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Laboratory Analytical Procedure (LAP)

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1. Introduction

- 1.1 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.
- 1.2 In addition, algal biomass samples may contain varying percentages of ash, depending on the species and the presence of growth media in the sample.
- 1.3 The following procedure 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 covered for total solids content, and a dry oxidation method at 575°C is covered for ash content.
- 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 amount of total solids remaining after either 60°C atmospheric pressure oven or 40°C vacuum oven drying of an algal biomass sample previously prepared (e.g., freeze drying, spray drying, etc.). 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 during either the 60°C atmospheric pressure or 40°C vacuum oven drying method.

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 60°C atmospheric pressure or 40°C vacuum drying) 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 0.5 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.

6. Apparatus

- 6.1 Analytical balance, accurate to at least 0.1 mg
- 6.2 Desiccator containing dry desiccant
- 6.3 Porcelain crucibles (ideally weighing less than 10 g) or equivalent, e.g., aluminum weigh boats (see section 10.1.5)
- 6.4 Vacuum oven, set to 40°C ± 1°C OR convection drying oven, set to 60°C ± 1°C
- 6.5 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)
- 6.6 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 None

8. 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 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 100 ± 5 mg (or appropriate quantity based on section 14.4) of prepared algal biomass into the pre-weighed crucible. 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.
- 10.1.5 Aluminum weigh boats 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 convection drying oven at $60^{\circ}\text{C} \pm 1^{\circ}\text{C}$ at atmospheric pressure or $40^{\circ}\text{C} \pm 1^{\circ}\text{C}$ under vacuum 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).

10.2 Ash

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 you will start a fire in the furnace (for crucibles only!).

- 10.2.1 Ashing 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 $575^{\circ}\text{C} \pm 25^{\circ}\text{C}$ for 24 ± 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 ashed samples from the muffle furnace and allow them to cool to room temperature in a desiccator.
- 10.2.1.6 Weigh the crucible and ashed sample and record the weight in a lab notebook to the nearest 0.1 mg to constant weight (section 3.5).
- 10.2.2 Ashing the samples using a muffle furnace equipped with a ramping program (for both crucibles and aluminum weigh boats)
- 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 ashed samples from the muffle furnace and allow to cool to room temperature in a desiccator.
- 10.2.2.4 Weigh the crucible and ashed sample 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:

$$RMS = x_m = mean = \sqrt{\frac{\sum_1^n x^2}{n}}$$

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

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 in duplicate, at minimum.
- 14.3 *RPD Criterion:* Each sample must reproduce total solids and ash content at $\pm 1\%$ wt.

14.4 *Sample Size*: 50 ± 2.5 mg or 100 ± 5 mg, based on available sample. If expected ash content is $>10\%$, then $25 \text{ mg} \pm 2.5$ mg of material can be used. Caution: the final ash weight must be above the minimum weight of the balance.

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

16. References

[1] Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D. *Determination of Total Solids in Biomass and Total Dissolved Solids in Liquid Process Samples: Laboratory Analytical Procedure (LAP)*. 9 pp.; NREL/TP-510-42621. Golden, CO: National Renewable Energy Laboratory, 2008. <http://www.nrel.gov/docs/gen/fy08/42621.pdf>.

[2] Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D. *Determination of Ash in Biomass: Laboratory Analytical Procedure (LAP)*. 8 pp.; NREL/TP-510-42622. Golden, CO: National Renewable Energy Laboratory, 2008. <http://www.nrel.gov/docs/gen/fy08/42622.pdf>.

[3] *2003 Annual Book of ASTM Standards*, Volume 11.05. Philadelphia, PA: American Society for Testing and Materials, International.