Overview

Identify critical factors for economic development of the algae bioeconomy

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

Reduce uncertainty around process inputs and outputs

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

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

Aim: Increase the value and opportunity for algal biomass

Today: No pathway to targeted and consistent biomass value and composition manipulation

Importance: Characterization critical to define bioproduct opportunities and quantify intrinsic value of biomass

Risk: Control over species and environmentally specific biochemistry leads to unpredictable value projections
Market Trends

Product
- Anticipated decrease in gasoline/ethanol demand; diesel demand steady
- Increasing demand for aviation and marine fuel
- Demand for higher-performance products
- Increasing demand for renewable/recyclable materials

Feedstock
- Sustained low oil prices
- Decreasing cost of renewable electricity
- Sustainable waste management
- Expanding availability of green H₂
- Closing the carbon cycle
- Risk of greenfield investments

Capital
- Challenges and costs of biorefinery start-up
- Availability of depreciated and underutilized capital equipment
- Carbon intensity reduction

Social Responsibility
- Access to clean air and water
- Environmental equity

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

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

Key Differentiators
- Enable assessment of strain performance and biomass quality under environmental stressors
- Integrate with cultivation and conversion research to optimize productivity
1. Management Strategy

1. Project progress tracked through milestones surrounding **quantitative compositional analysis and biomass value metrics**, quarterly progress reports and peer-reviewed publications

2. Publish open access **online standard procedures and reports**, tracking access and implementation across AAS platform

3. **Integrate research** with conversion (CPR), cultivation and strain selection (DISCOVR) R&D projects

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

5. **Industry outreach** through technical standards working group

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### Algal Biomass Composition

#### Task 1: Advanced Analytical Development

- **Standard Method Development and Training**
  - Standard Analytical Chemistry: Stefanie Van Wychen, Bonnie Panzak, Hannah Alt, Alicia Sowell
- **Mass Balance Accounting**
  - Novel Analytical Chemistry: Stefanie Van Wychen, Steven Rowland
- **Database Development**
  - Data Analytics: Madeline Lane, Kaitlin Lesco, NREL library
- **Industry & Community Liaison**
  - Communications outreach team: Stefanie Van Wychen, Madeline Lane, Working Group advisory board

#### Task 2: Experimental Valorization

- **Product Discovery and Isolation**
  - Mass Spectrometry Technology: Steven Rowland, Peter Shanta, Kaitlin Lesco
  - Separation and CAP integration: Tao Dong, Eric Knoshaug

- **Cost – Value Assessment**
  - TEA team (collaborative): Ryan Davis, Bruno Klein
  - Cultivation experimentation: Nick Sweeney, Allyson Malloy

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**Industry & Community Liaison**

- Stefanie Van Wychen, Madeline Lane, Working Group advisory board
Management: Impact across Research and Industry Community

- ARPA-E MARINER seaweed conversion
- Biotech Partnership
- Competitive awards
- Conv. Process Research
- TEA
- ABC

Industry outreach

• RACER (Sapphire, CSU, ASU)
• ECUA (CSU/NREL/Qualitas)
• GAI/NREL
• Lumen Biosciences
• ExxonMobil CRADA
2. Technical Approach

• Develop and apply advanced compositional analysis approach coupled with innovative cultivation approaches, covering biochemical diversity of algae phylogeny

• Implement intrinsic value calculations for novel components

• Develop accessible databases of biomass components for major reference species/biomass of algae

• Convene Technical Standards working group with industry-led experts guiding future trade metrics

Significance: This is a cross cutting project at the interface of cultivation, conversion and TEA, collaborative across the program
Approach: Algae-Specific Molecular Diversity Mapping for Products Discovery

- **Approach**:
  - Algae-Specific Molecular Diversity Mapping for Products Discovery
  - Varied Strain and Growth Conditions
  - Standard Composition Analysis
  - Data Analysis Software, Custom coding tools
  - Algae Component Databases
  - Algae Properties and Methods Database
  - Knowledgebase

- **Components**:
  - Gap Analysis
  - Dissemination

- **Outputs**:
  - Economic Impact:
    - Molecular Identification for Product Development
    - Strain / Cultivation Improvement & Modeling
    - Data Mining / Advanced Analytics

- **Knowledgebase**
  - Dissemination
  - Algae Properties and Methods Database
  - Algae Component Databases
  - Standard Composition Analysis
  - Data Analysis Software, Custom coding tools
  - Varied Strain and Growth Conditions
  - Gap Analysis

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### Approach: Critical Success Factors and Risks

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

- Applied technology to DISCOVR consortium strain outdoor deployment to understand **year-round outdoor cultivation manipulation impact on composition**

- New methods developed and made available to algae industry
  - Implement methods with rigorous QC protocol across algae community
  - Outreach for reference material NIST, AAFCO
  - Lead Technical Standards Committee at ABO

https://www.nrel.gov/bioenergy/microalgae-analysis.html
Impact: Analytical Web Page Shows Significant Impact Growth

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

Method downloads (>2,000/yr)

February 2019:
- > 14,000 method access
- > 5,000 downloads (since 2015)
- > 900 downloads yr⁻¹
- ~ 4,000 web visits yr⁻¹

February 2021:
- > 45,000 method access
- > 9,500 downloads (since 2015)
- > 2,000 downloads yr⁻¹
- > 15,000 web visits yr⁻¹

Total Downloads by method (since Sept 2018):

<table>
<thead>
<tr>
<th>Method Description</th>
<th>Total</th>
<th>FY19</th>
<th>FY20</th>
<th>FY21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summative Mass Analysis of Algal Biomass</td>
<td>575</td>
<td>236</td>
<td>271</td>
<td>68</td>
</tr>
<tr>
<td>Determination of Total Solids and Ash in Algal Biomass</td>
<td>299</td>
<td>131</td>
<td>131</td>
<td>37</td>
</tr>
<tr>
<td>Determination of Total Carbohydrates in Algal Biomass</td>
<td>497</td>
<td>224</td>
<td>201</td>
<td>72</td>
</tr>
<tr>
<td>Determination of Total Lipids as Fatty Acid Methyl Esters</td>
<td>503</td>
<td>209</td>
<td>227</td>
<td>67</td>
</tr>
<tr>
<td>Determination of Total Sterols in Microalgae</td>
<td>213</td>
<td>102</td>
<td>80</td>
<td>31</td>
</tr>
<tr>
<td>Determination of Total, Organic, and Inorganic Carbon</td>
<td>16</td>
<td>-</td>
<td>-</td>
<td>16</td>
</tr>
</tbody>
</table>
Impact: Technical Standards Focus
Group Identifies Priorities

Convened group of 15 key representatives:

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

Gap and Priority Analysis

<table>
<thead>
<tr>
<th>Biomass composition, elemental composition, energy content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identification methodology, contamination tracking and genetic analysis</td>
</tr>
<tr>
<td>Safety, toxins (e.g. mycotoxins), heavy metals</td>
</tr>
</tbody>
</table>

Labeling/reporting requirements

Industry + Contract Laboratories

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

4. Progress and Outcomes

Growth conditions provide unique and different lipid composition

Extraction of products ensures accurate representation of products

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

Spectral matching and via software and databases provides compound identification

Identification of algae-derived products provides insight for future innovation
Task 1: Algae Characterization Streamlining

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

Politis, A., Van Wychen, S., et al., 2021, *submitted*
Reported analytical data can be misleading

Carbohydrates are calculated by difference (absorbs all remaining components)

Methodology available at Contract Analytical Laboratories directly impacts reported compositional data

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

Task 2: Intrinsic Value Opens up Bioproduct Opportunities

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

Collaboration with Ryan Davis, Bruno Klein, Algae TEA, manuscript in preparation
Identifying unknowns adds to mass balance adjustments

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

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

Lipidomics allows for discovery of novel biobased surfactants

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

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

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

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

Tao Dong, collaboration with WBS 1.3.4.201, CPR

Lesco, K., Lane, M., Rowland, S. et al., 2021, in preparation
Summary
Optimize value of algae relying on metabolic plasticity in response to cultivation management and physiological stressors

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

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

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

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

NREL’s Bioenergy Program Is Enabling a Sustainable Energy Future by Responding to Key Market Needs
**Timeline**
- Start: FY2013
- Merit review cycle: FY2020-2022

<table>
<thead>
<tr>
<th>FY20</th>
<th>Active Project</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOE Funding</td>
<td>$700K</td>
</tr>
</tbody>
</table>

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

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

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

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

**Funding Mechanism**
AOP
Thank You

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

Lieve.Laurens@nrel.gov
Additional Slides
Responses to FY19 Reviewers’ Comments

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

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

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


Application to FTMS data Shows Species-specific Lipidome Profile

Monoraphidium

Nannochloropsis

Scenedesmus
Applied Lipidomics Database

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

Entries 11670
Species 128
Distinct Lipids 1108
Head Groups 38
Papers Cited 108
Molecular Complexity of Lipid Database

Frequency of Distinct Algal Lipids in Peer-Reviewed Literature

Head Groups

- DG
- DGCC
- DGDG
- DGMG
- DGTA
- DGTG
- DGTS
- DPG
- IPC
- LDGCC
- LDGDG
- LDGTS
- LMGDG
- LPA
- LPC
- LPE
- LPG
- LPI
- LSM
- LSQDG
- MG
- MGDG
- MGDG
- MGDG
- MGDG
- DMG
- DTA
- DGT
- DTS
- DPG
- IPC
- PDPT
- LDGCC
- PE
- LDGDG
- PG
- LDGTS
- PI
- LMGDG
- PS
- LPA
- SM
- LPC
- SQDG
- LPE
- SQMG
- LPC
- SQTG
- LPI
- TG
- LSM
- TGDG
- LSQDG
All biomass high protein, consistent with CPR sourced biomass for conversion research.
Protein Utilization

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

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