DOE Bioenergy Technologies Office (BETO) 2019 Project Peer Review

1.3.2.001 Algal Biomass Composition

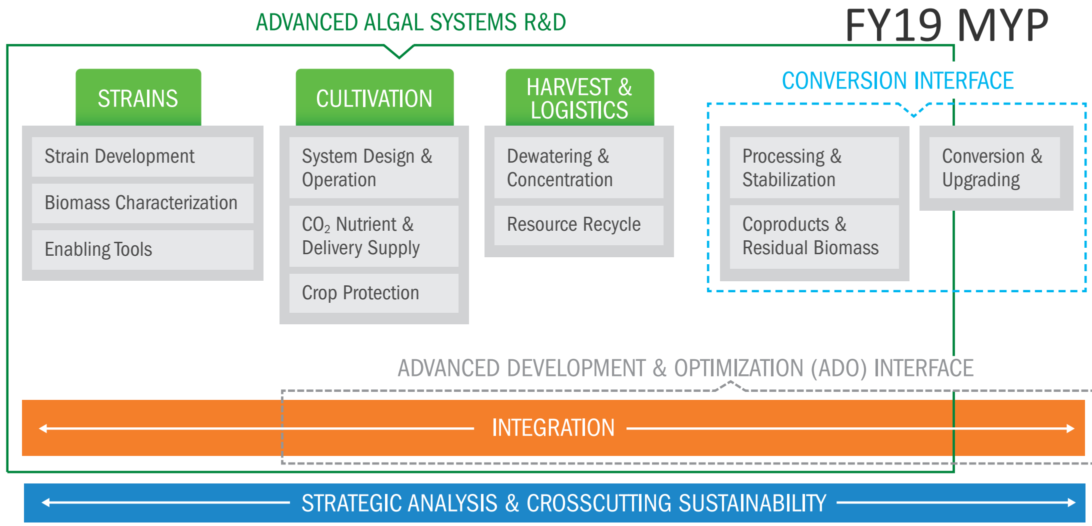
Advanced Algal Systems

March 4, 2019

Lieve M. Laurens

National Renewable Energy Laboratory

Goal Statement



1. Reduce cost of algal biofuels by increasing intrinsic algal biomass value

- Build quantitative assessment framework of biomass composition, energy, productivity for important model species
- Integrate scenarios of biomass production with in depth composition and co-product yields to identify a co-product portfolio in model species increasing the intrinsic value of biomass beyond the cost of production

2. Reduce uncertainty around process inputs and outputs

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

Outcome

- **Bioproduct portfolio** and respective pathways supporting process integration and increasing biomass intrinsic value
- **Suite of methods** allowing for unambiguous characterization of biomass implemented across research and commercial algae groups

Quad Chart Overview

Timeline

- Start: FY2013
- Merit review cycle: FY2017-2019
- 75% complete

	Total Costs Pre FY17**	FY 17 Costs	FY 18 Costs	Total Planned Funding (FY 19-Project End Date)
DOE Funded		$750K	$750K	$750K

End of Project Goal

Build a 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.

MYPP Barriers addressed

- AFt-B: **Sustainable Algae Production**, value of feedstock, seasonality, environmental variability
- AFt-E. **Algal Biomass Characterization**, Quality, and Monitoring: Chemical, biological and post-harvest physiological variation in harvested algae. *Need for standardized procedures to uniformly quantify major components*
- AFt-G **Algal Feedstock Material Properties**, study biomass *properties in relation to conversion process performance*

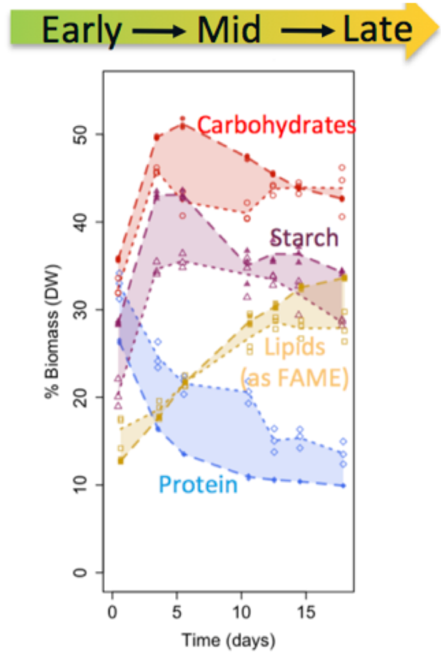
Objective

Build quantitative assessment framework of biomass composition, energy, productivity for important model species, integrating biomass production with in depth composition and co-product yields to ultimately identify a co-product portfolio in model algae species, that, when modeled as an integrated conversion framework with cultivation, is shown to increase the intrinsic value of biomass beyond the cost of production

Project Overview

Increase intrinsic value of algal biomass through manipulation of tunable algal biomass composition

- Identify and isolate high-value co-products in algal biomass grown under controlled conditions integrated with quantification of carbon and nitrogen balance
- Study species representing primary producers; *Chlorella*, *Scenedesmus* and *Nannochloropsis*, with transfer of experimental metabolic profiling approach to novel productive and commercially relevant species (FY17-19)
- Develop purification strategies for co-products in CAP process streams, to maximize derived value
- Establish quantitative cost-value framework between productivity, composition and energetic content (joint with TEA)

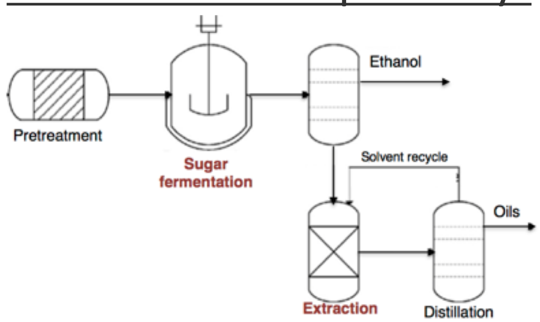


[Anal. Biochem. 2014(452)86-95]

Reduce uncertainty around major reported process inputs and outputs

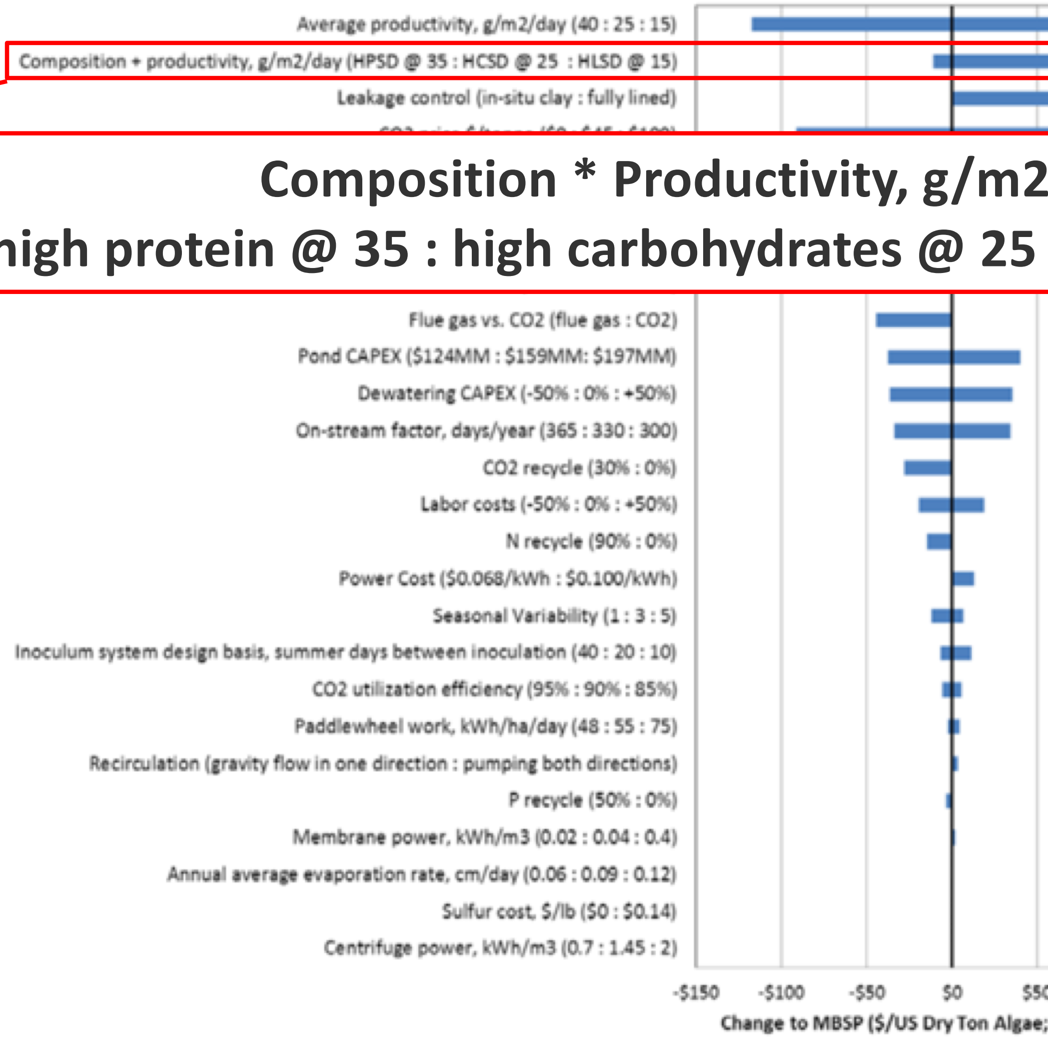
- Develop and maintain laboratory procedures for biomass analysis
- Train and support groups on the implementation of procedures
- Demonstrate and validate rapid high-throughput compositional analysis technologies

CAP conversion pathway:



TEA Tornado plot – Technical Hurdles with Large Pay-off

Composition * Productivity, g/m2/day
(high protein @ 35 : high carbohydrates @ 25 : high lipid @ 15)



MBSP = minimum biomass selling price:

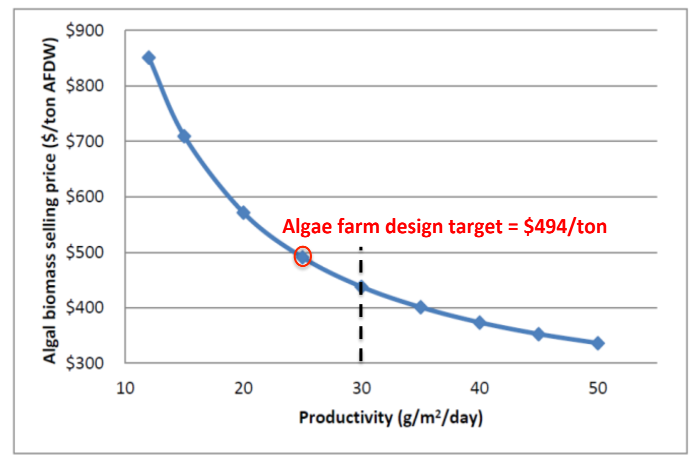


Figure 14. Tornado plot presenting results of the single-point sensitivity analysis on MBSP cost ($491/ton reference case)

https://www.nrel.gov/docs/fy14osti/62368.pdf
https://www.nrel.gov/docs/fy16osti/64772.pdf

Davis et al., 2014|2016 **Design Reports**

Algal Biomass Composition Drives Down MFSP

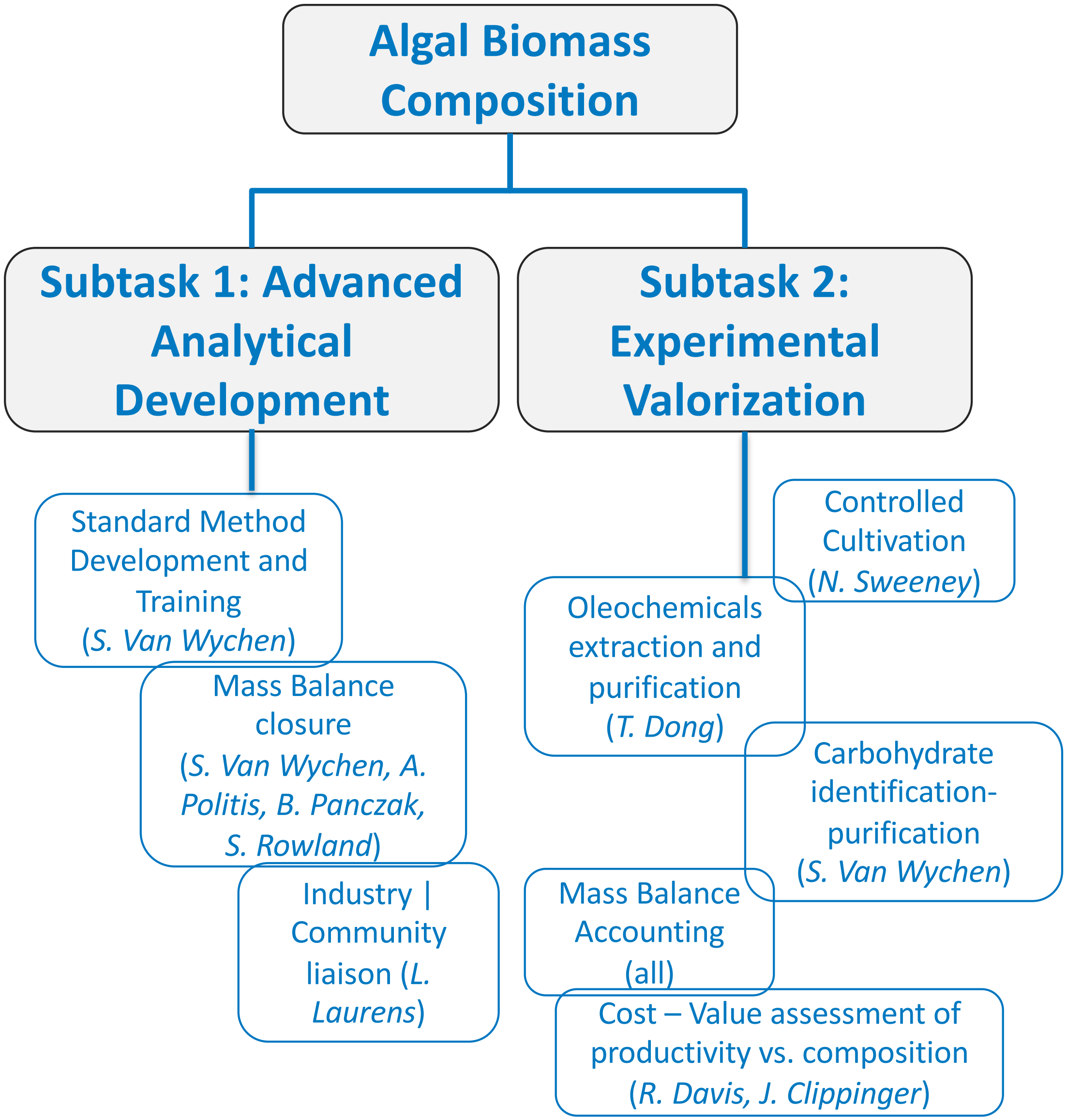
	Early →	Mid →	Late
Ferm Carbs	20.9	46.3	37.9
Lipids (as FAME)	6.6	26.5	40.9

Metric (%DW)	*Scenedesmus*			*Chlorella*			*Nannochloropsis*		
	Early	Mid	Late	Early	Mid	Late	Early	Mid	Late
Ash	5.6	2.3	2.1	4.7	2.1	2.6	14.2	13.6	5.1
Ferm Carbs	20.9	46.3	37.9	5.8	36.7	23.6	4.6	8	7.6
Mannitol	0	0	0	0	0	0	4	2.1	2.2
Other carbohydrates	3.4	1.6	1.3	5.9	5	3.5	2.9	1.5	2.1
Glycerol	0.7	2.9	4.5	1.4	2.5	4.5	1.4	2.8	6.4
Protein	34.5	12.8	8.9	40.2	13.2	12.7	32.7	23.1	9.4
Lipids (as FAME)	6.6	26.5	40.9	13	22.1	40.5	12.3	25.6	57.3
Sterols	0.9	0.7	0.4	0.2	0.4	0.3	0.4	0.6	0.2
Chlorophyll	3.0	1.2	1.2	5.8	2.4	2.1	3.0	1.8	0.3
non-FAME lipids	4.1	2.8	1.3	3.8	1.7	1.5	3.8	3.3	1.2
Nucleic acids	4.06	1.47	0.99	4.61	1.05	0.94			
Mass closure	**83.8**	**98.6**	**99.5**	**85.4**	**87.2**	**92.3**	**83.8**	**83.9**	**92.3**
Energy content, HHV (BTU/lb)	9,165	10,070	11,122	9,219	9,372	10,822	9,192	10,104	13,160
MFSP ($/GGE)	**$13.10**	**$5.80**	**$5.10**	**$14.50**	**$6.70**	**$5.30**	**$12.10**	**$7.10**	**$4.60**

MFSP ($/GGE)	**$13.10**	**$5.80**	**$5.10**

Approach - Management

1. Project progress tracked through **milestones and quantitative compositional analysis metrics**, quarterly progress reports and peer-reviewed publications
2. Yearly **update to online standard procedures**, tracking access and implementation across Advanced Algal Systems platform
3. **Research integrated** with TEA, conversion (CPR), cultivation and strain selection (DISCOVR) R&D projects
4. Close **collaboration with TEA** group on value, cost and productivity calculations



Approach - Technical

- Compositional analysis integrated with innovative cultivation approaches with impact calculations
- Develop cost-kinetics model linking biomass composition with productivity for important species, in process compatible with CAP pathway
- Build an experimentally and economically feasible and CAP-compatible bioproduct portfolio

Significance: *This is a cross cutting project at the interface of production, conversion and TEA, and collaborative across the program, with integration of compositional procedures at least under 5 different projects, with key partner at ASU BETO testbeds and DISCOVR consortium*

Approach - Technical

Critical Success Factor	Challenges	Strategy
Experimental demonstration of integration of co-products identified with CAP conversion pathway resulting in reduction of MFSP	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	Implement an outdoor cultivation scenario in small indoor 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 and in depth analytical approach for novel products and components to close mass balance gap

Accomplishments | Results

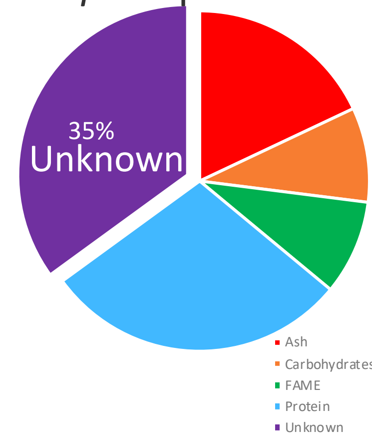
FY17-FY19 Accomplishments Overview

- Merit Review Cycle: Identifying critical factors for economic development and deployment of algal biofuels, biomass productivity and conversion efficiency and compositional characteristics
  – Build on the standardization effort for compositional characterization of algal biomass by supporting and validating the implementation of methods for full mass balance accounting (**Subtask 1**: Analytical Development)
  – Develop novel approach to maximize biomass value and conversion yields from dynamic biochemical composition and carbon allocation (**Subtask 2**: Experimental Valorization)

Task 1: Novel Analytical Tool Development

Task 1: Mass Balance accounting

Nannochloropsis sp.



- Existing methodology is robust but does not allow for closing mass balance of algal biomass
- Typically ~30-35% of the biomass remains unaccounted for and this causes inaccuracies in the TEA modeling of process flows

3 species selected as new, representative reference materials, high-protein biomass and basis for mass balance improvements:

Strain	Harvest	Ash	Carbs	Protein	FAME	Organic Acids*	Mass Balance
		---------------------------------- % ----------------------------------					
Monoraphidium sp.	Early	6.0	27.2	23.7	14.9	0	71.8
Nannochloropsis sp.	Early	17.8	8.8	32.5	9.6	0	68.7
Scenedesmus sp.	Early	18.9	9.3	42.5	5.4	0	76.1

- Challenges in implementing extraction-based quantitation is due to co-extraction and distribution of protein, lipid carbohydrate components or incomplete separation

	Lipid Extracted	Lipid Residual	Total
Chlorella sp.	18.93	38.08	102.7%
Nannochloropsis sp.	9.82	0.6	100.1%

Anal Bioanal Chem, 2012;403(1):167-78

New separations-based process is being implemented, based on quantitative extraction and fractionation to account for all products:

Extraction/Separation →
- Pretreatment of biomass
- Solvent type
- Temperature

Non-polar → Lipids/Pigments

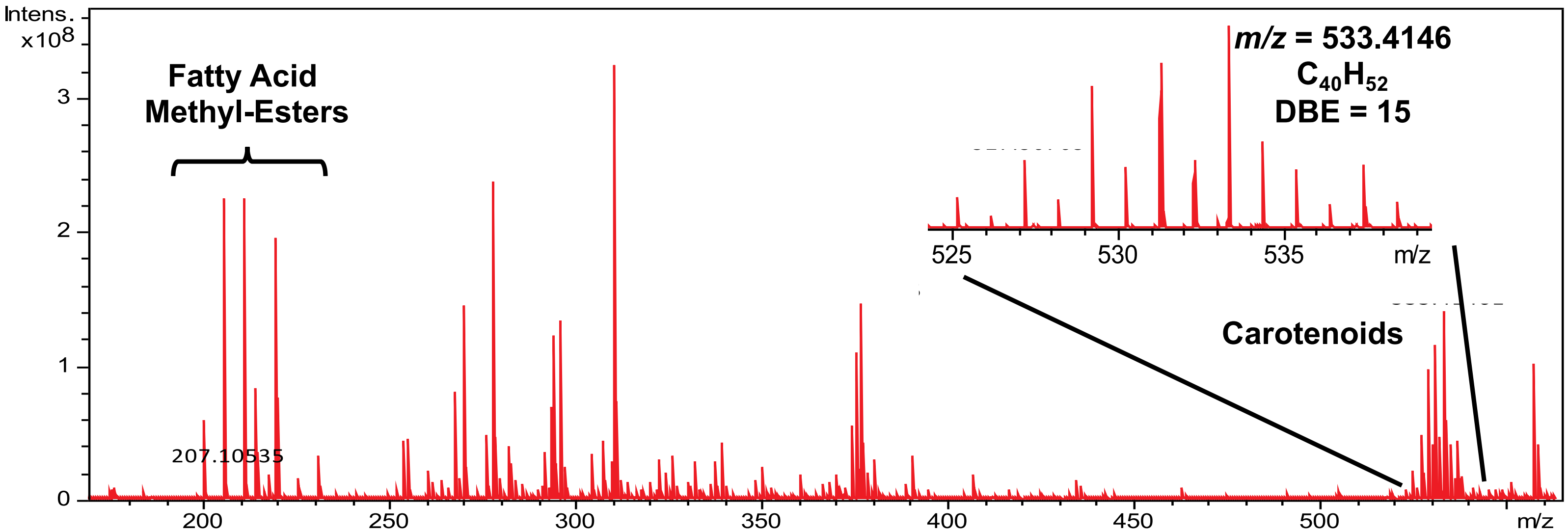
Polar → Metabolites/Polar lipids/?

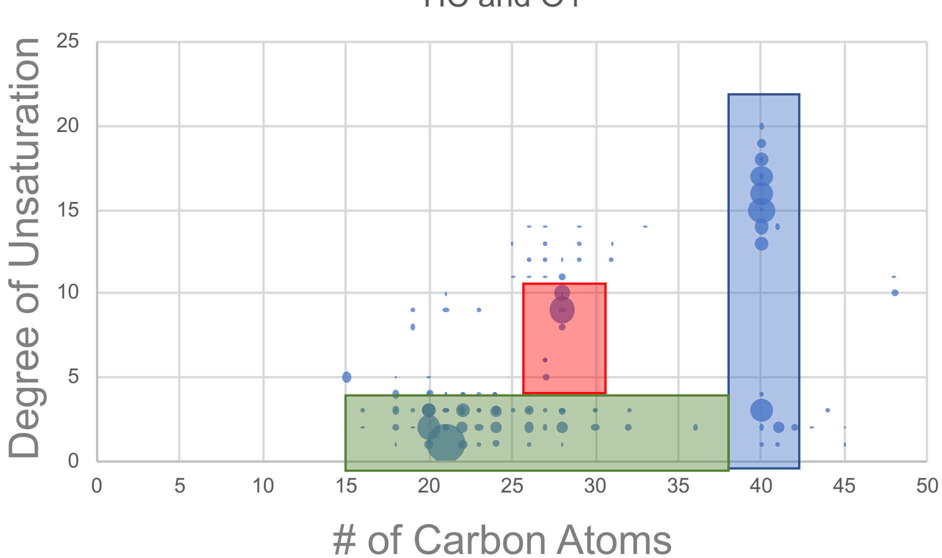
Residue → Proteins/Carbohydrates/Nucleic Acids
- Hydrolysis
- Enzymatic treatments
- NMR

Each fraction will be subjected to an in depth characterization to both identify missing components and search for potentially high-value novel components

Task 1: Novel product identification

Ultrahigh resolution MS was used for in depth mining of novel products in different fractions



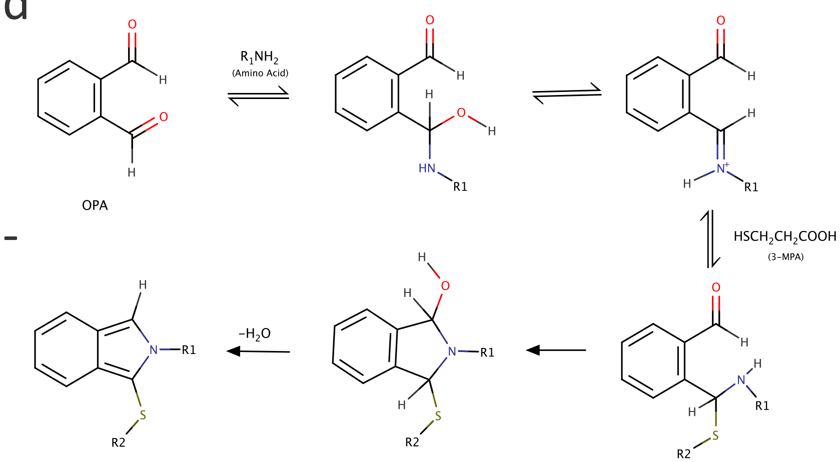


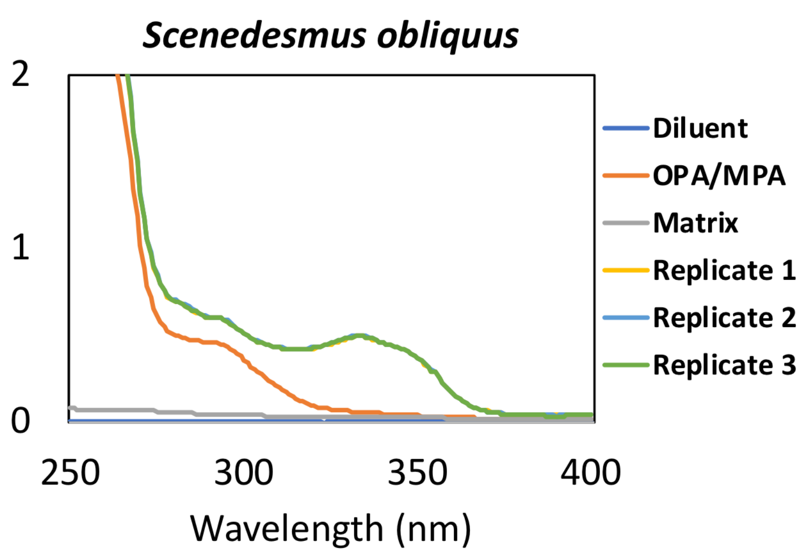
Over 170 compounds identified in lipids and assigned with molecular formulae:

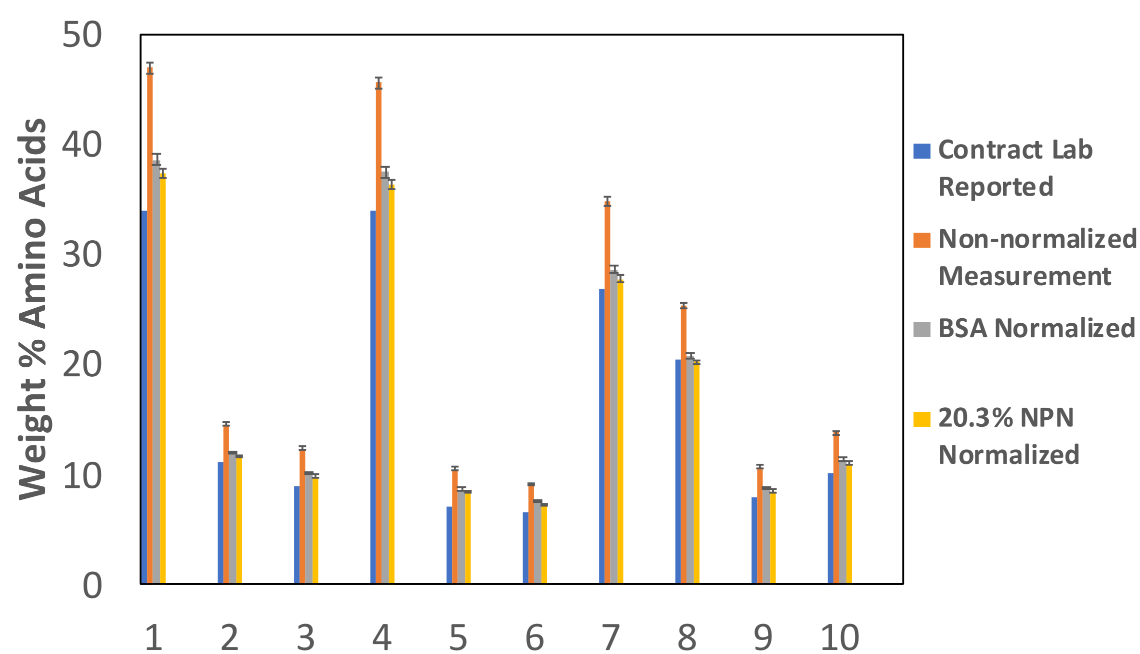
- Tetraterpenes (unidentified by GC-MS) 10 out of 12 matched in lipid database
- Sterol region (new sterols identified by FT-ICR MS and GC-MS)
- Cycloalkane/alkene/unsaturated ketones and alcohols (novel products, ideal fuel precursors)

Task 1: Novel Analytical Method – Protein Quantification

- ‘Traditional’ BCA spectrophotometric method highly interfered by algae biomass components, New method needed avoiding HPLC and post-column derivatization instrumentation and avoids need for species-specific N-protein conversion factor
- Based on selective derivatization of amino acids with spectrophotometric detection – **within 10% of standard amino acid analysis**







Manuscript submitted

Task 1: Standardization of Biomass Analytical Landscape

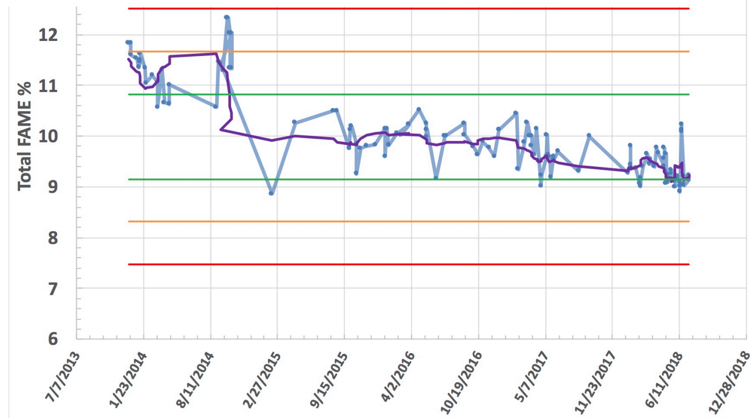
Approach: translation of R&D to reference methodology supporting common language

- Implemented methods with rigorous QC protocol across DISCOVR consortium and trained analysts
- Published new procedure for characterization of sterols in whole algal biomass
- Outreach to AOCS, ASTM, CEN, ISO to solicit input and fill niche areas of standard development
- Chair of Algae Biomass Organization Technical Standards Committee, outreach to and continuous feedback from Algae Industry – published compendium of Measurement Standards for Industry (IAM 8.0) in 2017

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

Task 1: Establishment of new QC material

Instability of previous reference algal biomass necessitated the need for new material:



New material (AzCATI grown) was characterized to determine a **Consensus Composition** determined at NREL by 3 analysts, 2 days and at least 5 replicate analyses (<5% RSD for all measurements):

% DW	Ash	N	Protein (N)	FAME	Carbs
Nannochloropsis sp.	18.01 ± 0.29	6.74 ± 0.10	32.21	9.75 ± 0.22	8.32 ± 0.51
Scenedesmus acutus	19.07 ± 0.74	8.71 ± 0.21	41.62	5.52 ± 0.21	9.13 ± 0.31

Amino acid content (% DW):

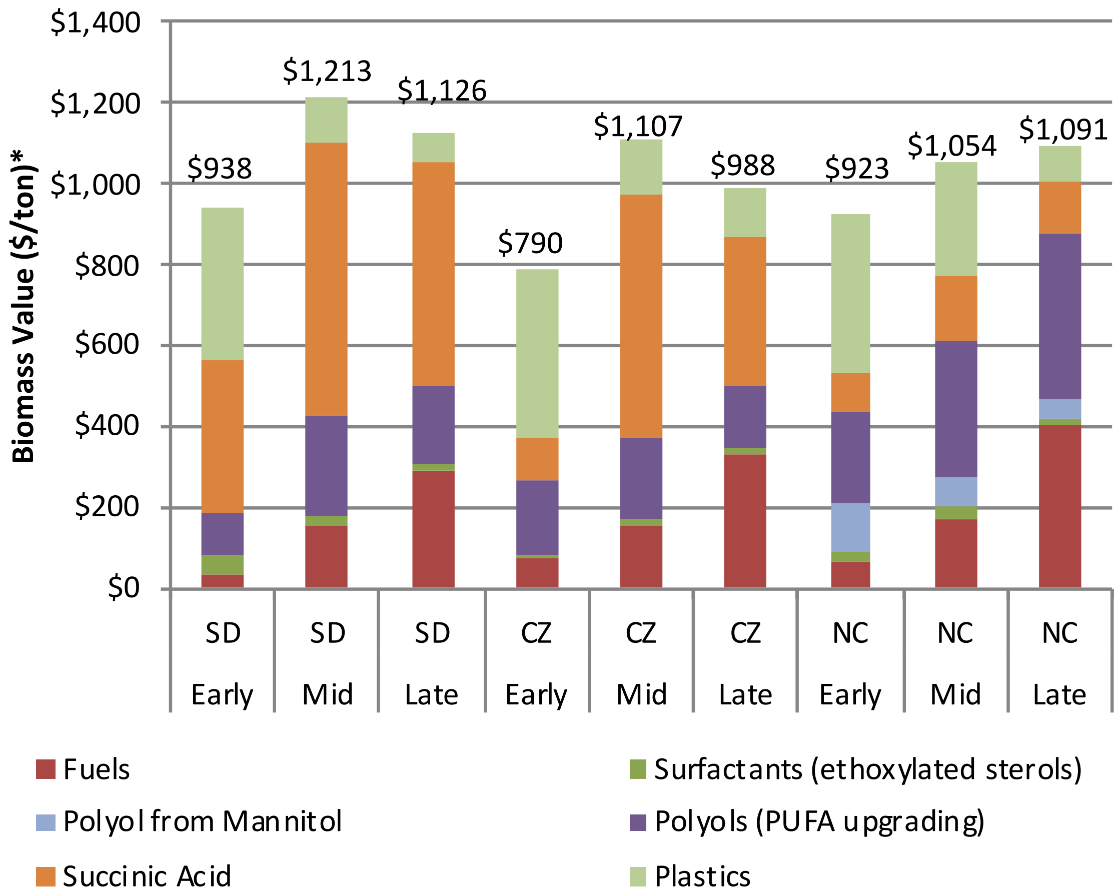
	Amino Acids
Nannochloropsis sp.	31.07
Scenedesmus acutus	36.50

Vacuum sealed, homogenized biomass **available for distribution** to partners and the community. Aliquots have been shipped to CalPoly and ASU and comparative data will be made available

Algal Biomass Valorization

Task 2: Framework Developed for Biomass Value Based on Fuel and Product Yields

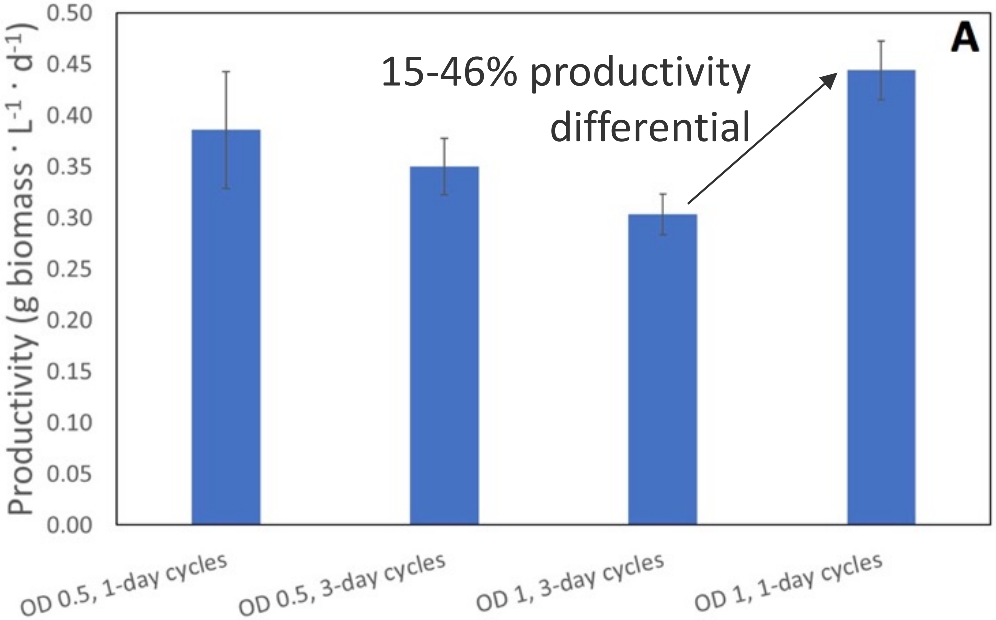
If after full TEA, if cumulative "value" exceeds MBSP → profitable

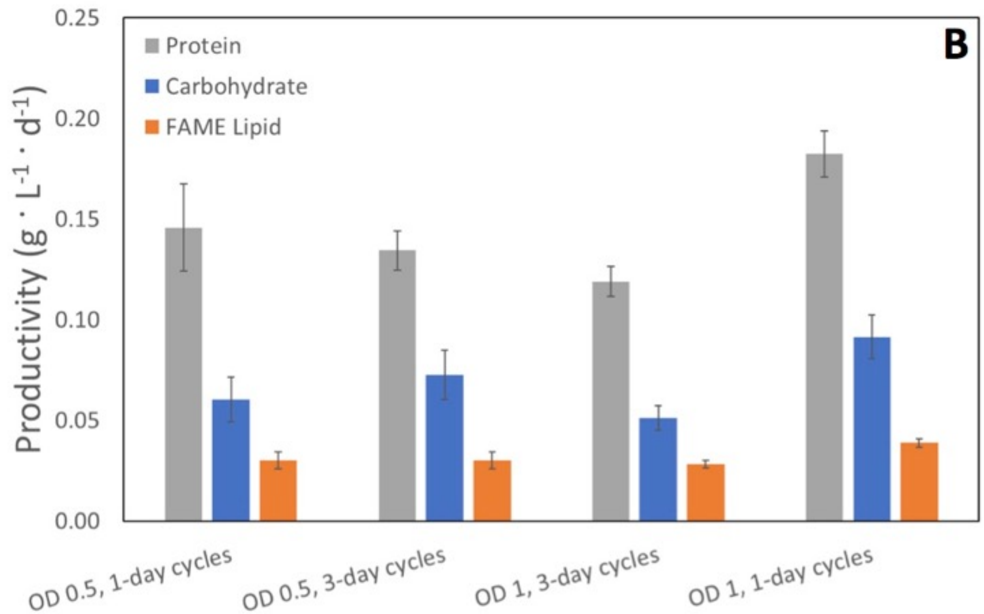


- Example results intended to show "how" to exercise framework model, but do not yet include fully-burdened processing costs (i.e. capex, purification power/chemicals) – will reduce $/ton values shown here
  - Yields and values adjusted for chemical co-feeds (i.e. CO2 for succinic acid, ethylene oxide for surfactants, etc.)
  - Fully-burdened processing costs will require full TEA, planned future work (FY19)
  - This approach allows for evaluating/optimizing best uses of variable biomass cost/quality across different options for fuels/products

[Joint effort with TEA project]

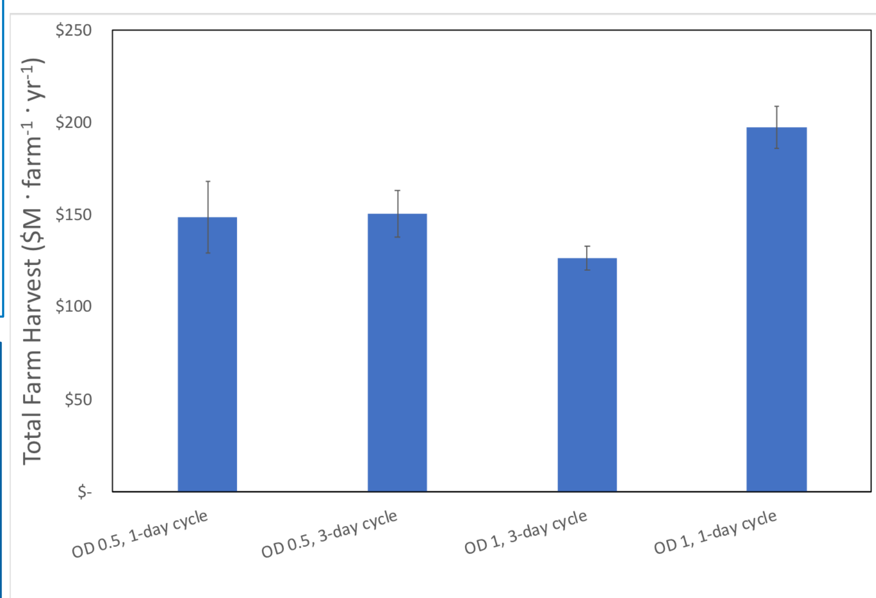
Subtask 2: Experimental Valorization
Biomass Productivity vs. Value



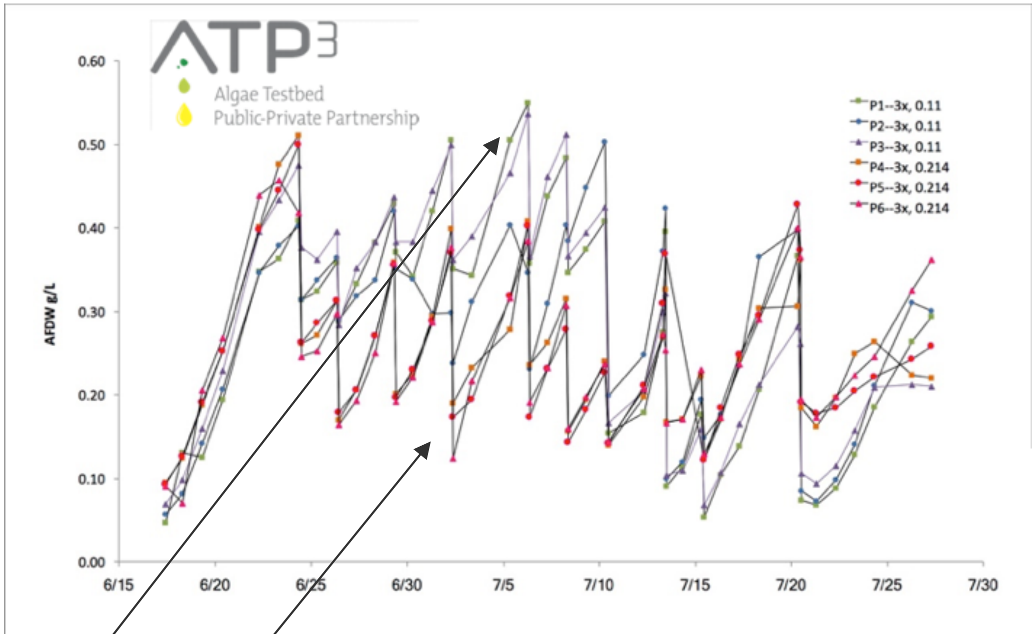


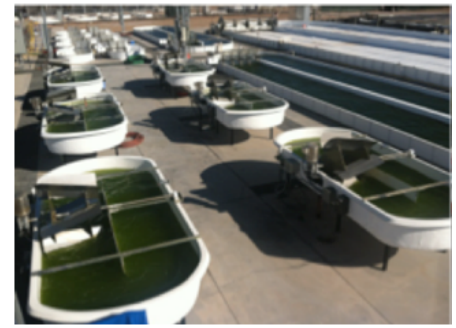
Milestone: Demonstrate at least 3 different cultivation scenarios with determination of composition at harvest as promising for TEA and conversion platform, to inform outdoor cultivation design for implementation at 1000L scale

Continuous cultivation at higher cell density may allow for more desirable compositional profile – at least 30% increase in output value – without reducing biomass productivity

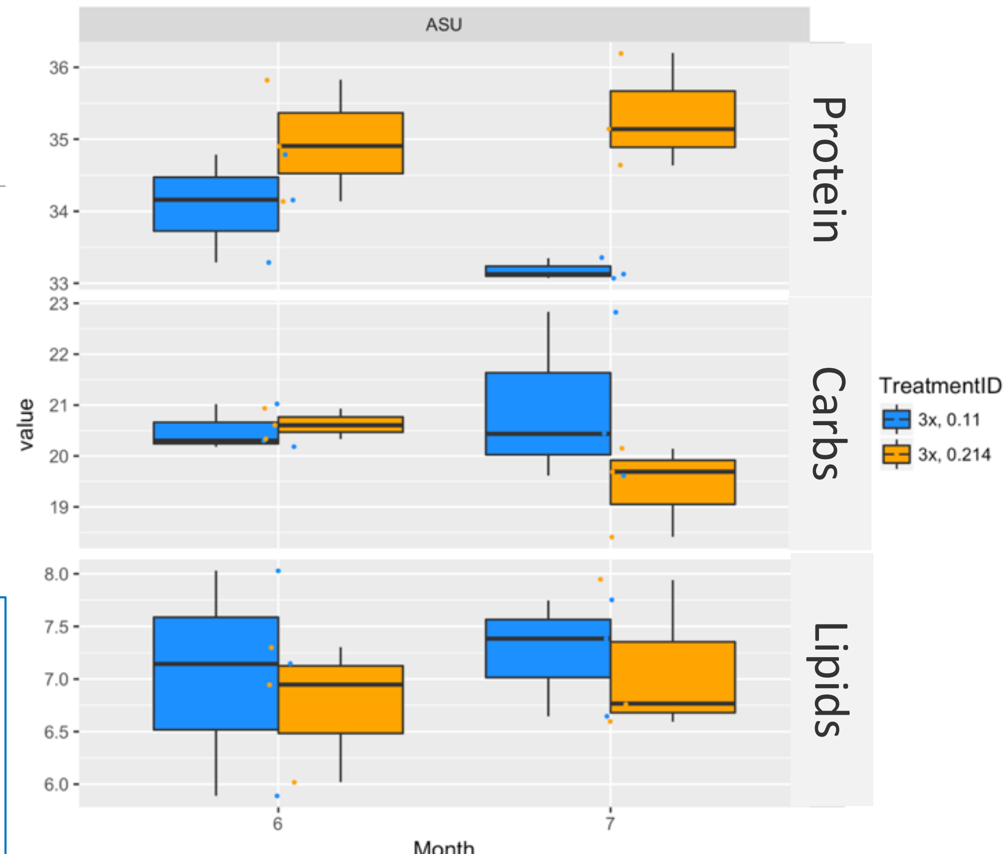


Subtask 2: Outdoor relevance





ATP[3] data from 2014-2015 cultivation at different dilution rates shows changes in protein, carbohydrate and lipid content:



Low dilution rate growth rate:
3x 0.11 **- 13.3 ± 1.1 g/m²/day**

High dilution rate growth rate:
3x 0.214 **- 12.4 ± 0.8 g/m²/day**

Outdoor deployment and cultivation manipulation in ATP3 testbed indicates composition impact without productivity penalty

Relevance

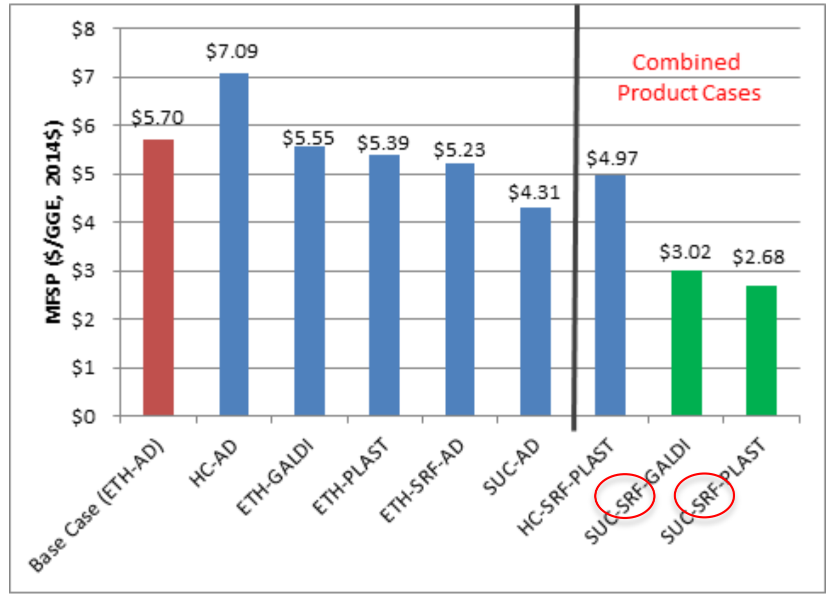
Relevance

Shifting biomass composition in relevant production environments unlocks product and cost-reduction potential

Co-product isolation and purification is **critical to accelerate commercialization of algae:**

- Algae farm of **5,000 acres** produces **190,000 ton** biomass:
  - 1,539 ton sterol/phytol* (~1% biomass) = **$10.75M revenue** potential ($2,900/ton SRF)
  - 10,260 ton PUFA (~5% biomass)* = **$27.72M revenue** potential ($2,300/ton Polyol)

Valorization R&D Commercial Relevance:



Expand Algae Market Potential

- Algal Biomass Valorization is highly relevant to BETO strategic goals, of identifying and removing critical barriers and uncertainties to accelerate creation of new domestic bioenergy and bioproduct industry
- Algal bioproducts identified in this task were made part of the Algal Biofuels Technology Review report and were the first bioproducts included in the 2016 Algae MYPP
- Components identified in this project have been used to demonstrate valorization integrated with CAP conversion process, compatible with fuels, e.g. development of renewable non-toxic surfactants could find a commercial application

Relevance

Stakeholder Outreach and Engagement:

- Enabling algae industry with guidance on uniform descriptions on biomass characterization and subsequent valorization and trading standards
- Methods have received > 5000 downloads and numerous groups are implementing integrated compositional analysis workflow and spreadsheet
- Harmonized characterization of materials of algae implemented across ATP^3 network
- Worked with and trained two commercial algae groups and hosted training workshop
- Created and characterized **novel standard reference material**, distributed to 2 collaborators for method and data aligning – *available to community via NREL website*
- Transferred methods to CPR, TEA, DISCOVR, ABP, ATP^3, PACE, and cross-platform (BETO Biochem and ARPA-E), e.g. lipid characterization, NIR high-throughput screening
- Compositional analysis methods included as guidance in two recent FOAs, which led to partnering requests


NREL

Determination of Total Carbohydrates in Algal Biomass

Laboratory Analytical Procedure (LAP)

Issue Date: December 2, 2013

Revision Date: December 29, 2015

Stefanie Van Wychen and Lieve M.L. Laurens

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

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

Future Work

FY19: Capture and model the **impact of cultivation parameters with biomass composition, products and value**

- Map productivity kinetics, carbon balance and composition and bioproducts for novel, commercially relevant species (in collaboration with DISCOVR) in outdoor-relevant conditions
- Develop novel approaches for mass balance accounting in early harvest replete algal biomass
- Expand product portfolio with protein-derivative products and polyamines as biopolymer crosslinkers
- Implementation of ultra-high resolution mass spectrometry platform for detailed metabolic and lipid fingerprinting of new species – make data on product composition in biomass openly available (web interface)
- Work with conversion interface project to identify and purify high-value co-products from process streams with aim to develop and integrate pathways for full valorization of products
- Maintain a set of easily applicable accurate compositional analysis procedures that are implemented across different groups and industry

Summary

- **Overview**: Algal Biomass Composition is set up to exploit the value of algae in a conversion pathway based on an in depth understanding of the biomass biochemical dynamics
- **Approach**: Robust and detailed quantitative compositional analysis of biomass to assign a portfolio of products from different species subjected to different environmental conditions to maximize the biomass value, targeting a 30% increase in value
- **Technical Accomplishments**:
  - Demonstrated high metabolic and compositional plasticity of algae in relevant productivity scenarios to manipulate intrinsic value of biomass
  - Developed and maintaining standard procedures providing basis for common language gaining traction in algae community and will enable and help drive commercialization
- **Relevance**:
  - Co-products are critical to achieve $3/GGE; Advanced Algal Systems economics need isolation and harnessing of high-value, large market products
  - Large biochemical diversity opens up routes for novel products with unique properties from separate product streams
  - Standard procedures fill a niche in the algae research community and are widely adopted and implemented
- **Future work**:
  - Identifying and isolate additional bio-products with high-value, large market components to further increase biomass value
  - Implement novel approaches, combining biomass separation and component identification and discovery to close biomass mass balance

Stefanie Van Wychen
Bonnie Panczak
Andy Politis
Steven Rowland
Peter Shanta
Nick Sweeney
Anna Fedders
Eric Knoshaug
Damien Douchi
Nick Nagle
Tao Dong
Ambarish Nag
Stefanie Van Wychen
Ryan Davis
Jenifer Clippinger
Philip Pienkos
Ryan Herold
Alyssa Ivankovich
Madeline Lane

Thank You

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

Lieve.Laurens@nrel.gov

NREL is a national laboratory of the U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy, operated by the Alliance for Sustainable Energy, LLC.

Response to Reviewers' comments (FY17)

Project Approach

- We appreciate the complimentary remarks by the review team and welcome the opportunity to respond. In particular we would like to add to the notes about the need for the inclusion of regulatory compliance in our cost projections. The respective value of the products we are developing is underpinned by rigorous techno-economic analyses (TEA), which are vetted and standardized throughout the DOE BETO portfolio. The scope of these TEAs focuses primarily on quantifying the economic ramifications of directly verifiable R&D/process attributes (i.e. conversions, yields, operating conditions, etc.) and historically may not have fully captured all regulatory compliance constraints for a given process, because the products, such as fuels, do not fall under the purview of the compliance agencies listed. However, moving forward we will continue to work towards better understanding those constraints for specific products and reflect the implications of such constraints on production costs through TEA modeling, to the extent that processing costs are impacted (and may be readily quantified) by those constraints.
- The current Good Manufacturing Practice (cGMP) and Good Agricultural Practice (GAP) regulatory considerations fall under the oversight of FDA and USDA respectively. While these are institutions and guidelines that primarily oversee the manufacture and production of agricultural and pharmaceutical products, we are specifically searching for the valorization of products that fall outside of the food and feed realm to allow for scalability with the ~200,000 ton/yr algae production. However, clean manufacturing of products and meeting purity specifications will be a priority for the commercial production of any of the potential products or derivatives that we have identified and it is likely that future more granular and refined assessments of a commercially proven process will define the needs and help shape regulatory compliance.
- On the note that the current TEA models do not capture purification costs, we want to elaborate. The preliminary analyses for "potential" product revenues calculated did not include downstream purification costs, as they were intended more to represent high-level value estimates for product options being considered. However, we have subsequently built more detailed TEA models for a number of those selected products, which do consider all process/purification costs (e.g. sterol purification and conversion to non-ionic surfactants). We recognize that for a number of the product opportunities those costs can be significant and we are tailoring our experimental plan to search for low cost and high efficiency product purification methods, e.g. the patented PUFA purification strategy presented. The costs associated with isolation of feedstock products will be included in future work following a similar approach to succinic acid purification pursued within the BETO Biochem platform, where the NREL TEA group placed a subcontract with an engineering consulting firm to establish processing costs for succinic acid production.
- Finally, we agree with the reviewers' comment that the methods, by virtue of covering advanced analytical approaches to characterize biomass with the necessary resolution and granularity to its individual constituents are not easy to implement. We have addressed this in the past by developing and disseminating high quality documentation around the methodology and provided training for groups are new to the implementation. This has helped with the implementation of the current methodology; however, we are continuously searching for additional simplified procedures that can be used as proxy for the methods currently in place. We have a FY18 milestone planned to specifically list and validate simplified methodology for rapid biomass fingerprinting and obtaining biomass compositional data with similar precision and accuracy.

Response to Reviewers' comments (FY17)

Accomplishments and Progress

- We would like to add clarification to the notes the reviewers brought up on the downstream conversion of sterols to surfactants. This is one aspect where the presentation of progress for this project was confounded with work done under the auspices of the Algal Biomass Conversion project. For this work, ABV identified, quantified and purified the sterol fraction from algae oils (as detailed in a FY16 Q3 milestone report, ABV project), while the ABC project, subsequently converted the purified sterol/phytol fraction to surfactants (FY16 Q4 milestone report, ABC project). We have supported the surfactant work with an in depth characterization of the resulting products by ultra-high resolution mass spectrometry instrument that we recently commissioned in the laboratory and the manuscript being developed from this work is also a joint effort between our tasks and reflects the close collaboration. We apologize for the blurring of the delineation between the projects and will make sure that in future presentations and reporting to BETO the distinction is made more clear.
- The focus of our work on extracellular polymeric substances finds a basis in the accounting of energy and carbon balance in a cultivation system to support maximizing productivity. Because recycling of >80% of the cultivation water is currently assumed in modeling reports, understanding the organic carbon load of the recycled water is of tremendous value to mitigate the subsequent contamination impact. If extrapolations of our measured data on EPS in algae cultures, at >150 mg/L, hold true to outdoor large-scale cultivation, this not only presents a significant loss of organic carbon that is not assimilated to more valuable biomass or products, rather lost to the media, and this organic load would have to be removed prior to recycling the water to a new inoculation pond to keep bacterial contamination under control. There are two options for mitigation the detrimental impact of EPS on the overall process, either manipulating the algae or cultivation methods (e.g. media composition) to reduce the secretion of EPS (Xu H, et al., Water Res 2013; 47: 2005–2014 and Domozych DS. Int J Plant Sci 2007; 168: 763–774), or harness the EPS in an online separation strategy prior to recycling. There are DOC reduction strategies that can be adopted from established waste water treatment methodologies (Pivokonsky M, et al. Crit Rev Environ Sci Technol 2016; 46: 291–335), that are scalable with an algae farm. Paramount to a number of these strategies, is a robust isolation and quantification protocol for EPS and structural identification of the polymers, with ultimately evaluating strategies for either selective utilization, reduced production implemented at scale or harvesting EPS for high-value applications, e.g. biodegradable emulsifiers used in fracking operations.
- Regarding the question on including the timing of nutrient stress in the productivity calculations and TEA to account for the cost reduction, we want to add some clarification. For each of the nine scenarios presented the respective productivity data was not available. The purpose of this work was to decouple the biomass productivity from the composition and just investigate the impact of the latter on fuel selling price. The minimum biofuel selling price (MFSP) is a BETO metric that is used to track year-over-year progress towards cost-competitive biofuels and therefore, we have used this metric here to quantify the impact of composition, in isolation and decoupled from productivity, on the bottom line MFSP of an envisioned biorefinery. For the underlying TEA models, the productivity was assumed to be consistent at (an optimistic outyear target of) 25 g/m2/day. We are fully aware that to achieve the shifts in composition, daily productivity will be reduced, however, the extent to which this happens is currently not available in the underlying cultivation data. This is not an oversight rather the start of a comprehensive approach in the current merit review cycle for this project to specifically address this during our experimental work in FY17 and FY18. The fundamental tradeoffs between productivity and value of the biomass and ultimately impact on the biofuel selling price is the subject of a framework assessment that is ongoing jointly between ABV and the TEA team at NREL. An initial milestone report (FY17 Q2 joint between ABV and TEA) specifically established the framework for this calculation and we are scheduled to populate fully developed TEA models with productivity and composition data in FY18 through collaboration with ATP3, and report on the quantitative impact of achieving the composition targets for conversion.

Publications

Peer reviewed manuscripts:

1. 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 Ag Food Chem**, Submitted
2. Fioroni, G., Katahira, R., Christensen, E. D., Pienkos, P. T., Laurens, L. ML. (2017) “Synthesis of Hydrophilic Derivatives of Algae Unsaponifiables as Potential High-Value Surfactants”, **ACS Sustainable Chemistry & Engineering**, under review
3. Li, Y., Leow, S., Dong, T, Nagle, N., Knoshaug, E. P., Laurens, L. ML., Pienkos, P. T., Guest, J., Strathmann, T., (2018) "Demonstration and Evaluation of Hybrid Microalgae Aqueous Conversion Systems for Biofuel Production”, **ACS Sustainable Chemistry & Engineering**, under review
4. Laurens, L. ML., Knoshaug, E. P., Rohrer, H., Van Wychen, S., Dowe, N., Zhang, M. (2016) “Solvent-free spectroscopic method for high- throughput, quantitative screening of fatty acids in yeast biomass” **RSC Analytical Methods**, in press
5. Arora, N., Laurens, L. M.L., Sweeney, N., Pruthi, V., Poluri, K. M., Pienkos, P. T. (2018) “Elucidating the unique physiological responses of halotolerant Scenedesmus sp. cultivated in sea water for biofuel production” **Algal Research**, 34, 260-268
6. Leow, S., Shoener, B. D., Debellis, J. L., Markham, J., Davis, R., Laurens, L. ML., Pienkos, P. T., Cook, S. M., Strathmann, T. J., Guest, J. S. (2017) “A Unified Modeling Framework to Advance Biofuel Production from Microalgae”, **Environmental Science & Technology**, 2018, 52 (22), 13591–13599
7. McKie-Krisberg, Z. M., Laurens, L. M.L., Huang, A., Polle, J.W. (2018) “Comparative energetics of carbon storage molecules in green algae”, **Algal Research**, 31, 326-333
8. Laurens L. ML., Markham, J., Templeton, D. W., Christensen, E. D., Van Wychen, S., Dong, T., Chen-Glasser, M., Davis, R., Pienkos, P. T. (2017) “Development of Algae Biorefinery Concepts for Biofuels and Bioproducts; a Perspective on the Molecular Identification of Process-compatible Bioproducts and Impact on Cost-Reduction” **Energy and Environmental Science**, 10, 1716—1738

Patent: Dong, T., Laurens, L. ML., Pienkos, P. T. (2017) " Renewable Polymers and Resins and Methods of Making the Same" USPTO provisional patent application No. 62/482238

Book chapters:

1. Laurens, L. ML., Olstad, J., Templeton, D. W. (2017) “Total Protein Content Determination of Microalgal Biomass by Elemental Nitrogen Analysis and a Dedicated Nitrogen-to-Protein Conversion Factor” ed. K. Spilling in “Biofuels from Algae: Methods and Protocol” in the series “Methods in Molecular Biology”, Publishers: Springer
2. Van Wychen, S., Laurens, L. ML. (2017) “Total Carbohydrate Content Determination of Microalgal Biomass by Acid Hydrolysis Followed by Spectrophotometry or Liquid Chromatography” ed. K. Spilling in “Biofuels from Algae: Methods and Protocol” in the series “Methods in Molecular Biology”, Publishers: Springer
3. Van Wychen, S., Laurens, L. ML. (2017) “Total Fatty Acid Content Determination from Whole Microalgal Biomass Using in situ Transesterifcation” ed. K. Spilling in “Biofuels from Algae: Methods and Protocol” in the series “Methods in Molecular Biology”, Publishers: Springer
4. Laurens, L. ML. and Chen-Glasser, M. (2017) “Research and Deployment of Renewable Bioenergy Production from Microalgae”, in “Microalgae as a Source of Bioenergy: Products, Processes and Economics”, ed. J. Pires, Publishers Bentham Science Publishers

Presentations

- "A Perspective on Algae for Chemical and Surfactant Production” (2018) Laurens, L. ML., invited presentation and panel participation at Cleaning Products US 2018 conference, Chicago, IL
- “Renewable bioenergy from algae; tying together biochemistry of storage carbon metabolism with biomass productivity” (2018) Fedders, A., Sweeney, N., Laurens, L.ML.* Poster Presentation, 8th Algae Biomass Summit, Houston, TX
- “New method for protein analysis in algae - derivatization and detection of amino acid o-phthalaldehyde 3-mercaptopropionic acid (OPA-3MPA) derivatives” (2018) Cuchiaro, H., Laurens, L. ML., Oral Presentation, 8th Algae Biomass Summit, Houston, TX
- “Extractability and Quality of Lipids in Algae, a Study of Species-specific Lipase Activation” (2018) Herold, R.A., Laurens, L.ML. Oral presentation and session chair “Industrial Oil Products, Biofuels”; “Compositional Analysis of Algal Biomass, An Emphasis of Unique Contribution of Algal Lipids”, Laurens, L. ML.; “Sterol Fingerprinting in Algae, a New Method for a New Feedstock”, Van Wychen, S., Laurens, L. ML., AOCS Annual Meeting, Minneapolis, MN
- “Sterol molecular fingerprinting in different algae provide options for high-value co-product development” (2017) Spinelli, P., Van Wychen, S., Duff, K., Palardy, O., Fioroni, G., Pienkos, P. T., Laurens, L. ML.*, Oral Presentation; “Extracellular Polymeric Substances in Algae:Problem or Opportunity?” (2017) Vandamme, D.*, Vadelius, E., Van Wychen, S., Laurens, L.ML., Oral Presentation, 8th Algae Biomass Summit, Salt Lake City, UT
- “Research and Deployment of Bioenergy Production from Algae, a State of Technology Review” Invited plenary presentation; “Sterol molecular fingerprinting in different algae provide options for high-value co-product development”, Oliver Palardy, Paris Spinelli, Stefanie Van Wychen, Gina Fioroni, Lieve Laurens*, Oral presentation, International Conference on Algal Biomass, Biofuels and Bioproducts, Miami, FL
- “Sterol molecular fingerprinting in different algae provide options for high-value co-product development”, Oliver Palardy, Paris Spinelli, Stefanie Van Wychen, Keegan Duff, Gina Fioroni, Lieve Laurens*, Oral presentation and session chair “Industrial Oil Products, Biofuels”, AOCS annual meeting, Orlando, FL

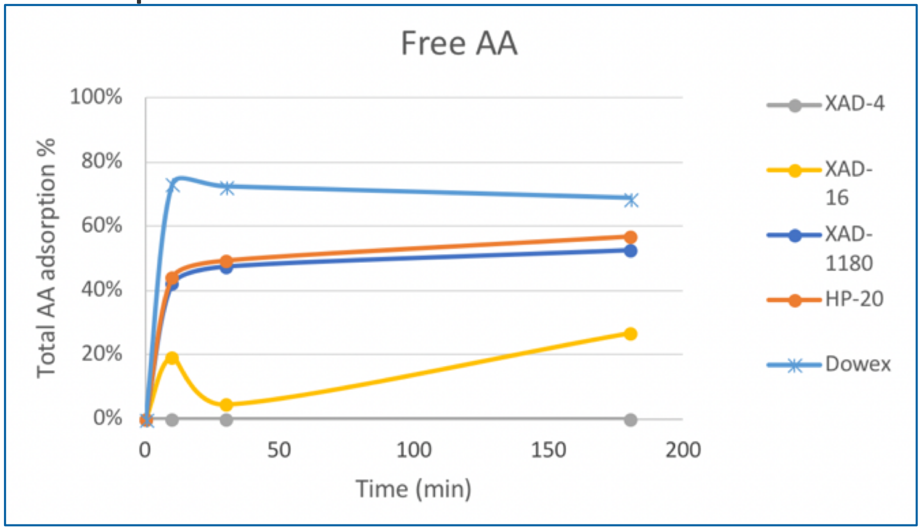
Task 2: Valorization and purification of Amino-acids

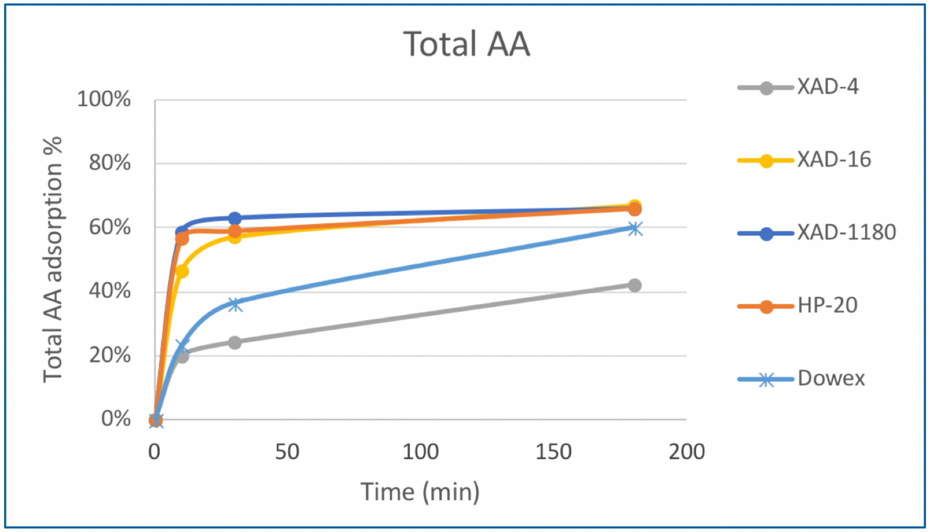
	Dissolved solids content g/L	Free AA g/L	Total AA g/L	Total AA in total solid %
Liquor	66.0	4.9	12.6	19.1

Developed HPLC-DAD quantification based on OPA derivatization

Milestone: Demonstrate yield and purity of at least 3 amino-acid and peptide purification strategies from CAP liquor to be used as feedstocks for fully renewable biopolyurethane synthesis from unsaturated fatty acids (non-isocyanate cross linked biopolymer synthesis)

Adsorption





XAD-16 allows for recovery of 62% of the AA in CAP liquor, at a purity of up to 90% (~5x increase from liquor) – opens up possibilities for valorization of amino acids from CAP process