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Fluorescence spectroscopy for wastewater monitoring: a review

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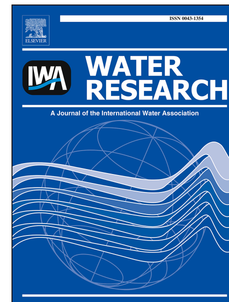

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1 # Fluorescence spectroscopy for wastewater
2 # monitoring: a review

3

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15

16 Abstract: Wastewater quality is usually assessed using
17 physical, chemical and microbiological tests, which are not
18 suitable for online monitoring, provide unreliable results, or use
19 hazardous chemicals. Hence, there is an urgent need to find a
20 rapid and effective method for the evaluation of water quality
21 in natural and engineered systems and for providing an early
22 warning of pollution events. Fluorescence spectroscopy has
23 been shown to be a valuable technique to characterize and
24 monitor wastewater in surface waters for tracking sources of
25 pollution, and in treatment works for process control and
26 optimization. This paper reviews the current progress in

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27 applying fluorescence to assess wastewater quality. Studies
28 have shown that, in general, wastewater presents higher
29 fluorescence intensity compared to natural waters for the
30 components associated with peak T (living and dead cellular
31 material and their exudates) and peak C (microbially
32 reprocessed organic matter). Furthermore, peak T fluorescence
33 is significantly reduced after the biological treatment process
34 and peak C is almost completely removed after the chlorination
35 and reverse osmosis stages. Thus, simple fluorometers with
36 appropriate wavelength selectivity, particularly for peaks T and
37 C could be used for online monitoring in wastewater treatment
38 works. This review also shows that care should be taken in any
39 attempt to identify wastewater pollution sources due to
40 potential overlapping fluorophores. Correlations between
41 fluorescence intensity and water quality parameters such as
42 biochemical oxygen demand (BOD) and total organic carbon
43 (TOC) have been developed and dilution of samples, typically
44 up to x10, has been shown to be useful to limit inner filter
45 effect. It has been concluded that the following research gaps
46 need to be filled: lack of studies on the on-line application of
47 fluorescence spectroscopy in wastewater treatment works and
48 lack of data processing tools suitable for rapid correction and
49 extraction of data contained in fluorescence excitation-emission
50 matrices (EEMs) for real-time studies.
51

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52 Key words: fluorescence spectroscopy, wastewater, organic
53 matter, monitoring

54

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83 ## 1 Introduction

84 Environmental monitoring is applied to determine the
85 compliance with ambient and discharge standards and to
86 identify areas with persistent issues for timely and effective
87 remediation (Cahoon and Mallin 2013). Wastewater quality
88 assessment is an essential part of environmental monitoring due
89 to the high anthropogenic impact of treated and untreated
90 discharges on water bodies (Suthar et al. 2010). There are two
91 important aspects of wastewater quality monitoring: the first
92 concerns the detection of pollution events for early warning and
93 rapid remedial responses of water bodies, while the second
94 aspect relates to wastewater treatment works where quality
95 monitoring is required for process control and compliance with
96 regulations at the effluent discharge point (Bourgeois et al.
97 2001, Michael et al. 2015, Rehman et al. 2015).

98 The quality of wastewater is generally assessed using
99 physical, chemical and microbiological tests. Among these
100 techniques, reliance is often placed on biological oxygen
101 demand (BOD), chemical oxygen demand (COD) and total
102 organic carbon (TOC) (Bourgeois et al. 2001, Bridgeman et al.
103 2013). However, these global parameters depend on expensive

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104 or time-consuming methods, offering only snapshots of
105 moments in time (Bourgeois et al. 2001, Chong et al. 2013,
106 Yang et al. 2015a), which makes them unsuitable for online
107 monitoring. Research conducted almost two decades ago
108 (Ahmad and Reynolds 1995, Tartakovsky et al. 1996, Reynolds
109 and Ahmad 1997, Ahmad and Reynolds 1999) has shown that
110 fluorescence spectroscopy could be used for wastewater quality
111 assessment as a tool for discharge detection in natural water
112 systems and for process control in wastewater treatment plants
113 (WwTPs). Fluorescence is the release of energy in the form of
114 light when molecules or moieties, named fluorophores, are
115 excited with a high-energy light source (Lakowicz 2006,
116 Reynolds 2014). The technique has been suggested for its
117 multiple advantages: it is fast, inexpensive, reagentless,
118 requires little sample preparation, is highly sensitive and non-
119 invasive (Reynolds 2003, Hudson et al. 2007, Cao et al. 2009,
120 Henderson et al. 2009, Hambly et al. 2010, Murphy et al. 2011,
121 Chong et al. 2013, Yang et al. 2015a). According to Reynolds
122 (2002) fluorescence monitoring could provide rapid feedback,
123 allowing dynamic, high spatial and temporal resolution studies.
124 In the past decades, more studies have proved the
125 potential of fluorescence spectroscopy as a monitoring and
126 detection tool in natural and engineered systems. This
127 technique has been used successfully to characterize organic
128 matter in seawater (Coble et al. 1990, Coble 1996, Conmy et al.

129 2004, Drozdowska 2007), freshwater (Baker 2001, McKnight
130 et al. 2001, Spencer et al. 2007b, Carstea et al. 2009) or
131 estuarine water (Huguet et al. 2009). Also, it has been used to
132 monitor riverine organic matter and diesel pollution (Downing
133 et al. 2009, Carstea et al. 2010), evaluate drinking water
134 treatment processes (Bieroza et al. 2009, Cumberland et al.
135 2012, Shutova et al. 2014) or detect pesticides (Ferretto et al.
136 2014). Fluorescence spectroscopy has been used to assess the
137 quality of raw sewage and effluents (Baker 2001, Boving et al.
138 2004, Pfeiffer et al. 2008), industrial (Santos et al. 2001,
139 Borisover et al. 2011, Li et al. 2015), or farm (Baker 2002b,
140 Old et al. 2012) discharges into natural systems. Moreover,
141 recent studies on short and long-term fluorescence monitoring
142 along the WwTPs process train have been undertaken, to
143 determine the potential of the technique for treatment processes
144 control (for example, (Murphy et al. 2011, Bridgeman et al.
145 2013, Cohen et al. 2014, Ou et al. 2014, Singh et al. 2015).
146 Although considerable work has been done so far in this field,
147 there are still issues with regard to the “matrix effects”, as
148 reviewed by Henderson et al. (2009), or with fouling (Reynolds
149 2002) that must be overcome to allow application of the
150 technique in WwTPs.

151 Other reviews proved the potential of applying
152 fluorescence spectroscopy to water quality monitoring (Hudson
153 et al. 2007, Henderson et al. 2009, Fellman et al. 2010, Ishii

154 and Boyer 2012, Yang et al. 2015b). However, none of them
155 focused only on wastewater, which requires a specific
156 discussion due to its complexity in composition and impact on
157 the environment. Moreover, a growing number of studies are
158 published each year on the application of fluorescence
159 spectroscopy to wastewater quality evaluation, proving its
160 scientific and industrial importance. In this paper, we review
161 the current progress in applying fluorescence spectroscopy to
162 assess wastewater quality. The technique's capabilities as a
163 detection and early warning tool of pollution with treated or
164 raw wastewater from different sources are discussed. Also, its
165 potential for process control in WwTPs is presented.

166

167 2 Fluorescence assessment of wastewater components

168 2.1 Organic matter fluorescence assessment

169 The most common methods of recording fluorescence
170 spectra for wastewater are excitation – emission matrices
171 (EEM) and synchronous fluorescence spectra (SFS). EEMs
172 represent fluorescence contour maps, which comprise a series
173 of repeated emission scans recorded in a range of excitation
174 wavelengths (Coble 1996). SFS are obtained by scanning
175 simultaneously both excitation and emission monochromators
176 at a fixed wavelength interval between them (Patra and Mishra
177 2002, Reynolds 2003). For many years, since the mid-1970s,
178 SFS were preferred as a multidimensional technique for the

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179 analysis of complex solutions, because it provided better peak
180 resolution, compared to emission spectra, and faster recording
181 time than EEMs (Ryder 2005). However, the improvement of
182 instrumentation allowed researchers to obtain fast, high-
183 resolution EEM collection, which increased the method
184 popularity in the research community. In addition, EEMs offer
185 varied possibilities of data interpretation, from simple peak-
186 picking and Fluorescence Regional Integration to the more
187 complex Parallel Factor Analysis (PARAFAC) and Self-
188 Organizing Maps. Among these methods, peak-picking and
189 PARAFAC are the most popular in the research community
190 and therefore only these two methods will be discussed in the
191 following sections.

192 The peak-picking method is a very simple tool to identify
193 components based on their maximum intensity and
194 corresponding excitation and emission wavelength pairs (Coble
195 1996). An example of peak-picking analysis is shown in Figure
196 1 (a). According to Goldman et al. (2012), peak-picking is a
197 viable analysis technique and can be employed for the
198 development and use of a real-time tool and may be related to
199 custom sensors available today. However, its applicability may
200 be limited due to peak shifts, possible overlapping and
201 interferences between peaks (Yang et al. 2015b). Moreover, it
202 may lead to misleading observations by associating each peak

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203 with a specific fluorophore, when two excitation wavelengths
204 are seen at fluorescent components (Fig. 1).

205 PARAFAC is a mathematical tri-liniar model that
206 deconvolutes EEMs into chemically meaningful components
207 (Fig. 1b). It separates the contribution of different fluorophores
208 without additional assumptions about their excitation and
209 emission spectra (Cohen et al. 2014). A thorough description of
210 PARAFAC method and components in wastewater is given by
211 Yang et al. (2015b). PARAFAC has become common practice
212 in water quality studies, over the past 10 years (Murphy et al.
213 2014). Yang et al. (2015b) proposed that PARAFAC be
214 developed into a surrogate method for conventional water
215 quality parameters, treatability of organic matter (OM) and
216 performance of treatment processes. Yu et al. (2014) suggested
217 that the PARAFAC tool, the EEMizer, developed by Bro and
218 Vidal (2011), could be implemented to monitor on-line the
219 WwTPs performance. The studies of Yu et al. (2015a) implied
220 that PARAFAC is able to identify contamination events and
221 can be used for early warning, but the component that indicates
222 contamination must be spectrally different from the existing
223 components, without major spectral overlap, which may
224 undermine the online monitoring strategy. Similarly, Murphy et
225 al. (2011) showed that at times PARAFAC had difficulties
226 distinguishing between components, returning hybridized
227 spectra. Also, in a comparison between chromatographic

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228 fluorescence fingerprints and EEM-PARAFAC, Li et al. (2014)
229 showed that the latter method could not reflect the variety of
230 organic matter species with similar fluorescence, but different
231 physico-chemical properties. In addition, PARAFAC is
232 currently applied only as post-processing technique, making it
233 unsuitable for continuous monitoring. Also, there is no
234 consensus regarding the optimum model in terms of sample
235 size and variability (Yu et al. 2015a).

236 All these techniques have been employed successfully to
237 analyse OM from various natural to engineered sources. A
238 thorough review on OM fluorescence is provided by Hudson et
239 al. (2007) and Fellman et al. (2010). Crude sewage is a
240 combination of domestic waste, industrial discharges, surface
241 runoff and storm flow. Its composition varies depending on the
242 age and type of sewerage, time of day, weather conditions and
243 type of incoming sewer (Ahmad and Reynolds 1995, Hudson et
244 al. 2007). Ellis (2004) showed that the general organic
245 composition of wastewater is 50 % proteins, 14 %
246 carbohydrates, 10 % fats and oils and trace amounts of priority
247 pollutants and surfactants, which are present in detergents,
248 soaps, shampoo and similar consumer products. More recently,
249 Huang et al. (2010) found that fibres, proteins and sugars are
250 the largest groups of OM in wastewaters, accounting for 20.64
251 %, 12.38 % and 10.65 %, respectively, of the total TOC.
252 According to the researchers, food related substances are the

253 main source of OM in wastewaters (Huang et al. 2010). Using
254 gas chromatography/mass spectrometry, Huang et al. (2010)
255 detected 90 compounds from the groups of alkyls and aromatic
256 hydrocarbons, alkenes, alcohols, organic acids, ketones,
257 phenols, nitrogenous compounds, ethers, amines and esters. In
258 addition, they found lipids, volatile fatty acids, humic acids,
259 DNA + RNA, tannic acids and linear alkylbenzene sulfonates.
260 Within the organic composition, there are numerous
261 overlapping fluorophores that contribute to the EEMs (Aiken
262 2014). Due to the difficulty of assigning specific fluorophores
263 to the peaks identified in EEMs, the fluorescence of wastewater
264 will be discussed as two regions based on the classification
265 provided by Li et al. (2014): the region Em < 380 nm is
266 associated with fluorophores containing a limited number of
267 aromatic rings and the indole moiety of free tryptophan whilst
268 the region > 380 nm is associated with polycyclic aromatic
269 fluorophores.

270 **2.2 Region Em < 380 nm**

271 Based on the peak-picking method, fluorescence in this
272 region is represented by peak T ($\lambda_{excitation}$ / $\lambda_{emission}$ *~225 (~280) /*
273 *~350 nm*) and peak B ($\lambda_{excitation}$ / $\lambda_{emission}$ *~225 (~280) / ~305*
274 *nm*) (Fig. 1a). Peaks T and B have been observed in all studies
275 that used the peak-picking method for EEM processing,
276 irrespective of the wastewater source (Table SM1). These
277 peaks have been associated with living and dead cellular

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278 material and their exudates and indicate microbial activity
279 (Bridgeman et al. 2013) and material derived from
280 anthropogenic activities (Yu et al. 2014). In PARAFAC, the
281 region Em < 380 nm is generally identified as components with
282 2 excitation wavelengths and 1 emission wavelength (Fig. 1b)
283 in the same wavelength ranges as peaks T and B in the peak-
284 picking method. These components are identified in both
285 municipal and industrial wastewater samples; however, the
286 component similar to peak T is more common in wastewater
287 compared to other components in this region (Table SM2).

288 By examining the list of wastewater organic components
289 (Dignac et al. 2000, Huang et al. 2010, Navalon et al. 2011),
290 and the literature review of Aiken (2014), Stedmon and Cory
291 (2014) and Baker et al. (2014), the following components were
292 considered as contributors to the fluorescence in the region Em
293 < 380 nm: phenols (for example cresols), indoles, mono and
294 polyaromatic hydrocarbons, DNA, aromatic amino acids
295 (phenylalanine, tyrosine), degradation products of lignin (lignin
296 phenols, vanillic acid, syringic acid etc.). These compounds are
297 derived from domestic waste, chemical, pharmaceutical,
298 plastic, petrochemical, paper, leather or textile industries (del
299 Olmo et al. 1996, Pokhrel and Viraraghavan 2004, He et al.
300 2007, Tchaikovskaya et al. 2007, Tertuliani et al. 2008). The
301 potential contributing fluorophores to this region are presented
302 in Table 1.

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303

304 ## 2.3 Region Em > 380 nm

305 The peak-picking method classifies this region as
306 follows: Peak A ($\lambda_{excitation}$ / $\lambda_{emission}$ *~225 / 400 - 500 nm)*, peak C
307 ($\lambda_{excitation}$ / $\lambda_{emission}$ *300 - 350 / 400 - 500 nm)* and peak M
308 ($\lambda_{excitation}$ / $\lambda_{emission}$ *310 - 320 / 380 – 420 nm)* (Fig. 1a). All
309 studies done so far on wastewater OM have identified peak C
310 and most studies found peak A (Table 1); however, peak M
311 was analysed only by Yu et al. (2014) at municipal wastewater.
312 Most of the studies that employed PARAFAC for EEM
313 analysis identified a maximum of 4 components associated and
314 microbially and terrestrially derived DOM (example of two
315 components in Fig 1b). However, Ishii and Boyer (2012) have
316 identified the PARAFAC components common in natural and
317 engineered water systems: Component 1 similar to peak A with
318 excitation in the region < *230 – 260 nm* and emission between
319 *400* and *500 nm*; Component 2 similar to peaks A + C found in
320 excitation region < *240 – 275 (339 – 420 nm)* and emission
321 within *434 – 520 nm*; and Component 3 similar to peak A + M
322 appearing in the excitation domain *<240 – 260 nm (295 – 380*
323 *nm)* and within the *374 – 450 nm* emission range. According to
324 Ishii and Boyer (2012), component 1 is found mostly in OM
325 sources dominated by terrestrial precursor material. Component
326 2 was defined as reduced quinone-like and was identified in
327 OM from a wide variety of aquatic systems, including those

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328 dominated by terrestrial and microbial inputs. While,
329 component 3 fluorophores were defined as oxidised quinone-
330 like and were similar to those with terrestrial and marine
331 precursors. Component 1 has not been reported in wastewater
332 studies, but components 2 and 3 were seen at studies made on
333 municipal and industrial wastewater (Table SM2). Additional
334 components were observed in wastewater (Table SM2), but
335 they vary depending on source.

336 As shown in Table 1, there are several fluorophores that
337 could contribute to the fluorescence of region Em > 380 nm:
338 lignins, PAHs, flavonoids, humic acids, quinones, aromatic
339 ketones, fluorescent whitening agents (FWAs),
340 pharmaceutically active compounds (Dignac et al. 2000, Huang
341 et al. 2010, Aiken 2014, Baker et al. 2014, Stedmon and Cory
342 2014). Among these components, FWAs have been proposed
343 as an indicator of human faecal contamination (Assaad et al.
344 2014), sewer misconnections (Chandler and Lerner 2015) and
345 presence of landfill leachates (Graham et al. 2015). FWAs are
346 highly soluble and poorly biodegraded, and therefore likely to
347 pass through biological treatment in WwTPs (Kramer et al.
348 1996, Poiger et al. 1998, Assaad et al. 2014). Research has
349 shown that these components can be detected with handheld
350 fluorometers, which enhances the capability for in situ water
351 monitoring (Hartel et al. 2007). Nevertheless, issues with
352 detecting FWAs in waters have been reported: the fluorescence

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353 of other peak C fluorophores overlap the peaks of FWAs, these
354 components are easily photodegraded and DOM hinders the
355 reaction of FWAs (Kramer et al. 1996, Baker 2002a, Hartel et
356 al. 2007, Assaad et al. 2014). Solutions to overcome
357 fluorescence overlap have been proposed, yet the other issues
358 identified may limit the method's applicability in detecting
359 sewage. The following solutions have been proposed: a) to use
360 the photodegradation rate to separate FWAs from organic
361 matter (Hartel et al. (2007); b) to_take into account the
362 differences in shape of the photodecay curve between FWAs
363 and natural organic matter (Cao et al. (2009)); c) to use a
364 baseline correction method to compare the differences in
365 fluorescence intensity of FWA, between the regions *320 nm –*
366 *345 nm* and *345 nm – 360 nm*, with the same values for the
367 water samples (Takahashi and Kawamura (2006)); and d) to
368 apply three-way analysis of EEMs assisted by second-order
369 chemometric analyses (Gholami et al. 2015). Discrimination
370 between humic substances and FWAs was achieved by Boving
371 et al. (2004), who analysed FWAs in solution with humic acid
372 and tannic acid. FWAs were recorded at *344 nm* and *422 nm*
373 emission wavelength, and *250 nm* excitation wavelength. The
374 authors found that the second peak of the FWAs was separated
375 from humic acids by 22 nm, but there was a 4 nm separation
376 from tannic acid. Therefore, the $\lambda_{excitation} / \lambda_{emission} = 250 / 422$

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377 *nm* peak could be used for FWAs detection without
378 interference from humic acid.

379 As shown above, there are several fluorophores that
380 contribute to the < 380 nm > Em regions, but the list is not
381 exhaustive. More studies are needed to identify new fluorescent
382 components and especially those specific to source with the
383 highest contribution to EEMs. Since the regions exhibit the
384 fluorescence of xenobiotic compounds, both can be used for
385 wastewater quality assessment. In particular, peaks T and C,
386 and the PARAFAC analogous components, are present in all
387 wastewater studies (Tables SM1 and SM2) and may be applied
388 to the control of wastewater treatment processes. However, it
389 may be difficult to identify the source and type of sewage
390 pollution in receiving water bodies. In this sense, Baker et al.
391 (2014) advise caution and stress the importance of using a good
392 sampling framework combined with an appropriate
393 multivariate analysis of data for successful investigation of
394 water pollution.

395

396 **3 Correlation of the fluorescence peaks with BOD, COD**
397 **and TOC**

398 In order to assess the capability of fluorescence
399 spectroscopy to act as a monitoring tool it is important to
400 consider the correlations between fluorescence peaks and BOD,
401 COD and TOC, commonly used indicators of OM

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402 concentration in natural waters and wastewater. As reviewed by
403 Bourgeois et al. (2001) and (Jouanneau et al. 2014), BOD is a
404 desirable measurement in treatment processes, it presents
405 several disadvantages, which make this technique unsuitable
406 for on-line monitoring and process control: it is slow to yield
407 information, it is labour intensive, toxic substances affect
408 bacteria, it may not reflect conditions in the treatment
409 processes, it is insensitive and imprecise at low concentrations
410 and has an uncertainty of 15-20% in the results. COD takes less
411 time to give a result than BOD (2-4 h) and is not affected by
412 toxic substances. However, it is still not suitable for on-line
413 monitoring and process control due to the measuring time and
414 because it requires hazardous chemicals. Also, COD is able to
415 discriminate between biodegradable and biologically inert
416 organic matter only in conjunction with BOD and not on its
417 own (Bourgeois et al. 2001, Chen et al. 2014). TOC is very
418 fast, as triplicates can be analyzed in minutes. However, it
419 cannot differentiate between biodegradable and
420 nonbiodegradable OM (Orhon et al. 2009). Also, conflicting
421 results have been reported between different techniques of
422 measuring TOC (Bourgeois et al. 2001).

423 Correlation between fluorescence and standard
424 parameters revealed that peaks T and C relate to BOD, COD
425 and TOC, as reviewed by (Henderson et al. 2009). Slightly
426 better correlation with BOD is seen at peak T compared to peak

427 C. An exception to the above observation is found at the study
428 of Wang et al. (2007) who obtained better correlation with the
429 PARAFAC component exhibiting fluorescence in the peak C
430 region, compared to the peak T component (Table 2). They
431 observed the best correlation with BOD at the component
432 similar to peak M (0.73). The researchers concluded that this
433 component contributed the most to BOD for wastewater-
434 impacted lakes. Nevertheless, these results highlight the
435 complexity of the source and that there are potentially several
436 fluorophores, which display fluorescence in the peak T/C
437 regions. It also shows that both regions could contribute to
438 BOD. The difference in correlation coefficients could also be
439 determined by the low sample sizes in some studies, which
440 might under or overestimate the relationship between
441 fluorescence and BOD, COD and TOC (Table 2). Another
442 cause of the difference could be the method used for data
443 processing, as PARAFAC offers better separation of
444 overlapping components compared to peak-picking.

445 Based on the correlation between BOD and peak T
446 fluorescence, Hur and Kong (2008) tried to estimate, using SFS
447 and first derivative spectra, the concentration of BOD of
448 samples from urban rivers affected by treated sewage. They
449 found that the relative fluorescence intensity, at *283 nm* to *245*
450 *nm* from SFS, is the optimum estimation index as it has the best
451 positive correlation with BOD values (0.91). It has been

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452 reported that the multiple regression method, using the light
453 scattering intensity at *633 nm* or turbidity, greatly enhances the
454 correlation between measured and predicted BOD values. Hur
455 and Kong (2008) also observed that filtered samples presented
456 enhanced correlation; however, Bridgeman et al. (2013)
457 reported slightly higher correlation coefficient between BOD
458 and fluorescence at unfiltered samples compared to filtered
459 with 0.45 or 0.2 µm. These differences could be site specific
460 and may depend on the sizes of OM components.

461 As reviewed by Baker et al. (2014), the correlation
462 between BOD and peak T fluorescence suggests a direct link
463 with microbiological activity in this region of fluorescence,
464 although the source of peak T fluorescence is generally
465 unknown. It was also implied that handheld instruments could
466 be used in the future to investigate the temporal variability of
467 BOD (Baker et al. 2014). Due to the relation with
468 microbiological activity, peak T fluorescence was suggested as
469 indicator of the presence / absence faecal coliforms (Sorensen
470 et al. 2015, Sorensen et al. 2016). Pfeiffer et al. (2008) obtained
471 excellent correlation (0.90 – 0.95) with faecal coliforms on
472 samples from a wastewater polluted river and (Tedetti et al.
473 2012) found a good correlation (0.78) between the PARAFAC
474 component and Escherichia Coli + enterococci on wastewater
475 impacted coastal water samples. More recently, (Baker et al.
476 2015) obtained a log correlation of 0.74 between fluorescence

477 and E. Coli measurements. These findings are encouraging, but
478 more work should be done to explore the link between
479 fluorescent components and faecal coliforms and its potential
480 use in on-line monitoring applications. In a comparison with
481 flow cytometer measurements, peak T intensity correlated with
482 an increase of total live and dead bacteria numbers (Bridgeman
483 et al. 2015). The researchers found that four bacteria isolated
484 from a potable water tap sample showed different responses in
485 the fluorescence signal, although the intensity of peak T
486 fluorescence did not correlate with the bacteria counts.
487 Nevertheless, peak T fluorescence could be used to assess the
488 microbiological activity in a water system.
489

490 4 Fluorescence detection of wastewater pollution

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

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502

503 **4.1 Sources of wastewater**

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

510

511 ***4.1.1 Domestic wastewater***

512 Wastewater is the flow of water used by a community
513 and includes household wastes, commercial and industrial
514 waste stream flows, and stormwater (Drinan and Spellman
515 2012). Domestic wastewater contains the solid and liquid
516 discharges of humans and animals, contributing with millions
517 of bacteria, virus, and non-pathogenic and pathogenic
518 organisms. It may also contain sanitary products, cleaners and
519 detergents, trash, garbage and any other substances that are
520 poured or flushed into the sewer system (Drinan and Spellman
521 2012). Public treatment facilities may also collect industrial
522 effluents and thus chemicals, dyes, acids, alkalies, grit or
523 detergents can be found in municipal wastewater (Drinan and
524 Spellman 2012). Stormwater runoff, if collected by WwTPs,
525 may bring into the system large amounts of sand, gravel, road-
526 salt and other grit (Drinan and Spellman 2012).

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527 As discussed in the previous sections, there are numerous
528 compounds that may contribute to the fluorescence peaks.
529 Generally, fluorescence spectra of untreated and treated
530 domestic wastewater are characterized by intense peaks in the
531 region Em < 380 nm, especially peak T, associated with high
532 microbial abundance, and by significantly lower intensity peaks
533 A and C fluorescence (Baker 2001, Hudson et al. 2007, Hur
534 and Cho 2012, Bridgeman et al. 2013). In some studies, the
535 fluorescence spectra of effluents showed a higher prevalence of
536 peaks A and C, compared to peaks T and B (Ghervase et al.
537 2010a, Riopel et al. 2014). Among peaks, T and C seem to be
538 present at most municipal wastewater samples (Tables SM1
539 and SM2) and may serve as indicators of wastewater
540 contamination. Peak B is rarely analysed at wastewater EEMs
541 due to the potential interferences from scattering; however, this
542 fraction could indicate the proximity of the measurement point
543 to the discharge point or freshness of the contamination.
544 According to Pfeiffer et al. (2008), the fluorescence of both
545 peak T and peak B decreases in intensity with increasing
546 distance from the release point, but peak B is completely
547 removed at longer distances, due to dilution or breakdown of
548 the organic fraction. For peak B removal, seasonal shifts should
549 also be taken into account as rainfall could contribute to
550 dilution, sunlight irradiation could cause photodegradation or
551 increase microbial uptake during summer (Meng et al. 2013).

552 From the myriad of fluorophores, FWAs may display
553 distinctive features in the EEMs for municipal wastewater
554 samples (Bridgeman et al. 2013). However, this fraction is not
555 specific to domestic wastewater, as it has been detected at
556 paper mill effluents (Baker 2002a, Ciputra et al. 2010,
557 Bassandeh et al. 2013) or landfill leachates (Graham et al.
558 2015). Therefore, peaks T and C seem to be the best tools of
559 monitoring domestic wastewater quality.

560 In addition to fluorescence intensity increase, it has been
561 shown that discharge of domestic sewage may change the
562 properties of OM from the receiving water bodies. For
563 example, Xue et al. (2011) found that sewage effluents change
564 the capacity of OM to form disinfection by-products and
565 decrease its sensitivity to UV light. Also, changes in
566 aromaticity and hydrophobicity of OM have been reported.
567 These OM characteristics have been assessed after discharge,
568 using the emission wavelength of peak C. In two studies
569 undertaken by Goldman et al. (2012) on OM wastewater
570 effluent and by Ghervase et al. (2010b) on untreated sewage
571 discharge, it was found that the fluorescence signal of the two
572 types of samples presented lower peak C emission wavelength,
573 indicating lower aromaticity compared to natural OM. While,
574 Spencer et al. (2007a) reported higher aromaticity of the OM
575 from an estuarine sample with anthropogenic impact from
576 domestic wastewater effluents, compared to the estuarine OM.

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577 Goldman et al. (2012) found that the mixture of effluent and
578 river waters produce midrange values and, therefore, a potential
579 increase in aromaticity with distance from discharge could be
580 expected. In marine environments, fluorescence measurements
581 on wastewater discharges showed great complexity of the
582 mixing properties. Petrenko et al. (1997) observed 4 layers in
583 the seawater column, 2 layers being affected by sewage
584 representing the "old" and "new" plume waters and 2 layers
585 unaffected by effluent. According to the researchers, the release
586 of wastewater increased 2 fold to the concentration of
587 ammonium, silicate and phosphate in sewage affected plumes
588 and could stimulate the growth of phytoplankton. Baker and
589 Inverarity (2004) also found an increase in nitrate and
590 phosphate concentrations downstream of discharge into urban
591 rivers.

592

593 ***4.1.2 Animal wastewater***

594 Animal wastes represent an important source of water
595 pollution, through the release of untreated wastewater or
596 surface runoff from farms. This type of wastewater produces
597 BOD values that are 1 to 3 times higher than sewage BOD
598 (Baker 2002b). Most meat processing units treat the wastewater
599 prior to release, however animal wastewater varies temporally
600 in composition, requiring continuous monitoring for effective
601 detection and removal of pollutants. Relatively few studies

602 have looked at the potential of using fluorescence spectroscopy
603 to monitor the quality of animal wastewater. However, data
604 gathered so far can help define particular characteristics of
605 animal wastewater OM. The fluorescence of animal wastewater
606 is generally dominated by the region Em < 380 nm. In
607 particular, peak T fluorescence seems to be common to all
608 samples, as it has been detected at farmyard runoff (Old et al.
609 2012), pig and cattle slurry, silage liquor, sheep barn waste
610 (Baker 2002b), poultry processing unit (Ghervase et al. 2010b)
611 and cattle slaughter house (Louvet et al. 2013). The researchers
612 also observed a low peak C fluorescence relative to peak T.
613 Baker (2002b) calculated the ratio between the fluorescence
614 intensity of these two peaks and found that peak T intensity
615 was 2 to 25 times higher than that of peak C, the highest ratio
616 being obtained for silage liquor, while the lowest was seen at
617 the sheep barn waste. A similar peak T/C ratio was obtained by
618 Old et al. (2012) at farmyard runoff samples. The ratio of peaks
619 T and C fluorescence intensity shows that farm waste pollution
620 events could leave a signature in river waters (Baker 2002b)
621 and confirm the potential of using fluorescence as a low cost
622 and rapid technique for tracing animal derived pollutants (Old
623 et al. 2012). Interestingly, pig and cattle slurry presented peak
624 B fluorescence at a similar intensity to that of peak T. Peak B
625 was also detected at poultry wastewater (Ghervase et al.
626 2010b), having even higher fluorescence than that of peak T.

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627 Ghervase et al. (2010b) suggested using the ratio of peak T and
628 peak B to detect poultry wastewater pollution in rivers.
629 However, this ratio applicability could be limited only to
630 certain types of animal wastewaters.

631 Cattle slaughterhouse wastewater may contain albumin
632 and haemoglobin that would contribute to the Em < 380 nm
633 fluorescence region (Louvet et al. 2013). Also, bovine serum
634 albumin may contribute to the fluorescence region of Em > 380
635 nm. Louvet et al. (2013) found another fluorescence peak that
636 could belong to metalloporphyrins ($\lambda_{excitation}$ / $\lambda_{emission}$ = *400 -*
637 *440 nm / 450 - 510 nm)*. These components are attributed to red
638 blood, which is a major pollutant in slaughterhouse wastewater.
639 Again, the ratio of peaks T and C fluorescence intensity was
640 found to be an effective indicator of biodegradation of
641 slaughter house wastewater (Louvet et al. 2013). Nevertheless,
642 the composition of animal derived pollutants is highly variable
643 in time and depends on the animal species, physiological state
644 and diet (Baker 2002b, Louvet et al. 2013). Therefore, more
645 studies are needed to better understand the properties of OM
646 from animal derived wastewater and set clear characteristics for
647 enhanced detection of pollution events.

648

649 ***4.1.3 Industrial sources of wastewater***

650 Industrial wastewater is primarily derived from the
651 manufacturing and processing of chemicals, textiles, wood,

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652 pulp mill or paper. The composition of effluents varies
653 depending on the raw materials used, the type of process and
654 the efficiency of material removal (Sánchez Rojas and Bosch
655 Ojeda 2005). Studies on continuous monitoring and evaluation
656 of industrial wastewater using fluorescence spectroscopy are
657 scarce, limiting identification of particular features of
658 wastewater fluorescence spectra. Few studies focussed on
659 wastewater from petrochemical, chemical and biochemical
660 industry (Borisover et al. 2011), brewery (Janhom et al. 2009,
661 Janhom et al. 2011), textile (Li et al. 2015), pulp mill and paper
662 processing (Baker 2002a, Ciputra et al. 2010, Cawley et al.
663 2012, Bassandeh et al. 2013) computer components
664 manufacturing (Cohen et al. 2014) and coke industry (Ou et al.
665 2014). In one short-term monitoring study, Yang et al. (2015a)
666 analysed and compared the fluorescence spectra of samples
667 from the effluents of 57 facilities belonging to 12 industrial
668 categories (non-alcoholic drinks, electronic devices, food,
669 leather and fur, meat, organic chemicals, pulp and paper,
670 petrochemical, resin and plastic, steel, steam-power and textile
671 dyeing) aiming to evaluate the potential of fluorescence
672 spectroscopy to identify wastewater sources. The researchers
673 were able to characterise and differentiate industrial effluents
674 using cluster analysis, EEM-PARAFAC and FT-IR.
675 Components from both < 380 nm > regions were observed, but
676 no component dominated over all samples. For instance, the

677 peak T component presented the highest fluorescence intensity
678 at leather and fur wastewater, while peak C components
679 dominated the EEMs of food wastewater samples. Therefore,
680 Yang et al. (2015a) concluded that, without additional analyses
681 it may be difficult to identify an industrial source with
682 fluorescence spectroscopy. However, Borisover et al. (2011)
683 observed a bathochromic shift of the peak T component
684 induced by polarity and composition of local environment.
685 They studied samples collected from rivers impacted by
686 industrial effluents of oil refineries, petroleum and chemical
687 and biochemical plants. The researchers recommended using
688 this component as fluorescent tracer of non-specific industrial
689 pollution.

690 Studies that evaluated wastewater samples from
691 particular industries have identified specific fluorophores. For
692 example, at pulp mill wastewater effluents, Cawley et al.
693 (2012) found a component that was attributed to lignosulfonic
694 acid or to a mixture of fluorophores from the many lignin
695 degradation products. However, the authors highlighted that
696 this component may exhibit different emission maxima
697 depending on variations in the actual chemical moieties present
698 in each sample. A similar component was found by Bassandeh
699 et al. (2013) at samples collected from the biologically treated
700 effluent of a newsprint mill and the authors attributed it to
701 lignins or chemicals involved in the paper making process.

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

723 A distinctive feature was also detected at textile industry
724 effluents by Li et al. (2015), who found a triple excitation
725 component with emission wavelength at *460 nm*. They
726 considered this feature as specific to textile-derived

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727 components, because most fluorophores in region Em > 380
728 nm present dual excitation peaks at emission wavelength
729 between *400* and *500 nm*. The triple excitation peaks were
730 associated with 1-amino-2-naphtol structure, based on a
731 spectral comparison with the standard solution and were
732 suggested to be used as specific indicators in textile effluents.
733 Li et al. (2015) also found that for peak T fluorescence there
734 were much more species with varying emission wavelengths,
735 which could relate to azo dyes as these substances emit similar
736 fluorescence in this region.

737 As shown in section 2.2 and Table 1, peak B fluorescence
738 could represent phenol-like matter, hydrocarbons or cresols as
739 found by Ou et al. (2014) at coke wastewater samples. In
740 addition to peak B and peak C fluorophores, Ou et al. (2014)
741 identified a component associated with heterocyclic
742 components and polycyclic aromatic hydrocabons (PAHs),
743 such as fluoranthene or naphtol. PAHs were also detected by
744 Cohen et al. (2014) at samples collected from a WwTPs that
745 receives 50% of its crude wastewater from a computer
746 component factory. Based on spectral similarities, Cohen et al.
747 (2014) suggested that this component contains a pyrene-like
748 moiety.

749 While for textile, pulp mill or coke wastewater,
750 distinctive components have been identified, brewery
751 wastewater has been shown to contain only the typical peaks T,

752 A and C (Janhom et al. 2009, Janhom et al. 2011), generated by
753 the cleaning and washing of raw materials. They also showed
754 that the fluorescence of brewery wastewater samples belonged
755 primarily to hydrophobic acids and hydrophilic bases OM
756 fractions.
757

758 4.2 Wastewater tracking in aquatic systems

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

768 In particular for industrial wastewater it may be more
769 difficult to separate sources due to the varied composition of
770 the solution. The release of industrial effluents in water bodies
771 may lead to the production of fluorescent fractions formed of a
772 mixture of proteinaceous and non-proteinaceous substances,
773 which generates a bathchromic shift in the typical peak T
774 fluorescence emission wavelength. According to Borisover et
775 al. (2011) this component may be used as a tracer of non-
776 specific industrial pollution. However, various industrial

777 wastewaters produce high quantities of particular fluorophores
778 like PAHs or heterocyclic compounds, differentiating them
779 from domestic wastewater. As shown by Cohen et al. (2014)
780 the pyrene-like components separated the wastewater with 50%
781 industrial input from the more domestic wastewater sources.
782 Also, the devices, developed by Tedetti et al. (2013) and Puiu
783 et al. (2015), that separate PAHs from other peak T
784 fluorophores, hold great promise in detecting both domestic
785 and industrial sources of pollution. Additionally, chemical
786 separation can be undertaken by the use of time resolved laser
787 induced fluorescence, which is capable to identify components
788 based on their lifetimes. PAHs have a relatively long
789 fluorescence lifetimes and great quantum efficiency, which
790 help at distinguishing PAHs from the OM background
791 (McGowin 2005).

792 However, the question remains as to how to differentiate
793 between wastewater from domestic, animal farms and industry
794 sources, which are characterized by intense Em < 380 nm
795 region. Domestic wastewater contains PAHs (Huang et al.
796 2010), which have a distinctive fluorescence signal; however,
797 the quantities could be too low in comparison to other
798 fluorophores and therefore the fluorescence of PAHs could be
799 exceeded by other compounds.

800 Component distinction can also be undertaken by
801 PARAFAC, which may be able to separate overlapping

802 components or identify specific pollutant indicators (Cohen et
803 al. 2014, Yang et al. 2015b). However, in case of low
804 concentrated pollutants, such as detergents, peak picking has
805 been shown to be more effective than PARAFAC (Mostofa et
806 al. 2010). Therefore, a combination of these techniques could
807 better provide a thorough view of the sample composition and
808 OM interaction with pollutants. Fluorescence spectroscopy
809 could be used as an early warning system in case of accidental
810 pollution and could serve as a quick method in initial
811 identification of the source of wastewater, before more
812 complex and expensive analyses would be employed.
813

814 5 Control of wastewater treatment processes using 815 fluorescence spectroscopy

816 Two decades ago, the studies of Reynolds and Ahmad
817 (1995) and Tartakovsky et al. (1996) demonstrated the potential
818 of using fluorescence spectroscopy for both off- and on-line
819 monitoring in wastewater treatment. Recent studies have
820 suggested that this technique could be applied to process
821 control and optimization (Bridgeman et al. 2013). With
822 increasingly stringent regulation it will be more difficult to
823 control treatment efficiency with current techniques, (BOD,
824 COD and TOC), which are expensive, time-consuming and
825 unreliable (Bridgeman et al. 2013, Rehman et al. 2015). More
826 pressure is put on WwTPs when other environmental

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827 implications, such as energy and chemical consumption or
828 greenhouse gases emissions are considered (Wang et al. 2015).
829 Fluorescence spectroscopy offers a robust technique available
830 for a rapid and low cost estimation of effluent quality.
831 However, studies on fluorescence monitoring of WwTPs
832 processes are scarce and only one long-term study at 5
833 municipal WwTPs has been achieved (Cohen et al. 2014).
834 Also, only one real-time monitoring study has been published
835 on two recycled water systems (Singh et al. 2015). According
836 to Reynolds (2002), WwTPs are hostile environments, making
837 continuous and dynamic monitoring of wastewater quality
838 difficult due to problems associated with fouling. This would
839 require regular cleaning, which is time consuming. In addition,
840 the fluorescence signal could be affected by pH, IFE,
841 temperature and metal ions, requiring subsequent corrections.
842 However, recent development of devices, already on market,
843 show great promise since they convert the on-line peak T
844 fluorescence signal into BOD equivalent values, using an
845 internal calibration factor or a multispectral approach
846 (ChelseaInstruments 2015, ModernWater 2015,
847 ZAPSTechnologies 2015). This type of instruments could
848 provide an immediate estimation of changes in wastewater
849 quality, displaying capabilities of effective process control.
850

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851 **5.1 Monitoring of fluorescent OM**

852 Fluorescence real-time monitoring of wastewater quality
853 is difficult to implement due to multiple potential factors that
854 may interfere with the signal. The only real-time monitoring
855 study was undertaken by (Galinha et al. 2011a) on a pilot scale
856 membrane bioreactor system to predict performance
857 parameters. EEMs were recorded for 10 months and processed
858 with multivariate techniques. They concluded that although
859 fluorescence was able to describe total COD for influent and
860 effluent, it could not accurately predict other performance
861 parameters and hence, fluorescence cannot totally replace
862 conventional monitoring of membrane bioreactors (Galinha et
863 al. 2011a). Nevertheless, real-time monitoring studies at full-
864 scale WwTPs should be undertaken in order to assess the
865 feasibility of the method and the issues that can arise from its
866 implementation. The studies done on the monitoring of surface
867 waters identified major issues and offered solutions, which
868 could be used to build a strategy for wastewater on-line
869 monitoring. The issues reported so far include: biofilm
870 formation, temperature, turbidity, inner filter effect, calibration
871 procedure, presence of quenching elements. Most of these
872 problems are thoroughly reviewed by Henderson et al. (2009).
873 Therefore, only the recent studies will be discussed. Before the
874 study of Carstea et al. (2010) no long-term, real-time
875 monitoring experiments were done due to fouling issues.

876 Carstea et al. (2010) showed that over a period of 11 days of
877 continuous EEM recordings on an urban river, biofilm
878 formation on the water extraction system had no influence on
879 the fluorescence signal. However, higher rates of biofilm
880 formation are expected in wastewater, compared to surface
881 water, due to the large quantities of extracellular polymeric
882 substances that enhance cell adhesion to solid surfaces
883 (Tsuneda et al. 2003).

884 Regarding temperature, Chen et al. (2015) tested a newly
885 developed, portable laser induced fluorescence system, for its
886 monitoring capabilities, on estuarine water and found that
887 temperature changes affected the fluorescence results.
888 Yamashita et al. (2015) and Khamis et al. (2015) also reported
889 the impact of temperature on the fluorescence of OM, at
890 monitoring studies on open ocean and urban river. Carstea et al.
891 (2014) have shown that peak T fluorescence suffers more
892 thermal quenching at samples with higher urban anthropogenic
893 impact compared to natural sources. Therefore, temperature
894 could have a major impact on OM fluorescence from
895 wastewater. However, a temperature-compensating tool has
896 been proposed and tested by Watras et al. (2011). Khamis et al.
897 (2015) also proposed a compensating tool for turbidity, which
898 can have a great impact on the fluorescence signal when large
899 particles are present. It is yet to be tested on wastewater
900 samples.

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901 The inner filter effect (IFE) is another major issue at
902 wastewater samples. The IFE is the apparent decrease in the
903 emitted fluorescence intensity or a distortion of the band-shape
904 resulting from the absorption of the excited and emitted
905 radiation (Henderson et al. 2009). Kothawala et al. (2013)
906 found that the best correction tool for the IFE is the absorbance
907 based approach, proposed by Lakowicz (2006). This approach
908 can be applied to samples with absorbance values of up to 1.5
909 cm^{-1}; at samples above this value a dilution of 2x is
910 recommended (Kothawala et al. 2013). However, the study of
911 Kothawala et al. (2013) was undertaken on lake water samples
912 and it is not known if these rules apply to wastewater
913 monitoring. As seen in Tables SM1 and SM2, for the
914 wastewater evaluation studies there are two preferred methods
915 for reducing the IFE: dilution and post-measurement
916 mathematical correction. A dilution factor of 10 was used in
917 some studies, while in others the samples were diluted until a
918 specific absorbance value was achieved. Most studies report the
919 absorbance values at wavelengths within the excitation region
920 of peak T. In specific studies, no dilution was used to analyse
921 samples as this procedure in not applied to on-line
922 measurements (for example, (Baker and Inverarity 2004,
923 Louvet et al. 2013, Li et al. 2014). However, IFE could be a
924 serious issue for monitoring studies, as this factor might lead to
925 an underestimation of the degree of pollution and poor

926 prediction of BOD, COD or TOC. In this case, dilutions to a
927 certain absorbance value (< 0.05 cm^{-1}, as used in most studies,
928 Tables SM1 and SM2) or post-measurement IFE correction are
929 recommended. However, other solutions should be found to
930 counteract IFE, as the use of UV absorbance measurements, in
931 addition to fluorescence spectroscopy, reduces the practicality
932 of the method for on-line monitoring.

933 In addition, Yamashita et al. (2015) proposed
934 fluorescence sensors calibration for dark blanks and/or
935 sensitivity. Solutions of L-tryptophan (Tedetti et al. 2013,
936 Khamis et al. 2015, Sorensen et al. 2015) and quinine sulphate
937 (Conmy et al. 2004, Chen et al. 2015, Yamashita et al. 2015)
938 are generally used as calibration standards for the two
939 fluorescence regions. However, Khamis et al. (2015) mention
940 that uncalibrated systems may be used if qualitative data is
941 needed.

942 Finally regarding the presence of quenching components,
943 Wang et al. (2014) have proved that the presence of humic-like
944 components could reduce the fluorescence of peak T in effluent
945 organic matter. However, even more complex interactions
946 could occur in wastewater samples. Galinha et al. (2011b)
947 found that the addition of bovine serum albumin to domestic
948 wastewater samples determined a decrease with 31-58 % of
949 peak T fluorescence. They concluded that the complexity of
950 interferences on the fluorescence signal might not allow the

951 simple and direct quantitative measurement of specific
952 fluorophores in complex biological systems, such as
953 wastewater. Also, in a study aiming to identify the contribution
954 of extracellular polymeric substances to dye removal, Wei et al.
955 (2015) showed that methylene blue has a substantial quenching
956 effect on peaks T and C fluorescence. Several studies (Baker
957 2001, 2002b, Spencer et al. 2007a, Xue et al. 2011) have
958 stressed that, although peak T is dominant in fluorescence
959 spectra of wastewater, it is very likely that sewage generates
960 high quantities of other components, which may significantly
961 impact peak T fluorescence. Nevertheless, a study conducted
962 by Zhou et al. (2015) on a drinking water source contaminated
963 with domestic wastewater, showed that all peaks were sensitive
964 to pollutant concentration, especially peak T, which could be
965 used as an early warning tool for contamination. Moreover,
966 Goldman et al. (2012) were able to predict the percentage of
967 municipal wastewater in rivers with 80 % confidence, by the
968 use of multivariate linear regression and the fluorescence of
969 both peak T and peak C. They recommended applying this
970 model to develop in situ instruments, inform monitoring
971 progress and develop additional water quality indicators. Also,
972 Hur and Cho (2012) recommended the use of absorbance
973 values at 220 nm and 254 nm, and PARAFAC components
974 similar to peaks T and C, as estimation indices for BOD and
975 COD in wastewater effluent contaminated river.

976

977 5.2 Monitoring of treatment processes with fluorescence 978 spectroscopy

979 Typical wastewater treatment begins with a series of 980 physical operations (pre-treatment and primary treatment), such 981 as screening and sedimentation to remove the floating and 982 settleable solids. These steps are followed by biological 983 processes, which are used to convert the finely divided and 984 dissolved OM from wastewater into flocculant settleable 985 biological solids (Tchobanoglous et al. 1991). Biological 986 processes include the suspended growth activated sludge 987 process, anaerobic/anoxic/oxic, sequencing batch reactor, 988 membrane reactor, trickling filter, etc. Activated sludge is the 989 most common process, involving the entrainment of air for 990 microbial degradation of OM. In the final steps of the 991 biological treatment, the sludge flocs are separated from the 992 treated effluent, through sedimentation, before the effluent is 993 discharged to a water body. In some WwTPs, additional 994 treatment processes (tertiary and quaternary), such as filtration, 995 chlorination, UV disinfection or reverse osmosis are adopted 996 after the biological treatment and subsequent sedimentation 997 (Yang et al. 2015b).

998 Few studies have focused, so far, on wastewater quality 999 monitoring in treatment works, using fluorescence 1000 spectroscopy, to understand the behavior of OM along the

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1001 process train, the removal of components and the potential of
1002 applying fluorescence as a control tool. Among these studies,
1003 some looked into the treatment of specific domestic/industrial
1004 wastewater (Janhom et al. 2009, Janhom et al. 2011, Zhu et al.
1005 2011, Yu et al. 2013), the removal and behavior of refractory
1006 OM in treatment works (Hur et al. 2011), characterization of
1007 reverse osmosis permeates (Singh et al. 2009, Singh et al. 2012,
1008 2015) or compared fluorescence EEM-PARAFAC and
1009 HPLC/HPSEC techniques (Li et al. 2014). Fluorescence
1010 monitoring of wastewater quality was performed at time frames
1011 spanning from 1 month to 20 months, by collecting samples
1012 from the inlet and outlet (Reynolds 2002, Riopel et al. 2014) or
1013 along different treatment steps (Singh et al. 2009, Hambly et al.
1014 2010, Murphy et al. 2011, Singh et al. 2012, Bridgeman et al.
1015 2013, Cohen et al. 2014, Ou et al. 2014, Singh et al. 2015). The
1016 longest monitoring study was undertaken by Cohen et al.
1017 (2014), who analyzed the wastewater quality from municipal
1018 treatment plants during 20 months. _ENREF_23_ENREF_126Most
1019 of the monitoring studies involved WwTPs that employed
1020 activated sludge, as biological treatment process. Nevertheless,
1021 a few long-term and short-term monitoring studies have proven
1022 the capacity of fluorescence to evaluate the treatment
1023 performance in plants that used trickling filters (Bridgeman et
1024 al. 2013), anaerobic/anoxic/oxic (Yu et al. 2014), a novel
1025 anoxic/aerobic/aerobic system (Ou et al. 2014) or other

1026 advanced biological treatments, such as phase isolated ditches,
1027 bio-Denipho process, sequencing batch reactors (Hur et al.
1028 2011). Hur et al. (2011) found no difference in OM
1029 fluorescence characteristics between conventional and
1030 advanced biological treatment, while Bridgeman et al. (2013)
1031 were able to show, using fluorescence spectroscopy, that
1032 activated sludge was more effective than trickling filters, in
1033 removing the organic fraction. Variations in the fluorescence
1034 signal among WwTPs were also observed by Murphy et al.
1035 (2011). Nevertheless, the general consensus is that the behavior
1036 of certain fluorescence peaks can be followed along treatment
1037 plants to test performance. Cohen et al. (2014) suggested using
1038 both peak T and peak C components as indicators of total
1039 microbial activity in wastewater. Therefore, varied
1040 instrumentation available on market or under development
1041 (Bridgeman et al. 2015) that measure both components may be
1042 applied to monitor treatment efficiency.
1043

1044 **5.3 Removal of fluorescence components along the**
1045 **treatment plant processes**

1046 Studies have shown that the OM, especially in the region
1047 Em < 380 nm is significantly removed after the biological
1048 treatment process (Fig. 2). This is to be expected since the
1049 biological treatment removes biodegradable material (Cohen et
1050 al. 2014). Riopel et al. (2014) reported a 60% reduction in the

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1051 peak T fluorescence. Within the Em < 380 nm region, peak T
1052 component experiences a different degree of removal compared
1053 to peak B component. Yu et al. (2013) found that peak T
1054 fluorescence decreases with 60 % in the anaerobic/anoxic zone,
1055 almost 40 % in the oxic zone and 5% in the final clarification
1056 process, whilst peak B fluorescence is reduced by 55%, almost
1057 100% and 0% in the respective zones. Yu et al. (2014) reported
1058 slightly higher reduction percentages for peak B in the
1059 anaerobic/anoxic/oxic system. They also observed that peak T
1060 remained relatively consistent in the treatment process (41 - 48
1061 %), but peak B decreased dramatically (33 - 7 %). However,
1062 Murphy et al. (2011) and Janhom et al. (2009) found a poor
1063 removal of peak B fluorescence. Janhom et al. (2009) stated
1064 that peak B substances are not considered refractory and
1065 suggested that these substances could be related to some
1066 humic-bound proteinaceous constituents, which may be
1067 biologically resistant. Nevertheless, Cohen et al. (2014) advises
1068 caution when comparing the sensitivity of fluorescent
1069 components to wastewater treatment due to possible multiple
1070 differences in the treatment system. In addition to the
1071 biological treatment, Cohen et al. (2014) found that soil-aquifer
1072 treatment causes a further significant decrease in the
1073 concentration of the OM fluorescing in the Em < 380 nm
1074 region. Murphy et al. (2011) and Hambly et al. (2010) also
1075 observed that chlorination generated a high removal rate of the

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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

1101 molecules degrade into smaller molecules that have a fulvic-
1102 like behavior, based on the polyphenol postulate of humic
1103 susbtances formation. They explain that due to the high
1104 microbial activity in WwTPs, the secreted exocellular enzymes
1105 will oxidize the polyphenols into quinones. The quinones will
1106 agglomerate with metabolites like amino acids or peptides,
1107 leading to the formation of humic polymers, which could be
1108 fulvic acids because they are smaller in size. Another
1109 explanation for the poor removal of these components is
1110 provided by Hur et al. (2011) who studied the fate of refractory
1111 OM in WwTPs. Refractory OM is not easily removed by the
1112 biological treatment process due to its recalcitrant nature.
1113 Moreover, Hur et al. (2011) showed that in most WwTPs, the
1114 percentage distribution of refractory OM increases in the
1115 effluents.

1116 Tertiary and quaternary treatment stages are responsible
1117 for removing most of the fraction that fluoresces in the region
1118 Em > 380 nm (Fig. 2). Hambly et al. (2010) observed that
1119 chlorination generated a higher reduction in peak C compared
1120 to previous treatment steps. Singh et al. (2012) found a
1121 minimum of 97 % removal of peak C fluorophores after the
1122 reverse osmosis process. Murphy et al. (2011) also reported
1123 almost complete removal of components following reverse
1124 osmosis treatment step.

1125 Removal of fluorescent compounds, like FWAs and
1126 PAHs, was also analysed. Bridgeman et al. (2013) found
1127 FWAs only in crude wastewater and not after other treatment
1128 steps, concluding that this fluorescent fraction associates with
1129 particulate matter, which is removed by the primary treatment
1130 stage. In addition, Tavares et al. (2008) stated that subsequent
1131 disinfection processes may further remove FWAs from
1132 wastewater. According to Hayashi et al. (2002), up to 80 % of
1133 FWAs are removed after the biological treatment, and thus
1134 these compounds could be used as molecular markers of less
1135 effective treatment processes. Ou et al. (2014) found that, for
1136 coke wastewater, the novel anoxic/aerobic/aerobic system
1137 successfully removed PAHs. While, Cohen et al. (2014)
1138 observed no reduction in the pyrene-like component along the
1139 treatment steps.

1140 In most monitoring studies, other changes in the
1141 fluorescence spectra with regard to peak shape and position
1142 were observed. However, the findings regarding peak position
1143 are not consistent across studies, potentially due to differences
1144 in the treatment process or source of wastewater. For example,
1145 Zhu et al. (2011) observed that peak C presented a blue shift of
1146 5 nm for the excitation wavelength and of 21 nm for the
1147 emission wavelength, from influent to effluent, at membrane
1148 bioreactor treated supermarket wastewater. Hur et al. (2011)
1149 reported a 20 nm excitation wavelength red shift between

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1150 influent and effluent, at refractory OM from municipal
1151 wastewater. Yet, Riopel et al. (2014), using PARAFAC, found
1152 no change in the peak C position or shape between sample
1153 locations. Riopel et al. (2014) observed that the PARAFAC
1154 component similar to peak T was elongated to longer
1155 wavelengths at influent samples compared to effluent. They
1156 attributed this elongation to the free or bound nature of the
1157 components. In the study of Zhu et al. (2011), peak T
1158 fluorescence displayed a red shift of 5 nm in the emission
1159 wavelength, from influent to effluent (Zhu et al. 2011).
1160 According to Zhu et al. (2011), the red shift is associated with
1161 the presence of carbonyl containing substances, hydroxyl,
1162 alkoxyl, amino groups and carboxyl constituents, while a blue
1163 shift is linked to a decomposition of condensed aromatic
1164 moieties and the break-up of the large molecules into small
1165 molecules.
1166

1167 **5.4 Fluorescence control and optimisation of treatment**
1168 **processes**

1169 Increasingly stringent regulation has put major pressure
1170 on water utilities to find new technologies and implement
1171 control concepts that would improve the overall performance of
1172 WwTPs (Rehman et al. 2015). As discussed in previous
1173 sections, fluorescence spectroscopy has the potential to be used
1174 as a highly effective monitoring technique of treatment quality.

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1175 This could be achieved through the use of peak T fluorescence,
1176 which could replace the out-dated and inaccurate BOD
1177 (Bridgeman et al. 2013). Consequently, fluorescence
1178 spectroscopy could provide the WwTPs with the optimum tool
1179 for real-time control and remediation of plant performance
1180 failures (Chong et al. 2013).

1181 Additionally, Bridgeman et al. (2013) and Ahmad and
1182 Reynolds (1995) suggested that fluorescence could improve the
1183 process control in activated sludge process. The bacteria and
1184 microorganisms that form the activated sludge are fed with
1185 wastewater containing organic waste. In order to sustain the
1186 biological activities into the activated sludge process for BOD
1187 reduction, air is pumped into the tanks to provide sufficient
1188 quantities of dissolved oxygen. Aeration is one of the most
1189 energy intensive operations from the WwTPs, almost 65 % of
1190 energy being consumed for the activated sludge process
1191 (Rehman et al. 2015). Water utilities often over aerate to
1192 ensure meeting discharge regulations (Bridgeman et al. 2013).
1193 It is estimated that, by monitoring OM in WwTPs, 40 % of the
1194 energy costs could be saved (Ahmad and Reynolds 1995).
1195 Thus, fluorescence may be used to optimize process control in
1196 treatment works and eliminate the unnecessary costs associated
1197 with overtreatment (Bridgeman et al. 2013).

1198 Promising results regarding online monitoring and
1199 process control were obtained by Singh et al. (2015), who

1200 published the first real-time study on two municipal recycled
1201 treatment plants. The researchers used a peak C sensor to prove
1202 the robustness of the technique in detecting reverse osmosis
1203 membrane fouling and integrity. They showed that the sensor
1204 was sufficiently sensitive to detect subtle differences between
1205 membrane permeates and identify underperformance issues.
1206 Also, no indication of fouling on probe and no deviation of
1207 probe performance were observed, during the experimental
1208 period. This study demonstrated the potential of using
1209 fluorescence for treatment process assessment and control.
1210

1211 **6 Conclusions and future considerations**

1212 The use of real-time fluorescence could lead to a positive
1213 change in the water industry, as operators would be able to start
1214 immediate remedial actions in case of accidental pollution
1215 events, cut costs associated with complex analytical approaches
1216 and comply with discharge regulation. Wastewater treatment
1217 processes reduce peak T fluorescence primarily by biological
1218 treatment, and peak C through chlorination and reverse
1219 osmosis. There are several simple probes or fluorometers
1220 available on market that measure these two components or
1221 more complex systems that convert the peak T fluorescence
1222 signal into BOD values.

1223 However, in case of monitoring surface waters
1224 contaminated with wastewater, the use of simple fluorometers

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1225 may not be the best solution to identify the exact source and
1226 take the appropriate remedial actions. Several fluorophores,
1227 with varied origins, were shown to contribute to peaks T and C,
1228 hindering the identification of the source of wastewater
1229 pollution in natural water systems. Single or double wavelength
1230 instruments could only be used as a time and cost effective first
1231 measure for early warning.

1232 Implementation of fluorescence instrumentation for on-line
1233 monitoring is relatively slow due to several factors, such as
1234 high quantities of suspended solids, temperature, fouling etc. In
1235 order to counteract these issues, dilution of samples is
1236 recommended: to a factor of 10 or to an absorbance value of <
1237 0.05 cm^{-1}, in the peak T absorbance region. However,
1238 wastewaters are highly variable in concentration and
1239 composition and therefore a general dilution factor may not be
1240 recommended. In addition, post-measurement mathematical
1241 correction could be applied to reduce the impact produced by
1242 external factors.

1243

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1249

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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.

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.

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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 hydrocarbons		Em < 380 nm	Short UV	Baker et al. (2014)	Municipal Ww (Guo et al. 2010, 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-	IHSS (2015)	

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			500) / 400-550		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

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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

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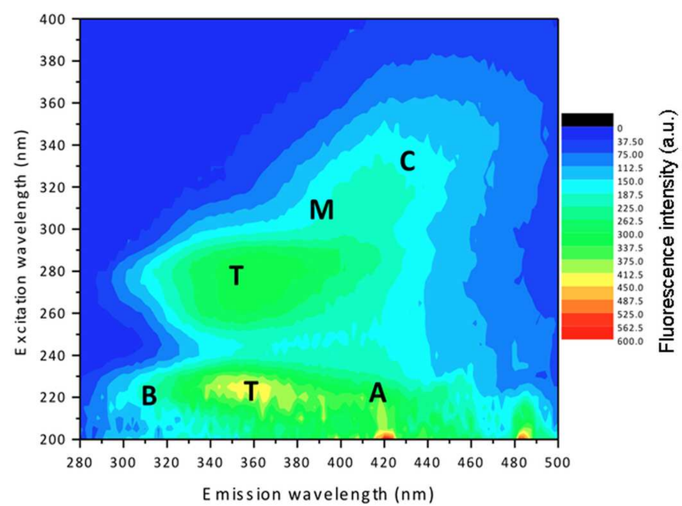

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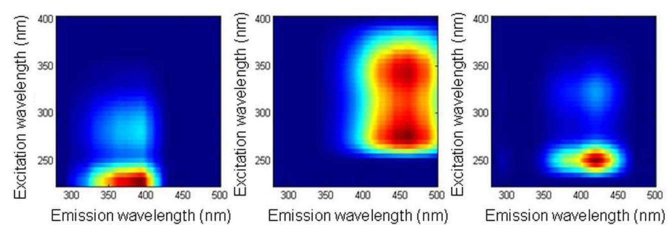

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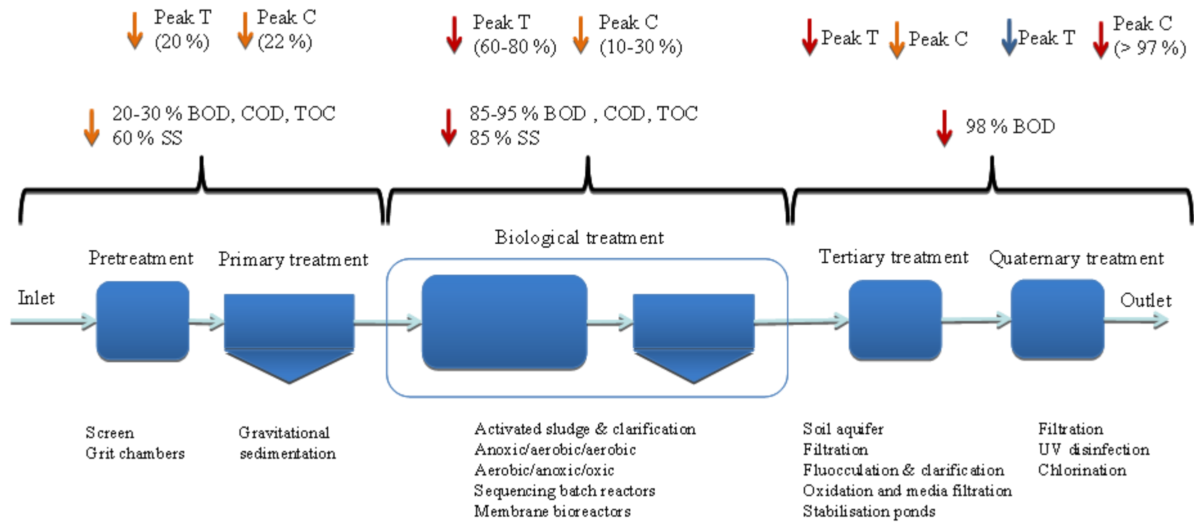

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- Several fluorophores contribute to common peaks hindering pollution source tracking
- Previous on-line studies may help build a strategy for wastewater analysis
- Dilution of samples, typically up to x10, useful to limit inner filter effect
- Calibration may not be needed for qualitative data
- Research gaps: online application of fluorescence and rapid data processing tools

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