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Laboratory Analytical Procedure (LAP)

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1. Introduction

- 1.1 This Laboratory Analytical Procedure (LAP) describes the methods used to determine the moisture-free total solids and ash content of a freeze-dried algal biomass sample. A traditional convection and vacuum oven drying procedure are included here for total solids content, and a dry oxidation method at 575°C is covered for ash and total inorganics content.
- 1.2 Algal biomass samples may contain a high and varying percentage of moisture, which can change rapidly when the sample is exposed to ambient humidity levels.
- 1.3 In addition, algal biomass samples may contain varying percentages of ash, or inorganics, depending on the species and the presence of growth media (e.g., seawater) in the sample.
- 1.4 This Laboratory Analytical Procedure is based on and is substantially similar to terrestrial feedstock analytical protocols, although at lower temperatures: *Determination of Total Solids in Biomass and Total Dissolved Solids in Liquid Process Samples* [1] and *Determination of Ash in Biomass* [2]. Minor algal-biomass-specific modifications were made.
- 1.5 Portions of the total solids method are similar to ASTM E1756-01 and T412 om-02 [3].
- 1.6 Portions of the ash method are substantially similar to ASTM E1755-01 [3].

2. Scope

- 2.1 This procedure is intended to determine the solids remaining after drying an algal biomass sample, that was previously prepared using one of the following methods: freeze drying, spray drying, etc., overnight at 40-60°C in an atmospheric pressure oven or vacuum convection oven. This method is not intended for biomass slurries or prepared samples with a moisture content greater than 10%.
- 2.2 This procedure is intended to determine the ash content of an algal biomass sample, corrected for moisture content determined after drying the sample at 40°C–60°C in an atmospheric pressure or vacuum convection oven drying method, and then combusted using a specific temperature ramp to a maximum temperature of 575°C.

3. Terminology

- 3.1 *Algal Biomass Sample*: Algal biomass prepared and dried by freeze drying, spray drying, etc., ensuring moisture is <10% and is ground/homogenized to a particle size <1 mm. This prepared biomass is referred to as “as-received.”
- 3.2 *Oven Dry Weight (ODW)*: The weight of biomass mathematically corrected for the amount of moisture present in the sample.

- 3.3 *Total Solids*: The amount of solids remaining after heating the sample as described in Section 10.1 until a constant weight is reached. Conversely, the moisture content is a measure of the amount of water (and other compounds volatilized at 40°C–60°C in an atmospheric pressure or vacuum drying oven) present in the sample.
- 3.4 *Ash*: The inorganic residue remaining after dry oxidation at 575°C.
- 3.5 *Constant Weight*: The weight that is achieved after sequential measurements that show a difference no larger than 0.1 mg after placing the sample back in the oven or furnace for at least 1 hour, and reweighing.

4. Significance and Use

- 4.1 The results of the chemical analyses of algal biomass samples are typically reported on a dry weight basis. The total solids content of a sample is used to convert the analytical results obtained from subsequent methods on an as-received basis to an ODW basis.
- 4.2 The ash content is a measure of the inorganic and mineral content of an algal biomass sample and is used in conjunction with the ODW to report analytical results on an ash-free, ODW basis.

5. Interferences

- 5.1 The total solids procedure is not suitable for algal biomass samples that have not been dried or that contain a significant amount of moisture (see Section 3.1).
- 5.2 The ash procedure is not suitable for samples that have not first been dried in an oven to correct for moisture content.
- 5.3 If measurements are taken before a constant weight is reached, data may be biased.
- 5.4 Ambient humidity can interfere with measurements. Ensure all desiccants are dry and that samples remain in a dry atmosphere before being weighed.

6. Apparatus

- 6.1 Analytical balance, accurate to at least 0.1 mg.
- 6.2 Desiccator containing dry desiccant.
- 6.3 Vacuum oven OR convection drying oven, set to 40°C–60°C.
- 6.4 Muffle furnace, equipped with a thermostat, set to 575°C ± 25°C or equipped with an optional ramping program (an alternative to pre-igniting the sample).
- 6.5 Ashing burner, ignition source, tongs, and clay triangle with stand (if not using a ramping program on the muffle furnace; see Section 10.2.1 for pre-ignition).

7. Reagents and Materials Needed

7.1 Reagents:

7.1.1 None.

7.2 Materials:

7.2.1 Porcelain crucibles (ideally weighing less than 10 g) or equivalent, e.g., aluminum weigh pans (see Section 10.1.5).

8. Environmental, Safety, and Health (ES&H) Considerations and Hazards

8.1 Use appropriate safety measures when handling an open flame.

8.2 When placing crucibles in an oven or furnace, use appropriate personal protective equipment, including heat-resistant gloves. ALWAYS use tongs to handle hot crucibles.

9. Sampling, Test Specimens, and Test Units

9.1 Samples must be dried (<10% moisture, Section 3.1) before being placed in a drying oven.

9.2 Care must be taken to ensure a representative and homogenous sample is taken for analysis.

9.3 The ash procedure should only be completed on samples that have first been dried in an oven to correct for moisture content.

10. Procedure

10.1 Determining total solids:

10.1.1 Pre-condition crucibles in the 575°C muffle furnace overnight to remove any combustible contaminants.

10.1.2 After conditioning is complete, remove crucibles from the 575°C furnace and cool to room temperature in a desiccator (preferably under vacuum).

10.1.3 Using gloves, tweezers, or tongs (to prevent adding weight from hand oils), weigh each crucible. Record the crucible weight in a lab notebook to the nearest 0.1 mg.

10.1.4 Weigh out 25 mg ± 2.5 mg (or appropriate quantity based on Section 14.4) of prepared algal biomass into the pre-weighed crucible. Less sample may be weighed out to accommodate for limited volume samples. Record the weight of the crucible and sample in a lab notebook to the nearest 0.1 mg. Include an empty crucible as the method control.

NOTE: It is recommended to include a quality control sample weighed out in triplicate with each set to monitor the success and repeatability of the method.

- 10.1.5 Aluminum weigh pans are acceptable as an alternative sample container; a ramping oven following the program shown in Section 10.2.2.1 should be used, and any pre-combustion steps (Section 10.2.1) for ash determination should be disregarded.
- 10.1.6 Place the samples into a vacuum or convection drying oven set at 40°C–60°C and dry for at least 18 hours. Remove the samples and allow them to cool to room temperature in a desiccator.
- 10.1.7 Weigh the crucible and oven-dried sample and record the weight in a lab notebook to the nearest 0.1 mg to constant weight (Section 3.5).

NOTE: The solids content of vacuum dried samples will sometimes recover at greater than 100%. This is likely due to a small amount of moisture being reabsorbed by the sample from the atmosphere during the weighing process. Use 100% solids for these samples and monitor that the % solids do not start recovering over 105%. If this happens, humidity control measures should be put into place.

10.2 Determining ash content:

NOTE: Use the same sample that was used for total solids determination for the ash procedure detailed below.

NOTE: If a muffle furnace with a ramping program is not available, samples must be pre-ignited first or sample combustion could start a fire in the furnace (for crucibles only, do not pre-ignite in aluminum pans).

- 10.2.1 Ash the samples using pre-ignition followed by dry oxidation in the 575°C muffle furnace (for crucibles only).
 - 10.2.1.1 Using an ashing burner and a clay triangle on a stand, heat the crucible containing the oven-dry sample until smoke appears.
 - 10.2.1.2 Immediately ignite the smoke and allow the sample to burn (re-ignite the smoke if necessary) until no more smoke or flame appears.
 - 10.2.1.3 Allow the crucible to cool on a suitable surface before placing it in the muffle furnace.
 - 10.2.1.4 Place the cool sample in the muffle furnace at 575°C ± 25°C for 24 hours ± 6 hours. Handle the pre-ignited samples with care while placing them in or taking them out of the furnace to prevent sample loss.

- 10.2.1.5 Remove the combusted samples from the muffle furnace and allow them to cool to room temperature in a desiccator.
- 10.2.1.6 Weigh the crucible and combusted sample and record the weight in a lab notebook to the nearest 0.1 mg to constant weight (Section 3.5).
- 10.2.2 Ash the samples using a muffle furnace equipped with a ramping program (for both crucibles and aluminum weigh pans).
- 10.2.2.1 Ramping program:
- Ramp from room temp to 105°C.
 - Hold at 105°C for 12 minutes.
 - Ramp to 250°C at 10°C/minute.
 - Hold at 250°C for 30 minutes.
 - Ramp to 575°C at 20°C/minute.
 - Hold at 575°C for 180 minutes.
 - Allow temperature to drop to 105°C.
 - Hold at 105°C until samples are removed.
- 10.2.2.2 Place the crucibles in the muffle furnace and start the ramping program.
- 10.2.2.3 Remove the combusted samples from the muffle furnace and allow to cool to room temperature in a desiccator.
- 10.2.2.4 Weigh the crucible with combusted sample residue and record the weight in a lab notebook to the nearest 0.1 mg to constant weight.

11. Calculations

11.1 Calculate the percent total solids on a dry weight basis as follows:

$$\%Total\ Solids = \frac{(Weight_{crucible + dry\ sample} - Weight_{crucible})}{weight_{sample\ as\ received}} \times 100$$

If desired, the percent moisture can also be calculated:

$$\%Moisture = 100 - \left(\frac{(Weight_{crucible+dry\ sample} - Weight_{crucible})}{weight_{sample\ as\ received}} \times 100 \right)$$

Calculate the ODW of a sample as follows:

$$ODW_{sample} = \frac{(Weight_{air\ dried\ sample} \times \% Total\ Solids)}{100}$$

11.2 Calculate and record the percent ash on an ODW basis as follows:

$$\%Ash = \frac{(Weight_{crucible+ash} - Weight_{crucible})}{ODW_{sample}} \times 100$$

11.3 To report or calculate the relative percent difference (RPD) between duplicates, use the following calculation:

$$RPD = \left(\frac{(X_1 - X_2)}{X_{mean}} \right) \times 100$$

where:

X_1 and X_2 = measured values

X_{mean} = the mean of X_1 and X_2

11.4 To report or calculate the root mean square deviation (RMS) or the standard deviation (STDEV) of the samples, use the following calculation:

$$RMS = x_m = mean = \sqrt{\left(\frac{\sum_1^n x}{n} \right)^2}$$

$$RMSdeviation = \sigma = stdev = \sqrt{\frac{\sum_1^n (x_i - x_m)^2}{n}}$$

where:

x_m = the root mean square of all x values in the set.

n = number of samples in set.

x_i = measured value from the set.

12. Report Format

- 12.1 Report the results as the percent total solids (or percent moisture), and cite the basis used in the calculations.
- 12.2 Report ash as a percent of the ODW of the sample.
- 12.3 For replicate analyses of the same sample, report the average, standard deviation, and %RPD.

13. Precision and Bias

- 13.1 An inherent error in any moisture determination involving drying of the sample is that volatile substances other than water may be removed from the sample during drying.

14. Quality Control

- 14.1 *Reported Results*: Report results with two decimal places. Report the average, standard deviation, and %RPD.
- 14.2 *Replicates*: Run all samples as at least duplicates, if possible, triplicates are preferred if enough material is available.
- 14.3 *RPD Criterion*: Each sample must reproduce total solids and ash content at $\pm 1\%$ wt.
- 14.4 *Sample Size*: It is recommended to use $25 \text{ mg} \pm 2.5 \text{ mg}$ or less, based on available sample, as well as sample homogeneity. Caution: the final ash weight must be above the minimum weight of the balance. Samples with predicted high ash content, such as macroalgae or seaweed samples, may be analyzed with less initial material, if the samples are well homogenized.
- 14.5 *Sample Storage*: All samples should be stored in an airtight container in a -20°C freezer.

15. Appendices

- 15.1 List of revisions/updates:
 - Distribution of May 16, 2013, DRAFT version.
 - Revision December 2, 2013, updated for public distribution.
 - Revision December 29, 2015, updated with minor revisions.
 - Revision September 13, 2023, updated with minor revisions.

16. References

- [1] A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, and D. Templeton. 2008. *Determination of Total Solids in Biomass and Total Dissolved Solids in Liquid Process Samples: Laboratory Analytical Procedure (LAP)*. Golden, CO: National Renewable Energy Laboratory. NREL/TP-510-42621. <http://www.nrel.gov/docs/gen/fy08/42621.pdf>.
- [2] A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, and D. Templeton. 2008. *Determination of Ash in Biomass: Laboratory Analytical Procedure (LAP)*. Golden, CO: National Renewable Energy Laboratory. NREL/TP-510-42622. <http://www.nrel.gov/docs/gen/fy08/42622.pdf>.
- [3] ASTM International. 2003. *2003 Annual Book of ASTM Standards, Volume 11.05*. Philadelphia, PA: ASTM International.