Effect of Growth Environment on Ultra-structure of a non-photosynthetic Diatom *Nitzschia alba*

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Introduction

Diatoms are microscopic unicellular algae with rigid cell walls (frustules) composed of silica. Found both in marine and freshwater habitats, the microorganisms are usually photosynthetic and pigmented; although the genera *Nitzschia* can live heterotrophically in the dark if supplied with a suitable organic carbon source.[1] The frustule consists of two valves that fit together like a petri dish. Each half of the frustule consists of a valve (the large outer surface) and a girdle (the circular band of silica attached to the edge of the valve). Diatom frustules also contain pores of nanometer scale that are uniform in dimension and species specific. Diatom frustules exhibit diversity in structure and morphology, which attracts the attention of materials scientists for its nanotechnology applications.

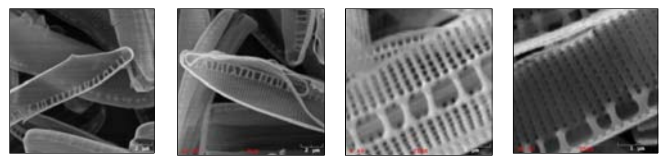
The diatom species *Nitzschia alba* was used in this study due to its ability to grow at room temperature, a characteristic that would allow for mass production without the space constraints of a cold chamber. Frustule formation has been shown to be affected by factors such as pH and temperature, which might explain changes in frustule formation when a single diatom species is grown under varying conditions.[2] The purpose of the present investigation was to compare and contrast the ultra-structure of the diatom species *N. alba* under varying growth conditions.

Procedure

N. alba was cultivated in an artificial saltwater (ASW) medium with the addition of Tryptone (1g/L). The cultures were grown at 16°C in a cold chamber, and at 26°C at room temperature. To isolate diatom cell walls, cells were harvested and centrifuged into two 50 ml test tubes. Upon final cycle, the ASW medium was decanted, and the cells were suspended in 5 ml of distilled water and 20 ml of nitric acid was added to each test tube, to ensure removal of organic material. The test tubes were boiled in a water bath for 30 minutes, and centrifuged once more for 10 minutes at 9000 rpm. The nitric acid was decanted; all cell wall contents were placed within one test tube and washed once more with distilled water. The *N. alba* cell walls were pipetted into eppendorf tubes, and centrifuged again. The eppendorf tubes were placed in an oven to dry the cell walls. Cell wall samples were prepared by suspending a small amount of the cell walls in 80 µl of methanol; 20 µl of the suspended substance was pipetted onto an aluminum stub and dried in an oven. Samples were coated in gold for examination by Scanning Electron Microscope (SEM).

Results and Discussion

There was a noticeably larger amount of diatoms grown in the refrigerator sample than in the room temperature sample; the refrigerated culture weighed 23.93 mg, the room temperature sample weighed 0.29 mg. (Both *N. alba* cultures were concurrently initiated and terminated.) The cells of both cultures were straight and needle-like in structure (Fig. 1a and 1b), with valves that appear to be straight. While not uniform in size, there was little difference between the morphology of the valves from both cultures. Pore size was also relatively similar between the two cultures (Fig. 1c and 1d), which suggests that no morphological changes occurred due to a change in growth environment.



(a) (b) (c) (d)

Figure 1. *Nitzschia alba* frustules from cultures grown at: **(a)** 16°C (cold chamber) **(b)** 26°C (room temperature) **(c)** 16°C **(d)** 26°C

Conclusion

There is little to no morphological discrepancies on the atomic level between the *N. alba* cultures that were grown at room temperature and those that were grown in the refrigerator. However, it was found that *N. alba* is more successfully grow at lower temperatures, similar to that of a refrigerator, than that of room temperature, as reported in the literature.

References

1. F.E. Round, R.M. Crawford, D.G. Mann; “The Diatoms: Biology & Morphology of the Genera,” Cambridge University Press, 1990.
2. J. Parkinson, R. Gordon; “Beyond micromachining: the potential of diatoms,” Tibtech, 1999.