Composition of Algal Biomass for Biofuels and Bioproducts: High Impact Data and Method Harmonization

Lieve Laurens

October 1st, 2013
Outline:

• Compositional Analysis
  o Productivity metrics and setting targets
  o Understanding process chemistry

• Consequences of lack of standardization on data
  o Computational models (TEA/LCA) and data integration

• Common language on measurements?
  o 2011 Baseline uncertainty quantification
  o Biological and physiological measurement variability
  o 2012-2013 ATP³ “Analytical Harmonization”
  o Round robin experiments
  o Conclusions and recommendations
Harmonization of Measurements

- Harmonized data and assumptions for LCA, TEA and RA models* after harmonization workshop, soliciting input from research and commercial community
- Consequence of lack of standardization is that measurement variability carries forward to computational models (TEA, LCA)
- Process and biomass improvement targets can only be set when measurement uncertainties are defined
  - Uncertainties in yield calculations
  - Cost estimates
  - Process Mass Balance

<table>
<thead>
<tr>
<th>Fuel</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipids (kg/ton)</td>
<td>255 ± 51</td>
</tr>
<tr>
<td>Green Diesel ($/gallon)</td>
<td>12.1 ± 2.4</td>
</tr>
</tbody>
</table>

Compositional Analysis in Algae

• “How hard can it be?”
  o Goal of 100% mass accounting
  o Show between 60 and 120% mass balance closure
  o Definitions of lipids, carbohydrates, protein

• Tracking metrics for target improvements in costs/productivity need better methods to robustly track progress at every step

• Empirical, historical methods utilized for algal biomass and process highly variable (12-18% RSD), often far from accurate, making comparison and modeling of data problematic

• Lack of standardized analytical procedures specifically tailored for algal biomass
2011 Method Uncertainties

- **Goal**: Quantify measurement uncertainty
- Survey of routinely used methods for proteins, lipids and carbohydrate measurements
- Comparison of yields, accuracy and precision of each method
- Design of Round Robin experiment
  - 8 analysts, 12 days, 9 methods, 5 replicates
  - 1 biomass sample (Chlorella sp.)
2012 Sample Matrix Variability

**Carbohydrates**
- $y = 0.69 x + 6.46$
- $R^2 = 0.77$

**Proteins**
- $y = 0.93 x + 5.08$
- $R^2 = 0.78$

**Lipids**
- $y = 0.86 x + 5.35$
- $R^2 = 0.94$
Biological vs Measurement Variability

**Chlorella sp.**

**Nannochloropsis sp.**

**Scenedesmus sp.**
Where we were a year ago...

- Significant bias between methods
  - Underlying measurement chemistry differences
  - Measurement interferences

- What are the best methods for compositional analysis of algae?
- What does the industry want to know?
- What data is needed to feed TEA/LCA models?
- Need for agreement on measurements for algal biomass constituents, and procedures for data collection
Standard Operating Procedures – Harmonization
Harmonizing Productivity and Analytical Measurements for High Quality Data

Goal
Develop and implement analytical pipeline for collection and dissemination of high-quality data to support computational models and experimental design

Specific Objectives
• Distribution of harmonized analytical procedures
• Implementation and alignment of methodology
• Consensus of procedures and data collection, QC and reporting
• Interface with and population of SDMS
Unified Field Studies – Harmonized Data

Goal
Eliminate Measurement Variability from Unified Field Study experimental effectors

Objectives:
• Need to lock down uncertainty around measurements
• Statistical analysis of UFS data should not be influenced by analytical variability
• Unified biochemical data ingest to data management system

Productivity metrics:
• Volumetric Dry Weight
• Ash Free Dry Weight
• Lipids
• Carbohydrates
• Protein
Harmonization Framework

Goal

• Alignment and uncertainty quantification of measurements
• Round Robin Experiment between participating laboratories using standard biomass sample (reference material, *Nannochloropsis* sp.)
• Statistical interpretation and setting QC requirements of measurements for the duration of ATP³

Questions

• Which methods should be used for compositional analysis?
• What data format is used for data collection?
• What are the checks/QC in place to make sure analytical methods performed ok?
Harmonization Framework

Algal Biomass Metrics Harmonization Framework (Phase 1)

<table>
<thead>
<tr>
<th>Activity ID</th>
<th>Activity Name</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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FY13 FY14

<table>
<thead>
<tr>
<th>Project Management</th>
</tr>
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<tbody>
<tr>
<td>PM001 Kick off meeting</td>
</tr>
<tr>
<td>PM002 Organize and conduct biweekly Analytical/Data group meetings</td>
</tr>
<tr>
<td>PM003 Organize and conduct biweekly Production/Data group meetings</td>
</tr>
<tr>
<td>PM004 Final harmonization report publishing - DELIVERABLE</td>
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<tr>
<td>PM005 Phase 1 Go/No Go Review</td>
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</table>

Analytical Method Implementation

Round Robin Experiment

Productivity Metrics Method Harmonization

Mini-pond operation and first Unified Field Study

Validation of productivity and composition measurements

Go/No Go Review
Jan 2014
Laboratory Analytical Procedures (LAP)

1. **Determination of Total Solids and Ash in Algal Biomass**
2. **Determination of Total Lipid as Fatty Acid Methyl Esters (FAME) by in situ Transesterification of Algal Biomass**
3. **Determination of Total Carbohydrate in Algal Biomass**
4. **Determination of Total Starch in Algal Biomass**
5. **Calculation of Nitrogen-to-Protein Conversion Factor**

10.2 **Preparation of the surrogate**
10.2.1 To make up a mixture of C13Me to a

11.1 Export the FAME concentrations (as µg mL⁻¹) for each individual fatty acid methyl ester (C4 – C24) from GC software and normalize for the quantity of the recovery standard C13. The outcome is the sum or total FAME content normalized for the recovery of the surrogate standard added at the start of the reaction (step 10.3.2):

\[
\text{Total FAME}_{\text{C13}} = \sum \text{Amount}_{\text{normalized FAME}_{\text{C13}}} \times \text{Amount}_{\text{surrogate}}
\]

11.2 Calculate the total FAME as a percent of the dry weight of the sample. For the FAME analysis, the dry weight refers to the weight after drying the sample overnight at 40°C in a vacuum oven:

\[
\% \text{Total FAME} = \frac{\text{Total FAME}_{\text{C13}}}{\text{ODW}_{\text{sample}}} \times 100
\]

11.3 To report or calculate the root mean square deviation (RMS) or the standard deviation (STDEV) of the samples, use the following calculation:

\[
\text{RMS} = x_n = \text{mean} = \left( \frac{\sum x_i}{n} \right)^{1/2}
\]

\[
\text{STDEV} = \sigma = \text{stdev} = \left( \frac{\sum (x_i - x_n)^2}{n} \right)^{1/2}
\]

Where:
- \( x_n \) is the root mean square of all values in the set
- \( n \) is number of samples in set
- \( x_i \) is measured value from the set
Laboratory Analytical Procedures – Standard spreadsheet (v. 7)

### Sample metadata

<table>
<thead>
<tr>
<th>Master Ref</th>
<th>Analyst</th>
<th>Laboratory</th>
<th>Sample Description</th>
<th>Testing ID</th>
<th>Material</th>
<th>Analysis start time</th>
<th>Replicate (1=Single, 2=Duplicate, 3=Triplicate)</th>
<th>Sample Comments</th>
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<tbody>
<tr>
<td>1</td>
<td>Xueyan Liu</td>
<td>TRL</td>
<td>ATP3 Nano</td>
<td>Y1</td>
<td>Freeze dried biomass</td>
<td>8/28/13</td>
<td>1</td>
<td>Nanochloropsis sp. QC biomass - replicate tests</td>
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<td>Nanochloropsis sp. QC biomass - replicate tests</td>
</tr>
</tbody>
</table>

- Protected calculations, only raw data to be entered
- Interface with data management system
Laboratory Analytical Procedures – Standard spreadsheet (v. 7)

<table>
<thead>
<tr>
<th>CATEGORICAL VARIABLES</th>
<th>MEASUREMENT VARIABLES, on total solids basis (% ODW)</th>
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<tbody>
<tr>
<td>Sample Description</td>
<td>Ash</td>
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<tr>
<td>Tracking ID</td>
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<td>ATP3 Nanno Y1</td>
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</tr>
<tr>
<td>ATP3 Nanno Y2</td>
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<tr>
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<td>Analyst</td>
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<tr>
<td>Xueyan Liu</td>
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<td>Material</td>
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<tr>
<td>freeze dried biomass</td>
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<td>Laboratory</td>
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<tr>
<td>Analysis Date</td>
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<tr>
<td>8/28/13</td>
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<td>Ash</td>
<td>20.42</td>
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<tr>
<td>Protein</td>
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<tr>
<td>FAME Lipids</td>
<td>13.38</td>
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<tr>
<td>Structural Sugars (MeTh)</td>
<td>8.55</td>
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<tr>
<td>Mass Closure</td>
<td>71.33</td>
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Method Implementation (pre-harmonization)

- Distribution of **reference biomass sample Nannochloropsis sp.**
- All laboratories were given methods to try for 2 months
- Goal to become familiar with procedures and spreadsheets and to downselect to final method best practice
- Feedback on inconsistencies or misinterpretation of the methods and standardized spreadsheet
- Improved methods and spreadsheet distributed before Round Robin start

<table>
<thead>
<tr>
<th></th>
<th>Moisture</th>
<th>Ash</th>
<th>N</th>
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<th>Carbs_MBTH</th>
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<td>X</td>
<td>X</td>
<td>X</td>
<td>?</td>
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<tr>
<td>Lab2</td>
<td>?</td>
<td>?</td>
<td>?</td>
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<tr>
<td>Lab4</td>
<td>X</td>
<td>X</td>
<td>?</td>
<td>?</td>
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</tr>
</tbody>
</table>
Phase I Round Robin (July 2013)

- Ash
  - RSD = 6.3%

- FAME
  - RSD = 22%

- Carbohydrates
  - RSD = 13.5%

- Protein
  - RSD = 2.6%
Phase II Round Robin (August 2013)

- **Ash**
  - RSD = 6.3%
  - Lab1 Lab2 Lab3 Lab4 Lab5 Lab6

- **FAME**
  - RSD = 11.7%
  - Lab1 Lab2 Lab3 Lab4 Lab5 Lab6

- **Carbohydrates**
  - RSD = 16.8%
  - Lab1 Lab2 Lab3 Lab4 Lab5 Lab6

- **Protein**
  - RSD = 2.5%
  - Lab1 Lab2 Lab3 Lab4 Lab5 Lab6
Conclusions

• All 5 testbeds are using the same methods for analytical characterization
• Trained and troubleshoot laboratory procedures to address inconsistencies
• Continued monitoring of reference material and tracking improvements
• Goal to achieve < 10% RSD
• Implementation of methods for productivity metrics in Unified Field Studies (October 2013)
Acknowledgements

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Eric Nicolai (CalPoly)
Tryg Lundquist (CalPoly)
Philip Lane (TRL)
Xueyan Liu (TRL)
Emily Knurek (Cellana)
Kate Evans (Cellana)
Steven Van Ginkel (GT)

Ed Wolfrum, David Crocker (NREL)
Questions?

Lieve.Laurens@nrel.gov

ATP³ booth, discussion of methods and analytical harmonization: Tomorrow 10:45 am

Posters

Van Wychen, S. #134 “Accurately Determining Microalgal Carbohydrates with MBTH”

Christensen, E. #107 “Dynamic Lipid Profiling in Algae: Application of High-Resolution Mass Spectrometry and Discovery of Novel Components of Advanced Biofuels Intermediates”
Standard Biomass Analytical Procedures

NREL has developed the following laboratory analytical procedures (LAPs) for stand American Society for Testing and Materials (ASTM) and the Technical Association of Industry (TAPPI) may have adopted similar procedures. ASTM and TAPPI versions n those organizations.

NREL Biomass Program researchers have also developed calculation workbooks (Ex automatically calculate desired compositional analysis and mass closure, based on measurement procedures in the pertinent LAPs. Workbooks are available for:

- Wood (hardwood or softwood)
- Corn stover (corn stover feedstock)
- Biomass hydrolyzate (liquid fraction produced from dilute-acid pretreatment)
- Corn stover Intermediate (solid fraction produced from dilute-acid pretreatment also be used for wood intermediates)
- Nitrogen-to-protein factor calculator

These workbooks are designed for use in conjunction with the appropriate NREL LAP to assist with the calculations necessary in the LAPs. Each workbook combines the LAPs, guiding the user to summative mass closure or summative analysis. Th workbook should be used prior to use, as it contains important instructions and lege the user to determine the most appropriate workbook to use, as each workbook is l Comments and suggestions are welcome.

Laboratory Analytical Procedure (LAP)

- Summative Mass Closure – Laboratory Analytical Procedure Review and Integration: Feedstock
- Summative Mass Closure – Laboratory Analytical Procedure Review and Integration: Pretreatment
- Determination of Structural Carbohydrates and Lignin in Biomass
- Determination of Extractives in Biomass
- Preparation of Samples for Compositional Analysis
- Determination of Total Solids in Biomass and Total Dissolved Solids in Liquid Process Samples
- Determination of Ash in Biomass
- Determination of Sugars, Byproducts, and Degradation Products in Liquid Fraction Process Sar
Relevant Publications


