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Filtration, extraction and analysis of chlorophyll *a* via fluorometric detection

Prepared By: David Velinsky and Jennifer DeAlteris

Approved By: Date:

Robin Davis
Quality Assurance Unit

FILTRATION, EXTRACTION AND ANALYSIS OF CHLOROPHYLL *A* VIA FLUOROMETRIC DETECTION

1.0 **Method Summary**

The most useful chemical method for determining the total quantity of phytoplankton in fresh and seawater is to estimate the amount of chlorophyll (as Chlor *a*). This value is used to determine the amount of plant biomass that is present in a waterbody. In general this method is 5 to 10 times more sensitive than the spectrophotometric method but may be less accurate if high concentrations of chlorophyll b are present. Replicate measurements at about 0.5 µg/L should be better than 10%. This procedure is modified from Parsons et al. 1984 (*A Manual of Chemical and Biological Methods for Seawater Analysis*) and Strickland and Parsons (Fish. Res. Bd. Canada, 1972).

2.0 **Method**

2.1 **Special Apparatus and Reagents:**

Filtration apparatus which accommodates 25mm Whatman GF/F filters or equivalent
15ml glass centrifuge tubes with caps
Low speed centrifuge (up to 3000 rpm)
5% (v/v) HCL
ACS quality acetone diluted with DI water (90% v/v)
1% (w/v) magnesium carbonate solution
Pasteur pipets and bulb
Chlorophyll *a* standard free of *b* and *c* forms (Sigma Chemicals)
Fluorometer (Turner Designs TD-700 or equivalent) and proper lamp (daylight white lamp) and filters (TD P/N 7000-961)

2.2 **Sampling and Filtration**

1. Filter water (volume dependent on expected concentration but typically around 50 to 200 mL) through glass fiber filter at a vacuum pressure of < 8psi. Add two drops of a saturated magnesium carbonate solution to the last few milliliters of sample (let solution settle). Record volume filtered. (Note: It is possible that membrane filters, methyl cellulose, may be used, and grinding step is not needed)

2. For storage up to 1 to 2 months, place folded filter in aluminum foil packet, label, and place packet in freezer at -20^{o}C.

2.3 **Extraction**

1. Extractions need to be done in darkened room.

2. Place filter in a 15 mL glass centrifuge tube with 5 ml of cold 90% acetone.

(Note: keep acetone in ice bath, helps keep sample from heating up during grinding step)

3. Use Teflon tissue grinder to break up filter, then using a Pasteur pipette, rinse grinder with remaining 5 mL of cold 90% acetone into centrifuge tube.

4. Place sample in refrigerator overnight to complete extraction (approx. 18 hours).

5. Set-up blank filter as above (i.e., wetted filter, magnesium carbonate and acetone).

2.3 **Fluorometric Determination**

1. Fluorometer should be fitted with the proper filters (for extractive acidification method) and a daylight white lamp for chlorophyll *a* analysis as per manufacturer specifications; both for excitation source and emitted light detection.

2. Calibrate meter prior to use (see below).

3. Centrifuge sample tubes at approximately 3000 rpm (500g) for 20 minutes or until solution is clear and filter in compacted on the bottom. Carefully take tubes out of centrifuge to minimize resuspension of filter into solution.

4. Using a Pasteur pipette, carefully transfer approximately 8 mL of the solution to the fluorometer cuvette. Make sure no glass fiber material comes across into cuvette.

5. Place the sample in the meter and record response. If the solution is too concentrated (e.g., > 50% on least sensitive setting), dilute sample with acetone. Record any dilution factor and meter response.

6. Add 2 drops 5% HCL, mix, wait two minutes, and re-measure sample after reading stabilizes. Record response.

2.4 **Calculations:**

mg Chlor a/m^3 = F_D x (T/T-1) (R_{before} -R_{after}) x (v/V)
mg Phaeo a/m3 = F_D x (T/T-1) (TR_{after}-R_{before}) x (v/V)

F_D = Calibration factor (i.e., slope)
T = R_{before}/R_{after} for a standard extract free of phaeophytin; average of samples. (see Step 5 below)
R = fluorometer reading (before and after acidification)
v = volume of acetone extract in ml
V = volume of water filtered
(Note correct samples for Blank value if measurable)

Appendix: Calibration

1. Need *b* and *c*-free Chlorophyll *a* (Sigma Chemicals C-6144)

2. Dissolve standard (e.g., 1 mg/ml) in 90% acetone (90 acetone: 10 DIW) in a 250 mL volummetric flask; 4000 µg/L (stock solution, SS). Then take 1 mL of SS and dilute to 100 mL; 40 µg/L (working solution, WS). (Note can use different volume flask but need to adjust calibration concentrations). Keep standard wrapped in Al foil, in dark and cold when not using.

3. Measure standard on spectrophotometer from assumed concentration 4000 µg/L stock solution:

Take 4000 µg/L standard and measure absorbance at 750, 664, 647, and 630nm. Then calculate Chlor *a* concentration:

C*a* = $11.85Abs664_b - 1.54\ Abs647_b - 0.08Abs630_b$ (UNESCO equation)

C*a* = concentration of Chlor *a* in stock solution .

4. Follow procedures for Calibration: Simple Mode on page 19 of TD-700 Laboratory Flourometer Operating Manual. This sets the optimal range and sensitivity of the instrument. When setting the typical sample, it should be about 400-500 µg/L.

Suggested dilution series (Can be changed):
4. Using 100 x 13mm test tubes or equivalent develop a dilution series similar to this (note: maximum concentration depends on expected concentrations in samples):

Chlor a conc (µg/L)	Solution (mL)	90% acetone (mL)
2.67	0.500 WS	7.0
5.34	1.00 WS	6.5
10.67	2.00 WS	5.5
21.33	4.00 WS	3.5
40.0	7.5 WS	0
53.3	0.10 SS	7.4
106.7	0.20 SS	7.3
213.3	0.40 SS	7.1
320.0	0.60 SS	6.9
426.7	0.80 SS	6.7
533.3	1.0 SS	6.5
640.0	1.2 SS	6.3

5. Measure fluorescence on meter before and after addition of 2 drops of 5% HCL. Determine slope of the linear fit to the regression. Calibration will be good for about one month. Check calibration periodically with 40 µg/L and Turner solid standard.