



Technical Standards Committee

September 2011 | Version 4.0



Algal Industry Minimum Descriptive Language: Guidance to Evaluate Life Cycle Inputs and Outputs

About the Algal Biomass Organization

Founded in 2008, the Algal Biomass Organization (ABO) is a non-profit organization whose mission is to promote the development of viable commercial markets for renewable and sustainable products derived from algae. Our membership is comprised of people, companies, and organizations across the value chain. More information about the ABO, including membership, costs, benefits, members and their affiliations, is available at our website: www.algalbiomass.org.

About the Technical Standards Committee

The Technical Standards Committee of the ABO is dedicated to the following functions:

- Developing and advocating algal industry standards and best practices
- Liaising with ABO members, other standards organizations and government
- Facilitating information flow between industry stakeholders
- Reviewing ABO technical positions and recommendations

For more information, please see: <http://www.algalbiomass.org/policy-center/technical-standards/>

Staff

Executive Director - Mary Rosenthal

General Counsel - Andrew Braff, Wilson Sonsini Goodrich & Rosati

Administrative Coordinator - Alexis Klassen

Administrative Coordinator - Barb Scheevel

Board of Directors

Chair - Mark Allen, Senior Advisor, BioProcess Algae

Vice Chair - John Pierce, DLA Piper

Secretary - Thomas Byrne, Byrne & Company, Ltd.

John Benemann, Benemann Associates

Keith Cooksey, Montana State University

Harrison Dillon, Solazyme

Bill Glover, The Boeing Company

Qiang Hu, Arizona State University

Ira "Ike" Levine, University of Southern Maine

B. Greg Mitchell, Scripps Institution of Oceanography

Margaret McCormick, Targeted Growth

Joel Murdock, FedEx Express

Herminia Rodriguez, University of Sevilla CSIC, Spain

Paul Woods, Algenol

Tim Zenk, Sapphire Energy

Committees

Events Committee

Chair - Philip Pienkos, National Renewable Energy Laboratory

Vice Chair - John Pierce, DLA Piper

Member Development Committee

Chair - Kimberly Kotovic, Targeted Growth

Vice Chair - Mark Allen, Senior Advisor, BioProcess Algae

Bylaw & Governance Committee

Chair - Mark Allen, Senior Advisor, BioProcess Algae

Vice Chair - Joel Murdock, FedEx Express

Government & Public Relations Committee

Chair - Tim Zenk, Sapphire Energy

Co-Chair - Gary Hopper, General Atomics

Technical Standards Committee

Chair - Jim Sears, A2BE Carbon Capture

Vice Chair - Keith Cooksey, Montana State University

Committee Administrator - Alexis Klassen

Director Recruitment Committee

Chair - Harrison Dillon, Solazyme

Vice Chair - Herminia Rodriguez, University of Sevilla CSIC, Spain

Peer Review Committee

Chair - Keith Cooksey, Montana State University

Vice Chair - John Benemann, Benemann Associates

About This Document

This Guidance Document recommends the Minimum Descriptive Language required for characterizing the economic and environmental inputs and outputs of an aquatic biomass operation. Voluntary adoption of uniform descriptive language will accelerate industry growth and unify research.

The Minimum Descriptive Language Scope of recommendations within this document are intended to be useful in characterizing a broad range of aquatic production operations. The Algal Biomass Organization (ABO) is primarily focused on the production of eukaryotic algae (both macro and micro) and cyanobacteria (blue-green algae); however, photosynthetic aquatic cultivation of crops such as duckweed and others will also benefit because many of the inputs and outputs will be common to all. The Minimum Descriptive Language in this document will equally serve heterotrophic algal cultivation, algae-bacteria binary systems and multi-trophic systems that employ higher organisms to harvest or otherwise enhance value.

ABO Technical Standards Committee Authors:

Jim Sears - Committee Chair, President and Chief Technology Officer, A2BE Carbon Capture LLC
Dr. Keith Cooksey - Vice Chair, Research Professor, Department of Microbiology, Montana State University
Dr. Rose Ann Cattolico, Professor of Algal Biology, University of Washington
Dr. James Collett, Chemical and Biological Process Development Group, Pacific Northwest National Laboratory
Dr. Harrison Dillon, President and Chief Technology Officer, Solazyme
Brice Freeman, Project Manager, Environmental Controls, Electric Power Research Institute
Dr. Laurie Locascio, Chief of Biochemical Science Division, National Institute of Standards and Technology
Adonis Neblett, Patent Attorney and Shareholder, Fredrikson & Byron
Dr. Philip Pienkos, Applied Scientist Group Manager, National Renewable Energy Laboratory
Dr. Lieve Laurens, Research Scientist, Algal Biomass Characterization, National Renewable Energy Laboratory
Dr. Robert McCormick, Principal Engineer, Fuels Performance, National Renewable Energy Laboratory

Additional Reviewers: ABO Board of Directors representing: Targeted Growth, A2BE Carbon Capture, Byrne & Company, Ltd., Benemann Associates, Montana State University, Solazyme, Boeing Commercial Airplanes, University of Southern Maine, Scripps Institution of Oceanography, FedEx Express, National Renewable Energy Laboratory, Wilson Sonsini Goodrich & Rosati, Raytheon Company, University of Seville, Mars Symbioscience, ABO Government and Public Relations Committee representing Sapphire Energy, Ron Pate representing Sandia National Labs and James Collett representing Pacific Northwest National Laboratory.

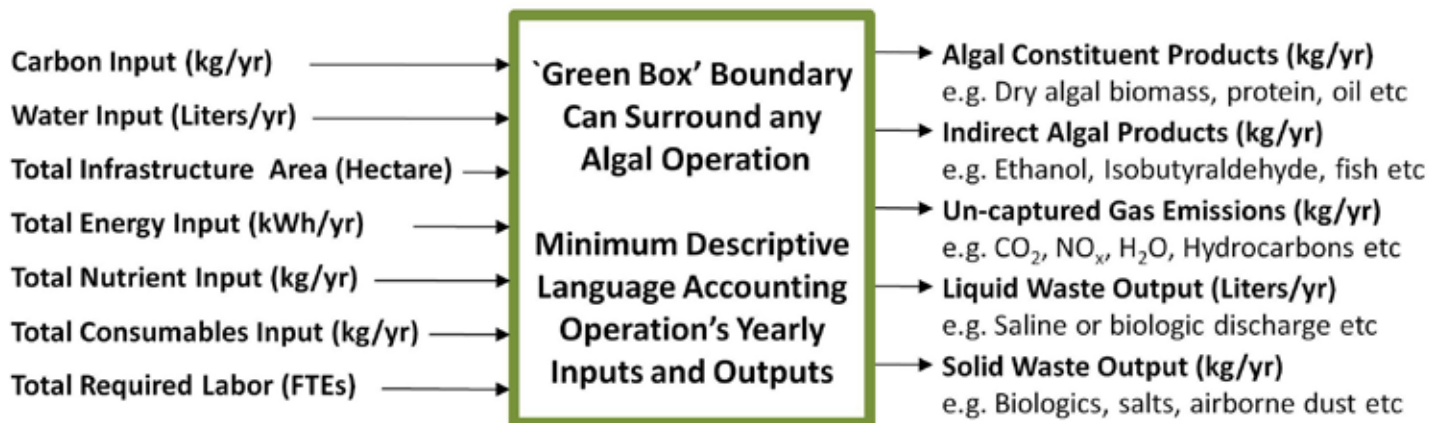


The Challenge

Algal operations will vary in size from small units producing specialty chemicals and nutraceuticals to large farming scale (hundreds to thousands of hectares) for production of food products and biofuels. Accurate assessment of their future economic and environmental footprint will be critical to financing development and performing environmental life cycle analysis. There is no harmonized minimum descriptive language set specifically developed to describe the diverse technologies being proposed for scaled algal farms. The lack of a suitable common language has created confusion in expressing attributes and has become a barrier to deployment of pilot demonstrations.

ABO's Solution

The ABO Technical Standards Committee proposes a set of Minimum Descriptive Language tailored to the needs of our industry that describes the footprint of any algal or similar production operation. This will be established by estimating, characterizing or measuring the magnitude of process inputs and outputs passing through an analytical "Green Box" arranged to surround the total algal operation. This boundary might encompass just an algal farm or fermentation facility with its support infrastructure, or could further include an appropriate portion of a biorefinery or power plant with which the farm is integrated. In this way, the Green Box descriptive method can be adapted to compare the environmental footprint of algal operations having different inner workings yet similar inputs and outputs. The minimum set of descriptive input and output variables needed to characterize the economic and environmental footprint of an operation are shown in the graphic below and are described in more detail through the balance of this document.



Above: Minimum Descriptive Language users will describe their operation by freely selecting which operational components to place within their Green Box and describing the whole via collective inputs and outputs.

Support of Life Cycle Analysis: The Minimum Descriptive Language will facilitate the conduct of Life Cycle Analysis (LCA) and techno-economic analysis while using a common language across the industry. Guidance on LCA studies may be found in ISO 1404xx series of documents that describe goals, scoping, quality, transparency and requirements for data collection, along with guidelines for the appropriate analysis and presentation of data. The EPA Renewable Fuel Standards (RFS2) guidance provides a template on how to conduct LCAs for biofuels to meet various renewable definitions. Further instruction on composing LCA studies may be found in the EPA report "LIFE CYCLE ASSESSMENT: PRINCIPLES AND PRACTICE" (EPA/600/R-06/060, May 2006).

Adoption into Peer-Reviewed Research: The Committee welcomes peer reviewed research of the scope and measurement methodologies best employed within the industry. Prospective authors may consult the "Minimum Information for Biological and Biomedical Investigations" collection of standards at www.mibbi.org for guidance on describing methods and results of research on the biology, biochemistry, or biotechnology of algal biomass production.

Process for Incorporating Stakeholder Comment: This Minimum Descriptive Language Guidance Document is designed to meet the evolving needs of the algal industry and its stakeholders. Accordingly, the ABO Technical Standards Committee invites formal stakeholder comment on furthering the scope and specifics of this document. Please email comments to technicalstandards@algalbiomass.org where they will be logged into the Technical Standards Committee database. The Committee will formally review comments prior to March 31, 2011 and recommend improvements to the Guidance Document on a periodic basis.

Recommended Minimum Descriptive Language for Scaled Algal Operations

The ABO Technical Standards Committee recommends that when large scale algal operations are proposed or analyzed, the following set of descriptive metrics are adopted to uniformly characterize these operations. By harmonizing upon a common set of descriptive metrics, the algal industry will accelerate its growth by eliminating confusion in the business and life cycle analysis arena of our industry. By identifying the sources and characteristics of inputs, and the intended fates and characteristics of outputs, we will make the necessary data available that will then allow for later upstream and downstream life cycle and techno-economic analysis.

Input Description:	Carbon Input – CO ₂ , regardless of source, is typically dissolved in the growth media of photosynthetic algae to accelerate growth although the carbon could come from another chemical source. Heterotrophic and mixotrophic algae obtain carbon from sugars or other organic compounds.
Measurement:	Quantify in Kg or tons per year the quantity of carbon or CO ₂ imported across the imaginary Green Box boundary regardless of source and mode of delivery.
Source Description:	Prospectively, pure CO ₂ from a pipeline or industrial storage system, dilute CO ₂ in flue gas, CO ₂ drawn directly from the atmosphere, chemically bound inorganic carbon in the form of bicarbonate, or sugars and other simple organic compounds, either pure or in mixtures, derived from lignocellulosic biomass.
Detail Characteristics:	Description of other compounds mixed with the carbon source, including possible heavy metals and other contaminants, micronutrients associated with flue gas (e.g., from coal-fired emitter sources), and input pressure levels if the carbon delivery is in gas or in a liquid form.

Input Description:	Water Input – Water in fresh, saline and wastewater forms is a basic component of algal growth media. See Appendix D for special wastewater considerations. Fresh water is typically lost through surface evaporation and may be gained from precipitation in open systems and may be lost in closed systems via their cooling apparatus. The concentrations of dissolved salts and other chemicals in cultivation media will increase with evaporation and recycling.
Measurement:	Input water volume is measured in liters or gallons brought into the Green Box per year. In operations employing “once-through” cooling systems, the downstream consumptive and environmental effects must be taken into account.
Source Description:	Well, pipeline, river, ocean, lake, surface or groundwater agricultural allotment, wastewater type, precipitation, etc.
Detail Characteristics:	Total dissolved solids, mineral type, total organic carbon, biological oxygen demand (BOD), major cations and anions, trace metals, bio or industrial contaminants.

Input Description:	Total Infrastructure Area – Significant amounts of land are typically needed to cultivate algae, process it into products and convey input and output materials (solids, liquids, gasses), power, energy, vehicles, equipment, and other machinery around the algal farm and processing facilities. Total infrastructure should include every necessary operational element that connects together with lifecycle or business analysis within the desired Green Box. These additional components may include operationally associated but off-site industrial elements, crop fields, wind farms, solar farms, and etc.
Measurement:	Data should include total footprint area in acres or hectares of infrastructure includes ponds, PBRs and fermenters, as well as evaporation ponds, access roads, pipes and other utilities, buildings and appropriate portions of offsite processing or generation facilities that may appropriately be characterized as part of the infrastructure of the overall Green Box analysis. For photosynthetic cultivation, the total direct solar areal capture area of ponds or PBRs should be broken out if a specific photosynthetic productivity is being proposed.
Source Description:	Infrastructure land under private ownership, leased, government, tribal, reclaimed, conservation reserve program, land use category, etc.
Detail Characteristics:	Latitude, altitude, climate zone, precipitation, slope, soil type and soil carbon content, indigenous plants and animals, etc.

Input Description: **Total Energy Input** – Algal operations use electricity, fossil fuels, other chemical energy, steam, etc. that are imported into the Green Box. Statements of incident solar radiation data should be based on the National Radiation Solar Database http://rredc.nrel.gov/solar/old_data/nsrdb/ with stipulation of specific NRSD data set and metrics that are being utilized. In the case where there is embodied energy in organic nutrients for heterotrophic algal production, the nutrient or chemical energy source should be fully characterized in the information from the Carbon Input or Nutrient Input sections to permit future calculations of upstream environmental footprint.

Measurement: KW-hours (kWh), BTUs, or other quantity depending of the type of imported energy should be used. These can be related to Joules for determination of overall energy balance. Note that if the algal operation is a net electricity or other energy type producer (beyond that energy embodied in the direct and indirect algal products) then the Total Energy Input can be adjusted or made negative to reflect this net non-algal energy export.

Source Description: Electricity sourced from fossil coal/gas, biopower or biomass co-generation, biogas, wind and solar generation or direct use of fossil resources as in gasification, etc. Transportation for energy input includes reference to transmission lines, pipelines, truck or rail and distance traveled.

Detail Characteristics: State input voltage level, phase, and frequency for electricity as well as the characteristics needed for both fossil and renewable energy footprint calculation.

Input Description: **Total Nutrient Input** – Algae, like plants, require macronutrients like nitrogen, phosphorous and potassium as well as a host of micronutrients specific to the species. Carbon has its own separate category in this Descriptive Language.

Measurement: Kilograms or tons of each individual nutrient and its chemical delivery form should be reported; for example, NH_4NO_3 for nitrogen or H_3PO_4 for phosphorus.

Source Description: Factory, mine, wastewater, agricultural waste or other recycled sources should be noted and specify the nutrient transportation distance for LCA accounting

Detail Characteristics: Provide fossil fuel-based manufacturing details, mined, renewable source, etc.

Input Description: **Total Consumables Input** – The parts or facilities that must be replaced in one year of operation. All subcategories that amount to 10% or more of the total consumable's monetary value or input mass should be broken out individually).

Measurement: Include dollar value, description and weight in Kg or tons of each generalized industrial component in a form allowing upstream lifecycle impact assessment.

Source Description: General identification of source, transportation distance and information is needed to calculate upstream footprint in future analysis.

Detail Characteristics: Cite ability to be recycled, toxic disposal load, imported vs. domestic sourcing.

Input Description: **Total Required Labor** – Algal operations will need laborers, technicians, engineers and other special classes of labor.

Measurement: Provide full time equivalents of each labor classification type, even if labor has seasonal variability so as to allow for economic impact assessments.

Source Description: Cite if workers are regionally housed commuters, on-site housing, locally recruited labor vs. imported labor, their training site locations, and worker demographics.

Detail Characteristics: Describe labor type, education type and source of required specialized education.

Output Description: **Algal Constituent Products*** – Extractable oil, total lipids, protein, carbohydrate, whole algae, diatomaceous material, chitin, any direct constituent of organism are identified.

Measurement: Provide productivity in kilograms or tons at a specified level of dryness; the composition of some products is expected to include ash unless specified ash-free.

Fate or Destination: Specific use in fuel, food, durable goods, nutraceutical, agriculture, industrial chemical, etc.

Detail Characteristics: Detail specific algal type, harvesting method, level of biological and chemical purity.

***See Appendix A:** Measuring/estimating algal dry weight and constituents like oil using small samples.

Output Description: **Indirect Algal Products** – Ethanol, extracellular oils, oxygen, hydrogen, hydrocarbons, remediation services, shrimp, fish, etc. produced by organism.

Measurement: Kilograms, tons, gallons or liters of products at specified dryness, quality or purity level that is exported from Green Box.

Fate or Destination: Refining into fuel, human food or nutraceuticals, animal feed compounding, durable goods agricultural applications, industrial chemicals produced.

Detail Characteristics: Species if appropriate, level of biological and chemical purity, applicable standards or specifications are to be documented.

Output Description: **Un-captured Gas Emissions**** – CO₂, NO_x, CO, H₂S, H₂, O₂, water vapor, volatile hydrocarbons, etc. that are emitted from any part of the infrastructure. Solid materials and biologic airborne emissions are accounted for under solid waste.

Measurement: Report PPM or PPB for regulatory or permitted limits and kilograms or tons of individual gas components emitted from whole operation on annual basis.

Fate or Destination: After emission to ambient air, what is the dispersion, deposition or chemical conversion that takes place with respect to environmental/atmospheric conditions?

Detail Characteristics: For example: Describe whether gas emissions expelled are components from a Green Box Input gas stream or are newly created by processes within the Green Box.

Output Description: **Liquid Waste Output***** – Water or other liquids containing levels of one or more pollutants: Saline/salts, nutrient, biologics, toxics, heat, suspended solids, sediment, microorganisms, BOD, TDS, trace metals or other contaminant-containing liquid. May also include spent solvents from extraction processes.

Measurement: Provide PPM or PPB for regulatory or permitted limits as well as liquid liters or gallons discharged or otherwise conveyed from algal production facility.

Fate or Destination: For water, point source and non-point source discharge to surface water, ground water, reinjection, water treatment, or conveyed/transported for recovery of useful constituents or disposal. For liquids such as organic solvents, appropriate industrial disposal, storage, or treatment, e.g. purification and recycling outside the Green Box.

Detail Characteristics: Composition and concentration of chemical, biological and other constituents, toxicity, and algal production process variations should be reported.

Output Description: **Solid Waste Output****** – Retired parts from Total Consumables input, sludge, viable biologics, airborne dust of organic or non-organic composition, etc.

Measurement: Note kilograms or tons whether stockpiled on site, buried on-site or moved offsite. In the case of viable biologics, note species, genetic modifications, the dispersion mechanism i.e. liquid or solid discharge, part of shipped product, airborne, etc., dispersion density, human/plant/animal health risks, and viability/risk of organism spreading to natural systems or other commercial aquaculture or agriculture systems residing outside the Green Box.

Fate or Destination: Use of landfill, burning, burial, chemical neutralization, storage, airborne or water dispersal and downstream effects.

Detail Characteristics: Provide chemical and biological composition, off gassing, toxicity, seasonal variations.

****See Appendix B:** Total Lifecycle Gaseous, Liquid, and Solid Waste Outputs

*****See Appendix C:** Government Environmental Programs for Regulation and Control of un-Captured Gas, Liquid, and Solid Outputs from Algae Operations

******See Appendix D:** Framework Issues for Use of Wastewater in Algae Cultivation

Appendix

Appendix A: Measuring/Estimating Algal Dry Weight and Constituents Like Oil Using Small Samples

This document highlights the need for methodologies to assess a wide variety of metrics within the industry. The Technical Standards Committee will work to provide standardized methods for these in the future. There is a pressing need for a standardized procedure to estimate and measure dry weight and algal oil content. This appendix discusses the challenge and the Committee is actively soliciting suggestions on improved methods applicable across the industry.

Dry weight, e.g. protein, oil, or carbohydrates, can be assessed by “primary measurements” in which the desired component is separated and directly weighed or by “indirect measurements” such as cell counting or fluorescence, where the measured quantity is calibrated to actual mass and used as an analog. Indirect measurements can be extremely useful because of their typical speed and sensitivity when calibrated and so both are discussed. The Technical Standards Committee is looking for relatively easy and inexpensive ways to perform primary measurement methods that work across a variety of algae and aquatic biomass.

Measuring “algal dry weight”: Comparison of methods for determining grams of algae/liter of culture

Note that the following methods for determining algal dry weight can be affected by the presence of significant quantities of contaminating microorganisms in the growth medium. Additionally, note that one is typically trying to assess the equivalent dry weight of algae in a small sample of liquid medium in order to indirectly assess the weight of biomass in a huge volume of that medium. For this reason, accurate measurement of sample volume and representative sampling is essential. The following methods include both “primary measurements” that directly determine the total weight of biomass and indirect methods, which can be expedient, yet must be calibrated against primary methods to yield accurate results.

Filtration: Filter a specific volume of growth media through a pre-weighed Whatman GF/F style ultrafine binder free glass fiber filter. Dry and re-weigh filter on a precision balance. Repeat drying and weighing until equal dry weights are obtained.

Advantages: Primary Direct Measurement. An easy and inexpensive test of total non-volatile mass.

Disadvantages: High resolution balance necessary. Vacuum or pressure filtration equipment needs to be operated at low pressure. Filtration can easily disrupt very soft bodied cells causing significant loss of material through the filter media. Unless sample and filter are fresh water rinsed before drying, salts from the media may become included as part of the measurement. A core assumption of a drying based methodology is that volatile components may be lost and unaccounted for after drying.

Centrifugation: Spin a volume of culture in a pre-weighed centrifuge tube. Remove supernatant. Dry and weigh pellet plus tube.

Advantages: Primary Measurement. Easy measurement of heavier-than-media cells and particles.

Disadvantages: Centrifuge and significantly high-resolution balance can be expensive and small pellet size can severely compromise accuracy of the results. Core assumption that all components to be measured are heavier than media may influence result if light weight components are poured off.

Turbidity: Measure with several instrument types (e.g., spectrophotometer, turbidimeter) at selected wavelengths. Note that one must compare turbidity measurements at several cell densities with a direct mass measurement method to determine the turbidity to weight relationship for each algal species.

Advantages: Good for small volume samples. Fast measurement and low cost sensor technology make this method useful for continuous monitoring of growth dynamics.

Disadvantages: Indirect Measurement. Algae change pigment density during different growth cycle phases and in response to nutrient changes. Presence of contaminating organisms or suspended solids can influence turbidity results. Presence of extracellular components could significantly alter results.

Cell Count: Count cells with hemocytometer, coulter counter, or flow cytometer. Note need to use a primary measurement method to calibrate cell count to weight for each alga species.

Advantages: Accurate when calibration method is accurate. Very small sample sizes are acceptable. Hemocytometers and microscopes are relatively inexpensive.

Disadvantages: Indirect Measurement. This can be time intensive if done manually. Coulter Counter and flow cytometer are easier but expensive and rely upon calibration with direct measurement method. Cell counting, especially with a coulter counter or flow cytometer, may be limited by cell size and complicated by large size distribution or by filamentous or a multicellular or colonial morphology.

Organic Carbon Content: A variety of C analyzers are available to measure total organic carbon in aqueous samples. Solid phase CHN analyzers will measure total carbon on a dried GF/F filter or a dried pellet. Need to use primary method to calibrate to cell weight for each algal species.

Advantages: Accurate even for small sample size if calibration is accurate.

Disadvantages: Indirect Measurement. Expensive equipment and C/N ratio changes with time of day and growth conditions.

Measuring Algal Lipids vs. Extractable Oils vs. Fuel Fraction, Proteins and Carbohydrates

Lipids are traditionally measured as a gravimetric solvent extraction yield. However, different procedures and types of solvents have resulted in inconsistent lipid yields¹. The completeness of extraction and composition depends on the biology of the alga and the physiological conditions experienced by the organism as well as the compatibility of the solvent polarity with the lipid molecule polarity and extraction conditions used. Inevitably, the extractable oil fraction will contain non-fuel components (e.g. chlorophyll, pigments, proteins, and soluble carbohydrates). Thus it may be necessary to assess the fuel fraction of these isolated oils (i.e. fatty acid content of extracted lipids) by transesterification followed by quantification of the fatty acid methyl esters (FAMES). Due to the large number of variables, it is difficult to standardize an extraction-based lipid quantification procedure.

As an alternative to extraction, there is a growing emphasis on quantification of lipids through a direct (or in situ) transesterification of whole algal biomass. The process consists of an acid hydrolysis of the biomass followed by a transmethylation of the fatty acids and quantification of the FAMES by gas chromatography. This procedure has been demonstrated to be robust across species and its efficacy is less dependent on the parameters listed above that influence an extraction process. The disadvantage is that no information on the origin of the lipids is available, e.g. relative level of neutral versus polar lipids. Several reports in the literature are suggesting in situ transesterification as the lipid quantification procedure of choice².

In addition to the procedures listed above, there has been a push to accelerate the quantification of lipids. Researchers want to tailor the analysis to the screening of thousands of individual strains in bioprospecting or metabolic engineering projects. These high-throughput methodologies are based on hydrophobic (lipophilic), fluorescent dyes, such as Nile Red³ and BODIPY. As the dyes are absorbed by the lipids the sample fluorescence intensity increases proportionally and this principle has been used extensively in the screening for higher lipid producing cells among thousands of candidates. An alternative technology that is gaining popularity is IR spectroscopy coupled with chemometrics to identify high lipid producing strains from a population⁴. One advantage of IR technology over the hydrophobic dyes is that the IR-based quantification is not dependent on the permeability of the cell walls to the dyes that has been reported to affect the fluorescence signal from lipids.

Protein percentage can be quantified using two common procedures; colorimetric and a nitrogen ratio calculation based on measuring elemental nitrogen and applying an algae-specific nitrogen-to-protein conversion factor. The colorimetric⁵ procedure is susceptible to interferences and is highly dependent on the protein standard used for calibrating the absorbance values. However, progress has been made by inclusion of NaOH digestion of algal biomass and proteins, where the colorimetric protein determination corresponds with the measured amino acid content.

¹ Guckert JB, Cooksey KE and Jackson LL J Microbiol Methods, 8: 139-149(1988); Bigogno C, Khozin-Goldberg I, Cohen Z (2002) Phytochem. 60:135-143

² Griffiths MJ, van Hille RP, Harrison ST (2010) Lipids. 45(11):1053-60; Bigelow, NW, Hardin, WR, Barker, JP, Ryken, SA, MacRae, AC, Cattolico, RA, (2011) J Am Oil Chem Soc

³ Cooksey KE, Guckert JB, Williams, SA, Callis, PR (1987) J Micro methods 6: 333-345,

⁴ Laurens LML, Wolfrum EJ (2011) Bioenerg. Res. 4: 22-35

⁵ Lowry, OH, NJ Rosbrough, AL Farr, and RJ Randall. (1951) J. Biol. Chem. 193: 265

Calculating protein content using a nitrogen-to-protein conversion factor has proven to be a more robust representation. Measuring elemental nitrogen is based on combustion and is not susceptible to interferences. An algal biomass-specific conversion factor⁶ was calculated from the typical amino acid composition of 12 species of algae grown under different conditions. An overall average ratio factor of 4.78 grams of algal protein to detected grams of elemental nitrogen has been used successfully for algal protein quantification.

Total carbohydrate content can be measured via a colorimetric phenol sulfuric acid method⁷ that is robust across species. However, not all sugars exhibit a similar colorimetric response and thus some carbohydrates could cause an over- or underestimation. Alternative carbohydrate quantification procedures involve sequential hydrolysis of sugar polymers and identification and quantification of the monomers via liquid or gas chromatography. There are reports in the literature, but a comprehensive comparison and test of robustness across strains is currently lacking.

Appendix B: Total Lifecycle Gaseous, Liquid, and Solid Waste Outputs

Gaseous emissions and liquid and solid discharges from commercial algal production operations, from a life cycle analysis perspective, include those incurred during facilities construction prior to the initiation of operations during the operating life of the facility and after operations cease and the facility is decommissioned. Emissions during site preparation and facilities infrastructure construction will include dust from construction and vehicle traffic, direct and indirect CO₂ emissions from fuel combustion for power generation and operation of vehicles and construction equipment. Additional CO₂, water, and other solid, liquid, and gaseous emissions would be embedded in the production of building materials, equipment, fuels, and other consumables (e.g., fertilizers and chemicals) used in facilities construction and algal production and processing operations. Similar emissions of dust and CO₂ will be embodied in the decommissioning and possible restoration of facilities sites to original condition. This document discloses that during algal production and processing operations, gaseous, liquid, and solid emissions can include indirect and direct greenhouse gases emissions (CO₂, NO_x, water vapor, etc.) associated with the production of input energy and materials and their consumption during operations, water vapor from evaporative loss, effluent waters with entrained organic and inorganic materials not otherwise suitable for recycled use within the operation, solid biomass residue fractions not included in algal constituent products or indirect products, and solid inorganic, organic, and biologic particulates that can become airborne emissions, suspended in liquid effluents. A typical total lifecycle analysis will consider the total impact of construction, operation and decommissioning.

Appendix C: Government Environmental Programs for Regulation and Control of Uncaptured Gas, Liquid, and Solid Outputs from Algae Operations

Most countries have an established body of environmental laws and regulations to which algal production operations will be subject. Such programs typically regulate at some level one or more of the following: gaseous emission or air pollutants, water pollution or discharges to water, solid and hazardous waste handling and disposal, facility siting and permitting, and handling of toxic substances. Additionally, some countries have regulatory requirements that relate to micro-organisms and algae with respect to their manufacture, importation, or processing for commercial activities, including R&D activities, as well as to their release to the environment.

Algal production operations will need to be mindful of the regulatory requirements of the jurisdictions in which they are sited and obtain the appropriate government issued permits, licenses or other authorization. As part of the process of obtaining permits, some jurisdictions may require decisions regarding siting and operation of a facility be subject to a public review process in order to identify the potential impact of the facility on the environment and to determine what must be done to avoid or mitigate any significant environmental impacts.

By accepting government issued permits or licenses, owners and operators of algal production operations are also accepting the responsibility to comply with permit conditions, limitations and statutory and regulatory requirements. In some jurisdictions, the consequences of non-compliance may include administrative remedies, civil court remedies, and criminal remedies, e.g., modification of permit conditions, permit revocation, fines and imprisonment.

⁶ Lourenço, SO, Barbarino, E, Lavin, PL, Marquez, UML, Aidar, E (2004) Eur. J. Phyc., 39(1) 17-32

⁷ Dubois, M, Gilles, KA, Hamilton, JK, Rebers, PA, and Smith, F (1956) Anal. Chem. 28(3)350-356

A fairly comprehensive list of U.S. environmental laws administered by the U.S. Environmental Protection Agency can be found at <http://www.epa.gov/lawsregs/laws/> with links to the listed laws. The primary federal regulations for protection of the environment are in the Title 40 Code of Federal Regulations (CFR); however, there are also state and local regulatory requirements to be considered.

The ABO believes that its members will conduct algal production operations responsibly and in compliance with the requirements of the nation or jurisdiction in which their facilities are sited.

Appendix D: Framework Issues for Use of Wastewater in Algal Cultivation

Municipal and agricultural wastewater may be rich in nitrogen and phosphorus which are primary nutrient requirements for algal cultivation. Some industrial wastewater sources also contain these constituents. Discharge of these, and other, pollutants in the United States is governed by USEPA through the National Pollutant Discharge Elimination System (NPDES) permit process. Most States have a permit process authorized under NPDES.

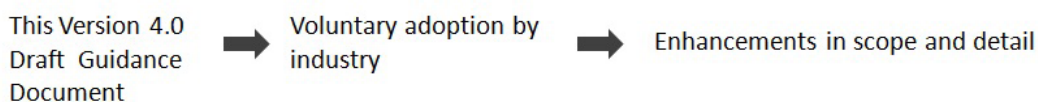
Generally speaking, these pollutants in a treated permitted discharge are low in concentration relative to algal cultivation interests. By design, these pollutants are to be removed to low levels in a treatment plant before discharge. In order to maximize the nutrient value of these otherwise pollutants, the wastewater or digester supernatant must be intercepted up stream in the treatment plant prior to traditional treatment and discharge.

If wastewater is diverted to algal cultivation prior to full treatment to NPDES permit requirements, it is highly likely that such water after use in algal cultivation could only be released under a NPDES permit. Either the water would have to be routed back to the permitted treatment plant for discharge under that NPDES permit, or the algal cultivation facility would have to operate under a NPDES permit.

The NPDES permit system has been in place for 40 years with many precedents, rulings, and some revisions that are well understood by practicing public and private sector professionals in this field. The ABO and the Technical Standards Committee welcome the participation of professionals in more fully understanding the implications of employing wastewater nutrients in the cultivation of algae and the implications of the NPDES permit process.

Document Revision:

This Minimum Descriptive Language document will be periodically revised based on ongoing stakeholder input.



Information for Stakeholder Comments:

To comment on this document or recommend improvements, please send suggestions to Alexis Klassen, Committee Administrator, via e-mail at technicalstandards@algalbiomass.org.

For further information on this document and the ABO's Technical Standards Committee, please contact Jim Sears, Chair of the Technical Standards Committee and President and Chief Technology Officer of A2BE Carbon Capture LLC, via e-mail at jimsears@algalbiomass.org or via phone at 303-541-9112.

Members | 2011

Platinum



Gold



Corporate

Algae Farm
 Algaedyne Corporation
 Algaeventure Systems
 Applied Chemical Technology
 Aquatic Energy
 Aurora Algae
 Austrade
 Battelle Pacific Northwest Division
 Bioalgene
 Colorado Lining International
 Combined Power Cooperative
 Donald Danforth Plant Science Center
 Earthrise Nutritionals
 Edison Materials Technology Center
 Electric Power Research Institute
 Endicott Biofuels

Evodos
 FedEx Express
 Greenwater Global
 Harris Group
 Incitor
 Independence Bio-Products
 Institut fuer Getreideverarbeitung
 International Air Transport Association
 Kent BioEnergy
 Kimberly-Clark
 Kuehnle AgroSystems
 Kuraray America
 Mars Symbioscience
 Mortenson Construction
 MTU Aero Engines
 Neste Oil

OpenAlgae
 OriginOil
 PetroAlgae
 Photon8
 Phyco Biosciences
 SFN Biosystems
 Solix Biosystems
 Siemens
 Spokane Industries
 Solution Recovery Services
 Stoel Rives
 Synthetic Genomics
 The Mitchell Family Corporation
 University of Minnesota
 Verno Systems
 Waste Management
 World Water Works

Supporting Organizations

Biotechnology Industry Organization

Phycological Society of America