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Application News

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Water Quality / RF-6000 with EEM Spectroscopy

Excitation-Emission Matrix (EEM) Fluorescence Spectroscopy for Analysis of Dissolved Organic Matter (DOM) in Natural Water and Wastewaters

❑ Introduction

Dissolved organic matter (DOM) are soluble organic materials in water from various sources such as soils, decomposed plant materials, living organisms and discharges from human activities. DOM is an important natural water quality indicator as it reflects the microbial activities, human activities and geological conditions around the area where the water sample has been collected. Excitation-emission matrix (EEM) fluorescence spectroscopy has been used to characterize the DOM and identify fluorescence emitting organic substances in natural water [1-2]. Here, we describe analysis examples of DOM in natural water and wastewater samples using the 3-dimensional (3D) EEM measurement on the RF-6000 Spectrofluorophotometer.

❑ Experimental

A few organic compounds standards are used for EEM measurement. The compounds, including tryptophan, tyrosine and humic acid, were purchased from Sigma Aldrich, USA. Type E-1 ultra pure water with resistivity of 18 MΩ was used.

The tryptophan and tyrosine were dissolved with ultra pure water to a concentration of 1 mg/L. The humic acid was dissolved with ultra pure water to a concentration of 20 mg/L and adjusted to a pH of 8 to 9 using sodium hydroxide solution. The samples used in this study were surface water from a local pond and wastewater samples from two different industrial sources. The samples were filtered through a 0.2 µm nylon filter prior to measurement.

EEM spectra of the samples were measured using the 3D spectrum mode in LabSolutions RF workstation on RF-6000. The measurement conditions are shown in Table 1.

Table 1. Instrument and Analytical Conditions

Instruments	: RF-6000 Spectrofluorophotometer
Spectrum Type	: 3D spectrum
Wavelength Range	: Excitation (Ex) 250 nm to 400 nm, Emission (Em) 250 nm to 600 nm
Wavelength Interval	: Ex 2.0 nm, Em 1.0 nm
Scan Speed	: 2000 nm/min
Bandwidth	: Ex 3 nm, Em 3 nm

❑ Results and Discussion

EEM spectra of standards:

Organic acids such as humic and fulvic acids, along with amino acids of proteins are the indicative components of DOM which can be characterized by the EEM of aquatic fluorophores. For example, tryptophan, tyrosine and phenylalanine have fluorescence due to the presence of an indole group, and can be used to indicate the presence of protein and peptides.

Table 2 lists the common aquatic fluorophores and their Ex and Em wavelength ranges [1]. Figure 1 shows the measured EEM spectra of tryptophan, tyrosine and humic acid in this study. The Em of tryptophan is in the range of 300~400 nm at Ex of 250~300 nm. The peak (λ_{Max}) at Em 350 nm / Ex 275 nm are in accordance with the literature data shown in Table 2 [1]. Likewise, the Em and Ex of tyrosine is in the range of 280~340 nm and 250~290 nm respectively. The peaks (λ_{Max}) are at Em 310 nm / Ex 275 nm. Both tryptophan and tyrosine EEM spectra indicate the presence of protein-like DOM in the aquatic sample.

The EEM spectrum of humic acid in water is less define. It has a broad range with Em of 400~600 nm at Ex of 250~370 nm, which is the similar as reported in literature [1]. The humic acid EEM is used to characterize the presence of humic-like or marine humic-like DOM in aquatic samples.

It is worth to note that humic acid has multi-chromophoric groups and its fluorescence behavior differs from a single chromophore molecule. The fluorescence of humic acid differs with source since there is a difference in the molecular components of humic acid from one source to another.

EEM analysis of natural water and wastewaters:

The EEM spectrum of the pond surface water (Figure. 2a) shows tyrosine-like fluorescence as well as a broad Em 350~500 nm with Ex 250~370 nm which could be attributed to humic-like fluorescence.

Table 2. Characteristic aquatic fluorophores published in literature [1]

	Fluorophore	Excitation (nm)	Emission (nm)
A	Tryptophan-like, protein-like	225-237	340-381
		275	340
B	Tyrosine-like, protein-like	225-237	309-321
		275	310
C	Humic-like	237-260	400-500
		300-370	400-500
	Marine humic-like	312	380-420

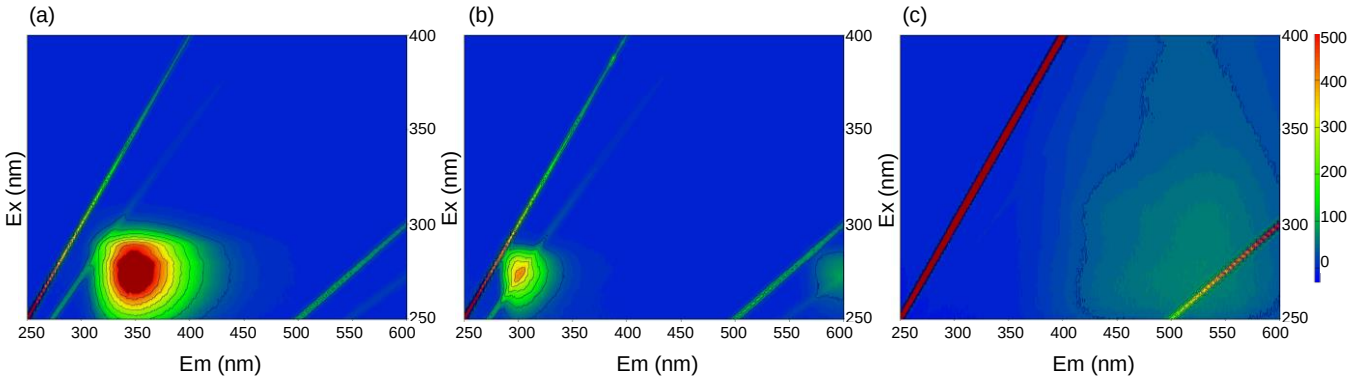

Figure 1. EEM 3D spectra of (a) tryptophan, (b) tyrosine, (c) humic acid in pure water

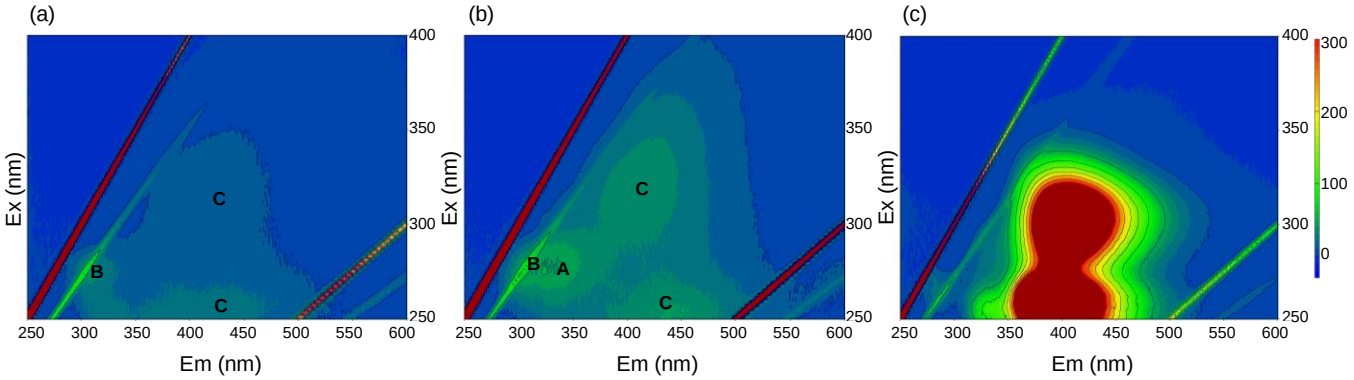

Figure 2. EEM spectra of (a) pond surface water, (b) wastewater from source X, (c) wastewater from source Y

The two wastewater samples have different EEM spectra. The wastewater from source X show tryptophan-like (A), tyrosine-like (B) and humic-like fluorescence (C) (Figure 2b). The wastewater from source Y shows the presence of unknown strong fluorophore of Em 350~480 nm at Ex 250~280 nm and 280~350 nm (Figure. 2c), which could not be identified directly to the above DOM (Table 2).

❑ Conclusions

The excitation-emission matrix (EEM) fluorescence spectroscopy can be used as a rapid analytical tool in the characterization of DOM in natural water and wastewater. Hence, it could be potentially used to assess water quality, wastewater treatment process and monitor pollution of natural water.

❑ References

1. Hudson, N., Baker, A. and Reynolds, D. (2007). *Fluorescence analysis of dissolved organic matter in natural, waste and polluted waters - a review.* River Research and Applications 23: 631-649.
2. Yan, Y., Li, H. and Myrick, M. L. (2000). *Fluorescence fingerprint of waters: excitation-emission matrix spectroscopy as a tracking tool.* Applied Spectroscopy 54(10): 1539-1542.
3. Matthews, B.J.H., Jones, A.C., Theodorou, N.K. and Tudhope, A.W. (1996). *Excitation-emission-matrix fluorescence spectroscopy applied to humic acid bands in coral reefs.* Marine Chemistry 55: 317-332.

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