A CONTRIBUTION TO THE ANALYTICAL DETERMINATION OF PHOTOSYNTHETIC PIGMENTS OF MARINE PHYTOPLANKTON

Nenad SMODLAKA[1] and Sergije KVEDER[2]
(1) Center for Marine Research, "Rudjer Bošković" Institute Rovinj, Yugoslavia
(2) Organic Chemistry and Biochemistry Deprtment, "Rudjer Bošković" Institute, Zagreb, Yugoslavia

SUMMARY

A fluorimetric method for simultaneous determination of chlorophylls and phaeophytins a and c is presented. The method is based on the measurement of the changes in the fluorescence of the pigments in solution, before and after acidification, at two wavelenghts ranges (620 and 650 nm, respectively).

RÉSUMÉ

Une méthod pour la détermination de la chlorophylle et phéophytine a et c est élaborée. La méthod est basée sur la mesure des changes de la fluorescence des pigments avant et apres l'acidification de la solution, a deux rangées de lonueur de vague (620 et 650 nm, respectivement).

INTRODUCTION

Today chlorophyll a standing crop is commonly used as a direct index of phytoplankton biomass. However, chlorophyll c is also present in the phytoplankton and in some species chlorophyll b. As the chlorophyll c is more stable *in vitro* than chlorophyll a it can be "measured" in nature in greater quantities than chlorophyll a, even though no algae is known which has a higher content of chlorophyll c than chlorophyll a (Jeffrey, 1974). A simple and fast method for chlorophyll c determination is needed because of its importance in phytoplankton photosynthesis.

MATERIALS AND METHODS

Chlorophylls used for instrument (fluorometer "Turner" 111) calibration were isolated from benthic algae and purified by thin-layer chromatography on silicagel HF plates. Concentrations were determined spectrophotometrically.

RESULTS AND DISCUSSION

The revised fluorimetric method for simultaneously determinating chlorophylls and phaeophytins a and c is based on the measurement of changes in the fluorescence of the pigments in solution before and after acidification at two wavelenght ranges (620 and 650 nm, respectively). To calibrate the instrument it is necessary to establish fluorescence per unit concentration. The general equation (1) represents the fluorescence (F) of a mixture of chlorophylls a and c, phaeophytins a and c:

$$F = aCa + bCb + paPa + pcPc \qquad (1)$$

where a, c, pa, pc are fluorescence per unit concentration of each component, and Ca, Cb, Pa, Pc their concentrations.

Using two filters and measuring the fluorescence before and after acidification with 0.1 N HCl allows equation (1) to be developed into 4 equations with 4 unknowns.

Substracting the fluorescence before and after acidification results in two equations with two unknowns. These can be solved as in equation (2):

$$Ca = \frac{\Delta F_2 \ (c_1 - pc_1) - \Delta F_1 \ (c_2 - pc_2)}{(a_2 - Pa_2) \ (c_1 - pc_1) - (a_1 - pa_1) \ (c_2 - pc_2)} \qquad (2)$$

Similar equation can be developed for chlorophyll c and for phaeophytin a and c.

Mathematically, it can be proven that the presence of chlorophyll b, in the concentrations normally encountered in the sea, would significantly interfere only with the determination of the phaeopigments. When this method was used for field determinations of phytoplankton chlorophylls a and c, the results obtained were in general satisfactory. Negative values for chlorophyll c were obtained in some instances, and may indicate the presence of some so far unidentified fluorecing substances.

References

JEFFREY, S.W., 1974. - Profiles of Photosynthetic Pigments in the Ocean Using Thin-Layer Chromatography. Mar. Biol. 26, 101-110.

DISCUSSION

Questions and comments:

1. What kind of new information we can add by measuring chlorophyll c ? (R. FUKAI, Monaco)

- Measured chlorophyll c values could be information on the stage of the natural phytoplankton population. Because of the increased stability of chlorophyll c comparing to chlorophyll a , ratio of chl a and chl c could be indication of the growing or declining phase of the phytoplankton population.

2. About the errors of determinations you said that they are within 10-15%. I think for real samples the errors may be much greter due to the accumulation of errors in each step of determinations. (R. FUKAI, Monaco)

- Errors of determinations within 10-15% are only for chlorophyll a real samples. The error is estimated relative to spectrophotometric method (Strickland and Parsons). For chlorophyll c errors are somewhat higher comparing to the same spectrophotometric method.