

The Laboratory Culture of Diatoms for Class Use

G. C. MARKS and W. W. BLOOM

Department of Biology

Valparaiso University, Valparaiso, Indiana 46383

Abstract

The study of living diatoms by beginning biology students has sometimes been difficult because relatively pure cultures of this organism have not been readily available. We have succeeded in isolating and culturing diatoms on a simple agar medium.

To the devoted general biologist an introductory course in biology cannot be considered complete without exposing the student to some meaningful experience with living diatoms. In many preparations the small size of the organism as well as their slow and erratic movements allow all but the most careful observer to overlook them. Until recently pure cultures were not available, and those obtained from supply houses tended to be little better than those that are collected from local standing or running water. Since too many organisms (or debris) are included in most cultures, we searched for a practical culture method for use in providing reasonably pure cultures of diatoms for our introductory courses.

A few earlier attempts to propagate a mixture of species in soil water ended in moderate success. Aware that some motile organisms are easily isolated and cultured on agar (*i.e.*, *Chlamydomonas*, *Amoeba*, Nematodes), we decided to try the following approach.

Stream water, moderately rich in diatoms, was applied as a thick streak on the surface of Bold's Basic Medium Agar (1) (BBMA) in glass Petri dishes. These plates and all those that followed were placed in a B.O.D. Box and incubated at 15°C under 16 watt cool white florescent tubes with 16 hours of light and 8 hours of darkness. In 3-4 weeks comparatively pure masses of dividing diatoms could be found among other algal clones. These diatoms had migrated away from the streak. It was then relatively easy with a sterile microscalpel or inoculating loop to transfer some individuals to a fresh plate. Again in a few weeks nearly pure clones of diatoms could be seen with the unaided eye. Microscopic examination of the plate revealed these organisms radiating out from the sites of inoculation.

Transfer of these organisms to a blank slide and subsequent microscopic examination were disappointing. They were difficult to remove, lacked the characteristic golden-brown color, and in many cases, the rigidity of the cell was lost. Those that retained their typical form did not exhibit motility. At this point a little reflection produced an awareness that although BBMA was fairly complete for mineral nutrient requirements for many algae, it is devoid of silicon essential for the formation of diatom cell walls. The previous luxuriant growth could presumably be explained by the probable inclusion of necessary silica in the original inoculum. Transfer to a second culture dish resulted in diminishing the silicon content of the culture below minimal requirements for sustained healthy formation and growth of cell walls.

It seems probable, that other nutritional requirements were met as the protoplasmic contents prospered. The silicon deficiency was easily overcome by adding 5 ml of 2% silical gel to 1 liter of BBM. This adjustment of the medium seemed to promote a rise in the rate of cell division. Comparable populations in the silica gel medium were attained in less than 2 weeks whereas it had taken almost 3 weeks in the agar medium lacking the silica gel.

The organisms were easily removed by flooding the plate with deionized water but once again we experienced disappointment because of the subsequent loss of motility. Close microscopic examination revealed the cell contents were disturbed in a manner similar to plasmolysis. Further experimentation quickly indicated that we had created an osmotic gradient too steep for the organism to overcome without trauma. A substitution of soil water for the deionized water in the removal of the organisms from the agar of the culture dish to the water of the observation slide quickly circumvented this final pitfall and eventually, healthy motile organisms could be transferred from the culture medium to the observation slides in nearly pure culture. Most organisms not eliminated by the above procedures and all less prominent than the diatoms, were minute flagellates and large bacteria. For teaching purposes we do not feel a single species culture is necessary.

We have had occasion to use diatom plates about 12 weeks old. The organisms when washed off with soil water had lost none of their vigor and made excellent classroom material. We are currently trying to isolate larger and less motile species by flooding plates with a thin film of pond water and transferring clones to fresh BBMA dishes.

Literature Cited

1. BOLD, HAROLD C. 1967. A laboratory manual for plant morphology. Harper and Row, New York, N.Y. 123 p.

