Fluorescence spectroscopy for wastewater monitoring: a review

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Abstract: Wastewater quality is usually assessed using physical, chemical and microbiological tests, which are not suitable for online monitoring, provide unreliable results, or use hazardous chemicals. Hence, there is an urgent need to find a rapid and effective method for the evaluation of water quality in natural and engineered systems and for providing an early warning of pollution events. Fluorescence spectroscopy has been shown to be a valuable technique to characterize and monitor wastewater in surface waters for tracking sources of pollution, and in treatment works for process control and optimization. This paper reviews the current progress in applying fluorescence to assess wastewater quality. Studies have shown that, in general, wastewater presents higher fluorescence intensity compared to natural waters for the components associated with peak T (living and dead cellular material and their exudates) and peak C (microbially reprocessed organic matter). Furthermore, peak T fluorescence is significantly reduced after the biological treatment process and peak C is almost completely removed after the chlorination and reverse osmosis stages. Thus, simple fluorometers with appropriate wavelength selectivity, particularly for peaks T and C could be used for online monitoring in wastewater treatment works. This review also shows that care should be taken in any attempt to identify wastewater pollution sources due to potential overlapping fluorophores. Correlations between fluorescence intensity and water quality parameters such as biochemical oxygen demand (BOD) and total organic carbon (TOC) have been developed and dilution of samples, typically up to x10, has been shown to be useful to limit inner filter effect. It has been concluded that the following research gaps need to be filled: lack of studies on the on-line application of fluorescence spectroscopy in wastewater treatment works and lack of data processing tools suitable for rapid correction and extraction of data contained in fluorescence excitation-emission matrices (EEMs) for real-time studies.

Key words: fluorescence spectroscopy, wastewater, dissolved organic matter, monitoring

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1 Introduction

Environmental monitoring is applied to determine the compliance with ambient and discharge standards and to identify areas with persistent issues for timely and effective remediation (Cahoon and Mallin 2013). Wastewater quality assessment is an essential part of environmental monitoring due to the high anthropogenic impact of treated and untreated discharges on water bodies (Suthar et al. 2010). There are two important aspects of wastewater quality monitoring: the first concerns the detection of pollution events for early warning and rapid remedial responses of water bodies, while the second aspect relates to wastewater treatment works where quality monitoring is required for process control and compliance with regulations at the effluent discharge point (Bourgeois et al. 2001, Michael et al. 2015, Rehman et al. 2015).

The quality of wastewater is generally assessed using physical, chemical and microbiological tests. Among these techniques, reliance is often placed on biological oxygen demand (BOD), chemical oxygen demand (COD) and total organic carbon (TOC) (Bourgeois et al. 2001, Bridgeman et al. 2013). However, these global parameters depend on expensive or time-consuming methods, offering only snapshots of moments in time (Bourgeois et al. 2001, Chong et al. 2013, Yang et al. 2015a), which makes them unsuitable for online monitoring. Research conducted almost two decades ago (Ahmad and Reynolds 1995, Tartakovsky et al. 1996, Reynolds and Ahmad 1997, Ahmad and Reynolds 1999) has shown that fluorescence spectroscopy could be used for wastewater quality assessment as a tool for discharge detection in natural water systems and for process control in wastewater treatment plants (WwTPs). Fluorescence is the release of energy in the form of light when molecules or moieties, named fluorophores, are excited with a high-energy light source (Lakowicz 2006, Reynolds 2014). The technique has been suggested for its multiple advantages: it is fast, inexpensive, reagentless, requires little sample preparation, is highly sensitive and non-invasive (Reynolds 2003, Hudson et al. 2007, Cao et al. 2009, Henderson et al. 2009, Hambly et al. 2010, Murphy et al. 2011, Chong et al. 2013, Yang et al. 2015a). According to Reynolds (2002) fluorescence monitoring could provide rapid feedback, allowing dynamic, high spatial and temporal resolution studies.

In the past decades, more studies have proved the potential of fluorescence spectroscopy as a monitoring and detection tool in natural and engineered systems. This technique has been used successfully to characterize organic matter in seawater (Coble et al. 1990, Coble 1996, Conmy et al. 2004, Drozdowska 2007), freshwater (Baker 2001, McKnight et al. 2001, Spencer et al. 2007b, Carstea et al. 2009) or estuarine water (Huguet et al. 2009). Also, it has been used to monitor riverine organic matter and diesel pollution (Downing et al. 2009, Carstea et al. 2010), evaluate drinking water treatment processes (Bieroza et al. 2009, Cumberland et al. 2012, Shutova et al. 2014) or detect pesticides (Ferretto et al. 2014). Fluorescence spectroscopy has been used to assess the quality of raw sewage and effluents (Baker 2001, Boving et al. 2004, Pfeiffer et al. 2008), industrial (Santos et al. 2001, Borisover et al. 2011, Li et al. 2015), or farm (Baker 2002a, Old et al. 2012) discharges into natural systems. Moreover, recent studies on short and long-term fluorescence monitoring along the WwTPs process train have been undertaken, to determine the potential of the technique for treatment processes control (for example, (Murphy et al. 2011, Bridgeman et al. 2013, Cohen et al. 2014, Ou et al. 2014, Singh et al. 2015). Although considerable work has been done so far in this field, there are still issues with regard to the "matrix effects", as reviewed by Henderson et al. (2009), or with fouling (Reynolds 2002) that must be overcome to allow application of the technique in WwTPs.

Other reviews proved the potential of applying fluorescence spectroscopy to water quality monitoring (Hudson et al. 2007, Henderson et al. 2009, Fellman et al. 2010, Ishii and Boyer 2012, Yang et al. 2015b). However, none of them focused only on wastewater, which requires a specific discussion due to its complexity in composition and impact on the environment. Moreover, a

growing number of studies are published each year on the application of fluorescence spectroscopy to wastewater quality evaluation, proving its scientific and industrial importance. In this paper, we review the current progress in applying fluorescence spectroscopy to assess wastewater quality. The technique's capabilities as a detection and early warning tool of pollution with treated or raw wastewater from different sources are discussed. Also, its potential for process control in WwTPs is presented.

2 Fluorescence assessment of wastewater components

2.1 Organic matter fluorescence assessment

The most common methods of recording fluorescence spectra for wastewater are excitation – emission matrices (EEM) and synchronous fluorescence spectra (SFS). EEMs represent fluorescence contour maps, which comprise a series of repeated emission scans recorded in a range of excitation wavelengths (Coble 1996). SFS are obtained by scanning simultaneously both excitation and emission monochromators at a fixed wavelength interval between them (Patra and Mishra 2002, Reynolds 2003). For many years, since the mid-1970s, SFS were preferred as a multidimensional technique for the analysis of complex solutions, because it provided better peak resolution, compared to emission spectra, and faster recording time than EEMs (Ryder 2005). However, the improvement of instrumentation allowed researchers to obtain fast, high-resolution EEM collection, which increased the method popularity in the research community. In addition, EEMs offer varied possibilities of data interpretation, from simple peak-picking and Fluorescence Regional Integration to the more complex Parallel Factor Analysis (PARAFAC) and Self-Organizing Maps. Among these methods, peak-picking and PARAFAC are the most popular in the research community and therefore only these two methods will be discussed in the following sections.

The peak-picking method is a very simple tool to identify components based on their maximum intensity and corresponding excitation and emission wavelength pairs (Coble 1996). An example of peak-picking analysis is shown in Figure 1 (a). According to Goldman et al. (2012), peak-picking is a viable analysis technique and can be employed for the development and use of a real-time tool and may be related to custom sensors available today. However, its applicability may be limited due to peak shifts, possible overlapping and interferences between peaks (Yang et al. 2015b). Moreover, it may lead to misleading observations by associating each peak with a specific fluorophore, when two excitation wavelengths are seen at fluorescent components (Fig. 1).

PARAFAC is a mathematical tri-liniar model that deconvolutes EEMs into chemically meaningful components (Fig. 1b). It separates the contribution of different fluorophores without additional assumptions about their excitation and emission spectra (Cohen et al. 2014). A thorough description of PARAFAC method and components in wastewater is given by Yang et al. (2015b). PARAFAC has become common practice in water quality studies, over the past 10 years (Murphy et al. 2014). Yang et al. (2015b) proposed that PARAFAC be developed into a surrogate method for conventional water quality parameters, treatability of organic matter (OM) and performance of treatment processes. Yu et al. (2014) suggested that the PARAFAC tool, the EEMizer, developed by Bro and Vidal (2011), could be implemented to monitor on-line the WwTPs performance. The studies of Yu et al. (2015a) implied that PARAFAC is able to identify contamination events and can be used for early warning, but the component that indicates contamination must be spectrally different from the existing components, without major spectral overlap, which may undermine the online monitoring strategy. Similarly, Murphy et al. (2011) showed that at times PARAFAC had difficulties distinguishing between components, returning hybridized spectra. Also, in a comparison between chromatographic fluorescence fingerprints and EEM-PARAFAC, Li et al. (2014) showed that the latter method could not reflect the variety of organic matter species with similar fluorescence, but different physico-chemical properties. In addition, PARAFAC is currently applied only as post-processing technique, making it unsuitable for continuous monitoring. Also, there is no consensus regarding the optimum model in terms of sample size and variability (Yu et al. 2015a). . ENREF_155

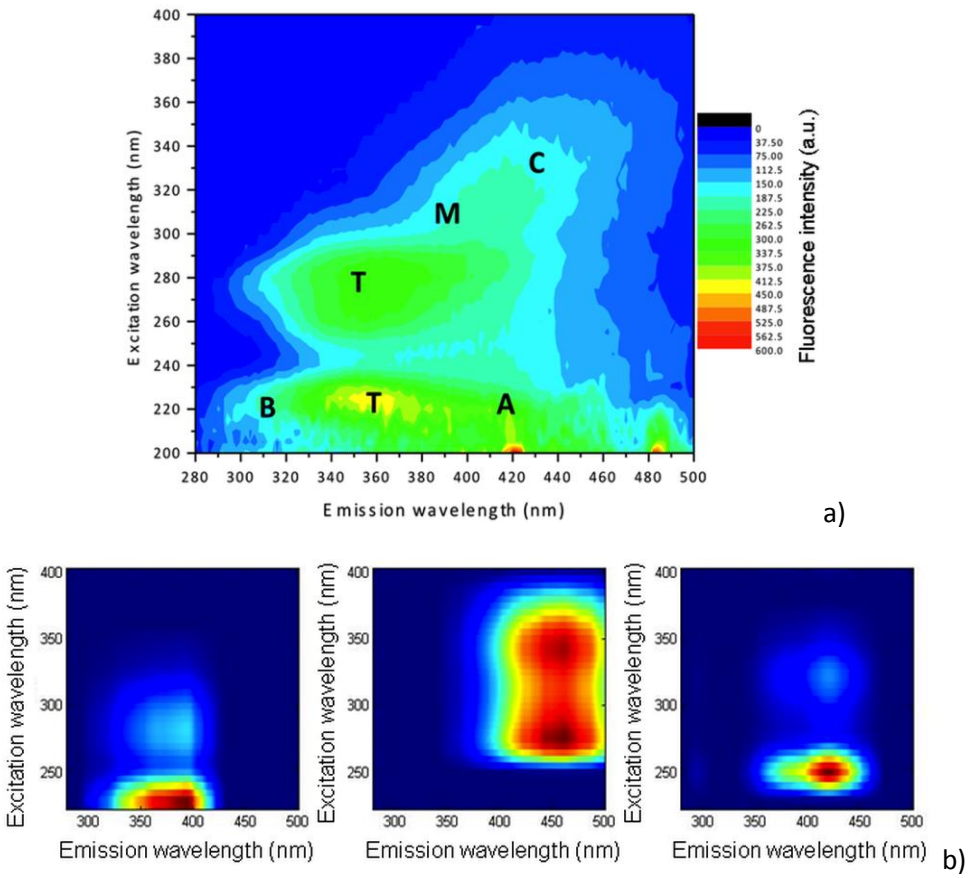

Figure 1. Main techniques of processing fluorescence EEMs. Examples of a) peaks identified with the peak picking method, and b) components identified with PARAFAC, for samples of water systems impacted by domestic wastewater.

All these techniques have been employed successfully to analyse OM from various natural to engineered sources. A thorough review on OM fluorescence is provided by Hudson et al. (2007) and (Fellman et al. 2010). Crude sewage is a combination of domestic waste, industrial discharges, surface runoff and storm flow. Its composition varies depending on the age and type of sewerage, time of day, weather conditions and type of incoming sewer (Ahmad and Reynolds 1995, Hudson et al. 2007). Ellis (2004) showed that the general organic composition of wastewater is 50 % proteins, 14 % carbohydrates, 10 % fats and oils and trace amounts of priority pollutants and surfactants, which are present in detergents, soaps, shampoo and similar consumer products. More recently, Huang et al. (2010) found that fibres, proteins and sugars are the largest groups of OM in wastewaters, accounting for 20.64 %, 12.38 % and 10.65 %, respectively, of the total TOC. According to the researchers, food related substances are the main source of OM in wastewaters (Huang et al. 2010). Using gas chromatography/mass spectrometry, Huang et al. (2010) detected 90 compounds from the groups of alkyls and aromatic hydrocarbons, alkenes, alcohols, organic acids, ketones, phenols, nitrogenous compounds, ethers, amines and esters. In addition, they found lipids, volatile fatty acids, humic acids, DNA + RNA, tannic acids and linear alkylbenzene sulfonates. Within the organic composition, there are numerous overlapping fluorophores that contribute to the EEMs (Aiken 2014). Due to the difficulty of assigning specific fluorophores to the peaks identified in EEMs, the fluorescence of wastewater will be discussed as two regions based on the classification provided by Li et al. (2014): region Em < 380 nm associated mostly with fluorophores containing a limited number of aromatic rings and the region > 380 nm with polycyclic aromatic fluorophores.

2.2 Region Em < 380 nm

Based on the peak-picking method, fluorescence in this region is represented by peak T ($\lambda_{excitation}$ / $\lambda_{emission}$ *~225 (~280) / ~350 nm*) and peak B ($\lambda_{excitation}$ / $\lambda_{emission}$ *~225 (~280) / ~305 nm*) (Fig. 1a). Peaks T and B have been observed in all studies that used the peak-picking method for

EEM processing, irrespective of the wastewater source (Table SM1). These peaks have been associated with living and dead cellular material and their exudates and indicate microbial activity (Bridgeman et al. 2013) and material derived from anthropogenic activities (Yu et al. 2014). In PARAFAC, the region Em < 380 nm is generally identified as components with 2 excitation wavelengths and 1 emission wavelength (Fig. 1b) in the same wavelength ranges as peaks T and B in the peak-picking method. These components are identified in both municipal and industrial wastewater samples; however, the component similar to peak T is more common in wastewater compared to other components in this region (Table SM2).

By examining the list of wastewater organic components (Dignac et al. 2000, Huang et al. 2010, Navalon et al. 2011), and the literature review of Aiken (2014), Stedmon and Cory (2014) and Baker et al. (2014), the following components were considered as contributors to the fluorescence in the region Em < 380 nm: phenols (for example cresols), indoles, mono and polyaromatic hydrocarbons, DNA, aromatic amino acids (phenylalanine, tyrosine), degradation products of lignin (lignin phenols, vanillic acid, syringic acid etc.). These compounds are derived from domestic waste, chemical, pharmaceutical, plastic, petrochemical, paper, leather or textile industries (del Olmo et al. 1996, Pokhrel and Viraraghavan 2004, He et al. 2007, Tchaikovskaya et al. 2007, Tertuliani et al. 2008). The potential contributing fluorophores to this region are presented in Table 1.

2.3 Region Em > 380 nm

The peak-picking method classifies this region as follows: Peak A ($\lambda_{excitation}$ / $\lambda_{emission}$ *~225 / 400 - 500 nm)*, peak C ($\lambda_{excitation}$ / $\lambda_{emission}$ *300 - 350 / 400 - 500 nm)* and peak M ($\lambda_{excitation}$ / $\lambda_{emission}$ *310 - 320 / 380 – 420 nm)* (Fig. 1a). All studies done so far on wastewater OM have identified peak C and most studies found peak A (Table 1); however, peak M was analysed only by Yu et al. (2014) at municipal wastewater. Most of the studies that employed PARAFAC for EEM analysis identified a maximum of 4 components associated and microbially and terrestrially derived DOM (example of two components in Fig 1b). However, Ishii and Boyer (2012) have identified the PARAFAC components common in natural and engineered water systems: Component 1 similar to peak A with excitation in the region *< 230 – 260 nm* and emission between *400* and *500 nm*; Component 2 similar to peaks A + C found in excitation region *< 240 – 275 (339 – 420 nm)* and emission within *434 – 520 nm*; and Component 3 similar to peak A + M appearing in the excitation domain *<240 – 260 nm (295 – 380 nm)* and within the *374 – 450 nm* emission range. According to Ishii and Boyer (2012), component 1 is found mostly in OM sources dominated by terrestrial precursor material. Component 2 was defined as reduced quinone-like and was identified in OM from a wide variety of aquatic systems, including those dominated by terrestrial and microbial inputs. While, component 3 fluorophores were defined as oxidised quinone-like and were similar to those with terrestrial and marine precursors. Component 1 has not been reported in wastewater studies, but components 2 and 3 were seen at studies made on municipal and industrial wastewater (Table SM2). Additional components were observed in wastewater (Table SM2), but they vary depending on source.

As shown in Table 1, there are several fluorophores that could contribute to the fluorescence of region Em > 380 nm: lignins, PAHs, flavonoids, humic acids, quinones, aromatic ketones, fluorescent whitening agents (FWAs), pharmaceutically active compounds (Dignac et al. 2000, Huang et al. 2010, Aiken 2014, Baker et al. 2014, Stedmon and Cory 2014). Among these components, FWAs have been proposed as an indicator of human faecal contamination (Assaad et al. 2014), sewer misconnections (Chandler and Lerner 2015) and presence of landfill leachates (Graham et al. 2015). FWAs are highly soluble and poorly biodegraded, and therefore likely to pass through biological treatment in WwTPs (Kramer et al. 1996, Poiger et al. 1998, Assaad et al. 2014). Research has shown that these components can be detected with handheld fluorometers, which enhances the capability for in situ water monitoring (Hartel et al. 2007). Nevertheless, issues with detecting FWAs in waters have been reported: the fluorescence of other peak C fluorophores overlap the peaks of FWAs, these components are easily photodegraded and DOM hinders the reaction of FWAs (Kramer et al. 1996, Baker 2002b, Hartel et al. 2007, Assaad et al. 2014).

Solutions to overcome fluorescence overlap have been proposed, yet the other issues identified may limit the method's applicability in detecting sewage. The following solutions have been proposed: a) to use the photodegradation rate to separate FWAs from organic matter (Hartel et al. (2007); b) to take into account the differences in shape of the photodecay curve between FWAs and natural organic matter (Cao et al. (2009)); c) to use a baseline correction method to compare the differences in fluorescence intensity of FWA, between the regions *320 nm – 345 nm* and *345 nm – 360 nm*, with the same values for the water samples (Takahashi and Kawamura (2006)); and d) to apply three-way analysis of EEMs assisted by second-order chemometric analyses (Gholami et al. 2015). Discrimination between humic substances and FWAs was achieved by Boving et al. (2004), who analysed FWAs in solution with humic acid and tannic acid. FWAs were recorded at *344 nm* and *422 nm* emission wavelength, and *250 nm* excitation wavelength. The authors found that the second peak of the FWAs was separated from humic acids by 22 nm, but there was a 4 nm separation from tannic acid. Therefore, the $\lambda_{excitation} / \lambda_{emission} = 250 / 422\ nm$ peak could be used for FWAs detection without interference from humic acid.

Table 1. Fluorophores contributing to regions Em < 380 nm >.

Potential fluorophores	**Component**	**Region**	**Peak position (nm)**	**Reference**	**Potential sources in Ww**
Lignins	Lignin phenols	Em < 380 nm	~ 245 (295) / 302	Walker et al. (2009)	Partially degraded food waste, undigested dietary fibre, toilet paper etc. Wastewater of paper and pulp industry (Pokhrel and Viraraghavan 2004) fibres from food (Huang et al. 2010)
			270-290 / 300-350	(Hernes et al. 2009)	
	Vanilic acid		/ 326	(Stedmon and Cory 2014)	
	Syringic acid		/ 338	(Stedmon and Cory 2014)	
	Breakdown products	Em > 380 nm	230-275 (300-390) / 400-520	(Baker 2002b, Ciputra et al. 2010, Osburn and Stedmon 2011, Cawley et al. 2012, Bassandeh et al. 2013)	Paper mill effluents (Baker 2002b, Ciputra et al. 2010, Cawley et al. 2012, Bassandeh et al. 2013)
Aromatic hydrocarbon	Toluene	Em < 380 nm	266 / 300 - 400	(Persichetti et al. 2013)	Municipal Ww (Huang et al. 2010, Mrowiec 2014); Ww with petrol derivatives (Mehdizadeh et al. 2011)
Phenols	Cresols		210-285 / 290-310	(del Olmo et al. 1996)	Pharmaceutical, fossil fuel or pesticide industries (Tchaikovskaya et al. 2007); Domestic Ww from disinfectants (Tertuliani et al. 2008)
Aromatic amino acids	Tyrosine		275 / 304	(Lakowicz 2006)	Proteins and peptides (Lakowicz 2006); Domestic Ww(Burleson et al. 1980, Dignac et al. 2000, Huang et al. 2010)
	Tryptophan		295 / 353	(Lakowicz 2006)	Proteins and peptides (Lakowicz 2006); Livestock Ww (Choi et al. 2013)
Indole			230 / 330-350	(Determann et al. 1998)	Municipal Ww (Dignac et al. 2000, Tertuliani et al. 2008, Huang et al. 2010); Coal tar, oil shale, personal care products, pesticides and pharmaceuticals (Gu and Berry 1991, Tertuliani et al. 2008, Aiken 2014)
DNA			267 / 327	(Vayá et al. 2010)	Proteins (Lakowicz 2006); Municipal Ww (Huang et al. 2010)
Polyaromatic		Em <	Short UV	(Baker et al. 2014)	Municipal Ww (Guo et al. 2010,

hydrocarbons		380 nm			Huang et al. 2010); Landfill leachate (Baker and Curry 2004)
	Phenanthrene, anthracene, pyrene, fluoranthene, benzo[a]pyrene	Em > 380 nm	220-300 / 370-430	(Schwarz and Wasik 1976, Patra and Mishra 2001, Yang et al. 2016)	Industrial Ww (Cohen et al. 2014, Ou et al. 2014); Municipal Ww (Huang et al. 2010)
Quinones		Em > 380 nm			Microbes, fungi, plants (Aiken 2014); Activated sludge (Hu et al. 2000)
Flavonoids					Plants (Aiken 2014); food (Egert and Rimbach 2011); olive oil mill Ww (Leouifoudi et al. 2014)
Humic acids			220-320 (400-500) / 400-550	(IHSS 2015)	Municipal Ww (Huang et al. 2010)
Pharmaceutically active compounds	Carbamazepine		308 / 410 (in 2 mol L^{-1} HCl, and 20 min irradiation time)	(Hurtado-Sanchez Mdel et al. 2015)	Faeces, urine (Zhang et al. 2008)
	Fluorquinolone		290 / 500		
	Piroxican		294 / 372 (in media with pH < 2)		
Fluorescent whitening agents			360-365 / 400 - 440	(Takahashi and Kawamura 2006, Tavares et al. 2008)	Laundry detergents, sanitary products, toilet paper and tissues; Papermaking industry (Takahashi and Kawamura 2006, Assaad et al. 2014)

Ww – wastewater

As shown above, there are several fluorophores that contribute to the < 380 nm > Em regions, but the list is not exhaustive. More studies are needed to identify new fluorescent components and especially those specific to source with the highest contribution to EEMs. Since the regions exhibit the fluorescence of xenobiotic compounds, both can be used for wastewater quality assessment. In particular, peaks T and C, and the PARAFAC analogous components, are present in all wastewater studies (Tables SM1 and SM2) and may be applied to the control of wastewater treatment processes. However, it may be difficult to identify the source and type of sewage pollution in receiving water bodies. In this sense, Baker et al. (2014) advise caution and stress the importance of using a good sampling framework combined with an appropriate multivariate analysis of data for successful investigation of water pollution.

3 Correlation of the fluorescence peaks with BOD, COD and TOC

In order to assess the capability of fluorescence spectroscopy to act as a monitoring tool it is important to consider the correlations between fluorescence peaks and BOD, COD and TOC, commonly used indicators of OM concentration in natural waters and wastewater. As reviewed by Bourgeois et al. (2001) and (Jouanneau et al. 2014), BOD is a desirable measurement in treatment processes, it presents several disadvantages, which make this technique unsuitable for on-line monitoring and process control: it is slow to yield information, it is labour intensive, toxic substances affect bacteria, it may not reflect conditions in the treatment processes, it is insensitive and imprecise at low concentrations and has an uncertainty of 15-20% in the results. COD takes less time to give a result than BOD (2-4 h) and is not affected by toxic substances. However, it is still not suitable for on-line monitoring and process control due to the measuring time and because it requires hazardous chemicals. Also, COD is able to discriminate between biodegradable and biologically inert organic matter only in conjunction with BOD and not on its own (Bourgeois et al. 2001, Chen et al. 2014). TOC is very fast, as triplicates can be analyzed in minutes. However, it cannot differentiate between biodegradable and nonbiodegradable OM (Orhon et al. 2009). Also,

conflicting results have been reported between different techniques of measuring TOC (Bourgeois et al. 2001).

Correlation between fluorescence and standard parameters revealed that peaks T and C relate to BOD, COD and TOC, as reviewed by (Henderson et al. 2009). Slightly better correlation with BOD is seen at peak T compared to peak C. An exception to the above observation is found at the study of Wang et al. (2007) who obtained better correlation with the PARAFAC component exhibiting fluorescence in the peak C region, compared to the peak T component (Table 2). They observed the best correlation with BOD at the component similar to peak M (0.73). The researchers concluded that this component contributed the most to BOD for wastewater-impacted lakes. Nevertheless, these results highlight the complexity of the source and that there are potentially several fluorophores, which display fluorescence in the peak T/C regions. It also shows that both regions could contribute to BOD. The difference in correlation coefficients could also be determined by the low sample sizes in some studies, which might under or overestimate the relationship between fluorescence and BOD, COD and TOC (Table 2). Another cause of the difference could be the method used for data processing, as PARAFAC offers better separation of overlapping components compared to peak-picking.

Table 2. Correlation coefficients for peaks T and C (or PARAFAC analogous components) with BOD, COD and TOC.

Reference	Samples	Sample size	Sample pH	Analysis temperature	Fluorescence Peak	BOD	COD	TOC
Reynolds and Ahmad (1997)	Raw, settled and treated Ww	129	N/A	Room temperature	280 / 340	0.94-0.97	N/A	N/A
Ahmad and Reynolds (1999)	Raw Ww	25	3 - 7	10-80^0 C	248 / 350	0.97	N/A	N/A
Reynolds (2002)	Raw Ww	56	6.8 ±0.4	26 ±10^{0}C	280 / 350	0.93	0.94	0.93
Baker and Inverarity (2004)	Ww effluents and effluent impacted rivers	434	N/A	N/A	220 / 350	0.85	N/A	N/A
Wang et al. (2007)	Ww impacted lake	26	N/A	Room temperature	294 / 320	0.54	0.16	N/A
					360 / 425	0.65	0.03	N/A
Hudson et al. (2008)	Ww effluents	141	N/A	20^0 C	280 / 350	0.71	N/A	0.77
					300-370 / 400-500-	0.34	N/A	0.75
Bridgeman et al. (2013)	Domestic Ww, raw and treated	48	N/A	20^0 C	275-285 / 340-360	0.92	0.56	N/A
					320-355 / 410-470	0.88	0.78	N/A
Cohen et al. (2014)	Domestic and industrial Ww, raw and treated	25-34	7.8 – 8.5	Room temperature	<240 (275) / 346	0.82	0.82-0.99	0.85-0.99
					<240 (305) / 422	0.72	0.91	0.99
Ou et al. (2014)	Industrial Ww, raw and treated	120	7 - 9	Room temperature	280 / 320	N/A	0.92	N/A

Ww – wastewater; N/A – not available

Based on the correlation between BOD and peak T fluorescence, Hur and Kong (2008) tried to estimate, using SFS and first derivative spectra, the concentration of BOD of samples from urban rivers affected by treated sewage. They found that the relative fluorescence intensity, at *283 nm* to *245 nm* from SFS, is the optimum estimation index as it has the best positive correlation with BOD values (0.91). It has been reported that the multiple regression method, using the light scattering intensity at *633 nm* or turbidity, greatly enhances the correlation between measured and predicted BOD values. Hur and Kong (2008) also observed that filtered samples presented enhanced correlation; however, Bridgeman et al. (2013) reported slightly higher correlation coefficient between BOD and fluorescence at unfiltered samples compared to filtered with 0.45 or 0.2 µm. These differences could be site specific and may depend on the sizes of OM components.

As reviewed by Baker et al. (2014), the correlation between BOD and peak T fluorescence suggests a direct link with microbiological activity in this region of fluorescence, although the

source of peak T fluorescence is generally unknown. It was also implied that handheld instruments could be used in the future to investigate the temporal variability of BOD (Baker et al. 2014). Due to the relation with microbiological activity, peak T fluorescence was suggested as indicator of the presence / absence faecal coliforms (Sorensen et al. 2015, Sorensen et al. 2016). Pfeiffer et al. (2008) obtained excellent correlation (0.90 – 0.95) with faecal coliforms on samples from a wastewater polluted river and (Tedetti et al. 2012) found a good correlation (0.78) between the PARAFAC component and Escherichia Coli + enterococci on wastewater impacted coastal water samples. More recently, (Baker et al. 2015) obtained a log correlation of 0.74 between fluorescence and E. Coli measurements. These findings are encouraging, but more work should be done to explore the link between fluorescent components and faecal coliforms and its potential use in on-line monitoring applications. In a comparison with flow cytometer measurements, peak T intensity correlated with an increase of total live and dead bacteria numbers (Bridgeman et al. 2015). The researchers found that four bacteria isolated from a potable water tap sample showed different responses in the fluorescence signal, although the intensity of peak T fluorescence did not correlate with the bacteria counts. Nevertheless, peak T fluorescence could be used to assess the microbiological activity in a water system.

4 Fluorescence detection of wastewater pollution

Fluorescence spectroscopy has shown its capabilities as a real-time assessment tool for wastewater quality due to its advantages and correlation with standard parameters. This technique could be very effective in detecting raw wastewater contamination in water bodies. Also, the impact of wastewater effluents on natural waters could be evaluated, since effluent organic matter has different composition and characteristics from naturally occurring OM (Wang et al. 2015). Therefore it is important to look at the different types of wastewater for particular characteristics that may facilitate identification in the receiving water bodies.

4.1 Sources of wastewater

Studies published so far on fluorescence spectroscopy have focused on domestic, farm and industrial wastewater, which includes textile, pulp mill, coke or brewery industries. More studies are needed on wastewater from oil refineries, metal processing, fermentation factories, pharmaceutical industry, chemical plants, meatpacking and processing etc.

4.1.1 Domestic wastewater

Wastewater is the flow of water used by a community and includes household wastes, commercial and industrial waste stream flows, and stormwater (Drinan and Spellman 2012). Domestic wastewater contains the solid and liquid discharges of humans and animals, contributing with millions of bacteria, virus, and non-pathogenic and pathogenic organisms. It may also contain sanitary products, cleaners and detergents, trash, garbage and any other substances that are poured or flushed into the sewer system (Drinan and Spellman 2012). Public treatment facilities may also collect industrial effluents and thus chemicals, dyes, acids, alkalies, grit or detergents can be found in municipal wastewater (Drinan and Spellman 2012). Stormwater runoff, if collected by WwTPs, may bring into the system large amounts of sand, gravel, road-salt and other grit (Drinan and Spellman 2012).

As discussed in the previous sections, there are numerous compounds that may contribute to the fluorescence peaks. Generally, fluorescence spectra of untreated and treated domestic wastewater are characterized by intense peaks in the region Em < 380 nm, especially peak T, associated with high microbial abundance, and by significantly lower intensity peaks A and C fluorescence (Baker 2001, Hudson et al. 2007, Bridgeman et al. 2013). In some studies, the fluorescence spectra of effluents showed a higher prevalence of peaks A and C, compared to peaks T and B (Ghervase et al. 2010a, Riopel et al. 2014). Among peaks, T and C seem to be present at most municipal wastewater samples (Tables SM1 and SM2) and may serve as indicators of wastewater contamination. Peak B is rarely analysed at wastewater EEMs due to the potential

interferences from scattering; however, this fraction could indicate the proximity of the measurement point to the discharge point or freshness of the contamination. According to Pfeiffer et al. (2008), the fluorescence of both peak T and peak B decreases in intensity with increasing distance from the release point, but peak B is completely removed at longer distances, due to dilution or breakdown of the organic fraction. For peak B removal, seasonal shifts should also be taken into account as rainfall could contribute to dilution, sunlight irradiation could cause photodegradation or increase microbial uptake during summer (Meng et al. 2013).

From the myriad of fluorophores, FWAs may display distinctive features in the EEMs for municipal wastewater samples (Bridgeman et al. 2013). However, this fraction is not specific to domestic wastewater, as it has been detected at paper mill effluents (Baker 2002b, Ciputra et al. 2010, Bassandeh et al. 2013) or landfill leachates (Graham et al. 2015). Therefore, peaks T and C seem to be the best tools of monitoring domestic wastewater quality.

In addition to fluorescence intensity increase, it has been shown that discharge of domestic sewage may change the properties of OM from the receiving water bodies. For example, Xue et al. (2011) found that sewage effluents change the capacity of OM to form disinfection by-products and decrease its sensitivity to UV light. Also, changes in aromaticity and hydrophobicity of OM have been reported. These OM characteristics have been assessed after discharge, using the emission wavelength of peak C. In two studies undertaken by Goldman et al. (2012) on OM wastewater effluent and by Ghervase et al. (2010b) on untreated sewage discharge, it was found that the fluorescence signal of the two types of samples presented lower peak C emission wavelength, indicating lower aromaticity compared to natural OM. While, Spencer et al. (2007a) reported higher aromaticity of the OM from an estuarine sample with anthropogenic impact from domestic wastewater effluents, compared to the estuarine OM. Goldman et al. (2012) found that the mixture of effluent and river waters produce midrange values and, therefore, a potential increase in aromaticity with distance from discharge could be expected. In marine environments, fluorescence measurements on wastewater discharges showed great complexity of the mixing properties. Petrenko et al. (1997) observed 4 layers in the seawater column, 2 layers being affected by sewage representing the “old” and “new” plume waters and 2 layers unaffected by effluent. According to the researchers, the release of wastewater increased 2 fold to the concentration of ammonium, silicate and phosphate in sewage affected plumes and could stimulate the growth of phytoplankton. Baker and Inverarity (2004) also found an increase in nitrate and phosphate concentrations downstream of discharge into urban rivers.

4.1.2 Animal wastewater

Animal wastes represent an important source of water pollution, through the release of untreated wastewater or surface runoff from farms. This type of wastewater produces BOD values that are 1 to 3 times higher than sewage BOD (Baker 2002a). Most meat processing units treat the wastewater prior to release, however animal wastewater varies temporally in composition, requiring continuous monitoring for effective detection and removal of pollutants. Relatively few studies have looked at the potential of using fluorescence spectroscopy to monitor the quality of animal wastewater. However, data gathered so far can help define particular characteristics of animal wastewater OM. The fluorescence of animal wastewater is generally dominated by the region Em < 380 nm. In particular, peak T fluorescence seems to be common to all samples, as it has been detected at farmyard runoff (Old et al. 2012), pig and cattle slurry, silage liquor, sheep barn waste (Baker 2002a), poultry processing unit (Ghervase et al. 2010b) and cattle slaughter house (Louvet et al. 2013). The researchers also observed a low peak C fluorescence relative to peak T. Baker (2002a) calculated the ratio between the fluorescence intensity of these two peaks and found that peak T intensity was 2 to 25 times higher than that of peak C, the highest ratio being obtained for silage liquor, while the lowest was seen at the sheep barn waste. A similar peak T/C ratio was obtained by Old et al. (2012) at farmyard runoff samples. The ratio of peaks T and C fluorescence intensity shows that farm waste pollution events could leave a signature in river waters (Baker 2002a) and confirm the potential of using fluorescence as a low cost and rapid technique for tracing

animal derived pollutants (Old et al. 2012). Interestingly, pig and cattle slurry presented peak B fluorescence at a similar intensity to that of peak T. Peak B was also detected at poultry wastewater (Ghervase et al. 2010b), having even higher fluorescence than that of peak T. Ghervase et al. (2010b) suggested using the ratio of peak T and peak B to detect poultry wastewater pollution in rivers. However, this ratio applicability could be limited only to certain types of animal wastewaters.

Cattle slaughterhouse wastewater may contain albumin and haemoglobin that would contribute to the Em < 380 nm fluorescence region (Louvet et al. 2013). Also, bovine serum albumin may contribute to the fluorescence region of Em > 380 nm. Louvet et al. (2013) found another fluorescence peak that could belong to metalloporphyrins ($\lambda_{excitation}$ / $\lambda_{emission}$ = *400 - 440 nm / 450 - 510 nm)*. These components are attributed to red blood, which is a major pollutant in slaughterhouse wastewater. Again, the ratio of peaks T and C fluorescence intensity was found to be an effective indicator of biodegradation of slaughter house wastewater (Louvet et al. 2013). Nevertheless, the composition of animal derived pollutants is highly variable in time and depends on the animal species, physiological state and diet (Baker 2002a, Louvet et al. 2013). Therefore, more studies are needed to better understand the properties of OM from animal derived wastewater and set clear characteristics for enhanced detection of pollution events.

4.1.3 Industrial sources of wastewater

Industrial wastewater is primarily derived from the manufacturing and processing of chemicals, textiles, wood, pulp mill or paper. The composition of effluents varies depending on the raw materials used, the type of process and the efficiency of material removal (Sánchez Rojas and Bosch Ojeda 2005). Studies on continuous monitoring and evaluation of industrial wastewater using fluorescence spectroscopy are scarce, limiting identification of particular features of wastewater fluorescence spectra. Few studies focussed on wastewater from petrochemical, chemical and biochemical industry (Borisover et al. 2011), brewery (Janhom et al. 2009, Janhom et al. 2011), textile (Li et al. 2015), pulp mill and paper processing (Baker 2002b, Ciputra et al. 2010, Cawley et al. 2012, Bassandeh et al. 2013) computer components manufacturing (Cohen et al. 2014) and coke industry (Ou et al. 2014). In one short-term monitoring study, Yang et al. (2015a) analysed and compared the fluorescence spectra of samples from the effluents of 57 facilities belonging to 12 industrial categories (non-alcoholic drinks, electronic devices, food, leather and fur, meat, organic chemicals, pulp and paper, petrochemical, resin and plastic, steel, steam-power and textile dyeing) aiming to evaluate the potential of fluorescence spectroscopy to identify wastewater sources. The researchers were able to characterise and differentiate industrial effluents using cluster analysis, EEM-PARAFAC and FT-IR. Components from both < 380 nm > regions were observed, but no component dominated over all samples. For instance, the peak T component presented the highest fluorescence intensity at leather and fur wastewater, while peak C components dominated the EEMs of food wastewater samples. Therefore, Yang et al. (2015a) concluded that, without additional analyses it may be difficult to identify an industrial source with fluorescence spectroscopy. However, Borisover et al. (2011) observed a bathochromic shift of the peak T component induced by polarity and composition of local environment. They studied samples collected from rivers impacted by industrial effluents of oil refineries, petroleum and chemical and biochemical plants. The researchers recommended using this component as fluorescent tracer of non-specific industrial pollution.

Studies that evaluated wastewater samples from particular industries have identified specific fluorophores. For example, at pulp mill wastewater effluents, Cawley et al. (2012) found a component that was attributed to lignosulfonic acid or to a mixture of fluorophores from the many lignin degradation products. However, the authors highlighted that this component may exhibit different emission maxima depending on variations in the actual chemical moieties present in each sample. A similar component was found by Bassandeh et al. (2013) at samples collected from the biologically treated effluent of a newsprint mill and the authors attributed it to lignins or chemicals involved in the paper making process. Cawley et al. (2012) and Bassandeh et al. (2013) both

identified distinctive PARAFAC peaks for the lignin derived components. However, Santos et al. (2001) observed very intense peaks and additional shoulders at the peak C for samples collected from rivers downstream of pulp mill effluent discharge. Also, compared to samples upstream, the researchers detected an additional peak at $\lambda_{excitation} / \lambda_{emission}$ *~290 / ~340 nm*, which coincides with the peak T fluorescence. Baker (2002b) suggested that peak T fluorescence results from the lignin and sugars produced by the pulping process, which are likely to be rich in aromatic proteins. This component correlated with TOC (r=0.62, N=18), indicating that peak T fluorescence was a significant contributor to the TOC at paper mill effluents, as this correlation was not seen at the river samples. In addition to lignin derived components, Baker (2002b) identified a peak associated with FWAs, which are commonly used in papers. The differences in results, found by these studies, could be attributed to variations in chemical moieties or to the fact that Cawley et al. (2012) and Bassandeh et al. (2013) used PARAFAC for data processing to provide better separation between lignin and other peak T or peak C fluorophores.

A distinctive feature was also detected at textile industry effluents by Li et al. (2015), who found a triple excitation component with emission wavelength at *460 nm*. They considered this feature as specific to textile-derived components, because most fluorophores in region Em > 380 nm present dual excitation peaks at emission wavelength between *400* and *500 nm*. The triple excitation peaks were associated with 1-amino-2-naphtol structure, based on a spectral comparison with the standard solution and were suggested to be used as specific indicators in textile effluents. Li et al. (2015) also found that for peak T fluorescence there were much more species with varying emission wavelengths, which could relate to azo dyes as these substances emit similar fluorescence in this region.

As shown in section 2.2 and Table 1, peak B fluorescence could represent phenol-like matter, hydrocarbons or cresols as found by Ou et al. (2014) at coke wastewater samples. In addition to peak B and peak C fluorophores, Ou et al. (2014) identified a component associated with heterocyclic components and polycyclic aromatic hydrocabons (PAHs), such as fluoranthene or naphtol. PAHs were also detected by Cohen et al. (2014) at samples collected from a WwTPs that receives 50% of its crude wastewater from a computer component factory. Based on spectral similarities, Cohen et al. (2014) suggested that this component contains a pyrene-like moiety.

While for textile, pulp mill or coke wastewater, distinctive components have been identified, brewery wastewater has been shown to contain only the typical peaks T, A and C (Janhom et al. 2009, Janhom et al. 2011), generated by the cleaning and washing of raw materials. They also showed that the fluorescence of brewery wastewater samples belonged primarily to hydrophobic acids and hydrophilic bases OM fractions.

4.2 Wastewater tracking in aquatic systems

Discrimination between sources using fluorescence spectroscopy may be challenging since domestic wastewater can be mixed with industrial effluents and agricultural runoffs (Andersen et al. 2014). Industrial wastewater could also contain domestic discharges from the toilets and kitchens within factories (Reynolds and Ahmad 1995). Moreover, organic pollutants like optical brighteners, PAHs or lignins have widespread application and thus can be found in any type of wastewater.

In particular for industrial wastewater it may be more difficult to separate sources due to the varied composition of the solution. The release of industrial effluents in water bodies may lead to the production of fluorescent fractions formed of a mixture of proteinaceous and non-proteinaceous substances, which generates a bathchromic shift in the typical peak T fluorescence emission wavelength. According to Borisover et al. (2011) this component may be used as a tracer of non-specific industrial pollution. However, various industrial wastewaters produce high quantities of particular fluorophores like PAHs or heterocyclic compounds, differentiating them from domestic wastewater. As shown by Cohen et al. (2014) the pyrene-like components separated the wastewater with 50% industrial input from the more domestic wastewater sources. Also, the devices, developed by Tedetti et al. (2013) and Puiu et al. (2015), that separate PAHs from other peak T fluorophores, hold great promise in detecting both domestic and industrial sources of pollution. Additionally,

chemical separation can be undertaken by the use of time resolved laser induced fluorescence, which is capable to identify components based on their lifetimes. PAHs have a relatively long fluorescence lifetimes and great quantum efficiency, which help at distinguishing PAHs from the OM background (McGowin 2005).

However, the question remains as to how to differentiate between wastewater from domestic, animal farms and industry sources, which are characterized by intense Em < 380 nm region. Domestic wastewater contains PAHs (Huang et al. 2010), which have a distinctive fluorescence signal; however, the quantities could be too low in comparison to other fluorophores and therefore the fluorescence of PAHs could be exceeded by other compounds.

Component distinction can also be undertaken by PARAFAC, which may be able to separate overlapping components or identify specific pollutant indicators (Cohen et al. 2014, Yang et al. 2015b). However, in case of low concentrated pollutants, such as detergents, peak picking has been shown to be more effective than PARAFAC (Mostofa et al. 2010). Therefore, a combination of these techniques could better provide a thorough view of the sample composition and OM interaction with pollutants. Fluorescence spectroscopy could be used as an early warning system in case of accidental pollution and could serve as a quick method in initial identification of the source of wastewater, before more complex and expensive analyses would be employed.

5 Control of wastewater treatment processes using fluorescence spectroscopy

Two decades ago, the studies of Reynolds and Ahmad (1995) and Tartakovsky et al. (1996) demonstrated the potential of using fluorescence spectroscopy for both off- and on-line monitoring in wastewater treatment. Recent studies have suggested that this technique could be applied to process control and optimization (Bridgeman et al. 2013). With increasingly stringent regulation it will be more difficult to control treatment efficiency with current techniques, (BOD, COD and TOC), which are expensive, time-consuming and unreliable (Bridgeman et al. 2013, Rehman et al. 2015). More pressure is put on WwTPs when other environmental implications, such as energy and chemical consumption or greenhouse gases emissions are considered (Wang et al. 2015). Fluorescence spectroscopy offers a robust technique available for a rapid and low cost estimation of effluent quality. However, studies on fluorescence monitoring of WwTPs processes are scarce and only one long-term study at 5 municipal WwTPs has been achieved (Cohen et al. 2014). Also, only one real-time monitoring study has been published on two recycled water systems (Singh et al. 2015). According to Reynolds (2002), WwTPs are hostile environments, making continuous and dynamic monitoring of wastewater quality difficult due to problems associated with fouling. This would require regular cleaning, which is time consuming. In addition, the fluorescence signal could be affected by pH, IFE, temperature and metal ions, requiring subsequent corrections. However, recent development of devices, already on market, show great promise since they convert the on-line peak T fluorescence signal into BOD equivalent values, using an internal calibration factor or a multispectral approach (ChelseaInstruments 2015, ModernWater 2015, ZAPSTechnologies 2015). This type of instruments could provide an immediate estimation of changes in wastewater quality, displaying capabilities of effective process control.

5.1 Monitoring of fluorescent OM

Fluorescence real-time monitoring of wastewater quality is difficult to implement due to multiple potential factors that may interfere with the signal. The only real-time monitoring study was undertaken by (Galinha et al. 2011a) on a pilot scale membrane bioreactor system to predict performance parameters. EEMs were recorded for 10 months and processed with multivariate techniques. They concluded that although fluorescence was able to describe total COD for influent and effluent, it could not accurately predict other performance parameters and hence, fluorescence cannot totally replace conventional monitoring of membrane bioreactors (Galinha et al. 2011a). Nevertheless, real-time monitoring studies at full-scale WwTPs should be undertaken in order to assess the feasibility of the method and the issues that can arise from its implementation. The studies done on the monitoring of surface waters identified major issues and offered solutions,

which could be used to build a strategy for wastewater on-line monitoring. The issues reported so far include: biofilm formation, temperature, turbidity, inner filter effect, calibration procedure, presence of quenching elements. Most of these problems are thoroughly reviewed by Henderson et al. (2009). Therefore, only the recent studies will be discussed. Before the study of Carstea et al. (2010) no long-term, real-time monitoring experiments were done due to fouling issues. Carstea et al. (2010) showed that over a period of 11 days of continuous EEM recordings on an urban river, biofilm formation on the water extraction system had no influence on the fluorescence signal. However, higher rates of biofilm formation are expected in wastewater, compared to surface water, due to the large quantities of extracellular polymeric substances that enhance cell adhesion to solid surfaces (Tsuneda et al. 2003).

Regarding temperature, (Chen et al. 2015) tested a newly developed, portable laser induced fluorescence system, for its monitoring capabilities, on estuarine water and found that temperature changes affected the fluorescence results. (Yamashita et al. 2015) and (Khamis et al. 2015) also reported the impact of temperature on the fluorescence of OM, at monitoring studies on open ocean and urban river. Carstea et al. (2014a) have shown that peak T fluorescence suffers more thermal quenching at samples with higher urban anthropogenic impact compared to natural sources. Therefore, temperature could have a major impact on OM fluorescence from wastewater. However, a temperature-compensating tool has been proposed and texted by (Watras et al. 2011). (Khamis et al. 2015) also proposed a compensating tool for turbidity, which can have a great impact on the fluorescence signal when large particles are present. It is yet to be tested on wastewater samples.

The inner filter effect (IFE) is another major issue at wastewater samples. The IFE is the apparent decrease in the emitted fluorescence intensity or a distortion of the band-shape resulting from the absorption of the excited and emitted radiation (Henderson et al. 2009). (Kothawala et al. 2013) found that the best correction tool for the IFE is the absorbance based approach, proposed by Lakowicz (2006). This approach can be applied to samples with absorbance values of up to 1.5 cm^{-1}; at samples above this value a dilution of 2x is recommended (Kothawala et al. 2013). However, the study of Kothawala et al. (2013) was undertaken on lake water samples and it is not known if these rules apply to wastewater monitoring. As seen in Tables SM1 and SM2, for the wastewater evaluation studies there are two preferred methods for reducing the IFE: dilution and post-measurement mathematical correction. A dilution factor of 10 was used in some studies, while in others the samples were diluted until a specific absorbance value was achieved. Most studies report the absorbance values at wavelengths within the excitation region of peak T. In specific studies, no dilution was used to analyse samples as this procedure in not applied to on-line measurements (for example, (Baker and Inverarity 2004, Louvet et al. 2013, Li et al. 2014). However, IFE could be a serious issue for monitoring studies, as this factor might lead to an underestimation of the degree of pollution and poor prediction of BOD, COD or TOC. In this case, dilutions to a certain absorbance value (< 0.05 cm^{-1}, as used in most studies, Tables SM1 and SM2) or post-measurement IFE correction are recommended. However, other solutions should be found to counteract IFE, as the use of UV absorbance measurements, in addition to fluorescence spectroscopy, reduces the practicality of the method for on-line monitoring.

In addition, Yamashita et al. (2015) proposed fluorescence sensors calibration for dark blanks and/or sensitivity. Solutions of L-tryptophan (Sorensen, Khamis, Tedetti) and quinine sulphate (Chen, Conmy, Yamashita) are generally used as calibration standards for the two fluorescence regions. However, (Khamis et al. 2015) mention that uncalibrated systems may be used if qualitative data is needed.

Finally regarding the presence of quenching components, Wang et al. (2014) have proved that the presence of humic-like components could reduce the fluorescence of peak T in effluent organic matter. However, even more complex interactions could occur in wastewater samples. Galinha et al. (2011b) found that the addition of bovine serum albumin to domestic wastewater samples determined a decrease with 31-58 % of peak T fluorescence. They concluded that the complexity of interferences on the fluorescence signal might not allow the simple and direct quantitative measurement of specific fluorophores in complex biological systems, such as wastewater. Also, in a

study aiming to identify the contribution of extracellular polymeric substances to dye removal, (Wei et al. 2015) showed that methylene blue has a substantial quenching effect on peaks T and C fluorescence. Several studies (Baker 2001, 2002a, Spencer et al. 2007a, Xue et al. 2011) have stressed that, although peak T is dominant in fluorescence spectra of wastewater, it is very likely that sewage generates high quantities of other components, which may significantly impact peak T fluorescence. Nevertheless, a study conducted by Zhou et al. (2015) on a drinking water source contaminated with domestic wastewater, showed that all peaks were sensitive to pollutant concentration, especially peak T, which could be used as an early warning tool for contamination. Moreover, Goldman et al. (2012) were able to predict the percentage of municipal wastewater in rivers with 80 % confidence, by the use of multivariate linear regression and the fluorescence of both peak T and peak C. They recommended applying this model to develop in situ instruments, inform monitoring progress and develop additional water quality indicators.

5.2 Monitoring of treatment processes with fluorescence spectroscopy

Typical wastewater treatment begins with a series of physical operations (pre-treatment and primary treatment), such as screening and sedimentation to remove the floating and settleable solids. These steps are followed by biological processes, which are used to convert the finely divided and dissolved OM from wastewater into flocculant settleable biological solids (Tchobanoglous et al. 1991). Biological processes include the suspended growth activated sludge process, anaerobic/anoxic/oxic, sequencing batch reactor, membrane reactor, trickling filter, etc. Activated sludge is the most common process, involving the entrainment of air for microbial degradation of OM. In the final steps of the biological treatment, the sludge flocs are separated from the treated effluent, through sedimentation, before the effluent is discharged to a water body. In some WwTPs, additional treatment processes (tertiary and quaternary), such as filtration, chlorination, UV disinfection or reverse osmosis are adopted after the biological treatment and subsequent sedimentation (Yang et al. 2015b).

Few studies have focused, so far, on wastewater quality monitoring in treatment works, using fluorescence spectroscopy, to understand the behavior of OM along the process train, the removal of components and the potential of applying fluorescence as a control tool. Among these studies, some looked into the treatment of specific domestic/industrial wastewater (Janhom et al. 2009, Janhom et al. 2011, Zhu et al. 2011, Yu et al. 2013), the removal and behavior of refractory OM in treatment works (Hur et al. 2011), characterization of reverse osmosis permeates (Singh et al. 2009, Singh et al. 2012, 2015) or compared fluorescence EEM-PARAFAC and HPLC/HPSEC techniques (Li et al. 2014). Fluorescence monitoring of wastewater quality was performed at time frames spanning from 1 month to 20 months, by collecting samples from the inlet and outlet (Reynolds 2002, Riopel et al. 2014) or along different treatment steps (Singh et al. 2009, Hambly et al. 2010, Murphy et al. 2011, Singh et al. 2012, Bridgeman et al. 2013, Cohen et al. 2014, Ou et al. 2014, Singh et al. 2015). The longest monitoring study was undertaken by Cohen et al. (2014), who analyzed the wastewater quality from municipal treatment plants during 20 months. Most of the monitoring studies involved WwTPs that employed activated sludge, as biological treatment process. Nevertheless, a few long-term and short-term monitoring studies have proven the capacity of fluorescence to evaluate the treatment performance in plants that used trickling filters (Bridgeman et al. 2013), anaerobic/anoxic/oxic (Yu et al. 2014), a novel anoxic/aerobic/aerobic system (Ou et al. 2014) or other advanced biological treatments, such as phase isolated ditches, bio-Denipho process, sequencing batch reactors (Hur et al. 2011). Hur et al. (2011) found no difference in OM fluorescence characteristics between conventional and advanced biological treatment, while Bridgeman et al. (2013) were able to show, using fluorescence spectroscopy, that activated sludge was more effective than trickling filters, in removing the organic fraction. Variations in the fluorescence signal among WwTPs were also observed by Murphy et al. (2011). Nevertheless, the general consensus is that the behavior of certain fluorescence peaks can be followed along treatment plants to test performance. Cohen et al. (2014) suggested using both peak T and peak C components as indicators of total microbial activity in wastewater. Therefore, varied

instrumentation available on market or under development (Bridgeman et al. 2015) that measure both components may be applied to monitor treatment efficiency.

5.3 Removal of fluorescence components along the treatment plant processes

Studies have shown that the OM, especially in the region Em < 380 nm is significantly removed after the biological treatment process (Fig. 2). This is to be expected since the biological treatment removes biodegradable material (Cohen et al. 2014). Riopel et al. (2014) reported a 60% reduction in the peak T fluorescence. Within the Em < 380 nm region, peak T component experiences a different degree of removal compared to peak B component. Yu et al. (2013) found that peak T fluorescence decreases with 60 % in the anaerobic/anoxic zone, almost 40 % in the oxic zone and 5% in the final clarification process, whilst peak B fluorescence is reduced by 55%, almost 100% and 0% in the respective zones. Yu et al. (2014) reported slightly higher reduction percentages for peak B in the anaerobic/anoxic/oxic system. They also observed that peak T remained relatively consistent in the treatment process (41 - 48 %), but peak B decreased dramatically (33 - 7 %). However, Murphy et al. (2011) and Janhom et al. (2009) found a poor removal of peak B fluorescence. Janhom et al. (2009) stated that peak B substances are not considered refractory and suggested that these substances could be related to some humic-bound proteinaceous constituents, which may be biologically resistant. Nevertheless, Cohen et al. (2014) advises caution when comparing the sensitivity of fluorescent components to wastewater treatment due to possible multiple differences in the treatment system. In addition to the biological treatment, Cohen et al. (2014) found that soil-aquifer treatment causes a further significant decrease in the concentration of the OM fluorescing in the Em < 380 nm region. Murphy et al. (2011) and Hambly et al. (2010) also observed that chlorination generated a high removal rate of the peak T fraction at recycled treatment plants.

Compared to peaks T and B components, peaks A and C are removed to a lower extent in the first stages of the treatment works (Fig. 2). Riopel et al. (2014) reported a reduction in the peak C component of 28 % and an increase in peak M with 4 % from influent to effluent. Cohen et al. (2014) found that one component in the Em > 380 nm region, sensitive to microbial activity, was removed, while other two components could not be removed by the biological treatment. Yu et al. (2013) observed a reduction in peak C - like component below 10 %. Later, Yu et al. (2014) showed that one component in the region Em > 380 nm increases from 6 % in the primary treatment to 19 % after the biological treatment. An increase in the fluorescence of this component was observed by Ou et al. (2014) in anoxic and aerobic treatments. Poor degradation of these components was also reported by Janhom et al. (2011) at an activated sludge treatment process. Yu et al. (2015b) found that with increasing retention times at sequencing batch reactor the peak C components increase in the soluble microbial products. These products are generated by substrate utilization or biomass decay and cell lysis, and are regarded as autochthonous matter. Cohen et al. (2014) and Riopel et al. (2014) suggest that these fluorescent components are either potentially produced during the process or are recalcitrant to decomposition. Riopel et al. (2014) mention that large molecules degrade into smaller molecules that have a fulvic-like behavior, based on the polyphenol postulate of humic susbtances formation. They explain that due to the high microbial activity in WwTPs, the secreted exocellular enzymes will oxidize the polyphenols into quinones. The quinones will agglomerate with metabolites like amino acids or peptides, leading to the formation of humic polymers, which could be fulvic acids because they are smaller in size. Another explanation for the poor removal of these components is provided by Hur et al. (2011) who studied the fate of refractory OM in WwTPs. Refractory OM is not easily removed by the biological treatment process due to its recalcitrant nature. Moreover, Hur et al. (2011) showed that in most WwTPs, the percentage distribution of refractory OM increases in the effluents.

Tertiary and quaternary treatment stages are responsible for removing most of the fraction that fluoresces in the region Em > 380 nm (Fig. 2). Hambly et al. (2010) observed that chlorination generated a higher reduction in peak C compared to previous treatment steps. Singh et al. (2012) found a minimum of 97 % removal of peak C fluorophores after the reverse osmosis process.

Murphy et al. (2011) also reported almost complete removal of components following reverse osmosis treatment step.

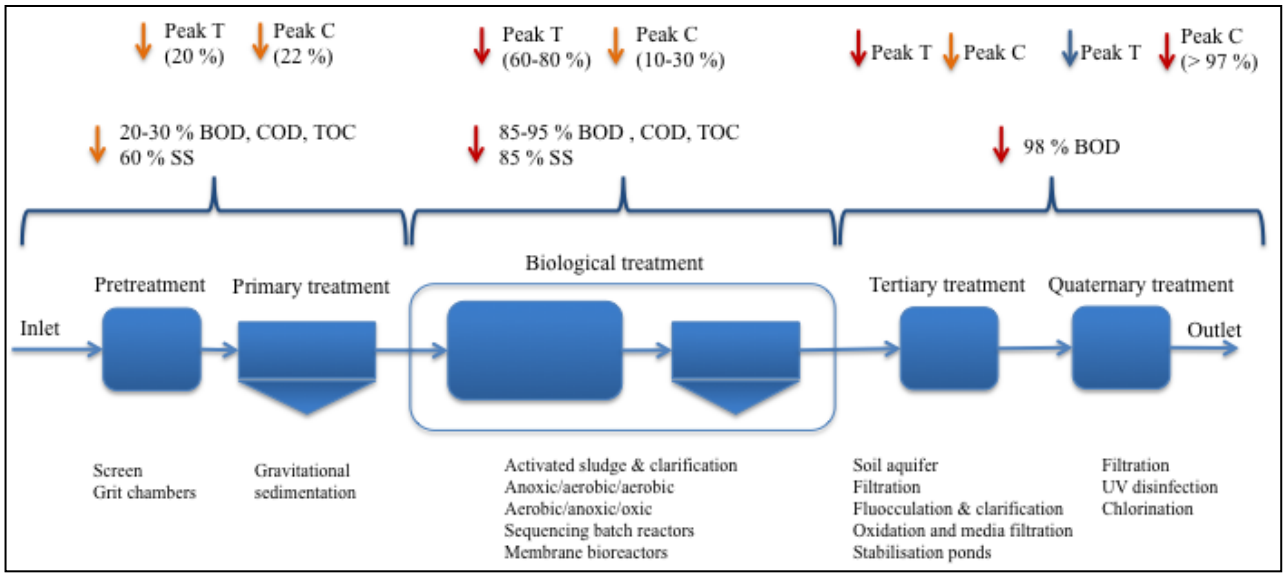

Figure 2. Removal of fluorescent components during treatment; the removal percentages represent collective values from several studies (Tchobanoglous and Burton 1991, Reynolds 2002, Hambly et al. 2010, Janhom et al. 2011, Murphy et al. 2011, Singh et al. 2012, Cohen et al. 2014, Ou et al. 2014, Riopel et al. 2014, Yu et al. 2014) and unpublished data. Blue arrow – low decrease, Orange arrow – moderate removal, red arrow – high removal.

Removal of fluorescent compounds, like FWAs and PAHs, was also analysed. Bridgeman et al. (2013) found FWAs only in crude wastewater and not after other treatment steps, concluding that this fluorescent fraction associates with particulate matter, which is removed by the primary treatment stage. In addition, Tavares et al. (2008) stated that subsequent disinfection processes may further remove FWAs from wastewater. According to Hayashi et al. (2002), up to 80 % of FWAs are removed after the biological treatment, and thus these compounds could be used as molecular markers of less effective treatment processes. Ou et al. (2014) found that, for coke wastewater, the novel anoxic/aerobic/aerobic system successfully removed PAHs. While, Cohen et al. (2014) observed no reduction in the pyrene-like component along the treatment steps.

In most monitoring studies, other changes in the fluorescence spectra with regard to peak shape and position were observed. However, the findings regarding peak position are not consistent across studies, potentially due to differences in the treatment process or source of wastewater. For example, Zhu et al. (2011) observed that peak C presented a blue shift of 5 nm for the excitation wavelength and of 21 nm for the emission wavelength, from influent to effluent, at membrane bioreactor treated supermarket wastewater. Hur et al. (2011) reported a 20 nm excitation wavelength red shift between influent and effluent, at refractory OM from municipal wastewater. Yet, Riopel et al. (2014), using PARAFAC, found no change in the peak C position or shape between sample locations. Riopel et al. (2014) observed that the PARAFAC component similar to peak T was elongated to longer wavelengths at influent samples compared to effluent. They attributed this elongation to the free or bound nature of the components. In the study of Zhu et al. (2011), peak T fluorescence displayed a red shift of 5 nm in the emission wavelength, from influent to effluent (Zhu et al. 2011). According to Zhu et al. (2011), the red shift is associated with the presence of carbonyl containing substances, hydroxyl, alkoxyl, amino groups and carboxyl constituents, while a blue shift is linked to a decomposition of condensed aromatic moieties and the break-up of the large molecules into small molecules.

5.4 Fluorescence control and optimisation of treatment processes

Increasingly stringent regulation has put major pressure on water utilities to find new technologies and implement control concepts that would improve the overall performance of WwTPs (Rehman et al. 2015). As discussed in previous sections, fluorescence spectroscopy has the potential to be used as a highly effective monitoring technique of treatment quality. This could be achieved through the use of peak T fluorescence, which could replace the out-dated and inaccurate BOD (Bridgeman et al. 2013). Consequently, fluorescence spectroscopy could provide the WwTPs with the optimum tool for real-time control and remediation of plant performance failures (Chong et al. 2013).

Additionally, Bridgeman et al. (2013) and Ahmad and Reynolds (1995) suggested that fluorescence could improve the process control in activated sludge process. The bacteria and microorganisms that form the activated sludge are fed with wastewater containing organic waste. In order to sustain the biological activities into the activated sludge process for BOD reduction, air is pumped into the tanks to provide sufficient quantities of dissolved oxygen. Aeration is one of the most energy intensive operations from the WwTPs, almost 65 % of energy being consumed for the activated sludge process (Rehman et al. 2015). Water utilities often over aerate to ensure meeting discharge regulations (Bridgeman et al. 2013). It is estimated that, by monitoring OM in WwTPs, 40 % of the energy costs could be saved (Ahmad and Reynolds 1995). Thus, fluorescence may be used to optimize process control in treatment works and eliminate the unnecessary costs associated with overtreatment (Bridgeman et al. 2013).

Promising results regarding online monitoring and process control were obtained by Singh et al. (2015), who published the first real-time study on two municipal recycled treatment plants. The researchers used a peak C sensor to prove the robustness of the technique in detecting reverse osmosis membrane fouling and integrity. They showed that the sensor was sufficiently sensitive to detect subtle differences between membrane permeates and identify underperformance issues. Also, no indication of fouling on probe and no deviation of probe performance were observed, during the experimental period. This study demonstrated the potential of using fluorescence for treatment process assessment and control.

6 Conclusions and future considerations

Fluorescence has been shown to be a valuable technique to characterize and monitor wastewater in surface waters for tracking sources of pollution and in treatment plants for process control and optimization. The use of real-time fluorescence could lead to a positive change in the water industry, as they would be able to start immediate remedial actions in case of accidental pollution events, cut costs associated with complex analytical approaches and comply with discharge regulation.

In general, wastewater presents higher fluorescence intensity compared to natural waters, especially for peaks T and C. Several fluorophores, with varied origins, were shown to contribute to peaks T and C, hindering the identification of the source of wastewater pollution in natural water systems.

Wastewater treatment processes reduce the OM fluorescence . In particular, peak T is mostly reduced by the biological treatment, while peak C is removed through chlorination and reverse osmosis. Therefore, simple fluorometers with appropriate wavelength selectivity, particularly for peaks T and C could be used for online monitoring, in WwTPs.

In response to these findings, there are several simple probes or fluorometers available on market that measure these two components or more complex systems that convert the peak T fluorescence signal into BOD values. However, in case of monitoring surface waters contaminated with wastewater, the use of simple fluorometers may not be the best solution to identify the exact source and take the appropriate remedial actions. Single or double wavelength instruments could only be used as a time and cost effective first measure for early warning.

Implementation of fluorescence instrumentation for on-line monitoring is relatively slow due to several factors, such as high quantities of suspended solids, temperature, fouling etc. There are

fewer issues when dealing with effluents, but it is more difficult to extract quantitative data in the case of untreated or poorly treated wastewater.

In order to counteract these issues, dilution of samples is recommended: to a factor of 10 or to an absorbance value < 0.05 cm^{-1}, in the peak T absorbance region. Considering the high concentration of components it is very likely that the fluorescence of peaks T and C will be quenched. Moreover, wastewaters are highly variable in concentration and composition and therefore a general dilution factor may not be recommended. Also, post-measurement mathematical correction could be applied to fluorescence spectra of wastewater samples. The correction and compensation tools, developed in the recent years, for the fluorescence signal could correct the impact produced by external factors. Some correction methods have been added to PARAFAC and it is expected that more uniform tools would generate another significant leap forward into the development of fluorescence based monitoring tools.

Highlights

- Wastewater shows higher peak T and peak C fluorescence compared to natural waters
- Peak T reduced after biological treatment and peak C after chlorination and RO
- Fluorometers measuring peaks T and C may be used for online wastewater monitoring
- Dilution of samples, typically up to x10, useful to limit inner filter effect
- Research gaps: online application of fluorescence and rapid data processing tools

Acknowledgements

The work of E.M. Carstea was supported by the European Commission Framework Programme 7, through a Marie Curie Intra-European Fellowship (PIEF-GA-2012-329962).

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1301 Table SM1. Fluorescence peaks identified with the peak-picking method.

Samples	Dilution factor	Em < 380 nm region components (nm)						Em >380 nm region components (nm)						Other peaks (nm)		Reference
		B (nm)		T1 (nm)		T2 (nm)		A (nm)		C (nm)		FWA (nm)				
		Ex	Em	Ex	Em	Ex	Em	Ex	Em	Ex	Em	Ex	Em	Ex	Em	
Ww	N/A		300		350				400		500					Ahmad and Reynolds (1995)
Raw, settled and treated Ww	N/A			280	340											Reynolds and Ahmad (1997)
Ww impacted river	N/A			276-281	340-370					326-339	416-422					Baker (2001)
Paper mill discharge	N/A			277	351					344	433	300	425			Baker (2002b)
River sample downstream of Ww discharge				280	351					344	436			386	472	
Single Ww works, synthetic sewage	N/A			280	350											Reynolds (2002)
River samples with Ww effluents	0			280	350	220	350	220-260	400-460	300-350	400-460					Baker and Inverarity (2004)
River samples with Ww effluents	x 5 – x 100			280	350-360											Baker et al. (2004)
River samples downstream of domestic WwTPs	N/A											320, 345, 360	430			Takahashi and Kawamura (2006)
Ww effluents	x 1 – x 10			276-296	330-378	221-247	331-378	217-253	395-443	318-347	405-445					Hudson et al. (2008)
Municipal effluent	N/A			280	335	235	335			315	410					An et al. (2009)
Brewery Ww	N/A	230, 275	315	285	365	230	365	255	455	335-355	405-465			290, 500	400, 525	Janhom et al. (2009)
Recycling plant with RO	N/A					235-250	340-382	235-260	400-440	305-340	406-430					Singh et al. (2009)
Paper mill Ww effluent	x 10	230	300	280	300			230	400-450	320-330	410-450			290	425	Ciputra et al. (2010)
Recycled water treatment plant	N/A			300	350	225	350	235	426	325	426					Hambly et al. (2010)
Raw and effluent domestic Ww	Dilution to absorbance of < 0.1 cm^{-1} at 254 nm					250 - 310	280 - 340			350	380			370, 390	400, 420	Hur et al. (2011)
Brewery Ww	Dilution of fractions from resin fractionation			265-295	315-390	230	340-365	255-265	435-455	330-335	395-410			290, 365	400, 455	Janhom et al. (2011)

Ww	N/A			280-285	320-330	235-240	335-350			315-330	410-420					Zhu et al. (2011)
RO water recycling plant	N/A			285	350	235	350	240	400-426	340	400-426					Singh et al. (2012)
Paper mill Ww effluent	x 10	230	300	280	300			230	425	325	425			290	425	Bassandeh et al. (2013)
Municipal WwTPs	x 10			275	340	225-237	340-380	237-260	400-500	300-370	400-500	370	410			Bridgeman et al. (2013)
River contaminated with Ww and fishery effluents	Ww samples x 10 Fishery effluent x 20	270-280	300-320	270-280	320-350			250-260	400-500	330-350	420-480					Chiarandini Fiore et al. (2013)
Slaughterhouse Ww	0					260-290	300-375	250	460	320-370	410-460			400-440	450-510	Louvet et al. (2013)
Domestic and industrial WwTPs	IFE correction			275-296	340-380	216-237	340-380	237-260	400-500	300-370	400-500					Yu et al. (2013)
Downstream of municipal WwTPs	N/A			285	335					355	405			325	375	Gutierrez et al. (2014)
Municipal WwTPs	0	280	310			235	340	240	430	340	435					Li et al. (2014)
Coke Ww	x 10	275	310	275	340	210	340	260	380-460	350	420-480					Ou et al. (2014)
Municipal WwTPs	N/A	225-235	290-310	265-285	330-370	225-235	330-370	230-260	430-470	290-320	380-420			290-320	380-420	Yu et al. (2014)
Textile Ww effluents	N/A			280	325	230	340	250	460	310	460			365	460	Li et al. (2015)

Ww – wastewater; WwTPs – wastewater treatment plants; RO – reverse osmosis; IFE – inner filter effect; N/A – not available; FWAs – fluorescent whitening agents.

1315 Table SM2. Fluorescence peaks identified with PARAFAC method.

Samples	Dilution factor	Em < 380 nm region components (nm)			Em >380 nm region components (nm)						Reference
		Ex/Em	Ex/Em	Ex/Em	Ex/Em	Ex/Em	Ex/Em	Ex/Em	Ex/Em	Ex/Em	
Ww effluents impacted lake	N/A			294 / 320	322 / 407	360 / 425					Wang et al. (2007)
Municipal WwTPs	Dilution of concentrated samples to absorbance at 350 nm < 0.08 cm^{-1}	220, 275 / 339	230 / 345	220, 275 / 300	240, 280 / 370	260, 380 / 467	230, 340 / 422				Guo et al. (2010)
Ww effluents impacted river	N/A	275-280 / 337-351	225-230 / 340-347		300-310 / 428-447 235-255 / 425-447						Mostofa et al. (2010)
Household sewerage drainage		230 / 338-351 280 / 339-346			335-345 / 432-437; 240-250 / 425-443						
Washing machine sample		280 / 344 235 / 348			345 / 437 250 / 441						
Industrial effluent impacted river	N/A		280 / 368			<250, 345 / 438					Borisover et al. (2011)
Recycled WwTPs	IFE correction Samples with absorbance > 0.3 cm^{-1} were eliminated from database	<250 / 348 290 / 352		250 / 304 270 / 300	<250, 320 / 400	<250, 370 / 464				350 / 428	Murphy et al. (2011)
WwTPs	IFE correction	230, 275 / 345		220 / 305, 345	245, 323 / 425						Yu et al. (2013)
Pulp mill Ww effluent	Dilution to absorbance of <0.2 cm^{-1} at 254 nm	230 / < 350			250, 370 / 450	<250, 320 / 454	<250 / 436	270, 390 / 512	<250, 305 / 396	275, 330 / 436	Cawley et al. (2012)
Municipal effluent	IFE correction	<230, 270 / 346	<235, 275 / 306		280 / 386				235, 340 / 410	255, 365 / 444	Tedetti et al. (2012)
Municipal and industrial WwTPs	Dilution to absorbance of <0.05 cm^{-1} at 240 nm	<240, 275 / 346			<240, 305 / 422	255, 380 / 468	270, 350 / 432		245, 270, 280, 350, 375 / 384, 402	<240, 300 / 368, 444	Cohen et al. (2014)
Municipal WwTPs	0	235, 290 / 340	225, 280 / 330	225, 280 / 300; 230, 280 / 310	250, 350 / 440						Li et al. (2014)
Coke WwTPs	x 10			280 / 320	280, 300 / 380					250, 345 / 450	Ou et al. (2014)

Treated and untreated Ww	N/A	280-290 / 305-350			325 / 380-410	350 / 410-420					Riopel et al. (2014)
WwTPs	N/A	235, 275 / 354			235, 320 / 411	254, 360 / 450					Qin et al. (2015)
Industrial Ww effluents	Dilution to absorbance of <0.05 cm^{-1} at 254 nm	< 250, 285 / 347		280 / 316	< 250, 275 / 385	265, 360 / 447		<250 / 445	335 / 413		Yang et al. (2015a)
Domestic Ww	Dilution to absorbance of <0.05 cm^{-1} at 254 nm	280 / 336		270 / 316	240, 290 / 392	260, 365 / 444					Yu et al. (2015b)
Treated municipal Ww effluents	N/A	280 / 334	225 / 338	270 / 316					245, 300 / 402	260, 360 / 444	Yu et al. (2015a)

1316 Ww – wastewater; WwTWs – wastewater treatment works; IFE – inner filter effect; N/A – not available.