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Strain, biochemistry, and cultivation-dependent measurement variability of algal biomass composition

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ABSTRACT

Accurate compositional analysis in biofuel feedstocks is imperative; the yields of individual components can define the economics of an entire process. In the nascent industry of algal biofuels and bioproducts, analytical methods that have been deemed acceptable for decades are suddenly critical for commercialization. We tackled the question of how the strain and biochemical makeup of algal cells affect chemical measurements. We selected a set of six procedures (two each for lipids, protein, and carbohydrates): three rapid fingerprinting methods and three advanced chromatography-based methods. All methods were used to measure the composition of 100 samples from three strains: *Scenedesmus* sp., *Chlorella* sp., and *Nannochloropsis* sp. The data presented point not only to species-specific discrepancies but also to cell biochemistry-related discrepancies. There are cases where two respective methods agree but the differences are often significant with over- or underestimation of up to 90%, likely due to chemical interferences with the rapid spectrophotometric measurements. We provide background on the chemistry of interfering reactions for the fingerprinting methods and conclude that for accurate compositional analysis of algae and process and mass balance closure, emphasis should be placed on unambiguous characterization using methods where individual components are measured independently.

The composition of algal biomass is important to many algal biofuels and bioproducts processes. Robust chemical characterization of the biomass helps in reducing the overall uncertainties around individual process steps and allows for identification of improvements. Even production schemes centered around the so-called "feedstock agnostic" hydrothermal liquefaction process depend on the compositional makeup of the feedstock biomass for the quality (and upgrading demands) of the intermediate "green crude." Analytical tools developed for algal biomass need to be accurate and precise and must be strain agnostic instead of measurement values obtained being influenced by the physiological and environment-induced changes in the biomass. Failing that, it will be necessary to understand the limitations of each method and to apply them with care under novel situations.

The current assumptions used for baseline techno-economic calculation of algal biofuels costs take into account a standard yield of approximately 13.2 g m^{-2} day^{-1} with a fixed oil content of 25% of the biomass dry weight [1]. However, algal biomass biochemical composition varies greatly depending on the strain and the nutrient status of the algal culture medium. Thus, timing of harvest can greatly affect the overall reported fuel yields and downstream processing characteristics. The observation that algal cells change their metabolic composition throughout the growth and in response of environmental and physiological stimuli is decades old [2], and here we take advantage of these exact physiological changes to demonstrate the challenges involved in measurement accuracy.

Accurate analytical characterization is necessary to determine the value of algal biomass. In the context of developing a lipid-based biofuels process, the actual fraction of fuel-relevant components (i.e., fatty acids) in biomass needs to be determined. If a simultaneous process for converting the carbohydrate fraction to fuel is implemented, a measure of fermentable carbohydrates is needed. We previously reported on the precision and accuracy of routinely used methods for compositional analysis of algae after a round-robin experiment among three different laboratories using nine literature-based and routinely used analytical methods for three constituents [3]. That work demonstrated considerable differences among values obtained by the respective methods for

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common constituents: lipids, carbohydrates, and protein. A review of recent literature reporting on algal biomass composition points to a lack of consistency in methods used between different groups of algal biologists and even between commercial laboratories engaging in algal biomass analysis [4–8]. The implication of the study reported in Ref. [3] is that major biochemical components of biomass cannot be compared across different laboratories with a precision of less than approximately 20% of the value measured as long as consistency of measurement is disregarded. This uncertainty carries forward in calculations of biomass and even oil and sugar market value. It also severely circumscribes strain or process improvement work that is geared toward incremental increases in lipid (or other biochemical component) content. There are significant differences among the nine respective methods used for characterization with respect to accuracy and precision, with empirical historically used methods having consistently reduced precision and the greatest difference from the reference measurements used. Although empirical methods are in widespread use, a comparative analysis of the precision and accuracy across model and commercially relevant strains has not been carried out until now.

The degree to which the whole biomass can be described (i.e., mass balance closure) is important to determine the applicability and fate of the biomass and to draw conclusions on process economics and efficiency parameters. If all separate constituents are measured individually, they should add up to 100%. The closer this value gets to 100%, the more confidence we have that we are not missing any significant components of the biomass and the more confidence we will have in the economic and technical models derived from these data. We demonstrated previously, by variation in mass balance calculations for one sample, that these reports need to be critically evaluated because of the measurements' dependence on the methods used and the inherent likelihood that components might be double-counted. For example, hydrophobic proteins might be coextracted along with lipids during a classical solvent extraction and will contribute to the weight of the lipid fraction. However, the protein is also typically measured directly on the intact biomass. Thus, summation of the gravimetric extractives and the direct protein measurement will bias the mass balance calculations high.

The objective of the work presented here was to study the effect of biochemical variability on the agreement of six down-selected analytical characterization methods used. This article deals less with within-method measurement uncertainty than with between-method differences caused by the underlying chemistry of the measurement reactions. Expanding on a round-robin biomass analysis effort between two separate laboratories, we have measured the biochemical composition of 100 algal biomass samples harvested from three strains—*Scenedesmus* sp., *Chlorella* sp., and *Nannochloropsis* sp.—grown under varying conditions as well as under an extended period of nitrogen starvation. The three strains used for this study are routinely cultivated in our laboratories and were chosen as representative model organisms for current research and development in algal biomass for biofuels and bioproducts research. We used a subset of six analytical procedures: two for each of three main biochemical compounds (lipids, carbohydrates, and protein). For a detailed study of the biochemical interferences, samples were taken from triplicate bioreactors at periodic intervals over the course of the production runs for each strain, representing controlled variation in biochemical composition. The within-strain composition indicates a trend of increased oil content throughout the nitrogen starvation period of nearly 7-fold. The carbohydrate content, on the other hand, shows a distinct maximum midway through the period of nitrogen starvation before the onset of the high lipid concentration. At final harvest points, the carbohydrate content decreased with an accompanying increase in lipid content to more than 40% of the biomass. This data set allows us to tease apart the respective characterization method comparison by strain and biochemical composition.

Materials and methods

Algal biomass production for compositional variability

Algae were cultivated in indoor and outdoor photobioreactors (PBRs)[1] in a batch process at Arizona State University (ASU, Mesa, AZ, USA) as part of the Sustainable Algal Biofuels Consortium (SABC) consortium. The three different species used in this study are part of ASU's Arizona Center for Algae Technology and Innovation's (AzCATI) culture collection: *Scenedesmus* sp. (LRB-AP-0401), *Chlorella* sp. (LRB-AZ-1201), both locally isolated strains, and *Nannochloropsis granulata* (LRB-MP-0209). Seed cultures for outdoor production are maintained under nutrient replete conditions using either a modified BG-11 medium for freshwater strains (*Scenedesmus* sp. and *Chlorella* sp.) or a modified f/2 medium for saltwater strains (*Nannochloropsis* sp.). Typical starting nitrate concentrations for indoor cultures are 1.5 g L^{-1} $NaNO_3$ for modified BG-11 and 0.75 g L^{-1} for modified f/2. Inocula for outdoor cultivation are maintained in an array of indoor and greenhouse flat panel photobioreactors (FP–PBRs) before transferring to the outdoor pilot PBR site to produce the biomass used for this study. Inoculum cultures are maintained under nutrient replete conditions at a pH of 7.9 ± 0.2 and mixed with an air bubbler supplemented with 2% (v/v) CO_2. Culture temperatures are maintained in a range between 20 and 25 °C indoors and under constant illumination and in a range between 19 and 28 °C in the greenhouse in PBRs exposed only to natural sunlight.

The outdoor FP–PBRs used are 1.2 × 14.6 m (height × length) with a 1500- or 650-L working volume (10- or 3.8-cm light path, respectively) and oriented facing east/west. For this study, the process used outdoors was a two-stage process where the volume and concentration were increased and nutrients were depleted in the longer light path 1500-L reactors prior to transfer to the shorter light path 650-L reactors as the finishing stage, previously shown to shift biomass composition in both *Chlorella* sp. and *Scenedesmus* sp. [5,6]. By timing the harvest, biomass of different composition can be obtained in a controlled fashion. Nitrogen levels were set to allow complete depletion after 4 to 6 days of additional growth and after achieving a final biomass concentration of approximately 0.8 g/L. Nitrogen levels were measured via daily sampling of the culture medium using a Lachat QuickChem 8500 workstation (Lachat Instruments, Milwaukee, WI, USA); when deplete, cultures were transferred from the 1500-L FP–PBRs into the 650-L FP–PBRs. Typical batch size for this work was 4500 L, using three 1500-L FP–PBRs in the nutrient replete stage and seven 650-L FP–PBRs in the nutrient deplete stage (as biological replicates). Typical biomass concentration for the start of the nutrient deplete stage was 0.75 ± 0.1 g/L (ash free dry weight). Cultivation time under nutrient deplete conditions depended on final target biomass composition desired, which, depending on season, typically was 3 to 5 days for high carbohydrate (midpoint harvest) biomass and 6 to 9 days for high lipid (late harvest) biomass. High protein (early harvest) biomass was obtained by harvesting prior to nutrient depletion from the 1500-L reactors. Final biomass concentrations at harvest were between 2.0 and 2.5 g/L for the high carbohydrate samples and between 3.0 and 4.0 g/L for the high lipid samples. Biomass was harvested using a two-stage process where the culture was transferred to a membrane filtration unit for primary dewatering and then on to a continuous centrifuge achieving a final solids

[1] *Abbreviations used:* PBR, photobioreactor; ASU, Arizona State University; FP–PBR, flat panel photobioreactor; FAME, fatty acid methyl ester; DMSO, dimethyl sulfoxide; NREL, National Renewable Energy Laboratory; HPLC–RID, high-performance liquid chromatography with refractive index detection.