On the use of fluorescence measurements to characterize wastewater

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Abstract This paper examines the correlations between some water quality parameters and fluorescence intensities and spectra in filtered wastewater using a 280 nm excitation wavelength. We did not obtain satisfying linear relationships between fluorescence and COD or TOC ($r^2 \cong 0.4$) for any of the emission wavelengths used between 320 and 400 nm (especially at 355 nm, the average emission $\lambda_{max.}$). The relationships with N_K were better ($r^2 \cong 0.7$); leading us to suggest that one evaluates mainly the organic nitrogen content at $\lambda_{ex.}$ = 280 nm. Whole spectra processing did not improve the correlations. Gel permeation chromatography yielded markedly different TOC and fluorescence chromatograms, which explains the difficulty of correlating organic content and fluorescence. Other investigations are necessary before spectrofluorimetry can be used as a reliable technique for on-line wastewater pollution estimation.
Keywords Fluorescence; gel permeation chromatography; Kjeldahl nitrogen; organic matter; PLS; wastewater

Introduction

Over the last three decades various methods have been suggested and examined to evaluate water pollution rapidly and directly. These include the widely known UV spectrophotometry (Mrkva, 1975; Thomas *et al*., 1993, 1996; El Khorassani *et al*., 1999). Fewer attempts relying on fluorescence measurements have been made, most of them for estimating organic and humic compounds in surface, sea and fresh waters (Smart *et al*., 1976; Vodacek, 1992; Green and Blough, 1994; Mopper *et al*., 1996). The literature also reports various relationships between COD, TOC or BOD and the fluorescence of organic compounds contained in wastewaters, and sometimes the variables influencing this fluorescence. Bari and Farooq (1985), who investigated chemical treatment levels in terms of organic matter removal for various wastewaters, showed that fluorescence ($\lambda_{ex.}$ = 365 nm and $\lambda_{em.}$ = 400–600 nm) was a good parameter for estimating specific classes of organic matter as well as their time trends. Fluorescence and UV measurements were well correlated with the corresponding COD values. Comber *et al*. (1996) tried unsuccessfully to correlate fluorescence (λ_{ex} = 250 and 350 nm; λ_{em} = 430 nm) in a large set of sewage and industrial effluents with their respective BOD. Recently, Reynolds and Ahmad made a significant contribution to the field in studying the fluorescence of surface and waste water and the influence of parameters such as temperature, pH or metal ions on the readings (Ahmad and Reynolds, 1995; Reynolds and Ahmad, 1995). These researchers observed (Reynolds and Ahmad, 1997) good linear relationships ($r^2 \cong 0.9$–0.94) between BOD and fluorescence intensities ($\lambda_{ex.}$ = 280 nm; $\lambda_{em.}$ = 340 nm) normalized to the water Raman signal for various raw domestic and industrial wastewaters, even though they admitted that the biodegradability of the potential fluorescing chromophores in the wastewaters was generally low or nil, except for amino acids.

In our study we decided to investigate the correlations between fluorescence intensities or spectra and water quality parameters other than BOD in a municipal filtered raw sewage at an excitation wavelength of 280 nm. Using gel permeation chromatography (GPC), we will also try to compare the "organic content" and "fluorescence" chromatograms and

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suggest some explanations about the organic content of the fractions and their corresponding fluorescence.

Material and methods

Sampling and analysis

UV spectrophotometry and water quality parameters of a raw sewage from a municipal wastewater treatment plant in Belgium (Arlon) were characterized from April to September 2000 within the framework of a technical study related to continuous monitoring of wastewaters. During this study, the feasibility of fluorescence measurements on this wastewater was also investigated. For this highly specific exploratory work the raw sewage samples were collected downstream from the plant's bar screen at 30-minute time intervals (between 17h00–06h00) over a 5-week period. Every morning, 90 min composite samples were made up from the collected samples, transported to the laboratory and analyzed immediately. The composite samples were filtered through a Whatman GF/C glass fiber filter and pH, conductivity, nitrate, nitrite, ammonia, Kjeldahl nitrogen (N_K), Total Organic Carbon (TOC) and Chemical Oxygen Demand (COD) were measured by standard methods (Standard Methods, 1995). Fluorescence analyses were performed using a commercial spectrofluorimeter (Perkin Elmer, LS 50) and high-grade quartz cuvettes with a 10 mm path length.

Operating conditions for routine fluorescence measurements

Before the 5-week intensive measurements period, four filtered sewage samples collected on the same day were examined to define the best operating conditions for routine fluorescence analysis. Figure 1a shows the excitation spectra of a typical filtered sample (COD = 120 mgO_2/l) for an emission wavelength at 340 nm and various dilutions (scan rate = 500 nm/min.; excitation slit width = 2.5 nm; emission slit width = 5 nm; number of scans by spectrum = 3).

According to Reynolds and Ahmad's (1997) observations, the optimum excitation wavelength for the maximum fluorescence emission is near 280 nm. Figure 1b shows the corresponding emission spectra. These spectra are typical of the preliminary samples (diluted or not) and indicate an emission band centered around 355 nm, without other relevant peaks, except the 309 nm water Raman peak.

The correlation between raw fluorescence intensities at 355 nm and reciprocal of dilution has a coefficient of determination (r^2) greater than 0.99, which corresponds to a 0–80 mgO_2/l COD range. If COD above 80 mgO_2/l are taken into account the coefficient of determination starts to decrease. This trend was observed for the various preliminary samples under the above-mentioned operating conditions.

As the upper COD of filtered raw water was usually near 400 mgO_2/l, a systematic dilution factor of 5 was applied for further routine fluorescence analyses. Each spectrum would then be multiplied by the dilution factor for data processing. Moreover, this systematic dilution procedure enabled us at the same time to adjust the samples' pH automatically

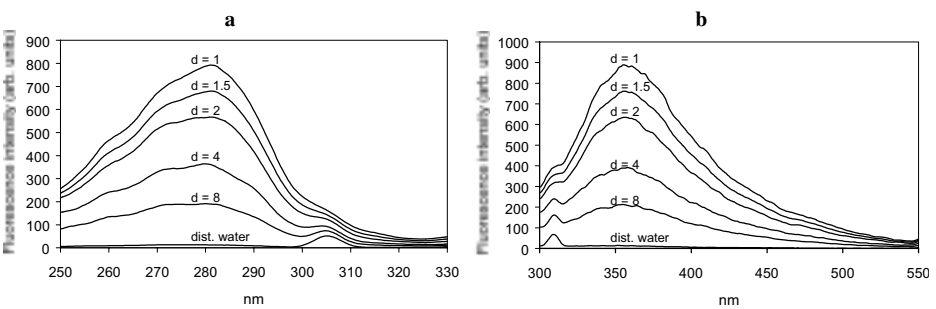

Figure 1 Excitation (a: $\lambda_{em.}$ = 340 nm) and emission (b: $\lambda_{ex.}$ = 280 nm) spectra for various dilutions (d)

around 7. We must also point out that the wastewater examined had low and rather constant potentially quenching metal ion concentrations that were not expected to affect the measurements. In our case the correlation between COD and fluorescence intensities was not improved when fluorescence intensity (I_{355nm}) was normalized with respect to the water Raman signal (I_{309nm}) and the transmittances (T_{355nm} and T_{309nm}) at the considered wavelengths, according to the procedure established by Reynolds and Ahmad (1997), where $I_{corrected} = (I_{355nm} \times T_{355nm})/(I_{309nm} \times T_{309nm})$.

No significant difference in the emission spectra quality was observed for the different scan rates between 50 and 500 nm/min.

The following operating conditions were selected for the routine fluorescence analysis step:

- sample dilution factor: 5
- scan rate: 500 nm/min.
- number of scans by spectrum: 3
- data interval: 0.5 nm
- excitation wavelength: 280 nm
- emision spectra: between 300 and 550 nm
- excitation slit width: 2.5 nm
- emission slit width: 5 nm

Conditions of GPC

The conditions were as follows: column Pharmacia (∅: 26 mm; height: 1,000 mm); gel height ≅ 840 mm; gel Sephadex G25 fine Pharmacia – MW: 500–5000 –; fraction collector Gradifrac; eluent: distilled water + NaN_3 (0.2%); flow rate: 2 ml/min; exclusion volume measured by blue dextran: 195 ml; injection volume: 10 ml of filtered, undiluted sewage. UV absorbance (280 nm) was recorded continuously at the column's outlet. The emission spectra ($\lambda_{ex.}$ = 280 nm) and TOC concentrations of GPC fractions (15 ml) were measured as soon as the raw fractions were collected. The GPC had to be carried out on samples with organic carbon concentrations greater than 100 mgC/l in order to obtain reliable chromatograms, for with smaller initial TOC concentrations most of the fractions' TOC measurements would be inaccurate.

Results

Water quality characteristics

Table 1 summarizes the main analytical characteristics of the filtered samples (n = 93). This table shows that the sewage's characteristics varied widely during this period. Moreover this sewage was almost nitrate and nitrite free and had a rather stable pH close to 8. Some of the 93 samples had COD higher than 400 mgO_2/l and were diluted 10 times before spectral analysis, the results of which are presented later. Table 2 gives the coefficients of determination of the linear regressions relating the main parameters of the sewage. Unsurprisingly, COD is well correlated with TOC, and to some extent there is also a good correlation between NH_4-N and N_K. Conductivity indicates some water quality trends, especially as regards N_K and NH_4-N. BOD_5 was also measured in some samples, from which we calculated a BOD_5/COD ratio of 0.58 (σ = 0.08; n = 10).

Fluorescence measurements

Single correlations. Contrary to what was observed in preliminary tests, the main emission

Table 1 Main characteristics of the filtered sewage samples (n = 93)

	pH	Conductivity µS/cm	$[NO_x$-N] (mg/l)	$[NH_4$-N] (mg/l)	N_K (mg/l)	COD (mgO_2/l)	TOC (mg/l)
Mean	8.05	868.8	0.4	14.6	20.4	238.3	73.8
Std. dev.	0.28	208.3	0.5	5.3	7.0	123.8	40.6
Range	7.54–8.81	267–1386	0–2	3.1–31	3.1–38.2	40–567	13.1–184.2

Table 2 r^2 values for the linear regressions between the main parameters of the sewage

	COD	TOC	N_K	NH_4-N	Conductivity
COD	1	0.96	0.21	0.21	0.26
TOC		1	0.22	0.18	0.23
N_K			1	0.81	0.61
NH_4-N				1	0.68
Conductivity					1

band of the 93 samples was not always centered on 355 nm. Indeed Figure 2 shows a 340–380 nm range for the maximum fluorescence intensity wavelengths, but with an average still at 355 nm ($\sigma = 6$). For each emission wavelength in the 309–420 nm range, coefficients of determination of the linear regressions relating fluorescence intensities to COD, TOC, NH_4-N and N_K values, respectively, were plotted for all samples (Figure 3). The curves corresponding to the sewage's organic content (TOC and COD) are very close due to the high correlation between COD and TOC. On the other hand, the coefficients of determination of the linear regressions relating those parameters to fluorescence intensity were always below 0.5, whatever the emission wavelength considered. Similar trends were observed for the available BOD_5 measurements. With N_K the coefficients of determination are markedly better although they remain moderate ($r^2 \cong 0.65$ in the 350–390 range). To some extent, the same can be said for NH_4-N, as a consequence of its relation with N_K.

This graph indicates therefore that at an excitation wavelength of 280 nm the exhibited fluorescence in the 340–390 nm range seems more related to the organic nitrogen content than to the organic carbon content, thereby confirming the observations of Mopper *et al.* (1996), who found that the fluorescence emitted near 330 nm after excitation at 270 nm was probably due to the tested seawater's protein content. Linear regressions with the different analytical parameters are not improved by normalizing the fluorescence intensities with the water Raman signal or using emission spectra areas. Still within the framework of simple regressions, Table 3 shows that a multilinear regression using fluorescence intensities at 309 nm (Raman) and 355 nm (mean λ_{max}) improves the correlation with the organic carbon content slightly but also weakens the correlation with the Kjeldahl (organic) nitrogen content slightly. Adding conductivity as an independent variable improves the correlation with the nitrogen parameters noticeably.

We should also mention that our sample set included fifteen sequential samples (corresponding to a 3-day period) presenting fluorescence that was highly correlated with COD or TOC ($r^2 \cong 0.97$ with I_{355nm}). This can probably be explained by sewage with a rather constant organic composition and for which the amount of fluorescing organic molecules was proportionate to the total organic content. On the other hand, the calculated coefficient of

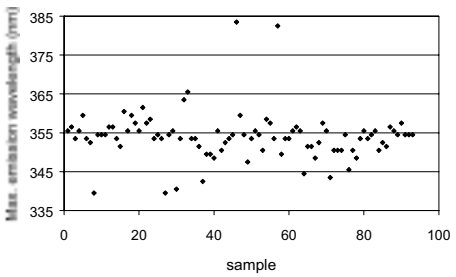

Figure 2 Wavelengths of maximum intensity for the various samples

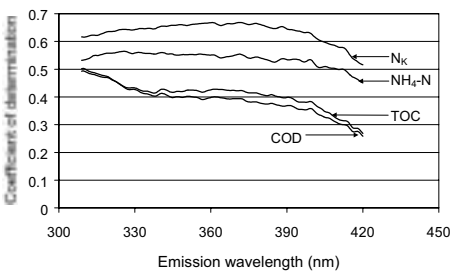

Figure 3 r^2 values for linear regressions of N_K, NH_4, TOC and COD vs. fluorescence intensity at each $\lambda_{em.}$

Table 3 r^2 values for multilinear regression using I_{309nm}, I_{355nm} and conductivity

Parameters	COD T	OC	N_K	NH_4-N
I_{309nm}, I_{355nm}	0.50	0.48	0.61	0.51
I_{309nm}, I_{355nm}, conductivity	0.55	0.49	0.78	0.77

determination between nitrogen and fluorescence (355 nm) for those fifteen samples was 0.77, that is, a value close to the one calculated for the entire sample set.

Attempts to use the complete fluorescence spectrum. Given that at a given excitation wavelength the fluorescence spectrum is the result of the contributions of all the fluorescing chromophores contained in the sewage, one can envision exploiting the wholeness of the spectrum. Similar procedures are often used in the field of UV spectra processing (Gallot and Thomas, 1993; Karlsson et al., 1995; El Khorassani et al., 1999). For fluorescence spectra processing we focused on using PLS (partial least squares) chemometric data processing, which allows one to define mutivariate calibration models (MCMs) for spectra processing. The software used was MATLAB version 5.0 (PLS Toolbox 2.0).

The experimental data base contains 93 lines – corresponding to the 93 samples – composed of "explicative data" (fluorescence intensities at $\lambda_{em.}$ between 300 and 450 nm; step: 1 nm) and variables to be estimated (TOC, COD, NH_4-N, and N_K). The data lines, which are chronologically ordered, were first time mixed randomly, and then separated into two equidata sets, i.e., a calibration set (46 lines) and a validation set (47 lines). MCM evaluation was performed by applying the models to the validation set and calculating the following prediction error:

$$E = \sqrt{\frac{\sum((yi - \overline{yi}) / yi)^2}{N}}$$

where : yi = analytical value (validation set)
$\overline{yi}$ = predicted value (validation set)
N = number of validation data

Table 4 gives the r^2 (calculated from) and prediction error values obtained for the validation set using multivariate calibration models implicating n (10, 20, 30, 40 and 46) of the 46 data lines of the calibration set (number of principal components: 5). We observe that the prediction errors for COD and TOC vary between 37–56% and 36–50%, respectively, depending on the number of lines employed in the calibration procedure of the PLS models. Errors obtained by single regression with fluorescence intensities at 355 nm are included in those ranges of errors. In this case, the PLS method did not improve the correlation between fluorescence and the quality parameters representative of organic matter.

Although they did not exhibit any particular trends in relation to *n*, the prediction errors

Table 4 Comparison of different MCMs using *n* of the 46 data lines of the calibration set (46). Validation on the complete validation set (47)

	COD		TOC		NH_4-N		N_K	
	r^2	*E* (%)	r^2	*E* (%)	r^2	*E* (%)	r^2	*E* (%)
Single regression with I_{355nm} (all 47 validation set values)	0.42	42.7	0.41	40.3	0.65	51.1	0.69	52.8
MCM (n = 10)	0.63	37.7	0.57	39.1	0.49	65.4	0.58	54.9
MCM (n = 20)	0.59	36.8	0.55	39.7	0.79	31	0.73	32.4
MCM (n = 30)	0.56	38.2	0.53	35.8	0.87	27.3	0.76	31.7
MCM (n = 40)	0.54	56	0.52	50.1	0.81	33	0.74	35.2
MCM (n = 46)	0.59	52.9	0.56	47	0.75	33.1	0.72	39.2

for NH_4-N (27–33%) and N_K (32–39%) were relatively stable for $n \geq 20$ and show that the PLS models tended to improve the correlations appreciably.

Identical attempts were made taking a larger calibration set ($n = 63$) and a validation set of 30 data lines. Table 5 provides the prediction errors obtained on the validation set for various n data lines used for multivariate calibration. The prediction errors for NH_4-N and N_K ($\cong$ 10–20%) are similar to those resulting from single regressions. Note that the latter were already low (16.1 and 10.7%) for the 30-point validation set. The observations made for COD and TOC remain valid. The PLS regressions confirm the correlation between fluorescence spectra and N_K and, indirectly, NH_4-N for the tested sewage. The correlations are less relevant, however, for the parameters corresponding to organic carbon.

These first attempts also suggest that, if fluorescence is to be used to evaluate one or several parameters characteristic of sewage quality, attention must paid to the calibration methodology (what kind of "reference sample", how many samples, etc.). Finally, we must add that various types of preliminary data processing (Savitsky–Golay smoothing, Savitsky–Golay derivation, etc.) did not yield better results.

Gel Permeation Chromatography

A GPC separation was performed on two samples with relatively high TOC values (> 100 mg/l). The trends and results presented for one sample were confirmed overall for the second sample. The sample in question had a TOC of 120 mg/l and a maximum fluorescence intensity at $\lambda_{em.}$ = 355 nm ($\lambda_{ex.}$ = 280 nm), i.e., the mean emission λ_{max} mentioned above. Figure 4 illustrates the sample's TOC, fluorescence ($\lambda_{ex.}$ = 280 nm; $\lambda_{em.}$ = 355 nm) and UV absorbance (280 nm) profiles after GPC separation.

During this GPC the total amount of organic carbon recovered was 1.26 mg, which was very close to the amount of injected organic carbon (1.2 mg). At a cumulative volume of 195 ml which corresponds to the exclusion volume, we observe a first relevant TOC peak representing already 1/3 of the total TOC. This peak is also detected by UV but not by fluorescence. That means that the large molecules with MW>5,000 absorbs in UV but are not directly fluorescing at the operating wavelengths. Next, the TOC chromatogram reveals an organic group representing about 60% of the total TOC between $\cong$ 300 and 460 ml. This peak corresponds to the first relevant fluorescence peak detected (F1). In the same elution zone, we did however observe three small UV absorbance peaks. The difference in the UV and TOC chromatograms' shapes in this zone could be partly explained by the fact that UV absorbance was continuously monitored at the column outlet, contrary to TOC, which was measured at each 15 ml eluted. Beyond 460 ml, when TOC and UV absorbance decrease, two main peaks were still revealed by fluorescence at $\cong$ 470 ml (F2) and especially at 550 ml (F3). These peaks were characterized by very high

Table 5 Comparison of different MCMs using n of the 63 data lines of the calibration set (63). Validation on the complete validation set (30)

	COD		TOC		NH_4-N		N_K	
	r^2	E (%)	r^2	E (%)	r^2	E (%)	r^2	E (%)
Single regression with I_{355nm} (all 30 validation set values)	0.34	45.2	0.34	49	0.65	16.1	0.85	10.7
MCM (n = 10)	0.59	38.4	0.52	40.9	0.58	27.7	0.72	21.1
MCM (n = 20)	0.56	36.2	0.50	38.3	0.83	13.8	0.84	12.3
MCM (n = 30)	0.53	38.8	0.49	37	0.89	11.5	0.89	11.2
MCM (n = 40)	0.53	54.3	0.51	53.8	0.81	18	0.87	14.4
MCM (n = 50)	0.58	52.1	0.54	51.6	0.74	20.7	0.78	19.2
MCM (n = 60)	0.57	46.5	0.53	46.4	0.78	18.8	0.76	19.8

fluorescence/ TOC ratios. So, F3 could be considered representative of an "inorganic" fluorescence. The F1, F2 and F3 fractions of the fluorescence chromatogram present spectra that are quite different from that of the raw sewage before GPC. For example, F1 has a band centered on 375 nm (355 nm for the sewage) whereas F2 and F3 show (besides the Raman peak) two bands at ≅ 340 nm and 405 nm.

Precise identification of the molecules responsible for the fluorescence spectra of the collected GPC fractions requires a sophisticated analytical investigation that was outside the scope of the present study. This identification cannot be performed beforehand due to the numerous potential fluorescing chromophores in sewage (the aromatic amino acids – particularly tryptophan, which has a $\lambda_{ex.}$ at ≅ 287 nm and a $\lambda_{em.}$ at ≅ 350 nm and an elution volume much higher than 500 ml, according to Bruchet (cited in Clement and Thomas, 1995), the humic and lignin compounds, whitening agents, etc.) but also because the relationship between a molecule's MW and elution volume is not always reliable, as indicated by Clement and Thomas (1995). This elution volume depends on various factors, including the type of wastewater. However, our experimental use of GPC combined with fluorescence measurements has shown that significant differences between the "fluorescence" (and even UV) and "organic" (TOC) chromatograms can exist. So far, the GPC performed on one kind of sewage is insufficient to yield definitive conclusions but suggests that the relevant chromatographic fractions' fluorescence/TOC ratios differ greatly. This also suggests that the rather poor correlations previously observed between organic matter and fluorescence can be explained by a relevant modification in the proportions of the different fluorescing groups in the sewage.

Conclusion and discussion

We did not obtain satisfying linear relationships between fluorescence intensity and COD or TOC (r^2 ≅ 0.4 for COD), using an excitation wavelength of 280 nm, for any of the emission wavelengths used between 320 and 400 nm (especially at 355 nm, the average emission $\lambda_{max.}$). The linear relationships with Kjeldahl nitrogen (and indirectly ammonia)

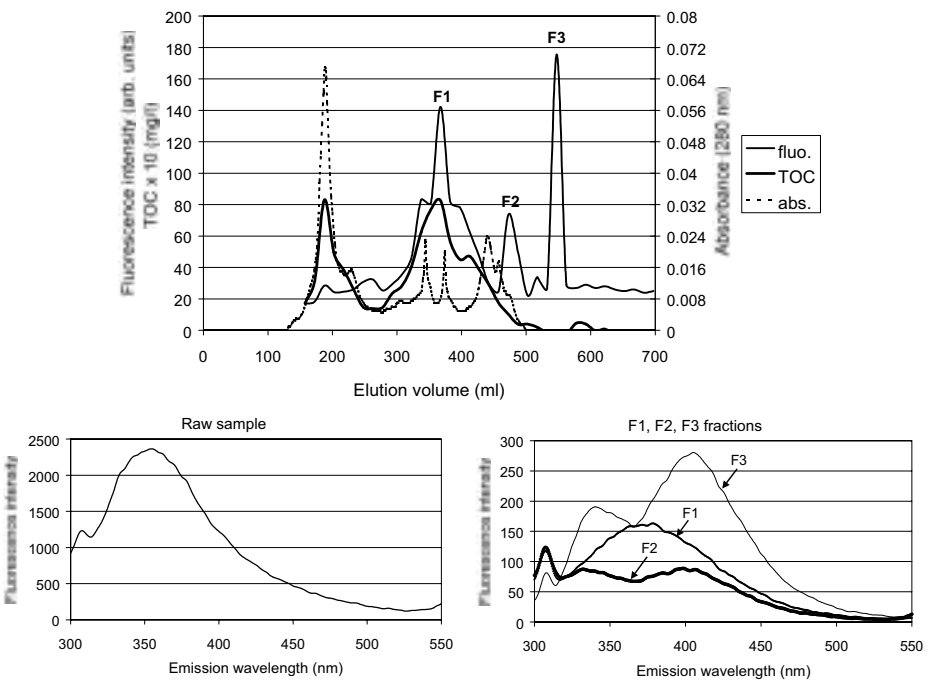

Figure 4 TOC, UV_{280nm} and fluorescence intensity (355 nm) chromatograms of the tested sewage and fluorescence spectra of the raw sample and the F1, F2, F3 fractions

were better ($r^2 \cong 0.7$ for N_K), leading us to suggest that at an excitation wavelength of 280 nm, one is evaluating mainly the organic nitrogen (protein) content. Attempts to process whole spectra did not improve the correlations significantly.

The GPC of sewage samples led to the fact that the TOC and fluorescence chromatograms were markedly different. So far, the only way to efficiently correlate fluorescence intensity to the organic content would be therefore when the ratio "fluorescing organic content/total organic content" varies little or none. Clearly this is not the case in this study except for 15 successively-collected samples, as mentioned.

The present conclusions are valid only for examined sewage, i.e. with filtered samples, and do not invalidate the conclusions of Reynolds and Ahmad (1997), who studied unfiltered sewage samples and observed that the suspended matter contributed significantly to the fluorescence.

In any case, utilizing a specific technique to estimate general environmental parameters needs precautions. More investigations are undoubtedly necessary before reliably using spectrofluorimetry as autonomous or complementary (i.e. coupled with physico-chemical probes or UV measurements-based devices) technique for on-line wastewater pollution estimation. The use of complementary excitation wavelengths (especially between 300–400 nm for the "humic" fluorescence), synchronous fluorescence as well as characterization and separation techniques like GPC would probably help to better define the potential scope of this technique for wastewater pollution control.

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