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OPTICS AND SPECTROSCOPY

STORAGE EFFECT ON FLUORESCENCE SIGNAL OF DISSOLVED ORGANIC MATTER COMPONENTS

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Abstract. This article presents a study concerning the storage effect on the fluorescence properties of dissolved organic matter fractions, obtained by ultrafiltration. Protein-like peak, γ, and recent production of organic matter, β, were evidenced through fluorescence spectroscopy in the 0.5 kDa subfractions, while humic substances, α and α', appeared predominantly around 1–3 kDa. Results have shown that storage at 4^0C, in darkness, for three months, did not affect the fluorescence properties of water samples.

Key words: fluorescence, ultrafiltration, dissolved organic matter.

1. INTRODUCTION

Dissolved organic matter (DOM) is directly connected to water quality and has been the subject of many scientific studies in the past decades [1–2]. Amongst these, a special emphasis has been put on DOM characterization through optical investigation methods, such as fluorescence spectroscopy [3–8], which gives a fingerprint of DOM present in water samples. However, sometimes it can be more suitable to study DOM based on its components, limiting overlapping effects in the fluorescence signal. Ultrafiltration (UF) can be employed for the size discrimination of DOM components [3, 4]. It divides the water sample in two fractions: the permeate and the retentate, both containing molecules that are smaller than the pores of the membrane. In addition, the retentate comprises molecules that are larger than the pores of the membrane and can not pass. Ultrafiltration is used in many areas, varying from water and wastewater treatment processes, to the dairy and chemical industry [9–11].

The aim of this study was to investigate the storage effect on the chromophoric dissolved organic matter of a bulk estuarine water sample and its subfractions, obtained by ultrafiltration. For this purpose, ultrafiltration was performed on a water sample, and the fluorescence signals, of the bulk sample and the obtained UF subfractions were measured at the beginning of the experiment and after three months of storage in darkness, at 4°C.

2. EXPERIMENTAL

The working protocol for the UF experiment is presented in Fig. 1. The bulk sample (BS) was ultrafiltered using a Millipore Prep/Scale^{TM} – TFF device equipped with regenerated cellulose membrane of 3 kDa cut off (Millipore membrane cartridge PLBC), resulting in the permeate P3 and retentate R3. Afterwards, P3 was ultrafiltered using a similar device, with 1 kDa cut off membrane (Millipore membrane cartridge PLAC), which gave the permeate P1 and retentate R1. The last step involved ultrafiltration of P1 with an Amicon TCF 10 tangential ultrafiltration device, equipped with a 0.5 kDa cut off membrane disc made of cellulose acetate (Amicon YC05; Millipore), resulting in the permeate P0.5 and retentate R0.5.

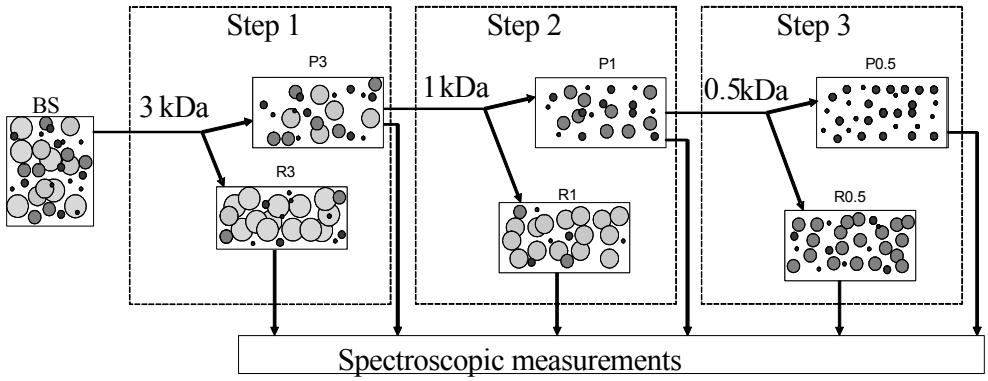

Fig. 1 – Working scheme for the ultrafiltration process.

All samples were analyzed using fluorescence spectroscopy, recorded as excitation-emission matrices (EEMs), with a Jobin-Yvon FL3-22 spectrofluorometer. EEMs were obtained by collecting 17 emission spectra between 260–700 nm, for excitation wavelengths between 250–410 nm, as previously described [12].

3. RESULTS AND DISCUSSION

The DOC analysis revealed variations of the carbon content and the fact that during each fractionation step, the highest amount of carbon remained within the retentate. The total carbon mass was divided unevenly between the subfractions (Fig. 2). Subfractions R3 and R1 contained 16 and 10 %, respectively, of the total carbon mass. The decrease in DOC concentration after the first fractionation step was probably due to the adsorption on the membrane. The molecules with molecular weight between 0.5 and 1 kDa represent the largest part of the total carbon mass, of approximately 43 %, close to that found by [4] for surface estuarine waters. The variation of DOC content can be related with the fact that ultrafiltration membranes are known to retain and afterwards randomly release organic molecules [13, cited by 4].

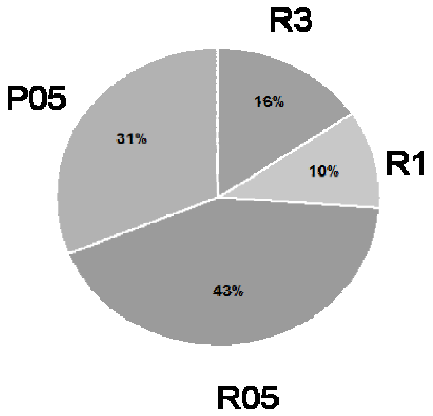

Fig. 2 – Dissolved organic carbon mass balance.

The EEMs of the UF subfractions (R3, R1, R0.5 and P0.5), are presented in Fig. 3, at the same intensity scale. The major chromophoric DOM components are illustrated: α, α' (humic-like molecules), β (attributed to the recent production of organic matter) and γ, representing the protein-like fraction. The mass balance for the entire UF process showed a fluorescence recovery percentage of approximately 78% for α, α' and β fluorophores. The γ component showed higher instability, with a recovery rate of 183 %, much higher than that reported in literature so far [4].

Much higher fluorescence concentration was found for subfraction R05, probably related with its DOC content, as this sample had the highest concentration of carbon as compared with the rest of the subfractions.

The humic-like molecules, α and α', were found in all subfractions. The α components showed maximum fluorescence signal for excitation wavelength of 340 nm, and emission around 434 nm, while the α' molecules had the strongest fluorescence emission around 445 nm, when excited with 250–270 nm. It appears

that for the water sample analyzed here, the molecular size of these fluorophores is around 1–3 kDa. For subfractions R3 and R1, the peaks associated with humic molecules, α and α', are better defined than for samples R05 and P05, suggesting that The P0.5 subfraction showed the lowest concentration of humic substances, which means that most of these molecules have been retained by the larger molecular weight cut-off filters. This is different than the results obtained by [15], which found these molecules between 13 and 150 kDa, and more appropriate with the earlier findings of [16], indicating the presence of humic-like molecules between 0.5–2 kDa.

It can be seen that R0.5 and P0.5 exhibit a more pronounced fluorescence maximum, attributed to the protein-like fraction, γ, around 330-340 nm emission, for excitation wavelength of 280 nm. This confirms that these fluorophores are present in the bulk sample (as a small shoulder) but their signal is overlapped by the other components. After refining the spectra through UF, the fluorescent signal of γ molecules could be detected. This type of molecules have been found between 0.5 and 2 kDa for rural river and urban lake samples [3], between 1 and 5 kDa for estuaries [15] and in the 0.5 kDa fractions for fresh and brackish water samples [4].

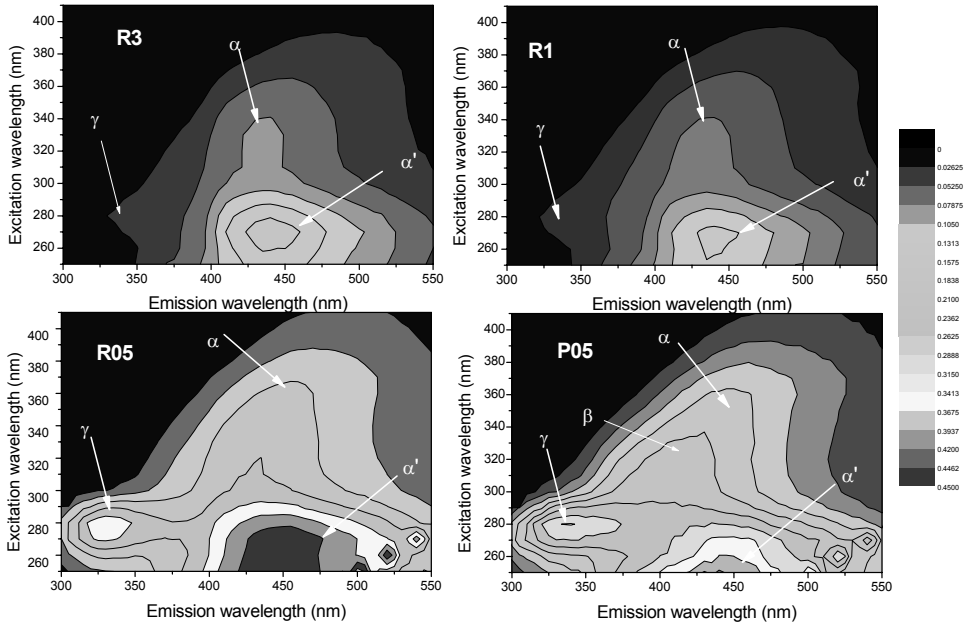

Fig. 3 – Fluorescence EEMs of ultrafiltration subfractions, following the steps in Fig.1.

In the EEM of P0.5 subfraction, another peak can be noticed, at λ_{ex} / λ_{em} = = 315 / 410 nm, corresponding to the recent production of organic matter (β). This means that β is not specific just for marine water samples, as originally discovered

[16], but is also common for brackish waters. Just like γ, it has smaller molecular weight than the other DOM fluorophores and its signal is covered by them.

The broad molecular size domains in which DOM components were reported indicates that they are very heterogeneous, subjected to external factors that can influence their size distribution.

The influence of storage at 4°C in darkness was evaluated by recording the fluorescence response of the bulk sample and its subfractions. As an example, the EEM of the bulk sample, measured at the beginning and three months after the start of the experiment are presented in Fig. 4. Both matrices are characterized by two large humic-like fluorescence bands, one at 260 nm excitation and emission around 420–450 nm and another one, for excitation wavelength of 340 nm and emission between 420–445 nm. A small shoulder can be observed in the emission domain 320–340 nm, with 280 nm excitation wavelength, attributed to the protein-like fraction. No important changes were noticed between the initial measurements and those performed after three months of storage at 4°C in darkness, except for minor variation of the intensity values. This means that even after such a long period, the samples kept their original properties, still being representative for the experiment. This is an important result, because in real practice, it is often not possible to measure samples within a few hours after collection, and storage for a period of time is necessary. Many studies used freezing as storage method [5, 6]. However, it was recently demonstrated by [7] that freezing/thawing is not the most adequate method for storage, as it leads to unpredictable changes of the original properties of water samples. Furthermore, storage at room temperature is not favorable, because it can generate high microbial activity within the water samples [17].

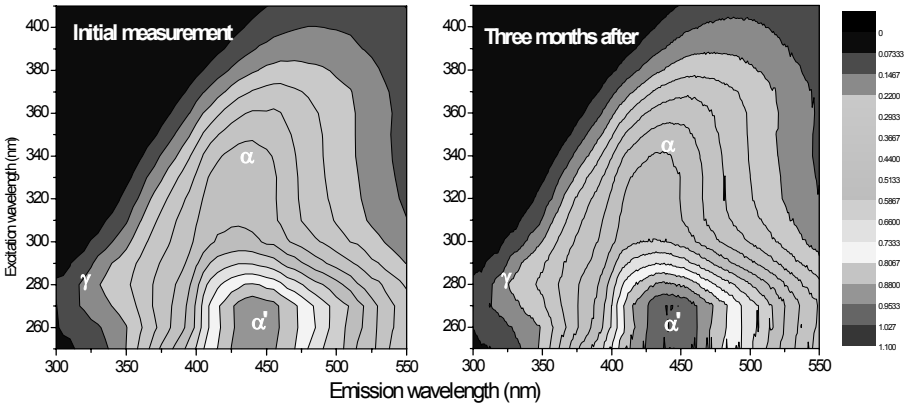

Fig. 4 – Comparison between the initial and the second measurement of the bulk sample.

To better evidence the changes which occur during storage, fluorescence intensity fractions, $I_{\alpha'}/I_{\alpha'}^{0}$, $I_{\alpha}/I_{\alpha}^{0}$, I_{β}/I_{β}^{0} and $I_{\gamma}/I_{\gamma}^{0}$ (Fig. 5), were calculated. The terms $I_{\alpha'}$, I_{α}, I_{β} and I_{γ} represent the fluorophores intensities, measured after storage, and

$I_{\alpha'}^{0}$, I_{α}^{0}, I_{β}^{0} and I_{γ}^{0} are those measured at the beginning of the experiment. In ideal conditions, these ratios should be equal to 1.

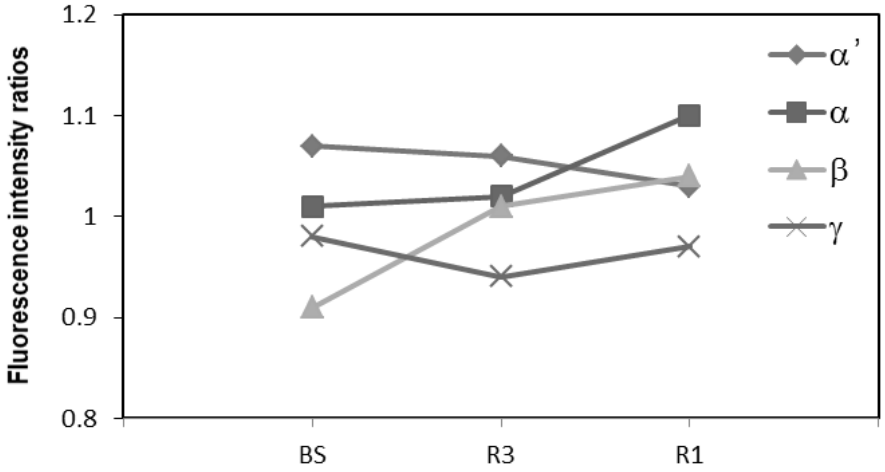

Fig. 5 – Fluorescence intensity ratios of samples measured after three months storage and at the beginning of the experiment.

However, in reality there are small variations due to, either the loss of organic material through sedimentation, or the gain caused by the activity of DOM components. After storage for three months, the fluorescence intensities of DOM components showed minor variations, as compared with the initial measurements, with an average of ± 4 %. This shows that storage at 4ºC, in darkness, for three months, does not affect the fluorescence response, both for bulk and the ultrafiltration subfractions.

4. CONCLUSIONS

The study presented an evaluation of the storage effect on the fluorescence properties of a bulk water sample and its ultrafiltration subfractions. The results show that protein-like molecules, γ, had molecular weight around 0.5 kDa. The contribution from the recent production of organic matter, β, was higher in the 0.5 kDa subfractions. The humic-like molecules, α and α', were mostly retained in the 1 and 3 kDa retentates. No important variations were noticed between the initial fluorescence measurements and those performed after three months of storage, at 4ºC, in darkness, on the bulk sample and the subfractions, except for minor variations of the intensity values, of approximately ± 4%. Thus, this could be the best option for storage of water samples, when measurements can not be performed as soon as possible after collection.

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