Analysis of the Effects of Changing Nutrient Status on Microalgae Using FT-IR Spectroscopy

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The effects of nutrients on algae can have environmentally important consequences: such as the initiation of algal blooms by nutrient-excess or the limitation of oceanic primary productivity by nutrient-deficiency. Algae react to changes in nutrient status by redistributing resources so that the reproductive and growth potentials are affected as little as possible, often resulting in a massive re-organisation of cellular components (Geider& Osborne 1989). The study of the changes in the pools of macromolecules in algal cells, especially in relation to each other, is therefore essential for understanding the response of these organisms to alterations in environmental conditions. Unfortunately, most of the methods used for the assessment of the size of cellular pools of macromolecules and of their variations are invasive and require the disruption of the cell itself. This of course introduces both large experimental errors and major perturbations of the system. Also, work of this kind is technically challenging and time-consuming, and often requires large numbers of cells, which are not always available in phytoplankton populations.

We have demonstrated, by comparing chemical measurements with the relative intensity of spectral bands attributed to protein, silica, and lipids, that FT-IR is a reliable way to accurately measure the relative concentration of these classes of compounds in the marine diatom *Chaetoceros muelleri*, under conditions of nitrogen limitation (Giordano et al., submitted). FT-IR has the advantage over conventional methods in that it minimises the disturbance of the intracellular environment by reducing the manipulation of the sample and, consequently, the introduction of experimental artefacts. We have conducted similar experiments with a number of freshwater and marine species under phosphorous limitation (Beardall et al., 2000), that indicate FT-IR can be applied as an analytical tool for the determination of macromolecular composition in a wide range of phytoplankton species. Moreover, have also demonstrated that, FT-IR spectroscopy combined with microscopy permits examination of the larger individual cells (>20μm) in a population, obviating interference from contamination by bacteria and other organisms, as well as allowing intercellular variability in molecular composition to be quantified. In this area we have been successful in recording high quality FT-IR spectra of single cells of *Scenedesmus* in work done with the co-operation of Bruker Optik at the GAP 99 workshop in Switzerland (Beardall et al., 2000). We are continuing with this work, growing *Scenedesmus* cultures under varying levels of phosphorus limitation and have begun to compare spectra, recorded from single colonies and masses of cells, with conventional chemical analyses. Furthermore, we are attempting to record FT-IR spectral images of *Scenedesmus* colonies using a focal-plane array detector. This approach may allow differences in intra and inter-colonial levels of macromolecular components to be detected and localised.

References

Beardall J, Berman T, Heraud P, Kadiri M, Light B, Mertens F, Patterson G, Roberts S, Sulzberger B, Sahan E, Urlinger U, Wood B (2000) Aquatic Sciences. Accepted.

Geider RJ, Osborne BA (1989) New Phytologist 112: 327-394.

Giordano M, Kansiz M, Heraud P, McNaughton D, Beardall J unpublished, submitted to Journal of Phycology.