

DOE Bioenergy Technologies Office (BETO) 2017 Project Peer Review



1.3.4.300 Algal Biomass Valorization (ABV)

BETO Advanced Algal Systems
Denver, CO
March 8th, 2017

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National Renewable Energy Laboratory

Goals

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

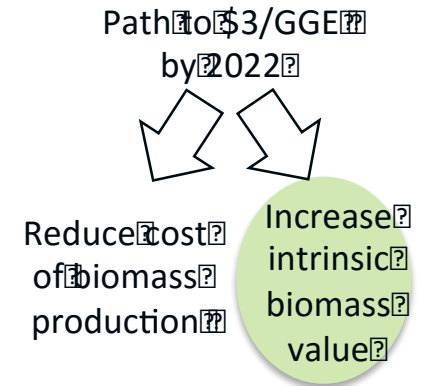
- Identify and isolate **scalable, high-value key products** in biomass and process streams to improve algal biofuels economics
- Quantitatively integrate biomass composition and carbon efficiency with cultivation parameters in a **multi-product algae biorefinery model**

2. Reduce uncertainty around process inputs and outputs

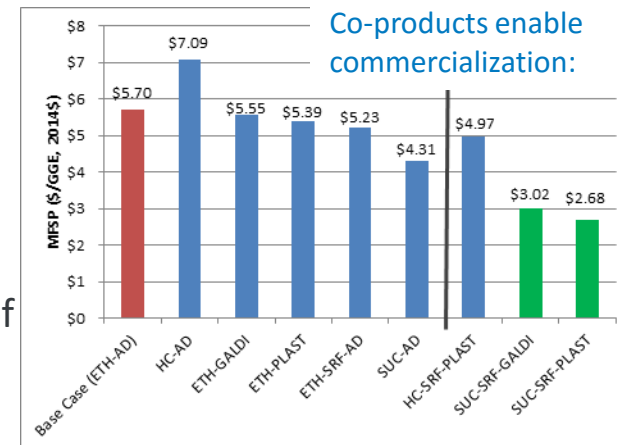
- Establish **common language** for characterization of range of species and process configuration
- Solicit input on **commercial need for standards** and route to adoption and implementation

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



Relevance



Quad Chart Overview

Timeline

- Start: 10/2012 (FY13)
- Merit Review: 2016 (FY17-FY19) - 16% Complete
- Ongoing project

Budget

	Total Costs FY 12 – FY 14	FY 15 Costs	FY 16 Costs	Total Planned Funding (FY 17-Project End Date)
DOE Funded	1,511K	800K	850K	2,250K

Barriers

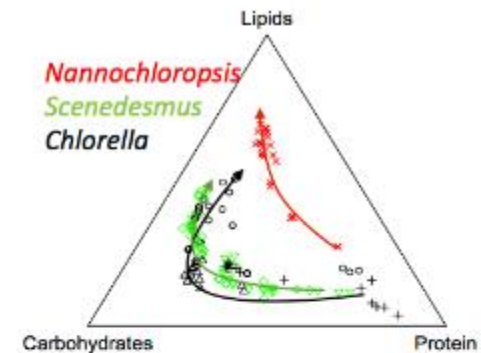
- Aft-B: **Sustainable Algae Production**, value of feedstock, seasonality, environmental variability
- Aft-E: **Algal Biomass Characterization**, Quality, and Monitoring: Chemical, biological and post-harvest physiological variation in harvested algae. Need for standardized procedures to uniformly quantify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

Partners

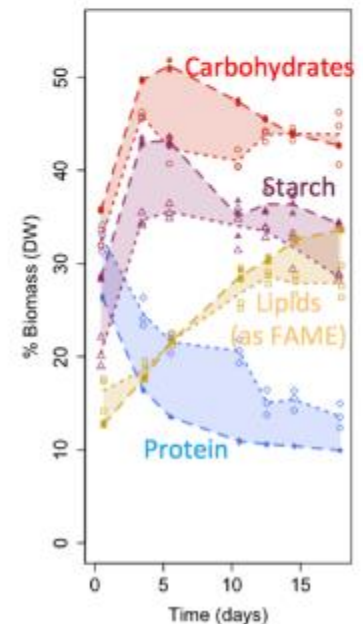
- **Partner (shared funding)**
 - ASU subcontract – outdoor cultivation
- **Interactions/collaborations:**
 - Algae Testbed Public-private partnership (**ATP³**)
 - Algal Biomass Conversion (**ABC**)
 - Algae Techno-economic Analysis (**TEA**)
 - Development of Integrated Screening, Cultivar Optimization and Validation Research (**DISCOVER**), Algae Biotech Partnership (**ABP**), Producing Algae for Coproducts and Energy (**PACE**)

Project Overview

- Early focus on developing **tools for characterization**
 - Biomass composition definitions poorly described in literature and lack validation, resolution and mass balance
 - component discovery, method development and mass balance closure in 3 dominant species (fresh – salt water)
- Observed **metabolic plasticity of algae**, presents gap between modeling and observed composition data
- Influenced the **adoption of a common language of biomass composition** through Sustainable Algal Biofuels Consortium (SABC), Algae Testbed Public Private Partnership (ATP³), Algal Biomass Organization (ABO) Technical Standards and International Energy Agency (IEA)
- **Combined Algal Processing (CAP)** pathway, opening up routes to valorize components through fractionation
- FY17-19 focus on establishing a **cost-value framework for additional species**, relevant to increasing value from production and novel conversion approaches



Early → Mid → Late



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

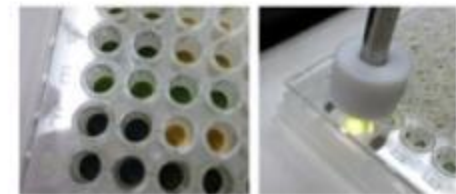
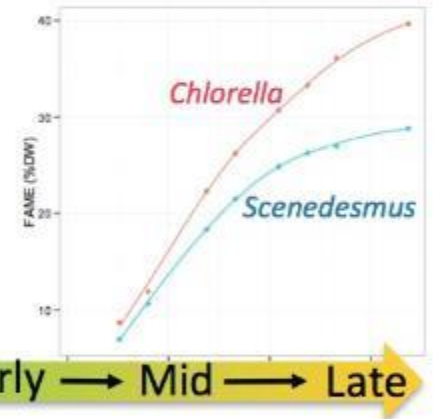
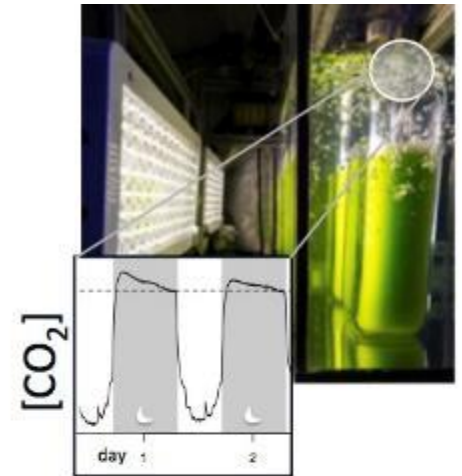
Approach - Technical

Increase intrinsic value of algal biomass

- 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)

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



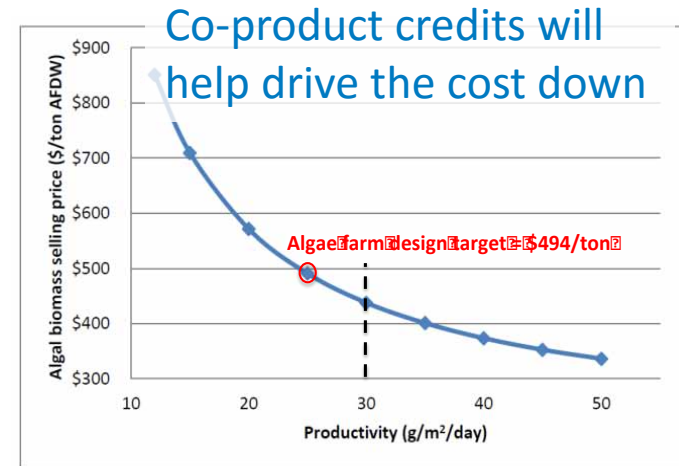
Approach – Technical

Critical success factors:

- **Experimental demonstration of integration** of co-products identified here with CAP conversion pathway showing reduction in minimum fuel selling price (MFSP)
- Achieve an **increase of the biomass intrinsic value** by adding co-products identified here to CAP pathway

Challenges:

- **Co-products are strain and process dependent** and may not be applicable across entire algae value-chain
- **Analytical methods difficult to implement** across laboratories -> increase uncertainty in biomass value assessment
- **Mass balance accounting** short of 100%, making process component flow modeling challenging



Species diversification, to cover phylogeny and cultivation environment (fresh/saline)



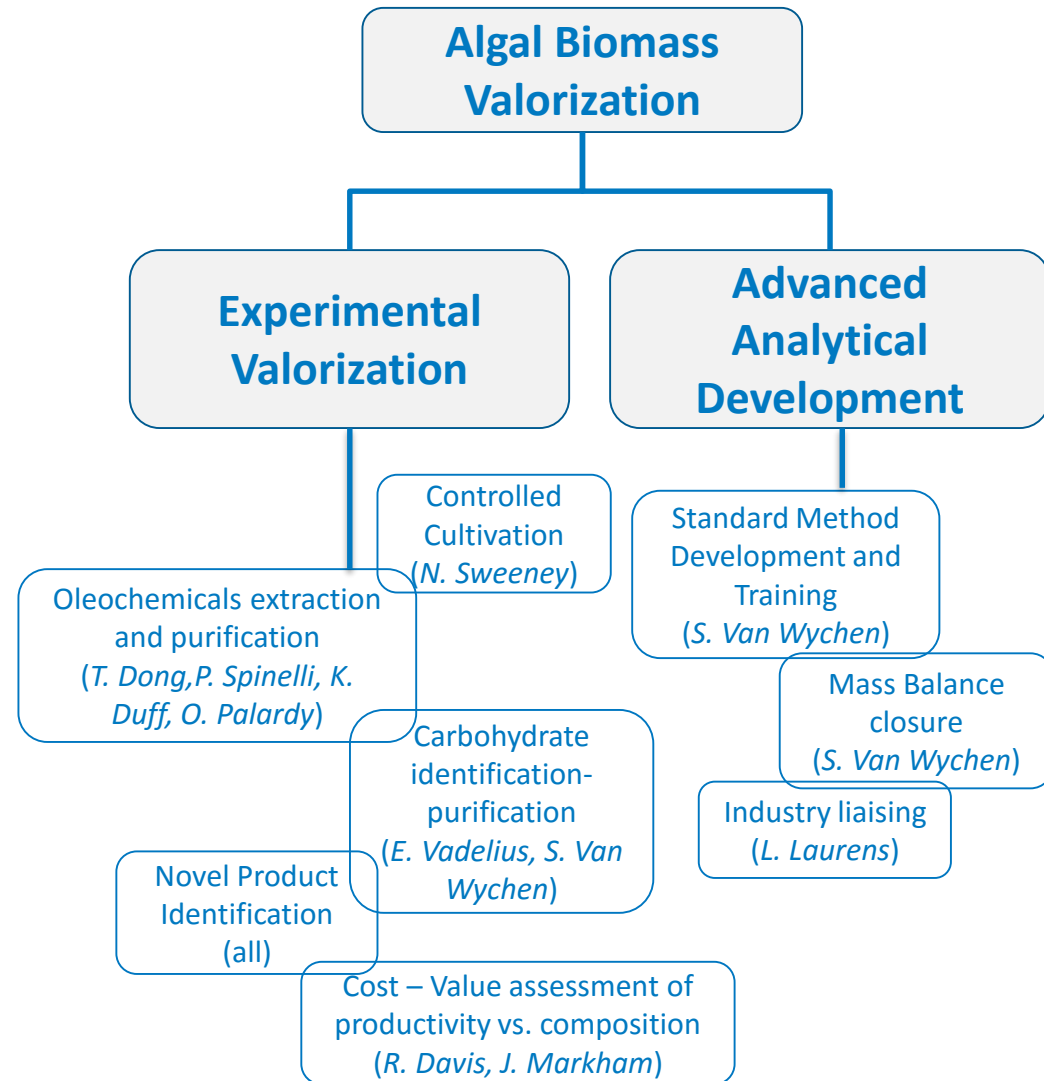
Search and validate simplified procedures for rapid biochemical fingerprinting



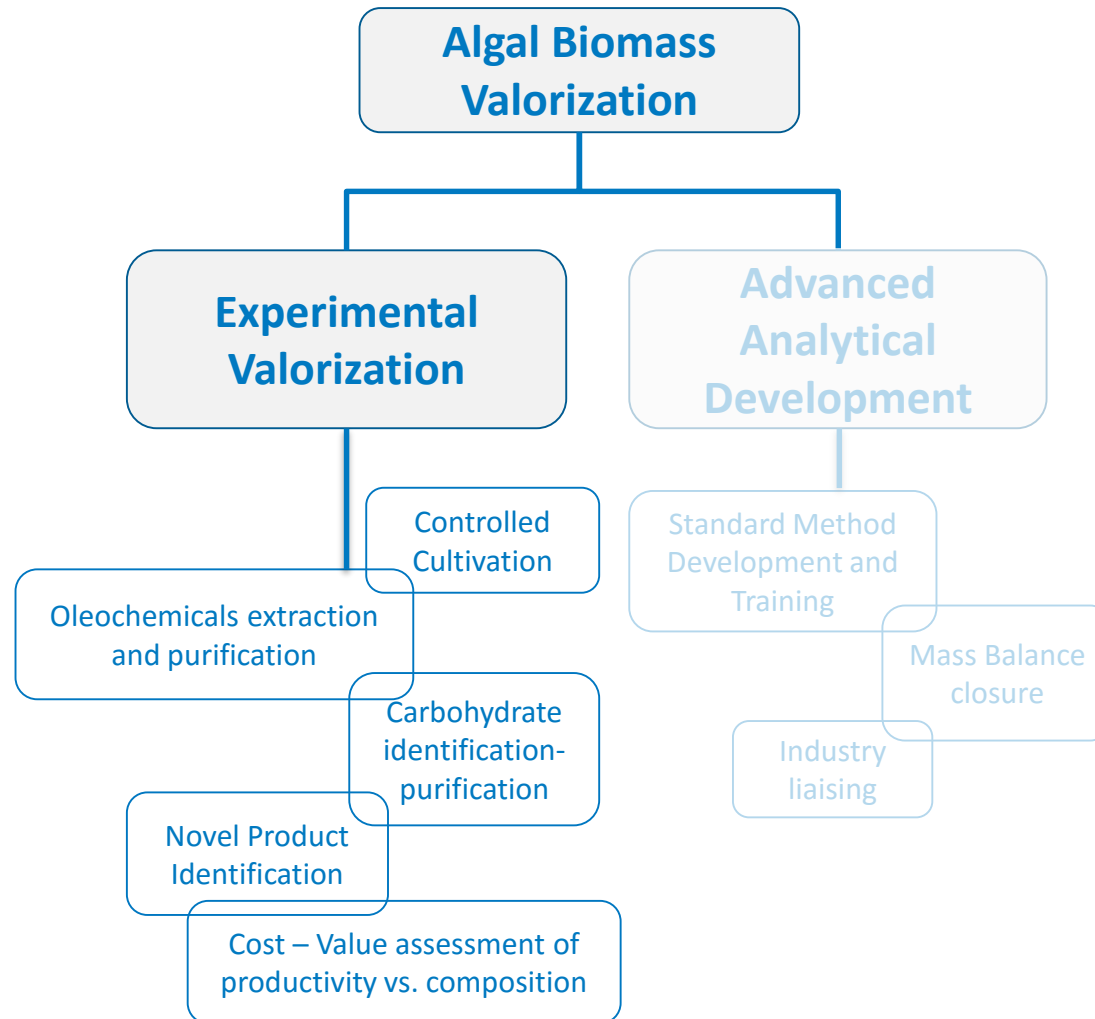
Quantify and add novel products to mass balance for novel species

Approach – Management

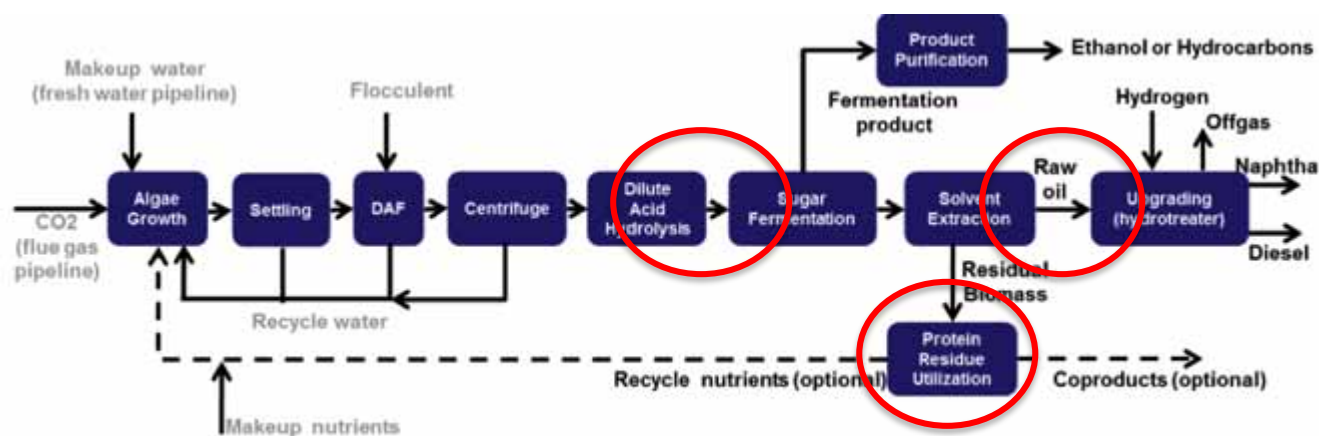
1. Project progress tracked through **milestones and quantitative compositional analysis metrics**, quarterly progress reports and peer-reviewed publications
2. Yearly **update to online standard procedures**, tracking access and implementation across Advanced Algal Systems platform
3. **Research integrated** with conversion (ABC), cultivation (ATP³) and strain selection (DISCOVER) R&D projects
4. Close **collaboration with TEA** group on value, cost and productivity calculations



Approach



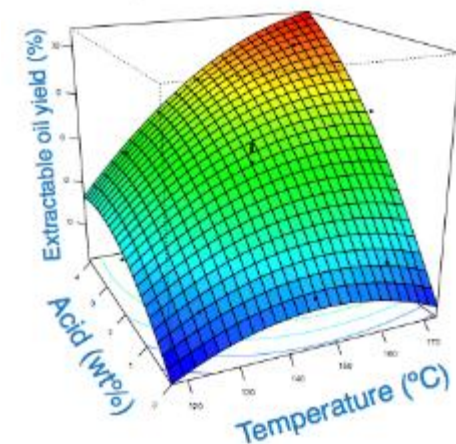
CAP – Dependent on Biomass Composition



- Non-destructive fractionation, **Combined Algal Processing (CAP)** allows for high-quality product recovery from lipids, carbohydrates and protein
- Potential for 35% reduction in MFSP by adopting **multi-product algae biorefinery**

Approach:

- **Refine co-product opportunities from product streams** will further aid economics and add to maximize biomass utilization
- Refine **unknown structure-function link of biomass composition** with conversion metrics will aid with conversion optimization



www.nrel.gov/docs/fy14osti/62368.pdf
[Green Chemistry (2014), 17, 1145-1158]
[Algal Research (2016)(, 19, 316-323]

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)	<i>Scenedesmus</i>			<i>Chlorella</i>			<i>Nannochloropsis</i>		
	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
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[FY16 Q2 Milestone]

Co-Products Provide Additional Revenue to Biorefinery

Product	Revenue Potential (\$MM/yr)	Yield (ton/yr)	Global Market Volume (ton/yr)	Process Complexity	Industry Involvement
Primary fuel product for reference					
Hydrocarbon fuels (from lipids)	\$40.7	12.8 MM gal/yr	56,900 MM gal/yr (U.S. Consumption)	Low	Cellana, GAI
Sugars					
Succinic acid (from sugars + glycerol)	\$136.3	78,000	441,000	High	Myriant, Bioamber, Succinity/BASF, Reverdia
Hydrocarbon fuels (from sugars + glycerol)	\$20.7	6.4 MM gal/yr	56,900 MM gal/y (U.S. Consumption)	Medium	Amyris, LS9
Lipids					
Surfactants from sterols	\$16.6	7,000	6,414,000	Medium	BASF, TerraVia
Polyols via polyunsaturated fatty acids	\$24.1	15,200	8,047,000	High	Cargill, Dow, Urethane Soy Systems, Bio-Based Technologies, Algenesis
Protein					
Protein → C4+ OH (SNL/Liao process)	\$9.7	9,100	734,400 as isobutanol 36,400 as plasticizer	Medium	Early R&D
Animal/fish feed	\$4.0 - \$16.0	45,700	16,538,000 - 190,126,000	Low	Mars, GAI, Cellana
Bioplastics	\$41.8	69,900	1,545,000	Medium	Algix
<i>Galdieria</i> via mixotrophic growth on protein stillage + HTL	\$20.3	6.4 MM gal/year	56,900 MM gal/year (U.S. Consumption)	Medium	Early R&D

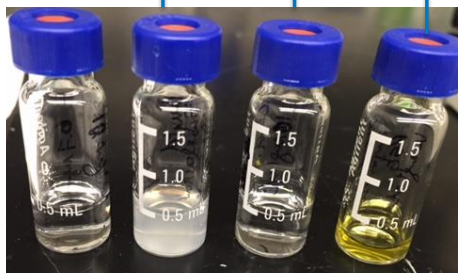
*Based on unit farm and mid-harvest *Scenedesmus* biomass and demonstrated composition, theoretical recovery

[R. Davis (NREL) FY16 milestone report]

Sterols Isolated and Converted to Surfactants

- Unsaponifiable lipids present in **CAP-oils**, not valorized in current HDO/HI process
- Isolated sterols and phytol providing value beyond basic CAP process
- **Non-ionic surfactant markets growing exponentially ~ 6,400,000 ton/yr**
 - e.g. alkylphenols used as antioxidants, lubricating oil additives, detergents, emulsifiers, paints ... (346,000 ton/yr)
- Commercial demand increasing for **bio-derived and bio-degradable surfactants (e.g. BASF)**

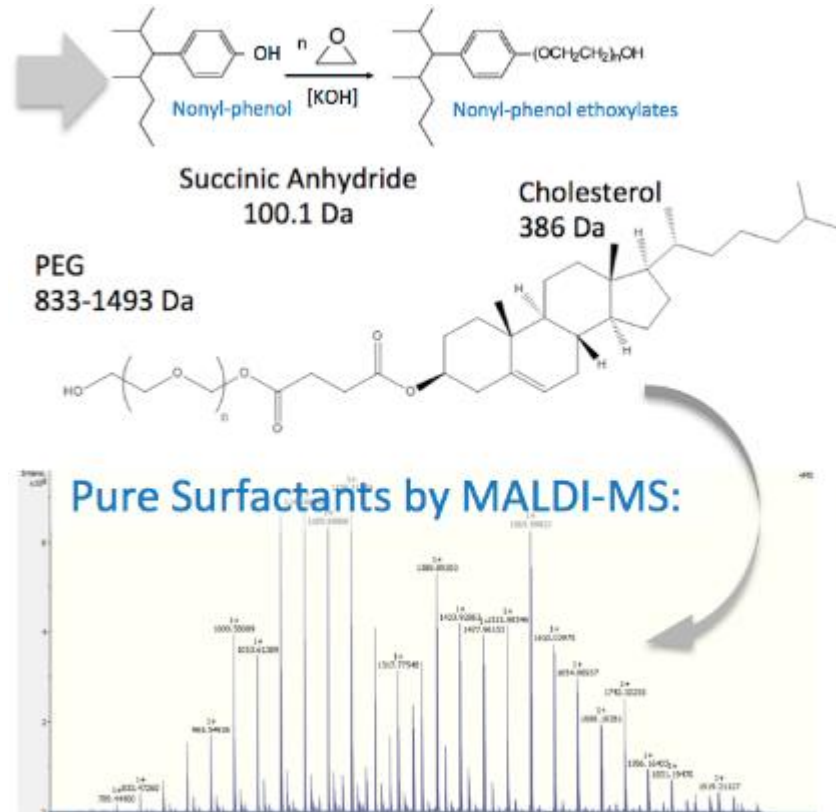
PEG₁₀₀₀
Phytol-SA-PEG
Cholesterol-SA-PEG
Algae-sterols-SA-PEG



[FY16 Q3 Milestone]

	% sterols	% Phytol
Scenedesmus CAP oil	2.8	1.4

Ergosterol	Stigmasterol	Ergosterol-like
3.68 ± 0.11	1.6 ± 0.1	29.6 ± 0.5



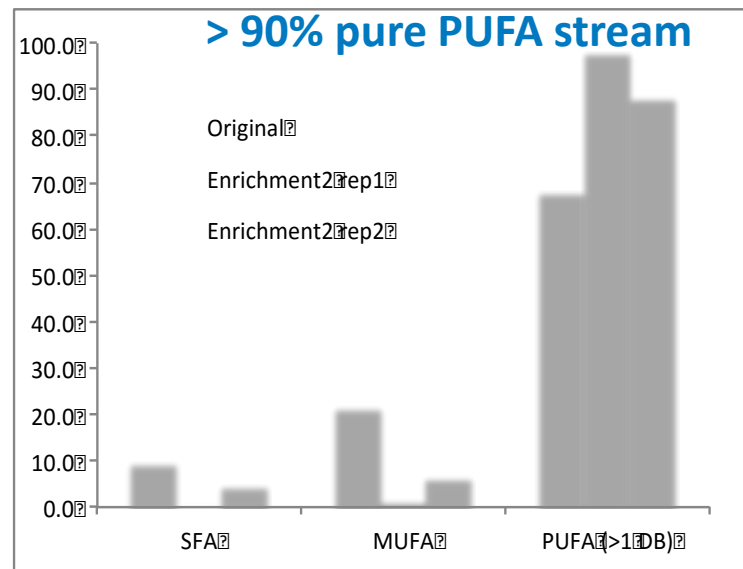
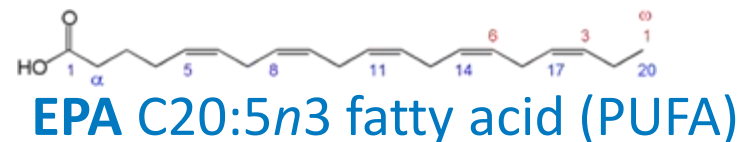
Polyunsaturated Fatty Acids Enriched from CAP Oils

- Oil fatty acid composition drives both fuel specifications and value of oleochemicals
- Removing long chain fatty acids from HDO stream, reduces yields, but improves oil properties and provides high-value product stream
- Polyunsaturated fatty acids (PUFAs) for polyol and polymer production can be purified for novel properties

PUFA concentrations are dynamic over growth:

	C16:0	C16:4	C18:1n9	C18:2n6	C18:3n3	C18:4n3
<i>Chlorella</i> [early]	15.9	12.4	3.7	9.6	34.0	0.0
[late]	22.0	1.4	26.0	20.1	12.1	0.0
<i>Scenedesmus</i> [early]	16.9	12.3	6.2	7.8	38.2	2.5
[late]	22.1	2.3	31.2	11.7	14.8	1.5

	<i>Scenedesmus</i>	<i>Chlorella</i>	<i>Nannochloropsis</i>	Linseed	Soy
C14	1.3	1.1	5.4	0	0.5
C16	18.4	11.5	15.6	5.4	8.5
C16:1n9	3.6	0.7	19.4	0	0
C18	1.3	1.1	0.3	3.5	4.0
C18:1n9	5.9	3.5	5.2	19.0	28.2
C18:2	14.1	11.4	4.1	24.0	49.2
C18:3	31.5	34.9	0	47.0	7.4
C20	1.0	0	0	0	0
C20:4	0	0	6.1	0	0
C20:5	0	0	38.7	0	0
C22	1.9	0	0	0	0
C22:1n9	1.2	0.8	0	0	0
C24	1.6	1.1	0	0	0



ROI 17-29 submitted, 2017: "Method Of Purification Of Polyunsaturated Fatty Acids From Complex Mixtures To Generate Novel Oil Compositions"

Extracellular Polymeric Substances, Problem or Opportunity?

- Extracellular polymeric material largely unknown (unaccounted for) carbon sink in growth media, consists of polysaccharide/protein **heterogeneous co-polymer**, containing valuable sugars (rhamnose, galactose, ...)
- **Problem:** Loss of productivity and complicates water recycling by fostering bacterial contamination
- **Opportunity:** Could be valorized as high-value biodegradable emulsifier, e.g. in fracking fluids

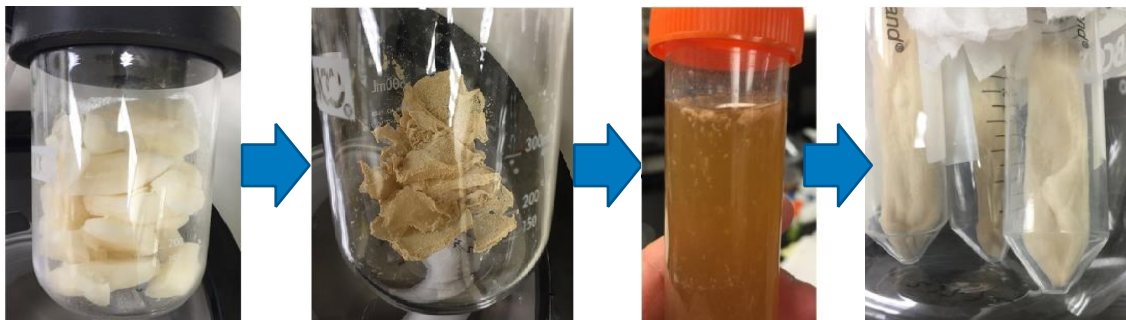
13% of assimilated carbon lost to cultivation media



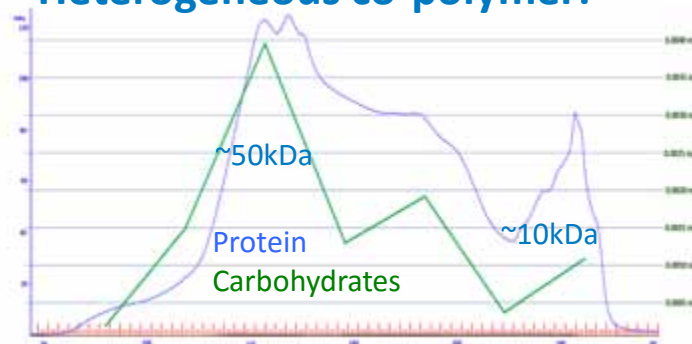
Species	mg/L	% biomass
<i>Chlorella vulgaris</i> UTEX 395	134 - 167	10 - 18
<i>Desmodesmus</i> sp.	165 - 181	6 - 7
<i>Desmodesmus abundans</i> UTEX 2976	19 - 40	1.8-2.1
<i>Scenedesmus obliquus</i> UTEX 393	22	2.4

Elemental Composition of *Chlorella* EPS

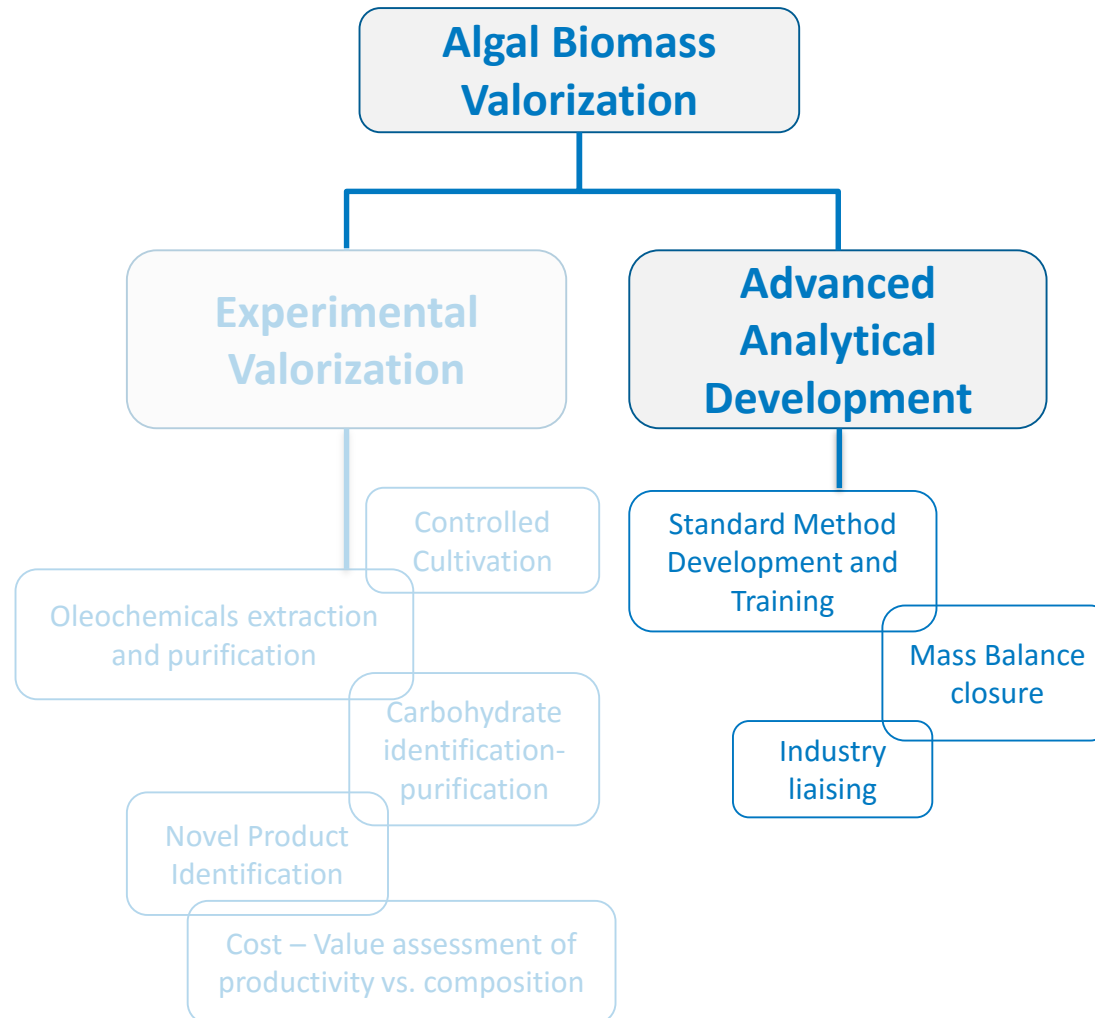
C %	H	N	O
35.3	5.8	3.3	43.5



Heterogeneous co-polymer:



Approach

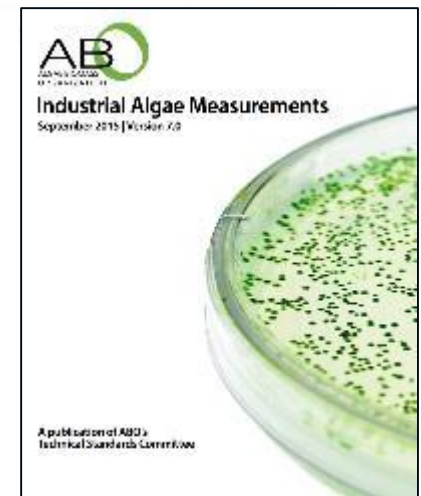
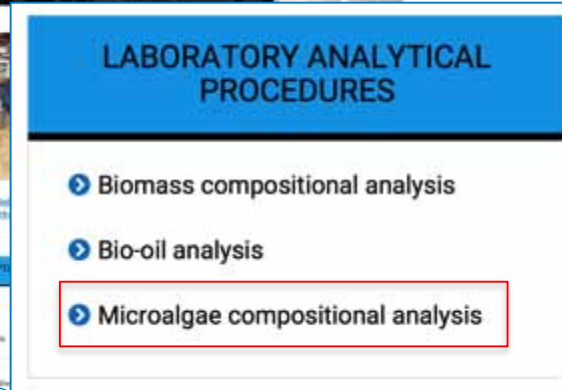


Standard Methods for Algae

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

- Implemented methods with rigorous QC protocol across ATP³ consortium and trained analysts from 5 different laboratories
- Open Access methods updated in 2015 to reflect improvements
- November 2016 – Organized a 4-day Analytical Training workshop for newly awarded PACE consortium
- Developed 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 7.0) in 2015

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



Relevance

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

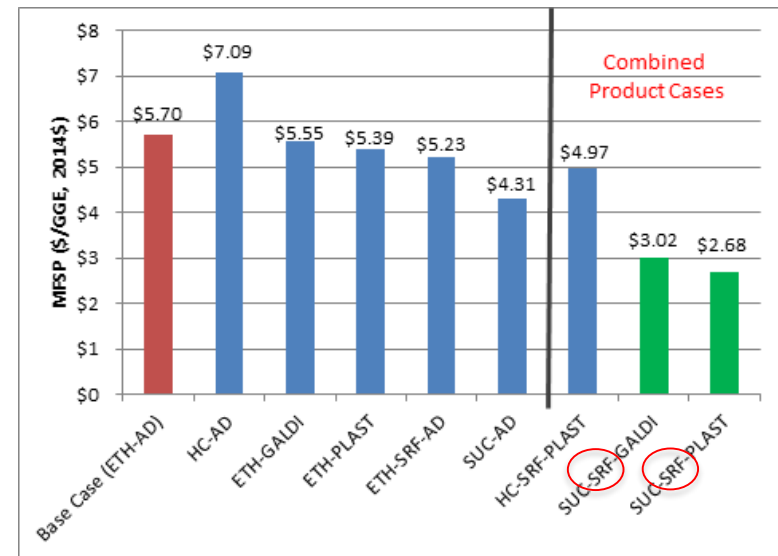
- Algae farm of **5,000 acres** produces **190,000 ton** biomass:

- 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)

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 the first bioproducts included in the Algae MYPP (2016)
- 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

Valorization R&D Commercial Relevance:



* Based on experimental recovery data

Relevance

Stakeholder Outreach and Engagement:

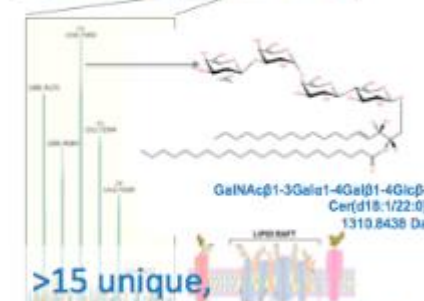
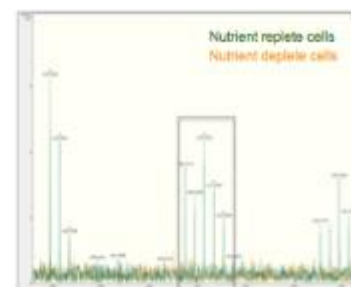
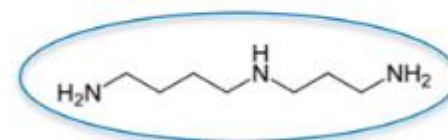
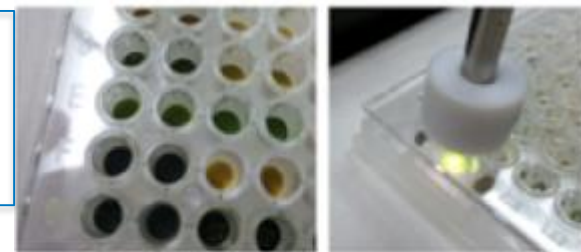
- Enabling algae industry with guidance on uniform descriptions on biomass characterization and subsequent valorization and trading standards
- Methods have received > 3,500 downloads and numerous groups are implementing integrated compositional analysis workflow and spreadsheet
- Harmonized characterization of materials of algae implemented across ATP³ network
- Worked with and trained two commercial algae groups and hosted training workshop
- Transferred methods to ABC, TEA, DISCOVER, ABP, ATP³, 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
- Published 9 papers + 3 submitted, 4 book chapters (1 published, 3 submitted), State of Technology of Algae Bioenergy (IEA) report, and filed 2 records of invention

The collage features several documents and images related to algae bioenergy research. At the top left is the NREL logo. Below it is a document titled "Determination of Total Carbohydrates in Algal Biomass Laboratory Analytical Procedure (LAP)" with an issue date of December 2, 2013. To the right of this document is a large blue text box stating: "> 8,600 method access" and "> 3,500 downloads (December 2016)". Below the LAP document is another NREL logo and a document titled "Industrial Algae Measurements" dated September 2015. At the bottom left is a report titled "State of Technology Review - Algae Bioenergy" with a green and yellow cover. At the bottom right is a photograph of a petri dish containing a green, textured substance, likely algae biomass.

Future Work

FY17-19: Capture and model the **impact of cultivation parameters with biomass composition, products and value**

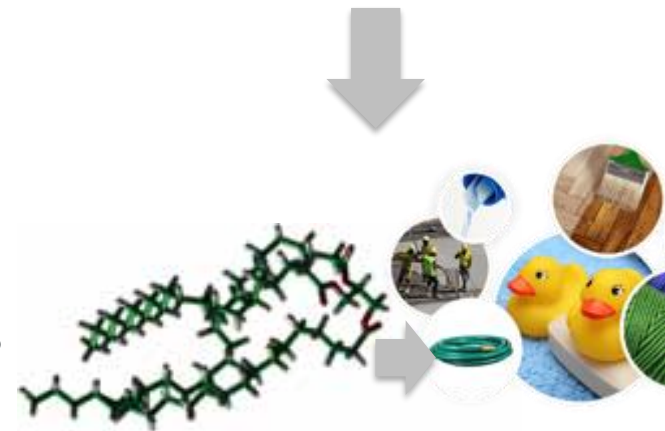
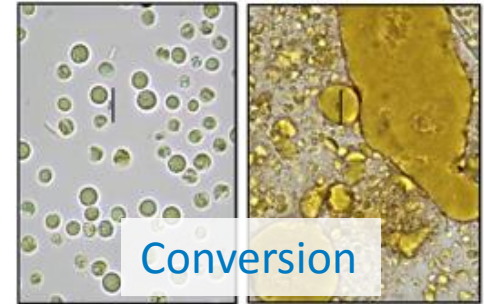
- Map productivity kinetics, carbon balance and composition and bioproducts for novel, commercially relevant species (DISCOVER, ABP, ATP³)
- Expand product portfolio with protein-derivative products and polyamines as biopolymer crosslinkers
- Implementation of ultra-high resolution mass spectrometry platform for detailed metabolic fingerprinting of new species – make data on product composition in biomass openly available (web interface)
- Work with ABC 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
- **Integrate co-product concepts within 2022 state of technology demonstration**



>15 unique, differentially expressed cell-wall ceramides discovered

Summary

- 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
 - Novel **bio-derived surfactants**; isolated algae-based sterols and phytol and identified route to novel synthesis route
 - Novel process developed for **PUFA enrichment** and purification for biopolymer evaluation
 - Purification and characterization of **extracellular polymers** for improved cultivation or co-product opportunities
- On track to identify and isolate additional bio-products with high-value, large market components to further **increase biomass value**
- **Standard procedures** providing basis for common language gaining traction in algae community and will enable and help drive commercialization





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Thank You!



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www.nrel.gov



<http://www.nrel.gov/bioenergy/algal-biofuels.html>

Responses to Reviewers' Comments from 2015

COMMENT:

The establishment of standard methods is very valuable as long as they are not too onerous, and should have been emphasized more, with some indication of how much more (or less) complex they are than the 'standard methods' that are being replaced, and how much more or less time and money will be required to do them. To my mind, as an experimentalist, it is important to use the right level of characterization at the right time - thorough quantitative work is only worthwhile after scoping studies tell us which are the important samples

RESPONSE:

We agree that in not all cases the same depth of molecular characterization is needed. In most applications, basic biomass compositional information is sufficient to understand the biomass quality and ultimately the approximate value, assuming routine methods allow for higher than 80-90% mass balance closure, without double counting. However, as the discussion in the algae industry and academic environment is shifting from fuels-only to fuels-and-coproducts approaches, the discovery of high-value products in algal biomass and the methods to be able to characterize those products is critical to allow this part of the industry to move forward and thus the methods we have developed will be needed. As metabolic engineering ramps up the in-cell rewiring of networks to produce more or different lipids, there is an ongoing requirement for both rapid assessment of the crude composition as well as the detailed molecular fingerprinting, the two approaches pursued in this project need to be developed simultaneously but not necessarily implemented on the same samples.

COMMENT:

Dr. Laurens' work has cemented its position as the best and only analytical approach within BETO's portfolio, which allows comparison between different projects to see which technologies do indeed yield better results. It would benefit the program to develop an implementation and quality control plan for other labs that use these methods. The measurement development and distribution will enable many programs by providing credible methods for quantifying progress. Communicating the methods critical to all algal BETO programs. It is how we measure success.

RESPONSE:

We agree that there is a need to establish unity across the BETO platform and the algae industry as a whole to at least discuss a common language approach to the issues at large. The requirement for measuring progress will be supported by the availability of detailed carbohydrate, lipid and protein content and profile. We have in the past two years implemented and analyzed the results of an across-platform implementation of standard compositional analysis methods and data management for the ATP³ network. This implementation was successful in that quality metrics accompanied the data and made the analysis of inter laboratory trends highly accessible. The results from this program are disseminated in peer review publications to make the framework accessible to other groups.

COMMENT:

When a facility is continuously harvesting (daily), the early/mid/late distinction will be homogenized. These results were getting at a critical question -- when to harvest biomass for maximum conversion of energy content -- but the content became lost in translation. Resolving optimal time of harvest in a continuously harvesting facility is a contribution Dr. Laurens' project is quite capable of, and that BETO desperately needs.

RESPONSE:

In the context of developing co-product options, maximizing the value of biomass to help support the economics of a processing plant is critical. It is with this in mind that the early/mid/late harvesting scenarios and the relative compositional differences were presented. Answering the question on whether it makes sense to add the additional time/cost to the process to achieve biomass with a compositional ratio of major components more suitable for the recovery of high-value co-products is highly complex. The uncertainty around the optimal time to harvest is due to the highly complex and integrated nature of the production process with downstream conversion. This is a challenge that should be solved as a collaborative effort between the techno-economic analysis and the production groups (e.g. ATP³) through data mining of growth versus productivity and composition data) and this will be part of future collaborative work that this task will be driving.

Publications

1. Laurens, L. ML., Van Wychen, S., Pienkos, P.T., Harmon, V. L., McGowen, J. "Harmonization of Experimental Approach and Data Collection to Streamline Analysis of Biomass Composition of Algae in an Inter-Laboratory Setting" **Algal Research**, *Submitted*
2. Jarvis, J., Corillo, Y., Sudhasinghe, N., Dadamudi, K. P., Laurens, L. M.L., Schaub, T. (2016) "Multiple-pass Elemental Composition Assignment for FT-ICR MS Analysis of Lipids" **J. American Society for Mass Spectrometry**, *Submitted*
3. Laurens, L. ML., Templeton, D.W., Christensen, E., Van Wychen, S., Vadelius, E., Markham, J., Chen-Glasser, M., Dong, T., Davis, R., Pienkos, P.T. (2017) "Development of Algae Biorefinery Concepts for Biofuels and Bioproducts; a Perspective on Process-compatible Components" **Energy and Environmental Science**, *Submitted*
4. Van Wychen, S., Long, W., Black, S., Laurens, L. ML. (2017) "MBTH: A novel approach to rapid, spectrophotometric quantitation of total algal carbohydrates", **Analytical Biochemistry**, 518, 90-93
5. Dong, T., Knoshaug, E.P., Pienkos, P. T., Laurens, L. ML.* (2016) "Lipid recovery from wet oleaginous microbial biomass for biofuel production: a critical review", **Applied Energy**, 177, 879-895 doi:10.1016/j.apenergy.2016.06.002
6. Dong, T., Knoshaug, E., Davis, R., Laurens, L. ML., Van Wychen, S., Pienkos, P.T., Nagle, N. (2016) "Combined Algal Processing: A novel integrated biorefinery process to produce algal biofuels and bioproducts" **Algal Research**, 19, 316-323 doi:10.1016/j.algal.2015.12.021
7. Dong, T., Van Wychen, S., Nagle, N., Pienkos, P.T., Laurens, L. ML.* (2016) "Impact of Biochemical Composition on Susceptibility of Algal Biomass to Acid-Catalyzed Pretreatment for Sugar and Lipid Recovery" **Algal Research**, 18, 69-77 doi:10.1016/j.algal.2016.06.004
8. Ma, J., Zhao, Q., Laurens, L. ML., Jarvis, E., Nagle, N., Chen, S., Frear, C. (2015) "Mechanism, kinetics and microbiology of inhibition caused by long chain fatty acids in anaerobic digestion of algal biomass", **Biotechnol Biofuels** 2015, 8:141
9. Vandamme, D., Beuckels, A., Vadelius, E., Depraetere, O., Noppe, W., Dutta, A., Foubert, I., Laurens, L. ML., Muylaert, K. (2015) "Inhibition of alkaline flocculation by algal organic matter for *Chlorella vulgaris*" **Water Research**, 88(1), 301-307 doi:10.1016/j.watres.2015.10.032
10. Christensen, E., Sudasinghe, N., Dandamudi, K.P.R., Sebag, R., Schaub, T., Laurens, L. ML.* (2015) "Rapid Analysis of Microalgal Triacylglycerols with Direct-Infusion Mass Spectrometry" **Energy and Fuels**, 29(10) 6443-6449 doi:10.1021/acs.energyfuels.5b01205
11. Laurens, L. ML.*, Slaby, E. F., Clapper, G., Howell, S., Scott, D. (2015) "Algal Biomass for Biofuels and Bioproducts; Overview of Boundary Conditions and Regulatory Landscape to Define Future Algal Biorefineries" **Industrial Biotechnology**, 11(4), 221-228
12. Templeton, D. W., Laurens, L. ML.* (2015) "Nitrogen-to-protein conversion factors revisited for applications of microalgal biomass conversion to food, feed and fuel" **Algal Research** 11:359-367 doi:10.1016/j.algal.2015.07.013
13. 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", Bookchapter, ed. J. Pires, Publishers Bentham Science Publishers, *Accepted for publication*
14. 3 Bookchapters in **Methods in Molecular Biology**: "Total fatty acid content determination from whole microalgal biomass using in situ transesterification", "Total carbohydrate content determination of microalgal biomass by acid hydrolysis and liquid chromatography" & "Total protein content determination in microalgal biomass by whole biomass combustion and elemental nitrogen analysis" *Submitted*

Presentations

1. “Renewable Bioenergy, Biofuels and Bioproducts Applications at NREL – Discoveries Driven by Ultrahigh-Resolution Mass Spectrometry” Invited presentation at Bruker Daltonics VIP event, Dallas, TX, 2017
2. “State of Technology Review on Algae Bioenergy” Laurens, L., McMillan, J. **Webinar**, IEA Bioenergy, 2017
3. “Algae Biorefinery Development for Biofuels and Bioproducts: A Perspective on Renewable Bioenergy” Laurens, L. Invited Oral presentation, Stevens Institute, Hoboken, NJ, 2016
4. “Co-products Value Proposition” Laurens, L. Invited Oral presentation, DOE **BETO Algae Biology Toolbox Workshop**, San Diego, CA, 2016
5. “Algae Biorefinery Development for Biofuels and Bioproducts” Laurens, L. Invited Oral plenary presentation, **DOE Bioenergy 2016**, Washington DC
6. “Sterol Molecular Fingerprinting for High-Value co-Product Synthesis” Palardy, O., Laurens, L. & “Dynamic Composition of *Nannochloropsis* sp. High-Value Omega-3-Fatty Acids at 5 Locations” Laurens, L., Harmon, V., Knoshaug, E., Dempster, T., Pienkos, P., McGowen, J. Oral presentations at 7th **Algae Biomass Summit**, Phoenix, AZ, 2016
7. “Dynamic Composition of *Nannochloropsis* sp. High-Value Omega-3-Fatty Acids at 5 Locations” Laurens, L., Harmon, V., Knoshaug, E., Dempster, T., Pienkos, P., McGowen, J. & “Biochemical Composition Impact on Acid Catalyzed Pretreatment of Algal Biomass for Fuels” **AOCS annual meeting**, Salt Lake City, UT, 2016
8. “Review of 2013-2015 Unified Field Studies of Algal Composition at ATP3 testbeds”, Laurens, L., Harmon, V., Knoshaug, E., Dempster, T., Pienkos, P., McGowen, J. Panel Oral presentation, **BIO World Congress**, San Diego, CA, 2016
9. “Dynamic composition of *Nannochloropsis* sp. biomass with an emphasis on high-value omega-3 fatty acids”, Laurens, L., Harmon, V., Knoshaug, E., Dempster, T., Pienkos, P., McGowen, J., Oral presentation, **International Conference on Algal Biomass, Biofuels and Bioproducts**, San Diego, CA, 2015
10. “Combined Algal Processing: A Novel Process for Biofuel and Bioproducts from Algal Biomass”, Pienkos, P., Dong, T., Knoshaug, E., Laurens, L., Davis, R., Nagle, N., Oral presentation, **International Conference on Algal Biomass, Biofuels and Bioproducts**, San Diego, CA, 2015
11. “The interplay between microalgal biology and flocculation”, Vandamme, D., Foubert, I., Laurens, L., Muylaert, K., Oral presentation, **International Conference on Algal Biomass, Biofuels and Bioproducts**, San Diego, CA, 2015
12. “Computational Data Mining and Visualization of Ultra-high Resolution Mass Spectrometry Data Sets for Microbial Lipids” Nag, A. and Laurens, L. Poster presentation at **37th Symposium on Biotechnology for Fuels and Chemicals**, San Diego, CA, 2015
13. Session chair, “Algal and Other Non-traditional Oil Characterization”, **AOCS annual meeting**, Orlando, FL, 2015
14. “Recovery of Biofuel Precursors from Methanotrophic Bacteria via an Efficient Two-stage Pretreatment Approach”, Dong, T., Laurens, L., Pienkos, P., Oral presentation, **AOCS annual meeting**, Orlando, FL, 2015
15. “Radiant energy vs. organic carbon: Algal lipid profile diversity in relation to cultivation and conversion parameters”, Black, B., Christensen, E., Dong, T., Schaub, T., Laurens, L., Oral presentation, **AOCS annual meeting**, Orlando, FL, 2015
16. "Algal Biomass and Product Yields as Critical Elements of a Growing Industry", Oral presentation, **3rd European Workshop**, Life Cycle Analysis of Algal based Biofuels & Biomaterials, Brussels, Belgium, 2015

Commercialization Outreach

- **Record of Invention:**
 - ROI 17-29 *submitted*, 2017: “Method Of Purification Of Polyunsaturated Fatty Acids From Complex Mixtures To Generate Novel Oil Compositions” Spinelli, P., Laurens, L.
 - ROI 17-11 "Using amino acid (peptide)-based crosslinkers to produce novel renewable polyurethane products” Dong, T., Laurens, L., Pienkos, P.
- **State of Technology Review – Algae Bioenergy**, an IEA Bioenergy Inter-Task Strategic Project (2017), reviewing the status and prospects for using micro- and macroalgae as feedstocks for biofuels and bioenergy. The report includes international activities advancing algae-derived bioenergy applications, and conversion pathways: <http://www.ieabioenergy.com/publications/state-of-technology-review-algae-bioenergy/>
- Procedures on current best practice of microalgal compositional analysis, implemented and adopted across algae industry:
 - Summative Mass Analysis of Algal Biomass – Integration of Analytical Procedures www.nrel.gov/docs/fy16osti/60943.pdf
 - Determination of Total Solids and Ash in Algal Biomass www.nrel.gov/docs/fy16osti/60956.pdf
 - Determination of Total Lipids as Fatty Acid Methyl Esters (FAME) by *in situ* www.nrel.gov/docs/fy16osti/60958.pdf
 - Determination of Total Carbohydrates in Algal Biomass www.nrel.gov/docs/fy16osti/60957.pdf
 - Calculation of N-to-Protein Conversion Factor
- Chair of **ABO’s Technical Standards Committee**, interaction with leading industry partners (www.algaebiomass.org/resource-center/technical-standards/introduction)
- In depth analytical training carried out for **Cellana** analytical team (joint with ATP³) in 2016
- Advanced characterization agreement between NREL and **Sapphire Energy** inc. and partnering for ABY project, tailoring HTL oil characterization linked with biomass composition
- Planning future integration of physiological cultivation tool and data to BAT model
- **Outstanding Contribution Award – Algae Biomass Organization** (2015) for leading the Technical Standards development of the Industrial Algae Measurements v. 7.0

Back up slides

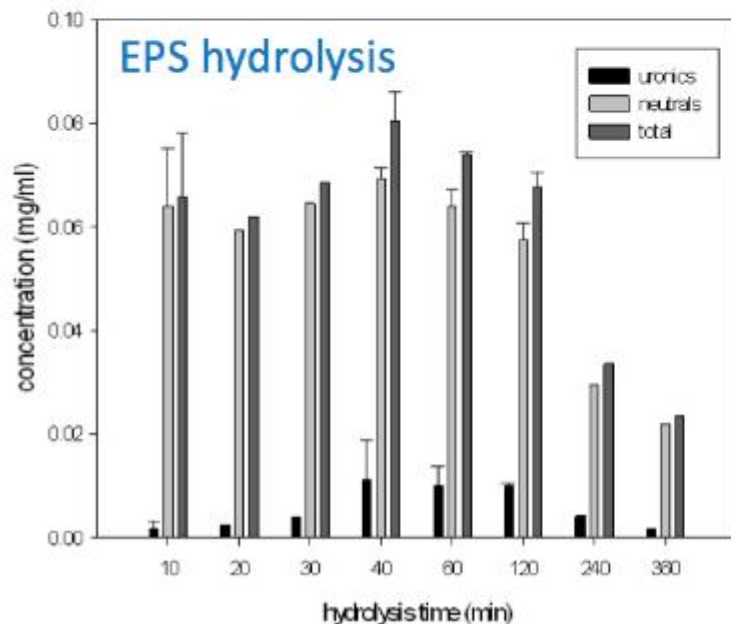
Algae Biorefinery Potential – high volume products

Biomass components	wt %	Product	Market* (ton/yr)
Fatty acids	10-45	Hydrocarbon fuel products (U.S. consumption)	16,000,000
Omega-3-fatty acids	3-10	Polyols – epoxy resin – polyurethane	8,000,000 – 11,000,000
	3-10	Nutraceuticals	22,000
Hydroxy-, branched-, fatty acids/alcohols	~1	Surfactants, fuel additives	3,500,000
Sterols	2-4	Surfactants	6,400,000
	2-4	Phytosterol nutra-/pharmaceuticals	25,000
	2-4	Emulsifiers	N/A
Phytol	3-4	Raw material for vitamin E, fragrance, soaps...	1
	3-4	Surfactants, fuel additives	3,500,000
Polar lipids	10-35	Ethanolamine	600,000
	10-35	Phosphatidylcholine, phosphoinositol and phosphatidyl ethanolamine (lecithin)	20,000-30,000
Glycerol	2-6	Di-acids for nylon production	2,500,000
	2-6	Feed, pharmaceuticals	25,000
Fermentable sugars (glucose, mannose)	10-45	Polylactic acid (PLA) polymers	300,000
	10-45	Di-acids (e.g. adipic acid)	2,500,000
	10-45	Ethanol	60,000,000
Mannitol	3-6	Polyether polyols	2,300,000 [¶]
Alginate	~3-5	Alginate additives	N/A
Starch	5-40	Polysaccharide-derived bioplastics	2,000,000
Protein	19-40	Thermoplastics	5,000,000
Amino acids/peptides	19-20	Polyurethane	11,000,000
Amino acids/peptides	19-20	Biobutanol, mixed alcohol fuels	740,000
Whole biomass	100	Animal/Fish feed	16,000,000 –
			190,000,000

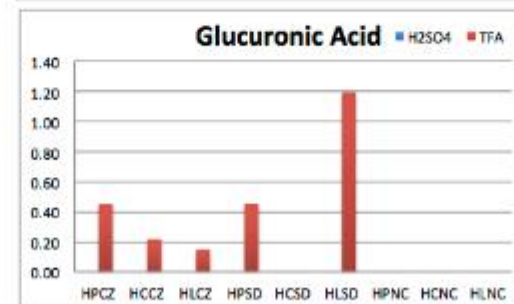
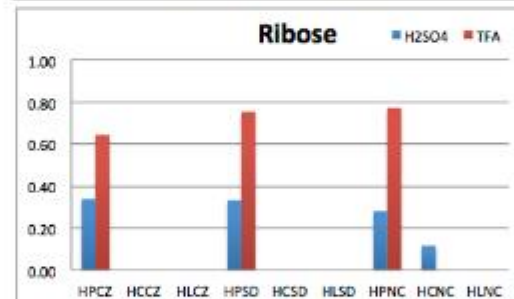
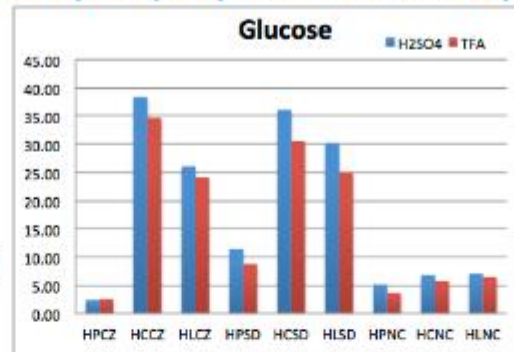
* Market size estimated based on displacement volumes ¶ based on sorbitol market size

Carbohydrates Structure-Function

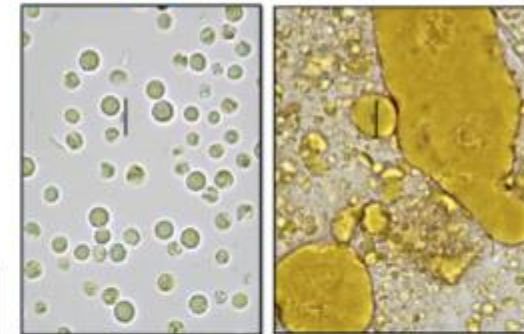
- Polymeric carbohydrates represent large component of biomass, structural, storage, excreted polymers
- Largest challenge on quantification
- Standardization agencies use 'subtraction' method to represent the carbohydrate fraction – doesn't inform on potential for conversion or valorization



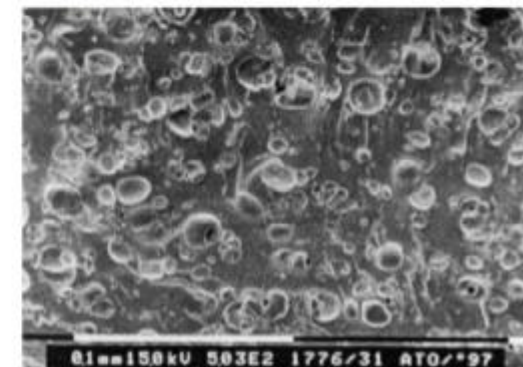
Hydrolysis polymer selectivity:



Structure-function of carbohydrates during conversion process, e.g. *Chlorella* indicates harsh pretreatment needed to access oils

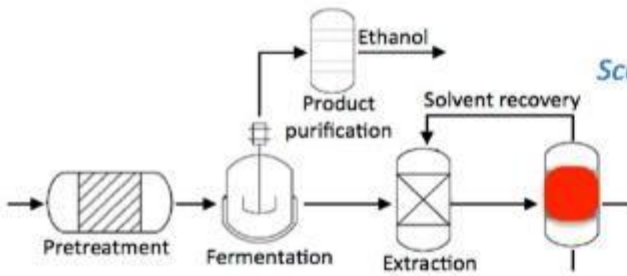


Stable non-separable starch-oil composites, with embedded oil droplets, can form at conversion temperatures

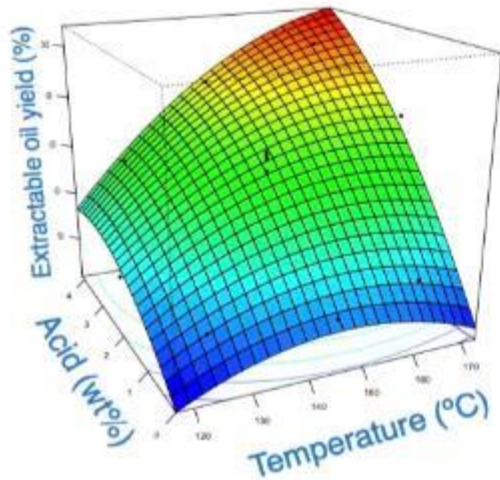


Eskins et al., (1996) Carbohydrate Polymers, 29:233-239

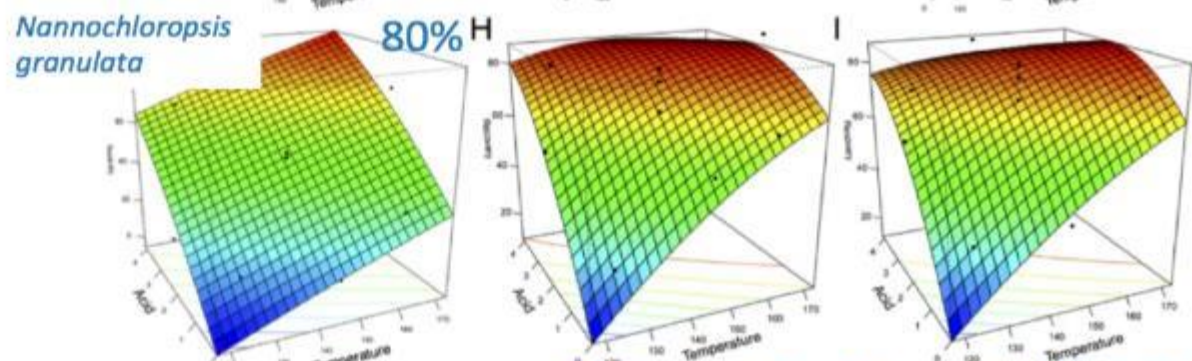
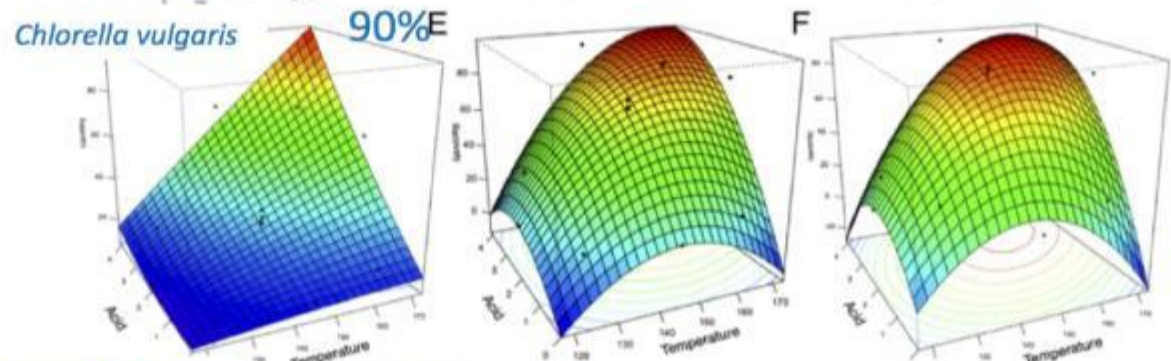
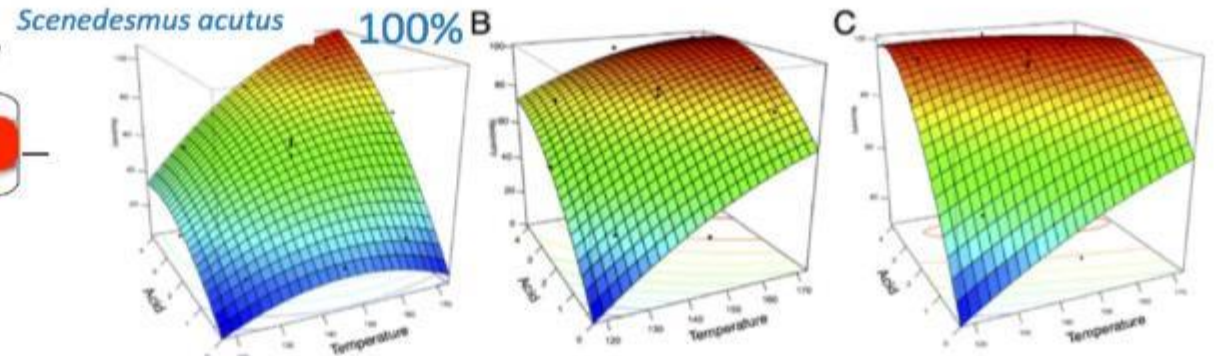
Impact of Biomass Composition on Conversion Susceptibility



Experimental Design of response of lipid extractability (mimic of CAP)



Early → Mid → Late harvest



T. Dong et al. Algal Research 18 (2016) 69–77

Analysis of compositional spaces favoring the selection of either CAP or HTL processing. No one process dominates across all feedstock compositions.

