Spectrofluorimetric applications for the study of extracellular algal exudates

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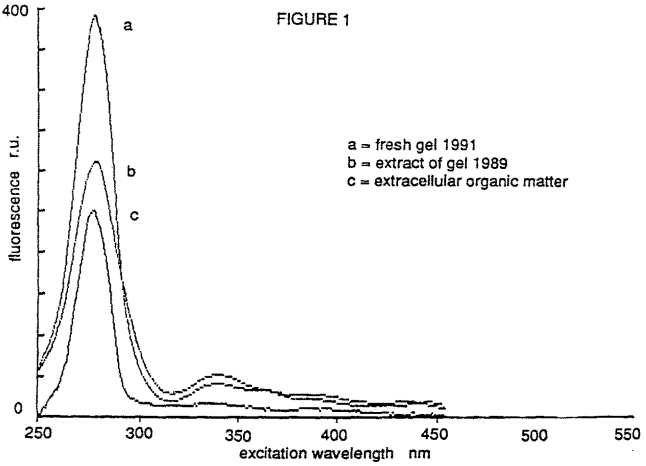
The phenomenon of the appearance of massive quantities of gel aggregates recently affected the Northern Adriatic Sea in the summer seasons 1988-89-91, and covered a surface water area estimated at over 10,000 km2. Such gel formation is attributed to the hyperproduction of extracellular algal exudates (MARCHETTI *et al.*, 1989). The ability of phytoplankton to produce gel-forming material is genetically determined, but its formation in large amounts depends on the physiological state of the cells (FOGG, 1990). The increase in the production of organic extracellular material by planktonic marine diatoms is attributed by some authors to nutritional stress, such as an unbalanced N/P ratio (MYKLESTAD, 1989). However, the environmental factors which regulate the release of these substances have not yet been clearly defined. A need for further investigation aimed at a better understanding of the mechanism of potentially gel-forming exudation is generally recognized. With this aim, in the present study, the use of fluorimetric techniques for the analysis of both the gel material taken in the field and the extracellular substances produced in laboratory cultures was considered. The technique chosen was the synchronous fluorescence spectroscopy, which involves the stepping of both the excitation and emission monochromators of the spectrofluorimeter at the same time, while monitoring the emission, keeping a constant wavelength difference between the monochromators (LLOYD, 1971). This approach, compared to conventional spectrofluorimetry, allows the narrowing of spectral bands and for this reason it is appropriately employed for the analysis of multicomponent mixtures containing fluorescent compounds, and in particular for the determination of dissolved organic matter (VO-DINH, 1978; VODACEK, 1989).

An aliquot of the gel material taken in July 1991 in surface waters offshore from Cesenatico was analyzed following filtration of the sample (0.22 µm). At the same time, a gel sample taken in July 1989 from the same area, using the dried mass on the extracts of which chemical analyses had already been undertaken (MARCHETTI *et al.*, 1989) was also analyzed. This mass was in turn extracted with distilled water for fluorimetric analysis following filtration. The synchronous ex-em fluorescence spectra of both samples are given in Fig.1, and show an evident similarity of the peaks around the wavelength of about 280 nm. Batch laboratory cultures of *Skeletonema costatum*, a marine diatom from the Northern Adriatic coastal waters, were gently filtered and the analyzed suspension medium showed fluorescence in the same region (Fig.1). Furthermore, the spectral responses of various carbohydrate solutions fell precisely in the same region. These results are in agreement with the chemical characterization of both gel (MARCHETTI *et al.*, 1989) and algal exudates (MYKLESTAD, 1989). Although the present study does not take into consideration the individuation of the single exudated compounds, which are species or strain specific (FOGG,1990), the mentioned ex-em spectra reveal the presence of organic extracellular substances potentially capable of forming gels, in dissolved state. Thus, in order to verify the application to the study of the production trend in laboratory algal assays, aliquots of *S.costatum* cultures were taken at regular intervals along the algal growth curves for microscopic cell counts and fluorimetric analysis of the filtered medium. In replicated assays, results indicated an increase of organic matter dissolved in the medium during the log phase of the algal growth, with spectral characteristics identical to those in figure 1.

This series of preliminary verifications suggests that the synchronous fluorescence technique might be applied to study the factors which affect the production of extracellular organic substances in laboratory algal assays. A parallel field investigation monitoring the production trend is in progress. The proposed fluorimetric technique, new in the environmental studies, has important advantages: it is a simple method which can be used on a routine basis, without resorting to techniques which are more expensive and excessively time consuming (VO-DINH, 1978). Further investigations regarding both the quantitative and the analytical aspects, by parallel verifications with chemical analyses, are under way in order to confirm the validity of this method.

FIGURE 1



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