DOE Bioenergy Technologies Office (BETO) 2015 Project Peer Review

1.3.4.300 Algal Biomass Valorization

BETO Algae Platform – Peer review
Alexandria, VA
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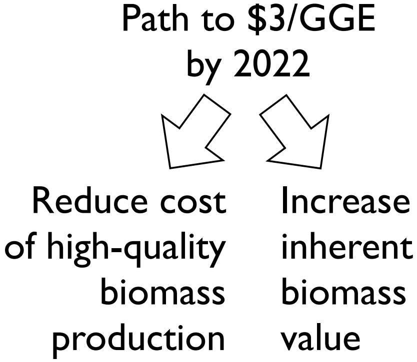
NREL is a national laboratory of the U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy, operated by the Alliance for Sustainable Energy, LLC.

Goal Statement

1. **Reduce cost of algal biofuels by increasing inherent algal biomass value**
   – Identify **key targets** to contribute to lowering the overall cost of algal biofuels production
   – Integrate biomass composition with cultivation parameters and conversion performance characteristics, guiding process improvements and product quality
   – Perform quantification of major components supporting a **multi-product algal biorefinery model** based on NREL Design Report and tracking the fate of potentially value-added products
2. **Reduce uncertainty around techno-economic analysis (TEA) major process inputs and outputs**
   – Establish, disseminate and maintain **analytical tools**, methods and datasets, integrated with research, NREL internal (conversion) and external (ATP[3]) supporting Algae Common Language across BETO algae platform
   – Outreach to **trade organizations** for adoption and implementation, e.g. support from Algal Biomass Organization (ABO) through technical standards committee



Quad Chart Overview

Timeline

- **Start: 10/2012**
- **End: 9/2017**
- **Percent complete: 50%**
- **Existing project**

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

Budget

	Total Costs FY 10 – FY 12	FY 13 Costs	FY 14 Costs	Total Planned Funding (FY 15-Project End Date)
DOE Funded	0	638 K	873 K	2,789 K

Partners

- Partner (shared funding)
  - New Mexico State University (Dr. Tanner Schaub)
- Interactions/collaborations:
  - Algae Testbed Public-private partnership (ATP^3)
  - Algal Biomass Conversion (ABC)
  - Algae Techno-economic Analysis (TEA)

Project Overview

Problem statement: High level of **uncertainty around composition and value of algal biomass and changes during cultivation and processing**

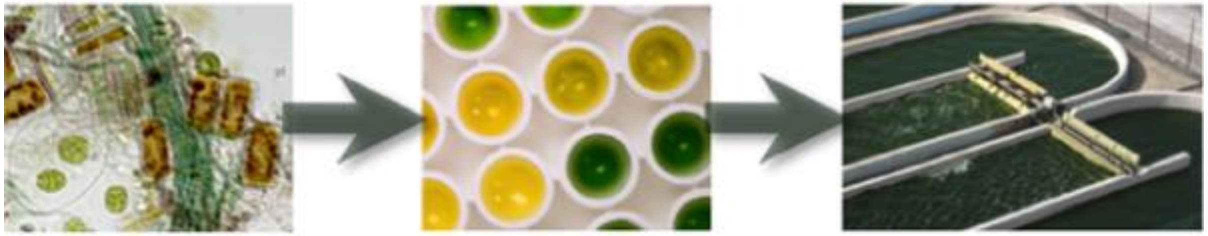
1. Impactful research into **algal biomass composition** for select algal strains
   - Algal biomass composition definitions poorly defined in literature, with highly disparate knowledge about composition between strains and growth conditions
   - FY13 and 14 focused on component discovery, method development and initial mapping of a baseline composition of biomass to establish a link between advanced analytical characterization and biochemical reshuffling of macromolecular components over cultivation
   - 30-40% lack of mass balance closure, translates to significant uncertainty regarding overall biomass value

2. Establish **relationship between biomass composition and cultivation and conversion** characteristics
3. Establishment of **standard methods** for transparent, harmonized data collection across BETO algae platform
   - Biomass methods used throughout BETO projects, including a collaborative multi-testbed site project like ATP^3

Approach - Technical

1. **Increase inherent value of algal biomass**
   - Demonstrate dynamic composition and quantitative short comings for **mass balance accounting**, lipids, protein and carbohydrate under controlled cultivation
   - Identify and isolate **high-value co-products**, scalable and compatible with novel multi-product biorefinery pathway established in ALU design case
   - Develop and validate rapid **high-throughput** compositional analysis technologies

2. **Reduce uncertainty around major process inputs and outputs**
   - Establish **laboratory procedures** for algal biomass analysis
   - Disseminate procedures online as freely downloadable documents
   - Collaborate with TEA group to quantify the impact of uncertainty on process and product cost



Approach – Management

1. Project progress tracked through milestones and quantitative target metrics, quarterly progress reports and peer-reviewed publications
2. Yearly update to online standard procedures
3. Research integrated with conversion and cultivation R&D projects
4. Close collaboration with TEA group

Critical success factors:

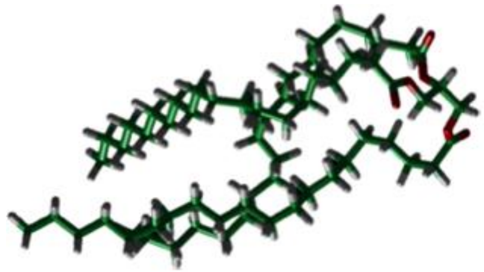
- Leverage knowledge of biomass composition to **tailor novel, highly efficient and economical conversion pathways**
- Identification of **novel high-impact components** that translate to commercial algal strains

Challenges:

- Potential risk that co-product options are highly **strain and process dependent** and do not represent entire algae value-chain
- Analytical methods difficult to implement across laboratories, which **compromises the value of data sets** collected -> increase uncertainty and biomass value assessment

Accomplishments – Biomass composition valorization

- Characterized primary and co-product components of algal biomass; **lipids, carbohydrates, protein**
- Selected strains relating to BETO projects:
  - ***Chlorella vulgaris***
  - ***Scenedesmus acutus***
  - ***Nannochloropsis granulata***
- Identified compatible and scalable co-products based on **oleochemistry** of novel lipids, carbohydrate and amino acid composition
- Demonstrated **high-throughput** biomass analysis technology
- Developed **standard methods** and implemented across collaborative ATP^3 project

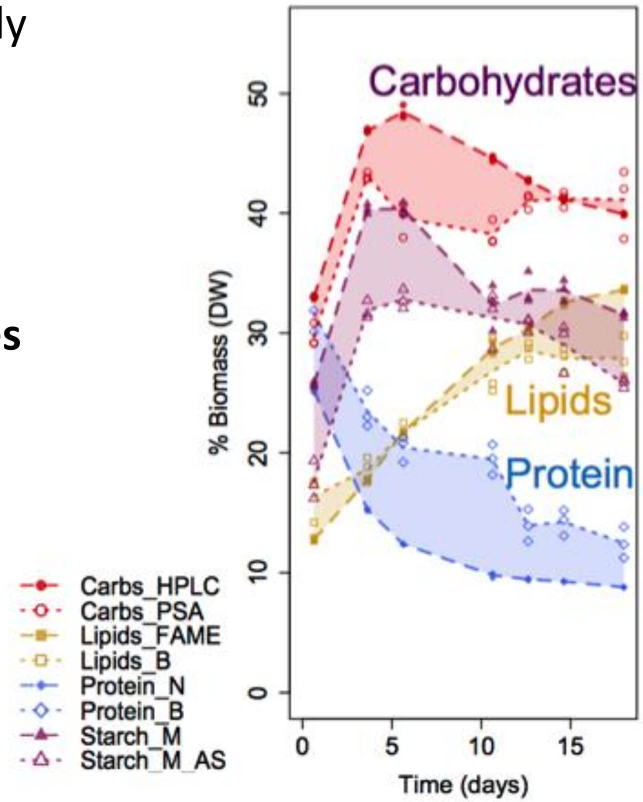


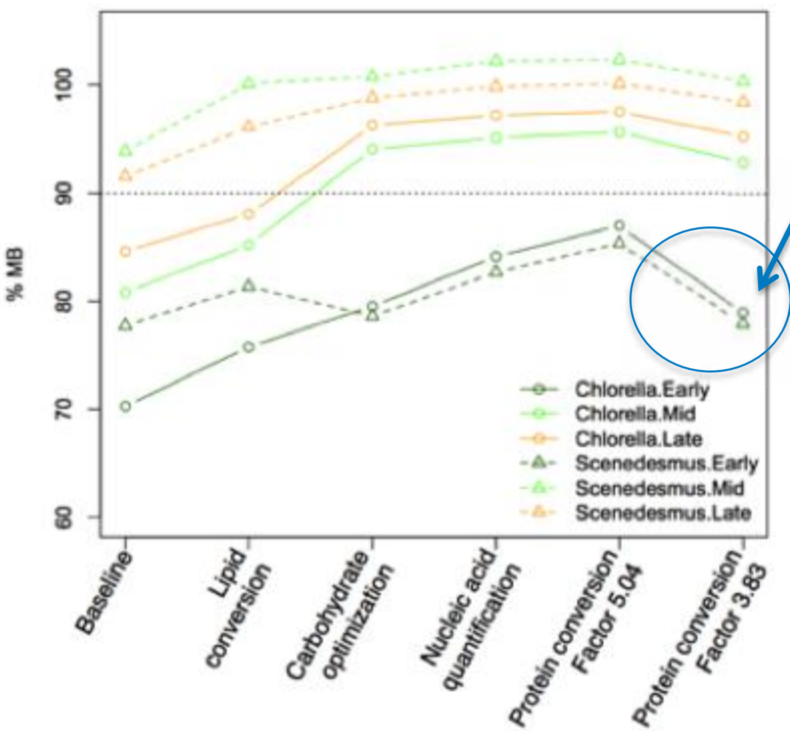
Biomass components:	**Wt %**	**Product ***
Raw Biomass	100%	Food/Feed products
Lipid (fatty acyl)	31%	Diesel fuel
Polyunsaturated fatty acids	3-4%	**Epoxies, polyols**
Branched chain fatty acids	~1%	**Fuel additives, surfactants**
Hydroxy fatty acids	~1%	**Surfactants, biopolymers**
Phytol	3-4%	**Surfactants, fuel additive**
Triglycerides	8-53%	**Biopolymers, coatings, Rubber**
Fatty alchohols	~2%	**Surfactants**
Sterols	2-10%	**Surfactants**
Glycerol	5%	**Di-acids for nylon production**
Carbohydrate monomers	25%	**Fermentation products**
Alginate	~10%	**Alginate additives**
Starch	5-40%	**Starch-derived bioplastics**
Protein	19-40%	**Thermoplastics**
Amino acids/peptides	19-20%	**Polyurethane**

** as scalable co-products in a biorefinery setting of a 10,000 acre algae farm*

Mass Balance Accounting

- Baseline mass balance missing 20-30%, found to be highly dependent on strain and physiological conditions
- Improved mass balance accounting through carbohydrates, lipids, nucleic acids, protein, pigments, inorganics
- 80% of biomass value derived from **Lipids, Carbohydrates and Protein**: higher resolution of individual components needed for full valorization

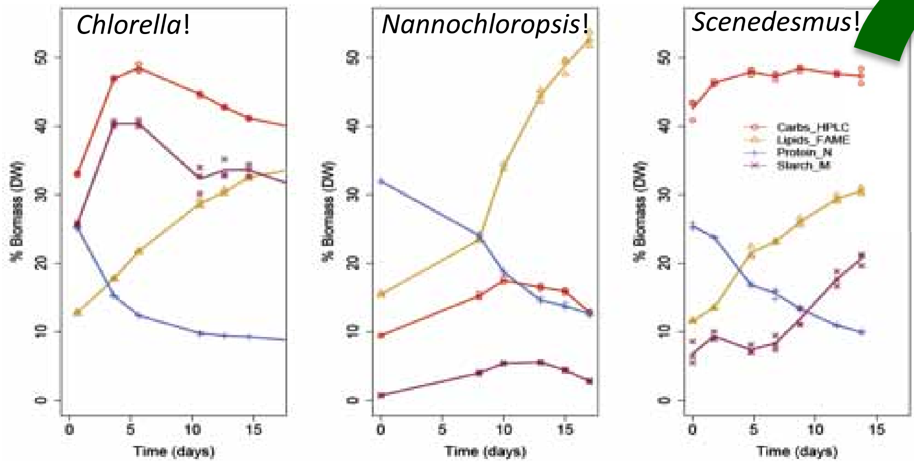


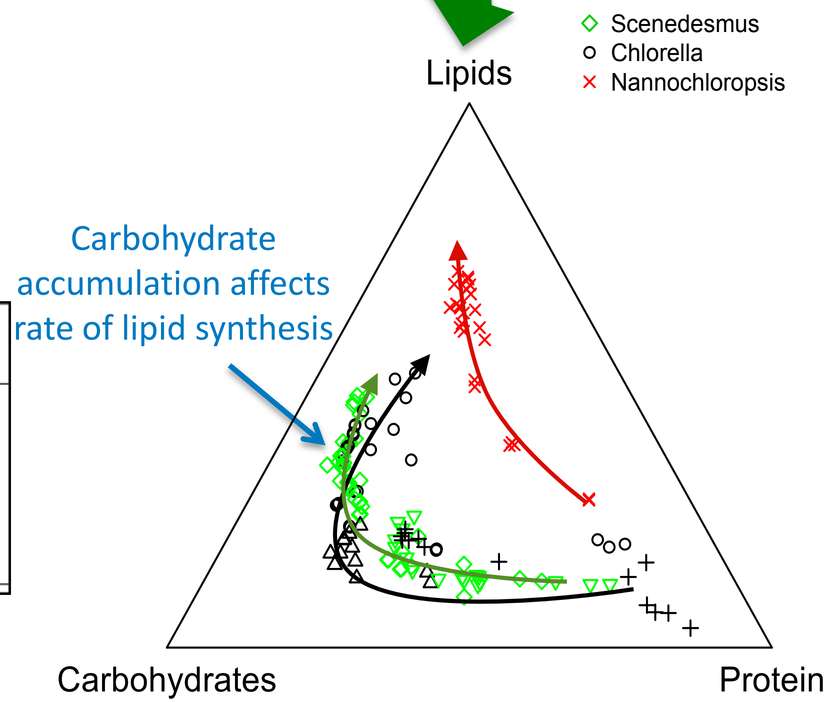


Strain	Harvest	Baseline Mass Balance (Oct. 2012)	Baseline Mass Balance (Sept. 2013)
Chlorella sp.	Early	70.3	78.96
Chlorella sp.	Mid	80.8	92.87
Chlorella sp.	Late	84.6	95.22
Scenedesmus sp.	Early	77.7	77.95
Scenedesmus sp.	Mid	93.9	100.31
Scenedesmus sp.	Late	91.5	98.45

Biomass Composition Trade-off

- Strain-specific macromolecular composition dependent on cultivation conditions, defining biomass quality
- Control over cultivation allows for improved biomass productivity by rigorous physiological control over biomass quality with CO_2 uptake and respiration

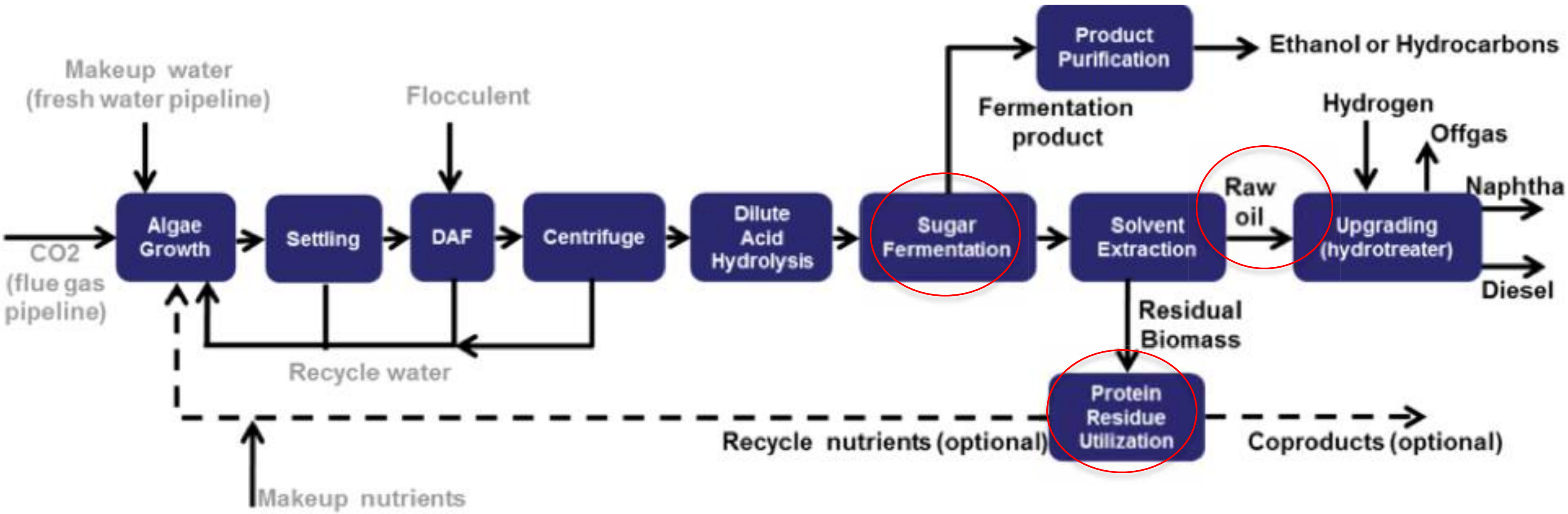




	Chlorella		*Scenedesmus*	
	early	late	early	late
Daily carbon uptake (mg)	54.7 ± 4.6	11.1 ± 1.6	155.1 ± 6.6	54.6 ± 4.2
Daytime carbon uptake (mg)	79 ± 2.5	19.3 ± 1.4	168.1 ± 7	58.6 ± 4.8
Nighttime respiration (mg)	-24.3 ± 4.3	-8.2 ± 0.5	-13.1 ± 1.7	-4 ± 0.6
Biomass accumulation (mg/day)	281	16	322	11
Biomass carbon (mg/day)	141	9	161	6

Scenedesmus appears to be more efficient at assimilating carbon dioxide from the influent gas stream compared with *Chlorella*

Process Flow Diagram – Design Report



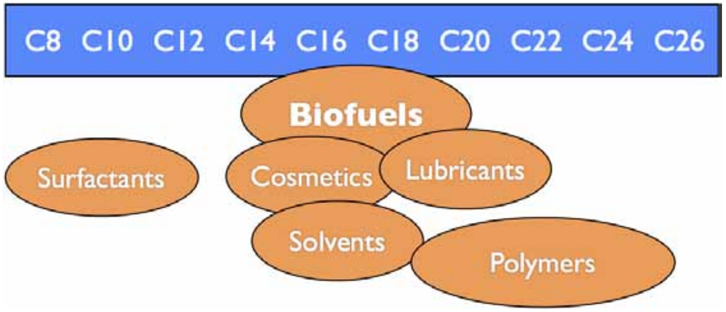
- **Non-destructive fractionation** allows for high-quality product recovery from lipids, carbohydrates and protein
- Potential for 40% reduction in MFSP by adopting multi-product algal biorefinery model
- Initial demonstration includes fermentative route to fuels, renewable diesel and ethanol
- Next stage of fractionation is additional component recovery from slipstreams, encompassing maximal biomass utilization

Davis et al 2014: www.nrel.gov/docs/fy14osti/62368.pdf
Laurens et al 2014: pubs.rsc.org/en/content/articlepdf/2015/gc/c4gc01612b

Lipids to Fuel and Bioproducts

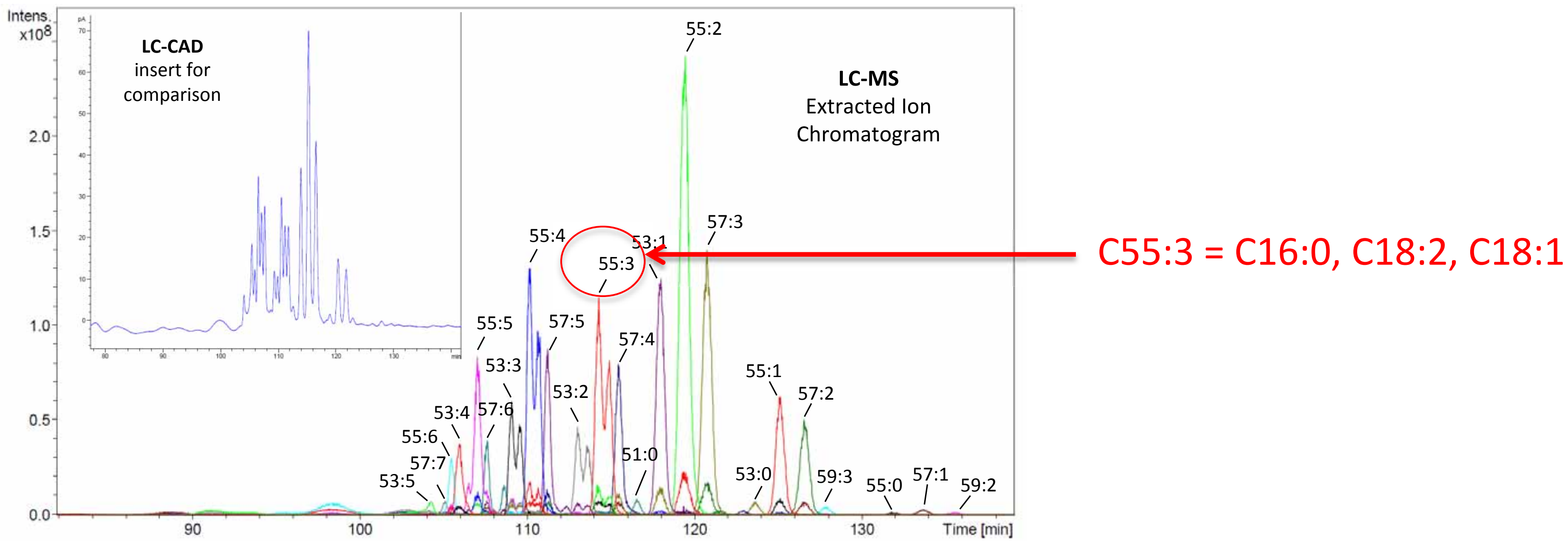
- Lipid composition impacts downstream fuel hydrotreating and oleochemical applications:
  - Efficiency and yield of catalytic fuel conversion
  - Presence of heteroatoms (N, P, S)
  - Polar lipid emulsification
  - Sterols and natural hydrocarbons
  - Triglyceride composition
  - Purification strategies to isolate valuable fractions
  - **Fatty acid content and profile**

Fatty acid chain length determines **oleochemical market** applications:



	Scenedesmus	*Chlorella*	*Nannochloropsis*	Linseed	Soybean
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

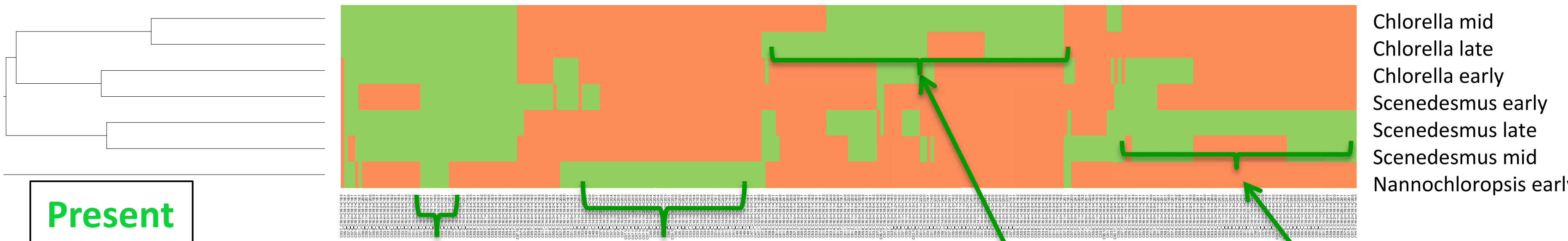
Algal Triglyceride Acyl Composition



- Triglycerides are **preferential feedstock for oleochemical industry** (mostly based on plant-derived oils), molecular make-up defines applications and feedstock/product quality
- Fatty acyl composition of TAG guide physico-chemical separations processes for PUFA-containing TAGs for co-product development
- **> 400 unique triglycerides** identified in 3 strains, enormous diversity not seen in plant oils

Nomenclature used: [55:3] = Total carbons : double bonds

Strain-specific TAG Molecular Diversity



Core set

C55:7_16:2.18:3.18:2
C55:7_16:1.18:3.18:3
C55:6_16:4.18:1.18:1
C55:6_16:3.18:3.18:0
C55:6_16:2.18:3.18:1
C55:6_16:0.18:3.18:3
C55:5_16:3.18:1.18:1
C55:5_16:0.18:3.18:2
C55:4_16:2.18:1.18:1
C55:4_16:1.18:2.18:1
C55:4_16:0.18:3.18:1
C55:3_16:1.18:1.18:1
C55:3_16:0.18:2.18:1
C55:2_16:1.18:1.18:0
C55:2_16:0.18:1.18:1
C55:1_16:0.18:1.18:0

Nannochloropsis

C49:1_C14:0>C14:0>C18:1
C57:9_C14:0>C20:5>C20:4
C57:8_C16:1>C18:2>C20:5
C57:8_C14:0>C18:2>C22:6
C57:7_C16:1>C18:2>C20:4
C57:6_C16:1>C18:1>C20:4
C57:6_C16:0>C18:1>C20:5
C57:4_C16:0>C18:1>C20:3
C57:12_C14:2>C18:5>C20:5
C57:11_C16:1>C18:5>C20:5
C57:11_C14:1>C20:5>C20:5
C57:10_C16:1>C18:4>C20:5
C57:10_C16:1>C18:3>C20:5
C57:10_C14:1>C20:5>C20:4
C57:10_C14:0>C20:5>C20:5

Chlorella

C63:4_18:2.18:2.24:0
C61:5_18:3.18:2.22:0
C61:4_18:2.18:2.22:0
C61:2_16:0.18:2.24:0
C59:3_18:2.18:1.20:0
C59:3_16:0.18:3.22:0
C59:2_16:0.18:2.22:0
C59:1_14:0.18:1.24:0
C57:5_18:2.18:2.18:1
C57:3_18:2.18:1.18:0
C57:3_16:0.18:2.20:1
C55:6_16:3.18:2.18:1
C55:6_16:2.18:2.18:2
C55:5_16:2.18:2.18:1
C55:5_16:1.18:2.18:2
C55:4_16:3.18:1.18:0
C55:4_16:0.18:2.18:2
C55:3_16:2.18:1.18:0
C55:3_16:2.16:1.20:0
C55:2_16:0.18:2.18:0
C53:9_16:4.16:3.18:2
C53:8_16:4.16:2.18:2
C53:8_16:3.16:3.18:2
C53:5_16:3.16:0.18:2
C53:4_16:2.16:0.18:2
C53:4_14:0.18:2.18:2
C51:6_16:3.16:3.16:0
C51:6_14:0.16:3.18:3
C51:5_14:0.16:3.18:2
C51:4_16:4.16:0.16:0
C51:3_14:1.16:0.18:2

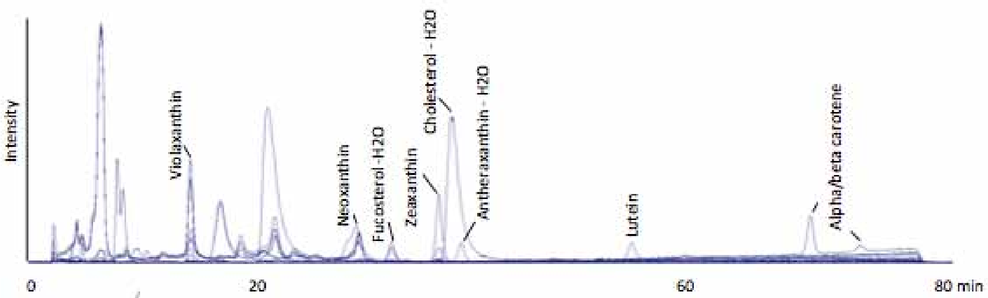
Scenedesmus

C65:4_16:3.18:1.28:0
C65:4_16:2.18:2.28:0
C65:4_16:1.18:3.28:0
C65:3_18:2.18:1.26:0
C65:3_16:2.18:1.28:0
C65:3_16:1.18:2.28:0
C65:3_16:0.18:3.28:0
C61:3_16:1.20:1.24:1
C61:3_16:1.18:1.24:1
C61:2_16:0.18:1.24:1
C59:4_18:3.18:1.20:0
C59:4_16:2.18:1.22:1
C59:3_16:2.18:1.22:0
C59:3_16:1.18:1.22:1
C59:2_16:0.20:1.20:1
C57:6_16:2.18:3.20:1
C57:5_16:3.18:1.20:1
C57:3_16:2.18:1.20:0
C57:1_16:0.18:0.20:1
C55:7_16:4.18:3.18:0
C55:7_16:2.18:4.18:1
C55:10_16:4.16:4.18:3
C53:2_14:0.18:1.18:1
C51:3_14:0.16:2.18:1
C53:0_16:0.16:0.18:0

Nomenclature used: [55:3] = Total carbons : double bonds

Natural Non-Fatty Acid Lipids

- Isolation of hydrocarbons, sterols and pigments from three strains using a novel, whole biomass saponification/extraction modification
- Contributes up to 10% of lipids, not previously accounted for, as potentially valuable substrates for oleochemicals



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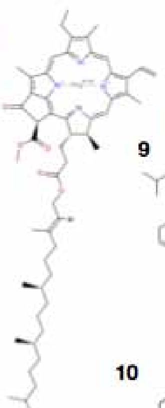

9 Phytol

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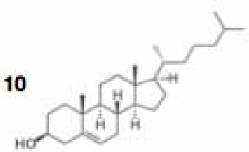
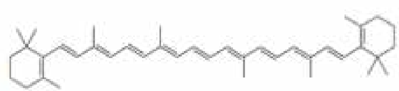


Cholesterol

Tocopherol, carotene, xanthophyll

11

12



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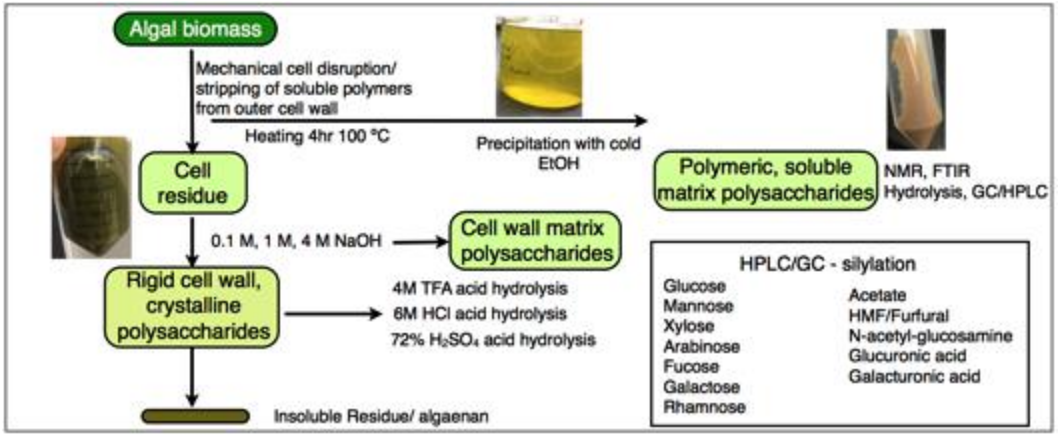
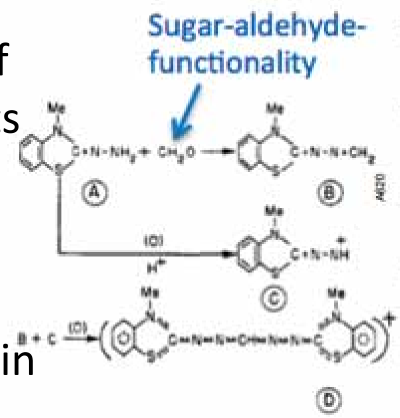
	Scenedemsus	*Chlorella*	*Nannochloropsis*
hexadecane		0.3	0.2
8-heptadecene		1.0	
heptadecane	0.4	0.5	
trimethyl 2-pentadecanone	0.4	0.2	0.1
n-Hexadecanoic acid	0.7	0.5	0.3
Nonadecane			0.2
Phytol	**68.5**	**82.1**	**41.1**
Phytol acetate	1.6	1.6	0.2
9-tricosene (z)	1.2		0.2
heptacosanol			0.3
1-nonadecene	0.3		
octadecenal			0.2
7-methyl (z,8,10 dodecadienal)			0.4
Eicosadiene			0.2
α-tocopherol			0.5
Cholesterol		**0.4**	**27.5**
Brassicasterol	0.7	0.4	0.9
unknown sterol		0.6	1.8
Ergosterol		**10.9**	
Campesterol	0.8	0.3	0.7
Stigmasterol	1.2	0.5	0.6
gamma-ergostenol	5.2	0.7	
Stigmast-7,16 dien-3-ol	**12.9**		
ß-sitosterol			3.7
Fucosterol			4.3
Unknown sterol 2	1.5		
Stigmast-7-en-3-ol	2.9		
Unknown hydrocarbon			3.6
Unknown hydrocarbon			1.8
Unknown hydrocarbon			α

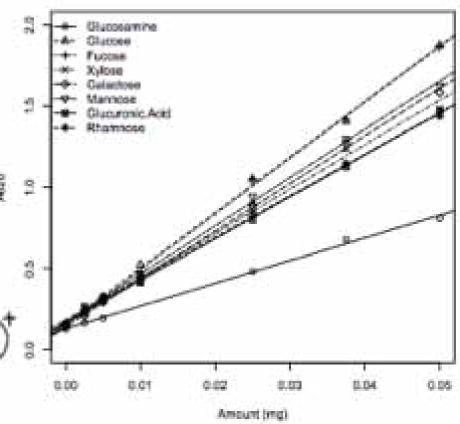
Carbohydrate Polymer Characterization



% DW	Bagasse	*Phaeodactylum tricornutum*	*Nannochloropsis sp.*	*Chlorella vulgaris*
Total Carbohydrates:	**62.2**	**19.6**	**8.6**	**20.5**
Glucose	38.5	2.7	4.2	4.8
Xylose	21.2	1.6	0.2	0.6
Rhamnose	ND	0.7	0.6	1.9
Galactose	0.4	1.5	1.4	5.7
Fucose	ND	2.1	0.4	0.3
Arabinose	1.7	ND	ND	0.7
Mannose	ND	6.6	1.3	2.0
Ribose	0.4	0.5	0.5	0.4
Glucosamine	ND	ND	ND	0.8
Glucuronic Acid	ND	4.0	ND	1.1
Galacturonic Acid	ND	ND	ND	2.1

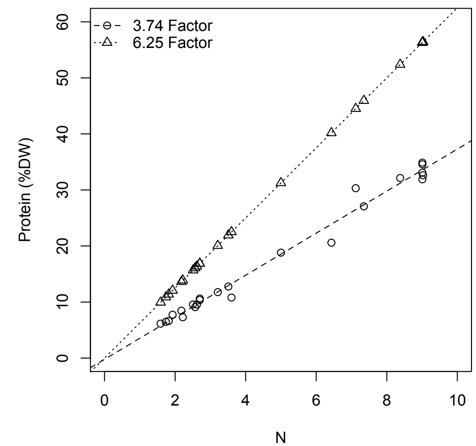
- Carbohydrates stored as both soluble, structural and storage polysaccharides – **Laminarin** (*Nannochloropsis*), **starch** (*Chlorella*) and **glucomannan** (*Scenedesmus*)
- Isolated and characterized charged **extracellular polysaccharides** associated with glycoprotein and excreted in algal growth medium, potential loss of assimilated carbon and substrate for contaminants
- Developed and implemented novel, accurate carbohydrate quantification procedure based on MBTH derivatization – spectrophotometric, compatible with hydrolysis procedures – no protein interference



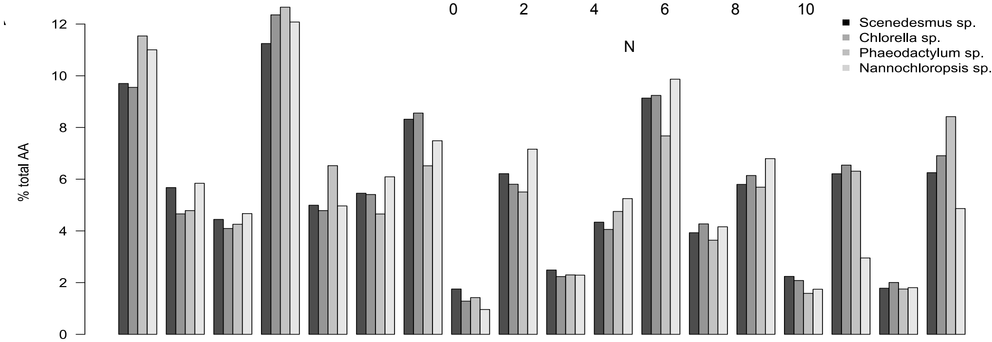


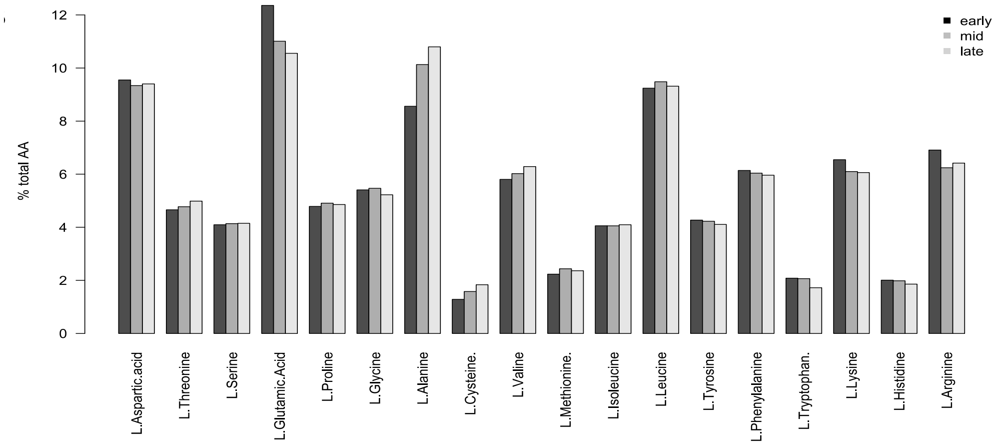
Protein Characterization

- Amino acid composition determines value of protein as bioproduct application and found to be consistent between strains and growth conditions
- Quantification based on nitrogen conversion factor most accurate, but highly dependent on non-protein-nitrogen content (higher than terrestrial biomass)

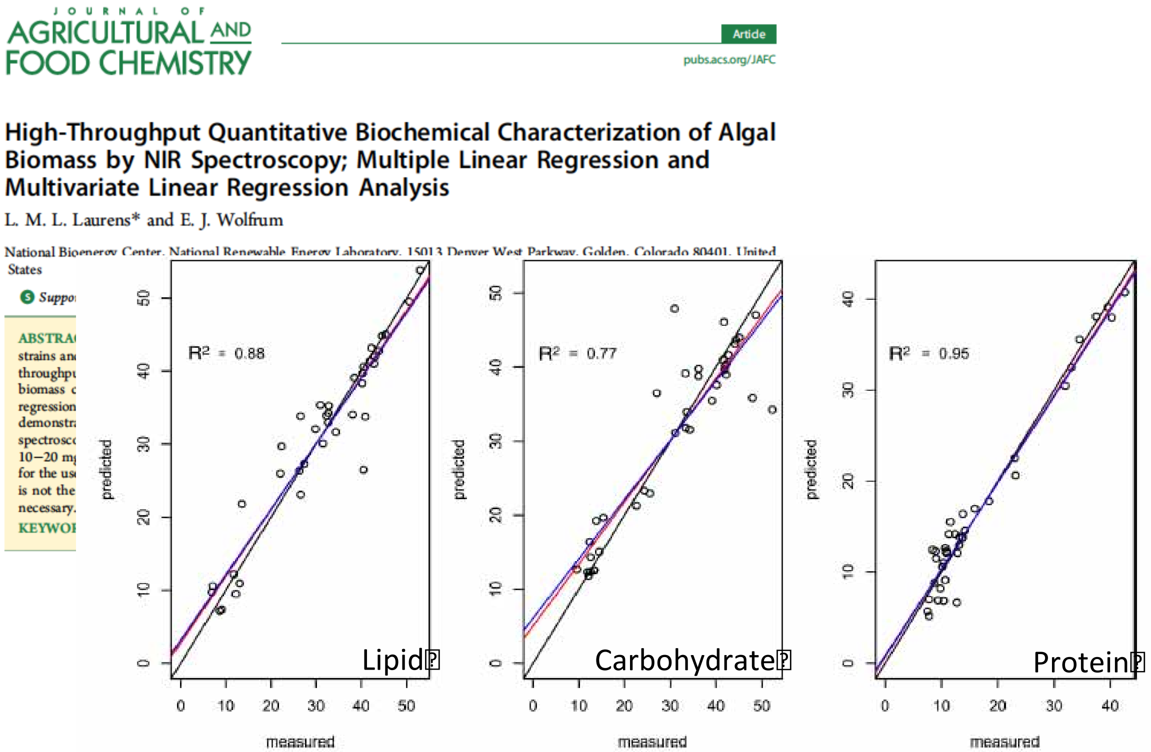


	Chlorella vulgaris			*Scenedesmus acutus*		
	early	mid	late	early	mid	late
N	***9.01***	***2.70***	***2.18***	***8.38***	***1.82***	***1.59***
Aspartic acid	3.33	0.98	0.80	3.15	0.60	0.57
Threonine	1.59	0.49	0.42	1.81	0.42	0.38
Serine	1.37	0.41	0.34	1.38	0.30	0.29
Glutamic acid	4.37	1.17	0.91	3.70	0.65	0.64
Proline	1.63	0.50	0.40	1.58	0.37	0.33
Glycine	1.66	0.50	0.39	1.56	0.32	0.30
Alanine	2.75	0.98	0.85	2.49	0.55	0.54
Cysteine*	0.44	0.16	0.15	0.56	0.17	0.15
Valine	1.98	0.62	0.53	1.97	0.44	0.39
Methionine*	0.79	0.26	0.21	0.82	0.21	0.17
Isoleucine	1.41	0.42	0.35	1.40	0.31	0.27
Leucine	3.21	0.99	0.79	2.96	0.65	0.56
Tyrosine	1.55	0.46	0.37	1.33	0.25	0.23
Phenylalanine	2.21	0.65	0.52	1.94	0.44	0.37
Tryptophan*	0.77	0.23	0.16	0.77	0.16	0.14
Lysine	2.31	0.65	0.52	2.04	0.33	0.34
Histidine	0.72	0.21	0.16	0.59	0.08	0.09
Arginine	2.50	0.68	0.57	2.10	0.36	0.38
TOTAL AA (anhydrous):	34.59	10.36	8.45	32.13	6.61	6.15
k_P factor	**3.84**	**3.84**	**3.88**	**3.83**	**3.63**	**3.87**





Whole Biomass High-Throughput Screening



JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

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High-Throughput Quantitative Biochemical Characterization of Algal Biomass by NIR Spectroscopy; Multiple Linear Regression and Multivariate Linear Regression Analysis

L. M. L. Laurens* and E. J. Wolfrum

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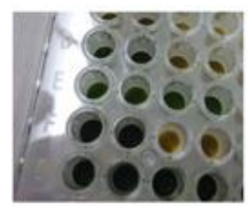
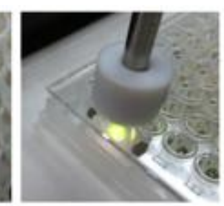
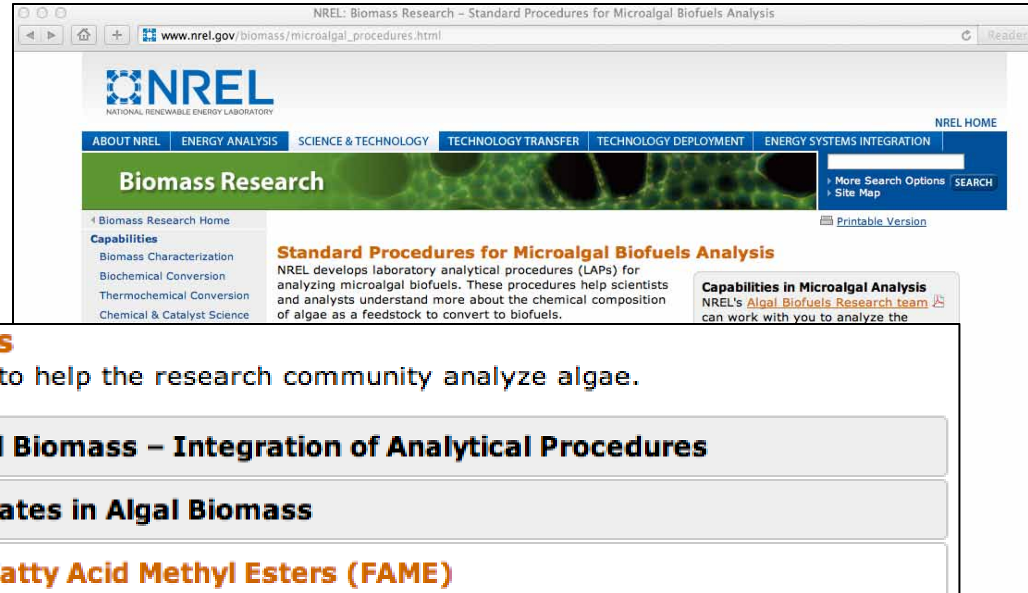
- Demonstrated <10% uncertainty on quantification lipids, protein and carbohydrate content predictions both on dry biomass and freshly harvest filter biomass
- Investigating implementation of this technology in **ATP³** consortium to rapidly increase throughput and frequency of compositional analysis – developing calibration transfer mechanism

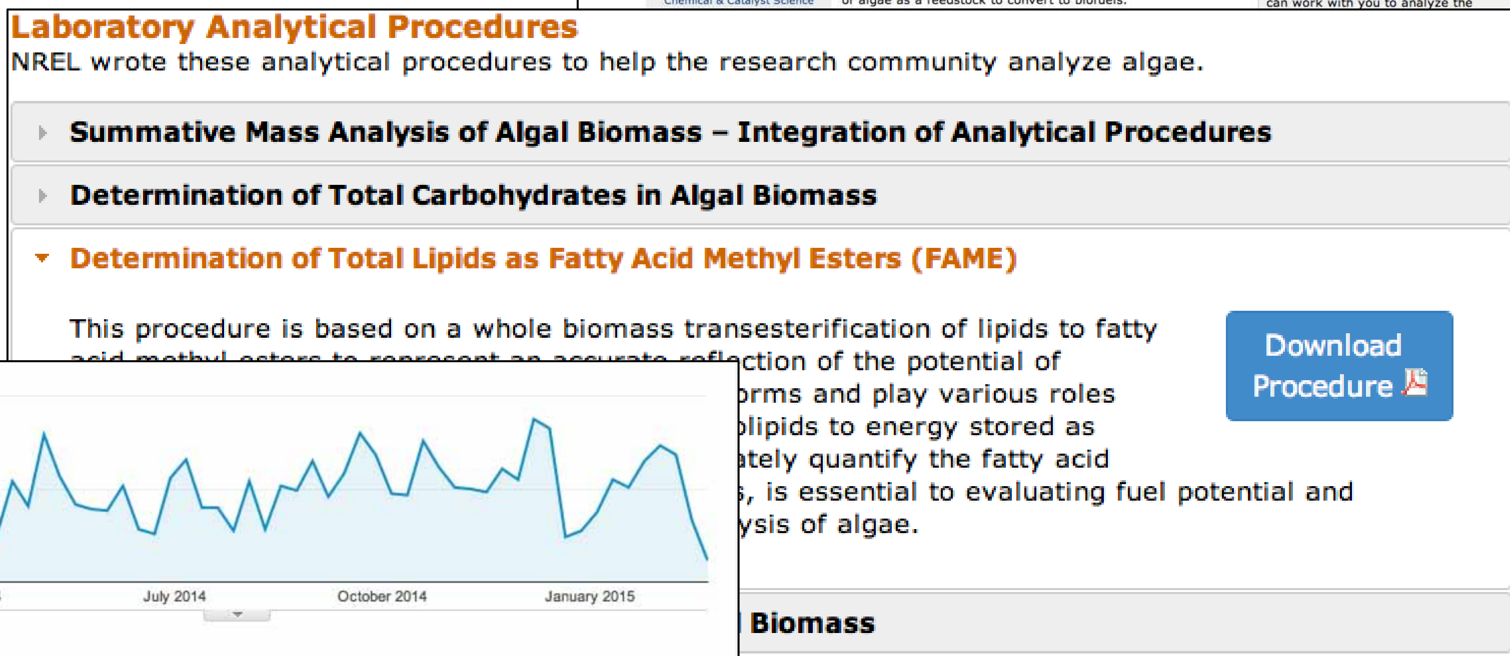


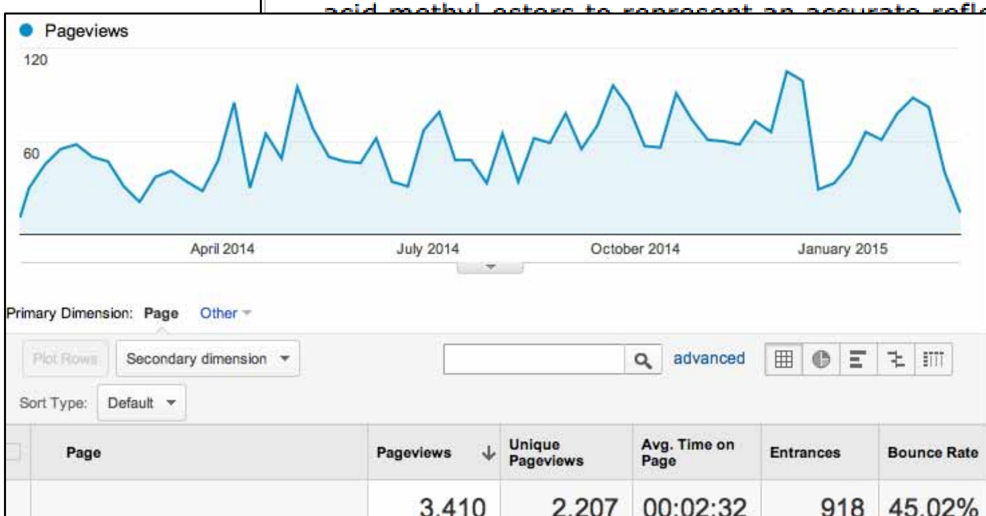


Dissemination of Microalgal Analysis Procedures

- Online published open-access procedures
- Recent outreach to **Algae Biomass Organization, AOCS and ASTM** for adoption and implementation







3,410 views with 1703 procedure downloads (since publication Dec. 2013)

Relevance

- **Algal Biomass Valorization** is highly relevant to BETO goals, of identifying critical barriers to the creation of new domestic bioenergy and bioproduct industry
- Directly **address MYPP barriers**:
  - **AFt-B. Sustainable Algae Production**: Comprehensive, unambiguous compositional characterization of dynamic algal biomass informing yields of existing processes and identify co-product pathway options
  - **AFt-E. Algal Biomass Characterization, Quality, and Monitoring**: Standardized (Open Access) procedures to uniformly quantify major components
  - **AFt-G Algal Feedstock Material Properties**: Biomass properties in relation to conversion process performance and cultivation systems

- **Identification of novel components scalable with fuels, adding value beyond estimates in current MYPP**

- Enabling algae industry with guidance on uniform descriptions on biomass characterization and subsequent valorization and trading standards for biomass
- Cross-platform (BETO Biochem and ARPA-E) implementation of technology developed, e.g. lipid characterization, NIR high-throughput screening

Future Work

- Upcoming key milestone will set the stage for supporting an **algae-based bioproducts industry**, compatible with a biofuels scenario
- **Crude lipid separation** into functional categories as lipid-co-product isolation and strategy for isolation of high value oleochemical feedstocks
- Application of characterization and performance assessment on **additional strains and poly-culture biomass**
- FY 16 go/no go milestone: Identification of **5 compositional factors that have the greatest impact on value and productivity** using chemometric data reduction methodology across wide range of organisms
- Detailed **mapping of potentially high-value co-products** identified in FY15 as influential for productivity and conversion parameters
- Continued standardization of compositional analysis with collaborative implementation of methods with **trade and standards organizations** (ASTM and ABO)

Summary

- **Algal Biomass Valorization** has provided significant contributions to algal biofuel developments:
  - Biomass compositional assessment for **>90% mass balance accounting** reduces uncertainty around biomass material properties
  - **Detailed characterization of major algal components** contributes to significant reduction of overall biofuel production costs
  - Enormous algae **triglyceride molecular diversity** defines novel oleochemical properties
  - Support of novel process design case conversion process pathway including **co-product development**
  - **Dissemination of procedures** and outreach to trade organization aids with standardizing compositional analysis
- Relevant to BETO algae platform goals of assessing **value and differences in feedstock quality** to support sustainable production
- Impact on algal biomass conversion projects to understand biomass **composition and conversion efficiency parameters**
- Future work focus on demonstration of **high-value, large market components** and mapping presence throughout cultivation and biomass conversion to advance algal bioenergy state of technology

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U.S. DEPARTMENT OF ENERGY

Questions?

Additional Slides

Responses to Reviewers' Comments from 2013

COMMENT:

The results shown look reasonably good but need to provide more evidence as to why the methods developed are better/more applicable than other methods. Evidence of a literature review being performed and pointing out how shortcomings in previously developed methods have been overcome would be helpful. Future calculations to prove mass balance closure will be significant way to show progress.

RESPONSE:

The first, establishing year of this project prioritized the need for evaluation of historical, literature-based and commercially implemented methods for compositional analysis of algae. We are basing our evaluation of accuracy and precision on established AOAC guidelines and these results were incorporated in online Laboratory Procedures and publications. A discussion of the shortcomings of existing methods used in leading laboratories in the field, with respect to underlying measurement chemistry and potential for interferences, was included in our recently published work in Analytical Chemistry and Analytical Biochemistry (Anal Chem 2012, 84:1879–87, Anal Biochem 2014, 452:86–95). In addition, we will continuously review the literature, commercial laboratory offered measurements, and our academic and commercial collaborators' data and methods along with historically published data and update or adapt our methods accordingly. As we demonstrate in this work we are able to achieve mass balance closure for two strains across at least two respective growth conditions

COMMENT:

NREL publications might not be adequate to fully leverage the value of this work across the industry. More detailed plans with the ABO Technical Standard Committee for wider acceptance and implementation may have value.

RESPONSE:

In addition to NREL publications and open access technical reports, the PI of this task is now leading the ABO Technical Standards Committee (since 2013) and this exposure has proven helpful for dissemination and wider publicity for the standard procedures developed under this task. The PI of this project has taken over the leadership of the technical committee and helped write a large portion of the IAM 6.0 document, and we are planning on developing this text further as a more thorough review of the literature. The technical standards committee is in the process of updating and expanding this report as a reference for current best practices in the industry.

COMMENT:

Attempts to develop predictive growth and composition models using laboratory PBRs to predict effects in outdoor cultivation appear to be redundant with other efforts. The use of lab PBRs to predict outdoor cultivation performance does not appear to be validated. Previous efforts by other researchers to screen strains using lab PBRs might not have been effective.

RESPONSE:

We include PBR development for cultivation in this task because controlled cultivation is critical to distinguish between biological, physiological and abiotic variability on biomass composition, biomass-specific method uncertainty and interferences. The custom-built PBR cultivation generates physiologically controlled biomass, as opposed to commercially available materials where cultivation information is hard to trace back. Through close interactions with the algae testbeds, we are establishing a strong connection to outdoor cultivation and by leading the analytical task for ATP^3, NREL is able to address large-scale biomass composition concerns, as well as harvesting, sampling and storage effects.

Publications

Publications:

1. 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** *under review*
2. 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** *under review*
3. Davis, R., Kinchin, C., Markham, J., Tan, E.C.D., Laurens, L. ML., Sexton, D., Knorr, D., Schoen, P., Lukas, J. (2014) “Process Design and Economics for the Conversion of Algal Biomass to Biofuels: Algal Biomass Fractionation to Lipid- and Carbohydrate-Derived Fuel Products” NREL-DOE Algae Pathway Design report: http://www.nrel.gov/docs/fy14osti/62368.pdf
4. Laurens, L. ML., Nagle, N., Davis, R., Sweeney, N., Van Wychen, S., Lowell, A., Pienkos, P. T. (2014) "Acid-catalyzed algal biomass pretreatment for integrated lipid and carbohydrate-based biofuels production" **Green Chemistry** doi: dx.dio.org/10.1039/C4GC01612B
5. Laurens, L. ML. (2014) “Whole Algal Biomass in situ Transesterification to Fatty Acid Methyl Esters as Biofuel Feedstocks”, in “BIOENERGY”, ed. Anju Dahiya, Publishers: Elsevier, Bookchapter
6. Zhao B., Ma J., Zhao Q., Laurens L., Jarvis E., Chen S., Frear C. (2014) "Efficient anaerobic digestion of whole microalgae and lipid-extracted microalgae residues for methane energy production" **Bioresource Technology**, 161:423-30
7. Laurens, L. ML., Van Wychen, S., McAllister, J., Arrowsmith, S., Dempster, T., McGowen, J., Pienkos, P. T. (2014) "Strain, Biochemistry and Cultivation-Dependent Measurement Variability of Algal Biomass Composition", **Analytical Biochemistry**, 1;452:86-95; dx.doi.org/10.1016/j.ab.2014.02.009
8. Laurens, L. ML. and Wolfrum, E. J. (2013) “High-Throughput Quantitative Biochemical Characterization of Algal Biomass by NIR Spectroscopy; Multiple Linear Regression and Multivariate Linear Regression Analysis”, **Journal of Agricultural and Food Chemistry**, 61(50): 12307-12314 doi: dx.doi.org/10.1021/jf403086f

Presentations

Presentations:

- "Technical Standards A case for a Common Language for Algae", Invited oral presentation, European Algal Biomass Association annual meeting, Florence, Italy
- "Algal Biomass Organization Technical Standards Committee, A Case for a Common Language for Algal Biomass and Bioproducts", Invited plenary presentation, 8th Algae Biomass Summit, San Diego, CA
- "Driving towards a Common Language for Characterization of Algal Biomass for Biofuels and Bioproducts: High Impact Data and Method Harmonization", Oral Presentation, Algal Biomass for Biofuels and Bioproducts annual meeting, Santa Fe, NM
- "Ultrahigh-resolution Mass Spectrometry for Discovery of Novel Components of Advanced Biofuels Intermediates", Oral Presentation, American Oil Chemists Society (AOCS) annual meeting, San Antonio, TX
- "Fractionation of Algal Biomass for Increased Biofuel Yields and Lower Costs" & "Composition of Algal Biomass for Biofuels and Bioproducts: High Impact Data and Method Harmonization" Oral presentations, Algal Biomass Organization, 7th Algae Biomass Summit, Orlando, FL
- "Characterization of Algae for Biofuels and Bioproducts" & "Optimization of conversion and Extraction of Algal Biomass" Invited Oral Presentations, Organization of "Algae Forum" at American Oil Chemists Society (AOCS) annual meeting, Montréal, Canada

Outreach publication:

1. NREL News Feature: "Unique Bioreactor Finds Algae's Sweet Spot" by Bill Scanlon, *February 2014,* (ww.nrel.gov/news/features/feature_detail.cfm/feature_id=8305)

Commercialization Outreach

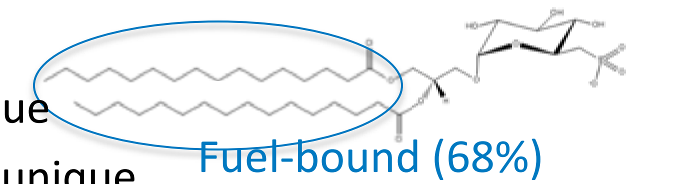
- Procedures on current best practice of microalgal compositional analysis, implemented and adopted across algae industry:
  - Summative Mass Analysis of Algal Biomass – Integration of Analytical Procedures http://www.nrel.gov/docs/fy14osti/60943.pdf:
  - Determination of Total Solids and Ash in Algal Biomasshttp://www.nrel.gov/docs/fy14osti/60956.pdf
  - Determination of Total Lipids as Fatty Acid Methyl Esters (FAME) by *in situ* Transesterificationhttp://www.nrel.gov/docs/fy14osti/60958.pdf
  - Determination of Total Carbohydrates in Algal Biomasshttp://www.nrel.gov/docs/fy14osti/60957.pdf
  - Calculation of N-to-Protein Conversion Factor
- Leading ABO's Technical Standards Committee, interaction with leading industry partners (www.algaebiomass.org/resource-center/technical-standards/introduction)
- Implementation and deployment of NIR model across ATP^3 network for high-throughput, higher frequency biomass compositional measurement
- Advanced characterization agreement between NREL and Sapphire Energy inc. and partnering for ABY project, tailoring HTL oil characterization linked with biomass composition
- Potential for future integration of physiological cultivation tool and data to BAT model
- Potential for future implementation of advanced characterization of polycultures (INL, Sandia) to assess impact on downstream processing
- In depth training scheduled for Cellana analytical team (joint with ATP^3)

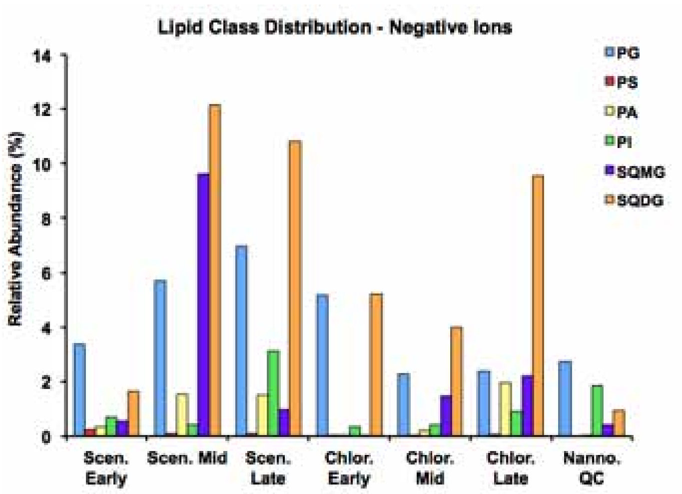
Backup Slides

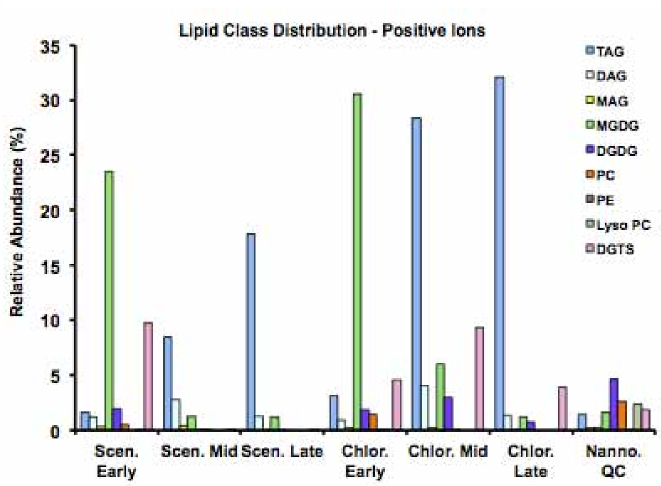
Strain-specific Lipid Molecular Diversity

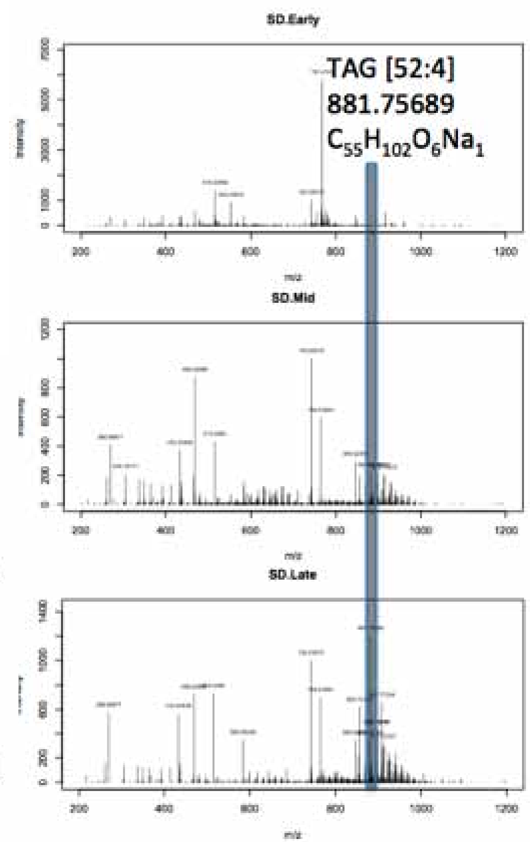
- Not all lipids have equal value as fuel precursor
- Lipid composition differs between strains and growth conditions impacting fuel feedstock parameters and value
- 2200 unique components identified in oil extracts with unique molecular elemental composition associated with exact mass through Fourier-transform Ion Cyclotron Resonance (FT-ICR) mass spectrometry
- Molecular weight of the ions can be interpreted as unique identifiers of lipid composition
    - Cross reference against LipidMAPS database and complement with algae-specific lipids as reference list

1,2-dihexadecanoyl-3-(6'-sulfo-α-D-quinovosyl)-sn-glycerol (MW = 794.5214)



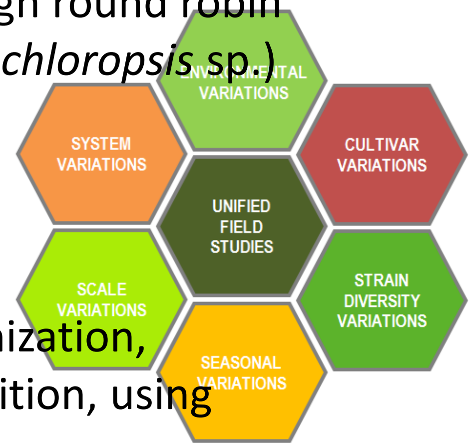






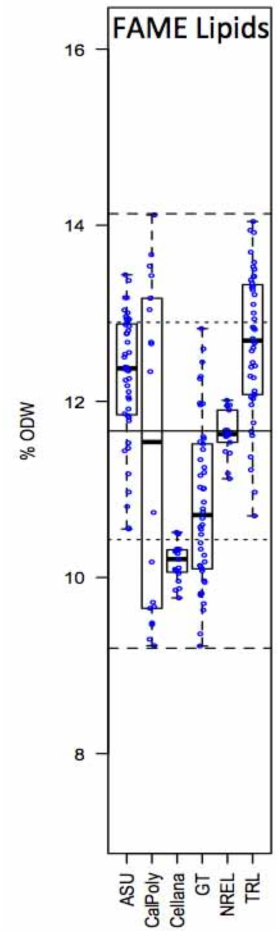
Implementation of procedures in ATP^3 consortium

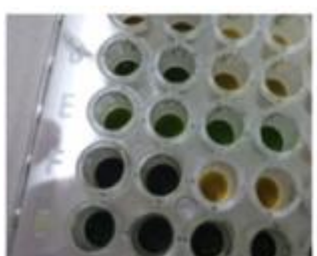
- Goal to develop and implement analytical pipeline for collection and dissemination of high quality data
- Establishment of statistical precision targets through round robin experiment on **standard biomass material** (*Nannochloropsis* sp.)



	mean	sd	RSD	*N*
Ash	18.8	1.2	6.4	*237*
Protein	28.2	0.8	2.7	*153*
FAME Lipids	11.6	1.2	10.6	*191*
Carbohydrates	7.7	1	12.5	*198*

- …y harmonization, standard biomass material and consensus composition, using validated procedures, spreadsheet
- Noticeable drain on resources for full compositional analysis, reduces number of harvest points and biochemical profile assessed – urgent need for higher throughput analysis technology – potential for low cost of implementation of NIR screening





Composition versus conversion parameters

Strain	Growth stage	FAME Lipids	Ash	Carb	Protein
	early	9.1	4.5	15.4	46.3
	mid	17	1.8	48.5	17.4
Scenedesmus	late	38.6	2.2	37.8	7.8
	early	12.1	6.7	9.8	43.2
Chlorella	mid	15	4.4	31.1	24
	late	23.1	5.3	35.8	15.2

Lipid extractability in relation to temperature and acid concentration for *Scenedesmus* and *Chlorella* indicates conversion susceptibility dependent on biomass composition *(Algal Biomass Conversion project)*

Chlorella

Scenedesmus