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UNITED STATES DEPARTMENT OF THE INTERIOR
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TECHNIQUES USED FOR THE CLEANING, CONCENTRATION AND OBSERVATION OF CHRYSOMONAD CYSTS FROM SEDIMENTS

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This report is preliminary and has not been edited or reviewed for conformity with U. S. Geological Survey standards or nomenclature

This report documents the techniques that we use to clean and concentrate chrysomonad cysts prior to examining them under a scanning electron microscope. Like diatoms, chrysomonad cysts are composed of opaline silica; the method we use is basically the same as one of the methods commonly used to prepare diatoms.

A small amount of sediment is first suspended in distilled water; it may be boiled if necessary to help disaggregate it. The sample is then passed through a 325-mesh sieve (43 micrometer openings) to remove coarse material. The material that passes through the screen is then transferred to a 250-ml Erlenmeyer flask, which is then filled with distilled water, stoppered, and allowed to settle for 24 hours. After 24 hours, the water is siphoned off, retaining the sample in the flask. If the water was cloudy, the water rinse is repeated until the water is clear after 24 hours; this process removes clay particles and colloids from the sample.

We have found it helpful to make a rigid siphon tube from glass tubing, with the short arm of the siphon just long enough to reach to the bottom of the Erlenmeyer flasks without sucking up the sediment from the bottom. The use of such a siphon minimizes the loss of sample material during the siphoning.

After the clear water has been siphoned off, about 50 ml of 30% hydrogen peroxide is added to the sample in a fume hood. The sample is then allowed to stand, unheated, for at least several hours. The reaction between the peroxide and the sample is exothermic and rather unpredictable; some samples react quite violently, and on occasion the violent boiling of the sample will begin quite some time after the peroxide has been added. After the initial reaction has ceased, the sample is gently boiled for 2-3 hours in the peroxide and allowed to cool. It is sometimes necessary to treat samples with peroxide before they will disaggregate enough to pass through the screen, particularly highly organic samples that have dried out

When the sample has cooled completely, potassium permanganate crystals are added very slowly and carefully to the peroxide (Van der Werff, 1965). We use crystals that pass through a #20 sieve (0.85 mm openings) but not through a #25 sieve (0.71 mm openings), and add them one at a time using tweezers. The reaction is a violent one, and must be done in a fume hood.

When the reaction between the peroxide and the permanganate is complete, crystalline oxalic acid is added to the sample until the pink permanganate color disappears.

The permanganate and oxalic acid are next removed

from the sample by serial dilutions with distilled water, allowing the sample to stand for 24 hours between siphonings. At least 3 dilutions are done, and the process is continued until the water is not cloudy after standing for 24 hours.

After the final siphoning, the sample is transferred to a glass vial and allowed to settle overnight. The water in the vial is then drawn off with a dropper and the vial is half filled with undenatured ethanol, which is required to keep bacteria from growing in the sample.

It is possible to concentrate the smaller cysts still further by passing the sample through a very fine sieve (10 micrometer openings), but we have not yet determined whether this results in the loss of the larger cysts.

Permanent microscope slides are prepared by placing several drops of water on a cover slip, adding a drop of the cleaned sample and allowing the suspension to evaporate dry. When the sample has dried out, a drop of Hyrax (1) mounting medium is placed on a clean microscope slide, and the cover slip is inverted and placed on top of the Hyrax. The entire slide is then placed on a hot plate and heated to about 100°C. The Hyrax melts, and the toluene that it contains boils away from beneath the cover slip Any residual bubbles under the cover slip disappear when the slide cools; the slide is only kept on the hot plate for about 30 seconds.

Samples for the scanning electron microscope are mounted on stubs to which round glass cover slips have been affixed with Lakeside. A few drops of water are placed on top of the stub so that the water covers most or all of the cover slip, and a drop of the cleaned sample is then added. The mixing of the alcohol in the sample with the water on the cover slip causes thorough mixing of the particles and distributes them evenly. The sample is then allowed to evaporate dry.

It is essential for both the microscope slides and the SEM stubs that not too much material be mounted together, and that the drying of the sample onto the glass surface be allowed to proceed slowly. When cover slips or stubs are heated to hurry things along, convection cells in the liquid cause the particles to bunch together, which hinders observation of the cysts.

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Any use of trade names is for descriptive purposes only, and does not constitute endorsement of these products by the U. S. Geological Survey.

We have found that the amount of sample added to the drop of water on the cover slip should be very small, so that it is very difficult to see that the water has anything in it. If the suspension of particles is visible before the drop evaporates, then the dried residue will be too dense for clear observations.

SEM samples are coated with a thin conductive layer of gold-palladium in a sputter coater, and are examined at an accelerating voltage of 30 KV and a working distance of 8-10 mm on a Cambridge S-180 scanning electron microscope. Searching for cysts is done at magnifications of 300x to 1000x; magnifications for photographs are chosen so that the image of the cyst nearly fills a 4"x5" negative, and generally range between 3500x and 25000x.

Whenever possible, the cysts are photographed with the pore showing, but we have no way to turn over a cyst that has settled onto the stub with the pore down. When a cyst must be photographed with some debris obscuring parts of it, we have elected to present the photograph complete, rather than cutting away the debris.

Our plates have been prepared by cutting out the photographs of cysts and mounting them on black poster board using rubber cement. Scale bars from the micrographs are then mounted beneath each cyst.

REFERENCES CITED

Van Der Werff, A., 1955, A new method for concentration and cleaning diatoms and other organisms: Proceedings of the International Association of Theoretical and Applied Limnology, vol. 12, p. 276-277.