Microalgae preparation for scanning electron microscopy: Dehydration

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GENERAL RECOMMENDATION:

Carefully follow safety procedures when working with fixatives!

If seawater is required to dilute fixatives, use sterile seawater, filtered over 0.22 µm filtration unit.

Algae: fixed natural sample, net sample or culture sample of all phytoplankton taxa

Apparatus:
sputter system, critical point dryer, tweezers

Solutions: series of alcohols (25%, 50%, 75%, 95%, 100% v:v), distilled water.

Plasticware: 10 ml syringes, Sweenex, filter nucleopore 25 mm in diameter, silicone seals

Sample fixation

Natural phytoplankton samples are usually fixed with neutralised formaldehyde. Formaldehyde is sold as a 37% solution (formol) and it has to be neutralised before use. Sodium carbonate (to be added in excess to the solution) or esamethylentetrammyne (10 g/1liter w:v) can be used to neutralise. The fixative must be filtered on blotting paper before use.

NB: prepare the fixative solutions under a flow-hood!

For culture material, or when peculiar species are present in the natural samples, more appropriate fixatives can be chosen:

Thecate dinoflagellates with strong thecal plates and diatoms are properly fixed with formaldehyde at a final concentration of 1.6% (equivalent to a 4% concentration of formol).

For thin-walled species, better results can be obtained with glutaraldehyde (final concentration of 1-2%), storing the fixed sample in the refrigerator overnight or, better for several days.

Naked dinoflagellates and flagellates are better fixed with osmium tetroxide (OsO_4). A 10% working solution of osmium tetroxide in distilled water can be used. Samples are fixed for 10-30 minutes with a final concentration of 1-2% of osmium tetroxide in seawater. Better results are obtained placing the tubes on ice during fixation.

Remember that osmium is highly toxic: work under the flow-hood and use proper protection (plastic gloves, mask and glasses). Carefully follow safety procedures and put the waste containing osmium in appropriate containers for toxic waste.

Additional information:

Hasle, G. R. (1978) – Diatoms. In Sournia A. (Ed.), Phytoplankton Manual. UNESCO Monograph on Oceanography Methodology, No 6, Paris: 136-142.

Hasle G. R. & Syvertsen E. E.(1997) – Marine Diatoms. In: Tomas C.R. (ed.), Identifying Marine Diatoms and Dinoflagellates, Academic Press, San Diego: 5-385

ASSEMBLE
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1. Set up a filtration apparatus consists of a 10 ml syringe connected to small opaque plastic containers (Swinnex), which was previously mounted a filter nucleopore 25 mm in diameter (with pores of 3 or more μm).
2. Filter 200-500 μl of culture or more if use natural sample by applying a light pressure on the plunger of the syringe, or simply by gravity to avoid damaging the cells.
3. Add a second filter nucleopore, separated from the first by a silicone seal
4. Wash 3 times in distilled water to remove the salt.
5. Dehydrate the samples through passages in a series of alcohols in increasing concentration (25%, 50%, 75%, 95%, 100% v:v).
6. Each dehydration step should last ca. 10 minutes.
7. The filter holder containing the dehydrated material can be stored for a few days in absolute ethanol. If you cannot finish the dehydration process it is possible to store the sample in the Swinnex covered by alcohol at 95% overnight.
8. The dried material undergoes the process of critical point (Critical Point Dry = CPD, critical point drying). In this way the ethanol is replaced with liquid CO_2 under controlled pressure and temperature conditions. When the pressure is reduced, CO_2 evaporates without causing surface tension forces on the cell surface. The sample is then dry and in a gaseous atmosphere and may be treated under atmospheric air condition. At this step, the filter with the sample can be taken out from the Swinnex and glued on the stub.
9. Before SEM examination, cells are coated with a metal layer in a sputter system. Gold, gold-palladium or platinum can be used.

Notes:

This method offers the advantages of "gently" rinsing and dehydrating the material without damaging the cells (e.g. by centrifugation); moreover, the filter holder can be directly placed in the critical point drying apparatus after the dehydration, thus preventing losses of material.

When dehydrating material fixed with osmium, it is better to carry out the first steps of dehydration by centrifugation. This allows washing the material from osmium, before placing it on the filter for the last steps of dehydration.