FLUORESCENCE OF AGEING EXTRACELLULAR PRODUCTS OF *SKELETONEMA COSTATUM*

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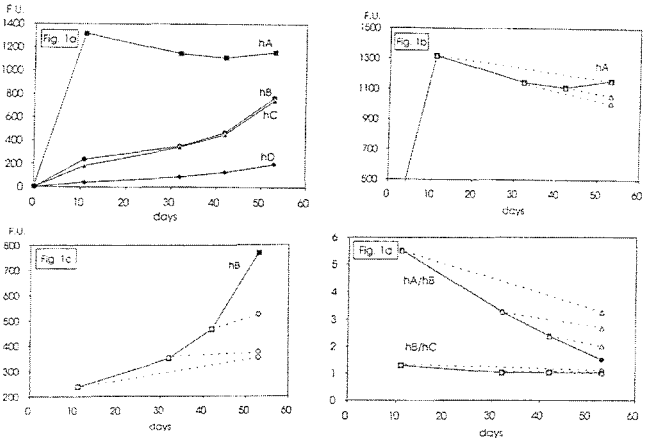
The extracellular organic matter (EOM) released in dissolved state in the water by phytoplankton was characterized using the synchronous fluorescence spectroscopy. This technique, due to its high sensitivity and selectivity, allows the spectral resolution of different compounds present in multicomponent mixtures of dissolved organic matter (VO-DINH, 1978; CABANISS and SHUMAN, 1987). The aim was to verify if quali-quantitative variations observed on the ageing EOM produced by algal cultures were related to changes in algal production rather than to chemical transformations of the released products. *Skeletonem costatum*, isolated from Adriatic Sea, was cultured in laboratory (EPA, 1974). The EOM produced was analyzed during a 53 days experiment, using a Spex-FluoroMax fluorimeter, scanning synchronously a wavelength range from 250 to 500 nm, with constant Δwl (25 nm) between ex and em monochromators. Samples were taken from the *S. costatum* culture at 11, 32, 42, 53 days of growth and filtered (0.45 µm). The filtered medium, containing the dissolved EOM, was kept ageing in the same light and temperature conditions of the producer culture. All samples were analyzed at the sampling time and at the 53rd day. The fluorimetric analysis provided spectra characterized by a main peak (A) at an excitation wavelength of 276 nm and a series of secondary peaks (B, C, D) located between 330 and 430 nm. Spectra of the differently aged culture-EOM (C-EOM) showed quantitative variations of the different components produced, as shown in Figure 1a (F.U.= fluorescence units). The fluorescence intensity of the first peak (hA), which reaches high values at the day 11, tends successively to decrease slightly, while the intensity of peaks >300 nm (hB, hC, hD) increase constantly during the 53 days (Fig 1a). The same trend in fluorescence was already described in previous ageing experiments made on a number of different algal species in culture (MINGAZZINI *et al.*, 1994; in press). In those cases, however, it was not clarified if the series of the higher wavelengths peaks, with respect to the first peak, may represent the fluorimetric response of different extracellular compounds produced in stationary growth phase, rather than a chemical transformation of the algal products already present in the medium.

The fluorescence values measured on the ageing filtered medium-EOM (M-EOM) are shown in Figure 1b and 1c. C-EOM and M-EOM are represented by uninterrupted and dotted lines, respectively. The decrease in fluorescence intensity of the first peak (Fig. 1b) is similar or even greater in the M-EOM compared to C-EOM. Conversely, the increase in fluorescence intensity of peaks >300 nm is constantly much lower in M-EOM than in C-EOM, as shown in Fig. 1c for hB. Since in M-EOM the decrease of hA is not accompanied by an increase of hB, hC, hD equal to that observed in C-EOM, the fluorescence enhancement is probably linked to the algal production in the stationary growth phase. The decrease of hA may be the result of slow photodegradation processes (CHEN and BADA, 1992) of products released by the actively growing culture.

The ratios hA/hB and hB/hC, calculated on C-EOM and on M-EOM in the 53-day experiment, are shown in Figure 1d. The ratio between the first two peaks (hA/hB), which decrease in time, was previously proposed (MINGAZZINI *et al.*, 1994; in press) to describe the quali-quantitative variation of the extracellular compounds released during the algal growth phases. The ratios between the last series of peaks (hB/hC in Figure 1d), which tend instead to remain constant in time, were used to describe the spectral features linked to the producer algal species. The comparison of the C-EOM and M-EOM supports the suggestions of MINGAZZINI *et al.*. The hB/hC ratio from both C-EOM and M-EOM remains in fact constant in time, indicating that the extracellular products released in the stationary phase from a monospecific culture do not vary qualitatively, while the decrease of the hA/hB ratio mostly reflects changes in production activity rather than chemical transformations of the released products. The differences observed comparing C-EOM to M-EOM hA/hB (Figure 1d) are in fact mainly related to the missing production in all M-EOM samples (from day 11 to 53), as shown in Figure 1c.



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