Periphyton Biomass Using Ash-Free Dry Weight

1. SCOPE AND APPLICATION

1.1. This method is used to estimate the biomass of periphyton in samples collected from freshwater and marine environments. Biomass (algae) is estimated using calculations based on a determination of dry weight and ash-free dry weight (AFDW) (also known as volatile suspended solids – VSS). The procedure can be modified for AFDW determination of phytoplankton.

1.2. This procedure is used for periphyton samples with LIMS test IDs of ALGAE-AFDW.

1.3. Method Detection Limits (MDL)

1.3.1. The minimum reporting limit for biomass is 1.0 mg dry wt/m^2. Results less than 1.0 mg dry wt/m^2 are reported as 1.0 with a K- qualifier.

2. SUMMARY OF METHOD

2.1. Sample (prepared per SOP BB-01) is homogenized. Sample is poured into a weighed crucible. Crucibles are placed in a drying oven to remove water. The resulting dry weight is the weight of both the organic and inorganic contents of the sample. The dried sample is then heated in a muffle furnace where combustion eliminates the organic content, leaving only inorganic ash. The AFDW is the dry weight (inorganic + organic contents) minus the weight of the ash (inorganic contents only), which equals the weight of the organic content of the sample.

2.2. Interferences

2.2.1. In order to ensure that all organic matter in the sample goes through the combustion process, the muffle furnace must remain at 550°C during analysis. Failure to maintain the temperature could result in organic matter persisting in the sample.

2.2.2. A sample may contain other organic matter, in addition to algae, that would be included in the final results. This analysis gives an estimate of the biomass of algae in a sample.

2.2.3. Body oils (e.g., fingerprints) and other body residues can contaminate beakers and crucibles, which then affects their weights. Disposable gloves must be worn when handling unheated crucibles. Furnace gloves must be worn when handling heated items. Also, any spilled algae or water should be wiped from the outside of the crucible before weighing.

2.2.4. Temperature can affect the stability of the weight measured by the balance. All samples taken out of the drying oven to be weighed should be allowed to

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equilibrate to room temperature before they are weighed. Depending on ambient temperature and humidity, equilibration takes approximately 30 minutes.

2.3. Definitions

2.3.1. Biomass – Total mass of organisms in a given area or volume.

2.3.2. Ash-Free Dry Weight (AFDW) – Used as an index for mass or percent of organic carbon in a quantified substrate.

2.3.3 Periphyton – The zoogleal and filamentous bacteria, attached protozoa and algae growing on submerged surfaces (sticks, stones, aquatic macrophytes, etc.).

2.3.4 Phytoplankton – Minute free-floating aquatic plants.

3. EQUIPMENT AND SUPPLIES

3.1. Mettler PB403-S/FACT balance, SN 1128150043 (Calibrated and used per SOP BB-037-1.1)
3.2. Large crucibles (200-250 ml capacity), 3 per sample (for 450ml of sample)
3.3. Rinse bottle containing deionized (DI) water
3.4. Graduated cylinder, 250 mL
3.5. Latex or vinyl gloves (DANGER!! to handle **cool** crucibles ONLY!)
3.6. Large crucible tongs
3.7. Crucible cooling pad
3.8. Furnace gloves
3.9. Isotemp 180L Drying Oven, SN 42073712, maintained at 105°C ± 2°C
3.10. Muffle furnace (Thermaline F-A1730, SN 4507) preheated to 550°C

3.10.1. To preheat the muffle furnace: Turn on the muffle furnace. Set the furnace control to 4 until it is near the desired temperature (about 2.5 hours), then turn to 2.

Note: The muffle furnace is not thermostatically controlled. The controls adjust the percentage of time the heating elements are on. This setting may vary slightly depending on outside temperature and changes in voltage levels and heating-element conditions.

4. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

4.1. See SOP FS 1000 for sample collection and preservation.

4.2. After collection, samples must be stored at ≤ 6°C but above 0°C until processed (holding time ≤ 48 hours). After tiles are scraped and sample is split, the AFDW aliquot can be held for 28 days at ≤ 6°C but above 0°C. If frozen, sample can be held up to 90 days (at < 0°C).

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5. SAMPLE PREPARATION

5.1. Refer to SOP BB-01.

6. SAMPLE ANALYSIS

6.1. If frozen, remove samples from freezer and allow to thaw at room temperature.

6.2. Place 3 crucibles per sample plus 3 extra for a duplicate in the drying oven for at least 1 hour to ensure they are dry.

6.3. Wear furnace gloves/autoclave gloves to remove crucibles from drying oven. Allow them to cool to room temperature, approximately 30 min.

6.4. Weigh one crucible. Record the crucible ID (found on the bottom of the crucible) and the weight of the crucible in the run log.

6.5. Homogenize the sample by vigorously shaking the sample container until thoroughly mixed.

6.6. Quickly pour 150 mL of sample into the graduated cylinder.

6.7. Pour the entire volume into the weighed crucible. Rinse any remaining sample into the crucible with a small amount of DI water.

6.7.1. Pouring the sample out in small amounts and then allowing the remaining sample to **gently** slosh back into the graduated cylinder helps keep the sample well mixed so that heavier material doesn't stick to the bottom.

6.7.2. If liquid is spilled while pouring sample or while transferring crucibles to the drying oven, make note of it. The final result will need a data qualifier (e.g., J – estimated value) and/or a nonconformance report.

6.8. Repeat steps 6.3.-6.6, two more times per sample until 450 mL of each sample has been placed in weighed crucibles. Rinse the cylinder with DI water between samples.

6.9. Place crucibles (use oven/autoclave gloves if oven is on) in the drying oven for at least 24 hours until all of the liquid has evaporated and the sample is dry.

6.10. Wear furnace gloves/autoclave gloves to remove the crucibles from the drying oven. Allow them to cool to room temperature, approximately 30 minutes. Weigh the crucible + dry sample and record the weight in the run log. (Refer to Section 9.3 for weighing technique.)

6.11. Wear furnace gloves and **use the tongs** to place the crucibles in the pre-heated (550°C ± 20°C) muffle furnace for 1 hour. (Up to 8 large crucibles will fit in the muffle furnace at one time.) After 1 hour, wear furnace gloves and **use the tongs** to remove the crucibles from the muffle furnace and place on crucible cooling pads. Place a sign nearby saying **Caution! Hot!**

Caution! Do NOT place hands inside of the heated muffle furnace, even if wearing protective gloves. Hold the tongs at the furthest point away from the tip, but where control of the tongs can still be maintained. This may mean that crucibles are not

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placed all the way in the back of the furnace and fewer than 8 crucibles are ashed at one time. This is OK. More than one batch can be ashed.

6.12. Allow the crucibles to cool for 20-30 min. Add just enough DI water to the remaining ash to re-wet it. Do not use too much force when squeezing the DI water bottle. It may result in loss of ash from the crucible. Make a comment in the run log regarding any lost sample.

6.13. Wear the furnace mitts/autoclave gloves and place the crucibles back in the drying oven for at least 24 hours until the sample is completely dry.

6.14. When samples have dried, remove them from the drying oven and allow them to cool, approximately 30 minutes.

6.15. Weigh the crucible + the ashed sample and record in the run log. (Refer to Section 9.3. for weighing technique.)

6.16. Return crucibles to the drying oven over night, then repeat steps 6.14.-6.15. Continue repeating until the difference in weight for the crucible + ashed sample is less than 0.010 g.

7. DATA ARCHIVAL

7.1. Open \\Fldep1\dear\Labs\Lab5\Biology\Bench Common\Periphyton\AFDW Template.

7.2. Rename the file and **Save As** AFDW-MM-DD-YY.

7.3. Enter data from the run log into the Data worksheet. The template will transfer calculations and results to the Table worksheet. The data entered into the "Crucible + Ashed Sample" column should be the last crucible + ashed weight recorded in the run log.

7.4. Print the Table worksheet and verify entries. Verification should be performed by two people. One person reads the data from the run-log while the other person checks it on the print-out. If there are any errors, draw a line through the incorrect entry, write in the correct information, and initial. After verification, make any corrections to the file, reprint it and re-verify everything. Draw a checkmark on the printout and write in the date and the initials of the two people who verified.

Verify:	Crucible Weight	Total Sample Volume
	Crucible + Dry Sample	AFDW Aliquot Volume
	Crucible + Ashed Sample	

7.5. On the Table printout, calculate the average and RPD for the duplicate samples. Have the calculations verified.

8. DATA UPLOAD AND AUTHORIZATION

8.1. When all corrections have been made and verified on the Table printout, open

LIMS/Technical/Results Editor.

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8.2. Enter **Analyst**, **Analysis Date** and **Analysis Time**. (The date and time crucibles were weighed and samples were poured should be used for the Analysis Date and Time.) Un-check the box next to "**Use Sig Figs?**" **Sort By** Sample ID. Under the heading **Analysis Group**…**Test Status**…, check "**V**" and select **Bio-Chl-a. Select Test…**ALGAE-AFDW. In **Select Sample IDs…**choose the appropriate sample numbers.

8.3. In the **Results** column, enter the **Biomass (g/m^2)** results from the printed and verified Table worksheet. Add any appropriate **Remarks** and **Comments**.

8.3.1. Duplicates:

8.3.1.1. If the duplicate average is being uploaded, add an "A" remark.

8.3.1.2. If the average is being uploaded, but the RPD failed, add a J-qualifier to the average ("AJ" remark) and the comment, "Result is J-qualified due to a lab duplicate RPD failure."

8.3.1.3. If the duplicate average is not being uploaded, enter the higher of the two individual results. Add a comment saying, "Laboratory duplicate result is XX g/m^2," where XX is the duplicate result not being uploaded. Also, if the average is not being uploaded, add a comment to all of the samples saying, "Precision data is unavailable due to the small amount of analyte in the QC sample."

8.4. Select **File/Save** or click the shortcut button that says **Save Changes to Results**. Under **Analysis Group…Test Status…**, uncheck V and check "**C**." Select **Bio-Chl-a**, **ALGAE-AFDW**, and the appropriate sample IDs. Print. Stamp the printout with the verification stamp. Initial and date the Data Entry and Uploaded lines.

8.5. Perform a two-person verification where one person reads the Biomass (g/m^2) results from the Table printout while the other person checks the entries in the Results Editor printout. Draw a line though any errors, write-in the correct data and initial. Initial and date the Verified line. Make corrections in the Results Editor file. (Select **Test Status…C** to make corrections.) Save, re-print, re-stamp and re-initial and date the Data Entry and Uploaded lines. Re-verify the corrected printout and initial and date the Verified line.

9. QUALITY CONTROL

9.1. Analyze a laboratory duplicate for 5% of samples and a minimum of one replicate per batch. The acceptance limit is ≤25% RPD. The duplicate would need to be prepared from a homogenized sample.

9.2. Due to consumption of the sample during processing, duplicates cannot be reanalyzed. Report duplicate results that exceed the acceptance limit with a J-qualifier

9.3. When weighing crucibles and dried samples, the weight of the first crucible must not vary more than +/- 0.01g when reweighed after every fifth crucible has been weighed (or last crucible if < 5 crucibles). If the weight varies more than 0.01 g, the crucibles should be dried for another hour (@105°C ± 2°C), cooled, and reweighed.

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10. SAFETY/HAZARDOUS WASTE MANAGEMENT

10.1. Refer to the Laboratory Health and Safety Manual and Hazardous Waste Management.

10.2. Read MSDS information for reagent handling and disposal.

11. REFERENCES

11.1. EPA Method 4805572010: Procedures for Handling and Chemical Analysis of Sediment and Water Samples

11.2. NELAC Quality Systems Manual

11.3. FDEP Bureau Quality Manual http://publicfiles.dep.state.fl.us/dear/labs/lab_qualitymanual_18.pdf

11.4. Laboratory Health and Safety Plan https://www.fldepnet.org/content/dear/bureau-laboratories-safety-documents

11.5. DEP SOP-001/01, *FS 1000 General Sampling Procedures*

11.6. DEP SOP BB-01, *Quantitative Periphyton Sample Preparation for Chlorophyll*-a, *Ash-free Dry Weight, and Taxonomic Identification.*

11.7. DEP SOP BB-030, *Sample Custody, Preparation Labels and Worksheet Instructions for Bench Biology Samples*

11.8. DEP SOP-BG-01 *Data-Entry Verification*

11.9. Standard Methods for the examination of Water and Wastewater 19TH Edition: Method 10200 I and 10300 C

11.10. In-house notes from Jennifer Weger, **Dry and Ash Free Dry Weight Measurements for the LakeWatch Phytoplankton Biomass**.

Appendix of Changes

06/13/2014	Created new SOP.
09/08/2014	Added thawing step for samples to coordinate with storage change from refrigeration to freezing in BB-01.
08/03/2015	Updated to reflect new file locations.
10/13/2017	Updated name and serial number of new drying oven. Section 7 re-formatted.
11/09/2018	Added correct link for the QA manual.
12/20/2019	Changed SOP reference for Mettler Toledo PB403-S/FACT Balance.
12/22/2020	Updated version numbers of referenced SOPs.

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