NOTES AND COMMENT

Method of Concentrating Phytoplankton Samples Using Membrane Filters

During studies of both lake and stream phytoplankton (Clark 1958, Clark and Sigler 1961) a phytoplankton separation method utilizing molecular filters was developed which may be of general interest.

Although molecular filters have had limited use for many years (Riley 1939, 1940; Cole and Knight-Jones 1949), widespread use has come only with commercial availability following World War II.

Recent applications of molecular filters to phytoplankton work (other than pigment analysis) have usually involved fixing the organisms and clearing and mounting the filter, a process which is useful but time consuming, and does not permit examination of the living organisms (Goldberg, *et al.* 1952; McNabb 1960). The present method accomplishes a considerable saving in separation time under many conditions and permits recovery of the living organisms from the filter for examination or for enumeration in a counting chamber.

The method of Cole and Knight-Jones (1949), in which the organisms are recovered from the filter surface by pipette, permits the recovery of living cells, but several trials in the present studies failed to develop a pipette method which was quantitative. As finally developed the present method involves:

1. Vacuum filtration of the water sample with vacuum released just as the last water passes through the membrane.
2. Cupping the filter either in the hand or in a special holder depending upon the type of filter used.
3. Washing the organisms from the filter into a petri dish with a compressed air atomizer and controlled water flow.

Several forms of hand atomizers were tried; none was satisfactory. The atomizer must deliver a fairly fine spray with considerable force.

The atomizer used in the present study was assembled by attaching a battery syringe bulb (Sears-Roebuck) to a Perkin Elmer Flame Photometer funnel (Fig. 1).

With spray intensity regulated by varying the distance from the filter, and with water supply controlled by pressure on the bulb, a little practice permitted recovery of the cells with a minimum of water used, usually 5–15 ml.

For some microplankton populations a brush-down with a small stiff camels hair brush followed by a second wash with the atomizer was necessary.

Filter holders for molecular filters are commercially available. However, the holder for the present studies was constructed locally for less than $15.00 from plastic and copper and functioned very satisfactorily (Fig. 2). After about 3 years the gasket seal compound between the copper and the plastic dried out and permitted some loss of suction. It would be more satisfactory if the lower section were constructed entirely of plastic.

In operation the filter is placed between the two sections and the clamps applied. The filter is supported below by a fritted glass disc set in the lower piece of plastic. Construction details will be supplied by the authors if desired.

Vacuum was applied in the laboratory by water aspirator and by electric vacuum pressure pump. The latter had the advantage of concurrently supplying the compressed air for the atomizer. On several occasions samples were filtered in the field using the windshield vacuum line from a car motor. The filters from field collections were placed in petri dishes and washed in the lab within an hour with no obvious damage to the river plankton sampled. This method was not tried with the more delicate lake plankton.

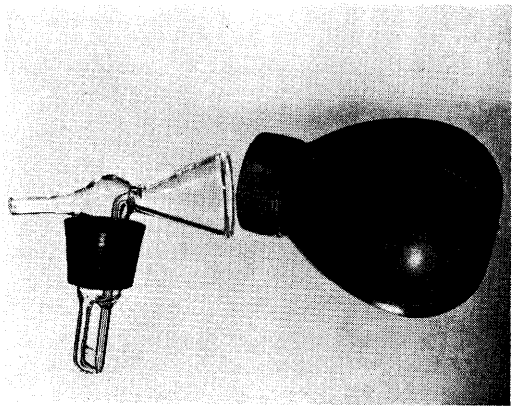

FIG. 1. Pressure atomizer.

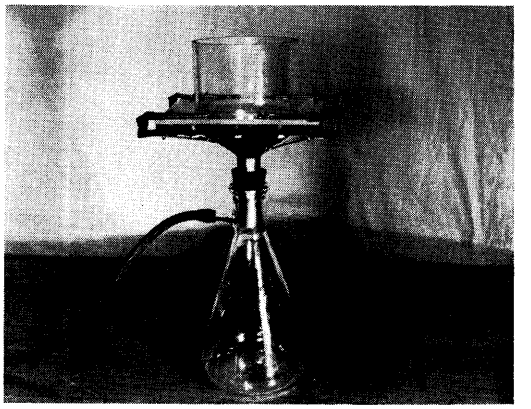

FIG. 2. Holder for molecular filters.

Since clogging is an obvious hazard, filters 150 mm in diameter were used to give what was considered to be the largest practical filtering area commensurate with filter cost.

Filters from two sources were used: Millipore Filter Corp.® (MF) Type RA, average pore size 1.2 μ; and Sleicher and Schuell (S & S) Membrane filters, coarse, coated, average pore size 0.5 μ.

Each filter has its advantages and disadvantages. The MF filter is extremely fast, passing a liter of clear water in less than 5 sec. Three liter samples with turbidities up to 15 ppm passed in less than 2 min. The MF filters are very fragile however because of the large pore size and high porosity. They cannot be cupped in the hand for washing but require a special holder. The holder was constructed by forming two sheets of plastic into tapered nesting troughs. A circle one-half inch smaller in diameter than the filters was cut from the upper piece before forming the trough, with the edge of the circle just touching the edge of the plastic. In operation the MF filter is placed on the lower trough, and the upper piece placed over the filter so that the edges of the filter are held down. The organisms can then be washed from the filter through the opening at the edge. Even with the special holder an MF filter rarely lasted for more than 3 samples.

The S & S filters are slower than the MF filters, because of smaller pore size and apparently lower porosity. One liter of clear water passed through the S & S filters in about 20 sec. In a typical series of separations with an S & S filter the first 3-L sample passed in 5 min, the sixth sample required 14 min. Turbidities were under 2 ppm.

The coated S & S filters consist of a standard membrane filter about 100 μ thick and bonded to a fairly stiff backing. The coated filters are very rugged and can stand repeated handling. They can be cupped easily in the hand for washing. In practice a filter can be used until clogging reduces the flow rate to an undesirable level.

The clogging point is reached suddenly with both makes of filters, and once reached, filtration becomes very slow, taking up to 30 min for the last liter of a very turbid 3-L sample. In general it was not practical to filter samples with a turbidity of over 3–4 ppm with S & S filters, or over 20 ppm with the MF filters. Both companies have recently announced filters with pore sizes up to 5 μ. If the phytoplankton population does not contain a significant number of very small organisms the larger porosities would reduce the turbidity problem.

Efficiency in recovery of organisms from the filter surface was tested in two ways: by repeated washings and by examination of the filter surface. For the lake population of micropankton a second wash of the filter surface resulted in recovery of 0.5 to

2% of the number given by the first wash when good technique was used, and rarely over 5% in any event. A third wash rarely gave more than an occasional cell. For this same microplankton population a sample run in a Foerst centrifuge at 10 min/L and 15,000 rpm and then re-run at 15 min/L had from 20 to 50% spill-over into the re-run.

On a comparison between centrifuge (10 min/L) and filtration, using aliquots from the same sample of lake water, the population estimate from the centrifuged sample was 50% of the estimate from the filtered sample. Precision of both counts was on the order of ± 10 to 15%, at the 95% level.

For the mountain stream phytoplankton population, which was composed of larger heavier cells, predominantly diatoms, one washing with the atomizer removed essentially all recoverable cells. Rarely were even single cells seen in a second wash.

Several of the uncoated filters were cleared with immersion oil after washing, and areas examined microscopically. Only an occasional cell was found on the filter surface.

Diatom chains and aggregates such as *Anacystis* occurred regularly in the concentrates, indicating that mechanical damage during recovery was not excessive.

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