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Review article

A new algae technical standards focus group: Summarizing and guiding the algae state of the art

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ABSTRACT

The value proposition of algae technical metrics was the subject of a recent, industry-led, Focus Group interactive discussion. The interactive platform allowed for real time capturing and priority ranking of the topics that are deemed critical challenges for a future successful algae-based bioeconomy. This work ultimately sets the stage for providing guidance, methods, and standard reference materials tailored to respective algae industry segments for the cultivation, processing and marketing of high-quality and safe algae-based products. The group solicits input from the broader algae community on the value proposition and presents a case here to build a consensus around algae-specific metrics. The value proposition of technical standards to the industry is that guidance obtained through these collaborations builds producers' and consumers' trust that algae and algae-derived products are safe, reliable, and may allow for expanded market access, and increased competitiveness and international trade.

1. Summary

To support an algae-based bioeconomy, there is a critical need to align language around technical metrics and standardization. A Technical Standards Committee [1,2], under the umbrella of the Algae Biomass Organization (ABO), has historically supported the algae-based (microalgae/macroalgae, phototrophic/mixotrophic/heterotrophic) production of: i) energetic bioproducts such as food, feed, fertilizer, nutraceuticals, cosmeceuticals, pharmaceuticals, medicines and biofuels; ii) environmental restoration approaches such as decarbonization, remediation of air, water and soils as well as ecosystem and biodiversity recovery; and iii) carbon-negative, biodegradable/regenerative substitutes of natural origin such as biotextiles, pigments, ink, biofertilizers, bioplastics, packaging, construction materials, concrete and asphalt.

An inaugural Technical Standards focus group convened representatives of the wider algae community to build a critical mass of standardization advocacy. The goals of this meeting were to identify gaps in current descriptive methodology and ultimately develop guidance for future trade metrics, develop and disseminate summary documents on state of technology of algal biomass characterization (Fig. 1). Ultimately, the metrics of success will be tied to influencing and implementing a common standard language, and tracking its adoption across the algae community, with acceptance by regulatory agencies with authority and oversight for approving licenses and certifications of consumer products from algae. Publishing the findings of this Focus Group is meant to invite and solicit broader industry engagement towards the goals of this technical standards effort. Representatives participating in this inaugural focus group event included commercial producing members of the algae community as well as contract analytical laboratory representatives and government representatives from the Department of Energy.

The major topics used to guide the discussion were selected based on an industry survey that was carried out and sent to the wider algae community in 2018. This survey indicated that there is interest and support for a guided Technical Standards effort. Results showed that currently most regulating standards (FDA GRAS and Kosher, Global Organization of EPA and DHA (GOED), etc.) are voluntary and not specific to algae. This lack of specificity has led to confusion around best practices as well as biomass selling price. In particular, the frequency of testing and prioritization of analyses are not clear in existing regulations. Beyond issues associated with reporting algae-biomass composition, companies with edible products had particular interest in both contamination and toxin and toxic metal contents, but were unsure what testing was most reliable for this. The working group discussion primarily covered topics in the categories of biomass composition, identification and safety of algae.

1.1. Biomass composition

Primary constituents, protein, lipid (fat), carbohydrate and ash

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content, are covered under general nutritional analyses by contract laboratories, however, there are some inconsistencies in the reporting and methods used to determine the biomass composition [3]. Since compositional characteristics are often the drivers of biomass quality, e.g. omega-3-fatty acid content and composition, protein and ash content have been reported on as important metrics for the nascent algae industry. In particular the protein content reporting in algae can be problematic, as this is often derived from elemental nitrogen determination and conversion with a generic conversion factor of 6.25. This factor is not appropriate for biomass materials that contain non-protein nitrogen components, and often represents a significant overestimation of the actual protein content of the biomass [4,5]. Beyond the nutritional analyses of algal biomass, compositional analytes of interest were included in the suggested methods of interest, e.g. elemental analysis (C, H, N, S, Na, P, K, Mg), acid- and neutral Detergent Fiber (ADF, NDF), fatty acid profile, lipid composition, sterols, amino acid profile, sugar composition, including a comprehensive profile to include sulfated polysaccharides (e.g. in macroalgae) and Nucleic acids.

The value proposition of technical standards to the industry and the consensus was highlighted in that such guidance ensures producers and consumers that algae and algae-derived products are safe, reliable, and may allow for expanded market access, and increased competitiveness and international trade.

The commercial laboratory representatives were specifically asked to present their viewpoint on interactions with clients, most of whom are producing members of the algae industry. In general there appears to be significant redundancy in the specific selection of methods for biomass characterization (in particular multiple methods for lipid determination), the contract laboratory representatives are unable to provide consistent guidance to clients with respect to differences in the measurements and what is recommended for those particular biomass matrices. In general it was noted that it is important to collect and report information on the different matrices (biomass sources representing different commercially produced materials) that are currently commercially deployed and the group identified a need for a specific list of types of biomass and products, and algae species that make up the biomass, that fall under the 'algae' definition with an accompanying list of suggested standard analytical procedures.

There was discussion on the need to standardize what is referred to as production system metrics, and associated measurement methods that are implemented for e.g. ash-free dry weight (AFDW), total organic carbon (TOC), water quality, etc. While this is a deviation on strictly compositional characterization of the biomass, productivity metrics are consistently at the basis of technical and economical analyses of any commercial farming operation. This deserves attention in a future iteration of the technical standards workshop, to ensure consistency across all production platforms in reporting a certificate of analysis. There is precedent for such methods to be derived from previously established standard methods, accepted and required by the Environmental Protection Agency (EPA) [6].

Questions posed by the members identified the need for a routine measurement around caloric value of the biomass, supporting the reporting towards bioenergy applications. In the context of rapidly changing biomass composition with varying physiological and nutritional environments of algae cultivation, there was a request to track biomass composition with temporal factors, as well as treatment, e.g. harvesting and storage procedures.

Specific technical questions surrounding methodology and reporting of biomass composition covered areas like temperature for moisture and ash determination. Other major comments included protein factor utilization and characterization of lipid fractions. In particular, one participant specifically discussed the need for fatty acids versus extractable lipid characterization and the need to standardize the definitions of some of the reported constituents. A prioritized impact ranking of the different constituents is shown in Fig. 2. Similarly, there

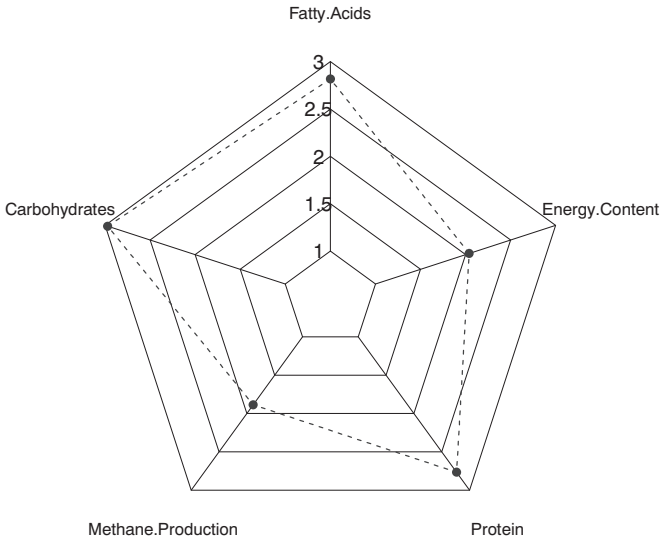

Fig. 2. Radar plot overview of relative ranking of impact of respective metrics on the composition aspect of Algae Technical Standards based on input from the participants in the Focus Group discussions.

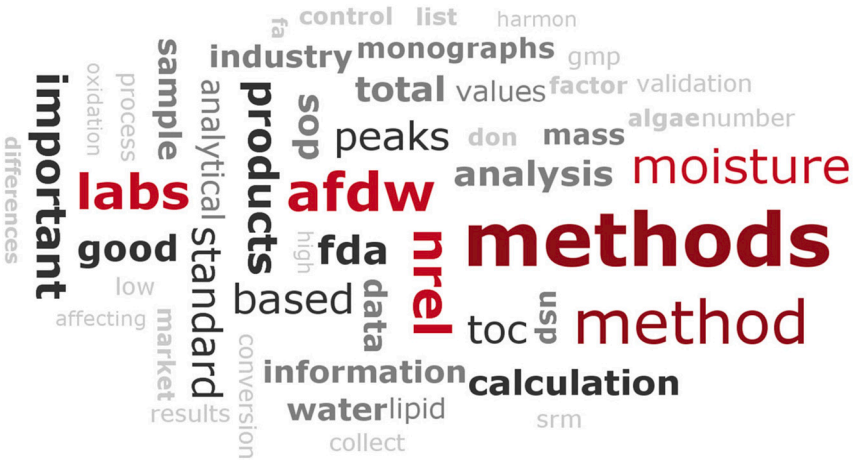

Fig. 1. Word cloud of topics and discussion points during the working group discussion, with font size reflecting the frequency each word was included in the discussion points.

are concerns highlighted around the reporting of carbohydrates on the basis of a difference calculation, subtracting ash, lipid, protein from the biomass to arrive at a total carbohydrate content. While this is a general methodology applied to food and nutritional labeling, the impact on mischaracterizing biomass material, in particular seaweeds or macroalgae with a majority of complex polysaccharides is considerable.

There are a number of different algae species that are currently used for commercial production ranging from food, feed and nutritional supplements to aquaculture and bioenergy application. Such a list is considered critical in further tailoring recommended characterization approaches and ultimately towards the development of reference materials. Reference materials along with a well-coordinated ring-test for validation of both methods and representative and relevant biomass or products. United States Pharmacopeial Convention (USP) Validation of Compendial Procedures [7] has good information about validating analytical procedures.

1.2. Identification

An underreported challenge in the algae community is the identification of algae, and contaminants (microbiological or chemical) in the culture or biomass source. It is generally accepted that DNA sequencing is the most robust approach for unambiguously characterizing algae. However, there are microscopic and biochemical fingerprints (e.g. unique products, such as gamma linolenic acid [GLA], or phycocyanin, astaxanthin, …) that are helpful secondary characteristics to use. Similarly, microbiological testing for contaminants is a common and well-accepted method for characterization. DNA-based identification relies on either PCR, shotgun sequencing or barcode sequencing. Primarily the working group highlighted the need for a more robust and generally accepted DNA sequencing approach to routinely classify species of algae [8,9]. There is a concerted effort to develop a more comprehensive whole genome sequencing approach by creating databases from samples that are analyzed and ideally this will be made available to the community and happen in concert with large, public, genome sequencing projects, such as the Joint Genome Institute (JGI) Community Sequencing Program. One of the highlighted challenges that was mentioned was that identification based on sequencing amplified DNA fragments may not be a quantitative representation of what is present in the sample, and that for example, when looking for a eukaryotic strain, the presence of important, and potentially harmful, prokaryotic contaminants may be missed.

The questions for identification are associated with the need for specific markers and also database coverage. In particular in the context of macroalgae DNA-based identification, there is a dearth of well-curated databases and thus the more standardized approach or distinguishable genetic markers are needed that are acceptable. There was a general request to compile, disseminate generally accessible databases with multiple characteristics of the strain and product, to help the industry credibly identify and assign their products.

1.3. Safety (toxins and toxic metals)

The discussion around safety and characterization of contaminants highlighted that contaminants present in algal biomass are typically of microbiological or environmental origin, pesticides, or comprise toxic (heavy) metals. Most of these contaminants are well-described in the literature and documented standard methods, derived from the Association of Analytical Communities (AOAC International); American Society for Testing and Materials (ASTM); American Oil Chemists' Society (AOCS); United States Pharmacopeia (USP) are available from the food-safety regulatory panel overseen by the U.S. Food and Drug Administration (FDA) or USP. However, one point that was noted that sometimes multiple methods are available and the specifications to meet prior to a 'safety certification' varies by market and industry segment. The analytes of interest identified by the working group: microbiological contaminants; biological toxins, aflatoxins, pesticides, pheophorbide and microcystins; pesticides; biogenic amines; sulfate; halogens (I, Br, Cl) and their halo-organic compounds; toxic metals (Pb, Hg, Cr, Ni, As); phenolic compounds.

This section received very few questions, though important points were raised by one of the contract laboratory representatives in the identification and reporting of toxins such as microcystins, where different methods can cause an up to two-fold difference in reported values (ELISA or LC-MS/MS methods). Such ranges are concerning in the context of certifying a biomass material as safe for the consumer, which points to the need to identify and disseminate industry-sanctioned specification targets for each of the prioritized toxins.

1.4. Labeling requirements

There is a general need to extend algae labeling beyond nutritional labeling prescribed by existing industries and agricultural products, to encompass the presence of active ingredients outside standard measurements. The participants during this section expressed interest in the development of additional consumer and marketing commitment to a set of quality metrics. In particular, there appears to be support for the development of a monograph for testing, akin to a previously established USP monograph for *Spirulina* characterization, which details the required testing [10].

The establishment of a consumer-recognizable seal would indicate quality factors such as purity, constituents, production methods, health metrics (e.g. toxins, toxic metals) of for example algal biomass. A number of existing monographs with appropriate targets for consumer safety and what is proposed here does not aim to replace or restart existing certifications, rather add where there are gaps or definitions of subject materials are not broad enough. The algae-producing industry is best positioned to contribute proposed criteria for inclusion and inclusion in a monograph. Such an initial step is usually followed by a meeting between users, scientists and the governing agencies to reduce the criteria to a list of common agreement and this is then further developed and validated using for example a standard reference material. However, this approach of monograph development may not applicable across different applications or products. In this context, the framework presented here, is primarily developed around the production of biomass for subsequent inclusion as an ingredient or a feedstock into a product pathway (e.g. nutritional supplements). In the latter case, we anticipate that the respective product markets and respective guiding organizations will take over the required safety and marketing parameters.

There are remaining questions on which governing organization is best suited to provide methodology and help come up with a suitability for purpose for each in the context of coming up with the criteria for prioritization. There was a recognized need for critical assessment of the applicability of different methods and ultimately a need for an independent set of reviewers who are in a position to bolster consumer confidence. A major question appears to be the lack of a central place that allows producers, consumers and purchasers to collect information and contribute knowledge and feedback.

2. Priority gaps

Perhaps the biggest identified priority is the need for establishment of definitions for each of the constituents, which would lead to at least a basis of a common language establishment that can then be followed up with a matching of existing standard methods. Furthermore, setting targets around the identification of toxins for specific biomass sources and matrices was listed as another of the major priority gaps.

A secondary recurring theme in the discussion forum was the need for a standard reference material (SRM), one each for the multiple different biomass and material matrices. The group recognized that several SRMs are already available through central distribution

mechanisms or partners (e.g. National Institute for Standards and Technology, NIST) but not necessarily tailored to specific constituents or characterized across a nationwide network. The establishment of an SRM needs a supply of a relevant biomass source of >10 kg DW and a statistical plan in place to assess and come up with a consensus composition. From the representative participation at the working group discussion, there is an anticipated need for a large quantity of microalgal biomass for at least 2 species, and a heterotrophic (high oil-accumulating) alga, as well as at least one or more seaweed (macroalgae) SRMs. There are established methods for interlaboratory validation through statistical analysis of ring tests on SRMs and ideally a set of methods should be selected to find sources of variance to align on a harmonized procedure, e.g. the Association of Official Analytical Chemists (AOAC) documents such procedures well [11].

3. Conclusions

The Technical Standards working group convened in March 2020 and set the stage for a ramped-up effort to align standardization of metrics and measurements for algal biomass characterization. In general, there was significant support for the establishment of a concerted effort on Technical Standards. There is also support for concerted effort in reference material establishment, followed by nation-wide ring-test for characterization. The group carried out a successful Gap and Priority Analysis and in general is planning a continuation with extended participation with wider representation across the industry segments to ensure broader applicability to the industry. The mission of this Working Group is to develop and disseminate a guidance document (monograph), in collaboration with the Algae Biomass Organization. We welcome feedback on this effort by the broader algae community.

CRediT authorship contribution statement

Lieve Laurens: Conceptualization, funding acquisition, writing, reviewing and editing, supervision.

Declaration of competing interest

No conflicts, informed consent, or human or animal rights are applicable to this study.

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