Algae as Pollution Indicators: Analysis Using the Membrane Filter

By BERNARD I. SOHN

In healthy ecosystems, bodies of water usually contain an abundance of different algae. When sewage or other nutrient-rich material pollutes a stream or other body of water, the species diversity is affected; that is, a notable change takes place in the numbers and types of algae that will be found. As the sewage or nutrients go through stages of bacterial decomposition in the stream, the numbers and kinds of algae continue to change. Eventually the purified water far downstream contains the same natural balance and species diversity as that found above the point of pollution. Thus by identifying variations in algal populations, one can get a good indication of water quality.

To science teachers familiar with the use of membrane filters in bacterial analysis of water, it will come as no great surprise to learn that these versatile filters lend themselves to a number of other analyses easily performed by students in assessing water quality. One such test is the analysis of algae.

Membrane filters are particularly suited to this because they screen out all particles larger than the filter pore size and retain them in a single microscopic plane on their surface. The filters are then easily rendered transparent with microscope immersion oil, and the trapped particles, including all algae in the sample, can be examined and identified microscopically. Furthermore, membrane filters are soluble in a number of organic solvents used in the analysis of algae pigment.

These techniques have given rise to a number of qualitative and quantitative tests now routinely employed by marine-research and public-health laboratories.

This article tells how some of these tests can be applied to an environmental-science program. The only specialized equipment required is a suitable vacuum filter-holder designed for use with membrane filters, a portable vacuum source (for field use), and a pair of nonserrated forceps for handling the delicate membranes without puncturing them. Membrane filters are available in a wide range of pore sizes. The type recommended here is the Millipore Corp.'s type HA (0.45-μ pore size): it is also the type used in bacterial analysis. Thus, a number of different tests (for bacteria, algae, suspended solids) can be performed on the same water sample using just one type of membrane.

The author is director, educational division, Millipore Corp., Bedford, Mass. 01730. He is a 1960 graduate of Suffolk University, where he majored in biology and chemistry, and has a master's degree in business administration from Northeastern University. From 1955 to 1959 he was a research chemist for the U.S. Fish and Wildlife Service. Before joining Millipore he was engaged in research on ion-exchange membranes for Ionics, Inc., and he holds several patents in membrane technology. Sohn has served as consultant in environmental education to the Biological Sciences Curriculum Study and the Tilton Water Pollution Program.

Collection of Samples

Samples of pond or river water should be collected from locations near lily pads or where there seems to be a heavy growth of algae or other vegetation. Clinging algae may be scraped from stumps and rocks by using a spatula or dull knife; free-floating algae can be collected in a net of fine mesh material (wire, plastic, cloth) or in a wide-mouthed collection

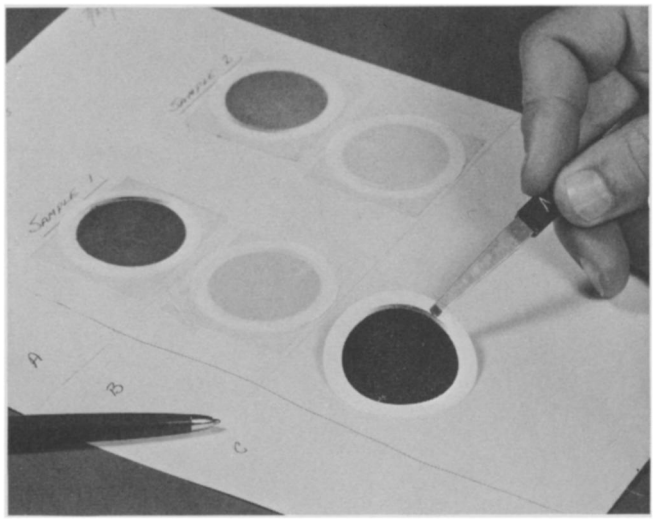

Fig. 1 Patch test for comparison of amounts of algae in different samples.

jar. If the water samples are to be brought back to the laboratory or kept for any length of time before analysis, it is advisable to keep them in capped jars filled only part-way, thus leaving an air space with a reserve oxygen supply. The jars should be kept in a cool dark place, such as a picnic cooler.

Patch Testing

A patch test is commonly performed to provide a visual comparison between the color of the material collected on the test filter and that of samples from other sources. Furthermore, a precalibrated reference chart can easily be prepared by filtering increasing volumes of test water through a series of preweighed membrane filters. Each filter is then dried, reweighed, and mounted on a chart in the order of increasing weight (for example, 5 mg, 10 mg, 20 mg, etc.). The result, of course, is an arrangement of filters of increasing color intensity, against which the test sample can be compared. This provides a simple way to compare visually the relative amounts of algal growth from different sources (fig. 1) or from the same source under different conditions, such as seasonal or nutritional fluctuations. Bear in mind, however, that for comparisons to be meaningful the same sample sizes must be used for each test.

1. Using smooth-tipped forceps, load the filter-holder with a membrane filter (fig. 2).
2. Add 250 ml of test water.
3. Apply vacuum with a few strokes of a hand vacuum-pump or some other suitable vacuum source, such as an aspirator. The water will flow through the filter, leaving the algae cells trapped on the filter surface.
4. When the filtration is completed, release the vacuum and place the filter on a clean surface to dry.
5. The dried filter can now be stored in a 47-mm petri dish or mounted on a chart along with other sample filters for comparison.

Counting Algae Populations

A more precise quantitative method of determining the population density of algae involves filtering a known volume of water through a membrane filter and then counting the collected cells with the aid of a microscope.

1. Collect and filter a sample of test water as above. If the water contains an abundance of algae it will be necessary to use a smaller sample size, say 10 to 50 ml. Be sure to record the volume of the sample.
2. When the filtration is completed release the vacuum and dry the test filter on a clean surface for about 30 minutes. This process can be accelerated if the filter is placed in a drying oven at 45 C for a few minutes.
3. Put about 5 ml of microscope immersion oil in a 47-mm petri dish or in a watch glass. Because membrane filters generally are made from mixed esters of cellulose, they have a refractive index of 1.5, the same as that of immersion oil. This is why a dry membrane filter will appear transparent when saturated with oil.
4. Pick up the dry filter with smooth-tipped forceps and float it in on the immersion oil. If the filter is completely dry it will immediately become transparent, as its pores become filled with oil of matching refractive index. (If the filter remains opaque, it means that there is still some water in the pore structure. Place the petri dish and the filter on a warm surface until the filter clears.)
5. Draw the filter across the edge of the petri dish to remove the excess oil (fig. 3).
6. Center the filter on a 5-by-7.6-cm (2-by-3-inch) microscope slide. (If you prefer to use the more common 2.5-by-7.6-cm (1-by-3-inch) slide, cut the filter evenly into four parts before beginning step 3.)
7. Place the slide on the stage of a microscope and, using a reflecting mirror or a substage light, scan the filter surface at low magnification (10× or 40×).

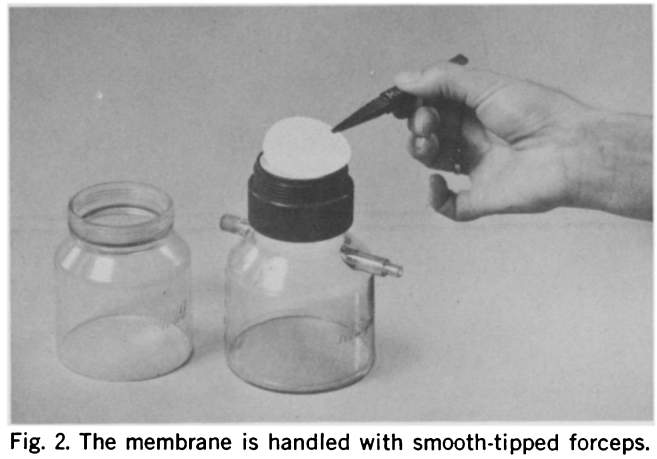

Fig. 2. The membrane is handled with smooth-tipped forceps.

8. Count the number of algae in each of 10 randomly selected fields on the filter (fig. 4). It is not necessary to count the entire filter: all the cells on the filter surface are evenly distributed.

9. Calculate the number of algae in the original sample by applying the following formulas:

$$\text{(i)}\quad \frac{1{,}380}{\text{area of field in mm}^2} \times \text{number of fields counted} = \text{factor}$$

$$\text{(ii)}\quad \text{number of algae counted} \times \text{factor} = \text{number of algae in original sample}$$

The value 1,380 is the filtration area in mm^2 of a 47-mm membrane filter when used in a Sterifil filter-holder. This value will vary for other kinds of filter-holders.

The area of the field of view is a variable that must be calculated for individual microscopes. It can be determined with the aid of a stage micrometer or simply by laying a transparent plastic rule across the diameter of the field of view and calculating the area using πr^2, the formula for the area of a circle. This procedure may be used for low- or high-power objectives.

10. Having calculated the number of algae cells in the original sample and knowing the volume of the original sample that was filtered, the student can calculate the number of algae per ml by the following formula:

$$\text{(iii)}\quad \frac{\text{number of algae in original sample}}{\text{volume of original sample in ml}} = \text{number of algae per ml}$$

Suppose, for example, the student filters a sample of 50 ml of pond water and, after drying and clearing the filter with immersion oil, he analyzes the filter microscopically at 100× magnification. He determines that the area of a field at 100× is 5 mm^2. He picks 10 fields at random and counts a total of 55 algae cells. His calculations would be (i) (1,380/5) × 10 = 2,760; (ii) 55 × 2,760 = 151,800 algae in the original, 50-ml sample; and (iii) 151,800/50 = approximately 3,000 algae per ml.

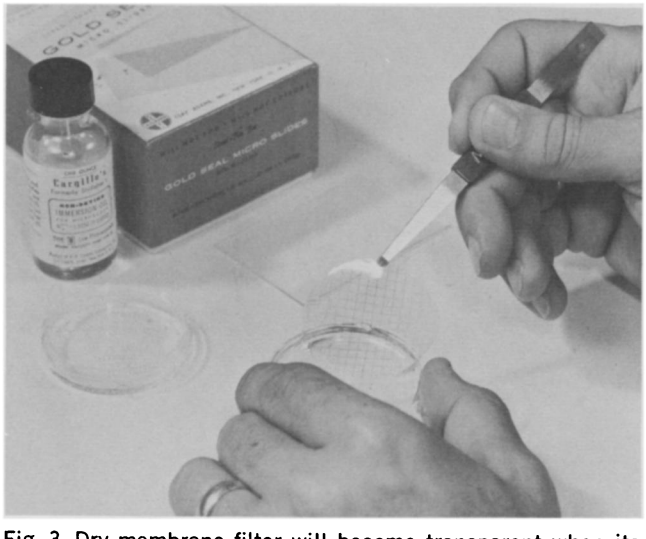

Fig. 3. Dry membrane filter will become transparent when its pores are filled with a fluid of matching refractive index.

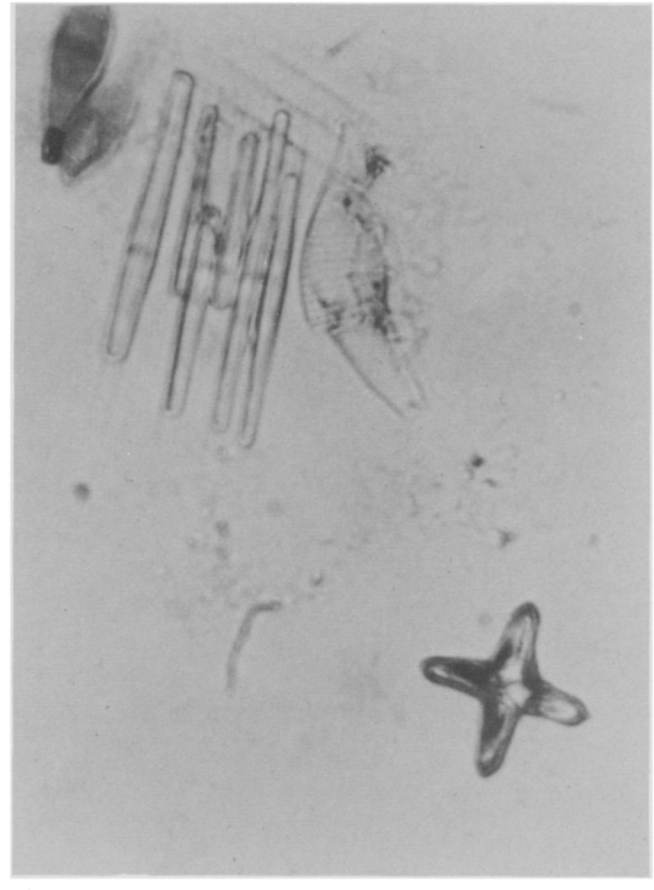

Fig. 4. Freshwater algae can be identified on a transparentized membrane filter. (400×.)

Several studies have shown that algae populations in excess of 1,000 per ml are evidence of overenrichment.

Identifying Characteristic Algae Groups

Another indicator of water quality is the kinds of algae found. Polluted water shows, for example, a sharp decline in the number of green algae and diatoms and an increase in the number of blue-green algae and flagellates. Students should use high magnification (100×) to identify the algae collected on the filter surface. The accompanying table gives the major characteristics of each group. With a little practice, these can easily be distinguished under the microscope.

Have students record the number of each of the groups seen in 10 fields at 400×. Use the formulas given above to determine the relative numbers of each group. Is there a healthy diversity of species or is there a predominance of blue-green algae? Do the findings indicate pollution or clean water?

Pigment Analysis

Another way of assessing water quality involves the analysis of algae pigment. Most algae contain

chlorophyll, which absorbs light waves (of specific wavelengths) and converts them to energy for photosynthesis. By measuring the absorption spectrum of chlorophyll-A in a water sample we are able to determine the amount of photosynthetic activity, and thus the density, of algae.

The following simple test provides a visual comparison of algae density:

1. Collect some samples of algae (as above). Use a sample size that will produce a heavy deposit on the filter without clogging it.
2. Place the test filter in a beaker containing 10 ml of solvent (90% acetone, 10% methyl alcohol). The filter, made of cellulose esters, will dissolve, leaving the algae pigment (chlorophyll-A and chlorophyll-B) in solution.
3. Transfer this to a test-tube and compare the color with other, similarly prepared samples.

Characteristics of some important groups of algae. For details consult Palmer (1959).

Characteristic	*Blue-green algae*	*Green algae*	*Diatoms*	*Flagellates*
Color	blue-green to brown	green to yellow-green	brown to light green	green or brown
Cell wall	not distinguishable	distinguishable (many have spines)	readily distinguishable; with regular markings	sometimes distinguishable
Nucleus	absent	present	present	present
Flagellum (tail)	absent	absent	absent	present

As a further activity, students can separate the solubilized chlorophylls by paper chromatography, as follows:

1. Concentrate the volume of solvent and pigments by heating the sample test-tube in a water bath or by allowing it to partly evaporate overnight. The final volume should be approximately 1 ml.
2. Cut a strip of standard filter paper about 12 cm wide and 70 cm long. With an eyedropper make repeated applications of the concentrated pigment sample at a spot about 25 cm from the end of the strip. Keep the spot as small as possible.
3. In a clean test-tube add 10 ml of a solvent system prepared by mixing equal parts of *n*-heptane, benzene, and ethyl acetate.
4. Use a paper clip to support and center the test strip in the solvent test-tube so that the end of the filter paper is immersed in the solvent system but the pigment spot is just above it. The filter-paper strip must not touch the sides of the test-tube.
5. Allow approximately 30 minutes for the various color bands to migrate.

Finally, if the students have access to a spectrophotometer they can quantify the amount of chlorophyll-A by determining the optical density of the pigment solution. The reading obtained at a wavelength of 750 mμ (peak of background scatter) is subtracted from that obtained at 670 mμ (peak of chlorophyll-A absorption). Researchers routinely use this value (optical density) when describing the relative pigment quality of water.

Additional Investigations

Once students have mastered the techniques of membrane filtration, they are prepared to conduct a wide range of independent investigations, which need not necessarily be limited to outdoor experiments. For example, by preparing beakers of mixed or pure cultures of algae they can determine how growth rate or species diversity is affected by storing in sunlight or shade and by adding plant nutrients.

Be sure to compensate for any evaporation that might take place by adding distilled water.

If students have access to a pond or stream, they can study the natural succession of algae through the seasons.

These are just a few of the many investigations that are possible with membrane filtration. The range of experiments is limited only by the resourcefulness and imagination of the student and the teacher.

Acknowledgment.—Fig. 1-3 photos are by Fasch Studio, Milton, Mass.; fig. 4 photo is courtesy of Millipore Corp., Bedford, Mass.

REFERENCES

AMERICAN PUBLIC HEALTH ASSN., *et al.* 1965. *Standard methods for the examination of water and wastewater,* 13th ed. American Public Health Assn., New York.

ANON. 1970. *Analyzing air and water pollution with millipore filters.* Publication ANE01, Millipore Corp., Bedford, Mass.

CREITZ, G. I., and F. A. RICHARDS. 1955. The estimation and characterization of plankton populations by pigment analysis, III: a note on the use of millipore membrane filters in the estimation of plankton pigments. *Journal of Marine Research* 14 (3): 211-216.

CHRISTENSEN, C. L. 1971. Diatoms, the neglected organisms. *American Biology Teacher* 33 (2): 98-99.

FRASER, W. C., and R. HULL, 1971. A habitat chart and key to some common fresh water algae. *Science Teacher* 38 (2): 28-32.

GOLDBERG, E. D., M. BAKER, and D. L. FOX. 1952. Microfiltration in oceanographic research, I: Marine sampling with the molecular filter. *Journal of Marine Research* 11: 194-204.

HERSHEY, J. T., *et al.* 1970. *A curriculum activities guide to water pollution and environmental studies.* Federal Water Quality Administration, U.S. Department of the Interior. Commercially available from Institute for Environmental Studies, Cleveland.

MCNABB, C. D. 1960. Enumeration of freshwater phytoplankton concentrated on the membrane filter. *Limnology and Oceanography* 5 (1): 57-61.

PALMER, C. M. 1959. *Algae in water supplies.* Public Health Service Publication No. 657, U.S. Department of Health, Education, and Welfare. U.S. Government Printing Office, Washington, D.C. (Price $1.00.)

SOHN, B. I. 1969. Membrane microfiltration for the science lab. *Science Teacher* 36 (8): 48-51.

Standard methods for the examination of water and wastewater, 13th ed. 1970. American Public Health Association.

STONG, C. L. 1971. The amateur scientist. *Scientific American* 224 (2): 118-122.