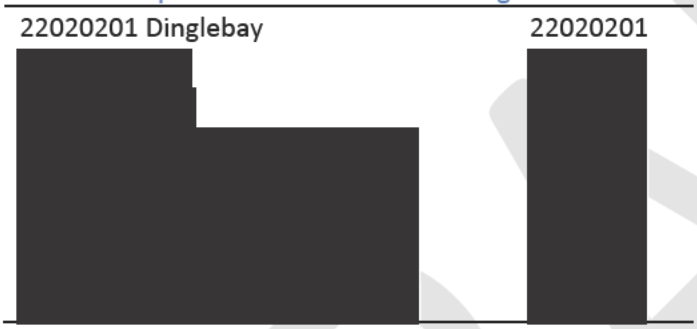
# Oceanium Seaweed samples and derived materials – Compositional Analysis Report

This report documents the compositional analysis of dried algal biomass based on standard analytical procedures to determine the proximate biochemical composition (ash, protein, lipid and total carbohydrate content) of the material. All data is reported as the mean ± standard deviation of triplicate analyses.

## Material Sourcing and Analysis

Macroalgae biomass was air dried and packaged at Oceanium, UK, and shipped to the National Renewable Energy Laboratory (NREL), in Golden, Colorado

Table 1: Sample identifiers and short designations




The biomass was homogenized to <2 mm particle size in the presence of dry ice and analyzed for full proximate composition using established procedures, including methods for total carbohydrates (including uronic acids after acid hydrolysis), lipids, as fatty acid methyl esters (FAME), protein and solids were determined based on previously published procedures:
https://www.nrel.gov/bioenergy/microalgae-analysis.html

Determination of Carbohydrates, Two-step sulfuric acid hydrolysis to hydrolyze the polymeric forms of carbohydrates in algal biomass into monomeric subunits. The monomers are then quantified by either high-performance liquid chromatography, in particular high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) [6] Uronic acids were analyzed in the same assay, but analyzed separated by HPAEC-PAD, as a control, alginic acid was included in the reaction and the recovery of the sum of the monomeric detected guluronic and mannuronic acid was 95.58% of the alginic acid dry weight [6]; Determination of Lipids as FAME, Following whole biomass, *in situ* transesterification of lipids to fatty acid methyl esters (FAME), followed by gas chromatography [8]; Determination of Solids, A convection oven drying procedure is used for total solids content, at 40°C under vacuum, and a dry oxidation method at 575°C for ash content; Determination of Protein, Protein content is determined via elemental nitrogen content and conversion via a 4.92 nitrogen-to-protein factor [4]; Summative Mass Analysis, Integration of LAPs to measure algal biomass constituents in an unambiguous manner and ultimately achieve mass balance closure [7]



15013 Denver West Parkway
Golden, CO 80401
Phone 303-275-3000

NREL is a national laboratory of the U.S. Department of Energy
Office of Energy Efficiency & Renewable Energy
Operated by the Alliance for Sustainable Energy, LLC

## Margin of Error

To minimize error in the analysis of the SRM, ISO guide 35 and IRMM guidelines were followed. Multiple analysts characterized this material and between batches, samples have been investigated for homogeneity.

Table 2: Mass fraction values for proximate biomass composition [g/100 g, ± stdev]

| | Ash | Carbohydrates (PAD) | Uronic acids (PAD) | Lipids (as FAME) | Protein (N x 4.92) | Mass Closure |
|---|---|---|---|---|---|---|
| 22020201 | 35.14 ± 0.21 | 19.98 ± 0.76 | 21.11 ± 0.54 | 1.17 ± 0.02 | 7.61 | 93.24 |

Table 2: Relative mass fraction values for monosaccharides comprising the carbohydrate fraction [g/100g total measured carbohydrates, or g/100g total measured uronic acids] <LOQ = below limit of quantification

| | 22020201 | 22020202 | 22020203 | 22020204 | 22020205 | 22020206 | 22020207 | 22020208 |
|---|---|---|---|---|---|---|---|---|
| Mannitol | 55.92 | 32.39 | 33.48 | 36.95 | 36.97 | 12.14 | 6.07 | 8.13 |

Table 3: Relative Mass Fraction Values for Fatty Acids Comprising the Lipid Fraction [% total measured fatty acids] ND = Not Detected

| | 22020201 | 22020202 | 22020203 | 22020204 | 22020205 | 22020206 | 22020207 | 22020208 |
|---|---|---|---|---|---|---|---|---|
| C8:0 Caprylic acid | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| C24:0 Lignoceric acid | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 3.37 | 2.43 |



## Contact

Lieve M.L. Laurens, PhD.; Algae Platform manager, Analytical Characterization Lead, National Bioenergy Center Lieve.Laurens@nrel.gov, Phone: +1 (303) 384-6196 or Stefanie.VanWychen@nrel.gov

# References

[1] Laurens, L. ML.*, Van Wychen, S., Pienkos, P.T., Harmon, V. L., McGowen, J. (2017) "Harmonization of Experimental Approach and Data Collection to Streamline Analysis of Biomass Composition of Algae in an Inter-Laboratory Setting" **Algal Research**, 25, 549-557

[2] Van Wychen, S., Long, W., Black, S., Laurens, L. ML.* (2017) "MBTH: A novel approach to rapid, spectrophotometric quantitation of total algal carbohydrates", **Analytical Biochemistry**, 518, 90-93

[3] Laurens, L. ML.*, Slaby, E. F., Clapper, G., Howell, S., Scott. D. (2015) "Algal Biomass for Biofuels and Bioproducts; Overview of Boundary Conditions and Regulatory Landscape to Define Future Algal Biorefineries" **Industrial Biotechnology**, 11(4), 221-228

[4] Templeton, D. W., Laurens, L. ML.* (2015) "Nitrogen-to-protein conversion factors revisited for applications of microalgal biomass conversion to food, feed and fuel" **Algal Research** 11:359-367

[5] 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**, 452:86-95

[6] Templeton, D. W., Quinn, M., Van Wychen, S., Hyman, D. and Laurens, L. M.L.* (2012) "Separation and Quantification of Microalgal Carbohydrates", **Journal of Chromatography A**, 1270: 225-234

[7] Laurens, L. ML.*, Dempster, T., Jones, H. D. T., Wolfrum, E., Van Wychen, S., McAllister, J., Arrowsmith, S., Parchert, K. J., and Gloe, L. M. (2012) "Algal biomass constituent analysis: method uncertainties and investigation of the underlying measuring chemistries", **Analytical Chemistry**, 84(4): 1879

[8] Laurens, L. ML.*, Quinn, M., Van Wychen, S., Templeton, D. T., Wolfrum, E. (2012) "Accurate and reliable quantification of total microalgal fuel potential as fatty acid methyl esters by in situ transesterification", **Analytical and Bioanalytical Chemistry**, 403(1):167-78.

**Analytical Procedures:**

Summative Mass Analysis of Algal Biomass – Integration of Analytical Procedures, www.nrel.gov/docs/fy16osti/60943.pdf

Determination of Total Solids and Ash in Algal Biomass, www.nrel.gov/docs/fy16osti/60956.pdf

Determination of Total Lipids as Fatty Acid Methyl Esters (FAME) by in situ Transesterification, www.nrel.gov/docs/fy16osti/60958.pdf

Determination of Total Carbohydrates in Algal Biomass, www.nrel.gov/docs/fy16osti/60957.pdf



15013 Denver West Parkway
Golden, CO 80401
Phone 303-275-3000

NREL is a national laboratory of the U.S. Department of Energy
Office of Energy Efficiency & Renewable Energy
Operated by the Alliance for Sustainable Energy, LLC