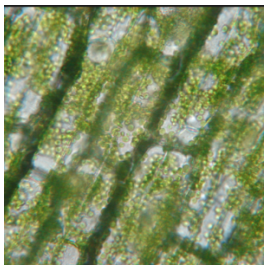
Jun 21, 2017

Total Chlorophyll a Measurements by Spectrophotometer



DOI
dx.doi.org/10.17504/protocols.io.ijpccmn

Dr. Steven Wilhelm

The Aquatic Microbial E... CyanoHABs

Steven W Wilhelm
The University of Tennessee, Knoxville

DOI: dx.doi.org/10.17504/protocols.io.ijpccmn

Protocol Citation: Dr. Steven Wilhelm 2017. Total Chlorophyll a Measurements by Spectrophotometer. **protocols.io** https://dx.doi.org/10.17504/protocols.io.ijpccmn

Protocol status: Working

Created: June 21, 2017

Last Modified: March 25, 2018

Protocol Integer ID: 6479

Abstract

Please contact Dr. Steven Wilhelm (wilhelm@utk.edu) for additional information regarding this protocol.

Adapted from Wetzel and Likens 2000. Limnological Analyses, Springer NY

Sample Preparation

1 Obtain a sample to be examined. This can be a culture or a field sample.

Note

For chlorophyll extractions, it is important to process at least duplicate samples, but triplicate samples are even better.

2 Collect materials on 0.2 µM polycarbonate membrane filters with gentle filtration

Note

Record the volume that is being filtered in L. The volume to be filtered is dependent on the sample in question. Typically, it will range from 25 mLs to 100 mLs. The more productive (eutrophic) the system, the less you will filter. You should avoid adding too much material as this will clog the filter and it will no longer select based on size exclusion.

3 Place the filter containing the sample into a 15 mL Falcon tube

4 Add 90% acetone to the tube

5 mL

5 Store sample and acetone at -20ºC overnight to allow for extraction of Chl a

Note

If -20 degree C is not available, 4 degree C will work. The main point is that the sample should be stored in the dark.

Determine Chl a Concentration in Extracts

6 Read the standards in a spectrophotometer at the following absorbance: 665 nm, 645 nm, 730 nm

7 Equation: mg/L Chl a in the extract = 11.75*(A665-A730)-1.31*(A645-A730)

Note

This equation gives results in mg/L, not ug/L.

8 To convert back to the original sample, use the following equation:

Chlorophyll in sample (mg/L)*acetone volume = total chlorophyll extracted

Total chlorophyll extracted (mg)/volume filtered (L) = concentration of chlorophyll in the original sample (mg/L)