1.3.4.300 Algal Biomass Valorization (ABV)

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

Lieve M. Laurens
National Renewable Energy Laboratory
Goals

1. Reduce cost of algal biofuels by increasing intrinsic algal biomass value
   o Identify and isolate *scalable, high-value key products* in biomass and process streams to improve algal biofuels economics
   o 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
   o Establish *common language* for characterization of range of species and process configuration
   o Solicit input on *commercial need for standards* and route to adoption and implementation

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

Path to $3/GGE by 2022

Reduce cost of biomass production

Increase intrinsic biomass value

Relevance

Co-products enable commercialization:
Quad Chart Overview

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

<table>
<thead>
<tr>
<th></th>
<th>Total Costs FY 12 – FY 14</th>
<th>FY 15 Costs</th>
<th>FY 16 Costs</th>
<th>Total Planned Funding (FY 17-Project End Date)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOE Funded</td>
<td>1,511K</td>
<td>800K</td>
<td>850K</td>
<td>2,250K</td>
</tr>
</tbody>
</table>

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 (DISCOVR), 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

[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

Co-product credits will help drive the cost down

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
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 (DISCOVR) R&D projects

4. Close **collaboration with TEA group** on value, cost and productivity calculations

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**Approach – Management**

- **Algal Biomass Valorization**
  - **Experimental Valorization**
    - Controlled Cultivation (*N. Sweeney*)
    - Oleochemicals extraction and purification (*T. Dong*, *P. Spinelli*, *K. Duff*, *O. Palardy*)
    - Carbohydrate identification-purification (*E. Vadelius, S. Van Wychen*)
    - Novel Product Identification (all)
    - Cost – Value assessment of productivity vs. composition (*R. Davis, J. Markham*)
  - **Advanced Analytical Development**
    - Standard Method Development and Training (*S. Van Wychen*)
    - Mass Balance closure (*S. Van Wychen*)
    - Industry liaising (*L. Laurens*)
Approach

Algal Biomass Valorization

Experimental Valorization
- Controlled Cultivation
  - Oleochemicals extraction and purification
  - Carbohydrate identification-purification
- Novel Product Identification
- Cost – Value assessment of productivity vs. composition

Advanced Analytical Development
- Standard Method Development and Training
- Mass Balance closure
  - Industry liaising
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

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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

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

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

[FY16 Q2 Milestone]
Focus on Algae Bio-Products Scaling with Farms

Biomass production cost: $491/ton*

+ Co-products Biomass Value $500-$800/ton

<table>
<thead>
<tr>
<th>Feedstock (%DW)</th>
<th>Product</th>
<th>Price ($/T)</th>
<th>Market (T/yr)</th>
<th>Produced (T/farm/yr)</th>
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</thead>
<tbody>
<tr>
<td>Fatty acids (10-45%)</td>
<td>Hydrocarbon Fuel</td>
<td>920</td>
<td>209,000,000</td>
<td>85,000</td>
</tr>
<tr>
<td>PUFA (3-6%)</td>
<td>Nutraceuticals</td>
<td>30,000-100,000</td>
<td>22,000</td>
<td>11,000</td>
</tr>
<tr>
<td></td>
<td>Polyols, polyurethane polymers</td>
<td>5,000-11,000</td>
<td>11,000,000</td>
<td>11,000</td>
</tr>
<tr>
<td>Sterol (1-2%)</td>
<td>Phytosterol nutra/pharmaceuticals</td>
<td>40,000-80,000</td>
<td>25,000</td>
<td>7,000</td>
</tr>
<tr>
<td></td>
<td>Surfactants/emulsifiers</td>
<td>1,500</td>
<td>2,000,000</td>
<td>7,000</td>
</tr>
<tr>
<td>Ferm Carbs (10-45%)</td>
<td>Di-acids (e.g. succinic acid)</td>
<td>2,250</td>
<td>2,500,000</td>
<td>83,000</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>780</td>
<td>68,000,000</td>
<td>83,000</td>
</tr>
</tbody>
</table>

DOE-EERE. 2016. National Algal Biofuels Technology Review
DOE-EERE. 2016. Strategic Plan for a Thriving Bioeconomy
Laurens, L. Energy and Environmental Science, *submitted*
## Co-Products Provide Additional Revenue to Biorefinery

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

*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)

<table>
<thead>
<tr>
<th></th>
<th>% sterols</th>
<th>% Phytol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scenedesmus CAP oil</td>
<td>2.8</td>
<td>1.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>% sterols</th>
<th>% Phytol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ergosterol</td>
<td>3.68 ± 0.11</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td>Stigmasterol</td>
<td>1.6 ± 0.1</td>
<td>29.6 ± 0.5</td>
</tr>
<tr>
<td>Ergosterol-like</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

[PEG 1000 Phytol-SA-PEG Cholesterol-SA-PEG Algae-sterols-SA-PEG]

[FY16 Q3 Milestone]
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:

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

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

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

Elemental Composition of *Chlorella* EPS

<table>
<thead>
<tr>
<th></th>
<th>C %</th>
<th>H</th>
<th>N</th>
<th>O</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPS</td>
<td>35.3</td>
<td>5.8</td>
<td>3.3</td>
<td>43.5</td>
</tr>
</tbody>
</table>

Heterogeneous co-polymer:
Approach

Algal Biomass Valorization

Experimental Valorization
  - Controlled Cultivation
  - Oleochemicals extraction and purification
  - Carbohydrate identification-purification
  - Novel Product Identification
  - Cost – Value assessment of productivity vs. composition

Advanced Analytical Development
  - Standard Method Development and Training
  - Mass Balance closure
  - Industry liaising
Standard Methods for Algae

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

- Implemented methods with rigorous QC protocol across ATP\(^3\) 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

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

* Based on experimental recovery data
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, DISCOVR, 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

Relevance

> 8,600 method access
> 3,500 downloads
(December 2016)
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 (DISCOVR, 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
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
Thank You!

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Collaborators:
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Howland Jones (Sandia, CA)
John McGowen (ASU, AZ)
Tom Dempster (ASU, AZ)
Valerie Harmon (HC, HI)
Dries Vandamme (KU Leuven, Belgium)

http://www.nrel.gov/bioenergy/algae-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.
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*


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
13. Session chair, “Algal and Other Non-traditional Oil Characterization”, AOCS annual meeting, Orlando, FL, 2015
Commercialization Outreach

• **Record of Invention:**
  - ROI 17-11 "Using amino acid (peptide)-based crosslinkers to produce novel renewable polyurethane products” Dong, T., Laurens, L., Pienkos, P.


• Procedures on current best practice of microalgal compositional analysis, implemented and adopted across algae industry:
  - Determination of Total Lipids as Fatty Acid Methyl Esters (FAME) by *in situ* [www.nrel.gov/docs/fy16osti/60958.pdf](http://www.nrel.gov/docs/fy16osti/60958.pdf)
  - Determination of Total Carbohydrates in Algal Biomass [www.nrel.gov/docs/fy16osti/60957.pdf](http://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](http://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
<table>
<thead>
<tr>
<th>Biomass components</th>
<th>wt %</th>
<th>Product</th>
<th>Market* (ton/yr)</th>
</tr>
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<tbody>
<tr>
<td>Fatty acids</td>
<td>10-45</td>
<td>Hydrocarbon fuel products (U.S. consumption)</td>
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<td>Omega-3-fatty acids</td>
<td>3-10</td>
<td>Polyols – epoxy resin – polyurethane</td>
<td>8,000,000 – 11,000,000</td>
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<tr>
<td></td>
<td>3-10</td>
<td>Nutraceuticals</td>
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<td>Hydroxy-, branched-, fatty acids/alkohols</td>
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<td>Surfactants, fuel additives</td>
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<td>Sterols</td>
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<td>Surfactants</td>
<td>6,400,000</td>
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<tr>
<td></td>
<td>2-4</td>
<td>Phytosterol nutra-/pharmaceuticals</td>
<td>25,000</td>
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<tr>
<td></td>
<td>2-4</td>
<td>Emulsifiers</td>
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<tr>
<td>Phytol</td>
<td>3-4</td>
<td>Raw material for vitamin E, fragrance, soaps…</td>
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<tr>
<td>Polar lipids</td>
<td>10-35</td>
<td>Ethanolamine</td>
<td>600,000</td>
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<tr>
<td></td>
<td>10-35</td>
<td>Phosphatidyicholine, phosphoinositol and phosphatidyl ethanolamine (lecithin )</td>
<td>20,000-30,000</td>
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<tr>
<td>Glycerol</td>
<td>2-6</td>
<td>Di-acids for nylon production</td>
<td>2,500,000</td>
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<tr>
<td></td>
<td>2-6</td>
<td>Feed, pharmaceuticals</td>
<td>25,000</td>
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<tr>
<td>Fermentable sugars (glucose, mannose)</td>
<td>10-45</td>
<td>Polylactic acid (PLA) polymers</td>
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<td></td>
<td>10-45</td>
<td>Di-acids (e.g. adipic acid)</td>
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<tr>
<td>Mannitol</td>
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<td>Polyether polyols</td>
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<td>Alginate</td>
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<tr>
<td>Starch</td>
<td>5-40</td>
<td>Polysaccharide-derived bioplastics</td>
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<tr>
<td>Protein</td>
<td>19-40</td>
<td>Thermoplastics</td>
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<tr>
<td>Amino acids/peptides</td>
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<td>Polyurethane</td>
<td>11,000,000</td>
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<tr>
<td>Amino acids/peptides</td>
<td>19-20</td>
<td>Biobutanol, mixed alcohol fuels</td>
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<tr>
<td>Whole biomass</td>
<td>100</td>
<td>Animal/Fish feed</td>
<td>16,000,000 – 190,000,000</td>
</tr>
</tbody>
</table>

* 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

Impact of Biomass Composition on Conversion Susceptibility

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

Scenedesmus acutus 100% B
Chlorella vulgaris 90% E
Nannochloropsis granulata 80% H

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

[Leow et al. In preparation.]