

Analysis of algae by the membrane filter-patch test method

Load the filter holder with a *white* 0.45 mm filter, handle the filter with forceps. Pour sample* into the funnel, apply vacuum. When the filtration is complete, release the vacuum, remove the filter with forceps, and set the filter down on a clean surface to *dry*. Compare the color of your filter with the other samples in the class. The intensity of color is proportional to the concentration of algae in the water. Rate the samples, the one with the most algae being a 4+, the next is 3+, 2+, + and no algae would be scored as -. Cut the filter in half and affix one preserved quarter to your result sheet using clear Contac paper.

Using forceps, float the other half in immersion oil in a watch glass. If the filter was dry, it will immediately become transparent as its pores fill with oil. Remove the filter from the oil drawing it across the edge of the watch glass to remove excess oil. Place the filter on a slide and examine under 100× and 400× magnification. To identify the algae in your sample compare the algae you observe to pictures in *Algae in Water Supplies*. List the algae you identify in your results. (Be sure to *clean* any oil from the microscope and lenses using lens paper.)

*The amount of sample required will vary with the productivity of the system. While 25 ml may be adequate for productive systems, as much as 100 mL may required for oligotrophic waters.

Determination of phosphate (PO_4^{3-}) concentration

Fill the test tube to the upper mark with the water sample. Using the 1 mL dropper add 2 mL (2 dropperfuls) of VM Phosphate Reagent to the test sample and mix the contents of the tube. Wait 5 minutes, then using the unmarked pipette add 2 drops of VM Reducing Reagent, and invert the contents. Color will develop immediately. Reading is made in the Axial Comparator by color comparison. Use a piece of white paper behind the comparator for a background. Record the PO_4^{3-} concentration in parts per million (ppm).

Analysis for nitrates (NO_3^-)

Nitrates in large amounts in drinking water can cause methemoglobinemia in infants under 6 months of age. Currently this is an important problem in livestock production. U.S. Public Health Service and Environmental Protection Agency Drinking Water Standards state the NO_3^- concentration limit as 10 ppm.

Fill the test tube to the line with the water sample. Using the 0.5 mL-pipette, add one pipette full of Nitrate Reagent 1. With the 1.0-mL pipette, add one measure of Nitrate Reagent 2. Shake the tube to mix. Add one 0.1 g spoonful of Reagent 3, shake to dissolve and let stand for 2 minutes. With a clean 0.05 g spoon, add 0.05 g of Reagent 4-R. Allow 5 minutes for the color to develop. The color is then compared with colors of known value on the Comparator. Multiply the comparator value by 4.4. Record the concentration of NO_3^- in ppm.

Chlorophyll

1. Water samples must first be filtered. The amount of sample required will vary with the productivity of the system. While 200 ml may be adequate for productive systems, as much as 1 liter may required for oligotrophic waters. We will filter 500 ml through a 0.45 μm membrane filter. Filter approximately 50 ml at a time and replace the filter as often as necessary.
2. Place the filters into a mortar and add approximately 3-4 ml of 90% alkaline acetone. Grind the samples thoroughly for 45 seconds, decant the homogenate into a graduated 15-ml conical tube. If some parts of filters still remain, add another 3 ml to the tissue grinder and grind for another 15 seconds. It is best to keep the acetone volume to a minimum so that it does not dilute the pigment. Combine the homogenates and record the total volume to the nearest 0.1 ml.
3. Centrifuge at maximum speed in a clinical centrifuge ($\sim 1000 \times g$) for 5 min. If possible, use a refrigerated centrifuge. Repeat the centrifugation if the supernatant is cloudy.
4. If the chlorophyll extracts are likely to be dilute, as in an unproductive system, increase the sensitivity of the assay by transferring 7 ml to a 20 mm light path length (rectangular) cuvette. (A 13×100 mm glass culture tube would be fine if working with a Spectronic 20.) Make a blank with 90% alkaline acetone.
5. Measure the absorbance at 750 and 665 nm using the 90% alkaline acetone as a blank.
6. Add 0.1 ml of 1 N HCl per ml of extract directly to the cuvet, cover, and invert to mix. Allow the tube to stand for 5 min.
7. Remeasure the absorbance in the acidified samples at 750 and 665 nm.

Calculations

$$\text{Chl a } (\mu\text{g/l}) = \frac{(k)(F)(E_{665o} - E_{665a})(v)}{(V)(Z)}$$

where

E_{665o} = turbidity-corrected absorption at 665 nm before acidification
= $A_{665o} - A_{750o}$, where A = absorption value

E_{665a} = turbidity-corrected absorption at 665 nm after acidification
= $A_{665a} - A_{750a}$

k = absorption coefficient of chlorophyll a = 11.0

F = factor to equate the reduction in absorbency to initial chlorophyll concentration
= 1.7:0.7, or = 2.43

R = maximum ratio of $E_{665o} : E_{650a}$ in the absence of phaeopigments, = 1.7

v = volume of extract in ml

V = volume of water filtered in liters

Z = length of light path through cuvet or cell in cm