WATER COLUMN CHLOROPHYLL *A* EXTRACTION

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Adapted from: ESS Method 150.1:Chlorophyll – Spectrophotometric, Environmental Sciences Section, Inorganic Chemistry Unit, Wisconsin State Lab of Hygiene, 465 Henry Mall, Madison, WI 53706. Equation for chlorophyll a from Jeffrey and Humphrey (1975).

1.0 Introduction

Chlorophyll *a*, a characteristic algal pigment, constitutes approximately 1% to 2% (dry weight) of planktonic algal biomass. This feature makes chlorophyll *a* a convenient indicator of algal biomass. This method is applicable to most surface waters.

2.0 Summary of Method

Algal cells are concentrated by filtering a known volume of water through a membrane filter (47 mm, 0.45 µm pore size nitrocellulose filter). The pigments are extracted from the concentrated algal sample in an aqueous solution of acetone. The chlorophyll *a* concentration is determined spectrophotometrically by measuring the absorbance (optical density - OD) of the extract at various wavelengths. The resulting absorbance measurements are then applied to a standard equation.

3.0 Sample Preservation and Preparation

1) Chlorophyll *a* samples are placed in a dark cooler and packed in ice at the time of collection.
2) Under low light conditions (light degrades chlorophyll pigments), set up the filtering manifold, seawater trap, and vacuum pump (or aspirator).
3) Place a 0.45 µm pore size nitrocellulose filter on each filtering funnel, and filter a known volume (measure with a graduated cylinder) of sample (in the dark), applying vacuum until the sample is dry. The amount of sample required depends on phytoplankton volume in the water sample. For coastal waters, filter in 50 ml increments. When water flow begins to slow, continue to filter small amounts of water until flow almost ceases.
4) Record the volume filtered for each sample.
5) If samples are to be run at a later date, fold the filter in half and wrap in pre-labeled aluminum foil or opaque tubes (or wrap test tube wrap with black plastic bag) and freeze. If samples are to be run immediately, proceed to step 4.0.

4.0 Procedure

1) Place the filter containing the concentrated algal sample in a pre-labeled test tube.
2) Add 5 mL of 90% acetone solution (i.e., 900 ml of acetone mixed with 100 ml of double distilled or ultrapure water).
3) Cap tightly, vortex until filter dissolves, and place in the dark box for 24 hr.

4) Repeat until the all samples are processed.
5) Create two blanks using 5 ml acetone solution and new filter (with no chlorophyll on it).
6) Wrap test tube rack in black garbage bag and place samples in freezer and allow extract to steep overnight (up to 24 hr).
7) Remove samples from freezer and remember to keep samples covered in low light conditions at all times.
8) Clarify extract by centrifuging samples for 15 minutes at approximately 500 g. Remember to balance the centrifuge (i.e., put equal number of samples on each side).
9) Turn on spectrophotometer and allow to warm up while samples are centrifuging.
10) Remove samples from centrifuge. DO NOT SHAKE! Rewrap test tube wrap in black plastic and take samples to spectrophotometer.
11) Carefully transfer the two blanks to the two 1.0 cm cuvettes. Pour using a continuous motion.
12) Set up the spec to measure the absorbance at: 750, 664, 647, 630, and 600 nm.
13) Autozero the spec with the blanks (make sure clear sides of cuvettes are facing away from you when you place in spec).
14) Remove closest cuvette. Empty contents into waste container. Pour first sample into cuvette (Do not shake and only pour once into cuvette after centrifuging).
15) Place cuvette into the slot vacated by the blank. Push Read Sample.
16) Repeat for remaining samples.
17) When finished save output file on computer hard drive and on a floppy disk.

5.0 Calculation
Subtract the absorbance at 750 nm from the 630, 647, and 664 nm values (turbidity correction

$$\text{ug chl a L}^{-1} = \frac{S\,[11.85\,(Abs664) - 1.54\,(Abs647) - 0.08\,(Abs630)]}{V}$$

Where S = The volume of acetone used for the extraction (mL)
V = The volume of water filtered (L)
L = The cell path length (cm)