

DETERMINING PERIPHYTON BIOMASS

General: There are essentially three different methods or approaches to estimate the biomass of periphyton algae. These are algae that are attached to a substrate such as rocks, logs or branches, higher aquatic plants or to other bottom substrates (sand or mud). The stuff is also often called “benthic algae” and the more general group of the algae, detritus, bacteria and fungi that are typically inextricably woven together is called the “aufwuchs” community. This term derives from the sound a person makes when they slip on slimy rock and crash into the water.

1. Ash-Free Dry Weight (AFDW) -This is a gravimetric approach that involves filtering the sample onto a pre-dried, pre-weighed filter then drying the sample to a constant weight. The sample is then ashed (oxidized) in a muffle furnace at high temperature and the ash residue (the inorganic remains) is re-weighed. An estimate of the organic matter that was vaporized is determined by difference. One of the major drawbacks to this method is that it measures the weight of all of the organic material in the sample including detritus, bacteria, insects, as well as periphyton. The final value is usually expressed as either a % of dry weight or as AFDW per unit area (that is, organic matter per unit area).
2. Pigment Analysis – This method involves extracting the chlorophyll from the algal cells. This method allows the differentiation of algal biomass from other organic constituents in the sample. The main disadvantage of this method is that the amount of chlorophyll within an algal cell is specific to its physiological state and ambient light conditions. The method also requires either a spectrophotometer or fluorometer.
3. Biovolume Measurement- This method determines the biovolume of individual algal cells. It is very time consuming and requires knowledge of algal taxonomy as well as the ability to be consistent in assigning complex volume formulae to even more complex microscopic shapes. This is quite a bit more simple when the sample is mostly unialgal such as for clumps of filamentous algae where there may be low species diversity..

Sample Collection: Many methods have been developed to collect periphyton from natural substrates. All involve quantitatively removing material from a known surface area. Different methods may be needed to collect periphyton from rocks, woody debris or docks, aquatic macrophytes, sand or other substrates. Substrate size will also determine how samples are collected. Large boulders require the use of a device that prevents the sample from being lost downstream. If the substrate is a fist-sized cobble the rock can be paced in a pan, scraped, then washed into a bottle with stream water.

One simple way to scrape a known area is to lay a plastic 35 mm slide (film removed) over the rock and scrape off the material within the slide area (scrub area = 2.3 cm x 3.5 cm = 8 cm²).

A known volume of the periphyton suspension can now be pulled by vacuum onto a pre-ashed (free of organic matter) glass fiber filter (Whatman GF/C or GF/A, Gleman A/E, Reeve–Angel 934 or 984, or equivalent) for Wet Weight (WW), DW, AFDW or chlorophyll determinations.

Precautionary notes: Wet weight determinations are required for estimating chlorophyll-a because of the need to treat subsamples for different analytical tests differently. Most chemical

analyses can be performed on dried (at ~ 103-105 °C) material. However, drying a sample will degrade the chlorophyll so this analysis must be done on “wet” frozen material. In order to compare the result performed on a small subsample to other surface area normalized measurements, such as DW or AFDW, you need to know the DW to WW ratio of your original sample. If multiplied by 100, this is the % moisture or %H₂O for the sample.

Sounds simple but it can be tricky because of the difficulties of estimating wet weight for a glob of muck that holds water like a sponge. How much of the water do you allow to run off or do you blot it? Its weight clearly can make a big difference and its removal is always subjective so at least be consistent. You can lay you blob on a waxed weighing paper or aluminum foil or even a clean paper towel to blot out some of the water. Then you can split the sample into a number of subsamples (also called *aliquots*). One set of these can then be weighed individually and then analyzed for chlorophyll or examined microscopically. Or, after weighing for WW, they can be frozen until analysis. The other aliquots can then be dried to obtain DW values and stored dry until later ashing for AFDW or subsequent analysis for nutrients, metals, etc.

By carefully keeping track of either a wet or a dry weight per aliquot and knowing the total wet weight of the original complete sample, and the area from which it was collected, you can then express various parameters, both biological and chemical, in similar units of surface area.

Methods:

See *Dry Weight and Ash-Free Dry Weight*
See *Chlorophyll Determination*

References:

Standard Methods for the Examination of Water and Wastewater, Method 10300 C,D. 20th Ed. 1998. American Public Health Association, Washington, D.C.

Steinman, A.D. and G. A. Lamberti. 1996. Biomass and pigments of benthic algae. P. 297. In *Methods in Stream Ecology*, Hauer, F.R. and G.A. Lamberti (eds). Academic Press, San Diego, CA.