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Harmonization of experimental approach and data collection to streamline analysis of biomass composition from algae in an inter-laboratory setting

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ABSTRACT

In order to establish and design long-term algae cultivation experiments, inter-laboratory projects need to harmonize the requirements of techno-economic and life-cycle analysis models, with standardized data inputs. In order to provide a consistent foundation and allow for integration and analysis of the results in computational technical and resource analysis models, we implemented closely coordinated, harmonized and objective analytical protocols along with a common language for measuring growth and productivity for the major algal components. We describe here the process by which we developed a harmonization framework for analysis across five geographically diverse testbed sites. Our goal was to align analytical procedures to ensure consistent reporting on biomass and lipid content, quality and yields to eliminate measurement variability as a source of uncertainty in production data. Developing standards for analysis that streamline reporting on composition and expected fuel yields from biomass is one of the major outcomes of this work and this provides a starting place for further advanced characterization of algae to support the techno-economical process analyses and account for the mass balance accounting of algal biomass. Initial analysis of data obtained from field studies shows trends in compositional shifts of lipid and protein content of the biomass that are in support of the physiological experiments demonstrated in the first geographically distributed unified outdoor cultivation trials.

1. Introduction

As algae operations progress from research to commercialization operations, a uniform language and common methodologies for characterization of process unit-operations is required. The Algae Testbed Public Private Partnership (ATP^3) interlaboratory consortium was originally designed to provide closely coordinated, harmonized, and objective standards for operational protocols, data collection and analysis, data management, quality control, modeling and assessment [1]. The partnership (http://atp3.org/about-us/) was designed to experimentally investigate the impact of geographical location on algal biomass productivity and composition, similar to the theoretical studies that have been performed in the literature [2–4]. Furthermore, the composition of algal biomass is inherently connected to the value of harvested material in a production scenario and opens avenues for valorization of the entirety of the biomass [5,6]. The influence of physical parameters on the bioenergetic yield and biomass composition is important to draw conclusions and verify resource assessment predictions that have been made and will ultimately guide the development and deployment strategy for an algae production plant [5,7].

The objective of this work was to set standards that streamline reporting on biomass production and composition and expected fuel yields from biomass throughout the project. The five testbed sites were selected as they offered geographic and climatic diversity and a spectrum of resources, including access to natural seawater and or wastewater and were equipped with a range of algae production and processing systems with demonstrated production, harvesting, and processing capacity in a variety of configurations (*e.g.*, open/closed ponds/PBRs). The sites represent diverse geographical and climatic locations; Southwest, desert (AzCATI, Arizona State University (ASU), Mesa, AZ); Western, coastal (California Polytechnic State University (CP), San Luis Obispo, CA); Southeast, inland (Georgia Institute of Technology (GT), Atlanta, GA); Pacific, tropical (Cellana LLC (CELL), Kona, HI), Midwest greenhouse (Touchstone Research Laboratory (TRL), Wooster, OH). The site staff was an integral part of this harmonization effort and ensured that the data collected and dissemi-

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nated was of the highest quality. At the onset of this project, there were no common procedures in place to implement and in this regard this project was breaking new ground. The analytical harmonization effort built procedures on existing analytical methodology and included the building of a data analysis and integration pipeline, by providing procedures and a coordination framework. The work and data pipeline included the implementation of a data management system and hosting of open-access data on the Open Energy Information (OpenEI) website (http://en.openei.org/wiki/ATP3) [8]. The establishment of a quality assurance and quality control (QA/QC) set of guidelines refers to the process or set of processes used to measure and assure the quality of a sample and the data generated by different groups. The implementation of QA/QC in analytical methodologies requires the following; i) Method Validation Standard (or standard reference material): A biomass sample that is in abundance and has been well characterized in all labs and designed to always be used in conjunction with analytical procedures to provide confidence in the data generated, ii) controls such as internal quantification standards, verification of calibration and instrumentation or procedures, iii) replicate analyses to determine confidence in analyses. Replicate analyses between different labs, researchers, and days on samples of the same material will provide information on the uncertainty associated with methods and reliable data on a reference material.

To provide an approach to get the best possible data within a consortium, there was a need to establish a baseline and set up checks consisting of the following best practices; i) designating a standard reference material for duration of project (homogenized, from one biomass stock and stored frozen nitrogen-purged aliquots, ii) standardizing sample storage and preparation, iii) establishing and distributing best practice methods – including iteration and optimization/updating methods/procedures, iv) determining uncertainty of methods between researchers/labs on standard reference material, through the statistical analysis of data from a round robin experiment. The rationale and emphasis of including quality control guidelines for methods is to verify that harvested and produced algal biomass material is analyzed in a consistent manner and that reported data is collected using the same methods and can thus be directly compared. It is not the objective of these methods to provide a complete characterization of the biomass material, rather provide information on the dynamic behavior of major biomass components and their relative biochemical compositional information over the course of unified cultivation experiments.

Accurate and precise determination of the composition in an interlaboratory project like ATP^3 will allow for observations in, for example, lipid productivity, to be related to testbed sites and ultimately geographical location. The harmonization framework pursued here will ascertain that the measurements and phenomena observed are not an artifact of analytical measurement uncertainties [9,10]. Within the framework of this consortium and cultivation studies, an initial validation experiment was established to study a shift in composition after nutrient depletion. Based on existing literature on *Nannochloropsis* [11,12] as well as for other species of eukaryotic algae [13–17] we anticipated a significant response of the cultures with respect to their composition, which provided a test to the analytical framework, as well as allow for the study of compositional shift response at different geographical sites. To our knowledge a study of the simultaneous controlled outdoor cultivation and physiological nutrient shift analysis has not been demonstrated and thus the description of the biomass composition shifts in coordinated framework is a unique contribution to the literature.

2. Materials and methods

2.1. Standard reference material

A flowchart of method integration is shown in Fig. 1 and details the analyses on dried biomass. The respective analytical procedures for each of the measurements were distributed to the testbed sites as detailed analytical procedures [18–21]. One biomass sample, *Nannochloropsis granulata* CCMP 535, was selected as a reference material because of its availability in large quantities of freeze-dried biomass. The biomass was grown under nutrient replete conditions (50 ppm N as nitrate) in a 30 m^2 open ponds at 20 cm depth at the AzCATI site in Mesa, AZ. The batch run was harvested at a cell concentration of 0.7 g/L by continuous centrifugation and stored as a frozen paste at − 20 °C prior to freeze drying in bulk and distribution to each of the ATP^3 partner sites. A total of 200 g of homogenized, freeze-dried biomass was distributed to each of 5 sites and stored at − 20 °C.

2.2. Compositional analysis methods

The methods for compositional analysis are as described before, with minor modifications to allow for the implementation at individual testbed sites [10,18–22]. Ash analysis was carried out as described before and as a single replicate for each of the samples [19]. In brief, crucibles were preconditioned in the 575 °C muffle furnace overnight to remove any combustible contaminants. Once the crucibles came to room temperature, their weights were recorded. In each crucible 100 ± 5 mg of freeze-dried algae was added and the weight of each sample was recorded. Samples were then placed in a 40 °C vacuum oven overnight and the oven dry weight of the sample was recorded, after which the samples were placed in the ramping 575 °C oven overnight. Lipids were measured as fatty acid methyl esters (FAMEs) in biomass, through an *in situ* FAME preparation method [23]. In brief, 4–7 mg of lyophilized microalgae biomass was added to a pre-weighted GC vial. An internal standard tridecanoic acid methyl ester, chloroform/methanol (2:1) and HCl methanol solution (5% v/v) was added the solution was heated at 85 °C for 1 h, extracted with 1 mL of hexane and analyzed by GC-FID with a DB-WAX column (Agilent, USA), 30 m 0.25 mm ID and 0.25 μm FT, temperature program 100 °C for 1 min, then 25 °C min^{-1} to 200 °C, hold for 1 min, then 5 °C to 250 °C and hold for 7 min, at a 1 mL min^{-1} He constant flow (GC instrumentation varied slightly between each of the sites and is not described in detail here). The individual FAME concentrations was quantified and normalized against the internal standard tridecanoic methyl ester [23]. Whole biomass carbohydrate content was determined as described previously in duplicate for each of the samples [18]. In brief; lyophilized biomass (25 mg) and 250 μL of 72% (w/w) sulfuric acid were added into a 10 mL glass vial. The first step hydrolysis was performed in 30 °C water bath for 1 h. Then, 7 mL of 18.2 MΩ water was added to the tube. The vial was sealed and autoclaved for 1 h at 121 °C. The concentration of monomeric carbohydrates after hydrolysis was quantified by methylbenzothiazolium hydrazine (MBTH) derivatization and subsequent spectrophometric analysis against a known glucose standard as described before [18,22]. The nitrogen content of the samples is determined by combustion using CHN analyzers (exact instrumentation varied between different testbed sites). The resulting values, measured as singlet analyses for each sample, but included a triplicate analysis for the reference material. Elemental nitrogen data was reported as weight percent of the sample. Protein concentration was calculated from the nitrogen content with a conversion factor of 4.78 [24]. All compositional analysis data was collected in a reference spreadsheet to automate and standardize the respective calculations and make quality control of the data straightforward. This spreadsheet is available upon request.

2.3. Cultivation field study

An initial validation Unified Field Study (UFS) was designed and implemented in the fall of 2013 and involved two phases of cultivation of a single strain; *Nannochloropsis oceanica* KA32, in six replicate mini-ponds comparing growth at different pH control set points and in nitrate replete and deplete media as described in detail elsewhere [1].