproduct Opportunities

- Developed novel concept of “value” of algal biomass based on its composition and valorization in a conversion process
- Applied to DISCOVR strains run under nutrient replete and deplete harvesting
- Correlations indicate <u>strong bias to carbohydrate content</u> (reflected by high value product succinic acid from sugars) – may be hard to achieve/engineer
- Transition to **high protein biomass** (CPR conversion) **emphasizes need for product identification in relevant biomass** (beyond AD)



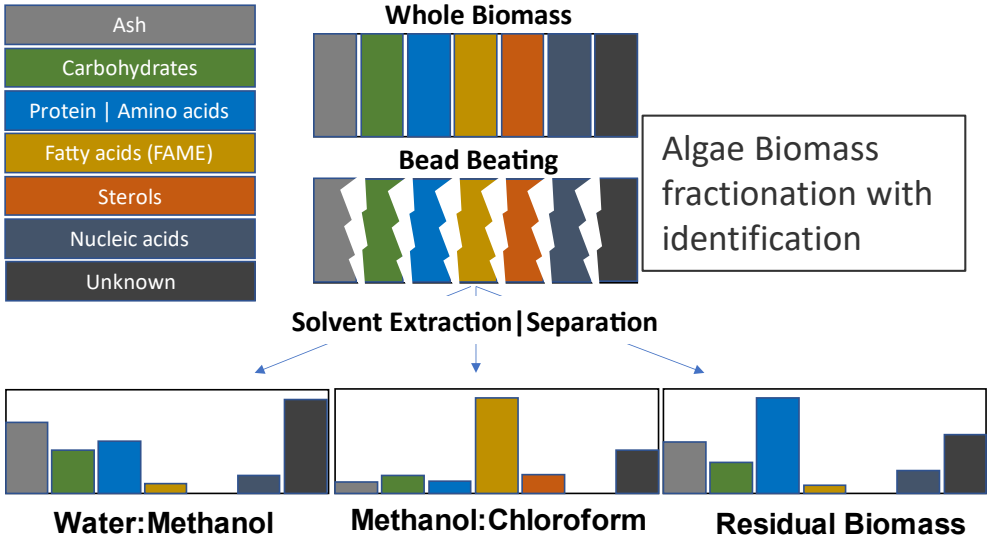


Cost correlations for algal biomass intrinsic value from carbs, lipids, protein fractions of biomass

Collaboration with Ryan Davis, Bruno Klein, Algae TEA, manuscript in preparation

Identifying unknowns adds to mass balance adjustments

- Algal biomass mass balance accounting of 70-80% leaving 20-30% unaccounted for potentially high-value products
- Identification of unknowns allows for method development and supports process economics
- Using solubility fractionation approach, greatest unknown fraction in insoluble residue after extraction



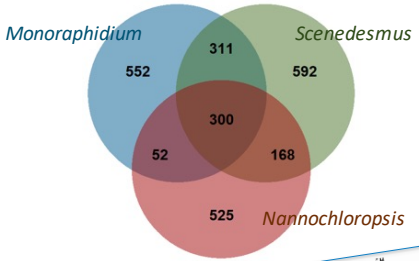
- 3% chlorophyll
- 4% nucleic acids
- 0.5% sterols
- 2-5% additional carbohydrates (insoluble fraction)
- Metabolites, precursors and other pigments – specifically for algae in exponential growth phase

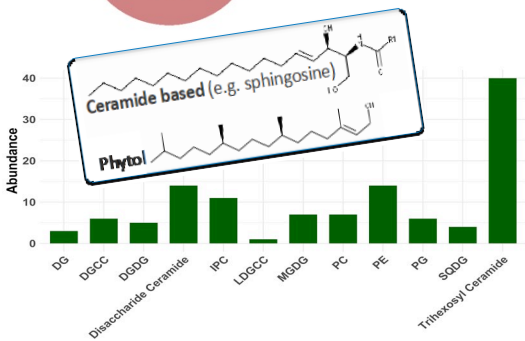
Added 10-12% to > 80% explained

Van Wychen, S., et al., "Advanced Mass Balance Characterization and Fractionation of Algal Biomass Composition", submitted to J. Appl. Phyc.

Lipidomics allows for discovery of novel biobased surfactants

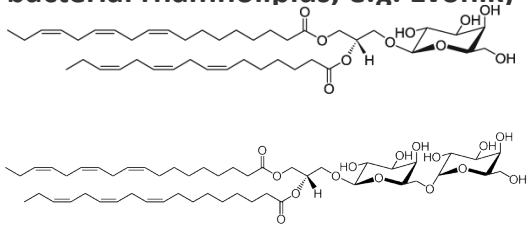
- Built novel, curated and validated algae-lipidomics database (>11,000 entries)
- Confirmed **unique lipidomics profile** by mass spectrometry for primary species deployed across algae program
- Identified **120 unique and naturally occurring algae lipids with bio-surfactant** potential

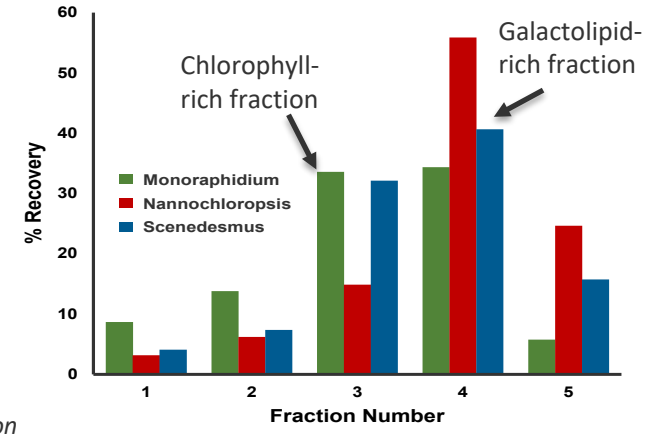




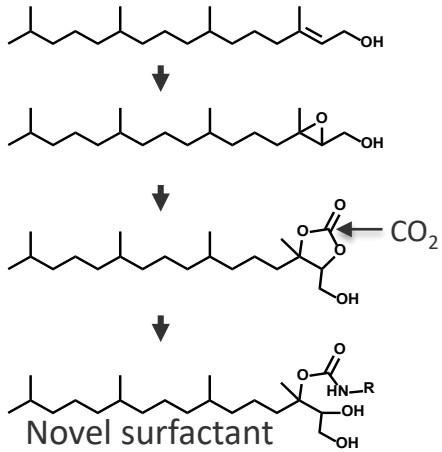
Lesco, K., Lane, M., Rowland, S. et al., 2021, *in preparation*

Isolation of galactolipids yields useful biosurfactants (akin to bacterial rhamnolipids, e.g. Evonik)





Novel synthesis technology applied to phytol creating unique class of nonionic surfactants



For phytol content of 1.6 wt%, phytol-based surfactant can reduce MFSP by $0.91/GGE (10%)

Tao Dong, collaboration with WBS 1.3.4.201, CPR

Summary

Optimize value of algae relying on metabolic plasticity in response to cultivation management and physiological stressors

Management

- Progress tracked by quantitative metrics around cost-value framework
- Publish and disseminate open access tools and data

Approach

- Apply and develop advanced analytical methodology
- Quantify improvements in algae value based on novel TEA valorization approach
- Implemented fractionation approach for advanced algae mass balance
- Curated literature-derived algae lipids database and applied to experimental bioproduct discovery pipeline

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

Impact

- Optimize value of algae in response to cultivation management and physiological stressors
- Disseminate of algae performance data through open access tools and databases for industry use

Progress & Outcomes

- Disseminated NREL-developed standard analytical methodology that is utilized throughout algae industry
- Developed novel concept for algae valorization based on co-product portfolio
- Implemented fractionation approach for advanced algae mass balance adjustments
- Curated applied lipidomics database and applied to experimental bioproduct discovery pipeline

Quad Chart Overview

Timeline

- Start: FY2013
- Merit review cycle: FY2020-2022

	FY20	Active Project
DOE Funding	$700K	$2.1M

Project Collaborators:

- DISCOVR consortium
- TEA team (Davis et al.)
- CAPSLOC (Kruger, Knoshaug, Dong, et al.)
- Sandia (Davis et al.)

Barriers addressed

Aft-E - Algal Biomass Characterization, Quality, and Monitoring, Aft-G - Algal Feedstock Material Properties

Project Goal

Build quantitative assessment framework of biomass composition, energy, productivity for important model species, integrating biomass production with in depth composition and co-product yields

End of Project Milestone

Co-product portfolio that, when integrated in a production and conversion pathway, can increase the intrinsic value of algal biomass by at least 30% of biomass cost value for a set of model species

Funding Mechanism

AOP

Core *#NRELAlgae* Team:

Stefanie Van Wychen
Steven Rowland
Peter Shanta
Bonnie Panczak
Hannah Alt
Madeline Lane
Alicia Sowell
Kaitlin Lesco
Tao Dong
Nicholas Sweeney
Mauro Lua
Allyson Malloy

DOE BETO project managers:

Devinn Lambert
Jamie Meadows
Colleen Tomaino

U.S. DEPARTMENT OF ENERGY

BioEnergy Technologies Office

Thank You

www.nrel.gov
www.nrel.gov/bioenergy/microalgae-analysis.html

Lieve.Laurens@nrel.gov

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NREL Transforming ENERGY

Additional Slides

Responses to FY19 Reviewers' Comments

"This project continues to have a clear benefit to the algal industry as a whole as well as an important link to BETO priorities. Dr. Laurens continues to be a leader in biomass compositional analysis, and the team regularly interacts with industry and the modeling community to make sure critical compositional assumptions are clear"

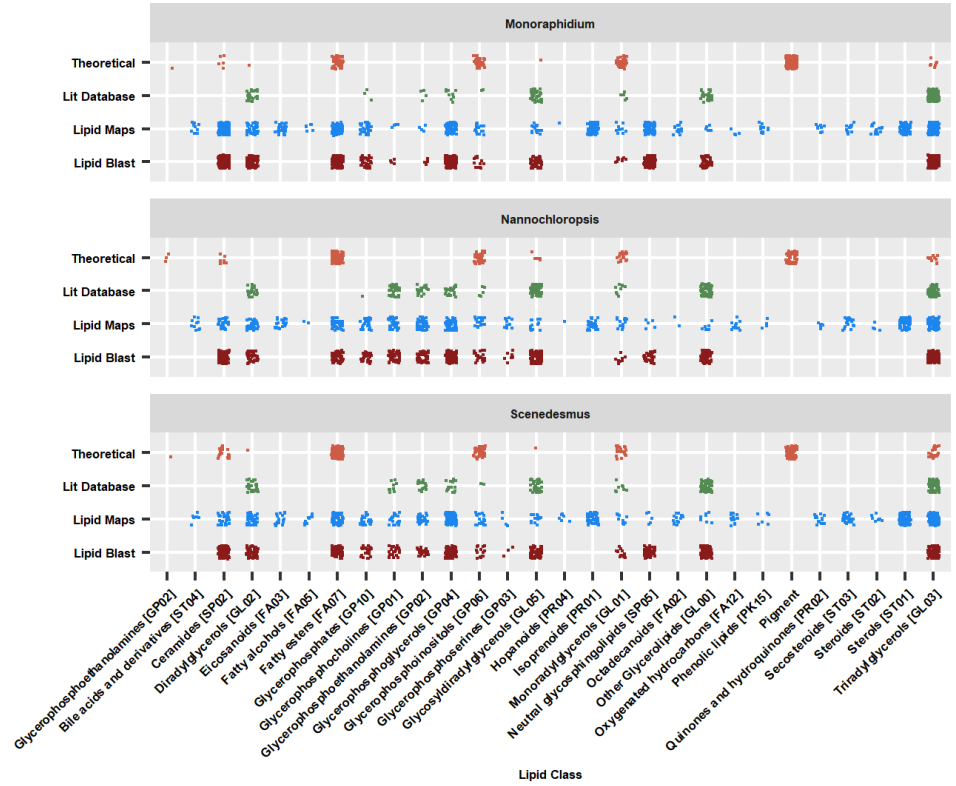
1. Some of the data can be considered strain dependent so interlocking data with different strains could be challenging.
2. Data was generated in a photobioreactor system and may not be outdoor-relevant, e.g. high light adding to the possibility that the light was the primary driver of culture stress
3. The team is encouraged to continue focus on appropriate identification and characterization of metabolites throughout the cultivation and production cycles, and to allow the data to drive potential valorization models that may be developed by other projects and collaborators.
4. Exploring cultivation experiments outdoors as soon as possible to understand how larger pond growth correlates with the biomass composition data already gathered

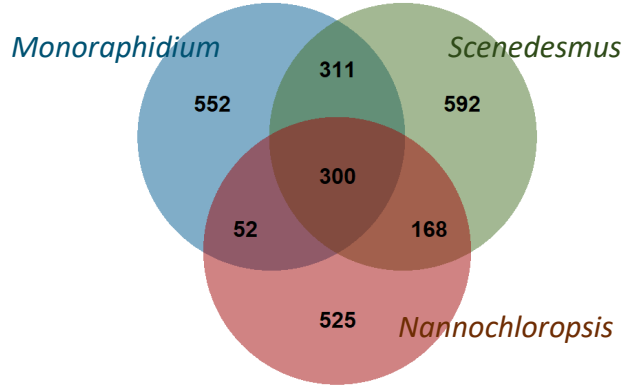
1. This project's goal is to establish the tools and techniques required to test whether there are cultivation conditions, light, temperature, cultivation operations that impact composition. If we can unlock this potential in indoor photobioreactors, we can implement relevant outdoor cultivation trials.
2. The growth experiments were carried out in photobioreactors, and thus high incident light may have been a primary driver, even though the conditions used did follow a mimicked diel light and temperature pattern. We are working on the DISCOVR project to study the cultivation-biomass quality connection and bias, and this aspect has been de-emphasized in the experimental approach for this project
3. We developed the experimental set up that allows us to test hypotheses in indoor photobioreactors and we are making progress in using these experiments to provide biomass for biochemical mapping of metabolites and thus validating different valorization models.
4. Aspects of the compositional dynamics of the outdoor cultivation will be covered in our expanded work within the DISCOVR consortium, while our own experiments in small ponds will allow for at least two species to be tested in terms of providing a baseline for the biochemical dynamics that are potentially achievable

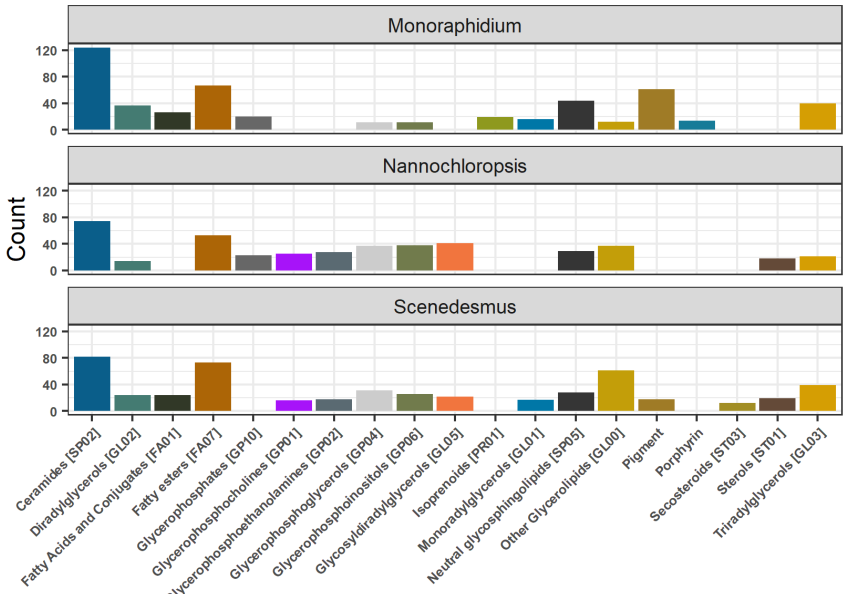
Publications, Patents, Presentations, Awards, and Commercialization

1. Lane, M., Van Wychen, S., Laurens, L. ML.* (2021) A Data-driven Comparison of Commercially Available Testing Methods for Algae Characterization, Algal Research, 53, 102134
2. Laurens, L. ML.* (2021) A New Algae Technical Standards Focus Group: Soliciting Input in Summarizing and Guiding the Algae State of the Art, Algal Research, 53, 102141
3. Savage, E., Nagle, N., Laurens, L. ML., Knoshaug, E. P. (2020) “Nitrogen derived from Combined Algal Processing supports algae cultivation for biofuels”, Algal Research, vol 50, 101987 https://doi.org/10.1016/j.algal.2020.101987
4. Cuchiaro, H., Laurens, L. ML.* (2019) “New Method for Protein Analysis in Algae: Optimization of Amino Acid Derivatization after Hydrolysis with O-Phthalaldehyde 3-Mercaptopropionic Acid (OPA-3MPA)” Journal of Agricultural and Food Chemistry, 67 (19), 5672-5679
5. Li, Y., Leow, S., Dong, T, Nagle, N., Knoshaug, E. P., Laurens, L. ML., Pienkos, P. T., Guest, J., Strathmann, T., (2019) "Demonstration and Evaluation of Hybrid Microalgae Aqueous Conversion Systems for Biofuel Production”, ACS Sustainable Chemistry & Engineering, 7, 6, 5835-5844
6. Panczak, B., Alt, H., Van Wychen, S., Sowell, A., Lesco, K., Laurens, L. ML. (2020), Determination of Total, Organic, and Inorganic Carbon in Biological Cultures and Liquid Fraction Process Samples, NREL/TP-2700-78622, https://www.nrel.gov/docs/fy21osti/78622.pdf
7. Laurens, L. ML. (2019) “Future Direction of Algae-based Fuels and Products” in “Future Directions on Bioenergy Technologies” eds. Thomas Foust and David Dayton, Publisher: Elsevier
8. Algal Biomass Production via Open Pond Algae Farm Cultivation: 2019 State of Technology and Future Research, Ryan Davis, Lieve Laurens: https://www.nrel.gov/docs/fy20osti/76569.pdf

Application to FTMS data Shows Species-specific Lipidome Profile

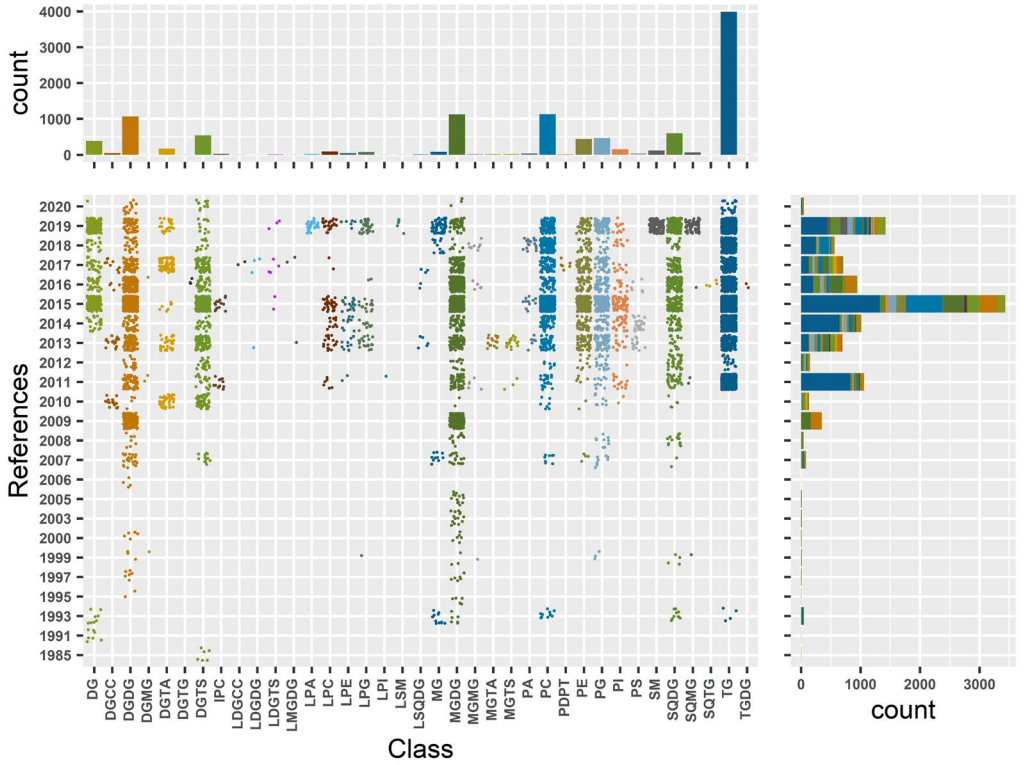






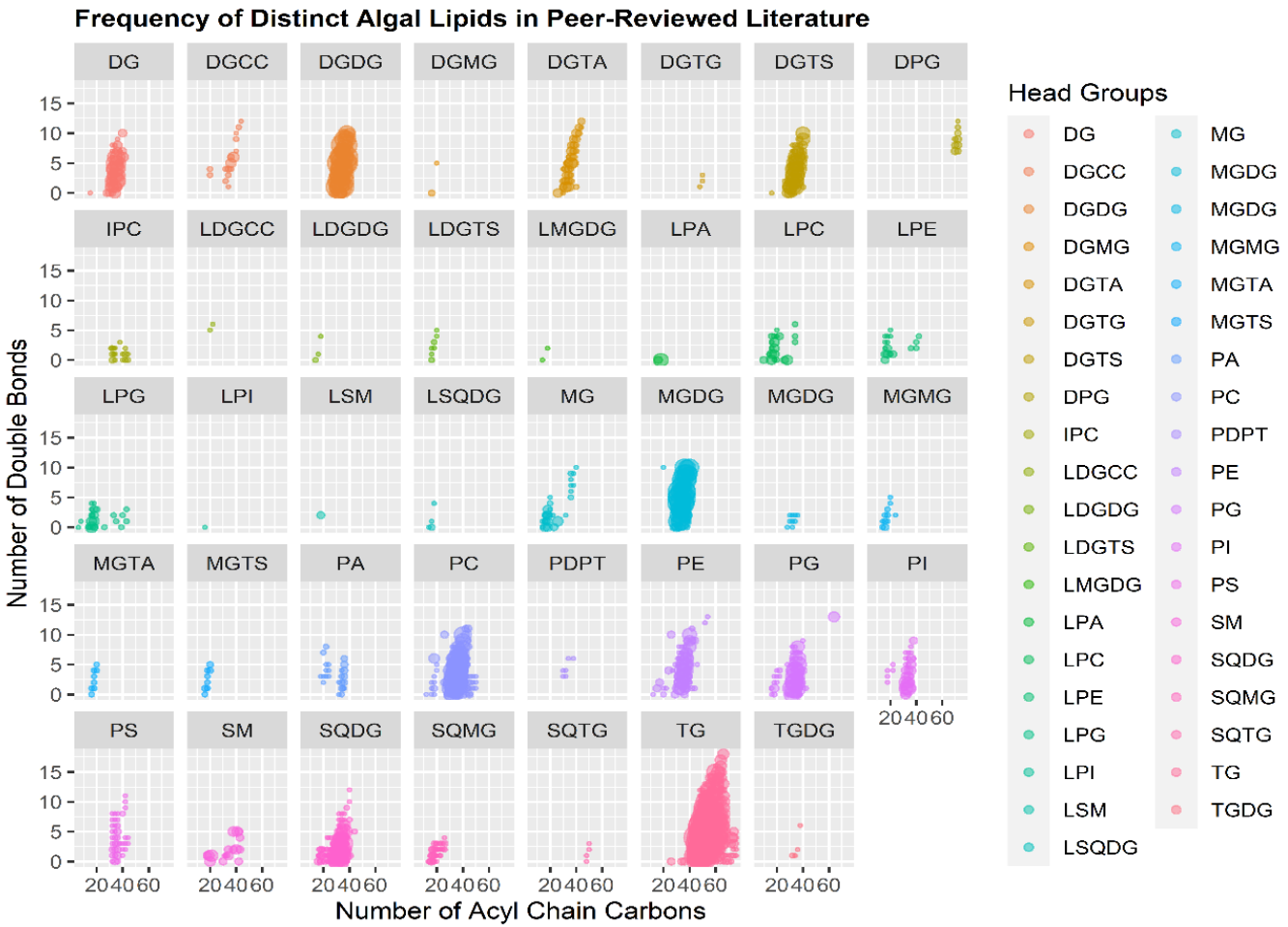
Applied Lipidomics Database

- Algae-specific lipid database established from deep literature survey (1985-2020); manuscript draft ready for submission
- Curation based on in house collected high-resolution MS data with bioinformatics pipeline for automated data assignments developed
- Utilization for high-value potential product discovery and mining (120 possible targets identified)

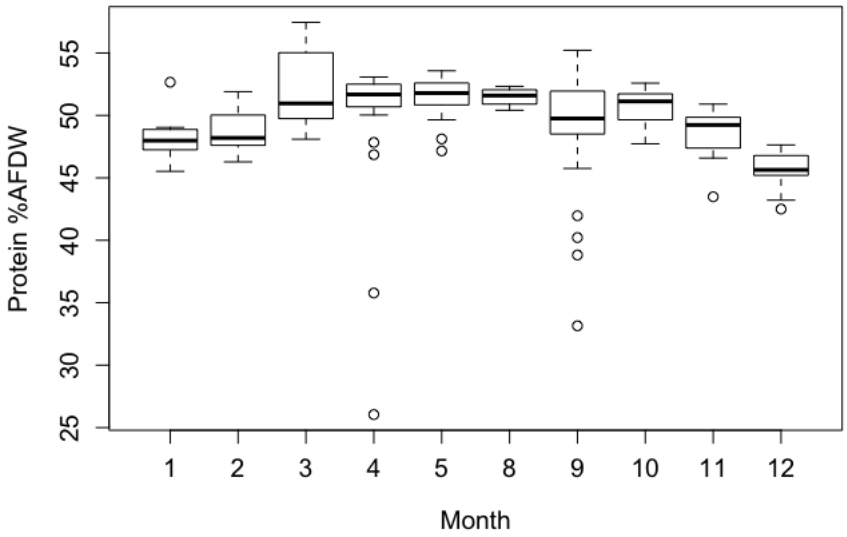


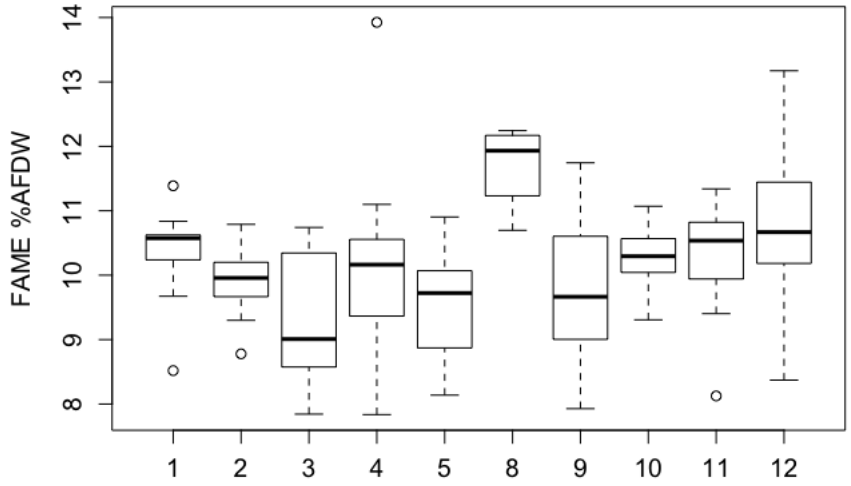
Entries	**11670**
Species	**128**
Distinct Lipids	**1108**
Head Groups	**38**
Papers Cited	**108**

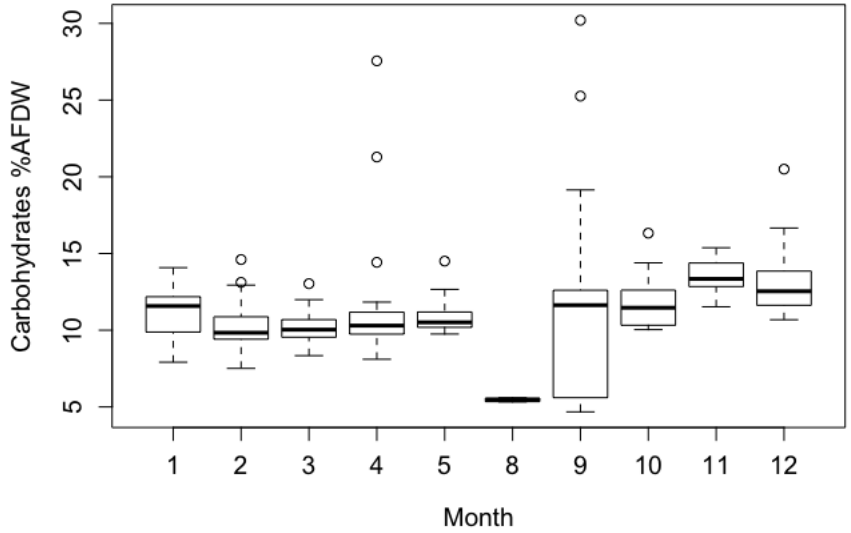
Molecular Complexity of Lipid Database



DISCOVR SOT Biomass Composition (FY20)



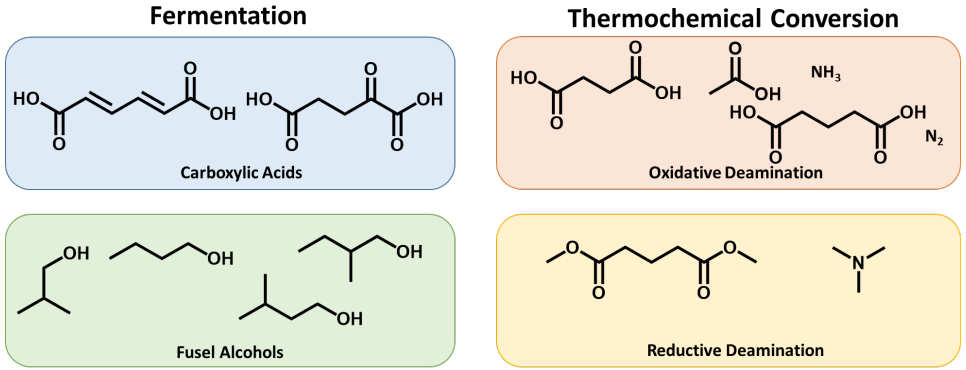


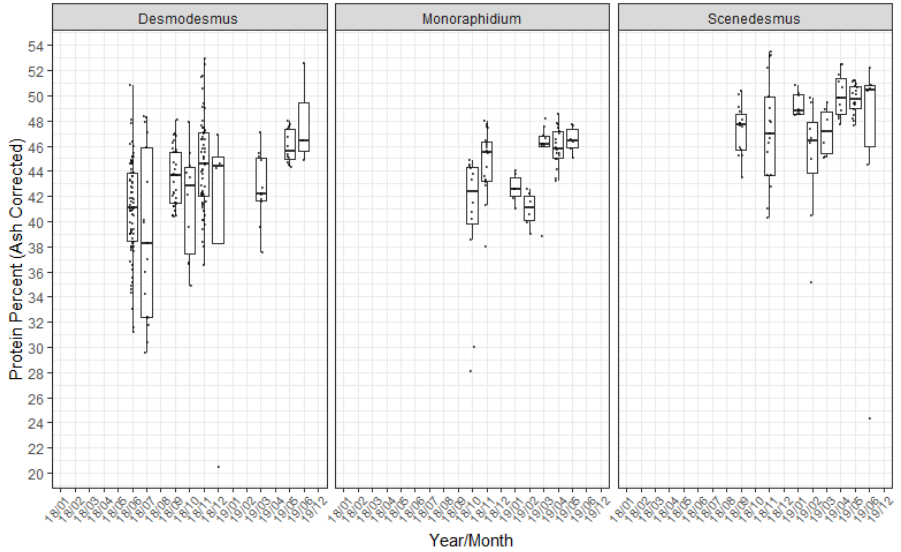


All biomass high protein, consistent with CPR sourced biomass for conversion research

Protein Utilization

Joint with SNL: Evaluate at least 3 process options for accommodating high-protein algal biomass including CAP process with MOT, muconate to fuels and chemicals and SNL process.





- Literature review: high protein algae composition, pretreatment fractionation options, 11 possible pathways and products
- Detailed analysis on animal feed and biopolymers by SNL
- Submitted to *Biotechnology Advances*