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25 detecting both minor changes in effluent quality and issues with treatment process
26 performance.

27 **Keywords:** real-time monitoring, fluorescence spectroscopy, wastewater treatment
28 plant, organic matter

29 **Introduction**

30 The most significant energy usage in wastewater treatment plants (WwTPs) arises from
31 the vigorous aeration of settled sewage in the activated sludge process (ASP, an aerobic
32 system involving entrainment of air for microbial degradation of organic matter - OM). This
33 process contributes to over 55% of the energy budget associated with wastewater treatment
34 (Environmental Knowledge Transfer Network 2008). Due to the diurnal variations in
35 wastewater flow and load, and lack of rapid and reliable effluent monitoring (Bourgeois et al.
36 2001; Jouanneau et al. 2014), treatment plants often over-aerate the settled sewage to be
37 certain of achieving regulatory compliance, leading to excessive energy consumption and
38 unnecessary operating costs.

39 In the past two decades, several studies have demonstrated, through off-line monitoring
40 experiments, the potential of fluorescence spectroscopy for treatment process control (Ahmad
41 and Reynolds 1995; Bridgeman et al. 2013; Cohen et al. 2014; Murphy et al. 2011; Ou et al.
42 2014; Singh et al. 2012, 2015; Tartakovsky et al. 1996). The technique offers practical
43 advantages, such as: fast measurements, cost-effectiveness, lack of need for reagents, and
44 high sensitivity (Coble et al. 1990; Yang et al. 2015). However, no on-line fluorescence
45 monitoring studies have been performed at WwTPs. To date, Galinha et al. (2011) have
46 undertaken the only real-time monitoring study of wastewater on a pilot scale membrane
47 bioreactor system to predict performance parameters. They found that fluorescence was able
48 to describe influent and effluent chemical oxygen demand (COD), but could not predict other
49 performance parameters. Singh et al. (2015) obtained promising results from an online

50 monitoring study on two water recycling sites. Using a single-wavelength fluorescence
51 sensor, they were able to prove the robustness of the technique in detecting reverse osmosis
52 membrane fouling and integrity. Moreover, Singh et al. (2015) showed that the sensor was
53 sufficiently sensitive to identify underperformance issues. Real-time monitoring of treated
54 wastewater at WwTPs has been hampered by numerous factors that can interfere with the
55 fluorescence signal: fouling, pH, inner filter effects, temperature and metal ion presence
56 (Henderson et al. 2009; Reynolds 2002). To counteract these issues, regular, time consuming
57 cleaning of contact surfaces or subsequent data corrections are recommended.

58 Here, we report the first *in-situ* and on-line monitoring of treated wastewater, using
59 three fluorescence portable devices, to test the robustness of the technique and the hypothesis
60 that we can obtain valuable results from a 1-month monitoring experiment without major
61 device cleaning or subsequent data correction. In addition, a laboratory scale activated sludge
62 system was constructed to establish, before the in-situ experiment, the relationship between
63 fluorescence and BOD.

64 **Methodology**

65 The real-time experiment was undertaken for 29 days, from the 10th of August until the
66 7th of September 2015, at a WwTP located in the West Midlands, UK. The treatment plant
67 serves a region of 450,000 population equivalent and collects on average 120 ML/day of
68 wastewater from various types of sources: household, surface runoff, industrial (soluble oil,
69 chemical laboratory waste, engine cleaning, painting wastes, laundering, meat processing,
70 slaughterhouse, print waste etc.). In addition, the WwTP receives activated sludge mixed
71 liquor from a nearby sewage sludge facility at periodic intervals. During the experiment,
72 liquor was pumped, before noon, on the: 13th, 15th, 21st and 24th of August 2015.

73 The treatment process train consists of coarse and fine screens at the inlet, six primary
74 sedimentation tanks, 3 activated sludge reactors and 12 final settlement tanks. The primary

75 treatment step removes solids as well as oil and grease, after which, the remaining wastewater
76 is delivered to the ASP, comprising three basic components: 1) a reactor in which
77 microorganisms are kept in suspension, aerated, and in contact with the wastewater they are
78 treating; 2) liquid-solid separation; and 3) a sludge recycling system for returning activated
79 sludge back to the beginning of the process.

80 ***Real-time monitoring***

81 *Laboratory scale ASP experiment*

82 Before the in-situ measurements were undertaken, a laboratory scale ASP was
83 constructed to check the feasibility of the method and the relationship with BOD. Settled
84 sewage and returned activated sludge (RAS) were collected twice a week from the WwTP and
85 stored at 4⁰ C prior to use. The setup consisted of a feed primary tank (30 L volume), aeration
86 tank (10 L volume) and final settling tank (4 L volume) (Fig. S1). The settled sewage was
87 pumped into the aeration tank at a rate of 11 mL/min. Two aquarium air stones were inserted
88 in the aeration tank to replicate the aeration process and two stirrers ensured a greater degree
89 of mixing. A stirrer was inserted in the final settling tank to ensure settlement of the sludge
90 flocs. The settled sludge was returned to the aeration tank via a peristaltic pump at a rate of 11
91 mL/min. An average mixed liquor suspended solids (MLSS) concentration of 3,300 mg/l was
92 maintained in the aeration tank. When the quantity of MLSS decreased, additional RAS was
93 added without changing the volume of liquor in the aeration tank. The health and population
94 of microorganisms in the activated sludge reactor were checked regularly via microscope. The
95 experiment ran for six weeks and samples were collected daily for fluorescence, BOD₅, COD
96 and total organic carbon (TOC) analyses. Dissolved oxygen concentration and pH were
97 monitored every 30 min in the ASP tank.

98 *In situ measurements*

99 Three portable fluorescence instruments were installed and left unattended at the WwTP

100 final effluent discharge point, before the discharge to the river. Specifically, these were two
101 submersible probes (Cyclops 7, Turner Designs; EXO1 sonde, YSI Xylem) and a cuvette-
102 based (DuoFluor; designed and manufactured at the University of Birmingham) (Bridgeman
103 et al. 2015). The Cyclops 7 and EXO1 were inserted directly into the final effluent channel.
104 Proprietary protective caps were placed over the two submersible sensors and they were not
105 cleaned for the duration of the experiment. The sensors were also secured with ropes to
106 prevent excessive movement caused by the fluid flow.

107 The cuvette-based DuoFluor device was installed in an adjacent shed for power
108 connection and protection from rainfall (Fig. S2). The final effluent was pumped to the
109 fluorimeter at a flow-rate of 340 mL/min. A mesh covered the pump end tube to prefilter the
110 water and prevent debris from entering the cuvette. However, biofilm growth was observed
111 with time on the cuvette walls and on the tubing. Consequently, the cuvette was washed (10
112 % nitric acid) and rinsed with de-ionised water on a weekly basis, and the tubing was replaced
113 after two weeks.

114 The measurement frequency was set at 15 min for all instruments. Cyclops 7 was
115 initially set up to measure every 30 min, however the number of data points was insufficient
116 to obtain an adequate assessment of water quality fluctuations. No problems occurred with the
117 submersible devices. However, operation of the DuoFluor ceased one week before the end of
118 the experiment due to power failure.

119 ***Measurements***

120 *Fluorescence peaks*

121 This study focused on specific fluorescence components, assigned to spectral regions T
122 ($\lambda_{\text{ex}}/\lambda_{\text{em}} - \sim 280 \text{ nm} / 350 \text{ nm}$) and C ($\lambda_{\text{ex}}/\lambda_{\text{em}} - \sim 330 \text{ nm} / 425 \text{ nm}$), which can be used to
123 assess the quality of wastewater (Carstea et al. 2016). Peak T is generally associated with
124 living and dead cellular material and their exudates and indicates microbial activity

125 (Bridgeman et al. 2013). Peak T is also widely associated with material derived from
126 anthropogenic activities (Yu et al. 2014). Several fluorophores could contribute to these
127 regions (Carstea et al. 2016; Coble et al. 2014). Considering the variety of wastewater
128 discharges received by the WwTP and the wavelengths used by the devices, the following
129 components could fluoresce in the peak T region: lignins, aromatic hydrocarbons and indoles
130 originating from domestic waste (partially degraded foods, undigested dietary fibre, toilet
131 paper, proteins and peptides), petrochemical, pharmaceutical and paper industries. Peak C is
132 defined as reduced quinone-like and was identified in OM from a wide variety of aquatic
133 systems, especially those dominated by terrestrial and microbial inputs (Ishii and Boyer
134 2012). Potential contributors to the fluorescence of peak C could be: lignin breakdown
135 products, quinones, flavonoids, humic acids and fluorescent whitening agents (FWAs)
136 originating from municipal wastewater (food, plants, microbes, fungi, laundry detergents,
137 sanitary products, toilet paper and tissues) and paper making industry (Carstea et al. 2016). In
138 a recent study, it was shown that the removal rates of peaks T and C correlated with the
139 removal of pharmaceuticals, such as gemfibrozil, ibuprofen and naproxil, and with personal
140 care products, such as triclosan or caffeine (Sgroi et al. 2016). Thus, the exact composition of
141 fluorophores cannot be determined by the measurement of peaks T and C, however, these
142 peaks are highly effective in showing the removal of wastewater OM. Apart from these two
143 peaks, the common fluorescence regions reported for FWAs, at excitation wavelength 370 nm
144 and 400 nm (Coble et al. 2014), were also considered, due to the proximity of EXO1
145 excitation wavelength to one of the FWAs peaks. Past studies (Assaad et al. 2014; Chandler
146 and Lerner 2015; Graham et al. 2015), proposed FWAs as indicators of human faecal
147 contamination, sewer misconnections and landfill leachates.

148 *Fluorescence measurements*

149 Fluorescence was measured with three portable fluorimeters. Cyclops 7 measures the

150 fluorescence intensity at the excitation / emission wavelengths of 285 nm / 350 ± 55 nm, with
151 a limit detection range of 3 ppb to 5,000 ppb tryptophan standard. EXO1 sonde houses three
152 sensors: fDOM (fluorescence dissolved OM), conductivity/temperature and pH. The fDOM
153 sensor records at 365 ± 5 / 480 ± 4 nm (excitation / emission wavelength pair). The detection
154 range is 0 ppb - 300 ppb quinine sulphate units. DuoFluor is capable of detecting fluorescence
155 in real time at 280/350 nm (Peak T) with minimum limit of detection 1.5 ppb of L-tryptophan
156 and at 330/425 nm (Peak C) with minimum limit of detection 1.5 ppb of quinine sulphate. The
157 linearity between the portable devices and a benchtop spectrofluorimeter (Varian Cary
158 Eclipse) was checked with a series of dilutions of L-tryptophan and quinine sulphate
159 standards (Fig. S3). L-tryptophan solutions were varied between 50 ppb and 250 ppb, while
160 quinine sulphate was prepared in concentrations of 10 ppb to 700 ppb. The linearity of the
161 EXO1 was checked up to 400 ppb of quinine sulphate, as recommended by the manufacturer.
162 R² values exceeded 0.98 for all instruments.

163 Excitation-emission matrices were produced using the benchtop spectrofluorimeter: by
164 scanning excitation wavelengths from 200 to 400 nm in 5 nm steps, and detecting the emitted
165 fluorescence in 2 nm steps between 280 and 500 nm. Excitation and emission slit widths were
166 set to 5 nm. Instrument stability was checked by recording the Raman values (at excitation
167 wavelength 348 nm and emission wavelength 395 nm) before each set of measurements. The
168 average Raman value was 9.94 a.u. with a standard deviation of 0.24. The fluorescence peaks
169 were extracted using the peak-picking method, in accordance with previous studies (Coble et
170 al. 2014).

171 *Ancillary analyses*

172 Rainfall, temperature, total phosphorus, iron, ammonia and suspended solids were
173 measured daily on-site at the WwTP outfall. In addition, samples were collected twice a week
174 for BOD₅, COD, TOC, nitrate and turbidity (Table S1). Low values were observed for all

175 parameters, indicating effective treatment of the wastewater. BOD₅ was measured based on
176 the standard method for wastewater testing using a HQ40d portable meter (Hach) with an
177 IntelliCAL LBOD101 LDO probe. The accuracy of the BOD₅ measurements was checked
178 using a 300 mg/L glucose-glutamic acid standard, and a coefficient of variation of 3.6 % was
179 observed. COD and nitrate were measured using a DR890 Hach colorimeter, following
180 standard procedures: *viz.* Reactor Digestion Method (USEPA) for COD, and Chromophoric
181 Acid Method (high range, Test 'N Tube) for water and wastewater for nitrate. Turbidity was
182 recorded using a Hach 2100N turbidimeter. TOC measurements were undertaken using a
183 Shimadzu TOC-Vcpn analyser, using the non-purgeable organic carbon determination
184 method.

185 **Results and discussion**

186 ***Laboratory scale ASP***

187 Before the *in-situ* study, a laboratory-based experiment was undertaken replicating the
188 ASP to establish the relationship with BOD and to determine the potential of using
189 fluorescence spectroscopy for real-time measurements. WwTPs measure BOD on a daily
190 basis; however, a qualitative method is used, which provides ranges of BOD values and the
191 result cannot be compared with fluorescence intensity. The regulatory 5-day BOD test is
192 performed only once per month. Therefore, the laboratory scale ASP was designed to identify
193 this fluorescence/BOD relationship.

194 Figure 1 shows the fluorescence intensity of peaks T and C measured with the benchtop
195 fluorimeter plotted against BOD. The Kendall correlation coefficients with BOD₅ are: 0.71
196 ($p < 0.001$ – 2-tailed test of significance, $N=87$) for peak T; and 0.43 ($p < 0.001$ – 2-tailed test of
197 significance, $N=87$) for peak C. The correlation between BOD and fluorescence is
198 challenging to identify at low BOD concentrations (Hudson et al. 2008), thus the values
199 quoted above were determined using a combination of data from final effluent and settled

200 sewage samples. An improved correlation was observed for BOD with peak T compared to
201 the peak C/BOD relationship was reported in other studies (Bridgeman et al. 2013; Hudson et
202 al. 2007). The various types of fluorophores that contribute to the peaks T and C fluorescence
203 region explain the difference in correlation values. In addition, Reynolds (2002) found that
204 peak T is more representative for the biodegradable organic matter than peak C. Considering
205 the strong correlation between peak T and BOD, obtained in this study, and the relationship
206 reported in other studies (Bridgeman et al. 2013; Carstea et al. 2016; Coble et al. 2014;
207 Hudson et al. 2008), it is clear that peak T fluorescence can detect some of the components
208 measured with BOD. Furthermore, fluorescence spectroscopy provides more information on
209 the nature of OM than the BOD test does and may be used as an independent indicator test for
210 the presence of bioavailable OM (Hudson et al. 2008).

211 Similar relationships were obtained between fluorescence and COD and TOC. The
212 Kendall correlation coefficients with COD are: 0.72 ($p < 0.001$ – 2-tailed test of significance,
213 $N=87$) for peak T; and 0.44 ($p < 0.001$ – 2-tailed test of significance, $N=87$) for peak C. While,
214 the Kendall correlation coefficients with TOC are: 0.82 ($p < 0.001$ – 2-tailed test of
215 significance, $N=81$) for peak T; and 0.49 ($p < 0.001$ – 2-tailed test of significance, $N=81$) for
216 peak C. The good correlation between peak T and TOC may be attributed to the sugars and
217 lignin (Baker 2002) degraded from sanitary products. However, the relationship between
218 fluorescence peaks and BOD, TOC and COD varies depending on the ratio of fluorescent to
219 non-fluorescent OM in a sample (Henderson et al. 2009).

220 *In situ measurements*

221 Peaks T and C data provided by the 3 devices are shown in Figure 2. Kendall
222 correlation analysis showed an association between EXO1 data and DuoFluor peaks T and C
223 ($R^2=0.49$ & 0.48 , $p < 0.001$), while Cyclops 7 data presented a slight correlation with the
224 DuoFluor peak T ($R^2=0.28$, $p < 0.001$) (Table 1). The analysis also revealed that the EXO1 and

225 the DuoFluor data correlated with peaks T, C and FWAs measured with the Varian benchtop
226 spectrofluorimeter. The variation in correlation coefficients might be explained by the
227 differences in excitation and emission wavelengths used by the devices. For instance, the
228 EXO1 excitation wavelength is closer to the optical region of FWAs, compared to the region
229 where peak C is generally reported (Coble et al. 2014), and compared to the peak C excitation
230 wavelengths measured with the DuoFluor and Varian Cary Eclipse. In addition, the
231 correlations with Varian Cary Eclipse data were established using a small sample size (N=8),
232 a larger dataset being needed to obtain statistically significant correlations. However, the
233 results are sufficient to provide an indication of devices potential to measure peaks T and C *in*
234 *situ*.

235 During the experiment, the DuoFluor system recorded a constant decrease in peak C
236 fluorescence intensity (Fig. 2D) due to biofilm formation on the cuvette. Regular cuvette
237 cleaning (twice per week) was required to ensure adequate DuoFluor fluorescence results.
238 The EXO1 and the Cyclops 7 sensors were not cleaned during the entire experiment and no
239 substantial reduction in fluorescence intensity was observed. However, further studies are
240 needed to test the time span until fouling interferes with the fluorescence signal. This
241 experiment shows that submersible instruments are more practical at WwTPs. The advantages
242 of needing less frequent cleaning (no cleaning for at least 1 month) and being battery powered
243 make them preferable for effluent monitoring. Fluorescence data were not corrected for
244 thermal quenching as little impact was expected for a decrease of 0.5⁰ C from day to night and
245 of 3⁰ C change over the entire period (Fig. S4A). Based on previous work (Carstea et al.
246 2014), it is estimated that the fluorescence intensity would increase by 0.3 % for a decrease in
247 temperature of 0.5⁰ C and by 2.6 % for a 3⁰ C temperature change. Temperature correction
248 may be needed in areas with high seasonal variation. Inner filter effect is also known to
249 impact the fluorescence measurements. However, Henderson et al. (Henderson et al. 2009)

250 showed that the inner filter effect is unlikely to occur in surface and wastewater samples with
251 a TOC concentration below 25 mg/l. The final effluent TOC concentrations measured within
252 the current experiment varied between 6.29 mg/L and 9.28 mg/L. Moreover, the same
253 samples showed absorbance values below 0.20 at 254 nm, this being the threshold
254 recommended by Aiken (Coble et al. 2014) for optically dilute samples. Metal ions have been
255 shown to affect the fluorescence intensity and peak position of OM components (Coble et al.
256 2014). The average iron concentration measured at the WwTP final effluent discharge point
257 was 0.30 mg/L (Table 2). Poulin et al. (Poulin et al. 2014) found that an iron:organic carbon
258 ratio of 0.3 would reduce the fluorescence intensity between 7 % and 23 % depending on the
259 type of water sample. In this study, an average value of 0.03 for the iron:organic carbon ratio
260 was observed. Suspended solids have been shown to influence the results from in situ
261 fluorimeters (Coble et al. 2014). However, Belzile et al. (2006) found a strong correlation
262 between a submersible fluorimeter and a benchtop spectrofluorimeter, at unfiltered samples
263 with suspended solids concentrations below 35 mg/L. In the current study, the effluent
264 suspended solids concentrations varied from 4.5 mg/L to 20.7 mg/L. Filtration, which would
265 reduce the quantity of suspended solids, may also contaminate the sample and remove a large
266 fraction of fluorescent components that are found in particulate or colloidal form (Coble et al.
267 2014). Furthermore, one aim of this study was to test the robustness of fluorescence
268 spectroscopy to monitor effluent quality without major intervention during or after
269 measurement. For this purpose, a qualitative analysis of effluent OM, i.e. without correction
270 for inner filter effect or extensive calibration, was sufficient to detect changes in effluent
271 water quality.

272 Peaks T and C displayed a diurnal variation with a cycle of approximately 12 h, the
273 highest intensity being recorded around midnight and the lowest intensity at noon (Fig. 2).
274 During dry weather days, peak T displayed a decrease in fluorescence intensity of < 9 % for

275 the Cyclops 7 and 16 % for the DuoFluor between midnight and noon, while peak C
276 decreased by < 10 % for the EXO1 sensor and 17 % for the DuoFluor over the same period.
277 The diurnal variation in fluorescence intensity was consistent with the changes in effluent
278 flow rate, conductivity and pH (Fig. S4 (B) and (C), and Fig. S5). However, fluorescence
279 intensity was not directly proportional to the degree of increase in flow rate. The effluent flow
280 rate presented 2 peaks, every day, of almost equal intensity (Fig. S5). We also observed two
281 peaks in the fluorescence data; the first peak being recorded at midnight and the second peak
282 at approximately 2 pm (Fig. 3). This 2 pm peak was substantially lower in intensity compared
283 with the midnight peak, although high flow rate was recorded. It is concluded that these
284 midnight and 2 pm peaks correspond to intensive household water use during the mornings
285 and evenings. Considering the total wastewater retention time within the WwTP from inlet to
286 discharge point (12-16h) and the additional retention time in the sewerage network from
287 household to the WwTP, it is believed that the high values of peaks T and C observed at
288 midnight correspond to the previous day morning high wastewater input, while the 2 pm peak
289 represents the previous evening water usage.

290 Several rainfall periods, of different intensity and duration, were recorded during the
291 real-time experiment (Table 1). We divided the precipitation days into 4 events: event I – 13th
292 to 14th of August; event II – 19th of August; event III – 23rd to 27th of August; event IV – 30th
293 of August to 3rd of September. The WwTP is served by a combined sewerage system and
294 therefore rainfall increases the influent flow and modifies the properties of the influent
295 affecting process performance and effluent quality (Wilén et al. 2006). Therefore, it is
296 believed that the amount and frequency of precipitation affects most of the measured water
297 quality parameters, depending on the catchment and sewerage system. Rain events were seen
298 to trigger high ammonia and iron values (Table 2). Precipitation also increased the
299 concentration of total phosphorus; the highest value being recorded during or after the first

300 day of the rain event. Conductivity and pH decreased after each rain event, depending on the
301 intensity of the event (Fig. S4). Conductivity showed a significant decrease after events I and
302 IV, while pH was the parameter least affected by precipitation.

303 A decrease in fluorescence intensity was observed one day after the beginning of each
304 precipitation event (Fig. 2). Precipitation events I and IV generated the greatest decrease in
305 Peak C (32 % & 42 % respectively) measured using the EXO1. Cyclops 7 recorded Peak T
306 reductions of 25 % (event I) and 28 % (event IV). DuoFluor measured a 26 % decrease in
307 peak C and 25 % in peak T following event I. The full impact of event IV was not assessed
308 with the DuoFluor due to data loss following a power outage at the WwTP. However, the
309 same effect is observed on peaks T and C after the other rain events. Overall, the decrease in
310 fluorescence intensity is consistent with the quantity of rain per event. After each rain event
311 the fluorescence intensity increased progressively until the next rainfall. Previous studies on
312 urban river monitoring (Carstea et al. 2009) showed that peaks T and C intensity increased
313 after precipitation events, due to the release of higher quantities of OM with surface runoff
314 compared to the receiving water. Here, a dilution of the wastewater's heavily concentrated
315 OM was observed. Others (Mines et al. 2007) also reported a dilution effect, reflected in a
316 decrease in BOD values. Since BOD correlates with fluorescence (Bridgeman et al. 2013), a
317 rainfall-generated decrease in fluorescence intensity is anticipated.

318 In addition to the daily variation and impact from precipitation, two data anomalies
319 were identified on the 24th of August and 3rd of September, both immediately after midnight
320 (Fig. 2– circled with red). These anomalies are most evident from the EXO1 sensor data. The
321 data are higher than the normal daily variation, with or without precipitation, and may be
322 associated with changes in influent quality or treatment processes. The first anomaly is
323 explained by the release of liquor from the sewage sludge facility on the 24th of August at
324 12:00pm. The WwTP managers report that silt is occasionally released with the liquor,

325 resulting in elevated concentrations of ammonia in the effluent. Unusually high ammonia was
326 recorded at the same time as the high fluorescence intensity (Fig. S5). The high fluorescence
327 intensity during the first anomaly could indicate the production of autochthonous OM from
328 the sewage sludge liquor (Cohen et al. 2014; Riopel et al. 2014), as peak C components
329 increase in the soluble microbial products with increasing retention times (Yu et al. 2015).
330 Also, condensed polymerized humic-like material may form during biodegradation (Saadi et
331 al. 2006). Therefore, liquor may carry large quantities of autochthonous OM, some of it
332 biologically resistant, produced during the long retention times, along the stages of the
333 sewage sludge facility.

334 The second anomaly (Fig. 2) is a result of the power issues that occurred at the WwTP.
335 On the 3rd of September, low power caused the aeration tank air blowers to fail. Fluorescence
336 data can be used to identify the process failure. The increase of peak C fluorescence from the
337 second anomaly may represent FWAs present in the sewage. Peak C wavelengths coincide
338 with the fluorescence regions of FWAs (Henderson et al. 2009). However, FWAs were also
339 measured in the excitation/emission wavelengths region of 250 nm / 344 nm and 422 nm
340 (Boving et al. 2004). Almost 80 % of FWAs are removed after the biological treatment and
341 these compounds may be used as molecular markers of less effective treatment processes
342 (Hayashi et al. 2002). Therefore, temporary interruption of the ASP tanks would have led to
343 the presence of untreated FWAs, as seen in the second anomaly.

344 Thus, real-time, *in situ* analysis demonstrated the ruggedness of fluorescence
345 spectroscopy and the ability to detect minor changes in effluent quality. Fluorescence
346 spectroscopy could be used to identify underperformance issues, albeit with a time lag
347 between the failure and the feedback information. However, fluorescence spectroscopy still
348 represents a fast and effective control method, and a reliable alternative to BOD. The benefits
349 of improved treatment control via fluorescence spectroscopy go beyond CO₂ reductions and

350 climate change mitigation, as they will also facilitate environmental improvements, reduce
351 operating costs and improve the financial performance of the global wastewater industry.

352 **Conclusions**

- 353 • This study reported the first real-time monitoring of effluent wastewater using
354 fluorescence spectroscopy. Results show that fluorescence spectroscopy is a
355 robust technique for monitoring changes in effluent quality. It also shows that
356 portable devices can run continuously, for 1 month, without any cleaning
357 procedure in the case of submersible systems (or with limited regular cleaning
358 for cuvette-based fluorimeters). Further studies are needed to test the time span
359 until fouling interferes with the fluorescence signal. In addition, multiple sites
360 should be considered in future studies to account various peculiarities of
361 wastewater input.
- 362 • Fluorescence peaks T and C showed that OM varied diurnally depending on the
363 flow rate. Precipitation decreased the fluorescence intensity of both peaks due to
364 dilution of wastewater with runoff. The degree of decrease in fluorescence
365 intensity was found to be proportional to the quantity of rainfall.
- 366 • 15 min measurement frequency yielded sufficient data to obtain a detailed
367 assessment of daily variation, precipitation impact on influent quality and
368 treatment process.
- 369 • A qualitative analysis of effluent OM, i.e. without correction for inner filter
370 effect or extensive calibration can detect changes in effluent water quality.
371 However, temperature correction may be needed in areas with high seasonal
372 variation. Inner filter effect correction may be required when quantitative
373 measurements are needed.
- 374 • Submersible instruments proved to be a more practical tool for *in situ*

375 measurement compared to the cuvette-based device. The advantages of reduced
376 cleaning frequency (no cleaning for at least 1 month) and battery operation make
377 them preferable for effluent OM monitoring.

378 • Results showed that fluorescence intensity of peaks T and C was capable of
379 detecting minor changes in influent OM quantity and issues with treatment
380 process. The substantial impact on peak C fluorescence intensity with changes in
381 the system was attributed to the input of autochthonous OM from sewage sludge
382 liquor and the presence of untreated FWAs. Although the variation in
383 fluorescence was more clearly observed at peak C compared to peak T, it is
384 recommended that both peaks are monitored due to variations in wastewater
385 composition.

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394 **Supplemental Data**

395 Figs. S1-S5 and Table S1 are available online in the ASCE Library (ascelibrary.org).

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522

523 **Figure captions**

524 **Fig. 1.** Relationship between BOD and fluorescence. (A) peak T and (B) peak C (N=87).

525 **Fig. 2.** In situ fluorescence measurements. Peak T measured with (A) Cyclops 7 and (B)
526 DuoFluor. Peak C measured with (C) EXO1 and (D) DuoFluor. Rainfall events I-IV are
527 marked with blue and anomalies are circled with red. The DuoFluor stopped recording during
528 rain event IV due to a power failure at the WwTP. The large differences in the fluorescence
529 intensity observed at graph (D) for the dates Aug 17, Aug 20, Aug 24 and Aug 28 were
530 caused by cuvette cleaning on the DuoFluor.

531 **Fig. 3.** Examples of daily fluorescence variation for the 3 portable devices. (A) peak T
532 fluorescence and (B) peak C fluorescence. The 2 pm peak is marked with a blue square.

533 **Table 1.** Kendall Correlation Between the Data from Portable Devices and Varian Benchtop
 534 Spectrofluorimeter.

Device		Cyclops 7	EXO1	DuoFluor		Varian		FWA 370	FWA 400
				Peak T	Peak C	Peak T	Peak C		
Cyclops 7		1	0.19	0.28	0.02	0.21	0.21	0.29	0.36
EXO1		-	1	0.49	0.48	0.64	0.64	0.86	0.93
DuoFluor	Peak T	-	-	1	-	0.33	0.20	0.47	0.47
	Peak C	-	-	-	1	0.60	0.47	0.73	0.73

535 Note: Correlation coefficients in bold have p values below 0.001 (p-values < 0.001 are considered significant).

536 **Table 2.** Standard Parameters Measured by the WwTP.

Date	Precipitation (mm)	Temperature (°C)	Total Phosphorus (mg/L)	Iron (mg/L)	Ammonia (mg/L)
10.08	0.0	19.5	0.57	0.31	0.06
11.08	0.0	18.9	0.73	0.29	0.06
12.08	0.0	18.8	0.70	0.33	0.21
13.08	33.0	19.4	0.44	0.34	0.21
14.08	3.0	18.5	0.98	0.49	2.37
15.08	0.0	19.2	0.47	0.35	0.35
16.08	0.0	19.7	0.30	0.28	0.30
17.08	0.0	18.5	0.62	0.28	0.06
18.08	0.0	18.2	0.50	0.33	0.06
19.08	8.0	18.9	0.44	0.37	0.08
20.08	0.0	19.2	0.61	0.33	0.30
21.08	0.0	19.5	0.46	0.25	0.06
22.08	0.0	19.7	0.38	0.35	0.35
23.08	9.0	20.1	0.37	0.46	0.32
24.08	9.0	18.9	0.55	0.27	0.06
25.08	7.0	18.4	0.66	0.30	1.81
26.08	0.5	18.7	0.38	0.26	0.06
27.08	0.5	18.3	0.48	0.25	0.06
28.08	0.0	18.4	0.39	0.21	0.06
29.08	0.0	18.9	0.49	0.24	0.06
30.08	18.0	19.2	0.51	0.17	0.06
31.08	12.0	18.3	0.39	0.25	0.06
01.09	25.0	17.0	0.51	0.20	0.10
02.09	4.0	17.0	0.30	0.19	0.27
03.09	1.0	17.3	0.37	0.23	0.07
04.09	0.0	17.1	0.50	0.40	0.68
05.09	0.0	17.1	0.37	0.25	0.40
06.09	0.0	17.4	0.49	0.24	0.30
07.09	0.0	17.7	0.54	0.34	0.15

537

Supplementary data

Online fluorescence monitoring of effluent quality in wastewater treatment plants

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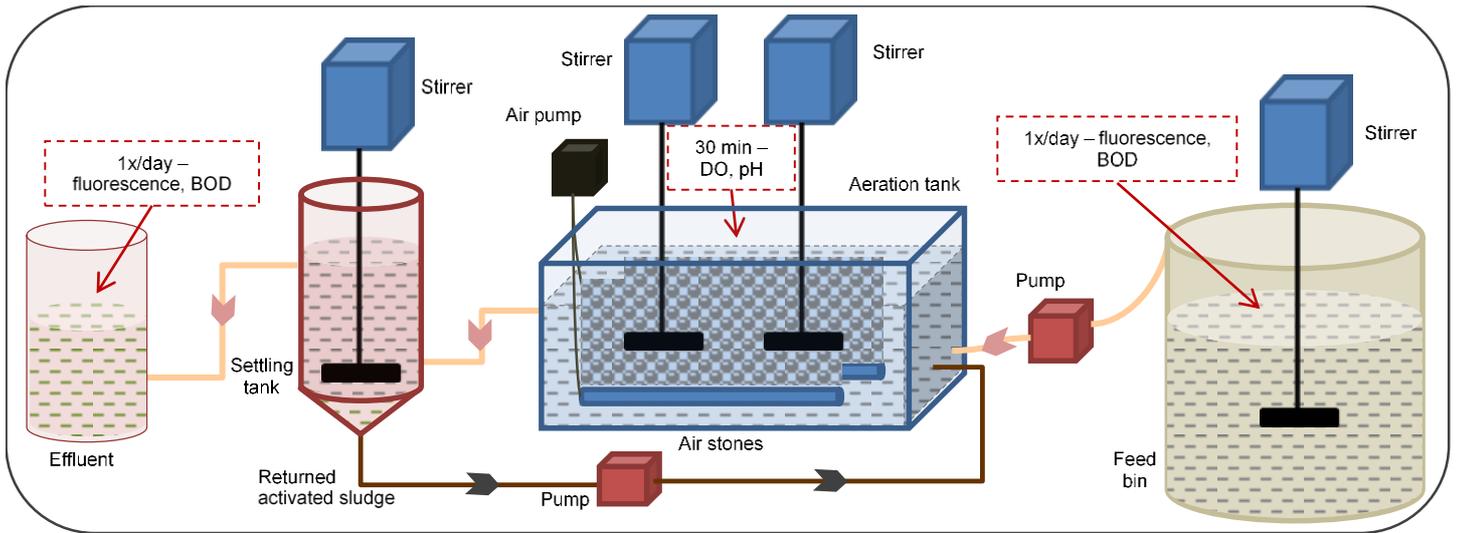


Fig. S1. The setup for the laboratory scale activated sludge process. BOD – biochemical oxygen demand; DO – dissolved oxygen. The orange and black arrows indicate the direction of the flow.

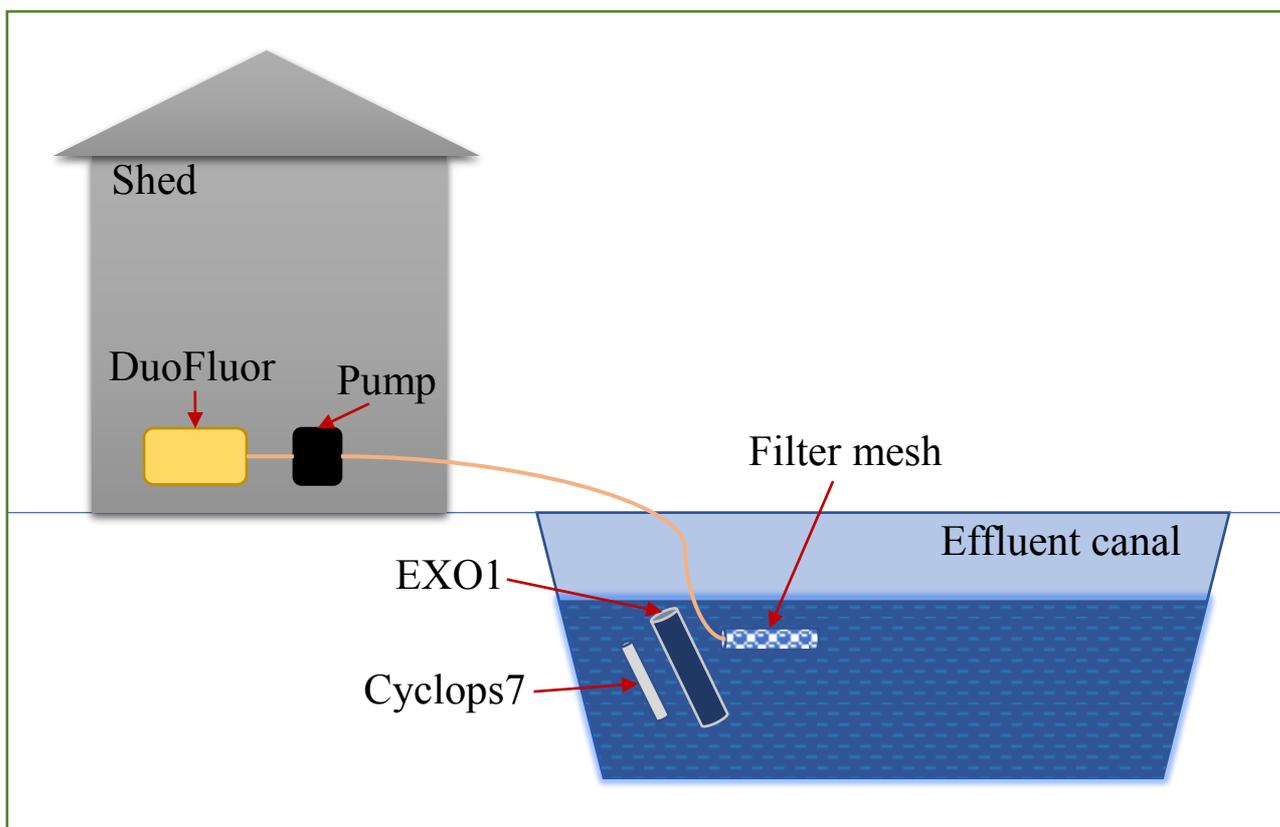


Fig. S2. The setup for the *in-situ* fluorescence measurements. The EXO1 and Cyclops 7 were connected to handheld devices and tightened with ropes to the cover grid above the effluent channel.

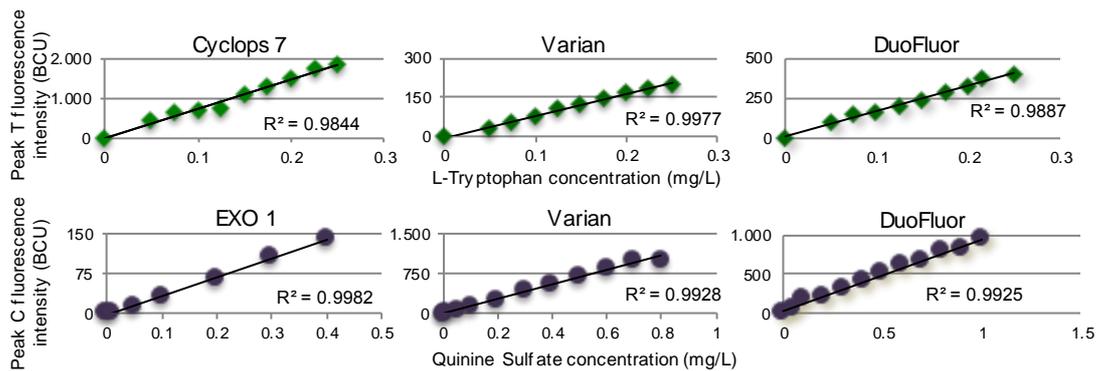


Fig. S3. Linearity check of the three portable devices and comparison with a benchtop spectrofluorimeter. The fluorescence intensity was corrected by extracting the blank spectrum.

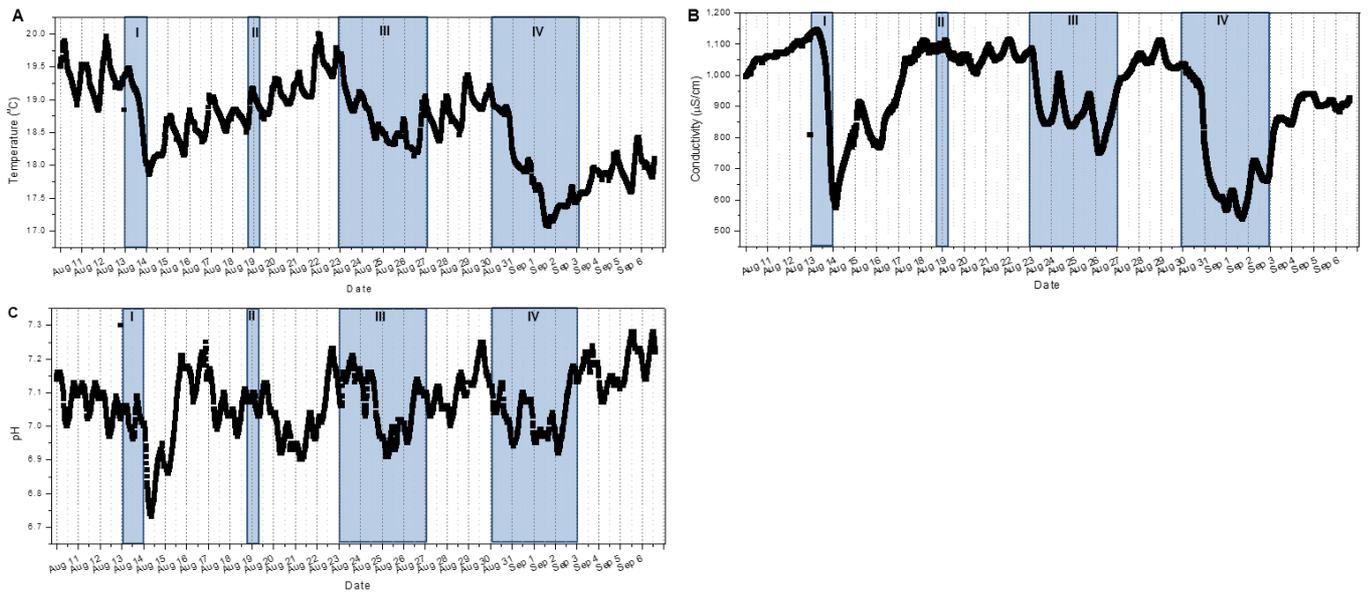


Fig. S4. In situ measurements with the EXO1 sonde. (A) temperature, (B) conductivity and (C) pH.

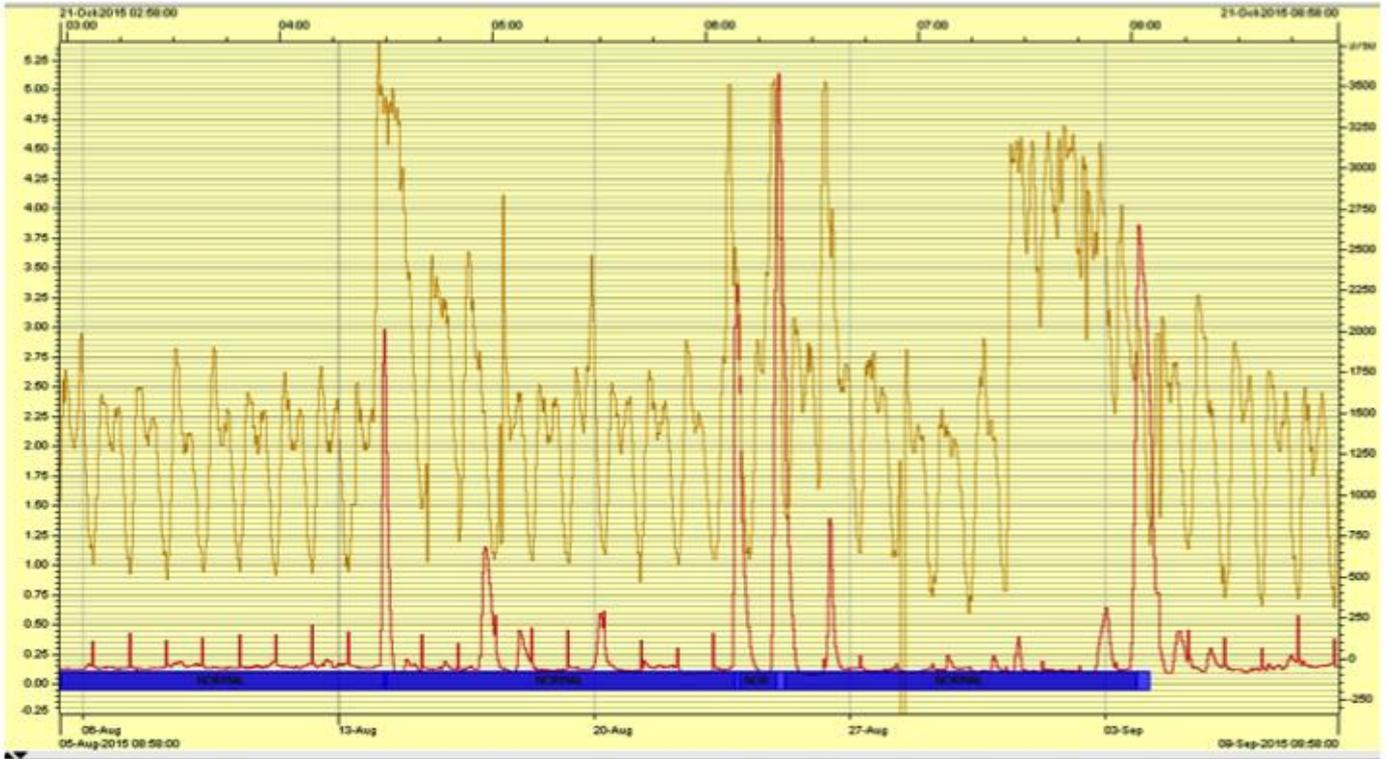


Fig. S5. Flow rate (brown line) and quantity of ammonia (red line) at the effluent. Graph provided by the WwTP.

Table S1. Standard parameters and peak T and peak C fluorescence for grab samples of final effluent. The fluorescence peaks were measured with a benchtop spectrofluorimeter.

Date	BOD	COD	TOC	Nitrates	Turbidity	Fluorescence intensity			
						Peak T	Peak C	FWA 370*	FWA 400*
	(mg/L)				(NTU)	(a.u.)			
13.08.2015	1.6	27	8.9	30.7	N/A	106	178	80	58
17.08.2015	2.9	23	8.5	23.8	2.18	100	166	68	51
20.08.2015	1.9	24	9.3	28.8	1.90	104	189	79	56
25.08.2015	3.0	30	7.9	19.0	1.85	94	148	67	50
28.08.2015	1.7	32	N/A	27.3	1.54	107	168	68	50
01.09.2015	2.7	15	6.3	14.9	2.18	76	129	58	38
03.09.2015	3.0	18	7.2	16.5	2.67	81	148	66	39
07.09.2015	1.7	17	8.5	22.1	1.96	97	159	68	48

* Fluorescence whitening agents' excitation wavelength